Selection, extraction, characterization and application of mucilage from cactus pear (*Opuntia ficus-indica* and *Opuntia robusta*) cladodes

by

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Alba du Toit

31 August 2016

“With your help I can advance against a troop; with my God I can scale a wall.”

(New International Version, Samuel 22:30)

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Summary/Abstract

Cactus pear plants (*Opuntia ficus-indica* and *Opuntia robusta*) are edible plants that grow and thrive in the semi-arid area of the Free State, South Africa, yet are unknown food sources. Cactus pear cladodes could be developed into a crop that could offer solutions in terms of food security, yet are ignored and underutilized in South Africa. Extensive research done abroad on the nutritional and therapeutic properties of cladodes and mucilage verified its high potential for human consumption. The development of mucilage into a commercial nutraceutical food product offers an opportunity to add value to the crop while providing a healthy product that could significantly enhance the well-being of the South African consumer. A successful, easy, inexpensive and chemical free extraction process was developed, yet cladodes were not equal in terms of the amount of quality of mucilage. Thus, the most appropriate cultivar for optimal mucilage yield and quality from forty-two local cultivars had to be selected and cladode harvesting time established.

It was found that mucilage yield was not a consequence of cladode size or moisture content, but of cultivar and viscosity. Seven cultivars were selected that proved to have the lowest viscosity while commercially viable in South Africa (*O. ficus-indica* cultivars namely Algerian, Meyers, Morado, Ficus-Indica, Gymno-Carpo, Tormentosa, Turpin). The eighth cultivar represented a different species (*O. robusta* Robusta). Further morphological and rheological investigations revealed that the dormant stage (dormant months) would be the optimal harvesting time, while the selection of cultivars were narrowed down to four (*O. ficus-ind*ica Algerian, Morado, Gymno-Carpo and *O. robusta* Robusta). Mucilage (native and freeze-dried powders) from all cultivars showed non-Newtonian, pseudoplastic tendencies. Higher viscosity mucilage was time dependent, rheopectic and had yield stress tendencies. Fluctuations in temperature, pH, concentration and electrolytes influenced the mucilage viscosity that could affect product texture.

In the analyses of the native mucilage, lower pH during summer was correlated to higher mucilage yields. The flow properties, water- and oil- related properties showed the high potential of mucilage powders as commercial nutraceutical product. The chemical analysis of freeze-dried mucilage powders showed high fibre and minerals, yet low fat and carbohydrates. Although the protein content was low, the presence of proteins was confirmed for the protein-lipid interaction necessary in the stabilization action of emulsions. The higher organic acids in summer concurred with the lower pH (as a consequence of CAM) that in turn affected the mucilage viscosity and yield. Mucilage was successfully applied to food products and is recommended for commercialization as a nutraceutical food product.

In terms of cultivar selection, ‘Robusta’ had the highest contents of protein, total fats (beneficial fatty acids), starch, potassium and phosphorous, while ‘Algerian’ mucilage had the lowest energy and insoluble fibre, but highest calcium, iron and copper contents. ‘Gymno-Carpo’ had high linoleic acid, magnesium and manganese and good fat ratios.

It is recommended that cladodes be harvested in the summer months, directly after the fruit had been harvested for optimal mucilage yield and quality. ‘Robusta’ emerged as the best cultivar for higher viscosity mucilage and Algerian for lower viscosity mucilage of the best quality. However, a careful selection of the most appropriate cultivar mucilage would be paramount for specific food applications.

Opsomming

Die turksvy (*Opuntia ficus-indica* en *Opuntia robusta*) is eetbare plante wat groei en floreer in die semi-droë area van die Vrystaat, Suid Afrika. Dit is egter ‘n onbekende voedsel bron. Turksvye kan ontwikkel as ‘n gewas waarvan die kladode ‘n oplossing kan bied in terme van voedselsekuriteit, tog word dit geïgnoreer en is onderbenut in Suid Afrika. Uitgebreide internasionale navorsing oor die voedings- en terapeutiese eienskappe van kladodes en slymgom het die potensiaal daarvan vir menslike gebruik bevestig. Die ontwikkeling van slymgom in ‘n kommersiële voedingsproduk bied ‘n geleentheid om waarde tot die gewas te voeg, terwyl dit ‘n gesonde produk kan lewer wat baie voordelig kan wees vir die welstand van die Suid-Afrikaanse verbruiker. ‘n Suksesvolle, maklike en chemies-vrye onttrekkingsproses is ontwikkel, tog het die kladodes nie dieselfde kwaliteit slymgom gelewer nie. Daarom moes vasgestel word watter kultivar die mees geskikste kultivar is in terme van die optimale slymgom opbrengs en kwaliteit uit 42 plaaslike kultivars. Die beste oestyd vir die kladodes moes ook bepaal word.

Daar is bevind dat die opbrengs van slymgom nie bepaal word deur die gewig of vog inhoud van die kladodes nie, maar deur die kultivar en viskositeit van die slymgom. Sewe kultivars met die laagste viskositeit, wat nogsteeds kommersieël lewensvatbaar is in Suid Afrika, is geïdentifiseer (*O. ficus-indica* kultivars: Algerian, Meyers, Morado, Ficus-Indica, Gymno-Carpo, Tormentosa, Turpin). Die agtste kultivar verteenwoordig ‘n ander spesie (*O. robusta* ‘Robusta’). Verdere morfologiese en rheologiese ondersoeke het getoon dat die dormante groeistadium die optimale oes tyd is, en dat die vier beste kultivars *O. ficus-indica*: Algerian, Morado, Gymno-Carpo en *O. robusta:* Robusta is. Slymgom (natuurlike slymgom en gevriesdroogde slymgom) van al die kultivars het nie-Newtoniese en pseudoplastiese neigings getoon. Slymgom met’n hoër viskositeit was tyd afhanklik, rheopeuties en het vloei spanning neigings getoon. Skommelings in temperatuur, pH, konsentrasie en elektroliete het die slymgom viskositeit beïnvloed, wat weer die voedselproduk se tekstuur kan affekteer.

Die lae pH-waardes van die plaaslike slymgom korreleer met die hoër slymgom opbrengste daarvan. Die vloei, water- en olie verwante eienskappe van die slymgom poeiers het potensiaal getoon as kommersiële nutraseutiese produkte. Die chemiese ontleding van die gevriesdroogde slymgom poeiers het hoë vesel en minerale inhoude getoon, maar ook lae vet en koolhidraat vlakke. Alhoewel die proteïen inhoud laag was, was die teenwoordigheid van proteïen bevestig

vir die proteïen-lipied interaksie, wat nodig was vir die stabiliserende aksie van die emulsies. Die hoër organiese suur inhoud van die slymgom, wat tydens die na-oes periode versamel is (as ‘n gevolg van CAM), het die slymgom viskositeit en opbrengs beïnvloed. Slymgom was suksesvol by voedselprodukte gevoeg en word aanbeveel vir die kommersialisering van ‘n nutraseutiese voedselproduk.

In terme van kultivar keuse, het ‘Robusta’ slymgom die hoogste proteïen, totale vette (voordelige vetsure), stysel, potassium en fosfor inhoude getoon, terwyl ’Algerian’ die laagste energie en onoplosbare vesel, maar die hoogste kalsium, yster en koper inhoude getoon het. ‘Gymno-Carpo’ het die hoogste linoleïensuur, magnesium, mangaan en goeie vet inhoud getoon.

Vir optimale slymgom opbrengs en kwaliteit word aanbeveel dat die kladodes direk nadat die vrugte geoes is in die somer (Februarie), geoes word. ‘Robusta’ het uitgestaan as die kultivar met ‘n hoër viskositeit slymgom en ‘Algerian’ met ‘n laer viskositeit slymgom met die beste kwaliteit. Kultivar keuse vir die beste slymgom produksie is egter van kardinale belang vir spesifieke aanwendings in voedselprodukte.

Key Terms

Opuntia ficus-indica

Opuntia robusta

Cactus pear

Prickly pear

Cladodes

Mucilage

Native mucilage

Freeze-dried mucilage

Food security

Arid and semi-arid

Rheological properties

Functional properties

Chemical properties

Nutritional content

Nutraceutical food products

Sustainable food source

Chapter 1

1 Introduction

In the semi-arid region of the Free-State, South Africa, grows a plant that is ignored and disregarded by most, however it is a healthy and unknown (in South Africa) food source for both humans and animals. In fact, this plant has the potential to reform agriculture in South Africa as it offers food security to semi-arid and arid regions (Potgieter, 2007). It could be developed into a profitable crop that would remain sustainable during droughts when no other forage or crop would survive (Russell and Felker, 1987). To animals it offers fodder, either in fresh or dried form and to humans it offers delicious fruit, high quality seed-oil, tasty side-dishes, health-improving properties and therapeutic potential. It is the ideal time for the potential of the cactus pear plant to be recognized and given an opportunity to take its rightful place in the agriculture and economy of this region.

Opuntia ficus-indica and *Opuntia robusta* (the cactus pear) are succulent species that are most intriguing plants due to their peculiar adaptations to survive during extreme heat, severe drought and poor soil conditions. In fact, cactus pear plants thrive during droughts and severe heat when only a few other plants could survive. The cactus pear uses an alternative form of photosynthesis, Crassulacean Acid Metabolism (CAM) that equips the plant with the means to use water very efficiently, that along with its succulence, results in the storing of large amounts of water that causes the plant to survive and thrive in harsh and dry conditions (Nobel and Castaneda, 1998; Potgieter, 2007; Stintzing and Carle, 2005).

The *Opuntia*'s adaptations to survive include:

- Crassulacean acid metabolism (CAM) is an alternative method of photosynthesis. Cactus pear plants have to retain water at all costs along with obtaining CO_2, however it has a special adaption to only open the stomata at night for the fixation of CO_2 when water-loss would be limited (temperatures are lower and humidity higher) (Salisbury and Ross, 1992)
- The presence of cuticular waxes that cover the cladodes and fruit (Salisbury and Ross, 1992)

- The ability to regenerate from the roots, cladodes, seeds, tissue grafting and grafting (Feugang et al., 2006)
- A widespread and shallow root system that obtains water from any source, such as mist or light rain (Feugang et al., 2006)
- The slimy fluid (mucilage) that is present in both the cladodes and the fruit (Feugang et al., 2006).

The entire plant could serve as a nutritious food source to humans and animals (De Wit et al., 2010; Inglese et al., 2002; Moßhammer et al., 2006a; Nefzaoui and Ben Salem, 2002; Russell and Felker, 1987; Sáenz, 2000, 1997). The fruit is cultivated and marketed in South Africa (De Wit and Fouché, 2015) but the young cladodes are an unknown food source although it is widely enjoyed in Mexico as a nutritious vegetable dish (Rodriguez-Felix and Cantwell, 1988; Sáenz, 2000). The whole plant is edible, thus the fruit and cladodes are a fundamental part of the Mexican culture and traditions. The fruit is eaten fresh, but the whole plant is used as human food and animal feed in arid regions during droughts, therefore it is known in some countries as “the bridge of life” (Sáenz, 2000). The fruit plays a major role in the diet of the people of Mexico and Chile therefore preserved and minimally processed cladodes are prepared in homes or are available to consumers. (Corrales-Garcia, 2009; Neeraj and Sirohi, 2015; Sáenz, 2002).

As the cladodes are safe for human consumption and readily available, they have always been considered an important nutritional food source in Latin America (Rodriguez-Felix and Cantwell, 1988). It has been nicknamed "the bread of the poor" and is often eaten as a green vegetable. In fact, the serving of the fresh young and tender cactus cladodes in a dish called "nopalitos" is very popular and is deeply embedded in the culture and local cuisine (Feugang et al., 2006). It is prepared either raw in dishes such as salads and salsas or cooked by means of boiling or frying. It is used with other ingredients in a variety of traditional culinary dishes including desserts, beverages, snacks, soups, stews, sauces and salads. As a result of its popularity, the cactus pear plant is commercially produced in Mexico, Southern California and Chile, mostly for the fruit and nopalitos (young edible cladodes).

The cactus pear grows and thrives in regions (Mexico, Israel and Morocco) where only limited vegetation grows, and crops could not grow without expensive interventions such as irrigation and fertilization (El-Mostafa et al., 2014; Potgieter and Mashope, 2009; Stintzing and Carle, 2005). Droughts are the norm rather than the exception in South Africa, while more than half of the land surface is already deemed arid or semi-arid climate regions, moreover larger parts of the land area are destined to become arid or semi-arid (Potgieter, 2007). In the future, global warming and climate change could cause greater pressure on the water resources making it more difficult to grow crops with high water requirements (Potgieter, 2007; Turral et al., 2011). Thus, plants that are naturally adapted to survive in dry and hot regions should be developed into sustainable crops. The cactus pear could offer these opportunities if it was developed into a "new" or alternative crop, as it is inherently drought-tolerant and could offer solutions and benefits for sustainable agriculture in less than ideal climate regions (Potgieter, 2007). As it produces masses of digestible energy, it could provide adequate sustenance to help ruminants survive through periods of severe drought in the form of fodder feed (Russell and Felker 1987). It requires low inputs, thus offers cheaper alternatives to expensive commercial fodder feed products. In fact, quite an effort has gone into finding ways to improve the usefulness of this ecologically adaptive plant (De Wit and Fouché, 2015; Moßhammer et al., 2006a).

In recent years, researchers have been investigating the nutritional and pharmacological potential of the cactus pear (El-Mostafa et al., 2014). Numerous studies showed that consumption of cactus pear products not only increased general wellness, but also treated ailments and therefore had valuable therapeutic potential (Fernández-López et al., 2010; Nazareno, 2013; Prakash and Sharma, 2014). Its high contents of fibre (insoluble and soluble), antioxidants (phenolics, ascorbic acid and carotene) and minerals (calcium, potassium, phosphorous and selenium) have proved its potential in health-promoting and nutritional benefits. The pharmacological potential include hypoglycaemic (Budinsky et al., 2001), antiulcer and hemo-protective (Tesoriere et al., 2009), anti-cancer (Sreekanth et al., 2007) anti-cholesterol (Sáenz et al., 2004), anti-osteoporosis (Aguilera-Barreiro et al., 2013), anti-depression (Park and Goins, 1994) properties and the enhancement of liver function (Brahmi et al., 2011).

The cactus pear cladodes are known for the production of a slimy substance that can be observed as soon as the outer cuticle of cladodes are damaged or cut. This slimy material is commonly referred to as "nopal dribble" (Sepúlveda et al., 2007) or mucilage. Mucilage is a soluble fibre that is classified as a hydrocolloid as it is a long-chain polymer that dissolves in water to give a thickening or viscosity producing effect (Glicksman, 1983). These interesting flow properties together with the nutritional and functional characteristics, compelled researchers to investigate the application of mucilage as a functional ingredient (Sáenz et al., 2004). The slimy substance that the cactus pear cladode contains is an unknown and unexplored by-product that has promising potential as a functional ingredient (Cárdenas et al., 1997; León-Martínez et al., 2011; Medina-Torres et al., 2000; Sáenz et al., 2004).

A market has emerged for functional foods that provide extended nutritional benefits besides the normal nourishment that a food product already offers. New and innovative functional food products have to be developed that would include health promoting compounds that will exert a positive influence in the body and thus on a person's overall health (Smith and Charter, 2010).

The aim of the study

The main objective for this study was for mucilage to be used commercially as a functional ingredient in innovative nutraceutical food products. Therefore is was necessary to select the optimal cultivars for use in mucilage applications. A complete characterization of mucilage in terms of morphological, rheological, functional and chemical properties had to be done to obtain the understanding and insight into making an informed selection from the 42 cultivars that were available. Not only the cultivar, but the season and month of harvest were imperative to be determined.

What complicated matters was that the only research available on the 42 local South African cactus pear cultivars focused on fruit production and seed oil quality. Very little data was available on the characterization and utilization of cladodes for animal fodder applications and none in human food applications. Therefore researchers were forced to begin anew, considering that the South African cultivars are different from those used in other countries. As cladodes have never been regarded as human food in South Africa, no former knowledge on the subject existed. To our knowledge there were no nopalito orchards in South Africa thus this research was conducted on mature cladodes (approximately six months old).

Another difficulty was the extraction of mucilage. Corrales-Garcia (2009) alleged that even though the technology for the extraction of mucilage was available, that it was too complex, expensive and the yield was too low for it to be a viable commercial produced product. Extraction procedures carried out by, Del-Valle et al. (2005), Goycoolea and Cárdenas (2003), Iturriaga et al. (2009a), Medina-Torres et al. (2000) and Sepúlveda et al. (2007) were laborious, expensive and unproductive.

The aims of this study were as follows:

1. To conduct a thorough study on all available literature on the history, morphological properties and the utilization of cactus pears, cladodes and mucilage. The nutritional and pharmacological properties were researched. Cladode flour and the acquisition thereof was investigated. Lastly, the composition of mucilage, its characteristics as well as the present extraction procedures were considered. Potential utilization of mucilage was obtained and listed.
2. To extract the mucilage. For mucilage to be economically viable, an optimal extraction procedure was developed that gave an optimal mucilage quality while delivering acceptable yield. However, it was clear to the researchers that for this project to have commercial success in South Africa, the extraction procedures needed to be uncomplicated. In fact, no chemicals or laboratory-restricted procedures would be feasible.
3. To identify the most suitable cultivars. The lack of knowledge concerning the local cultivars and the factors that influence mucilage yield was the basis for this part of the study, as the success and feasibility of commercialising would depend on selecting cultivars for the highest quality as well as acceptable mucilage yield. The available cultivars (42) was screened using rudimentary morphology, moisture content and viscosity methods to determine the factors that influence yield. Only eight cultivars were selected for further research for optimal mucilage yield and quality.
4. To investigate the differences in mucilage between summer and winter harvesting, through the study of the morphology of cladodes, mucilage viscosity and flow behaviour characteristics, of native (NM) and reconstituted freeze-dried (FM) mucilage. The quality and potential of the obtained freeze-dried mucilage powders was tested before further investigations were embarked upon. Only four cultivars and one growth stage were selected for further investigations.

5. To analyse the functional properties, mucilage from the four selected cultivars collected in the winter was included. The effect of the seasonal changes on the functional characteristics over a period of six months was investigated in order to identify a cultivar and a harvesting time for optimal mucilage functionality.
6. To conduct an in depth chemical analysis of the mucilage of the South African cactus pear cultivars as it was necessary to provide proof that mucilage in both its native or freeze-dried forms are equal in terms of its contents to those cultivars that have been analysed in other countries.
7. To explore and develop new ways of including native and freeze-dried mucilage in new and innovative food products. Products that already form part of normal dietary patterns, but address special dietary requirements were developed. Yet the products had to maintain quality, be convenient and be tasty. The viscosity, flow behaviour and functional properties of mucilage were used to the advantage of the products while nutrients had to be retained.
8. To present a summary of the most important findings. The potential of native and freeze-dried mucilage for commercialization was considered. Recommendations in terms of the most appropriate mucilage for applications was presented.

The workflow of this study is shown in Figure 1 in order to offer a schematic representation of the advancement through the chapters.

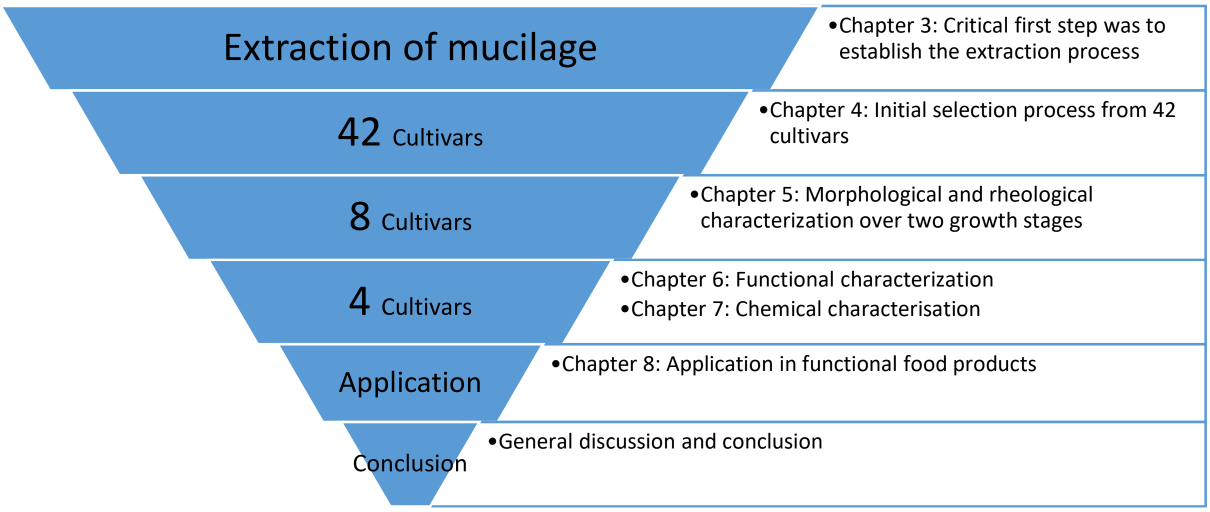


Figure 1: Schematic representation of the progression of the study on the selection, extraction, characterization and application from cactus pear cladodes

Chapter 2
Literature review

Abstract

The cactus pear is extremely efficient in its ability to obtain and retain water, thus it has adaptations to thrive where few other plants could survive. It had been known in South America long before its introduction to Europe. Once brought to South Africa, the spiny prickly pear thrived to the extent that it was declared to be a weed and had to be eradicated. Later, the spineless Burbank cactus pear cultivars were imported, distributed and studied. The cladodes have potential as a crop for emergency fodder and human consumption. The young cladodes could be made into delicious dishes and has health benefits as well as therapeutic properties. Cladodes have high contents of fibre (insoluble and soluble), antioxidants (phenolics, ascorbic acid and carotene) and minerals (calcium, potassium, phosphorous and selenium). The pharmacological potential include hypoglycaemic, anti-ulcer, anti-cancer, anti-cholesterol, anti-osteoporosis properties amongst others. However, calcium oxalate crystals are abundant in the cladode tissue. Cladode flour had been manufactured and tested in various baked products with relative success. The composition of the mucilage molecule enables it to absorb and hold huge amounts of water while the viscosity increase. When protein content was found in mucilage, its emulsifying capabilities were predicted. Mucilage could therefore be developed into a profitable functional ingredient that may be considered a GRAS (generally regarded as safe) product. The potential function of mucilage in food products include fat replacement, emulsifying, stabilizing, thickening and edible coatings and films.

2 Literature review

2.1 Introduction

The *Opuntia* genus belongs to the Cactaceae family and is indigenous to the Americas although the plant is most abundant in Mexico. It is extremely efficient in its ability to obtain and retain water, thus it grows predominantly in arid and semi-arid regions. In fact, it has adaptations that allow it not only to survive, but to thrive in poor soil with low pH levels, extremely high daytime temperatures and limited water supplies. Therefore, the *Opuntia* species grow in conditions where few other plants will grow. For this reason it is easily cultivated all over the world (Stintzing and Carle, 2005), in fact different *Opuntia* species has been observed as far north as 53˚N (United

States) and as far south as 50˚S in Argentina. One cold tolerant species (*Opuntia rafinesqeui*) is found on the snow-capped mountains of Switzerland (Russell and Felker, 1987).

Declining water sources and desertification in many parts of the world compelled researchers to take notice of edible, water efficient plants, such as *Opuntia ficus-indica*, not only to discover more efficient sources of human and animal nourishment but to explore its medicinal, cosmetic and nutritional potential as well (Yahia et al., 2009).

2.2 Cactus pears worldwide

2.2.1 Background and historical distribution

Evidence suggests that *Opuntia* was extensively harvested for 9000 years before the arrival of the Europeans to Central America. The Aztecs founded their capital city on a site indicated by an eagle sitting on a cactus pear, which is depicted on Mexico's coat of arms (Heinrich et al., 2014). It was valued not only as a source of nutritional food but also for its healing properties (Nazareno, 2013). The plant was taken to Europe by Columbus where plantations were established in Spain (Cadiz) in 1820. It was then introduced to the Canary Islands in 1824 where its cultivation became widespread to supply the rest of Europe with red dye. From Spain, the cactus pear spread east through Europe especially around the Mediterranean. When the Moors were expelled from Spain in 1610, the plant spread into Northern Africa and from there to India (Russell and Felker, 1987). It is from India that the Dutch brought it to their new settlement at the Cape of Good Hope (Medina et al., 2007; Van Sittert, 2002). The plant was used for several purposes; for the production of carminic acid (red dye), for food use and as medicine. Today, it is widely distributed in the semi-arid regions of Mexico, America, Africa, Australia and the Mediterranean basin (Piga, 2004).

2.3 Cactus pears in South Africa

2.3.1 The history of cactus pears in South Africa

In the Republic of South Africa and neighbouring countries, the spiny cactus pears quickly found highly favourable environmental conditions. Due to the plant's ability to propagate from seed, the spiny cactus pears grew and spread to a point where it was declared as an invader plant in South Africa. It invaded an estimated 900 000 ha of natural pastures mainly in the Eastern Cape and Karoo. Insect enemies like the cochineal insect and cactoblastis moth were used for the biological control of the plant starting in 1932. Infestations of the spiny prickly pear have now been eliminated due to a law prohibiting the uncontrolled growing of prickly pears (applicable only to the spiny plants). The spiny plant is officially declared a weed in South Africa, thus the commercial cultivation of *O. ficus-indica* in South Africa is limited to the spineless Burbank varieties (Van Sittert, 2002).

Luther Burbank was a researcher working in the USA who developed the spineless cactus pears (Van Sittert, 2002). The disadvantage of the spineless Burbank cactus pears was that it was not cold-tolerant (Russell and Felker, 1987). The Burbank type cactus pears did not survive for long in the cold climates of the USA and Europe yet it thrived in South Africa after being imported. These Burbank cultivars (imported from California in 1914 by the Agricultural Research Institute station of Grootfontein at Middelburg in the Karoo) have been allowed to grow on most farms in small numbers where they serve as a source of fruit in the hot summers. However, there was a growing realization amongst researchers that it can be a useful plant other than being used for fodder and fruit production (Brutch and Zimmerman, 1993).

There is strong evidence that cactus pear plants used to be part of the traditional food source in the arid Karoo. This is evident in the recipes of both the fruit and young cladodes in old recipe books written by Mrs. Winnie Louw and Mrs. Anne Schnell (Howell and Schnell, 1991) that included notes on the making of soap, using the cladodes in flower arrangements and preservation of the fruit in the form of jams, pickles and crystallized sweets.

At present, there are a few commercial farms where the cactus pear plant is cultivated for production of fruit for the local and European markets. The ripe fruit is harvested from December to March depending on the cultivar and climate. The fruit has a short shelf life of 8-10 days at ambient temperatures (15-30˚C), but it can be stored for six weeks at the correct temperature (10 ˚C) and humidity (90%) (Joubert, 1993). In Haenertsburg, in the Limpopo Province in the northern region of South Africa, Terence Untepertinger has more than 60 ha of cactus pear orchards on his farms and is presently expanding to include more land. From these orchards, fresh cactus pear fruit of *O. ficus-indica* cultivar Algerian are exported under the commercial name of “Consolata”. The Consolata Estates export cactus pears mainly to Europe ten months of the year. During peak season, that stretches from December to March, approximately 25 tons are harvested per day and of that, 19 tons are packed for the export market every day (Limpopo Independent Newspapers, 2011).

Another large cactus pear exporting business, “Afrigold” belongs to Mr. Doug Reed who farms on land that has been in the family since 1892 in the Mooketsi valley. Cactus pears were chosen as it was best suited to the dry climate and little artificial intervention would be needed to ensure a profitable business. The three cultivars that are planted on 65 ha on dry land as well as under drip irrigation are Algerian (pink-red coloured fruit), Gymno-Carpo (orange coloured fruit) and Morado (white-green coloured fruit). It has been marketed under the name “Afrigold” and “Sundance” for the past twenty years. Cactus pears are exported to Europe, Canada and the East as an exotic fruit (Reed, 2010).

The export of cactus pear fruit has been declining in the last five years in South Africa as the cultivation of cactus pears around the year has been improved in Italy, by making use of greenhouses and a method by which the ability of cactus plants to bear fruit more than once a year is utilized. This practice is known as scozzulatura. Scozzolatura involves the removal of the first flush of fruit and cladodes to ensure late-ripening fruit (three months after normal fruit ripening season) (Inglese et al., 2002). Consequently the importance of *Opuntia* spp. as an effective food production system both as fruit and as vegetable (cladodes) should be explored in the African market for human and animal use.

In a study done at an experimental orchard outside Bloemfontein, the cultivars Meyers, Roedtan, Gymno-Carpo and Robusta x Castillo cultivars of *O. ficus-indica* were recommended for fruit production, in fact Meyers was proven to be the most appropriate cultivar for economical purposes in the study. It was also found that there were large variations between cultivars, both as a result of genetics and the environment (De Wit et al., 2010).

Only the fruit is utilized in South Africa as the cladodes are not perceived to be a human food source. Therefore the potential use of the cladodes are enormous in the country where declining food sources and global desertification increase continuously. Maize in the form of flour is the staple diet in South Africa. This is counterproductive as large regions in South Africa is not ideal for growing maize without irrigation.

2.3.2 Research on cactus pear in South Africa

The spineless Burbank cactus pears are the only cultivars in South Africa on which research concerning fruit production has been conducted (Potgieter, 2007). The orchard was situated at Grootfontein in the Eastern Cape. Unfortunately, the orchard was not maintained and was almost destroyed. Thereafter, the Department of Agriculture in the Limpopo Province established a new germplasm bank at Mara and soon after that, secondary plantations were established in Oudtshoorn, Bloemfontein and Cradock. The ARC (Agricultural Research Council) embarked on the establishment and maintenance of the plantation outside Bloemfontein in 2003/2004. Research on cactus pear started in 2004 at the University of the Free State and focused mainly on livestock feed, management of cactus pear diseases and human food applications. The research on pre-harvest crop management has focused on fruit production and crop management (Potgieter and Mashope, 2009) while very little has been published on cladode production for human food applications. The existing information on cladodes are on its utilization for fodder in fresh or dried forms (Einkamerer et al. 2009; De Waal et al. 2006). Using cladodes for fodder is a foreign concept to the South African farmers, as maize and various grasses have always been cultivated for this purpose. Long term (± 12 years) research are being conducted on fruit and cladode production on 42 cultivars situated outside Bloemfontein. Very recently, in 2015, a new germplasm bank for the cactus pears was established when a collaboration between the ARC, UFS and DUT was established that took the first steps in a serious effort to support and fund cactus pear research at the UFS. Researchers at the University of the Free State believe that cactus pear has the potential to change the agriculture in the arid and semi-arid regions of the Free State, Northern Cape and North West provinces. Cladodes not only have the potential to become a human food source, but it also has enormous potential to supplement fodder in times of drought (De Waal, 2015; De Wit and Fouché, 2015). During an international workshop held in Bloemfontein, South Africa in 2015, it was established for the first time that cladodes are the most important and main product derived from the cactus pear plant (not the fruit). Cactus pear fruit could never effectively compete in a market of more popular fruit that is available year round such as apples, oranges and bananas. Cladodes, in contrast, could be harvested year round and be used fresh or dried as a food source for both animals and humans.

2.4 Morphological classification and description

The classification of the cactus pears studied in this work is briefly summarized as follows:

Order: Caryophyllales

Suborder: Potulacineae

Family: Cactaceae

Subfamily: Opuntioideae

Genus: *Opuntia*

Subgenus: *Opuntia*

Species: *ficus-indica* and *robusta* (L.) Mill. Gard. Dict. Abr. Ed. 8 No 2. 1768 (Scheinvar, 1995)

The plant may be divided into the root, vegetative part (cladode), flowers and fruit (Figure 2).

Figure 2: The *Opuntia ficus-indica* plant

2.4.1 Fruit (pulp)

The fruit is a fleshy berry and it has various shapes and sizes. The cactus pear fruit is known as prickly pear, cactus pear, tuna or fico d'india and comes in a rainbow of colours from white, green, yellow, orange, red, purple to brown. The pulp colour may or may not correspond with the peel and may range from yellow to red and to green. It is oval shaped and has a thick pericarp (peel) and juicy pulp with many small and hard seeds. The pulp (fruit) contains mainly water (84% to 90%) and reducing sugars (10 to 15%), glucose and fructose in almost equal amounts (Feugang et al., 2006). The large variety of cultivars cause a large variability in data collected from the fruit. In general, the thick pericarp accounts for 33 to 55% of the fruit and the soft and juicy pulp for 45 to 67% of the fruit. The weight of the whole fruit ranges from 67 to 216 g depending on cultivar, origin and climate (Piga, 2004). The fruit has a high pH value of 5.3 to 7.1 and thus has very low acidity (0.05 to 0.18% citric acid). The sugars range from 10-17 °Brix and as was mentioned earlier, are mainly reducing types, with glucose being the predominant sugar and fructose second, which is the reason for the very sweet taste of the fruit (Piga, 2004). The fruit contain high levels and various numbers of amino acids, such as proline, taurine and serine. Vitamin E and β-carotene are present in the lipid fraction of the fruit and seeds. The antioxidant content in the form of vitamins A and E improve the stability of the oil. Ascorbic acid is a major vitamin in cactus pears while smaller amounts of vitamin B1, B6, niacin, riboflavin and pantothenic acid are present in the fruit (Feugang et al., 2006). The fruit pulp is a good source of minerals especially calcium, potassium and magnesium. The total caloric value is 50 kcal/100 g, which is comparable to that of other fruit such as pears, apricots and oranges.

2.4.2 Peel

The thick pericarp is covered with small-barbed spines and glochids. The peel is usually between 36% and 48% of the weight of the whole fruit (Moßhammer et al., 2006a). El-Kossori et al. (1998) reported that the peel contained remarkable amounts of calcium (2.09%) and potassium (3.4%). The findings of Moussa-Ayoub et al. (2011) suggested that the bioactive compound isorhamnetin glycoside has only been found in the peels of *O. ficus-indica* fruit and therefore the peels are one of only a few sources of this phenol. The peel provides oil with appreciable amounts of polyunsaturated fatty acids, mainly linoleic acid, α– tocopherol, sterols, β-carotene and Vitamin K_1. Calcium and magnesium are also present in high amounts in the peel (Piga, 2004)

2.4.3 Seeds

There are considerable variations in form, size, structure, embryo characteristics and colour in cactus pear seeds. Seeds are 10 to 15% of the edible fruit (Feugang et al., 2006). They are described as hard and bony and may range in number from 120 to 350 per fruit. The seed weight ranges from 2.0 to 7.0 g per fruit (Nobel, 1988). The main ingredients found in the seeds are oils, proteins (sulphur containing amino acids), fibre and ash (minerals). In a study by Shongwe (2011) across three locations (Waterkloof, Cradock and Oudtshoorn) and seasons (2010 and 2011) it was found that of the 42 cultivars tested from Bloemfontein, the highest oil content was found in *O. ficus-indica* American cv. Giant (8.76%), while *O. ficus-indica* Meyers demonstrated good oil productivity (7.41%). In a study by De Wit et al. (2016a), Tormentosa was the best cultivar from an oil quality perspective as it had the highest yield together with the best oxidative stability while *O. robusta* spp. Montery and Robusta demonstrated the poorest oil stability. Cactus pear seed oils were found to be a good source of C18:2c9,12 (linoleic acid). The oils showed low oxidative stability as a result of the high content of oleic acid, stearic acid and other monounsaturated fatty acids (De Wit et al., 2016a).

2.4.4 Cladodes

Cladodes are the modified stems that fulfil the purpose that leaves usually do and are covered with large spines or small hair-like thorns (Feugang et al., 2006). These short, sharp deciduous glochids cover the *Opuntia* cacti and grow from the areoles which are the places where flowers and thus fruit can develop. Stintzing and Carle (2005) reported that the cladodes contain carbohydrates (64-71 g/100 g DM), ash (19-23 g/100 g DM), fibre (18 g/100 g DM), protein (4-10 g/100 g DM) and lipids (1-4 g/100 g DM). The cladodes are characterized by high malic acid content varying according to CAM rhythm. It contains calcium, magnesium, potassium, phosphorus and trace amounts of iron (Feugang et al., 2006). Younger cladodes contain higher carbohydrate, protein and water contents. The juice from cladodes typically has a pH of 4.6 with 0.45% titratable acids and 6.9 g/100 g dry matter (Stintzing and Carle, 2005). The high calcium and fibre content place cladodes higher than lettuce in nutritional value, but lower than spinach. Stintzing et al. (2005) concluded that cactus pad hydrocolloids constitute mainly hexoses and pentoses. During growth, the total protein content (9-16% DW) and fibre content (12.1-16.3% DW) decreased with age (Rodriguez-Felix and Cantwell, 1988). The water content of 88-95%

means that cladodes are classified as a low-calorie food with 27 kcal/100 g FM. Up to 36% of the cladode volume consists of mucilage and could reach up to 50% of the total weight (Stintzing and Carle, 2005). According to Nobel et al. (1992) the average sugar composition of the mucilage from cladodes is 42% arabinose, 22% xylose, 21% galactose, 8% galacturonic acid and 7% rhamnose.

2.4.4.1 The presence of calcium oxalate crystals in cladode tissue

Calcium oxalate crystals occur in Opuntioideae, a subfamily of Cactaceae and have a negative effect on nutrition as it binds with essential minerals such as calcium and cannot be absorbed in the human digestive system, moreover it could cause harm to the kidneys. It was suggested (He et al., 2012) that crystals may appear in the same plant tissue with various morphologies based on the chemical composition and could be present in all plant tissues. Many fruit and vegetables contain high oxalate levels. Fruit such as figs, berries, kiwis and red grapes have high oxalate content while vegetables such as rhubarb, spinach, leeks and beets are regarded as food containing high oxalates. As the presence of oxalates would reduce the availability of calcium in cladodes, McConn and Nakata (2004) determined the oxalate levels in young cladodes and compared it to the oxalate levels in kale and spinach. The highest oxalates were detected in spinach (136.8 mg/g DW) while virtually no oxalates were found in kale (2.03 mg/g DW). Cladodes contained substantial levels yet mostly in the insoluble material (34.5 mg/g DM).

Calcium oxalate crystals probably occur inside vacuoles. Vacuoles are the warehouses of the plant as any excessive substances can move in and out depending on its demand. When an excess of calcium occurs, it is absorbed into vacuoles, where it seems to become trapped in the form of calcium oxalate crystals (Salisbury and Ross, 1992). However, research showed that calcium may still be bioavailable in cladodes, especially in the more mature cladodes (135 days of growth) (Contreras-Padilla et al., 2011).

The presence of calcium oxalate crystals in the fresh cladode tissue has been reported by many authors (Contreras-Padilla et al., 2011; Ginestra et al., 2009; Malainine et al., 2003; McConn and Nakata, 2004; Sáenz et al., 2012; Trachtenberg and Mayer, 1982a). In a most recent study by Rojas-Molina et al. (2015), noticeably more crystals were observed in the insoluble dietary fibre, although it was seen in all tissues (cladode, soluble fibre and insoluble fibre). The total calcium oxalate content detected in samples were 6.71 mg/g DM in cladodes, 0.27 mg/g DM in soluble fibre and 5.39 mg/g DM in insoluble fibre. The low solubility of calcium oxalate (6.1 mg/l) was used to explain the lower occurrence of calcium oxalate in soluble fibre while calcium carbonate (that is more soluble), was more prevalent in the soluble fibre (49.67 mg/g DM) than insoluble fibre (21.95 mg/g DM). The bioavailability of calcium was specifically addressed in the study and it was found that the calcium would be available as a result of the positive molar oxalate: calcium ratios.

Contreras-Padilla et al. (2011) investigated the presence of oxalates and calcium at different maturity stages of the young cladodes of up to 135 days (nopalitos). It was observed that both the calcium oxalate content (11.4 mg/g to 4.25 mg/g DM) and the oxalate content (13.7 mg/g to 5.1 mg/g DM) reduced from 40 to 135 days while the calcium content increased from 17.9 mg/g DM to 30.7 mg/g DM. This proved a positive relationship between maturity and bioavailable calcium content, indicating that cladodes of advance maturity may be important sources of calcium and may have the least amount of calcium oxalate crystals. It was also suggested in this study that the molar ratios between oxalate and calcium indicated that calcium would be bioavailable in cladodes.

The presence of a large number of calcium oxalate crystals is known to be in all parts of the cladode tissue, powders and flours even when extensive effort was applied to purify the cladode samples (Malainine et al., 2003; Sáenz et al., 2012).

2.5 The utilization of the cactus pear

2.5.1 Non-food utilization of cactus pear plants

The cactus pear plant has always been cherished in Mexico for its various and peculiar uses. As the entire plant is a nutritious source of fresh produce it has been actively used in different types of home industry products. However, the traditional non-food products made from dried cladodes include woven mats, baskets, fabrics and home-made paper. The whole plant is used by growing it into fences to keep out any intruders. Plantings have also been made to control erosion in deforested areas (Le Houérou, 1996). Another very interesting use of the cladodes was found in Chile, where farmers traditionally used the liquid in which nopalitos were cooked (that contained the slimy mucilage) to clarify drinking water. More recently, studies confirmed that mucilage from the *O. ficus-indica* cladodes had not only a clarifying, but also a purifying ability similar to the purifying action of aluminium sulphate (Buttice et al., 2010) and could remove arsenic from drinking water (Fox et al., 2012). Other traditional applications of mucilage include the use in combination with lime to improve the adhesion properties of paint (Cárdenas et al., 1998). The spineless stems of the *Opuntia* have also played a significant role in providing valuable nutrients for farming animals by using it as fodder (Feugang et al., 2006).

There is a broad spectrum of possibilities for industrializing cactus pads, that could extend to different types of industry: handcrafts, cottage industry, extractive chemistry, food processing, pharmacology, cosmetology, bio-energy and fertilizers (Corrales-Garcia, 2009). Furthermore, the increasing demand for healthy and natural products could be seen as an opportunity for the cactus pear industry (Sáenz, 2002).

2.5.2 Fruit

Prickly pear fruit are usually eaten freshly picked, as it has a limited shelf life. The fairly high sugar content and low acidity provide for the delicious, sweet taste. As the pH values are reported to be between 5.3 and 7.1, it is classified as a non-acid fruit and therefore it is susceptible to microbial invasion (Piga, 2004). Thus, it has a very limited shelf life and should continuously remain in cold storage throughout the marketing process. The fruit is preserved by producing jams, syrups, canned fruit, dried fruit leathers and other products that are generally made at a cottage industry-scale and by farmers for own use (Moßhammer et al., 2006a). A unique preservation technique is “Tuna cheese” (Queso de tuna), a delicacy made from the concentrated cactus pear fruit juice, raisins, nuts and pine nuts. Other treats and sweets are also made from dried and concentrated pulp mixtures (Sáenz, 2000). A traditional Mexican homemade alcoholic drink called “Colonche” is obtained by fermenting the cactus pear pulp in wooden barrels (Sáenz, 2000).

Cactus pear juice is a common domestic drink and thus a common use of the fruit, however it was found by (Rodriguez-Felix and Cantwell, 1988) that due to the high amino acid content (proline and taurine) and the presence of minerals (calcium and magnesium), cactus pear juice could be commercialized into sports and energy drinks (Reyner and Horne, 2002; Seidl et al., 2000 cited in Moßhammer et al., 2006a). Specifically the taurine content is exceptional as it is rare in plants, especially fruit (El-Samahy et al., 2007).

While most other common fruit, especially red or pink coloured fruit (red grapes, cherry, raspberry, strawberry, peaches and apples) derive their colour from anthocyanin, cactus fruit pigments are betalains (Felker et al., 2008). Moreno et al. (2008) defined betalains as a water-soluble nitrogen-containing pigment, which comprise of the red-violet betacyanins and the yellow betaxanthins. Piga (2004) investigated the colouring range of betalains from cactus pears at near neutral pH and found that betacyanins and betaxanthins allow a very wide chromatic interval. Moßhammer et al. (2006b) stated that by mixing yellow-orange and purple juice as well as isolated betaxanthin and betacyanin fractions, they could produce tailor made hues that would cover the entire colour spectrum from bright yellow to blue-purple. Cactus pear concentrates are therefore suitable for colouring yoghurt, ice cream and other fruit preparations such as cereal bars, chocolates, instant products and even meat substitutes (Moreno et al., 2008). The purification of betalains is not required to produce different hues, in fact the pigment was more stable when the extracted juice was used. Moßhammer et al. (2006b) found acceptable overall pigment retentions of 71% to 83% after the reconstitution of semi-concentrated and concentrated juice. The use of cactus fruit powders could be used for colouring desserts, fruit or cereal bars, instant dishes and chocolates. It opens new fields of application for the cactus pear fruit as functional ingredient in food, pharmaceutical or cosmetic products (Feugang et al., 2006).

Another application of the fruit is as a frozen puree concentrate. Frozen concentrate could be used to flavour products like ice cream. As fruits have a high glucose and fructose content, it may also be considered for the manufacture of high fructose glucose syrup (Moßhammer et al., 2006a).

2.5.3 Seeds

From the earliest times Mexicans collected the seeds from the cactus pear fruits, dried and ground it into flour and used in combination with lucerne and hay for animal fodder (Nobel, 1988). According to Saenz (2002), oil can be extracted from the seeds. Cactus pear seeds have a high grade of unsaturated acids, with the highest content of linoleic acid (De Wit et al., 2016a; Shongwe et al., 2013). Thus it was compared to corn and grape seed oil (Labuschagne and Hugo, 2010).

2.5.4 Cladodes

In Mexico, the young cladodes (nopalitos) are enjoyed throughout the year as a green vegetable. In fact, the serving of the fresh young and tender cactus pads called "nopalitos" in a dish similar to green beans is deeply embedded in the culture and local cuisine (Feugang et al., 2006). It is prepared either raw in dishes such as salads and salsas or it is boiled or fried. It is used with other ingredients in a variety of traditional culinary dishes including desserts, beverages, snacks, soups, stews, sauces and salads. Nopalitos are preserved in brine or pickled (Corrales-Garćia et al., 2004; Rodriguez-Felix and Cantwell, 1988; Sáenz, 2000). Recipes and notes on how to use nopalitos are widely available in South and North America. The Cactus and Succulent Society's Cactus Cookbook compiled by Joyce L. Tate is an example thereof (Tate, 1978). The highest quality nopalitos are thin, have brilliant green colour and are turgid and fresh. It is harvested when it reaches a weight of 90 - 100 g and a length of 15 - 20 cm (Sáenz, 2000; Stintzing and Carle, 2005). The production of nopalitos are centered in Milpa Alta, a district in the south of Mexico that is known for its highly variable rainfall (Russell and Felker, 1987). Depending on the weather, the young cladodes (nopalitos) require from 20 to 30 days to grow to a harvestable size. After being harvested, it is cleaned, sliced, cut into small cubes and used raw in salsas or cooked in various traditional dishes (Rodriguez-Felix and Cantwell, 1988).

Regarding the harvesting of cladodes: it is customary to harvest the nopalitos midmorning after at least two hours of sunshine, when the level of acidity has decreased to a satisfactory level (Feugang et al., 2006; Rodriguez-Felix and Cantwell, 1988). The effect of CAM is that there is a significant difference in the acid content of the cladodes during the night and day. When harvested at the wrong time of day it causes the nopalitos to have an unacceptable astringent sourness.

Villarreal (cited in Saenz, 2000) made and tested candy made from the cladodes with or without chocolate coatings, with very good results. Crystallized cladodes that resembled crystallized melon peel was another product that was well liked by consumers (Sáenz, 2000).

Presently cactus pear cladodes are used in Mexico to produce a healthy high fibre breakfast cereal. The product is known as "Cactu Fibra" and is prepared only with wheat bran, cactus pear flour, sodium chloride and "NutraSweet" and is considered a weight reducing product. It claims not to contain any chemicals, colouring and preservatives (Sáenz, 2006, 2002).

The use of nopalitos to manufacture a low calorie marmalade has been investigated by Leopoldo et al. (2012). It was suggested that the marmalade could function as both a high nutritional value and low calorie sweetener considering the high antioxidant value of the marmalade. Surprisingly, the antioxidant properties of nopal was highly preserved after processing. In fact, the high temperatures used during manufacture increased the extractability of carotene and thus proved to be an even better source to the equivalent fresh young cladodes (Leopoldo et al., 2012). The increased antioxidant content of antioxidants and especially carotene has been reported before (Du Toit et al., 2015).

Cactus cladodes are used in an indirect way to produce high quality dairy products in the Mexican province of Milpa Alta. The local farmers rely on cladodes as fodder for dairy cows in order to ensure good lactation and thus high quality milk production. The milk, butter and other dairy products from these cladode-fed cows are highly desirable in markets as it is believed to have better flavour and colour than milk obtained from grain fed cows (Russell and Felker, 1987). Another interesting application of the cladodes entail (Ortiz-Rodriguez et al., 2013) the addition of minced cladode to raw milk in order to add commercial value to milk or cheese. The addition of the undried minced cladodes decreased the microbial growth in milk. It did not only lengthen the shelf-life of milk, but more practically, lowered microbial growth on artisan cheeses and thereby extended the shelf-life of the cheese noticeably.

2.6 Nutritional and pharmacological potential of cactus pear

In recent years, researchers have been reporting on the health benefits of the cactus pear (El-Mostafa et al., 2014). These benefits not only include a general increase in well-being, but also healing potential of fatal diseases (Fernández-López et al., 2010; Nazareno, 2013; Prakash and Sharma, 2014).

2.6.1 Nutritional benefits of cladodes and cladode flour

It is believed that cladodes have great potential in the food industry as a source of fibre, specifically soluble fibre (Ramírez-Tobías et al., 2007; Sáenz, 2002). The cladode contains both soluble and insoluble fibre. It is the mucilage that is contained inside the cladode that is classified as soluble fibre whereas the cellulose of the cell walls are the insoluble fibre. Therefore cladodes have been receiving a lot of research attention in recent years because of the potential source of fibre, together with the mucilage and pectin that it contains. It affords a very useful purpose for the older cladodes, as they seem to contain more dietary fibre as they mature (Sáenz, 1996). Fibre has numerous health promoting functions in the digestive system that depends on the type; soluble fibre will dissolve and swell in water and is then fermented by bacteria in the large intestine while insoluble fibre does not dissolve and is not metabolized by bacteria but cleans the digestive systems from the inside (Peña-Valdivia and Sánchez Urdaneta, 2006).

According to Sáenz (1997) consumers are aware of the link between fibre and cholesterol control and are looking for food products that is ready-to-eat, low in energy, low in cholesterol, low in fat and healthy.

High ash content has been found in cladodes by different researchers (Ayadi et al., 2009; López-Cervantes et al., 2011; Matsuhiro et al., 2006; Samia El-Safy, 2013; Stintzing and Carle, 2005). It may seem that the richness of minerals in the soil generally increased the ash content of cladodes. Ash content also increased significantly with the maturity of the cladodes (Ramírez-Tobías et al., 2007). Determining the ash content is a way to measure the total mineral content of a product. It indicates the total inorganic content that does not contribute energy to food. Thus, high ash values indicate high mineral content. A few studies have been conducted on specific minerals that cladodes of different maturities and cultivars contain.

Rodríguez-Garcia et al. (2007) investigated fresh and dried cladode flour at different stages of maturation in order to pinpoint the ideal age for optimal calcium content. The cladodes were harvested at 22, 40, 52 and 64 days in summer and spring, when they were at optimal human consumption sizes. It was found that calcium content increased during maturation, therefore more mature cladodes could be an important source of calcium. The daily consumption of cladode-related food products were especially recommended for use in developing countries, where dairy products are expensive, as well as for lactose intolerant consumers. In the same study, it was determined that phosphorous and sodium contents decrease with maturation while potassium contents varied with maturation. It was determined by Rodríguez-Garcia et al. (2007) that after cladodes have grown to reach a weight of more than 400 g, all mineral contents stabilized whether the cladodes were harvested in summer or spring. Therefore, cladodes could remain a good source of minerals at any growth stage (Rodríguez-Garcia et al., 2007). Cladodes consumed fresh or powdered, in the form of supplements, were recommended for the prevention of diabetes and osteoporosis. Ramírez-Moreno et al. (2011) concurred that young cladodes could be used as an alternative source of calcium to dairy products.

High levels of potassium (2.1 g/100 g DM) were reported by Sáenz (1997). It was reasoned that the high contents of potassium, together with the low content of sodium, justifies the use of cladodes as a nutraceutical ingredient.

The selenium content was studied and reported by Bañuelos et al. (2011). It was stated, that upon the known advantages of *Opuntia* as a sustainable food source, it has the added advantage of being a valuable source of selenium. In the thorough study it was estimated that the selenium contents of cladodes would render it an ideal candidate for utilization in selenium-enriched food products as well as in animal feed. The selenium distribution was homogeneous throughout the cladode with higher levels in the tips. The researchers conducted *in vitro* colon cancer inhibition analysis using the purple fruit. From the study it was concluded that the whole plant has potential to enhance well-being. It could provide organic, naturally enriched Se-fortified nutraceutical food products that could provide selenium as a cancer preventing substance to consumers through their daily diets.

The use of cladodes in phytochemicals has been investigated because of the high content of total phenolics and specifically flavonoids. In fact, some polyphenols are reportedly only found in cactus pear cultivars. These polyphenols include nicotiflorin (146.5 ml/100 g DM) and narcissin (137 mg/100 g DM), substances that have been indicated in the treatment of neurological deficits (El-Mostafa et al., 2014). Moussa-Ayoub et al. (2013) found that *O. ficus-indica* fruit, peel and cladodes contain isorhamnetin glycosides (a type of flavonoid) but that cladodes had higher flavonol contents compared to the fruit from the same plants.

Another study (Ginestra et al., 2009) confirmed the content of unique flavonoids in fresh cladodes and freeze-dried cladode flour of three commercial cultivars from Southern Italy. Although the total phenolic content was low, the predominant flavonoids observed were isorhamnetin, kaempferol and quercetin. Other studies that concurred these findings were done on cladodes of nine different varieties of *Opuntia* spp. (Santos-Zea et al., 2011), ten different varieties (Guevara-Figueroa et al., 2010) and dried cladode powders (León-Martínez et al., 2011; Sánchez et al., 2014).

Another antioxidant that has received attention from researchers is ascorbic acid. Stintzing and Carle (2005) reported that the total vitamin C in 100 g of fresh cladode was between 7 and 22 mg in *Opuntia* spp. Medina-Torres et al. (2011) first determined the ascorbic acid content in fresh cladodes (2.05 g/kg FM) before drying the cladodes (45˚C and 65˚C). There was a significant decrease in the ascorbic acid content before and after drying, as well as with the lower and higher drying temperatures. A loss of more than 80% of the ascorbic acid was observed after drying, that was attributed to the high temperatures necessary to dry cladodes.

Medina-Torres et al. (2011) determined total carotene for fresh and dried cladode samples and it was found that fresh cladodes contained 1.16 g/kg, but when dried at 45˚C, only 0.543 g/kg remained. This means that the β-carotene content was approximately 50% less than that of the fresh sample. The data suggested that fresh cladodes have a higher content of carotene than that found in other vegetables such as baby carrot, beetroot, spinach and lettuce. Bensadón et al. (2010) studied two cultivars from Mexico. Carotenoid content of 21.32 and 22.84 mg/g DM was reportedly found in the young cladodes of the two cultivars. In both the cladodes and fruit by-products (seeds), there were no significant differences in the carotenoid content between the cladodes from different coloured fruit varieties.

Jaramillo-Flores et al. (2003) tested cladodes from Mexico and identified three types of carotenoids in cladodes. The first had an orange colour (β-carotene), the second had a light yellow colour (cryptoxanthin) and the third was lutein. The total carotenoid content was 231.8 µg/g on a dry basis which corresponds to 36% β-carotene, 46% lutein and 18% cryptoxanthin. They found that the higher the temperature, the more extractable the carotenoids and the higher the antioxidant potential. Similar results were obtained in a study to determine the antioxidant content and potential in fresh and processed cactus fruit products (Du Toit, 2013; Du Toit et al., 2015). This was a surprising finding in both studies as it would be expected that processing would damage and thus decrease antioxidant content of products, however this seems not to be the case with carotene.

2.6.2 Pharmacological potential associated with cactus pear fruit and cladodes

Many studies have been done on the pharmacological potential associated with cactus pear fruit and cladodes. Stintzing and Carle (2005) compiled a pharmacological profile for the *Opuntia* spp. that described the antioxidant capacity, analgesic action, anti-inflammatory properties, antiulcerogenic effect, hypoglycaemic and antidiabetic effects. The anti-hyperlipidaemia, cholesterol lowering, anti-atherogenic and diuretical effects were also discussed. Further pharmacological effects that were elaborated on were the impact on uric acid metabolism, the antispermatogenic, the antiviral properties as well as the monoamine-oxidase inhibition.

Feugang et al. (2006) also elaborated on the therapeutic impact that cactus pear fruit and cladodes possess and discussed the anti-cancer effect, anti-oxidant properties, anti-viral, anti-inflammatory, anti-diabetic (type II), anti-hyperlipidaemia and hypercholesterolemia effects. The treatment of ulcers and rheumatism together with the use as anti-diuretic was reviewed as well.

Livrea and Tesoriere (2006) described the decrease of body oxidative stress in humans, the cardiovascular protective effects, the anti-ulcer and the hemoprotective effect. Extracts from cactus pear fruit were preventative against cancerous tumour growth and in alleviating damage to the brain. The inhibiting effect on lipid oxidation in human red blood cells and the treatment of ovarian, cervical and bladder cancer cells were also elaborated on.

Budinsky et al. (2001) provided proof of the anti-oxidative action in the human body and recommended cactus pear products as nutritional options that should be used more widely as natural, cheap remedies for hypercholesterolemia. As cladodes are rich in soluble dietary fibre it could be very effective in decreasing cholesterol levels. The soluble fibre in the prickly pear decreases plasma LDL levels (Nunez-Lopez et al., 2013). In research done by Frati-Munari et al. from 1983 to 1990 (cited in Sáenz et al., 2004) into the hypoglycaemic properties of *Opuntia ficus-indica*, it was found that mucilage acts as an interfering agent in the absorption of intestinal glucose. Raminez and Aguilar (1995, in Sáenz et al., 2004) also reported that *Opuntia* has a strong glucose reduction effect. As the control of glucose levels could not be explained by the presence and action of insoluble dietary fibre only, it was said to be the action of the soluble fibre (mucilage) that contributed to the extraordinary hypoglycaemic properties observed.

Results from a study done by (Galati et al., 2002) showed that consumption of nopalitos prevented gastric mucosa in the stomach. It was explained by Sáenz et al. (2004) that mucilage prevents the penetration of the necrotizing agent into gastric mucosa, therefore acting synergically with the natural defence factors of the gastric mucosa to prevent stomach ulcers from forming. Galati et al. (2003) indicated that dried cladode powders have significant anti-inflammatory properties and it was suspected that *Opuntia streptachanta* extracts will inhibit replication of DNA and RNA viruses, although the inhibitory component is unknown (Ahamd et al., 1996 cited in Sáenz et al., 2004). Mucilage could also be used in the treatment of wounds. As it forms a gel, it exerts a cooling effect, which will ease pain and accelerate healing (Stintzing and Carle, 2005).

Dried cladode powder, taken in a capsule form as a supplement, improved the bone mineral density of 817 Mexican woman who were included in a study, some who were diagnosed with low bone mass or osteoporosis. It was stated in the study (Aguilera-Barreiro et al., 2013) that the dried cladodes (cv. Redonda) contained formidable amounts of calcium (35.5 mg/g). The dried cladode supplement was therefore recommended for premenopausal woman with low bone density, who suffers from hypercalcaemia in their hip and spines in order to maintain normal calcium levels (Aguilera-Barreiro et al., 2013).

Hfaiedh et al. (2008) studied the protective effect of cactus cladode extract on cancerous growths in rats and found that regular consumption of cladodes (more particular the quercetin content found in cladodes) counteracted the peroxidative effect of nickel, proving its highly effective radical scavenger action.

Brahmi et al. (2011) analysed the consumption of young cladodes (2-3 weeks of age) and concluded that it should be considered as an accessible source of natural antioxidants that is hepatoprotective, as it enhances liver function, as it caused a total reduction of aflatoxins in the body. An interesting study by Park et al. (2010) indicated anti-depressant effects and another fascinating study by JongMin et al. (2010) suggested the use of cladode juice in order to improve long-term memory.

2.7 Cladode flour

2.7.1 Production of cladode flour

In light of the overwhelming evidence of the nutritional and pharmacological potential of the cactus pear as a whole, but specifically of the cladodes, it was self-evident that many researchers would embark on producing and evaluating cladode flour (Hernández-Urbiola et al., 2010). The drying of cladodes have been a topic of research in itself as a result of the problematic nature of dehydrating a plant that has an inherent disposition to invariably secure water. The cladode flour has been characterized and tested in different products to ensure its safety and application potential (Sáenz, 2000).

The effect of including cactus pear flour in baked products was investigated by different researchers because of the nutritional advantages and the ease of its addition to baked products. Cladode flour has been described as a very inexpensive product, but highly nutritional and beneficial to health, as it did not only contain dietary fibre but also high levels of calcium and potassium (Sáenz, 2002).

Right from the beginning, cladode flour showed potential in terms of total dietary fibre content (43%) of which 28.45% is insoluble and 14.54% is soluble fibre while the protein content was 3.9% (Sáenz, 2000, 1997). The cladode flour consisted of 52-53% carbohydrates, 20-22% ash, 15-16% protein, 9.75% water, 9.5% fibre and 0.25% lipids (Stintzing and Carle, 2005). The water activity of cladode flour was very low at 0.53, indicating good storage ability. The water absorption is important to determine as it would reflect the effect it would have in the gastrointestinal tract, where it would absorb and increase the bolus and create a satiety or satisfying effect. The water absorption ability of the flour particles once it reaches the gastrointestinal tract, depends on the size of the flour particle. Smaller particles would mean that more water absorption is able to take place in the bolus. Seventy seven present of the particles of the cladode flour produced in the study were between 150 and 190 µm (Sáenz et al., 2012).

It was observed that nopal flour increased viscosity in dough mixtures while viscosity increased with concentration and decreased with increased at higher temperatures. It was also implicated that the temperature at which the cladodes are dried had an adverse effect of the rheological behaviour of the final powdered product; more heat applied during drying, the more the viscosity of the dough decreased (Sáenz, 1997).

When the cladode flour was added to liquid foods, it was important to consider that the viscosity might increase as the concentration of mucilage increased (Sáenz, 2002). It had been suggested (Sáenz, 2000), that there is a possibility of using cladode flour in vegetable soups and gelled desserts. In a sensory test that was performed, 15% replacement by the flour had the greatest acceptance by the panel. Sáenz et al. (2000) investigated the addition of cladode flour to oatmeal cookies recipe and found that 3 cookies were enough to cover 6% of the recommended daily intake of dietary fibre (25 g/day). The cookies were harder and smaller, but had risen higher than the control. Cactus flour in processed products such as tortillas and other type of breads as a nutraceutical supplement also received research attention (Santos-Zea et al., 2011).

Cladode flour was used to prepare three types of baked products (health bread, oat biscuits and carrot cake). The appearance and texture was influenced by the incorporation of cactus flour, causing the quality parameters to differ significantly in terms of texture and volume. The colour of the products became darker however, from the overall acceptability ratings, the conclusion was made that 25% of wheat flour could be replaced with cladode flour in baked products (De Wit et al., 2015)

Cladode flour was made (Ayadi et al., 2009) using 2-3 year old cladodes with no purification and applied it in different concentrations (5, 10, 15 and 20%) in sponge cake. It was observed that with higher inclusion of cladode flour, the colour of the cake is affected, the crust was darker and the cake appeared green. Cake that contained more than 10% inclusion of cladode flour was unacceptable for a sensory panel. A 5% inclusion was recommended as well as the use of pistachio nuts in order to mask the green colour of the cake to some extent.

The cladode flour was said to regulate weight, increase fibre intake and manage diabetes mellitus. The effectivity has not been proven but has exciting prospects as it is a low kilojoule food ingredient with high fibre content. It could be considered a natural food supplement that may be used in solid or liquid food products (Sáenz, 2002; Sáenz et al., 2000; Stintzing and Carle, 2005). High levels of potassium do not occur in many foods, but dried cactus flour is a good natural source for this mineral (2.1 g/100 g DM) and together with the low sodium content it may potentially have significant impact on the nutrition of products that contain a certain percentage of cladode flour (Sáenz, 1997).

2.7.2 Drying cladodes for the production of cladode flour

Many different possibilities have been explored of drying cladodes effectively. The water holding properties that enables the plant to survive, causes a major challenge when it comes to drying the cladodes, especially since the effect of heat during the drying process seems to lower the functional and nutritional quality of the flour (Contreras-Padilla et al., 2012; Lahsasni et al., 2003; López et al., 2009; Medina-Torres et al., 2008; Ramírez-Moreno et al., 2013).

The effect of heat (boiling cladodes for 20 min in 1l water) on the nutritional composition of cladodes has been investigated (Ramírez-Moreno et al., 2013) and it was found that it was mostly the soluble contents (sugars, minerals, vitamin C and some phenolic compounds) that were negatively affected while the protein, lipid and fibre contents were mostly retained. Boiling also disrupted the structure of the mucilage and a less consistent and viscose gel was the result. Cooked cladodes showed minimum dispersion, thus more homogenous gel than raw cladodes. There was therefore a loss of the jellification capacity of mucilage after boiling the cladodes. The detection of the loss of the ability to retain glucose (57-75%) and thus control the glycaemic response was most alarming. Thus, the application of heat during processing of raw cladodes into functional ingredients in food (including the drying process) should be done with careful regulation of exposure to heat (Ramírez-Moreno et al., 2013).

Lahsasni et al. (2003) studied the adsorption-desorption isotherms of prickly pear cladode in order to shed light on the drying process and the stability of fresh and dried cladodes when stored. The graph showing the sorption isotherms have an s-shape profile that would be very typical when food is dried. Therefore desorption is not the equal opposite of adsorption demonstrating the hysteresis effect. Thus, it would be more difficult for the cladodes to lose moisture than to take moisture in. There was low capillary condensation, but it was hygroscopic nevertheless and once the humidity started to increase above 0.7% RH (relative humidity), there was a sharp increase in adsorption. It was found that temperature had an effect on the sorption isotherms since the equilibrium moisture content decreased with increase of temperature at constant equilibrium relative humidity.

Ruiz-Cabrera et al. (2008) air dried and osmodehydrated cactus pear and also found that higher temperatures were required for effective drying of cladodes, however higher heat caused vitamin C degradation and colour changes. Therefore, temperatures of 40 or 50˚C were recommended.

López et al. (2009) was exploring better ways of drying cladodes that did not jeopardize the quality and microbial security. A drying tunnel was tested at different temperatures of 35, 45 and 60˚C. It was recommended that the thorns as well as the cuticle that covers the cladode should be eliminated or partially removed, in order to shorten the drying process significantly (60%). The best temperature choice was 60˚C, considering that the duration of drying was reduced with 76%.

Gallegos-Infante et al. (2009) stated that it is important to find a drying method for cladodes that maintains most of its original properties. Convective drying was used at a temperature of 45˚C and air flow rates of 3 and 5 m/sec. The antioxidant capacity (2,2-diphenyl-1-picrylhydrazyl) (DPPH) and radical scavenging levels did not differ significantly at 3 and 5 m/sec although the phenolic content decreased. As the maintenance of antioxidant potential was a surprising finding, literature was used to prove that overall antioxidant properties were maintained or even enhanced in food product extracts. Therefore it was suggested that convective drying would be a suitable drying method for cladodes.

Contreras-Padilla et al. (2012) explored methods of drying older cladodes in an attempt to test its commercial value as cladode flour. Forced-air drying, freeze-drying and tunnel-drying were tested and freeze-drying proved the most nutritionally efficient way in all chemical analysis such as protein, ash, moisture, fat content, as well as colour retention tests.

2.8 Mucilage

The Cactaceae family, which *O. ficus-indica* is part of, is characterized by its production of a slimy substance that is observed as soon as the outer cuticle of cladodes are damaged or cut. This slimy material is commonly referred to as "nopal dribble" (Sepúlveda et al., 2007) or mucilage. When the slimy fluid was first extracted from cladodes, it was found to be a high molecular-weight polysaccharide material that was soluble in water (Trachtenberg and Mayer, 1982b). Since mucilage is soluble fibre, it was classified as a hydrocolloid as it consisted of long-chain polymers that dissolved in water. Moreover, it caused a thickening or viscosity producing effect (Glicksman, 1983). The non-Newtonian viscous colloids that form when mucilage is dissolved in water, is the result of its capacity to absorb and hold huge amounts of water in its structure (Sáenz, 2000). It was these interesting properties that compelled researchers to investigate its use as a functional ingredient (Sáenz et al., 2004).

The global trend towards healthier diets created a market for functional food products that provide extended nutritional benefits beyond normal nourishment that a product already offer (Smith and Charter, 2010). Cladode flour already offer potential therapeutic properties however, mucilage extractions have added potential for therapeutic, nutritional and functional use, therefore could be an ideal candidate for use as a functional ingredient in new innovative nutraceutical food products (Sáenz, 2006).

2.8.1 The composition of mucilage and the effect on viscosity and flow properties

Trachtenberg and Mayer (1981) did ground breaking research on mucilage using electron microscopy. It was found that mucilaginous cells in cladode tissue are specialized and contain acidic polysaccharides. Mucilage molecules were found to have high molecular weight (4.3 x 10^6 g/mol), while the sugars detected were arabinose 24.6%, galactose 40.1%, rhamnose 13.1% and xylose 22.2%. Mucilage was found to be a hetero-polysaccharide, meaning that it consists of different monosaccharides with varying uronic acid content (both a carbonyl and a carboxylic acid) (Trachtenberg and Mayer, 1982b).

The result of such an electrically charged molecule coming into contact with water, is that the hydrogen atoms split off, leaving negatively charged groups open along the chain. The result is that the long mucilage molecule will repel itself, causing it to uncoil and stretch out. All of these stretched out molecules in the fluid in turn, cause the viscosity of the fluid to increase. With the addition of cations (Na^+ or Ca^{2+}), the negative charges of the mucilage molecule will be neutralized, causing the viscosity of the fluid to decrease. Consequently, the viscosity of mucilage emphatically depends on the ion concentration of the solution (Trachtenberg and Mayer, 1982b).

The shape of the mucilage molecule was determined to be a prolate ellipsoid with the radius of gyration ca 850 Ȧ and the length at ca 2945 Ȧ indicating a long rod shape. The molecule at its longest is three times the length as in the contracted form thus indicating that it must be a very flexible molecule (Trachtenberg and Mayer, 1982b).

Medina-Torres et al. (2000) also found that the mucilage behaved as a polyelectrolyte and that it contained a molecular structure of up to 30 000 g/mol varying proportions of sugars. Nobel et al. (1992) reported that the average sugar composition of the mucilage from *Opuntia ficus-indica* was 42% arabinose, 22% xylose, 21% galactose, 8% galactonic acid, 7% rhamnose, and 18% D-galacturonic acid.

Cárdenas and Goycoolea (1997) determined that the polymer had an average molecular mass (MW) of 3 x 10^6 g/mol and a number average molecular mass of 2.4 x 10^6 g/mol, with a polydispersity index of 1.4. Medina-Torres et al. (2000) reported the molecular weight as 2.3 x 10^4 g/mol while Trachtenberg and Mayer (1982a) identified the molecular mass of 4.3 x 10^6 g/mol.

Although each researcher found different molecular masses for the mucilage molecule, it could be deduced that overall, the molecules are immense. The considerable size of the molecule is the reason for the unique rheological behaviour and the special functional properties that makes it ideal for applications in food and other products (Cárdenas et al., 1997). Cárdenas et al. (1997) described an entangled network of disorientated polymer coils that caused shear-thinning behaviour in solutions. At high concentrations the high weight mucilage molecules caused the system to have elastic solid characteristics during high oscillation tests. Mucilage solutions showed steady-shear flow curves in varying concentrations. Furthermore, all solutions showed non-Newtonian shear-thinning (pseudoplastic) behaviour. When more agitation was applied, the viscosity of the mucilage solutions decreased. The influence of agitation on mucilage solutions became more pronounced with increased mucilage concentration. Cactus mucilage was compared to okra gum as both form ropy solutions in water (Cárdenas et al., 1997).

As mucilage is a polyelectrolyte (Trachtenberg and Mayer, 1982b), cations are thus needed to bind to the open negative groups of the mucilage molecule, in order for the molecule to recoil back onto itself, causing the viscosity of the solution to decrease. The same influence of cations on the viscosity of solutions was obtained when a buffer (unchanged pH) was tested instead of water. When varying pH ranges were tested, the viscosity remained stable in acidic environments while it increased sharply in alkaline solutions. When Ca^{2+} was already dissolved in the mucilage, the viscosity remained stable at alkaline regions while it decreased sharply in acid regions (Trachtenberg and Mayer, 1982b). As calcium oxalate crystals are present in abundance in the plant tissue, calcium may be released from the oxalate and influence the viscosity of the mucilage, thereby controlling the water holding capacity of mucilage. The available calcium would not only provide the cations but also strongly influence the pH, causing the molecules to recoil and in turn influencing the viscosity of mucilage to decrease. Therefore the presence or absence of calcium may regulate water holding capacity in the plant (Trachtenberg and Mayer, 1982b).

2.8.2 The presence of mucilage and pectin in pulverized cladode pulp

Two distinct water-soluble high molecular-weight polysaccharide materials were found in the cladodes (as well as the fruit), namely mucilage and pectin. Goycoolea and Cárdenas (2003) were the first researchers to succeed in extracting the pectin with gelling capacity as well as non-gelling mucilage from the cladodes. The slimy fluid that is extracted from cladodes is the mucilage (non-gelling), while the pectin (gel-forming) is extracted from the solid cladode material using an alkaline process (Cárdenas et al., 2008). It is important to differentiate between the pectin and mucilage content as they have different properties in solutions and viscosity characteristics (Goycoolea and Cárdenas, 2003). Mucilage in *Opuntia* is not chemically associated, either covalently or otherwise to the structural cell-wall pectins (Cárdenas et al., 2008; Goycoolea and Cárdenas, 2003). It was proposed by Cárdenas et al. (1997) that mucilage is synthesized in specialized cells where its definitive physiological role is to bind water, thereby controlling the movement of water in different climatic conditions. The function that mucilage fulfils in the plant may be transferred as water-binding properties that would be valuable when applied in food products. The possibility that mucilage and pectin, because of their similar composition, co-extracted, as a result of the methodology of extraction was confirmed (Peña-Valdivia and Sánchez Urdaneta, 2006).

2.8.3 The presence of low-molecular weight compounds in mucilage extracts

Researchers found low-molecular weight compounds present in the mucilage extract yet were not quite sure what the explanation was (Trachtenberg and Mayer, 1982b). At first it was thought that it was the result of contamination during the extraction process however, other possibilities were investigated and it was found that protein molecules were present in the mucilage (Majdoub et al., 2001). The presence of protein in mucilage was unusual, as it was generally believed that mucilage would not contain any protein (Stintzing and Carle, 2005). It is known that xanthan and guar gum contain protein fractions (1-2%). It would therefore be possible that this protein fraction could provide mucilage with the properties needed for acting as a stabilizing agent (Iturriaga et al., 2009a).

Majdoub et al. (2001) identified the two polymeric components in the extract from the minced peeled cladodes. There was a high-molecular-weight fraction and a low-molecular-weight fraction. The two fractions were separated by ultra-filtration. The material retained had a high-weight average molar mass while the filtrate had a low molar mass. The high molar mass was a pure polysaccharide, without protein, which indicated the pectin fraction. The low molecular weight component contained a protein as nitrogen was detected (2.2%) (Majdoub et al., 2001). It was thus concluded that a major component of native mucilage extract is low-molecular proteins. It was also found that these proteins were not water soluble and similar to the albumin found in the seeds. The presence of protein would indicate that mucilage had the unique property of having the capacity to lower the oil-in-water interfacial tension and hence to create the emulsifying capacity in oil-in-water emulsions. Proteins are known to be effective emulsifiers but the surface properties of mucilage have not been tested so far (Goycoolea and Cárdenas, 2003). The conclusion made by Majdoub et al. (2001) was that the presence of protein may give emulsifying or stabilizing properties in the mucilage.

2.8.4 Mucilage extraction and purification methods

Given the significance and vast uses of extracted mucilage, it was necessary to investigate the various processes that have been used to extract mucilage from cladodes. Extracting high quality mucilage from cladodes has proved to be a challenging and laborious task, as the usual procedures such as pressing or filtering have not been successful. However, mucilage occurs in large enough volumes in the cladodes and peel of fruit that it could be extracted and processed for further use (Sáenz, 2000).

The first attempts to extract mucilage from cladodes were published as a patent that was registered by Gutzeit (1958) with the title “Method of making stable cactus mucilage”. In the patent three methods were described, yet in all three methods water was added first, after which the cladodes were boiled, macerated and pressed using a screw press. It was subsequently precipitated with water-soluble aliphatic alcohols. In the final steps of two of the described methods, activated carbon (Norit) was added and ultimately the mucilage was dried into a reportedly stable powdered product. In the third described method, mucilage was mixed with water in a 1 -2% mucilage concentration.

Cárdenas et al. (1998) investigated the use of cladode extracts as an impermeabilization agent in lime-mortar used to paint historical buildings. The extraction method used by these authors are mentionable as thicker, older pads were cut into small pieces and cooked in a steam bath without the addition of water, as it was believed that added water would dilute the extraction. After cooking, the pulp was centrifuged and the obtained mucilage was freeze-dried. The yield was 0.88% dry weight.

Initially, researchers extracted mucilage by homogenizing cladodes with water followed by precipitation with ethanol. Medina-Torres et al. (2000) reported a modified procedure by McGarvie and Parolis (1979) where the cladodes were macerated, the acquired pulp was centrifuged and precipitated using acetone, after which the cladode extract was finally washed with 2-propanol and dried.

Majdoub et al. (2001) went about extracting mucilage by shredding cladodes and blending the pulp before adding 1 litre of petroleum ether. Subsequently, the pulp was macerated a second time in deionized water, after which it was filtered, centrifuged and filtered once more. The obtained extraction was divided into three different batches, the first batch was freeze-dried as is (native sample), while the subsequent 2 batches were obtained by freeze-drying the filtrate (low weight) and retained solids (high weight) after a final ultra-filtration procedure. Therefore Majdoub et al. (2001) reportedly extracted an end product that had no salts and no compounds of low molecular weight. Ultimately, it was concluded in this study that proteins were present in the native sample that imparted important functional properties and interacted with polysaccharides, therefore purification (as done in the two subsequent batches) would be pointless.

Sepúlveda et al. (2007) investigated different variations in their methods. The first variation that was considered for their mucilage extraction procedure was the relationship between cladodes and water, secondly the optimal temperature and timing of heat application and lastly the specific type of alcohol that should be used for precipitation. The optimal extraction method was described as follows: Mature cladodes (2-3 years old) were macerated, mixed with water and filtered before it was centrifuged. An alcohol (such as ethanol) would be used to dissolve the chlorophyll and the precipitated extracted mucilage for the purpose of producing pale yellow colour mucilage. The best mucilage yield (1.5% FW) was achieved by mixing the cladodes and water to a 1:7 ratio. The optimal temperature for extraction was 40˚C and 4 hours of maceration was required. The use of Isopropyl alcohol was recommended in a ratio of 1:3 since it was commercially available and cheaper than ethanol.

The method used by León-Martínez et al. (2010) was as follows: 13 month old cladodes were grated, water was added (1:3) and agitated at 86˚C for 36 hours. The mucilage was separated from the solids by decantation, filtered using a sieve and stored at 4˚C. The obtained mucilage was dried by two different methods (spray-dried and freeze-dried). To reconstitute to 1, 3 and 6% concentrations, freeze-dried powder was dissolved in deionized water by scattering and stirring at 24˚C for 90 min.

Kim et al. (2013) aimed to extract low viscosity mucilage that could find more applications compared to high viscosity mucilage, thereby increasing the usefulness of cladodes. Polysaccharide degrading enzymes were used to remove the pectin from the cladodes in order to facilitate extraction of pure mucilaginous slime. Different enzymes were tested but a rapidaze/viscozyme (1:3, v/v) mixture was most effective in reducing the viscosity of the mucilage.

2.8.5 Drying techniques and factors that influence the drying process

Drying mucilage is difficult for the same reasons as has been explained with drying of cladodes (section 2.7.2), except that the challenges would be even more pronounced, mucilage being the component of cladodes that binds and retains the water molecules. As a consequence, the drying of mucilage has been another topic of research.

León-Martínez et al. (2010) tried to develop a cost effective commercial process for mucilage as powdered additive. Spray-drying (130-170˚C) and freeze-drying (-50˚C) was tested. The glass transition temperature of mucilage was determined to be 45˚C. Spray-drying was recommended and the conditions for effective spray-drying were specified.

Thereafter, León-Martínez et al. (2011) investigated the effects of different drying conditions on the properties of reconstituted mucilage solutions. The different drying techniques changed the rheological properties of the mucilage powder. It was thought that freeze-drying would most likely cause damage to the biopolymer structure of mucilage while spray drying would produce a more stable powdered product with lower rheological properties. It was found that spray drying caused the mucilage to decrease in viscosity. The flow behaviour tests showed non-Newtonian shear thinning behaviour that was directly influenced by the concentration. It was found that freeze-dried samples had a more pronounced shear-thinning behaviour than spray dried mucilage.

Medina-Torres et al. (2013) tested the possibility of microencapsulation of gallic acid by spray drying with mucilage from cactus cladodes. It was determined that mucilage had neutral and acidic fractions depending on the extraction method. Spray dried mucilage was a stable product with small particle size and a high viscosity and showed solid-like qualities. It was determined to be a promising food additive as it functioned as an effective encapsulating agent for the microencapsulation of gallic acid.

2.9 Potential uses for mucilage in food products as food additive

Mucilage is a natural ingredient that could enhance the nutritional benefits of food products (Sáenz, 2006). The transference of the properties of mucilage from its function in plants to food may be possible and could therefore be developed into a profitable functional ingredient (Stintzing and Carle, 2005). Mucilage could have the potential of being used in commercial products without limitation, as it may be considered a GRAS (generally regarded as safe) product (Glicksman, 1983). For the purpose of summarizing the potential uses of mucilage in food products, a very brief discussion follows. Chapter 8 offers a full discussion of the application of mucilage in functional foods.

Fat replacement

Public awareness about the health risks of dietary fat increased and the food industry responded by developing non-fat, low-fat and reduced-fat products. Considerable resources went into developing fat replacements that allow products to taste and function like high-fat foods (Akoh, 1998). Mucilage was first mentioned for its potential use as a fat substitute by Cárdenas and Goycoolea (1997) and Sáenz (2000).

Emulsifying agents

According to Glicksman (1985), hydrocolloids could be used to emulsify food systems as it emulsifies fat droplets through its ability to increase the viscosity of mixtures and thereby preventing the droplets to migrate and coalesce. Garti and Leser (2001) claimed that hydrocolloids are indeed true emulsifiers because they increase the viscosity and adsorb to the oil/water interfacial surface. In standard emulsifiers though, protein is an indispensable component as the protein is adsorbed at the interfacial surface and stabilizes the oil/water emulsion with high viscosity and mechanical strength. It was established in the literature (Majdoub et al., 2001; Stintzing and Carle, 2005; Teles et al., 1997) as well as in this thesis (chapter 7) that the dried mucilage contained crude protein that ranged between 2.52 and 5.74 g/100 g

DM. Mucilage should therefore be tested for use as a natural emulsifier in food systems (Garti and Leser, 2001; Iturriaga et al., 2009a).

Rwashda et al. (2001), cited by Garti (1999) studied the emulsifying capabilities of mucilage and found strong emulsifying action. Mucilage was capable of reducing surface interfacial tensions and stabilising oil-in-water emulsions. In addition, it formed small droplets, absorbed onto the oil-water interphase and the emulsion did not flocculate.

Stabilizing agent

Mucilage could be used as a stabilizing agent in food products as a result of its capacity to absorb and hold huge amounts of water in its structure (Sáenz, 2000). Non-Newtonian viscous colloids are formed when mucilage is dissolved in water therefore it causes a thickening or viscosity producing effect. Hydrocolloids stabilize food systems through its ability to increase the viscosity of mixtures and thereby preventing droplets to migrate and coalesce (Glicksman, 1983). Iturriaga et al. (2009a) compared cactus mucilage to commercial hydrocolloids and found that mucilage has stabilizing properties similar to those of xanthan and guar gums. The results showed the outstanding potential uses for mucilage due to its remarkably different behaviour as compared to other commercial hydrocolloids. Yahia et al. (2009) compared mucilage extracted from *Opuntia*, Maguey and Aloe Vera to find the best mucilage to be used as a hydro colloidal gum in the food industry.

Thickening agent

Mucilage has been investigated to see if it could be used as a thickening agent. The effect of pH over the viscosity is important to consider as it can reach values of 58.1 cps at pH 6.6 (Sáenz, 2002). Mucilage has been tested as a thickening agent in beverages, vegetable soup and gelled desserts with promising results (Sáenz, 2006). Mucilage has also been recommended as a thickening agent by other researchers (Stintzing and Carle, 2005).

Suspension agent

Gebresamuel and Gebre-Mariam (2013) found that *Opuntia* spp. mucilage could be used as a suspension agent and specifically for the suspension of paracetamol in pharmaceutical products. Mucilage proved to be effective as a suspension agent as the solutions tended to have the most essential property of being pseudoplastic. Mucilage was said to function as a natural drug delivery system that can disperse medicine uniformly when shaken and pour with ease. Mucilage was proposed as alternative to NaCMC (sodium carboxymethyl cellulose) as it is low cost, freely available, emollient, non-irritating and non-toxic. Mucilage suspensions had both thickening and flocculating ability.

Edible coatings

Edible films and coatings are thin layers of edible material on the surface of food for the purpose of extending shelf-life. It is made out of protein, polysaccharides, lipids and composites. An edible coating was made by combining mucilage with 5% w/w glycerol as a plasticizer. Strawberries were dipped in this mixture and it did not affect the taste or colour of the fruit but had a protective effect during storage and prolonged the shelf-life. This edible coating was reported to retard moisture migration and loss of volatile compounds, reduce respiration dates, form barriers to fats and oils, have high selective gas permeability and carry food additives (Del-Valle et al., 2005).

Edible film

Soluble packaging is useful where portion control or premeasured amounts may be an important consideration. This edible package is then eaten by consumers after it has been dissolved in the moisture used for cooking or other preparation (Glicksman, 1983).

In a study by Espino-Díaz et al. (2010), an edible film was made by mixing powdered mucilage, glycerol and water in 2:1:50 ratio. Films were formed simply by pouring the mixture into a glass petri dish coated with Teflon and allowing it to dry for 24 h. The films that were formed between pH 4 and 8 were uniform, smooth and translucent. It worked much better without the added calcium as the films had a more compact structure. In fact, after several tests, it was attained that the best results were obtained when no calcium was added and the pH was not adjusted, yet the addition of the plasticizer (glycerol) was essential for the films to form.

Foaming agent

Foams are formed by liquid films separating the gas cells (Glicksman, 1983). The production of the edible film, as described above (Espino-Díaz et al., 2010), by adding glycerol to mucilage solutions may be used to increase the foaming ability of mucilage through the strengthening of the film forming ability of mucilage and creating a whipping agent. When mucilage was added to egg foams, it was found that it produces an increase of the foam stability (Espinosa, 2002, cited in Sáenz et al. 2004).

Holographic material

Olivares-Pérez et al. (2012) used mucilage to obtain a holographic material. According to the study mucilage contains substances that come from the degradation of pectic substances and chlorophyll that, when mixed with polyvinyl alcohol matrix, can be used as a recording medium with photosensitizer properties. The mucilage was fermented up to 18 days, filtered and used as long as the pH was less than 7. The film was made at room temperature and the method involved mixing polyvinyl alcohol (12%) with distilled water at 85˚C, combining it with mucilage (32%) and allowing it to set in darkness for 24 hours. The holographic material obtained was established to be excellent material for building transmission holographic gratings.

Impermeabilization agents

Mucilage was historically added to lime mortar to prevent it from drying too quickly and help retain moisture. The study investigating the traditional claim, however did not prove the functionality of mucilage in the paint. Yet a connection could be made to the film-forming concept explained above. It seems that mixing mucilage and plasticizer formed a protective coating. This type of material could be described as impermeabilization agents (Cárdenas et al., 1998).

Encapsulation

Encapsulation is the coating of individual solid or liquid particles while microencapsulation occurs when minute particles are enrobed by a protective wall isolating it from its surroundings. Medina-Torres et al. (2013) tested the possibility of using mucilage for microencapsulation. Mucilage was recommended as an effective encapsulating agent for the microencapsulation of gallic acid.

Nanocomposite materials

Malainine et al. (2003) studied the cellulosic residue of the spines and cladodes after all mucilaginous contents were washed away and found that nanocomposite materials could be prepared from the micro fibrils and that high mechanical performances could be expected from such matrix systems.

2.10 Conclusion

The cactus pear plant has been thriving in South Africa since it was first imported. The entire plant is edible to both humans and animals and should be used to broaden the food base for humans and to provide affordable fodder to animals in South Africa. The fruit and cladodes not only provide the necessary nutrition for survival, but increase well-being as it contains nutrients that has valuable therapeutic potential as well. Its high contents of fibre (insoluble and soluble), antioxidants (phenolics, ascorbic acid and carotene) and minerals (calcium, potassium, phosphorous and selenium) may offer health-promoting and nutritional benefits. The pharmacological potential include hypoglycaemic, antiulcer and hemoprotective, anti-cancer, anti-cholesterol, anti-osteoporosis, anti-depression properties and the enhancement of liver function.

Calcium oxalate were found in cactus pear cladodes that could have a negative effect on nutrition. However, calcium oxalate crystals occur in many fresh vegetable and fruit, moreover calcium may still be bioavailable in cladodes, especially in the more mature cladodes.

The effect of including cactus pear flour in baked products was investigated by different researchers because of the nutritional advantages and the ease of its addition to baked products. Yet, two distinct water-soluble high molecular-weight polysaccharide materials were found in the cladodes (as well as the fruit), namely mucilage and pectin. Since protein were present in the thickening or viscosity producing mucilage, it may be used commercially as a functional ingredient in innovative nutraceutical food products. The transference of the properties of mucilage from its function in plants to food may be possible and it could therefore be developed into a profitable functional ingredient. Mucilage could have the potential of being used in commercial products without limitation, as it may be considered a GRAS (generally regarded as safe) product. The potential function of mucilage in food products include fat replacement, emulsifying, stabilizing, thickening and suspensions while edible coatings and films had been developed already.

Chapter 3
Evaluation of different extraction conditions for optimal mucilage extraction and yield from cactus pear cladodes

Abstract

Opuntia ficus-indica cladodes contain a slimy liquid (mucilage) that has commercial potential owing to its functional, nutritional and medicinal properties. Mucilage is a hydrocolloid gum that could be developed into a nutraceutical product that would promote and add value to the cactus pear as a crop in South Africa. Conventional extraction methods did not fulfil the requirements for a fast, inexpensive, easy and natural procedure that would deliver the yields necessary for commercial viability. It was thus the objective of the present invention. Subsequently an optimized extraction method was proposed by this study. No chemicals and minimal heat were applied thus the natural properties of mucilage would remain intact. The process for the highest quality, untarnished and pure mucilage involves peeling the cladodes, slicing into a size easy to handle, cooking only until cooked through (± 4 min at 100% power) in the microwave oven, coarsely macerating the cooked pieces, centrifuging at 8000 rpm for 15 min and decanting the extracted mucilage. Alternatively, the cladodes could be left unpeeled as well as the cooking and maceration steps switched around. In establishing an easy and inexpensive extraction process, many possibilities for research into the nature of mucilage and the application in food products were opened up.

3 Evaluation of different extraction conditions for optimal mucilage extraction and yield from cactus pear cladodes

3.1 Introduction

Opuntia ficus-indica is a succulent plant from the genus Cactaceae that originated in South America. It grows and thrives in water scarce and extremely hot climates mainly because of its highly effective adaptation of crassulacean acid metabolism (CAM) and highly effective water retaining abilities due to the presence of mucilaginous slime in the thick cladodes (Salisbury and Ross, 1992)

When the cladodes are cut through, the slimy fluid that is observed is commonly referred to as "nopal dribble" in South America (Sepúlveda et al., 2007), but is henceforth referred to as mucilage. Mucilage is a complex polysaccharide with a highly branched molecular structure that is classified as a gum or hydrocolloid (Cárdenas et al., 1997; Goycoolea and Cárdenas, 2003; Medina-Torres et al., 2000). Hydrocolloids are water-soluble dietary fibres that often display functional abilities in food systems and thus could be applied as healthy additives for commercial food products (Glicksman, 1983).

The slimy and thick appearance of mucilage is attributed to the high molecular weight polysaccharides that behave as polyelectrolytes (Trachtenberg and Mayer, 1982b). Mucilage has intriguing rheological properties (Figure 3) that has potential uses as additives for several industrial products in the food industry (Cárdenas et al., 1997; Sáenz, 2002).

Figure 3: Mucilage has intriguing rheological properties

Mucilage has the potential to be used as a fat mimic (Akoh, 1998), thickening, gelling and stabilising agent and a commercial emulsifier in food products (Prakash and Sharma, 2014). It is also recommended for its nutritional contribution (Hernández-Urbiola et al., 2010; Ramírez-Tobías et al., 2007; Sáenz, 2002; Yousfi et al., 2013) and medicinal properties (Feugang et al., 2006; Stintzing and Carle, 2005).

Cladodes are very good sources of both insoluble and soluble fibre (Sáenz, 1997). It is advantageous to the human digestive system because the insoluble fibre binds to toxins and the soluble fibre increases stool bulk (Sáenz et al. 2004). Mucilage, extracted or contained in cladodes is not hydrolysed nor absorbed by the human digestive system therefore the fibre it supplies to the digestive system is beneficial in more ways than one (Goycoolea and Cárdenas, 2003; López-Palacios et al., 2011; Sáenz, 2002, 2000, 1997; Sáenz et al., 2012).

Soluble dietary fibre is associated with decreasing circulating cholesterol and lipid levels and improved glycaemic control, satiety, and bone health (Smith and Charter, 2010). It reduces the risk of cancer, specifically colon cancer because of the capacity to hold water that insures stool bulk. Soluble fibre also contributes to the anti-cancer and improved immune system responses through its prebiotic effect (Smith and Charter, 2010). The fermentation of soluble fibre during digestion also produces rapid intestinal transit (Sáenz et al. 2004). Furthermore, due to the presence of lignin, dietary fibre also has anti-oxidation properties through the prevention of free radical formation (Sáenz, 2002). Other medicinal uses of mucilage include better utilization of glucose at a cellular level, preventing the replication of DNA and RNA viruses (Ahamd et al., 1996 cited in Sáenz et al. 2004), acting as a prebiotic to promote the growth of probiotic bacteria (Yahia et al., 2009), the prevention of stomach ulcers (Galati et al., 2003), acting as an anti-inflammatory agent and in the treatment of wounds (Feugang et al., 2006; Galati et al., 2003; Stintzing and Carle, 2005).

3.1.1 Extraction procedures

Given the significance and vast uses of extracted mucilage, it is necessary to investigate the various processes that have been used to extract it from cladodes and fruit.

One of the most used extraction procedures was published by Sepúlveda et al. (2007). Three variables that influenced yield in the extraction process were investigated namely the relationships between cladode and water, the temperature conditions and the type of alcohol used for precipitation. It was determined that the best yields of mucilage were obtained with cladode/water ratio at 1:7, temperature of 40±2˚C and by allowing 4 hours of extraction. The results of the study showed that the average yield after drying was 1.48% based on fresh weight and 19.4% based on dry weight. Results indicated that yield is very dependent on climatic conditions, such as cold and rain due to the ability of the mucilage to absorb water as a defence against stress conditions (Sepúlveda et al., 2007). It was found in this study that rain and temperature had very little impact on the extactability of mucilage.

Medina-Torres et al. (2000) used acetone to precipitate the mucilage, washed it with 2-propanol and finally dried it. Majdoub et al. (2001) used petroleum ether for degreasing and purifying mucilage after filtration and centrifugation. The extraction method proposed by Goycoolea & Cárdenas (2003) involved heating cladodes at 85˚C for 20 min, then liquidizing and centrifuging to obtain the mucilage supernatant. The mucilage was then filtered and precipitated with ethanol. It was freeze-dried and yield of approximately 0.7 g/kg of cladodes were reported.

The aim of the present investigation was to seek a simple, chemical free and more effective process for the extraction of mucilage as conventional solvent extraction methods do not produce the yields necessary for commercial viability. These existing extraction processes are long, expensive, complicated and required the addition of chemicals.

3.2 Materials and methods

3.2.1 Sample collection

Waterkloof is a ten year old cactus pear orchard in the Free State, South Africa, located in the Bloemfontein district. It is 1 348 m above sea level and receives 556 mm rainfall on average. The GPS coordinates are 29°10’53” S, 25°58’38” E. It hosts 40 *Opuntia ficus-indica* cultivars and two *Opuntia robusta* cultivars laid out in a randomized complete block design (RCBD) with two replications for each cultivar. Each replication consists of five data plants.

During the year of 2010, several cladodes were obtained from the orchard at Waterkloof for the extraction of mucilage. Once the extraction procedure was established, four cultivars namely three from *O. ficus-indica* (Algerian, Ficus-Indica and Nepgen) and one from *O. robusta* (Robusta) were harvested in September 2010 for the determination of mucilage yield (%). Three cladodes from the previous growth season that have not produced any fruit, from each cultivar were ramdomly collected from the first, third and fifth data plant. The cladodes were collected from the north side of the plant, the cladode had to be north/south orientated, in the middle of the plant, hip height and of a good quality. Cladodes were stored in paper bags at 4˚C.

3.2.2 Evaluation of the maceration method for the mucilage extraction

Fresh cladodes (*O. ficus-indica* cv. Ficus-Indica) were washed in chlorinated water, the spines were removed and peeled. In order to peel the cladodes, the whole cladode is cut into ± 3 cm strips. The strips are peeled one by one by placing it on a cutting surface and using a very sharp knife. The blade of the knife is placed horizontally on top of the slice and pulled across the surface of the strip to remove only the green outer peel. The peeled cladode pieces were divided into sixteen 100 g samples (four replications/method). The three different maceration applications of a multi food processor (Milex 4-in-1 multi-purpose Mean Juice Machine, model MMJ004) were tested to macerate the samples however, a hand-held Kenwood stick-blender was also used. During the process, 50 ml water was added to the blending process in order to bring the cladodes in contact with the blades. The macerated pulp was then centrifuged using a 12 Hettich centrifuge at 8000 rpm, 4˚C, for 15 minutes.

The four different methods were used to macerate the peeled samples:

- Moulinex juice extractor (Figure 4)
- Moulinex liquidizer fitting (Figure 5)
- Moulinex mincer (Figure 6)
- Kenwood hand held stick blender (Figure 7)

Figure 4: Moulinex juice extractor

Figure 5: The liquidizer fitting for the Moulinex food processor

Figure 6: The mincer fitting for the Moulinex food processor

Figure 7: The Kenwood hand-held stick-blender

3.2.3 Evaluation of methods to separate solids from mucilage

Several different attempts were made to separate the mucilage from the solids namely pressing, filtration, vacuum-filtration and sieves of different shapes and sizes. Finally, centrifugation was attempted at different speeds and durations.

3.2.4 Effect of heating the cladodes on the yield of mucilage

To determine the effect of cooking on the extractability of mucilage, the following experiment were conducted. Eight 100 g samples (four replications) of peeled cladode material were prepared. A Moulinex 4-in-1 multi-purpose Mean Juice Machine, model MMJ004 mincer was used to macerate the peeled cladodes. The minced pulp was cooked without adding water by placing it in a Defy 28 L Electronic microwave oven (900-Watt) for 4 minutes at 100% power. The cooked pulp was then centrifuged at 8000 rpm, 4 ˚C, for 15 minutes. For obtaining raw samples, cladodes were washed, peeled, minced, centrifuged and weighed. For cooked samples, cladodes were washed, peeled, minced, cooked in the microwave oven, centrifuged and weighed.

3.2.5 *Evaluation of the combined treatments for optimal extraction procedures*

Nine individual extraction procedures were carried out on nine cladodes of cv. Algerian to determine the operation and the sequence of procedures in pursuit of the optimal extraction procedure.

- raw or cooked cladodes
- mincing of cladode pieces before or after cooking
- immediate extraction or aged for five days (emulating storing)
- cooking by steaming (placing cladodes in a steaming basket above boiling water in direct contact with steam) or in a microwave oven

Fresh, raw, peeled 'Algerian' cladodes were washed, peeled and sliced. The Moulinex 4-in-1 multi-purpose Mean Juice Machine, model MMJ004 mincer was used to macerate the cladodes into pulp. Cooking was done in a Defy 900-Watt microwave oven at full power for 4 minutes or steaming over boiling water for 4 minutes. Ageing of mucilage took place by refrigeration at 4˚C. Centrifugation of the samples was executed at 8000 rpm, at 4˚C for 15 minutes.

The trial layout was as follows:

Sample 1. Cladode was minced and centrifuged to obtain mucilage. The raw mucilage was weighed on the day of extraction.

Sample 2. Cladode was cooked by steaming before being minced and centrifuged to obtain mucilage. The cooked mucilage was weighed on the day of extraction.

Sample 3. Cladode was minced before being cooked in the microwave oven, and centrifuged to obtain mucilage. The cooked mucilage was aged and weighed on the day of extraction.

Sample 4. Cladode was minced and refrigerated for 5 days and centrifuged to obtain mucilage. The raw mucilage was weighed on the 5th day after extraction.

Sample 5. Cladode was minced and centrifuged to obtain mucilage and refrigerated for 5 days. The raw mucilage was weighed on the 5th day after extraction.

Sample 6. Cladode was cooked by steaming before being minced, centrifuged and refrigerated for 5 days. The cooked mucilage was weighed on the 5th day after extraction.

Sample 7. Cladode was cooked in the microwave oven before being minced, refrigerated for 5 days and centrifuged. The cooked mucilage was weighed on the 5th day after extraction.

Sample 8. Cladode was minced before being cooked in the microwave oven, centrifuged, and refrigerated for 5 days. The cooked mucilage was weighed on the 5th day after extraction.

Sample 9. Cladode was minced before being cooked in the microwave oven, refrigerated for 5 days and centrifuged. The cooked mucilage was weighed on the 5th day after extraction.

The cladodes were weighed before being peeled, cooked and minced according to the treatments. The mucilage and pulp were both individually weighed.

3.2.6 Additional tests to verify the extraction procedure

1. Different small-scale tests were carried out in order to optimize the extraction procedure. Mucilage was extracted using the Moulinex mill and a Kenwood meat mill to determine whether the more finely ground pulp resulting from the meat mill would produce a higher mucilage yield.
2. Three drying methods namely sun-, oven- and freeze-drying were attempted in order to indicate the most effective method of drying mucilage. Sun-drying involved placing thinly sliced whole cladodes on a plastic sheet and allowing it to dry for five days before it was milled. Oven-drying was done by placing the whole sliced cladodes in the oven at 50˚C for 48 hours. For freeze-drying, frozen sliced cladode pieces were placed in the Perano freeze-drier at vacuum at -60˚C and dried for 72 hours. The freeze-dried samples were immediately sieved (Kitchen Craft stainless steel fine mesh 7.5 cm sieve), weighed, vacuum packed (Genesis vacuum sealer) and stored frozen (-18˚C).
3. Peeling the cladode is a tedious task that involves very sharp blades and skill. An assessment was done in order to investigate the effect of peeling the cladodes on mucilage quality and yield.

4. A final test was done using every possible combination of unpeeled, peeled, finely minced and minced, pulp or centrifuged and three drying methods in order to maximize the solubility of the powder once it was in a dried powder form. When the powder is used, it would have to be reconstituted. The rate and ease at which the powder is able to dissolve would be paramount for its functionality as a food additive.

The experimental design is set out in Table 1.

Table 1: Experimental design to maximize the extraction of mucilage and solubility of dried mucilage

	Peel		**Maceration method**	**Plant material**	**Centrifuged**		**Drying Method**
Sample	**With**	**Without**			**No**	**Yes**	
1	X		Kenwood	Pulp	X		Sun-dried
2	X		Moulinex	Pulp	X		Sun-dried
3	X		Moulinex	Pulp	X		Freeze-dried
4		X	Moulinez	Pulp	X		Freeze-dried
5		X	Kenwood	Pulp	X		Freeze-dried
6	X		Kenwood	pulp	X		Freeze-dried
7		X	Moulinex	Mucilage		X	Freeze-dried
8	X		Moulinex	Mucilage		X	Oven-dried
9	X		Kenwood	pulp	X		Oven-dried

3.2.7 Determination of mucilage yield using different cultivars

After the extraction procedure was established, the mucilage yield of four different cultivars was determined. Mucilage was extracted using the extraction procedure established in the preliminary tests. Three cladodes from four cultivars (Algerian, Ficus-Indica, Nepgen and Robusta) were harvested and prepared. The yield was determined by weighing each of the whole cladodes, and weighing again after it was peeled, cooked and minced. After the mucilage was extracted during centrifugation, the extracted mucilage was weighed again.

3.3 Results

3.3.1 Evaluation of the maceration method for the mucilage extraction

Maceration of the cladodes was tested using four different methods. The three different maceration applications of a multi food processor (Moulinex 4-in-1 multi-purpose Mean Juice Machine, model MMJ004) and the handheld Kenwood rotary blender.

Juice extractor: The juice extractor minced the cladodes into a very fine pulp, but the mucilage did not separate or extract during centrifugation. The finely macerated pulp was extremely thick and slimy and the mucilage did not separate from the solids during centrifugation. It was deduced that the juice extractor minced the cladodes too finely. No mucilage was extracted.

Blender with added water: The liquidizer could not function without the addition of water. It was successful in the maceration of the cladode with the addition of water but the supernatant was very watery and thin. The addition of water during maceration diluted the mucilage and displayed no viscosity. This was not desirable as it would require additional processing to remove the added water. After 50 ml distilled water was added, the total mucilage yield from the 100 g sample was 60 ml (10 ml mucilage and 50 ml water).

Mincer: The mincer macerated the cladodes into a rough pulp. The yield of the extracted mucilage (20.84 ml) was higher than the bender (10 ml) and the rotary blender method (14.34 ml). No water was added to the pulp during maceration, thus the supernatant was viscous but separated readily from the solids during centrifugation and was easily decanted. On average 20.84 ml mucilage was extracted from the 100 g sample.

Hand held rotary blender: The hand held blender was only effective when the cladodes were manually finely diced. The pulp was rough and lower yields of mucilage (14.34 ml) compared to the mincer method (20.84 ml) were extracted from the pulp. It required the most manual labour and was therefore deemed an ineffective method. On average 14.34 ml mucilage was extracted from the 100 g sample. It was deduced that the Moulinex mincer (Figure 8) would be used to macerate the cladodes for optimal mucilage extraction.

Figure 8: The Moulinex mincer

3.3.2 *Evaluation of methods to separate solids from mucilage*

Methods that were tested to separate mucilage from solids were ineffective. These methods included pressing (applying pressure using a manual handheld press to extract mucilage), filtration (allowing only liquids to pass through a porous medium such as filter paper), vacuum-filtration (allowing only liquids to pass though filter paper while being drawn by pressure, created by a vacuum) and sieving (placing in a porous (2 mm perforation) sieve for allowing liquids to pass through by means of gravity). The mucilage strongly associated with the solids and no mucilage would sieve through. Different speeds and durations were tested using a centrifuge. It was established that centrifugation at 8000 rpm for 15 minutes (4˚C) is necessary for the mucilage to separate effectively from the solids (data not shown).

3.3.3 *Effect of cooking (heating) of the cladodes on the yield of mucilage*

In an attempt to cook the sliced cladodes or cladode pulp, a microwave oven was used. It was cooked until the bright green colour changed into the olive green colour of cooked green vegetables. It looked and smelled cooked through. In fact, upon removing the bowl from the microwave, a slimy liquid was observed in the bowl. The slimy liquid was identified as mucilage. Cooking in the microwave oven caused the mucilage to be released from the pulp and mucilage was more easily extracted compared to raw cladode pulp. Formal testing revealed this observation to be confirmed. The yield of mucilage after extraction of raw or microwave oven cooked pulp is indicated in Table 2.

Table 2: Effect of microwave cooking on the yield of native mucilage

Sample	**Treatment**	**Average Yield (%)**
Raw samples	100 g cladode pieces, minced, centrifuged and weighed.	46.54
Cooked samples	100 g cladode pieces, minced, cooked in the microwave oven, centrifuged and weighed	58.70

It was evident that cladodes that were cooked in the microwave oven released more mucilage during separation in the centrifuge.

As microwave cooking could be 10 times faster than conventional cooking (Bennion, 1985), the microwave cooking method fulfilled another purpose in inactivating deterioration in tissues and served as a quick pasteurization (blanching) process to destroy microorganisms and inactivate enzymes. A short cooking process such as was used in these extraction procedures would have a minimal effect on the nutritional content of the mucilage as it was not exposed to high temperatures for an extended period of time. The effect of heat on the properties of mucilage would be minimal when using the microwave oven as the exposure to heat is limited compared to the extended exposure to heat applied in several reported extraction procedures (Cárdenas et al., 1998; Gutzeit, 1958; León-Martínez et al., 2010; Ramírez-Moreno et al., 2013).

3.3.4 Evaluation of the combined elements for optimal extraction procedures

After it was established how to macerate the cladodes and that cooking would be required, there was still uncertainty whether the cooking had to be conducted by using the microwave oven (microwaves) or if steaming (placing the cladodes in a steaming basket above boiling water in direct contact with steam) would deliver similar results. Another question that needed attention was the issue of allowing the pulp (minced) to age for several days before the extraction of the mucilage using the centrifuge would result in higher mucilage yield. Therefore the pulp was refrigerated for five days in an air-tight container and compared to immediately extracted mucilage to determine the extractability of mucilage after time passed during storage. The results for the tests are indicated in Table 3.

Table 3: Determination of the applicable and sequence of procedures for optimal mucilage yield of peeled "Algerian" cladodes

Sample	Whole cladode	Peeled cladodes	Minced pulp	Extracted mucilage	Yield of mucilage from pulp	Yield of mucilage from whole cladode
	g	g	g	g	g/100g	g/100g
1	975.00	341.25	273.00	136.50	50.00	14.0
2	805.25	281.84	223.21	125.00	56.00	15.5
3	840.01	294.00	232.85	144.60	62.10	17.2
4	1009.52	353.33	282.67	127.20	45.00	12.6
5	918.81	321.58	257.27	118.60	46.10	12.9
6	839.32	293.76	232.66	91.90	39.50	10.9
7	919.50	321.82	254.88	143.50	56.30	15.6
8	909.63	318.37	252.15	134.90	53.50	14.8
9	869.94	304.48	241.15	151.20	62.70	17.4
Average	898.55	314.49	249.98	≈130.4	≈52.4	≈14.6

Sample 1. Cladode, minced, and centrifuged. **(Raw)**

Sample 2. Cladode, cooked by steaming, minced, and centrifuged. **(Steamed)**

Sample 3. Cladode, minced, cooked in the microwave oven, and centrifuged. **(Microwaved)**

Sample 4. Cladode, minced, refrigerated for 5 days, and centrifuged. **(Raw)**

Sample 5. Cladode, minced, centrifuged, and refrigerated for 5 days. **(Raw)**

Sample 6. Cladode, cooked, minced, centrifuged, and refrigerated for 5 days. **(Steamed)**

Sample 7. Cladode, cooked, minced, refrigerated for 5 days and centrifuged. **(Microwaved)**

Sample 8. Cladode, minced, cooked in the microwave oven, centrifuged, and refrigerated for 5 days. **(Microwaved)**

Sample 9. Cladode, minced, cooked in the microwave oven, refrigerated for 5 days and centrifuged. **(Microwaved)**

Comparing raw to cooked samples, it was seen that raw samples (1, 4 and 5) had lower yields than cooked samples, except for sample 6 that was steamed. Cooking (any cooking method) of the samples before or after mincing it, allowed for higher extraction of mucilage.

When comparing steaming and microwaving as cooking methods, it is was observed that the microwaved samples had on average higher yields (samples 3, 7, 8 and 9). Steamed samples (2 and 6) had on average lower yields, in fact sample 6 had the lowest yield.

Aged samples (4, 5, 6, 8 and 9) displayed no observable trends. In fact sample 6 had the lowest yield and sample 9 the highest. Whether extraction of mucilage takes place immediately after mincing and cooking, or after a few days, did not affect the yield of mucilage.

In terms of the order that procedure should take place, samples that were minced first (3, 8 and 9) before cooking had good results, in fact samples 3 and 9 presented the highest yields. Samples that were cooked first gave low (sample 6) and intermediate results (samples 2 and 7).

The two samples that had the highest yields were samples 3 and 9. These samples had in common the order of work where the cladodes were first minced and then cooked in the microwave oven.

The average yield in this experiment from macerated pulp to mucilage was 52.36 g/100g and the average yield from whole cladodes to native mucilage was 14.56 g/100g. In Figure 9 the yield from the individual samples from pulp and from whole cladodes were illustrated together with the average yields.

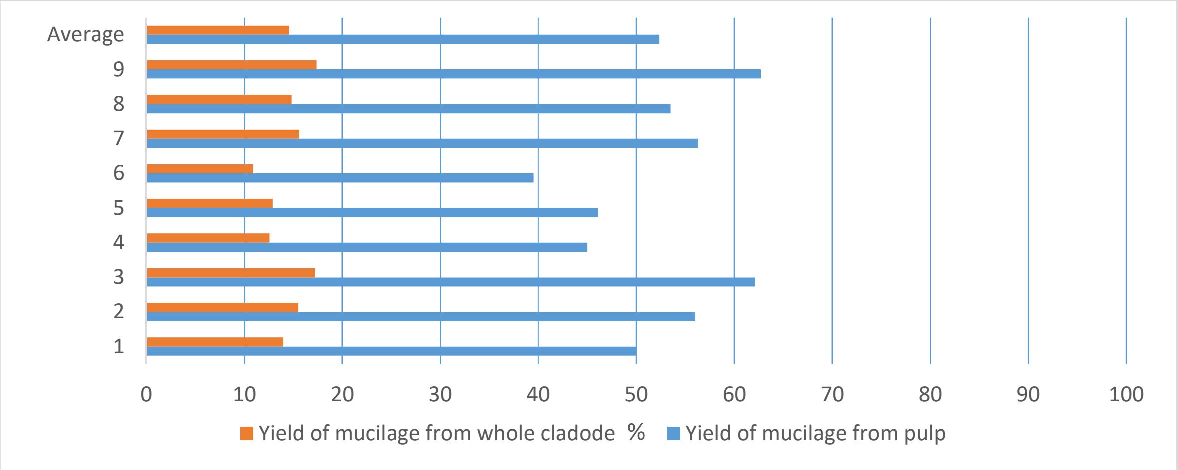


Figure 9: The % yield of mucilage from pulp and from whole 'Algerian' cladodes

The samples are presented as they were completed on the day of extraction (Figure 10) and the 5th day after extraction (Figure 11).

Figure 10: Samples of cactus pear mucilage centrifuged on the day of extraction

Figure 11: Samples of cactus pear mucilage centrifuged on the 5th day after extraction

The yield obtained through the use of this simple extraction procedure was similar to that obtained by other authors (Cárdenas et al., 2008; Del-Valle et al., 2005; Goycoolea and Cárdenas, 2003; Iturriaga et al., 2009a; Majdoub et al., 2001; Medina-Torres et al., 2000; Peña-Valdivia and Sánchez Urdaneta, 2006; Sepúlveda et al., 2007).

3.3.5 Additional tests to verify extraction procedure

The small-scale tests done on mucilage extraction offered the following results:

Very finely ground pulp does not produce more mucilage as the fine pulp seems to have an increased ability to hold on more closely to the mucilage. The recommended Moulinex mill (as described in section 2.2) was in fact the best way of mincing the cladode.

Sun-drying was time-consuming, unreliable and it was difficult to control and duplicate as a method for a scientific investigation. Oven drying of mucilage caused the dried mucilage to dry into a glass-like structure tightly attached to the drying container. Mucilage had to be chiselled loose creating glasslike flakes that had to be ground in order to form a powder. Very low drying temperatures had to be employed as the mucilage quickly developed a burned aroma and the colour darkened considerably. Freeze-drying caused the water to extract and a sponge-like brittle structure formed that was easily removed from the container and easily crushed into a powder. Oven drying was not a practical drying method and freeze-drying of mucilage was the preferred method of drying.

Peeling of cladodes before extracting mucilage delivered both positive and negative results. The argument against peeling was that it required skill, sharp blades, was time consuming and the yield of mucilage reduced, while; peeled cladodes delivered smaller quantities of mucilage with fewer impurities that proved to be an advantage during the centrifugation process.

The ability of dried mucilage to dissolve in distilled water is set out in Table 4. The viscosity of a fluid is a physical manifestation of the molecular weight and the distribution of molecules (Brookfield Engineering Laboratories, 2014).

Table 4: Determination of solubility of differently acquired dried cactus pear mucilage powders

Sample	**Treatments**				Stirred (30 s)				Heated (80 ˚C)			
	With Peel	**Without Peel**	**Maceration method**	**Drying Method**	Dissolved	Sediment	Dispersed	Viscosity	Dissolved	Sediment	Dispersed	Viscosity
1	X		Kenwood	Sun-dried		X		None		X		None
2	X		Moulinex	Sun-dried		X		None			X	Low
3	X		Moulinex	Freeze-dried			X	None			X	None
4		X	Moulinez	Freeze-dried	X		X	None	X		X	None
5		X	Kenwood	Freeze-dried	X		X	None	X		X	None
6	X		Kenwood	Freeze-dried		X		None			X	None
7		X	Moulinex	Freeze-dried	X			High	X			Medium
8	X		Moulinex	Oven-dried			X	None	X			Low
9	X		Kenwood	Oven-dried			X	None		X		None

The best solubility was observed in the freeze-dried powders. The peeled pulp dissolved somewhat after stirring and heating but there were undissolved pieces that floated and were dispersed throughout the solvent. The sun-dried and oven-dried powders did not dissolve readily and the viscosity was not affected. Applying heat for increased solubility only had a positive effect on the unpeeled and oven dried pulp, but the viscosity of the solvent did not reflect that of native mucilage. Consequently the only dried powder that dissolved readily without heating, and affected the viscosity of the solvent sufficiently, was the extracted mucilage that was freeze-dried.

The only samples that resulted in completely dissolved, viscous reconstituted mucilage, was the peeled, roughly minced (using the Moulinex mincer), centrifuged and freeze-dried mucilage powder. Therefore the extraction and drying method used henceforth was validated.

3.3.6 Determination of mucilage yield using different cultivars

It was suspected that not all cactus cultivars would yield equivalent amounts of mucilage. Therefore an investigation was launched to give researchers an indication of the cultivar that could be used for food application purposes. The cultivars used were Algerian (Figure 12), Ficus-Indica (Figure 13), Nepgen (Figure 14) and Robusta (Figure 15).

Figure 12: Cladodes from the cactus pear cultivar Algerian

Figure 13: Cladodes from the cactus pear cultivar Ficus-Indica

Figure 14: Cladodes from the cactus pear cultivar Nepgen

Figure 15: Cladodes from the cactus pear cultivar Robusta

The results of the investigation is presented in Table 5.

Table 5: Comparison of the cactus pear mucilage yield from four cultivars during the extraction procedure

Cultivar	Weight of whole cladode g	Peeled cladode g	Weight of minced pulp g	Mucilage g	Peeled cladode from whole cladode g/100 g	Minced pulp from peeled cladode g/100 g	Minced pulp from whole cladode g/100 g	Mucilage from minced pulp g/100 g	Mucilage from whole cladode g/100 g
Algerian									
Algerian 1	1087	438	375.98	148.04	40.29	85.84	34.59	39.37	13.62
Algerian 2	1123	423.98	350.1	192.43	37.75	82.57	31.18	54.96	17.14
Algerian 3	900	357.55	285.26	100.77	39.73	79.78	31.7	35.33	11.2
Average	**1036.67**	**406.51**	**337.11**	**147.08**	**39.26**	**82.73**	**32.49**	**43.22**	**13.98**
Robusta									
Robusta 1	1002	298.63	199.41	57.68	29.8	66.77	19.9	28.93	5.76
Robusta 2	715	251.8	202.99	65.41	35.22	80.62	28.39	32.22	9.15
Robusta 3	1075	359.3	294.48	119.14	33.42	81.96	27.39	40.46	11.08
Average	**930.67**	**303.24**	**232.29**	**80.74**	**32.81**	**76.45**	**25.23**	**33.87**	**8.66**
Nepgen									
Nepgen 1	719	227	159.57	49.77	31.57	70.3	22.19	31.19	6.92
Nepgen 2	1007	355.99	289.53	39.94	35.35	81.33	28.75	13.79	3.97
Nepgen 3	788.2	228.32	160.99	17.95	28.97	70.51	20.43	11.15	2.28
Average	**838.07**	**270.44**	**203.36**	**35.89**	**31.96**	**74.05**	**23.79**	**18.71**	**4.39**
Ficus-Indica									
Ficus-Indica 1	518	213.42	147.98	57.58	41.2	69.34	28.57	38.91	11.12
Ficus-Indica 2	700	224.8	169.94	74.28	32.11	75.6	24.28	43.71	10.61
Ficus-Indica 3	720	211.52	170.85	74.54	29.38	80.77	23.73	43.63	10.35
Average	**646**	**216.58**	**162.92**	**68.8**	**34.23**	**75.24**	**25.52**	**42.08**	**10.69**
Overall average	**862.85**	**299.19**	**233.92**	**83.13**	**34.57**	**77.12**	**26.76**	**34.47**	**9.43**

It was confirmed that mucilage from different cultivars inherently had particular characteristics which affected the extractability of the mucilage. 'Algerian' cladodes were the biggest (1036 g) in this study, while 'Ficus-Indica' were the smallest (646 g). The average weight of cladodes were 862.85 g. Once the cladodes were peeled, an average of 34.57 g/100 g of the cladodes remained. The highest amount of peeled pieces (406.51 g) remained in 'Algerian' while 'Ficus-Indica' had the least (216.19 g). After mincing, there was another loss in weight, with the remaining pulp weighing an average of 77.12 g/100g of the peeled cladode pieces. The profile remained unchanged with 'Algerian' having the highest amount of minced pulp and 'Ficus-Indica' the least. After the mucilage was extracted, 'Algerian' had the highest yield (147.08 g) but 'Nepgen' had the lowest yield (35.89 g). Only 18 g/100 g of the original pulp remained of the 'Nepgen' mucilage. In comparison to the whole cladode, 'Nepgen' had the lowest yield (4.39 g/100 g) while 'Algerian' had the highest yield (13.98 g/100g). 'Ficus-Indica' mucilage yield was slightly lower than 'Algerian' (10.69 g/100 g). The lowest yield was that of 'Nepgen' (4.39 g/100 g). Overall, the native mucilage yield from whole cladodes was 9.43 g/100 g. 'Robusta' yields remained intermediate throughout. The mucilage yield from the whole cladode (80.74 g or 8.66 g/100 g) was higher than 'Nepgen', but lower than' Algerian' and 'Ficus-Indica'. 'Algerian' proved to be supreme in providing mucilage. 'Algerian' mucilage was therefore suggested for food application purposes.

3.4 Conclusion

3.4.1 Method of mucilage extraction

An effective, easy and inexpensive method of mucilage extraction has been established in this study. No chemicals have been used in this method and as such, the extracted mucilage is natural and unadulterated by chemicals.

Coarsely mincing the cladodes into pulp using the Moulinex mill and freeze-drying the mucilage proved to be the most effective method of obtaining larger yields of mucilage. The task of peeling the cladodes was tedious and impaired the yield of pulp obtained from the cladodes but peeling the cladodes first may provide an unadulterated, superior quality mucilage product. Unpeeled cladodes produced a higher yield of mucilage. Freeze-drying was imperative for the dried mucilage powder to dissolve readily.

The optimized extraction method as proposed by this study is as follows:

Step 1: Peeling

The cladodes has to be readied for the procedure by washing it in chlorinated water (Figure 16). It is important to cut away all the hard and unnecessary fibres that do not yield any mucilage. The green part of the cladode as well as the hard fibres inside the green peel must be cut away. Only the light green slimy inside part of the cladode should remain.

Figure 16: Cactus pear cladodes washed and ready or the extraction procedure

Figure 17: Peeled cactus pear cladodes

It is important to note that peeling the cladodes is the most effective procedure for obtaining the highest quality, untarnished and pure mucilage. However, lacking an efficient automated or electronic device to reduce the skilled manual labour involved in the time consuming hand peeling process that required skill and sharp knives, the peeling of cladodes will be omitted in later mucilage extraction procedures (chapters 4, 5, 6 & 7).

The argument against peeling was that it and the yield of mucilage reduced, while; peeled cladodes delivered smaller quantities of mucilage with fewer impurities that proved to be an advantage during the centrifugation process.

Step 2: Slicing

The inside part of the cladodes cut into a size that is easy to handle (Figure 18).

Figure 18: Cactus pear cladodes peeled and cut into pieces

Step 3: Microwave cooking

The cladodes pieces are cooked for the purpose of softening it before the mincing process and for the cladodes to release more mucilage. Cooking is done in the microwave oven (900-Watt) at 100% power for 4 minutes or until the cladode pieces are soft and are cooked through and the mucilage is visible (Figure 18). Cooked pieces are softer and therefore take less energy and time to mince. Cooking cladodes first before maceration is therefore the most productive and easiest procedure.

Figure 19: Cactus pear mucilage visible after cooking in the microwave oven

Step 4: Maceration

Macerating the cladode pieces into a rough pulp is most effective for extraction of mucilage.

Figure 20: Cactus pear mucilage visible in the macerated pulp

The rotary blade of a Moulinex 4-in-1 multi-purpose Mean Juice Machine model MMJ004 was used. It has a fast moving rotating blade that macerates the cladode pieces without the addition of water. The cooked cubes were easily and quickly macerated compared to raw pieces.

Step 5: Centrifuge

The mucilage pulp observed in Figure 21 was divided into the centrifuge tubes. Cladode pulp act as a solid and has to be cut using a sharp knife or scissors for it to be divided in centrifuge tubes.

Figure 21: Flow behavior of cactus pear cladode pulp

After dividing the pulp into tubes, it was centrifuged at 8000 rpm for 15 minutes at 4˚C to separate the mucilage from the solids and obtain the mucilage supernatant (Figure 22).

Figure 22: Cactus pear mucilage and solids separate during centrifugation

The mucilage was decanted and weighed while the solid material left in the tubes were discarded (Figure 22).

Consequently, the method for extracting mucilage from *Opuntia ficus-indica* and *Aloe barbadensis* was patented in 2011 by the University of the Free State (Du Toit and De Wit, 2011).

Figure 23: Extracted mucilage

It is important to note that the extraction process is conducted without the addition of water. In the determination of yield using different cultivars, it was observed that cladodes were not equal in terms of mucilage yield. Out of the four cultivars investigated, 'Algerian' yielded the most mucilage.

3.5 Recommendations for further study

Extracted mucilage has enormous potential for its utilization as an additive that is natural and inexpensive. The numerous possibilities of obtaining different products and by-products from cactus pear cladodes opened new opportunities for the semi-arid regions. It could add value to cactus pear as a crop and give purpose to the unused mature cladodes. This crop has much to contribute to human food and can be a potential source for human medicine, a source of natural additives to the food industry and a potential food source for the inhabitants of Southern Africa. Nevertheless, many aspects require further research.

All the cultivars (42) available at Waterkloof farm need to be screened for mucilage extraction. The cultivars to be used in future food applications need to be identified and characterized.

- Size and weight of cladodes have to be compared to mucilage yield.
- The role of viscosity and water content on the extractability of mucilage has to be determined.
- Not only the cultivars but the time of cladode harvesting for the purpose of extracting mucilage should be determined.

- The flow properties of mucilage will shed light on how it will act when present in food and predict its behaviour and performance.
- A process for the drying of mucilage needs to be established.
- Once cultivars have been selected, analysis of the functional and chemical properties of native and dried mucilage has to be undertaken.
- Native as well as dried mucilage has to be successfully included in food products and thus diets of local South Africans. These preliminary studies have revealed that vast differences between mucilage yield and viscosity earmarks certain cultivars more useful as food additives and others as food source.

Chapter 4
Morphological, moisture, mucilage yield and- viscosity evaluation of cladodes from forty-two available South African cactus pears cultivars

Abstract

Opuntia ficus-indica cladodes contains a slimy liquid (mucilage) that has potential to add value and promote the cactus pear as a crop in South Africa. Extracted mucilage could be developed into a commercial nutraceutical product that could find many different applications in food products owing to its capacity to absorb water. The aim was to evaluate and select amongst the 42 available cultivars, those that will produce optimal mucilage yield and quality. Investigations were conducted on the cladodes (weight, size and moisture content) and the native mucilage (yield and viscosity) after which the relationships amongst attributes were correlated. Cladodes weights were mostly between 400 and 800 g. Nepgen had the lowest yield (3.21%/3.64 g) while Algerian had the largest mucilage yield (37.41%/42 g), the average yield was 17.5%. The average moisture content was 87.08% while solids made up 12.92% of whole cladodes. The cultivars were grouped according to viscosity into the five classes (low, medium-low, medium, medium-high and high) of viscosity. The correlations indicated that there were no discernible relationship between mucilage yield and cladode weight while a moderate positive correlation was indicated between mucilage yield and cladode moisture content. The strongest correlation was found between mucilage yield and mucilage viscosity. A weak positive relationship was indicated between cladode moisture content and mucilage viscosity. Thus, cultivars that inherently contain low viscosity mucilage would contribute the highest yield of mucilage. The eight identified cultivars that were recommended for further studies were Turpin, Gymno-Carpo, Algerian, Malta, Van As, Ficus-Indica, Morado, Tormentosa, Meyers and Robusta (different species).

4 Morphological, moisture, mucilage yield and- viscosity evaluation of cladodes from forty-two available South African cactus pears cultivars

4.1 Introduction

The cactus pear plant thrives all over the world and is especially valued in arid and semi-arid regions such as Africa, South America, Australia, Asia and the Mediterranean basin (El Kossori et al., 1998). The cactus pear plant has received a surge of research interest for its significance as a crop that uses water very efficiently (Yahia et al., 2009). It is valued as animal fodder as it is highly palatable to ruminants, contain high moisture and is an appropriate plant for sustainable agricultural systems in arid areas where crops have to withstand drought, high temperatures and poor soil (Nefzaoui and Ben Salem, 2002). It is one of Mexico's most valued resources as it has such a wide germplasm variability as well as many potential uses. Furthermore it is seen as an emergent fruit crop in countries like Morocco, Tunisia, Ethiopia, Yemen and Turkey where the fruit has become more popular than other common fruit such as oranges or bananas (Yahia and Mondragon-Jacobo, 2011).

As the stems or pads are safe for human consumption, they are considered an important and healthy food source in Latin America (Sáenz, 2000). It has been nicknamed "the bread of the poor" (Feugang et al., 2006) and “the bridge of life” (Sáenz, 2000) as it is a readily available food source and is enjoyed as an appetizing vegetable dish (Sáenz, 2000). In fact, the serving of the fresh young and tender cactus pads called "nopalitos" sliced into a salad or stir-fried and served with cheese, is deeply embedded in the culture and local cuisine (Feugang et al., 2006). Mucilage, a slimy liquid that oozes from the cladodes, is a complex polysaccharide carbohydrate, with a highly branched structure (Trachtenberg and Mayer, 1982b). Mucilage is classified as a hydrocolloid seeing that it contains long-chain polymers that dissolve in water to give a thickening or viscosity producing effect. Mucilage extracted from the cladodes could be used in many different ways in food products as it has the capacity to absorb large amounts of water and is a non-Newtonian liquid (Sáenz, 2000). It could lead to the commercialization of cactus products and turn the cultivation, processing and development of new products into new business opportunities (Sumaya-Martínez et al., 2011).

The aim was to evaluate the 42 cultivars of the *Opuntia* spp. available at Waterkloof farm (the experimental orchard outside Bloemfontein) in order to select cultivars with the highest mucilage yield for further investigation. The cultivars (cladodes and mucilage) will be evaluated according to morphological characteristics in order to determine the cultivars that would produce optimal yield and quality of extracted mucilage. The most important consideration for selecting cultivars for further investigation was first and foremost the quantity of mucilage, as abundance of mucilage would increase productivity and profit if mucilage is to be commercialized. It is essential to investigate primary traits such as cladode size, weight, moisture content and viscosity in relation to yield, in order to identify cultivars with the ideal characteristics. The optimal mucilage extraction procedure and method of drying as well as the ability of dried mucilage to dissolve was included in this study.

4.2 Materials and methods

4.2.1 Sample collection

Waterkloof is a ten year old cactus pear orchard in the Free State, South Africa, located in the Bloemfontein district. It is 1 348 m above sea level and receives 556 mm rainfall on average. The GPS coordinates are 29°10’53” S, 25°58’38” E. It hosts 40 *Opuntia ficus-indica* cultivars and two *Opuntia robusta* cultivars (Robusta and Monterey). The orchard is laid out in a RCBD with two replications for each cultivar. Each replication consists of five data plants.

The first screening of all cultivars was done during the dormant phase (July) in 2013. From the five data plants per replication, one cladode from the first and the third plant was sampled. Thus four cladodes of every cultivar (two from each replication) amounted to a total of 168 samples.

Harvesting was done on 17 July 2013 between 9:00 and 11:15. In order to standardize the collection of cladodes, cladodes were collected from the north side of the plant, the cladode had to be north/south orientated, in the middle of the plant, hip height and of a good quality. Only the youngest cladodes of the previous growth season (December 2012-April 2013) were sampled. These cladodes have not produced any fruit in previous seasons.

4.2.2 Sample preparation

The cladodes were bagged in absorbent paper bags, labelled and transported. The cladodes were weighed, measured and photographed whole. They were refrigerated at 4˚C until the extraction and analysis commenced (no longer than one week). Since carrying out extracting procedures on the whole cladodes would have been unnecessary for the purposes of this initial selection process, a standard size circular segment was used for analysis. This segment was from the middle of the cladode and another smaller standard size circular segment from the top end of the cladode was used for the moisture content determinations.

4.2.3 Morphological determinations

The size (length, width and diameter in mm), weight (g), volume (mm^3) and surface area were obtained as these are important tools when judging cultivars, seeing that it demonstrates the yield from each cladode and thus the productivity of the cultivar. Hernández et al. (2010) developed a fast, easy and inexpensive method for estimating cladode surface area. This protocol is not entirely accurate for all cultivars but is useful for practical purposes. The elliptical mathematical formula is as follows:

$$x = \left(\frac{W}{2}\right) \times \left(\frac{L}{2}\right) \times \pi$$

Where:
X = estimated area
W = width (minor axis)
L = length (major axis)
π = 3.141516 (Hernández et al., 2010).

Weight, volume and surface area are three different ways of indicating size and weight of cladodes. Nevertheless, the three different measurements were compared in order to determine whether they correlate to each other and determine which means of measure could be indicative of all three values.

4.2.4 Yield determination

The mucilage was extracted according to the patented method described in chapter 3 of this thesis (Du Toit and De Wit, 2011). The unpeeled cladode segments were weighed and sliced before it was cooked in the microwave oven. It was minced using the household Moulinex food mill. The minced and cooked pulp (M&C) was weighed again for yield recording purposes. No water was added during the extraction process. The supernatant that dissociated from the solids during the centrifugation process was decanted and weighed using a Radwag PS 750/C/2 scale in order to obtain the yield (g) of native mucilage.

$$Yield\ of\ mucilage\ (\%) = \frac{supernatant\ liquid\ (g)}{original\ cladode\ segment\ (g)} \times 100$$

4.2.4.1 The correlation of mucilage yield and cladode weight

Mucilage yield was correlated to cladode weight in order to observe the interrelationship between the weight of a cladode and the amount of mucilage that was extracted from the segment of cladode.

4.2.5 Waste determination

The solid material that precipitated in the bottom of the centrifuge tube was also weighed in order to obtain a percentage of solid wastage.

$$Yield\ of\ solid\ waste\ (\%) = \frac{precipitated\ solids\ (\mathrm{g})}{original\ cladode\ segment\ (g)} \times 100$$

4.2.6 Moisture and Solids determination

The circular segment of cladode obtained by the method described in Section 3.2.2 was cut into small pieces by dividing the segment firstly in thirds horizontally and secondly vertically into thirds. The metal petri dishes were weighed with and without sample by using a Metter AE 200 scale and the weight was recorded to three decimal points. The samples were placed inside an Eco Therm oven at 102˚C for 24 hours. It was placed in a desiccator vacuum containing silica crystals for 1 hour and the dried samples were weighed (Reeb and Milota, 1999). Moisture content (%) and solid content (%) were determined for every sample. It was calculated as follows:

$$Moisture\ content\ (\%) = \frac{original\ sample\ (g) - dry\ sample(g)}{original\ sample(g)}\ x\ 100$$

$$Solids\ content\ (\%) =\ 100\% - moisture\ content$$

4.2.6.1 The correlation of mucilage yield and cladode moisture content

Mucilage yield was correlated to cladode moisture content in order to observe the interrelationship between the moisture levels of a cladode and the amount of mucilage that was extracted from the cladode segment.

4.2.7 Viscosity determinations

Three uncomplicated and rudimentary methods were used to judge the consistency of the mucilage in terms of the viscosity. These three methods were used due to the diversity of the mucilage consistency in that a single method may not sufficiently indicate the nature of the mucilage consistency. In order to categorize the cladodes, the viscosity of mucilage of each cladode was candidly described as low, medium and high.

4.2.7.1 The Line-spread test

The line-spread test is a method of comparing the relative viscosity of a viscous liquid such as gels, hydrocolloids and other thickened liquids in a quick, reliable and inexpensive way (Kim et al., 2014)

The test was performed using a sheet of paper, covered with glass, marked with concentric circles; each circle was 0.5 cm from the other, evenly measured off. It was marked from 1 to 13 cm. The circle was divided into eight parts and covered with a glass plate in order to provide an even and level surface. A 1 cm open ended metal cylinder that corresponded with the smallest circle was placed on the chart and filled with 5 ml of mucilage. The cylinder containing mucilage was lifted in order for the mucilage to flow freely. When the mucilage stopped flowing, the distance it flowed was recorded (cm) on the eight lines dividing the circle. The distance values were added up to indicate the line-spread measurement value. The higher the reading of the line-spread test, the lower the viscosity of the mucilage as a result of it spreading further (Kim et al., 2014).

4.2.7.2 The Cylinder test

The cylinder test is an easy and inexpensive test to measure viscosity of liquids by determining the time it takes a plastic marble to fall through a measured amount of mucilage. It involves a measured cylinder tube and a marble with the correct weight for the viscosity of the liquid, in this case the mucilage. A 10 ml measuring cylinder was filled to the 5 ml mark with mucilage. The moment the marble was dropped, the time was recorded for the marble to reach the bottom of the cylinder, using a stopwatch (Sciencebuddies.org, 2013). Time was recorded in seconds (s).

4.2.7.3 The Separating funnel test

The separating funnel test was developed by the researchers of the University of Nebraska-Lincoln describing a technique to measure viscosity using a modified Beral pipette (University of Nebraska-Lincoln, 2013). In the experiment, a separating funnel was used instead of a Beral pipette. The measured 5 ml of mucilage was poured into the separating funnel, the stop tap opened, and the time recorded for the mucilage to run through the funnel using a stopwatch. Time was recorded in seconds (s).

4.2.8 Categorization of the cultivars according to the viscosity of mucilage

In an attempt to classify and arrange the cultivars according to viscosity, the results for all three tests (line-spread, separating funnel and cylinder), together with the visual physical descriptions on the appearance of the mucilage, were considered. A value of low, medium or high viscosity was given to each sample. The value was hence assigned: low = 1, medium = 2 and high = 3. The scores for each of the four samples of each cultivar was added up, the subsequent total scores were interpreted for each cultivar as follows: 4-5 Low, 6-7 medium low, 8-9 medium, 10-11 medium high and 12 was high viscosity. The cultivars were grouped according to the total values in order to organize them into the five classes.

4.2.8.1 The correlation of mucilage yield and viscosity

Yield was correlated to viscosity (line-spread values) in order to observe the interrelationship between the viscosity of the mucilage and the amount of mucilage that was extracted from the cladode segment.

4.2.8.2 The correlation of cladode moisture content and mucilage viscosity

A final correlation between moisture content and viscosity was necessary to investigate the interrelationship between the amount of moisture found in a cladode and the viscosity of the mucilage that was extracted therefrom.

4.3 Statistical Methods

A one way analysis of variance (ANOVA) procedure (NCSS, 2007) was used to determine the effect of cultivar on the morphological, yield, viscosity, moisture and solid content of mucilage. The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to determine whether significant differences exist between treatment means (NCSS, 2007). The Analysis Toolpak add-in in Excel was used to find the correlation coefficient between two variables (Excel easy, 2016).

4.4 Results and discussion

4.4.1 Morphological determinations

The cladode weight, size, volume and surface area measurements were important observations as it increased knowledge and understanding of cladodes originating from different cultivars and could play a meaningful role when selecting cultivars for mucilage production.

The weight of the whole cladodes ranged between 335.5 g ('Ofer') and 924.5 g ('Arbiter') (Table 6). The average weight for cladodes was 578.5 g. Most of the cladodes were not significantly different from each other as far as weight was concerned. 'Arbiter' was significantly heavier than 'Ofer', 'R1251' and 'Turpin' and 'Skinners Court' was significantly heavier than 'Ofer'.

Wessels (1989) described three shapes of *Opuntia ficus-indica* plants; the bushy-type (Figure 24), the columnar shape that has elongated cladodes (Figure 25) and the round-cladode shaped plants (Figure 26).

Figure 24: The bushy-type cactus pear plant has oval shaped cladodes that grow outwards

The longest cladode was 'Skinners Court' (434.25 mm) while the shortest was 'Robusta' (280 mm) that corresponded with the plant and cladode shapes described by Wessels (1989). 'Arbiter' had long and narrow cladodes while 'Robusta' had a round shape. Average length of all the cultivar's cladodes was 347.9 mm. ‘Amersfoort’, ‘Arbiter’, ‘Meyers’, ‘Postmasburg’ and ‘Skinners Court’ had significantly longer cladodes than all other cladodes. Four cultivars had cladodes longer than 400 mm namely ‘Meyers’ (400 mm), ‘Amersfoort’ (404 mm), ‘Skinners Court’ (434.25 mm) and ‘Postmasburg’ (408 mm).

Figure 25: The columnar cactus pear type plant

Figure 26: The round-shaped cladodes of *O. robusta*

The cultivar with the widest measurement for cladodes in Table 6, was ‘American Giant’ with 255.25 mm and the narrowest cladode was ‘Roly Poly’ (134.75 mm). The average width of cladodes of all cultivars were 188.94 mm. ‘Monterey’, ‘R1260’, ‘Robusta’ and ‘Turpin’ together with ‘American Giant’ had significantly wider cladodes, while ‘Blue Motto’, ‘Direkteur’ and ‘Roly Poly’ had significantly narrower cladodes than all other cultivars. In fact, it is clear from Table 6 that most of the cultivars did not significantly differ from each other in terms of width.

The exact length and width measurements of cladodes may be important when standard sizes may be required for industrial peelers in which case the very long or very wide cladodes may cause excessive waste (Table 6).

The diameter of the thickest cladode was 16.5 mm (‘Mexican’) and the thinnest was ‘Ofer’ with 6.75 mm and the average diameter for all the cultivars was 10.36 mm (Table 6). ‘Mexican’ cladodes had significantly shorter as well as significantly thicker cladodes that add up to average volume and surface area. ‘Nepgen’, ‘Postmasburg’, ‘Robusta’ and ‘Ofer’ had significantly thinner cladodes, while ‘Mexican’ and ‘Arbiter’ had significantly thicker cladodes than all other cultivars.

Thicker cladodes may produce more mucilage when cladodes are peeled as more mucilage rich tissue may be accessible.

In terms of volume (Table 6), only 'Ofer' with a volume of 38,1953 mm^3 was significantly lower than 'Skinners Court' with a volume of 99,3193.5 mm^3. The average volume (68,2070.96 mm^3) corresponded with the size of the cladodes. 'Ofer' had the lightest and shortest cladodes while 'Arbiter'had the heaviest and 'Skinners Court' the longest cladodes.

'Tormentosa' had the widest cladodes while 'Skinners Court' and 'Roly Poly' had the thickest diameter (Table 6). 'Ofer' (the second lowest surface area) cladodes were consistently the smallest cladodes and were among the cultivars with the lowest surface area together with cultivars 'Amersfoort', 'Malta', 'Nepgen', 'Nudosa' and 'R1251'. The two cultivars with significantly larger surface areas were 'Arbiter' and 'Skinners Court' of which 'Arbiter' was the highest. The average surface area of all the cultivars was 2852.96 mm^2. In summary, most of the cladodes did not differ significanlty in terms of weight, in fact most cultivars weighed between 400 and 800 g. The exceptions were 'Arbiter' and 'Skinner Court' which had signicantly heavier cladodes and 'Ofer' which had significanlty lighter cladodes (significance level $p = 0.002$) than all other cultivars.

Table 6: The morphological properties of the whole cladodes of 42 cactus pear cultivars harvested in July 2013

Cultivar	**Weight (g)**	**Length (mm)**	**Width (mm)**	**Diameter (mm)**	**Volume (mm^3)**	**S. area (mm^2)**
Algerian	655.50^{abc}	319.00^{abcd}	206.25^{cdef}	12.00^{abc}	869768.50^{ab}	3137.39^{ab}
American Giant	589.00^{abc}	309.00^{abcd}	255.25^{f}	8.00^{ab}	629640.00^{ab}	1964.82^{a}
Amersfoort	707.00^{abc}	404.25^{cde}	175.50^{abcde}	11.75^{abc}	827548.25^{ab}	3713.47^{ab}
Arbiter	924.50^{c}	395.00^{bcde}	182.50^{abcde}	15.50^{bc}	1087846.25^{b}	4740.74^{b}
Berg x Mexican	503.50^{abc}	358.75^{abcde}	192.25^{abcdef}	12.75^{abc}	866215.50^{ab}	3576.42^{ab}
Blue Motto	513.50^{abc}	376.50^{abcde}	148.00^{abc}	12.25^{abc}	685639.50^{ab}	3627.27^{ab}
Corfu	680.50^{abc}	341.25^{abcde}	189.00^{abcde}	11.75^{abc}	767509.50^{ab}	3172.15^{ab}
Cross X	555.00^{abc}	354.00^{abcde}	180.75^{abcde}	10.00^{abc}	674323.00^{ab}	2808.52^{ab}
Direkteur	491.00^{abc}	362.25^{abcde}	138.75^{ab}	12.25^{abc}	641902.00^{ab}	3512.80^{ab}
Ficus-Indica	471.50^{abc}	339.50^{abcde}	179.75^{abcde}	11.75^{abc}	751832.50^{ab}	3193.16^{ab}
Fresno	762.50^{abc}	350.25^{abcde}	186.75^{abcde}	13.50^{abc}	901240.00^{ab}	3721.72^{ab}
Fusicaulis	777.50^{abc}	360.00^{abcde}	195.75^{abcdef}	12.75^{abc}	896555.00^{ab}	3575.44^{ab}
Gymno-Carpo	487.50^{abc}	342.00^{abcde}	189.00^{abcde}	10.00^{abc}	661165.00^{ab}	2721.34^{ab}
Malta	442.00^{abc}	296.50^{abc}	196.00^{abcdef}	8.50^{ab}	491878.25^{ab}	1975.82^{a}
Messina	514.00^{abc}	349.75^{abcde}	168.25^{abcd}	10.00^{abc}	591292.00^{ab}	2774.74^{ab}
Mexican	720.00^{abc}	295.75^{abc}	167.50^{abcd}	16.50^{c}	855018.75^{ab}	3937.69^{ab}
Meyers	575.00^{abc}	400.50^{cde}	199.00^{abcdef}	8.25^{ab}	652615.00^{ab}	2581.54^{ab}
Montery	719.00^{abc}	317.50^{abcd}	233.50^{ef}	12.25^{abc}	900047.50^{ab}	3036.67^{ab}
Morado	558.25^{abc}	361.50^{abcde}	193.75^{abcdef}	8.75^{abc}	613353.75^{ab}	2474.73^{ab}
Muscatel	784.00^{abc}	383.75^{abcde}	170.75^{abcde}	11.25^{abc}	771011.75^{ab}	3464.90^{ab}
Nepgen	571.50^{abc}	382.50^{abcde}	190.00^{abcde}	7.00^{a}	521334.00^{ab}	2148.80^{a}
Nudosa	530.00^{abc}	304.00^{abcd}	190.25^{abcde}	8.00^{ab}	448150.00^{ab}	1887.66^{a}
Ofer	335.50^{a}	324.75^{abcd}	174.00^{abcde}	6.75^{a}	381953.00^{a}	1719.59^{a}
Postmasburg	551.50^{abc}	408.50^{de}	178.75^{abcde}	7.50^{a}	552624.50^{ab}	2409.15^{ab}
R1251	396.50^{ab}	291.75^{ab}	173.50^{abcde}	8.50^{ab}	435964.50^{ab}	1958.15^{a}
R1259	472.50^{abc}	316.75^{abcd}	191.25^{abcdef}	9.25^{abc}	560016.00^{ab}	2299.59^{ab}
R1260	650.50^{abc}	362.00^{abcde}	225.25^{def}	10.50^{abc}	895697.50^{ab}	3048.45^{ab}
Robusta	476.00^{abc}	280.50^{a}	218.00^{def}	7.00^{a}	426461.00^{ab}	1542.09^{a}
Robusta xCastillo	696.00^{abc}	330.50^{abcde}	208.25^{cdef}	10.50^{abc}	741030.00^{ab}	2759.63^{ab}
Roedtan	505.50^{abc}	331.00^{abcde}	204.25^{cdef}	10.75^{abc}	718563.75^{ab}	2760.02^{ab}
Roly Poly	443.00^{abc}	301.25^{abcd}	134.75^{a}	13.75^{abc}	555967.75^{ab}	3236.55^{ab}
Rossa	621.00^{abc}	364.50^{abcde}	207.75^{cdef}	10.25^{abc}	782588.75^{ab}	2940.07^{ab}
Santa Rosa	577.00^{abc}	354.50^{abcde}	200.00^{bcdef}	11.25^{abc}	799129.00^{ab}	3124.43^{ab}
Schagen	574.50^{abc}	335.75^{abcde}	195.50^{abcdef}	10.50^{abc}	686178.25^{ab}	2774.55^{ab}
Sharsheret	522.67^{abc}	374.67^{abcde}	190.00^{abcde}	9.00^{abc}	660556.33^{ab}	2641.75^{ab}
Sicilian Indian Fig	524.50^{abc}	314.75^{abcd}	175.75^{abcde}	9.75^{abc}	573985.00^{ab}	2468.84^{ab}
Skinners Court	899.50^{bc}	434.25^{e}	167.25^{abcd}	13.75^{abc}	993193.50^{ab}	4678.70^{b}
Tormentosa	578.50^{abc}	392.25^{bcde}	205.00^{cdef}	8.25^{ab}	671315.00^{ab}	2552.68^{ab}
Turpin	403.50^{ab}	304.75^{abcd}	213.50^{def}	8.75^{abc}	583262.50^{ab}	2102.07^{a}
Van As	521.50^{abc}	363.25^{abcde}	195.75^{abcdef}	8.25^{ab}	582511.75^{ab}	2334.74^{ab}
Vryheid	577.50^{abc}	378.00^{abcde}	167.25^{abcd}	8.25^{ab}	533346.00^{ab}	2489.65^{ab}
Zastron	439.00^{abc}	345.75^{abcde}	181.50^{abcde}	8.25^{ab}	514179.75^{ab}	2235.97^{ab}
Average	**578.53**	**347.93**	**188.95**	**10.37**	**682070.96**	**2852.96**
Significance level	$p = 0.002$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.015$	$p < 0.001$

Cultivar means with different superscripts in the same column differ significantly

4.4.2 Yield determination

The utmost productivity of mucilage can only be accomplished when optimal mucilage could be extracted from minimal raw plant material. Therefore, the assessment of the amounts and percentage yields of mucilage extracted from different cultivars is one of the most important objectives of this investigation.

A standard size segment/portion/fragment of each cladode was separated from the whole cladode (unpeeled segment) and the weight of this segment ranged from 82.4 g ('Meyers') to 141.27 g ('Arbiter') as a result of the diameter differences (Table 7). There were little significant differences in terms of segment weight: 'Arbiter' segment weighed significantly more than cultivars 'Meyers', 'Ofer', 'Robusta' and 'Zastron' segments. The average weight of the segments for all cultivars were 102.10 g which culminated to 13.55% loss of weight during the preparation of the pulp. The loss occurred when the diced segment pieces were cooked in the microwave oven and minced. 'Arbiter' still had significantly more pulp than 'Robusta', nevertheless the amount of pulp was within acceptable boundaries of between 63 g and 120 g. The average for all cultivars was 88.26 g. The mucilage recovered after extraction was between 3 and 42 g (Table 7), therefore it was observed that the amount of mucilage that was extractable from the pulp differed among cultivars. The average mucilage extracted for all cultivars were 17.76 g and 17.45% of the original cladode segment. The cultivars that yielded significantly less mucilage than 'Algerian' were 'American Giant' (10.55 g/11.59%), 'Blue Motto' (9.6 g/9.53%), 'Corfu' (8.12 g/6.28%), 'Messina' (6.87 g/6.96%), 'Postmansburg' (10.25 g/10.36%), 'Robusta' (6.61 g/7.57%), 'Zastron' (6.32 g/6.91%) and' Nudosa' (4.35 g/4.35%), while 'Nepgen' yielded the least (significant different $p < 0.001$) mucilage with 3.64 g that was 3.21% of the original segment. It was therefore demonstrated that the greatest differences in yield occurs when mucilage is separated from the solids (during centrifugation) rather than the mincing and cooking processes.

Table 7: Yield of extracted mucilage from cladodes of 42 cactus pear cultivars harvested in July 2013

Cultivar	**Unpeeled segment (g)**	**Pulp (g) (Minced & Cooked)**	**Mucilage yield from segment (g)**	**Mucilage yield from segment (%)**
Algerian	107.17^{ab}	98.28^{ab}	42.39^{b}	37.41^{e}
American Giant	92.21^{ab}	78.43^{ab}	10.55^{a}	11.59^{abcd}
Amersfoort	109.12^{ab}	94.14^{ab}	22.65^{ab}	19.56^{abcde}
Arbiter	141.27^{b}	122.91^{b}	20.66^{ab}	15.43^{abcde}
Berg x Mexican	89.30^{ab}	77.03^{ab}	19.76^{ab}	21.49^{abcde}
Blue Motto	99.69^{ab}	85.87^{ab}	9.60^{a}	9.52^{abcd}
Corfu	116.13^{ab}	99.23^{ab}	8.12^{a}	6.28^{abc}
Cross X	101.06^{ab}	92.07^{ab}	29.39^{ab}	29.87^{cde}
Direkteur	109.94^{ab}	98.60^{ab}	13.68^{ab}	12.16^{abcd}
Ficus-Indica	92.62^{ab}	75.36^{ab}	30.79^{ab}	33.62^{de}
Fresno	99.12^{ab}	87.66^{ab}	13.78^{ab}	13.80^{abcde}
Fusicaulis	105.16^{ab}	95.59^{ab}	10.97^{ab}	10.34^{abcd}
Gymno-Carpo	95.76^{ab}	78.29^{ab}	19.39^{ab}	18.02^{abcde}
Malta	90.37^{ab}	78.29^{ab}	18.09^{ab}	18.95^{abcde}
Messina	97.87^{ab}	88.05^{ab}	6.87^{a}	6.96^{abc}
Mexican	125.19^{ab}	110.54^{ab}	15.18^{ab}	10.33^{abcd}
Meyers	82.40^{a}	68.17^{ab}	16.50^{ab}	18.95^{abcde}
Montery	124.27^{ab}	109.33^{ab}	15.41^{ab}	10.53^{abcd}
Morado	94.78^{ab}	78.40^{ab}	25.11^{ab}	24.92^{abcde}
Muscatel	110.86^{ab}	90.49^{ab}	13.00^{ab}	11.50^{abcd}
Nepgen	98.08^{ab}	74.81^{ab}	3.64^{a}	3.21^{a}
Nudosa	90.01^{ab}	76.60^{ab}	4.35^{a}	4.35^{ab}
Ofer	82.61^{a}	77.59^{ab}	12.68^{ab}	15.40^{abcde}
Postmasburg	104.12^{ab}	92.58^{ab}	10.25^{a}	10.36^{abcd}
R1251	100.38^{ab}	90.77^{ab}	16.21^{ab}	16.15^{abcde}
R1259	99.66^{ab}	89.45^{ab}	21.39^{ab}	21.45^{abcde}
R1260	103.59^{ab}	92.72^{ab}	20.06^{ab}	18.52^{abcde}
Robusta	84.82^{a}	63.93^{a}	6.61^{a}	7.57^{abc}
Robusta x Castillo	109.91^{ab}	99.25^{ab}	32.02^{ab}	28.71^{bcde}
Roedtan	90.53^{ab}	76.47^{ab}	14.20^{ab}	15.25^{abcde}
Roly Poly	101.87^{ab}	84.54^{ab}	12.35^{ab}	12.42^{abcd}
Rossa	96.92^{ab}	84.33^{ab}	22.83^{ab}	22.44^{abcde}
Santa Rosa	108.06^{ab}	90.91^{ab}	16.41^{ab}	14.14^{abcde}
Schagen	93.48^{ab}	80.01^{ab}	18.49^{ab}	17.57^{abcde}
Sharsheret	91.77^{ab}	79.19^{ab}	24.09^{ab}	25.85^{abcde}
Sicilian Indian Fig	101.08^{ab}	87.84^{ab}	21.37^{ab}	20.77^{abcde}
Skinners Court	130.52^{ab}	116.18^{ab}	26.06^{ab}	19.09^{abcde}
Tormentosa	98.56^{ab}	83.37^{ab}	23.97^{ab}	24.54^{abcde}
Turpin	98.57^{ab}	92.05^{ab}	26.31^{ab}	26.78^{abcde}
Van As	111.12^{ab}	101.47^{ab}	27.17^{ab}	24.39^{abcde}
Vryheid	122.67^{ab}	109.60^{ab}	13.92^{ab}	10.43^{abcd}
Zastron	85.53^{a}	73.41^{ab}	6.32^{a}	6.91^{abc}
Average	**102.10**	**88.26**	**17.76**	**17.45**
Significance level	$p = 0.004$	$p = 0.008$	$p < 0.001$	$p < 0.001$

Cultivar means with different superscripts in the same column differ significantly

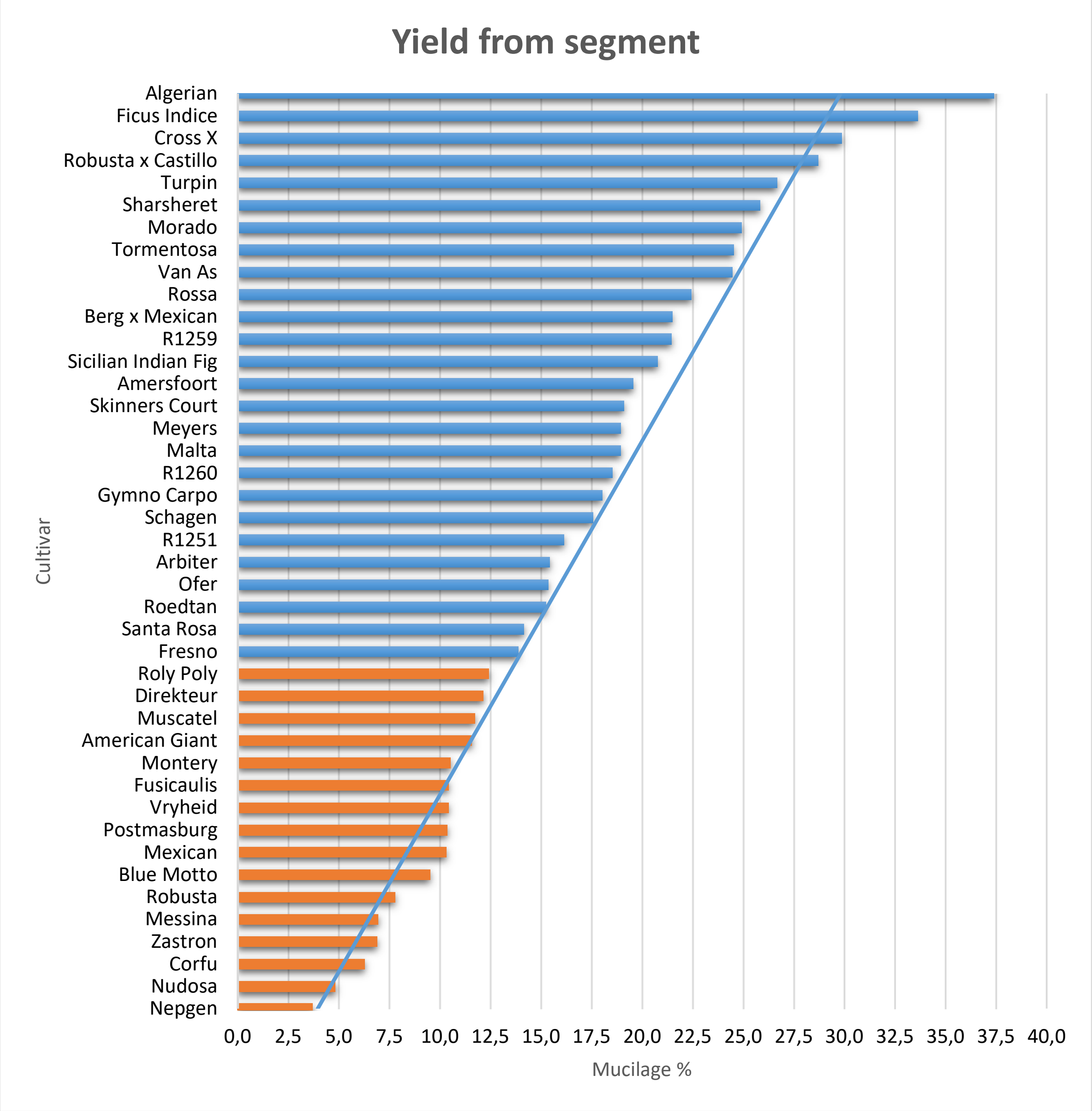


Figure 27: The yield of mucilage (%) from 42 cactus pear cultivar's cladode segments harvested in July 2013 from lowest to highest yielding cultivars

In Figure 27 the cultivars were arranged from lowest to highest percentage yield. 'Nepgen' had the lowest yield (3.21%/3.64 g) while 'Algerian' had the largest mucilage yield (37.41%/42 g). The average yield was 17.5%. Cultivars 'Van As', 'Sharheret', 'Morado', 'Turpin', 'Cross X', 'Robusta x Castillo', 'Ficus-Indica' and 'Algerian' all had yields above 25% from a single cladode segment.

'Nepge'n and 'Nodusa' had yields of less than 5%. The cultivars that yielded significantly less mucilage (%) than 'Algerian' are indicated in orange in Figure 27.

4.4.2.1 The correlation of mucilage yield and cladode weight

In Figure 28 the mucilage yield and cladode weight were correlated in order to determine whether more mucilage is extracted from heavier cladodes.

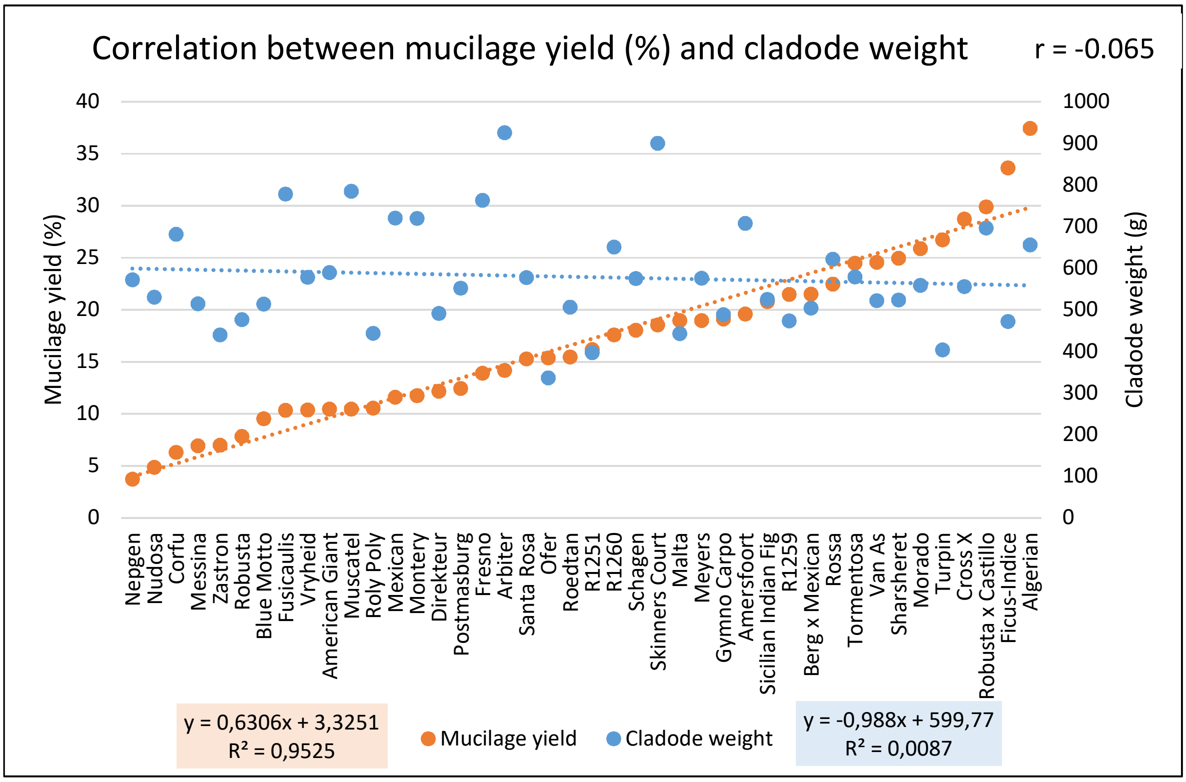


Figure 28: Correlation of the mucilage yield and weight of whole cladodes from 42 cactus pear cultivars harvested in July 2013

In Figure 28 the yield values were sorted from lowest to highest in order to visualize the correlation with the weight values. The correlation coefficient showed no linear relationship between cladode weight and mucilage yield (r =-0.065). It was evident that weight of the cladodes (blue line) did not correspond to the yield (orange line). It had been presumed that heavier cladodes would provide more mucilage, but from Figure 28 it was clear that the weight of cladodes was not an indication of the yield of mucilage. The trend line for weight of cladodes actually decreased slightly as yield increased rapidly (indicating the slight negative correlation).

Figure 28 showed therefore that weight of cladode had no correlation and no relationship to the mucilage yield. Heavier cladodes with more surface area and higher volume will not be an indication of more mucilage and smaller cladodes of less mucilage yield.

4.4.3 Waste determination

An important consideration for the choice and selection of cultivars to use for the extraction of mucilage is the wastage that could occur when only a part of the pulp (mucilage) is extracted for use. The solids that are a product of the extraction process could be utilized in future but in this study it was regarded as waste. In Table 8 the waste (sediment left after the centrifugation process) ranged between 42 and 70 g and was 56 to 91% of the original pulp sample. In Figure 29 the percentage yield is shown in relationship to the percentage waste from highest to lowest yield of mucilage. Algerian had yield above 40% and waste under 60%. Negpen had the lowest value for yield with 4.4% and the most waste (98%).

4.4.4 Moisture and Solids determination

This analysis was undertaken to determine variations between cultivars and whether the moisture and solid ratio of the cladode would influence the mucilage yield. The moisture and solids content of all the cultivars are indicated in Table 9. The moisture content in cladodes harvested in July (winter) was between 80.39% (‘Nepgen’) and 90.73% (‘Amersfoort’) (Table 9). There was only a 10% difference between the lowest and the highest moisture content. The average was 87%. ‘Amersfoort’ had significantly higher moisture content as well as the lowest solid content ($p < 0.001$) than ‘Robusta’, ‘Nepgen’ and ‘Montery’ (Table 9). It is important to consider that these specific cactus pear plants grow in a summer rainfall area and that they were harvested in the coldest and driest month of the year (July). Having an average moisture content of above 85% illustrates the extraordinary water holding capacity of the cladodes. The ratio of the constant high percentage of moisture compared to low solids in all the cultivars are shown in Figure 30. Solid material that include all other material in the cladode except for water, ranged between 9.27% (‘Amersfoort’) and 19.61% (‘Nepgen’). Usually plant material used for human food, such as vegetables, have between 70 and 95% water. The solid material includes carbohydrates, protein, fats, minerals and antioxidants that provide nourishment (Brown, 2007).

Table 8: Yield of wastage (%) after mucilage extraction of cladodes from 42 cactus pear cultivars harvested in July 2013

Cultivar	Pulp (M&C) (g)	Sediment (pellet) (g)	Waste (%)
Algerian	98.28^{ab}	54.89^{abcd}	58.52^{ab}
American Giant	78.43^{ab}	64.94^{abcde}	82.63^{abc}
Amersfoort	94.14^{ab}	66.47^{abcde}	72.17^{abc}
Arbiter	122.91^{b}	99.33^{e}	80.16^{abc}
Berg x Mexican	77.03^{ab}	54.47^{abcd}	71.72^{abc}
Blue Motto	85.87^{ab}	72.52^{abcde}	84.56^{abc}
Corfu	99.23^{ab}	67.69^{abcde}	73.39^{abc}
Cross X	92.07^{ab}	58.69^{abcd}	62.87^{abc}
Direkteur	98.60^{ab}	81.46^{abcde}	82.79^{abc}
Ficus-Indica	75.36^{ab}	42.97^{a}	56.20^{a}
Fresno	87.66^{ab}	70.89^{abcde}	80.79^{abc}
Fusicaulis	95.59^{ab}	81.14^{abcde}	84.88^{abc}
Gymno-Carpo	78.29^{ab}	45.62^{a}	66.22^{abc}
Malta	78.29^{ab}	51.44^{abc}	68.68^{abc}
Messina	88.05^{ab}	78.31^{abcde}	89.00^{abc}
Mexican	110.54^{ab}	91.64^{de}	85.06^{abc}
Meyers	68.17^{ab}	48.95^{ab}	73.50^{abc}
Montery	109.33^{ab}	90.68^{cde}	85.36^{abc}
Morado	78.40^{ab}	51.42^{abc}	67.91^{abc}
Muscatel	90.49^{ab}	73.50^{abcde}	82.49^{abc}
Nepgen	74.81^{ab}	66.70^{abcde}	89.69^{bc}
Nudosa	76.60^{ab}	69.65^{abcde}	91.86^{c}
Ofer	77.59^{ab}	62.02^{abcde}	79.97^{abc}
Postmasburg	92.58^{ab}	78.85^{abcde}	84.59^{abc}
R1251	90.77^{ab}	70.72^{abcde}	77.94^{abc}
R1259	89.45^{ab}	67.44^{abcde}	74.59^{abc}
R1260	92.72^{ab}	61.46^{abcde}	65.12^{abc}
Robusta	63.93^{a}	55.99^{abcd}	87.38^{abc}
Robusta x Castillo	99.25^{ab}	62.73^{abcde}	63.49^{abc}
Roedtan	76.47^{ab}	58.40^{abcd}	77.28^{abc}
Roly Poly	84.54^{ab}	69.96^{abcde}	82.44^{abc}
Rossa	84.33^{ab}	57.29^{abcd}	69.66^{abc}
Santa Rosa	90.91^{ab}	71.52^{abcde}	80.53^{abc}
Schagen	80.01^{ab}	54.34^{abcd}	68.25^{abc}
Sharsheret	79.19^{ab}	48.15^{ab}	62.28^{abc}
Sicilian Indian Fig	87.84^{ab}	63.73^{abcde}	73.05^{abc}
Skinners Court	116.18^{ab}	85.87^{bcde}	74.78^{abc}
Tormentosa	83.37^{ab}	56.78^{abcd}	68.41^{abc}
Turpin	92.05^{ab}	62.37^{abcde}	67.56^{abc}
Van As	101.47^{ab}	70.47^{abcde}	69.25^{abc}
Vryheid	109.60^{ab}	93.15^{de}	86.42^{abc}
Zastron	73.41^{ab}	64.94^{abcde}	88.99^{abc}
Average	**88.26**	**67.25**	**76.25**
Significance level	$p = 0.008$	$p < 0.001$	$p < 0.001$

Cultivar means with different superscripts in the same column differ significantly

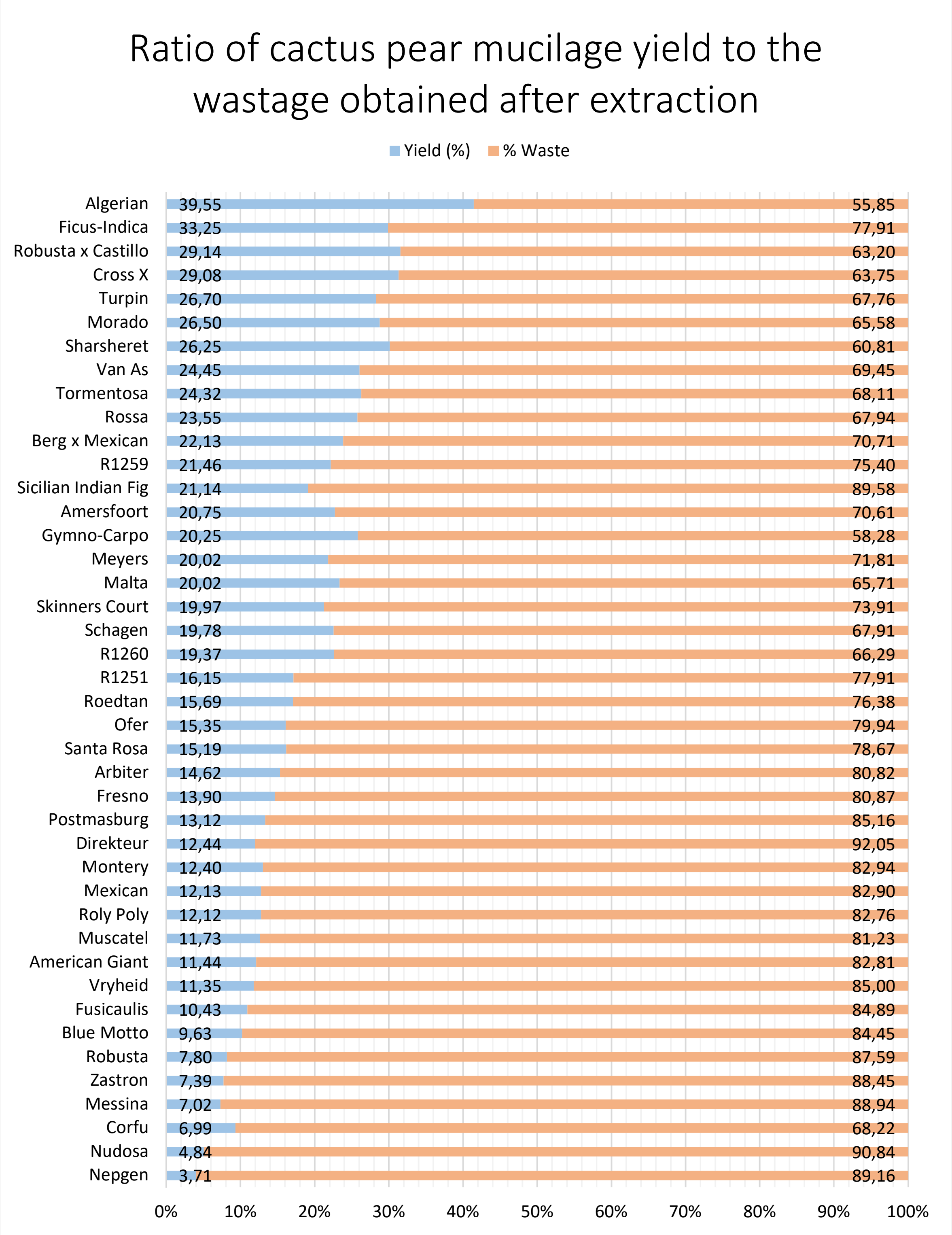


Figure 29: Ratio of mucilage and waste obtained in the mucilage extraction process from 42 cactus pear cultivars harvested in July 2013

Table 9: The moisture (%) and solids (%) content of cladodes from 42 cactus pear cultivars harvested in July 2013

Cultivar	Moisture Content (%)	Total Solids (%)
Algerian	88.12^{bcd}	11.88^{abc}
American Giant	86.77^{abcd}	13.23^{abcd}
Amersfoort	90.73^{d}	9.27^{a}
Arbiter	88.54^{bcd}	11.46^{abc}
Berg x Mexican	88.14^{bcd}	11.86^{abc}
Blue Motto	86.70^{abcd}	13.30^{abcd}
Corfu	86.94^{abcd}	13.06^{abcd}
Cross X	87.92^{bcd}	12.08^{abc}
Direkteur	86.36^{abcd}	13.64^{abcd}
Ficus-Indica	88.96^{bcd}	11.04^{abc}
Fresno	88.31^{bcd}	11.69^{abc}
Fusicaulis	88.38^{bcd}	11.62^{abc}
Gymno-Carpo	88.23^{bcd}	11.77^{abc}
Malta	87.00^{bcd}	13.00^{abc}
Messina	86.57^{abcd}	13.43^{abcd}
Mexican	85.43^{abcd}	14.57^{abcd}
Meyers	87.27^{bcd}	12.73^{abc}
Montery	83.52^{abc}	16.48^{bcd}
Morado	88.66^{bcd}	11.34^{abc}
Muscatel	88.34^{bcd}	11.66^{abc}
Nepgen	80.39^{a}	19.61^{d}
Nudosa	85.30^{abcd}	14.70^{abcd}
Ofer	84.86^{abcd}	15.14^{abcd}
Postmasburg	87.49^{bcd}	12.51^{abc}
R1251	87.75^{bcd}	12.25^{abc}
R1259	88.11^{bcd}	11.89^{abc}
R1260	87.42^{bcd}	12.58^{abc}
Robusta	82.84^{ab}	17.16^{cd}
Robusta x Castillo	89.63^{cd}	10.37^{ab}
Roedtan	88.28^{bcd}	11.72^{abc}
Roly Poly	88.00^{bcd}	12.00^{abc}
Rossa	88.66^{bcd}	11.34^{abc}
Santa Rosa	86.50^{abcd}	13.50^{abcd}
Schagen	86.52^{abcd}	13.48^{abcd}
Sharsheret	87.38^{bcd}	12.62^{abc}
Sicilian Indian Fig	87.39^{bcd}	12.61^{abc}
Skinners Court	87.72^{bcd}	12.28^{abc}
Tormentosa	87.21^{bcd}	12.79^{abc}
Turpin	86.19^{abcd}	13.81^{abcd}
Van As	87.88^{bcd}	12.12^{abc}
Vryheid	86.59^{abcd}	13.41^{abcd}
Zastron	84.41^{abcd}	15.59^{abcd}
Average	**87.08**	**12.92**
Significance level	$p < 0.001$	$p < 0.001$

Cultivar means with different superscripts in the same column differ significantly

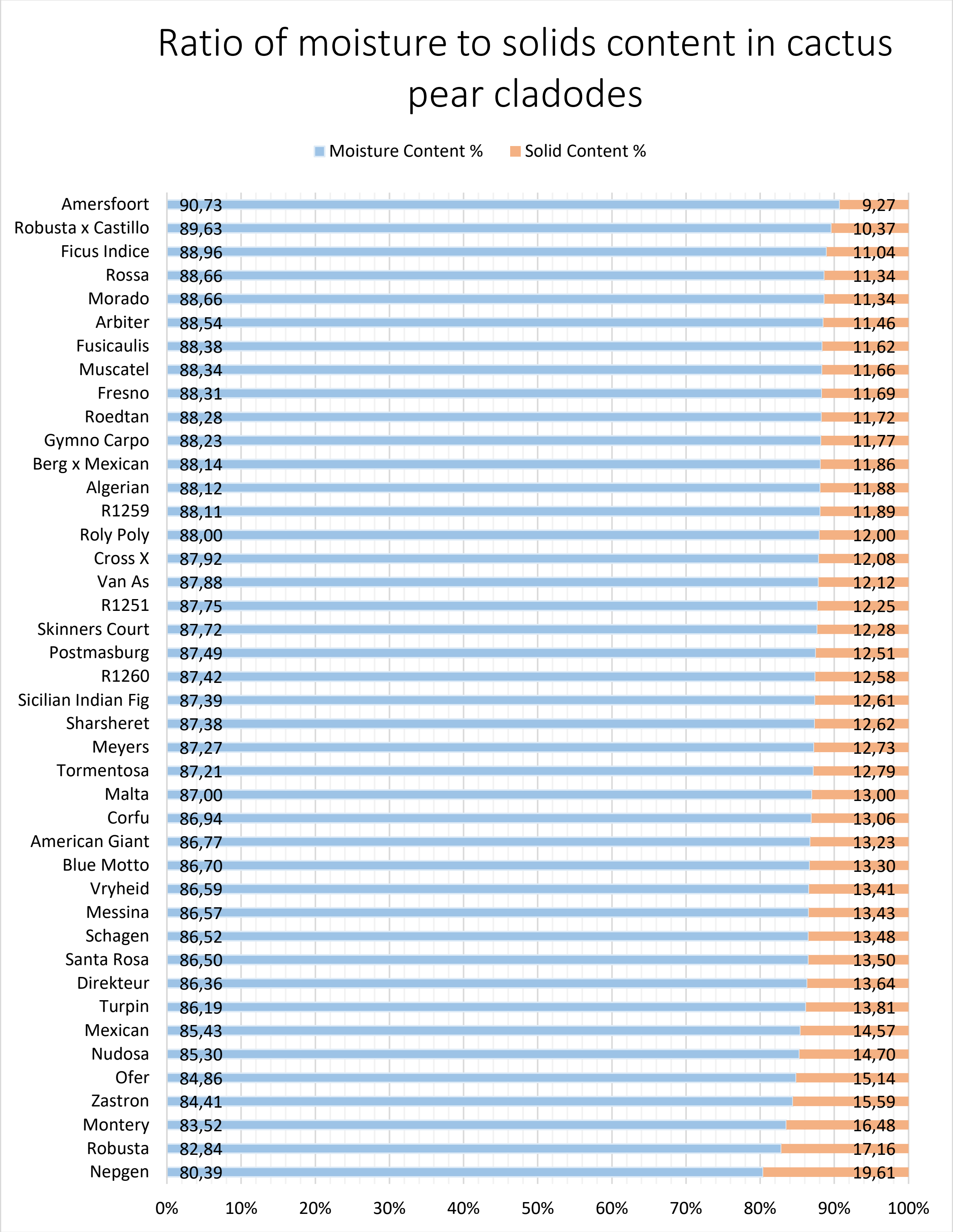


Figure 30: Ratio of moisture and solids content of cladodes from 42 different cactus pear cultivars harvested in July 2013

4.4.4.1 The correlation of cactus pear mucilage yield and cladode moisture content

Mucilage yield was correlated in Figure 31 to cladode moisture content in order to observe the interrelationship between the moisture levels of a cladode and the amount of mucilage that was extracted from the cladode segment.

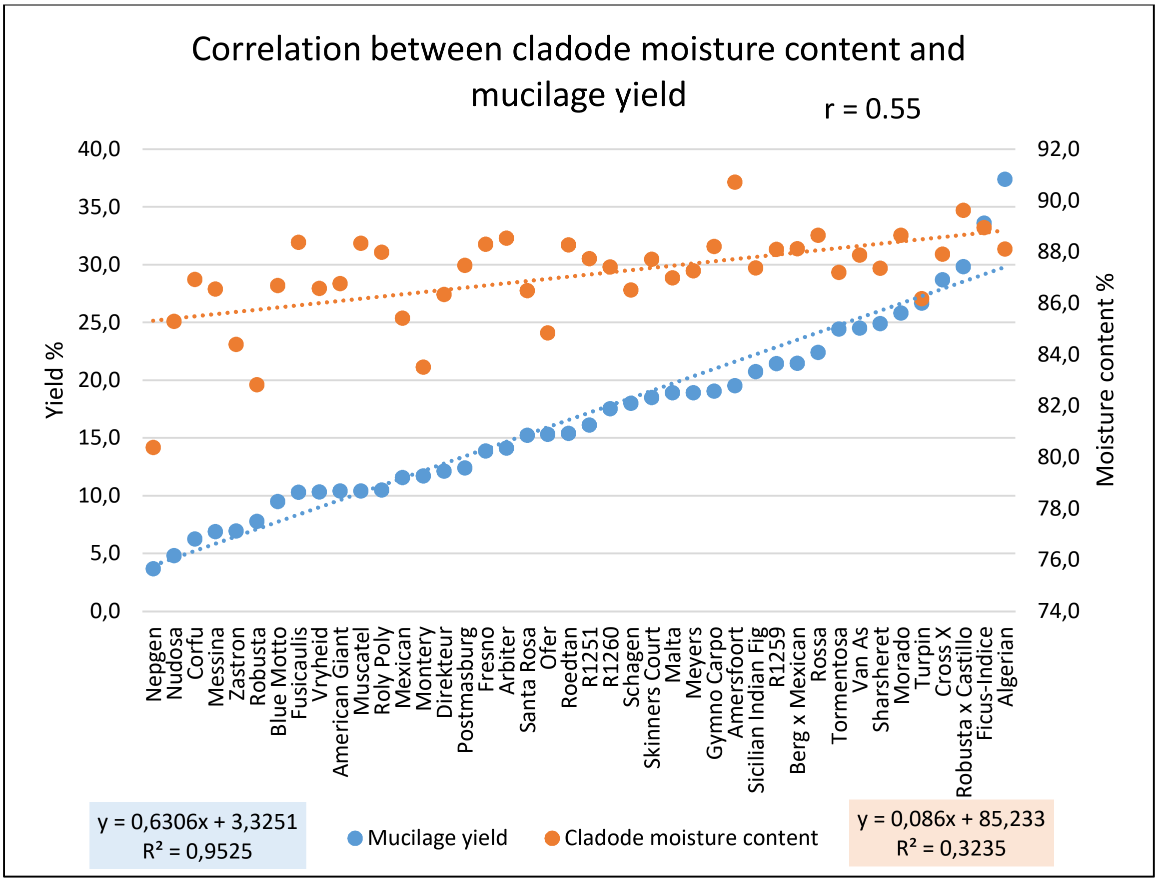


Figure 31: Correlation of mucilage yield and cladode moisture content of 42 cactus pear cultivars harvested in July 2013

It was observed that all cladodes had high moisture contents and could therefore serve as a source of water to humans and animals alike. It was thought that high moisture content would correlate strongly with high mucilage yield, however the correlation seen in Figure 31 demonstrated a weak positive relationship and no general discernible relationship between the moisture content of a cladode and the yield of mucilage. The moisture content of cladodes was sorted from the lowest to the highest in Figure 31. The % mucilage yield (blue line) showed a weak positive correlation to moisture content (orange) line, however moisture content of an individual cladode would not automatically be indicative of the amount of mucilage it would yield. For example, 'Algerian' had similar yield to other cultivars but the moisture content was significantly higher. Therefore it can be established that % moisture content had little correlation to the % yield of mucilage.

4.4.5 Viscosity determinations

The internal forces that hold liquid particles together could cause thicker consistencies and resistance to flow. It is referred to as viscosity (Lewis, 1987). The viscosity of a fluid is a physical manifestation of the molecular weight and the distribution of molecules (Brookfield Engineering Laboratories, 2014). As macerated cladode pulp and native mucilage demonstrated viscosity, analysis of the viscosity of mucilage was undertaken to determine variations between cultivars and whether the viscosity would have an influence on the mucilage yield.

4.4.5.1 Line-spread test

Viscosity was determined using three methods of which the line-spread test provided the most quantifiable data. The line-spread measurement method refers to the distance that mucilage flowed when allowed to run freely, therefore the higher the value, the lower the viscosity.

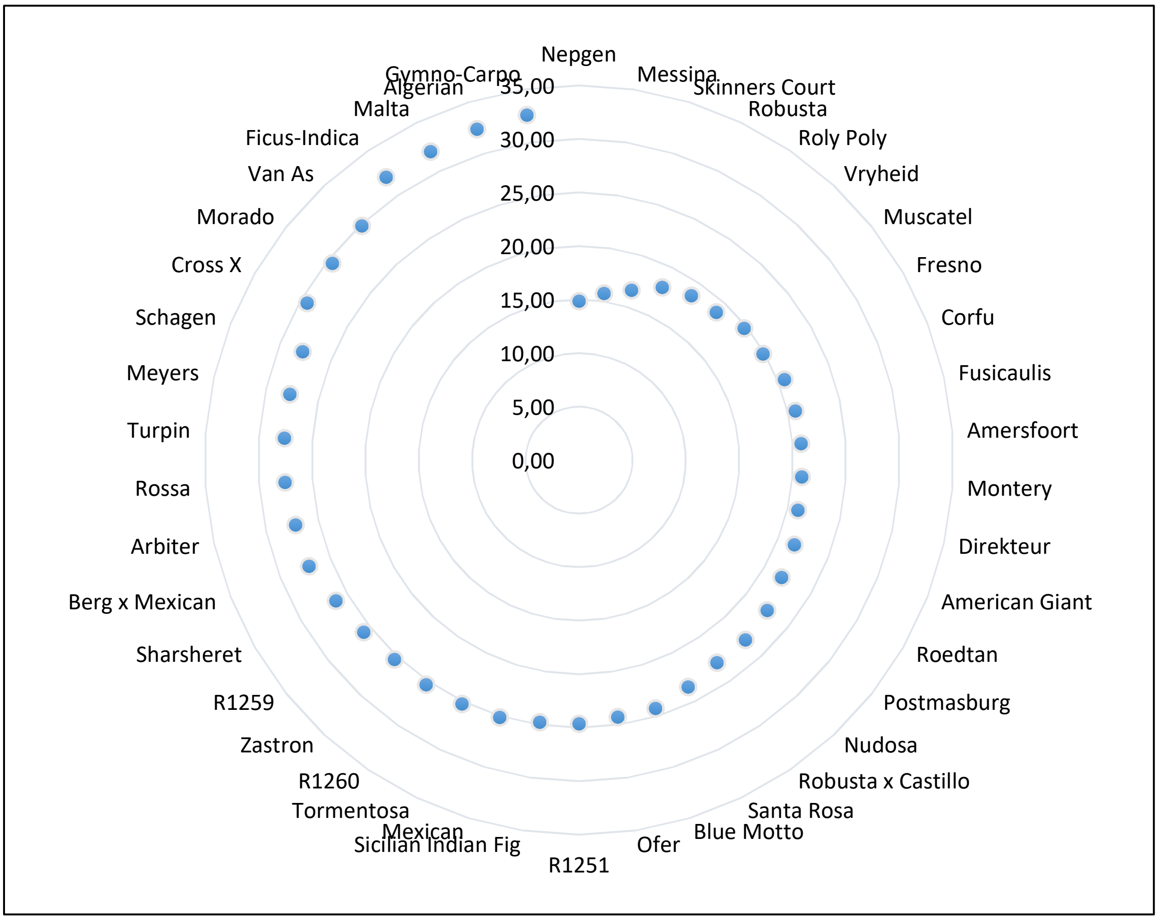


Figure 32: Viscosity of mucilage according to the line-spread test results from 42 cactus pear cultivars harvested in July 2013

The line-spread test produced distance values that ranged from 14.88 cm (‘Nepgen’) for the highest viscosity to 32.63 cm (‘Gymno-Carpo’) according to Figure 32 for the lowest viscosity. The difference in readings was 19 cm. This large range indicated the considerable diversity in viscosity.' Gymno-Carpo' (32.63 cm) was the cultivar with the lowest line-spread value followed by 'Algerian' (32.38 mm), 'Ficus-Indica' (32.00 cm) and 'Malta' (32.00 cm) had significantly low viscosity ($p < 0.001$) that spread the furthest. The cultivar 'Nepgen' (14.88 cm) had the highest viscosity that spread the least (significant difference $p < 0.001$) and 'Messina' (15.75 mm), 'Skinners Court' (16.63 cm) and 'Robusa' (17.94 cm) also demonstrated extremely low viscosity. The average spread reading was 24.17 cm, therefore the cultivars 'Blue Motto' (24.25 cm), 'R1251' (24.63 cm), 'Sicilian Indian Fig' (24.75 cm) and 'Ofer' (24.25 cm) can be regarded as having average viscosity.

4.4.6 Categorization of the cultivars according to the viscosity of mucilage

The viscosity of each of the four cladodes of each of the 42 cultivars was measured according to three methods: line-spread, cylinder test and separating funnel. The results for all three methods, together with the visual physical descriptions of the appearance of the mucilage, were considered for each sample (four samples per cultivar) in order to apply a value of low, medium or high viscosity, was assigned to each sample. The value was hence assigned: low = 1, medium = 2 and high = 3. The scores for each of the four samples of each cultivar was added up, the subsequent total scores were interpreted for each cultivar as follows: 4-5 Low, 6-7 medium low, 8-9 medium, 10-11 medium high and 12 was high viscosity. The cultivars were grouped according to the total values in order to organize them into the five classes. All of the data was considered and cultivars were arranged using all the averaged data (not shown) in order to ascertain the appropriate category (Table 10).

Table 10: Categorization of 42 cactus pear cultivars according to viscosity (harvested in July 2013)

Low 4-5	Medium low 6-7	Medium 8-9	Medium high 10-11	High 12
Algerian	American Giant	Amersfoort	Corfu	Fusicaulis
Ficus-Indica	Arbiter	Blue Motto	Direkteur	Messina
Gymno-Carpo	Berg x Mexican	Mexican	Fresno	Robusta
Meyers	Cross X	Ofer	Montery	Roly Poly
Rossa	Malta	R1251	Muscatel	Skinners Court
Turpin	Morado	Robusta x Castillo	Nudosa	Vryheid
Van As	R1259	Santa Rosa	Postmasburg	
	R1260	Sicilian Indian Fig	Roedtan	
	Schagen		Zastron	
	Sharsheret		Nepgen	
	Tormentosa			

4.4.6.1 The correlation of mucilage yield and viscosity

Mucilage yield was correlated to mucilage viscosity in order to observe the interrelationship between the viscosity of mucilage and the amount of mucilage that was extracted from the cladode segment Figure 33.

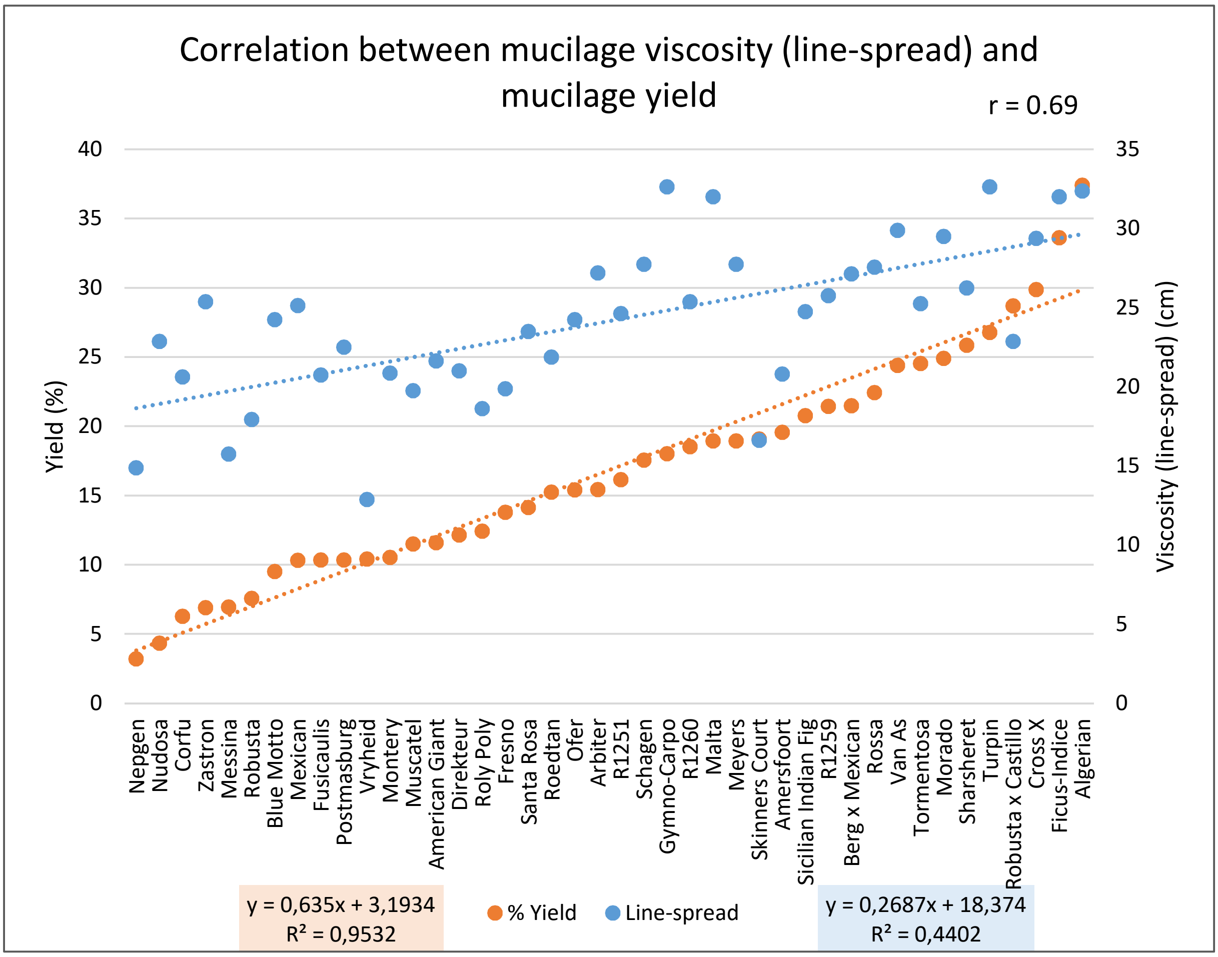


Figure 33: Correlation between mucilage yield and viscosity (line-spread) from 42 cactus pear cladodes harvested in July 2013

There was a strong positive relationship between mucilage yield and viscosity (r = 0.69). There was a general increase in the line-spread viscosity trend line (blue) that coincided with the increase in yield (orange) in Figure 33. 'Vryheid' and 'Skinners Court' were outliers that demonstrated low line-spread values and higher yield. 'Nepgen' had low line-spread (14.88 cm) as well as low yield (3.21%) while 'Algerian' demonstrated high line-spread values (32.38 cm) and high yield (37.41%). As a strong positive (r = 0.69) correlation between yield and viscosity was indicated, it could be presumed that cultivars with higher line-spread values (low viscosity) would produce higher mucilage yields.

4.4.6.2 The correlation of cladode moisture content and mucilage viscosity

A final correlation was necessary to investigate the relationship between moisture content and viscosity (Figure 34).

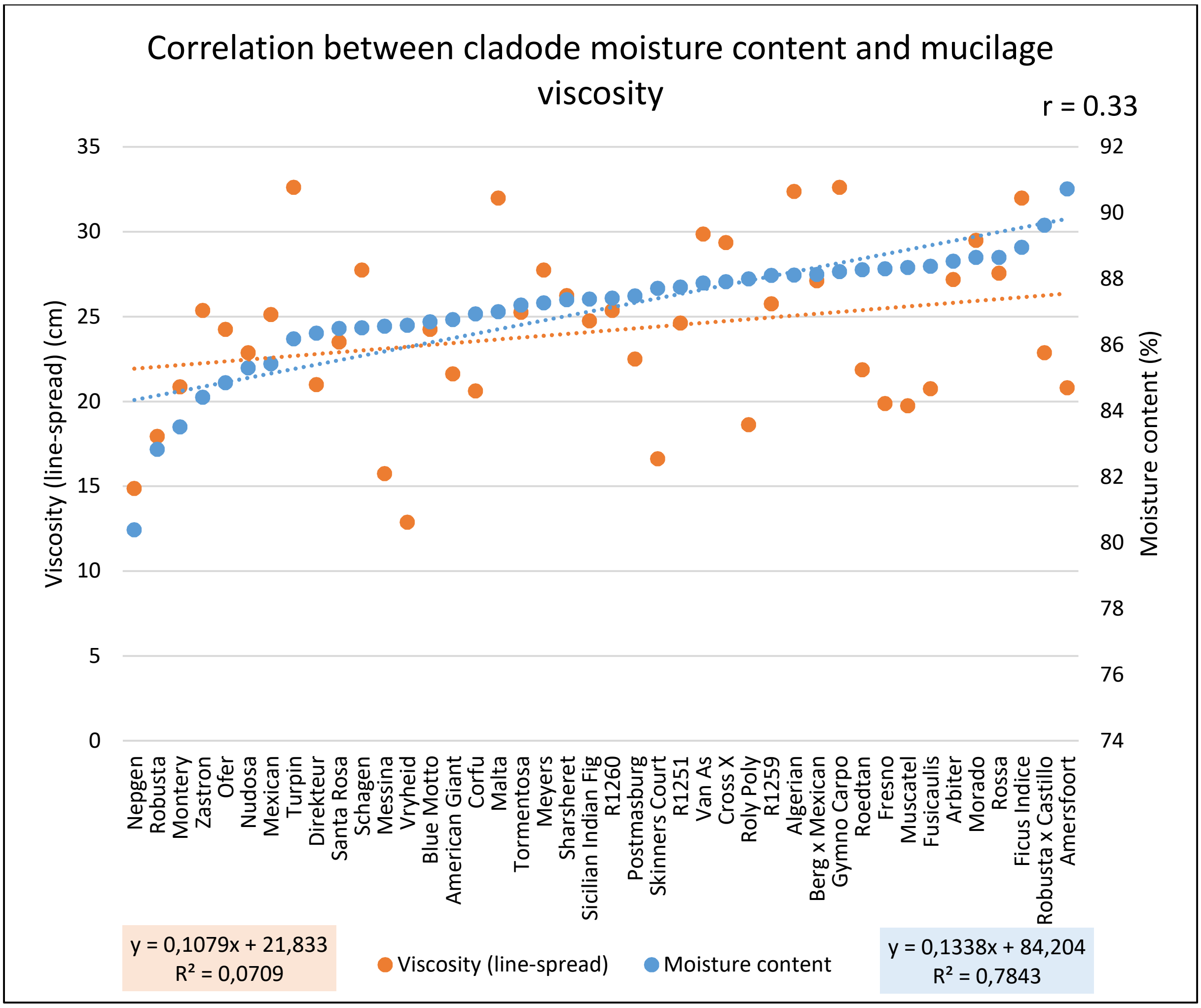


Figure 34: Correlation between mucilage viscosity and cladode moisture content from 42 cactus pear cladodes harvested in July 2013

In Figure 34, a weak positive correlation (r = 0.329) was observed between the amount of moisture that a cladode contains (blue) and the viscosity of the mucilage (orange). Moisture contents of an individual cladode would not automatically be indicative of the viscosity it would demonstrate. Therefore moisture content values are not truly relevant to the viscosity of the mucilage.

4.5 Conclusion

Morphological characteristics such as weight, size, volume and surface area are important considerations when cladodes are harvested, transported and processed as they are bulky and heavy. From the 42 investigated cultivars, Ofer, Amersfoort, Malta, Nepgen, Nudosa and

R1251 cladodes were the smallest cladodes in terms of weight, volume and surface area while Arbiter and Skinners Court cladodes were the biggest. The average weight for cladodes used in this study was 578.53 g, surface area was 2852.96 mm^2 and volume was 68,2070.96 mm^3. In terms of size the average length was 347.93 mm, width was 188.95 mm and diameter was 10.37 mm. Cladodes weights were mostly between 400 g and 800 g.

The yield of native mucilage ranged from 5%/3.64 g ('Nepgen') to 25%/42 g ('Algerian'). The average yield was 17%. The average moisture content was 87.08% while solids made up 12.92% of whole cladodes. The solid waste was the greater part of the pulp and could contain useful ingredients and may prove to be used in food products in future.

The 42 cultivars were categorized into 5 classes that indicated the viscosity (low, medium-low, medium, medium-high and high) of the specific cultivar as was determined by three different methods.

In order to determine the influence of the cladode and mucilage attributes on the extractability of mucilage for optimal yield, correlations were conducted. The correlation of r= -0.06 indicated that there were no discernible relationship between mucilage yield and cladode weight, thus cultivars that inherently produce bigger and heavier cladodes are not necessarily the cultivars that would be selected for greater mucilage yield.

A moderate positive (r= 0.55) correlation was indicated between mucilage yield and cladode moisture content while a weak positive relationship was indicated between mucilage viscosity and cladode moisture content (r= 0.33). Higher moisture content of a specific cladode would therefore not strongly suggest higher mucilage yield or lower viscosity. It should however be investigated whether cladode moisture content in general varies according to seasonal rainfall. Substantially more rain may influence cladode moisture content, which could, in turn, influence mucilage viscosity and thus mucilage yields.

The strongest correlation was found between mucilage yield and mucilage viscosity (r = 0.7). Thus, cultivars that inherently contain low viscosity mucilage would contribute the highest yield of mucilage. Future investigations should focus on determining the factors that influence the viscosity of mucilage in growing cladodes.

As high yields were paramount in the selection process, cultivars with high mucilage yield and low viscosity will be used in the future investigation. The identified cultivars were Turpin, Gymno-Carpo, Algerian, Malta, Van As, Ficus-Indica, Morado, Tormentosa and Meyers. Robusta will be included in further studies in order to identify and compare the properties of different species.

Chapter 5

Morphological and rheological evaluation of extracted mucilage from eight selected commercial cactus pear cultivars

Abstract

Mucilage has potential for use in nutraceutical products, thus the cultivars and time of harvest (post-harvest or dormant stage) had to be selected for optimal mucilage yield and quality. Morphological (size, weight, moisture content, pH and yield) and rheological (viscosity and flow behaviour) analysis of native (NM) and freeze-dried mucilage (FM) from eight cactus pear cultivars (Algerian, Meyers, Morado, Ficus-Indica, Gymno-Carpo, Tormentosa, Turpin and Robusta) were conducted. The dormant stage cladodes were bigger thus more advantageous for extraction. Although higher yields of NM were obtained from the post-harvest stage, the yields of FM was similar for both stages. Robusta, Algerian and Gymno-Carpo had the most consistently high yields of NM and FM while Turpin had the lowest yields in both stages. The pH (4.5) and moisture content (86.5%) were similar between cultivars and stages. Consistently higher viscosities were detected in dormant stage NM and the FM showed higher abilities to influence viscosity of solutions (Morado FM was most consistent). The potential of FM for commercial use was confirmed with the results obtained for the functional properties, namely water solubility index (60.96%), water absorption capacity (3.65 ml/g), water holding capacity (14.94 ml/g) and swelling capacity (2.79 g/g). Mucilage (NM and FM) from all cultivars showed non-Newtonian, pseudoplastic tendencies. Higher viscosity mucilage is time dependent, rheopectic and had yield stress tendencies. Fluctuations in temperature, pH, concentration and electrolytes influenced mucilage viscosity. The dormant stage and four cultivars (Algerian, Morado, Gymno-Carpo and Robusta) were selected for further investigation and characterization purposes.

5 Morphological and rheological evaluation of extracted mucilage from eight selected commercial cactus pear cultivars

5.1 Introduction

In the midst of the global food security dilemma (Tweeten, 1999), *Opuntia ficus-indica* and *Opuntia robusta* thrive in arid lands and could provide nourishment to humans and animals alike (De Waal, 2015). The slimy substance that the cactus pear cladode contains has the potential to be developed into commercial nutraceutical products that could add value to cactus pear crops (Cárdenas et al., 1997; Sáenz, 2002). Mucilage is classified as a hydrocolloid due to the large molecules that are soluble in water and produce viscous colloids with extraordinary rheological behaviour (Cárdenas et al., 1997; Medina-Torres et al., 2000; Sáenz et al., 2004; Sepúlveda et al., 2007; Trachtenberg and Mayer, 1981).

As mucilage has unique flow characteristics (Cárdenas et al., 1997; León-Martínez et al., 2011; Medina-Torres et al., 2000) it is important to determine the flow behaviour in order to predict the way it will behave in food products during processing, preparation and consumption (Brookfield Engineering Laboratories, 2014). The interactions that occur between particles as well as between the product and the environment, could impact the viscosity of the products. Rheological data is helpful in predicting product performance and behaviour (Brookfield Engineering Laboratories, 2014). Thus, determining the rheology of a fluid involves studying the reasons why liquids change form and move from one place to another. The intermolecular attractions that hold liquid particles together, may be stronger in certain liquids, causing the liquid to resist changing, moving or flowing when external forces are applied. These attractions, friction and resistance to flow is found in liquids that have a thicker consistency and is referred to as viscosity. In order to measure the exact amount of friction in such a liquid, a Viscometer is used (Brookfield Engineering Laboratories, 2014).

“Shear” is the action of disturbing a liquid and could take place when the liquid is poured, stirred, or moved in any way. Any movement in liquid causes friction between the molecular layers in the fluid. At higher viscosities, the friction between molecules are greater and thus more force will be necessary to cause the liquid to move from its original state (Lewis, 1987). Isaac Newton described viscosity as the movement of layers of fluid, separated by a specific distance in the liquid in the same direction but at different velocities (speeds). The speed at which these layers move in relation to one another is “shear rate”. The force needed for the movement to occur is the “shear stress”. Viscosity (poise) is determined by measuring the percentage torque using standard spindles of the Brookfield Viscometer. The percentage torque is converted into centipoise by multiplying the percentage torque by the appropriate factor (the factor finder table supplied with the Brookfield viscometer) for the spindle and speed (Brookfield Engineering Laboratories, 2014).

Sometimes, fluids demonstrate solid elastic tendencies. These viscoelastic properties are observed when deformation of the liquid and applied forces do not correspond, for example when shear stress lingers after the force has been removed, or when the Weissenberg effect (fluid that coils around and creeps up the spindle) is witnessed (Lewis, 1987).

In chapter 4, it was concluded that mucilage extracted from different cultivars naturally had diverse viscosities, therefore selecting the appropriate cultivar is paramount. From the previous study in chapter 4, eight cultivars were identified on which further investigation in this chapter is based. However, the samples in chapter 4 were harvested in winter during the pruning of the cladodes, in the dormant stage. It has been observed that the viscosity of mucilage is quite different in summer compared to winter months, consequently it was imperative to determine the differences in the yield, viscosity and other basic characteristics between the post-harvest stage in summer (after the fruit harvest) and the winter (dormant stage) mucilage. The viscosity and flow behaviour was thus analysed in both the growth and dormant stage in order to select the most appropriate time for cladode harvest for the purpose of extracting mucilage. Moreover, the freshly extracted native mucilage and the reconstituted freeze-dried mucilage were examined in order to compare the characteristics before and after drying. One harvesting stage and four cultivars had to be identified for further investigation and characterization purposes. For native or freeze-dried mucilage to be used effectively in commercial food products, the behaviour and flow of mucilage amidst diverse environments, manipulation and packaging conditions should be understood.

The overall aim of this study was to select the most appropriate cultivar and harvesting time for optimum mucilage yield and quality; therefore, the selection process was continued through the further evaluation of the eight cultivars previously (chapter 4) selected (Algerian, Meyers, Morado, Ficus-Indica, Gymno-Carpo, Tormentosa, Turpin and Robusta). The morphological characteristics of cladodes and rheological properties of native (NM) and reconstituted freeze-dried (FM) mucilage during two harvesting stages were determined. In order to obtain evidence of the quality and potential of freeze-dried mucilage powders, for further investigations to be warranted (chapters 5, 6 and 7), the water-related functional properties of the dried powders were analysed.

5.2 Materials and Methods

The eight selected cultivars (Algerian, Meyers, Morado, Ficus-Indica, Gymno-Carpo, Tormentosa, Turpin and Robusta) were investigated over two growth stages. In summer the cactus pear plant will grow vegetatively and also bear fruit when in full production (after five years) (Potgieter, 2007). Harvesting of cladodes for the purpose of extracting mucilage would only take place after the fruit has been harvested (post-harvest). In winter, the cactus pear plant is dormant therefore no growth will occur during this period. Both the extracted native mucilage (NM) and reconstituted freeze-dried mucilage (FM) were analysed. Mucilage was extracted according to the standard patented extraction procedure (Du Toit and De Wit, 2011). For each cultivar, six cladodes were harvested from six different cactus pear plants for the two growth stages, winter (dormant) and summer (post-harvest). Viscosity and flow behaviour were determined by using the Brookfield RVTD2 Viscometer at 5, 10, 20 50 and 100 rpm using disk spindles at 20°C.

5.2.1 Cladode collection

The eight selected cultivars (Algerian, Meyers, Morado, Ficus-Indica, Gymno-Carpo, Tormentosa, Turpin and Robusta) were harvested in two growth stages. The first samples were collected in late January directly after the fruit had been harvested (post-harvest stage), while the second harvesting took place in July (dormant stage). With each collection, six cladodes were harvested from different cactus pear plants of the same cultivar. That is, one cladode from the first, third and fifth tree from each of the two replications.

5.2.2 Sample preparation

After each cladode was labelled, the samples were placed in absorbent paper bags and transported to the laboratories where they were refrigerated at 4˚C until the mucilage was extracted (Du Toit and De Wit, 2011) within five days of harvest.

Cladodes were weighed and measured, the rough outer edge and any imperfections discarded. A small but standard size circle, at the top end of the cladode was used for moisture content determinations as it was the thickest part of the cladode. After extraction, the mucilage samples were decanted and forced through a 16 cm Kitchen Craft stainless steel sieve in order to thoroughly separate the viscous mucilage liquid from any last remaining solid particles. The analyses were done on native mucilage after which the samples were placed in individual containers, covered and frozen (-18˚C). The frozen samples were placed in the Perano freeze-drier at vacuum at -60˚C and dried for 72 hours. The freeze-dried samples were immediately sieved (Kitchen Craft stainless steel fine mesh 7.5 cm sieve), weighed, vacuum packed (Genesis vacuum sealer) and stored frozen (-18˚C).

The concentration for reconstituting the freeze-dried powder with distilled water for testing purposes was different for the two stages. The concentration used for post-harvest stage was

10%, while it was 5% for the dormant stage. The moisture and solids content for the freeze-dried mucilage was determined after freeze-drying and it showed different results. Post-harvest freeze-dried mucilage contained on average 97.01% moisture and 2.99% solids, while freeze-dried mucilage from the dormant stage had 92.76% moisture and 7.23% solids (data not shown). Therefore, the concentration of the dormant stage reconstituted mucilage was lower (5%) than the reconstituted post-harvest mucilage in order to compensate for the difference in percentage solids. Even at 10% concentration the mucilage showed lower viscosity than the dormant stage mucilage. The explanation for the low viscosity during the post-harvest stage was determined during this study and will be discussed in later chapters. In short, it was seen from this study that long summer days favours Crassulacean Acid Metabolism (CAM) and thus the production of more organic acids. These acidic substances provides cations that neutralize polyelectrolyte mucilage molecules. Neutralized mucilage molecules curl up and the viscosity of the fluid decrease (Trachtenberg and Mayer, 1982b). Therefore, cladodes harvested in January after the fruit harvest (hottest summer month) had lower viscosity mucilage.

5.2.3 Morphology

Cladodes were weighed and measured in length, width and diameter. In order to calculate volume (mm^3), the three before-mentioned measurements were multiplied. The method previously described by Hernández et al. (2010) was used for estimating cladode surface area. The elliptical mathematical formula is as follows:

$$surface\ area\ \ x^2 = \left(\frac{W}{2}\right) \times \left(\frac{L}{2}\right) \times \pi$$

Where:
W = width, minor axes
L = length, major axis
π = 3.141516

5.2.4 Yield determination

The supernatant liquid that separated during the centrifugation process is the native mucilage (NM) and was decanted and weighed using a Radwag PS 750/C/2 scale in order to obtain the yield (g).

$$Yield\ of\ NM\ (\%) = \frac{supernatant\ liquid\ (g)}{original\ cladode\ segment\ (g)} \times 100$$

The freeze-dried mucilage (FM) was also weighed in order to determine the yield for every sample of every cultivar separately.

$$Yield\ of\ FM\ (\%) = \frac{freeze\ dried\ powder\ (g)}{original\ cladode\ segment\ (g)} \times 100$$

5.2.5 Moisture content

The standardized round piece of cladode material obtained by the method described in Section 3.2.2 was diced into small (approximately 10 mm^2) pieces. The metal petri dish was weighed without and with the sample by using a Metter AE 200 scale and the weight was recorded to four decimals. The samples were placed inside an Eco Therm oven at 102˚C for 24 hours. It was placed in the desiccator containing silica crystals, for 1 hour and the dried samples were weighed again.

5.2.6 pH

A calibrated Eutech pH 2700 pH/mV/˚C/˚F instrument was used to determine pH at 22˚C of the native mucilage immediately after extraction.

5.2.7 Viscosity determinations

Both the native and reconstituted freeze-dried mucilage were used to perform the line-spread test as well as the Viscometer assessments of both the stages (post-harvest and dormant stages). The NM was tested immediately after extraction while the reconstituted mucilage (FM) was tested after it had been thoroughly dissolved (method described in Section 5.2.8.1). Post-harvest FM was reconstituted to 10% concentration in order to compensate for the higher moisture content of the freeze-dried powder. The dormant FM was reconstituted only to a 5% concentration (higher solids content).

5.2.7.1 Line-spread

The line-spread test is a method of comparing the relative viscosity of a viscous liquid such as gels, hydrocolloids and other thickened liquids. It is a quick, reliable and inexpensive way of determining and comparing viscosity (Kim et al., 2014).

The line-spread determination is described in Section 4.2.7.1.

5.2.7.2 Viscometer

A rotational digital RV Brookfield laboratory Viscometer was used with disc spindles. It measured the torque needed to rotate the immersed disc spindle in the mucilage. Disc spindles are general purpose spindles that ensures accurate, reproducible apparent viscosity determinations in most fluids. The Viscometer is easy to operate especially when it is necessary to measure apparent viscosity over a range of shear rates. When the rate is accelerated or decelerated, the spindle experiences increasing or decreasing drag (resistance to flow) which means that a variety of viscosity ranges can be measured. Measurements made using the same spindle at different speeds indicate the flow properties of a fluid (Brookfield Engineering Laboratories, 2014).

The viscosity and flow determinations where the Viscometer was used, were repeated on three different samples. Thus each reported viscosity point is the result of the averaged data from three sets. Spindle number six was used in these determinations since it produced on scale readings at all required speeds (rpm). The full scale range for spindle 6 is 8000000 cP (centipoise) or millipascal-second (mPa-s) and the minimum is 1000 cP (mPa-s). The sample size was smaller than the required 600 ml, therefore a smaller sample of 15 ml was established and standardized together with a standard small container. As long as the same size container was used for all tests, there would be no correlation problems and the probable effect on calibration could be ignored (Brookfield Engineering Laboratories, 2014). The spindle was immersed to the middle of the shaft indentation. Speeds used were 5 to 100 rpm where laminar flow was possible using the number six spindle.

Shear rate is the change in speed in which intermediate layers of fluid move with respect to each other and is measured in reciprocal seconds (s^{-1}) (Lewis, 1987). Determining shear rate and shear stress is no easy task using the standard type Viscometer and standard disk spindles (Mitschka, 1982). Measurements were therefore taken in percentage torque and converted to centipoise using the factor finder chart. The procedure was followed to recalibrate the range as a different container was used and therefore the guard leg could not be used (Brookfield Engineering Laboratories, 2014).

5.2.7.2.1 Single point viscosity

The viscosity of a non-Newtonian liquid is very difficult to determine as any reading obtained on the Viscometer would be different when using different speeds and spindles. Therefore viscosity measured using the Viscometer would be different depending on the rate of agitation. Yet a value indicating the viscosity of a substance is important to obtain, as it allows for comparisons. Thus a single viscosity reading was taken for each sample by using spindle 6 of the RV spindle series, at 50 rpm, 20˚C. The reading was taken after 60 seconds. Both the NM (10%) and FM (5%) were used to perform the single point viscosity test in both the post-harvest and dormant stages. It is essential to control the surrounding environment as well as the temperature of the mucilage especially when comparisons between mucilage of different cultivars have to be made (Brookfield Engineering Laboratories, 2014).

5.2.7.2.2 Controlled rate ramp

The controlled rate ramp test is the simplest way to determine whether a fluid is Newtonian or non-Newtonian. The viscosity will not change when the fluid is Newtonian while it will change with increased spindle rotation rates when the fluid is non-Newtonian. With the temperature at 20˚C, the rate of rotation is increased from 5 to 10, 20, 50 and 100 rpm and the torque reading taken after 60 seconds at each speed setting. Both the NM (10%) and FM (5%) were used to perform the controlled rate ramp test on the mucilage from the post-harvest and the dormant stage. The fluctuations in viscosity should be determined at different shear rates in order to understand and predict the flow behaviour of mucilage during processing and consumption (Brookfield Engineering Laboratories, 2014).

5.2.7.2.3 Controlled rate on the up-down ramp

The controlled rate on the up-down ramp is an efficient test to determine whether fluids are time dependent. If a fluid is time independent, the up and down curve will coincide. If not, the fluid is time dependent. In the test, the rate was progressively increased as indicated with the controlled rate ramp beginning on the slowest speed setting (5 rpm). From the fastest rate (100 rpm) the rate was progressively adjusted downwards to the lowest setting. With every rate increase or decrease the reading was taken at 30 s. In this test the rate adjustments are made without stopping the spindle rotation (Brookfield Engineering Laboratories, 2014).

Both the NM (10%) and FM (5%) were used to perform the test on the mucilage from the post-harvest and dormant stages.

5.2.7.2.4 Dynamic yield point

A certain amount of force is necessary in order to get mucilage flowing. The force needed for the fluid to start moving has to be determined, as the amount of force would influence the pressure that would be needed during processing. When consumers use a product that has yield stress tendencies, the product may be difficult to access or pour. The dynamic yield is determined by using a controlled rate ramp chart. The torque readings are log-log on the y-axes and the speed (rpm) on the x-axes. A best fit line is calculated and explorated to where torque or shear stress is equal to “0” (Brookfield Engineering Laboratories, 2014). The NM (10%) and FM (5%) data were used to attain the dynamic stress point.

5.2.7.2.5 Time sensitivity test

In the time sensitivity test, the spindle rotation is maintained at a constant speed for an extended period and the viscosity monitored. When the constant shear is applied, some substances will progressively become thinner and runnier while others will become thicker and stickier (Brookfield Engineering Laboratories, 2014). Time sensitivity indicates the tendency of the substance to change viscosity at a constant shear rate over a time period (Lewis, 1987). This test involved setting the Viscometer on a chosen rate and recording the torque reading at different time intervals. The rates were set on 5, 10, 20, 50 and 100 rpm and the reading recorded at 15, 30, 60, 90 and 120 seconds. The temperature was controlled at 20˚C. Therefore there was a 120 seconds time lapse between changes in rotational speeds (Brookfield Engineering Laboratories, 2014). Both the NM (10%) and FM (5%) were used to perform the time sensitivity test on the mucilage from the post-harvest and dormant stages.

5.2.7.2.6 Temperature sensitivity

A small change in temperature can often have an adverse effect on viscosity. As temperature changes are usually part of processing or usage, the evaluation of viscosity fluctuations occurring during temperature changes is essential (Brookfield Engineering Laboratories, 2014). The effects of different temperatures on mucilage viscosity at a steady rate (50 rpm), and time (30 s) were tested. The samples were cooled or heated to 5, 10, 20, 40, 60 and 80˚C (Brookfield Engineering Laboratories, 2014). NM (10%) and FM (5%) from the post-harvest and dormant stages were tested for temperature sensitivity.

5.2.7.2.7 *Viscosity at different pH levels*

The effects of pH fluctuations on mucilage viscosity have been reported before (Medina-Torres et al., 2000; Trachtenberg and Mayer, 1982b), thus it was imperative to confirm them in this study. The pH was lowered with 1 M HCl and increased using 1 M NaOH in order to determine the effect thereof on viscosity. The viscosity of the samples was determined using spindle 6 at 50 rpm. This test was only carried out on the dormant stage NM as the purpose was to indicate differences between cultivars and not stages.

5.2.7.2.8 *Effect of concentration on viscosity*

It would be expected that an increase in mucilage concentration would cause an increase in viscosity, nevertheless the specifics have to be determined. As the concentration of FM can be manipulated (not NM), the concentration was increased from 4 to 6, 8, 10, 12 and 14% in order to observe the increase in viscosity as influenced by mucilage concentration.

5.2.7.2.9 *Influence of ionic strength on viscosity with addition of monovalent (NaCl), divalent ($CaCl^{2+}$) and trivalent ($FeCl^{3+}$) cations*

The influence of ionic strength on mucilage viscosity has been reported before (Medina-Torres et al., 2000; Trachtenberg and Mayer, 1982b), thus it was imperative to confirm the effects of monovalent, divalent and trivalent cations in this study. The three reagents (sodium chloride, calcium chloride and iron(III) chloride powder) were dissolved in 1 ml distilled water in different concentrations (0.1, 1.0, 10, 100 and 1000 mM) and added to the native mucilage (Majdoub et al., 2001; Medina-Torres et al., 2000; Trachtenberg and Mayer, 1982b). This test was carried out on dormant stage NM as the purpose was to indicate differences between cultivars and not stages.

5.2.7.2.10 *Effect of a combination of variations between pH and $CaCl^{2+}$*

A combination of variables could shed light on the viscosity of mucilage as the pH of a cactus pear cladode varies not only over seasons and days, but hourly, as a consequence of CAM. Calcium is abundant in the cladodes in the form of calcium oxalate crystals. It has been speculated that these two properties cooperate in the regulation of mucilage viscosity, thus the plant's ability to retain water (Trachtenberg and Mayer, 1982a). The viscosity changes were observed when $CaCl^{2+}$ was constant (1mM) and the pH varied after which the pH was kept constant (pH 7), then $CaCl^{2+}$ added in different concentrations (0.1, 1.0, 10, 100 and 1000 mM). These tests were carried out on the dormant stage NM as the purpose was to indicate differences between cultivars and not stages.

5.2.8 Water-related properties of dried mucilage powder

The water-related properties were carried out on the freeze-dried powder obtained from the dormant stage mucilage. These were preliminary analyses that were done to evaluate the obtained freeze-dried powders for quality and functionality. These determinations were only conducted on freeze-dried mucilage of a single stage (the dormant stage) as the explicit purpose was to compare cultivars and not stages.

5.2.8.1 Water Solubility Index (WSI)

WSI was determined according to published methods (Ayadi et al., 2009; Gebresamuel and Gebre-Mariam, 2013) with slight modifications. One gram of dormant stage freeze-dried mucilage was dissolved in 20 ml distilled water. The powder was dissolved by making a paste using only 2 ml of water and shaking at a high speed using the Genie vortex for 10 s. This procedure was followed in order to form a lump-free paste that would integrate and dissolve more readily. The rest of the distilled water (18 ml) was then added and vortexed again using a Genie vortex mixer for 10 s. Thereafter, the reconstituted mucilage was homogenized for 30 s using a Kenwood stick blender and allowed to stand for an hour in order to dissolve fully. It was centrifuged for 10 min at 8000 rpm. The supernatant that was decanted was measured in terms of volume and weight and subsequently dried. The metal petri dishes were weighed before and after the supernatant was dried in order to obtain the amount of freeze-dried powder that dissolved in the supernatant (Ayadi et al., 2009; Gebresamuel and Gebre-Mariam, 2012). It was calculated using the following equation:

$$WSI\ \% = \frac{dried\ solids\ in\ supernatant\ (g)}{original\ sample\ (g)}$$

5.2.8.2 Water Absorption Capacity (WAC)

Water absorption capacity is described as the amount of water (ml) that was missing from the initial water used and thus absorbed by the powder. WAC was calculated using the following equation and expressed as ml/g (Oladele and Aina, 2007; Samia El-Safy, 2013).

$$WAC\ ml/g = \frac{water\ (ml) - supernatant\ (ml)}{sample\ (g)}$$

5.2.8.3 Water Holding Capacity (WHC)

Ayadi et al. (2009), López-Cervantes et al. (2011) and Traynham et al. (2007) described the method that was used with minor modifications. One gram of dormant stage freeze-dried mucilage was dissolved in 20 ml distilled water (method described in 5.2.8.1). The mucilage was homogenized for 30 s using a Kenwood stick blender in a 70 mm glass beaker (tight fitting) and allowed to stand for an hour in order to dissolve fully. It was centrifuged using a Hettich EBA 12 centrifuge for 10 min at 8000 rpm. The supernatant was decanted and the centrifuge tube inverted for 30 min. The amount of water held by the powder was calculated using the following equation:

$$WHC\ (ml/g) = \frac{wet\ precipitate\ (g) - dried\ precipitate(g)}{dried\ precipitate\ (g)}$$

5.2.8.4 Swelling power

The swelling capacity of a powder is the actual weight of the water that remained in the actual amount of precipitate. The swelling ability of the dormant stage mucilage powder was determined by slightly adapting the methods used by several researchers (Ayadi et al., 2009; Gebresamuel and Gebre-Mariam, 2012; Sáenz et al., 2012; Samia El-Safy, 2013; Sepúlveda et al., 2013). One gram of powder was inserted into a pre-weighed centrifuge tube and dissolved in 20 ml distilled water (method described in 5.2.8.1). It was centrifuged (Hettich EBA 12) at 8000 rpm for 10 minutes and after it was decanted, the weight of the tube containing the paste was determined. The supernatant was dried in order to obtain the amount of powder that actively dissolved in the water. The swelling power was calculated by dividing weight of the wet precipitate with the actual weight of solids (after subtracting the amount of powder that was dissolved in the supernatant from the initial sample size). Swelling power is measured in g/g (gram of water/gram of solids). The following equation was used:

$$Swelling\ power\ (g/g) = \frac{precipitate\ (g)}{sample\ (g) - dried\ supernatant\ (g)}$$

5.3 Statistical Methods

A two way analysis of variance (ANOVA) procedure (NCSS, 2007) was used to determine the effect of growth stage (post-harvest and dormant stages) and cultivar on the morphological, yield and other properties of mucilage. The Tukey-Kramer multiple comparison test (α = 0.05) was carried out to determine whether significant differences exist between treatment means (NCSS, 2007).

5.4 Results

5.4.1 Morphology

The cladode size, weight and surface area measurements were important observations as they increased knowledge and understanding of cultivars and could play a meaningful role when selecting cultivars and are reported in Table 11.

Table 11: Morphological information: Comparison of cactus pear cladodes from 8 different cultivars harvested in the dormant and post-harvest stages

	Cultivar	**Weight (g)**	**Length (mm)**	**Width (mm)**	**Diameter (mm)**	**Volume (mm^3)**	**Surface area (mm^2)**
Post-harvest stage	Algerian	676.7^{ab}	390.7^{ab}	213.0^{ab}	10.7^{ab}	89,5159.0^{ab}	3274.5^{ab}
	Meyers	565.3^{a}	394.5^{ab}	189.7^{a}	9.8^{ab}	75,5475.0^{a}	3064.9^{ab}
	Morado	594.2^{ab}	374.3^{ab}	211.7^{ab}	9.7^{ab}	76,9615.0^{a}	2839.1^{ab}
	Ficus-Indica	746.3^{ab}	446.2^{b}	210.5^{ab}	10.3^{ab}	97,9578.3^{ab}	3635.3^{ab}
	Tormentosa	670.0^{ab}	399.7^{ab}	215.7^{ab}	9.8^{ab}	85,9748.3^{ab}	3118.9^{ab}
	Turpin	588.7^{ab}	360.8^{ab}	209.8^{ab}	8.1^{a}	64,5388.7^{a}	2336.3^{a}
	Gymno-Carpo	808.7^{ab}	390.7^{ab}	223.3^{abc}	10.5^{ab}	91,3187.5^{ab}	3206.2^{ab}
	Robusta	1129.3^{bc}	332.5^{a}	257.3^{bc}	11.5^{ab}	102,7271.2^{ab}	3035.6^{ab}
	Average	**722.4**	**386.18**	**216.36**	**10.05**	**85,0037.7**	**3063.74**
Dormant stage	Algerian	829.3^{ab}	417.7^{ab}	212.7^{ab}	9.5^{ab}	84,9457.3^{ab}	3118.1^{ab}
	Meyers	893.2^{abc}	450.3^{b}	224.3^{abc}	9.8^{ab}	99,1104.3^{ab}	3483.9^{ab}
	Morado	699.3^{ab}	368.0^{ab}	204.7^{ab}	9.0^{ab}	68,2163.0^{a}	2603.9^{ab}
	Ficus-Indica	838.0^{ab}	391.8^{ab}	205.5^{ab}	11.5^{ab}	94,3723.7^{ab}	3562.1^{ab}
	Tormentosa	1002.8^{abc}	421.3^{ab}	228.3^{abc}	10.3^{ab}	100,5595.8^{ab}	3434.3^{ab}
	Turpin	515.3^{a}	343.2^{a}	199.2^{ab}	9.0^{ab}	60,3249.0^{a}	2385.2^{a}
	Gymno-Carpo	749.2^{ab}	368.3^{ab}	204.3^{ab}	11.0^{ab}	83,6436.7^{ab}	3189.9^{ab}
	Robusta	1415.8^{c}	398.8^{ab}	277.2^{c}	12.5^{b}	143,2038.3^{b}	3949.0^{b}
	Average	**867.86**	**394.93**	**219.53**	**10.33**	**91,7970.98**	**3215.79**
	Overall Average	**795.13**	**390.56**	**217.95**	**10.19**	**88,4004.34**	**3139.77**
Significance levels	**Two-way ANOVA**	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.04$	$p = 0.003$	$p = 0.017$
	Dormant X Post-harvest Interaction	$p = 0.393$	$p = 0.0125$	$p = 0.3396$	$p = 0.8064$	$p = 3680$	$p = 0.598$

Means with different superscripts in the same column differ significantly

The weight of cladodes ranged between 565.3 g and 1129.3 g in post-harvest stage and 515.3 g to 1415.8 g in dormant stage (Table 11). Cladodes generally weighed more during the dormant stage yet did not differ significantly in terms of the morphological observations. In both stages 'Robusta' had significantly heavier cladodes (1129.3 g post-harvest stage and 1415 g dormant stage) than all the other cultivars and also proved to have the largest volume for both stages. Interestingly, Robusta was not the cultivar with the largest cladode surface area in the post-harvest stage. 'Turpin' cladodes in both stages were significantly lower in weight, volume and surface area than 'Robusta' in the dormant stage (515.3 g) (Table 11).

The lengths of the post-harvest cladodes ranged from 332.5 mm ('Robusta') to 446.2 mm ('Ficus-Indica') and from 343.2 mm ('Turpin') to 450.3 mm ('Meyers') in the dormant stage (Table 11). 'Ficus-Indica' cladodes were significantly longer in the post-harvest stage (446.2 mm) than 'Robusta' in the same stage and were the only cladodes over 400 mm. In the dormant stage three cultivars had average lengths over 400 mm. 'Turpin' (dormant stage) had significantly shorter cladodes than 'Meyers', mirroring the rounder shape of the cladodes (Wessels, 1989).

In the post-harvest as well as the dormant stage 'Robusta' had significantly wider cladodes than most other cultivars except for 'Gymno-Carpo' (post-harvest stage), 'Meyers' and 'Tormentosa' in the dormant stage, as this cultivar has rounder shaped cladodes (Wessels, 1989) (Table 11).

'Meyers' in the post-harvest stage had the narrowest cladodes while 'Turpin' had the narrowest cladodes in the dormant stage (not significant).

The diameter ranged from 8.1 to 11.5 mm in the post-harvest stage and 9.0 to 12.5 mm in the dormant stage. 'Robusta' in the dormant stage was the thickest (12.5 mm) and 'Turpin' in the post-harvest stage (8.1 mm) was the thinnest cladodes in diameter (Table 11). The cladodes were in general thicker in the dormant stage than the post-harvest stage. In practical terms, thicker cladodes were easier to peel.

In terms of volume, the 'Robusta' cladodes had the highest volume in both the stages while only the dormant stage volume of 'Robusta' was statistically significantly more than 'Turpin' (both stages), 'Morado' (both stages) and 'Meyers' (post-harvest stage) (1432038 mm^3, p = 0.003) (Table 11). 'Turpin' and 'Morado' cladodes were the smallest cladodes in terms of volume in both the stages and 'Meyers' cladodes were also in the smaller category in the post-harvest stage.

'Turpin' cladodes had significantly smaller surface areas (2336.3 mm^2 and 2385.2 mm^2) in both the stages than 'Robusta' in the dormant stage (Table 11). Interestingly, 'Robusta' cladodes had the highest volume in the dormant stage (3949 mm^2), however in terms of surface area, it did not have the highest surface area. In fact, the cultivar with the largest surface area in the post-harvest stage was 'Ficus-Indica' (3635.3 mm^2).

It was thus found that cladodes harvested during the dormant stage were bigger and heavier than cladodes harvested in the post-harvest stage (Table 11). 'Robusta' cladodes were consistently at the highest and 'Turpin' consistently at the lowest end in terms of weight and size in the dormant stage. Cladodes had particular shapes depending on the cultivar and although all cultivars had different lengths, widths and diameters cladodes, there were few outliers in terms of surface area and volume. Only 'Turpin' cladodes were consistently the smallest and lightest in terms of volume and surface area.

In a practical sense, the bigger cladodes are easier to use in terms of the effort that each cladode requires for harvesting, cleaning, peeling and slicing, when one cladode is handled at a time. Therefore, the dormant stage was selected, since bigger cladodes were harvested than in the post-harvest stage.

5.4.2 Yield determination

Calculating and comparing the percentage mucilage yield extracted from cultivars over different stages (post-harvest and dormant) (Table 12) is of paramount significance as ideally the maximum native mucilage should be extracted from the least possible raw plant material for maximum productivity.

In Table 12, the average weight of the cladodes in the post-harvest stage was lower than in the dormant stage. In both the stages, 'Robusta' had the heaviest cladodes. It is important to note that during this investigation whole cladodes were used for yield determination and not a segment as was used in chapter 4. Whole cladodes were studied in this part of the study as the investigation intensified and the sample pool was significantly smaller. Once the cladodes had been prepared for extraction by peeling and cubing, there were no significant differences between the weights of any post-harvest stage pulps except for 'Robusta' that had a larger yield of peeled and cut pieces (Table 12). In both stages, 'Turpin' had the lowest yield of peeled and cut pieces. The 'Turpin' cladodes were smaller and thinner, which made it more difficult to peel without disturbing the inner mucilaginous parts of the cladode, therefore the yield was negatively affected. Once the pieces were minced and cooked, the amount of pulp that was ready for centrifugation was not significantly different, except for 'Robusta' that had significantly more pulp (both stages).

Table 12: Comparison of the yield properties of native mucilage extracted from cladodes of eight different cactus pear cultivars harvested in the dormant and post-harvest stages

	Cultivar	**Weight of whole cladode (g)**	**Peeled and cut cladode pieces (g)**	**Pulp (minced and cooked) (g)**	**Extracted Mucilage (NM) (g)**	**Freeze-dried powder mucilage (FM) (g)**	**% NM Yield from minced and cooked pulp (%)**	**% NM Yield from peeled pieces (%)**	**% NM Yield from whole cladode (%)**	**FM from cladode (%)**
Post-harvest stage	Algerian	676.7^{ab}	246.2^{ab}	219.4^{ab}	142.6^{abc}	3.3^{a}	62.8^{g}	55.9^{f}	20.9^{f}	0.49
	Meyers	565.8^{ab}	221.0^{ab}	191.0^{ab}	105.7^{abc}	3.2^{a}	54.2^{fg}	47.0^{ef}	18.5^{def}	0.56
	Morado	594.2^{ab}	233.2^{ab}	202.5^{ab}	96.2^{abc}	2.9^{a}	45.5^{cdefg}	41.2^{cdef}	15.7^{bcdef}	0.49
	Ficus-Indica	746.3^{ab}	313.8^{ab}	279.5^{ab}	143.3^{abc}	3.3^{a}	49.1^{efg}	43.1^{def}	17.9^{cdef}	0.45
	Tormentosa	670.0^{ab}	260.2^{ab}	228.6^{ab}	94.3^{abc}	3.4^{a}	41.8^{bcdefg}	37.2^{bcdef}	14.2^{abcdef}	0.51
	Turpin	592.0^{ab}	207.0^{ab}	173.8^{ab}	69.2^{ab}	2.5^{a}	33.6^{abcdef}	28.4^{abcde}	10.5^{abcdef}	0.43
	Gymno-Carpo	805.7^{ab}	348.3^{ab}	307.7^{abc}	170.1^{bc}	4.0^{ab}	53.3^{efg}	46.9^{ef}	20.3^{ef}	0.49
	Robusta	1087.8^{bc}	545.3^{bc}	487.4^{bc}	209.8^{c}	8.9^{b}	46.6^{defg}	41.2^{cdef}	19.7^{ef}	0.82
	Average	**717.31**	**296.88**	**261.24**	**128.91**	**3.95**	**48.34**	**42.60**	**17.21**	**0.53**
Dormant stage	Algerian	829.3^{ab}	392.3^{abc}	271.5^{ab}	90.6^{abc}	4.7^{ab}	29.3^{abcde}	22.4^{abcd}	9.8^{abcde}	0.57
	Meyers	893.2^{abc}	309.7^{ab}	343.3^{abc}	65.4^{ab}	3.9^{ab}	21.5^{abc}	20.8^{abc}	7.2^{abc}	0.44
	Morado	699.7^{ab}	263.5^{ab}	211.2^{ab}	60.2^{ab}	3.1^{a}	22.6^{abcd}	18.8^{ab}	7.5^{abc}	0.44
	Ficus-Indica	838.0^{ab}	363.2^{ab}	316.7^{abc}	102.4^{abc}	5.3^{ab}	30.5^{abcdef}	25.9^{abcde}	11.8^{abcdef}	0.64
	Tormentosa	1002.8^{abc}	462.0^{abc}	397.8^{abc}	96.2^{abc}	6.7^{ab}	23.4^{abcd}	19.9^{abc}	9.4^{abcde}	0.67
	Turpin	515.3^{a}	177.0^{a}	140.3^{a}	27.2^{a}	2.1^{a}	14.1^{a}	11.4^{a}	4.4^{a}	0.41
	Gymno-Carpo	749.2^{ab}	375.7^{ab}	327.8^{abc}	69.1^{ab}	3.6^{ab}	20.5^{ab}	17.8^{ab}	8.8^{abcd}	0.49
	Robusta	1415.8^{c}	731.8^{c}	644.7^{c}	74.9^{ab}	5.0^{ab}	12.9^{a}	10.6^{a}	5.3^{ab}	0.35
	Average	**867.91**	**384.40**	**331.66**	**73.25**	**4.30**	**21.85**	**18.45**	**8.03**	**0.50**
	Overall Average	**792.61**	**340.64**	**296.45**	**101.08**	**4.13**	**35.10**	**30.53**	**12.62**	**0.50**
Significance level	**Two-way ANOVA**	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.003$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.051$
	Dormant X Post-harvest Interaction	$p = 0.3412$	$p = 0.6744$	$p = 0.6976$	$p = 0.2126$	$p = 0.0671$	$p = 0.3414$	$p = 0.3689$	$p = 0.3029$	$p = 0.0652$

Means with different superscripts in the same column differ significantly

In the dormant stage ‘Turpin’ had significantly less pulp than ‘Robusta’ in both stages (Table 12). Therefore, the conclusion was made that the loss that occurred from the whole cladode to pulp was generally equivalent in terms of percentage loss.

The yield results of the extracted mucilage from the dormant stage were quite contrary to that of the post-harvest stage (Table 12). In the post-harvest stage the yield of mucilage ranged from 96.2 g (‘Morado’) to 209.8 g (‘Robusta’) while the dormant stage yield ranged from 27 g (‘Turpin’) to 102 g (‘Ficus-Indica’). It was apparent that the average yield of extracted mucilage for all the cultivars were higher in the post-harvest than in the dormant stage. In the post-harvest stage, only three cultivars yielded less than 100 g mucilage per cladode while in the dormant stage, only one cultivar yielded more than 100 g.

To merely compare the amount of mucilage extracted is trivial, as each cladode differed according to size and weight. The percentage yield of each step should be monitored in order to compare the progressive loss that occurred during each stage.

The average yield from the minced and cooked pulp (42.6%) during the post-harvest stage was much larger than in the dormant stage (18.45%) (Table 12). The yield ranged from 33.6% (post-harvest, ‘Turpin’) to 62% (post-harvest, ‘Algerian’) while it ranged from 12.9% (dormant ‘Robusta’) to 30.5% (dormant ‘Ficus-Indica’). ‘Algerian’ yield (62.8%) of minced and cooked pulp in post-harvest stage was significantly more than the other cultivars while the ‘Turpin’ (14.1%) and ‘Robusta’ minced and cooked pulp (12.9%) was significantly less compared to other cultivars. The results of NM from peeled pieces mirrored the results from pulp values in terms of losses.

When comparing the mucilage yield (%) to the whole cladodes it was apparent the yield (%) was superior in the post-harvest stage (Table 12). It ranged from 10.5% (‘Turpin’) to 20.0% (‘Algerian’) with the average of 17.2% in post-harvest stage and from 4.4% (‘Turpin’) to 9.8% (‘Algerian’) with the average at 8.03% in dormant stage. Therefore, the average yield of NM from cladodes in the post-harvest stage was more than double that of the dormant stage (Table 12).

The results in terms of the freeze-dried mucilage yield were contrary to all the results thus far, as the yield of freeze-dried powder in the dormant stage was almost double that of the powder yield from the post-harvest stage (Table 12). ‘Algerian’ and ‘Ficus-Indica’, that before had the largest native yield results, now had the lowest % FM yield from NM in the post-harvest stage. Moreover, once more, 'Algerian' and 'Morado' had the lowest FM yields in the dormant stage. 'Robusta', that had the highest FM yield (0.8%) in the post-harvest stage had the lowest FM yield (0.3%) in the dormant stage. FM yield (%) from the whole cladodes for 'Algeria', 'Turpin' and 'Gymno-Carpo' were similar (0.5%) in both stages. 'Ficus-Indica' and 'Tormentosa' FM yields were higher in the dormant stage than in the post-harvest stage.

Initially the post-harvest stage (average 128.9 g) had higher yields than the dormant stage (73.25 g), yet after freeze-drying, there were no significant differences (p = 0.003) between the eight cultivars of the post-harvest (3.94 g/ 0.53%) and the dormant stage (4.3 g/ 0.50%) (Table 12). In conclusion, the average yield of NM in the post-harvest stage was higher in all cultivars, therefore it seemed that the post-harvest stage would offer the best option for harvesting cladodes, nevertheless after freeze-drying, the yields of the two stages were similar. As it is believed that the FM would find greater commercial use, no stage was selected here. In terms of yield of FM, the cultivars offered similar yield results and no specific cultivar emerged as an outlier (Table 12). It is evident that only approximately 0.5% of the original weight of the cladode was converted into the dried mucilage product.

In chapter 3 it was established that the viscosity of mucilage had the largest influence on mucilage yield and that the viscosity of mucilage is inherent to the cultivar. There seemed to be a problem with very high viscosity for the method of extraction of mucilage used in this study. Thus, very high viscosity mucilage could not be separated from the solids during centrifugation.

Sepúlveda et al. (2007) found that the best yields of mucilage were obtained after 4 hours of extraction (1.48% fresh weight or 19.4% dry weight). It was pointed out that yield was very dependent on climatic conditions, such as cold and rain, due to the ability of the mucilage to absorb water as a defence mechanism against stress conditions. It was stated that the mucilage content increased during low temperatures and dryer periods. According to Sepúlveda et al. (2007) the yield of mucilage was dependent on the climatic conditions at the time of the collection of the cladodes. The mucilage obtained during very dry climatic conditions would be more viscous (Sepúlveda et al., 2007). Nobel and Castaneda (1998) affirmed that mucilage yield could be influenced by climatic conditions as well as the management of the orchard.

Cárdenas and Goycoolea (1997) investigated freeze-dried mucilage and found a dried mucilage yield of approximately 0.7 g/kg from cladodes. In a study done by Peña-Valdivia and Sánchez Urdaneta (2006), the average mucilage content of nopalitos was 6.35%, which contrasts sharply with the amount found in fruit (0.91%). Interestingly, it was observed that cooking nopalitos allow for the release of mucilage into the cooking media. A yield of 1.48% was reported by Sepúlveda et al. (2007) and 0.7 g/kg reported by Cárdenas and Goycoolea (1997). It correlates well with the yields found in this study (0.5%) (Table 12).

5.4.3 Moisture content

Moisture content indicates the hydration status of cladodes. It indicates the degree of stress that the cladodes experienced either in drought (winter conditions) or heat stress (summer conditions) (Mullan and Pietragalla, 2012). It was therefore imperative to compare the moisture content during the two growth stages (Table 13).

Table 13: Moisture content (%) of eight different cactus pear cladodes harvested in the post-harvest and the dormant stages

	Cultivar	**Moisture Content (%)**
Post-harvest stage	Algerian	85.3
	Meyers	82.8
	Morado	88.7
	Ficus-Indica	93.6
	Tormentosa	88.3
	Turpin	89.5
	Gymno-Carpo	94.4
	Robusta	93.6
	Average	**89.53**
Dormant stage	Algerian	87.0
	Meyers	88.3
	Morado	86.4
	Ficus-Indica	88.4
	Tormentosa	87.7
	Turpin	85.1
	Gymno-Carpo	86.5
	Robusta	87.7
	Average	**87.14**
	Overall Average	**88.33**
Significance level	**Two-way ANOVA**	p = 0.465
	Dormant X Post-harvest Interaction	p = 0.4665

Means with different superscripts in the same column differ significantly

Average moisture content levels during the dormant stage were slightly lower (87.14%) than in the post-harvest stage (89.53%) (Table 13). In the post-harvest stage there were bigger differences between cultivars in terms of the moisture levels although not significantly different. The lowest moisture content was ‘Meyers’ (82.8%) and the highest was ‘Gymno-Carpo’ (94.4%), the difference being 11.6%. In the dormant stage the moisture levels only differed with 3.3% between ‘Ficus-Indica’ (88.57%) and ‘Turpin’ (85.1%) (Table 13). The average moisture content of both stages was 88.33%. From these results no significant differences for the moisture content of cladodes from the post-harvest and the dormant stages were detected. It has been established in chapter 3 that the moisture content and the yield of native mucilage showed a weak relationship.

5.4.4 pH

The pH of the two stages was determined to observe differences that may occur between cultivars and growth stages (Table 14).

Table 14: pH of native mucilage extracted from cladodes of eight different cactus pear cultivars harvested in the post-harvest and the dormant stages

	Cultivar	**pH**
Post-harvest stage	Algerian	4.0^{a}
	Meyers	4.2^{abcd}
	Morado	4.2^{abc}
	Ficus-Indica	4.2^{ab}
	Tormentosa	4.2^{abc}
	Turpin	4.2^{ab}
	Gymno-Carpo	4.2^{ab}
	Robusta	4.5^{abcd}
	Average	**4.21**
Dormant stage	Algerian	4.5^{abcd}
	Meyers	4.5^{abcd}
	Morado	4.6^{bcd}
	Ficus-Indica	4.6^{bcd}
	Tormentosa	4.5^{bcd}
	Turpin	4.7^{cd}
	Gymno-Carpo	4.6^{bcd}
	Robusta	4.7^{d}
	Average	**4.59**
	Overall Average	4.4
Significance level	**Two-way ANOVA**	$p < 0.001$
	Dormant X Post-harvest Interaction	$p = 0.7907$

Means with different superscripts in the same column differ significantly

The importance of pH in the preservation of food (shelf life) is well documented. When the pH values are low (pH 4.5), bacteria will not survive or grow. Factors such as cultivar, seasonal variations, maturity of the plant, geographical areas, post-harvest handling and storing could have adverse effects on the pH values of a plant (Rahman, 2007). The pH levels in the post-harvest stage were slightly lower (average 4.21) than in the dormant stage (average 4.59) (Table 14), yet the cultivars did not differ significantly from each other in any growth stages. The highest pH recorded from the dormant stage was for 'Turpin' and 'Robusta' (pH 4.7). The pH of 'Turpin' was significantly higher in the dormant stage than in the post-harvest stage. The lowest pH was 4 ('Algerian', post-harvest stage) and the highest was 4.7 ('Robusta', dormant stage). The pH of 'Algerian' in the post-harvest stage was significantly lower than 'Morado', 'Ficus-Indica', 'Tormentosa', 'Turpin', 'Gymno-Carpo' and 'Robusta' in the dormant stage. 'Robusta' had the highest pH in both stages (Table 14). The important cut-off point of pH of 4.5 was just missed by some of the cultivars in the dormant stage, yet the post-harvest stage mucilage was comfortably below it (Table 14). Thus, the post-harvest stage mucilage is very well protected against bacteria growth. The organic acids responsible for the relatively low pH in mucilage was mainly malic acid. Analyses on organic acids will be discussed in chapter 7.

5.4.5 *Viscosity determinations*

Viscosity data could serve as a "window" to other characteristics of the material. The viscosity of a fluid is a physical manifestation of the molecular weight and the distribution of molecules (Brookfield Engineering Laboratories, 2014). The fundamental differences in viscosity between the two stages as well as between the cultivars was observed in the line-spread test. A more sophisticated measurement was conducted using the RV Brookfield Viscometer.

5.4.5.1 *Line-spread*

The line-spread test quantifies the spreading of a substance therefore the higher the reading the lower the viscosity of the mucilage (Kim et al., 2014) as reported in Table 15. It was overall observed in Table 15 that the NM from the dormant stage had higher viscosities in all cultivars than the NM of the post-harvest stage. The higher line-spread values indicated that the post-harvest NM samples spread further and thus the post-harvest stage mucilage had the lowest viscosity. Therefore 'Robusta' and 'Turpin' were the only mucilage with an average line-spread score under 30 mm (higher viscosity) in the post-harvest NM results. The post-harvest stage had an NM average of 32.30 mm (Table 15) and the dormant stage 20.1 mm. The highest value and therefore the lowest viscosity was 'Algerian' from the post-harvest stage (36.00 mm) and the lowest value with the highest viscosity was 'Robusta' (19.90 mm) for the dormant stage. It was established in chapter 3 that viscosity influenced yield and in this data, it was confirmed that the post-harvest stage NM had higher yields (Section 5.4.2) and lower viscosities (section 5.4.5). It was later deduced from this study that the different summer (post-harvest) and winter (dormant) daytime temperatures influence CAM. CAM had an adverse effect on cladode pH, which influenced the mucilage viscosity, which had an effect on the yield.

Table 15: Line-spread averages of native and reconstituted freeze-dried mucilage extracted from the cladodes of 8 different cactus pear cultivars harvested in the post-harvest and dormant stages

	Cultivar	**Line-spread NM (Native) (mm)**	**Line-spread FM (Freeze-dried) (mm)**	
Post-harvest stage	Algerian	36.0^{c}	36.4^{ef}	10%
	Meyers	33.6^{c}	35.4^{cdef}	
	Morado	30.8^{c}	29.7^{abcd}	
	Ficus-Indica	33.5^{c}	36.2^{def}	
	Tormentosa	32.2^{c}	32.2^{bcdef}	
	Turpin	29.6^{bc}	31.7^{abcdef}	
	Gymno-Carpo	33.4^{c}	37.4^{f}	
	Robusta	29.3^{bc}	25.1^{a}	
	Average	**32.30**	**33.01**	
Dormant stage	Algerian	23.0^{ab}	29.8^{abcde}	5%
	Meyers	19.4^{a}	25.4^{a}	
	Morado	16.8^{a}	26.0^{ab}	
	Ficus-Indica	23.1^{ab}	32.1^{bcdef}	
	Tormentosa	18.7^{a}	28.5^{ab}	
	Turpin	18.7^{a}	28.3^{ab}	
	Gymno-Carpo	21.2^{a}	29.1^{abc}	
	Robusta	19.9^{a}	28.1^{ab}	
	Average	**20.1**	**28.41**	
	Overall Average	26.20	28.71	
Significance level	**Two-way ANOVA**	$p < 0.001$	$p < 0.001$	
	Dormant X Post-harvest Interaction	$p = 0.6275$	$p < 0.001$	

Means with different superscripts in the same column differ significantly

When comparing FM (10% concentration) and NM from the post-harvest stage, the line-spread results corresponded in terms of the viscosity of mucilage. Even the average of 33.01 mm (post-harvest, FM) was quite similar to the average of 32.3 mm (post-harvest, NM). It was only the FM (post-harvest) results from 'Robusta' (25.1 mm) that indicated higher viscosity than 'Robusta' NM (29.3 mm) (post-harvest). There was significant interaction ($p < 0.001$)

observed between the two stages as seen in the significant differences indicated between ‘Gymno-Carpo’ and ‘Meyers’. ‘Gymno-Carpo’ (37.4 mm) FM in the post-harvest stage had significantly lower viscosity than ‘Gymno-Carpo’ NM (29.1 mm) in the dormant stage while Meyers in the post-harvest stage had significantly lower viscosity than in the dormant stage. Therefore the stage of growth during harvest had significant influence on the viscosity of the mucilage.

The results for FM of the dormant stage (average 28.4 mm) corresponded more with the results from the FM post-harvest stage (average 33.01 mm) and even NM post-harvest stage (average 32.3 mm) than the dormant stage NM viscosities (average 21.10 mm), as the overall viscosity results from the dormant NM indicated noticeably higher viscosities. The viscosity of the NM in the dormant stage was generally significantly higher than in the post-harvest stage, except for ‘Ficus-Indica’ and ‘Algerian’ from the dormant stage, that did not differ significantly from ‘Turpin’ and ‘Robusta’ from the post-harvest stage. Nevertheless, when this mucilage was freeze-dried and reconstituted at 5% concentration, the viscosities of FM dormant stage almost matched those of the post-harvest FM.

One of the aims of this study was to identify the stage for harvesting cladodes for optimal mucilage, therefore it was deduced from these results that the dormant stage NM produced FM mucilage that had higher potential for influencing the viscosities of solutions at lower concentrations.

5.4.5.2 Viscometer

The flow behaviour of a fluid is essential in that it predicts the behaviour of a fluid in a certain situation. For mucilage to be used effectively in commercial food products, the behaviour and flow of mucilage amidst diverse environments, manipulation and packaging conditions should be understood. The differences detected in the flow behaviour in specific situations is indicative of changes that occur at molecular level and can be detected and quantified without conducting complex molecular weight measurements (Brookfield Engineering Laboratories, 2014). Changes in flow behaviour of a specific material could also indicate the occurrence of chemical reactions. Therefore, determining the rheological behaviour could be useful when chemical, mechanical or heat treatments are applied. It also proves useful in quality control or to track the course of a reaction (Brookfield Engineering Laboratories, 2014). In order to establish an end product that performs in a consistent way during processing, storing and consuming, the viscosity and flow behaviour should be understood (Brookfield Engineering Laboratories, 2014).

The amount of NM extracted from ‘Turpin’ during the dormant stage was not sufficient for the Viscometer test, as a minimum amount (30 ml) of mucilage was required for reliable readings. After the ‘Turpin’ NM was freeze-dried, the FM was sufficient for reconstitution and the Viscometer tests were completed on ‘Turpin’ FM.

5.4.5.2.1 *Single point viscosity*

When a fluid is non-Newtonian is it very difficult to define a single point of viscosity since a value attained would be different at another speed and spindle used. Single point viscosity is an apparent viscosity in the sense that the moment the spindle starts rotating, the viscosity reading will change. Therefore viscosity measured using the Viscometer would be different depending on the rate of agitation. In Table 16, the viscosity of mucilage is shown as determined at 50 pm at 20˚C after 60 seconds.

Table 16: Viscosity of native and reconstituted freeze-dried mucilage extracted from the cladodes of 8 different cactus pear cultivars [cP (centipoise)] at 50 rpm after 60 s (1 min) harvested in the post-harvest and dormant stages

	Cultivar	Viscosity NM (Native) (cP)	Viscosity FM (Freeze-dried) (cP)	
Post-harvest	Algerian	53.3	100.0	10%
	Meyers	70.0	108.0	
	Morado	173.3	360.0	
	Ficus-Indica	50.0	93.3	
	Tormentosa	93.3	130.0	
	Turpin	260.0	73.3	
	Gymno-Carpo	70.0	80.0	
	Robusta	126.7	760.0	
Dormant	Algerian	420.0	150.0	5%
	Meyers	832.0	244.0	
	Morado	637.0	328.0	
	Ficus-Indica	430.8	106.7	
	Tormentosa	630.0	136.7	
	Turpin		160.0	
	Gymno-Carpo	845.0	151.7	
	Robusta	1014.0	220.0	
Post-harvest average		112.1	213.1	
Dormant average		686.9	187.1	

The mucilage extracted from cladodes harvested in the post-harvest stage had lower viscosity. ‘Turpin’ (260 cP) had the highest viscosity, while ‘Morado’ (173 cP) and ‘Robusta’ (126 cP) also demonstrated a tendency toward higher viscosity. ‘Ficus-Indica’ (50 cP) and ‘Algerian’ (53 cP) had the lowest viscosity. ‘Meyers’ (70 cP), ‘Tormentosa’ (93 cP) and ‘Gymno-Carpo’ (70 cP) had intermediate viscosity.

The overall viscosity of the FM post-harvest stage followed the same trends in terms of viscosity compared to the overall post-harvest stage NM (Table 16). ‘Robusta’ was the only FM that demonstrated much higher results compared to NM. ‘Robusta’ FM had the highest viscosity (760 cP) and ‘Morado’ the second highest (360 cP) for the FM post-harvest stage.

The viscosity of NM dormant stage was considerably higher compared to post-harvest stage (Table 16), (as was seen in the line-spread tests). ‘Robusta’ had the highest average viscosity (1014 cP) and ‘Algerian’ the lowest (420 cP). It was thus observed that a substantial difference occurred between the average viscosity of native mucilage from the two different growth stages, owing to the low viscosity of the post-harvest stage NM and the high viscosity of the dormant stage NM (Table 16).

When FM from the two different stages was compared, the results were quite comparable (Table 16). The trends in viscosity was duplicated in all *O. ficus-indica* cultivars. Only ‘Robusta’ viscosity was unpredictable and seemed to be irregular. It was deduced that reconstituted mucilage (FM) gave more consistent results in terms of viscosity. When using freeze-dried mucilage in solutions, the viscosity could be predetermined by selecting appropriate cultivars and by controlling the concentration of the reconstituted mucilage solution. These results compared well with the line-spread results (Table 15). The line-spread and single point Viscometer results showed comparable results in terms of cultivars that generally showed higher or lower viscosities and showed important differences between the cladode harvesting seasons.

5.4.5.3 Controlled rate ramp

The controlled rate ramp observed in Figure 35 was the simplest test to determine whether a fluid is Newtonian or non-Newtonian. A Newtonian fluid such as water will always have constant viscosity, regardless of the rate of the movement. Therefore, by increasing the rate of the spindle, and plotting the viscosity readings, it was possible to observe the changing viscosity in non-Newtonian fluids. With the temperature at 20˚C, the rotation rate was set at

5, 10, 20, 50 and 100 rpm and the torque reading taken after 60 seconds at each speed setting. Both the NM and reconstituted freeze-dried mucilage (FM) of the post-harvest and the dormant stages were tested on all eight cultivars. The discussion will focus on the readings obtained at 50 rpm after 60 s, as it coincides with the single point viscosity as discussed in Table 16. The controlled rate ramp results are presented in Figure 35. It was evident that mucilage of all seasons and cultivars (regardless of viscosity) were non-Newtonian as the viscosity reading consistently decreased when the rate of the rotating spindle was increased (Lewis, 1987).

In Figure 35A 'Turpin' displayed the highest viscosity (260 cP) and 'Morado' viscosity at 50 rpm was noticeably high (173 cP). There were little differences observed in the viscosities of the other cultivars (Figure 35A).

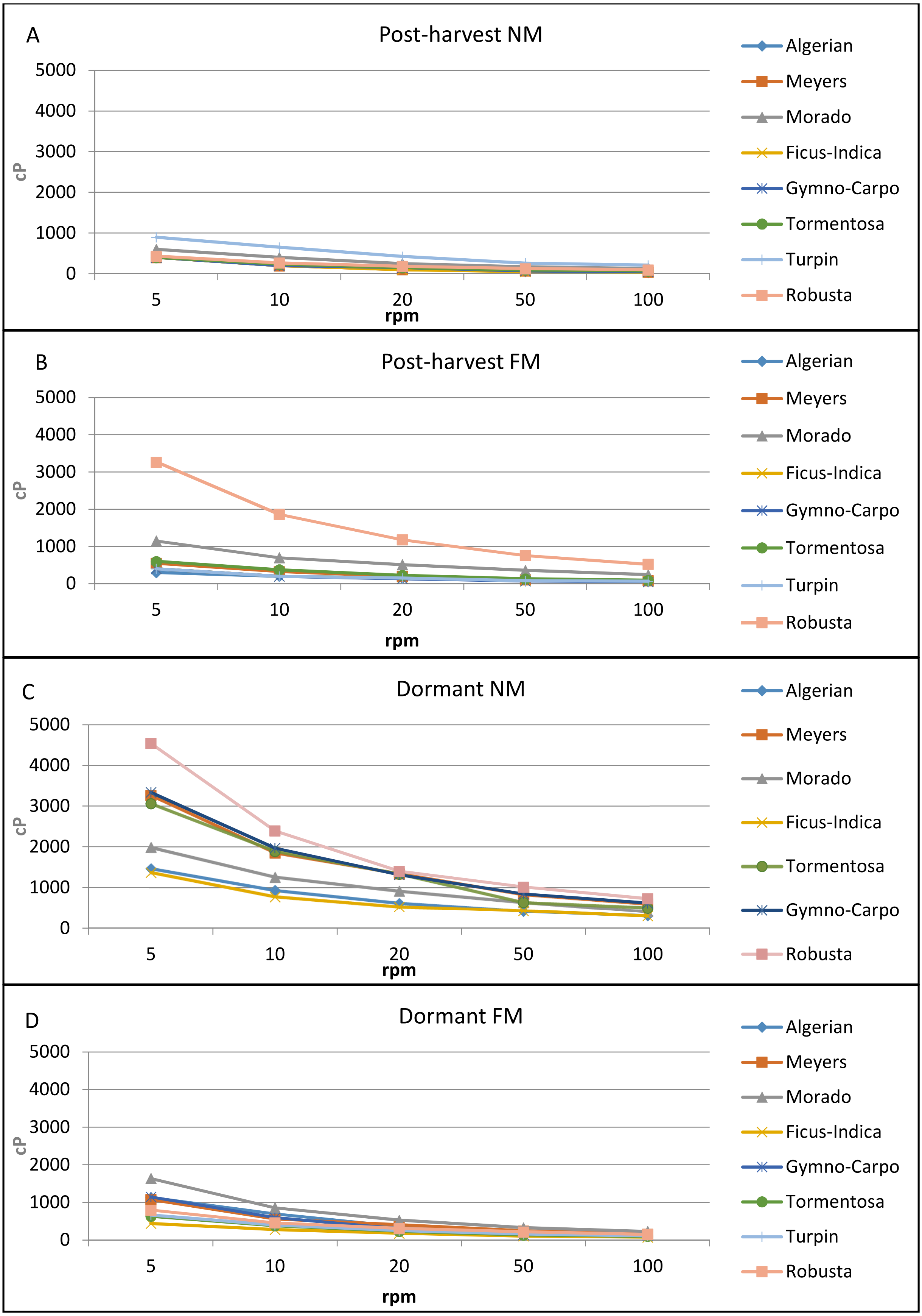


Figure 35: Controlled rate ramp: A Post-harvest NM; B Post-harvest FM; C Dormant NM; D Dormant FM

‘Robusta’ reconstituted mucilage (post-harvest NM) displayed the highest viscosity while ‘Morado’ was slightly more viscous than the other cultivars (Figure 35B). The viscosity of ‘Algerian’ was barely detected (Figure 35B). Incorporating low viscosity mucilage in food products could be possible and beneficial as it would increase the fibre content while remaining undetected in low viscosity liquid products such as milk and beverages.

During the dormant stage (Figure 35C), the viscosity of NM at 50 rpm was between 420 cP (‘Algerian’) and 1014 cP (‘Robusta’). These values were considerably higher than NM of the post-harvest stage (Figure 35A).

The cultivars from the dormant stage (FM) that showed the highest viscosity at 50 rpm were Morado, Algerian and Meyers (Figure 35D). The viscosity ranged from 106 cP (‘Ficus-Indica’) to 328 cP (‘Morado). The viscosity of cultivars was noticeably lower than that of the dormant stage NM, as discussed in Sections 5.2.7.1 and 5.2.7.2.1. In fact, it was consistent to the lower readings obtained from the post-harvest NM (Figure 35).

Figure 35A-D verified that mucilage from all the cultivars, whether the samples were NM or FM, or had high or low viscosity, had a tendency to change viscosity depending on the rate of agitation. In fact, it is difficult to tell the viscosity of the fluid, as it was different at each rate and could not be indicated using a stationary spindle. In Newtonian fluids, the viscosity remained constant as the shear rate increased and the line on a graph will be a straight line running parallel with the x-axes. In Figure 35A-D it was apparent that the lines indicating the relationship between viscosity and shear rate were diagonal and curved. It indicated that mucilage from any cultivar and any viscosity displayed decreasing viscosity with increasing rotation rates (Figure 35). Trachtenberg and Mayer (1982b) described that whenever a linear relationship between the viscosity and shear rate was not observed such as is seen in Figure 35A-D, the fluid was pseudoplastic. The moment that the spindle started moving, the long and flexible molecular structures of the mucilage would have been destroyed, and the molecules orientated in the direction, or parallel to the turning of the spindle as the hindering of the spindle rotation decreased. This caused the friction that the spindle encountered to decrease and the viscosity reading would be lower. The phenomenon is called shear thinning behaviour (Trachtenberg and Mayer, 1982b). The decrease in viscosity observed in Figure 35A-D was as a result of the decreasing resistance that the spindle experienced when the molecule formation changed. The faster the spindle turned, the more molecular structure was destroyed causing the spindle to encounter less resistance, and therefore lower viscosity readings were obtained.

The implication for mucilage inclusion in food products is that when products are chewed, shaken or stirred the viscosity will decrease, allowing better mouthfeel, easy stirring, mixing, sipping or pouring, after which the product will recover to the original viscosity (Glicksman, 1983). Shear-thinning or pseudoplastic behaviour was reported for mucilage solutions before (Cárdenas et al., 1997; León-Martínez et al., 2011; Medina-Torres et al., 2000).

5.4.5.4 Controlled rate on the up-down ramp

Analysing time-dependent fluids involved commencing at a low speed and noting the torque percentage reading at each successive higher speed without halting the Viscometer, followed by reducing the speed to the starting point. If the fluid is time independent, the up and down curve will coincide. If not, the fluid is time dependent. In Figure 36, the time-dependent tendency of all cultivars, both stages (the post-harvest stage and the dormant stage) as well as the NM and FM were presented.

In Figure 36A-D, the viscosities of all samples were identical to the single point viscosity as discussed in Figure 36 and the controlled ramp tests (Section 5.4.5.3), therefore the discussion concerning the viscosities of specific cultivars as well as the similarities and differences between the stages in Section 5.4.5.3 suffice.

The viscosity decreased as the rate was increased and recovered likewise when the rate was decreased. When Figure 36A-D was studied, it was evident that the data points of the up curves were slightly higher than on the down curves, therefore the mucilage was thixotropic and time dependent. The higher the viscosity of the mucilage, the more the up and down curves differed. Therefore the thixotropic tendency was more pronounced when the viscosity was higher. This occurrence is known as hysteresis (Lewis, 1987). At any specific rate of shear, there were two possible apparent viscosities that depended on the shearing history. The up curve exhibited higher viscosity than on the down curve, therefore the mucilage increased in viscosity over time (Lewis, 1987). This tendency was more pronounced in NM than in FM.

In food products, mucilage will decrease in viscosity when products are shaken or stirred, allowing easy stirring, mixing, sipping or pouring. In this up-down ramp analysis, it was observed that the viscosity would recover to the original viscosity when the agitation ceased.

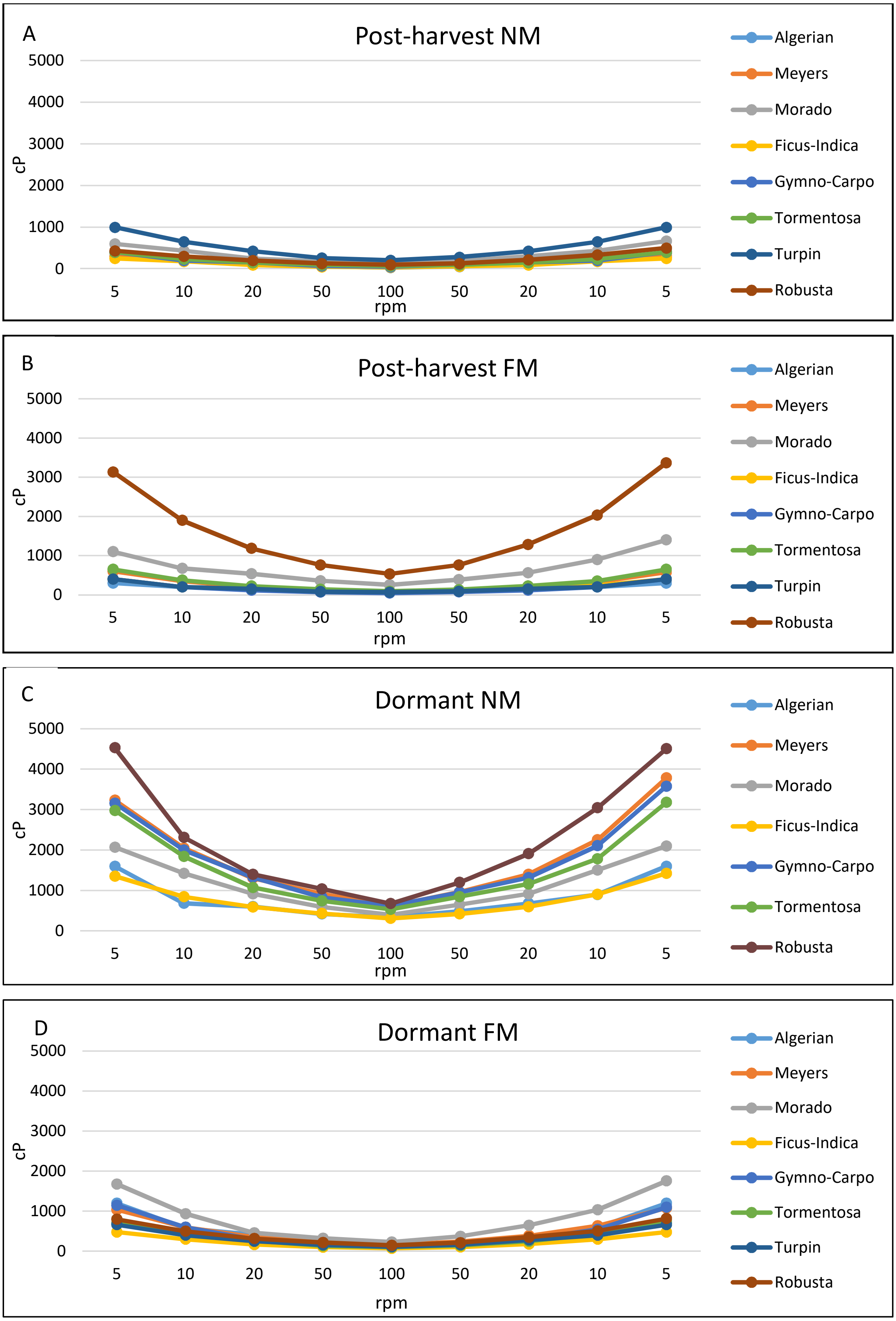


Figure 36: Controlled rate on the up-down ramp: A Post-harvest NM; B Post-harvest FM; C Dormant NM; D Dormant FM

5.4.5.5 Dynamic yield point

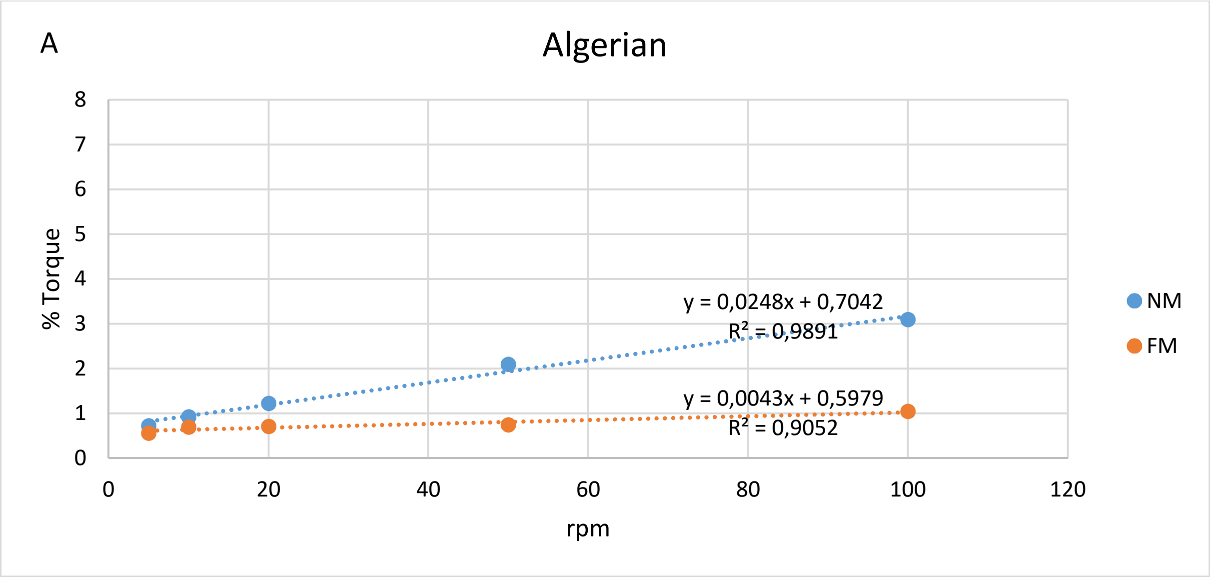
Yield stress is a complex matter and as a consequence, no universal method for determining yield stress, static yield stress (the point at which material changes from a solid to a liquid state) or dynamic yield stress (the point at which a material changes from a liquid to a solid state) has been developed (Malvern Instruments Worldwide, 2012; Moller et al., 2009). Yet a rudimentary analysis on the yield stress tendencies observed in mucilage was adequate for the purpose of this study. A certain amount of force was necessary in order to get mucilage flowing. Before the right amount of force was applied, the mucilage would not start flowing and would behave like a solid, while as soon as the necessary stress was applied and succeeded, flow would begin (Lewis, 1987). This behaviour needed to be measured as it would influence the pressure needed during product manufacture. It would also influence the type of packaging of food products to ensure ease of access and pouring.

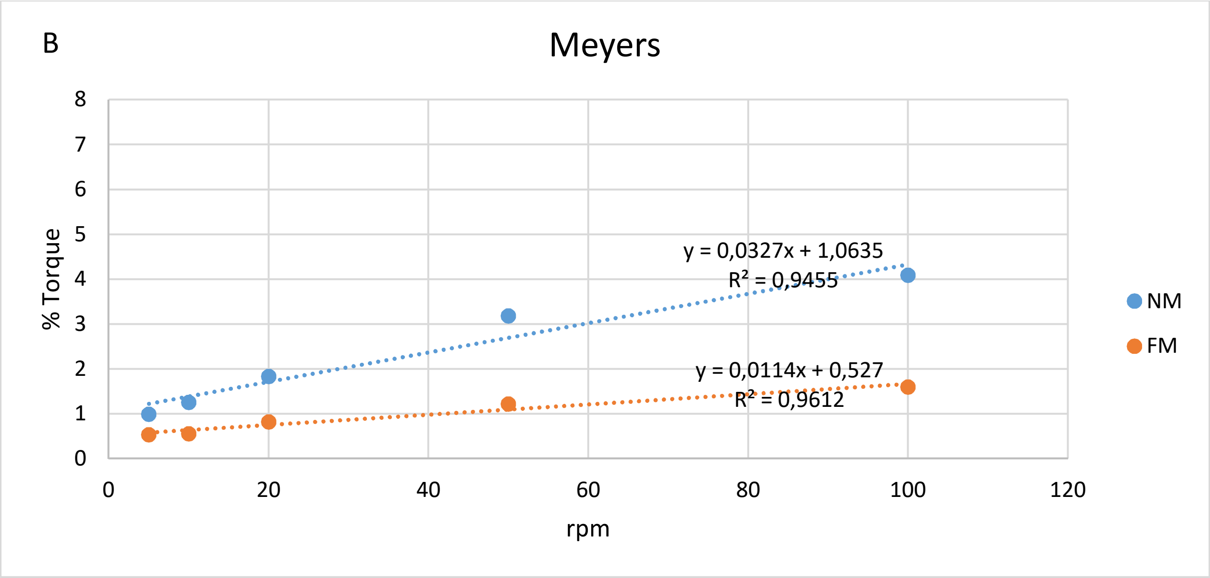
The dynamic yield value [torque value at which flow began (%)] was determined in order to indicate the pourability of mucilage. The dynamic yield was determined by using the torque values (%) attained, using the Viscometer readings during the controlled rate ramp test. A best fit line was explorated to where torque or shear stress was equal to “0” on the y-axes (Figure 37). A Newtonian fluid such as water, would flow the instant (y-axes at 0.1%) that force was applied (such as when water was poured), while mucilage remained static until enough force was applied to begin flow. Dynamic yield point was reported as % torque (Table 17) and was obtained as presented in Figure 37.

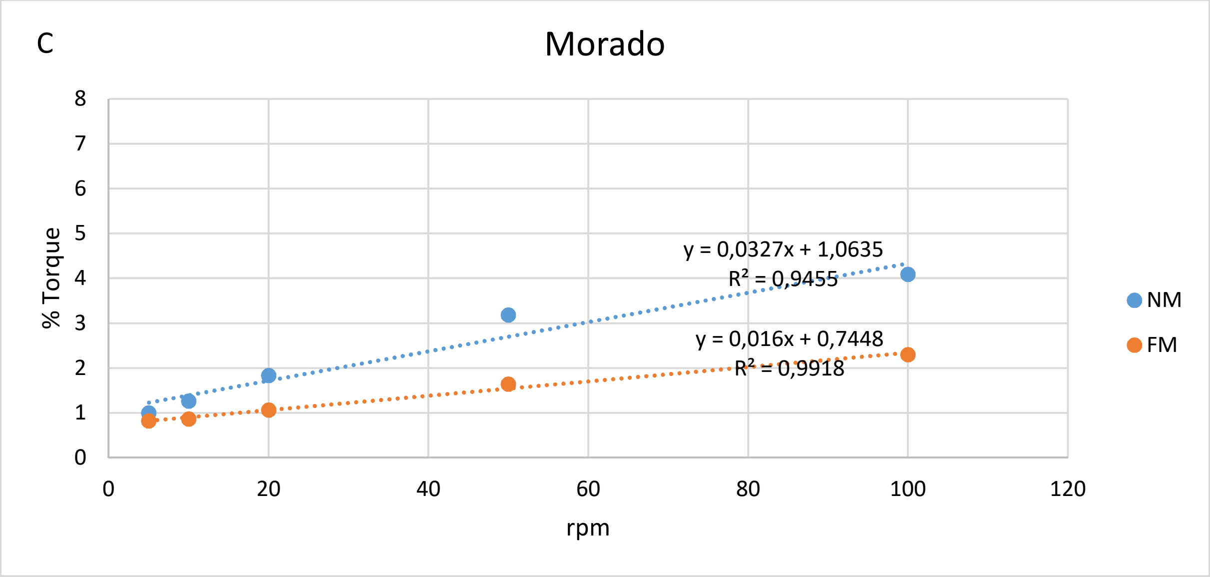
Table 17: The dynamic yield values of native and reconstituted freeze-dried mucilage extracted from the cladodes eight different cactus pear cultivars harvested in the dormant stages

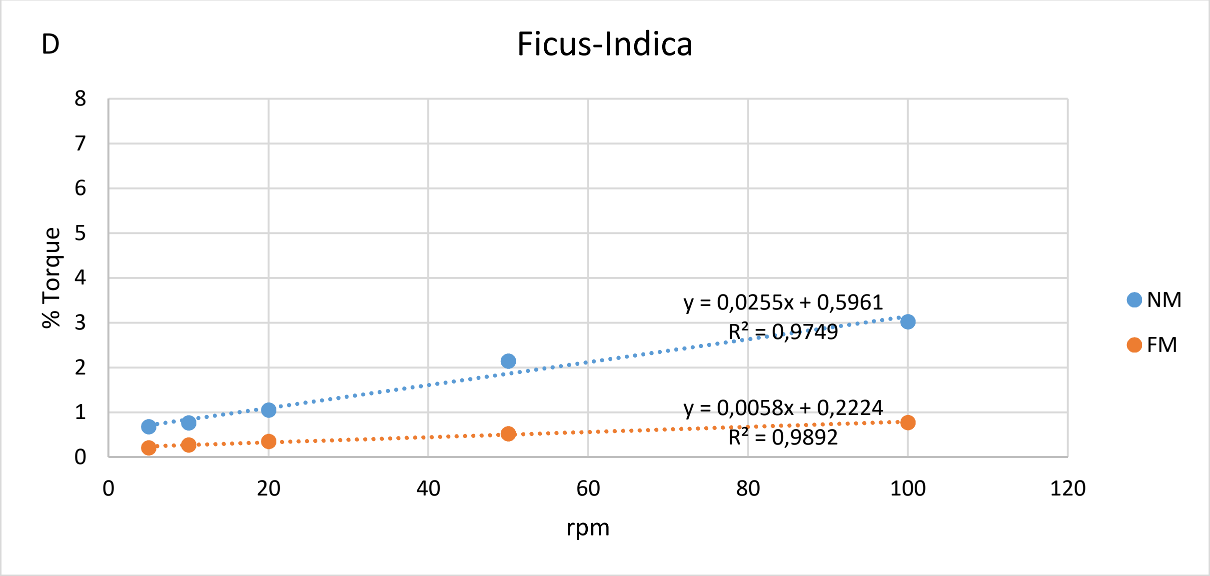
Cultivar	NM y-axes	FM y-axes
Algerian	0.70	0.60
Meyers	1.57	0.53
Morado	1.06	0.74
Ficus-Indica	0.60	0.22
Gymno-Carpo	1.59	0.52
Tormentosa	1.59	0.31
Robusta	1.93	0.38

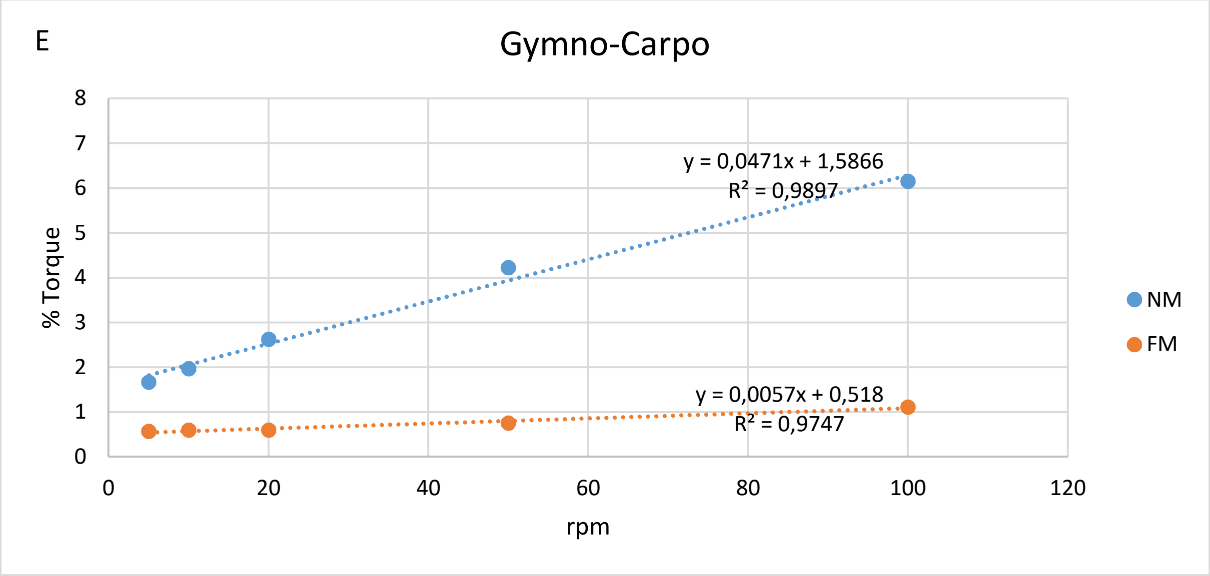
As none of the y-axes values in Table 17 were zero, none of the mucilage samples would start to flow as soon as force was applied. The amount of force necessary for flow was indicated on the y-axes when the trend lines were explorated to the y-axes as seen in Figure 37.

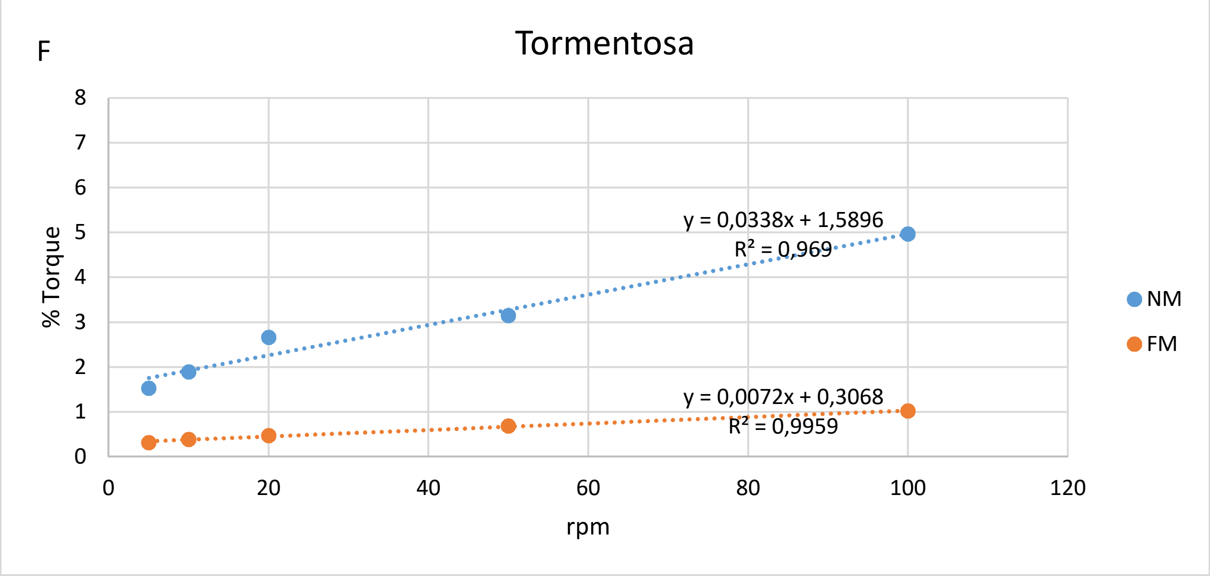

A
Algerian
% Torque
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
120
rpm
y = 0,0248x + 0,7042
R² = 0,9891
y = 0,0043x + 0,5979
R² = 0,9052
● NM
● FM


B
Meyers
% Torque
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
120
rpm
y = 0,0327x + 1,0635
R² = 0,9455
y = 0,0114x + 0,527
R² = 0,9612
● NM
● FM


C
Morado
% Torque
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
120
rpm
y = 0,0327x + 1,0635
R² = 0,9455
y = 0,016x + 0,7448
R² = 0,9918
● NM
● FM


D
Ficus-Indica
% Torque
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
120
rpm
y = 0,0255x + 0,5961
R² = 0,9749
y = 0,0058x + 0,2224
R² = 0,9892
NM
FM


E
Gymno-Carpo
% Torque
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
120
rpm
y = 0,0471x + 1,5866
R² = 0,9897
y = 0,0057x + 0,518
R² = 0,9747
NM
FM


F
Tormentosa
% Torque
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
120
rpm
y = 0,0338x + 1,5896
R² = 0,969
y = 0,0072x + 0,3068
R² = 0,9959
NM
FM

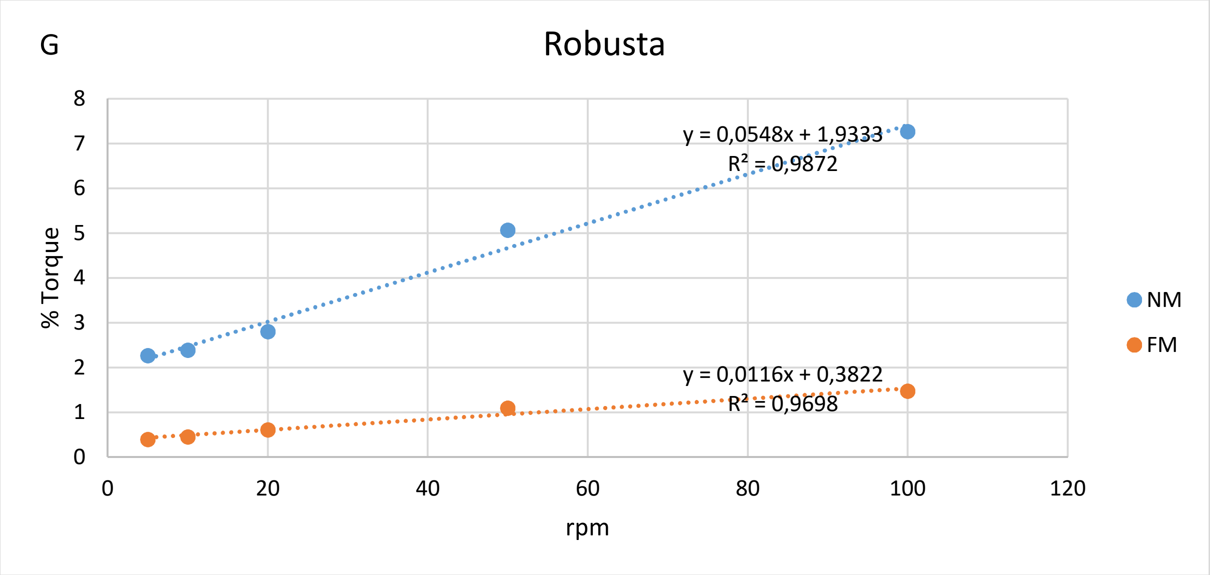


Figure 37: Determination of dynamic yield stress of native and reconstituted mucilage extracted from cladodes of eight different cactus pear cultivars harvested in the dormant stage

Post-harvest NM yield values all computed to 0.0 values, except for 'Turpin' that had a yield value of 0.1, therefore in low viscosity mucilage the yield point was not observed (data not shown). The dynamic yield point was thus only observed in higher vicosity mucilage. From post-harvest FM, it was only 'Robusta' that demonstrated yield stress (0.5) (data not shown).

On the other hand, the dormant stage NM demonstrated considerably higher viscosity than that of the post-harvest stage, therefore the yield values for the dormant stage NM and FM were substantial and reported as demonstrated in Figure 37.

Dynamic yield was higher in the dormant stage NM (as a result of the higher viscosity mucilage) and ranged between 0.5 ('Ficus-Indica') and 1.9 ('Robusta'). The lower viscosity FM ranged from 0.32 for 'Robusta' to 0.7 for 'Morado' (Figure 37). The higher viscosity mucilage ('Robusta', 'Tormentosa', 'Gymno-Carpo' and 'Meyers'), that exhibited dynamic yield points, could further be classified as plastic fluids as the dual tendencies to behave as a solid as well as a liquid were the main characteristic of the mucilage (Lewis, 1987).

Packaging would need consideration as enough force would have to be applied for mucilage to begin flowing. Thicker consistency products such as sauces or paste will not initially flow when it is poured. Consumers would need to knock on the side, shake or otherwise apply force to the packaging before the contents will start to flow.

5.4.5.6 Time sensitivity test

Time sensitivity is necessary to determine, as it indicates the tendency of the substance to increase or decrease viscosity at a constant shear rate over a time period (Lewis, 1987). When constant shear is applied, some substances will progressively become thinner and runnier, while others will become thicker and stickier (Brookfield Engineering Laboratories, 2014). This test involved setting the Viscometer on a chosen rate and recording the torque reading at different time intervals. The rates were set on 5, 10, 20, 50 and 100 rpm and the readings recorded at 15, 30, 60, 90 and 120 s. The findings are reported in Figure 38.

In Figure 38A-D the time sensitivity of mucilage was observed by analysing the entire line that each sample demonstrated, as it indicated that the viscosity of mucilage decreased as shear rate increased (shear thinning behaviour). Secondly, it was important to analyse the viscosity as time lapsed (120 s) at the constant shear rate.

The low viscosity post-harvest mucilage (Figure 38A) displayed shear thinning behaviour (as was previously established (Figure 35A) but the viscosity did not appear to change during the time period when the rate was constant. Therefore, low viscosity NM (all post-harvest NM cultivar samples as well as all FM, except for ‘Robusta’) was time independent (Lewis, 1987).

Likewise, reconstituted mucilage (all cultivars) of both post-harvest and dormant stages (Figure 38B & D) did not change over the considerable time lapse, therefore reconstituted mucilage viscosity was deemed as more stable and predictable.

Native mucilage from the dormant stage (Figure 38C) showed several occurrences where the viscosity increased as time lapsed. This may indicate that after the initial drop in viscosity there was a slight recovery of viscosity, as soon as the shear rate was increased. It was especially evident in high viscosity mucilage (‘Robusta’), that time dependency was observed, therefore rheopectic behaviour occurred. This behaviour showed a build-up in structure and is known as rheopexy.

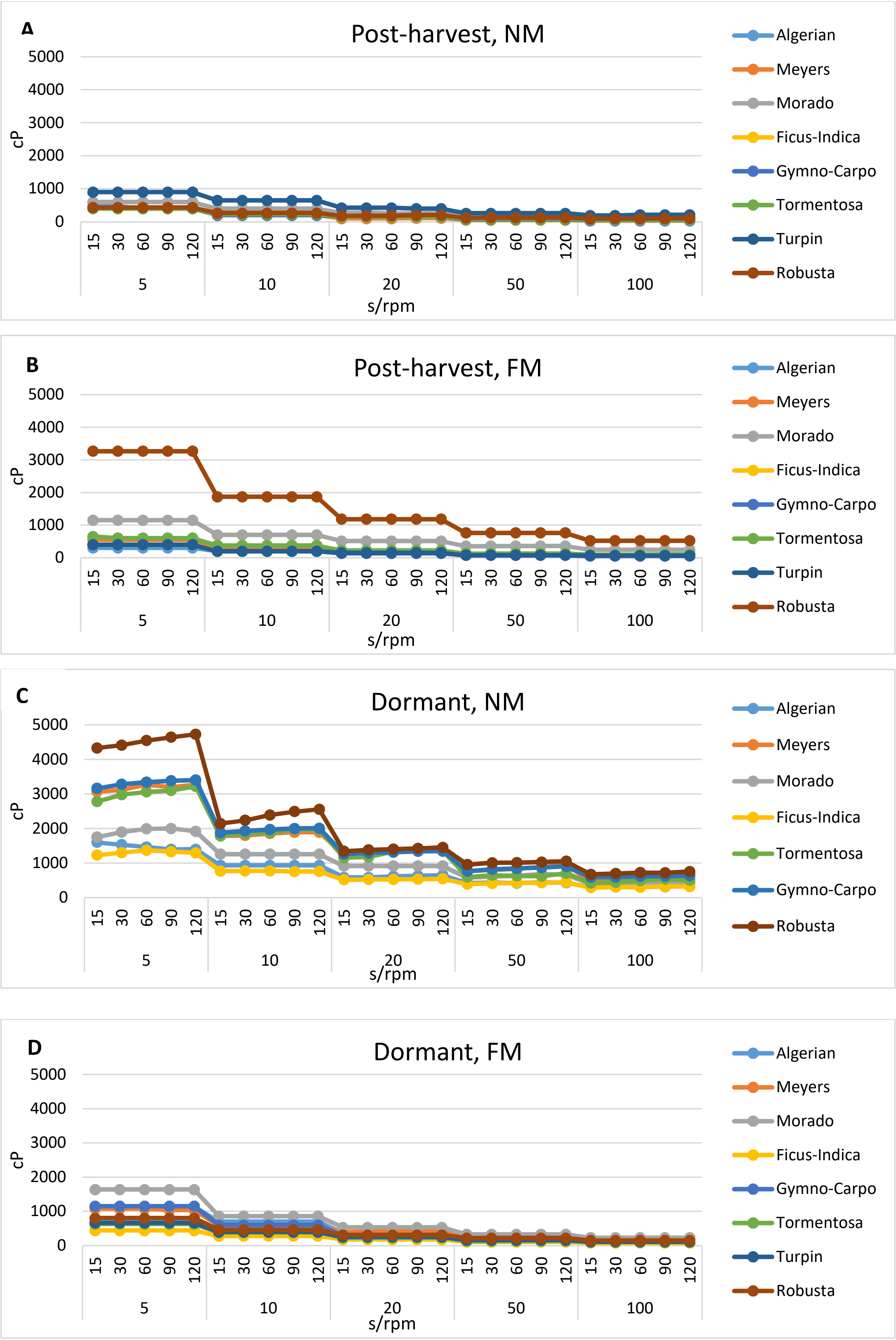


Figure 38: Controlled rate ramp with time sensitivity: A Post-harvest NM; B Post-harvest FM; C Dormant NM; D Dormant FM

5.4.5.7 Temperature sensitivity

Temperature often has an effect on the viscosity of materials. A small change in temperature can often have an adverse effect on viscosity. As temperature changes are usually part of processing or usage, it was an important consideration and the evaluation of changes occurring during temperature changes was essential (Brookfield Engineering Laboratories, 2014). In order to determine the effect of temperature on mucilage viscosity at a steady rate (50 rpm) and time (30 s), mucilage samples were cooled and heated to different temperatures (5, 10, 20, 40, 60 and 80 ˚C). The data obtained is presented in Figure 39.

In Figure 39A 'Turpin' viscosity ranged from 330 cP at 5˚C to 130 cP at 80˚C, which was a 200 cP drop for the high viscosity mucilage. Low viscosity mucilage such as 'Ficus-Indica' ranged from 80 cP at 5˚C to 45 cP at 80˚C, which was a 35 cP drop. Therefore, the higher viscosity mucilage displayed a more significant change in viscosity ('Robusta', 'Turpin' and 'Morado').

In Figure 39B it was observed that 'Robusta', which had the highest viscosity, displayed the most significant drop in viscosity with increased temperature. In fact, in the low viscosity mucilage cultivars the differences were barely observable (Algerian, Ficus-Indica, Meyers, Gymno-Carpo, Turpin and Tormentosa).

The NM dormant stage (Figure 39C) mucilage that had the highest viscosity showed interesting results. For the first time in these graphic representations lines intersected. 'Gymno-Carpo' had the highest viscosity at 5˚C while at 80˚C, 'Meyers', 'Robusta' and 'Morado' had higher viscosity readings. Therefore, 'Meyers' and 'Robusta' were not as adversely affected by temperature as 'Gymno-Carpo' and 'Tormentosa'.

Low and high viscosity mucilage (Figure 39A-D) exhibited similar tendencies. The high viscosity mucilage experienced a more pronounced effect when the temperature was manipulated while the low viscosity mucilage lost its non-Newtonian tendencies and became Newtonian. It would be imperative to select cultivars carefully when matching mucilage with a product as each cultivar had a unique sensitivity to temperature changes.

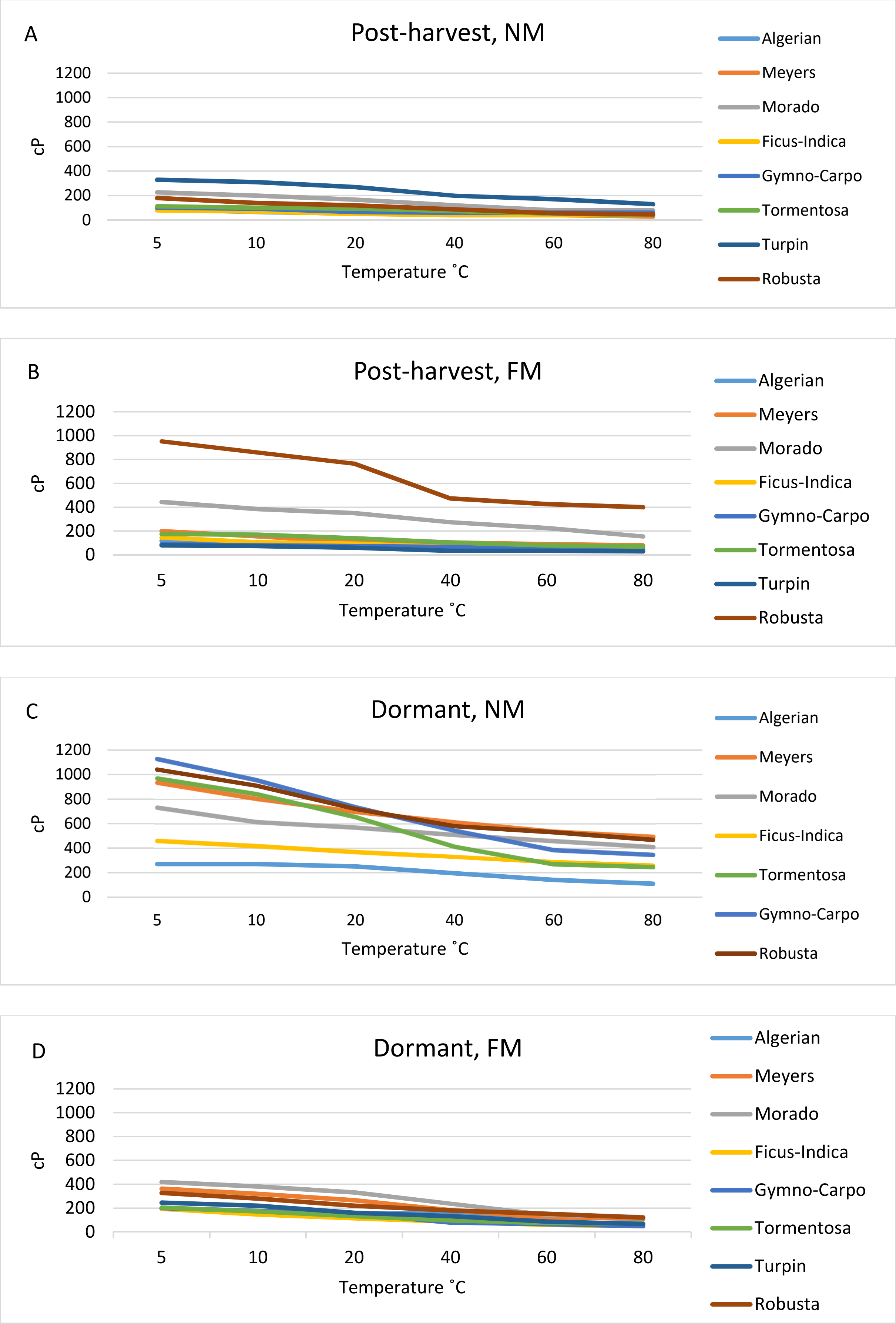


Figure 39: Temperature sensitivity: A Post-harvest NM; B Post-harvest FM; C Dormant NM; D Dormant FM

It was thus observed that temperature had a profound effect on the viscosity of mucilage. The viscosity was higher at colder temperatures and lower at warmer temperatures. This would be important to consider when developing products that need either heating or cooling in the processing stages. In addition, it will have an adverse impact on the consistency of products when consumed. Not only will the mucilage become less slimy and more palatable when heated up, products will become runnier, causing stirring, mixing, shaking or sipping to be easier. When products are cold or frozen it may set or will be solid enough to hold its form when scooped up or shaped. For applications in beverages, selecting a cultivar with low viscosity mucilage would be imperative as it could affect the viscosity of drinks during refrigeration.

Effect of different pH levels on viscosity

It was reported that mucilage viscosity was affected by changes in pH (Trachtenberg and Mayer, 1982b). The influence of pH on mucilage had extensive consequences that began with the environmental conditions where the cactus pear plant grows (this phenomena will be discussed in subsequent chapters). In short, as cactus pear plants are succulents with CAM, organic acids (such as malic acid) are synthesized. When more organic acids are present a drop in the pH of the cladodes takes place (Trachtenberg and Mayer, 1981), causing a subsequent drop in mucilage viscosity. In Figure 40, the influence of pH on NM (dormant stage) viscosity is observed.

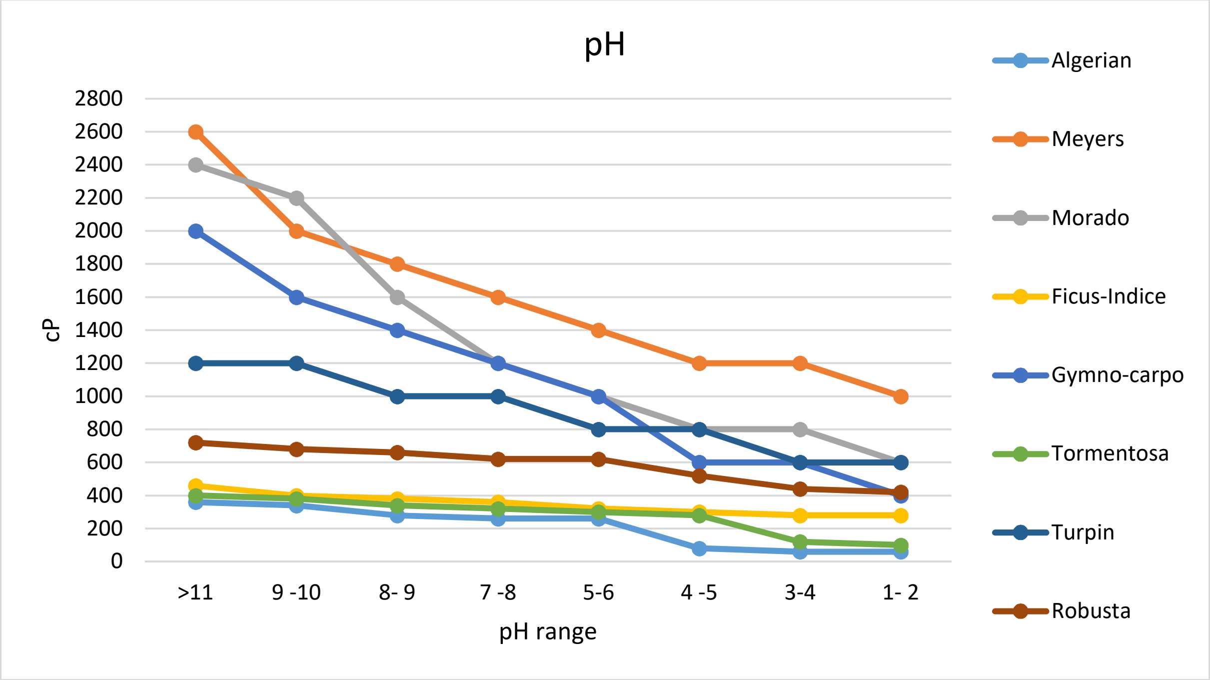


Figure 40: Viscosity of native mucilage extracted from cladodes of eight different cactus pear cultivars harvested in the dormant stage with variations in pH

The viscosity of the mucilage samples was influenced by varying the pH, as it was seen that the viscosity of mucilage in the alkaline region (7-11) was higher, while it was lower in the acidic region (Figure 40). This effect was more pronounced on the higher viscosity mucilage from cultivars Meyers, Morado, Gymno-Carpo and Turpin (Figure 40). Corresponding results have been reported (Medina-Torres et al., 2000; Trachtenberg and Mayer, 1982b).

The influence of pH on viscosity would have to be considered when mucilage is applied in food products, as it could influence the consistency of the product. Products that are naturally acidic such as fruit juices, dairy products, foods containing high oil contents and beverages may cause mucilage to remain low in viscosity.

5.4.5.8 Effect of concentration on viscosity

It would be expected that an increase in concentration of the freeze-dried mucilage would cause an increase in viscosity. Yet it has become more apparent that several factors (pH, temperature, ionic strength and speed and length of agitation) affect mucilage viscosity. For this analysis, freeze-dried mucilage was used. The freeze-dried mucilage that was reconstituted for this test was of the cultivars Algerian, Morado, Gymno-Carpo and Robusta. The viscosity values obtained with increased concentration of freeze-dried mucilage is presented in Figure 41.

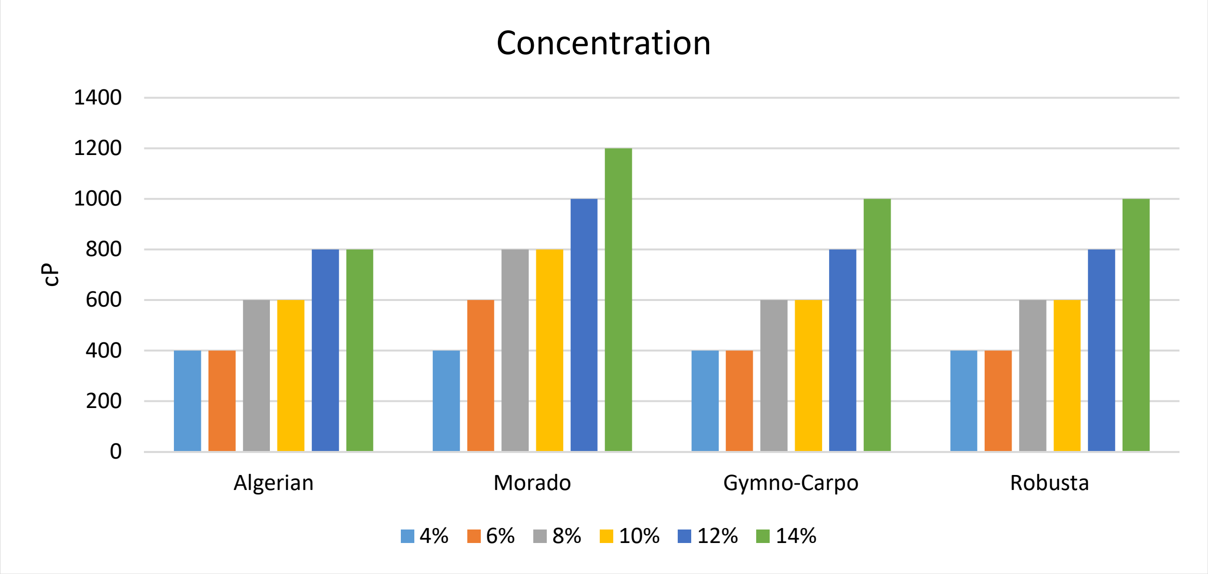


Figure 41: Viscosity of reconstituted freeze-dried mucilage extracted from cladodes of four different cactus pear cultivars harvested in the dormant stage with increased concentrations of freeze-dried mucilage

The viscosity steadily increased from the 4% to 14% increase in concentration (Figure 41). It was interesting to observe that at 4%, all the reconstituted mucilage solutions were similar in viscosity (400 cP). ‘Morado’ was the only sample that showed an increase in viscosity from 4% (400 cP) to 6% (600 cP) inclusion. The ‘Algerian’ sample did not show any increase from 8 to 10%, and again from 12 to 14% (1200 cP). The ‘Morado’ sample increased viscosity with every addition except for 8 to 10% and showed the highest viscosity at 14%. ‘Gymno-Carpo’ and ‘Robusta’ showed the same increases in viscosity, with no increase from 8 to 10% (Figure 41).

It was thus observed that increased concentration of mucilage caused an increase in viscosity (Figure 41). The concentrations of freeze-dried mucilage had to be increased greatly (4%) before any difference in viscosity was observed. Even though the dried mucilage was obtained from cultivars, which in previous tests, demonstrated vast differences in NM viscosity, it exhibited limited differences in viscosity here. Also, the origin (cultivar) of mucilage seemed to be inconsequential to the influence on viscosity. Another observation that could be made is that although increased mucilage concentration caused increase in viscosity, it never caused the formation of a gel. According to Stintzing and Carle (2005), with increasing mucilage concentration, the solution tended to form a weak gel and at 10% resulted in a gel-like network. Gel formation may be a property of pectins extracted from cactus pear cladodes

(Goycoolea and Cárdenas, 2003), but it was certainly not observed in mucilage used in this study.

5.4.5.9 *Influence of ionic strength on viscosity with addition of monovalent (NaCl), divalent (CaCl^{2+}) and trivalent (FeCl^{3+}) cations*

Medina-Torres et al. (2000) and Trachtenberg and Mayer (1982b) reported that mucilage molecules are negatively charged polyelectrolytes. Negative charges produce strong intermolecular repulsions and thus more expanded molecules. Majdoub et al. (2001) described the potential of mucilage molecules to interact with divalent cations such as Ca^{2+} and Mg^{2+}. The negative polarity of mucilage molecules cause the increase the viscosity of solutions. Thus, with the addition of positive ions, the expanded molecules will contract causing the viscosity to reduce significantly. In Figure 42, Na^{+} (monovalent) was added to NM (dormant stage) in different concentrations (0.1, 1, 10, 100 and 1000 mM).

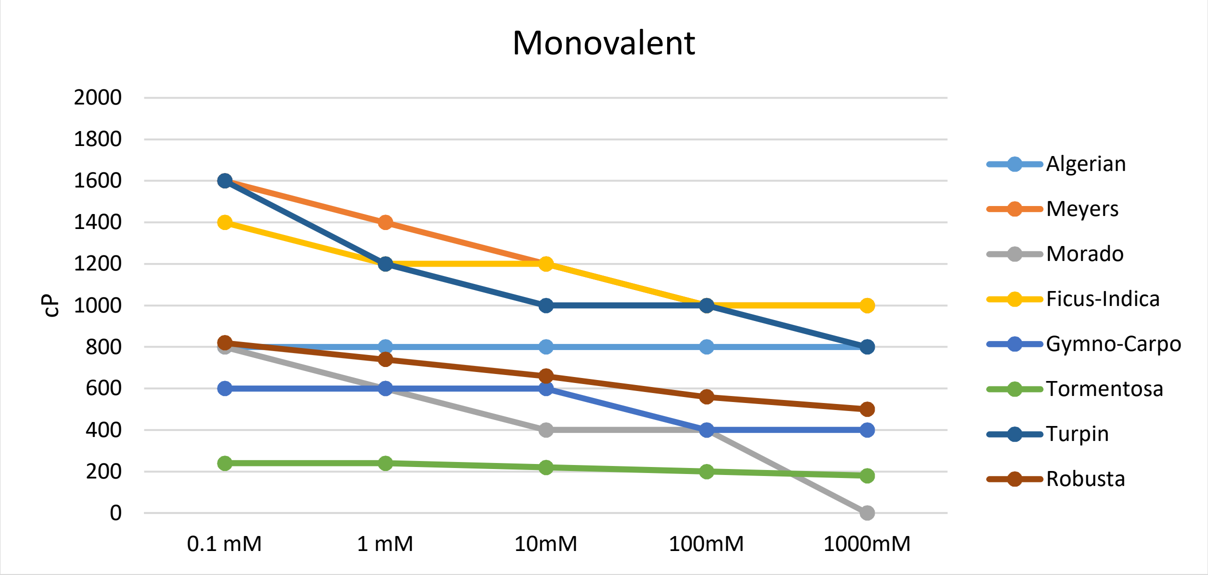


Figure 42: Viscosity of native mucilage extracted from cladodes of eight different cactus pear cultivars harvested in the dormant stage with increasing concentrations of NaCl

The monovalent NaCl had very little influence on the viscocities of the mucilage, since higher concentration additions of NaCl either had no effect on NM viscosity ('Algerian') or slight decreases in viscosity were observed ('Tormentosa' and 'Robusta'). A more pronounced influence was seen in the higher viscosity mucilage cultivars ('Meyers', 'Ficus-Indica' and 'Turpin'). There was a sudden drop seen in 'Gymno-Carpo' NM between 10 and 100 mM and 'Morado' (0.1, 1 and 10 mM).

In Figure 43, the influence of addition of divalent CaCl^{2+} on viscosity was indicated. CaCl^{2+} was added to NM (dormant stage) in different concentrations (0.1, 1, 10, 100 and 1000 mM).

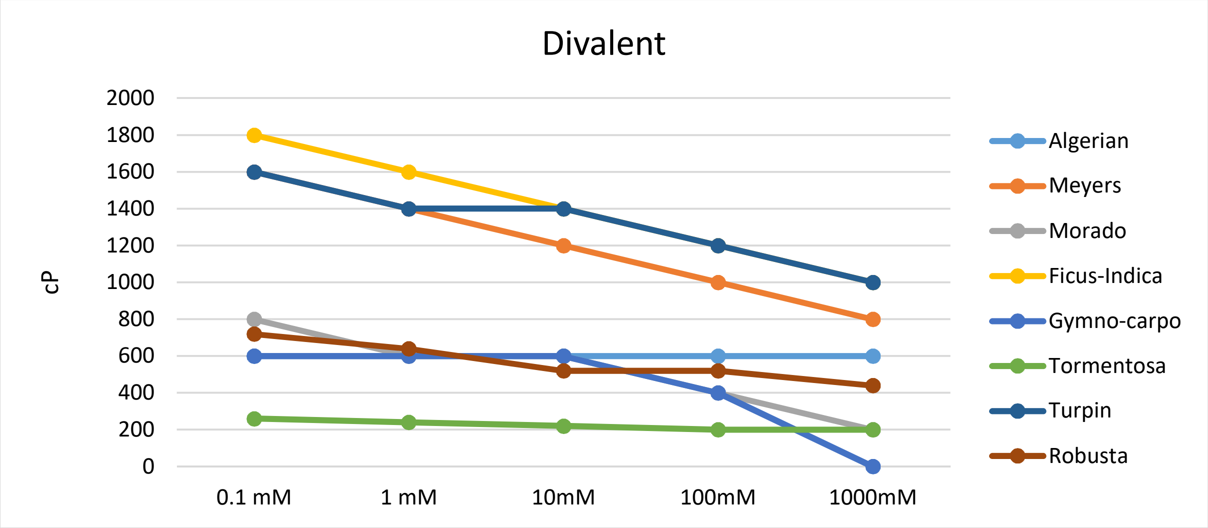


Figure 43: Viscosity of native mucilage extracted from cladodes of eight different cactus pear cultivars harvested in the dormant stage with increasing concentrations of CaCl^{2+}

The influence of divalent CaCl^{2+} was more pronounced than the monovalent NaCl (Figure 43). As observed for NaCl, there was a more noticeble influence on the higher viscosity mucilage cultivars (Ficus-Indica, Turpin and Meyers), while the low viscosity NM showed limited decreasing adjustments (Tormentosa, Robusta and Morado). The sudden drop observed in 'Gymno-Carpo' NM before (Figure 42), was observed again and continued to decrease to 1000 mM. 'Algerian' NM was not affected by the addition of CaCl^{2+} in different concentrations (Figure 42).

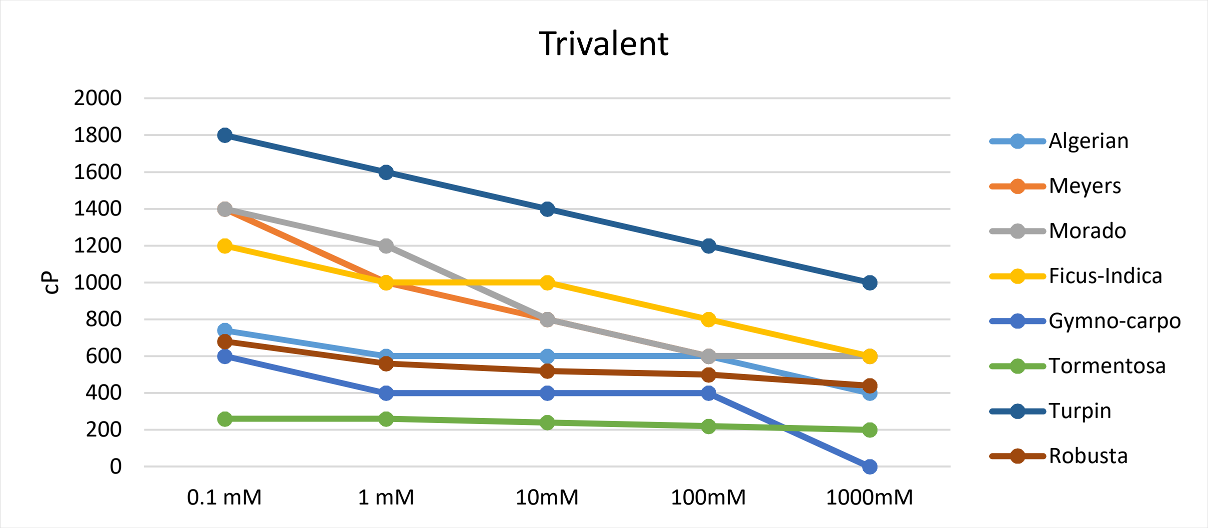


Figure 44: Viscosity of native mucilage extracted from cladodes of eight different cactus pear cultivars

harvested in the dormant stage with increasing concentrations of $FeCl^{3+}$

In Figure 44, the effect of trivalent ions on the viscosity of NM (dormant stage) is indicated. $FeCl^{3+}$ (trivalent) was added to NM in different concentrations (0.1, 1, 10, 100 and 1000 mM). All of the NM samples showed decreased viscosity with the addition of $FeCl^{3+}$ (Figure 44). The cations seemed to affect the higher viscosity mucilage cultivars more adversely (Turpin, Ficus-Indica, Meyers and Morado).'Gymno-Carpo' NM showed the sudden drop in viscosity again as was observed in Figure 42 and Figure 43, while this time at 1000 mM. 'Algerian' NM had small decreases in viscosity at 1 and 1000 mM additions. 'Tormentosa' and 'Robusta' NM showed very little decrease in viscosity with increased $FeCl^{3+}$ addition.

Majdoub et al. (2001) stated that mucilage did not show much sensitivity to calcium as no viscosity changes were observed, while pectins were sensitive to divalent cations and formed gels with the addition of calcium. Medina-Torres et al. (2000) found that viscosity of mucilage depended on ionic strength and added that it was more pronounced when divalent cations were used.

It was seen in this analysis that cations caused a decrease in mucilage viscosity, while higher ionic strength had more pronounced effects. The higher viscosity mucilage was most adversely affected.

The effect of addition or presence of cations on viscosity in the application thereof in food products would have to be considered as mucilage will become less viscous in savoury and dairy products, since the addition of electrolytes, such as calcium and magnesium, react to the negatively charged mucilage molecule, causing it to lose its thickening power.

5.4.5.10 Effect of combination of variations of pH and ionic strength

The effect of pH on NM has been demonstrated (Section 5.4.4), nevertheless, the effect of pH in the presence of calcium ($CaCl^{2+}$) could be quite different as seen in Figure 45 and Figure 46. Only NM from three cultivars is shown in Figure 45. The highest viscosity mucilage cultivars for this test (Meyers, Turpin and Robusta) were used.

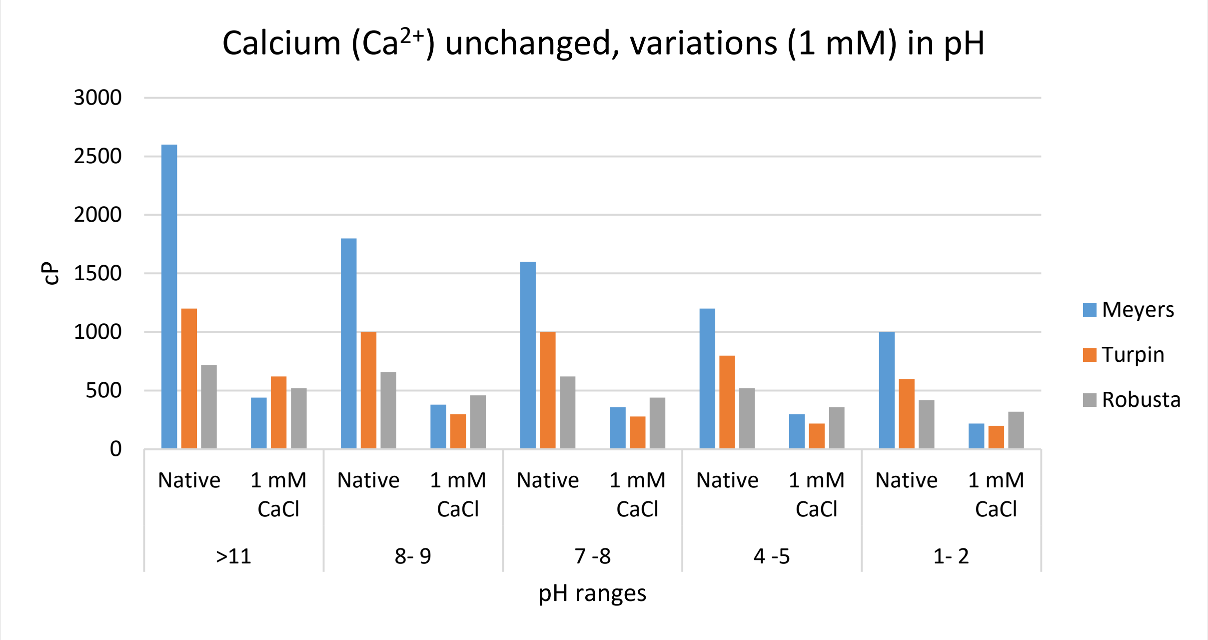


Figure 45: Viscosity of native mucilage extracted from cladodes of eight different cactus pear cultivars harvested in the dormant stage (containing 1 mM $CaCl^{2+}$) with variations in pH

Variations in pH had a more severe effect on NM (without Ca^{2+}) than on NM with an added 1 mM calcium concentration (Figure 45). Even at extremely alkaline pH ranges (>11) or acidic ranges (1-2), the viscosity of the calcium-added mucilage only slightly varied from approximately 530 to 250 cps. It was thus observed that the presence of calcium subdued the effect of pH variations on the viscosity of mucilage.

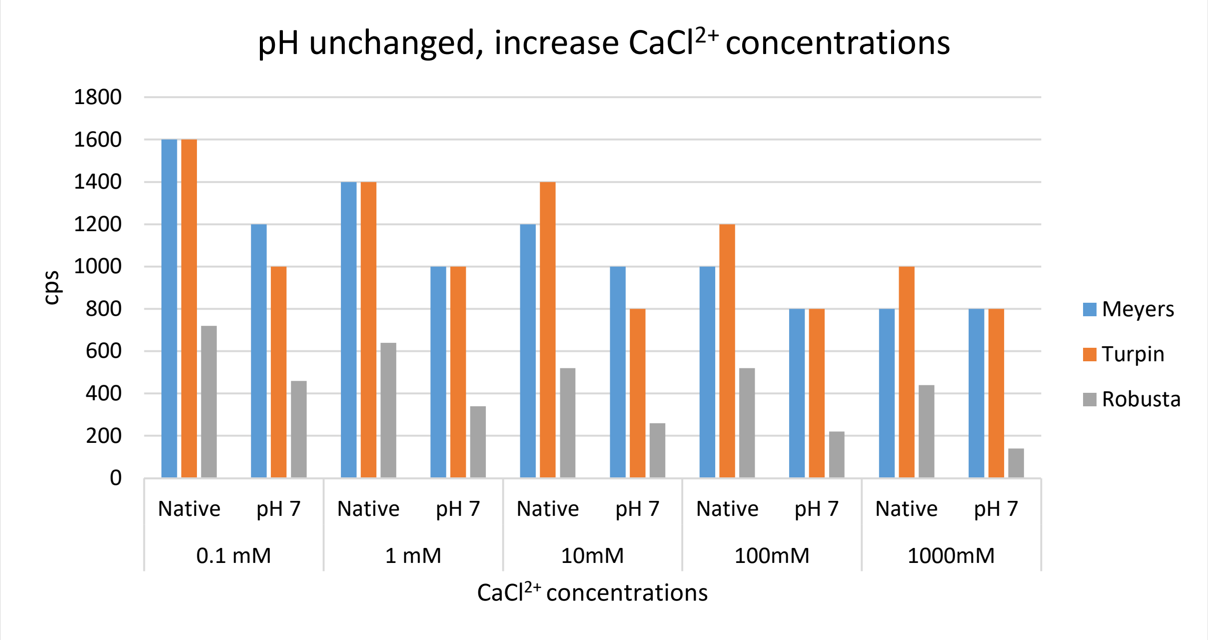


Figure 46: Viscosity of native mucilage extracted from cladodes of eight different cactus pear cultivars harvested in the dormant stage (pH neutral) with increasing additions of $CaCl^{2+}$

The viscosity of NM was more affected by increases in $CaCl^{2+}$ concentrations than when the pH was controlled at a constant pH 7 (Figure 46). The viscosity was lower at neutral pH because the cations supplied by the Ca^{2+} bound to a greater extend to the negatively charged mucilage molecules in a neutral pH environment and the viscosity subsequently decreased. It was thus observed and concluded that the combination of Ca^{2+} and pH influenced the viscosity of mucilage.

5.4.6 Water-related properties

Investigation of the functional properties of the dehydrated powder of the eight cultivars were undertaken as a preliminary evaluation of the powder obtained after freeze-drying of the mucilage, as it was paramount to understand and predict the hydrophilic tendencies of mucilage powder (Table 18). It was imperative at this stage of the study to establish the functionality of the freeze-dried powder in order to evaluate the quality of the obtained freeze-dried powder to warrant further investigations. These analyses were only done on the freeze-dried powder from the dormant stage as it was a preliminary evaluation. This issue will be discussed in more detail in Chapter 6. According to Ayadi et al. (2009), the water solubility index (WSI) is related to the presence of soluble molecules, therefore it gives an indication of the solubility of a substance in water. WSI (Table 18) ranged from 56.35% ('Meyers') to 71.8% ('Robusta'). 'Robusta' had significantly higher WSI than 'Algerian', 'Meyers', 'Turpin' and 'Gymno-Carpo' (p = 0.006). The average water solubility was 60.96% (Table 18).

Table 18: The effect of cultivar on the functional properties of freeze-dried mucilage powders extracted from the cladodes of 8 different cactus pear cultivars harvested in the dormant stage

Cultivar	**Water solubility index (%)**	**Water absorption capacity (ml/g)**	**Water holding capacity**	**Swelling power (ml/g)**
Algerian	57.20^{a}	4.17^{b}	12.91^{ab}	1.80^{a}
Meyers	56.35^{a}	4.00^{ab}	21.25^{b}	1.99^{a}
Morado	60.43^{ab}	3.73^{ab}	12.83^{ab}	3.97^{ab}
Ficus-Indica	63.40^{ab}	3.50^{ab}	10.99^{a}	2.35^{ab}
Gymno-carpo	56.75^{a}	4.53^{b}	16.97^{ab}	2.22^{ab}
Tormentosa	62.87^{ab}	4.17^{b}	12.28^{ab}	4.66^{b}
Turpin	58.89^{a}	2.89^{ab}	16.45^{ab}	2.51^{ab}
Robusta	71.80^{b}	2.22^{a}	15.82^{ab}	2.80^{ab}
Average	**60.96**	**3.65**	**14.94**	**2.79**
Significance level	p = 0.006	p = 0.005	p = 0.045	p = 0.010

Means with different superscripts in the same column differ significantly

Water absorption capacity (WAC) is the ability of the powder particles to retain water in its structure. The ability to retain water is strongly related to particle size, as water molecules bind more tightly to finer particles (Fenema, 1996). The WAC of the freeze-dried powder was between 2.22 ml/g and 4.53 ml/g (Table 18). 'Robusta' powder's absorption ability was significantly lower than (p = 0.005) cultivars 'Algerian', 'Gymno-Carpo' and 'Tormentosa'. The absorption ability of the cultivars 'Algerian', 'Gymno-Carpo', 'Tormentosa' did not differ significantly. 'Robusta' had the lowest water absorption capacity but was not significantly different from the other cultivars. The average WAC was 3.65 ml/g.

Water holding capacity (WHC) is the ability of a matrix of molecules to entrap large amounts of water to such an extent that the loss of the water is prevented (Fenema, 1996). The ability of a powder particle to hold water is important in terms of texture of products, as it improves the texture of baked products or desserts (Samia El-Safy, 2013). WHC (Table 18) ranged between 10.99 ml/g ('Ficus-Indica') and 21.25 ml/g ('Meyers'). 'Ficus-Indica' was significantly lower than 'Meyers' (p = 0.045) but did not significantly lower than the other cultivars. The average WAC was 14.94 ml/g.

Samia El-Safy (2013) explained swelling power as the hydration capacity of a dried powder. The eating quality of cladode flour in baked foods could be correlated to the retention of water of the swollen dried cladode granules. The swelling capacity (Table 18) of freeze-dried powder was low and ranged from 1.8 ml/g ('Algerian') to 4.66 ml/g ('Tormentosa'). 'Algerian' and 'Meyers' values were significantly lower than 'Tormentosa' (p = 0.010) but not significantly lower than the other cultivars. The average swelling capacity was 2.79 ml/g (Table 18).

These results correlated well with literature as reported on mucilage (Gebresamuel and Gebre-Mariam, 2012), although the reported investigations were mostly done on cladode flour (Ayadi et al., 2009; Samia El-Safy, 2013). As most studies were conducted on cladode flour and not on mucilage powder, the higher WHC and lower swelling power is appreciable. As these preliminary results were promising, a full investigation was launched into the functional properties of freeze-dried powder and reported in chapter 6.

The functional properties of the FM (dehydrated powder) of the eight cultivars were undertaken, as it was paramount to understand and predict the hydrophilic tendencies of mucilage powder. As expected, as mucilage consists of soluble fibre, the association with water showed potential. The average water solubility was 60.96% and the average water absorption was 3.65 ml/g. Water holding capacity showed good results at 14.94 ml/g and swelling capacity was low at 2.79 ml/g. It was evident that the quality of the mucilage powder showed potential. As a result of these promising results, a full investigation was conducted into the functional properties of freeze-dried powders (chapter 6).

5.5 Conclusion

There were positive attributes in both stages, nevertheless as cladode size and weight, pH and moisture content did not offer substantial advantages for either of the stages, mucilage yield and viscosity were the factors that offered substantial differences between stages and therefore had to be considered. The yield obtained from post-harvest stage cladodes was significantly higher than dormant stage, while after drying, the yields were equal. Though, the ability of dormant stage FM to influence viscosity was superior. Therefore, the dormant stage was identified in this study as the best time to harvest cladodes and extract mucilage for freeze-drying purposes, although the investigation should be conducted over several years to come to a final conclusion.

Analysis of the eight cultivars investigated in the study showed that mucilage extracted from different cultivars intrinsically had diverse viscosities, therefore selecting the appropriate cultivar for a specific purpose and product would be paramount. Certain cultivars consistently had low viscosity mucilage (such as Ficus-Indica), while other cultivars consistently had high viscosity mucilage (such as Robusta). Mucilage (NM and FM) from all cultivars showed non-Newtonian, pseudoplastic tendencies. Higher viscosity mucilage was determined to be time dependent, rheopectic and had yield stress tendencies. It was found that fluctuations in temperature, pH, concentration and electrolytes influenced the mucilage viscosity. These tendencies would have to be considered when mucilage is applied in food products, as it could have an adverse effect on the textural properties.

As further studies on the freeze-dried powders were continued due to the promising results of its functional properties, only four cultivars could be identified in this study for the further analysis (due to constrained resources) and the selection was made as follows:

'Algerian' was chosen as the low end viscosity as it additionally had the advantage of consistently having high yields. 'Algerian' is commercially popular in South Africa for its pink fruit.

‘Morado’ was included as it demonstrated high viscosity and yield and as it is a highly commercial cactus pear plant in South Africa, because of the quality green fruit.

‘Gymno-Carpo’ had medium-high viscosity and good yield and generally good results. It is also a tasty orange fruit that is commercially grown in South Africa.

‘Robusta’ is not a commercial crop in South Africa but has substantial potential as a fodder. In fact, as a result of its resistance to cochineal (pest), it was recommended for fodder during times of drought, resulting in a great deal of ‘Robusta’ plantations on South African farms (De Kock, 1980). Another advantage is that the purple fruit contains promising quantities of the colourant and antioxidant betalain (Du Toit, 2013) that could be a value add for this cultivar. As it is a different species and has the highest viscosity mucilage it is paramount to be studied further.

5.6 Recommendation

It is recommended that cladodes harvested during the dormant stage and mucilage from Algerian, Morado, Gymno-Carpo and Robusta cultivars be investigated further. The functional properties of the freeze-dried powders have to be investigated (chapter 6) and the chemical properties of the selected cultivars will be explored in full (chapter 7).

In future research the combinations of factors that influence mucilage viscosity should be investigated in full.

Chapter 6
Functional characterization of native (liquid) and freeze-dried powdered mucilage from four selected cultivars

Abstract

Mucilage from four cultivars (Algerian, Morado, Gymno-Carpo and Robusta) were extracted and evaluated over six months (February, April, May, June, July, August). Native, freeze-dried and reconstituted mucilage were analysed for functional characteristics. The aim was to identify a cultivar and a preferred harvesting time for optimal mucilage functionality. Algerian cultivar was recommended for optimal yield while for month, no specific month could be identified, yet the summer months after the fruit harvest were recommended. Conductivity and pH were influenced by daytime temperatures. Freeze-dried mucilage was the brightest and had the most yellow tones, while the reconstituted mucilage appeared to be greener. Green colour development of O. ficus-indica (Algerian, Morado and Gymno-Carpo) and O. robusta Robusta was discrepant. The bulk and tapped density values of freeze-dried powders were comparable with cladode flour and the flow characteristics were rated as fair. Water solubility index (WSI) (78.8%) of mucilage powder was higher, the swelling power (2.19 g/g) and water absorption capacity (WAC) (0.89 ml/g) were lower while water holding capacity (WHC) (8.04 ml/g) compared well with cladode flour. There could be very little degradative activities in freeze-dried powders as the a_w for freeze-dried mucilage (FM) was below 0.5 (0.34). The freeze-dried powders demonstrated good oil absorption and oil holding capabilities along with emulsification ability (especially Robusta) while it did not have foaming ability. Overall though, none of the cultivars or months emerged as producing exceedingly better functional properties or superior quality of native or freeze-dried mucilage.

6 Functional characterization of native (liquid) and freeze-dried powdered mucilage from four selected cultivars

6.1 Introduction

The cactus pear plant has specialised adaptations for retaining water that allows it to survive in severely dry areas. The mucilage from the cactus pear plant functions as a very complex water preservation system that facilitates the effective use of water. These properties may be present and active in the extracted mucilage and could be utilized in food products. It was suggested by Goycoolea and Cárdenas (2003) and Gebresamuel and Gebre-Mariam (2012) that mucilage could be used as a thickening agent in food products and that it could modify viscosity and stabilize emulsions (Medina-Torres et al., 2000).

It is customary to evaluate a new food ingredient for its functional performance in food systems (Traynham et al., 2007). Cladode flour is an exceptional source of dietary fibre and antioxidants and therefore should be evaluated for its nutritional content, physico-chemical properties and technological parameters (Ayadi et al., 2009). The dried mucilage powders should be considered to be a health promoting natural food substance that should be used to supplement food in dry or liquid forms (Sáenz et al., 2010).

During 2014, mucilage from eight cultivars that consisted of seven *Opuntia ficus-indica* cultivars and one *Opuntia robusta* cultivar were investigated over two growth stages (post-harvest stage and dormant stage). Cladodes from cultivars Algerian, Morado, Meyers, Ficus-Indica, Gymno-Carpo, Tormentosa, Turpin and Robusta (*O. robusta*) were included in the study. Subsequently, the cultivars were narrowed down to four cultivars (Algerian, Morado, Gymno-Carpo and Robusta) while the best growth stage for mucilage extraction was identified as the dormant stage.

In order to further investigate and identify the best cultivar and time of harvest, native mucilage powder from four cultivars (Algerian, Morado, Gymno-Carpo and Robusta) were extracted and evaluated over a period of months that include the post-harvest stage (February, March and April) as well as the months (May, June, July, August) of dormancy. To determine the influence of seasonal changes on mucilage yield, mucilage from the four selected cultivars over a six month period will be analysed in order to identify the factors that influence mucilage yield and to pinpoint a preferred harvesting time for optimal mucilage yield in order to make it economically viable.

The native mucilage was freeze-dried and the effect of the seasonal changes that took place over the specific period of time in terms of the functional characteristics, were identified and analysed in the four selected cultivars. The aim was to identify a cultivar and a preferred harvesting time for optimal mucilage functionality.

6.2 Material and methods

6.2.1 Sample collection

The cladodes of four cultivars namely Algerian, Morado, Gymno-Carpo and Robusta were harvested for the purpose of mucilage extraction and characterization. The orchard is laid out in a RCBD with two replications for each cultivar. Each replication consists of five data plants. From the five data plants per replication, one cladode from each of the five plants was sampled. Thus ten cladodes of every cultivar (five from each replication) amounted to a total of 40 samples per month.

The harvesting of sample cladodes was done between 9:00 and 11:15 on 25 February, 15 April, 20 May, 10 June, 15 July and 12 August. In order to standardize the collection of cladodes, cladodes were collected from the north side of the plant, the cladode had to be north/south orientated, in the middle of the plant, hip height and of a good quality. Only the youngest cladodes of the previous growth season were sampled. These cladodes have not produced any fruit in previous seasons. The cladodes were marked, packaged and transported to the laboratory where they were refrigerated immediately.

Native mucilage was obtained from every cladode sample (ten samples/cultivar/month). These cladodes were not peeled prior to mucilage extraction in order to simplify the extraction process, as explained in chapter 3. Extraction of mucilage proceeded according to the patented method discussed in chapter 3 (Du Toit and De Wit, 2011). Evaluations of native mucilage were completed before it was frozen in aliquots. It was freeze-dried for 72 hours in a Perano freeze-drier at -60˚C. It was subsequently immediately vacuum packed and stored in a freezer at -18˚C. The dried powders from all ten samples were pooled in order to accumulate at least 20 g dried powder per cultivar (4) per month (6). Consequently, 24 freeze-dried mucilage samples of 20 g were collected.

6.2.2 Dried mucilage powder preparation

Hygroscopic tendencies of various flour and powders have been a common problem for the food industry for a long time. Consequently the substance absorbs moisture from the environment that causes problems such as lumps and caking, that spoil the product for use. Dried calcium citrate is an edible salt of an organic acid that can be added in suitable quantities to hygroscopic substances in powder form, in order to stabilize it and thus prevent moisture absorption (Aeckerle, 1941).

In order to stabilize the dried mucilage powder by preventing it from forming lumps and absorbing moisture, calcium citrate was added. One gram calcium citrate was added to 20 g of powder (5%) before it was repackaged in tightly sealed containers. It was kept in the freezer at -18˚C.

6.2.3 Climate data

Waterkloof experimental/sample collection site has an automatic weather service station (De Brug Weather Station) that collects data such as daily maximum and minimum temperatures and daily rainfall. The data was obtained courtesy of Dr. Herman Fouche (Agricultural Research Council – Animal production Institute, Old Olifantsfontein Road, Irene, South Africa 0062).

6.2.4 Native mucilage: Morphological evaluation

6.2.4.1 Native mucilage yield

Extraction of mucilage followed the patented procedure (Du Toit and De Wit, 2011) described in chapter 3 (PA 153178/P). The cladodes were cleaned but not peeled before extraction. The mucilage was weighed and the percentage yield calculated according to the original cladode weight.

$$yield\ of\ mucilage\ (\%) = \frac{supernatant\ liquid\ (g)}{cladode\ weight\ (g)} \times 100$$

6.2.4.2 Cladode weight

The weight of each sample cladode (ten per cultivar) was recorded.

6.2.4.3 Moisture and solids content

A segment of cladode was cut into small pieces and mixed into a representable sample portion of the cladode. A metal petri dish was weighed with and without sample by using a Mettler AE 200 scale and the weight was recorded to three decimal points. The samples were placed inside an Eco Therm oven at 102˚C for 24 hours. They were placed in a desiccator containing silica crystals for 1 hour after which the petri dishes containing the dried samples were weighed again (McClements, 2003).

Moisture content (%) and solid content (%) were determined for every sample (ten samples per cultivar). It was calculated as follows:

$$Moisture\ content\ (\%) = \frac{original\ sample\ (g) - dry\ sample(g)}{original\ sample(g)}\ x\ 100$$

$$Solids\ content\ (\%) =\ 100\% - moisture\ content\ (\%)$$

The solids in a plant material consist of all its components excluding the water. Therefore deducting the moisture content from the original sample constitutes the solids content. The solids content include the fat, carbohydrates, proteins, minerals and all other constituents of the plant material (Brown, 2007).

6.2.4.4 Viscosity

The line-spread test is a method of comparing the relative viscosity of a viscous liquid such as gels, hydrocolloids and other thickened liquids. It is a quick, reliable and inexpensive way of determining and comparing viscosity (Kim et al., 2014).

The line-spread determination is described in Section 4.2.7.1.

6.2.4.5 pH

A calibrated Eutech pH 2700 pH/mV/˚C/˚F instrument was used to determine pH at 22˚C. The tests were executed on each native mucilage sample (ten samples per cultivar) as soon as the extraction procedure was completed. For the reconstituted samples, one gram of freeze-dried sample was dissolved in 10 ml water by first forming a paste with 2 ml of water. The remaining water was added slowly while stirring continuously after which the samples were allowed to dissolve fully for 24 hours before continuing with the pH tests.

6.2.4.6 Conductivity

A calibrated Eutech pH 2700 pH/mV/˚C/˚F instrument was used to determine the conductivity (mV) at 19-22˚C. Conductivity is measured in millivolt (mV) which is equal to millimho (mmho) and millisiemens (mS). Conductivity was measured concurrently with pH determinations on the samples as described in section 6.2.4.5.

6.2.5 Colour

Colour of the native mucilage was determined by taking six readings on each of the ten native mucilage samples per cultivar, using a Konica Minolta Chroma CR-400 meter. This instrument collects colour data in terms of three coordinates (L*, a*, b*). The three coordinates of CIELAB represent the lightness of the colour (*L** = 0 indicates black while L* = 100 indicates white), red and green (*a**, negative values indicate green while positive values indicate red) and yellow and blue (*b**, negative values indicate blue while positive values indicate yellow).

The chroma (C*) and hue (h˚) was calculated from the a* and b* coordinates using an online colour parameter converter (ColorMine.org, 2014). Chroma represent the relative saturation of a colour and starts at 0 where 0 indicate unsaturated colour (dullness) and 100 indicate colour purity (brightness). In fact, hue indicate the position of a colour on the colour wheel in degrees; h˚ = 0˚ indicates red, h˚ = 90˚ indicates yellow, h˚ = 180˚ indicates green, h˚ = 270˚ indicates blue and back to 0˚.

The five different values (L*, a*, b*, C* and h˚) indicated by the CIELAB and CIELCH colour scales describes the colour accurately and can be plotted on a colour wheel as indicated in Figure 47.

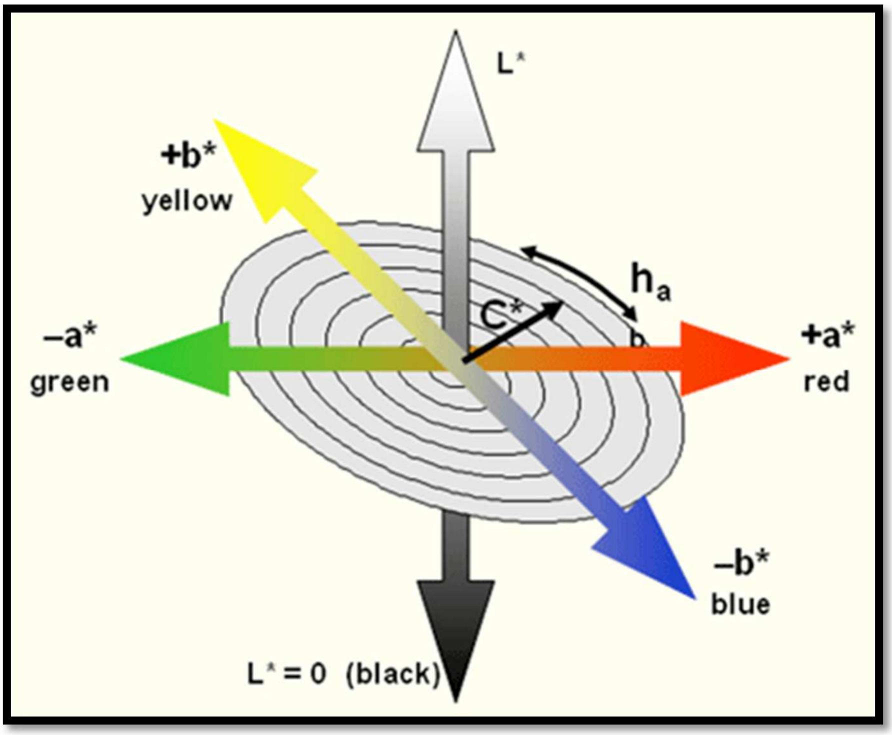


Figure 47: The CIELAB and CIELCH colour values indicated on the colour wheel (Engineering 360, 2017)

For colour determinations on dried powders, the powders were used as such and for the reconstituted mucilage samples, 1 g of freeze-dried mucilage powder was dissolved in 20 ml distilled water (5% concentration). The freeze-dried powder contained 5% calcium citrate (white powder) that could have an influence on the colour measurements, yet it is the form in which the powder would be marketed and sold.

6.2.6 Freeze-dried mucilage powder

6.2.6.1 Bulk density and tapped density

The method described by Oladele and Aina (2007) and Ayadi et al. (2009) was applied and adapted for practical reasons. Bulk density was measured by placing 3 g of mucilage powder into a 10 ml tarred measuring cylinder and the volume that the powder filled was recorded in millilitres. Subsequently the cylinder containing the powder was tapped 20 times on a soft cloth in order to pack the powder as densely as possible. The difference before tapping (bulk density) and after tapping (tapped density) was taken and calculated as follows:

$$Bulk\ and\ tapped\ density\ (g/ml) = \frac{weight\ of\ powder\ (g)}{volume\ of\ powder\ (ml)}$$

The compressibility index and Hausner ratio reflects the ability of a powder to settle (Particle Analytical, 2007). The compressibility index and Hausner ratio was calculated using the following equations:

$$Compressibility\ index\ \% = \frac{100\ (V0 - Vf)}{V0}$$

$$Hausner\ ratio = \frac{V0}{Vf}$$

Where
V0 = Bulk volume (ml)
Vf = Tapped volume (ml)

6.2.6.2 Water-related properties of dried mucilage powder

6.2.6.2.1 Water Solubility Index (WSI)

WSI was determined according to methods followed by Ayadi et al. (2009) and Gebresamuel and Gebre-Mariam (2013) with slight modifications. One gram of freeze-dried mucilage was dissolved in 20 ml distilled water by firstly making a paste using only 2 ml of water and mixing using a Genie vortex for 10 s. This procedure was followed in order to form a paste that could dissolve more easily without the formation of lumps in the larger amount of water. The rest of the distilled water (18 ml) was then added and vortexed again for 10 s. The mucilage was homogenized for 30 s using a Kenwood stick blender and allowed to stand for an hour in order to dissolve fully. It was centrifuged using a 12 Hettich centrifuge for 10 min at 8000 rpm. The supernatant that was decanted was measured in terms of volume and weight and subsequently dried. The metal petri dishes were weighed before and after the supernatant was dried in order to obtain the amount of freeze-dried powder that was dissolved in the supernatant (Ayadi et al., 2009; Gebresamuel and Gebre-Mariam, 2012). It was calculated using the following equation:

$$WSI\ \% = \frac{dried\ solids\ in\ supernatant\ (g)}{original\ sample\ (g)}$$

6.2.6.2.2 Swelling power

Swelling capacity of a powder is the actual weight of the water remaining in the actual amount of precipitate that remained after dissolution. Swelling power of mucilage powder was determined by slightly adapting the methods used by Ayadi et al. (2009); Gebresamuel and Gebre-Mariam (2012); Sáenz et al. (2012); Samia El-Safy (2013) and Sepúlveda et al. (2013). One gram of powder was inserted into a pre-weighed centrifuge tube and dissolved in 20 ml distilled water. It was centrifuged (12 Hettich centrifuge) at 8000 rpm for 10 min, and after it was decanted, the weight of the tube containing the paste was determined. The supernatant was dried in order to obtain the amount of powder that actively dissolved in the water. Swelling power was calculated by dividing the weight of the wet precipitate with the actual weight of solids (after subtracting the amount of powder that was dissolved in the supernatant from the initial sample size). Swelling power is measured in g/g (gram of water/gram of solids). The following equation was used:

$$Swelling\ power\ (g/g) = \frac{precipitate\ (g)}{sample\ (g) - dried\ supernatant\ (g)}$$

6.2.6.2.3 Water Holding Capacity (WHC)

Ayadi et al. (2009), López-Cervantes et al. (2011) and Traynham et al. (2007) described the method that was used with minor modifications. One gram of freeze-dried mucilage was dissolved in 20 ml distilled water by firstly making a paste using only 2 ml of water and blending it using a Genie vortex for 10 seconds. This procedure was followed in order to form a paste that could dissolve more easily without the formation of lumps in the larger amount of water. The rest of the distilled water (18 ml) was then added and vortexed again for 10 s. The mucilage was homogenized for 30 seconds using a Kenwood stick blender and allowed to stand for an hour in order to dissolve fully. It was centrifuged (12 Hettich centrifuge) for 10 min at 8000 rpm. The supernatant was decanted and the centrifuge tube inverted for 30 min. The amount of water held by the powder was calculated using the following equation:

$$WHC\ (ml/g) = \frac{wet\ precipitate\ (g) - dried\ precipitate\ (g)}{dried\ precipitate\ (g)}$$

6.2.6.2.4 *Water Absorption Capacity (WAC)*

Water absorption capacity is described as the amount of water (ml) that was missing from the initial water and thus absorbed by the powder and that remained in the powder after being centrifuged. WAC was calculated using the following equation and expressed as ml/g (Oladele and Aina, 2007; Samia El-Safy, 2013).

$$WAC\ \ ml/g = \frac{water\ (ml) - supernatant\ (ml)}{sample\ (g)}$$

6.2.6.2.5 *Water Activity (a_w)*

The water activity of the freeze-dried samples was determined using a Novasina thermoconstanter. The container was filled and levelled to the halfway mark with mucilage powder. The thermoconstanter was set at 22˚C and the a_w determined as ERH/100 (equilibrium relative humidity).

6.2.6.3 Oil related properties

6.2.6.3.1 *Absorption Capacity (OAC)*

Oil absorption capacity (OAC) was determined by using the method described by Samia El-Safy (2013) with slight modifications. Freeze-dried mucilage powder (0.1 g) was added to 2 ml canola oil and shaken for 5 min using a Vortex Genie. It was centrifuged (12 Hettich centrifuge) for 30 min at 5000 rpm. Similar to the method used for WAC, the amount of supernatant oil was deducted from the initial amount of oil added. This was divided by the sample (g) added and expressed as ml/g:

$$OAC\ \ ml/g = \frac{oil\ (ml) - supernatant\ (ml)}{sample\ (g)}$$

6.2.6.3.2 *Oil Holding Capacity (OHC)*

The method of Ayadi et al. (2009) was slightly modified to determine the oil holding capacity (OHC). Freeze-dried mucilage powder (0.1 g) was added to 2 ml canola oil and shaken for 5 min using a Vortex Genie. It was centrifuged (12 Hettich centrifuge) for 30 min at 5000 rpm. The supernatant oil was separated carefully using a pipette and the centrifuge tube inverted for 12 hours. The precipitate was weighed and the amount of oil held by the powder was calculated using the following equation:

$$OHC\ (ml/g) = \frac{precipitate\ (g) - original\ sample\ (g)}{original\ sample\ (g)}$$

6.2.6.4 Functional properties

6.2.6.4.1 Foaming ability

The methods of Kaur and Singh (2005) and Oladele and Aina (2007) were followed with slight differences. One gram of freeze-dried mucilage was dissolved in 20 ml distilled water by first forming a paste with 2 ml of water using a Genie vortex mixer for 10 s. The remaining water was then added slowly while continuously mixing using the vortex mixer for 10 s. The 20 ml of reconstituted mucilage was transferred into a 250 ml glass beaker and homogenized using a Kenwood stick blender for 30 s. The glass beaker was held at a 40˚ angle and moved around in order to produce the maximum amount of foam. The content of the beaker was immediately poured into a graduated cylinder and the volume of foam was recorded immediately (10 s), then after 2 minutes and repeated at 10 minute intervals at 12 and thereafter at 22, 32 and 42 minutes. The last reading was taken an hour later at 102 minutes. The data was recorded as volume of foam and expressed as percentage increase in volume. Foam capacity is defined as the amount of foam formed immediately after agitation while foam stability is determined over predetermined time intervals.

6.2.6.4.2 Emulsifying capacity

Kaur and Singh (2005) and Iturriaga et al. (2009a) described the method for this procedure. It was adapted by dissolving 2 g powder in 20 ml water by first forming a paste using 2 g as previously described, left overnight and homogenized using a Kenwood stick blender for 15s. The 20 ml sunflower oil was added in two stages by first adding 10 ml during the first 30 s of homogenizing and adding the remaining 10 ml during the second 30 s of homogenizing. It was immediately transferred to a tarred cylinder and the volume of emulsion at the top or creamed layer (level of the separation) read immediately (20 s) and repeated after 1 min, 2, 5, 10, 20, 60 min and 24 hours later to calculate % creaming for an indication of the stability of the emulsion (Ercelebi and Ibanoglu, 2009).

$$Creaming\ \% = \frac{volume\ of\ the\ serum\ layer\ (ml)}{total\ volume\ of\ the\ emulsion\ (ml)}\ x\ 100$$

6.3 Statistical Methods

A one way analysis of variance (ANOVA) procedure (NCSS, 2007) was used to determine the effect of month and cultivar on the morphological, yield and functional properties of mucilage. The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to determine whether significant differences exist between treatment means (NCSS, 2007). Correlations analysis were determined using the Analysis ToolPak in Excel.

6.4 Results

In order for mucilage to be economically viable, the yield of mucilage extracted from cladodes must be optimal. Therefore the factors that influence the yield of native mucilage need to be identified and correlated. Correlations between yield, the other morphological information and environmental (climate) data will indicate which factors were involved in influencing mucilage yield.

6.4.1 Climate data

Waterkloof farm that hosts the experimental cactus pear orchard, is situated west of Bloemfontein in the Free State Province. It is a semi-arid climatic region, where the mid-day temperature is hot (26 to 29˚C) in summer (January to April) months and rapidly cools down in May to temperatures that at night, are close to 0˚C in the winter (June, July) months (World Weather and Climate Information, 2016). Daytime temperatures indicate that December, January and February are the warmest months of which January is the warmest while June has the lowest daytime temperatures (World Weather and Climate Information, 2016).

In Figure 48 the data is arranged and presented from July to June the next year, following the growth of the cactus plants from winter to winter (The data was obtained courtesy of Dr. Herman Fouché).

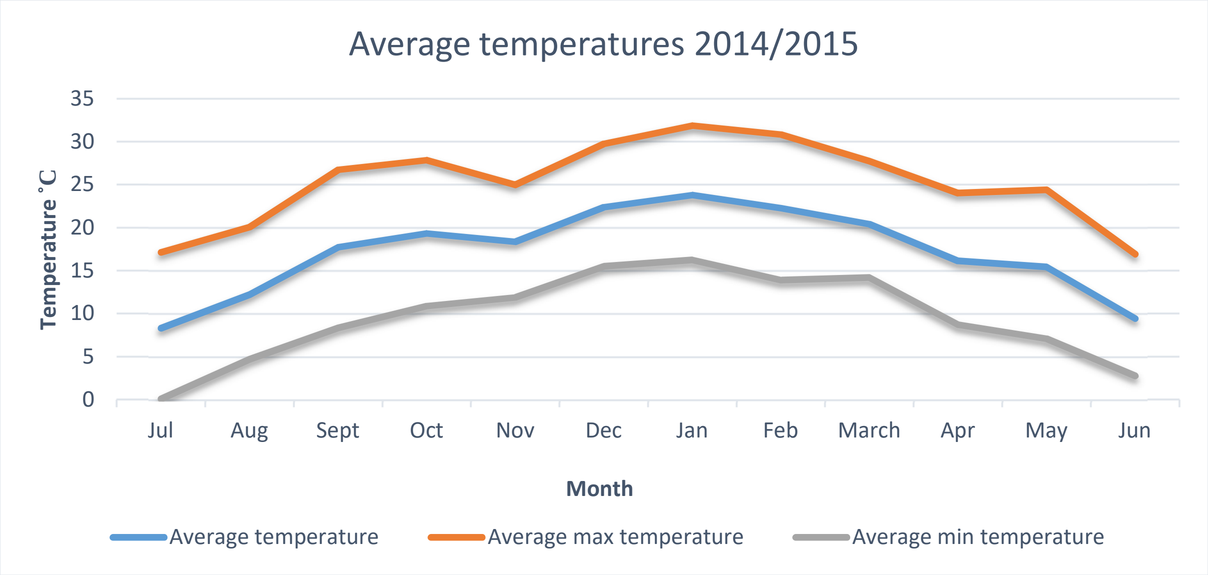


Figure 48: Average temperatures recorded at Waterkloof farm from July 2014 to June 2015

Average temperatures in Figure 48 (average of absolute minimum and absolute maximum) ranged from 23.8˚C in January 2015 in the summer to 8.3˚C in July 2015 in the winter. Average maximum temperatures were above 30˚C in January and February and then lowered to 16.9˚C in June. July had the coldest average minimum temperatures (0.1˚C) and January the warmest minimum temperatures (16.2˚C). The extreme minimum and maximum temperatures are depicted in Figure 49.

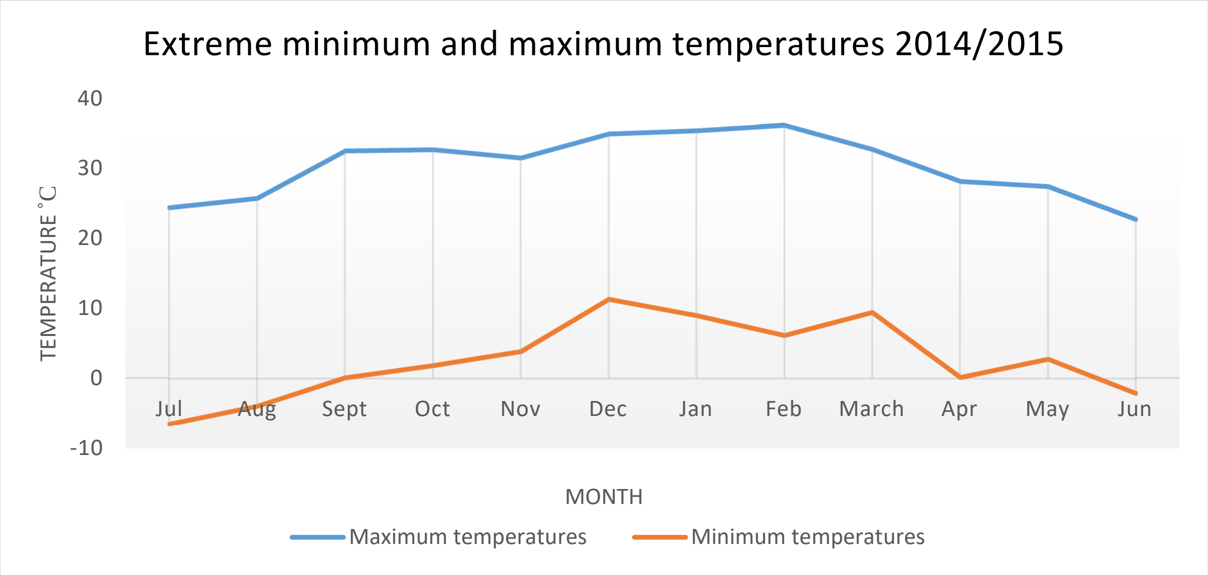


Figure 49: Extreme temperatures recorded at Waterkloof farm from July 2014 to June 2015

The extreme minimum temperatures of below 0˚C were observed only in June (-2.13˚C), July (-6.5˚C) and August (-3.94˚C) and reached 11.3˚C in December (Figure 49). Extreme maximum temperatures above 30˚C were recorded from September (32.5˚C) to March (32.7˚C).

As Bloemfontein lies within a summer rainfall area, it is common for very little rain to fall during the winter months. In Figure 50, the cumulative rainfall (orange line) is shown from July 2014 to June 2015.

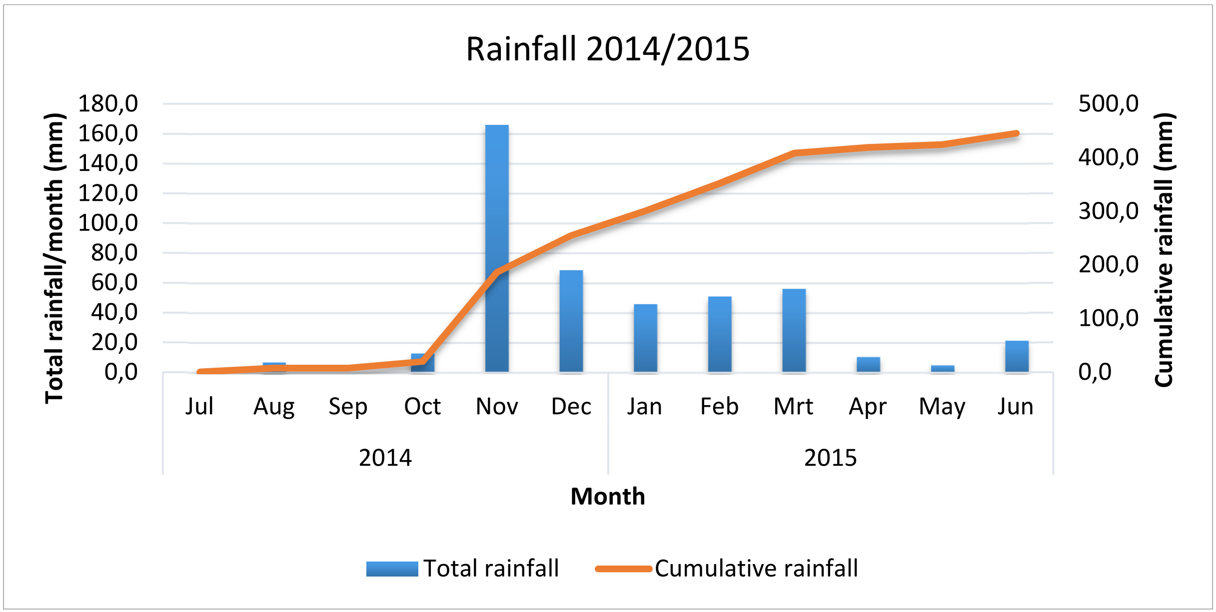


Figure 50: Total rainfall/month and cumulative rainfall/year recorded at Waterkloof farm from July 2014 to June 2015

From July to October only 20.3 mm rainfall was recorded while in November 2014, 165.9 mm rain fell. The average rainfall during December, January, February and March was ± 50 mm, yet for April and May even lower rainfall was recorded (Figure 50). For the year from July 2014 to June 2015 the cumulative rainfall was 445.7 mm (Figure 50). It is common in Bloemfontein for the driest months to be May, June, July, August, September and October of which July is the driest month. March is known as the wettest month (World Weather and Climate Information, 2016).

6.4.2 Native mucilage: Morphological evaluation

The previous chapters focused on the physical differences of mucilage between individual cultivars and growth stages. In this chapter the emphasis will shift to observing the functional and chemical differences between the harvesting months and the four chosen cultivars.

Morphological data on cladode weight and mucilage yield has been reported in chapters 3 and 4, nevertheless it was repeated on the four specific cultivars over the six months (10 samples per month per cultivar) used in this section of the study, in order to obtain reliable statistically analysed morphological data that is relevant to the functional and chemical determination sections of this work.

6.4.2.1 Native mucilage yield

Comparing the percentage mucilage yield extracted from cultivars over a period of six months is of utmost importance, as ideally the maximum amount of native mucilage should be extracted from the least possible amount of raw plant material (cladodes) for maximum productivity. The yield of native mucilage according to cultivar and month obtained is presented in Table 19.

Table 19: The effect of month of harvest and cultivar on yield (%) of native mucilage extracted from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p < 0.001)**
Algerian	58.08^{I}	48.53^{HI}	45.87^{GHI}	41.56^{EFGH}	42.21^{FGH}	35.58^{EFGH}	**45.31^{c}**
Morado	39.52^{EFGH}	46.24^{GHI}	30.62^{DEF}	32.32^{DEFG}	32.13^{DEFG}	29.65^{CDEF}	**35.08^{b}**
Gymno-Carpo	42.88^{FGH}	48.31^{HI}	32.09^{DEFG}	34.9^{EFGH}	43.28^{FGH}	32.38^{DEFG}	**38.97^{b}**
Robusta	27.01^{BCDE}	31.87^{DEFG}	18.48^{ABCD}	14.98^{ABC}	12.3^{A}	12.78^{AB}	**19.57^{a}**
Month Means (p < 0.001)	**41.87^{b}**	**43.74^{b}**	**31.77^{a}**	**30.94^{a}**	**32.48^{a}**	**27.60^{a}**	**34.73** **C x M: (p = 0.038)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
Interaction means with different superscripts in capital letters differ significantly ($p < 0.05$)
☐ **= All means**

The interaction between month and cultivar was significant (p = 0.038) in Table 19. The cultivar with the highest average yield of native mucilage was 'Algerian' (45.31 %). In fact, 'Algerian' had significantly higher (p < 0.001) % yield of native mucilage than the other cultivars. Algerian mucilage yield from cladodes harvested in February not only had significantly higher yield than the other months for Algerian but for all other cultivars. For Morado, the month that the highest yield was recorded was April although the yield was not significantly more than February, but significantly more than the other months. For Gymno-Carpo April mucilage also had the highest yield, although it was not significantly higher than February, June or July. 'Robusta' had significantly lower % yield of native mucilage (19.57%) than the other cultivars. In July, the lowest yield of mucilage was observed, not only for Robusta but for all the cultivars and months. April yielded the most mucilage for Robusta, yet it was not significantly more than February or May. The % mean yields for 'Morado' (35.08%) and 'Gymno-Carpo' (38.97%) were very similar and thus not statistically different from each other (Table 19). Significantly more (p < 0.001) mucilage was extracted from cladodes harvested in

April (43.74%) and February (41.87%) than in August (27.60%), July (32.48%), June (30.94%) and May (31.77%). Yield results from May were not statistically different to June (Table 19). The overall average native mucilage extracted was 241.92 g (34.73%) per cladode.

6.4.2.2 Cladode weight

The weight of cladodes was regarded as fundamental and essential information as it increased the knowledge pool of each investigated cultivar and could play a meaningful role when selecting cultivars for different applications. The average weight of cladodes according to cultivar and month obtained is presented in Table 20.

Table 20: The effect of month and cultivar on the weight (g) of cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p < 0.001)**
Algerian	533.80	709.00	648.10	710.40	877.40	789.10	**711.30^{a}**
Morado	494.30	618.60	609.30	660.30	693.40	745.50	**636.90^{a}**
Gymno-Carpo	473.10	612.90	539.30	564.60	818.80	809.60	**636.38^{a}**
Robusta	896.00	1040.00	655.70	775.10	983.10	1178.00	**921.32^{b}**
Month Means (p < 0.001)	**599.30^{a}**	**745.13^{abc}**	**613.10^{a}**	**677.60^{ab}**	**843.18^{bc}**	**880.55^{c}**	**726.48** **C x M: (p = 0.144)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

It was evident that 'Robusta' cladodes were significantly heavier (average 921.32 g), in fact 'Robusta' cladodes were consistently heavier during the six months of the study (Table 20). 'Gymno-Carpo' (636.38 g) and 'Morado' (636.90 g) had lower (not significantly different) cladode weights than 'Algerian' cladodes (711.3 g). Cladodes of all the cultivars were the heaviest in August with an average of 880.55 g (statistically significant $p < 0.001$).

July average weight (843.18 g) and April average weight (745.13 g) were slightly lower than in August while May (613.10 g) and February (599.30 g) cladodes weighed significantly less ($p < 0.001$) than July and August. April cladodes (745.13 g) were not significantly different from any of the other months, while June cladodes (677.60 g) weighed significantly lower than cladodes harvested in August (880.55 g). The interaction between month and cultivar was not significant (Table 20).

In observing cladode weight patterns (Table 20), it was interesting to observe that the weight of cladodes seemed to have increased considerably from June to August, perhaps as the season starts to change from winter to spring. It could be speculated that the cladodes were heavier in April and July following the substantial rainfall in March and June (Figure 50). The cladodes were lighter in weight in February although ample rain fell during February (51.1 mm) as well as the previous months (68 mm December and 46 mm January) (Figure 50).

In order to make conclusions concerning the weight of specific cultivars the data of at least three years would be necessary, but it was perceived that cladodes belonging to a specific cultivar may inherently grow to be of a specific weight and shape. 'Robusta' cladodes were round and heavy while 'Algerian', 'Morado' and 'Gymno-Carpo' cladodes were not equally substantial. As already described in chapter 4 (section 4.1), Wessels (1989) described three shapes of cactus pear cladodes and plants; the bushy-type plant, the columnar shape cladodes that have elongated cladodes and the round-cladode shaped plants. The cultivars Algerian, Morado and Gymno-Carpo all belong to the bushy-type plant category while Robusta is an example of the round-shaped cladode category.

Figure 51 depicts the average mucilage yield and cladode weight obtained according to cultivars.

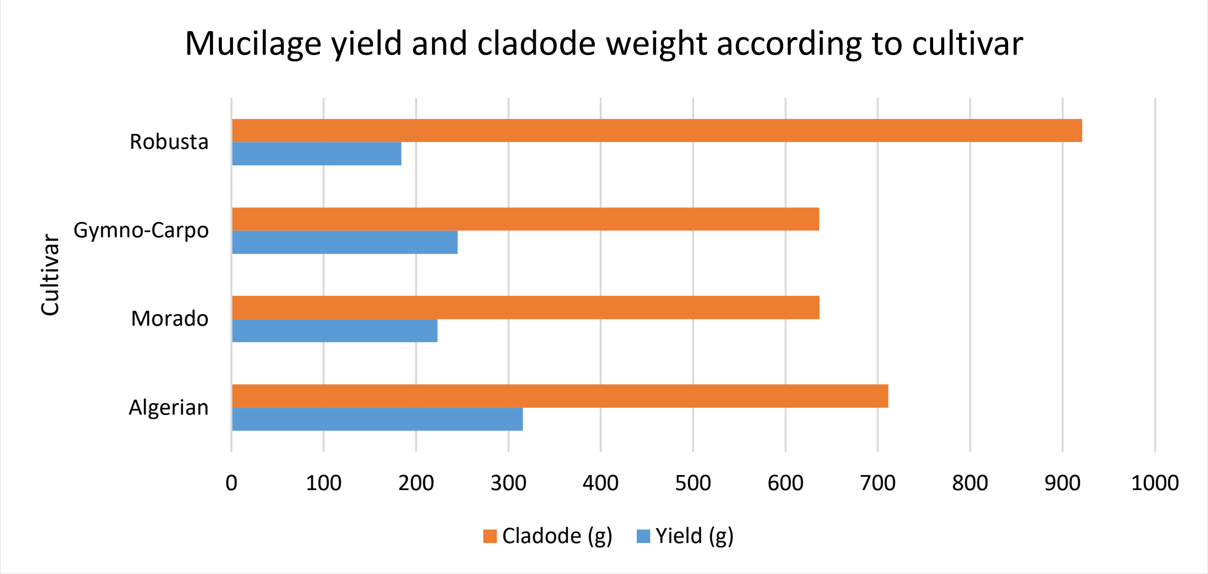


Figure 51: Comparison of cactus pear cladode weight and native mucilage yield extracted from the cladodes according to cultivar

In Figure 51 the contradicting results were clearly seen: 'Robusta' had the heaviest cladodes and delivered the least mucilage while 'Algerian' cladodes were lighter in weight than 'Robusta' cladodes, yet delivered the most mucilage. Accordingly, 'Algerian' seems to be the recommended cultivar for the largest % yield of native mucilage.

In order to identify factors that influence mucilage yield, a correlation between cladode weight and native mucilage yield according to cultivar is presented in Figure 52.

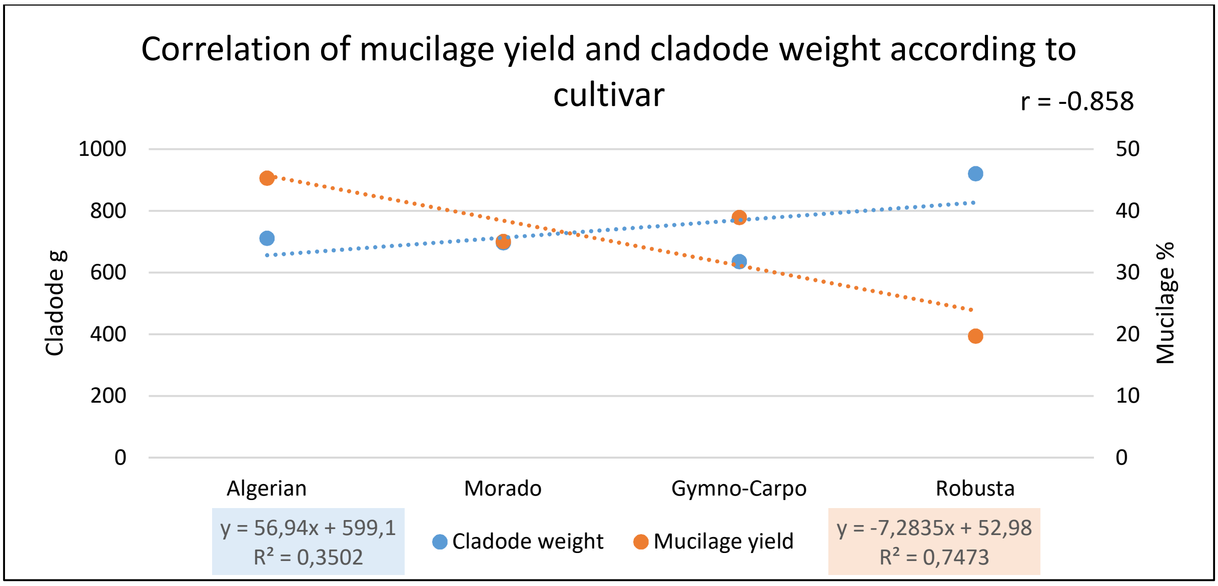


Figure 52: Correlation of weight of cactus pear cladodes and native mucilage yield extracted from the cladodes according to cultivar

The correlation between mucilage yield and cladode weight showed a very strong negative (r= 0.858) relationship, which reiterated the finding in chapter 4 that the heavier cladodes do not yield higher percentage of mucilage, in fact, the opposite was true.

In Figure 53, the average mucilage yield (g) and cladode weight (g) obtained according to month is depicted.

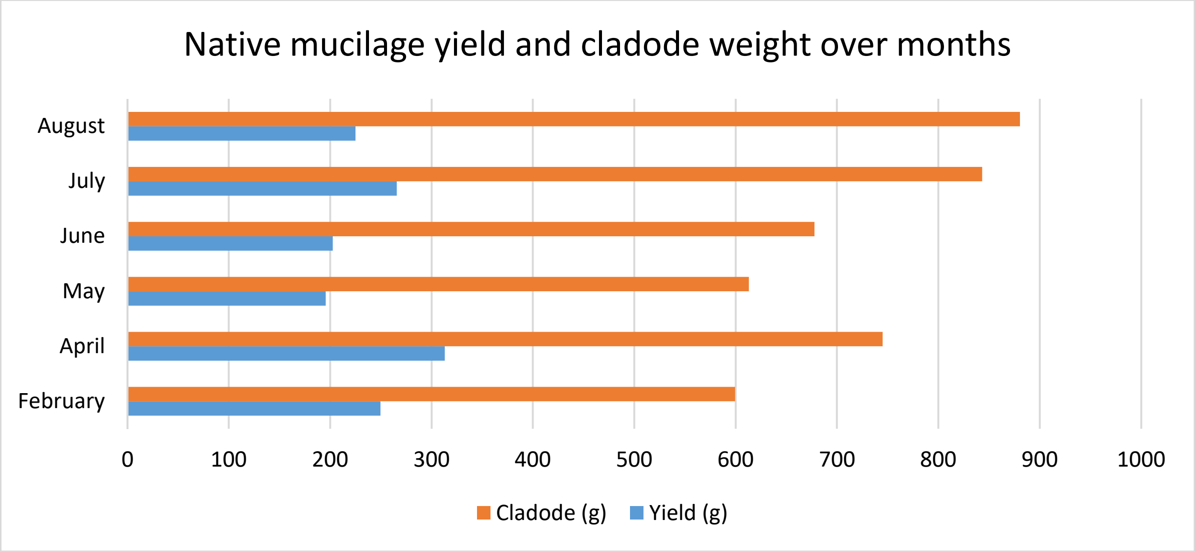


Figure 53: Comparison of cactus pear cladode weight and native mucilage yield extracted from cladodes over months

In Figure 53 it was observed that February (599.3 g) and April (745.125 g) cladodes were lighter in weight while it yielded more native mucilage (February 249.7 g and April 313.2 g) in comparison to August cladodes (880.6 g), which were heavy and large, yet yielded average mucilage (225 g). July cladodes also yielded above average mucilage (265.7 g) yet the cladodes were above average weight (843.2 g). Therefore February to April would be the best months to harvest cladodes for the largest yield of mucilage extraction purposes. A correlation between mucilage yield and cladode weight over months is presented in Figure 54.

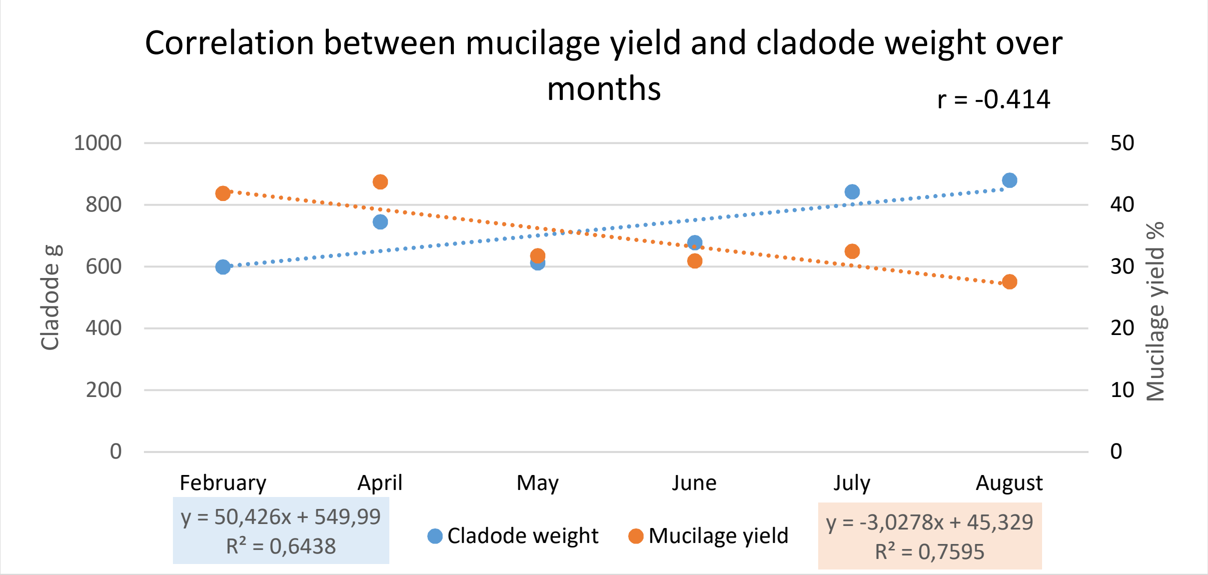


Figure 54: Correlation of weight of cactus pear cladodes and native mucilage yield extracted from the cladodes over months

There was a weak to moderate negative correlation (r = -0.414) between cladode weight and mucilage yield (%) obtained over months (Figure 54). Therefore the size of cladodes as harvested over months did not affect the mucilage that was extracted from the cladodes.

'Algerian' cladodes were smaller than 'Robusta' cladodes, yet yielded the highest percentage mucilage. Bigger and heavier cladodes did not yield the most mucilage. Accordingly, 'Algerian' was the recommended cultivar for the largest yield of native mucilage compared to the weight of the cladodes. There were no specific month that could be identified as the optimal harvesting time, yet the summer months generally had the best results when cladode and mucilage weights were compared.

In order to identify factors that influence mucilage yield, a correlation between mucilage yield and daytime temperatures (Figure 55) were conducted to determine whether there was a connection between the general decline in mucilage yield over months and the decrease of daytime temperatures as summer changed into winter.

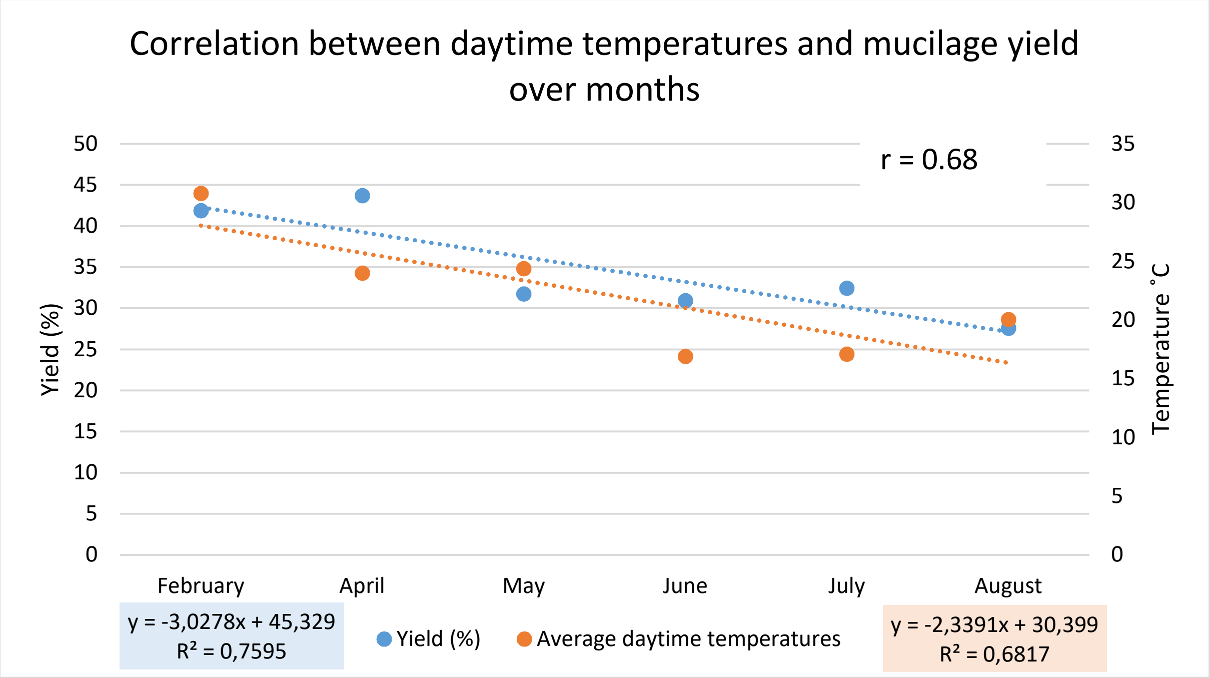


Figure 55: Correlation of cactus pear mucilage yield and daytime temperatures over months

When correlating % mucilage yield to the climate data, a positive correlation was found (r = 0.68) (Figure 55). Thus more mucilage was extracted during February and April. It could be theorised that the extractability of mucilage was higher during the warmer, higher rainfall months (February and April).

It was thus established that cladode weight did not influence mucilage yield, yet there was a positive correlation between the daytime temperatures and mucilage yield. The influence that temperature has on mucilage yield shall be investigated and clarified in this section.

6.4.2.3 Moisture and solids content

Water is the major component in food and influences its taste, texture and appearance. Vegetables consists of between 74 and 95% water, fruit between 80 and 95% and meat products between 53 to 81% (Fenema, 1996). Consequently, there was sufficient basis to determine the moisture content of cladodes. It is important to note that water may be present in different forms, it may be chemically bound, physically bound, trapped, capillary or bulk water. Water molecules can exist in a variety of different environments and physicochemical structures (McClements, 2003). It is the extraordinary ability of cactus pear cladodes to retain water under unfavourable climatic conditions due to the mucilage content that causes the cactus pear to thrive when other plants cannot survive (Feugang et al., 2006). Thus, the ability of mucilage to absorb and hold huge amounts of water (Sáenz, 2000) causes the high water content in cladodes, however the water seems to be bound and mostly unavailable.

The % moisture content of cladodes according to cultivar and month obtained is presented in Table 21.

Table 21: The effect of month and cultivar on the moisture content (%) of cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.114)**
Algerian	92.00	92.61	91.75	91.41	92.13	91.81	**91.95**
Morado	91.11	88.37	89.33	90.01	90.61	91.19	**90.10**
Gymno-Carpo	92.45	92.03	89.97	86.18	84.23	91.19	**89.34**
Robusta	89.03	91.35	88.27	88.81	89.22	88.68	**89.23**
Month Means (p = 0.575)	**91.15**	**91.09**	**89.83**	**89.10**	**89.05**	**90.72**	**90.15** **C x M: (p = 0.782)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ = All means

Comparison of the month means in Table 21 showed the highest moisture content in February (91.15%). It could be argued that this was because of ample rain and hot summer days. The average moisture content progressively declined after February but in total with only 2 percentage points, in fact the moisture content remained high (89%) during the dry and cold winter months (Table 21). There were no significant statistical differences found between months of harvest or cultivars and the interaction between month and cultivar was not significant (p = 0.782). In August, it only rained 1 mm, yet the moisture content of most of the cladodes increased, in fact it was only 'Robusta' that did not increase substantially from July to August. 'Algerian' had the highest moisture content every month as well as the highest average (91%). The lowest moisture content was observed in 'Gymno-Carpo' (89.34%) and 'Robusta' (89.23%). The overall average moisture content measured was 90.15% (Table 21). It seems that the characteristics inherent to the cultivar influenced the moisture content of the cladode to a greater extent than the season, rainfall or temperature.

Ayadi et al. (2009) reported moisture content of 90.67% for spiny cladodes and 91.04% for spineless cladodes. Cladodes had a moisture content of 93.5 g/100 g according to López-Cervantes et al. (2011). Samia El-Safy (2013) found moisture content of 94.097% in fresh cladodes. Calvo-Arriaga et al. (2010) detected varied moisture content of 89.93% in Milpa Alta and 94.35% in COPENA VI (both *O. ficus-indica)* cultivars. Stintzing and Carle (2005) came to the conclusion that moisture content for cladodes ranged between 88% and 95%.

The solids include all material other than moisture. Solid material in a vegetable refer to the carbohydrates, protein, fats, fibre and other compounds that could provide sustenance. Solids content of vegetables is usually in the range of 5 to 30% (Brown, 2007). The % solids according to cultivars and over months are observed in Table 22.

Table 22: The effect of month and cultivar on solids content (%) of cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.114)**
Algerian	8.00	7.39	8.25	8.59	7.87	8.19	**8.05**
Morado	8.89	11.63	10.67	9.99	9.39	8.81	**9.90**
Gymno-Carpo	7.55	7.97	10.03	13.82	15.77	8.81	**10.66**
Robusta	10.97	8.65	11.73	11.19	10.78	11.32	**10.77**
Month Means (p = 0.575)	**8.85**	**8.91**	**10.17**	**10.90**	**10.95**	**9.28**	**9.85** **C x M: (p = 0.782)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ = All means

There were no significant statistical differences detected between months of harvest or cultivar for % solids content and the interaction between month and cultivar was not significant (Table 22). It was observed that the average solids content was the highest in July (10.95%) and the lowest in February (8.85%). In terms of cultivar, 'Algerian' (8.05%) had the lowest and 'Robusta' (10.77%) the highest solids content. The overall average solids content for cladodes was 9.85% (Table 22).

In Figure 56 the ratio of moisture to solids content is shown in order to observe the relationship between the values and to observe the contribution of each to the total content in cladodes.

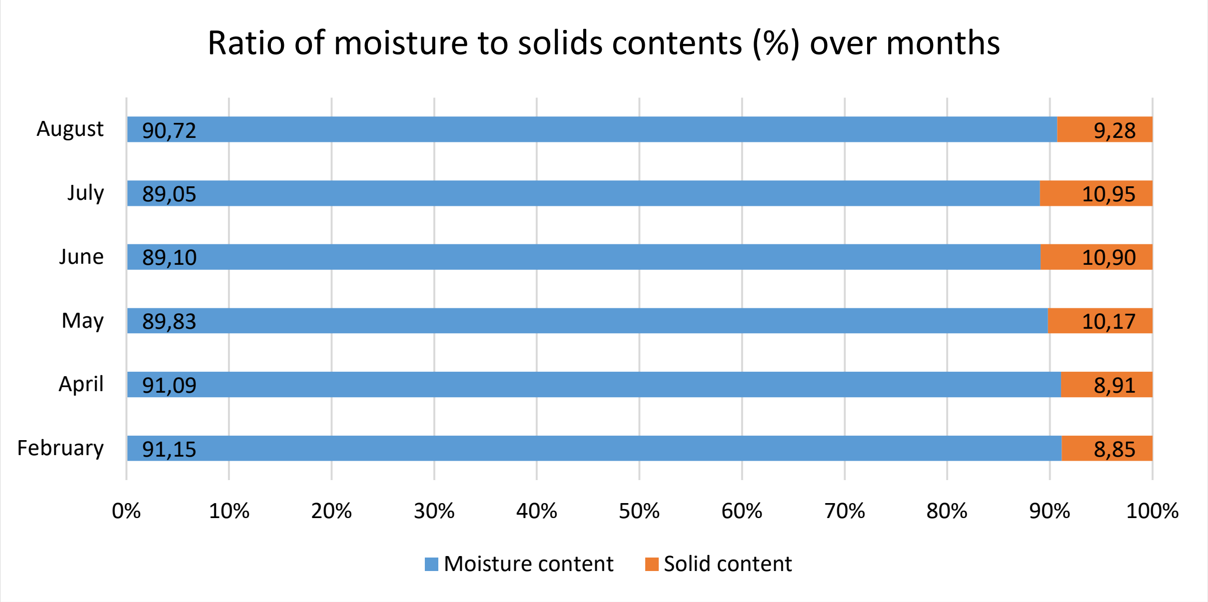


Figure 56: Ratio of moisture and solids content in cactus pear cladodes over months

In Figure 56 there was only a slight drop in cladode moisture content during the months when less than 25 mm of rain was reported (April to August) (Figure 50) compared to the summer months (January to March) when more than 45 mm rain fell.

In order to identify factors that influence mucilage yield, the influence of rainfall on cladode moisture content was correlated in Figure 57.

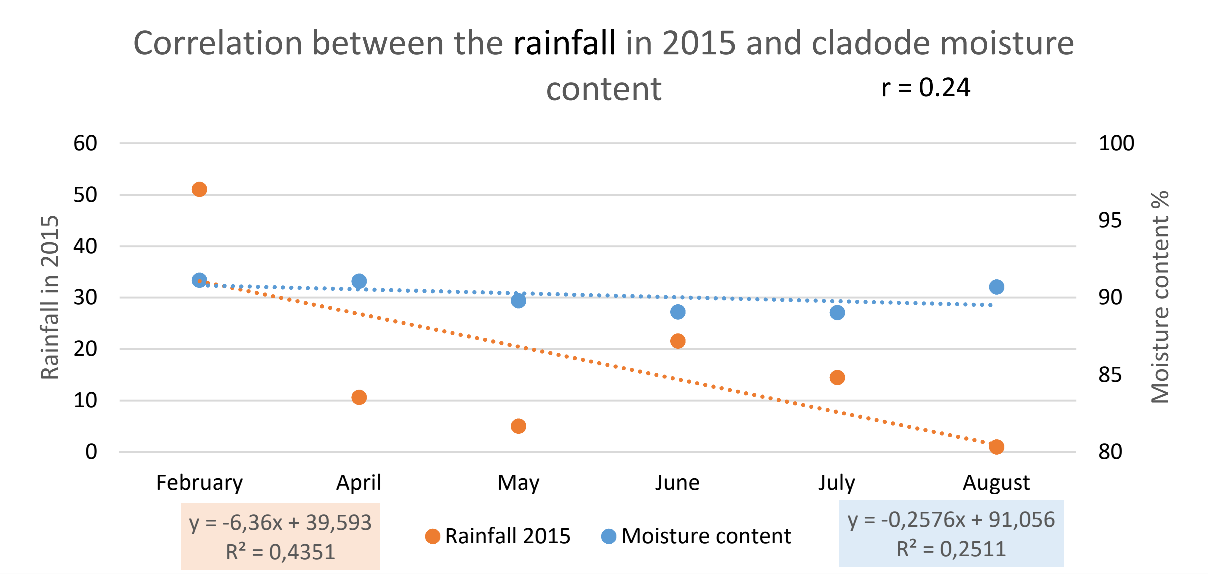


Figure 57: Correlation of rainfall 2015 and cladode moisture content over months

No discernible relationship between rainfall and cladode moisture content could be found (r=0.24). Therefore, it was established that cladode moisture content was not influenced by the amount of rain that was recorded on the farm where the orchard is situated. The daytime temperatures were correlated with cladode moisture content in order to determine the influence of seasonal temperatures in Figure 58.

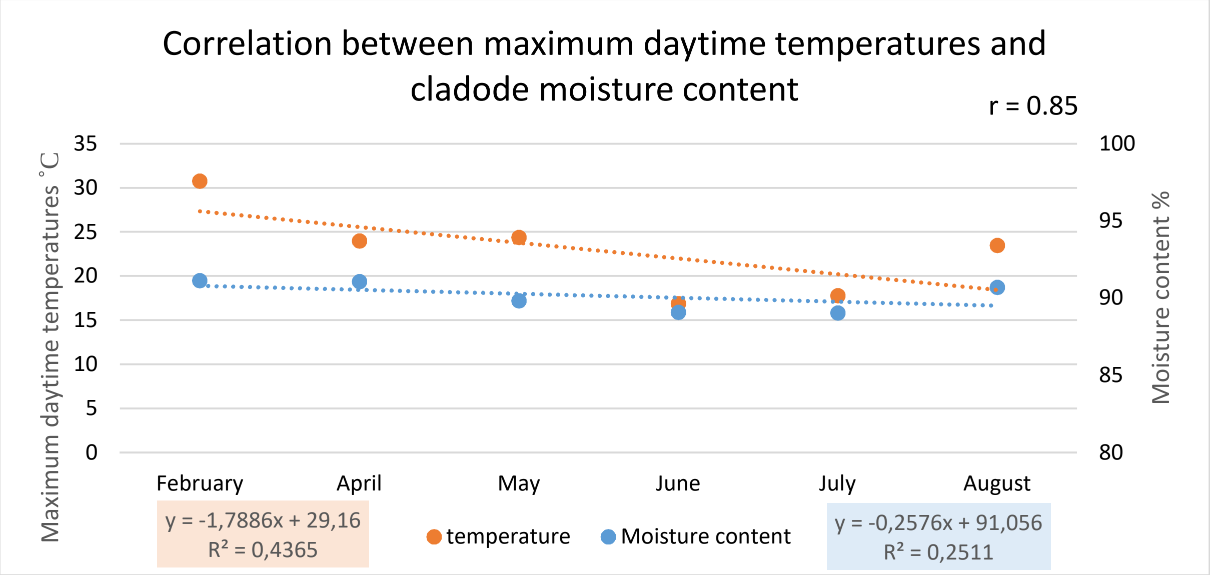


Figure 58: Correlation between maximum daytime temperatures and cladode moisture content over months

There was a strong positive correlation between daytime temperatures and cladode moisture content (r=0.85). This result was interesting as it was expected that higher rainfall would increase cladode moisture content, while hot, dry summer days would cause the moisture content of cladodes to decrease. However, the opposite was proven. The moisture and solids content of cladodes do not depend on rainfall and remains relatively constant despite the availability of rain water. Yet, maximum daytime temperatures had a strong positive relationship with moisture content, therefore when the temperatures during the day were high, the moisture content of cladodes was higher.

The influence of cladode moisture content on mucilage viscosity and mucilage yield was correlated in Figure 59 and Figure 60 in order to determine whether cladode moisture content influences the consistency and extractability of mucilage from cladodes.

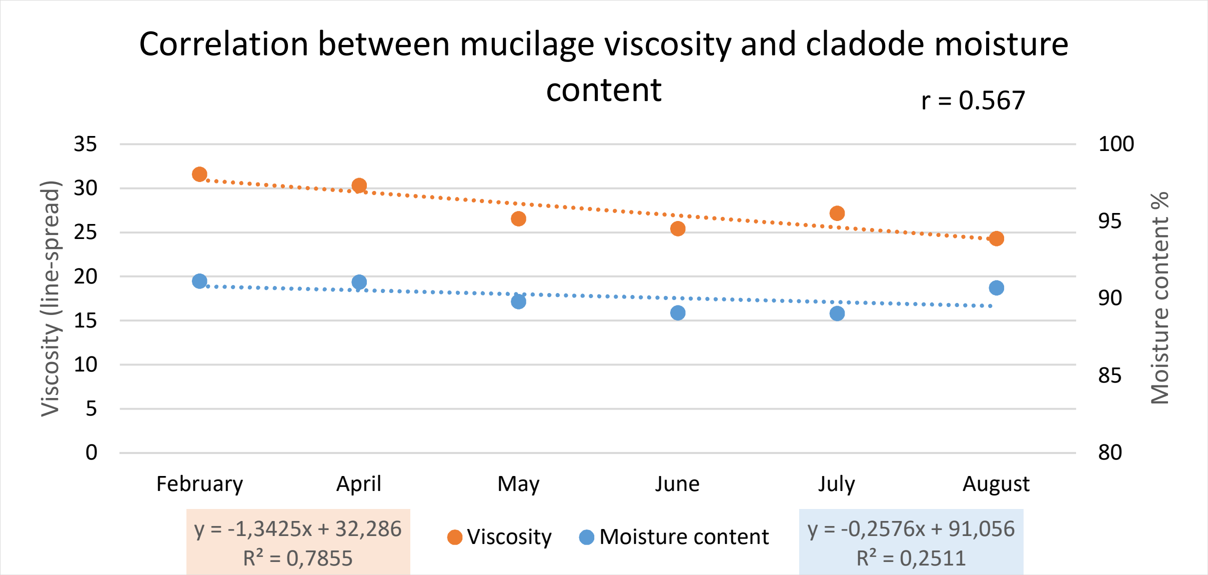


Figure 59: Correlation between mucilage viscosity and cladode moisture content over months

A moderate positive relationship (r = 0.567) was observed between mucilage viscosity and cladode moisture content. Therefore the higher the moisture content, the lower the viscosity of mucilage (high line-spread values indicate low viscosity).

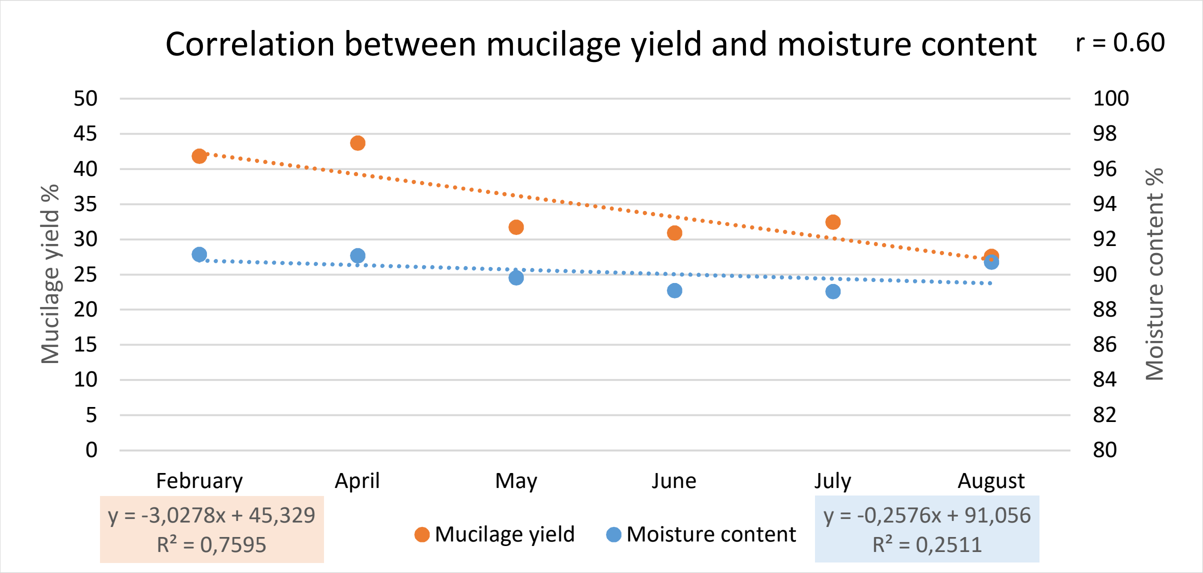


Figure 60: Correlation between mucilage yield and cladode moisture content over months

A moderate positive relationship was observed between mucilage yield and moisture content (r=0.60). It was thus observed that although the moisture content fluctuation in cladodes are relatively small, it had a moderate influence on the mucilage viscosity and yield. No general discernible relationship of moisture content and mucilage yield or viscosity could be established in chapter 4, yet in these tests, a moderate correlation could be observed.

6.4.2.4 Viscosity

It was established in chapter 3 that viscosity influenced yield and confirmed in chapter 5. The pH values of native mucilage according to cultivar and month obtained is presented in Table 23.

Table 23: The effect of month and cultivar on line-spread value of native mucilage

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p < 0.001)**
Algerian	35.47	31.39	30.03	26.26	28.54	25.89	**29.60^{bc}**
Morado	31.62	31.88	27.16	25.98	28.32	25.39	**28.39^{b}**
Gymno-Carpo	34.18	32.12	28.84	28.65	31.11	28.29	**30.53^{c}**
Robusta	25.18	25.96	20.35	20.90	20.89	17.70	**21.83^{a}**
Month Means (p < 0.001)	**31.61^{b}**	**30.34^{b}**	**26.60^{a}**	**25.45^{a}**	**27.22^{a}**	**24.32^{a}**	**27.59** **C x M: (p = 0.241)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
☐ = All means

The viscosity of 'Gymno-Carpo' was significantly lower than that of 'Morado' and 'Robusta'. 'Robusta had the highest viscosity (significantly different) compared to the other cultivars. 'Morado' viscosity was significantly lower than 'Robusta' and significantly higher than 'Gymno-Carpo'. The viscosity of native mucilage from 'Algerian' did not differ significantly from 'Morado' and Gymno-Carpo'. The mucilage extracted from cladodes harvesed in the hotter months (February and April) had significantly lower viscosity than the cooler months. There was no statistical difference between viscosity of mucilage in May, June, July and August although a general decline in viscosity values was observed. The interaction between month of harvest and cultivar was not significant. The mean viscosity according to the line spread method was 27.59.

It was already established (chapter 4) that the viscosity of mucilage influenced the extractability of mucilage from cladodes and therefore the yield of mucilage. A correlation between mucilage yield and viscosity (line-spread) were conducted to determine the influence of mucilage viscosity on yield of extracted mucilage.

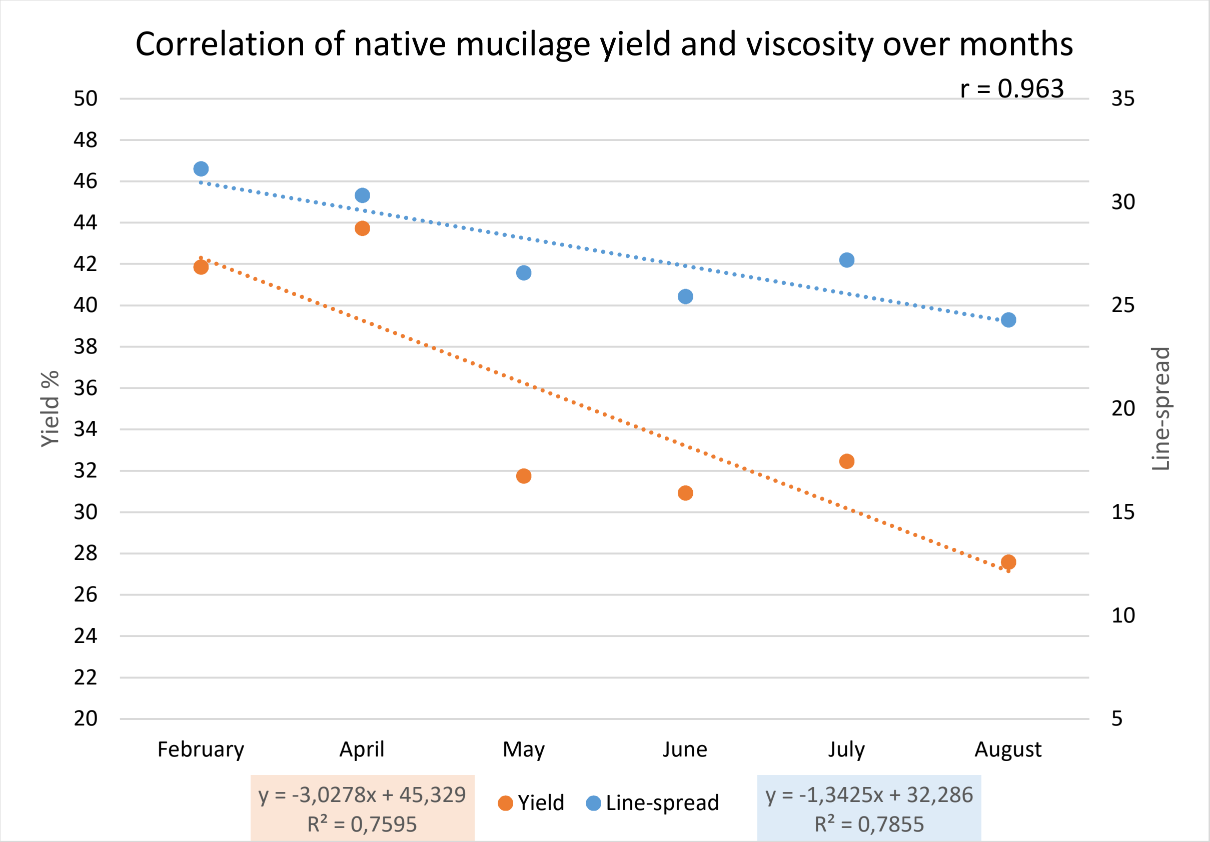


Figure 61: Correlation between mucilage yield and mucilage viscosity over months

The correlation of % mucilage yield and viscosity was an almost perfect positive correlation (r = 0.963). The viscosity of mucilage therefore had a direct influence on the yield of mucilage from cladodes. The notion that the extractability of mucilage was higher when the viscosity of the mucilage was lower was confirmed. Thus, viscosity is a factor that influences mucilage yield.

6.4.2.5 pH

Cactus pear plants are succulents with CAM metabolism, as such, it is a remarkable characteristic (of CAM plants) that malic acid is constructed at night and then deconstructed the following day. The malic acid is synthesized from starch or other carbohydrates. This accumulation of malic acid at night, causes the pH to drop towards dawn and the deconstruction during the day, causes it to rise again towards dusk (Salisbury and Ross, 1992). As a result of the high concentration of acids (H^+ ions), the osmotic potential is negative, causing the absorption and storage of water, which is an enormous benefit for a plant that grows in semi-arid land with poor soil quality (Salisbury and Ross, 1992).

Therefore the cactus pear has adaptations that equip it to thrive during extremely hot days with high irradiance levels, cold nights and poor soil. CAM is the reason why cactus pears frequently grow in deserts (Salisbury and Ross, 1992).

The acidic taste of cladodes that were harvested early in the morning is a confirmation of the high concentration of acids in cladodes, before the presence of sunlight was able to reverse the process, as a high acid content in young cladodes caused an undesirable sour taste. It is customary in Mexico to harvest later in the day, after at least two hours of sunshine, when the level of acidity has decreased to a satisfactory level (Rodriguez-Felix and Cantwell, 1988). The pH values of native mucilage according to cultivar and month obtained is presented in Table 24.

Table 24: The effect of month and cultivar on the pH of native mucilage extracted from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.422)**
Algerian	3.96^{AB}	3.98^{AB}	4.20^{BC}	4.34^{C}	5.15^{EF}	5.54^{H}	**4.53**
Morado	3.89^{A}	3.97^{AB}	4.04^{AB}	4.68^{D}	5.24^{EFG}	5.35^{FGH}	**4.53**
Gymno-Carpo	3.99^{AB}	4.02^{AB}	4.11^{ABC}	5.12^{EF}	5.07^{E}	5.46^{GH}	**4.63**
Robusta	4.07^{AB}	4.15^{ABC}	4.14^{ABC}	5.15^{EF}	5.26^{EFGH}	5.36^{FGH}	**4.69**
Month Means (p < 0.001)	$\mathbf{3.97^{a}}$	$\mathbf{4.03^{ab}}$	$\mathbf{4.12^{b}}$	$\mathbf{4.82^{c}}$	$\mathbf{5.18^{d}}$	$\mathbf{5.42^{e}}$	**4.59** **C x M: (p < 0.001)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
Interaction means with different superscripts in capital letters differ significantly ($p < 0.05$)
C x M = Interaction between cultivar and month.
☐ = All means

The pH of native mucilage increased significantly progressively from April (4.03) to August (5.42) (Table 24), though there was an increase from February (3.97) to April (4.03), it was not significant. The interaction between months of harvest and cultivars was significant. For Algerian, the pH for June was significantly higher than February, April and May while July was significantly higher than June and August significantly higher than July. For Morado, February, April and May mucilage had lower pH (significantly different) than June while July and August was significantly higher than June. Gymno-Carpo mucilage also showed a progressive increase, though February, April and May were not significantly different from each other. June and July pH were significantly higher than the first three months and August pH was significantly higher than June and July. Robusta had a similar tendency to increase progressively every month, yet February, April and May pH did not differ significantly from each other. June, July and August mucilage demonstrated higher pH than the first three months but was not significantly different from each other. The overall average pH was 4.59.

There were no significant differences between cultivars. Products with pH levels below 4.5 are required for the food to be considered safe, as it prevents the growth of bacteria. However yeast and moulds may still occur at a pH lower than 4.5.

In order to identify factors that influence mucilage yield a correlation between pH of native mucilage and yield of native mucilage was conducted in Figure 62.

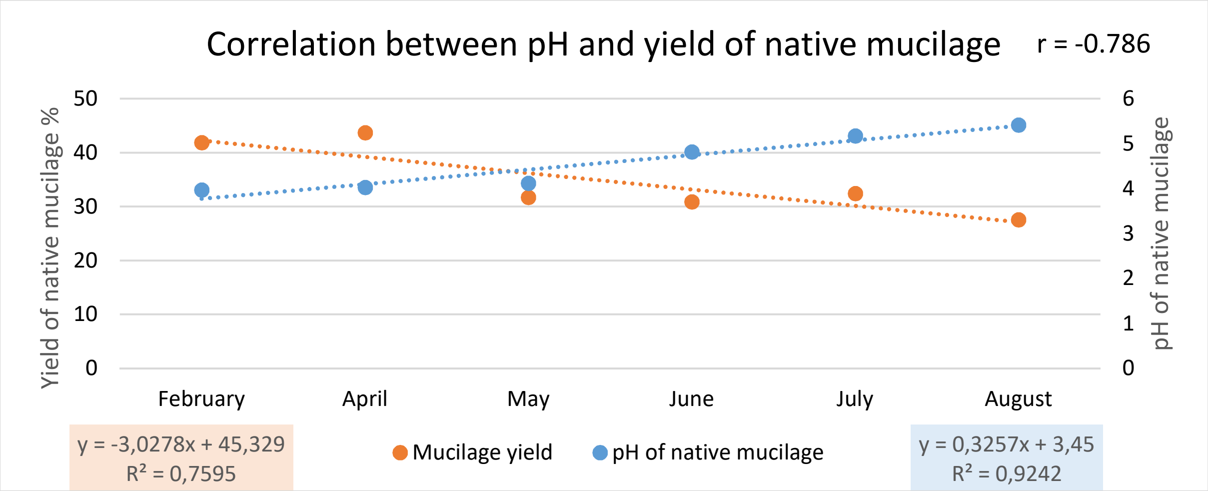


Figure 62: Correlation between native mucilage pH and yield of native mucilage over months

A strong negative relationship was observed (r=-7.9) in Figure 62, therefore the lower the pH, the higher the yield. This correlation thus indicated that pH is a factor that influenced mucilage yield. The correlation between pH and viscosity is presented in Figure 63.

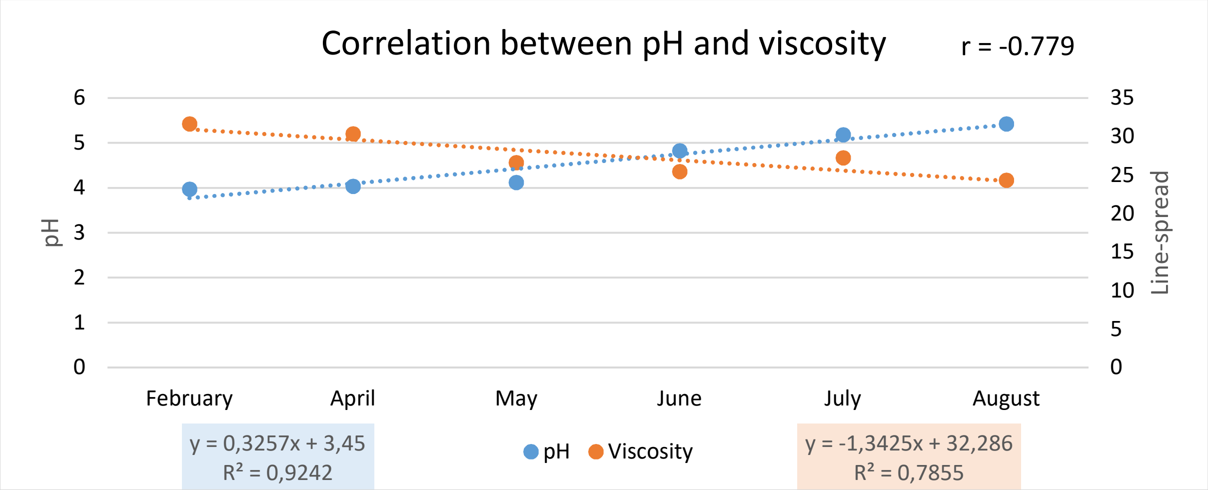


Figure 63: Correlation between native mucilage pH and viscosity (line-spread) over months

A strong negative relationship (r=-0.779) was seen between pH and viscosity of native mucilage. When the pH of mucilage was low, the viscosity was low (high line-spread results indicate low viscosity).

In order to clarify the factors that influence mucilage pH, it was correlated to maximum daytime temperatures for the period that cladodes were harvested in Figure 64.

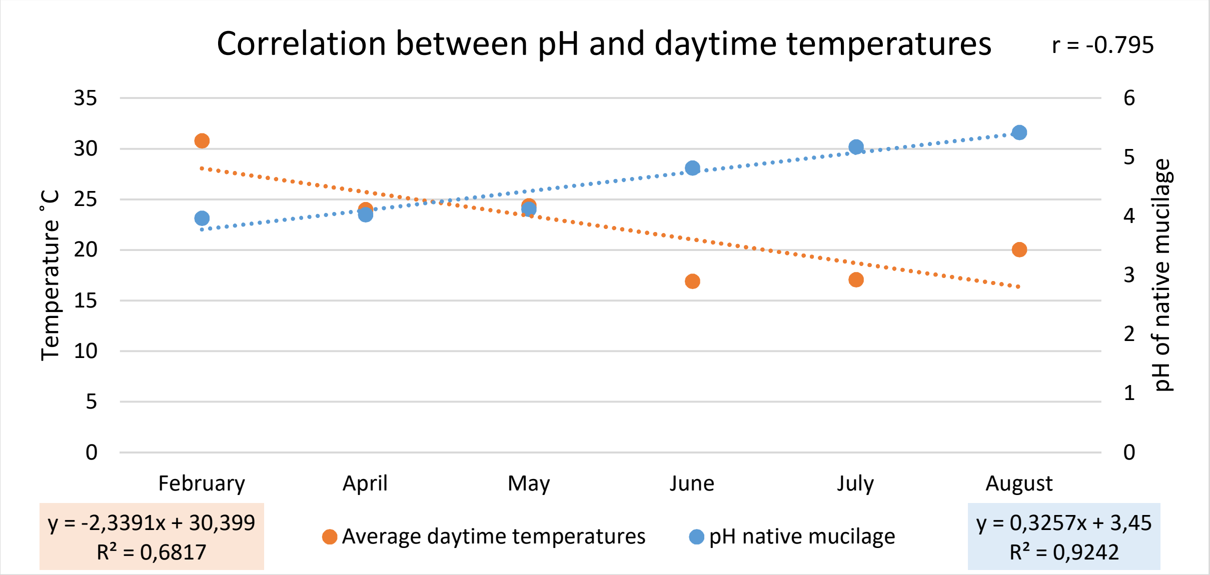


Figure 64: Correlation between pH of native cactus pear mucilage and daytime temperatures over months

A strong negative correlation (r=-0.795) between pH and daytime temperatures indicate that acidic substances (such as malic acid and uronic acid) increased during hot summer months and decreased during the colder and shorter days of winter.

The native mucilage was dried and reconstituted to a 10% concentration after which the pH was determined again. The pH values of reconstituted mucilage according to cultivar and month obtained is presented in Table 25.

Table 25: The effect of month and cultivar on pH of reconstituted freeze-dried mucilage extracted from cactus pear cladodes

	Feb	**April**	**May**	**June**	**July**	**Aug**	**Cultivar Means (p = 0.246)**
Algerian	3.96	3.96	4.17	4.28	4.08	4.50	**4.16**
Morado	3.86	3.89	4.05	4.20	4.20	4.29	**4.08**
Gymno-Carpo	3.96	4.04	4.09	4.06	4.07	4.25	**4.08**
Robusta	4.14	4.16	4.25	4.23	4.26	4.39	**4.24**
Month Means (p = < 0.001)	**3.98^{a}**	**4.01^{a}**	**4.14^{ab}**	**4.19^{ab}**	**4.15^{ab}**	**4.36^{b}**	**4.14 C x M: (p = 0.241)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ = All means

pH varied between 3.86 in February for 'Morado' and 4.5 for 'Algerian' in August. In Table 25 it is evident that the average pH increased in all four cultivars from February to August. The pH values for February and April were significantly lower than the pH for August (p < 0.001). There were no statistical differences between cultivars (Table 25) and the interaction between months of harvest and cultivar was not significant (p = 0.151). It is assumed that the pH of reconstituted mucilage reflect the pH values of the freeze-dried mucilage. With pH values below 4.6 (pH < 4.5), the freeze-dried mucilage will not be spoiled by bacteria but will be susceptible to spoilage by yeasts and moulds (Brown, 2007). The average pH value of freeze-dried mucilage powders (4.14) were lower than that of native mucilage (average of 4.59). This difference was mostly due to the progressive increase in pH that was seen from June to August. Both the native and reconstituted mucilage pH values were plotted in Figure 65 in order to observe the differences in pH values and the increase in average pH values that was observed in native mucilage.

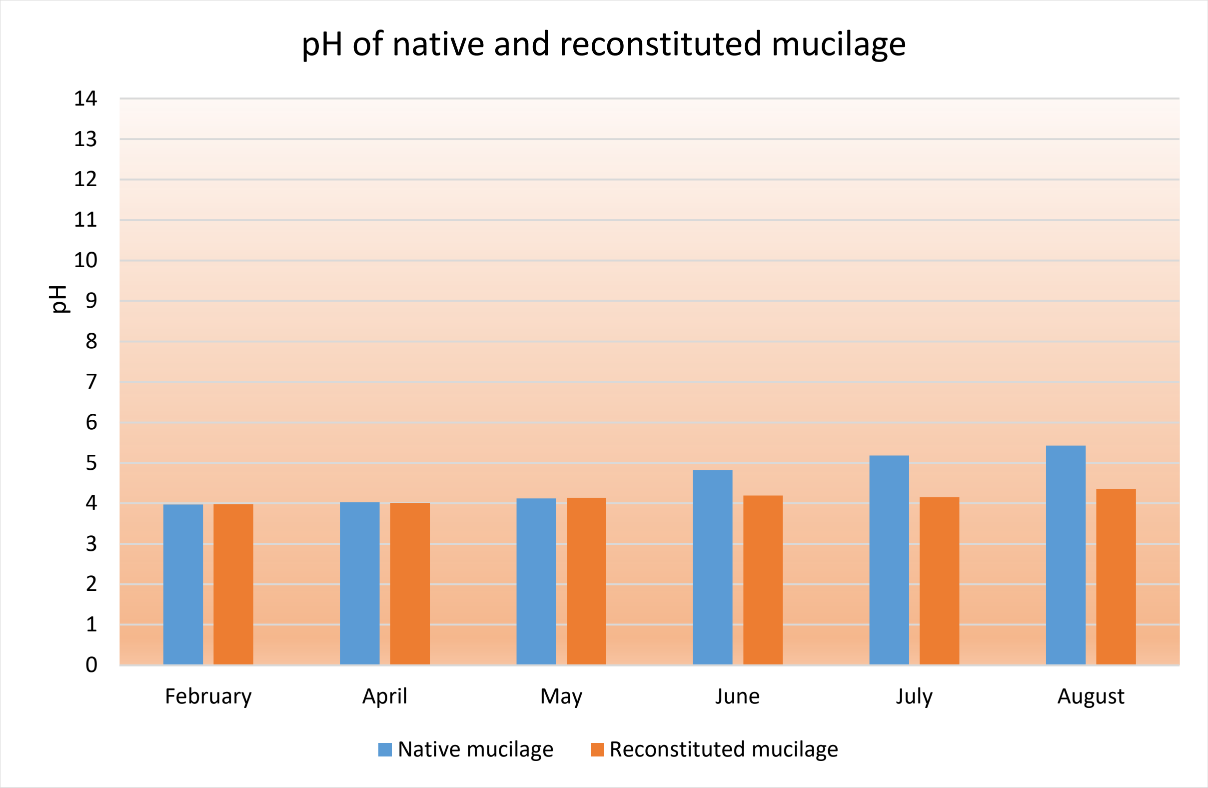


Figure 65: Comparison of pH from native and reconstituted cactus pear mucilage according to cultivar and over months on a pH scale

The pH levels significantly increased in native mucilage, progressively from February (pH 3.97) to August (pH 5.42) (Figure 65). The presence of uronic acids had been reported in cladodes (Majdoub et al., 2001; Ribeiro De Oliveira et al., 2010) and was especially detected in older cladodes in the dry season (Ribeiro De Oliveira et al., 2010). More acid sugars provide more hydrogen ions and thus a drop in the pH takes place when organic acids increase. It will be shown in chapter 7 that malic acids decreased steadily from February to August, therefore the pH increased likewise.

According to the low pH found by different researchers, cladodes were indicated as being a low pH vegetable (Samia El-Safy, 2013). Ayadi et al. (2009) reported pH of 4.02 in spiny cladodes and 3.84 in spineless cladodes. Stintzing and Carle (2005) stated that the juice from cladodes typically had a pH of 4.6. Gebresamuel and Gebre-Mariam (2012) reported pH values of close to neutral in different low concentrations (1-12%) mucilage dispersions (*O. ficus-indica*) of 5.57 to 6.43. Rodriguez-Felix and Cantwell (1988) reported the pH of 4.6 and Samia El-Safy (2013) at 3.27, both determined in cladode flour. Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had pH of between 4.07 and 4.35 that did not differ significantly with cladode age (Rodríguez-Garcia et al., 2007).

6.4.2.6 Conductivity

Specific conductance is a direct indication of the electrolyte levels and thus the levels of free moving ions in a substance. As mucilage molecules are polyelectrolytes, the conductivity was measured in order to determine the concentration of the free moving ions. The intrinsic viscosity of mucilage depends on the ionic strength and electrostatic interactions between the macromolecules. Conductivity also indicates the extent to which the viscosity and flow behaviour could be manipulated by the addition of substances containing cations (Benoit, 2004; Dickinson, 2003). The viscosity of mucilage could be influenced by the addition of cations such as Ca^{2+} as was demonstrated in chapter 5. Conductivity is expressed in units of millivolts (mV) per centimetre which is equal to millimho and millisiemens (mS). The conductivity (mS) values according to cultivar and month are observed in Table 26.

Table 26: The effect of month and cultivar on conductivity (mS/cm) of native mucilage extracted from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p =0.396)**
Algerian	167.68^{FG}	166.59^{FG}	152.50^{EF}	148.02^{EF}	95.80^{BC}	74.01^{A}	**134.10**
Morado	172.01^{G}	169.98^{FG}	162.88^{EFG}	125.30^{D}	85.87^{ABC}	85.75^{ABC}	**133.63**
Gymno-Carpo	166.12^{FG}	164.35^{EFG}	157.74^{EFG}	100.28^{C}	100.73^{C}	80.44^{AB}	**128.28**
Robusta	161.53^{EFG}	156.93^{EFG}	156.58^{EFG}	97.83^{BC}	84.74^{ABC}	85.89^{ABC}	**123.92**
Month Means (p < 0.001)	**166.84^{e}**	**164.46^{de}**	**157.43^{d}**	**117.86^{c}**	**91.79^{b}**	**81.52^{a}**	**129.98 C x M: (p < 0.001)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
Interaction means with different superscripts in capital letters differ significantly ($p < 0.05$)
C x M = Interaction between cultivar and month.
☐ = All means

Conductivity of native mucilage samples decreased progressively from February to August (Table 26). The mean conductivity values were significantly higher in February (166.84 mS/cm), April (164.45 mS/cm) and May (157.43 mS/cm) compared to June (117.86 mS/cm), while June was significantly higher than July (91.79 mS/cm) and August (81.52 mS/cm) was significantly lower than July. The interaction between months of harvest and cultivar was significant ($p < 0.001$). For Algerian the conductivity values decreased from February to June (not significantly), while July values were significantly lower than the first three values and August values significantly lower than July. For Morado mucilage the conductivity values decreased from February to May (not significantly). June values were significantly lower than the first months and significantly higher than July and August (not significantly different from each other). Gymno-Carpo mucilage demonstrated a similar decrease in conductivity. February, April and May values decreased, but were not significantly different from each other. June and July values were significantly lower than the first three months, but did not differ significantly from each other. August mucilage was significantly lower than all the other months. June, July and August conductivity values for Robusta did not differ from each other although it was significantly lower than February, April and May values (not significantly different). In terms of cultivars, there were no statistical differences detected. The overall average was 129.98 mS/cm (Table 26). In order to determine the correlation between pH and conductivity, a correlation was done as presented in Figure

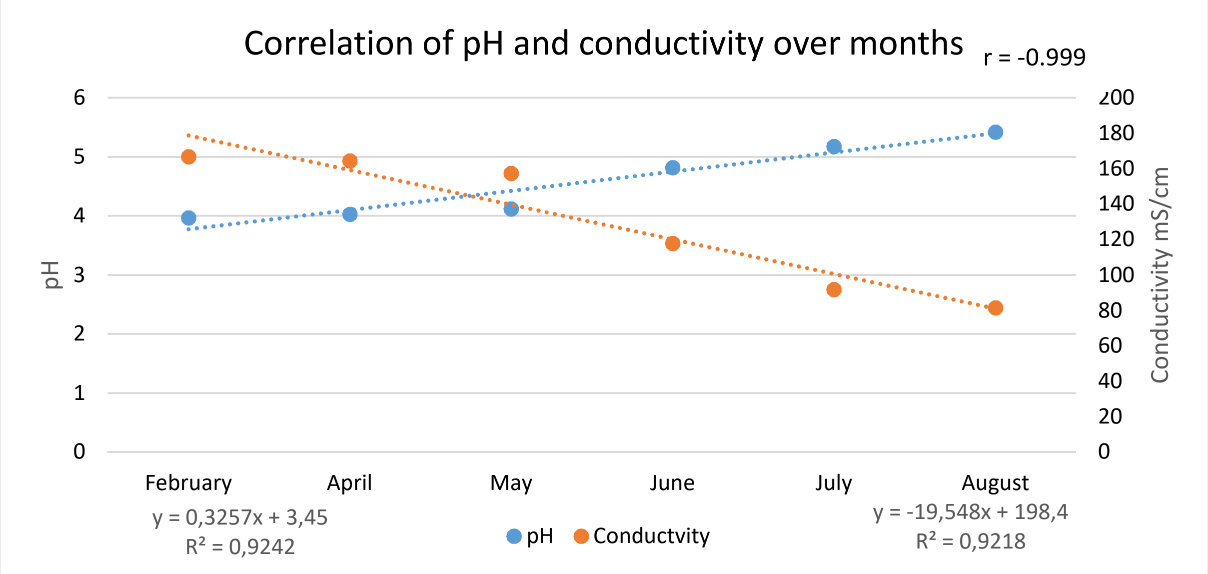


Figure 66: Correlation of pH and conductivity of native mucilage from cactus pear cladodes over months

The correlation between pH and conductivity was almost a perfect positive relationship (r = 0.999). The stronger ionic solution (February) that occurred as a consequence of the increased hydrogen (supplied by sugar acid and organic acids), resulted in higher conductivity and lower pH values. As the acidic substances (organic acids) decreased from February to August, the pH increased and thus the conductivity (lower ion concentration) decreased.

Thus, a correlation between hot summer days, low pH and high conductivity was established in this section of the study. Higher yields and lower viscosity were previously correlated to high daytime temperatures. Therefore, a connection between hot summer days and high mucilage yields had been established as well.

The conductivity values obtained in reconstituted mucilage according to cultivar and month is presented in Table 27.

Table 27: The effect of month and cultivar on conductivity (mS/cm) of reconstituted freeze-dried mucilage extracted from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.198)**
Algerian	105.70	106.40	96.20	89.60	102.10	77.80	**96.30**
Morado	111.20	110.60	102.70	94.40	94.90	89.80	**100.60**
Gymno-Carpo	107.20	101.80	100.70	102.30	102.00	92.80	**101.13**
Robusta	96.80	96.10	91.50	93.30	91.40	84.10	**92.20**
Month Means (p = 0.003)	**105.23^{b}**	**103.73^{b}**	**97.78^{ab}**	**94.90^{ab}**	**97.60^{ab}**	**86.13^{a}**	**97.56** **C x M: (p = 0.183)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

Conductivity varied between 84.1 mS/cm for ‘Robusta’ in August to 111.2 mS/cm for ‘Morado’ in February. The conductivity in February (105.23 mS/cm) and April (103.73 mS/cm) was significantly higher than August (86.13 mS/cm). No statistical differences were detected between cultivars (Table 27) and the interaction between month of harvest and cultivar was not significant.

Both the native and reconstituted mucilage conductivity values were plotted in Figure 67 in order to observe the differences in values and the decrease in average conductivity values that was observed.

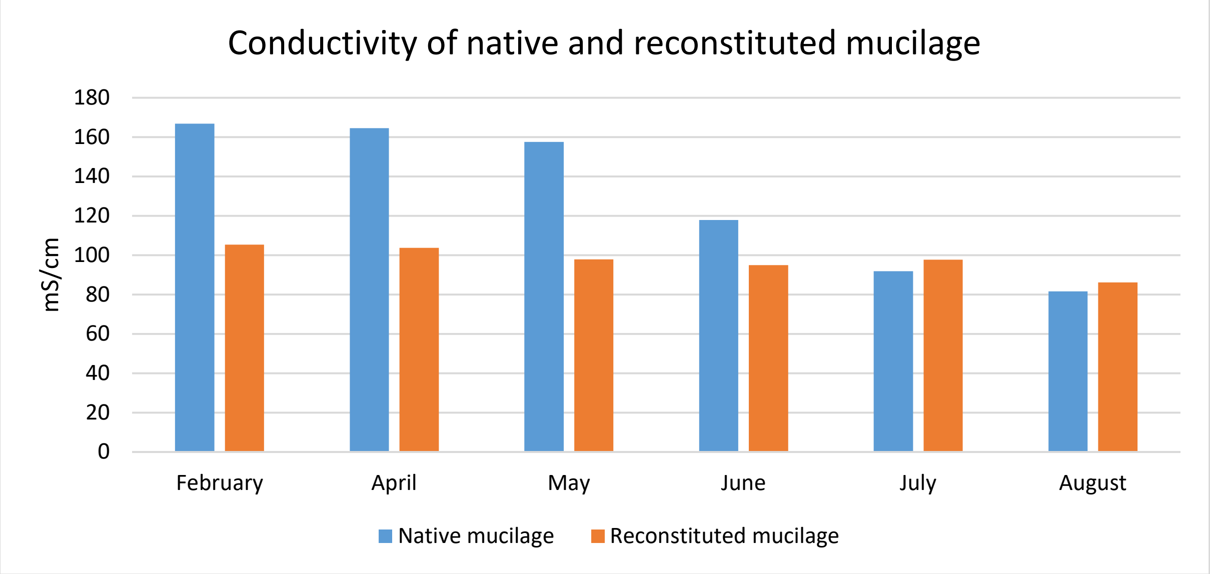


Figure 67: Comparison of conductivity from native and reconstituted freeze-dried cactus pear mucilage over months

Conductivity values of both native and reconstituted mucilage samples decreased progressively from April to August (Figure 67). The conductivity of native mucilage (166.84 mS/cm) was higher than reconstituted in February (105.23 mS/cm) after which it decreased every month to almost similar (native 81.52 and reconstituted 86.13 mS/cm) values in July and August.

In Figure 66, the pH and conductivity of reconstituted mucilage was correlated in order to shed light on the relationship between the properties. A strong negative relationship was observed between pH and conductivity (r = -0.998). The trend line for conductivity (Figure 66) had the opposite tendency to that of pH: the conductivity reduced in all cultivars from February to August while the pH increased (Figure 66). This was as a direct result of the increase in cations.

Conductivity serves as an indication of flour quality (Clements, 1975). Very little is found on conductivity in literature except for Gebresamuel and Gebre-Mariam (2012) who reported conductivity in mucilage dispersions in different low concentrations (1, 4, 8 and 12%) as between 2.73 and 13.12 mS/cm. The large discrepancy between their values were attributed to the abundancy of calcium (divalent) and potassium (monovalent) content. The conductivity values were very different to the values reported in this study, however in this study, the pH values were lower as well.

When % yield was correlated to daytime temperatures of months, a positive correlation was found (r=0.7), therefore it was theorised that the extractability of mucilage was higher (viscosity of the mucilage was lower) during the warmer, higher rainfall months (February and April). The moisture and solids content of cladodes did not differ according to rainfall and remained constant despite the abundance of water during summer months. The pH however, increased significantly over months in both native and reconstituted mucilage, while the conductivity decreased significantly. A strong negative relationship was observed between pH and conductivity (r=-0.999). The increased concentration of ions (high conductivity) observed in February, thus occurred as a consequence of the increased availability of hydrogen atoms, caused by the higher acidity levels, supplied by organic acids and sugar acids.

In conclusion, CAM is more efficient during hotter summer days in producing acids that lower the pH. The lowered pH means an increase in ion concentrations. With the addition of the hydrogen atoms, the negative charges of the mucilage molecules will be neutralized, causing the viscosity to decrease. Mucilage that is lower in viscosity is more readily extracted leading to higher yields of native mucilage. It could therefore be concluded that higher yields of native mucilage may be extracted from cladodes that were harvested during hotter weather.

Cladode moisture content is not influenced by rainfall, but by temperature. Moisture content only had a moderate influence on mucilage viscosity and yield.

The influence of ions on the viscosity of mucilage is an important property of mucilage that should demand considerable consideration in the application of mucilage in food products. Mucilage could become less viscous in savoury and dairy products since the addition of positive ions in the form of salt (NaCl) and calcium ($CaCl_2$) containing positive ions react to the negatively charged mucilage molecule, causing it to lose its thickening power. The consistency of the mucilage could be influenced by the properties inherent of the food products.

6.4.3 Colour

The three coordinates of CIELAB represent the lightness of the colour (*L** = 0 indicates black and L* = 100 indicates white), red and green (*a**, negative values indicate green while positive values indicate red) and yellow and blue (*b**, negative values indicate blue and positive values indicate yellow) (HunterLab, 2007).

6.4.3.1 Native mucilage

The L* value that indicate lightness according to cultivar and month is presented in Table 28. The interaction between months of harvest and cultivar was not significant. February mucilage (48.22) was significantly more luminous than April (38.99) mucilage while the luminosity of the other months did not differ significantly from each other.

Table 28: The effect of month and cultivar on colour L value of native mucilage from cactus pear cladodes*

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.047)**
Algerian	51.15	36.34	38.48	41.07	45.74	42.96	**42.62^{ab}**
Morado	43.66	36.52	40.74	42.74	43.85	40.89	**41.40^{a}**
Gymno-Carpo	48.32	44.59	44.48	43.62	40.22	40.50	**43.62^{b}**
Robusta	49.74	38.50	42.15	42.94	42.71	41.70	**42.96^{ab}**
Month Means (p < 0.001)	**48.22^{c}**	**38.99^{a}**	**41.46^{b}**	**42.59^{b}**	**43.13^{b}**	**41.51^{b}**	**42.65** **C x M: (p < 0.134)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

After April luminosity remained statistically stable from May to August where the luminosity was respectively 41.46, 42.59, 43.13 and 41.51 (Table 28). ‘Gymno-Carpo’ L* value was significantly different from ’Morado’. The a* value that indicate red and green according to cultivar and month is presented in Table 29.

Table 29: The effect of month and cultivar on colour a value of native mucilage from cactus pear cladodes*

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p < 0.001)**
Algerian	-3.42	-3.07	-5.24	-5.52	-5.13	-6.27	**-4.78^{b}**
Morado	-3.71	-2.94	-6.01	-6.87	-4.97	-5.87	**-5.06^{b}**
Gymno-Carpo	-3.28	-3.41	-5.31	-5.48	-3.82	-4.49	**-4.30^{b}**
Robusta	-5.71	-7.39	-7.00	-5.96	-8.16	-7.08	**-6.88^{a}**
Month Means (p < 0.001)	**-4.03^{b}**	**-4.20^{b}**	**-5.89^{a}**	**-5.96^{a}**	**-5.52^{a}**	**-5.93^{a}**	**-5.25** **C x M: (p< 0.103)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
▭ **= All means**

The lower the a* value, the more intense the green colour of the mucilage. The green colour was on average statistically similar from May to July, as it ranged between -5.52 and -5.96 but was significantly different from the green colour in February (-4.03) and April (-4.2) (Table 29). ‘Robusta’ was (-6.88) significantly greener than the other cultivars. The interaction between months of harvest and cultivar was not significant. When native 'Robusta' mucilage is used in food products, this could have a negative effect on the final colour of the product. As 'Robusta' is a different species (*O. robusta*) the cladodes have a grey-green colour that is easily noticed as being different from *O. ficus-indica* cladodes of which the other cultivars investigated here, are examples. The a* values were correlated to daytime temperatures in order to investigate a connection between summer days and the green colour of cladodes (Figure 68).

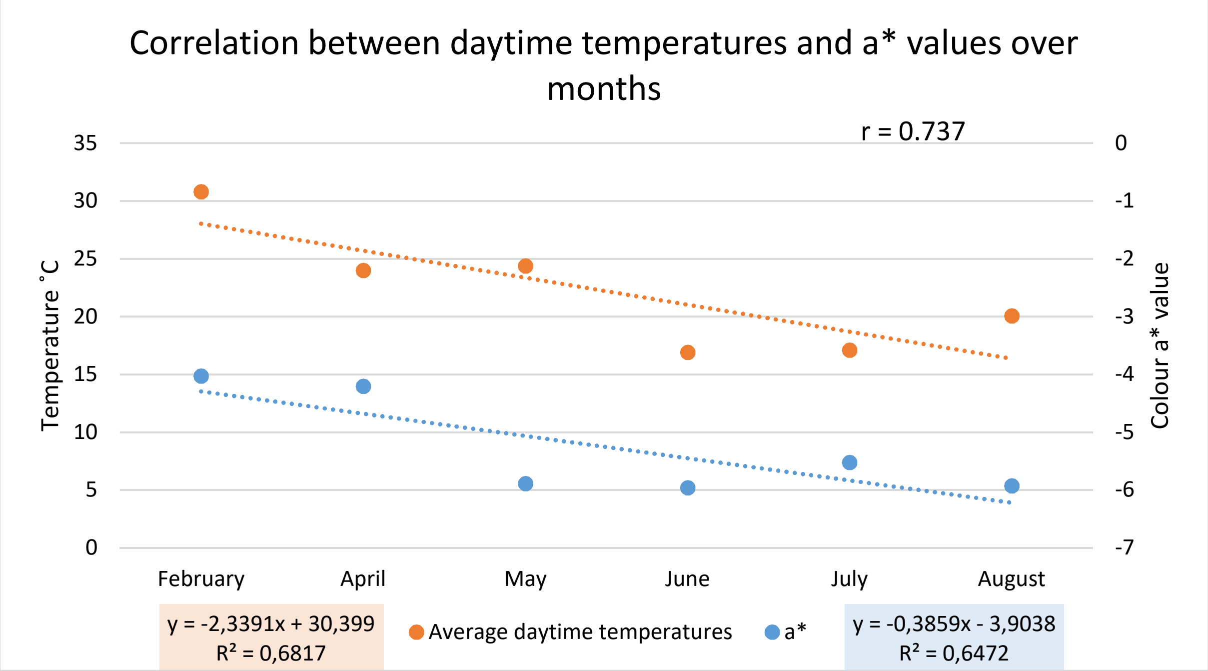


Figure 68: Correlation of daytime temperatures and a* values for native mucilage from cactus pear cladodes over months

A positive correlation (r= 0.7) was observed between the a* values and the average daytime temperatures (Figure 68). The higher the temperature (February, April) the higher the a* values. However, when the daytime temperatures decreased, the a* values decreased, indicating a darker, deeper green colour. Therefore, the green colour of mucilage became duller during winter months.

The b* values that indicate yellow and blue according to cultivar and month are observed in Table 30.

Table 30: The effect of month and cultivar on colour b value of native mucilage from cactus pear cladodes*

	Feb	**April**	**May**	**June**	**July**	**Aug**	**Cultivar Means (p < 0.001)**
Algerian	12.15	11.39	17.39	17.32	15.06	17.44	**15.13^{a}**
Morado	13.28	10.79	18.05	17.09	14.59	15.85	**14.94^{a}**
Gymno-Carpo	10.89	10.23	18.45	17.03	12.8	14.76	**14.03^{a}**
Robusta	20.27	14.58	20.07	18.48	18.17	15.05	**17.77^{b}**
Month Means (p < 0.001)	**14.15^{b}**	**11.77^{a}**	**18.49^{d}**	**17.48^{cd}**	**15.16^{b}**	**15.78^{bc}**	**15.47** **C x M: (p = 0.234)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

'Robusta' (17.47) mucilage had significantly more yellow and less blue tones than the other cultivars (Table 30). The yellow tones of the other cultivars did not differ significantly. The intensity of yellow tones were significantly different month to month. It decreased significantly from February (14.55) to April (11.77) whereupon it increased significantly in May (18.49) and June (17.48). The interaction between month of harvest and cultivar was not significant. The average b* value was 15.47 (Table 30).

The C* values that indicate Chroma (colour intensity or saturation) according to cultivar and month are observed in Table 31.

Table 31: The effect of month and cultivar on colour C value of native mucilage from cactus pear cladodes*

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar means (p = 0.083)**
Algerian	12.62	11.80	18.16	18.18	15.91	18.53	**15.87**
Morado	13.79	11.18	19.03	18.42	15.41	16.90	**15.79**
Gymno-Carpo	11.37	10.78	19.20	17.89	13.36	15.43	**14.67**
Robusta	21.06	16.35	21.26	19.42	19.92	16.63	**19.11**
Month means (p = 0.012)	**14.71^{ab}**	**12.53^{a}**	**19.41^{b}**	**18.48^{b}**	**16.15^{ab}**	**16.87^{ab}**	**16.36** **C x M: (p = 0.568)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

Colour intensity (C* values) was not significantly different between cultivars although 'Robusta' had the most intense C* colour value (19.11). May (19.41 and June (18.48) native mucilage had significantly more intense green colour than April mucilage. The interaction between month of harvest and cultivar was not significant. The average C* value for native mucilage was 16.36.

The h˚ values that indicate the hue (the position around the colour wheel) according to cultivar and month are observed in Table 32.

Table 32: The effect of month and cultivar on colour h˚ value of native mucilage from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar means (p = 0.045)**
Algerian	105.73	105.09	106.77	107.68	108.81	109.78	**107.31**^{a}
Morado	105.61	105.25	108.42	111.90	108.82	110.33	**108.39**^{a}
Gymno-Carpo	106.77	108.44	106.06	107.84	106.62	106.92	**107.11**^{a}
Robusta	105.74	116.88	109.23	107.88	114.19	115.20	**111.52**^{b}
Month means (p = 0.423)	**105.96**	**108.91**	**107.62**	**108.83**	**109.61**	**110.56**	**108.58** **C x M: (p = 0.234)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

'Robusta' mucilage was significantly different in terms of hue (h˚) than the other cultivars while there was no significant difference between the other cultivars. In terms of month, no significant difference was observed. The interaction between month of harvest and cultivar was not significant. The average h˚ for native mucilage was 1.858.

In conclusion, for native mucilage, the luminosity (L*) indicated that the brightness of the mucilage was medium (average 42.65). The a* values indicated green of various intensities with an average value of -5.25. The b* showed a yellow undertone (15.47). The Chroma (C*) values (16.36) indicated low colour saturation and the mean h˚ (108) was indicative of yellow-green. Therefore the native mucilage had various shades of a light straw-green colour.

It was observed that the three *O. ficus-indica* (Algerian, Morado and Gymno-Carpo) cultivars had similar values and similar trends, therefore the values were combined for comparison to *O. robusta* Robusta. In Figure 69 the mean a* (green-red) and b* (blue-yellow) values for *O. ficus-indica* over months were correlated with a* values (indicated on the left vertical axes) and b* values (on the right).

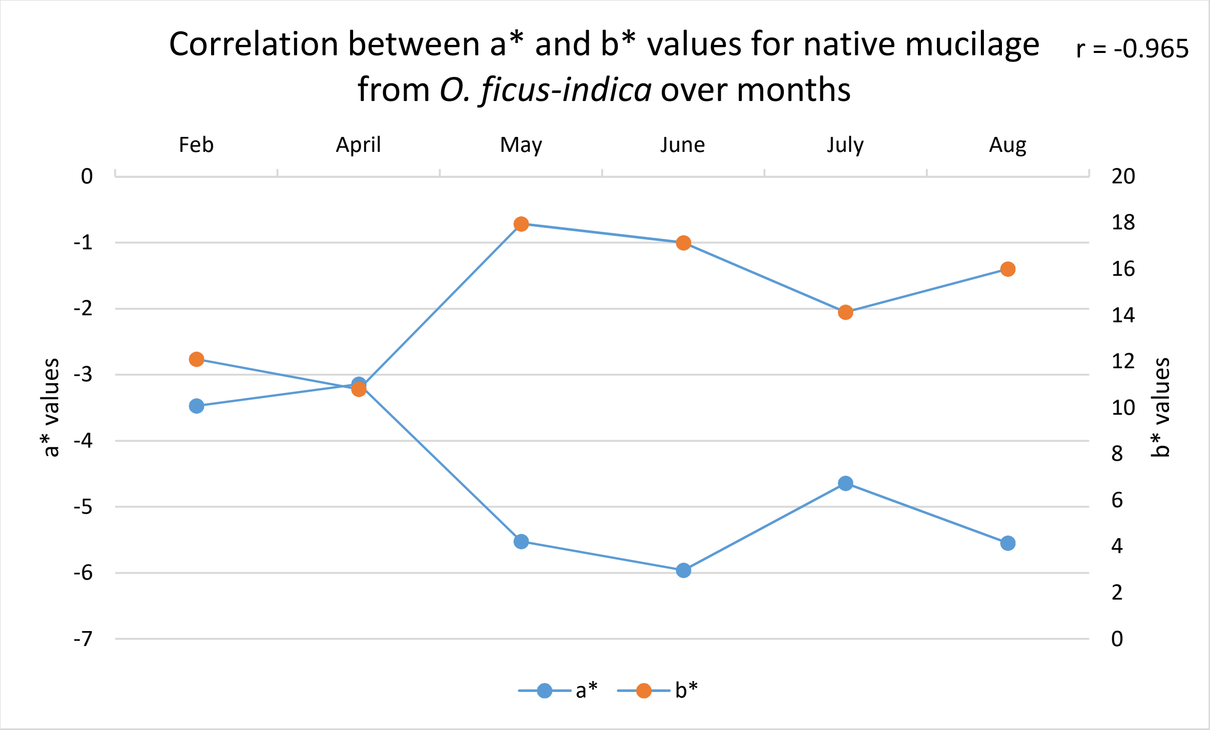


Figure 69: Correlation of *O. ficus-indica* a* and b* values for native mucilage from cactus pear cladodes over months

An almost perfect negative correlation (r = -0.965) between the a* and b* values emerged. In fact, when a* values increased, the b* values decreased to form a mirror image of one another. This correlation indicated that the greener the cladodes, the lower the blue colouration, causing a more brilliant green, however when the blue colouration increased, the green decreased, causing a duller green colour.

In Figure 70, 'Robusta' a*(green-red) and b* (blue-yellow) values over months were plotted on a graph with a* values indicated on the left vertical axes and b* values on the right.

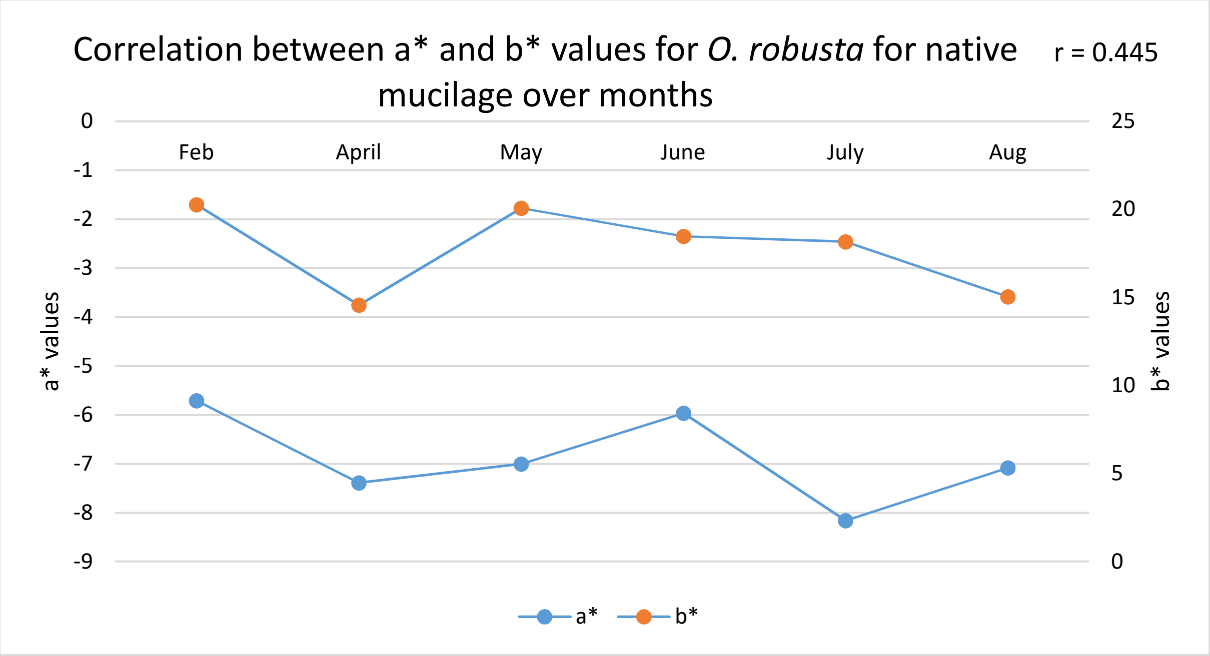


Figure 70: Correlation of *O. robusta* a* and b* values for native mucilage from cactus pear cladodes over months

Robusta a*(green-red) and b* (blue-yellow) values did not follow a similar trend as was seen with *O. ficus-indica* a* and b* values (Figure 69). The correlation coefficient showed a relatively weak positive correlation at r= 0.445. Therefore, in ‘Robusta’, there was an association between the colours that prevented the green colouration from appearing overly bright green and rather maintaining a duller “green-blue” colour. This correlation remained intact over the months that were tested.

Colour trends and correlations for *O. robusta* Robusta were thus observed to be contrary to the *O. ficus-indica* cultivars.

In Figure 71 the mean a* (green-red) values for *O. ficus-indica* (OFI) where plotted against the mean a* *O. robusta* Robusta values.

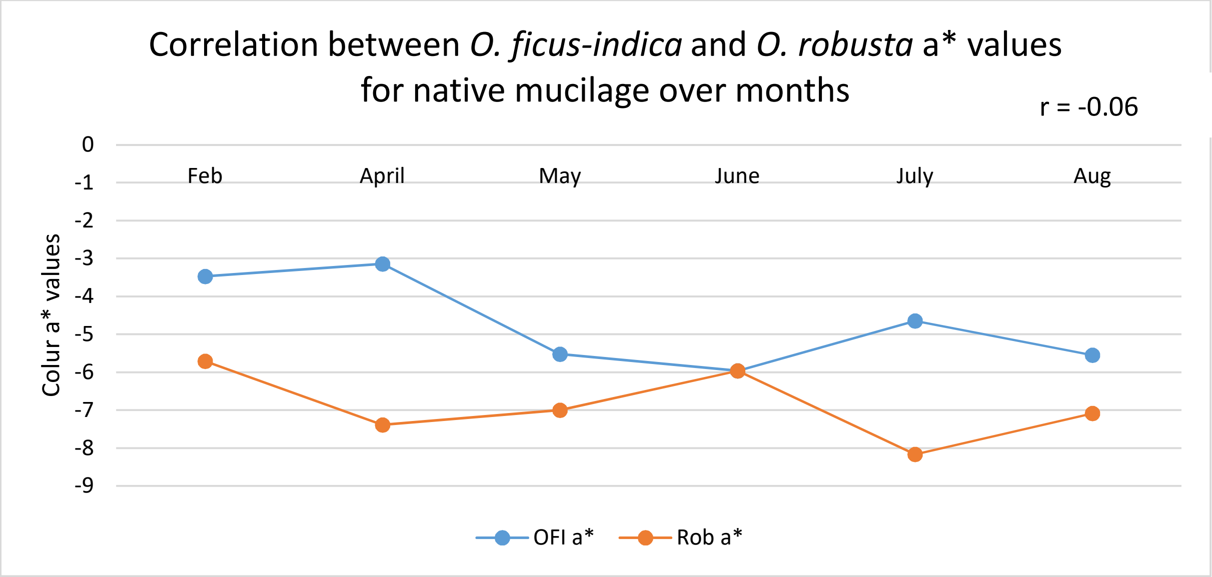


Figure 71: Correlation between a* values for *O. ficus-indica* and *O. robusta* native mucilage from cactus pear cladodes over months

The mean a* (green-red) values for *O. ficus-indica* cultivars displayed a moderate negative correlation to the 'Robusta' a* values (r=-0.06). It was observed that the intensity of green contrasted each other almost perfectly. Thus when OFI a* values increased (mucilage became more green), it decreased in 'Robusta' (mucilage became less green) and vice versa. It was observed that the development of green colouration of 'Robusta' cladodes was different and contrary to that of OFI cladodes.

In Figure 72 the mean b* (blue-yellow) values for OFI were plotted against the mean b* 'Robusta' values.

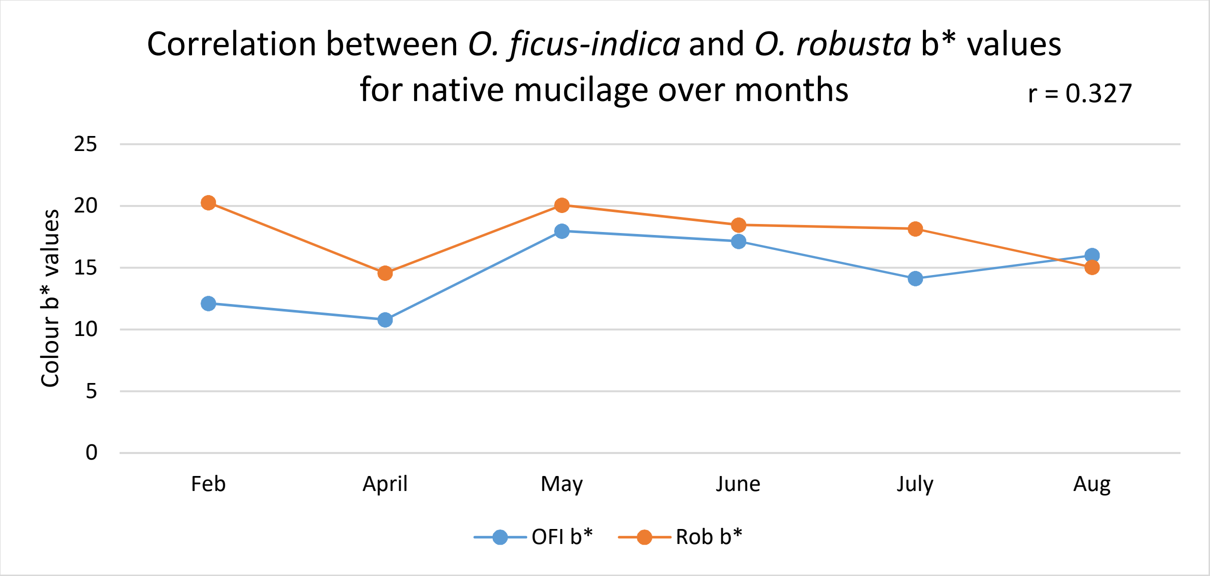


Figure 72: Correlation between b* values for *O. ficus-indica* and *O. robusta* native mucilage from cactus pear cladodes over months

The b* (blue-yellow) values showed a weak correlation (r = 0.327) but the corresponding tendencies were observed that was in contrast with the negative correlation seen in the a* values. In b* values, both lines increased and decreased at the same time (Figure 72). It was attained that the development of blue colouration was similar in all cladodes. In conclusion the 'Robusta' mucilage was observed to look different (more greenish) than the mucilage from the other cultivars, proved to have different colour values (not always significantly different) and seemed to have different colour development to other cultivars. The factors that influence cladode colour development are not known.

6.4.3.2 Colour of freeze-dried mucilage

The L* value that indicate lightness according to cultivar and month is presented in Table 33.

Table 33: The effect of month and cultivar on colour L value of freeze-dried mucilage from cactus pear cladodes*

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.424)**
Algerian	92.43	91.39	79.45	77.95	74.52	74.46	**81.70**
Morado	93.17	89.77	76.82	75.98	80.05	77.07	**82.14**
Gymno-Carpo	93.04	92.27	74.07	75.89	84.55	78.19	**83.00**
Robusta	79.53	76.15	75.73	75.81	78.37	75.09	**76.78**
Month Means (p < 0.001)	**89.54^{b}**	**87.39^{b}**	**76.52^{a}**	**76.41^{a}**	**79.37^{ab}**	**76.20^{a}**	**80.91** **C x M: (p = 0.625)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

In comparison to the L* values observed in native mucilage, the powdered mucilage was considerably more luminous (Table 33) as native mucilage had an average L* value of 42.65 and freeze-dried mucilage an average L* value of 80.91 (indicating lightness closer to white). February and April samples were significantly brighter than May, June and August. No significant differences were detected between cultivars (Table 33). The interaction between months of harvest and cultivar was not significant. The a* value that indicate red and green according to cultivar and month is presented in Table 34.

Table 34: The effect of month and cultivar on colour a value of freeze-dried mucilage from cactus pear cladodes*

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.189)**
Algerian	-3.83	-4.35	-2.42	-5.05	-3.73	-7.57	**-4.49**
Morado	-3.26	-5.16	-3.95	-4.40	-4.08	-6.73	**-4.60**
Gymno-Carpo	-2.94	-3.28	-2.27	-5.06	-6.32	-5.50	**-4.23**
Robusta	-3.39	-6.57	-6.86	-5.12	-7.56	-6.94	**-6.07**
Month Means (p = 0.036)	**-3.36^{b}**	**-4.84^{ab}**	**-3.87^{ab}**	**-4.91^{ab}**	**-5.42^{ab}**	**-6.68^{a}**	**-4.85** **C x M: (p = 0.174)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

The varying negative a* values observed indicated that the green colour of freeze-dried mucilage varied (Table 34). The average a* values were not significantly different from April to July while February powders were significantly less green than the August powders. 'Robusta' showed greener colouration in July and August although it was not significant according to Table 34. The interaction between month of harvest and cultivar was not significant. In fact, this tendency was also seen in native mucilage. When freeze-dried 'Robusta' mucilage is used in food products, the deep green colouration could have a negative effect on the final colour of the product. No statistical differences were found between cultivars. The average a* value for freeze-dried mucilage was -4.85.

The b* values that indicate yellow and blue (b*, negative values indicate blue and positive values indicate yellow) are indicated in Table 35. The average b* value for July was significantly lower than August values and indicated less yellow and more blue tones. In fact, the values decreased from February to July (not significantly), but increased in August to higher values than February.

Table 35: The effect of month and cultivar on colour b value of freeze-dried mucilage from cactus pear cladodes*

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.876)**
Algerian	24.05	21.93	22.04	23.94	21.73	23.78	**22.91**
Morado	22.92	27.15	24.54	22.22	19.33	26.58	**23.79**
Gymno-Carpo	23.83	22.13	22.68	25.50	21.15	25.17	**23.41**
Robusta	25.81	21.69	24.83	23.52	21.63	24.43	**23.65**
Month Means (p = 0.038)	**24.15^{ab}**	**23.22^{ab}**	**23.52^{ab}**	**23.79^{ab}**	**20.96^{a}**	**24.99^{b}**	**23.44** **C x M: (p = 0.236)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

The yellow tones of the cultivars did not differ significantly. The interaction between month of harvest and cultivar was not significant. The results were interesting, as it was expected that ‘Robusta’ would differ from the other cultivars. In native mucilage, the ‘Robusta’ b* (blue-yellow) values had significantly higher yellow tones but showed corresponding tendencies to the other cultivars. Yet, no statistical differences between cultivars were noted in Table 35.

The C* values indicate Chroma (C*), low values indicate dullness (unsaturation) and high values indicate bright (colour purity). The Chroma values (C*) for freeze-dried mucilage are indicated in Table 36.

Table 36: The effect of month and cultivar on colour C value of freeze-dried mucilage from cactus pear cladodes*

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar means (p = 0.798)**
Algerian	24.36	22.36	22.17	24.47	22.05	24.96	**23.39**
Morado	23.15	27.64	24.86	22.65	19.76	27.42	**24.25**
Gymno-Carpo	24.01	22.37	22.80	26.00	22.08	25.77	**23.84**
Robusta	26.03	22.67	25.76	24.07	22.92	25.40	**24.47**
Month means (p = 0.041)	**24.39^{ab}**	**23.76^{ab}**	**23.90^{ab}**	**24.30^{ab}**	**21.70^{a}**	**25.89^{b}**	**23.99** **C x M: (p = 0.682)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

The Chroma did not differ significantly between cultivars. July mucilage had significantly duller C* values compared to August mucilage. The mucilage harvested from February to June did not differ significantly in terms of C* values. The interaction between month of harvest and cultivar was not significant. The mean C* value for freeze-dried mucilage was 23.99.

The hue degrees (h˚) that indicate the position of a colour on the colour wheel are observed in Table 37.

Table 37: The effect of month and cultivar on colour h˚ value of freeze-dried mucilage from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar means (p = 0.173)**
Algerian	99.15	101.23	101.92	101.92	99.75	107.66	**101.94**
Morado	98.10	100.77	99.15	101.21	101.92	104.21	**100.89**
Gymno-Carpo	97.04	98.44	95.72	101.23	106.64	102.33	**100.23**
Robusta	97.49	106.86	105.45	102.29	109.29	105.86	**104.54**
Month means (p = 0.042)	**97.94**^{a}	**101.82**^{ab}	**100.56**^{ab}	**101.66**^{ab}	**104.40**^{ab}	**105.02**^{b}	**101.90** **C x M: (p = 0.234)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ = All means

The average hue angle values were the lowest in February (97.94˚) whereafter it increased to 105.02˚ in August (significantly different). The months from April to July did show increased h˚ values but was not statistically significantly different from each other. The overall average for hue was 101.9, indicating a yellow-green colour. The average hue for Algerian (101.93˚), Morado (100.89˚) and Gymno-Carpo (100.23˚) was very similar and indicated a yellow-green hue that was more yellow than the native mucilage (108˚). Robusta showed a slightly greener hue at 104.53˚ but the difference was not statistically significant to the other cultivars.

The colour of cladode powder is influenced by the age of the cladode as well as the drying conditions. The lower the temperature during drying, the better the colour of the cladode flour (López-Cervantes et al., 2011). Ayadi et al. (2009) observed that when light green cladode powders were used in sponge cake formulations, the colour of the cake changed. The crust of the cake became green while the crumb appeared darker. The colour of the cake was only acceptable to panellists when they were lead to believe that the cake contained green ingredients, such as pistachios (Ayadi et al., 2009). A continual decrease in appearance was observed with increased addition of cladode flour to carrot cakes (De Wit et al., 2015). According to Sáenz (1997) the green colour is not intense and powders could easily be added to products without it influencing the colour of food products. Colour parameters of mature cladode flour that was dried in an air force tunnel was L* 73.37, a* -5.20 and b* 26.1. These values indicated pale, yellow-greenish colour and thus care should be taken to mask the green colour in baked products (Sáenz et al., 2010). It is expected that the use of the freeze-dried mucilage that was produced in this study would similarly have little effect on applied food products in terms of the colour.

6.4.3.3 Colour of reconstituted mucilage

The colour analysis would not be complete without investigating the colour of reconstituted mucilage. These determinations were carried out using 5% concentration solutions. The L* value that indicate lightness according to cultivar and month is presented in Table 38.

Table 38: The effect of month and cultivar on colour L value of reconstituted freeze-dried mucilage from cactus pear cladodes*

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.701)**
Algerian	49.35	58.49	56.38	50.24	51.39	50.70	**52.76**
Morado	49.30	50.96	50.76	53.63	52.46	49.87	**51.16**
Gymno-Carpo	53.54	52.31	48.60	54.89	50.52	52.48	**52.06**
Robusta	61.54	62.30	48.87	50.69	48.40	50.97	**53.80**
Month Means (p = 0.359)	**53.43**	**56.01**	**51.15**	**52.36**	**50.69**	**51.00**	**52.44** **C x M: (p = 0.462)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

There were no significant differences found for months or cultivars and similar trends were seen to that of the native mucilage as well as the mucilage powder (Table 38). The lightness of the reconstituted mucilage ranged between 49 and 58. It was observed in Table 38 that the average L* value was 52.44. The reconstituted mucilage therefore appeared to be darker compared to the powder average (L* 80) while it was lighter than native mucilage (average 42.65). The interaction between month of harvest and cultivar was not significant. The a* value that indicate red and green according to cultivar and month is presented in Table 39.

Table 39: The effect of month and cultivar on colour a value for reconstituted freeze-dried mucilage from cactus pear cladodes*

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.048)**
Algerian	-4.23	-5.35	-6.40	-7.06	-4.76	-7.86	**-5.94**^{b}
Morado	-3.47	-4.56	-5.14	-6.03	-5.97	-6.35	**-5.25**^{b}
Gymno-Carpo	-2.17	-4.42	-5.47	-6.57	-5.07	-6.52	**-5.03**^{b}
Robusta	-5.83	-8.42	-7.64	-6.92	-6.91	-7.16	**-7.16**^{a}
Month Means (p = 0.598)	**-4.88**	**-5.69**	**-6.16**	**-6.64**	**-8.63**	**-6.97**	**-5.84** **C x M: (p = 0.528)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

No significant differences were observed between months, but 'Robusta' had significantly greener colouration than the other cultivars. The average a* value of -5.8 indicated that the mucilage had a more greenish colour than the powder (-4.85). Dried mucilage average a* value was -4.85 and native mucilage was -5.25 suggesting that reconstituted mucilage (-5.8) had a darker green colour compared to both native and dried mucilage (Table 39). The interaction between month of harvest and cultivar was not significant. The b* values that indicate yellow and blue according to cultivar and month are observed in Table 40.

Table 40: The effect of month and cultivar on colour b value for reconstituted freeze-dried mucilage from cactus pear cladodes*

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.624)**
Algerian	15.14	16.75	17.03	20.35	12.44	21.69	**17.23**
Morado	13.05	12.61	21.12	15.93	18.74	16.86	**16.38**
Gymno-Carpo	5.98	13.28	18.34	16.95	11.97	17.71	**14.04**
Robusta	20.29	20.87	20.59	21.65	16.32	17.59	**19.55**
Month Means (p = 0.535)	**27.50**	**15.88**	**19.27**	**18.72**	**14.87**	**18.46**	**19.12** **C x M: (p = 0.628)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

No significant differences were detected for b* values in either month or cultivar (Table 40). The yellowness of 'Gymno-Carpo' mucilage tested very high in February but the yellow values obtained during the rest of the months were comparable with other cultivars (negative values for b* indicate blue and positive values indicate yellow) (Table 40). The interaction between month of harvest and cultivar was not significant. The average b* value for reconstituted mucilage was 19.12 which was less yellow than dried mucilage (23.44) while it was more yellow than native mucilage (15.47).

The Chroma values (C*) for reconstituted freeze-dried mucilage are indicated in Table 41. 'Gymno-Carpo' reconstituted mucilage (14.93) was significantly duller from 'Robusta' (20.97) mucilage in terms of colour intensity. There were no significant differences observed between months. The interaction between month of harvest and cultivar was not significant. The mean C* value for reconstituted mucilage was 17.84.

Table 41: The effect of month and cultivar on colour C value of reconstituted freeze-dried mucilage from cactus pear cladodes*

	February	April	May	June	July	August	Cultivar means (p = 0.041)
Algerian	15.72	17.58	18.19	21.54	13.32	23.07	**18.24^{ab}**
Morado	13.50	13.41	21.74	17.03	19.67	18.02	**17.23^{ab}**
Gymno-Carpo	6.36	14.00	19.14	18.18	13.00	18.87	**14.93^{a}**
Robusta	21.11	22.51	21.96	22.73	17.72	19.80	**20.97^{b}**
Month means (p = 0.142)	**14.18**	**16.87**	**20.26**	**19.87**	**15.93**	**19.94**	**17.84** **C x M: (p = 0.432)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

The hue angles (h°) that indicate the position of a colour on the colour wheel are observed in Table 42.

Table 42: The effect of month and cultivar on colour h° value of reconstituted freeze-dried mucilage from cactus pear cladodes

	February	April	May	June	July	August	Cultivar means (p = 0.453)
Algerian	105.61	107.72	110.60	109.14	110.94	109.92	**108.99**
Morado	104.90	109.89	103.68	110.74	107.67	110.64	**107.92**
Gymno-Carpo	109.95	108.41	106.61	111.19	112.96	110.22	**109.89**
Robusta	106.04	111.98	110.36	107.73	112.95	111.20	**110.04**
Month means (p = 0.078)	**106.63**	**109.50**	**107.81**	**109.70**	**111.13**	**110.50**	**109.21** **C x M: (p = 0.268)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

There were no statistically significant differences between cultivars or month of harvest for reconstituted mucilage. The mean h° was 109.21. The interaction between months of harvest and cultivars was not significant. The mean h° values for native mucilage (108 h°) and reconstituted mucilage (109 h°) was more similar in comparison to freeze-dried mucilage (101 h°).

The L* a*, b*, C* and h° values for native, freeze-dried and reconstituted mucilage is presented in Figure 73, Figure 74, Figure 75, Figure 76 and Figure 77 in order to observe the differences in values.

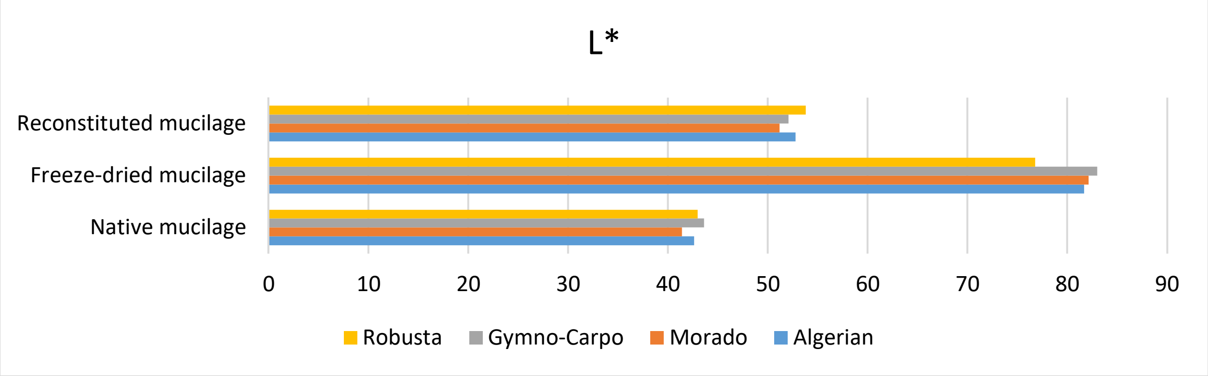


Figure 73: Comparison of the L* values for the different mucilage types from cactus pear cladodes

In comparison, the powder was a great deal lighter than the reconstituted and the native mucilage (Figure 73). The colour values of the reconstituted were more comparable with the native mucilage with the L* value averages 42 and 52 respectively, the a* values -4 and -6, and the b* values 17 and 19 for native mucilage and reconstituted mucilage.

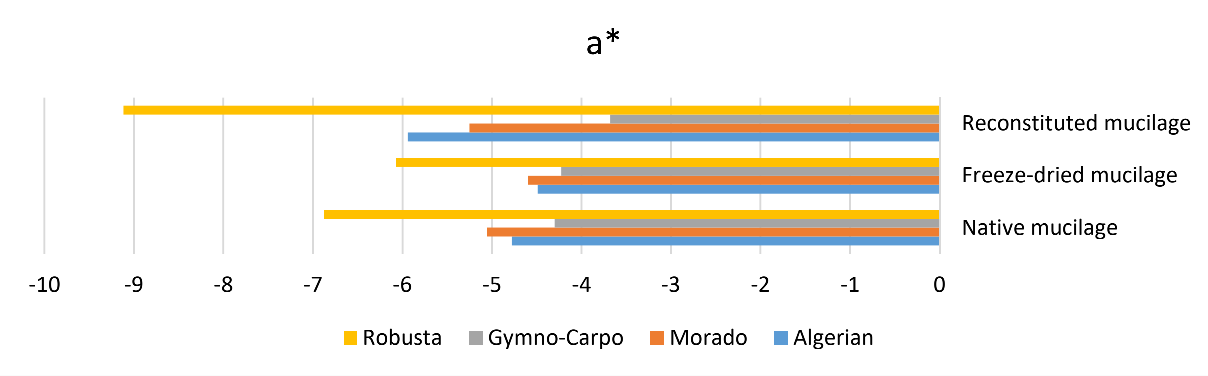


Figure 74: Comparison of the a* values for the different mucilage types from cactus pear cladodes

It is demonstrated in Figure 74 that the a* values for 'Robusta' (mucilage samples) indicated that the mucilage was consistently more green in native, freeze-dried and reconstituted mucilage.

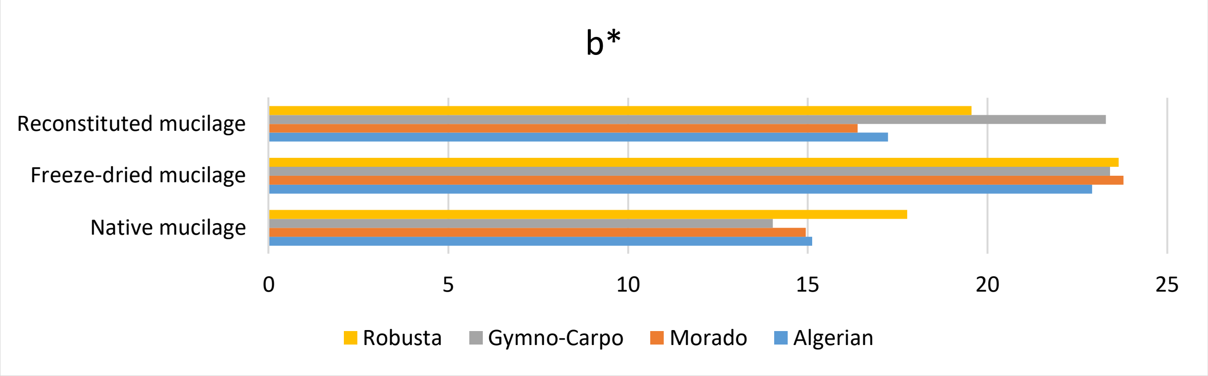


Figure 75: Comparison of b* values for the different mucilage types from cactus pear cladodes

The b* values were relatively consistent for freeze-dried and native mucilage (Figure 75), but in reconstituted mucilage, the b* values indicated inconsistent yellow tones. The freeze-dried powder was more yellow than the other types of mucilage (Figure 75).

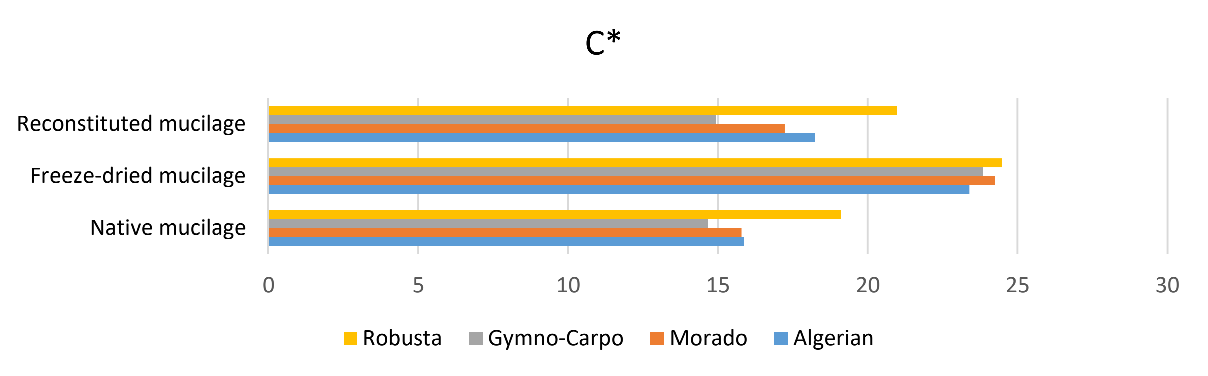


Figure 76: Comparison of C* values for the different mucilage types from cactus pear cladodes

The C* values were relatively consistent for freeze-dried mucilage (Figure 76), but in native and reconstituted mucilage, the C* values indicated inconsistent intensities of colour and ‘Robusta’ powders had higher Chroma values that indicated purer green colour. The freeze-dried powder was more intense (less dull) than the other types of mucilage.

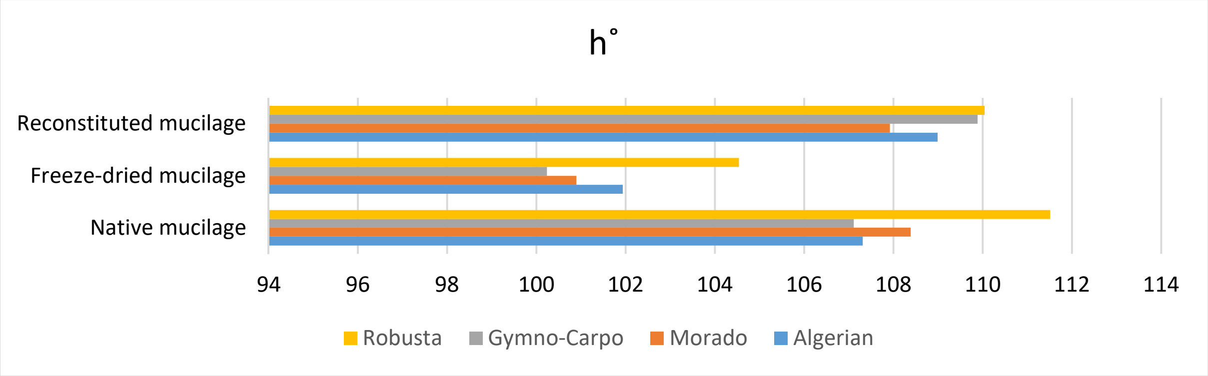


Figure 77: Comparison of h˚ degrees for the different mucilage types from cactus pear cladodes

In Figure 77 the h˚ degrees (yellow-green colour) for native and reconstituted mucilage were relatively comparable while the hue degrees for freeze-dried mucilage were more yellow in comparison. ‘Robusta’ mucilage was in the same range of hue than the other cultivars in the reconstituted mucilage’s while mucilage from ‘Robusta’ was a greener yellow-green than the other cultivars in freeze-dried and native mucilage.

Finally, the overall averages of the mucilage types are presented in Figure 78. It was observed that freeze-dried mucilage was the brightest and had the yellowest tones of the different types of mucilage, while the reconstituted mucilage appeared to be slightly deeper green than the other types of mucilage.

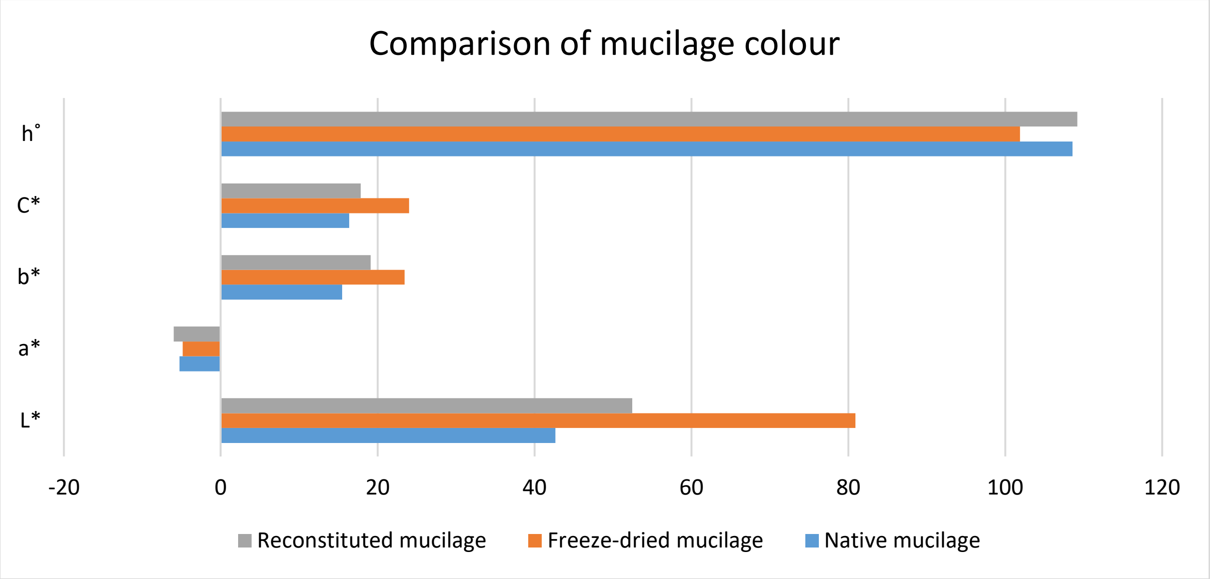


Figure 78: Comparison of L*, a*, b*, c* and h˚ values for the different mucilage types from cactus pear cladodes

Mucilage in all its forms was green, freeze-dried mucilage was the lightest as well as the dullest and appeared yellower. 'Robusta' mucilage was a darker green than the other cultivars in all three of the mucilage types.

It was observed that the green colouration of cladodes were influenced by daytime temperatures, thus the green colour of mucilage became duller during winter months. At close inspection of the data, it was observed that the three *Opuntia ficus-indica* cultivars (OFI) namely Algerian, Morado and Gymno-Carpo, followed similar trends in terms of colour, while *Opuntia robusta* Robusta colour development was different. For OFI, a strong negative correlation was found between the a* and b* values, indicating that the greener the cladodes, the lower the blue colouration, causing a more brilliant green, however when the blue colouration increased, the green colouration decreased, causing a duller green cladode colour. For 'Robusta' there was a more direct link between the colouration changes for both green and blue, preventing the green colouration from appearing overly bright green and rather maintaining a duller "blue-grey" colour throughout the months tested. The green colouration (a* values) between OFI and 'Robusta' showed a negative correlation indicating that the development of green colouration of 'Robusta' cladodes, as observed in the mucilage, is different to that of OFI cladodes. However, the blue colouration (b* values) showed a weak correlation indicating that similar factors influence the development of the duller (blue) colouration. The factors that would have an influence on cladode (mucilage) colour have not been studied. Overall, freeze-dried mucilage was the brightest and had the yellowest tones of the different types of mucilage, while the reconstituted mucilage appeared to be slightly greener than the other types of mucilage. All mucilage was green, freeze-dried mucilage was a lighter green colour, 'Robusta' mucilage appeared darker green while OFI mucilage was a more yellow, straw-green colour.

6.4.4 Freeze-dried mucilage

6.4.4.1 Bulk density and Tapped density

Powder particles are important to characterise in order for the user to understand it, as changes in the stability and properties of the powder could have consequences in the manufacturing stage or in the final food product. The physical properties of a powder enable the product developer to understand the solubility, dissolution rate and the stability of the powder. The rate at which a powder dissolves has a direct influence on its food application possibilities, methods of inclusion in formulations and eventually the bioavailability of the product in the human body (Particle Analytical, 2007). Bulk density is defined as the mass (g) of uncompressed powder divided by the volume (ml) that is taken up in a tarred cylinder. The bulk density depended on the density of the powder as well as the inter-particle spaces (arrangement of particles) that formed when the powder was poured carefully into a cylinder without compacting it. The bulking properties of a powder depended on how the sample was treated, prepared and stored. The interactions between particles indicate the interactions and interferences in the ability of the powder to flow. (Particle Analytical, 2007).

Table 43: The effect of month and cultivar on bulk density (g/ml) on freeze-dried mucilage powder from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.546)**
Algerian	0.47	0.45	0.65	0.65	0.68	0.65	**0.59**
Morado	0.33	0.48	0.48	0.56	0.52	0.63	**0.50**
Gymno-Carpo	0.29	0.60	0.58	0.58	0.63	0.68	**0.56**
Robusta	0.35	0.68	0.48	0.60	0.61	0.61	**0.56**
Month Means (p < 0.001)	**0.36^{a}**	**0.55^{b}**	**0.55^{b}**	**0.60^{b}**	**0.61^{b}**	**0.64^{b}**	**0.55** **C x M: (p = 0.322)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

The bulk density (g/ml) according to cultivar and month obtained is presented in Table 43. The bulk density ranged from 0.294 to 0.682 g/ml (Table 43). The overall average was 0.55 g/ml. It was observed that the bulk density was significantly lower ($p < 0.001$) in February (0.36 g/ml) than all the other months up to August. The values steadily increased to August although there were no statistical differences detected between the other months. There were no statistical differences between cultivars and between the months of April to August. The interaction between months of harvest and cultivar was not significant.

These results were comparable to results in other studies where the bulk density was determined as 0.68 ±0.02 g/ml and 0.69 ±0.01 g/ml (Gebresamuel and Gebre-Mariam, 2012). León-Martínez et al. (2010) found bulk density values between 0.593 ± 0.033 g/ml and 0.769 ± 0.014 g/ml for differently treated powders. Ayadi et al. (2009) reported bulk density values of 0.703 and 0.647 g/cc for spiny and spineless cladode powders. When Sáenz et al. (2012) tested purified cladode powders, the bulk density values ranged between 0.35 and 0.45 g/ml. Cladode flour that was dried using a spray dryer at eleven different temperatures, speeds and flow rates had bulk density values between 0.570 and 0.769 g/ml. It was noted that the bulk density values decreased as the drying temperature increased (León-Martínez et al., 2010).

Powder particles with higher moisture content would tend to have higher bulk and weight as the particles would be bigger (León-Martínez et al., 2010). Therefore, the lower the moisture content and the smaller the particles, the higher the quality of the dried powder. It was established that there were no differences between cultivars therefore no cultivar could be singled out as having the best bulk density, however February mucilage powders had the best bulk density properties.

When the powder was compressed by mechanically tapping it with intent to compact the particles, the volume recorded was expressed as tapped density (Particle Analytical, 2007). Tapped density (g/ml) according to cultivar and month is presented in Table 44. Tapped density ranged from 0.357 g/ml for February (‘Gymno-Carpo’) to 0.833 g/ml for July and August (‘Algerian’). The overall average was 0.677 g/ml (Table 44). As was the finding in the bulk density values, there was a rise (significantly different) in results from February to August as seen in Table 44. February average values were significantly lower ($p < 0.001$) than values for April, June, July and August, however these months did not differ significantly from each other. There were no significant differences between cultivars. The interaction between months of harvest and cultivar was not significant. Dried mucilage powder had tapped density values of 0.85 ± 0.02 g/ml in *Opuntia ficus-indica* and 0.81 ±0.03 in *Opuntia stricta* (Gebresamuel and Gebre-Mariam, 2012).

Table 44: The effect of month and cultivar on tapped density (g/ml) on freeze-dried mucilage powder from cactus pear cladodes

	Feb	**April**	**May**	**June**	**July**	**Aug**	**Cultivar Means (p = 0.576)**
Algerian	0.60	0.57	0.79	0.75	0.83	0.83	**0.73**
Morado	0.47	0.57	0.58	0.70	0.64	0.79	**0.62**
Gymno-Carpo	0.36	0.75	0.67	0.71	0.75	0.79	**0.67**
Robusta	0.48	0.79	0.59	0.73	0.75	0.77	**0.68**
Month Means (p = < 0.001)	**0.48^{a}**	**0.67^{b}**	**0.66^{ab}**	**0.72^{b}**	**0.74^{b}**	**0.80^{b}**	**0.68** **C x M: (p = 0.219)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
☐ = All means

The compressibility index and Hausner ratio reflects the ability of a powder to settle (Particle Analytical, 2007). The flow character of a powder is measured according to the compressibility index and the Hausner ratio. These ratios indicate the ability of a product to settle and flow and is rated from excellent to very, very poor. The compressibility index and Hausner ratios (Table 45) were used to estimate the flow characteristics of dried mucilage powders.

Table 45: The flow character of freeze-dried mucilage powders from cactus pear cladodes as indicated by the Compressibility index (%) and the Hausner ratio

Compressibility index (%)	Flow character	Hausner ratio
1-10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
>38	Very, very poor	>1.6

In a powder that is free-flowing, the bulk and tapped densities will be closer in value while in powders that flow poorly, the interparticular interactions are bigger, thus bigger differences will be observed between bulk and tapped densities.

The flow character of the 24 dried powders was determined and is presented in Table 46. According to Table 46, none of the mucilage powders were excellent, very poor or very very poor. In fact, the flow characteristics ranged between poor (February,’ Morado’ and ‘Robusta’) and good (April ’Robusta’, May ‘Gymno-Carpo’ and June ‘Algerian’). Passable powders were February ‘Algerian’, August ‘Algerian’ and ‘Morado’. The freeze-dried mucilage obtained for April, May, June and July only had ratings of either good or fair (Table 46). Mostly though, the flow characteristics were indicated at being either passable or fair. The overall average of the compressibility index was 18.79, while overall average for the Hausner ratio was 1.23. These ratings indicate that the powders would handle with relative ease when it was poured or measured out. According to the Compressibility index, 'Algerian' had the lowest ratings on average (18.65), while 'Morado' had the highest ratings (average 20.5). The best powders were obtained in May (average 17.7), while February powders received the lowest rating overall (24). The overall flow characteristics of mucilage powder could be rated as being fair (Table 46).

Table 46: The flow character of 24 freeze-dried mucilage powder samples from four different cultivars cactus pear cladodes harvested over six months as indicated by the Compressibility index and the Hausner ratio

Month	Cultivar	Compressibility index	Hausner ratio	Flow character
February	Algerian	22	1.28	Passable
	Morado	30	1.42	Poor
	Gymno-Carpo	18	1.21	Fair
	Robusta	27	1.37	Poor
April	Algerian	20	1.25	Fair
	Morado	16	1.19	Fair
	Gymno-Carpo	20	1.25	Fair
	Robusta	14	1.16	Good
May	Algerian	17	1.21	Fair
	Morado	17	1.21	Fair
	Gymno-Carpo	13	1.16	Good
	Robusta	18	1.22	Fair
June	Algerian	13	1.15	Good
	Morado	20	1.26	Fair
	Gymno-Carpo	19	1.24	Fair
	Robusta	18	1.22	Fair
July	Algerian	18	1.22	Fair
	Morado	19	1.23	Fair
	Gymno-Carpo	17	1.20	Fair
	Robusta	18	1.23	Fair
August	Algerian	22	1.28	Passable
	Morado	21	1.26	Passable
	Gymno-Carpo	14	1.16	Good
	Robusta	20	1.26	Fair
Average		18.79	1.23	Fair

The correlation was attained in order to observe the relationship between bulk and tapped density. The results are presented in Figure 79.

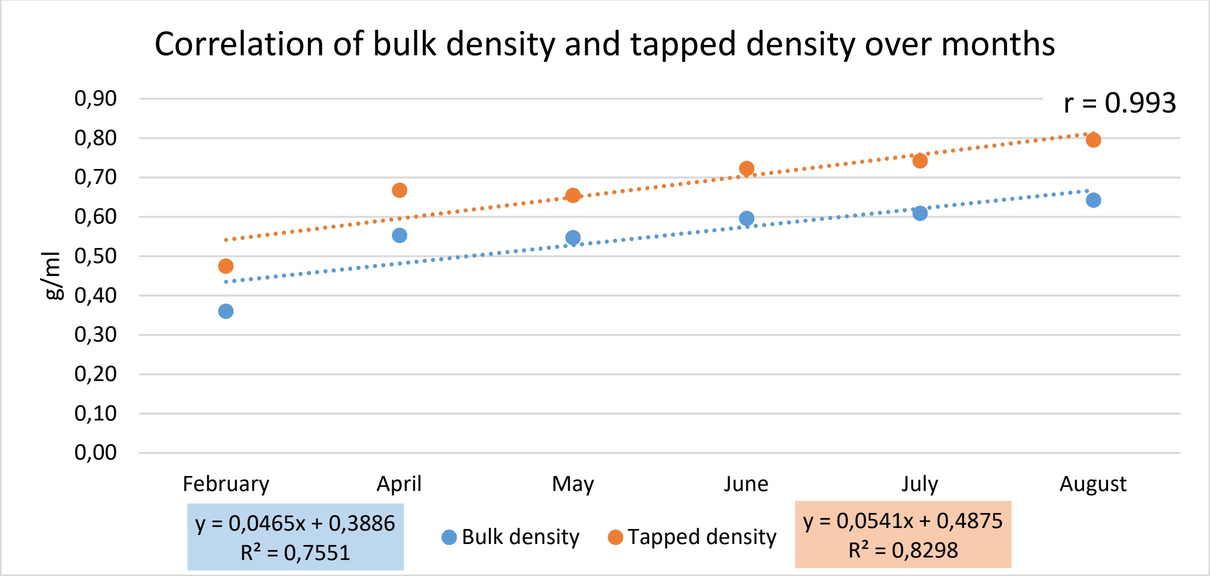


Figure 79: Correlation of bulk and tapped density of freeze-dried mucilage powders from cactus pear cladodes over months

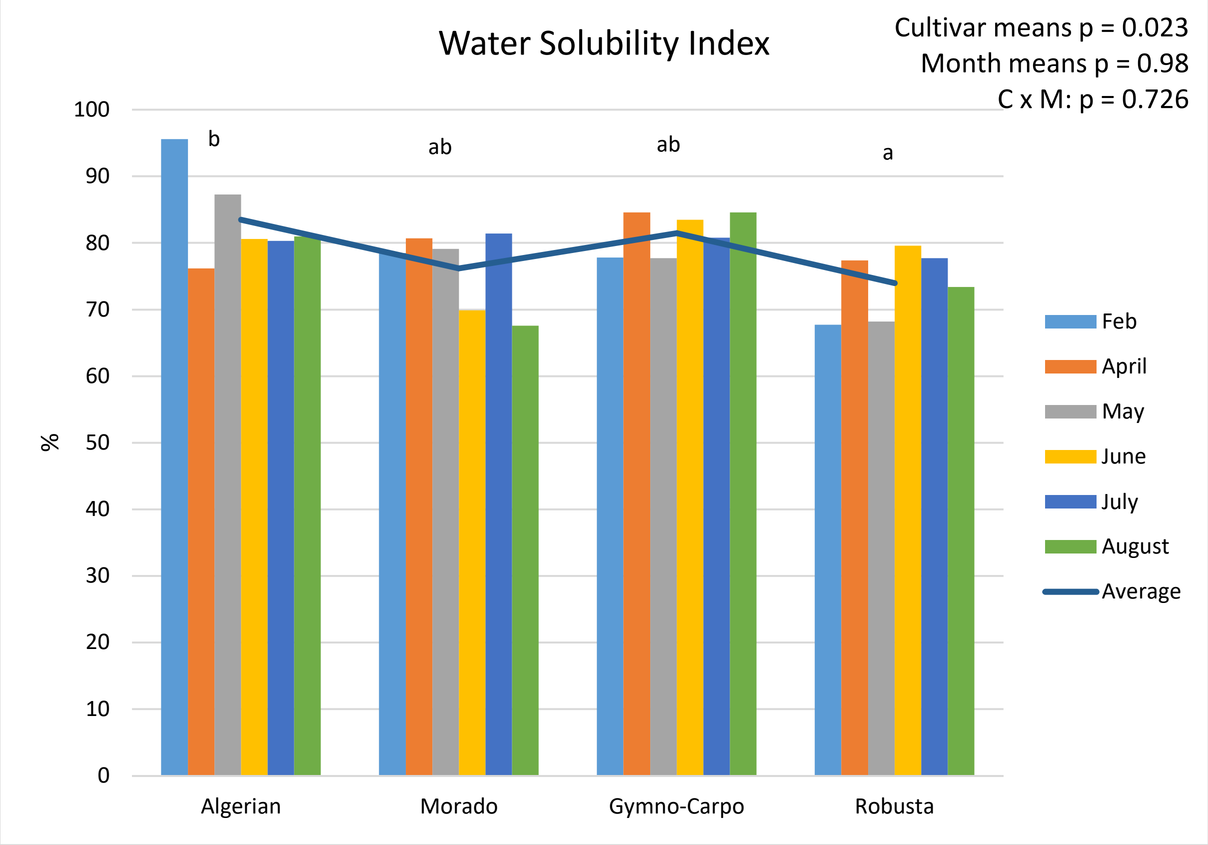
In Figure 79 an almost perfect correlation between bulk and tapped density was observed (r= 0.993). The general tendencies in both bulk and tapped density to gradually and continuously increase over months are observed in Figure 79. These findings were in agreement with the results obtained for Compressibility index and the Hausner ratio that indicated that the general quality of freeze-dried mucilage powder were the poorest in February, yet increased in April and subsequent months. The flow characteristics of freeze-dried powders were either passable or fair, indicating that the powders would be handled with relative ease when used.

6.4.4.2 Water-related properties

An analysis of the water-related properties of freeze-dried mucilage powders was undertaken as it was paramount to understand and predict the hydrophilic tendencies of mucilage powder. It was imperative at this stage in the study to compare the influence of the different cultivars and harvesting months in order to determine the quality and functionality of the obtained freeze-dried powder.

6.4.4.2.1 Water Solubility Index (WSI)

Compounds in dried particles are only available for reactions and absorption when the molecules are separated and surrounded by water, thus dissolved. Dissolution of a substance is dependent on the particle size as well as the actual solubility of the molecules in water (Particle Analytical, 2007). According to Ayadi et al. (2009), the water solubility index (WSI) is related to the presence of soluble molecules therefore it gives an indication of the solubility of a substance in water. The WSI (%) according to cultivar and month obtained is presented in Figure 80. As mucilage is essentially a soluble fibre, higher WSI values were expected in mucilage powders than in cladode flour. Gebresamuel and Gebre-Mariam (2012) determined solubility in *Opuntia ficus-indica* and *Opuntia stricta* mucilage powders and reported WSI between 49.63% for *Opuntia stricta* and 75.19% for *Opuntia ficus-indica*. Sepúlveda et al. (2007) determined WSI to be between 69.79 and 78.88 % for mucilage obtained and dried by different methods. Ayadi et al. (2009) found that spiny cladode flour only had a solubility index of 5.23%, while the spineless cladode flour had a much higher WSI of 27.84%. It was speculated that highly soluble sugars and starch could be responsible for the difference between dried mucilage from spineless and spiny cladodes.



Different superscripts above bar-sets for each cultivar indicates significant differences between cultivars

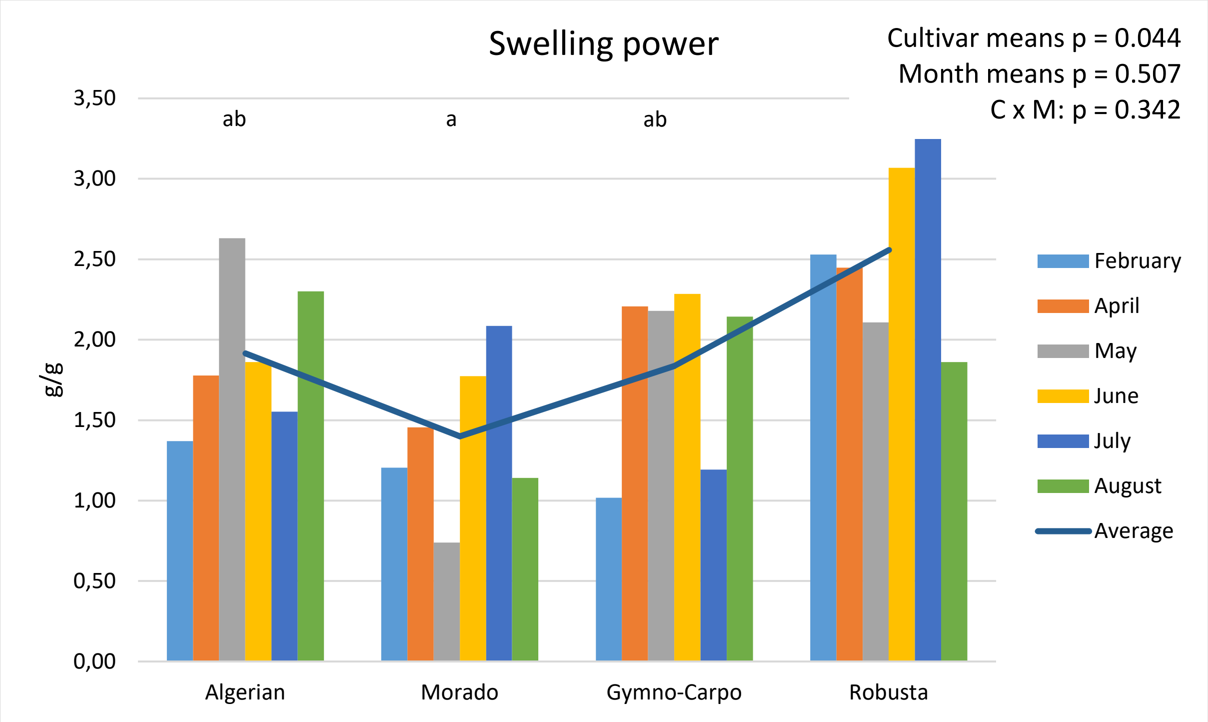
Figure 80: The effect of month and cultivar on the water solubility index (WSI) (%) of freeze-dried mucilage from cactus pear cladodes

In July the mucilage had the highest % WSI and in August the lowest (Figure 80) although no significant differences were found between months. In terms of cultivars, ‘Algerian’ and ‘Robusta’ differed significantly (p = 0.023) as ‘Algerian’ powders had the highest WSI (83.5%) while ‘Robusta’ (74%) powders had the lowest ability to dissolve (Figure 80). The interaction between month of harvest and cultivar was not significant. Overall though, the average of

78.8% WSI in mucilage powder was higher than values found by other authors who studied cladode flour (Ayadi et al., 2009; Sepúlveda et al., 2007).

6.4.4.2.2 Swelling power

Samia El-Safy (2013) explained swelling power as the hydration capacity of a dried powder. The eating quality of cladode flour in baked foods could be correlated to the retention of water of the swollen dried cladode granules. The swelling power results contrasted the WSI (as discussed above); as WSI is calculated from the undissolved fraction of the powder that remained behind as solids. Starch is insoluble, yet when heated in water it becomes soluble as the amylose leaches out of the starch granule. In this study, the mucilage powder was never exposed to heat and it is therefore accepted that the starch is insoluble. Therefore, the insoluble fibre, starch and other constituents that are not water soluble and that remained behind in the centrifuge tube as solids were being tested for its capacity to swell. The swelling power (g/g) according to cultivar and month obtained is presented in Figure 81.



Different superscripts above bar-sets for each cultivar indicates significant differences between cultivars

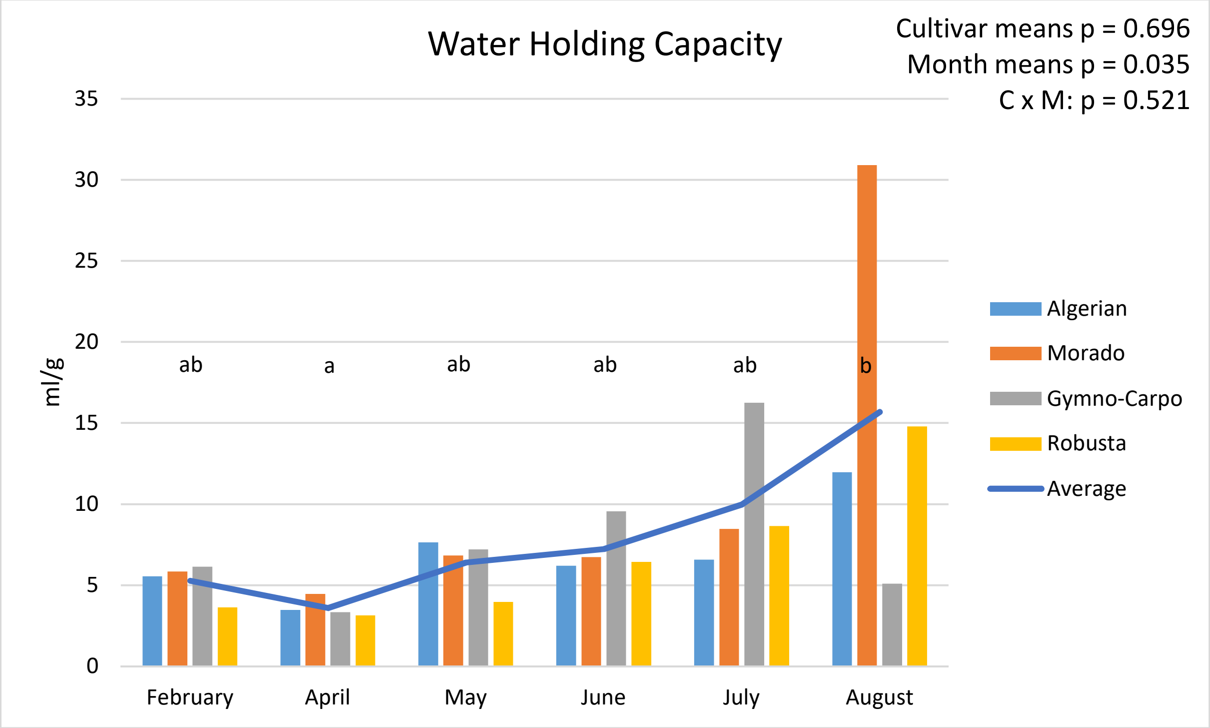
Figure 81: The effect of month and cultivar on swelling power (g/g) of freeze-dried mucilage from cactus pear cladodes

In Figure 81 it is apparent that 'Morado' mucilage had significantly lower swelling power (p = 0.044) than 'Robusta'. No significant differences were observed between 'Algerian', 'Gymno-Carpo' and 'Morado'. In the previous analysis (section 6.2.6.2.1), 'Robusta' had the lowest WSI (Section 6.2.6.2.1), yet had high ability to swell. In fact, June and July 'Robusta' mucilage demonstrated the best ability to swell and hold water. Furthermore, 'Robusta' had the highest values over all the months studied (Figure 81). There were no significant differences observed between months. The interaction between months of harvest and cultivar was not significant. The overall average of 2.19 g/g was lower than was found by other authors (Ayadi et al., 2009; Gebresamuel and Gebre-Mariam, 2012; Sáenz et al., 2012; Samia El-Safy, 2013; Sepúlveda et al., 2013). This could generally be attributed to the amount of powder that actively dissolved into the solvent as mucilage consists of soluble fibre molecules that would have dissolved in the supernatant and was therefore not involved in this analysis.

The swelling power of mucilage powder from two species of cactus pear (*Opuntia ficus-indica* and *Opuntia stricta*) was between 3.15 and 9.01 ml/g (Gebresamuel and Gebre-Mariam, 2012). Samia El-Safy (2013) reported the swelling power value of 6.0 g/g in cladode flour. Sepúlveda et al. (2013) found 5.86 ml/g for unpeeled and 6.49 ml/g for flour produced from peeled cladodes. Sáenz et al. (2012) determined 7.02 to 8.27 ml/g in purified cladode flour. Ayadi et al. (2009) explained that swelling ability of flours were related to the polysaccharide content and determined the average of spiny and spineless cladode flour at 7.5 cm^3/g. As mucilage molecules are soluble, lower swelling power values were expected in mucilage powders than what was obtained in cladode flour as was reported in the literature.

6.4.4.2.3 Water holding capacity (WHC)

Water holding capacity (WHC) is the ability of a matrix of molecules to entrap large amounts of water to such an extent that the loss of the water is prevented (Fenema, 1996). The ability of a flour or powder to hold water is important in terms of the textural quality of products (Samia El-Safy, 2013). Traynham et al. (2007) stated that WHC is important in food systems as it demonstrates the ability of protein matrix to absorb and retain entrapped water against gravity. The WHC obtained according to cultivar and month is presented in Figure 82.



Different superscripts above bar-sets for each month indicates significant differences between months

Figure 82: The effect of month and cultivar on water holding capacity (ml/g) of freeze-dried mucilage from cactus pear cladodes

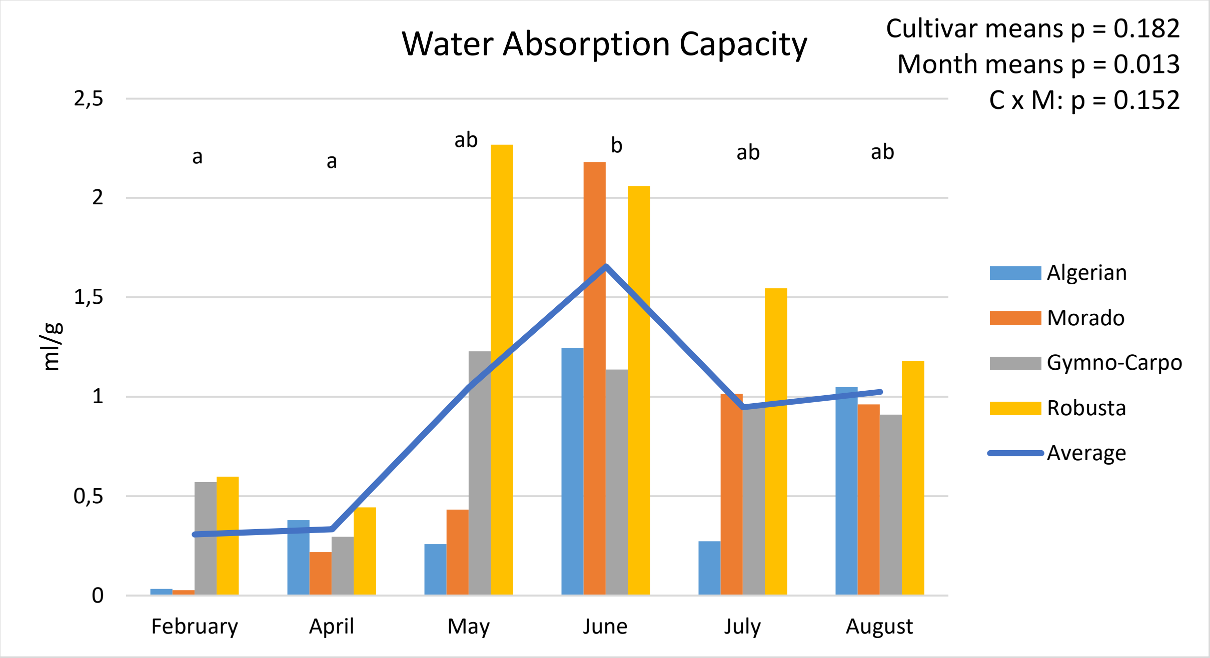
On average August mucilage had significantly high WHC (p = 0.035), mostly because of the exceptionally high WHC seen in ‘Morado’ (Figure 82), while ‘Robusta’ and ‘Algerian’ also had high WHC in August. In April, the WHC was significantly lower (p = 0.035) than August. There were no statistical difference between cultivars, although ’Morado’ had the highest WHC, especially in June and July. ‘Algerian’ and ‘Robusta’ both had lower average WHC values, however both these cultivars showed increased capacities in the August mucilage. ‘Morado’ was the best cultivar for WHC (Figure 82). The interaction between month of harvest and cultivar was not significant. The overall average for freeze-dried mucilage was 8.04 ml/g which compared well with values obtained in other studies. According to Ayadi et al. (2009), WHC is the ability of dried powder to associate with water and may be related to the fibre content. It was reported that spiny cladodes contained higher total dietary fibre, insoluble fibre and soluble fibre than spineless cladodes. Spiny cladode flour also demonstrated the highest water holding capacity with 6.74 g/g in comparison with 2.97 g/g for spineless cladode flour. This difference was explained as the result of the high fibre content of spiny cladodes, as flour with high WHC usually contain more hydrophilic constituents (Ayadi et al., 2009). López-Cervantes et al. (2011) agreed that the structure and the composition of fibre in powders affect its water holding capacity. Their results ranged between 6.48 and 14.44 g/g. It was claimed that the drying conditions as well as the fibre source affected the WHC. Cladode flour demonstrated exceptional WHC and could be used to improve the texture of foods such as baked goods and desserts (López-Cervantes et al., 2011; Samia El-Safy, 2013). Sepúlveda et al. (2013) obtained values of 10.22 and 10.08 g/g for whole and peeled cladode flour respectively while Sáenz et al. (2012) reported 5.41 to 5.86 g/g after different purifying treatments.

6.4.4.2.4 Water absorption capacity (WAC)

Water absorption capacity (WAC) is the ability of the powder particles to retain water in its structure when water is withdrawn. The ability to retain water is strongly related to particle size as water molecules bind more tightly to finer particles (Fenema, 1996). Kaur and Singh (2005) stated that WAC depends on the hydrophilic constituents that may be present in the flour, such as the polysaccharides or the protein content. It was mentioned before that mucilage molecules readily dissolve into water, therefore it was expected that the WAC would be low.

The WAC according to cultivar and month is presented in Figure 83. Mucilage powder from June had significantly high WAC (1.66 ml/g) compared to February and April (0.31 ml/g) WAC values (Figure 83). The WAC values ranged from February 'Morado' (lowest WAC at 0.027 ml/g) to May 'Robusta' (highest WAC at 2.267 ml/g). 'Algerian' had the lowest average value (0.54 ml/g) while 'Robusta' was in general the cultivar with the highest WAC (1.35 ml/g) (Figure 83). No statistically proven differences between cultivars were observed. The overall average (0.89 ml/g) was lower than WAC determined by Samia El-Safy (2013) namely between 4.5 ml/g and 7.1 ml/g in cladode flour. Sáenz et al. (2012) tested four different purified cladode powders and obtained an average value that was lower than that of this study (0.24 g/g).

In conclusion, mucilage is the water soluble fraction that had been separated from the solids during the extraction process. Thus the results for WSI was higher in mucilage powders than for cladode flour while the swelling power, WHC and WAC was lower.

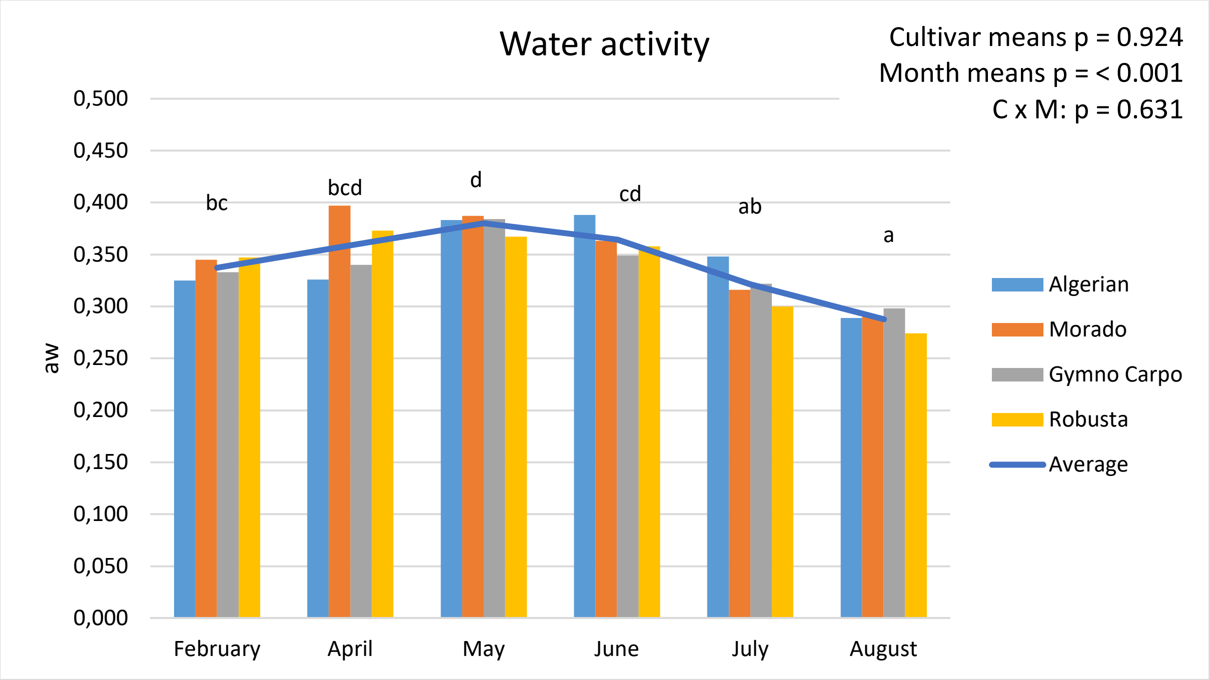


Different superscripts above bar-sets for each month indicates significant differences between months

Figure 83: The effect of month and cultivar on water absorption capacity (ml/g) of freeze-dried mucilage from cactus pear cladodes

6.4.4.2.5 Water activity

Water activity signifies the safety of a product as it is used to predict the spoiling rate of food. Thus it suggests the shelf life duration of a food product. Water activity and water content is not similar; even when the water content of a product is high, water molecules could be restricted from serving as a source of nourishment to pathogenic microorganisms by being bound to the substances dissolved in the water (Fenema, 1996). Therefore, the lower the a_w, the longer the shelf life of a product. The shelf life stability of foods depends on the a_w and is classified according to the perishability rate. Foods with a_w of 0.8 to 1 are highly perishable, 0.6 to 0.8 are intermediate foods such as jams, sweets and dried fruit, while < 0.5 are dried products such as pasta, biscuits and powders that have no microbial reproduction and thus extremely low spoiling rates (Fenema, 1996). Water activity data obtained is presented in Figure 84.



Different superscripts above bar-sets for each month indicates significant differences between months

Figure 84: The effect of month and cultivar on water activity of freeze-dried mucilage of freeze-dried mucilage from cactus pear cladodes

Water activity (Figure 84) ranged from 0.27 in August ('Robusta') to 0.39 in June ('Algerian'). The overall average was 0.34. Water activity was significantly lower in August ($p < 0.001$) than in all the other months. There were no significant differences between cultivars and the interaction between months of harvest and cultivar was not significant. The presence of dissolved substances such as protein and carbohydrates would render water molecules unavailable for other reactions, thereby impacting the water activity of mucilage. Nevertheless, at these levels reported in Figure 84 (average of 0.34), there could be no or very little degradative activities along with no yeast and mould growth. Therefore, at a_w levels below 0.5, the freeze-dried mucilage is safe and cannot support microbial reproduction (Fenema, 1996). It could be speculated that a_w continually declined from June to August as a result of the low rainfall in the winter months (Figure 3).

Ayadi et al. (2009) determined water activity at 0.762 for spiny and 0.767 for spineless cladode powders and it was determined as 0.53 in cladode flour (Sáenz et al., 2010). No literature was found on water activity levels in mucilage powders.

Mucilage powders were more soluble than cladode flour, as the water solubility index was 78.8%. The swelling power (average of 2.19 g/g) of undissolved powders was lower than results that were reported by other authors, however more powder dissolved in the water compared to cladode flour. The water holding capacity (8.04 ml/g) compared well with values obtained for cladode flour in other studies, indicating its high potential for use for commercial products containing high water contents. Water absorption capacity of mucilage powders was lower (0.89 ml/g) than reported in cladode flour, however lower values could be expected with soluble substances (such as mucilage). The water activity of mucilage powders showed that there would be very little or no degradative activities and no yeast or mould growth. Thus, the water-related properties of mucilage powder showed potential for commercial use for products of high water content.

6.4.4.3 Oil related properties

6.4.4.3.1 Oil absorption capacities (OAC)

Oil absorption capacity (OAC) is a parameter to evaluate hydrophobic nature of particles and can be related to emulsifying properties (López-Cervantes et al., 2011). Fat absorption properties are important as they lead to the positive mouth feel and better flavour of food products (Samia El-Safy, 2013). The OAC as determined in mucilage powders according to cultivar and month obtained is presented in Table 47.

Table 47: The effect of month and cultivar on oil absorption (g/g) capacities of freeze-dried mucilage from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.950)**
Algerian	4.49	4.08	2.82	3.46	3.03	3.40	**3.55**
Morado	5.13	3.49	4.19	3.04	2.86	3.50	**3.70**
Gymno-Carpo	4.99	3.26	2.63	2.80	3.35	3.73	**3.46**
Robusta	4.55	2.95	3.81	2.96	3.18	3.67	**3.52**
Month Means (p < 0.001)	**4.79^b**	**3.45^a**	**3.36^a**	**3.07^a**	**3.11^a**	**3.58^a**	**3.56** **C x M: (p = 0.732)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

In February the mucilage demonstrated significantly high (p < 0.001) oil absorption capacity (4.79 g/g) compared to all the other months (Table 47). June freeze-dried mucilage had the lowest oil absorption capacity (3.07 g/g) although May, April, July and August values were not significantly different. In terms of cultivar, 'Morado' had the best capacity overall while 'Gymno-Carpo' had the lowest average although no significant differences were observed for cultivars (Table 47). The interaction between months of harvest and cultivar was not significant. With the overall average at 3.56 g/g, it may be stated that freeze-dried mucilage powders analysed in this study had high oil absorption capacity compared to results in studies that focused on cladode flour. The interaction between months of harvest and cultivar was not significant.

Samia El-Safy (2013) determined the OAC for cladode flour as 2.8 ml/g and Sepúlveda et al. (2013) reported results of 1.48 and 1.34 g/g for whole and peeled cladode flour respectively. Sáenz et al. (2012) determined values between 1.29 and 1.95 g/g for four differently purified powders and Ayadi et al. (2009) reported 1.29 g/g for spiny and 1.31 g/g for spineless cladode flour. Fernández-López et al. (2010) showed oil absorption to be just over 2 g/g and concluded that these values are too low and that cladode flour would be unsuitable for emulsification purposes.

6.4.4.3.2 Oil Holding Capacity (OHC)

The oil holding capacity (OHC) measured the retention ability of the swollen mucilage particles that were precipitated in the centrifuge tube after the supernatant oil was separated carefully using a pipette and the centrifuge tube inverted for 12 hours. High OHC of flour particles is associated with binding flavour in food systems where optimum oil absorption is required, for example the production of sausages. Flour particles that bind and hold oils contain the hydrophobic constituents and amino acids that associate with lipids (Ayadi et al., 2009; Chandra, 2013; Kaur and Singh, 2005). The OHC as was determined in mucilage powders according to cultivar and month obtained is presented in Table 48.

Table 48: The effect of month and cultivar on oil holding capacity (ml/g) of freeze-dried mucilage from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.835)**
Algerian	1.52	1.44	0.65	1.12	0.63	1.26	**1.10**
Morado	1.89	0.52	1.21	0.75	1.29	1.72	**1.23**
Gymno-Carpo	1.72	1.03	0.82	1.05	1.29	1.50	**1.24**
Robusta	1.99	1.07	1.08	1.05	1.49	1.25	**1.32**
Month Means (p = 0.003)	**1.78^{b}**	**1.02^{a}**	**0.94^{a}**	**0.99^{a}**	**1.18^{ab}**	**1.43^{ab}**	**1.22** **C x M: (p = 0.732)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between cultivar and month.
☐ **= All means**

According to Table 48, February mucilage powders had significantly higher ability to hold oil than April, May and June mucilage powders (p = 0.03). An increase in OHC was observed from June to August and was not significantly different than April and May powders. The values ranged from February 'Robusta' (1.99 g/g), which had the highest values, to 0.52 g/g for April

‘Morado’, which had the lowest oil holding capacity (Table 48). Robusta was the cultivar with the best holding capacity while ‘Algerian’ had the lowest holding capacity on average over six months. However, statistically there were no differences detected between cultivars. The overall average for freeze-dried mucilage powder was 1.22 g/g (Table 48). The interaction between the months of harvest and cultivars was not significant.

A correlation between OHC and OAC (Figure 85) showed a remarkable similarity in terms of trends over months. A non-linear regression was observed between the two measurements as both decreased from February to April and remained relatively unchanged until July and increased in August. According to Ayadi et al. (2009) the OHC of cladode powders compared with the values found in flour produced from vegetable sources such as wheat, apples, peas and carrots. Very similar values were reported for spiny and spineless cladode powder at 1.29 and 1.21 g fat/g (dry basis).

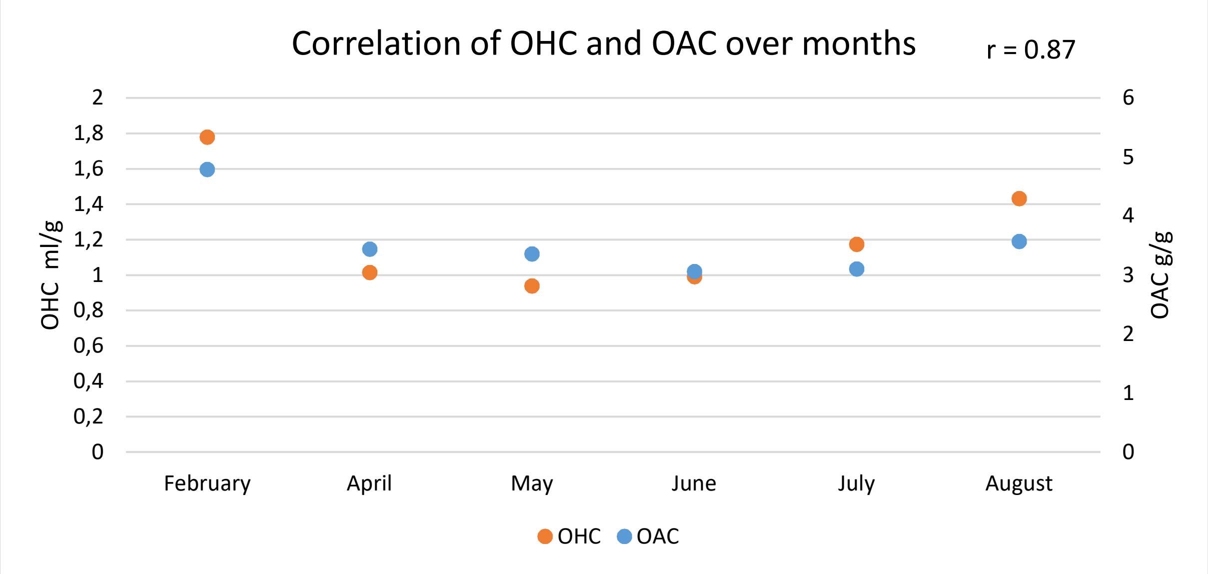


Figure 85: Correlation between oil holding- and oil absorption capacities of freeze-dried mucilage from cactus pear cladodes over months

It could be derived from these results that the freeze-dried powders in this study certainly demonstrate oil absorption and holding capabilities. The use of mucilage powders in high fat food products are therefore recommended. Mucilage powders are also recommended for use in emulsions although Kaur and Singh (2005), who studied chickpea flour, commented that OAC values below 2 g/g are too low to impart sufficient emulsifying capacity to food products.

6.4.4.4 Functional properties

6.4.4.4.1 Foaming ability

A foam is formed when gas bubbles are dispersed into a fluid or any semi-solid substance. A condition for foam formation is the presence of a surface active ingredient. Such an ingredient is usually a soluble protein that holds the water phase in position and creates relatively stable air pockets (Fenema, 1996).

The possibility that mucilage may have foaming abilities have been mentioned but as far as the researchers are aware of have not been proven. Nevertheless, the ability of mucilage to act as stabilizer in food products (similar to gelatin), may offer an explanation of its apparent ability to act as a foaming agent. Its function as stabilizing agent has been described before. Majdoub et al. (2001) speculated that the presence of protein may impart stabilizing properties to mucilage and Iturriaga et al. (2009a) mentioned that it would be possible that the protein fraction could provide mucilage with stabilizing properties. In fact, Iturriaga et al. (2009a) compared cactus mucilage to commercial hydrocolloids and found that both native and freeze-dried mucilage had outstanding potential as stabilizing agents similar to those of xanthan and guar gums. When mucilage was added to egg foams, it was found that it produced an increase of the foam stability (Espinosa, 2002, cited in Sáenz et al. 2004). Yet there is very little literature available on the foaming abilities of mucilage.

The foaming ability and stability of a 5% concentration reconstituted mucilage solution was determined according to cultivar and month and is presented in Table 49.

Table 49: The effect of month and cultivar on % foam layer after indicated time lapse of freeze-dried mucilage from cactus pear cladodes

Month	Cultivar	10 s (%)	2 min (%)	12 min (%)	22 min (%)	32 min (%)	42 min (%)	102 min (%)
Feb	Algerian	15	10	0	0	0	0	0
	Morado	20	20	0	0	0	0	0
	Gymno-Carpo	15	0	0	0	0	0	0
	Robusta	15	5	0	0	0	0	0
April	Algerian	5	7.5	0	0	0	0	0
	Morado	15	2.5	0	0	0	0	0
	Gymno-Carpo	15	10	5	0	0	0	0
	Robusta	20	20	2.5	0	0	0	0
May	Algerian	15	5	5	2.5	2.5	2.5	0
	Morado	5	2.5	5	0	0	0	0
	Gymno-Carpo	20	2.5	0	0	0	0	0
	Robusta	50	10	7.5	2.5	0	0	0
June	Algerian	10	2.5	0	0	0	0	0
	Morado	22.5	5	15	5	5	0	0
	Gymno-Carpo	20	17.5	15	5	5	2.5	1.25
	Robusta	20	5	2.5	0	0	0	0
July	Algerian	10	0	0	0	0	0	0
	Morado	15	0	0	0	0	0	0
	Gymno-Carpo	20	0	0	0	0	0	0
	Robusta	20	0	0	0	0	0	0
Aug	Algerian	0	0	0	0	0	0	0
	Morado	0	0	0	0	0	0	0
	Gymno-Carpo	0	0	0	0	0	0	0
	Robusta	100	5	0	0	0	0	0

Statistical analysis was done on the above data (Table 49) obtained at 10 s, 2 min and 12 min in order to ascertain the significant differences for months and cultivars. After 12 min, most of the foam had dissipated and foam only remained in samples from May ‘Algerian’ (2.5%) until 42 min, May ‘Robusta’ for 22 min and June ‘Gymno-Carpo’ (5 to 1.25%) for 102 min (Table 49).

The foaming stability was determined after a 10 s time lapse and was presented according to cultivar and month in Table 50.

Table 50: The effect of month and cultivar on % foam layer after 10 sec of freeze-dried mucilage from cactus pear cladodes

Cultivar	**February**	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.04)**
Algerian	15.00	5.00	15.00	10.00	10.00	0.00	**9.17^{a}**
Morado	20.00	15.00	5.00	22.50	15.00	0.00	**12.92^{a}**
Gymno-Carpo	15.00	15.00	20.00	20.00	20.00	0.00	**15.00^{a}**
Robusta	15.00	20.00	50.00	20.00	20.00	100.00	**37.50^{b}**
Month Means (p = 0.978)	**16.25**	**13.75**	**22.50**	**18.13**	**16.25**	**25.00**	**18.65** **C x M: (p = 0.523)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between cultivar and month
☐ **= All means**

After an only 10 second lapse after agitation of the mucilage solution in order to form a foam, only an average of 18.65 % of the volume was foamy. No significant differences were detected between months, although it was observed that August mucilage had the highest % foam. 'Robusta' samples had noticeably more foam volume (significantly different) than the other cultivars after only 10 seconds. The interaction between month of harvest and cultivar was not significant. The foaming stability was again determined after a 2 min time lapse according to cultivar and month and is presented in Table 51.

Table 51: The effect of month and cultivar on % foam layer after 2 min of freeze-dried mucilage from cactus pear cladodes

Cultivar	**February**	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.834)**
Algerian	10.00	7.50	5.00	2.50	0.00	0.00	**4.17**
Morado	20.00	2.50	2.50	5.00	0.00	0.00	**5.00**
Gymno-Carpo	0.00	10.00	2.50	17.50	0.00	0.00	**5.00**
Robusta	5.00	20.00	10.00	5.00	0.00	5.00	**7.50**
Month Means (p = 0.114)	**8.75**	**10.00**	**5.00**	**7.50**	**0.00**	**1.25**	**5.42** **C x M: (0.318)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between cultivar and month.
☐ **= All means**

Two minutes after agitation, the average foam volume that remained intact was 5.42%. No significant differences or interaction were detected between months or cultivars, although it was observed that April mucilage and 'Robusta' tended to have better foaming abilities. The foaming stability determination was repeated after 12 min according to cultivar and month and is presented in Table 52.

Table 52: The effect of month and cultivar on % foam layer after 12 min of freeze-dried mucilage from cactus pear cladodes

Cultivar	**February**	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.755)**
Algerian	0.00	0.00	5.00	0.00	0.00	0.00	**0.83**
Morado	0.00	0.00	5.00	15.00	0.00	0.00	**3.33**
Gymno-Carpo	0.00	5.00	0.00	15.00	0.00	0.00	**3.33**
Robusta	0.00	2.50	7.50	2.50	0.00	0.00	**2.08**
Month Means (p = 0.028)	**0.00^{a}**	**1.88^{ab}**	**4.38^{ab}**	**8.13^{b}**	**0.00^{a}**	**0.00^{a}**	**2.40** **C x M: (0.427)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between cultivar and month.
☐ **= All means**

After 12 minutes (Table 52), the average volume of foam that remained was only 2.4%. In fact, most of the samples had no visible foam. There were no significant differences detected between cultivars, although 'Algerian' had the lowest foam volume. June mucilage had significantly higher foaming ability after 12 minutes (8.13%) than February, July and August mucilage. All the months except for June did not differ significantly from each other. The interaction between month of harvest and cultivar was not significant.

According to observations, July and August mucilage had no foaming abilities. 'Robusta' was the cultivar with the best ability to foam, in fact it was observed that August 'Robusta' foamed very well initially while the foam bubbles were large and unstable and disappeared instantly. June 'Gymno-Carpo', had better foaming abilities as well as better foaming stability as a small amount of foam was still observed after 100 minutes. It was concluded that overall, the freeze-dried mucilage at 5% concentration did not have foaming ability. It was thus concluded from these results that freeze-dried mucilage had no meaningful ability to foam. Higher concentration of the mucilage may increase the ability to foam, as increased mucilage content will increase the protein content as well as the viscosity of the mucilage.

It was remarked that the foam ability of tigernut flour was related to the protein and the lipid content when Oladele and Aina (2007) reported foam capacity of 10.28% for yellow tigernut flour and 11.07% for brown tigernut flour. The foam stability was between 50 and 60% after 1 hour. Kaur and Singh (2005) investigated the foaming capacity of chickpea flour and proved that foaming capacity progressively increased with increased concentration. The chickpea flour had very high foam ability and good stability after 120 min.

Freeze-dried powders at a 5% concentration had no foaming stability. It is recommended that higher concentrations of mucilage should be tested. Another suggestion would be to add high protein substances such as milk-, egg- or soya powders. Addition of glycerol or gelatine to mucilage solutions may be investigated to increase the foaming ability of mucilage through the strengthening of the film forming ability of mucilage. Edible films have been produced using mucilage and glycerol before.

6.4.4.4.2 Emulsifying capacity

Emulsions are formed when one liquid is divided into small droplets (± 1 μm) and dispersed in another liquid. Any one of the liquids must be water-based and the other liquid be oil-based. For the two liquids to be emulsified permanently, an emulsifying agent is necessary and a substantial amount of energy in the form of vigorous agitation is the last requirement for a permanent emulsion to be produced (Fenema, 1996).

The ability of mucilage to have emulsifying properties has been a topic of discussion by different researchers. Especially when a protein fraction was discovered in mucilage, emulsifying ability of mucilage became a matter of contention. Majdoub et al. (2001) proved the presence of protein in mucilage and speculated that it would indicate that mucilage had the unique property of having the capacity to lower the oil-in-water interfacial tension and hence to create the emulsifying capacity in oil-in-water emulsions. In a study done by Iturriaga et al. (2009a) mucilage showed potentially good emulsification properties as well. Native mucilage (2% solution) reportedly had 90.77% emulsion capability and 90% stability while freeze-dried mucilage (10% solution) was given 100% values in both tests. There are three ways in which an emulsion can become unstable, creaming (a layer forms on top due to higher density), flocculation (droplets aggregate) and coalescence (droplets merge) however, these destabilization processes tend to take place concurrently (Fenema, 1996). Emulsion stability is assessed by monitoring the emulsified or creamed layer (% creaming) over time at ambient temperatures (Ercelebi and Ibanoglu, 2009).

The emulsifying ability and creaming stability of a 5% concentration reconstituted mucilage solution was determined according to cultivar and month and is presented in Table 53.

Table 53: The effect of month and cultivar on % emulsified layer in relation to the original volume of freeze-dried mucilage from cactus pear cladodes

Month	Cultivar	20 s (%)	1 min (%)	2 min (%)	5 min (%)	10 min (%)	20 min (%)	60 min (%)	24 h (%)
	Algerian	91.3	94.5	92.6	91.4	88.5	86.4	83.8	82.1
	Morado	97.3	95.9	94.8	92.1	89.7	87.2	83.9	82.1
	Gymno-Carpo	88.6	93.2	90.3	88.9	87.6	85.7	82.5	85.5
Feb	Robusta	100.0	100.0	100.0	100.0	100.0	99.5	97.0	90.7
	Algerian	94.1	96.3	90.1	86.7	85.0	82.4	58.7	71.0
	Morado	91.4	93.4	91.4	90.2	88.4	86.4	83.8	82.1
	Gymno-Carpo	97.1	91.8	87.5	84.6	82.9	81.9	57.3	70.3
April	Robusta	100.0	100.0	100.0	100.0	100.0	100.0	99.0	90.9
	Algerian	100.0	100.0	100.0	99.0	98.0	97.4	93.8	88.8
	Morado	100.0	100.0	100.0	100.0	100.0	100.0	99.5	94.0
	Gymno-Carpo	100.0	100.0	100.0	100.0	99.5	99.0	97.5	92.8
May	Robusta	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.0
	Algerian	100.0	100.0	100.0	99.5	98.5	97.5	93.8	87.2
	Morado	100.0	100.0	100.0	100.0	100.0	100.0	99.0	89.9
	Gymno-Carpo	100.0	100.0	99.5	99.0	98.0	95.9	91.7	84.7
June	Robusta	100.0	100.0	100.0	100.0	100.0	100.0	98.5	86.8
	Algerian	100.0	100.0	100.0	99.5	99.0	97.5	92.8	86.0
	Morado	99.7	99.9	99.9	99.9	99.9	99.5	97.5	87.7
	Gymno-Carpo	100.0	100.0	100.0	99.9	99.0	97.5	92.3	89.2
July	Robusta	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.0
	Algerian	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.0
	Morado	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.0
	Gymno-Carpo	100.0	100.0	100.0	100.0	100.0	98.0	92.9	87.1
Aug	Robusta	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.0

Statistical analyses were done on the data obtained at 20 s, 5 min, 60 min and 1 hour in order to ascertain the significant differences for months and cultivars. It was observed that the emulsified (creamed) layer volume was between 91.1% and 100% after 10 seconds and was relatively stable over 24 hours (70.3% - 98%). The average emulsified volume after 24 hours was 87.79%.

The volume of the emulsified layer was determined after a 20 s time lapse to determine the creaming stability of the emulsions and is presented according to cultivar and month in Table 54.

Table 54: The effect of month and cultivar on % emulsified layer after 20 sec of freeze-dried mucilage from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.585)**
Algerian	91.25	94.12	100.00	100.00	100.00	100.00	97.56
Morado	97.33	91.43	100.00	100.00	99.71	100.00	98.08
Gymno-Carpo	88.57	97.14	100.00	100.00	100.00	100.00	97.62
Robusta	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Month Means (p = 0.014)	**94.29^{a}**	**95.67^{a}**	**100.00^{b}**	**100.00^{b}**	**99.93^{b}**	**100.00^{b}**	**98.31** **C x M: (p = 0.231)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between cultivar and month.
☐ = All means

The overall average emulsifying capacity was 98.31% 20 s after homogenization (Table 54). Mucilage from February and April (94.29 and 95.67%) had significantly lower emulsifying capacities than the other months (May to August). Although no significant differences between cultivars were observed, all ‘Robusta’ samples were fully emulsified (100%). The interaction between month of harvest and cultivar was not significant. The creaming stability was determined after time lapse of 5 min and is presented according to cultivar and month in Table 55.

Table 55: The effect of month and cultivar on % emulsified layer after 5 min of freeze-dried mucilage from cactus pear cladodes

Cultivar	**Feb**	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.412)**
Algerian	91.36	86.69	99.00	99.50	99.50	100.00	**96.01**
Morado	92.09	90.16	100.00	100.00	99.90	100.00	**97.02**
Gymno-Carpo	88.93	84.57	100.00	98.99	99.90	100.00	**95.40**
Robusta	100.00	100.00	100.00	100.00	100.00	100.00	**100.00**
Month Means (p = 0.002)	**93.09^{ab}**	**90.35^{a}**	**99.75^{b}**	**99.62^{b}**	**99.82^{b}**	**100.00^{b}**	**97.11** **C x M: (p = 0.282)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between cultivar and month.
☐ = All means

Five minutes after homogenization the overall average volume of emulsified fluid was 97.11%. There were no significant differences between cultivars, while ‘Robusta’ emulsions remained at 100% (Table 55). April samples had significantly lower emulsification capacity at

90.35% than May (99.75%), June (99.62%), July (99.82%) and August (100%). The interaction between month of harvest and cultivar was not significant.

The creaming stability determination was repeated after 60 min by measuring the emulsified layer and comparing it to the original volume. The results are presented according to cultivar and month and is presented in Table 56.

Table 56: The effect of month and cultivar on % emulsified layer after 60 min of freeze-dried mucilage from cactus pear cladodes

Cultivar	**February**	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.171)**
Algerian	83.80	58.71	93.84	93.84	92.82	100.00	**87.17**
Morado	83.94	83.80	99.50	99.00	97.49	100.00	**93.95**
Gymno-Carpo	82.50	57.26	97.47	91.66	92.31	92.86	**85.68**
Robusta	96.98	99.00	100.00	98.50	100.00	100.00	**99.08**
Month Means (p = 0.013)	**86.81^{ab}**	**74.69^{a}**	**97.70^{b}**	**95.75^{b}**	**95.65^{b}**	**98.21^{b}**	**91.47** **C x M: (p 0.182)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between cultivar and month.
☐ **= All means**

The overall average emulsified volume that remained after 60 minutes decreased somewhat to 91%. There were no significant differences between the cultivars, 'Robusta' showed the highest emulsification capacity (99.08%), followed by 'Morado' (93%). The May to August mucilage had higher values (significantly different) compared to April mucilage samples. February and April mucilage powders did not differ significantly from each other. The interaction between month of harvest and cultivar was not significant.

After 24 hours, the creaming stability determination was repeated once again according to cultivar and month and is presented in Table 57.

Table 57: The effect of month and cultivar on % emulsified layer after 24 hrs of freeze-dried mucilage from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.126)**
Algerian	82.10	71.05	88.81	87.21	85.99	95.00	**85.03**
Morado	82.13	82.10	93.97	89.90	87.69	92.00	**87.96**
Gymno-Carpo	85.46	70.31	92.82	84.73	89.17	87.08	**84.93**
Robusta	90.72	90.91	98.00	86.80	98.00	95.00	**93.24**
Month Means (p = 0.011)	**85.10^{ab}**	**78.59^{a}**	**93.40^{b}**	**87.16^{ab}**	**90.21^{ab}**	**92.27^{b}**	**87.79** **C x M: (p = 0.321)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between cultivar and month.
☐ **= All means**

The overall average volume of the emulsified layer that had remained intact after 24 hours was 87.79% (Table 57). There were no significant differences between cultivars and again 'Robusta' had the highest values (average 93.24%). May and August mucilage emerged as the months with significantly high creaming stability (93.40% and 92.27%) compared to April mucilage creaming stability (78.59%) (Table 57). The interaction between month of harvest and cultivar was not significant.

In the emulsification ability and creaming stability (% creaming) test it was observed that the emulsion was immediately and completely formed in most of the samples. In terms of months, in February and April, 'Algerian', 'Morado' and 'Gymno-Carpo' mucilage did not fully emulsify after the specified homogenization time and the creaming stability of those emulsions deteriorated quickly. Overall, April mucilage showed the lowest emulsification ability. August mucilage had the highest emulsification ability as the emulsions from all cultivars remained at 100% for the first 10 minutes.

'Robusta' had the highest emulsification ability as the emulsions were completely stable for 20 minutes. 'Algerian' and 'Gymno-Carpo' emulsions had the lowest emulsification ability as it consistently showed the first signs of increased separation. 'Morado' emulsions showed higher emulsification ability and consequently was the cultivar that performed second best after 'Robusta'.

Reconstituted mucilage at 5% concentration had promising emulsification ability and stability, thus had the potential to be applied for emulsions. Mucilage is recommended to be tested for commercial use as emulsifying agent in high fat food products. August 'Robusta' showed the most promising results. For the emulsion ability of mucilage powders to be understood and justified, the protein content should be determined.

6.5 Conclusion

Bigger and heavier cladodes did not yield the most mucilage. All mucilage was green, freeze-dried mucilage was a lighter green colour, Robusta mucilage appeared darker green while OFI mucilage was a more yellow, straw-green colour.

The water and oil-related properties of mucilage powder showed potential for commercial use for high-water and high-fat food products. Reconstituted mucilage at a 5% concentration had no foaming stability while it had promising emulsification ability and stability.

February mucilage had lower viscosity, lower pH and higher conductivity than other months while Robusta had the highest oil absorption capacity and emulsifying capabilities, yet yielded the least mucilage. For the selection of cultivars, Algerian (best WSI and highest yield), Morado (best WHC) and Robusta (best swelling power and emulsification capacity) performed better than Gymno-Carpo. Overall though, none of the cultivars or months emerged as producing exceedingly better functional properties or superior quality native or freeze-dried mucilage.

When % yield was correlated to daytime temperatures of months, a positive correlation was found (r = 0.7), therefore it was theorised that the extractability of mucilage was higher (viscosity of the mucilage was lower) during the warmer, higher rainfall months (February and April) as was indicated in this study. The moisture and solids content of cladodes did not differ according to rainfall and remained constant despite the abundance of water during summer months. However, these results may be different in other years with other climatic conditions. It is recommended that this study be repeated over the same growth stages in order to fully understand the influence of the climate on the mucilage yield.

The pH however, increased significantly over months in both native and reconstituted mucilage, while the conductivity decreased significantly. A strong negative relationship was observed between pH and conductivity (r= -0.998). The increased concentration of ions (high conductivity) observed in February, thus occurred as a consequence of the increased availability of hydrogen atoms, caused by the higher acidity levels, supplied by organic acids and sugar acids.

Overall though, none of the cultivars or months emerged as producing exceedingly better functional properties or superior quality native or freeze-dried mucilage. The final selection would have to rely on the outcome of the chemical analysis.

Chapter 7

Chemical characterization of freeze-dried powdered mucilage from four selected cactus pear cultivars

Abstract

In an effort to select the optimal cultivar and month of harvest, chemical analysis of freeze-dried mucilage from four cactus pear cultivars (Algerian, Morado, Gymno-Carpo and Robusta) was conducted. Several chemical analyses including scanning electron microscopy (SEM) were employed. The gross energy content was low (10.24 kJ/g DM), while the moisture content (15.76%) was relatively high but acceptable. The protein content for the three O. ficus-indica cultivars was not significantly different (2.74-3.23 g 100 g^{-1}*) while it was significantly higher in 'Robusta' (4.79 g 100 g*$^{-1}$*) (O. robusta). The average fat content was 0.61% while Robusta had the highest fat content (0.9%). The most important fatty acids were palmitic acid (14.03%), stearic acid (2.95%), oleic acid (19.12%) and linoleic acid (62.14%). PUFA were higher (± 60%) than SFA and MUFA, while omega-6 predominated over omega-3 fatty acids. The total carbohydrates were (62.48%) while the sugar contents (sucrose 14.64 mg/g, glucose 198.51 mg/g and fructose 37.52 mg/g) and the starch contribution (5.9 g/100 g) were low. Insoluble fibre (NDF 2.1 g/kg and ADF 1.43 g/kg) values proved the purity of mucilage as a soluble fibre. Mucilage is a good source of calcium (3.01%), potassium (2.75%) and magnesium (2.48%), phosphorous (109.46 mg/kg), manganese (188.38 mg/kg), iron (22.1 mg/kg), and zinc (24.54 mg/kg). Many large crystals were observed in O. ficus-indica cultivars, yet fewer and smaller calcium oxalate crystals were found in Robusta cladode tissue, furthermore none were observed in freeze-dried mucilage. Organic acid content for O. robusta was different from the O. ficus-indica cultivars, furthermore the malic acid content (average 2.62 g/L) decreased continuously from February to August. This analysis assisted in revealing the valuable potential of freeze-dried mucilage powders obtained in this study.*

7 Chemical characterization of freeze-dried powdered mucilage from four selected cactus pear cultivars

7.1 Introduction

Researchers are expediting new natural ingredients in the pursuit of innovative functional foods that provide extended nutritional benefits besides the normal nourishment that a food product already offers (Bensadón et al., 2010). Mucilage in its native and freeze-dried form is an ideal candidate for such innovative functional food products. Mucilage could enhance the nutritional content of food products while replacing ingredients that add undesired and unnecessary kilojoules and chemicals. Mucilage has been reported to have high nutritional value, dietary fibre and antioxidant value (Ayadi et al., 2009; Hernández-Urbiola et al., 2010; Sáenz, 2002, 2000, 1996, Sáenz et al., 2012, 2004; Stintzing and Carle, 2005)

Understanding the chemistry as well as obtaining knowledge of the functional properties should lead to better selection of appropriate gums for specific products. Mucilage occurs in large enough volumes in the cladodes that it could be extracted and processed for further use. Mucilage could be utilized in food products as it is a highly complex polysaccharide that dissolves and absorbs water and forms viscous non-Newtonian liquids (Sáenz, 2000).

The composition of cladodes are highly dependent on the age of the plant, the season and the climatic conditions. Nutritional and chemical data will therefore vary even within species or cultivars (Stintzing and Carle, 2005). Younger and older cladodes have different nutritional profiles and soil fertilization also play an important role in the levels of certain minerals (Stintzing and Carle, 2005). Young cladodes are generally considered to be superior in nutritional value to mature cladodes (López-Cervantes et al., 2011).

The cactus pear is a Crassulacean Acid Metabolism (CAM) plant that evolved to survive in arid conditions by keeping the stomata in the leaves shut during the day to prevent evaporation and consequently opening the stomata at night to continue the carbon fixation pathway and thus to collect carbon dioxide. Therefore the cactus pear has adaptations that equip it to thrive during extremely hot days with high irradiance levels, cold nights and poor soil. In fact, cactus pears frequently grow in deserts as a consequence of CAM (Salisbury and Ross, 1992). All cells absorb O_2 and release CO_2, but for the process of respiration to occur, substrates such as starch, fructans, sucrose, fats and other products such as organic acids and proteins are required (Salisbury and Ross, 1992). These components constitute the nutrients that are essential in the daily diets of humans and animals alike (Whitney and Rolfes, 2015).

Mucilage in its native and freeze-dried form is an ideal candidate for use in functional food products as it could enhance the nutritional content of food products (De Wit et al., 2015) while the nutritional and functional properties (chapter 6) could be useful when it replaces unhealthy ingredients such as fats and oils. Mucilage contains virtually no fat (Bensadón et al., 2010; Hernández-Urbiola et al., 2011; Rodríguez-Garcia et al., 2007; Sáenz, 1997; Stintzing et al., 2005), while it would considerably increase the soluble fibre (Bensadón et al., 2010; Hernández-Urbiola et al., 2011; Sáenz et al., 2012; Stintzing and Carle, 2005), minerals (Fuentes-Rodriguez et al., 2013; Hernández-Urbiola et al., 2011; Sáenz, 2002) and antioxidants (Bensadón et al., 2010; Brahmi et al., 2011; Gallegos-Infante et al., 2009; Sánchez et al., 2014; Stintzing and Carle, 2005) in food products.

An in depth chemical analysis of the South African cactus pear cultivars has not been conducted and is necessary to provide proof that mucilage in both its native or freeze-dried forms originating from the South African cactus pear cultivars, are equal to those cultivars that have been analysed in other countries. In confirming that these mucilage products fulfil the requirements, value could be added to the by-products of the crop. Additional applications would render it more useful as a crop. The advantage of mucilage to the food industry is that it is natural, healthy and inexpensive (Medina-Torres et al., 2000).

During 2014, mucilage from eight cultivars that consisted of seven *Opuntia ficus-indica* and one *Opuntia robusta* cultivars were investigated over two growth stages (post-harvest stage and the dormant stage). Cladodes from the cactus pear cultivars Algerian, Morado, Meyers, Ficus-Indica, Gymno-Carpo, Tormentosa, Turpin and Robusta (*O. robusta*) were included in the study. Subsequently, the cultivars were narrowed down to four (Algerian, Morado, Gymno-Carpo and Robusta) while the best growth stage for mucilage extraction was identified as the dormant stage.

In the previous discussion (chapter 6), the mucilage according to cultivar and month (time of harvest) was investigated for optimal and functional properties. In chapter 6, 'Algerian' was identified as the cultivar that would consistently produce the highest mucilage yield. Yet no specific month of harvest could be identified, though the post-harvest months (February to April) were recommended to collect the cladodes bound for mucilage extraction. Mucilage powders had promising functional properties and dissolved more readily in water than cladode flour. For cultivar, Algerian (best WSI), Morado (best WHC) and Robusta (best swelling power and emulsification capacity) performed better than Gymno-Carpo while for month, February and August mucilage had the most positive attributes. Overall though, none of the cultivars or months emerged as producing significantly better functional properties or superior quality native or freeze-dried mucilage. The final selection would have to rely on the outcome of the current chemical analysis.

7.2 Materials and methods

7.2.1 *Sample collection*

The sample collection and sample preparation methods were described in chapter 6 (Section 2.1). Freeze-dried mucilage powders from four cultivars (Algerian, Morado, Gymno-Carpo and Robusta) and six months (February, April, May, June, July, August) (24 samples) were employed for the chemical investigations that follow.

All the values in this work is presented on “as is” basis on the freeze-dried mucilage powder that was obtained. It is believed that the dried product obtained in this study would, if ever, be marketed and sold in its present form and as such, the label on the product would contain the necessary information concerning the specific content. It is thus stated emphatically that none of the data has been expressed on a “dry matter” basis.

7.2.2 *Scanning Electron Microscopy (SEM)*

7.2.2.1 *Preparation of fresh cladode samples for SEM investigation*

Scanning electron microscopy (SEM) was conducted on fresh cladodes in order to inspect the typical cell structures of cladodes and occurrences of oxalate crystals for observable differences between cultivars (Algerian, Morado, Gymno-Carpo and Robusta). For SEM on fresh cladodes, samples were collected from fresh cladodes of the four cultivars in February 2016.

Freshly cut samples were placed into the primary fixative fluid (glutaraldehyde) for several hours before it was washed for 10 minutes in a sodium phosphate buffer at pH 6.8 – 7.2. The secondary fixation was done in osmium tetroxide for 1-2 hours. The samples were then rinsed twice with the same buffer for 5-10 minutes. Dehydration was done in an ethanol series of 30, 50, 70 and 95% for 10-30 minutes in each stage. The final dehydration stage of 100% was for two changes of 15-30 minutes each. The last step was to fully dehydrate the samples using a critical point dryer at 31.5˚C for 1.5 hours. The dehydrated fresh cladode samples were mounted on a metal stub and a sputter coater was used to coat it with gold (Glauert, 1974). The four prepared cultivar samples were inspected using a Joel scanning electron microscope.

7.2.2.2 Preparation of freeze-dried mucilage powder for SEM investigation

In order to observe dissimilarities between freeze-dried mucilage samples of which the cladodes were harvested in different months, only samples from the first month (February) and the last month (August) were included in the SEM investigation. The images obtained were inspected for differences between the four cultivars (Algerian, Morado, Gymno-Carpo and Robusta) and between months (February and August) in the particle size and form and for the presence and type of crystals.

Thoroughly dried powders did not require any preparation and the samples were mounted onto metal stubs and gold was sputtered on to coat the sample. The four February and four August samples (eight samples) were inspected using a Joel scanning electron microscope.

7.2.2.3 Particle size of mucilage powder

Dried particles were measured during the SEM analysis and approximate sizes (μm) were calculated to indicate average sizes of particles of the particular cultivar.

7.2.3 Gross energy

The Leco Automatic Calorimeter AC-500 series Oxygen Combustion Vessel was used for determining the Gross energy. Thoroughly mixed samples (0.35 g) were weighed into crucibles which were connected with fuse wires to two electrodes. The crucibles were carefully placed in the electrode under the fuse wire and placed in the bomb cylinder. The bomb was placed in the gas filling site and filled to 3000 kPa (30 atmospheres) pressure with oxygen. After it cooled down, it was placed into the calorimeter. The energy content appeared as MJ/kg. No calculations were necessary as the instrument provided the correct reading (Leco, 2001).

7.2.4 Moisture content

The moisture content of the mucilage was determined during the fatty acid analysis by subtraction [100% - % lipid - % Fat Free Dry Matter (FFDM)] and expressed as % moisture (w/w) per 100 g mucilage (Folch et al. 1957).

7.2.5 Crude protein content

The protein content was determined by thermal combustion. Nitrogen (N) was determined in a Leco Nitrogen analyser (Leco, 2001) and crude protein (CP) was calculated automatically by the Leco machine by multiplying the N content by a factor of 6.25 (AOAC, 2000).

For the determination of crude protein, 0.6 g of the freeze-dried samples were weighed into glass pill vials (in duplicate) and dried overnight (100˚C). Between 0.09 and 0.25 of the dry matter was placed in foil containers. The correct masses of samples were determined and verified and placed in the carousel of the Leco machine. After 3 minutes the sample had been analysed and the crude protein values were displayed as g CP/kg DM.

7.2.6 SDS – PAGE: Protein characterization

SDS-PAGE was performed on a C.B.S. DSG-200-02 instrument at 16 ˚C. The protocol described by Laemmli (1970) was used. Sample preparation of mucilage was done as follows: 0.2 g of each sample was weighed into a 1.5 ml Eppendorf tube. Buffer I (0.3 g SDS, 5 ml β-mercaptoethanol, 0.444 g TrisHCL, 0,266 g Tris-base in 10 ml deionized water) (85 ml) was added and it was vortexed thoroughly and placed in a 80˚C water bath for 5 min. Buffer II [100 ml 60 mM TrisHCL (pH 8.0), 80 g glycerol (1 ml = 1.25 g)] was added together with 2 g of SDS and 0.02 g Bromophenolblue. It was thoroughly vortexed again and placed back into the water bath at 80˚C for 10 min. It was then centrifuged at 13000 rpm for 10 min. A 100 ml of supernatant was mixed with 100 ml TD: TS mix in a new Eppendorf tube. It was vortexed again and placed in a water bath (80˚C, 5 min) (Laemmli, 1970). The samples (25 ml) were loaded into the gel matrix together with the Precision plus protein all blue (Bio-Rad catalogue # 161-0373) standard. Samples included the selected months (February, May, June and August) of all four cultivars (Algerian, Morado, Gymno-Carpo and Robusta). These samples were chosen to ascertain differences in proteins between cultivars and over selected months.

7.2.7 Total lipid and fatty acids analysis

7.2.7.1 Total extractable fat content (EFC) and fat free dry matter (FFDM)

Total lipid content from mucilage samples was quantitatively extracted, according to the method of Folch et al, (1957), using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene, was added at a concentration of 0.001% to the chloroform: methanol mixture. A rotary evaporator was used to dry the fat extracts under vacuum and the extracts were also dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as moisture adsorbent.

Total extractable fat content (EFC) was determined gravimetrically and expressed as % fat (w/w) per 100 g mucilage (Folch et al, 1957).

The fat free dry matter (FFDM) content was determined by weighing the residue on a pre-weighed filter paper, used for Folch extraction, after drying. By determining the difference in weight, the FFDM could be expressed as % FFDM (w/w) per 100 g mucilage (Folch et al, 1957). The extracted fat was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at -20°C until further analysed.

7.2.7.2 Fatty acids analysis (FAME) (% of total fatty acids)

Approximately 10 mg of total lipid (from Folch extraction) was transferred into a Teflon-lined screw-top test tube by means of a disposable glass Pasteur pipette. Fatty acids were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14% boron trifluoride in methanol (Park and Goins, 1994).

Fatty acid methyl esters (FAME) were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, chrompack CPSIL 88 (100 m length, 0.25 μm ID, 0.2 μm film thickness). Column temperature was 40-230°C (hold 2 minutes; 4°C/minute; hold 10 minutes). Fatty acid methyl esters in hexane (1μl) were injected into the column using a Varian CP 8400 Autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas (Park and Goins, 1994).

Galaxy Chromatography Data System Software recorded the chromatograms. FAME samples were identified by comparing the relative retention times of FAME peaks from samples with those of standards obtained from SIGMA (189-19). Fatty acids were expressed as the relative percentage of each individual fatty acid as a percentage of the total of all fatty acids present in the sample.

7.2.7.3 Fatty acids ratios

The following fatty acid contents and ratios were calculated by using the fatty acid data: total saturated fatty acids (SFA), total mono-unsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA) and PUFA/SFA ratio.

7.2.8 Total carbohydrate content

Carbohydrates consist of monosaccharides, disaccharides, oligosaccharides and polysaccharides. Total carbohydrates include sugars, starch and fibre. Determining the total carbohydrate content is possible when the total moisture, protein, fats and ash contents are known, thus the carbohydrate content (%) was determined accordingly.

$$Total\ carbohydrates\ (\%) = 100\% - moisture\ (\%) - protein\ (\%) - fats\ (\%) - ash\ (\%)$$

7.2.8.1 Starch analysis

Native starch in freeze-dried mucilage was determined according to the UV-method assay by Boehringer Mannheim / R-Biopharm Enzymatic Bioanalysis/ Food Analysis (BOEHRINGER MANNHEIM /R-BIOPHARM Enzymatic BioAnalysis, 2016a). Freeze-dried mucilage had to be pre-treated before the starch analysis in order to convert it to a soluble form. It was pre-treated by adding 20 ml dimethylsulfoxide (DMSO) and 5 ml hydrochloric acid (8 M) was added to one gram of homogenized and filtered (0.2 mm pore diameter sieve) samples and incubated for 30 minutes at 60˚C. It was cooled, 50 ml distilled water added and the pH adjusted to pH 4-5 using sodium hydroxide (5 M) under vigorous shaking. It was transferred to a 100 ml volumetric flask, filled up to mark and the sample was taken from the top of the solution by means of a pipette.

According to the instructions of the UV-method for the determination of starch in foodstuffs Cat. No. 10 207 748 035, the reagents (solution 1 & 2 and suspension 3) were prepared and the procedure was followed. A reagent blank and samples were pipetted into 1.00 cm light path (Elisa plate) mixed and incubated for 15 minutes at 55-60˚C, before the addition of solution 2 (1 ml) and redistilled water (1 ml). The absorbance was read using a microplate reader before the reaction was started by adding suspension 3. It was mixed for 15 minutes and the second absorbance reading was done.

The following formula was used for the calculation of starch content:

$$\Delta A = (A2 - A1)\ sample - (A2 - A1)\ reagent\ blank$$

$$c\ (\mu mol) = \frac{V\ x\ MW}{\varepsilon\ x\ D\ x\ V\ x\ 1000}\ x\ \Delta A\ (g/l)$$

Where:
V = final volume (ml)
v = sample volume
MW = molecular weight of the substance to be assayed (g/mol) (for starch 162.1 g/mol)
D = light path (cm)
ε = extinction coefficient of NADPH

The starch content μmol/g was converted to g/100 g.

7.2.8.2 Sucrose/D-Glucose/D-Fructose analysis

The sugars namely sucrose, D-glucose and D-fructose were determined in freeze-dried mucilage according to the standard assay procedure by Boehringer Mannheim / R-Biopharm Enzymatic Bioanalysis/Food Analysis (BOEHRINGER MANNHEIM /R-BIOPHARM Enzymatic BioAnalysis, 2016b). Firstly, the freeze-dried samples were prepared by boiling 1 g powder at 80˚C for 15 min in 80% ethanol. According to the instructions of the UV-method for the determination of sucrose/D-Glucose/D-fructose in foodstuffs Cat. No. 10 716 260 035, the reagents (solutions 1 & 2 and suspension 3 & 4) were prepared and the procedure instructions were followed:

Freeze-dried mucilage (tested for sucrose, glucose and fructose) samples (10 μl) were pipetted into 1.00 cm microplate wells together with the blank samples. Solution 1 (20 μl)) was added to sucrose samples and mixed. It was incubated for 5 min at 35 ˚C before the addition of solution 2 (100 μl) and redistilled water in amounts indicated. The absorbance was read (A1) after 3 min. Suspension 3 was added (2 μl) and after 15 min was read again (A2). Suspension 4 was added and the absorbance read for the third time (A3).

Glucose content is determined before and after the enzymatic hydrolysis of sucrose while fructose is determined afterwards. The absorbance differences were determined as follow:

$$\Delta A = (A2 - A1)\ sample - (A2 - A1)\ blank$$

ΔA Sucrose = ΔA glucose (from the sucrose sample)- ΔA glucose (from the glucose sample). Fructose is determined by the absorbance differences of A3- A2 (both blank and sample). The general equation for calculating concentrations:

$$c = \frac{V\ x\ MW}{\varepsilon\ x\ d\ x\ d\ x\ 1000}\ x\ \Delta A\ (g/l)$$

Where:

V = final volume
v = sample volume (ml)
MW = molecular weight of the substance to be assayed
d = light path (1 cm)
ε = extinction coefficient of NADPH in micro plate reader (5.4099)

The values for substitution in the equation for glucose, sucrose and fructose calculations are indicated in Table 58.

Table 58: The values substituted for sucrose, D-glucose and D-fructose calculations

	Final volume **ml**	**Molecular weight** **g/mol**	**Extinction coefficient**	**Light path** **cm**	**Sample volume** **ml**
Glucose	0.302	180.16	5.4099	1	0.01
Sucrose	0.302	342.3	5.4099	1	0.01
Fructose	0.304	180.16	5.4099	1	0.01

Glucose = 1.005 x $\Delta A_{glucose}$ g $litre^{-1}$

$$\frac{1.005}{MW}\ x\ \frac{1000000}{g\ fresh\ weight}\ x\ \Delta Aglucose$$

= $\Delta A_{glucose}$ µmol fresh $weight^{-1}$

Sucrose = 1.91 x $\Delta A_{sucrose}$ g $litre^{-1}$

$$\frac{1.91}{MW}\ x\ \frac{1000000}{g\ fresh\ weight}\ x\ \Delta Asucrose$$

= 557.99 x $\Delta A_{sucrose}$ µmol fresh $weight^{-1}$

Fructose = 1.012 x $\Delta A_{\ fructose}$ g $litre^{-1}$

$$\frac{1.012}{MW}\ x\ \frac{1000000}{g\ fresh\ weight}\ x\ \Delta Afructose$$

= ΔA fructose µmol fresh $weight^{-1}$

The sucrose, D-glucose and D-fructose content µmol/g was converted to mg/g.

7.2.9 Crude fibre

Neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) were determined as described by Goering and Van Soest, (1970) and Roberson and Van Soest, (1981). To determine both neutral and acid detergent fibre, 1 gram of the air-dried and sieved samples (in duplicate) was weighed into glass pill vials and dried overnight. It was transferred to sintered glass crucibles, the weight determined accurately and placed in the hot extraction unit. The applicable acid or neutral detergent solution (100 ml) was added to the samples and boiled for 60 minutes. The enzyme α-amylase was added to the neutral detergent fibre samples. It was filtered with suction and washed three times with hot distilled water after which it was rinsed twice with acetone. Samples were then dried overnight at 100˚C, cooled in a desiccator and weighed accurately. Then the samples were incinerated in a muffle furnace and allowed to cool before removing it, placed in the desiccator for 30 minutes and weighed again. ADF/NDF is expressed as ADF g/kg DM and NDF g/kg DM.

$$ADF\ and\ NDF\ = \frac{RCD - RCA}{original\ sample\ (g)}$$

Where:
RCD = residue (g) in crucible after drying
RCA = residue (g) in crucible after ashing

7.2.10 Chemical minerals

7.2.10.1 Crude ash

Ash was determined using two grams freeze-dried powder of each sample in individually weighed crucibles. It was placed in a furnace capable of maintaining temperature of 600˚C (Hesse, 1971). As minerals are not destroyed by heat, the ash remaining (g) is used to determine the percentage ash of the sample.

$$Ash\ \% = \frac{sample\ after\ incineration\ (g)}{sample\ before\ incineration\ (g)}$$

Ash could also be expressed as g ash/kg DM.

7.2.10.2 Dietary minerals

The mineral analyses were performed on two g of freeze-dried mucilage powder of each sample in duplicate and according to standard methods (The Non-Affiliated Soil Analysis Work Committee, 1990): exchangeable Ca, Mg, K and Na (1 mol dm^{-3} NH_4OAc at pH 7) and extractable Cu, Fe, Mn and Zn (DTPA solution) was determined by atomic absorption. P was determined calorimetrically (430 nm) according to the Venado-Molebdate method (Hesse, 1971).

7.2.11 Organic acids (Chromatographic analysis)

Freeze-dried samples were first pre-treated by boiling 1 g at 80˚C for 15 min in 80% ethanol.

7.2.11.1 HPLC quantification

Samples were diluted 1:4, and centrifuged twice to remove all insoluble matter. Analysis was performed on a Thermo Surveyor HPLC with UV/Vis detection at 202 nm. The analytical column was a BioRad Aminex HPX 87H and the mobile phase was 5 mM H_2SO_4. Flow rate was 0.6 ml/min (Kupiec, 2004).

7.3 Statistical Methods

A one way analysis of variance (ANOVA) procedure (NCSS, 2007) was used to determine the effect of month and cultivar on the chemical properties of mucilage. The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to determine whether significant differences exist between treatment means (NCSS, 2007).

7.4 Results

7.4.1 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is the high resolution scanning of surfaces using electrons in order to provide images with high magnification and remarkable depth in field (Thomas and Ducati, 2014).

7.4.1.1 Scanning electron microscopy on fresh cladodes

A typical leaf is depicted in Figure 86 where the upper epidermis, the palisade and spongy mesophyll is illustrated (Schoolbag.info, 2016). Similar cell structures and arrangements are observed in the SEM images of the cladode cross sections that follow.

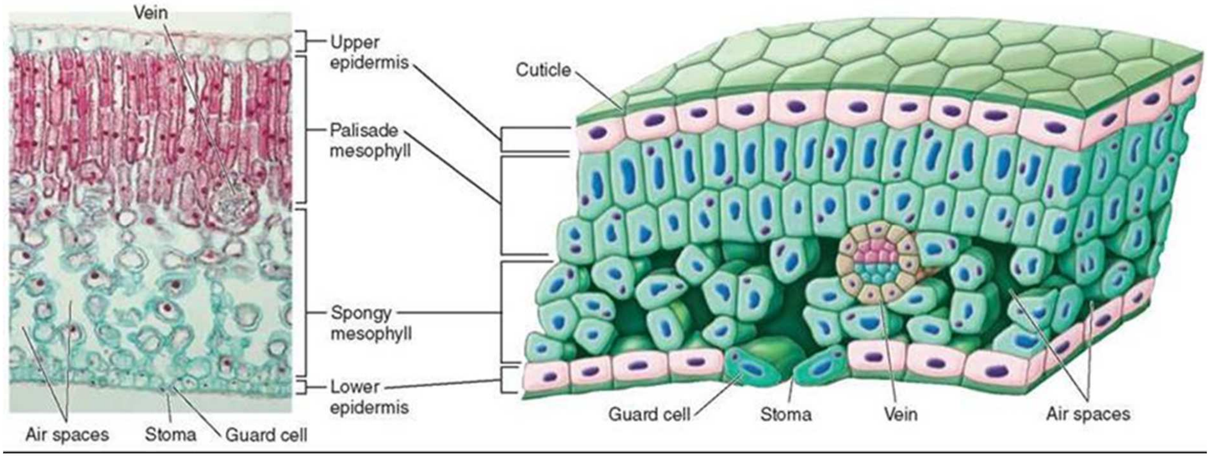


Figure 86: The cross section of a typical leaf

7.4.1.1.1 ‘Algerian’

In the SEM images, the cuticle, the multiple epidermis and the palisade parenchyma cells were clearly seen in the mesophyll layer images of cladodes.

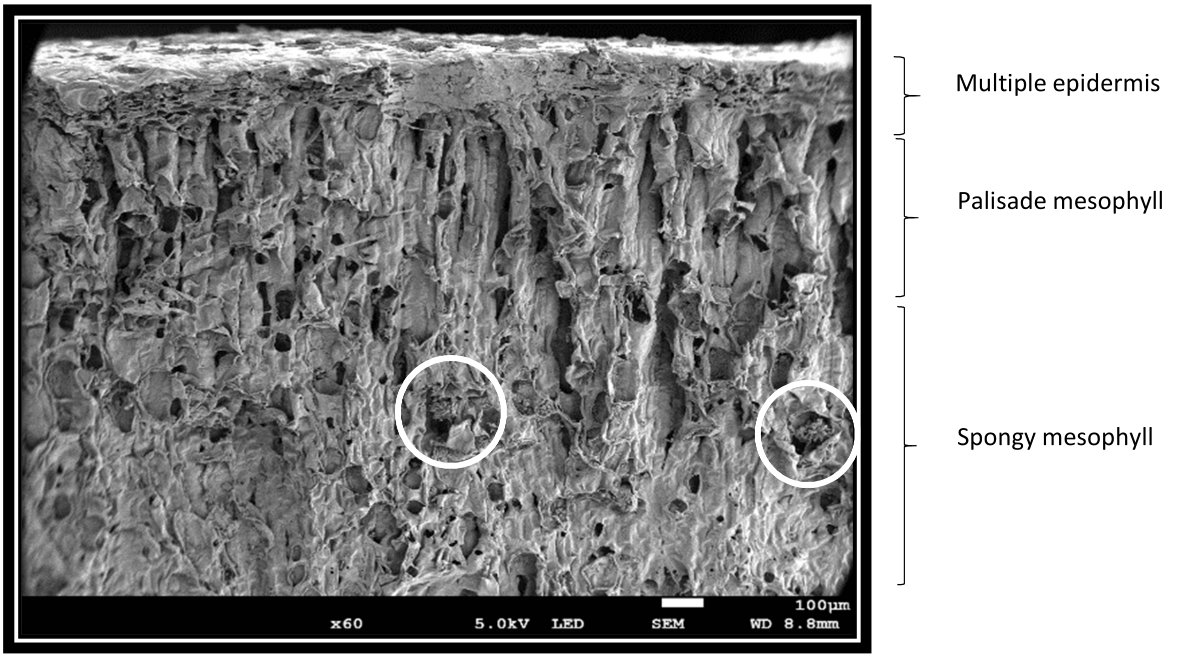


Figure 87: Two crystals (± 100 μm) visible in ‘Algerian’ tissue at X60 magnification

It was observed that calcium oxalate crystals were abundant in the tissue of ‘Algerian’ cladodes (Figure 88). The average size of the crystals was 70 μm. The sizes ranged from 55 to 100 μm. Sliminess was observed although it was less evident than what was observed in ‘Robusta’ tissue.

In ‘Algerian’ tissue (as was seen in all the cultivars), several crystals were visible (Figure 87 and Figure 88) mostly directly under the epidermis and in the interior tissue. The crystals have been identified as calcium oxalate crystals by a number of authors (Contreras-Padilla et al., 2011; McConn and Nakata, 2004; Ramírez-Moreno et al., 2011; Trachtenberg and Mayer, 1982a). Oxalic acid may occur in dissolved form but is mostly present in its crystalline form as whewellite or weddelite crystals (Ginestra et al., 2009; Rojas-Molina et al., 2015; Stintzing and Carle, 2005). The crystals were present in great abundance, were large (range from 30 to 100 µm) and had sharp edges.

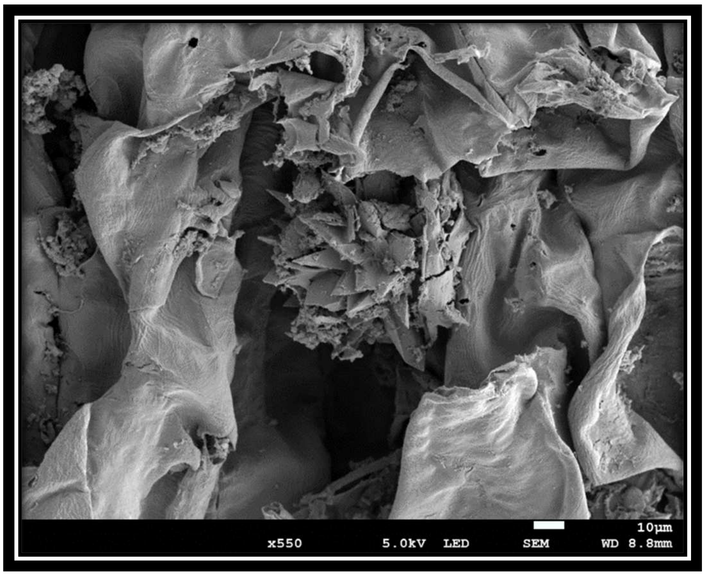


Figure 88: Magnification of a crystal in 'Algerian' tissue (72 µm) at X550

7.4.1.1.2 'Morado'

The cuticle, upper epidermis and the palisade mesophyll as explained in Figure 86 is clearly seen in the SEM image of 'Morado' (Figure 89). Several crystals were visible at X60 magnification of this image (Figure 89).

In the 'Morado' SEM images (Figure 90), large and abundant crystals were observed. It was found mostly in and just under the epidermis layer but crystals were also found in the mesophyll. The crystals ranged from 31 to 85 µm and the average size observed was 50 µm.

7.4.1.1.3 'Gymno-Carpo'

SEM images of Gymno-Carpo were comparable with 'Algerian' and 'Morado' images. The crystals were visible in the palisade mesophyll (Figure 91). The sliminess of the mucilage was observed in the palisade mesophyll. The crystals in the 'Gymno-Carpo' tissue were mostly observed under the epidermis but was also found in the spongy mesophyll. The crystals ranged between 40 and 60 µm and the average size was 47 µm.

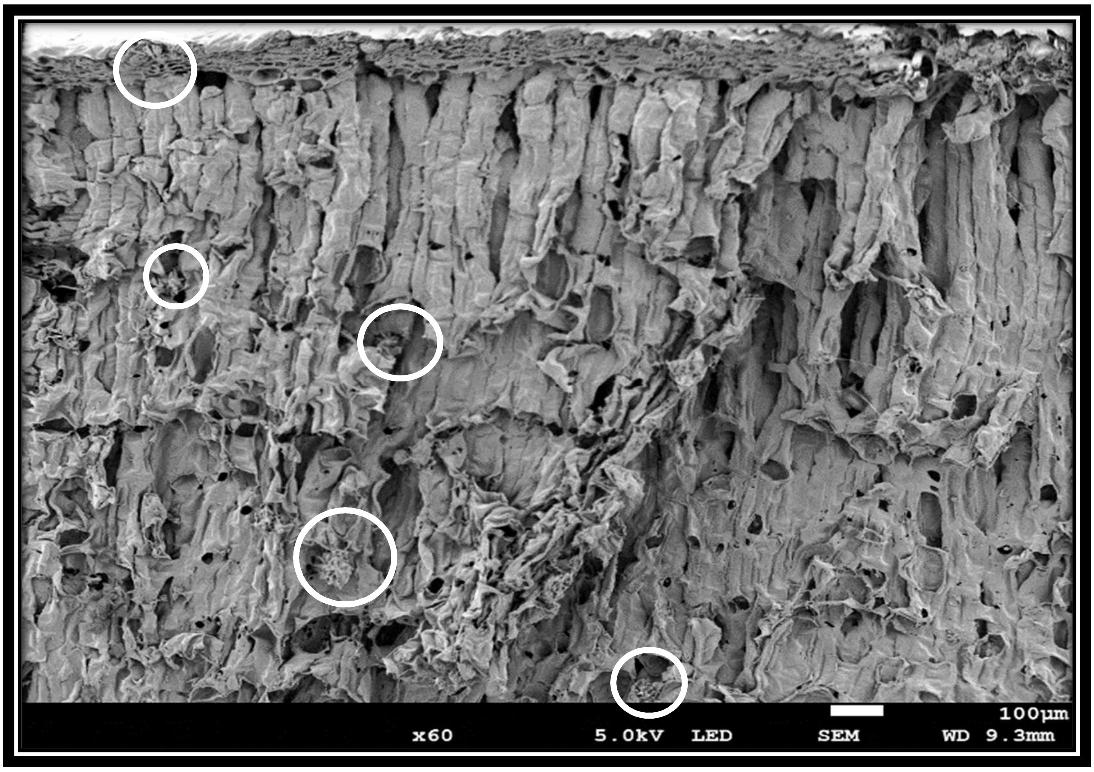


Figure 89: Five crystals visible in 'Morado' mesophyll (30-85 µm) at X60

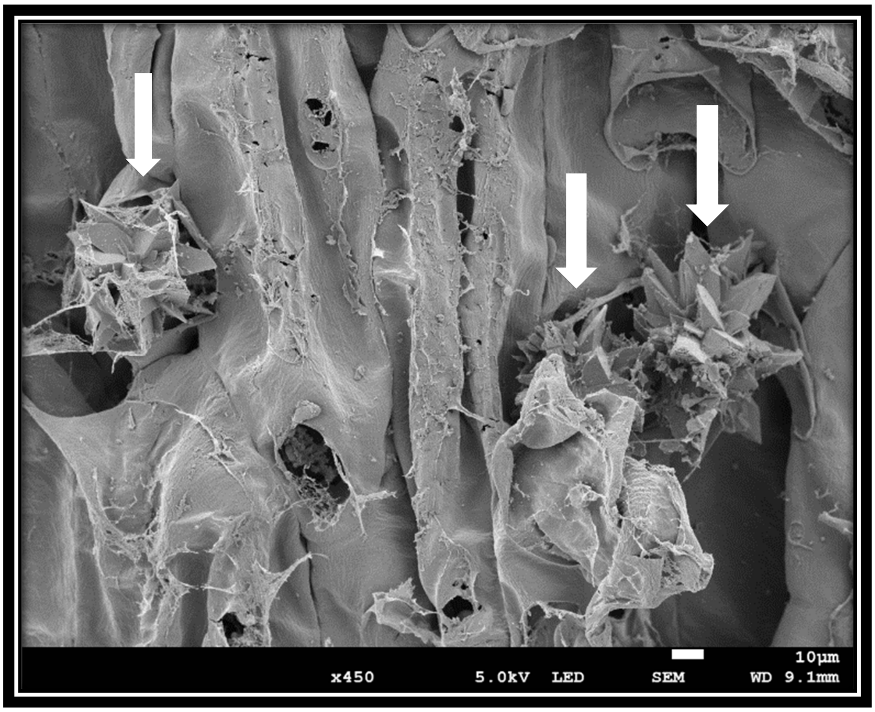


Figure 90: Three large (40-60 µm) crystals in parenchyma cells at X450

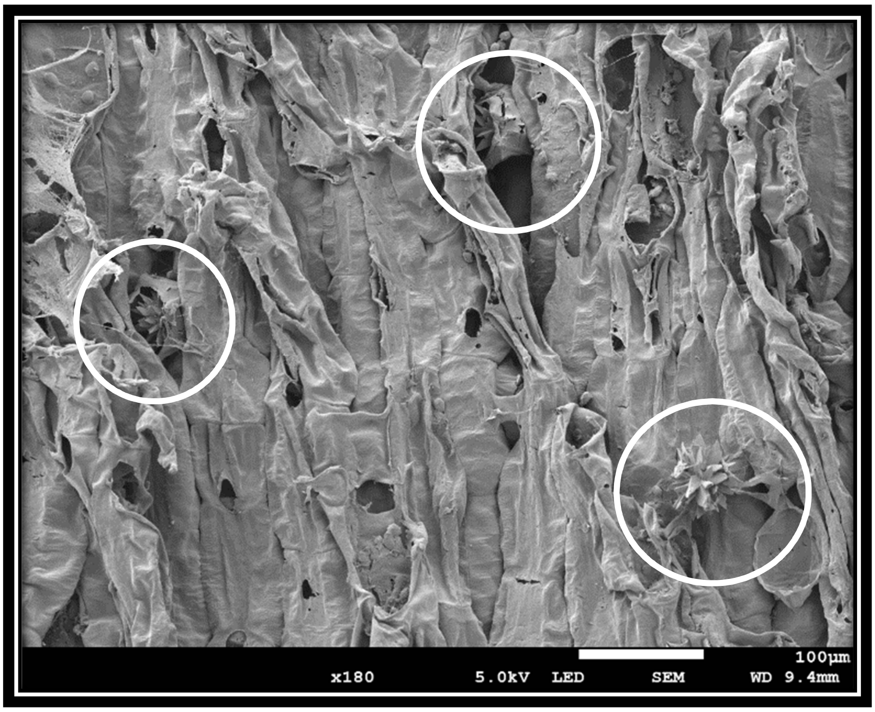


Figure 91: Three crystals (40-55µm) visible in 'Gymno-Carpo' palisade mesophyll at X180

7.4.1.1.4 'Robusta'

In observing the 'Robusta' SEM image (Figure 92), it was apparent that the crystal morphology was different. At first glance, no crystals were found therefore the 'Robusta' samples were sliced diagonally and remounted for SEM imaging. Thus the anatomy of the epidermis and mesophyll, which was clearly observed in the samples before is not as noticeable in these images although it was similar. In the subsequent SEM imaging of 'Robusta' tissue (Figure 92), crystals were observed although it was observed that the form and occurrence of crystals in the 'Robusta' tissue were noticeably different. Crystals were more abundant close to the cuticle in the epidermis (Figure 92). The forms of crystals were different in that they were not sharp. The average size of crystals found were 16 µm, and the crystals observed in 'Robusta' tissue were smaller and ranged from 6 µm to 35 µm (Figure 92) and very scarce in the interior tissue. The sliminess was more apparent in the 'Robusta' tissue compared to the previously discussed cultivars (Figure 93).

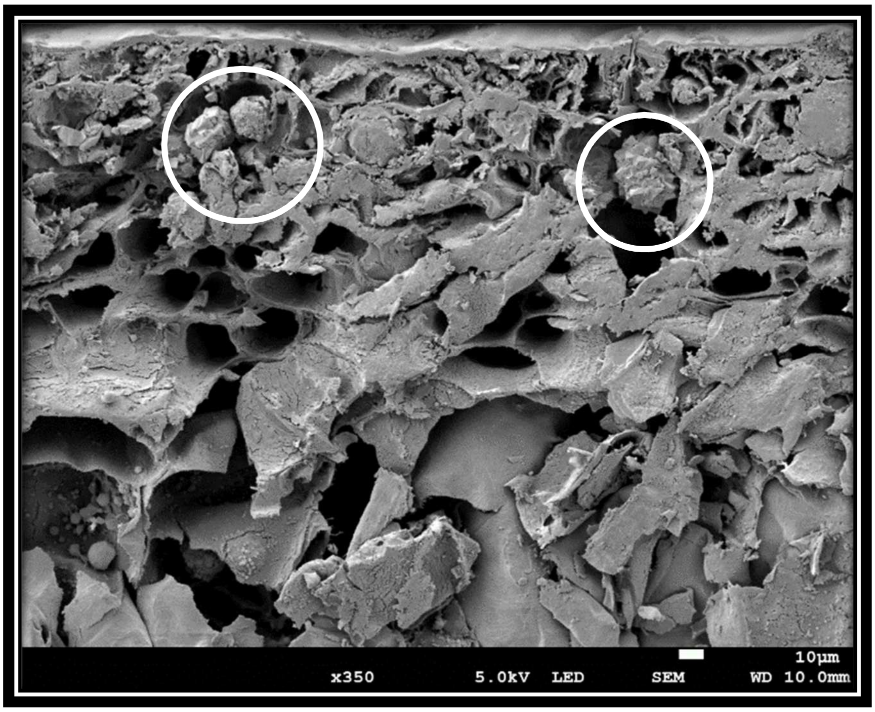


Figure 92: Three crystals (15-30 µm) observed directly under the cuticle of 'Robusta' at X350

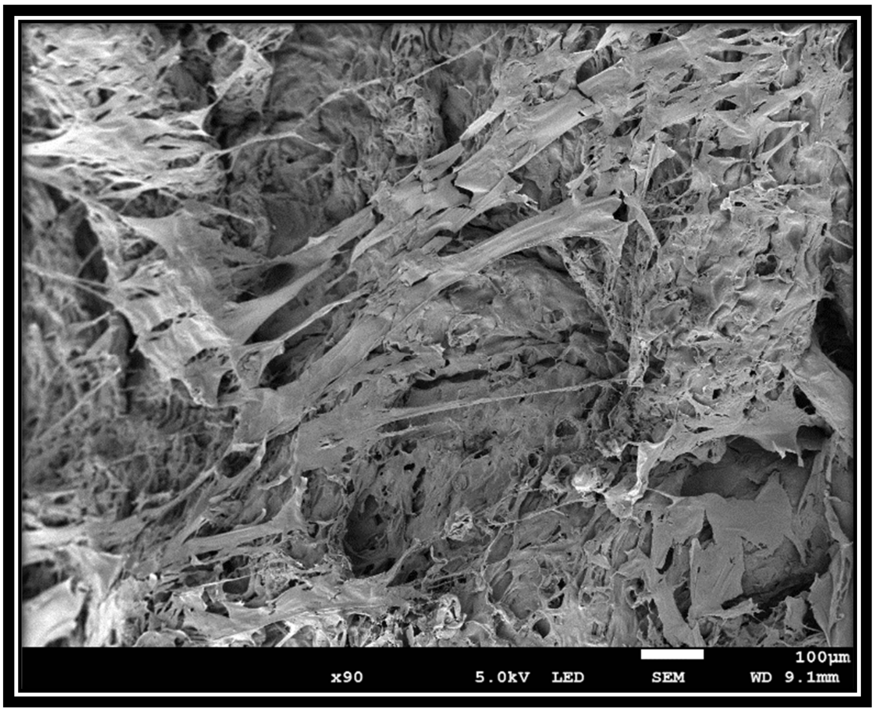


Figure 93: Sliminess in the interior tissue of 'Robusta'

7.4.1.1.5 Calcium oxalate crystals

Calcium oxalate crystals probably occur inside vacuoles. Vacuoles are known as the dumping ground and a warehouse, as excessive substances can move in and out depending on demand.

Excess calcium that would be toxic to cytoplasm is absorbed into vacuoles where it becomes trapped in the form of calcium oxalate crystals (Salisbury and Ross, 1992).

The presence of calcium oxalate crystals in the fresh cladode tissue has been reported by many authors (Contreras-Padilla et al., 2011; Ginestra et al., 2009; McConn and Nakata, 2004; Sáenz et al., 2012; Trachtenberg and Mayer, 1982a). Corresponding results are reported in this study as the druse crystals were abundantly present in the epidermis and palisade layer in the three *O. ficus-indica* cultivars (Algerian, Morado and Gymno-Carpo). In 'Robusta' (*O. robusta*) the crystals were present but not as abundant, in a different form and smaller.

In a most recent study by Rojas-Molina et al. (2015), calcium oxalate crystals were observed in dried cladode material that was suspended in water. The oxalate crystals observed were identified as whewellite (crystals with acute sharp points emerging from the centre) and wheddellite (conglomeration of tetragonal crystals) druses. In their study, noticeably more crystals were observed in the insoluble dietary fibre although it was seen in all tissues (cladode, soluble fibre and insoluble fibre). Total calcium oxalate detected in samples was 6.71 mg/g DM in cladodes, 0.27 mg/g DM in soluble fibre and 5.39 mg/g DM in insoluble fibre. The low solubility of calcium oxalate (6.1 mg/l) most probably caused the lower occurrence of calcium oxalate in soluble fibre while calcium carbonate (that is more soluble), was more prevalent in the soluble fibre (49.67 mg/g DM) than insoluble fibre (21.95 mg/g DM). Furthermore, the sizes of crystals were very large (considerably larger than what was observed in this study), as they were reported to be between 150 and 250 μm. It was concluded by the authors that the growth of the crystals is a function of the age of the plant. The bioavailability of calcium was specifically addressed in the study and it was found that the calcium would be available as a result of the molar oxalate: calcium ratios.

When Ginestra et al. (2009) investigated cladode tissue by SEM, calcium oxalate crystals were mostly observed in the alcohol-insoluble residue (interpreted as the insoluble fibrous structures), although the crystals were scattered throughout the cladode tissue. In fact, a continuous layer or calcium oxalate crystals in the epidermis was reported.

Contreras-Padilla et al. (2011) looked at the presence of oxalates and calcium at different maturity stages of the young cladodes up to 135 days and found that both calcium oxalate content (11.4 mg/g to 4.25 mg/g) and oxalate content (13.7 mg/g to 5.1 mg/g) reduced from 40 to 135 days while the calcium content increased from 17.9 mg/g DM to 30.7 mg/g DM.

This proved a positive relationship between maturity and bioavailable calcium content indicating that cladodes of advance maturity may be important sources of calcium and may have the least amount of calcium oxalate crystals. The molar ratios indicated that calcium would be bioavailable in young cladodes harvested between 2 and 4.5 months old.

Calcium oxalate crystals were found in dehydrated cladode powder at different stages of cladode development (60, 100 and 200 g), however the crystal size increased while the calcium oxalate content decreased with maturity (7.95 to 3.94 mg/g) (Rodríguez-Garcia et al., 2007). Crystal sizes increased with maturity (weight of cladodes) and was reported as 40 µm in 60 g, 55 µm in 100 g, 72 µm in 150 g and 100 µm in 200 g cladodes (Rodríguez-Garcia et al., 2007).

The presence of a large number of calcium oxalate crystals is known to be in all parts of the cladode tissue, powders and flours even after extensive efforts to purify the cladode samples (Malainine et al., 2003; Sáenz et al., 2012). How and why the crystals form is not known but it has been attributed to the hydration form of the calcium oxalate. It was stated by He et al. (2012) that crystals could be present in all plant tissues while different types of crystals may appear in the same plant tissue, based on its chemical composition.

The presence of oxalates in cladodes, spinach and kale was investigated by McConn and Nakata (2004). The highest oxalates were detected in spinach (136.8 mg/g DW) while virtually no oxalates were found in kale (2.03 mg/g DW). Cladodes contained substantial levels (35.11 mg/g DW) but mostly in the insoluble material (34.5 mg/g DM).

7.4.1.2 Scanning electron microscopy of freeze-dried mucilage powder

Interestingly, dissimilarities between freeze-dried mucilage samples of which the cladodes were harvested February and August were observed.

7.4.1.2.1 February

The freeze-dried powder particles were observed as having random forms and sizes varying from 150 to 200 µm. February dried mucilage powders from 'Algerian' (Figure 94 and Figure 95) were similar to 'Morado', 'Gymno-Carpo' and 'Robusta'. The particles had porous structures with a regular pattern of small holes. The mucilage displayed randomly sized and highly disorganized fractured pieces. The three dimensional structure may have formed during the freeze-drying and then broke when it was mechanically crushed into powder. The porousness of the powder differed slightly as it was observed that all February dried mucilage had a hard sponge-like structure with regular perforations. It was noticed that 'Algerian' perforation openings were bigger and therefore it can be assumed that moisture penetrated the structure more readily. It could be observed that the perforations of the 'Robusta' structure were smaller. It could be speculated that the porous structure of February dried mucilage caused viscosity to be lower as the solvent (water) more readily penetrated through the tissue.

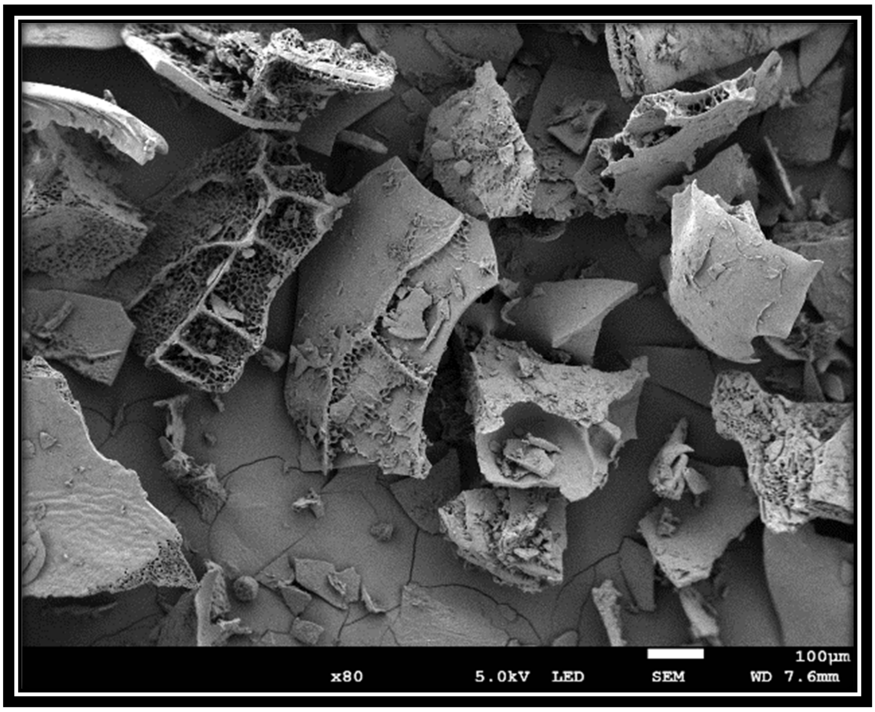


Figure 94: Broken perforated (porous) fragments of freeze-dried mucilage powder from the cultivar 'Algerian'

7.4.1.2.2 August

The perforations in the dried mucilage particles disappeared altogether in the August powder of all cultivars as seen in 'Algerian' (Figure 96 and Figure 97). More compact, solid structures were observed in all August samples (all cultivars). The particles were equally random in size and shape compared to the February powder. It is speculated that in the August dried mucilage the solvent (water) did not penetrate the cladode structure but was absorbed onto the mucilage structure and thus the viscosity of the solution increased.

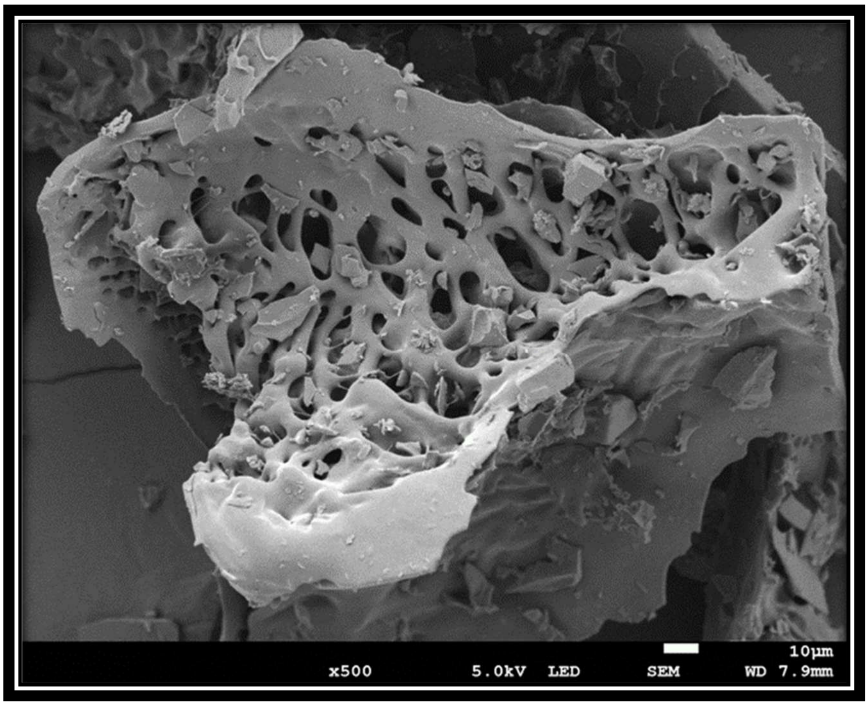


Figure 95: Magnification (X500) of a perforated particle of freeze-dried mucilage powder from the cultivar 'Algerian'

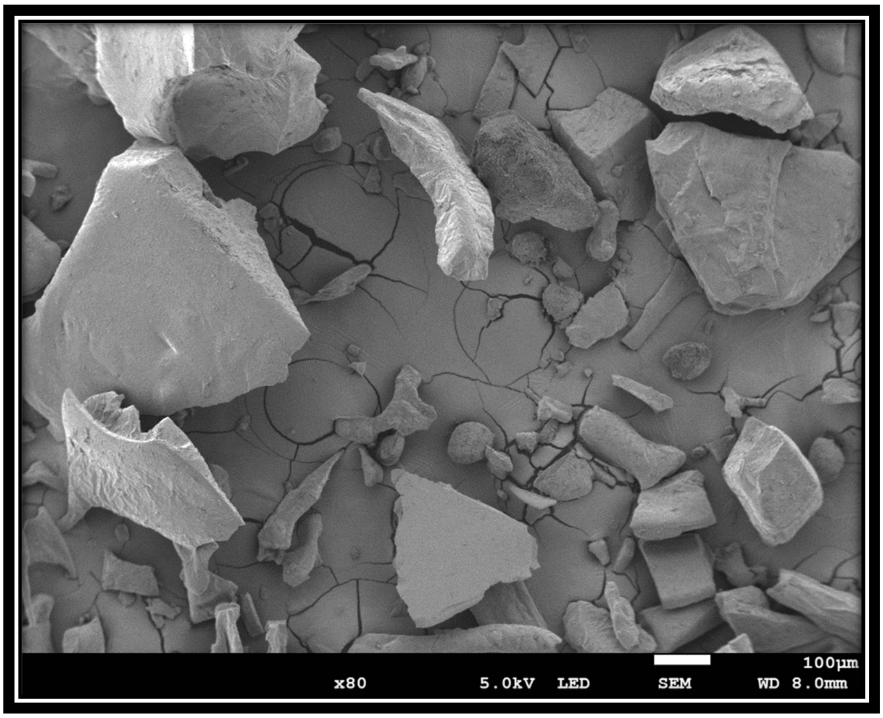


Figure 96: Broken solid fragments of mucilage particles of freeze-dried mucilage powder from the cultivar 'Algerian'

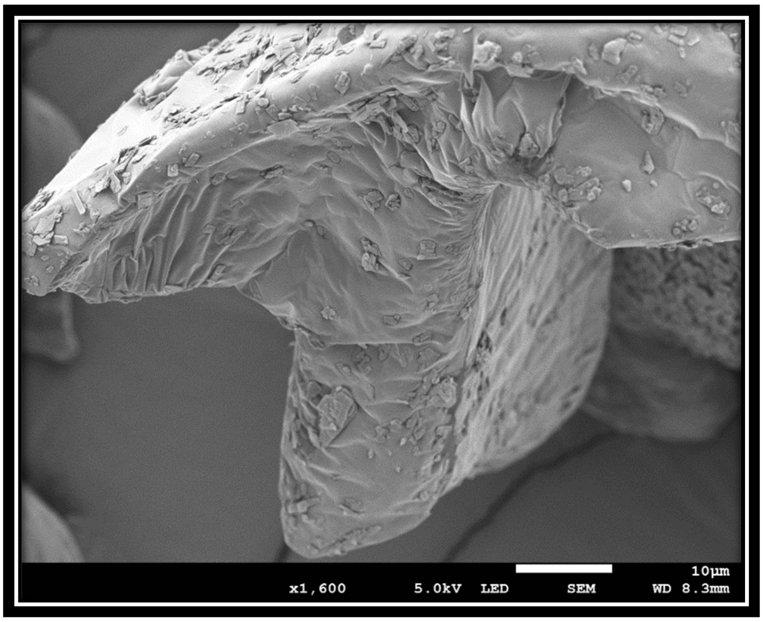


Figure 97: Magnification (X200) of a solid particle of freeze-dried mucilage powder

Calcium citrate was added to the mucilage powder according to Aeckerle (1941) in order to stabilize the freeze-dried powder and prevent hygroscopic activity. These crystals were of different sizes and shapes but all had a similar flaky appearance (Figure 98). Calcium citrate crystals were observed scattered throughout the powder structures (Figure 99). The calcium citrate crystals were mostly perfectly round or oval shaped or had round projections that caused the crystals to appear more like rounded globules.

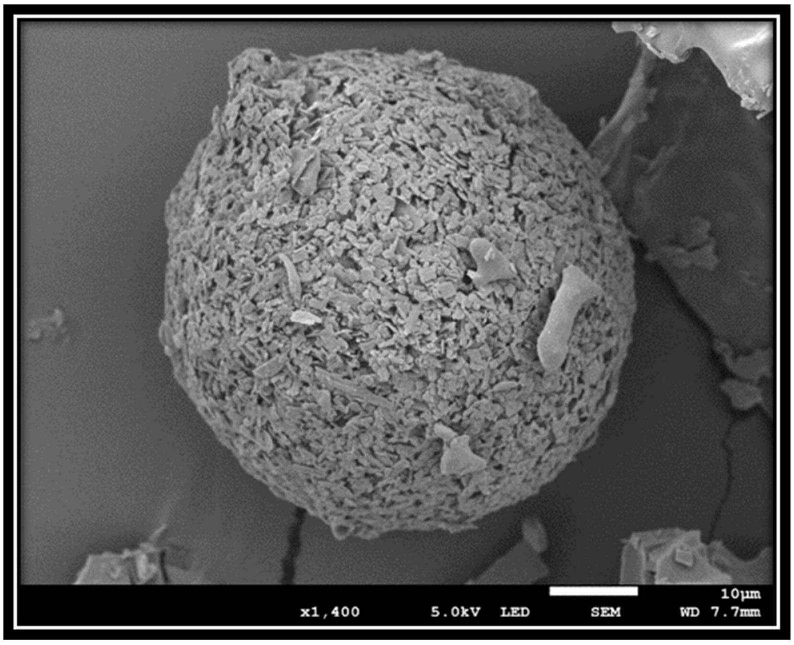


Figure 98: High magnification of a typical calcium citrate crystal found in the freeze-dried mucilage powder

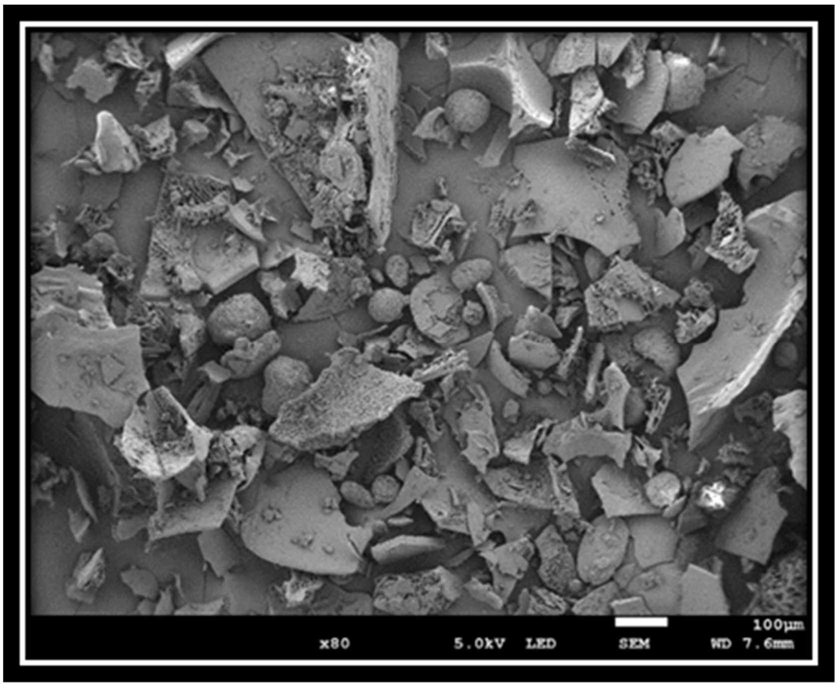


Figure 99: Calcium citrate crystals scattered between freeze-dried mucilage particles

Calcium citrate crystals were added to the mucilage powder, and were seen scattered between the mucilage particles.

Calcium oxalate crystals were not observed in any of the freeze-dried samples in these studies as described by Sáenz et al. (2012). It may be speculated that calcium oxalate crystals did not co-extract when the mucilage was separated from the solids during the extraction process. The low solubility of calcium oxalate and the relatively low concentration of calcium oxalate found in soluble fibre (Rojas-Molina et al., 2015) may be a factor in the absence of the crystals in the predominantly soluble fibre of mucilage. It may also be possible that calcium oxalate crystals were eliminated in the filtration or drying phase of the freeze-dried mucilage used in this study.

When 13 month old cladodes were used by León-Martínez et al. (2010) to extract mucilage that was to be spray-dried, the SEM micrographs showed very different particles that had sphere-like structures and that was collapsed and agglomerated. In fact, no crystals seemed to have been observed and no mention was made of the presence of such crystals in the stable mucilage powder that was investigated.

7.4.1.3 Particle size

The particle size of the powder is important because it has an influence on the rheological behaviour. Larger particles may be influenced by gravity and show a tendency to sediment whereas when the particles are very small, it retains water very effectively in the bowels and causes a satiety effect (Sáenz et al., 2010). The particle size must be between 150 and 190 µm. The particle size can easily be controlled in the milling process (Sáenz, 1996).

Particle sizes averages for 'Algerian' was 410 x 260 µm (average 335 µm), 'Morado' was 375 x 275 µm (average 326 µm), 'Gymno-Carpo' was 477 x 375 µm (average 430 µm) and 'Robusta' was 265 x 230 µm (average 246 µm). Thus the particle sizes of 'Robusta' powdered mucilage was smaller than that of the other cultivars while 'Gymno-Carpo' had the largest size particles (as seen in SEM images). It was therefore determined that the particle sizes were too big, and should be ground to a smaller size for optimal functionality. It is assumed that the particle sizes differed as a consequence of the manual grinding procedure however it could be the result of biological differences. It is recommended for further studies.

Future research could be done to find a milling process that produces smaller mucilage particles while the milling temperatures are controlled. These powders were manually ground to prevent destroying the nutrients through the application of heat when mills are used.

7.4.2 Gross energy

The gross energy content of a food product is a theoretical value, as not all the energy determined by an Oxygen Combustion Vessel could be used in the human body for energy purposes. Foods are usually not effectively digested or absorbed in the digestive system and therefore some of the energy may be lost. The content of the food product and how it was prepared could influence the digestibility thereof. Thus, the gross energy values are only an indication of a theoretical maximal energy content and do not reflect the energy ultimately utilized by humans (WHO/FAO, 2002).

Energy is obtained mostly from the fats, carbohydrates, protein and fibre that the sample contain. Water, minerals and antioxidants would not contribute towards energy (Whitney and Rolfes, 2015).

Gross energy (GE) is the heat, measured in joule (J) that is released when a sample is completely oxidized in an adiabatic bomb calorimeter. Results are given as mega Joules (MJ =

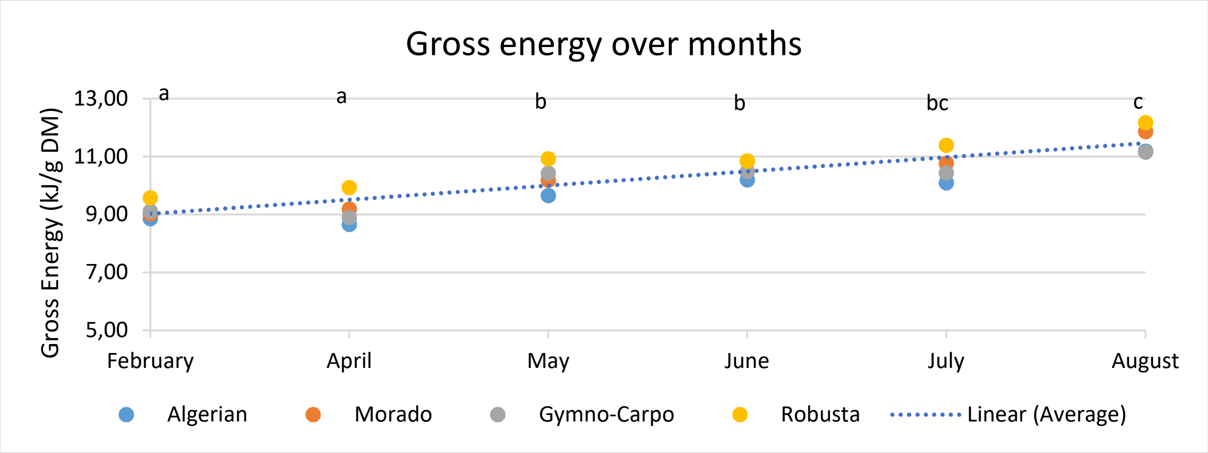
10^6J). The cross energy of freeze-dried mucilage according to cultivar and month obtained is presented in Table 59.

Table 59: The effect of month and cultivar on gross energy (kJ/g DM) on freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.332)**
Algerian	8.85	8.66	9.65	10.19	10.09	11.19	**9.77**
Morado	9.02	9.19	10.18	10.84	10.76	11.86	**10.31**
Gymno-Carpo	9.11	8.90	10.42	10.48	10.43	11.15	**10.08**
Robusta	9.57	9.92	10.92	10.84	11.38	12.17	**10.80**
Month Means (p < 0.001)	**9.14^{a}**	**9.17^{a}**	**10.29^{b}**	**10.59^{bc}**	**10.67^{bc}**	**11.59^{c}**	**10.24** **C x M: (p = 0.541)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between month and cultivar.
▭ = All means

The average energy content of freeze-dried mucilage was 10.24 kJ/g DM. The gross energy content of 'Robusta' (10.8 kJ/g) was slightly higher than that of the other three cultivars (Algerian' 9.77 kJ/g, Morado 10.31 kJ/g and Gymno-Carpo 10.08 kJ/g) although there were no significant differences between cultivars. The higher energy content in 'Robusta' could be attributed to the significantly higher fat content that was observed in 'Robusta', especially in July and August (Section 7.4.6.1.1). Yet there were significant differences seen between months.



Different superscripts above each month indicates significant differences between month means

Figure 100: Gross energy content and the trend over months of freeze-dried mucilage powders from cactus pear cladodes according to cultivar

It was evident that the gross energy increased constantly as demonstrated by the trend line in Figure 100, from February (9.14 kJ/g) to August (11.59 kJ/g) making February (9.14 kJ/g) and April (9.17 kJ/g) the months when mucilage (all cultivars) had significantly the lowest energy value than in August when the highest (11.59 kJ/g) energy content was reported for all cultivars. The rise in gross energy can be attributed to the increases that were observed both in the total fat (section 7.4.6.1.1) and starch (section 7.2.8.1) content over months. While fat content steadily increased from February to August, starch content increased more sharply in July and August. The interaction between months of harvest and cultivar was not significant.

Slightly lower values were reported in cladode four. It was reported to contain 6.9 kJ/g by Sáenz et al. (2010) which was reportedly 40% of the energy provided by wheat flour.

7.4.3 Moisture content

Food products may contain water in different forms, it may be chemically bound, physically bound, trapped, capillary or bulk water. Water molecules can exist in a variety of different environments and physicochemical structures. Therefore water may exist in products without it being visible (McClements, 2003). In fact, wheat flour has 13-15% water. The moisture content in dried flour products is important to determine as it influences the microbial safety. Also, the higher the moisture content, the lower the solids in the dried product (NDSU Department of Plant Sciences, 2014). The moisture content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 60.

Table 60: The effect of month and cultivar on moisture content (%) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.889)**
Algerian	14.71	18.97	14.45	13.86	15.70	15.90	**15.60**
Morado	16.44	20.62	15.34	14.50	14.32	16.14	**16.23**
Gymno-Carpo	15.65	18.51	14.91	13.60	16.03	16.31	**15.83**
Robusta	17.54	17.07	14.57	13.90	16.35	12.92	**15.39**
Month Means (p < 0.001)	**16.09^{a}**	**18.79^{b}**	**14.82^{a}**	**13.97^{a}**	**15.60^{a}**	**15.32^{a}**	**15.76** **C x M: (p 0.634)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between cultivar and month.
☐ **= All means**

The moisture content in freeze-dried mucilage was on average 15.76%. Although there were no significant differences between cultivars, 'Gymno-Carpo' demonstrated the highest moisture content (15.83%). 'Robusta' had the lowest average moisture content (15.76%) (Table 60). There were however significant differences observed between months, April mucilage had significantly higher moisture than all the other months. There were no trends observed between months, however June mucilage had the lowest moisture content (13.97%). The interaction between months of harvest and cultivar was not significant.

In the only study available on mucilage powders, the moisture content found in dried mucilage powders (no mention of the drying technique used) from cultivars in Ethiopia was an average of 11.57% (Gebresamuel and Gebre-Mariam, 2012). Different studies have been reported on the drying of cladode flour, where whole cladodes were spray-dried at eleven different temperatures, speeds and flow rates and the moisture contents were between 4.42 and 24.81%, in fact most drying treatments delivered dried cladode flours with moisture contents of between 7 and 10%. It was seen that moisture content decreased as inlet temperature increased (León-Martínez et al., 2010). Mature cladode flour that was dried in a forced air tunnel by Sáenz et al. (2010) had moisture content values of 7.14%. Sepúlveda et al. (2013) reported values of 6.77-5.93 g/100 g. López-Cervantes et al. (2011) dried cladodes at 60, 70 and 80˚C and determined the moisture content in cladode flour as 1.63, 1.93 and 2.00%. Cladodes flour produced from cladodes between 40 and 135 days of age had between 5.03 g/100 g and 4.18 g/100 g moisture content that decreased with cladode maturity (Hernández-Urbiola et al., 2011). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 4.06 and 7.31% moisture content (Rodríguez-Garcia et al., 2007).

Compared to the moisture content of cladode flour reported in the literature, the freeze-dried mucilage powder had relatively high values. The high moisture content is explained on account of the method of drying used in this study, as freeze-drying is a technique that does not employ heat and therefore mucilage molecules do not release all of the bound water molecules even under immense pressure. Also, as whole cladode flour would contain many more constituents that would also influence moisture content, comparing dried mucilage and dried cladode values is not entirely appropriate. Furthermore, it is known that water activity and water content is not similar; even when the water content of a product is high, water molecules could be restricted from serving as a source of nourishment to pathogenic microorganisms by being bound to the substances dissolved in the water (Fenema, 1996). In chapter 6 (section 8.2.5) water activity in mucilage powders were discussed in full, as a consequence, the overall average water activity was determined at 0.34. This value is well below the safe level of 0.5. Therefore, there could be no degradative activities, including yeast and mould growth meaning that freeze-dried mucilage is safe and cannot support microbial growth (Fenema, 1996).

Nevertheless, as the freeze-dried mucilage powders were relatively high in moisture content and had hygroscopic tendencies, it will be advisable to store the powder under vacuum packaging and frozen storage.

7.4.4 Crude protein content

The composition of the twenty amino acids that a protein consist of appropriates its specific role in plants or food. Protein serves different purposes in food products; not only does it have a nutritional role, it also plays a useful role as a functional ingredient in food products. Examples of the numerous functions that proteins contribute to include viscosity, gelation, emulsification, foaming and flavour binding (Fenema, 1996). The crude protein content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 61.

Table 61: The effect of month and cultivar on protein content (g/100 g DM) on freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p < 0.001)**
Algerian	2.70	2.52	2.72	2.94	2.61	2.97	**2.74**^{a}
Morado	2.79	2.83	3.56	3.45	3.17	3.60	**3.23**^{a}
Gymno-Carpo	2.90	2.52	3.76	2.94	3.08	2.84	**3.01**^{a}
Robusta	5.20	5.33	5.74	4.05	4.28	4.14	**4.79**^{b}
Month Means (p = 0.937)	**3.40**	**3.30**	**3.94**	**3.35**	**3.28**	**3.39**	**3.44** **C x M: (p = 0.732)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M: Interaction between cultivar and month.
☐ **= All means**

Crude protein was calculated from the nitrogen content determined by thermal combustion. Nitrogen is an indication of the protein content because nitrogen is an element of which amino acids are made up of. It is a fact that not all nitrogen is converted to protein and is referred to as non-protein nitrogen. This might be problematic when human metabolism is involved (Sosulski and Imafidon, 1990).

The crude protein averages for cultivars ranged from 2.52 to 5.74 g/100 g DM. The protein content for the three *O. ficus-indica* cultivars was not significantly different (2.74-3.23 g/100 g) from each other while protein content was significantly higher in 'Robusta' (4.79 g/100 g) (*O. robusta*) (Table 61). Consistently every month, over the six month period studied, 'Robusta' proved to contain the highest crude protein and ranged from 4.05 g/100 g June to 5.74 g/100 g May although it was not significantly different. The interaction between month of harvest and cultivar was not significant. In fact, it was almost double than the other cultivars. Both 'Algerian' and 'Gymno-Carpo' mucilage had low crude protein content in April causing April to have the lowest average protein content (3.30 g/100 g). The only data available on mucilage in (Gebresamuel and Gebre-Mariam, 2012), reported protein content of 6.82% in mucilage extracted from cladodes from Northern Ethiopia.

Ayadi et al. (2009) reported total protein content in cladode flour as 8.74 g/100 g DM for spiny cladodes and 8.88 g/100 g DM for spineless cladodes. Cladode flour had a protein content of 7.26% according to Samia El-Safy (2013). Sáenz et al. (2010) tested mature cladode flour and found 3.87 g/100 g protein. Crude protein was 7.52, 7.42 and 7.24 g/100 g DM at three drying temperatures for cladode flour tested by López-Cervantes et al. (2011). Padron-Perreira et al. (2009) found protein values of 9.5% in cladode flour and 9.71% in enzymatically hydrolysed flour. Rodriguez-Felix and Cantwell (1988) determined crude protein average of 13.25 g/100 g DM in dehydrated cladodes of the Redonda cultivar. Cladode flour made from cladodes of 40 to 135 days old had an average protein content of 7.8 g/100 g (Hernández-Urbiola et al., 2010). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 11.39% and 14.22% protein content (Rodríguez-Garcia et al., 2007). Crude protein contents in oven-dried and freeze-dried whole cladodes from Brazil was 4.82 and 4.98% DW respectively (Teles et al., 1997).

The crude protein content results reported in this study was lower than what was found by other researchers. Gebresamuel and Gebre-Mariam (2012), found higher values in mucilage that ranged from 5.18 to 6.82% in cultivars from Ethiopia. Older cladodes (as were used in this study) have been reported to have lower protein content than younger cladodes (Ramírez-Tobías et al., 2007). Sepúlveda et al. (2007) detected between 6.1 g/100 g and 7.9 g/100 g crude protein in mucilage precipitated with ethanol and isopropyl alcohol. Even though the protein content was low, the presence of protein in mucilage was a significant finding. When Iturriaga et al. (2009a) studied the use of cactus mucilage in emulsions; they discovered a protein fraction in mucilage. It is known that xanthan and guar gum also contains protein fractions (1-2%). It is thus possible that this protein fraction could provide mucilage with the properties needed for acting as an emulsifying agent.

Glutamine is the major amino acid (17.3 g/100 g), followed by valine (3.7 g/100 g) and serine (3.2 g/100 g) (El-Mostafa et al., 2014; Feugang et al., 2006) in cladodes. In fact, it contains higher lysine, methionine and tryptophan than most cereals (Stintzing and Carle, 2005). López-Cervantes et al. (2011) found low protein values and low amino acid content in young cladode flour dried at different temperatures. The amino acids found at higher concentrations were tyrosine, proline, aspartic acid and glutamic acid. Leucine concentrations were very low and methionine was not detected. It was stated that methionine is a limited amino acid in cladodes. Cladode flour obtained from cladodes harvested at different days of age (40-135 days) showed a presence of 17 amino acids, the highest values were for glutamic acid, threonine and phenylalanine while the lowest were for methionine, tryptophan, histidine and arginine (Hernández-Urbiola et al., 2010). Data obtained by Samia El-Safy (2013) indicated that glycine, arginine, glutamic acid, aspartic acid and lysine were the most abundant amino acids in cladode flour. Histidine content (important amino acid for children) was higher than the FOA/WHO guidelines. Threonine was low and methionine was not detected. Lysine was pointed out as being the most indispensable amino acid in cladode flour, glycine was 13% and arginine 10% of the protein content. As a result cladode flour could be utilized as a good complement to other protein products in a balanced diet due to its essential amino acid content. The limiting amino acids in cladode flour were threonine, tyrosine and leucine (Samia El-Safy, 2013). Lysine and aspartic acid were found to be most abundant in the study by Teles et al. (1997) while it was reported that the biological value of protein was 72.6 (related to egg protein).

7.4.5 SDS – PAGE: Protein characterization

A protein fingerprint was determined using SDS-PAGE (Figure 101). Differences between cultivars and months were observed as well as the different sizes and types (classes) of proteins in mucilage. The first band (largest protein) that was detected in the samples were at ~ 100 kD, detected in February 'Robusta' (Lane 4) and May ['Algerian' (lane 5), 'Morado' (lane 6) and 'Gymno-Carpo' (lane 7)] as well as June ['Algerian' (lane 9), 'Morado' (lane 10), 'Gymno-Carpo' (lane 11)] and August ('Morado') (lane 14).

Prominent bands were observed between 37 and 75 kD in February 'Robusta' (lane 4), June ['Algerian' (line 9), 'Morado' (lane 10), 'Gymno-Carpo (lane 11)], in fact it was very prominent in August 'Morado' (lane 14), while May 'Robusta' (lane 8) was very faint. These bands indicate the presence of prolamins (high in glutamine and proline).

Between 20 and 37 kD, more bands were observed in all samples while the bands were more prominent in February and August.

The most prominent bands were observed at 15 and 10 kD (smallest proteins) in all samples except for 'Robusta' (February (lane 4), May (lane 8), June (lane 12) and August (lane 16).

Therefore it was observed that the proteins that were present in the three *Opuntia ficus-indica* (OFI) cultivars (Algerian, Morado and Gymno-Carpo) were not present in *Opuntia robusta* samples. The bands located at ~ 10 - 15 kD indicated the presence of 2S albumins.

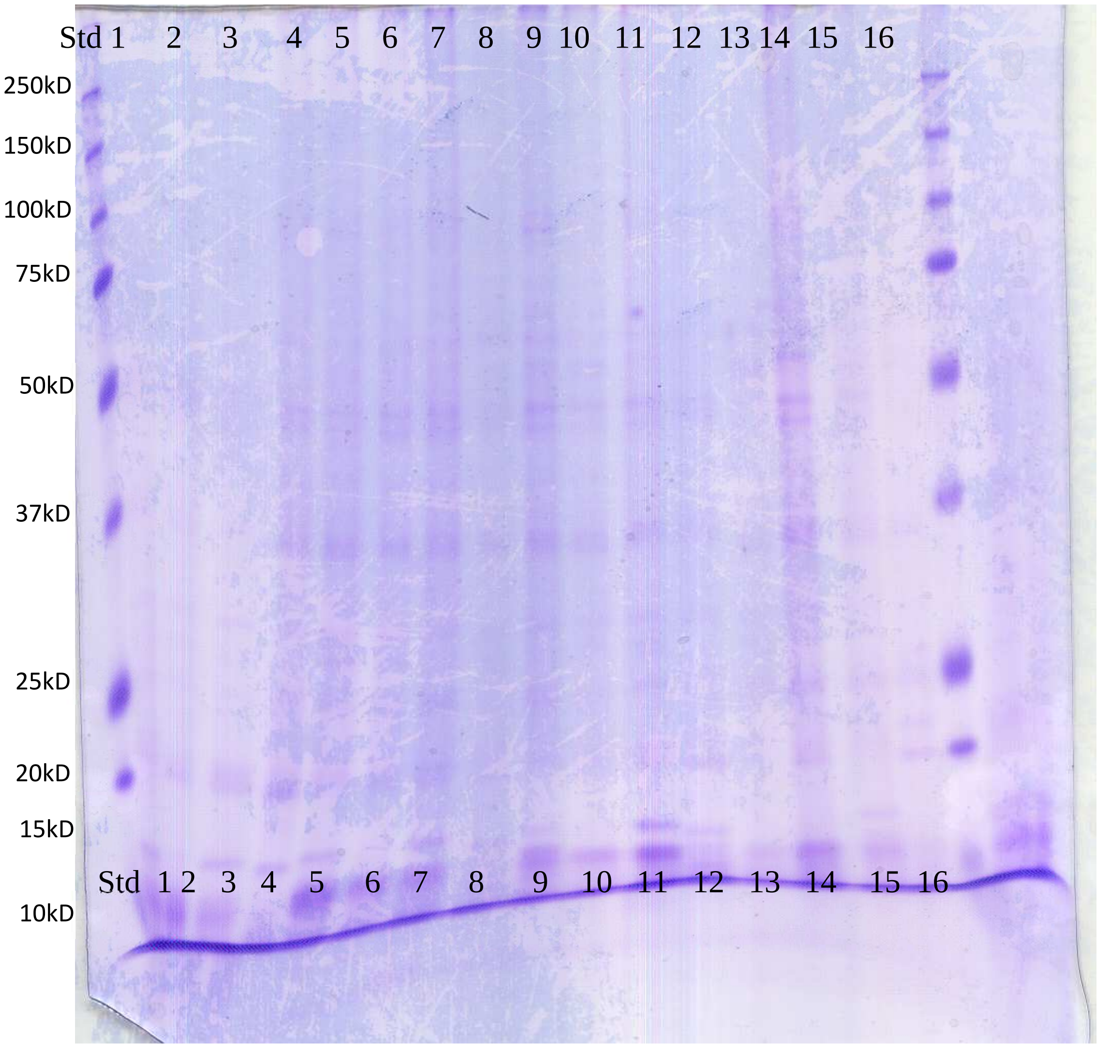


Figure 101: SDS-PAGE analysis of protein in freeze-dried mucilage powders from cactus pear cladodes for the four cultivars over four months

Lane 1: February, Algerian
Lane 2: February, Morado
Lane 3: February, Gymno-Carpo
Lane 4: February, Robusta
Lane 5: May, Algerian
Lane 6: May, Morado
Lane 7: May, Gymno-Carpo
Lane 8: May, Robusta
Lane 9: June, Algerian
Lane 10: June, Morado
Lane 11: June, Gymno-Carpo
Lane 12: June, Robusta
Lane 13: August, Algerian
Lane 14: August, Morado
Lane 15: August, Gymno-Carpo
Lane 16: August, Robusta

The only ‘Robusta’ sample that resembled OFI bands, was in lane 4 (February), which was more consistent with May (‘Algerian’ (lane 5), ‘Morado’ (lane 6) and ‘Gymno-Carpo’ (lane 7). The ‘Robusta’ sample in lane 16 (August) also seemed to be slightly more prominent compared to the *O. robusta* samples from May (lane 8) and June (lane 12).

The prominence of glutamine in prolamines (Nassar, 2008) and albumin proteins have already been reported in cactus pear seed research. In fact, the 2S albumins (14-19 kD), 11S globulins (40 kD) and prolamins (37, 50 and 75 kD) rich in glutamine and proline amino acids found in cactus pear seeds were reported in a study done at this University (Lebeko, 2010). The prominence of glutamine in cladodes has been reported by different researchers (El-Mostafa et al., 2014; Feugang et al., 2006; Hernández-Urbiola et al., 2010; Samia El-Safy, 2013; Teles et al., 1997), as well as the presence of albumin-like proteins (Majdoub et al., 2001).

Therefore, it was concluded that ‘Robusta’ samples differed from OFI samples in terms of the presence of protein although the differences could not be quantitatively distinguished. The presence of protein in mucilage was confirmed, thus mucilage would participate in the formation of emulsions by reducing the surface activity at the interphase and stabilizing emulsions.

7.4.6 Total lipid and fatty acids analysis

Analysis of the fatty acids detected in cladodes have shown that palmitic acid, oleic acid, linoleic and linolenic acid contribute up to 90% of the total fatty acid while polyunsaturated fat content was higher than mono- or saturated fats (El-Mostafa et al., 2014). Fat content of cladode flour made from cladodes between 40 and 135 days was on average 1.8 g/100 g (Hernández-Urbiola et al., 2010). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 1.96% and 3.00% fat content (Rodríguez-Garcia et al., 2007).

7.4.6.1 Total extractable fat content (EFC) and fat free dry matter (FFDM)

7.4.6.1.1 Total fat content

Lipids are primary compounds in plants that are needed for survival and growth as they serve an important role in reserving energy. The fats are only stored in specialized tissue and cells and only at certain times in the life cycle of the plant (Salisbury and Ross, 1992). Fats are rarely stored in the leaves, roots and stems, however it is usually stored in the seeds (for example sunflower, cottonseed or corn) and sometimes the fruit (for example coconut or avocado).

The leaves only produce fatty acids in their membranes, not actual fats. The fatty acids synthesized in the organelles of leaves or stems are mainly palmitic acid and oleic acid and most other fatty acids are formed merely by modification of their structures. The plant material that is used for human food purposes mostly consist of starch and only contains diminutive amounts of fat (Salisbury and Ross, 1992).

Invisible fats occur naturally in food and contribute to the flavour, mouthfeel, palatability and colour of dishes. The specific chemical configuration of the lipid molecules cause different outcomes in food preparation and nutrition. In modern food products, fats impart unique characteristics in food products such as textures, plasticity, solubility, satiety, appearance and nutrients. The fats in food are a source of concentrated energy and carries the fat-soluble vitamins (Bennion, 1985; Brown, 2007). Lipids consists of a glycerol molecule to which the three fatty acids are attached, but the degree of saturation, the length of the three fatty acids and the number of carbons of the fatty acids differ and determine its function in food (Brown, 2007).

Fats contain larger amounts of carbon, hydrogen and less oxygen compared to carbohydrates, therefore it has much greater energy potential (Salisbury and Ross, 1992). The fat content (%) of freeze-dried mucilage according to cultivar and month obtained is presented in Table 62.

Table 62: The effect of month and cultivar on % fat of freeze-dried mucilage powders from cactus pear cladodes

Cultivar	**February**	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.012)**
Algerian	0.15	0.57	0.40	0.82	0.42	0.47	**0.47^{a}**
Morado	0.62	0.33	0.67	0.67	0.43	1.00	**0.62^{ab}**
Gymno-Carpo	0.39	0.15	0.81	0.48	0.42	0.41	**0.44^{a}**
Robusta	0.66	0.58	0.84	0.92	1.13	1.28	**0.90^{b}**
Month Means (p = 0.359)	**0.45**	**0.41**	**0.68**	**0.72**	**0.60**	**0.79**	**0.61** **C x M: (p= 0.451)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
☐ **= All means**

It was evident from Table 62, that the freeze-dried mucilage contained very small amounts of fat. It was mostly less than 1%. Only 'Robusta' values were above 1% in July and August. In fact, 'Robusta' had the highest values for total fat content and was significantly higher than 'Gymno-Carpo' (0.44%) and 'Algerian' (0.47%) (p = 0.012) consistently throughout the six months (average 0.90%). The fat content ranged from 0.15% ('Algerian', February) to 1.28% ('Robusta', August). The overall average fat content in freeze-dried mucilage powder was

0.61%. There were no significant differences detected between months but the fat content increased from February (0.45%) to August (0.79%). The interaction between month of harvest and cultivar was not significant.

The fat content values found in this study corresponds to those reported by Gebresamuel and Gebre-Mariam (2012) who also found low fat content in mucilage powder (0.42%). Ayadi et al. (2009) reported fat content as 3.95 g/100 g DM for spiny cladodes and 4.69 g/100 g DM for spineless cladodes. Sáenz et al. (2010) found values of 1.85 g/100 g in mature cladode flour. López-Cervantes et al. (2011) determined total lipid content as 2.3 g/100 g DM. Crude lipids in cladode flour was 2.206% (Samia El-Safy, 2013) of which saturated fatty acids were 32.21% and unsaturated fatty acids were 58.44%. Consequently, the fat content of mucilage power in this study was lower than that reported in cladode flour.

7.4.6.1.2 Total fat free dry matter (FFDM) content

When the composition of food is determined, considering the moisture content alone does not give an accurate indication of the moistness (in terms of the sensory experience) of the product. The presence of fats also contribute to the texture of foods. Therefore FFDM represents the portion of a food product that is void of water as well as fats. Most fruit and vegetables are classified as "high water and low fat content" food (Chen and Rosenthal, 2015). The fat free dry matter (%) of freeze-dried mucilage according to cultivar and month obtained is presented in Table 63.

Table 63: The effect of month and cultivar on % fat free dry matter of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p < 0.001)**
Algerian	79.46	76.66	74.89	77.95	73.09	77.44	**76.58^{b}**
Morado	75.80	73.01	76.98	71.77	74.90	79.51	**75.33^{ab}**
Gymno-Carpo	76.39	71.27	76.08	71.65	70.52	67.92	**72.31^{a}**
Robusta	84.98	77.12	83.73	77.01	78.63	77.84	**79.89^{b}**
Month Means (p = 0.399)	**79.16**	**74.52**	**77.92**	**74.59**	**74.29**	**75.68**	**76.03** **C x M: (p = 0.278)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

The FFDM ranged from 67.92% (August,' Gymno-Carpo') to 84.98% (February, 'Robusta'). There were significant differences between cultivars. 'Gymno-Carpo' had significantly lower FFDM (72.31%) than 'Algerian' (79.16%) and 'Robusta' (79.89%). The overall average FFDM was 79.03%. Average FFDM content over months were not significantly different. The interaction between months of harvest and cultivar was not significant.

7.4.6.2 Fatty acid analysis (FAME) (% of total fatty acids)

Different fatty acids have different functions in food products and determine the nutritional contribution of the food product (Brown, 2007; Charley, 1982; Whitney and Rolfes, 2015). The different fatty acids that exist are the result of the differences in the length of the carbon-chain, the degree of saturation and the number and location of their double bonds (Whitney and Rolfes, 2015). The environment has an effect on the types of fatty acids that are found in plants. It has been reported that heat could have an influence on the saturation degree of the fatty acids (Salisbury and Ross, 1992).

Analysis of the fat content indicated the presence of fatty acids in freeze-dried mucilage powder (Table 64) and was expressed as % of total fatty acids content.

Table 64: Fatty acids content (%) of four cultivars of freeze-dried mucilage powders from cactus pear cladodes

Fatty acids	Abbreviation	Algerian	Morado	Gymno-Carpo	Robusta	Average
Myristic	C14:0	0.07	0.06	0.05	0.06	0.06
Palmitic	C16:0	14.14	13.84	12.97	15.15	14.03
Palmitoleic	C16:1c9	0.66	0.73	0.61	0.60	0.65
Margaric	C17:0	0.02	0.02	0.02	0.02	0.02
Heptadecenoic	C17:1c10	0.04	0.05	0.06	0.06	0.05
Stearic acid	C18:0	3.13	2.94	2.91	2.82	2.95
Oleic	C18:1c9	15.95	20.91	16.13	23.49	19.12
Linoleic	C18:2c9,12 (n-6)	65.28	60.59	66.49	56.21	62.14
Arachidic	C20:0	0.13	0.18	0.14	0.51	0.24
Eicosenoic	C20:1c11	0.17	0.16	0.16	0.15	0.16
α-Linolenic	C18:3c9,12,15 (n-3)	0.16	0.19	0.14	0.27	0.19
Behenic	C22:0	0.13	0.17	0.16	0.22	0.17
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.09	0.12	0.12	0.39	0.18
Lignoceric	C24:0	0.03	0.04	0.03	0.06	0.04

The main fatty acids that were present at higher concentrations included linoleic, oleic, palmitic and stearic acid. The average content of the fatty acids found in very low concentrations (less than 1% of total fatty acids) included palmitoleic, arachidic, α-linolenic, eicosatrienoic, behenic, eicosenoic while myristic, heptadecenoic, lignoceric and margaric were almost not detected. The contents were very similar for most fatty acids, although 'Robusta' contents were notably higher in arachidic, α-linolenic, behenic and eicosatrienoic fatty acids. The fatty acids that were more abundant in the freeze-dried mucilage were palmitic (14.03%), stearic acid (2.95%), oleic acid (19.12%) and linoleic acid (62.14%). These fatty acids contributed 98.24% of the total fatty acids.

The abundant fatty acids found in the analysis of freeze-dried mucilage such as, palmitic, stearic, oleic and linoleic fatty acids are those fatty acids that usually occur in plant membranes (Salisbury and Ross, 1992). It is characteristic of fatty acids pertaining to a plant source such as cactus pear cladodes, to have longer carbon-chains and for a high proportion of unsaturated fats to occur. The long-chain (12-24 carbons) fatty acids that originates from certain meats, seafood and vegetables oil are most common in the diet (Charley, 1982).

López-Cervantes et al. (2011) determined that the most abundant fatty acids in cladode flour were palmitic acid, linoleic acid and oleic acid. Cladode flour was thus concluded to be rich in essential fatty acids although the total lipids were too low to be considered a good source of essential fatty acids (López-Cervantes et al., 2011). Samia El-Safy (2013) found oleic acid to be the most prominent fatty acid found in cladode flour at 28.05% and that linoleic acid (18.9%) and palmitic acid (16.23%) were present. Thus, it was concluded that cladode flour could be considered nutritious and health promoting, as it contained such high amounts of essential fatty acids (Samia El-Safy, 2013). Mucilage powder fatty acid content corresponds to that of cactus flour. Only these main fatty acids will be discussed.

A very recent study by (De Wit et al., 2016b) reported the fatty acid composition of seed oil of six of the South African cultivars that included Algerian, Morado and Robusta. Comparison of the average fatty acids contents (as % of total fatty acids) of seeds oil and dried mucilage samples (Table 65) gave surprisingly similar results.

Table 65: Comparison of fatty acids composition (%) of seed oil and freeze-dried mucilage powders from cactus pear fruit and cladodes

Fatty acid	Abbreviation	Seed oil	Mucilage
Myristic	C14:0	0.05	0.06
Palmitic	C16:0	15.39	14.03
Palmitoleic	C16:1c9	0.69	0.65
Margaric	C17:0	0.03	0.02
Heptadecenoic	C17:1c10	0.05	0.05
Stearic acid	C18:0	2.95	2.95
Oleic	C18:1c9	18.77	19.12
Linoleic	C18:2c9,12 (n-6)	61.42	62.14
Arachidic	C20:0	0.20	0.24
Eicosenoic	C20:1c11	0.22	0.16
α-Linolenic	C18:3c9,12,15 (n-3)	0.23	0.19
Behenic	C22:0	0.12	0.17
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.14	0.18
Lignoceric	C24:0	0.03	0.04

It was observed that average fatty acid composition was comparable and very few differences were observed for the most abundant fatty acids, as well as the fatty acids that were detected in miniscule amounts.

7.4.6.2.1 Palmitic acid (C16:0)

Palmitic acid is a saturated fatty acid that is the most abundant in nature and the most widely distributed saturated fatty acid in food (Charley, 1982; Salisbury and Ross, 1992). The C16:0 content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 66.

Table 66: The effect of month and cultivar on the C16:0 content (% of total fat content) of freeze-dried mucilage powders from cactus pear cladodes

Cultivar	**February**	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.094)**
Algerian	16.81	12.56	11.60	14.47	14.50	14.91	**14.14**
Morado	14.25	11.55	13.90	14.39	14.57	14.41	**13.84**
Gymno-Carpo	11.08	11.73	15.42	13.44	12.97	13.18	**12.97**
Robusta	13.78	16.74	14.76	14.76	15.79	15.10	**15.15**
Month Means (p = 0.882)	**13.98**	**13.14**	**13.92**	**14.26**	**14.46**	**14.40**	**14.03** **C x M: (p = 0.418)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
☐ = All means

Palmitic acid ranged between 11.08% (Gymno-Carpo, February) and 16.81% (Algerian, February) of the total fat content. The average for all samples was 14.03%. There were no significant differences for cultivars or months but Robusta demonstrated the highest values and Gymno-Carpo the lowest. The C16:0 content showed a slight increase from February to August although no significant differences between the months were indicated. The interaction between months of harvest and cultivar was not significant.

7.4.6.2.2 Stearic acid (C18:0)

Stearic acid is a saturated fatty acid that is the simplest of the 18-carbon fatty acids, with the bonds between its carbons being alike (Whitney and Rolfes, 2015). Stearic acid is characteristic of the fat molecules of the fat in meat and is generally low in plant sources (Charley, 1982). The C18:0 content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 67. The C18:0 content ranged from 2.54% (June, Algerian) to 5.11% (February, Algerian). The average C18:0 content was 2.95% of the total fatty acid content. Except for 'Algerian', February (5.11%), the values were very even for cultivars and there were no significant differences between cultivars. The interaction between months of harvest and cultivar was not significant. February values were significantly higher (3.77%) than May to August. On average 'Algerian' had the highest C18:0 content (3.13%) but it was not significantly higher than the contents of the other cultivars.

Table 67: The effect of month and cultivar on the C18:0 content (% of total fat content) of freeze-dried mucilage powders from cactus pear cladodes

Cultivar	**February**	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.823)**
Algerian	5.11	2.98	2.69	2.54	2.75	2.70	**3.13**
Morado	3.74	3.03	2.69	2.79	2.84	2.58	**2.94**
Gymno-Carpo	3.28	3.16	2.89	2.69	2.81	2.63	**2.91**
Robusta	2.94	3.47	2.74	2.79	2.45	2.53	**2.82**
Month Means (p = 0.007)	**3.77^{b}**	**3.16^{ab}**	**2.75^{a}**	**2.70^{a}**	**2.71^{a}**	**2.61^{a}**	**2.95** **C x M: (p = 0.431)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
▭ **= All means**

7.4.6.2.3 Oleic acid (C18:1c9)

Oleic acid is an 18-carbon, monounsaturated fatty acid that is abundant in nature (Salisbury and Ross, 1992) and is omnipresent in plant food sources. It is found in large amounts in vegetable oils such as olive and canola oil (Whitney and Rolfes, 2015). It is therefore also the most ingested unsaturated fatty acid in the human diet (Charley, 1982). The C18:1 content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 68.

Table 68: The effect of month and cultivar on the C18:1 content (% of total fat content) of freeze-dried mucilage powders from cactus pear cladodes

	February	April	May	June	July	August	Cultivar Means (p = 0.023)
Algerian	11.63	12.96	11.82	21.95	17.28	20.05	**15.95^{a}**
Morado	23.09	15.09	23.61	25.02	16.33	22.33	**20.91^{ab}**
Gymno-Carpo	15.16	9.19	25.82	16.11	15.91	14.56	**16.13^{ab}**
Robusta	18.77	29.07	21.39	20.93	28.16	22.60	**23.49^{b}**
Month Means (p = 0.838)	**17.16**	**16.58**	**20.66**	**21.00**	**19.42**	**19.89**	**19.12 C x M: (p = 0.621)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
▭ = All means

The C18:1 values varied a great deal and ranged between 9.19% (April, 'Gymno-Carpo') and 29.07% (April, 'Robusta'). 'Robusta' had significantly higher (p = 0.023) average occurrence of oleic acid (23.49%) than only 'Algerian' (15.95%). Oleic acid values were low as the average for all samples was 19.12%, compared to linoleic acid (62.14%). It ranged from 9.19 ('Gymno-Carpo', April) to 29.07 ('Robusta', April). Although there were no significant differences reported over months, the contents increased slightly from April to May, remained high to June and decreased again to July and August. The interaction between months of harvest and cultivar was not significant.

7.4.6.2.4 Linoleic acid (C18:2c9,12 (n-6))

Linoleic acid contributes to the omega-6 content and is abundant in nature (Salisbury and Ross, 1992). In fact, most fats of vegetable origin contain significant amounts of it (Charley, 1982). It is a polyunsaturated, 18-carbon fatty acid that has two double bonds and is common in vegetable oils and seeds such as seed oils from corn, cottonseeds and soybeans (50 – 53%)(Bennion, 1985). Usually oleic acid predominates in plant materials and it is rare for linoleic acid to predominate, as is evident in these current findings. This phenomenon is only seen in a limited amount of plant materials (Charley, 1982). Linoleic acid in seed oils determined in six cultivars collected from the same orchard outside Bloemfontein (De Wit et al., 2016a) ranged from 55.82% ('Nudosa') to 67.32% ('Monterey') while the average of linoleic acids in six cultivars was 61.42%. The C18:2 content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 69.

Table 69: The effect of month and cultivar on the C18:2c9,12 (n-6) content (% of total fat content) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.018)**
Algerian	65.07	70.10	72.63	59.42	63.91	60.57	**65.28**^{ab}
Morado	57.14	68.89	58.25	56.12	64.60	58.55	**60.59**^{ab}
Gymno-Carpo	69.22	74.55	53.97	66.15	67.28	67.80	**66.49**^{b}
Robusta	62.26	48.75	59.17	59.04	50.99	57.05	**56.21**^{a}
Month Means (p = 0.810)	**63.42**	**65.57**	**61.00**	**60.18**	**61.69**	**60.99**	**62.14** **C x M: (p = 0.345)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
☐ **= All means**

The linoleic acid content was high compared to oleic acid. It ranged from 48.75% ('Robusta', April) to 74.55% ('Gymno-Carpo', April) of the total fatty acids (%). There were significant differences observed between cultivars (p = 0.018). 'Gymno-Carpo' had significantly higher C18:2c9,12 content (66.49%) than 'Robusta' (56.21%). The overall average was 62.14%. It seems as if the three *O. ficus-indica* species had higher contents than *O. robusta* although it was not always significantly different. The interaction between months of harvest and cultivar was not significant. This corresponds to the trend observed for cactus pear seed oils. Furthermore, it seems as if hot daytime temperatures had an effect on the contents, as seen with the higher values (although not significant) during February and March. The opposite effect was seen for oleic acid.

7.4.6.2.5 Omega-6 Fatty Acids (n-6) and omega-3 Fatty Acids (n-3)

The polyunsaturated fatty acids known as omega-3 and omega-6 fatty acids have exceptional significance in fat-related human nutrition (Whitney and Rolfes, 2015). Both are 18-carbon polyunsaturated but the double bonds are at different positions. Both are essential fatty acids that the body cannot synthesise and have to be ingested. They are fundamental in a range of longer-chain fatty acids that play important roles in regulating blood pressure, preventing blood clotting and other body functions important to health (Whitney and Rolfes, 2015).

In fact, the human cells synthesise all fatty acids except for linoleic (omega-6) and α-linolenic (omega-3) acids. Both are indispensable in brain development, growth and in cardiac health. The correct ratio between omega-3 and omega-6 has to be maintained in the diet (4:1 to 10:1). Omega-3 is considered to reduce inflammation while omega-6 promotes it. Omega-6 usually occurs in plant and animal sources while the source for omega-3 is primarily found in fish (Whitney and Rolfes, 2015).

Omega-6 improves cardiac health by lowering LDL cholesterol and improving insulin resistance. There is usually enough omega-6 in a normal diet, as sources are animal fats and vegetable oils. Therefore it is recommended to reduce omega-6 ratios in favour of omega-3. An oversupply of omega- 6 in the diet may lead to the formation of blood clots, inflammation and the narrowing of blood vessels (Whitney and Rolfes, 2015).

Two fatty acids contribute to the omega-6 content. These fatty acids are linoleic acid C18:2c9,12 (n-6) (discussed in section 7.4.6.2.4) and eicosatrienoic acid (C20:3c8,11,14 (n-6) which were detected in minuscule amounts (average 0.18%). The fatty acid that represents the omega-3 content is α-Linolenic acid (C18:3c9,12,15 (n-3).

7.4.6.2.6 α-Linolenic (C18:3c9,12,15 (n-3)

The omega-3 portion of the fat content was α-linolenic acid (C18:3c9,12,15 (n-3). Omega-3 fatty acids provide more health benefits than omega-6 fatty acids. Omega-3 fatty acids are highly concentrated in the brain and eyes. It is important for cognitive (memory and performance) and behavioural functions. Symptoms of deficiencies include fatigue, poor memory, dry skin, heart problems and depression (Whitney and Rolfes, 2015). The C18:3 content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 70. The content of C18:3 was very limited. Nonetheless, it ranged between 0.05% (‘Gymno-Carpo’, February) to 0.32% (‘Robusta’, July). The overall mean content was 0.19%. ‘Robusta’ had significantly higher C18:3 content (0.27%) than all the other cultivars. However, ‘Algerian’ (0.16), ‘Morado’ (0.19) and ‘Gymno-Carpo’ (0.14) did not differ significantly from each other. There seems to be an increasing trend between the months, as August mucilage seemed to contain more than February and April but no significant differences between months were indicated. The interaction between months of harvest and cultivar was not significant. It is common to find limited amounts of omega-3 in plant sources such as cactus cladodes and therefore also the mucilage. Mucilage is thus a better sources of omega-6 fatty acids than omega-3 fatty acids.

Table 70: The effect of month and cultivar on the C18:3c9,12,15 (n-3) content (% of total fat content) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.018)**
Algerian	0.07	0.12	0.11	0.24	0.18	0.24	**0.16^{a}**
Morado	0.10	0.11	0.24	0.18	0.19	0.29	**0.19^{a}**
Gymno-Carpo	0.05	0.06	0.21	0.18	0.13	0.23	**0.14^{a}**
Robusta	0.23	0.21	0.28	0.30	0.32	0.29	**0.27^{b}**
Month Means (p = 0.035)	**0.11^{a}**	**0.12^{a}**	**0.21^{b}**	**0.23^{b}**	**0.20^{b}**	**0.26^{b}**	**0.19** **C x M: (p = 0.454)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

7.4.6.2.7 Total saturated fatty acids (SFA) content

Fatty acids are saturated when there are no double bonds between carbons. Thus, every carbon on the fatty acid chain is linked to two other carbon atoms and to two hydrogen atoms (Brown, 2007; Charley, 1982; Salisbury and Ross, 1992). Saturated fats are deemed as unhealthy as the uncontrolled intake of such fats are associated with high cholesterol, overweight, obesity and could be the cause of innumerable chronic diseases (Bennion, 1985; Whitney and Rolfes, 2015). There are limited plant sources of fats that are mostly saturated (such as palm oil and coconut oils), usually saturated fats in plants are diminutive (Brown, 2007). The SFA content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 71.

Table 71: The effect of month and cultivar on saturated fatty acids content of freeze-dried mucilage powders from cactus pear cladodes

Cultivar	**Feb**	**April**	**May**	**June**	**July**	**Aug**	**Cultivar Means (p = 0.117)**
Algerian	22.33	15.93	14.55	17.40	17.62	18.09	**17.65**
Morado	18.53	14.89	16.93	17.66	17.85	17.65	**17.25**
Gymno-Carpo	14.65	15.17	18.87	16.57	16.16	16.32	**16.29**
Robusta	17.65	20.92	18.15	18.45	19.21	18.63	**18.84**
Month Means (p = 0.919)	**18.29**	**16.73**	**17.12**	**17.52**	**17.71**	**17.67**	**17.51** **C x M: (p = 0.513)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

Compared to the total % fat, the saturated fat content in freeze-dried mucilage powders ranged between 14.55 and 22.33%. The average was 17.51%. There were no trends or significant differences observed for months or cultivars although this content followed the same trend as observed for C16:0, being higher in *O. robusta*. It also showed correlation with

C18:0 being higher during the hot summer months (February and April). The interaction between months of harvest and cultivar was not significant.

7.4.6.2.8 Total monounsaturated fatty acids (MUFA) content

When the fatty acid is unsaturated, double bonds are present in the fatty acid chain. Fatty acids with one double bond occurring in the fatty acid chain is monounsaturated. One carbon atom is thus linked to a second carbon by a double bond. Monounsaturated fatty acids occur primarily in plant sources of food such as olives, peanuts and avocado (Brown, 2007; Charley, 1982). Monounsaturated fats are healthier than saturated fats and is present in a variety of foods and oils. Foods rich in monounsaturated fatty acids improve blood cholesterol levels, which can decrease the risk of heart disease and type 2 diabetes (Whitney and Rolfes, 2015). The main MUFA found in mucilage is oleic acid. The MUFA content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 72.

Table 72: The effect of month and cultivar on monounsaturated fatty acids content on freeze-dried mucilage powders from cactus pear cladodes

Cultivar	**February**	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.023)**
Algerian	12.47	13.79	12.70	22.83	18.18	20.91	**16.81**^{a}
Morado	24.16	16.04	24.50	25.93	17.26	23.23	**21.85**^{ab}
Gymno-Carpo	16.02	10.18	26.77	16.96	16.38	15.43	**16.96**^{a}
Robusta	19.56	29.83	22.24	21.78	28.90	23.40	**24.29**^{b}
Month Means (p = 0.840)	**18.05**	**17.46**	**21.55**	**21.88**	**20.18**	**20.74**	**19.98** **C x M: (p = 0.348)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
☐ = All means

The ratio of MUFA to total fatty acids was higher than SFA. It ranged from 10.18% ('Gymno-Carpo', May) to 29.83% ('Robusta', April). The average MUFA was 19.98%. 'Algerian' (16.81%) and 'Gymno-Carpo' (16.96%) both had significantly lower MUFA content than 'Robusta' (24.29%). Over months, no significant trends were observed, although May and June had higher values. The same trend was seen for oleic acid (C18:1) which is the main MUFA. The highest values were observed for *O. robusta.* The interaction between months of harvest and cultivar was not significant.

7.4.6.2.9 Total polyunsaturated fatty acids (PUFA)

When more than one double bond occurs in the carbon-chain, the fatty acid is polyunsaturated. The location and the number of double bonds and the length of the fatty acid chain differentiates them and influences its function in food and nutrition (Charley, 1982). Polyunsaturated fatty acids are primarily from plant sources such as vegetable oils or from fish (Brown, 2007; Charley, 1982). Foods rich in PUFA improve blood cholesterol levels, which can decrease the risk of cardiovascular diseases and diabetes (Whitney and Rolfes, 2015). The PUFA content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 73.

Table 73: The effect of month and cultivar on polyunsaturated fatty acids content of freeze-dried mucilage powders

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.022)**
Algerian	65.20	70.28	72.76	59.77	64.20	61.00	**65.53**^{ab}
Morado	57.31	69.07	58.57	56.41	64.89	59.12	**60.90**^{ab}
Gymno-Carpo	69.33	74.65	54.36	66.47	67.46	68.25	**66.75**^{b}
Robusta	62.79	49.25	59.61	59.77	51.88	57.96	**56.88**^{a}
Month Means (p = 0.913)	**63.66**	**65.81**	**61.32**	**60.61**	**62.11**	**61.58**	**64.39** **C x M: (p = 0.731)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

The PUFA content in comparison to the total fatty acid (%) content was significantly higher than that of SFA and MUFA. It ranged from 49.25% ('Robusta', April) to 74.65% ('Gymno-Carpo', April). The average PUFA content was 62.51%. *O. robusta* Robusta content (56.88%) was significantly lower than 'Gymno-Carpo' (66.75%). There were no significant differences seen between months. The same trends were observed for linoleic acid (the main PUFA) as lower values were observed for 'Robusta'. The interaction between months of harvest and cultivar was not significant. Considering that the PUFA content was higher than SFA or MUFA, it can be concluded that the fats that occur in freeze-dried mucilage are healthy and would contribute to human health.

7.4.6.3 Fatty acids ratios

The total fatty acids content could therefore be divided into SFA, MUFA and PUFA content. A full presentation of the contents over months and per cultivar gave an informative perspective (Figure 102). Over 50% of the total fats were polyunsaturated and under 20% were saturated. The average ratios per cultivar showed that SFA were very similar. In fact, it was the relatively big differences in the MUFA contents that caused the PUFA content to differ. On average, 'Robusta' had the lowest content of PUFA and the highest of MUFA. 'Gymno-Carpo' and 'Algerian' had very similar contents with the lowest content of SFA and the highest of PUFA.

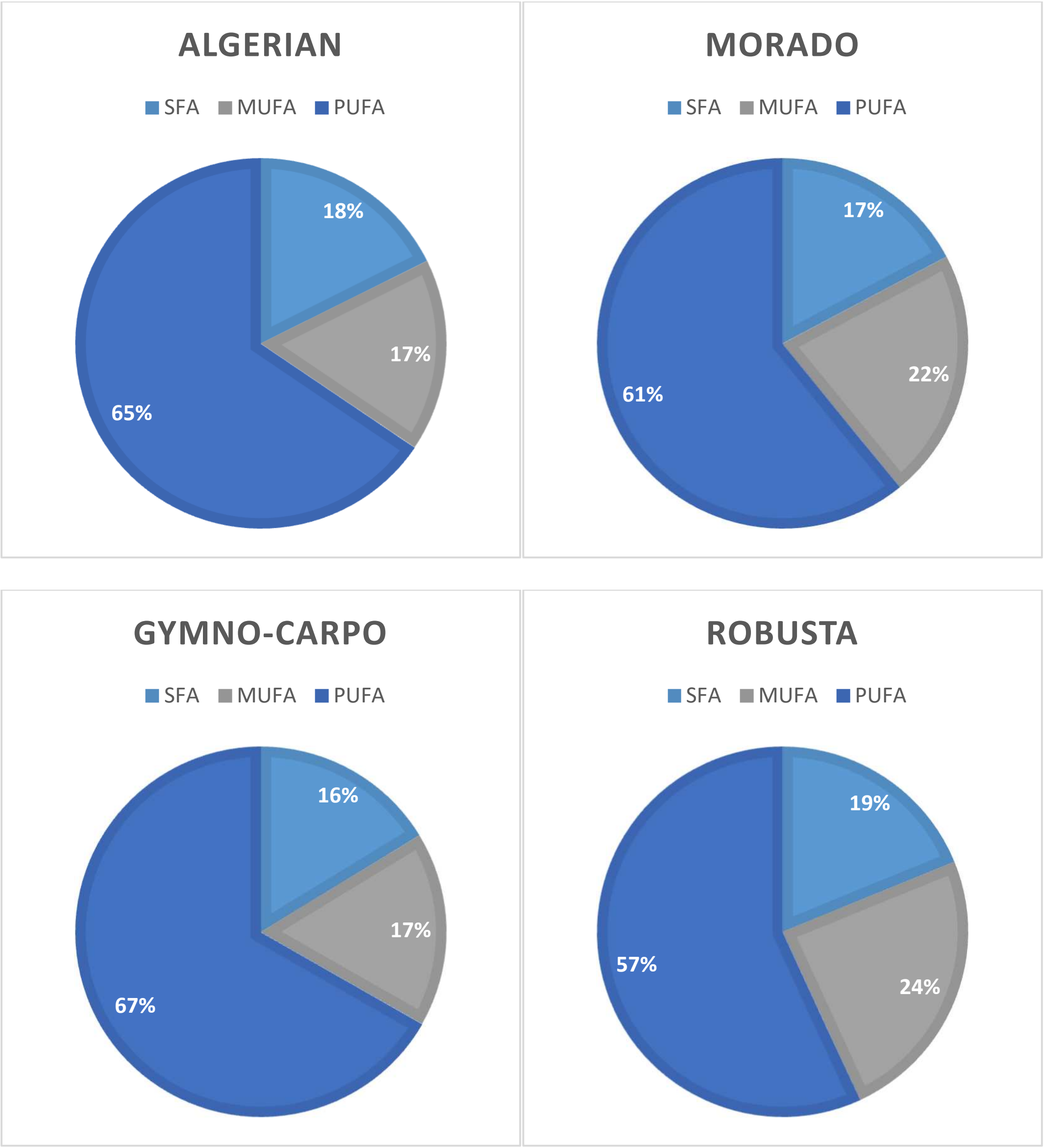


Figure 102: Comparison of average saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) content of freeze-dried mucilage powders

The mucilage fatty acid contents (ratios) in comparison to the seed oil ratios reported by De Wit et al. (2016a), determined in six cultivars collected from the same orchard outside Bloemfontein, were very similar as seen in Table 74.

Table 74: Fatty acids ratios of seed oil extracted from cactus pear seeds in fruit and freeze-dried mucilage powders from cactus pear cladodes

Fatty acid ratios	Seed oil	Mucilage
Total saturated fatty acids (SFA)	18.70	17.51
Total monounsaturated fatty acids (MUFA)	19.61	19.98
Total polyunsaturated fatty acids (PUFA)	61.68	62.51
Total omega- 6 fatty acids (n-6)	61.55	62.32
Total omega- 3 fatty acids (n-3)	0.18	0.19
PUFA:SFA	3.37	3.64

The ratio of polyunsaturated fatty acids and saturated fatty acids (PUFA: SFA) showed high polyunsaturated fat content and good ratios of healthy fatty acids. Meat commonly has a 50:50 (polyunsaturated: saturated) ratio while food originating from plants customarily have a ratio of 85:15 or 6:1 (polyunsaturated: saturated). A higher ratio means that the food is highly polyunsaturated (Brown, 2007). The PUFA: SFA content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 75.

Table 75: The effect of month and cultivar on PUFA:SFA ratio of freeze-dried mucilage powders from cactus pear cladodes

Cultivar	**February**	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.044)**
Algerian	2.92	4.41	5.00	3.44	3.64	3.37	**3.80^{ab}**
Morado	3.09	4.64	3.46	3.19	3.64	3.35	**3.56^{ab}**
Gymno-Carpo	4.73	4.92	2.88	4.01	4.17	4.18	**4.15^{b}**
Robusta	3.56	2.35	3.29	3.24	2.70	3.11	**3.04^{a}**
Month Means (p = 0.882)	**3.58**	**4.08**	**3.66**	**3.47**	**3.54**	**3.50**	**3.64 C x M: (p = 0.484)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
☐ **= All means**

The PUFA:SFA ratio ranged from 2.35 (‘Robusta’, April) to 5.00 (‘Algerian’, May). The overall ratio was 3.64. ‘Gymno-Carpo’ ratio was significantly higher (4.15%) than ‘Robusta’ (3.04%), but no significant differences were observed between ‘Robusta’ (3.04%), ‘Algerian’ (3.80%) and ‘Morado’ (3.56%). April had the highest ratios overall but there were no significant differences or trends observed between months. The interaction between months of harvest and cultivar was not significant. These ratios are somewhat lower than that reported by Brown (2007) for highly PUFA foods.

It was observed in the limited fat content of freeze-dried mucilage powder that PUFA were more abundant than SFA and MUFA, and that linoleic acid, better known as omega-6, predominated over omega-3 fatty acids. Although the polyunsaturated fatty acids contribute to well-being, the percentage of fats were too inconsequential to consider mucilage powder as a good source of essential fatty acids. In fact, freeze-dried mucilage is mostly fat free.

7.4.7 Total carbohydrate content

CAM plants store energy by producing carbohydrates during the day. When the sun is shining, malic acid that has been produced at night, is carboxylated (Salisbury and Ross, 1992). Energy is usually stored in the form of sucrose and starch. Bright light and long days (summer) are favourable for photosynthesis and the formation of starch (Salisbury and Ross, 1992).

Food containing some form of carbohydrates originating from plants is the main food source for humans. Carbohydrates provide the energy and heat that the human body commonly utilizes for energy. A constant supply is needed for the optimal functioning of the brain and muscles. When food containing carbohydrates are consumed, the body receives glucose for immediate energy and the unused energy is reserved for later use. In excess, carbohydrates such as sugars are detrimental to health and cause overweight, obesity, dental cavities, diabetes, hypertension and heart disease (Whitney and Rolfes, 2015).

Carbohydrates are composed of three major constituents namely monosaccharides, disaccharides and polysaccharides. Monosaccharides found in mucilage are glucose and fructose and will be discussed shortly. Sucrose is a disaccharide present in mucilage and the discussion on sucrose will follow. In terms of polysaccharides, two types are recognised, namely, the digestible and the indigestible carbohydrates. Starch found in mucilage would form part of the digestible polysaccharides while the fibre is indigestible. Indigestible fibre includes the following substances found in cladodes: cellulose, hemicellulose, pectin, gums (mucilage), inulin and lignin. Of the indigestible fibre, some are insoluble (cellulose, hemicellulose, lignin and resistant starch) and some are soluble (gums (mucilage), fructans, inulin and pectin). Cladodes contain carbohydrates and thus when the whole cladodes are dried (such as when cladode flour is made), it would contain all the carbohydrates and fibre mentioned here in various amounts. In this study though, mucilage (gum) was extracted from the cladode and it is thus expected that it would contain mostly soluble indigestible fibre and only trace elements of any other substance. Thus, mucilage is classified as an ingestible, soluble fibre that forms part of total carbohydrates, although it contains fractions of protein, fats and carbohydrates (sugars, starch and insoluble fibre) (Brown, 2007; Whitney and Rolfes, 2015).

Mucilage content in cladodes is influenced by temperature and irrigation and the carbohydrate profiles may vary according to individual sugar contents and polysaccharide structures (Ginestra et al., 2009). The total carbohydrate content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 76 .

Table 76: The effect of month and cultivar on the total carbohydrate content (%) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.989)**
Algerian	60.43	55.94	64.43	64.37	64.77	64.16	**62.35**
Morado	60.65	56.71	63.93	65.88	65.59	64.26	**62.84**
Gymno-Carpo	60.06	57.32	63.53	66.47	64.97	63.45	**62.63**
Robusta	55.10	56.53	62.36	66.63	64.24	67.66	**62.09**
Month Means (p < 0.001)	**59.06^{a}**	**56.63^{a}**	**63.56^{b}**	**65.84^{b}**	**64.89^{b}**	**64.88^{b}**	**62.48** **C x M: (p = 0.325)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

The carbohydrate contents ranged between 55.10% (February, 'Robusta') to 67.66 (August, 'Robusta'). The overall average was 62.48%. In terms of cultivar, there were no significant differences. February (59.06%) and April (56.63%) had significantly lower carbohydrates than the other months (May, June, July and August). April mucilage (56.63%) had the lowest content of carbohydrates while June had the highest (65.84). The interaction between months of harvest and cultivar was not significant. No information on carbohydrate content of mucilage is available. These values correlated very well with total carbohydrates in fresh cladodes and cladode flour reported by other authors.

Ayadi et al. (2009) reported high total carbohydrates as 60.36 g/100 g DM for spiny cladodes and 60.93 g/100 g DM for spineless cladodes. Total carbohydrates were 67.6 to 68.23 g/100 g DM in López-Cervantes et al. (2011) detected in young cladodes. Samia El-Safy (2013) found 45.062% carbohydrates in cladode flour. Ginestra et al. (2009) found 36% of whole cladodes (dry weight) and 37% of the alcohol insoluble cladode extract to be carbohydrates.

7.4.7.1 Starch analysis

Carbohydrates are mostly stored in plant cells as starch. It is concentrated in the chloroplasts where it is formed from photosynthesis and exists in the form of plastids. Amylose and amylopectin are the two types of starch that are present in most grains and which are composed of D-glucose chains. These chains can consist of 2000 to 500 000 glucose units (Salisbury and Ross, 1992).

Starch is constructed by plants in the interest of storing energy, as starch is the form in which plant cells store glucose. When starch is ingested by humans, it is hydrolysed in the body to form glucose to be used for energy. Starchy food is the main food source for humans (Whitney and Rolfes, 2015). The starch content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 77.

Table 77: The effect of month and cultivar on the starch content (g/100 g DW) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p < 0.586)**
Algerian	5.38	3.62	6.64	5.85	6.32	6.29	**5.69**
Morado	5.53	5.53	4.39	5.37	5.03	7.34	**5.53**
Gymno-Carpo	5.66	6.22	5.74	4.72	7.12	6.99	**6.08**
Robusta	4.92	5.42	5.28	6.69	6.45	10.14	**6.49**
Month Means (p = 0.032)	**5.38^{ab}**	**5.12^{a}**	**5.51^{ab}**	**5.66^{ab}**	**6.24^{ab}**	**7.69^{b}**	**5.95** **C x M: (p = 0.321)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
☐ = All means

Starch values ranged from 3.62 g/100 g (April, 'Algerian') to 10.14 g/100 g (August, 'Robusta'). The cultivar means ranged from 5.53 ('Morado') to 6.49 g/100 g ('Robusta') although there were no statistical significant differences between cultivars. The average starch content was 5.95 g/100 g (5.9%). Statistical differences were observed between months. In February the starch content was almost the same in all the cultivars (average 5.38 g/100 g) and do not differ significantly from each other. A significant drop took place in April for 'Algerian' (3.62 g/100 g), causing lower means than in February (5.39 g/100 g). The starch content of 'Algerian' increased drastically to May (6.64 g/100 g). However, from May, the starch values were consistent and no significant differences were observed between months until August. The interaction between months of harvest and cultivar was not significant.

The formation of starch in plants has never been completely understood but the effect of the environment on starch formation has been confirmed. Longer days and the presence of sunlight encourage more photosynthesis and increased translocation, therefore starch accumulates in both the chloroplasts and amyloplasts. Starch formation is thus influenced by seasons, as the longer days of August activated the formation of more starch. Plant growth is particularly influenced by changes in season as changes in temperature initiates critical processes in the life cycle of the plant (Salisbury and Ross, 1992).

Ginestra et al. (2009) observed (using SEM analysis) that the parenchyma from the central core of the cactus pear cladode tissue stained blue/brown with iodine/potassium, therefore starch was present in the freshly harvested cladode tissue. Ayadi et al. (2009) found significantly different starch content in spiny cladodes (7.63 g/100 g) and spineless cladodes

(13.9 g/100 g). Fluctuations in carbohydrate contents as a result of season were reported before by Sutton et al. (1981). There is very little information available on starch content of cladodes as it seems that researchers focus their attention on the determination of total carbohydrates and sugar composition. (No information is available on starch content of mucilage).

7.4.7.2 Sucrose/D-Glucose/D-Fructose analysis

7.4.7.2.1 Sucrose

Sucrose is a disaccharide that consists of a fructose and a glucose molecule. It tastes sweet because it contains fructose that has an intensely sweet taste and is the natural sweetness tasted in fruit, vegetables and grains (Whitney and Rolfes, 2015). In human food, sugars are not only used for its sweet taste in food and food products, it also contributes to browning, caramelization, to feed yeast cells and help to achieve a fine, even texture in baked products (Bennion, 1985).

Sucrose is common and abundant in plants as it acts as an energy source in photosynthetic cells and can be easily transported by the phloem. Free glucose and fructose are not precursors of sucrose, but for sucrose to undergo respiration, it first has to be split into glucose and fructose (Salisbury and Ross, 1992).

Fats that are stored in the stems (cladodes) of the plant contain much greater amounts of energy compared to carbohydrates. During the respiration of fats, the energy that is released is used for growth and development. Fats cannot be transported to areas where growth is commencing. Consequently, fats are converted to sucrose in the glyoxysomes which are micro bodies that occur in plant cells. Lipases hydrolyse fats into glycerol and fatty acids which is converted to sucrose through the cooperation of the glyoxysomes, cytosol and mitochondria. Sucrose is then transported by the phloem to growing hotspots in order to provide the carbon required for growth and development (Salisbury and Ross, 1992).

When sucrose had been hydrolysed to fructose and glucose, the sucrose content became zero (but negative values are obtained during analysis). High sucrose content indicated that energy was being transported by the phloem to the areas where it is required for growth and development. When abundant glucose and fructose were available for glycolysis and respiration purposes, sucrose content decreased. The sucrose content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 78. The sucrose content ranged from 0 mg/g to 137.23 mg/g (June, ‘Algerian’). The average sucrose content was 14.64 mg/g (1.4%). The sucrose content fluctuated greatly between the months and cultivars. Consequently neither the average contents for cultivars nor months were indicative of sucrose content as it fluctuated greatly and was zero at times.

Table 78: The effect of month and cultivar on the sucrose content (mg/g DW) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (NSA)**
Algerian	0.00	33.33	16.95	137.23	0.00	0.00	31.25
Morado	17.72	0.00	0.00	0.00	0.00	0.00	2.95
Gymno-Carpo	25.74	0.00	19.00	0.00	0.00	10.36	9.18
Robusta	53.38	0.00	0.00	0.00	0.00	37.72	15.18
Month Means (NSA)	24.21	8.33	8.99	34.31	0.00	12.02	14.64

NSA = Not statistically analysed.

☐ = All means

The most appreciable effect of the seasons and differences between months were observed in ‘Algerian’ while the other cultivars showed fewer fluctuations (Table 78). ‘Algerian’ showed contrary sucrose results to the other cultivars. ‘Algerian’ sucrose levels were the lowest (0.00 mg/g) in February when the other cultivars had values above 15 mg/g. When sucrose levels decreased to April for the other cultivars, ‘Algerian’ increased considerably. In April, ‘Robusta’ (that had the highest sucrose levels in February) had no sucrose (0.00 mg/g) while ‘Algerian’ sucrose levels went from the lowest to the highest sucrose content (33.33 mg/g) (Table 78). Another interesting difference in ‘Algerian’ results was seen in June samples, where ‘Algerian’ sucrose levels increased sharply (137.23 mg/g) while those of the other cultivars decreased or remained zero (0.00 mg/g). In July all cultivars had no sucrose until August when ‘Gymno-Carpo’ and ‘Robusta’ sucrose levels increased (Table 78). The interaction between months of harvest and cultivar was not significant.

7.4.7.2.2 D-Glucose (immediate energy)

Glucose is one of the main products of photosynthesis, which takes place in the cladodes, thus glucose is abundant in cladodes. Glucose performs different functions in cladodes and thus would be present in larger amounts than other sugars. Starch also supplies the glucose for glycolysis (Salisbury and Ross, 1992). Glucose is a monosaccharide, with only a mild sweet flavour in human food. It serves as an immediate source of energy source for all forms of life (Whitney and Rolfes, 2015). The glucose content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 79. The glucose content detected in this test would include all glucose, also the glucose broken down from other disaccharide sugars. Glucose content ranged from 112.46 mg/g (February, ‘Morado’) to 240 mg/g (August, ‘Algerian’). The average glucose content was 198.51 mg/g (19.85%). No significant differences were detected between cultivars with ‘Gymno-Carpo’ having the highest glucose content (206.19 mg/g). The interaction between months of harvest and cultivar was not significant.

There were statistically different values between months (p = 0.012). In February, the mean glucose values were the lower (152.09 mg/g) than July 214.09 mg/g) and August (219.10 mg/g). The highest mean values were detected in April, July and August, with August having the highest mean glucose content (219.10 mg/g), but it was not significantly higher than May and June. No information on glucose content of mucilage is available.

Table 79: The effect of month and cultivar on the glucose content (mg/g DW) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.766)**
Algerian	141.53	212.55	223.18	135.57	195.52	240.85	**191.53**
Morado	112.46	223.26	179.97	212.43	214.89	204.31	**191.22**
Gymno-Carpo	185.37	190.62	208.96	209.92	230.49	211.80	**206.19**
Robusta	169.01	214.99	195.22	216.52	215.47	219.44	**205.11**
Month Means (p = 0.012)	**152.09^{a}**	**210.36^{b}**	**201.83^{ab}**	**193.61^{ab}**	**214.09^{b}**	**219.10^{b}**	**198.51** **C x M: (p = 0.432)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

Ayadi et al. (2009) reported glucose content in cladode flour as 24.9% in spiny cladode flour and 7.47% in spineless cladode flour of soluble sugars. Ginestra et al. (2009) reported glucose content in different extractions as being between 117.31 mg/g and 208.72 mg/g. In fact, glucose content of 127.87 mg/g was reported in water soluble cladode extract and 122.42 mg/g in alcohol insoluble cladode extract while 153.15 mg/g was reported in whole dry cladodes. The total monosaccharides composition was 362.89 mg/g in whole cladodes, 375.11 mg/g in alcohol insoluble extracts and 455.63 mg/g in water soluble cladode extracts. Consequently, Ginestra et al. (2009) reported high (41%) content of glucose in extracts from cladodes. It was described as a prevalence of glucose in all samples tested.

7.4.7.2.3 Fructose (stored energy)

Fructose is a monosaccharide with an intensely sweet flavour like that of honey. The structure differs from glucose, the arrangement of the atoms stimulates the taste buds and produces a sweet sensation (Charley, 1982).

Fructans in plants are fructose polymers and are smaller than the glucose polymers of starch as the polymer chains only contain between three and a few hundred units. These water-soluble polymers are amalgamated and stored in vacuoles. They are formed by bonding more fructose units to the fructose of a sucrose molecule. Therefore the primary role of fructans in plants is carbohydrate storage and is quickly available as fructose can be respired straightaway (Salisbury and Ross, 1992). The fructose content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 80.

Table 80: The effect of month and cultivar on the fructose content (mg/g DW) of freeze-dried mucilage powders

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.828)**
Algerian	4.86	51.13	35.57	40.93	39.89	47.26	**36.61**
Morado	4.48	24.97	46.52	47.64	44.42	44.75	**35.46**
Gymno-Carpo	39.67	67.12	19.32	51.46	57.72	26.82	**43.68**
Robusta	14.62	26.89	29.10	39.26	77.84	18.14	**34.31**
Month Means (p = 0.031)	$\mathbf{15.91^{a}}$	$\mathbf{42.53^{ab}}$	$\mathbf{32.63^{ab}}$	$\mathbf{44.82^{ab}}$	$\mathbf{54.97^{b}}$	$\mathbf{34.24^{ab}}$	**37.52** **C x M: (p = 0.522)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ = All means

The mean fructose content was 37.52 mg/g (3.75%). The content of fructose ranged between 4.48 mg/g and 77.84 mg/g. 'Gymno-Carpo' fructose levels dropped sharply in May, while 'Robusta' levels peaked in July. No statistical differences were detected between cultivars, although 'Gymno-Carpo' had the highest and 'Robusta' the lowest means. Statistically meaningful lower mean levels were detected in February while significantly higher means were found in July. The interaction between months of harvest and cultivar was not significant. No information on fructose content of mucilage is available.

Ayadi et al. (2009) found fructose to be the major sugar in cladodes as 54.15% of soluble sugars were found in spiny cladode flour and 90.33% (of soluble sugars) in spineless flour.

An analysis of the trends of carbohydrate (sucrose, glucose and fructose) contents fluctuations observed over months and per cultivar follows as indicated in the graphs (Figures

93-96). This analysis sheds an interesting light on the influence of the environment on carbohydrate metabolism and the stresses on the cactus pear plant.

7.4.7.2.4 'Algerian'

When sucrose had been hydrolysed to fructose and glucose, the sucrose content became zero. Negative values were obtained during analysis as a trademark of this specific kit used for analysis. These negative values allowed for interesting analysis in terms of finding trends and comparisons between sucrose levels and the fructose and glucose levels, however, when the sucrose levels dropped below zero, there was no sucrose to be detected. In Figure 103 an inverse correlation emerged between glucose and sucrose that was evident in all cultivars; when glucose levels decreased, sucrose increased (June) and the opposite was seen when glucose increased (May, July and August). Fructose and starch remained unchanged over months.

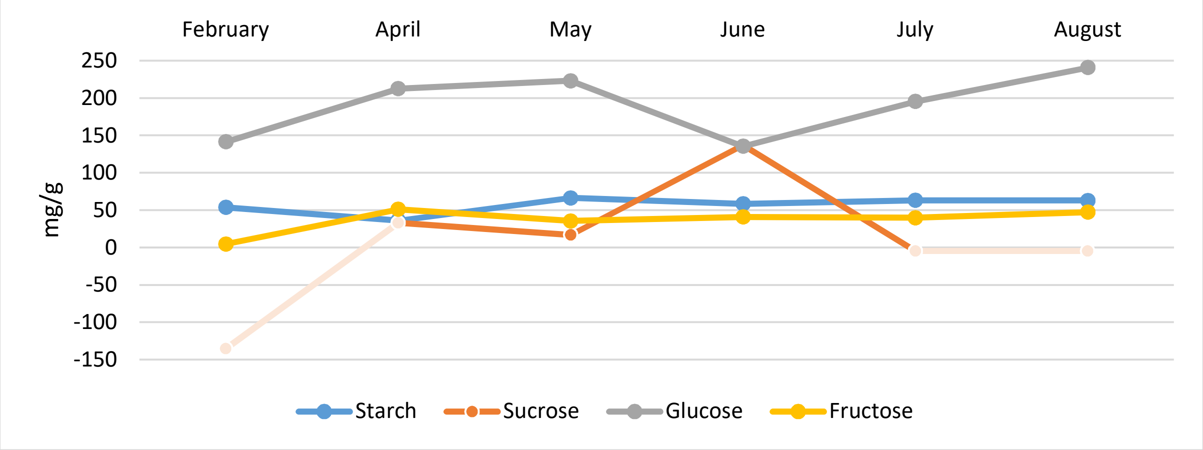


Figure 103: Trends of carbohydrate content fluctuations over months in the freeze-dried powder of the cultivar Algerian

7.4.7.2.5 ''Morado'

In Figure 104, the same trend was observed for 'Morado', the trend lines show that glucose and sucrose levels were the inverse of each other, when one increased the other decreased and vice versa. As was seen in 'Algerian', fructose and starch remained level from month to month.

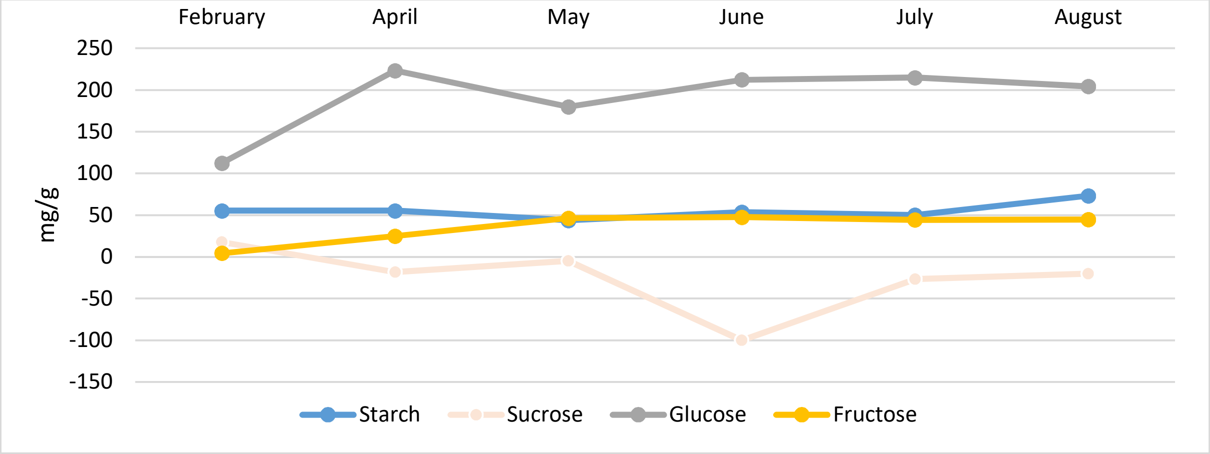


Figure 104: Trends of carbohydrate content fluctuations over months in the freeze-dried powder of the cultivar Morado

7.4.7.2.6 'Gymno-Carpo'

As was seen in 'Morado', the glucose and sucrose trend line remain the inverse of each other. Interestingly, for 'Gymno-Carpo' (Figure 105) there was also an inverse correlation between fructose and sucrose, as can be seen from the trend lines above. Therefore sucrose and fructose seemed to follow the same inverse correlation pattern as sucrose and glucose.

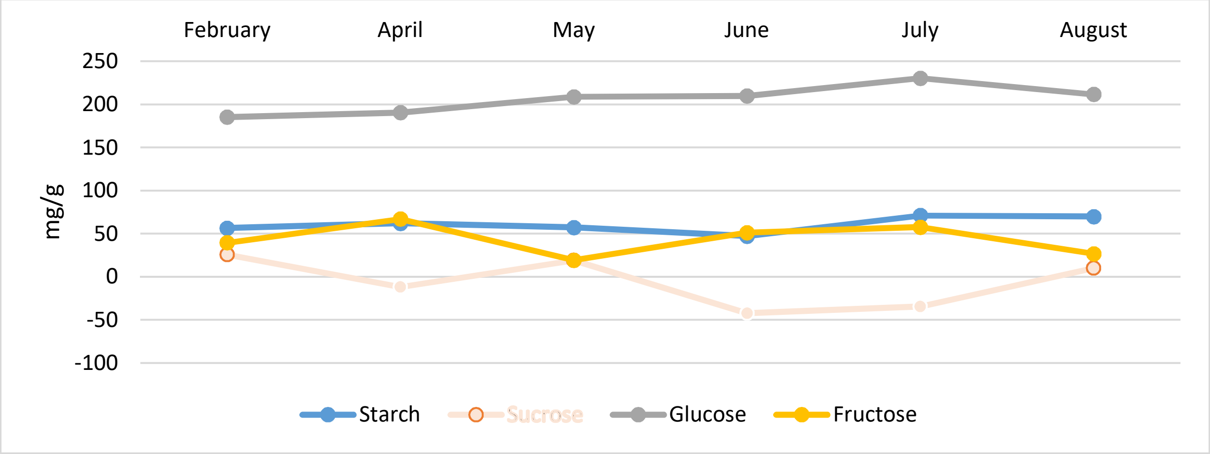


Figure 105: Trends of carbohydrate content fluctuations over months in the freeze-dried powder of the cultivar Gymno-Carpo

7.4.7.2.7 'Robusta'

In 'Robusta', a different pattern was observed. Sucrose showed a sharp decline in May and June, while glucose stayed relatively constant (Figure 106). As was seen in 'Gymno-Carpo', the sucrose and fructose contents showed an inverse correlation.

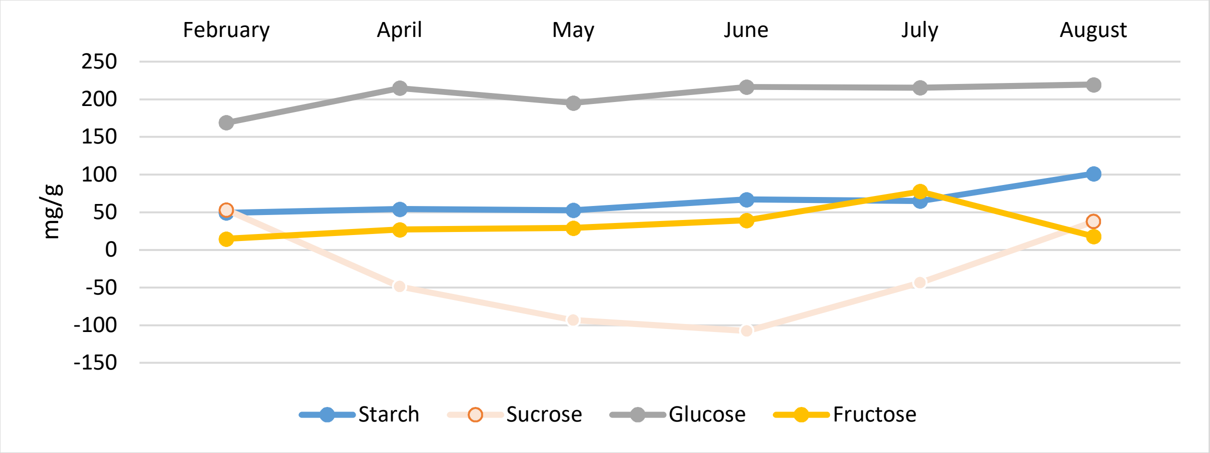


Figure 106: Trends of carbohydrate content fluctuations over months in the freeze-dried powder of the cultivar Robusta

When carbohydrates are being transported in a plant, it is usually in the form of sucrose. In the summer months, the ‘Algerian’ cactus pear plant was secure but during these winter months, ‘Algerian’ was struggling for survival although the plants were in its dormant stage. In the coldest month (June) sucrose was being transported in order to supply the plant with energy. Therefore the glucose levels were lower as it was being used, while simultaneously the fructose levels were high, as energy was being stored for its immediate availability. The lack of sharp fluctuations observed in the other three cultivars indicated that those cultivars are hardier and thus did not experience stress during the extreme temperatures in Bloemfontein from summer to winter. It is important to note that these observations were only done on one season while data from at least three seasons would need to be available for the confirmation of this observation.

Freeze-dried mucilage powder contained too little sugar and starch to make a meaningful contribution to human nutrition. The sugar content was too low to impart a sweet flavour to the products and the starch content was too low to influence products (to which it was added) in any way. The energy contribution from starch and sugars would be low.

Total soluble sugars were reported by Ayadi et al. (2009) in cladode flour as 2.49 g/100 g DM for spiny cladodes and 6.01 g/100 g DM for spineless cladodes. Saccharose was 29.95% for spiny and 2.2% of soluble sugar for spineless cladode flour of total sugars, glucose was 24.9% for spiny and 7.47% of total soluble sugars for spineless cladode flour and fructose 54.15% for spiny and 90.33% for spineless cladode flour of total soluble sugars.

Majdoub et al. (2001) determined sugar content in extensively ultra-filtrated pectin extracted from whole cladodes and found 1.0% glucose, 18.7% xylose, 20.3% galacturonic acid, 6.9% rhamnose and 33.1% arabinose. The charged sugar was found and estimated at 20% and therefore evidence was found for interaction with divalent cations (Ca^{2+} and Mg^{2+}). The negative charges (galacturonic and glucuronic acid) that exist along the polymer chain interacts with the salts, which in turn affect the viscosity of the pectin. Rodriguez-Felix and Cantwell (1988) found soluble sugars to be 9.87%. In this study, mucilage was not hydrolysed therefore the sugar composition was not determined.

7.4.8 Crude fibre

Fibre refers to material that is formed in plants for its structure and protection. Plant cells are surrounded by primary and sometimes secondary cell walls that have tensile strength comparable to that of a steel cable. Cellulose micro fibrils are embedded through crosslinking in hemicellulose and are closely related to the pectic substances which are hydrated and are part of the primary cell walls. As long as the fibrous substances are part of a growing plant it stretches and allows the plant to grow. It is porous enough for water and minerals to move through, yet rigid enough to give the plant strength (Salisbury and Ross, 1992).

Once the plant becomes human food, the fibre cannot be broken down by the human digestive enzymes and is called roughage or bulk. It fulfils an important role in nutrition and digestive tract health. Fibre is composed of cellulose, hemicelluloses, beta-glucans, pectins and gums, which are all polysaccharides (Bennion, 1985).

Cellulose and hemicelluloses are insoluble in water while beta-glucans, pectic substances and gums are soluble and are the substances responsible for binding water and thus making it difficult for dispersed substances to escape. Foods rich in soluble fibre lower blood cholesterol by binding with bile acids in the gastrointestinal tract and thus causing it to be excreted. Consequently, the body has to use the available cholesterol to manufacture new bile acid. Fermentation of fibre in the colon also contributes to lower cholesterol. Lowering of cholesterol helps to decrease the risk of heart disease. Soluble dietary fibre such as mucilage has also been linked to managing type 2 diabetes, the health of the large intestine, protection against cancer of the colon and weight management (Whitney and Rolfes, 2015).

Fibre is characterised by the undigested portion of carbohydrates, furthermore fibre is classified according to its solubility in water. NDF (neutral detergent fibre) and ADF (acid detergent fibre) represents the insoluble fibre portion that is the roughage or bulk. Mucilage is soluble fibre therefore it was expected that only minute amounts of insoluble fibre (NDF and ADF) would be detected.

7.4.8.1 Neutral detergent fibre (NDF)

Neutral detergent fibre analysis only measures the structural components of plant cells such as cellulose, lignin and hemicellulose. Since mucilage forms part of the soluble gums, very little to no structural components were expected. The NDF content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 81.

Table 81: The effect of month and cultivar on the neutral detergent fibre (NDF) content (g/kg DW) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.014)**
Algerian	1.38	0.31	0.64	0.00	0.61	1.49	**0.74^{a}**
Morado	1.36	0.62	0.70	2.60	0.75	2.48	**1.42^{a}**
Gymno-Carpo	2.23	0.05	5.51	1.24	0.00	1.12	**1.69^{ab}**
Robusta	1.46	1.30	6.41	4.14	9.39	4.54	**4.54^{b}**
Month Means (p = 0.694)	**1.61**	**0.57**	**3.32**	**2.00**	**2.69**	**2.41**	**2.10** **C x M: (p = 0.431)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ = All means

NDF contents were low as the overall average was 2.10 g/kg. The NDF values ranged from 0.00 ('Algerian', June and 'Gymno-Carpo', July) to 9.39 g/kg ('Robusta', July). There were no statistical significant differences between months detected, nevertheless April had the lowest values (means 0.57 g/kg) and May the highest (means 3.32 g/kg). May demonstrated high mean NDF values, mostly as a result of the high 'Gymno-Carpo' and 'Robusta' values. There were significant differences between cultivars. 'Robusta' had significantly higher NDF contents (4.54 g/kg) than 'Algerian' and 'Morado' (0.74 and 1.42 g/kg), but 'Gymno-Carpo' (1.69 g/kg) did not differ significantly from any of the other cultivars. The interaction between months of harvest and cultivar was not significant.

It could be speculated that the structures of *O. robusta* differ from that of *O. ficus-indica*. In the SEM analysis (Figure 93), it was observed that the tissue samples of the 'Robusta' cladodes were slimier than the other cultivars and that the crystal morphology was different. There may exist a difference in the interactions and bonds between the fibres that may be indicated by the disparity in the yield, viscosity (discussed in chapters 4 and 5) and in the significant differences in the NDF values. It was demonstrated that very little of the total fibre content found in mucilage, contained NDF.

7.4.8.2 Acid detergent fibre (ADF)

Acid detergent fibre also measures the woody fibre that is highly indigestible but it does not include hemicellulose. The ADF content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 82.

Table 82: The effect of month and cultivar on the acid detergent fibre (ADF) content (g/kg DW) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.321)**
Algerian	1.37	0.80	0.00	2.14	0.00	0.98	**0.88**
Morado	1.28	0.85	0.78	2.38	0.00	1.15	**1.07**
Gymno-Carpo	2.77	0.19	3.14	2.81	0.00	0.18	**1.52**
Robusta	0.00	0.00	3.16	2.30	4.65	3.46	**2.26**
Month Means (p = 0.523)	**1.36**	**0.46**	**1.77**	**2.41**	**1.16**	**1.44**	**1.43** **C x M: (p = 0.321)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ **= All means**

ADF ranged from 0 g/kg seen in six different samples (Table 82), to 4.65 g/kg ('Robusta', July). The average ADF in mucilage was 1.43 g/kg. There were no statistically different values for months or cultivars. As was observed in NDF values, 'Algerian' had the lowest average ADF values (0.88 g/kg) and 'Robusta' the highest (2.26 g/kg). As was evident in NDF values, the amount of total fibre content that consist of ADF, was very low. The interaction between months of harvest and cultivar was not significant.

The NDF and ADF together constitute the insoluble fibre content of freeze-dried mucilage. In Figure 107 the low totalled NDF and ADF values showed that the mucilage that was extracted from cladodes were mostly purely soluble fibre (mostly mucilage and possibly pectin) and contained very little insoluble fibre.

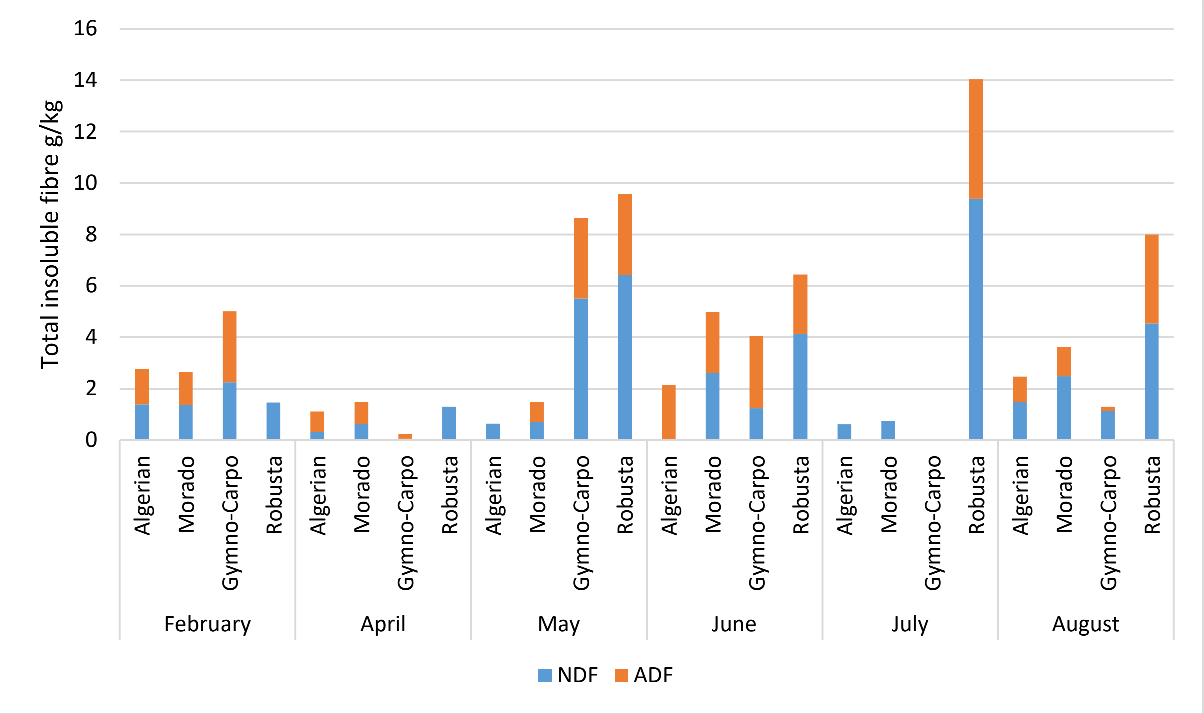


Figure 107: Total NDF and ADF contents of freeze-dried mucilage powders from cactus pear cladodes according to cultivars over months

These findings correlated with that of Gebresamuel and Gebre-Mariam (2012) as equally low insoluble fibre was found. In fact, the extremely low crude fibre content (0.06%) was reported in mucilage powders from Ethiopian cladodes. Ayadi et al. (2009) determined the total dietary fibre (TDF) in cladode flour as 51.24% for spiny cladodes and 41.83% of total carbohydrates for spineless cladode flour. Insoluble dietary fibre was 34.58% for spiny and 30.36% for spineless cladode flour and soluble fibre was 12.98% for spiny and 8.78% for spineless cladode flour. According to López-Cervantes et al. (2011) the crude fibre content was 6.0 – 6.15 g/100 g in cladode flours dried at different temperatures. Cladode flour investigated by Samia El-Safy (2013), contained 18.468% DM fibre, consequently it was seen as a good source of fibre. Padron-Perreira et al. (2009) reported 22.07% and 20.51% NDF, 14.81% and 13.76% ADF in cladode flour and enzymatically hydrolysed flour. Sáenz (1997) reported the dietary fibre content of cladode flour as 42.99% of which 28.45% was insoluble and 14.54% soluble fibre and later tested mature cladode flour dried in a forced air tunnel and found the crude fibre content to be 6.76 g/100 g (Sáenz et al. 2010). The ratio of insoluble and soluble fibre was 2:1, which is the norm when high fibre flour is used in food products (Sáenz et al., 2010). Sepúlveda et al. (2013) was looking for differences between cladode flour from cladodes with and without peel and found 7.25/100 g and 6.03/100 g respectively.

7.4.9 Chemical minerals

Just like animals and humans, plants need minerals for survival and growth. Plants require minerals such as calcium, phosphorous, sulphur, potassium and iron. Various species absorb various amounts from the soil, depending on what it needs to complete its life cycle. All the minerals, in a soluble form, contribute to osmotic pressure and additionally some have an essential role in the structure of the plant and others form part of essential enzymes that contribute to chemical reactions (Salisbury and Ross, 1992).

In order to feed humans, plants need to be properly fertilized first (Salisbury and Ross, 1992). By the time plants have been transformed into sources of food, it is important to handle it in such a way that minerals are protected, as they are easily lost through the handling, fractioning, leaching, blanching and non-bioavailability. In food, the elements are found in soluble form (Na^+, K^+, Cl^- and SO_4^{2-}), colloidal form (Ca^{2+} and PO_4^{2-}), chelated (oxalic acid) and in interaction with other ingredients (e.g. Fe, Zn, Ca and fibre). The behaviour and capacity for absorption are often determined by other components present in the food, for example fibre, Fe, Zn and Ca (Fenema, 1996).

Both the major and the trace minerals are required by the human body for survival. They are needed in larger amounts (major elements) and in smaller amounts (trace elements), but both types are essential not only to maintain the body's fluid balance but also to play important roles in bone growth and health in general (Whitney and Rolfes, 2015).

The mineral content of cladodes is highly variable and depends on the climatic region, species, age and condition of the studied cladodes (Stintzing and Carle, 2005).

7.4.9.1 Crude Ash

Ash is a way to measure the total mineral content of a product. It indicates the total inorganic content and it does not contribute energy to food. High ash values indicate high mineral content.

Minerals are inorganic and as such always retain its identity, even when digested. It may be bound and temporarily inactivated, but it is never changed or broken down. Consequently, care should be taken in the handling of food as its solubility in water is the primary manner in which minerals are lost from raw material to prepared food (Whitney and Rolfes, 2015).

It is important to note that only the minerals that normally would be deemed important for nutrition was determined. The major minerals that were not determined (chloride and sulphate) and the minor minerals (iodine, selenium, fluoride, chromium and molybdenum) would be part of the mucilage powder make-up. In fact, there is very little published information on the values of these minerals except for selenium that was present in high quantities (8.88 µg/g) in cladodes (Bañuelos et al., 2012, 2011).

The ash content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 83.

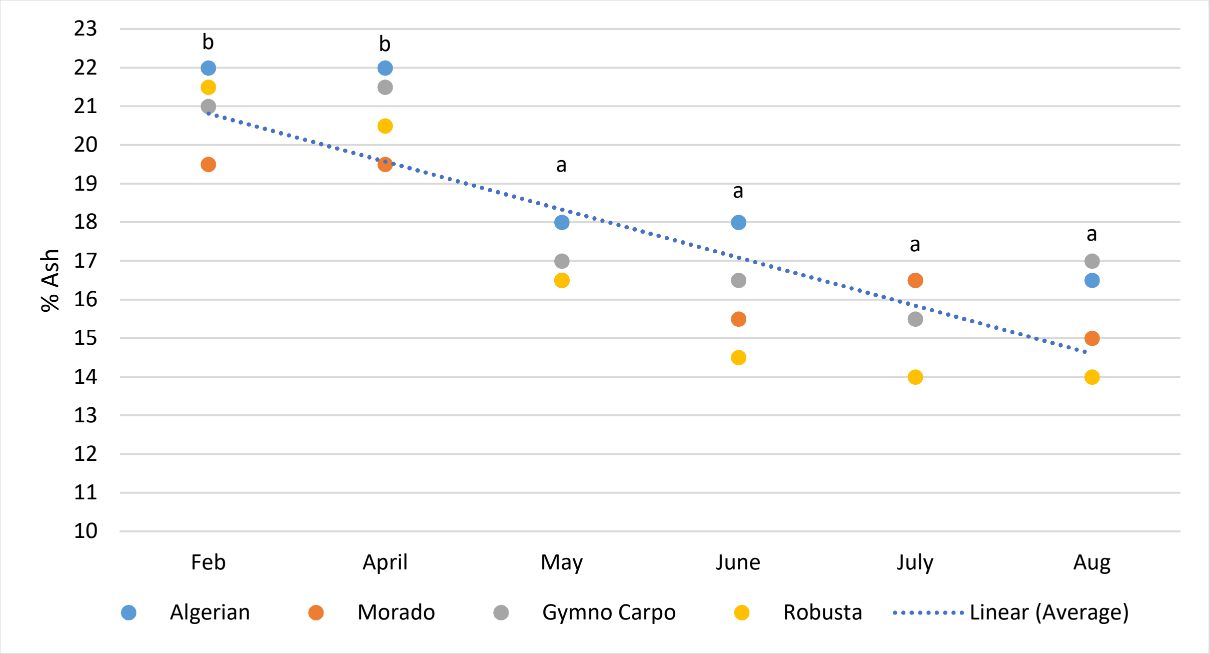
Table 83: The effect of month and cultivar on the crude ash content (%) of freeze-dried mucilage powders from cactus pear cladodes

	Feb	April	May	June	July	Aug	Cultivar Means (p = 0.545)
Algerian	22.00	22.00	18.00	18.00	16.50	16.50	**18.83**
Morado	19.50	19.50	16.50	15.50	16.50	15.00	**17.08**
Gymno-Carpo	21.00	21.50	17.00	16.50	15.50	17.00	**18.08**
Robusta	21.50	20.50	16.50	14.50	14.00	14.00	**16.83**
Month Means (p < 0.001)	**21.00^{b}**	**20.87^{b}**	**17.00^{a}**	**16.12^{a}**	**15.63^{a}**	**15.62^{a}**	**17.71** **C x M: (p = 422)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

On average, 'Algerian' had the highest and 'Robusta' the lowest values but overall there were no significant differences between cultivars. Ash ranged between 14.4% for 'Robusta' June and 22% for February and April 'Algerian' with an overall average ash content of 17.71%. 'Algerian' ranged from 16.5 to 22%, 'Morado' from 15 to 19.5%, 'Gymno-Carpo' from 15.5 to 21% and 'Robusta' from 14 to 21.5% ash.

A trend was observed in the % ash values (Figure 108), as it was significantly higher in the summer months of February (21%) and April (20.87%), than in the winter months (May to August). The period from February to April is an important post-harvest stage for the plants to prepare for the dormant (winter) and early spring growth. As very hot daytime temperatures prevail during the months of February and April, it could be speculated that the climate had an influence on the ash values as a consequence of higher absorption of minerals from the soil that took place during summer.



Different superscripts above months indicate significant differences between month means

Figure 108: Trend of % ash content fluctuations of freeze-dried mucilage powders from cactus pear cladodes over months

The values in this study for mucilage, corresponded very well with what was found in the literature; Gebresamuel and Gebre-Mariam (2012) determined ash at 33.96% for dried mucilage from Ethiopian cultivars. Sepúlveda et al. (2007) detected higher values between 34.9 g/100 g and 39 g/100 g ash in mucilage precipitated with ethanol and isopropyl alcohol. Ayadi et al. (2009) reported ash values of 25.65 g/100 g DM for spiny cladode flour and 23.3 g/100 g DM for spineless cladode flour. While it was reported to be 20.12-20.78 g/100 g DM by López-Cervantes et al. (2011). Samia El-Safy (2013) found 27.004% ash in cladode flour. Padron-Perreira et al. (2009) reported ash of 20.34% in cladode flour and 23.25% in enzymatically hydrolysed flour. Sáenz et al. (2010) dried mature cladodes using a forced air tunnel and determined ash as 15.86%. Stintzing and Carle (2005) reported that ash in cladodes were 19.6 g/100 g. Ramírez-Tobías et al. (2007) reported that the richness of minerals in soil generally increased the ash content of cladodes. The ash increased significantly with the maturity of the cladodes. Cladode flour from cladodes between 40 and 135 days of age had between 17.65 and 24.3 g/100 g ash content that increased with cladode age (Hernández-Urbiola et al., 2010). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 18.41% and 23.24% ash content (Rodríguez-Garcia et al., 2007)

7.4.9.2 Dietary minerals

Major and minor dietary elements are equally necessary in the body, major elements are present and needed in larger amounts. The minor dietary elements are discussed later in this chapter. The first three major elements discussed here are the elements that are needed to be taken in by means of food. Failure of adequate intake of these elements lead to deficiencies that can be fatal (Whitney and Rolfes, 2015). Phosphorous and sodium (that will be discussed later) are essential major dietary elements but are unlikely to be deficient in the human body.

Minerals have electrical charges that account for another vital function in the behaviour of water molecules (Fenema, 1996). Therefore it was theorized in this study that the ability of the major minerals to supply ions and anions, would have a pronounced effect on the nature and movement of mucilage. As mucilage has high water content, the salts will dissolve and the electrolyte solution formed will be attracted to both ions and anions and thus be attracted to the electrolytes.

Trachtenberg and Mayer (1982b) determined that mucilage molecules are polyelectrolytes. The result of such an electrically charged molecule coming into contact with water, is that the hydrogen atoms split off, leaving the molecule negatively charged. The result is that the long mucilage molecule will repel itself, causing it to uncoil and stretch out. All of these stretched out molecules in the fluid in turn, cause the viscosity of the fluid to increase. With the addition of cations such as Na^+ or Ca^{2+}, the negative charges of the mucilage molecule will be neutralized, causing the viscosity of the fluid to decrease. Consequently, the viscosity of mucilage emphatically depends on the ion concentration and thus the mineral content has an influence on mucilage viscosity (Trachtenberg and Mayer, 1982b).

7.4.9.3 Major minerals

7.4.9.3.1 Calcium

Calcium is absorbed as divalent Ca^{2+}. Calcium is necessary for normal membrane functions in all plant cells, and acts as a binder for proteins. Calcium is abundant in most plants and is in central vacuoles and bound in cell walls. Calcium is often precipitated as insoluble crystals of oxalates. It also plays a role in activating enzymes through its association with calmodulin (Salisbury and Ross, 1992).

Many researchers have reported the high content of calcium in cladodes. Values of 3.4 g/100 g (Sáenz, 1997) and 35.3 mg/g (Aguilera-Barreiro et al., 2013) compare well with values reported in this study. Many researchers have suggested the consumption of young cladodes (nopalitos) to increase calcium intake (Hernández-Urbiola et al., 2010; Morales et al., 2012; Moßhammer et al., 2006b; Rodríguez-Garcia et al., 2007; Russell and Felker, 1987; Sáenz, 2002, 1997).

The presence of calcium oxalate crystals in the fresh cladode tissue has been reported by many authors (Contreras-Padilla et al., 2011; Ginestra et al., 2009; McConn and Nakata, 2004; Sáenz et al., 2012; Trachtenberg and Mayer, 1982a) and has been shown (SEM images) and discussed in Section 7.4.1.1.5. In fact, in this study it was shown that crystals were abundantly present in the epidermis and palisade layer in three of the cultivars ('Algerian', 'Morado' and 'Gymno-Carpo''). In 'Robusta' the crystals were present but not as abundant, in a different form and smaller. How and why the crystals form is not known but it has been attributed to the hydration form of the calcium oxalate. The occurrence of calcium oxalate crystals was discussed in section 7.4.1.1.5.

Calcium has a profound effect on the mucilage molecule as a result of the cations that it supplies. It affects the dimensions as well as the rigidity of the mucilage molecules. In the absence of calcium, the molecule experiences strong repulsion, causing it to fully expand and the mucilage molecules has been reported to become three times as long as in their "normal" coiled-up contracted state (Trachtenberg and Mayer, 1982b). As large amounts of calcium is available in the form of calcium oxalate in the plant, and the pH changes constantly as a result of CAM, calcium may be retrieved from the calcium oxalate crystals to regulate the viscosity of the mucilage molecules and thus control the movement of water.

Calcium is the mineral that is the most abundant in the body. It builds, strengthens and provides rigidity to bones to enable it to support weight. The presence of calcium in the intracellular fluids of the body is essential for survival through its role in maintaining blood pressure, its role in blood clotting and by activating enzymes that releases energy for muscle contractions. It also plays an indirect role in the transmitting of nerve impulses, discharge of hormones and activation of other enzymes (Whitney and Rolfes, 2015).

Adults need between 1000 and 1200 mg of calcium per day that translates to 3 to 4 cups of milk a day. Although vegetables are not the best source and the bioavailability can be low, green vegetables such as kale, parsley, watercress and broccoli are amicable sources. It is unfortunate that while green leafy vegetables may appear to be good sources of calcium, the calcium may be in a bound form that limits or prevents the absorption thereof (Whitney and Rolfes, 2015).

Calcium has an important role in the retention of water in cladodes (Stintzing and Carle, 2005). The calcium content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 84.

Table 84: The effect of month and cultivar on the calcium content (%) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.854)**
Algerian	2.95	5.08	2.48	3.26	3.03	2.84	**3.27**
Morado	3.49	4.59	2.21	2.27	3.00	2.56	**3.02**
Gymno-Carpo	3.24	4.25	2.28	2.50	2.23	3.35	**2.97**
Robusta	4.30	4.28	1.59	1.99	2.29	2.26	**2.78**
Month Means (p < 0.001)	**3.49^{bc}**	**4.55^{c}**	**2.14^{a}**	**2.50^{ab}**	**2.64^{ab}**	**2.75^{ab}**	**3.01** **C x M: (p = 0.561)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
▭ **= All means**

The mean content for calcium in mucilage powder was 3.01%. The values ranged from 1.59 (‘Robusta’, May) to 5.08 (‘Algerian’, April). In fact April mucilage powder had across cultivars, the highest values of calcium (4.55%), only to drop in May to the lowest values for all cultivars (2.14%). February (3.49%) and April (4.55%) had significantly higher calcium contents than May (2.14%) and did not differ significantly from each other. February (3.49%), June (2.50%), July (2.64%) and August (2.75%) powders did not differ significantly from each other. No significant differences were detected for cultivars. ‘Algerian’ (3.27%) and ‘Morado’ (3.02%) had the highest average calcium values, with ‘Gymno-Carpo’ (2.97%) slightly lower, ‘Robusta’ (2.78%) had the lowest mean values over the six month period. Similar to the ash values (section 7.4.9.1), the values were higher in the summer months (February and April). The interaction between months of harvest and cultivar was not significant. Sepúlveda et al. (2007) detected between 10.35 g/100 g and 12.67 g/100 g calcium in mucilage precipitated with ethanol and isopropyl alcohol.

These results were consistent with the findings by Ayadi et al. (2009) who reported higher calcium values in spiny cladode flour (3.03 g/100 g DM) than in spineless (1.4 g/100 g DM). In cladodes (dry weight), 5.64 g/100 g (Feugang et al., 2006) and 5.64-17.95 mg/100 g (El-

Mostafa et al., 2014) was reported. Samia El-Safy (2013) found 1335 parts per million. Ramírez-Moreno et al. (2012) found that young cladodes could be a source of calcium for people unable to eat dairy products and for people in developing countries. Cladode flour made from cladodes that were 40-135 days old had approximately 15 mg/g to approximately 35 mg/g calcium that showed a positive correlation with cladode maturity (Hernández-Urbiola et al., 2010). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 1.35% and 3.3% calcium content and also increased with maturity (Rodríguez-Garcia et al., 2007). The calcium oxalate was mostly detected in the insoluble dietary fibre fraction of the cladode. The calcium content in soluble fibre was higher and calcium carbonate levels were 50% higher in soluble fibre. The bioavailability of calcium in cladodes were determined through calculating the oxalate: calcium ratio, as the ratio was ≥1, calcium would be bioavailable in cladodes (Rojas-Molina et al., 2015).

7.4.9.3.2 Potassium

Potassium is essential for photosynthesis and respiration in plants (amongst other essential activities for plants) as it activates many enzymes. K^+ is easily distributed in plants and is a major contributor of turgor pressure through the maintenance of osmotic pressure of cells. Soil are often deficient in potassium therefore it forms part of the fertilizer applied to plants (Salisbury and Ross, 1992).

Like calcium, potassium is a cation and as such it plays an important role in plants, maintaining fluid and electrolyte balance in cells (Salisbury and Ross, 1992). It can therefore be assumed that K^+ would have an influence on the viscosity of mucilage as a result of its electrolytic nature as was described before.

Potassium is located inside cells in the human body, thus playing an essential role in maintaining electrolyte balance. It has a function during nerve impulse transmission and muscle contraction (Whitney and Rolfes, 2015). Most adults consume too little potassium and too much salt, a habit that raises blood pressure and may lead to very serious health issues. Fresh vegetables and fruit are the main sources of potassium. Foods rich in potassium reduces blood pressure and therefore the risk of strokes and heart disease (Whitney and Rolfes, 2015). The potassium content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 85.

Table 85: The effect of month and cultivar on the potassium content (%) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.043)**
Algerian	4.05	2.45	2.65	3.10	2.55	2.30	**2.85**^{ab}
Morado	2.65	1.40	2.50	2.00	2.55	2.45	**2.26**^{a}
Gymno-Carpo	3.45	2.75	2.45	2.10	2.10	2.60	**2.58**^{ab}
Robusta	4.45	3.75	3.50	2.40	3.00	2.75	**3.31**^{b}
Month Means (p = 0.090)	**3.65**	**2.59**	**2.78**	**2.40**	**2.55**	**2.53**	**2.75** **C x M: (p 0.183)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
▭ **= All means**

The mean potassium % of total ash was 2.75% and it ranged from 1.4% (‘Morado’, April) to 4.45% (‘Robusta’, February). ‘Robusta’ was the cultivar with the highest content of potassium consistently every month (average of 3.31%, statistically meaningful) while Morado (2.26%) had significantly lower potassium levels than Robusta (3.31%). These results may once again show similarity with daytime temperature data. The potassium values were higher in the warmer months (February, April, May and August) and were the lowest in June (coldest month) although the values were not significantly different. The interaction between months of harvest and cultivar was not significant. Sepúlveda et al. (2007) detected between 1.61 g/100 g and 2.01 g/100 g potassium in mucilage precipitated with ethanol and isopropyl alcohol.

In cladodes, 2.35 g/100 g (Feugang et al., 2006) and 2.35-55.2 mg/100 g (El-Mostafa et al., 2014) were reported. Cladode flour made from cladodes that were 40-135 days old had 55 to 72 mg/g potassium that showed no correlation with cladode maturity (Hernández-Urbiola et al., 2010). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 5.52% and 6.84% sodium content that increased with cladode age (Rodríguez-Garcia et al., 2007). Ayadi et al. (2009) reported 3.5 g/100 g DM while Stintzing and Carle (2005) concluded in a review that 60% of total ash was potassium (166 mg/g). Samia El-Safy (2013) determined 950 ppm in cladode flour. The highest potassium content in all cultivars were observed in February while it reached its lowest point in June.

High levels of potassium do not occur in many foods, but nopal flour is a good natural source for this mineral (2.1 g/100 g) and together with the low sodium content it may potentially have significant impact on the nutrition of products that contain a certain percentage of nopal flour (Sáenz, 1997).

7.4.9.3.3 Magnesium

In plants, magnesium is absorbed in its divalent Mg^{2+} form. Its presence in chlorophyll renders it critical for ATP and for activating enzymes necessary for the formation of DNA and RNA, photosynthesis and respiration (Salisbury and Ross, 1992). The electrolytic nature of Mg^{2+}, could decrease the viscosity of mucilage when the cations bind with the negatively charged mucilage molecule.

In the human body, magnesium functions in bone mineralization, enzyme activation, muscle contraction, immune system, blood sugar regulation and nerve impulse transmission. The average intake of magnesium generally fall below recommended intake amounts, as foods that are good sources, such as green leafy vegetables, legumes and nuts are not favourite food items (Whitney and Rolfes, 2015). Magnesium deficiency causes amongst other, severe symptoms of chronic heart disease, tetany, confusion and hallucinations (Whitney and Rolfes, 2015). The magnesium content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 86.

Table 86: The effect of month and cultivar on the magnesium content (%) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p < 0.001))**
Algerian	2.95	2.40	2.65	2.90	2.30	2.10	**2.55**^{b}
Morado	2.80	2.45	2.75	2.55	2.70	2.45	**2.62**^{b}
Gymno-Carpo	3.20	2.65	2.60	2.70	2.35	2.80	**2.72**^{b}
Robusta	2.25	2.20	1.80	2.00	2.00	1.95	**2.03**^{a}
Month Means (p = 0.463)	**2.80**	**2.43**	**2.45**	**2.54**	**2.34**	**2.33**	**2.48** **C x M: (p = 0.322)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month
▭ **= All means**

The magnesium mean value was 2.48% of the total % ash. The % range was 1.95% (‘Robusta’, May) to 3.2% (‘Gymno-Carpo’, February). ‘Robusta’ levels of magnesium was significantly lower than the other cultivars with a mean value of 2.03% in comparison with values above 2.5% for the cultivars Algerian, Morado and Gymno-Carpo. Magnesium levels were slightly elevated in February (hottest daytime temperatures) but remained mostly stable during the rest of the months of analysis. The interaction between months of harvest and cultivar was not significant. There were no significant differences between months. No values for magnesium content in mucilage was found in literature.

In cladodes, 0.19 g/100 g DW (Feugang et al., 2006) and 8.80 mg/100 g (El-Mostafa et al., 2014) was reported. Cladode flour made from cladodes that were 40-135 days old had 8.8 mg/g to 11.2 mg/g magnesium that showed no correlation with cladode maturity (Hernández-Urbiola et al., 2010).

When average values for cultivars were compared, it was evident that 'Robusta' had the highest average potassium content, 'Gymno-Carpo' the highest magnesium and 'Algerian' the highest average calcium content. In 'Robusta', the potassium content was higher than the calcium but in the other three cultivars the potassium content was lower than the calcium. In both 'Robusta' and 'Algerian' the magnesium content was the lowest while in 'Gymno-Carpo' and 'Morado', the potassium content was the lowest.

7.4.9.3.4 Phosphorous

Phosphorous is absorbed by the roots and converted to organic forms but never undergoes reduction. It remains in plants as free or bound esters. It is one of the elements that is often deficient in soil. It is involved in photosynthesis, RNA, DNA and phospholipids (Salisbury and Ross, 1992). Phosphorous is usually bound to Ca^{2+} and acts as a buffer system therefore it may reduce the effects of cations on the viscosity of mucilage.

Phosphorous is abundant in food but protein rich food are the best sources. Deficiencies are unlikely but toxicity can occur (rarely) and can disrupt hormonal imbalances, cause kidney problems and heart disease (Whitney and Rolfes, 2015).

Phosphorous is paramount in the metabolism of energy and it activates B-vitamins and enzymes. It has major roles in cells and cell membranes and acts with calcium in the formation and maintenance of bones and teeth. It is also part of phospholipids, it is used in energy transfer and maintains the acid-base balance (Whitney and Rolfes, 2015). The phosphorous content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 87.

Table 87: The effect of month and cultivar on the phosphorous content (mg/kg) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.808)**
Algerian	149.75	119.10	80.00	123.75	115.20	95.20	**113.83**
Morado	100.00	100.95	97.85	71.60	92.95	100.20	**93.93**
Gymno-Carpo	145.95	113.50	97.95	72.05	79.45	92.50	**100.23**
Robusta	105.20	78.35	67.90	397.50	67.10	63.15	**129.87**
Month Means (p = 0.498)	**125.23**	**102.98**	**85.93**	**166.23**	**88.68**	**87.76**	**109.46** **C x M: (0.659)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ **= All means**

The average phosphorous content decreased steadily from its highest point in February to May and remained steady until August. No significant differences were detected for months or cultivars, although the highest contents were observed in June (lowest daytime temperatures). The interaction between months of harvest and cultivar was not significant.

Vegetables usually have phosphorous values of below 100 mg/kg but mucilage powder maintained higher levels and had an average of 109.46 mg/kg (0.01%). It ranged from 63.15 mg/kg (‘Robusta’, August) to 149.75 mg/kg (‘Algerian’, February). ‘Robusta’ (129.87 mg/kg) had the highest average value over the six month period and ‘Morado’ (93.93 mg/kg) the lowest.

Cladode flour made from cladodes that were 40-135 days old had 0.2 mg/g to 0.4 mg/g phosphorous that showed no correlation with cladode maturity (Hernández-Urbiola et al., 2010). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 0.29% and 0.38% phosphorous content (Rodríguez-Garcia et al., 2007).

7.4.9.3.5 Sodium

Sodium is not considered to be an essential element for the survival of plants, yet there are some species that may require it more than others. Sodium is regarded as important for plant growth on the basis that if all forms of sodium contamination could be completely removed from plants, it would probably not survive. Yet it is unlikely that in nature, the sodium content in plants will ever be in such severe absence. CAM plants, such as the cactus pear, grow better with sodium and it is therefore regarded as beneficial for such plants. The main role of sodium in plants is to substitute the potassium needed for optimal growth (Salisbury and Ross, 1992).

Sodium possesses an electron that is quickly donated thus it is a cation and plays a major role in maintaining fluid balance and regulating fluid volume. In its capacity as a cation, and as it is abundant, it would influence the viscosity of mucilage.

Sodium is the primary regulator of extracellular volume in the human body and is paramount for nerve transmission and muscle contraction. A normal diet always provides enough sodium as the body only needs small amounts. Because high sodium intake is an antecedent for high blood pressure, the daily recommendations are set very low and people are warned to lower their sodium intake (Whitney and Rolfes, 2015). The sodium content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 88

Table 88: The effect of month and cultivar on the sodium content (mg/kg) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.311)**
Algerian	150.00	65.50	63.00	56.50	89.50	93.00	**86.25**
Morado	195.00	107.50	95.50	66.50	126.50	185.00	**129.33**
Gymno-Carpo	165.00	89.50	73.00	58.50	160.00	140.00	**114.33**
Robusta	170.00	270.00	87.50	100.50	76.00	155.00	**143.17**
Month Means (p = 0.034)	**170.00^{b}**	**133.13^{ab}**	**79.75^{ab}**	**70.50^{a}**	**113.00^{ab}**	**143.25^{ab}**	**118.27** **C x M: (p = 0.285)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
▭ = All means

Sodium in mucilage powder ranged from 56.5 mg/kg (‘Algerian’, June) to 270 mg/kg (‘Robusta’, April). The average sodium content was 118.27 mg/kg (0.01%). June (70.50 mg/kg) values were significantly lower than sodium values in February (170 mg/kg). There were no statistically different values found between cultivars although ‘Robusta’ had the highest sodium contents (143.17 mg/kg) amongst cultivars and ‘Algerian’ the lowest (86.25 mg/kg). The interaction between months of harvest and cultivar was not significant.

Samia El-Safy (2013) reported 465 ppm of sodium in cladode flour. In cladodes 0.4 g/100 g (Feugang et al., 2006) and 0.3-0.4 g/100 g (El-Mostafa et al., 2014) was reported. Cladode flour made from cladodes that were 40-135 days old had 0.2 mg/g to 0.55 mg/g sodium that showed no correlation with cladode maturity (Hernández-Urbiola et al., 2010). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 0.21% and 0.12% sodium content that decreased with cladode age (Rodríguez-Garcia et al., 2007).

7.4.9.3.6 Minor dietary elements

Plants need trace elements for survival as much as the macro nutrients, only in smaller amounts. Plants cannot complete its life cycle without it or if the element form part of any molecule that is in itself important to the survival of plants. In general, plants absorb these elements from the soil and employ or store it. If a deficiency of the trace element should develop, characteristic symptoms will develop that an orchard manager could recognize and rectify (Salisbury and Ross, 1992).

Trace minerals are needed in the human diet and food originating from plants could serve as a significant source. A variety of foods provide trace minerals but protein rich food usually are better sources and the elements tend to be more bioavailable. Trace elements are as essential to human life as it is to plants and are involved in vital bodily functions but is only needed in minute amounts in the body. Trace elements include iron, zinc, iodine, selenium, copper, manganese, fluoride, chromium and molybdenum (Whitney and Rolfes, 2015).

The average manganese, iron, copper and zinc content per cultivar and the overall average contents (mg/kg) are indicated in Table 89.

'Gymno-Carpo' had the highest manganese content (220.67 mg/kg) and 'Robusta' the lowest (156 mg/kg). The average manganese content was 188.38 mg/kg. Manganese content in cladodes have been reported as 0.19-0.29 mg/100 g (El-Mostafa et al., 2014). Cladode flour made from cladodes that were 40-135 days old had 0.03 mg/g to 0.29 mg/g manganese that showed no correlation with cladode maturity (Hernández-Urbiola et al., 2010)

Table 89: Mean trace element contents of freeze-dried mucilage powders from cactus pear cladodes collected in the post-harvest (February and April) and dormant (May, June, July and August) stages

Trace Element mg/kg	Algerian	Morado	Gymno-Carpo	Robusta	Average	% of Ash
Manganese	190.92	185.75	220.67	156.17	188.38	0.01
Iron	32.33	18.25	15.67	22.17	22.10	0.002
Copper	6.17	5.67	5.42	4.92	5.54	N/A
Zinc	23.67	25.67	24.33	24.50	24.54	0.002

'Algerian' had the highest iron content (32.33 mg/kg) and 'Gymno-Carpo' the lowest (15.67 mg/kg). The average iron content was 22.10 mg/kg. Iron content in cladode flour has been reported as 1.6 mg/100 g (Stintzing and Carle, 2005), 0.14 mg (Nefzaoui and Ben Salem, 2002) and 0.6185 ppm (Samia El-Safy, 2013). In cladodes 0.14 µg/100 g (Feugang et al., 2006) and

0.09 g/100 mg (El-Mostafa et al., 2014) was reported. Cladode flour made from cladodes that were 40-135 days old showed an increase from 0.09 mg/g to 0.22 mg/g iron that positively correlated with cladode maturity (Hernández-Urbiola et al., 2010).

The copper content was very even between cultivars and very low. The highest content was detected in 'Algerian' and the lowest in 'Robusta' (4.92 mg/kg) while the average copper content was 5.54 mg/kg. Ayadi et al. (2009) also reported very low copper content in cladode flour.

The average zinc content was 24.54 mg/kg and the contents were very similar in all four cultivars. The highest zinc content was observed in Morado (25.67 mg/kg) and the lowest in 'Algerian' (23.67 mg/kg). In cladodes 0.08 mg/100 g was reported (El-Mostafa et al., 2014). Cladode flour made from cladodes that were 40-135 days old had 0.03 mg/g to 0.08 mg/g zinc that showed no correlation with cladode maturity (Hernández-Urbiola et al., 2010)

Ayadi et al. (2009) found that calcium, iron and magnesium values were high in cladode flour but that sodium, phosphorous, zinc and copper contents were present but low. Samia El-Safy (2013) determined the mineral content of cladode flour and concluded that it was considered to be a good source of minerals and an important food for humans; especially the calcium, potassium and magnesium values were significantly high. In fact, it was stated that cladode flour or fresh nopalitos could serve as an alternative source of calcium when dairy products cannot be consumed.

7.4.10 Organic acids

Organic acids are produced in guard cells that are located in the upper epidermis on the surface of a leaf. It regulates the openings (stomates) that allow water and gasses to pass through the waxy cuticle of the leaf. As cactus pear plants are succulents with CAM metabolism, the stomates only open at night, when the temperature would be lower and humidity higher, in order to prevent water loss through transpiration. Thus, CO_2 taken from the air is bound with PEP carboxylase (an enzyme), to synthesise malic acid while oxygen is released back into the air (Salisbury and Ross, 1992). The most common organic acid that is produced is malic acid. Acids provide hydrogen ions and thus a drop in the pH in the cladode takes place. When an excess of hydrogen ions (acids) occur, they are pumped into the vacuoles (an organelle in the cell) where excessive substances can move in and out depending on demand, thereby maintaining the pH of the surrounding cytoplasm. Organic acids are synthesized from starch or other carbohydrates available in the guard cells. During the day, the decarboxylated malic acid is converted to glucose. Oxalic acid may occur dissolved but is mostly present in its crystalline form as whewellite or weddelite crystals (Rojas-Molina et al., 2015; Stintzing and Carle, 2005) as previously discussed in section 7.4.1.1 (SEM on fresh cladodes).

The effect of CAM is that there is a significant difference in the acid content of the cladodes early in the morning and late afternoon (that influences the acceptability of the taste). Malic acid contributes to sourness of fresh vegetables and fruit, as a matter of fact, malic acid sourness has been described as extreme tartness. As a high acid content in young cladodes causes an undesirable sour taste, it is customary in Mexico to harvest young cladodes later in the day after at least two hours of sunshine, when the level of acidity has decreased to a satisfactory level (Rodriguez-Felix and Cantwell, 1988).

The importance of acids in the preservation of food is well documented. When the pH values are low (below pH 4.5), bacteria will not survive or grow. Acids are used in the body to regulate the narrow range of pH acidity of its fluids (Whitney and Rolfes, 2015).

Stintzing and Carle, (2005) reported a decrease of malic acid content at 6 a.m. (985 g/100 g) and 6 p.m. (95 g/100 g), as well as citric acid content at 6 a.m. (178 g/100 g) and 6 p.m. (31 g/100 g) due to the effect of CAM. The presence of malonic, citric, succinic, eucomic and tartaric acids were detected along with piscidic and phorbic acids. Pisidic acid increased rapidly with age while phorbic acid reduced with age. It was stated that these organic acids found in cladodes were rare and are only found in succulent CAM plants. Therefore, the reported organic acids present in cladodes are mostly uncommon and are not routinely tested for during standard HPLC analysis.

7.4.10.1 HPLC quantification analysis

HPLC analysis was performed on the soluble material of the mucilage powder. From the approximately twenty organic acids of which the standards were available for comparison, only malic acid could be positively identified. This indicates that the other organic acids present in mucilage are not common.

The malic acid content of freeze-dried mucilage according to cultivar and month obtained is presented in Table 90.

Table 90: The effect of month and cultivar on the malic acid content (g/L) of freeze-dried mucilage powders from cactus pear cladodes

	February	**April**	**May**	**June**	**July**	**August**	**Cultivar Means (p = 0.740)**
Algerian	4.63	3.71	2.30	3.20	2.77	0.01	**2.77**
Morado	4.09	3.16	3.16	2.55	2.89	0.22	**2.68**
Gymno-Carpo	3.23	3.45	2.50	2.64	2.77	2.55	**2.86**
Robusta	2.78	2.63	2.98	2.19	2.39	0.01	**2.16**
Month Means (p < 0.001)	**3.68^{b}**	**3.24^{b}**	**2.73^{b}**	**2.64^{b}**	**2.70^{b}**	**0.69^{a}**	**2.62** **C x M: (p = 0.462)**

Cultivar means with different superscripts in the same column differ significantly.
Month means with different superscripts in the same row differ significantly.
C x M = Interaction between cultivar and month.
☐ = All means

On average, ‘Gymno-Carpo’ had the highest and ‘Robusta’ the lowest malic acid values but overall there were no significant differences between cultivars. Malic acid contents ranged between 0.01 g/L (‘Algerian’ and ‘Robusta’, August) to 4.63 g/L (‘Algerian’ February) with an overall average of 2.62 g/L. In terms of months, it was observed that the malic acid contents was high in February (average 3.68 g/L) from where it consistently and continually decreased every month (not significantly) until August when the contents were significantly lower than all the other months, however no significant difference occur between February, April, May, June and July.

A trend was thus observed in the malic acid contents, as it was higher in the summer months of February (3.68 g/L) and April (3.24 g/L), while it decreased continuously (not significantly) to the significantly lower values observed in August (0.69 g/L). The interaction between months of harvest and cultivar was not significant. As very hot daytime temperatures prevail during the months of February and April, it could be speculated that the seasons had an influence on the malic acid contents as a consequence of CAM. Longer days favour CAM, thus longer days of sunshine caused the production of more malic acids that caused the decrease in cladode pH as was observed and discussed in chapter 6. In fact, the NM and FM mucilage reflected the lower pH values in the summer months.

In the HPLC analysis, several peaks indicating the presence of organic acids were detected. Unfortunately, using the standard organic acid HPLC analysis, only malic acid could positively be identified and the concentrations determined.

In Figure 109 and Figure 110 only one profile for each species is shown for detailed analysis. The organic acids that were detected in the HPLC quantification analysis are presented in Figure 111 over months and per cultivar.

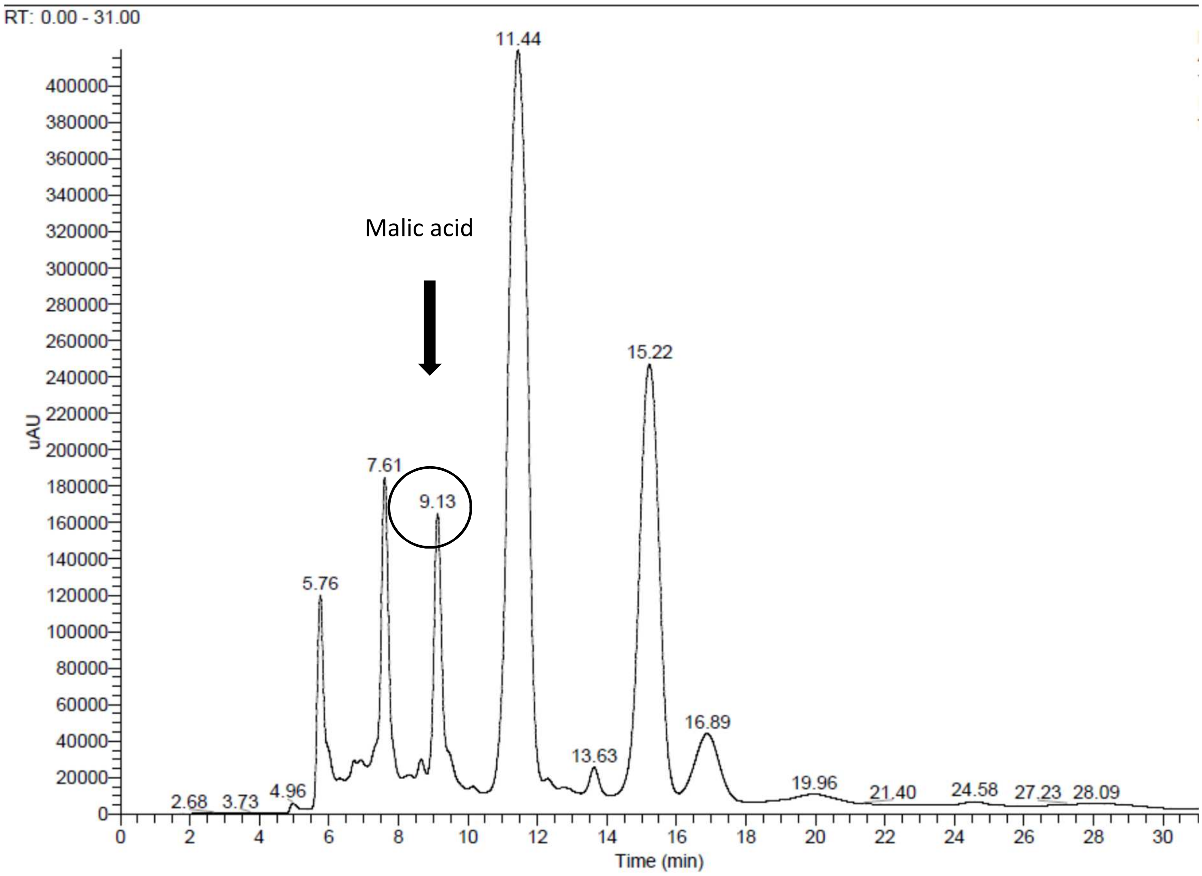


Figure 109: HPLC quantification profile for the freeze-dried powders from the cladodes of *O. ficus-indica* cactus pear cultivars (Algerian, Morado and Gymno-Carpo)

A typical HPLC image for the three *O. ficus-indica* cultivars (Algerian, Morado and Gymno-Carpo) is seen in Figure 109. Similar peaks were observed in all the *O. ficus-indica* cultivars (Algerian, Morado and Gymno-Carpo) (Figure 111). The peak at 9.13 min (identified as malic acid) remained throughout the months though it disappeared in August. The 11.44 min peak was the highest peak and remained high throughout all the months although it could not be identified. The peak noticed at 15.22 was higher in February than the other months as it seemed to decrease continuously until it was barely detected in August. When Figure 109 is compared to Figure 111, the *O. ficus-indica* cultivars HPLC images are all matched while the images for 'Robusta' are different. The organic acids profile as detected using HPLC analysis in *O. robusta* Robusta is shown in Figure 110.

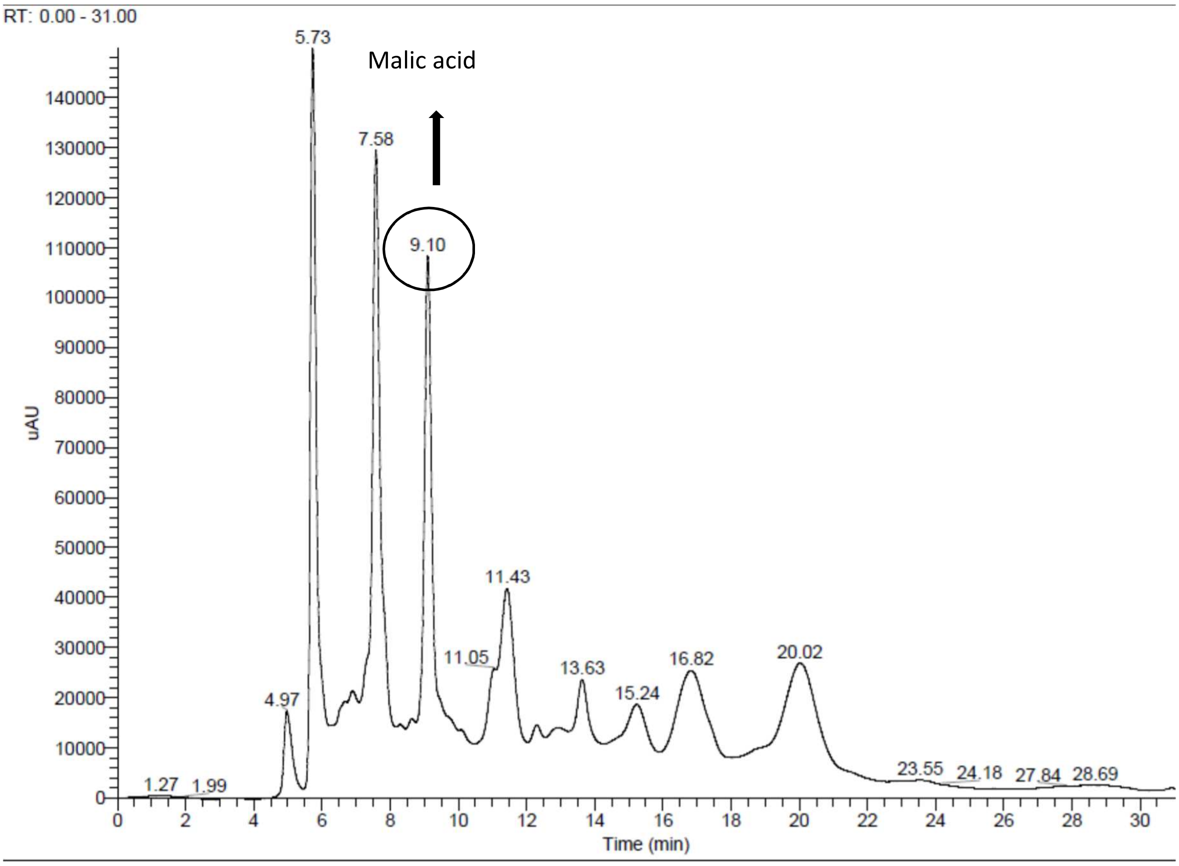
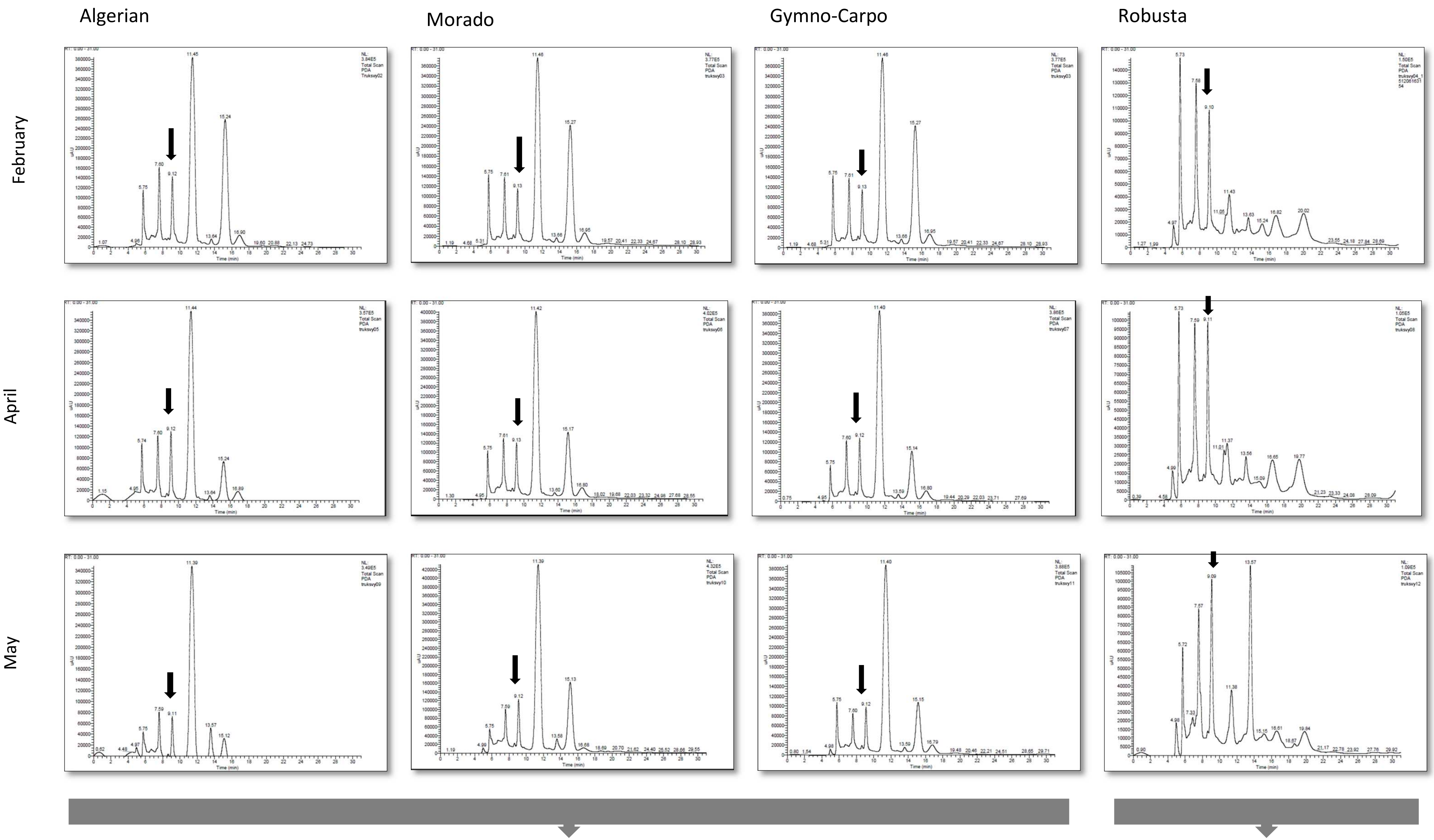


Figure 110: HPLC quantification profile for the freeze-dried powders from the cladodes of *O. robusta* cactus pear cultivar Robusta

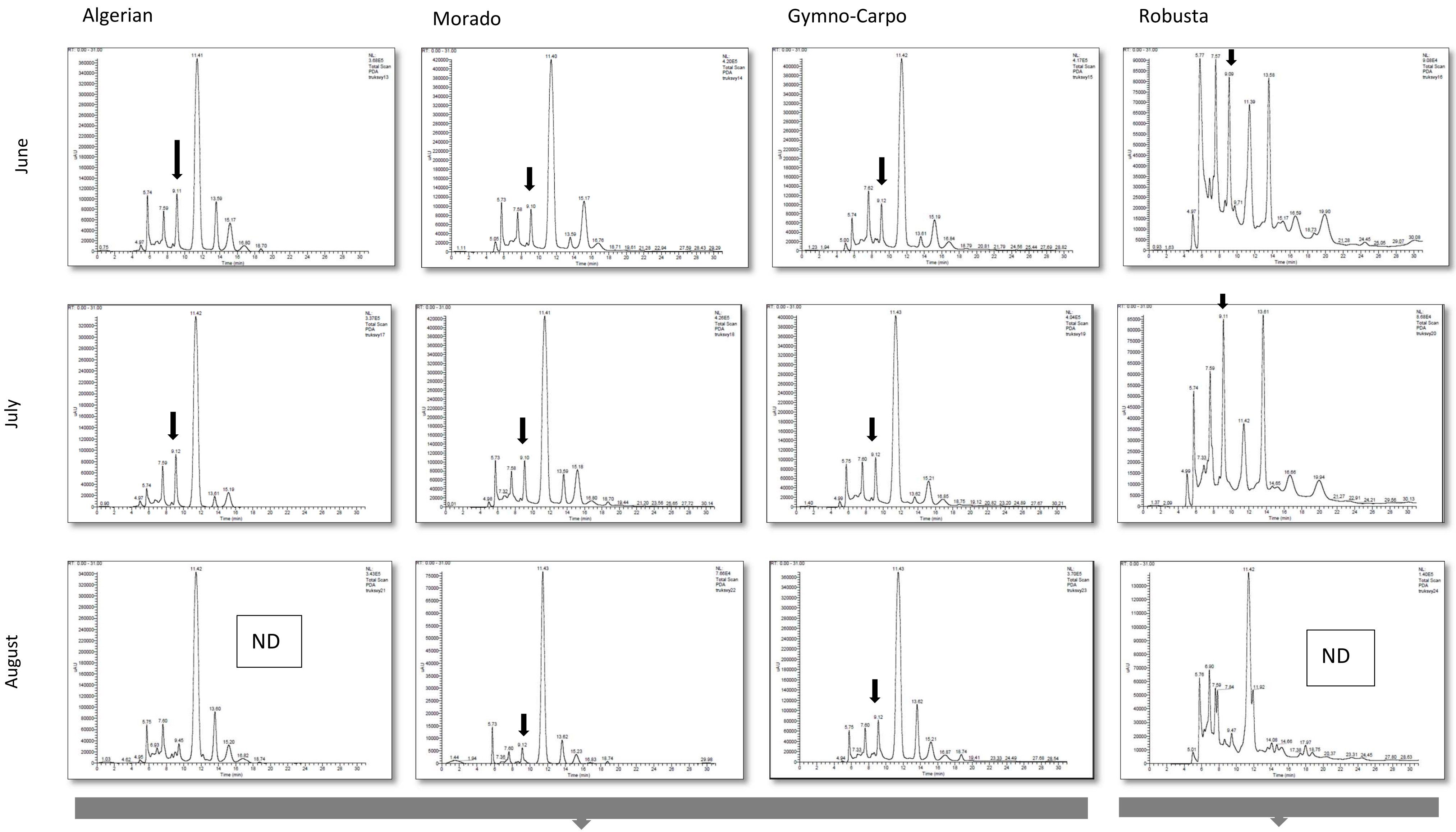
In the HPLC 'Robusta' profiles (Figure 110) (for all the months), there were important differences compared to the *O. ficus-indica* profiles. The first peak at 5.73 min was similar to *O. ficus-indica* however the peak remained considerably higher throughout the months tested (February, April, May, June, July and August). The peak at 9.10 min that was identified as malic acid was substantially higher than in *O. ficus-indica* profiles and remained very high until July after which it disappeared in August (Figure 111).

Figure 111 contains all the HPLC images for the cultivars (from left to right) as well as the months (from top to bottom) where it is observed that there were differences between the *O. ficus-indica* (all cultivars) profiles and the *O. robusta* Robusta profiles. Another feature that is shown in Figure 111 is the decrease in malic (and other) peaks from February (high peaks) consistently every month until August (low peaks).



Cultivars are similar in terms of the shown peaks

Robusta peaks are different



Indicating malic acid peak
ND: Malic acid not detected

Cultivars are similar in terms of the shown peaks

'Robusta' peaks are different

Figure 111: Images from HPLC quantification analysis for organic acids of freeze-dried mucilage from cactus pear cladodes according to cultivars over months

Of the 24 samples, it was observed that the *O. ficus-indica* cultivars (Algerian, Morado and Gymno-Carpo) HPLC images match in terms of the peaks detected while the peaks for *O. robusta* Robusta were different. The HPLC profiles showed that the organic acid content of *O. robusta* was different from the *O. ficus-indica* cultivars. In fact, the profiles for the three cultivars from *O. ficus-indica* cultivars corresponded remarkably while Robusta was noticeably different. Malic acid was identified as peak 9.13 min and show high peaks for all cultivars in February and decreased every month until it was barely detected in August, this corresponds with the values of malic acid reported in Table 90. In future research, the standards of more organic acids, such as pisidic and phorbic acids will be included in order to identify the unknown peaks.

An important finding was made during this study regarding the relationship between CAM, organic acids, pH, and viscosity of mucilage and yield of mucilage. In the first section of this study (chapter 4), it was reported that higher yield of mucilage correlated with lower mucilage viscosity. The connection between mucilage viscosity and conductivity of the solution was discussed in chapter 2. The mucilage molecule is a polyelectrolyte that has negatively charged groups open along the long molecule that will repel each other. Therefore the molecule will uncoil and stretch out, causing the viscosity to increase. The viscosity of the mucilage solution will thus strongly depend on the ion concentration of the solution (Medina-Torres et al., 2000; Trachtenberg and Mayer, 1982b). In chapter 6, the correlation between conductivity and pH was shown (r = 0.0998), as well as the correlation between daytime temperatures and pH. In this section, the relationship between the abundance of organic acids (low pH) and season was shown as a consequence of CAM. Thus, it could be concluded that the higher yields and lower viscosity of native mucilage that was found in the post-harvest stage (late summer months) (chapter 5) was caused by the abundance of organic acids in late summer months, while the opposite is true with lower yields, higher viscosity and the lack of organic acids in winter (dormant stage) months.

7.5 Conclusion

From data presented and discussed above it can be concluded that for cultivars, 'Robusta' had the most beneficial chemical properties, while 'Algerian' would be rated as second and 'Gymno-Carpo' as third best. 'Robusta' had the highest contents of protein, total fats (beneficial fatty acids), starch, potassium and phosphorous, while 'Algerian' mucilage had the lowest energy contribution and was the purest (lowest insoluble fibre content). Moreover 'Algerian' had the highest calcium, iron and copper contents. 'Gymno-Carpo' mucilage did well with its high linoleic acid, PUFA and good fat ratio, had high magnesium and manganese contents. 'Morado' only performed well with its high MUFA and zinc contents.

In terms of selecting the optimal month of cladode harvest for the production of mucilage, February had the best results, after which August was the second best month. February mucilage had the lowest moisture content, highest minerals and highest organic acids (lowest pH). August mucilage had the highest energy content and performed well with the beneficial fatty acids.

Overall though, 'Robusta' emerged as the best cultivar and February as the best month in this study for chemical properties, even though it cannot be claimed that the selected cultivar or harvesting had exceedingly superior quality freeze-dried mucilage. The final selection would have to rely on careful consideration of the rheological, functional and chemical properties obtained. It was found that the chemical composition of the cladodes influence the mucilage yield and viscosity and should be taken into consideration when cladodes are harvested for mucilage extraction.

The chemical analysis of freeze-dried mucilage powder showed that it would considerably increase the soluble fibre, mineral and antioxidant content of any food product that it forms part of since it would extend the nutritional benefits beyond the normal nourishment that the product already offers. It contains virtually no fat and carbohydrates and will contribute very little in terms of energy values. Innovative ways should be explored to include mucilage in food products that will promote health and well-being by virtue of its protective and preventative properties.

7.6 Future research

Future research is necessary to verify and complete the chemical characterization of mucilage. It will also be interesting to determine the influence of environmental and seasonal changes on the chemical composition of mucilage. It is paramount that the individual amino acids should be identified, the proteins isolated and characterised and the unknown peaks in the HPLC profiles be isolated and characterised. Hydrolysis of the freeze-dried mucilage power, in order to obtain information about the individual sugars, are imperative to be obtained for a chemical characterization to be complete.

Chapter 8

Application of liquid native and powdered freeze-dried mucilage in functional food product development – a sensory perspective

Abstract

The global trend towards healthier diets created a market for functional food products that provide extended nutritional benefits beyond the normal nourishment that a product already offers. Mucilage (native and freeze-dried) is an ideal candidate for innovative food products, as it has promising functional properties and could significantly enhance the nutritional content of food while replacing unhealthy ingredients. Ways were explored in which mucilage could be applied in familiar, convenient food products while maintaining product quality. Sorbet and fat-free ice-cream were made containing therapeutic amounts of native mucilage, however the "green" taste of mucilage and the inability of the products to freeze completely negatively affected the acceptability thereof. When freeze-dried mucilage was added to home-made yoghurt it affected the flavour negatively, however the more mucilage was added, the higher the acceptability became. Excellent results were obtained when native mucilage replaced oil or egg in mayonnaise formulations. It could effectively replace up to 75% of egg yolk and 30% of oil and deliver highly acceptable mayonnaise. Equally satisfactory results were obtained with the inclusion of native mucilage to replace pork fat in polony formulations. Native mucilage could replace 100% of pork fat in polony. Thus, mucilage performed extremely well as a fat-replacer in both of the emulsified products (polony and mayonnaise) while an adverse effect on crystallization of ice crystals renders its use in frozen products to be limited. It was thus concluded that mucilage has a strong potential for application in functional food products when correctly applied in appropriate products.

8 Application of liquid native and powdered freeze-dried mucilage in functional food product development – a sensory perspective

8.1 Introduction

Consumers are becoming more aware that food has an influence on short term as well as long term health. There is a global trend towards healthier diets and thus the demand for healthier food products is increasing. A market has emerged for functional foods that provide extended nutritional benefits besides the normal nourishment that a food product already offers. New functional food products have been developed to include increased health-promoting compounds that will exert a positive influence on the body and thus on a person's overall health. Functional food products are not medical food (specific formulations used for specific disorders or diseases used under medical supervision) (Smith and Charter, 2010), nor are they dietary supplements. They are products that already form part of normal dietary patterns but address special dietary requirements. Nevertheless, there is a precondition to these innovative functional food products; they have to maintain quality, be convenient and be tasty (Smith and Charter, 2010).

Mucilage in its native and freeze-dried form is an ideal candidate for such innovative functional food products, as it has been proven to enhance the nutritional content of food, while replacing ingredients that add undesired and unnecessary kilojoules and chemicals (De Wit et al., 2015). Mucilage contains virtually no fat (Bensadón et al., 2010; Hernández-Urbiola et al., 2011; Rodríguez-Garcia et al., 2007; Sáenz, 1997; Stintzing et al., 2005) and carbohydrates (Sáenz et al., 2004; Stintzing and Carle, 2005), while it would considerably increase the soluble fibre (Bensadón et al., 2010; Hernández-Urbiola et al., 2011; Sáenz et al., 2012; Stintzing and Carle, 2005), mineral (Fuentes-Rodriguez et al., 2013; Hernández-Urbiola et al., 2011; Sáenz, 2002) and antioxidant (Bensadón et al., 2010; Brahmi et al., 2011; Gallegos-Infante et al., 2009; Sánchez et al., 2014) content of food products.

The development of mucilage-containing functional food products would not only benefit consumers by providing natural remedies and treatments of ailments without the use of medication, but would also benefit the cactus pear industry. Exploring ways in which mucilage could be included in everyday food is directed towards adding value to cactus pear by-products that would otherwise be regarded as waste (Bensadón et al., 2010). By virtue of obtaining an effective, suitable and practical purpose for cladodes, other than animal feed, would enhance the farming viability of the cactus pear plant as a crop.

8.1.1 Potential cladode-based food products

In the pursuit of viable cladode-based food products, quite a significant amount of research has been done and some of the promising results on many different products will be discussed in short.

8.1.1.1 Cladode flour

Cladode flour is the most popular cladode by-product that has been tested in food products. Obtaining cladode flour is a straightforward process that only requires drying and grinding of cladodes. Cladode flour has been investigated by different researchers for the benefits that the high fibre, minerals and antioxidants content provide to increase the nutritional value of baked products. Cladode flour was described (Sáenz et al., 2012) as a natural progressive source of high fibre flour that could be added to processed food products in order to increase the fibre content.

Purified flour was developed (Sáenz et al., 2012) by taking care to wash and filter the dried cladode powder. The purification process lowered the ash content but increased the total fibre content from 54.7 to 82.3 g/100 g. Consequently, the insoluble fibre content also increased from 30 to 63.6 g/100 g compared to unpurified cladode flour. When the functional properties of the two flours were compared, the water retention, water absorption, fat absorption and cation exchange capacity was not significantly different in the unpurified compared to the purified flour. In earlier research, Sáenz et al. (2010) dried mature cladodes in a forced air tunnel and tested it by including it in baked products. It was recommended that only 5% inclusion in fluid formulations could be used successfully as the increased viscosity of the batters caused by the cladode flour was problematic.

De Wit et al. (2015) sun-dried mature cladodes and milled it into a fine powder. It was used to replace different concentrations of cake flour (wheat) in carrot cake, health bread and oat biscuits. The products were evaluated physically, chemically and sensorically. There was a decrease in the volume of baked products, the texture became firmer and the colour of the products darkened. The sensory panel analysis results showed that baked products containing cactus pear flour were significantly different (as acceptability decreased) compared to the control products. The fibre content of the products increased considerably with increased additions of the cladode flour. It was concluded that cladode flour could replace 25% of regular cake flour and still provide overall acceptable baked products.

Ayadi et al. (2009) washed, dried and ground 2-3 year old cladodes in order to produce flour that was intended to create an outlet for surplus cladode production. The cladode flour was added to cake ingredients in order to study its influence on the characteristics of the dough and the finished product. The chemical analysis of the cladode flour revealed its high antioxidant, ash, fibre and low protein and fat contents. However, it was found that it affected the sensory properties of baked products to such an extent that only 5% could be added to formulations for products to remain within acceptable standards.

8.1.1.2 Reduced/replaced fat products

Public awareness about the health risks of dietary fat had been increasing during the last decade. The food industry responded and took action towards the development of non-fat, low-fat and reduced-fat products. Considerable resources went into developing fat replacements that allow products to taste and function like high-fat foods (Akoh, 1998).

According to Akoh (1998), a substance such as mucilage (hydrocolloid) would be categorized as a fat-mimic as it functions by absorbing a substantial amount of water and would not be stable when heated. Fat-mimics could replace mouthfeel and bulk in food products but was not capable to replace fat on a one-to-one, gram-for-gram basis. Very often, products that contain fat-mimics lack in terms of the original characteristics of high fat products (Akoh, 1998).

It was deduced for the purpose of this study that since mucilage is a hydrocolloid, it should be tested as a fat-mimic as it had the required tendency to increase the viscosity of water-based mixtures. Mucilage would also be able to perform well in a product where its main role would be the improvement of the textural quality of the water-phase, the production of a creamy, smooth consistency and a fattier mouthfeel. The main drawback of fat-mimics is the difference in taste and texture. This would be the expected drawback of mucilage-containing products as mucilage possesses a vegetative-green taste and slimy, gummy or sticky mouthfeel. It is therefore recommended, as in similar hydrocolloids, that it would merely be used as a partial replacer of the fat in food products (Akoh, 1998).

8.1.1.3 Emulsified food products

According to Glicksman (1985), hydrocolloids could be used to emulsify food systems but they are not true emulsifiers. In fact, the emulsifying function of hydrocolloids could more accurately be described as stabilizing, as it only appears to emulsify fat droplets through its ability to increase the viscosity of mixtures and thereby preventing the droplets to migrate and coalesce. Garti and Leser (2001) disagreed with this statement, in fact they claimed that hydrocolloids are indeed true emulsifiers because it fulfills the required properties of emulsifiers to increase the viscosity and to adsorb to the oil/water interfacial surface.

In standard emulsifiers, protein is an indispensable component as the protein is adsorbed at the interfacial surface and stabilizes the oil/water emulsion with high viscosity and mechanical strength. It was established in the literature (Majdoub et al., 2001; Stintzing and

Carle, 2005; Teles et al., 1997) as well as in this thesis (chapter 7) that the dried mucilage contained crude protein that ranged between 2.52 and 5.74 g/100 g DM. Unpurified mucilage thus contains proteineous matter, therefore it could possess similar properties to gum Arabic. It is known that gum Arabic has excellent emulsification properties for oil-in-water emulsions, due to its integral proteinaceous matter. Thus, as a protein-containing hydrocolloid, mucilage could have the potential of having strong emulsifying properties and as such, could be used in commercial products without limitation, as it will be considered a GRAS (generally regarded as safe) product. Mucilage should therefore be tested for use as a natural emulsifier in food systems.

Rwashda et al. (2001), cited by Garti (1999) studied the emulsifying capabilities of mucilage and found strong emulsifying action. Mucilage was capable of reducing surface interfacial tensions and stabilizing oil-in-water emulsions. In addition, it formed small droplets, absorbed onto the oil-water interphase and the emulsion did not flocculate. Cactus mucilage was compared to commercial hydrocolloids (Iturriaga et al., 2009a) and it was detected that it had similar stabilizing properties to xanthan and guar gums. These results showed the outstanding potential uses for mucilage due to its remarkably different behaviour as compared to other commercial hydrocolloids.

The aim of this chapter was to find more applications for the mucilage extracted from cladodes by investigating its performance in food products in terms of the functional properties and sensory acceptability. Only four products are described here but in the larger investigative studies at this University department, many successful products have been developed and tested using the cactus pear fruit, seed and cladodes under the direction of Dr. Maryna de Wit. These products include fruit juice and jellies (De Wit et al., 2014), cladode flour in the preparation of carrot cake, heath bread and oats cookies (De Wit et al., 2015), seed oils (De Wit et al., 2016b), traditional indigenous fermented beverages (maguey and “platpit” beer) and candies (Turkish Delight and marshmallows) (De Wit and Fouché, 2015).

8.2 Materials and Methods

Native and freeze-dried mucilage, obtained by methods described in chapters 6 and 7 were applied in food products in 2016 in order to test its functionality and performance in a practical manner. Specific cultivars were selected according to their strongest properties suitable for specific products as was determined in chapters 6 (functional properties) and chapter 7 (chemical properties). It is important to note that the four products described here serve only as examples to demonstrate the massive potential of the application of mucilage in functional food products. Only sensory testing results are discussed in full but further objective tests were carried out and reported.

8.2.1 Ice-cream

The objective for this product was to study the performance of mucilage during cold processing and freezing storage temperatures. Native mucilage replacements were high as a therapeutic nutraceutical product was conceptualized that would contain significant quantities of soluble fibre. As gelatine is an animal protein and not suitable for kosher products, it was omitted, as it was believed that mucilage would stabilise the ice-cream products sufficiently. Mucilage was used in altered formulas in order to make dairy free sorbet, as well as fat free ice-cream using skimmed milk. The dairy free products (sorbet) were developed for consumers on a dairy-free or vegan diet and the fat-free ice-cream for consumers in the low-kilojoule or kosher product markets.

The cultivar that was used in ice-cream products was Morado. Morado was selected for use in all ice-cream replacement products, as it demonstrated high water holding properties and low viscosity, along with high protein and calcium contents, which would offer beneficial nutritional value to sorbet and low-fat ice-cream products.

8.2.1.1 Formulation for making ice-cream

The control ice-cream (Food and Cookery, 1991) recipe (ingredients, amount and method) was standardized. The ingredients and amounts are seen in Table 91.

Table 91: The ingredients and amounts used in the making of the control ice-cream product

Control	**Content**		
	ml	**g**	**%**
Cream	500	500	81
Gelatine	6	4	1
Boiling water	50	50	8
Sugar	70	56	9
Vanilla essence	5	5	1
Salt	1	1	0
Total	632	616	100

8.2.1.1.1 Method

Step 1: Hydrate and disperse the gelatine by adding it to cold water and heating it slowly in a 1000 watt microwave oven [100% power] for 30 seconds in order to disperse the hydrated gelatine.

Step 2: Measure the sugar, vanilla essence and salt into a glass bowl and mix well. Transfer the mixture to a saucepan, add the cream and heat slowly on mark 3 while whisking until the sugar dissolves (± 5 minutes).

Step 3: Add the dispersed gelatine to the hot milk mixture, mix well and pour it into an electric ice-cream machine. Allow the machine to cool and agitate slowly for 30 minutes until the ice-cream is firm and smooth. Place into an air-tight container and freeze (-18˚C) until further use.

8.2.1.2 Mucilage replacements

8.2.1.2.1 Sorbet

Sorbet is per definition dairy-free. In the sorbet formulations, gelatine was omitted together with a portion of the cream (25% and 100% respectively). Pink cactus pear ('Algerian') fruit puree was added to the formulation in order to replace a portion of the cream and to add flavour and colour to the ice-cream. The replacement of cream with mucilage and fruit puree is given in Table 92.

Table 92: The ingredients and amounts used in the making of mucilage inclusion sorbet products

		19% inclusion			**55% inclusion**		
Control	**Sorbet**	**ml**	**g**	**%**	**ml**	**g**	**%**
Cream	Mucilage	125	125	19.3	500	500	55.7
	Fruit puree	400	400	61.7	275	275	30.6
Gelatine	Gelatine	0	0	0.0	0	0	0.0
Hot water	Hot water	0	0	0.0	0	0	0.0
Sugar	Sugar	140	112	17.3	140	112	12.5
Vanilla essence	Vanilla essence	10	10	1.5	10	10	1.1
Salt	Salt	1	1	0.2	1	1	0.1

8.2.1.2.2 Fat replacement ice-cream

In order to preserve a measure of creaminess in ice-cream and maintain some milk solids to improve the smooth texture, skimmed milk was used in combination with mucilage and cactus fruit puree to develop fat-free ice-cream. The replacement of cream with mucilage, skimmed milk and fruit puree is given in Table 93.

Table 93: The ingredients and amounts used in the making of mucilage inclusion fat replacement ice-cream products

		15% inclusion			30% inclusion		
Control	**Ice-cream**	**ml**	**g**	**%**	**ml**	**g**	**%**
Cream	Mucilage	125	125	15.3	250	250	30.6
	Skimmed milk	375	375	45.9	250	250	30.6
	Fruit puree	250	250	30.6	250	250	30.6
Gelatine	Gelatine	0	0	0.0	0	0	0.0
Hot water	Hot water	0	0	0.0	0	0	0.0
Sugar	Sugar	70	56	6.9	70	56	6.9
Vanilla essence	Vanilla essence	10	10	1.2	10	10	1.2
Salt	Salt	1	1	0.1	1	1	0.1

8.2.1.3 Sensory panel

The sensory panel for sorbet and ice-cream consisted of 50 panellists (ASTM Manual Series: MNL 13, 1992) as seen in Table 94. Eighty percent of the panellists were female and 54% were between the ages of 20 and 30.

Table 94: Demographic profile of the consumer panel for the ice-cream and sorbet products

Gender:	% of Total	Age:	% of Total
Female	80	< 20	6
Male	20	20-29	54
		30-39	16
		40-49	6
		50-59	16
		>60	2

8.2.2 Yoghurt

Since yoghurt is an everyday food product for countless and diverse consumers, it was believed that yoghurt would be an ideal functional food product. The addition of small amounts of freeze-dried mucilage would increase the soluble fibre content considerably while remaining invisible and undetected to the consumer. At the same time, mucilage would contribute its water holding properties and act as a stabiliser to prevent syneresis.

The cultivar selected for the addition of freeze-dried mucilage was Robusta. This cultivar had high viscosity while displaying no gelling properties, high protein, high fibre and amical amounts of calcium, potassium, iron and phenolic compounds.

8.2.2.1 Formulation for making yoghurt

The control yoghurt product recipe (ingredients, amount and method) was standardized (Hugo and De Wit, 1999). The ingredients and amounts are seen in Table 95.

Table 95: The ingredients and amounts used in the making of the control yoghurt product

Control	**Ml**	**g**	**%**
Full cream milk	500	500	95
Milk Powder		13	2
Sugar		13	2
Starter culture (Danisco)		0	0
Total	N/A	525	100

8.2.2.1.1 Method

Step 1: Mix all the dry ingredients with a little of the milk while stirring in order to dissolve it thoroughly. Pour the mixture through a fine sieve in order to remove the lumps. Allow the mixture to stand for 30 minutes for ingredients to dissolve further.

Step 2: Heat the milk at 90˚C for 5-10 minutes, then cool it down to 42˚C. First dissolve the starter culture in a small portion of the milk and then add it to the milk. Incubate the milk for 4 hours at 42˚C.

Step 3: Stir the yoghurt until it is smooth and shiny after coagulation or when a pH of 4.6 is reached. The yoghurt is cooled down to 20˚C and placed in sealed containers and cooled to under 8˚C for storage.

8.2.2.2 Mucilage additions to yoghurt

After the yoghurts were made, the freeze-dried mucilage powder was dissolved in 25 ml of the yoghurt before it was added to the larger batch. The addition of mucilage powder is given in Table 96.

Table 96: The ingredients and amounts used in the making of mucilage addition yoghurt products

	Mucilage additions for yoghurt							
Yoghurt	**1 g addition**		**2 g addition**		**3 g addition**		**4 g addition**	
Ingredients	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**
Milk	500	95.02	500	94.84	500	94.66	500	94.48
Mucilage	1	0.19	2	0.38	3	0.57	4	0.76
Milk Powder	12.5	2.38	12.5	2.37	12.5	2.37	12.5	2.36
Sugar	12.5	2.38	12.5	2.37	12.5	2.37	12.5	2.36
Starter culture (Danisco)	0.2	0.04	0.2	0.04	0.2	0.04	0.23	0.04

8.2.2.3 Sensory panel

The sensory panel consisted of 50 members (ASTM Manual Series: MNL 13, 1992) of which 62% were female and 38% were male as set out in Table 97. Seventy percent of the panellists were between 20 and 30 years of age.

Table 97: Demographic profile of the consumer panel for the sensoric evaluation of mucilage addition yoghurt products

Gender:	% of Total	Age:	% of Total
Female	62	< 20	6
Male	38	20-29	70
		30-39	10
		40-49	8
		50-59	6
		>60	0

8.2.3 Mayonnaise

Mayonnaise is presumably the best-known food emulsion. It was observed in chapter 6 and 7 that mucilage had potential as an emulsifying agent. As mayonnaise is an emulsion, contains a large amount of fat (70% to 80%), is easy to make and is never exposed to direct heat for cooking, it would serve as the ideal product for testing the fat-replacement and emulsification properties of mucilage.

Hydrocolloids, of which mucilage is an example, often displays fat-replacing properties, therefore not only the egg yolk (containing the emulsifying agent lecithin) but also the oil in mayonnaise was replaced with native mucilage. Mucilage was used in the native form, as it requires significantly less processing and is already in a liquid form similar to the ingredients that it would replace (egg and oil).

It is important that an emulsion must be formed in the early stages of making mayonnaise. As tiny droplets of oil is added to the emulsion and mixing is continued, it becomes more viscous and an oil-in-water emulsion is formed. In this study, mucilage would be tested as emulsifier in mayonnaise to determine its capacity to act as such (Bennion, 1985).

Native Robusta mucilage was used in the mayonnaise formulations. Robusta consistently displayed the best ability to emulsify, had the highest viscosity and proved to have very good water- holding, swelling and water- absorption properties.

8.2.3.1 Formulation for the making of mayonnaise

The control mayonnaise (Food and Cookery, 1991) recipe (ingredients, amount and method) was standardized. The ingredients and amounts are seen in Table 98.

8.2.3.1.1 Method

Step 1: Add egg yolks, mustard, sugar, salt and 10 ml lemon juice into a bowl, mix for 30 seconds at the 2nd speed setting using a handheld electric beater.

Step 2: Add the oil one drop at a time into the mixture, beating constantly at the highest speed setting.

Step 3: During the first 5 minutes 50 ml of oil should be added. After 10 minutes, 150 ml of oil should be added. After 15 minutes when the mixture has thickened, add 5 ml vinegar and mix at the first speed setting. Beat for 5 more minutes until smooth.

Step 4: Place into a glass jar, seal the lid airtight and keep in the refrigerator (4˚C) for up to a month.

Table 98: The ingredients and amounts used in the making of the control mayonnaise product

Control	**ml**	**g**	**%**
Egg, large	40	37.2	12.66
Sunflower oil	250	230	78.28
Mustard powder	5	3.3	1.12
Sugar	5	4.25	1.45
Salt	3	3.69	1.26
Lemon juice	10	10.31	3.51
White wine vinegar	5	5.05	1.72
Total	280	293.8	100

8.2.3.2 Mucilage replacements

8.2.3.2.1 Egg replacement mayonnaise

Egg-free products have always been in demand because of egg allergies and vegan consumers. The spread of avian influenza H5N2 has caused the wholesale prizes of eggs to increase. Therefore industrial food product producers are investigating the possibility of reducing the use of eggs in products or to replace eggs completely. The ingredients for egg replacement are often based on gums (such as mucilage). The advantages of using gums instead of egg in food products include moisture retention, increasing of shelf life, viscosity maintenance and (in the case of mucilage), a good emulsifying agent (Naschay, 2016).

When the egg-yolk is replaced with mucilage, the mucilage also has to take up the emulsifying function for the mayonnaise to be formed. The replacement of egg yolk with mucilage for the development of egg replacement mayonnaise is given in Table 99.

Table 99: The ingredients and amounts used in the making of egg replacement mayonnaise products using native mucilage

		25% replacement			**50% replacement**			**100% replacement**		
Control	**Egg replacements**	**ml**	**g**	**%**	**ml**	**g**	**%**	**ml**	**g**	**%**
Egg yolk	Egg yolk	28	27.9	9.50	18	18.6	6.33	0	0	0
	Mucilage	9	9.3	3.17	18	18.6	6.33	37	37.2	12.66
Sunflower oil	Sunflower oil	250	230	78.28	250	230	78.28	250	230	78.28
Mustard powder	Mustard powder	5	3.3	1.12	5	3.3	1.12	5	3.3	1.12
Sugar	Sugar	5	4.25	1.45	5	4.25	1.45	5	4.25	1.45
Salt	Salt	3	3.69	1.26	3	3.69	1.26	3	3.69	1.26
Lemon juice	Lemon juice	10	10.31	3.51	10	10.31	3.51	10	10.31	3.51
White wine vinegar	White wine vinegar	5	5.05	1.72	5	5.05	1.72	5	5.05	1.72

8.2.3.2.2 Fat replacement mayonnaise

The oil was replaced with native mucilage in order to develop fat replacement mayonnaise with the added advantage of the soluble fibre contained in native mucilage. The replacement of sunflower oil with mucilage for the development of fat replacement mayonnaise is given in Table 100.

Table 100: The ingredients and amounts used in the making of fat replacement mayonnaise products using native mucilage

		15% replacement			**30% replacement**		
Control	**Fat replacement**	**ml**	**g**	**%**	**ml**	**g**	**%**
Egg yolk	Egg yolk	40	37.2	12.66	40	37.2	12.66
Sunflower oil	Sunflower oil	210.5	195.5	66.54	173.5	161	54.80
	Mucilage	39.5	34.5	11.74	76.5	69	23.49
Mustard powder	Mustard powder	5	3.3	1.12	5	3.3	1.12
Sugar	Sugar	5	4.25	1.45	5	4.25	1.45
Salt	Salt	3	3.69	1.26	3	3.69	1.26
Lemon juice	Lemon juice	10	10.31	3.51	10	10.31	3.51
White wine vinegar	White wine vinegar	5	5.05	1.72	5	5.05	1.72

8.2.3.3 Sensory panel

The sensory panel consisted of 50 panellists (ASTM Manual Series: MNL 13, 1992) of which 80% were female and 20% were male as set out in Table 101. Seventy-four % were between 20 and 30 years of age.

Table 101: Demographic profile of the consumer panel for the sensoric evaluation of egg and oil replacement mayonnaise products using native mucilage

Gender:	% of Total	Age:	% of Total
Female	80	< 20	2
Male	20	20-29	74
		30-39	8
		40-49	4
		50-59	12
		>60	0

8.2.4 Polony

Polony is a processed, unfermented meat (beef) emulsion containing pork fat. The conception of this product was to evaluate the performance of mucilage as water-binder, stabiliser and emulsifier in another, very different type of emulsion. Also, to study the effect of high temperature processing on mucilage and lastly to lower the fat content of polony (8-15% fat content).

Polony is an affordable meat product that is popular in South Africa and a “vetkoek” (deep fried bread) filled with polony has been described as one South Africa’s favourite township dishes (South African Department of Tourism, 2016). In Cape Town, the Gatsby Sandwich is a cultural symbol. It is a monstrous fast-food meal that consists of a Portuguese roll, polony, “slap-chips” (French fries) and atchar (pickles) (CapeTownMagazine.com, 2016).

The formulation and manufacturing process of polony was done based on the personal communication, assistance and advice given by Prof. Arno Hugo, professor in Meat Science at the University of the Free State (Hugo, 2010). Each polony sample (5 kg) was made in the bowl cutter, the polony castings filled and cooked in the steam oven on-site at the University of the Free State meat processing facilities.

‘Algerian’ was selected as the appropriate cultivar for use in the polony formulations. ‘Algerian’ consistently had high native mucilage yield. It also proved to be high in protein, calcium, potassium and iron.

8.2.4.1 Formulation for the making of polony products

The control polony ingredients, amount and method was standardized. The ingredients and amounts are seen in Table 102.

Table 102: The ingredients and amounts used in the making of the control polony product

Control	**g**	**%**
Lean beef	2130	42.6
Soya Protein Isolate	120	2.4
Ice water	1845	36.9
Back fat (Pork)	600	12
Spice mixture	200	4
Salt mixture	90	1.8
Erythrosine (colour)	15	0.3
Total	5000	100

8.2.4.1.1 Method

Step 1: Mince beef using a 3 mm plate. Chop the beef, soya protein isolate, salt and sodium nitrite in the bowl cutter for three rounds at slow speed.

Step 2: Add a third portion of the ice water and chop at high speed until a strong binding is achieved and a temperature of 8˚C is reached.

Step 3: Add half the remaining ice water, spices and back fat/mucilage and chop until a temperature of 8.5˚C is reached.

Step 4: Add the rest of the ice water and chop until a temperature of 12.5˚C is reached.

Step 5: Fill into a French polony casing of desired diameter. Cook at 75˚C until an internal temperature of 68.5˚C is reached (Figure 112). Cool product and store in the refrigerator (4˚C).

Figure 112: The polony in the steam oven

8.2.4.2 Mucilage replacements

In the meat emulsion formulation, pork fat was replaced by 25%, 50%, 75% and 100% mucilage. The replacement of pork fat with mucilage to produce polony is given in Table 103.

Table 103: The ingredients and amounts used in the making of fat replacement polony products using native mucilage

		Replacement %							
		25		**50**		**75**		**100**	
Control	**Fat replacement**	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**
Lean beef	Lean beef	2130	42.6	2130	42.6	2130	42.60	2130	42.6
Soya Protein Isolate	Soya Protein Isolate	120	2.4	120	2.4	120	2.40	120	2.4
Ice water	Ice water	1845	36.9	1845	36.9	1845	36.90	1845	36.9
Back fat (Pork)	Back fat (Pork)	450	9.0	300	6	150	3.00	0	0.0
	Mucilage	150	3.0	300	6	450	9.00	600	12.0
Spice mixture	Spice mixture	200	4.0	200	4	200	4.00	200	4.0
Salt mixture	Salt mixture	90	1.8	90	1.8	90	1.80	90	1.8
Erythrosine (colour)	Erythrosine (colour)	15	0.3	15	0.3	15	0.30	15	0.3

8.2.4.3 Sensory testing

Twenty five panellists (ASTM Manual Series: MNL 13, 1992) participated in the polony consumer panel. Their ages ranged between 21 and 65. Fourteen panellists were female and 6 were male. Five panellists failed to mention their gender.

8.2.5 Sensory analysis

Sensory testing is a scientific method to analyse, interpret and measure how food smells, tastes and feels (Campbell et al., 1987). It depends on human judgement, which is subjective, but the general human reaction to food products will determine whether or not consumers will buy a product containing mucilage or not. This will tell the food product developer if the product is similar to existing food products and therefore it dictates the quality of food that is produced and sold (McWilliams, 1989).

Sensory analysis tests were performed at the Sensory Laboratory, Food Science division, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State.

A naïve consumer panel of 50 people were used for the ice-cream, mayonnaise and yoghurt while 25 panellists participated in the polony tasting test. The products were presented to the participants in random order without any indication of its contents (Figure 113). In order to prevent bias, each product was coded with a three digit randomized code and rotated as there were 6 potential serving orders. Members of the tasting panels did not have an opportunity to interact with each other as they were situated in separate tasting booths. A preference ranking test was applied that required panel members to indicate their acceptability and preference for the appearance, mouthfeel, taste and overall acceptability of the products. The products included the control sample (no mucilage) as well as the products containing mucilage. The products were scored using a nine point hedonic scale (1 = dislike extremely; 2 = dislike very much, 3 = dislike moderately; 4 = dislike slightly; 5 = neither like or dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely). The hedonic scale is regarded as the most used evaluation technique for measuring food preferences and functions by describing the like or dislike experienced by the panel member (McWilliams, 1989) (Figure 113).

Figure 113: Presentation of the sorbet and ice-cream products to the sensory consumer panel

8.3 Statistical Methods

After the data was collected, an analysis of variance test (ANOVA) as well as a multiple comparison test (Fisher LSD) were done. The Tukey-Kramer multiple comparison test (α = 0.05) was carried out to determine whether significant differences exist between treatment means (NCSS, 2007).

8.4 Results

8.4.1 Ice-cream

Frozen desserts include ice-cream, sorbet, sherbet and Italian gelato, and are extremely popular, especially during the summer. Sorbet only contains fruit puree that is sweetened and stabilised, thus sorbet does not contain fat (Bennion, 1985).

Ice-cream is a frozen crystalline mixture that is composed of cream or full cream milk and a flavoured sugar syrup. It is agitated in order to incorporate air into the ice-cream mixture. The milk fat and milk solids are responsible for the formation of numerous tiny ice crystals that cannot be detected on the tongue and that do not blemish the smooth velvety texture of ice-cream. True ice-cream is made exclusively from genuine dairy and may contain no fat other than milk fats. Ice-cream often contain commercial stabilizers and emulsifiers (Bennion, 1985).

Reduced-kilojoule, low-fat and sugar-free frozen desserts are in demand by consumers and the food industry is always trying to find new, innovative ways to supply it. In fact, since the mid 1990's the sales of "light" ice-cream products have increased by 50% and represents a large part of the market (Bennion, 1985).

8.4.1.1 Products

Product A: Control, cactus pear ice-cream

Product B: 19% native mucilage inclusion sorbet

Product C: 55% native mucilage inclusion sorbet

Product D: 15% native mucilage inclusion ice-cream

Product E: 30% native mucilage inclusion ice-cream

Figure 114: Products presented in random order to the panellists

Four products were developed (as observed in Figure 105) together with the control sample in random order as they were presented to panellists.

8.4.1.2 Sensory assessment

The sensory assessment will include a detailed description of each product as assessed by the researcher, together with a photo in order to facilitate making a clear distinction between the different products.

8.4.1.2.1 Product A: Control ice-cream product

A standard ice-cream formulation was used that contained more than 80% cream and dispersed gelatine. Pink 'Algerian' cactus pear fruit was pureed and added to the ice-cream in order to create a characteristically cactus pear flavoured ice-cream and to impart the pink colour to the ice-cream (Figure 115). The properties of the product are represented in Table 104.

Table 104: Description of the properties of cactus pear ice-cream (control) product

Appearance	
Solidity	Frozen solid, firm
Colour	Light pink, creamy
Texture	
Consistency	Soft but solid and did not melt immediately
Creaminess	Very creamy
Mouthfeel	Cold, smooth, slowly melted in the mouth
Taste	
Bland/Tartness	Rich and creamy
Sweetness	Pleasant sweet flavour of cactus pear fruit

Figure 115: Product A: Cactus pear ice-cream (control)

8.4.1.2.2 Product B: Sorbet, 25% native mucilage replacement

Numerous consumers are lactose-intolerant, overweight, kosher, vegan and prone to allergies (Smith and Charter, 2010). Sorbet does not contain dairy products, animal products eggs or fat and as such is a popular choice for those consumers mentioned here. Sorbet has the added benefit of significantly increased soluble fibre, antioxidant and mineral content.

The 500 g cream was replaced with 125 g mucilage and 400 g cactus pear fruit puree (Figure 116). The gelatine and water was omitted as mucilage was tested as the stabilising agent in the sorbet. The sugar and vanilla was increased in order to enhance the flavour of the sorbet. The properties of sorbet are described in Table 105.

Table 105: Description of the properties of 19% native mucilage inclusion sorbet

Appearance	
Solidity	The sorbet was thick and not frozen solid
Colour	Dark red-orange colour of fruit puree
Texture	
Consistency	Thick, high viscosity and soft but could flow
Creaminess	Melted quickly, watery around the edges
Mouthfeel	Watery consistency with larger ice crystals that melt easily, faster than the control
Taste	
Bland/Tartness	Flavour or fruit much more prominent
Sweetness	Light flavour of cactus pear fruit. Slight taste of the mucilage but overall acceptable

Figure 116: Product B: 19% native mucilage inclusion sorbet

The most important observation of this product was its inability to freeze solid. It remained soft and viscous.

8.4.1.2.3 *Product C: 55% native mucilage inclusion sorbet*

The 55% mucilage inclusion sorbet was conceptualised as a therapeutic product that contained an increased amount of mucilage while the fruit puree content was decreased (Table 92). The ice-cream product would offer solutions to consumers who would be prepared to compromise taste for therapeutic advantages of the product. The properties of this product are described in Table 106.

Table 106: Description of the properties of 55% native mucilage inclusion sorbet

Appearance	
Solidity	Thick but not frozen
Colour	Brown-pink
Texture	
Consistency	Viscosity was higher than product B. Product was not frozen but contained small ice crystals and started to melt immediately once removed from the freezer
Creaminess	Melted quickly, watery around the edges
Mouthfeel	Soft, slightly slimy, small ice crystals that melted quickly
Taste	
Bland/Tartness	Strong taste of mucilage detected, the flavour of fruit was weak. Unacceptable taste
Sweetness	Pleasant taste that changed into a strong mucilage aftertaste

Figure 117: Product C: 55% native mucilage inclusion sorbet

This product (Figure 117) would not be acceptable to most people as the colour was unattractive, the texture was uncharacteristically runny for sorbet and the flavour of mucilage was strong and it had an unpleasant aftertaste. For this product to succeed, the inclusion of mucilage would have to be lowered and stabilising agents, such as Xanthan gum, introduced.

8.4.1.2.4 Product D: 15% native mucilage inclusion ice-cream

This low-fat ice-cream was conceptualised to develop a high-fibre, fat-free product. Both the cream and gelatine was replaced in this recipe (Figure 118), yet it would be for consumers to enjoy a healthy snack. The 15% mucilage inclusion ice-cream was made using skimmed milk (45%), native mucilage (15%), cactus pear fruit puree (30%) and flavourings (sugar and vanilla). The properties of the 15% mucilage inclusion ice-cream product are described in Table 107.

Table 107: Description of the properties of 15% native mucilage inclusion ice-cream

Appearance	
Solidity	Firm but not frozen hard
Colour	Light pink with visible foam
Texture	
Consistency	Thick with noticeable ice crystals, looked frozen but melted quickly
Creaminess	Creamier than sorbet
Mouthfeel	Ice crystals on tongue that melted away quickly
Taste	
Bland/Tartness	Fruit flavour less strong
Sweetness	Milky with some hints of cactus pear flavour. The taste of mucilage was concealed

Figure 118: Product D: 15% native mucilage inclusion ice-cream

8.4.1.2.5 *Product E: 30% native mucilage inclusion ice-cream*

In this fat replacement ice-cream product, equal amounts of mucilage, skimmed milk and fruit puree was used (30%). Therefore the mucilage inclusion was double compared to the low mucilage low-fat ice-cream. The concept was to increase the mucilage and thus the soluble fibre content considerably while reducing the milk content. It was successful as the remaining milk contributed creaminess and provided a more acceptable flavour to the product (Figure 119). The properties of the product are described in Table 108.

Table 108: Description of the properties of 30% native mucilage inclusion ice-cream

Appearance	
Solidity	Thick, slimy, foamy
Colour	Light pink-brown
Texture	
Consistency	Lower viscosity, foamy, aerated texture with noticeable ice crystals
Creaminess	Creamier than sorbet
Mouthfeel	Creamy, bubbly, melted quickly
Taste	
Bland/Tartness	Overpowering flavour of mucilage with strong mucilaginous aftertaste
Sweetness	Fruity flavour detected but not strong enough to mask mucilage taste

Figure 119: Product E: 30% native mucilage inclusion ice-cream

8.4.1.3 Sensory analysis

The ice-cream products were presented to a sensory taste panel. The results are presented in Table 109.

Table 109: Sensory properties of different ice-cream products

Ice-cream	**A**	**B**	**C**	**D**	**E**
Product	**Control**	**Sorbet 19%**	**Sorbet 55%**	**Ice-cream 15%**	**Ice-cream 30%**
Appearance	$7.62^c \pm 1.07$	$4.66^{ab} \pm 1.86$	$4.14^a \pm 1.92$	$5.00^b \pm 1.70$	$5.18^b \pm 1.79$
Taste	$7.28^c \pm 1.43$	$4.04^a \pm 1.86$	$3.42^a \pm 1.95$	$4.92^b \pm 1.95$	$3.96^a \pm 1.73$
Mouthfeel	$7.68^c \pm 1.32$	$4.62^{ab} \pm 1.99$	$4.22^a \pm 1.91$	$5.26^b \pm 2.01$	$4.96^b \pm 1.75$
Overall acceptability	$7.50^c \pm 1.33$	$4.02^a \pm 1.85$	$3.70^a \pm 1.85$	$4.78^b \pm 1.87$	$4.18^{ab} \pm 1.64$

Significance level $p < 0.001$. Means with different superscripts in the same row differ significantly

The appearance, taste, mouthfeel and overall acceptability of the control sample was significantly higher than all the other products. The ice-cream products received better appearance scores than the sorbet products (Table 109), although the differences were not significant. The appearance was described as "neither like nor dislike" that indicated that the products did not look appetising to the panellists. The taste of the control sample was well liked, but the other products did not fare well on taste. The best rating in terms of taste was for Product D (significantly different $p < 0.001$). Products B, D and E received significantly lower taste scores than Products A and D, with Product C receiving the lowest scores for taste. It can be deduced that the high percentage inclusion of native mucilage affected the flavour of ice-cream noticeably. The mouthfeel values for all the replacement products were significantly lower ($p < 0.001$) than the control. As was seen before (Table 109), Product D had the highest scores and Product C the lowest. Product D and E had significantly higher scores than Product C while Product B was not significantly different than D and E. The products received scores equal to “dislike slightly” to “neither like nor dislike”. The inclusion of mucilage had a negative effect on the mouthfeel of all products. The overall acceptability followed the same trends as before: all the products received significantly lower scores than the control Product A. Product D had significantly higher values than Product B and C.

The control was overall acceptable but the other products did not receive good acceptability ratings. Panellists regarded Product A (control ice-cream) as “like moderately to like very much”. All of the attributes for all the replacement products were significantly lower ($p < 0.001$) than the control. Product D received the best ratings amongst all products except for appearance. The presence of milk and lower inclusion of native mucilage caused this product to have an acceptable appearance, better taste and had the best texture in terms of mouthfeel. In Figure 120 the products were compared in relation to the sensory property scores that were obtained for the attributes.

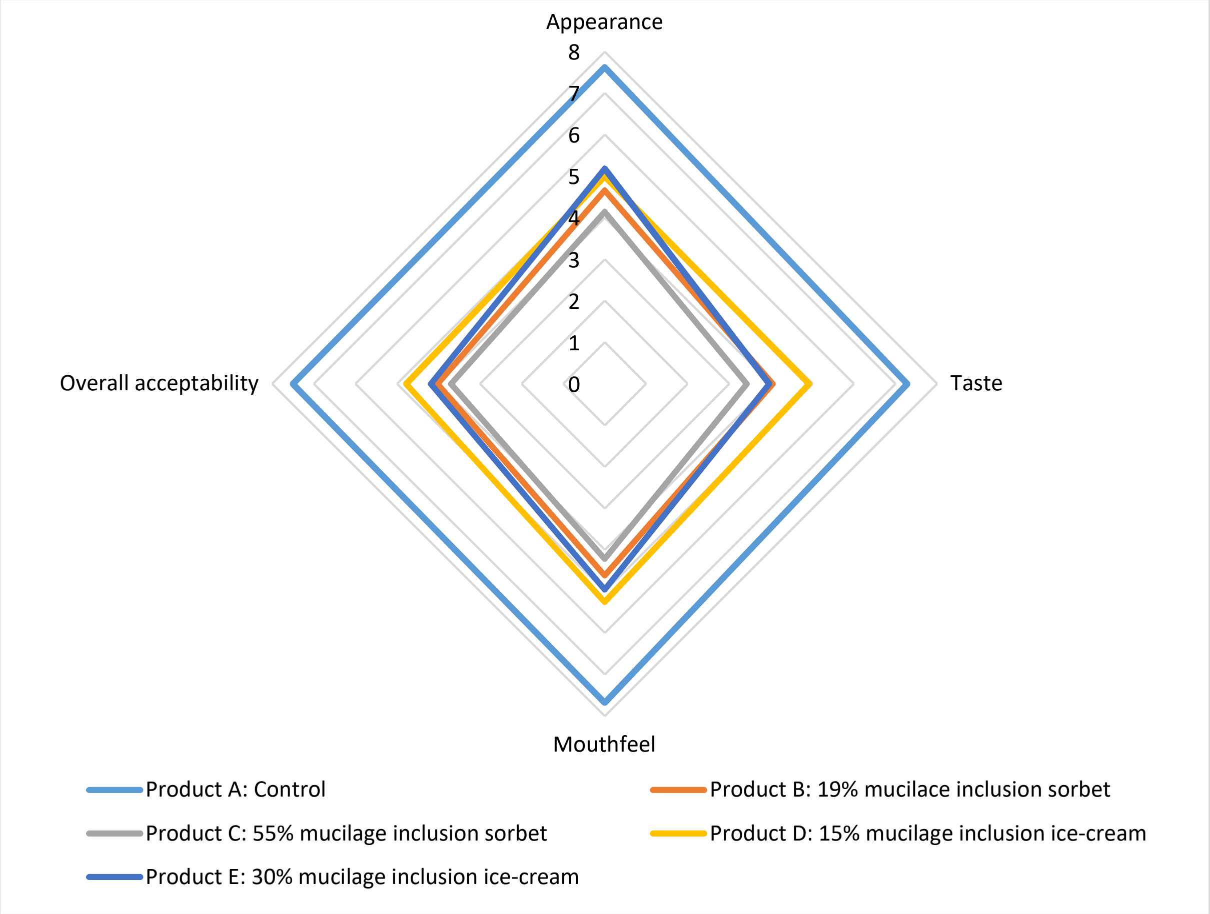


Figure 120: Spider plot of attributes for ice-cream and sorbet products from consumer panel data

From Figure 120 it is evident that the mucilage-containing products were rated much lower than the control ice-cream. The mucilage taste was detected in the products, therefore more care should be taken with the other flavourings to mask the "green" flavour more effectively. Yet seeing that these products contained high (therapeutic) amounts of mucilage, it was not rated as completely unacceptable by consumers. Patients in need of large amounts of soluble fibre in their daily diets, may find these products to be satisfactory.

The control was smooth, frozen and solid but none of the mucilage replacement products were solidly frozen. Consequently, it was observed that mucilage had an adverse effect on the ability of the products to freeze, thus the crystallization of the water. Ideally in the making of ice-cream products, many small crystals formed but instead, in the mucilage replacement products, large crystals were observed. The separated ice crystals that were observed in the products may indicate that mucilage prevented extensive nucleation and resulted in the products having a grainy icy texture. It is known that bound and strongly sorbed water cannot be frozen while the amount and kind of solutes influence the formation of ice crystals (Fenema, 1996). The degree to which the water is bound on a macroscopic level depends on the ability of a matrix of molecules (such as mucilage) to physically entrap a large amount of water (Fenema, 1996). Bound water often display features that are decidedly different from the norm, such as was reported and discussed in chapter 5. This phenomenon has to be investigated in future research and ways have to be found to incorporate this feature as an advantageous property in newly developed nutraceutical products.

8.4.2 Yoghurt

Yoghurt is a fermented dairy product and is produced by adding enzymes to milk. These are proteolytic enzymes naturally present in milk, microorganisms and rennin. Whole, low-fat or skimmed milk can be used to produce yoghurt. The bacteria cause lactic acid fermentation of lactose to form lactic acid causing the coagulation of the casein. This produces the distinctive taste and texture of yoghurt. The incubation period of 2-4 hours at 42-46˚C lasts until the pH reaches 4.6 and the milk is thickened (Bennion, 1985).

Mucilage did not replace ingredients in the yoghurt formulation, instead freeze-dried mucilage powder was added as a bioactive ingredient that could advance the preventative or protective properties of an already popular and healthy product (Smith and Charter, 2010). The addition of mucilage would not only increase the fibre content but also increase the mineral, antioxidant and protein content while binding the water and thus stabilizing the yoghurt to prevent syneresis.

8.4.2.1 Products

Product A: Control, natural yoghurt

Product B: 1 g freeze-dried mucilage powder addition yoghurt

Product C: 2 g freeze-dried mucilage powder addition yoghurt

Product D: 3 g freeze-dried mucilage powder addition yoghurt

Product E: 4 g freeze-dried mucilage powder addition yoghurt

8.4.2.2 Sensory assessment

The addition of the pink cactus pear fruit puree was omitted in the yoghurt samples, as it was suspected in the ice-cream that the fruit flavour interfered with the untarnished flavour of the samples. It is therefore mentioned that in the yoghurt, just as in the ice-cream, cactus pear fruit puree could be used to add flavour and colour to the yoghurt.

The sensory assessment will include a detailed description of each product as assessed by the researcher, together with a photo in order to facilitate making a clear distinction between the different products.

8.4.2.2.1 Product A: Control, natural yoghurt

The control yoghurt was home-made using lactic acid starter bacteria (Danisco). It was made using full cream milk (95%), milk powder (2.3%) and sugar (2.3%). This recipe produced a smooth, very acceptable product (Figure 121). The properties of the natural yoghurt are described in Table 110.

Table 110: Description of the properties of Product A: Control, natural yoghurt

Appearance	
Colour	Off-white, opaque, glossy
Texture	
Consistency	Medium viscosity, thick consistency
Mouthfeel	Smooth, creamy, full bodied
Taste	
Flavour	Rich, pleasant, tangy with a slightly sweet flavour

Figure 121: Product A: Control, natural yoghurt

8.4.2.2.2 Product B: 1 g freeze-dried mucilage powder addition yoghurt

It was deduced after numerous trials that mucilage, whether it was in the native form or the freeze-dried form, could not be successfully introduced either to replace ingredients (such as milk) or as an additional ingredient before pasteurization or incubation took place. The yoghurts were therefore completely finished and attained the desired flavour, acidity and set consistency that is characteristic to yoghurt before the additions were made. Fruit pulp or other flavours would normally be added at this point, after the yoghurt had set. Mucilage could not be introduced before the yoghurt was ready as it caused the yoghurt to separate and fail. Sour smelling, watery liquid formed that did not resemble the control product A. It was speculated that the sliminess contributed by mucilage, together with the formation of mucus that is inherent to the starter culture functioning, caused excessive liquid in yoghurt while too little milk solids remained for yoghurt to be produced. The effect of fermentation and high temperature pasteurization on mucilage is not known and would be a subject for future study. For native mucilage to succeed in a yoghurt formulation, milk solids and stabilising agents need to be added. Therefore, for the purpose of these studies, freeze-dried powder was added after the milk was pasteurized, together with the starter culture in order to maintain the original natural properties thereof. The freeze-dried powder had to be dissolved in a small amount of the yoghurt before it was added.

The rationale behind developing this product was to increase the health benefits of an already popular healthy product in such a way that it remains undetected to the consumer while at the same time the mucilage provides its functional properties to act as stabilizer in preventing syneresis. The properties of the product are described in Table 111.

Table 111: Description of the properties of product B: 1 g freeze-dried mucilage powder addition yoghurt

Appearance	
Colour	No differences in colour but appeared very runny
Texture	
Consistency	The yoghurt was thinner, pouring consistency
Mouthfeel	Smooth, creamy
Taste	
Flavour	A sharper tart flavour was detected but mucilage taste was undetected

Figure 122: Product B: 1 g freeze-dried mucilage powder addition yoghurt

The quality of Product B (Figure 122) was significantly reduced in comparison to Product A. The viscosity was lower and the taste was affected negatively. This drop in sensory properties could be attributed to the fact that the mucilage interfered with the composition of the yoghurt but the concentration of mucilage was not high enough to impart its water holding properties to the product.

8.4.2.2.3 Product C: 2 g freeze-dried mucilage powder addition yoghurt

With the addition of 2 g of mucilage powder, the product improved because the functional properties provided by the mucilage were starting to take effect on the yoghurt (Figure 123). The properties of the product are described in Table 112.

Table 112: Description of the properties of product C: 2 g freeze-dried mucilage powder addition yoghurt

Appearance	
Colour	No differences in colour but it looked more runny than Product A
Texture	
Consistency	Thicker than product B
Mouthfeel	Smooth and creamy
Taste	
Flavour	Taste of mucilage was undetected, but the yoghurt was less tasty than the control sample. The tangy flavour had changed into tartness

Figure 123: Product C: 2 g freeze-dried mucilage powder addition yoghurt

8.4.2.2.4 Product D: 3 g freeze-dried mucilage powder addition yoghurt

In product D, 3 g of mucilage powder was added. As was the case in product C, the product improved slightly (Figure 124). The properties of the product are described in Table 113.

Table 113: Description of the properties of product D: 3 g freeze-dried mucilage powder addition yoghurt

Appearance	
Colour	No differences in colour but it looked more runny than Product A
Texture	
Consistency	Thicker than Product C but thinner than the control sample. It had body with a firm consistency
Mouthfeel	Smooth and creamy
Taste	
Flavour	Taste of mucilage was undetected, but the yoghurt was less tasty than the control sample. It had a sharper tart flavour

Figure 124: Product D: 3 g freeze-dried mucilage powder addition yoghurt

8.4.2.2.5 Product E: 4 g freeze-dried mucilage powder addition yoghurt

In the final product, 4 g of mucilage was added (0.76%). The properties of the product are described in Table 114.

Table 114: Description of the properties of product E: 4 g freeze-dried mucilage powder addition yoghurt

Appearance	
Colour	It looked similar to the control sample
Texture	
Consistency	As thick as the control sample, it had a lot of body and a thick and firm consistency
Mouthfeel	Smooth and creamy
Taste	
Flavour	Taste of mucilage was undetected, but the yoghurt was less tasty than the control sample. The sharp taste disappeared and it tasted pleasantly tangy

Figure 125: Product E, 4 g freeze-dried mucilage powder addition yoghurt

Even though it was less than 1% mucilage addition, the quality of the yoghurt had improved to almost equal to the control sample (Figure 116). The functional properties of the mucilage contributed to the consistency of the yoghurt to such an extent that it restored the sensory properties of the yoghurt.

8.4.2.3 Sensory analysis

It should be mentioned that South African yoghurt consumers rarely purchase unflavoured yoghurt and are used to the taste, colour and flavour of flavoured yoghurt. The yoghurt products were presented to the panellists (Figure 126). The results of the sensory tests are presented in Table 115.

Figure 126: Presentation of the products to the consumer panel

Table 115: Sensory properties of different freeze-dried mucilage addition yoghurt products

Yoghurt	**A**	**B**	**C**	**D**	**E**
Freeze-dried powder additions	**Control**	**1 g**	**2 g**	**3 g**	**4 g**
Appearance	$7.12^{b} \pm 1.44$	$4.86^{a} \pm 2.08$	$5.08^{a} \pm 1.68$	$6.54^{b} \pm 1.45$	$6.72^{b} \pm 1.49$
Taste	$6.86^{c} \pm 1.54$	$4.62^{a} \pm 2.27$	$4.98^{ab} \pm 1.93$	$5.42^{b} \pm 2.03$	$5.20^{ab} \pm 2.21$
Mouthfeel	$6.98^{b} \pm 1.44$	$5.06^{a} \pm 2.18$	$5.26^{a} \pm 1.88$	$5.74^{a} \pm 1.93$	$5.72^{a} \pm 2.07$
Overall acceptability	$6.96^{b} \pm 1.52$	$4.88^{a} \pm 2.25$	$5.14^{a} \pm 1.96$	$5.56^{a} \pm 1.96$	$5.38^{a} \pm 2.14$

Significance level $p < 0001$. Means with different superscripts in the same row differ significantly

According to the sensory panel, there were no significant differences ($p < 0.001$) in the appearance between products D and E and the control yoghurt product A. Products B and C appeared significantly different ($p < 0.001$) to products A, D and E Product A (control) (Table 115). It is important to state that the differences in appearance were in terms of the viscosity of the product and not the colour as there were no colour differences.

All the products tasted significantly different from the control sample A. Products B and C received significantly lower scores from the panellists than Products A, while the taste improved in products D and E (not significantly).

The mouthfeel scores of all the addition products dropped significantly from the control sample A, in fact it improved in products D and E but not enough to be equivalent to that of the control yoghurt (product A).

The panellists scored the overall acceptability of Product A (control) “like moderately”, while the mucilage addition yoghurts were judged as “neither like not dislike”. This is a significant decrease in acceptability. In Figure 127 the products were compared in relation to the sensory property scores that were obtained.

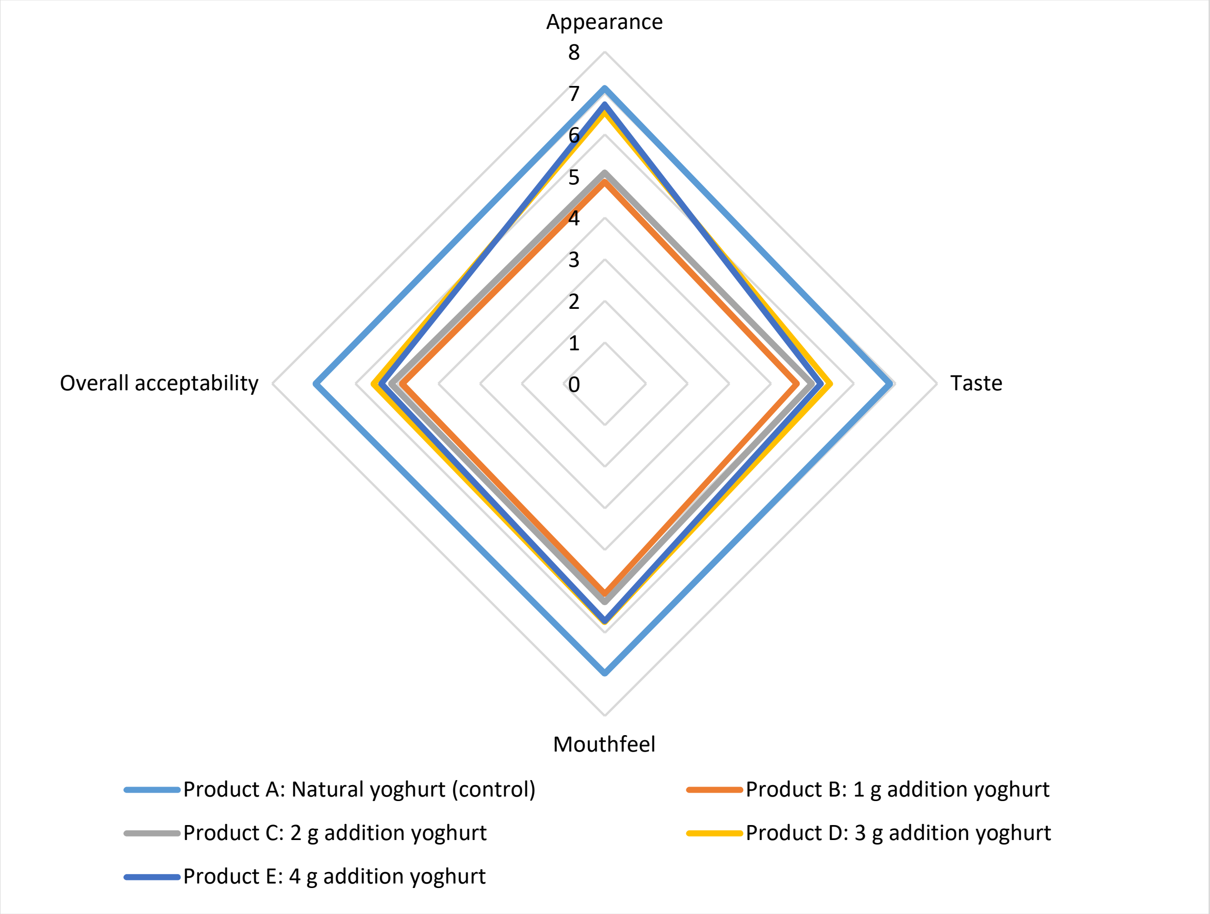


Figure 127: Spider plot of attributes in yoghurt products from consumer panel data

It was observed in Figure 127, that the lowest addition (1 g) product performed the worst in terms of all the attributes while the highest addition product (3 and 4 g) had the highest acceptability ratings apart from the control sample A. It is recommended that the addition of mucilage powder should be increased in further product development investigations. Even though the taste of mucilage was not detected, it affected the flavour of the yoghurt negatively. It is recommended that a flavouring, such as cactus pear fruit puree, be added in order to mask this effect. Adding dried mucilage to yoghurt would increase the soluble fibre content of yoghurt while remaining completely unnoticeable to the consumer as the characteristic mucilage "green" taste was not detected.

8.4.3 Mayonnaise

Mayonnaise is a popular condiment that is added to food to enhance the flavour, seasoning and moistness. It is often applied to food by the diner either as dressing or as spread, or it may be added to food prior to serving. Mayonnaise is a semisolid food prepared from vegetable oil, vinegar, egg yolk and flavourings. The edible oil content of mayonnaise must be more than 65% (Bennion, 1985). The intention of this product was to develop a mayonnaise-type product for those consumers who are avoiding fats (kilojoule control) or eggs (allergies or vegans) as well as food manufacturers looking for ways to reduce costs.

In chapter 6 high oil holding capacity (OHC) was determined (1.22 ml/g) in the freeze-dried mucilage powder that could indicate its application in high-fat products such as mayonnaise. The OHC measured the retention ability of the swollen mucilage particles that were precipitated in the centrifuge tube after the supernatant oil was separated carefully using a pipette and the centrifuge tube inverted for 12 hours. Thus, freeze-dried mucilage particles possess the ability to bind with oil and thus improve the flavour and consistency of the product it is applied to, in this case, mayonnaise.

8.4.3.1 Products

Product A: Control, mayonnaise

Product B: 25% egg with native mucilage replacement mayonnaise

Product C: 50% egg with native mucilage replacement mayonnaise

Product D: 100% egg with native mucilage replacement mayonnaise

Product E: 15% oil with native mucilage replacement mayonnaise

Product F: 30% oil with native mucilage replacement mayonnaise

8.4.3.2 Sensory assessment

Since mayonnaise is a condiment and as such is often part of delicatessen salads, potato salad was therefore made using the different mayonnaise products to evaluate acceptability and preference by the consumer panel. It was established that increasing the mucilage content in mayonnaise formulations beyond 30% causes serious quality deterioration of the product in terms of appearance, texture and taste. It is only Product F (30% oil replacement) that contains less than 65% oil (54%), thus it is not technically a mayonnaise.

The sensory assessment will include a detailed description of each product as assessed by the researcher, together with a photo in order to facilitate making a clear distinction between the different products.

8.4.3.2.1 Product A: Control, mayonnaise

Mayonnaise was produced using a standard home-made mayonnaise recipe. It consisted of egg yolks (12%) and 250 ml sunflower oil (78%). Egg and oil made up 90% of the total mayonnaise formula. The control sample was very thick, smooth, very creamy, very rich with only a slight tangy flavour (Figure 128). It was presented to consumers in the form of a potato salad (Figure 129). The properties of the product are described in Table 116.

Table 116: Description of the properties of product A: Control, mayonnaise

Appearance	
Colour	Light, pale yellow colour
Emulsion	No separation of phases
Foam	None
Texture	
Consistency	Very thick, creamy, smooth and good spreadability. It is solid and moldable like butter (Figure 129)
Taste	
Flavour	Very rich and creamy, comparable to butter/shortening. The home-made mayonnaise had richer, creamier flavour compared to commercial mayonnaise products. The tanginess from the lemon juice and vinegar did not manifest in the flavour of the home-made product

Figure 128: Product A: Control mayonnaise

Figure 129: Product A: Control mayonnaise potato salad

8.4.3.2.2 Product B: 25% egg with native mucilage replacement mayonnaise

The egg yolk was replaced by mucilage in order to observe the emulsifying properties of mayonnaise.

Table 117: Description of the properties of product B: 25% egg with native mucilage replacement mayonnaise

Appearance	
Colour	Light, pale yellow colour
Emulsion	No separation of phases
Foam	No foam
Texture	
Consistency	Slightly thinner than control sample, creamy, smooth and good spreadability (Figure 131).
Taste	
Flavour	Pleasant tangy flavour. The tartness of the vinegar was more evident in the taste than in the control sample.

Figure 130: Product B: 25% egg with native mucilage replacement mayonnaise

Figure 131: Product B: 25% egg with native mucilage replacement mayonnaise potato salad

Egg yolk contains the lipoprotein lecithin that imparts its emulsifying properties for the oil to be emulsified permanently in a mayonnaise. In Product B, 25% of the egg yolk was replaced with native mucilage (9.3 g). Product B had a good colour and spreadability as well as smell, taste and texture (Figure 121). The properties of the product are described in Table 109.

8.4.3.2.3 Product C: 50% egg with native mucilage replacement mayonnaise

In the 50% replacement mayonnaise, 50% of the egg yolk was replaced with mucilage. The larger concentration of the mucilage (18 g) had a more visible effect on the viscosity of the mayonnaise. The 50% egg replacement product had a pleasing smell, colour, texture and creamy taste (Figure 132). The properties of the product are described in Table 118.

Table 118: Description of the properties of product C: 50% egg with native mucilage replacement mayonnaise

Appearance	
Colour	Cream colour with tiny green spots
Emulsion	No separation
Foam	No foam
Texture	
Consistency	More runny, thinner, creamy and soft (Figure 133)
Taste	
Flavour	The tartness of the vinegar is more pronounced, but pleasant

Figure 132: Product C: 50% egg replacement with native mucilage mayonnaise

Figure 133: Product C: 50% egg with native mucilage replacement mayonnaise potato salad

8.4.3.2.4 Product D: 100% egg replacement with native mucilage mayonnaise

The vegan-friendly product D (Figure 134) contained no egg yolk and was only made using mucilage as replacement. The 100% egg replacement product was conceived in order to determine whether this vegan friendly product could be accepted by consumers. The properties of the product are described in Table 119.

Table 119: Description of the properties of product D: 100% egg with native mucilage replacement mayonnaise

Appearance	
Colour	Light yellow-green with green spots
Emulsion	No separation of phases
Foam	No foam
Texture	
Consistency	The viscosity was very low but the consistency was creamy. It could be compared to salad cream (Figure 135).
Taste	
Flavour	Astringent taste and mucilage aftertaste

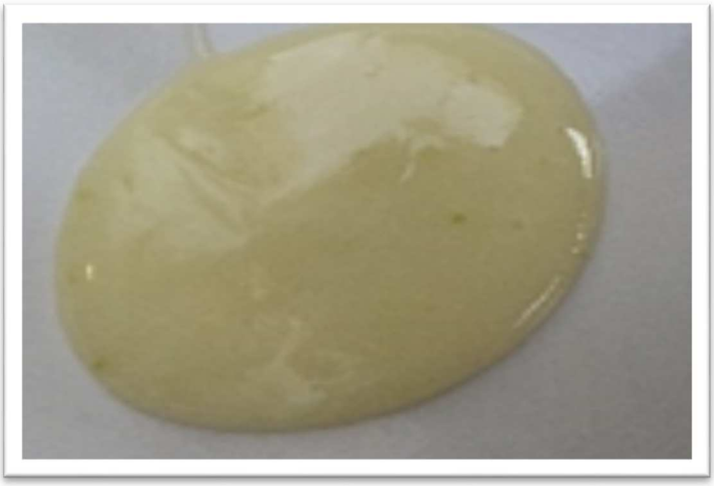


Figure 134: Product D, 100% egg with native mucilage replacement mayonnaise

Figure 135: Product D, 100% egg with native mucilage replacement mayonnaise potato salad

8.4.3.2.5 Product E: 15% oil with native mucilage replacement mayonnaise

Mayonnaise is a high-fat product. Many low-fat mayonnaise products exist as it is a product that most consumers prefer. In order to observe the fat-replacement properties of mayonnaise, 15% of the oil was replaced with native mucilage in product E (Figure 136). The properties of the product are described in Table 120.

Table 120: Description of the properties of product E: 15% oil with native mucilage replacement mayonnaise

Appearance	
Colour	Off-white colour
Emulsion	No separation of phases
Foam	Slight foam development, bubbles are small and few
Texture	
Consistency	Thick, creamy and soft with good spreadability (Figure 137)
Taste	
Tartness	Creamy, tangy taste

Figure 136: Product E: 15% oil with native mucilage replacement mayonnaise

Figure 137: Product E: 15% oil with native mucilage replacement mayonnaise potato salad

8.4.3.2.6 Product F: 30% oil with native mucilage replacement mayonnaise

The mucilage replaced 30% of the oil in this product and the mayonnaise produced was fully emulsified and acceptable to consumers. In fact, it was very similar to the 15% replacement product in terms of colour, texture and smell (Figure 138). It was not clear whether consumers would be able to distinguish between these two products and whether a clear preference would emerge from sensory analysis. The properties of the product are described in Table 121.

Table 121: Description of the properties of product F: 30% oil with native mucilage replacement mayonnaise

Appearance	
Colour	Off-white colour
Emulsion	No separation of phases
Foam	Slight formation of foam, bubbles were small but more abundant than product E
Texture	
Consistency	Thick, creamy and soft with good spreadability. It pulled threads and appeared to have plastic properties (Figure 139).
Taste	
Flavour	Creamy, more pronounced tangy taste but not astringent

Figure 138: Product F, 30% oil with native mucilage replacement mayonnaise

Figure 139: 30% oil with native mucilage mayonnaise replacement potato salad

8.4.3.3 Sensory analysis

The potato salad containing the different mayonnaise products was presented to the panellists (Figure 140). The results of the sensory tests are presented in Table 122.

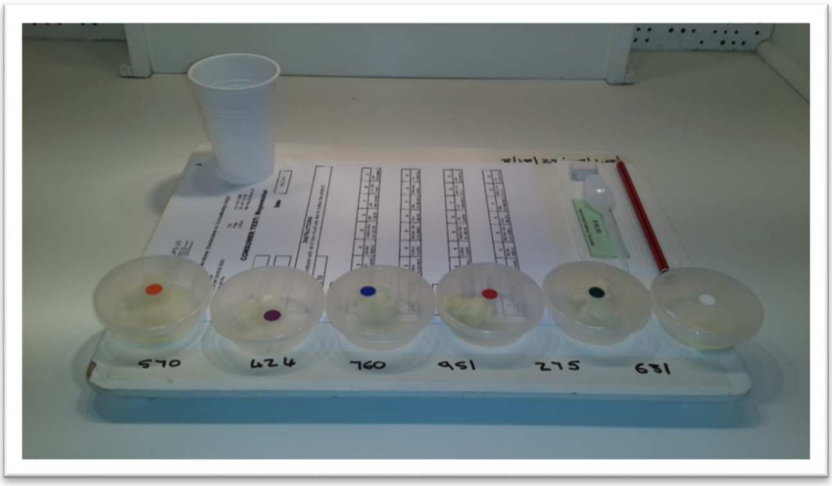


Figure 140: Presentation of the mayonnaise products to the consumer panel

Surprisingly product B received significantly higher scores from panellists than the control sample in terms of all attributes (Table 122). The appearance of product B was significantly higher than the control mayonnaise while products C and E were comparable with the control. The appearance of product D was rated significantly lower than all the other products.

Product B tasted significantly better than all the other samples, including the control. In fact, the control sample was rated lower than product B but these ratings were not always significantly different. These samples were perceived as having a more acidic flavour that was described as "tanginess". The panellists experienced the acidity of the products positively and rated the products higher. Product D was rated significantly lower as "dislike slightly".

The mouthfeel was rated in similar fashion with product B receiving significantly high scores with product D significantly lower. Products B and C received higher overall acceptability scores than product A (control), but not significantly different. Product B was overall rated as the best product and product D as the worst.

Products E and F were perceived as being very good products. Product F received slightly higher ratings for taste and mouthfeel than the control. Product E was comparable to the control in terms of appearance and mouthfeel. In terms of taste, a surprising result was observed. Product F was rated better than product E as well as the control sample A. In fact, the result was significantly higher than product E. Since the result was so unexpected, twenty additional consumers were asked to taste product A, E and F (without potatoes), rate them in terms of preferred taste and give a comment explaining their assessment of the products. The results were confirmed: product F and in some instances E, were preferred above product A (control). The reasons given by the consumers were that products E and F were not as rich they were tangier and thus more similar to commercial mayonnaise-type salad dressings. The unexpected outcome of these products was that the "green" mucilage flavour was not detected in any of the products.

Table 122: Sensory properties of different types of mayonnaise products

Mayonnaise	**A**	**B**	**C**	**D**	**E**	**F**
Sample	**Control**	**Egg 25 %**	**Egg 50 %**	**Egg 100 %**	**Oil 15 %**	**Oil 30 %**
Appearance	6.38^{c} ± 1.66	7.34^{d} ± 1.08	6.84^{cd} ± 1.35	2.76^{a} ± 1.72	6.26^{c} ± 1.59	5.28^{b} ± 1.98
Taste	5.68^{bc} ± 2.04	7.00^{d} ± 1.32	6.28^{c} ± 1.68	4.20^{a} ± 1.87	4.98^{b} ± 2.00	5.90^{c} ± 1.67
Mouthfeel	6.00^{bc} ± 2.04	7.04^{d} ± 1.40	6.54^{cd} ± 1.49	3.92^{a} ± 1.97	5.76^{b} ± 1.66	6.06^{bc}± 1.62
Overall acceptability	5.98^{bc} ± 1.87	7.18^{d} ± 1.10	6.46^{c} ± 1.47	3.74^{a} ± 1.99	5.40^{b} ± 1.86	5.80^{bc} ± 1.70

Significance level p < 0001. Means with different superscripts in the same row differ significantly

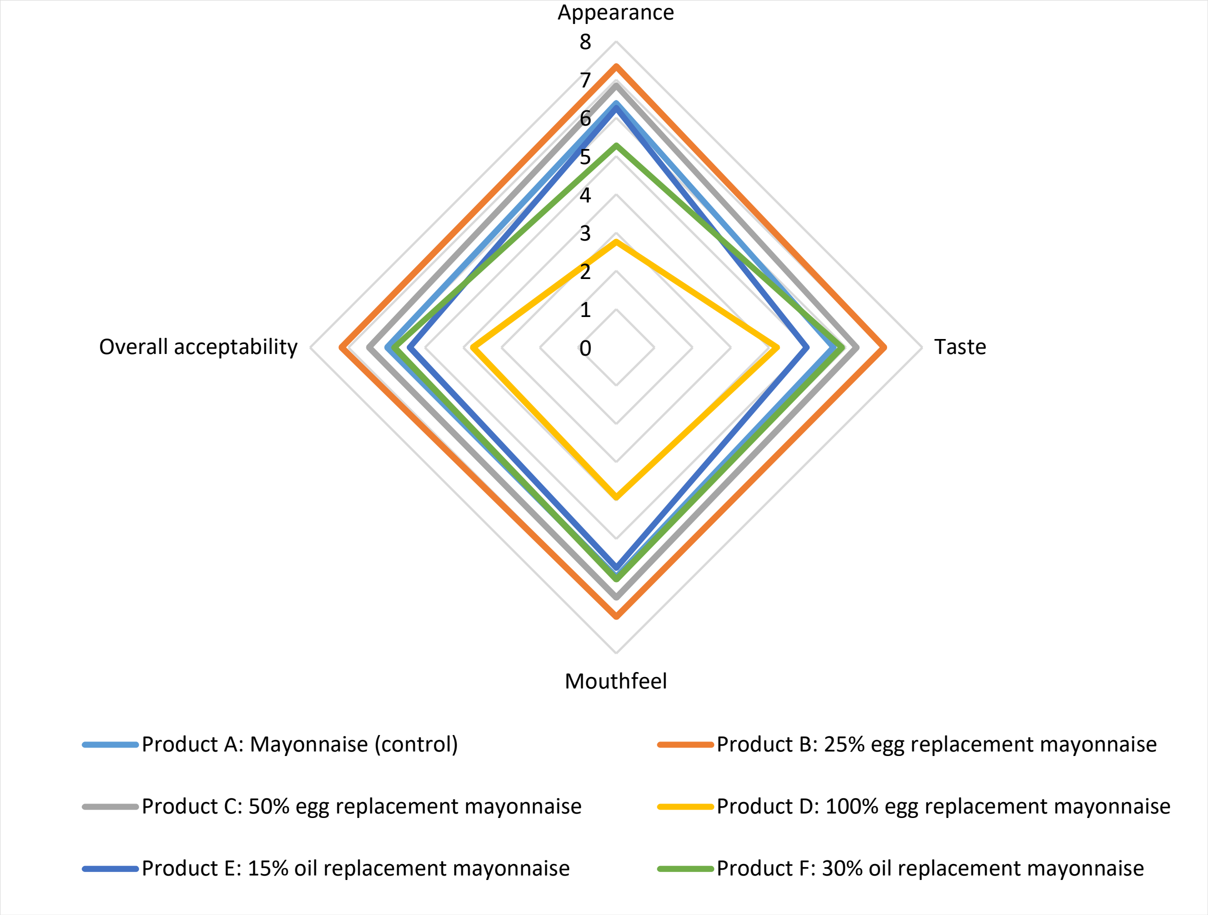


Figure 141: Spiderplot of attributes of mayonnaise products from consumer panel data

It was observed in Figure 141, that native mucilage could be used effectively in mayonnaise formulas to replace up to 50% of egg yolk as well as up to 30% of oil and still deliver highly acceptable products. It is recommended that another vegan friendly emulsifying agent such as soya be added in order to improve product D.

8.4.4 *Polony*

Polony is a meat emulsion that is a two-phase system, with the dispersed phase consisting of either solid or liquid fat particles, the continuous phase being the water containing salts and dissolved, gelled and suspended proteins. A meat emulsion is not a true emulsion since the two phases involved are not liquids and the fat droplets in a commercial emulsion are larger than 50 µm in diameter and thus do not conform to one of the requirement of a classical emulsion (Knipe, 1992).

In order to make high quality polony, the correct combination of ingredients are necessary, as well as the correct processing procedures applied, such as high speed grinding and chopping for emulsifying purposes. A stable emulsion would have to be prepared which will hold up well during the cooking process in a steam cooker. Polony and vienna sausages have such fine meat particles that they are not distinguishable on the smooth product surface. However, if either the quantity or quality of meat ingredients or the processing methods are inadequate, the meat mixture will be unstable and result in a poor quality product upon cooking (Knipe, 1992).

8.4.4.1 *Products*

Product A: Control, Polony

Product B: 25% fat with native mucilage replacement polony

Product C: 50% fat with native mucilage replacement polony

Product D: 75% fat with native mucilage replacement polony

Product E: 100% fat with native mucilage replacement polony

8.4.4.2 *Sensory assessment*

The sensory assessment will include a detailed description of each product as assessed by the researcher, together with a photo in order to facilitate making a clear distinction between the different products.

8.4.4.2.1 Product A: Control polony

Fat in the form of animal fat (in this instance pork fat) is usually added in the manufacturing of polony. The control sample was a high quality product (Figure 142). The properties of the product are described in Table 123.

Table 123: Description of the properties of product A: Control polony

Appearance	
Colour	Red-pink, consistent colour
Cutting edge	Excellent
Texture	
Consistency	Firm, set
Mouthfeel	Soft and smooth
Taste	
Savoury	Pleasant characteristic flavour
Aftertaste	None

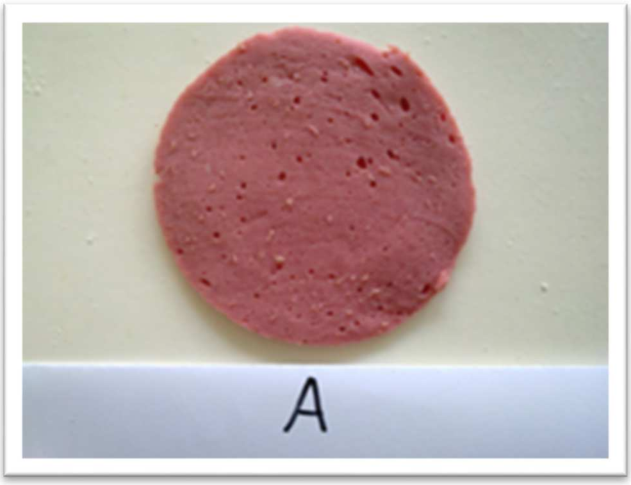


Figure 142: Product A, Control polony

8.4.4.2.2 Product B: 25% fat with native mucilage replacement polony

In product B, 25% of the fat was replaced with native mucilage (Figure 143). The properties of the product are described in Table 124.

Table 124: Description of the properties of product B: 25% fat with native mucilage replacement polony

Appearance	
Colour	Red-pink, consistent colour
Cutting edge	Excellent
Texture	
Consistency	Firm, set
Mouthfeel	Soft and smooth
Taste	
Savoury	Pleasant savoury flavour
Aftertaste	None

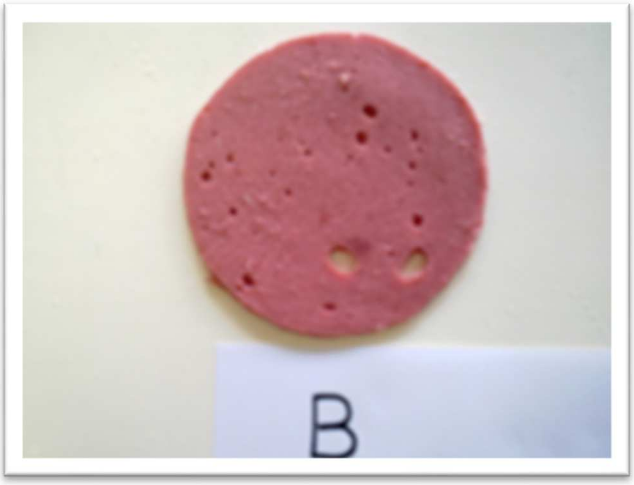


Figure 143: Product B: 25% fat with native mucilage replacement polony

8.4.4.2.3 Product C: 50% fat with native mucilage replacement polony

Product C was made using 50% (300 g) of native mucilage (Figure 144). The properties of the product are described in Table 125.

Table 125: Description of the properties of product C: 50% fat with native mucilage replacement polony

Appearance	
Colour	Red-pink, consistent colour
Cutting edge	Excellent
Texture	
Consistency	Firm, set
Mouthfeel	Soft and smooth
Taste	
Meaty	Pleasant savoury flavour
Aftertaste	None

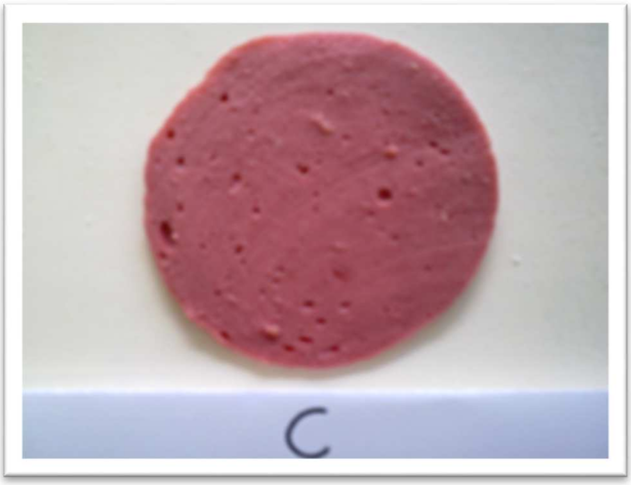


Figure 144: Product C: 50% fat with native mucilage replacement polony

8.4.4.2.4 *Product D: 75% fat with native mucilage replacement polony*

Product D contained 450 g mucilage (Figure 145). The properties of the product are described in Table 126.

Table 126: Description of the properties of product D: 75% fat with native mucilage replacement polony

Appearance	
Colour	Red-pink, consistent colour
Cutting edge	Excellent
Texture	
Consistency	Firm, set
Mouthfeel	Soft and smooth
Taste	
Savoury	The faint green flavour of mucilage was detected
Aftertaste	Faint aftertaste of mucilage was observed

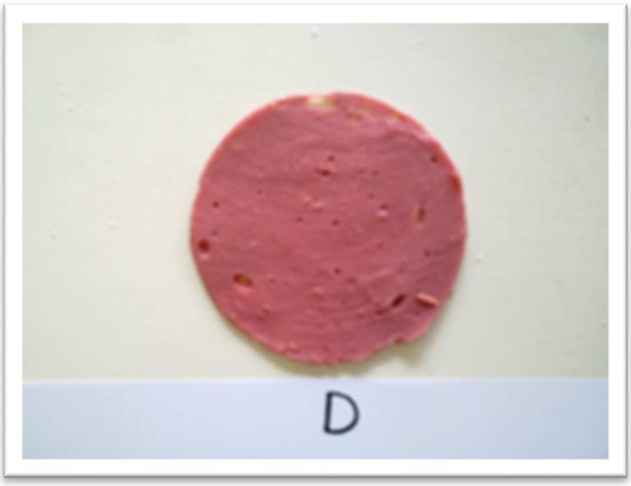


Figure 145: Product D: 75% fat with native mucilage replacement polony

8.4.4.2.5 *Product E: 100% fat with native mucilage replacement polony*

In product E, all of the fat in the polony recipe was replaced with mucilage (Figure 146). The properties of the product are described in Table 127.

Table 127: Description of the properties of product E: 100% fat with native mucilage replacement polony

Appearance	
Colour	Red-pink, consistent colour
Cutting edge	Excellent
Texture	
Consistency	Firm, set
Mouthfeel	Soft and smooth
Taste	
Savoury	A slight green flavour of mucilage was detected
Aftertaste	Slight aftertaste of mucilage was observed

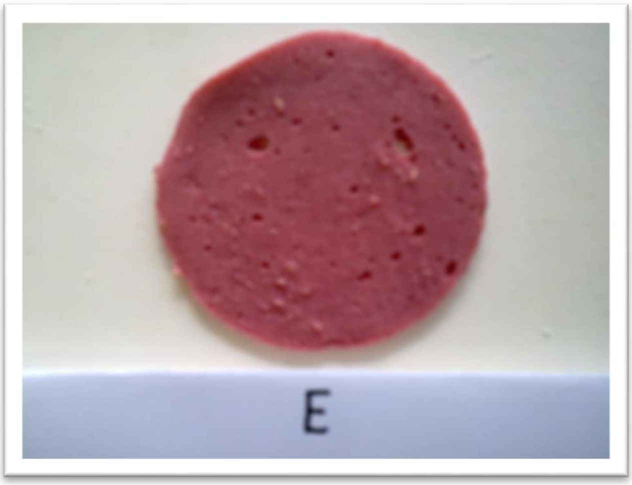


Figure 146: Product E: 100% fat with native mucilage replacement polony

8.4.4.3 Sensory analysis

The polony products were presented to the panellists in random order. The products are seen in Figure 147.

Figure 147: Products A to E (from left to right) displayed together

In Table 128, product B received similar scores (in terms of appearance) to product A (control). Products C, D and E had significantly lower scores than Product A for the appearance of the polony. Product B received lower scores in terms of taste than Product A, nevertheless the difference in taste was not significant. Products C, D and E received very similar scores for taste even though the mucilage inclusion in each product increased by 25% and it may seem that panellists could not distinguish between them in terms of taste. The mouthfeel of the products were comparable as no differences were picked up by the panellists. It was evident that the control sample was better liked than the other products. Product D was significantly less acceptable than Products A and B but not Products C and E.

Table 128: Sensory properties of different polony products

Polony Sample	**A Control**	**B 25%**	**C 50%**	**D 75%**	**E 100%**	**Significance level**
Appearance	7.24^{b}	7.08^{ab}	6.00^{a}	6.04^{a}	6.00^{a}	$p < 0.05$
Taste	7.32^{b}	6.72^{ab}	5.92^{a}	5.60^{a}	5.88^{a}	$p < 0.05$
Mouthfeel	7.2	7.16	6	6	6.32	NS
Overall acceptability	7.23^{c}	6.99^{bc}	5.97^{ab}	5.88^{a}	6.07^{ab}	$p < 0.05$

Means with different superscripts in the same row differ significantly. NS = Not significant

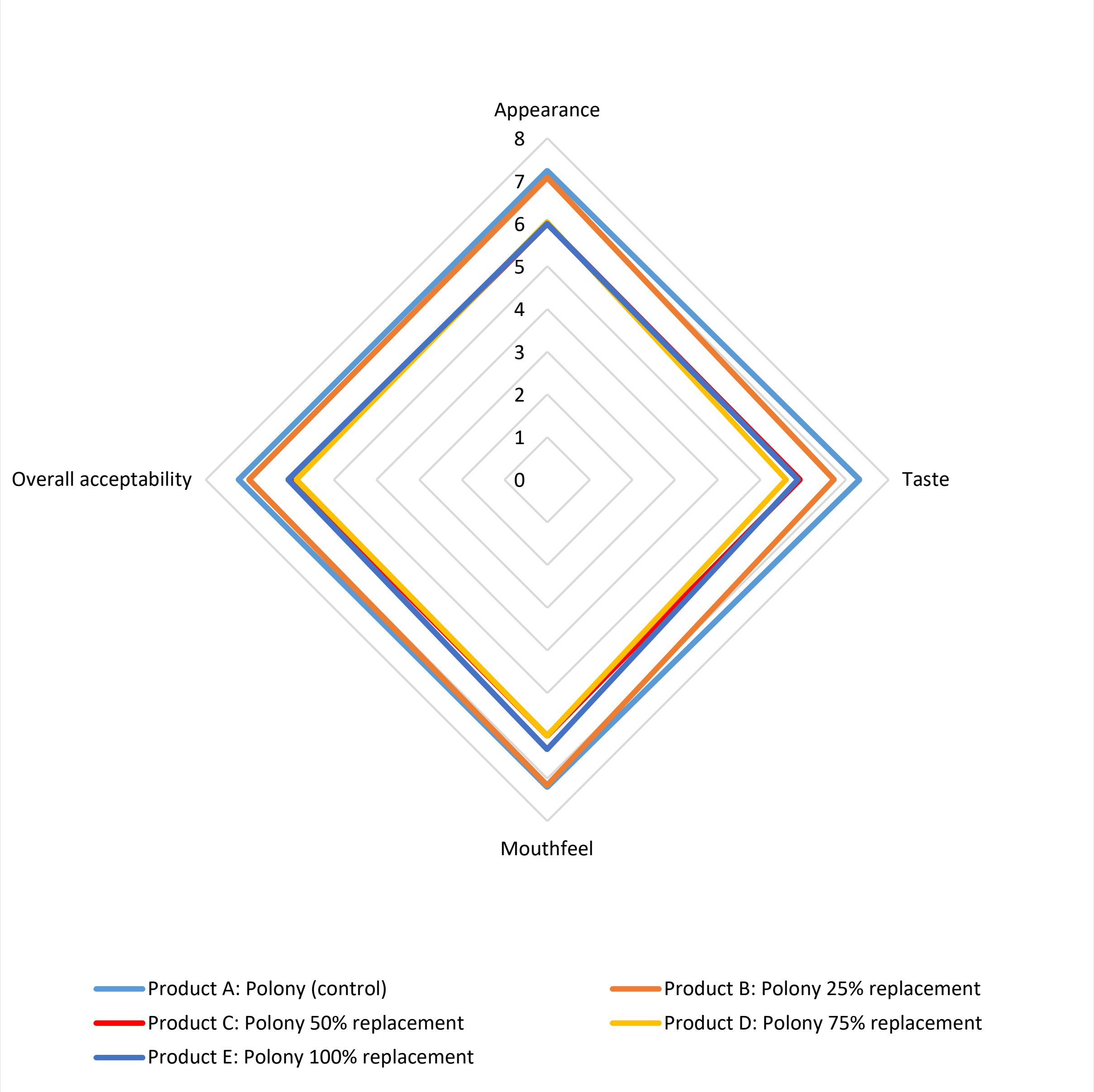


Figure 148: Spiderplot of attributes and polony products from consumer panel data

In Figure 148, it is evident that the panel was unable to detect a difference in the mouthfeel between products A and B. For taste and general impression, the panel chose the control and sample B (25% fat replacement) above samples C to E (50% to 100% fat replacement) (product C is hidden under product D and E in the spider plot). None of the products received low ratings, and it could be concluded that any of the mucilage replacement products would be accepted by consumers.

8.5 Conclusion

Mucilage in its native as well as freeze-dried form was successfully incorporated in different food products. It was observed that mucilage was most effective in fat-containing emulsified products as it possesses emulsification capacity and the characteristic mucilage taste is masked in the products. Mucilage also performed extremely well as a fat-replacer in the polony and the mayonnaise. In both products significant amounts of fat was replaced with mucilage and both products were accepted by consumer panels. The effect of heat (cooking) observed in polony may indicate that mucilage would be stable when included in products that undergo high temperature processing.

It was found that mucilage had an adverse effect on crystallization of ice crystals, the formation of few large ice crystals instead of many small crystals prevented the products from freezing effectively. Another negative property was that native mucilage was tasted in the products, therefore care should be taken with flavourings to mask the “green” flavour more effectively. The amount of mucilage should be regulated carefully in order to attain the optimum addition level.

When freeze-dried mucilage is added to everyday products, such as yoghurt, as a bioactive agent, it would increase its positive physiological effects in the body and thereby redefine the product as a functional food product. Freeze-dried mucilage was successfully added to yoghurt after pasteurization and fermentation in order to increase the soluble fibre, as well as nutrients such as antioxidants and minerals. The addition of the mucilage powders could be increased and addition of flavourings added.

The products that were tested in this paper has strong potential for commercialization and it is recommended that further tests be done in order to find optimum mucilage replacement and addition levels. Consequently, it was concluded in this study that native and freeze-dried mucilage could be applied in food products by means of replacing ingredients that are undesirable for multiple reasons or added to product formulations successfully. No mucilage-containing functional food products are commercially available in South Africa to the knowledge of the researchers.

Selection of the most appropriate cultivar mucilage for the application is paramount as the rheological, functional and chemical properties differ between cultivars and the required properties of the product should correspond with the capabilities of the mucilage.

8.6 Recommendations

It is recommended that further studies be done in order to find successful applications of mucilage in food products. The studies should be repeated and the products improved by changing the control formulas, the mucilage additions and replacements and the ingredients. Commercial stabilisers and preservatives could also be tested in products. Physical and chemical tests are necessary after manufacture and during the shelf-life analysis. Native and dried mucilage from different cultivars could result in better functional and chemical properties in products. Testing for microbial activity such as coliform, mould and yeast count, *E. coli* presence and total bacteria count, in order to determine shelf life of products, are suggested for future studies.

Chapter 9

9 General discussion and conclusion

This study was conceptualized based on the demand to add value to the cactus pear plant as a multi-purpose crop. The value of the cactus pear plant as a crop had been realised by researchers, however in order to convince South African farmers to plant cactus pear as a crop, more tangible uses had to be found. The evidence of the therapeutic and nutritional value as well as the rheological and functional advantages of dried mucilage in the literature existed, thus the objective of developing such a product emerged. Thus, for the purpose of assisting to transform cactus pear cladodes into a more valuable commodity, mucilage would be developed into a commercially viable human functional food ingredient.

The only research available on the 42 local cactus pear cultivars had focused on fruit production and seed oil quality, yet there has been a realization in the research community that the cladodes are the true value of the cactus pear plant because of the nutritional advantages and nourishment that it offers to both humans and animals, while the contribution of the fruit and the seeds are significant yet subservient.

With this shift in the paradigm, the research community in South Africa had to begin anew, considering that the South African cultivars are different from those used in other countries. As cladodes have never been regarded as human food in South Africa, no former knowledge on the subject existed. The lack of knowledge concerning using mucilage from local cultivars for human food consumption, became the basis for this study, as the success and feasibility of commercialising would depend on selecting the best cultivars for the highest quality and optimal yield of mucilage.

This investigation was the most comprehensive study ever performed of mucilage characteristics and properties of the 42 cactus pear cultivars that occur in South Africa.

An effective, easy and inexpensive method of mucilage extraction was developed in this study. No chemicals were used and as such, the extracted mucilage is natural and unadulterated by chemicals. In establishing the extraction process, many possibilities for research into the nature of mucilage and the application in food products were opened up.

It was observed that cultivars were not equal in terms of mucilage yield. In order to determine the influence of the cladode and mucilage attributes on the extractability of mucilage for optimal yield, correlations were performed on the data. There was a weak relationship between mucilage yield and cladode weight (r= -0.06), this indicated that cultivars that inherently produce heavier cladodes would not necessarily be the cultivars that would be selected for higher percentage mucilage yield. A moderate positive correlation was indicated between mucilage yield and cladode moisture content (r= 0.55) while a weak positive relationship was indicated between mucilage viscosity and cladode moisture content (r= 0.33). Higher moisture content of a specific cladode would therefore not strongly suggest higher mucilage yield or lower viscosity. A stronger correlation was found between mucilage yield and mucilage viscosity (r= 0.7). Thus, cultivars that inherently contain low viscosity mucilage would contribute the highest yield of mucilage. As high mucilage yields were paramount in the selection process, eight cultivars with high mucilage yields and low viscosity mucilage were selected for further investigation. The eight identified cultivars were Turpin, Gymno-Carpo, Algerian, Ficus-Indica, Morado, Tormentosa, Meyers and Robusta.

The influence of the climate such as differences rainfall between seasons and temperatures as well as the growth stage of the cladodes affect mucilage yield and viscosity. More than one season's data, preferable three seasons would be required to investigate the influence of climate changes on mucilage quality and yield. In the data available, it was seen that differences between cladode size and weight, pH and moisture content did not offer substantial advantages for either of the growth stages that sampling took place. However, the mucilage yield of cladodes sampled during the post-harvest stage was significantly higher than from cladodes harvested in the dormant stage, but after drying, the mucilage yields were equal. Though, the ability of dormant stage freeze-dried mucilage (FM) to influence the viscosity of solutions was superior. Therefore, the dormant stage was identified as the best time to harvest cladodes and extract mucilage for freeze-drying purposes.

Analysis of the viscosity and flow behaviour of eight cultivars showed that mucilage extracted from different cultivars intrinsically had diverse viscosities, therefore selecting the appropriate cultivar for a specific purpose and product would be paramount. Certain cultivars would consistently have low viscosity mucilage (such as Ficus-Indica), while other cultivars consistently had high viscosity mucilage (such as Robusta). Mucilage (NM and FM) from all cultivars showed non-Newtonian, pseudoplastic tendencies. Higher viscosity mucilage was determined to be time dependent, rheopectic and had yield stress tendencies. It was found that fluctuations in temperature, pH, concentration and electrolytes influenced the mucilage viscosity. These tendencies would have to be considered when mucilage is applied in food products, as it could have an adverse effect on the textural properties.

Further studies on the freeze-dried powder were continued only on the selected four cultivars that offered the best results. The selected cultivars were Algerian (high yields, low viscosity), Morado (high viscosity and yield), Gymno-Carpo (medium-high viscosity and good yield) and Robusta (high viscosity and different species).

The cultivar and time of harvest had to be considered for optimal mucilage utilization, thus the functional and chemical properties were determined for six months from February to August (except March). From the results obtained during the functional properties' investigation, 'Algerian' mucilage was recommended for optimal yield, while 'Robusta' mucilage was included for its high oil absorption capacity and emulsifying capabilities, however lower mucilage yields were obtained from Robusta. Overall though, none of the cultivars or months emerged as producing exceedingly better functional properties or superior quality native or freeze-dried mucilage. No specific month could be identified as the optimal harvest time, yet February powdered mucilage had lower viscosity, lower pH and higher conductivity amongst the months. The final selection had to rely on the outcome of the chemical analysis.

For cultivars, Robusta had the most beneficial chemical properties, while Algerian was rated as second and Gymno-Carpo as third best. 'Robusta' had the highest contents of protein, total fats (beneficial fatty acids), starch, potassium and phosphorous. 'Algerian' mucilage had the lowest energy contribution and was the purest (the lowest insoluble fibre content). In addition, 'Algerian' had the highest calcium, iron and copper contents. 'Gymno-Carpo' mucilage had high linoleic acid content, PUFA and good fat ratio, high magnesium and manganese and had the best antioxidant profile. 'Morado' had high MUFA and zinc contents.

In terms of selecting the optimal month for harvesting cladodes for the production of mucilage, February had the best results, after which August was the second best month. February mucilage had the highest mineral content and the highest organic acids (lowest pH). August mucilage had the highest energy content and higher beneficial fatty acids. It was thus found that the months after the fruit harvest (February) produced better quality mucilage.

An important connection between CAM and mucilage quality, viscosity and yield was made in this study. The abundance of organic acids caused by the increased effect of CAM in

February month, resulted in lower viscosity mucilage, that was easier to extract (higher yields), furthermore it was higher quality (more nutrients) mucilage.

Cladodes should be harvested in the summer months, directly after the fruit had been harvested for optimal mucilage yield and quality. 'Robusta' emerged as the best cultivar for higher viscosity mucilage and 'Algerian' for lower viscosity mucilage of the best quality.

Though, it cannot be claimed that the selected cultivar or harvesting had exceedingly superior quality freeze-dried mucilage. The final selection would have to rely on careful consideration of the rheological, functional and chemical requirements of the specific applied food products.

The chemical analysis of freeze-dried mucilage powder showed that it would considerably increase the soluble fibre, mineral and antioxidant content of any food product that it forms part of, since it would extend the nutritional benefits beyond the normal nourishment that the product already offers. It would contribute virtually no fat and carbohydrates, thus very little energy. Innovative ways should be explored to include mucilage in food products that will promote health and well-being by virtue of its protective and preventative properties.

Mucilage in its native as well as freeze-dried forms were successfully incorporated in different food products due to the emulsifying, fat-replacing and water-holding capacities. Mucilage was most effective in fat-containing emulsified products as the characteristic mucilage taste was masked in the products. Therefore, the most successful products were polony and mayonnaise. The products that had the least success were ice-cream and sorbet; it was attained that mucilage prevented the crystallization of ice crystals. Freeze-dried mucilage was successfully added to yoghurt after pasteurization and fermentation thereby increasing the antioxidant and mineral contents.

Therefore, it was found in this study that mucilage had strong potential for commercialization. Although, it is recommended that further tests be done in order to find optimum mucilage replacement and addition levels in food products, furthermore the careful selection of the most appropriate cultivar mucilage for the application would be paramount.

9.1 Recommendations for further study

The extraction, characterization and application of pectins from cactus pear cladodes for the purpose of developing another commercial product should be done. The solid waste obtained after the mucilage extraction process could find application in such a product.

Once the nopalito orchards have been established at the University of the Free State, research on the optimal cultivar for harvesting nopalitos should commence. Flour and mucilage produced from the young cladodes could be investigated in order to compare the quality of the obtained products with the results obtained in this study.

Changes over months in the functional and chemical contents in literature were often attributed to cladode maturity while changes could in fact rather be attributed to seasonal changes. Therefore, longer term studies on the composition of cladodes are necessary to determine the true cause of content fluctuations.

In this study, the post-harvest stage mucilage was eliminated in preference to the higher viscosity mucilage obtained during the dormant stage, yet a systematic year-round investigation for optimal mucilage yield and quality should be conducted on the cultivars selected in this study.

A financial analysis in terms of the cost of production, cladode yield and mucilage yield of the different cultivars would be necessary to select the most cost effective options for the commercialization of mucilage.

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