INFLUENCE OF THE PHYSICO-CHEMICAL CHARACTERISTICS OF NOPALITOS ON ITS EATING QUALITY

By

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Declaration

I declare that the dissertation hereby handed in for the qualification of M.Sc. Consumer Science at the University of the Free State, is my own independent work and that I have not previously submitted the same work for a qualification at/in another university/faculty.

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Abbreviations and acronyms

°C	Degree Celsius
°Brix	Degree Brix
%	Percentage
a*	Red/ green axis
ANOVA	Analysis of variance
am	Ante meridiem
Avg	Average
b*	Yellow/ green axis
C*	Chroma
CAM	Crassulacean acid metabolism
cm	Centimetre (s)
cm^2	Square centimetre (s)
cm^3	Cubic centimetre (s)
CO_2	Carbon dioxide
cv.	Cultivars
d	Diameter
FAO	Food and Agriculture Organization
Feb	February
g	Gram (s)
h^0	Hue angle
ha	hectare
HIV	Human immunodeficiency virus

IDF	Insoluble dietary fiber
Kg	kilogram (s)
L*	Lightness
L	Length
mg	Milligram (s)
Min	Minutes
ml	Millilitre (s)
mm	Millimetre (s)
mV	Millivolt (s)
NaOH	Sodium hydroxide
NCSS	Number Cruncher Statistical Systems
O	*Opuntia*
Oct	October
PAR	Photosynthetically Active Radiation
PCA	Principle component analysis
PEP	Phosphoenolpyruvate
pH	Potential Hydrogen
s	second
SAWS	South African Weather Services
SDF	Soluble dietary fiber
sp.	Specie
spp.	Species
TDF	Total dietary fiber

TSS	Total Soluble Solids
w	Width
WRZ	winter-rainfall zone

Abstract

More than 820 million people in the world are suffering from hunger and 1.3 billion from food insecurity. People experiencing food security have sufficient access to safe, sufficient and nutritious food in both quality and quantity. In Africa, food insecurity is on the rise because of population increase, global warming and desertification. There is a need for drought-resilient crops which will be less impacted by climate change as only climate-smart food systems can lead to food security.

The cactus pear is capable of providing sustainable food production in arid and semi-arid areas. Cactus pears (*Opuntia ficus-indica*) produces young and edible cladodes known as nopalitos. Nopalitos are a source of sustainable, economic and nutritious food and could be an important food source to livestock and human alike. In Mexico, nopalitos are a traditional food, eaten fresh or cooked in dishes enjoyed through many generations.

The aim of the study was to identify the most ideal size, cultivar and harvesting season for optimal eating qualities. The characteristics of nopalitos from the two cultivars (Fusicaulis and Morado) were compared to be able to select the most ideal eating cultivar. Nopalitos were also compared to different well-known vegetables to describe the texture, taste and juiciness. Consumers are reluctant to try unknown food, therefore, a description of how nopalitos taste was attempted in this study. The ideal post-harvest handling, the preferred cooking methods and the optimal cooking times to use in cooking instructions and recipes for South African consumers were determined in this study.

Physicochemical characteristics (length, weight, width, diameter, surface area, volume, colour, firmness, compressibility, TA, pH, TSS, mucilage, moisture and viscosity) of Fusicaulis and Morado nopalitos were determined and compared between size (9 cm, 12 cm, 15 cm, 18 cm, 21 cm and 24 cm), two seasons (autumn and spring) and over two years (2017 & 2018). The nopalitos were compared to different vegetables (baby marrow, carrot, celery, cucumber, green beans, green pepper, onion and tomato) in order to observe the differences and similarities of nopalitos compared to other popular vegetables. The influence of cooking methods and different time increments on the texture of nopalitos and green peppers was compared.

The important differences between the characteristics of the two cultivars were influenced by size and season as opposed to cultivar and year. The optimal size and cultivar were observed between 15 cm and 18 cm of Morado nopalitos. There were similarities observed between nopalitos and vegetables in the turgidity, texture and taste. The taste of nopalitos could be described as being more sour than tomatoes, and as having comparable sugar content compared to celery, green pepper and green beans. Nopalitos can be cooked using the following methods roasting, boiling and shallow frying over a shorter cooking time.

Both cultivars of *Opuntia ficus-indica* were observed to be suitable for use as fresh vegetable source between the size of 15 cm and 18 cm as they were thinner, brighter green, softer and less slimy. It is ideal to harvest nopalitos over the autumn season because nopalitos had highest quality attributes. Nopalitos can be prepared similarly to most vegetables using the simple cooking methods (roasting, boiling and shallow frying) for a limited cooking period.

Nopalitos have potential as a source of fresh vegetables in South Africa because of similarities in turgidity, texture and taste to popular vegetables. Cactus pear plants have been thriving in the world for decades and could contribute to decreasing the number of people suffering from food insecurity.

Key terms

Food security, food systems, arid and semi-arid plants, *Opuntia ficus-indica*, nopalitos, Fusicaulis and Morado cultivars, cactus pear, food source, cooking methods, fresh vegetable.

Chapter 1

1.1 Introduction

The Food and Agriculture Organization of the United Nations describe food security as daily access to sufficient food by individuals. By implication, food security is only achieved when affordable, safe and nutritious food products are widely available to all (FAO, 2010; Hart, 2009). South Africa is considered to be food secure at national level however, significant numbers of households in rural areas are food insecure (De Cock *et al*., 2013; Altman *et al*., 2009). Increases in prices of maize and wheat, which are the staple foods in South Africa, are the leading cause of food insecurity in South African households (Altman *et al*., 2009). As a result, the government of South Africa is working towards fighting food insecurity by providing support programmes such as agricultural opportunities to South Africans. In which individuals acquire skills and assistance to produce food to meet their basic food needs (Baiphethi & Jacobs, 2009).

Climate change has an impact on agricultural production, which affects food security (Kusangaya *et al*., 2014; Bryan *et al*., 2009). It is predicted that South Africa will become a desert due to global warming (Potgieter, 2007). Bryan *et al*. (2009) proposed adaptation strategies for crops to acclimate to climate change, such as the use of more drought-resilient crops, and changing planting and irrigation schedules.

Cactus pears have been consumed by humans for over 9000 years (Nobel, 2002). In Mexico, nopalitos have been considered a traditional Mexican vegetable for many years. Nopalitos are consumed fresh, cooked or processed. Different recipes are available to prepare delicious and nutritious meals and side dishes (Silos-Espino *et al*., 2003; Sáenz, 2000; Rodriguez-Felix & Cantwell, 1988).

The cactus pear plant can grow with low water availability; it does not require water for irrigation and grow under undesirable conditions and prevent soil erosion (Patel, 2013). Therefore, this plant can be helpful for sustenance in South Africa, since the availability of water is becoming a challenge as a result of the lack of rainfall (Bryan *et al*., 2009).

Cactus pear cladodes (nopalitos) should be brought to the attention of South Africans as an ideal food source (Du Toit *et al*., 2015) since it has only been used for animal feed (Betancourt-Dominguez *et al*., 2006; Middleton & Beinart, 2005).

The cactus pear plant has potential to improve the security status in poor South African households, due to its adaptability to hot and dry climates (Patel, 2013; Fouché, 2010; Betancourt-Domínguez *et al*., 2006; Stintzing & Carle, 2005). The cactus pear is capable of providing sustainable and valuable production in arid and semi-arid areas (Inglese *et al*., 1995). The whole cactus pear plant is edible, including the cladodes (nopalitos) and the fruit (Fouché, 2010; López-Palacios *et al*., 2010). In fact, cactus pear plants have been thriving in South Africa for many years. Commercial farms find markets for fruit locally and export fruit to Europe. The cladodes are processed and sold to livestock farmers for fodder (De Waal *et al*., 2015; Potgieter & Smith, 2006). Furthermore, the cladodes of the cactus pear serve many purposes, which includes the manufacturing of therapeutic and cosmetic products (Santos Díaz *et al*., 2017; Feugang *et al*., 2006; Stintzing & Carle, 2005; Nobel, 2002; Sáenz, 2000). This ideal food source could benefit agriculture and the economy of the country (Kalegowda *et al*., 2015; Patel, 2013). It will improve the nutrient intake of consumers from rural and urban areas (Patel, 2013; Feugang *et al*., 2006).

South African food and consumer scientists need to develop recipes in order to introduce this food source to South African consumers.

1.2 Objectives

The objective of this study was to develop nopalitos as a food source in South Africa. Consumers who are familiar with nopalitos in their daily diet use visual quality measures, to determine a good quality nopalito (López-Palacios *et al*., 2010). Hence, the nopalito must be young, bright green, thin, soft, not slimy and must have a fresh appearance (Ramírez-Moreno *et al*., 2013; Calvo-Arriaga *et al*., 2010; Corrales-García *et al*., 2004; Sáenz-Hernández *et al*., 2002; Rodriguez-Felix & Cantwell, 1988).

Two *Opuntia ficus-indica* cultivars were studied. Fusicaulis was included in the study because it proved to be one of the cultivars with high production of cladodes planted in South Africa (De Kock, 2001). Morado was included because it is the most commercially planted cultivar for fruit production in South Africa (Jansen, 2018; Coleman, 2013). Therefore, the physico-chemical characteristics of the two cultivars were observed.

1.3 The aims of this study were as follows:

1. To conduct a thorough study on all available literature on the background of cactus pear plants from different countries, the planting system, CAM pathway, physical characteristics of matured cladodes, chemical composition, including a study on the therapeutic application, characteristics of nopalitos, nopalitos as human food, pre-cooking preparation of nopalitos, processing of nopalitos and shelf life of nopalitos.
2. To identify the most ideal size, cultivar and harvesting season for optimal eating qualities. Two years of harvest was included in the study to determine if quality would vary when harvested during different years. The eating quality characteristics identified by consumers were translated to measurable attributes as follows:

Quality characteristic	Measurable attributes
Young and thin	Weight, length, width, diameter at different sizes, 9, 12, 15, 18 and 24 cm.
Bright green	Colour: L*, a*, b*, C* and h°
Soft	Texture: ability to penetrate and compress the nopalitos
Fresh	Turgidity: moisture content
Not slimy	Mucilage yield and viscosity
Taste	Gustatory properties: titratable acidity, pH and TSS

3. To compare the characteristics of nopalitos from the two cultivars (Fusicaulis and Morado) with those of different well-known vegetables in order to describe the texture, taste and juiciness of nopalitos. Consumers are reluctant to try unknown food, therefore, a description of how nopalitos taste was attempted in this study.
4. To determine the ideal post-harvest handling, the preferred cooking methods and the optimal cooking times to use in cooking instructions and recipes for South African consumers.

Chapter 2

2 Literature review

2.1 Introduction

Studies show that areas which already experience long and dry summer seasons will be affected severely by global warming in the future. In many countries, particularly North and South Africa, Middle East, Central and West Asia, it is estimated that the availability of water will reach levels which are below threshold (Rijsberman, 2006). Moreover, an increased population will require more water and food to survive. The world's population is expected to increase to approximately 9.7 billion by the year 2050 and could reach 11 billion by the year 2100 (United Nations, 2019). Hence, it is important to introduce new food production alternatives with high potential to sustain in arid and semi-arid areas (García-Hernández *et al*., 2006).

Opuntia ficus-indica, better known as the cactus pear, is a plant that grows under favourable or unfavourable climatic conditions. The plant grows in arid and semi-arid areas as it easily adapts to the temperature of the region (Chalak *et al*., 2014; Patel, 2013; Betancourt-Domínguez *et al*., 2006; Stintzing & Carle, 2005; Nobel, 2002). It grows in environments such as deserts, below sea level, high altitude regions, tropical regions with temperatures above 50 °C and regions where temperatures are as low as -40 °C (Yahia & Sáenz, 2011). The cactus pear plant could be an alternative to popular crops due to the plant's uniqueness as a drought-tolerant plant with a root system that is able to absorb the smallest amount of water in areas with low rainfall (Rodrigues *et al*., 2016; Snyman, 2005). It thrives in areas where the soil conditions are too poor for other plants to grow. Indeed, the plant has developed and was able to withstand many climatic conditions in most regions such as North, Central and South America, in the Mediterranean, North, Central, and South Africa, in the Middle East, Australia, and India without causing damage to the cladodes or fruit (Gheribi & Khwaldia, 2019; Ochoa & Barbera, 2017; Stintzing & Carle, 2005).

The structure of the cactus pear plant consists of flat-stemmed spiny cladodes, edible fruit and roots. The typical roots of cactus pear plants are shallow and grow horizontally in order to absorb water from light rain. The root system has a high capacity for water storage and is able to take up water at very low soil-water content (Snyman, 2014). The cactus pear plant has a jointed succulent pseudostem, succulent plants contain large cells for water storage (Griffiths & Males, 2017). The modified stems (cladodes) allow a huge amount of water to be stored in the parenchyma which is the inner part of cladode (Prat *et al*., 2017; Feugang *et al*., 2006). The inner part of the cladode is specifically known as the white medullar parenchyma, which is responsible for the storage of water that causes the increased thickness in diameter of cladodes (Feugang *et al*., 2006). Water is stored in the epidermis (single layer of cells on he outer chlorenchyma), also in the thick, waterproof cuticle (Pasiecznik, 2019). The cactus pear cladode contains a hydrophilic mucus-like compound known as mucilage that has water-holding capacity which allows the plant to thrive in unfavourable climatic conditions (Gheribi & Khwaldia, 2019; Du Toit *et al*., 2019; Monrroy *et al*., 2017; Tegegne, 2002; Cárdenas *et al*., 1998).

The cactus pear plant has been known for the production of fruit which is referred to as prickly pear, tuna, baraberry, nopal fruit, Indian fig, cactus apple and xoconostle in different parts of the world (Patel, 2013). More specifically, cactus pear fruit is referred to in Spain as *higo chumbo,* in Italy as *fico d'indica*, in France as *figue de Barbarie*, in Mexico as *tuna*, in the United States and South Africa as prickly pear, in Israel as *teen shawki*, in India as *nagphani*, in Colombia as *higo* and in Brazil as *palma forrageira* (Yahia & Sáenz, 2011). The fruit is found in an oval and elongated shape with colours that vary from white, green, yellow, orange, red, purple to brown (Feugang *et al*., 2006; Piga, 2004). It weighs from 67 g to 216 g depending on factors such as the place of origin, cultivar and soil conditions (Du Toit, 2013; Shongwe *et al*., 2013; Yahia & Sáenz, 2011; Piga, 2004). The whole fruit contains nutrients which are similar to some fruits, for instance, the fruit has 50 calories per 100 g, similar to pears, apricots and oranges (Feugang *et al*., 2006; Sáenz, 2000). The fruit has a high sugar content that ranges from 10 °Brix to 17 °Brix which is mostly glucose and fructose giving sweetness to the fruit (Du Toit, 2013; Yahia & Sáenz, 2011; De Wit *et al*., 2010; Piga, 2004). It is characterized by low acidity (0.05 % to 0.18 %) and high pH that ranges from 5.3 to 7.1. Moreover, it contains antioxidants such as ascorbic acid, phenolic and betalain compounds which are associated with the nutritional and health benefits of the cactus pear fruit. The antioxidant content of cactus pear fruit is similar to that of red grape, orange and grapefruit and is higher than that of pear, apple, tomato and grape (Du Toit *et al*., 2018b; Yahia & Sáenz, 2011). The whole fruit can be stored from 3-4 weeks due to its short shelf life and peeled fruit can be stored for eight days under controlled conditions (Feugang *et al*., 2006).

2.2 Background of cactus pear from different countries

Cactus pear plants have been produced in countries such as Mexico, Argentina, Bolivia, Brazil, Peru, Chile, Algeria, South Africa, Morocco, Australia and Italy for multiple purposes (Ochoa & Barbera, 2017 and Stintzing & Carle, 2005). Some of the species used for high-quality fruit production are *Opuntia ficus-indica*, *Opuntia hyptiacantha*, *Opuntia megacantha* and *Opuntia streptacantha*. *Opuntia robusta*, *Opuntia leucotricha* and *Opuntia ficus-indica* are some of the species used for nopalito production and mature cladodes as forage for animal feed (Sáenz, 2013). The biggest *Opuntia ficus-indica* producer in the world is Mexico, followed by Italy, South Africa and Chile. Other countries follow namely Brazil, Argentina, America, Peru Columbia, Jordan, Egypt, Tunisia, Algeria, Morocco, Israel, Turkey, Spain and Greece (Akça *et al*., 2017). *Opuntia ficus-indica* has been used for multiple purposes namely food and energy production, cattle feed, medicinal, environmental and colourants and ornamental uses (Table 2.1).

Table 2.1: The current and prospective uses of *Opuntia ficus-indica* (Pasiecznik, 2019; Bauman & Schmidt 2015; Inglese *et al*., 1995)

Commercial area	Specific use
Food production	Emergency (famine) food, fruits, nopalitos, fruit juice, oil extracted from seeds, miel de tuna (prickly pear honey), queso de tuna (prickly pear candy), jellies and marmalades
Energy production	Alcohol and fresh biomass
Cattle feeding	Forage, fodder, fruit wastes and invertebrate food for dye-containing insects
Medical uses	Tea made of flowers for diuretic purposes, cladodes for diabetes, mucilage for wound healing, treatment of digestive problems, mashed cladodes for relief of heat and inflammation and management of human immunodeficiency virus (HIV)
Environmental uses	Agroforestry, soil fixation, soil mulching, soil water supply, hedges, shade or shelter, wind breaks and drinking water purification
Colourant uses	Betanins in fruits and carminic acid (red cochineal insect dye)
Ornamental	Christmas tree, cut flowers, garden plant, potted plant and seed trade

2.2.1 Mexico

Mexico has a large production area of about 3 million ha that produces cactus pear species such as *O. ficus-indica*, *O. robusta*, *O. streptacantha*, *O. joconoxtle*, *O. megacantha*, *O. streptocantha*, *O. leucotricha*, *O. hyptiacantha* and *O. chavena* and specifically *Opuntia ficus-indica* for nopalito production. The production area around the town of Milpa Alta is known for the production of high quality and quantity nopalitos (Ochoa & Barbera, 2017). In the 1970s and early 1980s, the cactus pear plant was recommended as an alternative crop to replace dry beans and corn in drought stricken areas such as was experienced by farmers from the Milpa Alta region (Ochoa & Barbera, 2017). Since then, it has played a role in the agricultural economy of Mexico as the production of nopalitos is similarly as important to the economy as the production of corn and the agave plant (Graffith, 2004). The cactus pear plant has been rated in the sixth place based on volume compared to vegetables produced nationally in Mexico, after melon, onion, chilli pepper and red tomato and in the eighth place based on value, after red tomato, green pepper, potato, onion, green tomato, squash and asparagus (Berger *et al*., 2013; Yahia & Guevara Arauza, 2002).

2.2.2 South America

In Argentina, the cactus pear has been studied intensively by research institutions to promote household and small-scale industrial processing of cactus pear for the benefit of their nutrition, health and businesses (Ochoa & Barbera, 2017; Sáenz, 2013). *Opuntia ficus-indica* was first introduced during the extremely hot and dry summer seasons in Argentina, mainly as animal feed. In Brazil, matured cladodes are produced in large numbers for the use of fodder (fresh and dry mass). It has been thriving and known for its multiple uses in areas of Bolivia where rainfall can be 350-640 mm per year, which is similar to precipitation in Bloemfontein (South Africa). In Chile, cactus pear has been mainly developed for fruit production, however due to research over the years there is a growing interest in using nopalitos in the Chilean diet. In Peru, a thriving cactus pear industry developed for commercial production of carminic acid (red colourant made from dried cochineal insects) and fruit (Ochoa & Barbera, 2017).

2.2.3 North Africa

In Morocco, the cactus pear production area has increased from 50 000 ha to 120 000 ha due to drought control programmes established through government initiatives (Ochoa & Barbera, 2017). The cactus pears are classified into three groups based on the characteristics of the cladodes. The Christians' nopal, which is used as field fences, while Muslims' nopal with inermis cladodes, are used for green fodder. Moses' nopal with large cladodes is known for their production of bigger fruit (Bakour *et al.*, 2017; El-Mostafa *et al.*, 2014; Nuňez-López *et al.*, 2013). Algeria is renowned for having a cactus pear processing unit that is responsible for the packaging of cactus pear fruit and the production of essential oils, pharmaceuticals, juice, jam and livestock feed (Ochoa & Barbera, 2017).

2.2.4 South Africa

The spiny cactus pears were brought to South Africa in the seventeenth century (Middleton & Beinart, 2005). Around the nineteenth and early twentieth century, the cactus pears began to spread in the drier areas of Eastern Cape Province (Middleton & Beinart, 2005; Beinart & Wotshela, 2003). In the early twentieth century, the farmers identified the different species as "doornblad", "kaalblad" and "rondeblaar": *Opuntia ficus-indica*, which was called doornblad had long and narrow leaves, with many long white thorns. Rondeblaar had round leaves, with brown and short hair-like thorns. Rondeblaar referred to cladodes that were thick, round and had brown and short hair-like thorns (Beinart & Wotshela, 2003). In 1914, South Africa received twenty-two spineless Burbank *Opuntia ficus-indica* and *O. robusta* cultivars that were brought from California. This collection of cultivars are only found in South Africa because of its intolerance to extreme cold weather conditions (Du Toit *et al*., 2018a). These cultivars were first cultivated at Grootfontein Research Institution, Middelburg, Eastern Cape. The commercial farmers in the Middleburg district identified the importance of the plant for its multi-purposes as a fruit, as fodder and as hedging plant in the rural areas (Ochoa & Barbera, 2017; Middleton & Beinart, 2005; Beinart & Wotshela, 2003). There are more than 70 cultivars in South Africa, only a small number of cultivars are suitable for commercial fruit production (Potgieter & Mashope, 2009; Potgieter & Smith, 2006).

Nowadays, there are 42 cultivars of *Opuntia ficus indica* that are conserved in germplasm banks of Limpopo, Free State, Eastern Cape and Western Cape for research purposes. Interestingly, the research is not only based on the use of cactus pear fruit and the cladodes as fodder, but it also includes the production of the young cactus pear cladodes for human consumption (Ochoa & Barbera, 2017; De Wit *et al*., 2010). In addition, the main production areas in South Africa are in the summer rainfall areas where unfavourable climatic conditions such as long droughts are prevalent (Oelofse *et al*., 2006). However, in the winter rainfall regions of the South Western Cape, cactus pears are also successfully cultivated (Brutsh, 1997).

2.3 Cladodes

2.3.1 Planting system

The *Opuntia* plants can be cultivated in a variety of environments, however, for better quality production the following should be taken into consideration: the temperature and the availability of water in the region, the day or night length as well as soil characteristics (Liguori & Inglese, 2015). Before planting, the fields are ploughed for the purpose of having good drainage. The drainage is improved by cross ripping the field with a chisel, in caution to avoid altering the soil profile. The orientation of the rows should be north-south for Photosynthetically Active Radiation (PAR) to take place. The rows should be filled with cow manure and planted in an upright position with the cut end in the ground. Also, half of the cladode should be exposed outside. Pre-planting handling of cladodes includes using bordeaux paste and 0.4 ml methridathion to disinfect cuttings. Single and multiple cuttings are used depending on whether it is a one or two year-old cladode. The cladode cuttings determines where the cladodes will be planted in the orchard, in terms of the orientation of the rows. On the other hand, multiple cladode cuttings has an impact on early fruiting due to the rapid formation of plant structure, but this method may require a large amount of planting material (Liguori & Inglese, 2015).

The planting system of cladodes could affect the growth and production of nopalitos. There are few crucial aspects to consider when planting for the purpose of harvesting nopalitos. This includes aspects such as size of the cladode, the position of cladode, exposure of cladode to the sun, and the depth of planting. Bakali *et al*. (2016), concluded that horizontal side plantation would be a standard method for plantation appropriate for arid areas because it showed great potential for growth and production. Stambouli-Essassi *et al*. (2015), took another approach to observe the development of roots for vegetative multiplication by observing varying cladode cutting sizes. In the study, the whole cladode, cut in half, quarter and tenth cuttings were planted for observations. The root developed for all, but the results of cladodes cut in tenths showed more interest compared to the whole, quarter and half ones, implying that a reduced size of cladode cutting does not reduce its rhizogenesis and caulogenesis potentialities. Singh & Singh (2003) reported on planting methods of cladodes and concluded that in order to obtain a high rate of plant growth and dry weight including a large number of cladodes, one has to apply the upright planting system of 1 year-old full-size cladodes with a width of 8-15 cm.

Coleman (2013) reported on a farmer in the Bloemfontein district that plants cactus pear on his farm (Waterkloof), where average annual rainfall of about 480 mm with low temperatures (-6 °C) in winter and as high as 38 °C in summer can be expected. The planting of cladodes begins in the rainy season. However, cladodes can be planted in dry condition, where irrigation is supplied so that cladodes could thrive until the first rains. In the first year of planting, no fertilizers are used. Only a small amount of about 200 kg per hectare of a balanced fertilizer can be used in mother plants to promote maximum yield, a fertilizer of 10-10-10 that consists of 10 % nitrogen, phosphorus and potassium while, 90 % contains secondary, trace nutrients and fillers (Hemmer, 2019). When planting, the cladodes are placed with one-third underground, and the plant density can be about 800 to 1000 plants per hectare. The cladode yield is 50t/ha fresh weight and between 150 to 200 cladodes from the mother plant each year (Coleman, 2013).

2.3.2 CAM pathway

The cactus pear plant produces cladodes that are essential and responsible for its photosynthetic process (Feugang *et al*., 2006). During the night, CO_2 is taken up through the open stomata. The plant opens the stomata at night in a distinctive way for carbon dioxide fixation ensuring that there would be limited water loss, low temperatures and high humidity (Inglese *et al*., 2017; Larcher, 2001; Callaway, 1995; Salisbury & Ross, 1992). Crassulacean Acid Metabolism (CAM) allows the plant to continually thrive in deserts (Salisbury & Ross, 1992). The carbon dioxide fixation by CAM pathway allows the plant to be able to endure drought (Jaramillo-Flores *et al*., 2003). Carbon dioxide is fixed by the plant as malic acid. During the night when the stomata open, CO_2 disperses into the mesophyll's intracellular spaces and into the cytosol, where it is bound to phosphoenolpyruvate (PEP) through PEP carboxylase and where oxaloacetate is formed and transformed into malate. The malate is later transported from the cytosol to the vacuole and converted into malic acid. Oxygen is released to prevent water loss through transpiration. The guard cells and stomata close during the day; malic acid is decarboxylated releasing carbon dioxide which is converted into glucose through photosynthesis. Thus, CAM also causes the acid content of the cladodes to change throughout the day (Inglese *et al*., 2017; Feugang *et al*., 2006; Jaramillo-Flores *et al*., 2003; Salisbury & Ross, 1992). CAM is more effective in sunny summer weather than cold winter weather; thus the cactus pear plant seems to thrive during summer seasons (Du Toit *et al*., 2018a).

2.3.3 Physical characteristics of matured cladodes

The shape of cladodes can be described as oval or elongated, with cladodes that can grow up to 60-70 cm long depending on the availability of water and nutrients (Sáenz, 2013). Specifically, the shape of *Opuntia ficus-indica* cladodes can be described as bushy-type, columnar shape with elongated cladodes and round-cladode shape (Wessel, 1989). De Wit *et* al. (2017) studied cladodes of forty-two spineless cactus pear cultivars in South Africa; the weight ranged between 396.5 g and 924.5 g and the length was 29.17 cm to 40.85 cm. Its width ranged between 14.80 cm and 25.52, and the diameter was 0.67 cm to 1.65 cm (De Wit *et al*., 2017).

2.3.4 Chemical composition

The chemical composition of cladodes depends on the growth stages and species. The water content of cladodes increases as the cladodes develop (Rodriguez-Felix & Cantwell, 1988). The protein content is low (9 to 16 % dry weight) similar to other fresh vegetables. The lipid content remains consistently low (1.3 to 3 %) throughout the cladode development. There is an increase in the ash content, carotene content and chlorophyll content as the cladode develop (Rodriguez-Felix & Cantwell, 1988). Cladodes may contain β-carotene and lutein in levels higher than that of other vegetables (Hernández-Perez *et al*., 2009). The carotenoids ratio in nopalitos is β-carotene (36 %), lutein (46 %) and α-cryptoxanthin (18 %). When nopalitos, or any vegetables, are thermally treated with higher temperatures, it may result in higher extractability of carotenes leading to higher antioxidant activity compared to fresh produce (Jaramillo-Flores *et al*., 2003). The cladodes have high contents of fiber, pectin, lignin, mucilage, cellulose and hemicellulose, which are beneficial for human wellbeing. The mucilage (cladode juices) contains flavonoids and phenolic acid which provide the cactus mucilage with antioxidants (De Santiago *et al*., 2018).

The chemical composition of spiny and spineless cladodes is illustrated in Table 2.2 and cladodes before and after cooking are illustrated in Table 3. The cladodes have high percentages of moisture in which spiny cladodes contain (90.67 %), while, spineless cladodes contain (91.04 %) moisture. The low water activity of 0.76, allows the cladodes to remain stable during storage as the amount of free water is limited for use by microorganisms and chemical reactions (Sáenz, 1997). Cladodes have a low pH content, with spiny cladodes at pH 4.02 and spineless cladodes at pH 3.84. Spiny cladodes (8.74 g/100) and spineless (8.88 g/100 g) contain a low protein content (Table 2.2). Sáenz (1997) reported that cladodes obtained from Mexico contained a total dietary fiber content of 42.99 g/100 g, with soluble dietary fiber of 14.54 g/100 g and 28.45 g/100 g being insoluble dietary fiber, with a ratio of soluble dietary fiber to insoluble dietary fiber of 1:2. A similar ratio was observed for the raw cladodes in Table 3. While Ayadi *et al.* (2009) reported a ratio of soluble dietary fiber to insoluble dietary fiber of 1:3 for Tunisian cladodes (Table 2.2), a similar ratio to that of fried cladodes shown in Table 2.3.

Table 2.2: Proximate composition of cladodes from *Opuntia ficus-indica* f. amyloceae (spiny cladodes) and *Opuntia ficus-indica* f. inermis (spineless cladodes) (Ayadi *et al*., 2009)

PARAMETERS	SPINY CLADODES	SPINELESS CLADODES
Moisture (%)	90.67	91.04
Water activity	0.762	0.767
pH	4.02	3.84
TA (% of malic acid)	0.724	0.652
Protein (g/100 g)	8.74	8.88
TDF (%)	51.24	41.83
IDF (%)	34.58	30.36
SDF (%)	12.98	8.78
Soluble sugars (g/100 g)	2.49	6.01

De Santiago *et al*. (2018) reported on the changes of moisture percentage of cladodes, as high heat is applied during cooking and with the addition of oil, an expected decrease in moisture between raw (93.7 %) and fried cladodes (79.7 %) was observed. However, the moisture percentage was still high. The protein content was low and the cladode maintained a low protein content even after cooking, only with a slight increase between raw (1.1 g/100 g) and fried cladodes (1.9 g/100 g). An increase of total fibre content was observed between raw (3.1 g/100 g) and fried cladodes (4.8 g/100 g) because the applied heat causes depolymerisation and rupture of some glycosidic linkages in fibre polysaccharides, hence the increase illustrated in Table 2.3. Heat applied during cooking could cause changes in chemical and nutritional attributes of foods such as water loss, increased protein and dietary fibre content (De Santiago *et al*., 2018).

Table 2.3: Chemical composition of cladodes before and after cooking (De Santiago *et al*., 2018)

Sample	Moisture (%)	Protein (g/100 g)	Total fibre (g/100 g)	Soluble fibre (g/100 g)	Insoluble fibre (g/100 g)
Raw	93.7	1.1	3.1	1.0	2.1
Boiled	93.8	1.4	3.3	1.3	1.8
Microwaved	90.8	1.9	4.4	1.7	2.7
Fried	79.7	1.9	4.8	1.6	3.2

2.3.5 Therapeutic application

Cactus pear plants have been reported and used as a medicinal plant to treat several human diseases (Corrales-García *et al*., 2004). Some plants contain polysaccharides associated with the healing of wounds, and these polysaccharide fractions have been identified in *Opuntia* *ficus-indica* cladodes. Galati *et al*. (2003) reported on the use of a treatment containing *Opuntia ficus-indica* lyophilized (freeze-dried) cladodes which produced positive results with tissue reconstruction. According to El-Mostafa *et al*. (2014), freeze-dried extracts of cladodes could be utilized for the prevention of cartilage alterations and treatment of joint disease. Cladode extracts could regulate cholesterol levels and could be used to treat cough. Patel (2013); Stintzing & Carle (2005) reported on managing Diabetes Mellitus by lowering glucose levels in the diet through the consumption of food containing cactus pear cladodes. Betancourt-Domínguez *et al*. (2006) stated that cladodes could lower glucose levels, control renal water and handle sodium levels of diabetes mellitus patients. The *Opuntia* plant may be an important part of prevention regiments for cardiovascular diseases, cancer and diabetes, as well as inhibiting inflammation and viral infections (Chahdoura *et al*., 2018).

2.4 Nopalitos

2.4.1 Characteristics of nopalitos

Mexicans refer to cladodes as nopales or pencas and to young cladodes between 3 to 4 weeks old as nopalitos (Feugang *et al*., 2006; Sáenz-Hernández *et al*., 2002). Nopalitos are fresh young cladodes which are used for human food and form part of the vegetable food group in the Mexican population (Bensadón *et al*., 2010; Betancourt-Dominguez *et al*., 2006; Feugang *et al*., 2006; Graffith, 2004; Saénz, 1997). Nopalitos have a soft, flat stem with spines that can be easily removed (López-Palacios *et al*., 2012; Corrales-García *et al*., 2006; Feugang *et al*., 2006). The weight of nopalitos may vary from 40-100 g with a length of 11-20 cm, depending on species. Likewise, the chemical composition of nopalitos depends on the variety of species, the stage of development and the time of harvest (Rodriguez-Felix & Cantwell, 1988). In Table 2.4, the chemical composition of nopalitos that are 20 cm in length are demonstrated and consist of 91.7 g water, 1.1 g protein, 0.2 g lipid, 1.3 g ash, 1.1 g crude fibre, 4.6 g complex carbohydrates and 0.82 g simple sugars. The antioxidant content was 12.7 mg ascorbic acid and 28.9 µg carotenes. Twenty centimetres were indicated as being the most popular size to harvest nopalitos and can be obtained at 20 to 30 days of development (Rodriguez-Felix, 2002).

Table 2.4: Chemical composition of nopalitos that are 20 cm in length (Rodriguez-Felix & Cantwell, 1988)

Parameters	Average
Water (g/ 100 g)	91.7
Protein (g/ 100 g)	1.1
Lipid (g/ 100 g)	0.2
Ash (g/ 100 g)	1.3
Crude fibre (g/ 100 g)	1.1
Complex carbohydrates (g/ 100 g)	4.6
Simple sugars (g/ 100 g)	0.82
Ascorbic acid (mg/ 100g)	12.7
Carotenes (µg %)	28.9

2.4.2 Nopalitos as human food

Mexico is well known for its high nopalito production, however, there are other countries such as Argentina, Brazil, Tunisia, Italy, Israel and China that similarly enjoy the taste, nutritional and health benefits of nopalitos (El-Mastafa *et al.*, 2014; Feugang *et al.*, 2006; Sáenz *et al.*, 2004). Despite nopalitos being less valuable compared to the fruit production worldwide, nopalitos are available in commercial markets (Graffith, 2004). Consumers familiar with nopalitos make selections of which nopalitos to purchase in the market by looking at the noticeable visual qualities such as colour and size (López-Palacios *et al.*, 2010). Most importantly, in countries where nopalitos are considered as a staple food, consumers prefer nopalitos that are bright green, thin, soft and with a fresh-looking appearance (Corrales-García *et al.*, 2004; Sáenz-Hernandez *et al.*, 2002). These consumers are attracted by the appearance of these nopalitos and associate the physical appearance of the nopalitos with good quality and good taste.

In Mexico, the nopalitos are known as green vegetables and prepared in a variety of ways. Some of the dishes prepared using nopalitos are discussed here:

- Boiling nopalito strips, onion and garlic in water for 8-10 minutes (Figure 2.1). The cooked nopalitos can be used in salads, scrambled eggs or Mexican stews (Martínez, 2019)

Figure 2.1: Basic nopalito recipe (http://mexicoinmykitchen.com/how-tocook-cactus-paddles/)

- Mixing nopalitos, tomatoes, red radishes and red onion with lime juice and olive oil to prepare a refreshing salad in Figure 2.2 (Bauer, 2019).

Figure 2.2: Nopalito and tomato salad (http://simplyrecipes.com/recipes/nopalito_cactus_salad/)

- Stir-fried nopalitos, onions and chillies, sprinkled with cheese (Martínez, 2019).
- Stir-fried, with green onions, nopalitos and eggs for breakfast in Figure 2.3 (Martínez, 2019).

Figure 2.3: Nopalitos with eggs (http://mexicoinmykitchen.com/how-tocook-cactus-paddles/)

2.4.3 Pre-cooking preparation of nopalitos

The preparation of nopalitos includes washing and blanching for the following reasons; to inactivate enzymes, inactivate micro-organisms, softening nopalitos and removing mucilage. Nopalitos can be blanched by soaking nopalitos in boiling water for 30 minutes, removed and shocked in cold water to prevent further cooking (García & Sáenz, 2013).

2.4.4 Processing of nopalitos

Minimal processing of fruits and vegetables includes partial preparation of products without interfering with the fresh-like quality of the product in order to provide the consumer with convenience. It includes processes like washing, sorting, trimming, peeling, slicing, chopping, antioxidant treatments and packaging (Bansal *et al*., 2015). Minimally processed nopalitos are produced to preserve their freshness and provide consumers with a convenient product (Sáenz-Hernández *et al*., 2002).

2.4.4.1 Jams, marmalades and cladode chutney

Nopalitos can be used in the production of jams and marmalades (Corrales-García, 2009; Sáenz-Hernández *et al*., 2002; Sáenz, 2000). In Mexico, the nopalitos are mixed with different fruits (pineapples, pear, fig, peach and cactus pear fruit) in a syrup to make a variety of jams (Figure 2.4). The mucilage naturally found in nopalitos is conveniently used to improve the consistency of jams of different fruits such as blueberries, raspberries, blackberries, strawberries, peach, apple, pear, pineapple, apricots and plums (Corrales-García, 2009).

Figure 2.4: Mexican nopal (nopalito) jam (http://www.laneta.com.au/store)

Marmalade is prepared by cooking minced nopalitos with sugar and pectin depicted in Figure 2.5 (Sáenz-Hernández *et al*., 2002). Chutney is made by liquidizing nopalitos into a pulp and added to a stainless-steel saucepan with the following ingredients: sugar, cayenne pepper, minced onion, salt, ginger (powder), mustard (powder), garlic (powder) and white grape vinegar. The mixture is allowed to slowly boil until it is as thick as the consistency of jam. It is poured into hot, sterilised jars, sealed and stored at room temperature (Du Toit *et al*., 2018b; Du Toit, 2013).

Figure 2.5: Marmalade (http://tmnopal.com.mx/english/nopal-marmalade/)

2.4.4.2 Brined and pickled products

Popular products consumed in Mexico are nopalitos in brine and pickled nopalitos. The preparation includes removing the thorns, washing the nopalitos, dicing or cutting into pieces, scalding or cooking and conditioning the nopalitos. To produce nopalitos in brine, the brine is made of salt and water and is mixed with sautéed nopalitos and bottled in a sterilised jar, allowed to cool, air-dried and sealed for storage shown in Figure 2.6 (Du Toit *et al*., 2018b; Du Toit, 2013; Sáenz-Hernández *et al*., 2002; Sáenz, 2000).

Figure 2.6: Nopalitos in brine (www.azteca.co.za/product/nopal-natural-en-salmuera/)

For pickled nopalitos, nopalitos are washed and cut into strips. The nopalito strips are soaked in brine for 20 hours. It is removed from the brine and rinsed in cold water to be transferred into sterilised jars. The jars with nopalito strips are filled with pickling mixture. The pickling mixture contains that contains vinegar, mustard seeds, allspice, whole cloves, aromatic herbs and olive oil. The jars are sealed and left for 2 months at room temperature to mature shown in Figure 2.7 (Du Toit, 2013; Sáenz-Hernández *et al*., 2002; Sáenz, 2000).

Figure 2.7: Pickled nopalitos

2.4.4.3 Preserved nopalitos

To produce preserved nopalitos, the nopalitos are washed and cut into thin strips. The strips are dipped in boiling water and packed into hot, sterilised jars. The jars are filled with a boiling mixture of salt, vinegar, sugar and water. A space of 5 mm is left at the top of the jar so that the nopalito strips can expand and allow bubbling of the liquid during the preservation process. The lid is turned back by half a turn and the jar placed on the false bottom of the pressure cooker filled with hot water. The pressure cooker is sealed and the pressure allowed to rise slowly. After 25 minutes, the bottles are removed from the heat source and allowed to cool down slowly and stored at room temperature (Du Toit, 2013).

2.4.4.4 Nopalito juice

To produce a healthy nopalito drink, nopalitos and pineapple slices are chopped into pieces. The chopped pieces are placed into a blender, adding fresh orange juice or water, parsley and ice cubes. The ingredients are processed in a blender until smooth as illustrated in Figure 2.8 (Martínez, 2019).

Figure 2.8: Nopal cactus juice (http://mexicoinmykitchen.com/how-tocook-cactus-paddles/)

2.4.4.5 Dried nopalito products

Nopalitos are cut into thin lengths, washed and blanched at a temperature of 80 °C for 5 minutes. The cladodes are dried in a Convection oven at 90 °C for a period of 18 hours (Du Toit, 2013).

2.4.4.6 Crystallised nopalitos

Nopalitos are cut into small pieces, washed and treated with calcium hydroxide for the removal of mucilage. At this stage, the nopalitos are osmotically dehydrated with sucrose solutions and then dehydrated in a forced-air tunnel at a temperature of 60 ˚C. The crystallised nopalitos could be incorporated with other ingredients in the making of cakes or served on its own as crystallised sweets (Sáenz, 1996).

2.4.4.7 Nopalito flour

The nopalitos could be dried to produce flour, which is very rich in dietary fibre and could be used as an ingredient in the baking industry. It could be used for confectionery products and could substitute wheat flour in baked products such as carrot cake, seed bread, crunchy oats biscuits and cookies (De Wit *et al.,* 2015 and Sáenz, 1996). Nopalito flour could also be used in vegetable soups and gelled desserts (Sáenz, 2000). The downside about the nopalito flour is that it could affect the quality characteristics of dough and cakes. An unacceptable green interior colour and a dark crust were reported in cakes when cladode flour was included (Ayadi *et al.,* 2009). Chahdoura *et al*. (2018) made a conclusion based on physical characteristics and sensory analysis that cakes prepared with incorporation of cladode flour the recipe, with well-defined quantities, had higher acceptability scores than cakes prepared without the cladode flour.

2.4.5 Shelf life of nopalitos

It is important to monitor the post-harvest handling of nopalitos as continuous changes take place between harvest and consumption (Berger *et al*., 2013). Nopalitos should be stored at low temperatures post-harvest to retain vitamins and extend its shelf life (Sáenz-Hernández *et al*., 2002). It can be achieved under modified atmospheres, where there is little oxygen available for oxidation and browning (Sáenz-Hernández *et al*., 2002). Nopalitos which were stored in an open box at 5 °C with a relative humidity of 85-90% or at 10 °C with a relative humidity of 80-90%, for a period of 15 to 21 days, showed visible tissue injuries (Stintzing & Carle, 2005). While, when stored at 5 °C for a period of 4 weeks or at 10 °C for a period of 2 weeks packed in sealed 25 µm thick polypropylene bags, only slight changes in colour and firmness was observed, and the quality was not seriously affected (Corrales-García *et al*., 2006). Nopalitos stored below 10 °C for 3-4 weeks had chill injuries that may lead to decay.

On the other hand, nopalitos remained in good condition for up to 30 days when stored at 10 °C (Cantwell *et al*., 1992).

2.5 Conclusion

South Africa is a dry country with areas that are semi-desert. Climate change is causing high temperatures that are affecting water resources in the country. As a result, the future climate projections showed an even drier and more drought-prone climate. According to rainfall data, 2017 was the driest year in recent decades and that followed two already dry winters based on South African Weather Services (SAWS). Cactus pear plants grow in those dry and semi-desert areas and as a result, cladodes have been thriving in South Africa for the longest time and have been utilized as fodder. The young cladodes (nopalitos) of the cactus pear plant are a potentially sustainable and healthy food source in South Africa. Nopalitos have been an excellent food source in Mexico as they can be eaten raw or processed. It is important to develop durable and hardy plants that grow easily and could serve as a food source in South Africa. Nopalitos have the potential to support the South African population in facing climate change and food shortages as an alternative crop. Nopalitos present an opportunity to serve as an alternative food source in South Africa. It may contribute to sustainable agriculture and economical growth of the country. The whole cactus pear plant is edible for both humans and animals.

Chapter 3

3 The physico-chemical characteristics of nopalitos from two cactus pear cultivars over four harvesting times in two years

3.1 Introduction

Cactus plants are classified in the family of Cactaceae which consists of flat stem segments known as cladodes. A total of 1600 species are found in Cactaceae and approximately 180 species are of the genus *Opuntia*. Only certain *Opuntia* plants are important for commercial use. *Opuntia ficus-indica* has been developed for either fruit or nopalito production in over 20 countries such as Tunisia, Italy, Brazil, Algeria, Argentina, Chile, Mexico and South Africa to name a few (De Cortázar & Nobel, 1992). *Opuntia ficus-indica* species are the most cultivated, distributed and consumed as either the fruit or the vegetable source known as nopalitos (De Santiago *et al*., 2018). Mexico has two highly consumed *Opuntia ficus-indica* cultivars for the production of nopalitos, namely Milpa Alta and Atlixco (Ramírez-Moreno *et al*., 2013). The thin, soft and young cladodes called nopalitos have been consumed as a vegetable in Mexico for decades. When cactus cladodes reach a certain maturity they are no longer palatable or edible for humans and are only used for animal feed or other purposes.

Consumers are becoming more aware of their food intake, making food choices leaning towards healthier options (Sáenz *et al*., 2010). The nutritional value of nopalitos is found to be in-between lettuce and spinach (Nerd *et al*., 2001). Rodriguez-Felix (2002) reported that commercially matured nopalitos (*Nopalea cochenillifera*) could be harvested between 11 and 13 cm, while *Opuntia* nopalitos could be harvested at 20 cm. These studies were within a range recorded by Ramírez-Moreno *et al*. (2013) stating that commercial standard nopalitos are harvested between 7-30 cm long with a thin cuticle and a bright green colour. Lopez-Palacios *et al*. (2010) detected an increase in mucilage content throughout the development of nopalitos. Development of cactus pear plant in a country may contribute to the agricultural economy by developing new agricultural products (Chalak *et al*., 2014).

It is important to study the components found in young cladodes as interest increases for its use as a source of vegetable. The SEM images give a clear view of different structure crystals that develop on nopalitos at different growth stages (Contreras-Padilla *et al*., 2015). The presence and distribution of crystals in plants can be dependent on factors such as soil composition and location of the plantation (Contreras-Padilla *et al.,* 2015).

Calcium oxalate crystals may be traced in most parts of a plant such as roots, leaves, stems, seeds, floral organs and anthers (Konyar *et al*., 2014; Franceschi & Horner, 1980). Contreras-Padilla *et al.* (2015) identified calcium oxalate crystals in *Opuntia* plants. Different crystalline structures were present in cladodes including calcium oxalate monohydrate (whewellite), calcium oxalate dehydrate (weddelite), calcium carbonate (calcite), calcium magnesium bicarbonate, magnesium oxide and potassium peroxydiphosphate. Oxalate can be present in cladodes in different crystal shapes which are categorised according to their morphology; crystal sand, raphide, druse, styloid and prismatic (McConn & Nakata, 2004). Oxalate in plants has a negative effect on the nutritional quality of foods due to these compounds binding essential minerals such as calcium and forming complexes that decrease calcium absorption (Mc Conn & Nakata, 2004; Franceschi & Horner, 1980). Plants with high oxalate content can increase the risk of renal calcium oxalate when ingested by a certain group of people (McConn & Nakata, 2004).

3.2 Materials and Methods

3.2.1 Collection of nopalitos

The young cladodes of two *Opuntia ficus-indica* cultivars (Fusicaulis and Morado) were harvested at the experimental orchard on the west campus of the University of the Free State 29°6’27.08”S 26°11’32.90”E. Nopalitos are seasonal, they were harvested during two seasons (late summer-autumn and spring). The first collection was post-fruit harvest (February-March), because the plant begins to produce nopalitos after fruit harvest. The second collection was during the spring growth (September-October) due to the plant producing nopalitos in spring. The collection of nopalitos was repeated in a period of two years. In March and October 2017, and in February and October 2018. The cladodes were harvested using a sharp blade, cutting each one from the mother cladode to prevent cutting into its skin and avoid tissue injuries. It is important to be careful when cutting the cladodes to avoid damage to the base of cladode which might cause an opening for microorganisms (Berger *et al*., 2013). Each cladode was labelled and packaged according to the cultivar and the growth stage (length). It was packed in a crate and transported to the laboratory. The nopalitos were harvested at six different lengths 9 cm, 12 cm, 15 cm, 18 cm, 21 cm and 24 cm (Figure 3.1). There were six samples for each growth length. The cladodes were stored in the refrigerator at 4 °C for not more than 24 hours before further analysis if they were not analysed immediately after harvest.

Drought became a serious challenge in most of the winter-rainfall zone (WRZ) and other parts of South Africa which had extended to Bloemfontein in 2017. The year 2017 was described as the driest year and was compared to back to 1921 based on South African Weather Services (SAWS). The autumn and winter seasons of 2017 were discovered to be most dry compared to previous other years (Conradie, 2018).

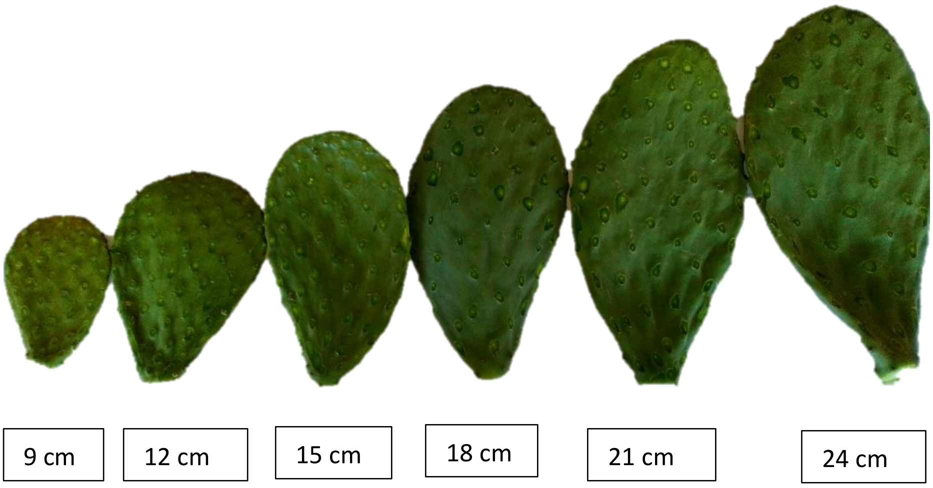


Figure 3.1: Cladodes at different growth lengths

3.2.1.1 Sample preparation

The young cladodes (nopalitos) were removed from the refrigerator and allowed to reach room temperature for 30 minutes. After reaching room temperature, the thorns were removed manually using a sharp blade. Morado nopalitos had more thorns and required a lot more time to remove thorns compared to Fusicaulis nopalitos.

The first collection was conducted over three days namely 8, 15 and 16 March 2017. Nopalitos were harvested midmorning, because that is the time of the day in which the acidity is decreased (Berger *et al.*, 2013). Therefore, the time of harvest was between 9:00 am and

10:00 am, 3 hours after sunrise. The time of sunrise was 6:12 am and 6:17 am with an average temperature of 25 °C at the time of harvest.

The second collection was on 13, 17 and 24 October 2017. The time of sunrise was 5:29 am and 5:40 am with an average temperature of 24 °C at the time of harvest. The harvest time was between 9:00 am and 10:00 am, 3 hours after sunrise on 13 October and between 10:00 am and 11:00 am, 4 hours after sunrise on 17 and 24 October 2017.

The third collection was on 12, 14, and 21 February and 01 March 2018. The time of sunrise was 5:55 am and 6:07 am with an average temperature of 21 °C at the time of harvest. The time of harvest was between 9:00 am and 10:00 am, 3 hours after sunrise on 14 February and 1 March and between 10:30 and 11:00 am, 4 hours after sunrise on 12 and 21 February 2018.

The fourth collection was on 15, 31 October and 7 November 2018. The time of sunrise was 5:37 am, 5:22 am and 5:18 am with an average temperature of 19.3 °C at the time of harvest. The time of harvest was between 9:00 am and 10:00 am, 3 hours after sunrise on 15 October and 7 November and between 10:30 and 11:30 am, 4 hours after sunrise on 31 October 2018.

3.2.1.2 **Determination of weight**

The nopalitos were weighed to three decimals on a Radwag Ps 750/c/2 scale to measure the weight in gram (g) (Figure 3.2).

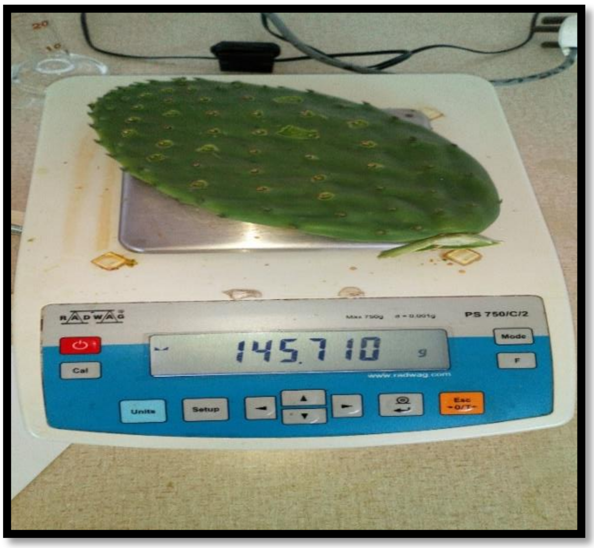


Figure 3.2: The nopalitos weighed on a Radwag Ps 750/c/2 scale

3.2.1.3 Determination of size

Each nopalito was placed on a flat surface to measure the size. A metric ruler was used to measure the length in cm. The metric measuring tape was placed horizontally on top of the nopalito to measure the width. A metric ruler was used to measure the diameter. All the measurements were in centimetre (Figure 3.3).

Figure 3.3: Size of the nopalito measured with a metric ruler and measuring tape

3.2.1.4 Determination of volume and surface area

The volume of cladodes was calculated using the following formula: length (l), width (w) and diameter (d).

$$\text{Volume (cm}^3\text{)} = (l \times w) \times d$$

The surface area was calculated using the following formula: (Du Toit, 2013; Hernández *et al.*, 2010).

$$\text{Surface area (cm}^2\text{)} = [(w \times l) \div 2] \times \pi$$

3.2.1.5 Determination of colour

A Konica Minolta colorimeter was used, placing it flat towards the edges of the cladode. This was done in three different sections that are not far from each other, receiving three readings from each cladode. Illustrated in Figure 3.4, the measurement readings were in L*, a* and b* of CIELAB. The three coordinates can be explained as follows: L* measures the lightness of colour, where L* = 0 shows black and L* = 100 shows white. The a* coordinate represent red and green, where negative values show green and positive values show red. The b* coordinate represent yellow and blue, where negative values show blue and positive values show yellow. The other two coordinates are C* and h° of CIELCH. Chroma (C*) represent the relative saturation of a colour, where 0 value shows unsaturated colour or dull colour and 100 value shows brightness of colour. Hue (h°) shows the position of colour in degrees on a colour wheel, where h° = 0° shows red, h°= 90° shows yellow, h° = 180° shows green and h° = 270° shows blue. Both C* and h° coordinates were calculated from a* and b* with the use of an online colour parameter converter (ColorMine.org, 2014). Chroma was calculated using the following formula $C^* = (a^{*2} + b^{*2})^{0.5}$ (Ripoll *et al.*, 2011). Hue angle was calculated using the following formula $h° = \tan^{-1} (b^*/a^*)$ (Ripoll *et al.*, 2011).

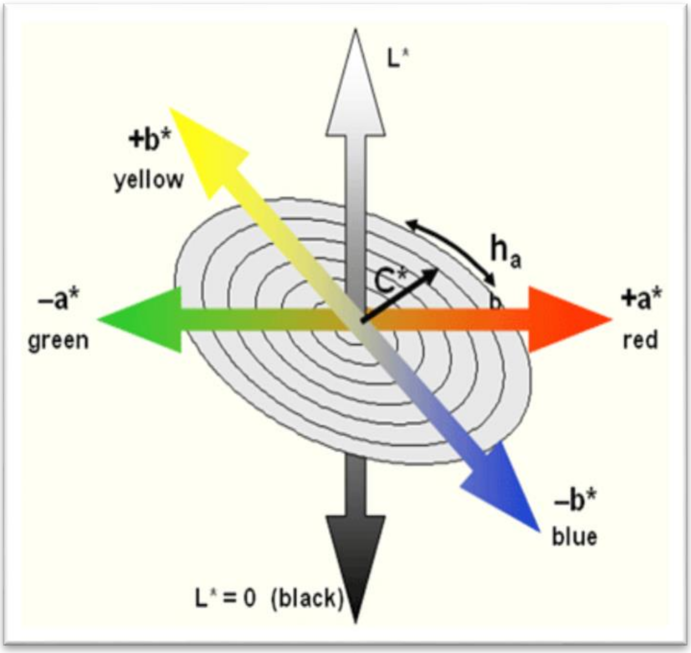


Figure 3.4: The hunter color scale indicating CIELAB and CIELCH colour values (Engineering 360, 2017)

3.2.1.6 Determination of firmness (ability to be penetrated)

The calibrated Stanhope-seta penetrometer was used to measure the firmness. The time was set for 5 s and three different readings were taken from each cladode in 10ths m/m. The nopalito was placed on the machine base table. The needle attachment was inserted in the slider which allows the penetration made by a needle inside the nopalito. The needle was adjusted close to the nopalito but may not touch its skin. A start button was pushed, where the slider allows the needle to free fall inside the nopalito. Once the penetration was made the rod was pushed downwards gently to take the readings (Figure 3.5). The low values indicate high firmness (hard tissue) and the high values indicate low firmness (soft tissue), firmness is associated with good quality in terms of texture, freshness and ripeness (García-Nava *et al*., 2015).

Figure 3.5: The needle of the penetrometer penetrating through the young cladode

3.2.1.7 Determination of compressibility

A Stanhope-seta penetrometer was used with a flat tip compressor. A weight of 200 g was used to assist with compressing the young cladode. The time was set for 5 s, and the reading was taken in 10ths m/m. The nopalito was positioned on the machine base table. A flat tip compressor was inserted in the lower part of the slider and tightened with the fixing screw. In the top part of the slider, the weight was applied. The start button was pushed, and after 5 s the reading was recorded (Figure 3.6). When the value of the reading is low, it indicates a hard tissue (little compression) and a high value is an indication of tender (soft) tissue as the product was easier to compress (Du Toit *et al*., 2016).

Figure 3.6: The young cladode compressed in a penetrometer with added weight

3.2.1.8 Determination of gustatory properties

To the consumer, taste is a factor in the assessment of quality. Nopalito acidity affects the taste of nopalitos. Thus three methods were used to determine the gustatory properties of nopalitos namely titratable acidity, pH and TSS.

3.2.1.8.1 Titratable acidity

In a conical flask, five ml mucilage was diluted with 35 ml of distilled water and 0.3 ml phenolphthalein was added with a pipette. The sample was titrated to a faint pink end product with 0, 1 M NaOH (Figure 3.7). This was repeated in triplicate to get accurate results (Mañara & Badilla, 2015). The value of T was obtained, and the following formulas were used for calculations of titratable acidity (TA) and titratable acidity expressed as % citric acid.

a) $\% \text{ TA} = 10 \times T$
b) $\% \text{ TA as citric acid} = (T \times 192) \div (3 \times 1000)$

T = mean titre (ml) of 0.1 M NaOH

Figure 3.7: The faint pink end product after titration

3.2.1.8.2 pH

The young cladode was cut into small pieces and macerated into a pulp using a Kenwood food processor. It was transferred into a small bowl and a Eutech pH 2700 pH/mV/°C/°F probe was inserted in the sample to measure the pH and temperature (Figure 3.8).

Figure 3.8: The Eutech pH 2700 pH/mV/°C/°F probe inserted in the sample

3.2.1.8.3 Total Soluble Solids (TSS) content

A hand-held Refractometer was used to determine the sugar content (measure of sweetness) and it was measured in °Brix (Figure 3.9). The plastic cover of the refractometer was opened, and using a pipette a few drops of mucilage were placed on the glass prism and was covered. The refractometer was held up to natural light and the reading was taken by looking through the eyepiece (Mañara & Badilla, 2015; Smith, 2010).

Figure 3.9: The few drops of mucilage sample in a refractometer

3.2.1.9 Determination of mucilage content

The macerated pulp was cooked in the microwave for 3 minutes (Figure 3.10). The pulp was weighed, divided into centrifuge tubes and was centrifuged at the speed of 8000 rpm for 15 min at 4 °C according to a patented method (Du Toit & De Wit, 2011). The mucilage yield obtained was weighed (Figure 3.11). The mucilage was used for further analysis and the solids were regarded as waste material and discarded.

Figure 3.10: The pale green cooked pulp with slight mucilage visible

Figure 3.11: The mucilage yield separated from the solids (waste material)

3.2.1.10 Determination of moisture content

A metal Petri dish was weighed without and with sample using a Mettler AE 200 scale and the weight measured was noted down in four decimal places. The weighed samples were put inside an Eco Therm oven at a temperature of 102 °C for 24 hours. A desiccator that contains silicon crystals was used to cool down the sample without any interferences for 20 minutes and then the Petri dish containing dried sample was weighed again shown in Figure 3.12 (Du Toit, 2017; McClements, 2003). The moisture content (%) was calculated using the following formula:

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

Figure 3.12: The weighed samples in Petri dishes before and after the drying process.

3.2.1.11 Determination of viscometer viscosity

A Brookfield DV3T Viscometer, spindle number sc4-21 was used to determine the viscosity of each sample of extracted mucilage. Measurements were obtained using the same spindle at different speeds to indicate the flow properties of the fluid. Viscosity measured using the viscometer would be different depending on the speed rate (Du Toit, 2013). The required sample size is 7 ml of extracted mucilage to run the system. The rates were set on 50, 75, 100, 150, 200, 225 and 250 rpm at a controlled temperature of 25 °C illustrated in Table 3.1. The viscosity measurements were taken from the highest torque percentage at different speeds if the sample did not reach 10% torque (Figure 3.13).

Table 3.1: The steps with increasing speed used to measure each sample (Brookfield Engineering Laboratories)

Step	Speed (rpm)	Time (s)	Temperature °C	Spindle
1	50	5	25	SC4-21
2	75	5	25	SC4-21
3	100	5	25	SC4-21
4	150	5	25	SC4-21
5	200	5	25	SC4-21
6	225	5	25	SC4-21
7	250	5	25	SC4-21

Figure 3.13: The Brookfield DV3T Viscometer.

3.2.2 Scanning electron microscopy

A scanning electron microscope (SEM) was used in order to study the cell structure of cladodes and the existence of crystals in different nopalito cultivars at different growth lengths. Fresh nopalitos samples (5 mm) from both cultivars, Morado and Fusicaulis, at different growth lengths (9 cm, 15 cm and 24 cm) were used. The fresh nopalito samples were fixed in 0.1M (pH 7.0) sodium phosphate-buffered glutardialdehyde (3%) for at least 3 hours. The samples were dehydrated in a graded ethanol series (50%, 70% and 95% for 30 minutes in each phase followed by two changes of 100% for 1 hour in each phase). The samples were dried using a critical point dryer (Tousimis, Maryland, U.S.A.). After drying, the samples were mounted on stubs (Aluminium pin type, 10 mm) using epoxy glue and gold coated with a Bio-Rad sputter coater (United Kingdom). Specimens were analysed with a JSM-7800F Extreme-resolution Analytical Field Emission SEM (Tokyo, Japan) (Glauert, 1974).

3.3 Statistical analysis

The effects of size, cultivar, season and year as well as their interactions on nopalito attributes were evaluated by means of analysis of variance (ANOVA) (NCSS 11 Statistical Software, 2016). One way Analysis of Variance (ANOVA) was performed to determine significant differences between the six length groups, the two cultivars, the two seasons, the two years, interaction between size and cultivar, interaction between size and season and interaction between size and year (NCSS 11 Statistical Software, 2016). The Tukey-Kramer multiple comparison test ($\propto = 0.05$) was carried out to determine significant differences between the treatment means (NCSS 11 Statistical Software, 2016). Pearson's correlation analysis was performed to determine the relationship between different quality attributes of nopalitos (NCSS 11 Statistical Software, 2016). The properties of nopalitos of the six different sizes were visualized in a 2-dimensional space by principal component analysis (PCA) (XLSTAT, 2018).

3.4 Results and discussion

3.4.1 ANOVA for the effect of size, cultivar, season and year on the characteristics of nopalitos

In Table 3.2, nopalito harvest size had a highly significant ($p < 0.001$) effect on the morphological properties (weight, width, diameter, volume and surface area), firmness ($p < 0.001$), colour parameters (only b*, C* and h°) ($p < 0.001$), mucilage yield ($p < 0.001$), gustatory properties (TA as % citric acid and pH) ($p < 0.001$). Size also had a significant ($p < 0.05$) effect on the L* ($p = 0.002$), a* ($p = 0.001$) and TSS ($p = 0.025$).

Cultivar had a highly significant ($p < 0.001$) effect on the weight, diameter, firmness, L*, a*, b*, C*, h°, and moisture content ($p < 0.001$). Cultivar also had a significant ($p < 0.05$) effect on the volume ($p = 0.001$) and mucilage yield ($p = 0.002$).

Season had a highly significant ($p < 0.001$) effect on the diameter, volume, surface area, L*, a*, b*, C*, viscometer viscosity and TSS content ($p < 0.001$), as well as a significant ($p < 0.05$) effect on the width ($p = 0.011$), firmness ($p = 0.002$), compressibility ($p = 0.001$), and TA as citric acid ($p = 0.002$).

Year had a highly significant ($p < 0.001$) effect on the diameter, surface area, mucilage yield and viscometer viscosity, TA as citric acid and TSS content ($p < 0.001$). Year also had significant effects on the weight ($p = 0.007$), volume, ($p = 0.007$), firmness ($p =0.009$), a* ($p = 0.033$), h° ($p =0.002$), moisture content ($p = 0.001$) and pH ($p = 0.007$).

Table 3.2: Analysis of variance (ANOVA) for the influence of size, cultivar, season and year on the characteristics of nopalitos

Properties:	Size (A)	Cultivar (B)	Season (C)	Year (D)
Weight (g)	p < 0.001	p < 0.001	p = 0.441	p = 0.007
Length (cm)	p = 1.000	p = 1.000	p = 1.000	p = 1.000
Width (cm)	p < 0.001	p = 0.174	p = 0.011	p = 0.487
Diameter (cm)	p < 0.001	p < 0.001	p < 0.001	p < 0.001
Volume (cm^3)	p < 0.001	p = 0.001	p < 0.001	p = 0.007
S. area (cm^2)	p < 0.001	p = 0.921	p < 0.001	p < 0.001
Firmness (10ths m/m)	p < 0.001	p < 0.001	p = 0.002	p = 0.009
Compressibility (10ths m/m)	p = 0.063	p = 0.214	p = 0.001	p = 0.086
L*	p = 0.002	p < 0.001	p < 0.001	p = 0.156
a*	p = 0.001	p < 0.001	p < 0.001	p = 0.033
b*	p < 0.001	p < 0.001	p < 0.001	p = 0.745
C*	p < 0.001	p < 0.001	p < 0.001	p = 0.274
Hue (h°)	p < 0.001	p < 0.001	p = 0.082	p = 0.002
Moisture content (%)	p = 0.200	p < 0.001	p = 0.455	p = 0.001
Mucilage yield (%)	p < 0.001	p = 0.002	p = 0.099	p < 0.001
Viscosity (cP)	p = 0.258	p = 0.288	p < 0.001	p < 0.001
TA as citric acid (%)	p < 0.001	p = 0.327	p = 0.002	p < 0.001
pH	p < 0.001	p = 0.273	p = 0.942	p = 0.007
TSS (°Brix)	p = 0.025	p =0.111	p < 0.001	p < 0.001

3.4.1.1 The effect of size on the characteristics of nopalitos

In Table 3.3, the weight of nopalitos at 9 cm (26.92 g) was significantly ($p < 0.001$) lower than at 24 cm (161.83 g). The weight of nopalitos was significantly ($p < 0.001$) different throughout development. No significant differences were observed between 12 and 15 cm, however, the weight significantly differed with other growth lengths. The width of nopalitos at 9 cm (5.84 cm) was significantly ($p < 0.001$) narrower than nopalitos at 24 cm (12.26 cm). The width of nopalitos was significantly ($p < 0.001$) different throughout development. No significant differences were detected between 12, 15, 18, 21 and 24 cm nopalitos in terms of diameter however, 9 cm (0.50 cm) nopalitos had significantly ($p < 0.001$) lower diameter values. Astello-García *et al*. (2015) reported that old cladodes have a thicker cuticle because of the expansion of the parenchyma (water storage) This is in agreement with the results of the current study that nopalitos at 9 cm had a substantially lower diameter than 24 cm nopalitos. The volume of nopalitos at 9 cm (36.94 cm^3) was significantly ($p < 0.001$) lower than nopalitos at 24 cm (347.85 cm^3). The volume of nopalitos was significantly ($p < 0.001$) different between 9, 15, 18 and 24 cm. The surface area at 9 cm (6.20 cm^2) was significantly ($p < 0.001$) lower than nopalitos at 21 cm (29.70 cm^2). The surface area of nopalitos was significantly ($p < 0.001$) different between 9, 15 and 24 cm.

The firmness of nopalitos at 15 cm (72.49 10ths m/m) had significantly ($p < 0.001$) higher values (less firm) than nopalitos at 24 cm (57.89 10ths m/m). The firmness of nopalitos was significantly ($p < 0.001$) different between 9 and 18 cm. Since it was found that more mature cladodes become more firm, it explains why only young and smaller nopalitos which are more tender are suitable for human food, and the more mature cladodes are only suitable for animal fodder. No significant differences were detected between the sizes of nopalitos for compressibility.

In terms of colour, the L* of nopalitos at 9 cm (50.84) was significantly higher ($p = 0.002$) than nopalitos at 24 cm (47.30). The a* value of nopalitos at 9 cm (-17.82) was significantly ($p = 0.001$) lower than nopalitos at 24 cm (-15.59), and the b* value at 9 cm (30.39) was significantly ($p < 0.001$) higher than nopalitos at 24 cm (22.81). The b* value of nopalitos was significantly ($p < 0.001$) different between 9, 12 and 24 cm. The C* value of nopalitos at 9 cm (35.32) was significantly ($p < 0.001$) higher than nopalitos at 24 cm (27.04). The C* value of nopalitos was significantly ($p < 0.001$) different between 9, 12 and 24 cm. The h° of nopalitos at 9 cm (120.76 °) was significantly ($p < 0.001$) lower than nopalitos at 24 cm (124.79 °). Thus, nopalitos at 9 cm had a lighter, brighter and greener colour than nopalitos at 24 cm. No significant differences were observed between the sizes of nopalitos for moisture content.

The moisture content was constantly high throughout development because nopalitos are known to absorb and store water. The mucilage yield at 9 cm (17.32 %) was significantly ($p < 0.001$) lower than at 24 cm (48.92 %). The mucilage yield was significantly ($p < 0001$) different between 9, 12, 18 and 24 cm. No significant differences were observed between the sizes of nopalitos on the viscometer viscosity, although the viscosity at 9 cm was lower. TA as % citric acid of mucilage at 9 cm (0.30 %) was significantly ($p < 0.001$) lower than TA of mucilage at 24 cm (0.44 %). The TA as % citric acid of mucilage was significantly ($p < 0.001$) different between 9, 15 and 21 cm. The pH of nopalitos at 9 cm (4.44) was significantly ($p < 0.001$) higher than nopalitos at 24 cm (4.05), thus 9 cm nopalitos were found to be less sour than nopalitos at 24 cm. The pH of nopalitos was significantly ($p < 0.001$) different between 9, 12 and 18 cm. TSS of mucilage at 9 cm (6.22 °Brix) was significantly ($p = 0.025$) higher than mucilage at 21 cm (5.63 °Brix).

Table 3.3: Analysis of variance (ANOVA) for the effect of size on the characteristics of nopalitos

Size groups	9 cm	12 cm	15 cm	18 cm	21 cm	24 cm	Sign. level
Weight (g)	$26.92^{a} \pm 6.41$	$51.91^{b} \pm 23.37$	$66.83^{b} \pm 17.55$	$100.02^{c} \pm 35.01$	$135.37^{d} \pm 41.97$	$161.83^{e} \pm 36.74$	$p < 0.001$
Width (cm)	$5.84^{a} \pm 0.56$	$7.24^{b} \pm 0.99$	$8.31^{c} \pm 1.12$	$9.87^{d} \pm 1.38$	$11.05^{e} \pm 1.04$	$12.26^{f} \pm 1.41$	$p < 0.001$
Diameter (cm)	$0.50^{a} \pm 0.23$	$0.71^{b} \pm 0.36$	$0.71^{b} \pm 0.31$	$0.73^{b} \pm 0.30$	$0.81^{b} \pm 0.36$	$0.81^{b} \pm 0.32$	$p < 0.001$
Volume (cm^3)	$36.94^{a} \pm 23.78$	$93.75^{ab} \pm 77.31$	$138.21^{b} \pm 121.24$	$249.75^{c} \pm 211.21$	$261.22^{cd} \pm 176.46$	$347.85^{d} \pm 203.61$	$p < 0.001$
S. area (cm^2)	$6.20^{a} \pm 4.16$	$11.33^{ab} \pm 9.21$	$17.01^{bc} \pm 14.76$	$26.83^{cd} \pm 25.34$	$29.70^{d} \pm 24.90$	$28.24^{d} \pm 18.13$	$p < 0.001$
Firmness (10ths m/m)	$61.09^{ab} \pm 5.70$	$65.93^{bc} \pm 9.44$	$72.49^{d} \pm 9.05$	$68.84^{cd} \pm 10.61$	$63.39^{abc} \pm 11.02$	$57.89^{a} \pm 10.37$	$p < 0.001$
Compressibility (10ths m/m)	30.15 ± 6.71	30.40 ± 7.18	27.00 ± 7.10	30.52 ± 6.90	30.71 ± 6.00	30.46 ± 6.56	$p = 0.063$
L*	$50.84^{b} \pm 4.71$	$48.67^{ab} \pm 5.61$	$48.48^{ab} \pm 4.11$	$48.30^{ab} \pm 4.55$	$47.48^{a} \pm 3.84$	$47.30^{a} \pm 3.82$	$p = 0.002$
a*	$-17.82^{a} \pm 2.45$	$-16.36^{ab} \pm 2.98$	$-16.85^{ab} \pm 2.43$	$-16.27^{ab} \pm 3.02$	$-15.77^{b} \pm 2.93$	$-15.59^{b} \pm 2.60$	$p = 0.001$
b*	$30.39^{c} \pm 6.17$	$26.47^{b} \pm 7.61$	$25.64^{ab} \pm 5.02$	$24.45^{ab} \pm 6.60$	$23.62^{ab} \pm 5.83$	$22.81^{a} \pm 5.66$	$p < 0.001$
C*	$35.32^{c} \pm 6.32$	$31.19^{b} \pm 7.91$	$30.71^{ab} \pm 5.41$	$28.98^{ab} \pm 6.83$	$28.53^{ab} \pm 6.32$	$27.04^{a} \pm 6.50$	$p < 0.001$
Hue (h°)	$120.76^{a} \pm 3.32$	$122.56^{ab} \pm 4.03$	$123.61^{bc} \pm 2.56$	$123.76^{bc} \pm 2.94$	$123.96^{bc} \pm 2.95$	$124.79^{c} \pm 3.18$	$p < 0.001$
Moisture content (%)	87.63 ± 1.39	88.15 ± 1.47	88.38 ± 1.49	88.13 ± 1.51	88.35 ± 2.22	87.92 ± 1.40	$p = 0.200$
Mucilage yield (%)	$17.32^{a} \pm 8.86$	$29.47^{b} \pm 12.68$	$35.46^{bc} \pm 11.75$	$41.65^{cd} \pm 9.78$	$45.46^{de} \pm 11.03$	$48.92^{e} \pm 10.67$	$p < 0.001$
Viscosity (cP)	71.77 ± 91.53	152.07 ± 203.48	143.84 ± 222.65	153.74 ± 238.65	170.56 ± 253.39	162.73 ± 247.02	$p = 0.258$
TA as citric acid (%)	$0.30^{a} \pm 0.09$	$0.31^{ab} \pm 0.08$	$0.36^{bc} \pm 0.07$	$0.39^{cd} \pm 0.10$	$0.43^{d} \pm 0.11$	$0.44^{d} \pm 0.08$	$p < 0.001$
pH	$4.44^{d} \pm 0.19$	$4.26^{c} \pm 0.21$	$4.18^{bc} \pm 0.23$	$4.13^{ab} \pm 0.21$	$4.09^{ab} \pm 0.14$	$4.05^{a} \pm 0.13$	$p < 0.001$
TSS (°Brix)	$6.22^{b} \pm 0.83$	$5.89^{ab} \pm 0.85$	$5.93^{ab} \pm 0.80$	$5.82^{ab} \pm 0.85$	$5.63^{a} \pm 0.83$	$5.77^{ab} \pm 0.93$	$p = 0.025$

Means with different superscripts in the same row differ significantly

3.4.1.2 The effect of cultivar on the characteristics of nopalitos

Fusicaulis nopalitos (108.47 g) were significantly ($p < 0.001$) heavier than Morado nopalitos (72.49 g) shown in Table 3.4. Interestingly, De Wit *et al*. (2017) found that the weight of mature cladodes from different cultivars did not differ significantly. No significant differences between cultivars were observed in nopalitos for length and width. Fusicaulis nopalitos had a significantly ($p < 0.001$) wider diameter of 0.87 cm than Morado nopalitos (0.55 cm). Rodriguez-Felix & Cantwell (1988) described *Opuntia ficus indica* nopalitos as long and thin compared to *Opuntia inermis* and *Opuntia amyclaea* nopalitos which were disc-shaped. In agreement with Wessel (1989), the shape of Fusicaulis nopalitos was observed to be columnar shape with elongated nopalitos while, Morado nopalitos had a round-nopalito shape. The volume of Fusicaulis nopalitos (224.80 cm^3) was significantly ($p = 0.001$) higher than Morado nopalitos (151.11 cm^3). Fusicaulis nopalitos (61.14 10ths m/m) were significantly ($p < 0.001$) firmer than Morado nopalitos (68.74 10ths m/m). No significant differences were detected between cultivars for compressibility.

In terms of colour, L* of Fusicaulis nopalitos (45.90) was significantly ($p < 0.001$) lower than that of Morado nopalitos (51.12). The a* value of Fusicaulis nopalitos (-15.28) was significantly ($p < 0.001$) higher than Morado nopalitos (-17.61), and the b* value of Fusicaulis nopalitos (21.70) was significantly ($p = 0.001$) lower than Morado nopalitos (29.43). The C* value of Fusicaulis nopalitos (26.32) was significantly ($p < 0.001$) lower than Morado nopalitos (34.27). The h° of Fusicaulis nopalitos (125.41°) was significantly ($p < 0.001$) higher than Morado nopalitos (121.07°). The colour parameters in the study therefore, showed that Morado nopalitos had more yellow-greener tone, higher lightness and more saturated in colour than Fusicaulis nopalitos. Also, both cultivars were between yellow and green on the colour wheel in terms of hue angle. In a study by Sáenz (1997), the colour of nopal flour was described as pale green with high lightness, the colour parameters were L* (73.37), a* (-5.20) and b* (26.1).

The moisture content of Fusicaulis nopalitos (88.46 %) was significantly ($p < 0.001$) higher than Morado nopalitos (87.72 %). Mucilage yield of Fusicaulis nopalitos (39.16 %) was significantly ($p = 0.002$) higher than Morado nopalitos (33.61 %). It was observed that there was a relation between moisture and mucilage content, as a result the higher the moisture content so was the mucilage content. No significant differences between cultivars were found for TA as % citric acid, pH and TSS.

Table 3.4: Analysis of variance (ANOVA) for the effect of cultivar on the characteristics of nopalitos

Cultivar	Fusicaulis	Morado	Sign. level
Weight (g)	108.47^{b} ± 60.30	72.49^{a} ± 43.49	p < 0.001
Length (cm)	16.50 ± 5.14	16.50 ± 5.14	p = 1.000
Width (cm)	8.90 ± 2.28	9.29 ± 2.64	p = 0.174
Diameter (cm)	0.87^{b} ± 0.30	0.55^{a} ± 0.27	p < 0.001
Volume(cm^{3})	224.80^{b} ± 197.64	151.11^{a} ± 163.82	p = 0.001
S. area (cm^{2})	19.77 ± 19.59	20.00 ± 20.16	p = 0.921
Firmness (10ths m/m)	61.14^{a} ± 10.15	68.74^{b} ± 9.70	p < 0.001
Compressibility (10ths m/m)	30.37 ± 7.66	29.37 ± 5.84	p = 0.214
L*	45.90^{a} ± 4.23	51.12^{b} ± 3.27	p < 0.001
a*	-15.28^{b} ± 2.90	-17.61^{a} ± 2.19	p < 0.001
b*	21.70^{a} ± 5.97	29.43^{b} ± 4.74	p = 0.001
C*	26.32^{a} ± 6.38	34.27^{b} ± 5.21	p < 0.001
Hue (h°)	125.41^{b} ± 2.78	121.07^{a} ± 2.50	p < 0.001
Moisture content (%)	88.46^{b} ± 1.47	87.72^{a}± 1.67	p < 0.001
Mucilage yield (%)	39.16^{b} ± 15.03	33.61^{a} ± 14.81	p = 0.002
Viscosity (cP)	128.83^{a} ± 204.78	156.07^{b} ± 228.86	p = 0.288
TA as citric acid (%)	0.38 ± 0.10	0.37 ± 0.11	p = 0.327
pH	4.21 ± 0.20	4.18 ± 0.25	p = 0.273
TSS (°Brix)	5.96 ± 0.81	5.79 ± 0.91	p = 0.111

Means with different superscripts in the same row differ significantly

3.4.1.3 The effect of season on the characteristics of nopalitos

No significant differences were found on the weight of nopalitos harvested during the two seasons. The width of nopalitos harvested in autumn (8.73 cm) was significantly (p = 0.011) lower than in the spring harvest (9.46 cm) illustrated in Table 3.5. The diameter of nopalitos harvested in autumn (0.80 cm) was significantly (p < 0.001) wider than spring harvest (0.62 cm). Thus thinner nopalitos could be harvested in spring. The volume of nopalitos harvested in autumn (145.13 cm^{3}) was significantly (p < 0.001) smaller than in spring harvest (230.78 cm^{3}). The surface area of nopalitos harvested in autumn (12.09 cm^{2}) was significantly (p < 0001) smaller than in spring harvest (27.68 cm ^{2}).

The firmness of nopalitos harvested in autumn (63.04 10ths m/m) was significantly (p = 0.002) higher than in spring harvest (66.83 10ths m/m), implying that nopalitos harvested in spring had a lower firmness (more easily penetrated by a penetrometer) than in autumn. The compressibility of nopalitos harvested in autumn (28.60 10ths m/m) was significantly (p = 0.001) higher than in spring harvest (31.14 10ths m/m), indicating that nopalitos harvested in spring were softer and more easily compressed than those harvested in autumn, hence it may be best to harvest during spring season.

In terms of colour, L* of nopalitos harvested in autumn (50.45) was significantly ($p < 0.001$) higher than in spring harvest (46.58). The a* value of nopalitos harvested in autumn (-17.75) was significantly ($p < 0.001$) lower than in spring harvest (-15.13), and the b* value of nopalitos harvested in autumn (27.46) was significantly ($p < 0.001$) higher than in spring harvest (23.67). The C* values of nopalitos harvested in autumn (32.45) was significantly ($p < 0.001$) higher than in spring harvest (28.14). The h° of nopalitos harvested in autumn (123.59°) was not significantly (p = 0.082) higher than in spring harvest (122.89°). This indicates that nopalitos harvested in autumn had a brighter yellow to green tone than in spring. In terms of colour, autumn harvest had better green nopalitos than spring harvest.

No significant differences were detected in the moisture content and mucilage yield between the two seasons. Viscometer viscosity of mucilage from the autumn harvest (31.07 cP) was significantly ($p < 0.001$) lower than in the spring harvest (253.83 cP). From the difference in viscosities it may be deduced that climatic conditions had an impact over the two seasons. TA as % citric acid of mucilage in autumn harvest (0.39 %) was significantly (p = 0.002) higher than in spring harvest (0.35 %). No significant difference was detected between the pH of the two seasons. TSS of mucilage in autumn harvest (5.53 °Brix) was significantly ($p < 0.001$) lower to the spring harvest (6.22 °Brix). Both measures of sourness (TA and pH) showed that nopalitos harvested in spring was less sour with more sugar content (TSS). It can be deduced that spring harvest could be more ideal for harvesting nopalitos because they are thinner, softer, less sour and with more sugar content than in autumn.

Table 3.5: Analysis of variance (ANOVA) for the effect of season on the characteristics of nopalitos.

Season	Autumn	Spring	Sign. Level
Weight (g)	93.00 ± 58.32	87.96 ± 52.59	p = 0.441
Width (cm)	8.73^{a} ± 2.31	9.46^{b} ± 2.57	p =0.011
Diameter (cm)	0.80^{b} ± 0.37	0.62^{a} ± 0.26	p < 0.001
Volume (cm^3)	145.13^{a} ± 122.19	230.78^{b} ± 223.62	p < 0.001
S. area (cm^2)	12.09^{a} ± 6.43	27.68^{b} ± 25.03	p < 0.001
Firmness (10ths m/m)	63.04^{a} ± 11.19	66.83^{b} ± 9.68	p = 0.002
Compressibility (10ths m/m)	28.60^{a} ± 7.31	31.14^{b} ± 6.05	p = 0.001
L*	50.45^{b} ± 4.37	46.58^{a} ± 3.96	p < 0.001
a*	-17.75^{a} ± 2.73	-15.13^{b} ± 2.25	p < 0.001
b*	27.46^{b} ± 7.05	23.67^{a} ± 5.59	p < 0.001
C*	32.45^{b} ± 7.53	28.14^{a} ± 5.79	p < 0.001
Hue (h°)	123.59 ± 3.43	122.89 ± 3.38	p = 0.082
Moisture content (%)	88.16 ± 1.74	88.02 ± 1.47	p = 0.455
Mucilage yield (%)	37.85 ± 16.88	34.91 ± 13.10	p = 0.099
Viscosity (cP)	31.07^{a} ± 31.50	253.83^{b} ± 262.12	p < 0.001
TA as citric acid (%)	0.39^{b} ± 0.11	0.35^{a} ± 0.09	p = 0.002
pH	4.19 ± 0.24	4.19 ± 0.21	p 0.942
TSS (°Brix)	5.53^{a} ± 0.82	6.22^{b} ± 0.75	p < 0.001

Means with different superscripts in the same row differ significantly

3.4.1.4 The effect of year on the characteristics of nopalitos

In Table 3.6, the weight of nopalitos in 2017 (99.19 g) was significantly ($p = 0.007$) higher than in 2018 harvest (81.77 g). There were no significant differences for the width of nopalitos over the two years. The diameter of nopalitos in 2017 (0.86 cm) was significantly ($p < 0.001$) wider than in 2018 harvest (0.57 cm). The volume of nopalitos in 2017 (158.92 cm^3) was significantly ($p = 0.007$) smaller than in 2018 harvest (216.99 cm^3). The surface area of nopalitos in 2017 (12.82 cm^2) was significantly ($p < 0.001$) smaller than in 2018 harvest (26.95 cm^2). The firmness of nopalitos in 2017 (63.32 10ths m/m) was significantly ($p = 0.009$) lower than the 2018 harvest (66.56 10ths m/m). There were no significant differences for the compressibility of nopalitos over the two years. The morphological characteristics had an effect on the year of harvest because of weather changes observed over the two years of harvest. Nopalitos harvested in year 2018 were smaller and thicker compared to those harvested in year 2017.

In terms of colour, no significant differences for the L* of nopalitos over the two years. The a* value of nopalitos in 2017 (-16.80) was significantly ($p = 0.033$) lower than in the 2018 harvest (-16.09), and there were no significant differences between the b* and C* values of nopalitos over the two years. The h° of nopalitos in 2017 (123.87 °) was significantly ($p = 0.002$) higher than in the 2018 harvest (122.61 °).

The moisture content of nopalitos in 2017 (88.40 %) was significantly ($p = 0.001$) higher than in the 2018 harvest (87.78 %). The mucilage content of nopalitos in 2017 (41.60 %) was significantly ($p < 0.001$) higher than in the 2018 harvest (31.16 %). The viscometer viscosity of mucilage in 2017 (25.49 cP) was significantly ($p < 0.001$) lower than in the 2018 harvest (259.41 cP). The TA as % citric acid of mucilage in 2017 (0.42 %) was significantly ($p < 0.001$) higher than in the 2018 harvest (0.33 %). The pH of nopalitos in 2017 (4.16) was significantly ($p = 0.007$) lower than in the 2018 harvest (4.23). The TSS of mucilage in 2017 (5.44 °Brix) was significantly ($p < 0.001$) lower than in the 2018 harvest (6.32 °Brix). The significant differences between the two years might be caused by the climatic changes over the years, as 2018 was perceived to yield best nopalitos than in the year 2017. Also the year 2017 was the driest year according to SAWS. It may be deduced that climatic conditions may affect the quality of nopalitos.

Table 3.6: Analysis of variance (ANOVA) for the effect of year on the characteristics of nopalitos

Year	2017	2018	Sign. Level
Weight (g)	$99.19^{b} \pm 57.34$	$81.77^{a} \pm 52.32$	$p = 0.007$
Width (cm)	9.20 ± 2.49	8.99 ± 2.45	$p = 0.487$
Diameter (cm)	$0.86^{b} \pm 0.34$	$0.57^{a} \pm 0.24$	$p < 0.001$
Volume (cm^3)	$158.92^{a} \pm 120.05$	$216.99^{b} \pm 229.18$	$p = 0.007$
S. area (cm^2)	$12.82^{a} \pm 6.83$	$26.95^{b} \pm 25.36$	$p < 0.001$
Firmness (10ths m/m)	$63.32^{a} \pm 11.08$	$66.56^{b} \pm 9.91$	$p = 0.009$
Compressibility (10ths m/m)	29.18 ± 7.82	30.56 ± 5.59	$p = 0.086$
L*	48.90 ± 4.02	48.13 ± 5.09	$p = 0.156$
a*	$-16.80^{a} \pm 2.34$	$-16.09^{b} \pm 3.20$	$p < 0.033$
b*	25.69 ± 6.48	25.44 ± 6.80	$p = 0.745$
C*	30.75 ± 6.61	29.84 ± 7.45	$p = 0.274$
Hue (h°)	$123.87^{b} \pm 3.68$	$122.61^{a} \pm 3.02$	$p = 0.002$
Moisture content (%)	$88.40^{b} \pm 1.58$	$87.78^{a} \pm 1.59$	$p = 0.001$
Mucilage yield (%)	$41.60^{b} \pm 15.74$	$31.16^{a} \pm 12.58$	$p < 0.001$
Viscosity (cP)	$25.49^{a} \pm 29.10$	$259.41^{b} \pm 257.47$	$p < 0.001$
TA as citric acid (%)	$0.42^{b} \pm 0.09$	$0.33^{a} \pm 0.10$	$p < 0.001$
pH	$4.16^{a} \pm 0.20$	$4.23^{b} \pm 0.25$	$p = 0.007$
TSS (°Brix)	$5.44^{a} \pm 0.77$	$6.32^{b} \pm 0.72$	$p < 0.001$

Means with different superscripts in the same row differ significantly

3.4.2 ANOVA for the various interactions between size, cultivar, season and year on the characteristics of nopalitos

The interaction between size and cultivar had a highly significant ($p < 0.001$) effect on the weight, width, volume, surface area, firmness, colour parameters only b* and on the pH of nopalitos (Table 3.7). The interaction between size and cultivar also had a significant effect on diameter ($p = 0.006$), and in terms of colour L* ($p = 0.001$), a* ($p = 0.003$), C* ($p = 0.001$), h° ($p = 0.001$), TA as % citric acid ($p= 0.028$) and TSS ($p = 0.020$). It was deduced that the interaction between size and cultivar had the most significant effect on the size, texture, colour, sliminess and gustatory properties.

The interaction between size and season had a highly significant ($p < 0.001$) effect on the surface area, viscometer viscosity, TA as % citric acid and pH ($p < 0.001$). The interaction between size and season also showed a significant effect on the width ($p = 0.002$), volume ($p = 0.003$), moisture content ($p= 0.020$) and mucilage yield ($p= 0.012$). It was deduced that the interaction between size and season had significant effects on size, turgidity, sliminess and gustatory properties.

The interaction between cultivar and season had a highly significant ($p < 0.001$) effect only on the mucilage yield. The interaction between cultivar and season also had significant effects on the weight ($p = 0.002$), h° ($p = 0.042$), viscometer viscosity ($p = 0.025$), TA as citric acid ($p = 0.012$), pH ($p = 0.002$) and TSS ($p = 0.018$). It was deduced that the interaction between cultivar and season had the least significant effects on size, only colour parameter h°, sliminess and gustatory properties.

The interaction between size, cultivar and season had a highly significant ($p < 0.001$) effect on the volume, surface area and TA as % citric acid. The interaction between size, cultivar and season also had significant effects on the a*($p = 0.018$), moisture content ($p = 0.015$), viscometer viscosity ($p = 0.041$) and TSS ($p = 0.009$). It was deduced that the interaction between size, cultivar and season had the least significant effects on size, only colour parameter a*, turgidity, sliminess and gustatory properties.

The interaction between size and year had a highly significant ($p < 0.001$) effect on the surface area, firmness, mucilage yield, and viscometer viscosity. The interaction between size and year had also showed significant effects on the weight ($p = 0.001$), diameter ($p = 0.041$), volume ($p = 0.026$), h° ($p = 0.007$), moisture content ($p = 0.001$), TA as % citric acid ($p = 0.007$) and TSS ($p = 0.024$). It was deduced that the interaction between size and year had significant effects on size, texture, only colour parameter h°, turgidity, sliminess and gustatory properties.

The interaction between cultivar and year had highly significant ($p < 0.001$) effects on the firmness, colour parameters L*, a*, b*,C*,h° and TA as % citric acid. The interaction between cultivar and year also had a significant effect on the mucilage yield ($p = 0.030$), viscometer viscosity ($p = 0.006$), pH ($p = 0.026$) and TSS ($p = 0.004$). It was deduced that the interaction between cultivar and year had significant effects on texture, colour, sliminess and gustatory properties.

The interaction between size, cultivar and year had a highly significant ($p < 0.001$) effect on the weight, diameter, surface area, only colour parameter a* and TA as % citric acid. The interaction between size, cultivar and year also showed significant effects on the volume ($p = 0.001$), firmness ($p = 0.003$), L* ($p = 0.042$), b* ($p = 0.003$), C* ($p = 0.004$), moisture content ($p = 0.008$), mucilage yield ($p = 0.001$), viscometer viscosity ($p = 0.028$) and TSS ($p = 0.002$). It was deduced that the interaction between size, cultivar and year had significant effect on size, texture, colour, turgidity, sliminess and gustatory properties.

The interaction between season and year had a highly significant ($p < 0.001$) effect on the diameter, volume, surface area, firmness, compressibility, L*, a*, b*, C*, viscometer viscosity and pH ($p < 0.001$). The interaction between season and year showed significant effects on the width ($p = 0.050$), mucilage yield ($p = 0.012$), TA as % citric acid ($p = 0.002$) and TSS ($p = 0.006$). It was deduced that the interaction between season and year had the most significant effect on the size, texture, colour, sliminess and gustatory properties of the nopalitos.

The interaction between size, season and year had a highly significant ($p < 0.001$) effect on the weight, volume, surface area, moisture content, mucilage yield, viscometer viscosity, TA as % citric acid and pH ($p < 0.001$). It was deduced that the interaction between size, season and year also had significant effect on size, turgidity, sliminess and gustatory properties.

The interaction between cultivar, season and year had a highly significant ($p < 0.001$) effect on the width, compressibility, L*, a*, b*, C*, h°, mucilage yield, and TA as % citric acid. The interaction between cultivar, season and year also had a significant effect on the weight ($p = 0.007$), volume ($p = 0.001$) and pH (0.005). It was deduced that the interaction between cultivar, season and year had significant effects on size, texture, colour, sliminess and gustatory properties.

The interaction between size, cultivar, season and year had a highly significant ($p < 0.001$) effect on the diameter, volume and surface area. The interaction between size, cultivar, season and year also showed significant effects on the compressibility ($p = 0.027$), L* ($p = 0.043$), a* ($p = 0.017$), b* ($p = 0.012$), C* ($p = 0.021$), viscometer viscosity ($p = 0.018$), TA as % citric acid ($p = 0.030$) and pH ($p = 0.001$). It was deduced that the interaction between size, cultivar, season and year had significant effects on size, texture, colour, sliminess and gustatory properties of the nopalitos.

The interaction between size and cultivar, the interaction between size and season and the interaction between size and year were discussed. These interactions were discussed in order to understand the relationship between the size and cultivar, size and time of harvest. The other interactions were not further discussed.

Table 3.7: Analysis of variance (ANOVA) for the interaction between size, cultivar, season and year on the characteristics of nopalitos

	A X B	A X C	B X C	A X B X C	A X D	B X D	A X B X D	C X D	A X C X D	B X C X D	A X B X C X D
Weight (g)	$p < 0.001$	$p = 0.113$	$p = 0.002$	$p = 0.073$	$p = 0.001$	$p = 0.700$	$p < 0.001$	$p = 0.489$	$p < 0.001$	$p = 0.007$	$p = 0.522$
Width (cm)	$p < 0.001$	$p = 0.002$	$p = 0.079$	$p = 0.785$	$p = 0.675$	$p = 0.276$	$p = 0.709$	$p = 0.050$	$p = 0.207$	$p < 0.001$	$p = 0.266$
Diameter (cm)	$p = 0.006$	$p = 0.582$	$p = 0.964$	$p = 0.229$	$p = 0.041$	$p = 0.296$	$p < 0.001$	$p < 0.001$	$p = 0.862$	$p = 0.763$	$p < 0.001$
Volume (cm^3)	$p < 0.001$	$p = 0.003$	$p = 0.503$	$p < 0.001$	$p = 0.026$	$p = 0.491$	$p = 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.001$	$p < 0.001$
S. area (cm^2)	$p < 0.001$	$p < 0.001$	$p = 0.902$	$p < 0.001$	$p < 0.001$	$p = 0.804$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.338$	$p < 0.001$
Firmness (10ths m/m)	$p < 0.001$	$p = 0.173$	$p = 0.122$	$p = 0.072$	$p < 0.001$	$p < 0.001$	$p = 0.003$	$p < 0.001$	$p = 0.634$	$p = 0.072$	$p = 0.049$
Compressibility (10ths m/m)	$p = 0.133$	$p = 0.062$	$p = 0.253$	$p = 0.071$	$p = 0.599$	$p = 0.284$	$p = 0.456$	$p < 0.001$	$p = 0.294$	$p < 0.001$	$p = 0.027$
L*	$p = 0.001$	$p = 0.434$	$p = 0.147$	$p = 0.181$	$p = 0.817$	$p < 0.001$	$p = 0.042$	$p < 0.001$	$p = 0.268$	$p < 0.001$	$p = 0.043$
a*	$p = 0.003$	$p = 0.217$	$p = 0.478$	$p = 0.018$	$p = 0.089$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.884$	$p < 0.001$	$p = 0.017$
b*	$p < 0.001$	$p = 0.202$	$p = 0.394$	$p = 0.147$	$p = 0.137$	$p < 0.001$	$p = 0.003$	$p < 0.001$	$p = 0.637$	$p < 0.001$	$p = 0.012$
C*	$p = 0.001$	$p = 0.350$	$p = 0.772$	$p = 0.061$	$p = 0.209$	$p < 0.001$	$p = 0.004$	$p < 0.001$	$p = 0.502$	$p < 0.001$	$p = 0.021$
Hue (h°)	$p = 0.001$	$p = 0.469$	$p = 0.042$	$p = 0.965$	$p = 0.007$	$p < 0.001$	$p = 0.045$	$p = 0.249$	$p = 0.124$	$p < 0.001$	$p = 0.202$
Moisture content (%)	$p = 0.323$	$p = 0.020$	$p = 0.497$	$p = 0.015$	$p = 0.001$	$p = 0.293$	$p = 0.008$	$p = 0.908$	$p < 0.001$	$p = 0.141$	$p = 0.162$
Mucilage yield (%)	$p = 0.092$	$p = 0.012$	$p < 0.001$	$p = 0.054$	$p < 0.001$	$p = 0.030$	$p = 0.001$	$p = 0.012$	$p < 0.001$	$p < 0.001$	$p = 0.447$
Viscosity (cP)	$p = 0.047$	$p < 0.001$	$p = 0.025$	$p = 0.041$	$p < 0.001$	$p = 0.006$	$p = 0.028$	$p < 0.001$	$p < 0.001$	$p = 0.100$	$p = 0.018$
TA as citric acid (%)	$p = 0.028$	$p < 0.001$	$p = 0.012$	$p < 0.001$	$p = 0.007$	$p < 0.001$	$p < 0.001$	$p = 0.002$	$p < 0.001$	$p < 0.001$	$p = 0.030$
pH	$p < 0.001$	$p < 0.001$	$p = 0.002$	$p = 0.145$	$p = 0.564$	$p = 0.026$	$p = 0.280$	$p < 0.001$	$p < 0.001$	$p = 0.005$	$p = 0.001$
TSS (°Brix)	$p = 0.020$	$p = 0.142$	$p = 0.018$	$p = 0.009$	$p = 0.024$	$p = 0.004$	$p = 0.002$	$p = 0.006$	$p = 0.515$	$p = 0.165$	$p = 0.172$

A = Size; B = Cultivar; C = Season; D = Year

3.4.3 Morphological characteristics

It was important to observe the measurements of weight, width, diameter, volume and surface area as it is helpful to provide insight into the two cultivars at different growth lengths. It could also help to identify a cultivar of a standard size suitable for consumer preference.

3.4.3.1 Interaction between size and cultivar

In Table 3.8, the weight of Fusicaulis nopalitos at 9 cm (32.5 g) was significantly ($p < 0.001$) lower than at 24 cm (185.33 g). The weight of nopalitos was significantly ($p < 0.001$) different between 9, 12, 18 and 21 cm. The weight of Morado nopalitos at 9 cm (21.33 g) was significantly ($p < 0.001$) lower than at 24 cm (138.33 g). The weight of nopalitos was significantly ($p < 0.001$) different between 9 cm, 15 cm, 18 cm, 21 cm and 24 cm. No significant differences were detected between the weight of the cultivars of nopalitos at 9 cm. Fusicaulis nopalitos were significantly ($p < 0.001$) heavier than Morado nopalitos from 12 cm to 24 cm as illustrated in Table 3.8. Fusicaulis nopalitos appeared bigger and heavier when visually observed compared to Morado nopalitos that looked lighter in mass and smaller. An increase in weight was observed as nopalitos developed in both cultivars, where, younger nopalitos had a lower weight and were visually smaller, older nopalitos had higher weight and appeared bigger. According to Feugang *et al*. (2006); Rodriguez-Felix (2002) young tender nopalitos that are frequently harvested and consumed commercially have a weight that ranges from 40-100 g and 11-20 cm in length. Similarly, Rodriguez-Felix & Cantwell (1988) reported an optimal weight being 50-80 g and length from 15-25 cm. Thus, Morado nopalitos were closer to the ideal weight and size as proposed by Feugang *et al*. (2006) and Rodriguez-Felix (2002).

The width of Fusicaulis nopalitos at 9 cm (6. 0 cm) was significantly ($p < 0.001$) lower than at 24 cm (11.61 cm). The width of nopalitos was significantly ($p < 0.001$) different between 9 cm, 12 cm, 18 cm and 24 cm. The width of Morado nopalitos at 9 cm (5.68 cm) was significantly ($p < 0.001$) lower than at 24 cm (12.9 cm). The width of nopalitos was significantly ($p < 0.001$) different between 9 cm, 12 cm, 15 cm, 18 cm and 24 cm. There were no significant differences between the two cultivars in terms of the width of nopalitos at 9 cm to 21 cm, only the width of Fusicaulis nopalitos at 24 cm was significantly ($p < 0.001$) lower than Morado nopalitos. It corresponded with visual appearance of nopalitos at 24 cm, Fusicaulis nopalitos had an elongated shape and Morado nopalitos had a round shape. The diameter of Fusicaulis nopalitos at 9 cm (0.65 cm) was significantly ($p = 0.006$) lower than 21 cm (1 cm). There were no significant differences in the diameter of nopalitos between 12 cm and 24 cm. The diameter of Morado nopalitos at 9 cm (0.36 cm) was significantly ($p = 0.006$) lower than 24 cm (0.72 cm). There were no significant differences in the diameter of nopalitos between 12 cm and 21 cm. The diameter of Morado nopalitos was significantly ($p = 0.006$) lower than Fusicaulis nopalitos in all the sizes. According to Lima *et al*. (2016), the difference in width and diameter of cladodes can be affected by physiological and biochemical processes such as photosynthesis, respiration and transpiration.

The volume of Fusicaulis nopalitos at 9 cm (50.54 cm^3) was significantly ($p < 0.001$) lower than at 24 cm (336.35 cm^3). The volume of Fusicaulis nopalitos between 9 cm and 15 cm was significantly ($p < 0.001$) different compared to the volume between 18 cm and 24 cm. The volume of Morado nopalitos at 9 cm (23.34 cm^3) was significantly ($p < 0.001$) lower compared to 24 cm (359.35 cm^3). The volume of Morado nopalitos between 12 cm and 15 cm was significantly ($p < 0.001$) lower compared to nopalitos between 18 cm and 21 cm. In terms of cultivars, the volume of Morado nopalitos was significantly ($p < 0.001$) lower than Fusicaulis nopalitos only at 18 cm. The surface area of Fusicaulis nopalitos at 9 cm (7.10 cm^2) was significantly ($p < 0.001$) smaller compared to 18 cm (30.22 cm^2) and 21 cm (26.68 cm^2). The surface area of Morado nopalitos at 9 cm (5.30 cm^2) was significantly ($p < 0.001$) smaller than at 24 cm (33.48 cm^2). The surface area of Morado nopalitos at 12 cm was significantly ($p < 0.001$) smaller compared to nopalitos at 21 cm. There were no significant differences observed between the two cultivars in terms of surface area. Nopalitos between 15 and 18 cm were found to have optimal results and could thus, be described as high quality and ready to be harvested.

Table 3.8: Analysis of variance (ANOVA) for the interaction between size and cultivar on the morphological characteristics of nopalitos

Size	**9 cm**		**12 cm**		**15cm**		**18cm**		**21cm**		**24cm**		**Sign.**
Cultivar	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**level**
Weight (g)	32.50^{a}	21.33^{a}	66.65^{cd}	37.17^{ab}	79.45^{d}	54.21^{bc}	119.84^{ef}	80.21^{d}	167.035^{g}	103.71^{e}	185.33^{g}	138.33^{f}	$p < 0.001$
Width (cm)	6.00^{a}	5.68^{a}	7.31^{b}	7.17^{b}	8.03^{bc}	8.60^{cd}	9.50^{de}	10.25^{ef}	10.94^{fg}	11.16^{fg}	11.61^{g}	12.90^{h}	$p < 0.001$
Diameter (cm)	0.65^{b}c	0.36^{a}	0.92^{de}	0.49^{ab}	0.90^{de}	0.51^{ab}	0.86^{cde}	0.60^{ab}	1.00^{e}	0.61^{abc}	0.90^{de}	0.72^{bcd}	$p = 0.006$
Volume (cm³)	50.54^{ab}	23.34^{a}	132.86^{abc}	54.64^{ab}	185.90^{bcd}	90.52^{abc}	332.79^{ef}	166.71^{bc}	310.36^{def}	212.09^{cde}	336.35^{ef}	359.35^{f}	$p < 0.001$
S. area (cm²)	7.10^{ab}	5.30^{a}	12.96^{abc}	9.70^{ab}	18.65^{abcde}	15.37^{abcd}	30.22^{de}	23.44^{bcde}	26.68^{cde}	32.72^{e}	23.01^{bcde}	33.48^{e}	$p < 0.001$

Means with different superscripts in the same row differ significantly

3.4.3.2 Interaction between size and season

In Table 3.9, no significant differences in the weight of nopalitos were observed between the two seasons, however, differences were detected between the growth sizes. The width of nopalitos harvested in autumn at 9 cm (5.61 cm) was significantly ($p = 0.002$) lower than nopalitos at 24 cm (11.45 cm). The width of nopalitos harvested in autumn was significantly ($p = 0.002$) different between 9 cm, 12 cm, 18 cm and 24 cm. The width of nopalitos harvested in spring at 9 cm (6.07 cm) was significantly ($p = 0.002$) lower than nopalitos at 24 cm (13.06 cm). The width of nopalitos harvested in spring was significantly ($p = 0.002$) different between growth sizes, however, no significant differences were obtained between 18 cm and 21 cm. It was observed that nopalitos (18 and 24 cm) harvested in spring had a significantly ($p = 0.002$) wider width than the autumn harvest. The diameter of nopalitos did not significantly differ between the two seasons, however, differences were observed between the growth sizes. These results were in disagreement with the work of *Opuntia excels* studied by Lerdau *et al*. (1992) implying that cladodes had a thicker diameter in the wet season (September) than in dry season (March) due to changes in the parenchyma.

The volume of nopalitos harvested in autumn at 9 cm (28.58 cm^3) was significantly ($p = 0.003$) smaller than nopalitos at 24 cm (288.71 cm^3). The volume of nopalitos harvested in autumn was significantly ($p = 0.003$) different between 9 cm, 18 cm and 24 cm. The volume of nopalitos harvested in spring at 9 cm (45.31 cm^3) was significantly ($p = 0.003$) smaller than nopalitos at 24 cm (406.99 cm^3). The volume of nopalitos harvested in spring between 9 cm and 12 cm were significantly ($p = 0.003$) different compared to nopalitos between 18 cm and 24 cm. The volume of nopalitos harvested in spring had a significantly ($p = 0.003$) larger volume than those in autumn harvest at 18 cm. The surface area of nopalitos harvested in autumn at 9 cm (3.97 cm^2) was significantly ($p < 0.001$) smaller than nopalitos at 24 cm (21.59 cm^2). The surface area of nopalitos harvested in spring at 9 cm (8.43 cm^2) was significantly ($p < 0.001$) smaller than nopalitos at 21 cm (41.41 cm^2). The surface area of nopalitos harvested in spring was significantly ($p < 0.001$) different between 9 cm, 15 cm and 18 cm. It could therefore be deduced that nopalitos (15 cm, 18 cm and 21 cm) harvested during spring had significantly ($p < 0.001$) larger surface area than those harvested during the autumn harvest. Inglese *et al*. (1999) had a different observation in which, there were no differences in surface area of cladodes between the spring and summer development.

Table 3.9: Analysis of variance (ANOVA) for the interaction between size and season on the morphological characteristics of nopalitos

Size	**9 cm**		**12 cm**		**15cm**		**18cm**		**21cm**		**24cm**		**Sign.**
Season	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**level**
Weight (g)	26.60	27.24	57.45	46.37	68.15	66.45	99.30	98.38	143.18	127.56	161.73	161.93	$p = 0.113$
Width (cm)	5.61^{a}	6.07^{a}	7.14^{b}	7.35^{b}	7.97^{bc}	8.70^{cd}	9.26^{d}	10.45^{e}	10.90^{ef}	11.20^{ef}	11.45^{f}	13.06^{g}	$p = 0.002$
Diameter (cm)	0.56	0.44	0.77	0.65	0.80	0.61	0.82	0.63	0.90	0.72	0.93	0.69	$p = 0.582$
Volume (cm^3)	28.58^{a}	45.31^{ab}	66.82^{ab}	120.69^{abc}	96.20^{abc}	185.69^{bcde}	173.73^{bcd}	324.52^{ef}	216.36^{cde}	306.09^{def}	288.71^{def}	406.99^{f}	$p = 0.003$
S. area (cm^2)	3.97^{a}	8.43^{ab}	6.46^{a}	16.21^{abc}	9.39^{ab}	25.16^{cd}	13.74^{abc}	40.53^{e}	17.98^{abc}	41.41^{e}	21.59^{bcd}	34.90^{de}	$p < 0.001$

Means with different superscripts in the same row differ significantly

3.4.3.3 Interaction between size and year

In Table 3.10, the weight of nopalitos harvested in 2017 at 9 cm (27.44 g) was significantly ($p < 0.001$) lower than nopalitos at 24 cm (171.46 g). There were significant ($p < 0.001$) differences in the weight of nopalitos between 9 cm, 12 cm, 18 cm and 21 cm in the 2017 harvest. The weight of nopalitos harvested in 2018 at 9 cm (26.39 g) was significantly ($p < 0.001$) lower than nopalitos at 24 cm (152.20 g). There were significant ($p < 0.001$) differences in the weight of nopalitos between 9 cm, 15 cm, 21 cm and 24 cm. Nopalitos (18 and 21 cm) harvested in 2017 were significantly ($p < 0.001$) heavier than those in the 2018 harvest. The width was not significantly different ($p = 0.675$), however, there were differences detected between the growth sizes. The diameter of nopalitos harvested in 2017 at 9 cm (0.60 cm) was significantly ($p = 0.041$) lower than nopalitos at 21 cm (0.96 cm). There were significant ($p = 0.041$) differences in the diameter of nopalitos between 9 cm and 12 cm. The diameter of nopalitos harvested in 2018 at 9 cm (0.40 cm) was significantly ($p = 0.041$) lower than nopalitos at 24 cm (0.69 cm). It was observed that nopalitos (12 cm, 15 cm and 21 cm) harvested in 2017 had a significantly ($p = 0.041$) wider diameter than those in the 2018 harvest. The volume of nopalitos harvested in 2017 at 9 cm (31.64 cm^3) was significantly ($p = 0.026$) smaller than nopalitos at 24 cm (309.79^3). There were significant ($p = 0.026$) differences in the volume of nopalitos between 12 cm and 18 cm harvested in 2017. The volume of nopalitos harvested in 2018 at 9 cm (42.25 cm^3) was significantly ($p = 0.026$) smaller than nopalitos at 24 cm (385.91 cm^3). The volume of nopalitos between 9 cm and 12 cm were significantly ($p = 0.026$) different compared to nopalitos between 18 cm and 24 cm. There were no significant differences in the volume of nopalitos between the two years. The surface area of nopalitos harvested in 2017 at 9 cm (4.07 cm^2) was significantly ($p < 0.001$) smaller than nopalitos at 24 cm (23.40 cm^2). The surface area of nopalitos harvested in 2018 at 9 cm (8.33 cm^2) was significantly ($p < 0.001$) smaller than nopalitos at 21 cm (40.97 cm^2). There were significant ($p < 0.001$) differences in surface area of nopalitos between 9 cm, 15 cm and 18 cm. It was observed that nopalitos between 18 cm and 21 cm harvested in 2017 had a significantly ($p < 0.001$) smaller surface area than those in the 2018 harvest.

Table 3.10: Analysis of variance (ANOVA) for the interaction between size and year on the morphological characteristics of nopalitos

Size	9 cm		12 cm		15 cm		18 cm		21 cm		24 cm		Sign.
Year	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**level**
Weight (g)	27.44^{a}	26.39^{a}	60.50^{bc}	43.33^{ab}	72.60^{c}	61.06^{bc}	113.82^{d}	86.23^{c}	149.34^{e}	121.41^{d}	171.46^{e}	152.20^{e}	$p < 0.001$
Width (cm)	5.75	5.93	7.28	7.21	8.51	8.11	10.04	9.69	11.17	10.93	12.41	12.10	$p = 0.675$
Diameter (cm)	0.60^{abc}	0.40^{a}	0.93^{de}	0.49^{ab}	0.85^{cde}	0.56^{ab}	0.86^{cde}	0.61^{abc}	0.96^{e}	0.65^{abc}	0.93^{de}	0.69^{bcd}	$p = 0.041$
Volume (cm^3)	31.64^{a}	42.25^{a}	81.93^{ab}	105.57^{abc}	105.87^{abc}	170.55^{abcd}	189.32^{bcd}	310.17^{de}	234.99^{cd}	287.46^{de}	309.79^{de}	385.91^{e}	$p = 0.026$
S. area (cm^2)	4.07^{a}	8.33^{ab}	6.86^{a}	15.80^{abc}	9.93^{abc}	24.08^{cd}	14.21^{abc}	39.45^{e}	18.42^{abcd}	40.97^{e}	23.40^{bcd}	33.09^{de}	$p < 0.001$

Means with different superscripts in the same row differ significantly

3.4.4 Texture

The texture of nopalitos was determined since it is one of the most important characteristics that play a role in the quality of vegetables, when the product is placed in the mouth and chewed the texture can be perceived (Barrett *et al.*, 2010). During the texture analysis with the penetrometer the depth that the needle was able to penetrate into the nopalito and flat attachment covered to compress was measured in 10ths m/m. The high penetration values indicated less firmness and more compression of nopalitos (softness) and low values are indicative of more firmness and little compression (hardness). Less firm nopalitos are accepted and associated with good quality. The higher the reading, the softer the sample as the attachment will penetrate deeper into a softer sample (Du Toit, 2017; McWilliams, 2016). The texture measured by the penetrometer showed the measurements of the interaction between size and cultivar (Table 3.11). The interaction between size and season on the texture of nopalitos (Table 3.12). Illustrated in Table 3.13 was the interaction between size and year on the texture of nopalitos.

3.4.4.1 Interaction between size and cultivar

In Table 3.11, the firmness of Fusicaulis nopalitos at 24 cm (53.6 10ths m/m) was significantly ($p < 0.001$) firmer than nopalitos at 15 cm (68.84 10ths m/m). There were significant ($p < 0.001$) differences between 15 cm and 21 cm. The firmness of Morado nopalitos at 9 cm (59.24 10ths m/m) was significantly ($p < 0.001$) firmer than nopalitos at 15 cm (76.13 10ths m/m). There were significant ($p < 0.001$) differences between 9 cm and 12 cm. Fusicaulis nopalitos between 18 and 24 cm had a significantly ($p < 0.001$) firmer texture than Morado nopalitos. At 18 cm, Morado nopalitos (75.94 10ths m/m) were significantly ($p < 0.001$) less firm than Fusicaulis nopalitos (61.75 10ths m/m). As the nopalitos developed, at 21 cm Morado nopalitos (70.17 10ths m/m) had a significantly ($p < 0.001$) less firm texture than Fusicaulis nopalitos (56.62 10ths m/m). When nopalitos reached 24 cm, Morado nopalitos (62.17 10ths m/m) had a significantly ($p < 0.001$) less firm texture than Fusicaulis nopalitos (53.60 10ths m/m). Interestingly, Fusicaulis nopalitos were more firm at 24 cm on the other hand, Morado nopalitos were more firm at 9 cm. It can be explained that, Fusicaulis nopalitos were less firm at early development, while, Morado nopalitos were more firm at early development. Another interesting observation was that both cultivars reached their highest penetration value (Fusicaulis 68.84 10ths m/m and Morado 76.13 10ths m/m) at

15 cm which is associated with less firm texture. In other words, this length could be an ideal length of harvest as both cultivars became more tender at this size. On the other hand, Morado nopalitos at 18 cm could be an ideal length of harvest compared to Fusicaulis nopalitos. No significant differences were observed for the compressibility of both cultivars, however, differences were perceived between the growth sizes.

Table 3.11: Analysis of variance (ANOVA) for the interaction between size and cultivar on the texture of nopalitos

Size Cultivar	9 cm Fusicaulis	 Morado	12 cm Fusicaulis	 Morado	15cm Fusicaulis	 Morado	18cm Fusicaulis	 Morado	21cm Fusicaulis	 Morado	24cm Fusicaulis	 Morado	Sign. level
Firmness (10ths m/m)	62.94^{bcd}	59.24^{ab}	63.06^{bcd}	68.79^{cde}	68.84^{cde}	76.13^{e}	61.75^{bc}	75.94^{e}	56.62^{ab}	70.17^{de}	53.60^{a}	62.17^{bc}	$p < 0.001$
Compressibility (10ths m/m)	30.30	30.00	30.38	30.42	27.21	26.80	29.83	31.21	31.68	29.74	32.83	28.08	$p = 0.133$

Means with different superscripts in the same row differ significantly

3.4.4.2 Interaction between size and season

In terms of texture, there were no significant differences on the firmness ($p = 0.173$) and compressibility ($p = 0.062$) over the two seasons, however, differences were perceived between the growth sizes (Table 3.12). It can be deduced that the time of harvest does not influence the texture of nopalitos. The texture of nopalitos harvested in either season would be comparable. In contrast, Inglese *et al*. (1999) observed that the spring harvest had thicker cladodes than those of summer harvest.

3.4.4.3 Interaction between size and year

The firmness of nopalitos harvested in 2017 at 9 cm (59.76 10ths m/m) was significantly ($p < 0.001$) firmer than nopalitos at 15 cm (69.47 10ths m/m). The firmness of nopalitos harvested in 2018 at 24 cm (57.41 10ths m/m) was significantly ($p < 0.001$) firmer than nopalitos at 15 cm (75.51 10ths m/m) illustrated in Table 3.13. There were significant ($p < 0.001$) differences in the firmness of nopalitos between 9 cm and 15 cm. Nopalitos (12 cm) harvested in 2017 had a significantly ($p < 0.001$) firmer texture than those harvested in 2018. It was interesting to observe that over the two years nopalitos at 15 cm had a less firm texture, which could be associated with high quality. No significant ($p = 0.599$) differences were observed for compressibility over the two years, however, differences were observed between the growth sizes. It was thus observed that at 15 cm the texture of nopalitos harvested in both years was of the highest quality and was comparable.

Table 3.12: Analysis of variance (ANOVA) for the interaction between size and season on the texture of nopalitos

Size	9 cm		12 cm		15cm		18cm		21cm		24cm		Sign.
Season	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	level
Firmness (10ths m/m)	61.32	60.87	62.55	69.31	69.89	75.27	67.82	70.21	61.40	65.39	55.56	60.22	p = 0.173
Compressibility (10ths m/m)	27.68	32.63	29.21	31.58	24.52	29.86	30.44	30.42	30.10	31.32	29.91	31.00	p = 0.062

Means with different superscripts in the same row differ significantly

Table 3.13: Analysis of variance (ANOVA) for the interaction between size and year on the texture of nopalitos

Size	9 cm		12 cm		15 cm		18 cm		21 cm		24 cm		Sign.
Year	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	level
Firmness (10ths m/m)	59.76^{ab}	62.43^{abcd}	60.87^{abc}	70.99^{de}	69.47^{cde}	75.51^{e}	66.49^{bcd}	71.20^{de}	64.96^{abcd}	61.83^{abc}	58.36^{ab}	57.41^{a}	p < 0.001
Compressibility (10ths m/m)	29.72	30.58	30.54	30.25	25.79	28.21	30.54	30.50	29.52	31.91	28.98	31.94	p = 0.599

Means with different superscripts in the same row differ significantly

3.4.5 Colour

The three colour coordinates of CIELAB represent lightness of colour (L*= 0 indicate black and L*= 100 indicate white), red and green (where a* negative values = green and positive values = red) and yellow and blue (b* negative values = blue and positive values = yellow). The other two coordinates of CIELCH represent saturation of colour (C*= 0 indicate unsaturated and C*= 100 indicate brightness of colour) and position of colour in degrees on a colour wheel (where h°= 0° indicate red, h°= 90° indicate yellow, h°= 180° indicate green and h°= 270° indicate blue).

3.4.5.1 Interaction between size and cultivar

In Table 3.14, L* values of Fusicaulis nopalitos ranged from 44.80 (12 cm) to 48.21 (9 cm) and there were no significant differences between the growth sizes. Morado nopalitos at 24 cm (49.22) had a significantly ($p = 0.001$) lower lightness (L*) than nopalitos at 9 cm (53.48). There were significant ($p = 0.001$) differences in the lightness of nopalitos between 9 cm and 21 cm. The lightness value of Morado nopalitos was significantly ($p = 0.001$) higher compared to Fusicaulis nopalitos. The a* values of Fusicaulis nopalitos at 9 cm (-17.14) was significantly lower than nopalitos at 21 cm (-14.50). There were significant ($p = 0.003$) differences between 9 cm and 12 cm. No significant differences were observed in a* values of Morado nopalitos between the growth sizes. It was observed that only Morado nopalitos at 12 cm, 18 cm and 21 cm that had a significantly ($p = 0.003$) greener tone than Fusicaulis nopalitos. The b* values of Fusicaulis nopalitos at 18 cm (20.17) was significantly ($p < 0.001$) lower than nopalitos at 9 cm (26.15). No significant differences were observed between the growth sizes, excluding nopalitos at 9 cm. The b* values of Morado nopalitos at 24 cm (25.32) was significantly ($p < 0.001$) lower than nopalitos at 9 cm (34.62). There were significant ($p < 0.001$) differences on the b* values of nopalitos between 9 cm and 15 cm. Fusicaulis nopalitos had a significantly ($p < 0.001$) less yellow tone than Morado nopalitos throughout growth sizes. It was perceived that the yellow tone decreased as nopalitos developed. Morado nopalitos at 9 cm were more yellow to greener in colour than Fusicaulis nopalitos. The C* values of Fusicaulis nopalitos at 18 cm (24.14) was significantly ($p = 0.001$) less bright than nopalitos at 9 cm (31.29). No significant differences were observed on Fusicaulis nopalitos between the growth sizes, excluding nopalitos at 9 cm which had more bright characteristics. The C* values of Morado nopalitos at 24 cm (29.67) was significantly ($p = 0.001$) less bright than nopalitos at 9 cm

(39.35). There were significant (p = 0.001) differences on the brightness of Morado nopalitos between 9 cm 15 cm. Morado nopalitos had significantly (p = 0.001) more brightness compared to Fusicaulis nopalitos throughout growth sizes. The h° of Fusicaulis nopalitos at 9 cm (123.44°) was significantly (p = 0.001) lower than nopalitos at 24 cm (126.69°). The h° of Morado nopalitos at 9 cm (118. 08°) was significantly (p = 0.001) lower than nopalitos at 24 cm (122.89°). Morado nopalitos between 9 cm and 12 cm had significantly (p = 0.001) less yellow-green colour compared to nopalitos between 15 cm and 24 cm.

Thus, Morado was found to have colour characteristics associated with higher quality than Fusicaulis, excluding the h° of Fusicaulis that was higher than Morado nopalitos.

3.4.5.2 Interaction between size and season

The interaction between size and season did not influence the colour (L*, a*, b*, C* and h°) of nopalitos, however, there were differences perceived between growth sizes (Table 3.15). In terms of colour, the interaction between size and season implied that nopalitos can be harvested either season.

3.4.5.3 Interaction between size and year

No significant differences observed in terms of colour parameters L*, a*, b* and C* for the interaction between size and year, however, there were differences perceived between the growth sizes. The h° of nopalitos harvested in 2017 at 9 cm (120.45°) was significantly (p = 0.007) lower than nopalitos at 24 cm (125.55°). The h° of nopalitos at 9 cm was significantly (p = 0.00) different between nopalitos at 15 cm and 24 cm (Table 3.16). The h° of nopalitos harvested in 2018 had no significant differences between growth sizes. No significant differences of h° were observed over the two years. Nopalitos at 24 cm had a significantly (p = 0.007) yellow to greener tone compared to nopalitos at 9 cm in both years.

Table 3.14: Analysis of variance (ANOVA) for the interaction between size and cultivar on the colour of nopalitos

Size	**9 cm**		**12 cm**		**15cm**		**18cm**		**21cm**		**24cm**		**Sign.**
Cultivar	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**level**
L*	48.21^{abc}	53.48^{e}	44.80^{a}	52.54^{de}	46.05^{ab}	50.91^{cde}	45.52^{a}	51.09^{cde}	45.45^{a}	49.51^{cd}	45.38^{a}	49.22^{bcd}	p = 0.001
a*	-17.14^{abc}	-18.50^{a}	-14.62^{e}	-18.10^{ab}	-15.79^{bcde}	-17.92^{ab}	-14.73^{de}	-17.82^{ab}	-14.50^{e}	-17.04^{abcd}	-14.88^{cde}	-16.31^{abcde}	p = 0.003
b*	26.15^{cd}	34.62^{f}	20.85^{ab}	32.08^{ef}	22.48^{abc}	28.80^{de}	20.17^{a}	28.73^{de}	20.22^{a}	27.03^{d}	20.31^{a}	25.32^{bcd}	p < 0.001
C*	31.29^{cd}	39.35^{f}	25.50^{ab}	36.89^{ef}	27.49^{abc}	33.94^{de}	24.14^{a}	33.82^{de}	25.11^{ab}	31.96^{cde}	24.42^{a}	29.67^{bcd}	p = 0.001
Hue (h°)	123.44^{bc}	118.08^{a}	125.64^{d}	119.47^{a}	125.32^{cd}	121.90^{b}	125.78^{d}	121.75^{b}	125.62^{d}	122.31^{b}	126.69^{d}	122.89^{b}	p = 0.001

Means with different superscript in the same row differ significantly

Table 3.15: Analysis of variance (ANOVA) for the interaction between size and season on the colour of nopalitos

Size	**9 cm**		**12 cm**		**15cm**		**18cm**		**21cm**		**24cm**		**Sign.**
Season	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**level**
L*	53.09	48.60	50.51	46.82	50.35	46.45	50.25	46.20	48.83	46.13	49.19	45.42	p = 0.434
a*	-19.53^{a}	-16.11^{bcde}	-17.44^{abc}	-15.28^{cde}	-18.20^{ab}	-15.45^{cde}	-17.40	-15.00	-16.74	-14.81	-16.93	-14.25	p = 0.217
b*	32.89^{c}	27.89	27.62	25.32	27.41	23.71	26.47	22.33	24.75	22.50	25.19	20.44	p = 0.202
C*	38.29	32.34	32.75	29.63	32.93	28.33	30.96	26.86	30.02	27.05	29.27	24.82	p = 0.350
Hue (h°)	121.08^{ab}	120.44^{a}	123.34^{abcd}	121.78	123.91	123.43	123.73	123.64	124.53	123.39	124.94	124.64	p = 0.469

Means with different superscript in the same row differ significantly

Table 3.16: Analysis of variance (ANOVA) for the interaction between size and year on the colour of nopalitos

Size	**9 cm**		**12 cm**		**15 cm**		**18 cm**		**21 cm**		**24 cm**		**Sign.**
Year	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**Level**
L*	51.55	50.14	48.96	48.37	49.05	47.91	48.59	48.02	47.86	47.10	47.39	47.22	p = 0.817
a*	-18.28	-17.36	-16.30	-16.42	-17.33	-16.38	-16.28	-16.27	-16.51	-15.03	-16.09	-15.09	p 0.089
b*	31.61	29.17	26.20	26.73	25.31	25.97	23.91	24.99	24.11	23.14	23.01	22.62	p = 0.137
C*	36.57	34.06	30.95	31.44	30.69	30.74	28.88	29.07	29.30	27.77	28.12	25.97	p = 0.209
Hue (h°)	120.45^{a}	121.07^{ab}	123.01^{abcd}	122.10^{abc}	124.61^{cd}	122.61^{abcd}	124.81^{cd}	122.72^{abcd}	124.81^{cd}	123.12^{abcd}	125.55^{d}	124.04^{bcd}	p = 0.007

Means with different superscripts in the same row differ significantly

3.4.6 Turgidity and sliminess (moisture content, mucilage yield and viscosity)

The turgidity and sliminess of nopalitos were analysed by measuring moisture content, mucilage yield and mucilage viscosity. The assessment of moisture and mucilage characteristics of the two cultivars is important to obtain knowledge for product acceptability. The quality of food regarding the texture, taste, appearance and stability is influenced by the moisture available in the food product (Du Toit, 2017; McClements, 2003). Nopalitos have the ability to retain water under unfavourable weather conditions and therefore, it was important to understand the available water in nopalitos. The mucilage content in nopalitos was important to determine because sliminess is an undesirable attribute for food products (García & Sáenz, 2013). Saénz (1996) stated that mucilage should be removed when cladodes are used in food product development. It was observed that mucilage extracted from mature cladodes can be extremely slimy and described as non-Newtonian (Du Toit, 2017). A method was used to test the consistency of the mucilage as regards to viscosity using a viscometer. Viscosity indicates the resistance to flow in a liquid. The low values indicated low viscosities and high values indicated high viscosities on the viscometer viscosity (Du Toit, 2017).

3.4.6.1 Interaction between size and cultivar

The interaction between cultivar and nopalito size had no significant effect ($p = 0.323$) on the moisture content of nopalitos (Table 3.17). The moisture content remained high for both cultivars at all growth sizes. Du Toit (2017) highlighted that the high moisture percentage in nopalitos could be a benefit to humans and animals as a source of water. The results of this study may imply that nopalitos could be a good source of water at any stage of development, this is in agreement with reports that cladodes are known for having an ability to store water (Bauman & Schmidt, 2015; Snyman, 2014). Betancourt-Dominguez *et al*. (2006), observed a different pattern in moisture content where there was a decrease in moisture content (94.2-91.5 %) in different cultivars over time however, only one cultivar had an increase. The moisture content for this study (87.47-88.85 %) was lower than that (89-94 %) reported by (Calvo-Arriaga *et al.*, 2010; Feugang *et al.*, 2006; Stintzing & Carle, 2005). There were no significant differences ($p = 0.092$) in the mucilage yield of the two cultivar nopalitos, however, a difference in the mucilage yield between the growth sizes was observed. Mucilage yield of younger cladodes is lower than matured cladodes (Gheribi & Khwaldia, 2019). No significant differences (p = 0.047) in the viscosity of the two cultivars nopalitos, were observed however, a difference in the viscosity content between growth sizes was observed.

3.4.6.2 Interaction between size and season

In Table 3.18, the moisture content of nopalitos 87.41 % (9 cm) significantly (p = 0.020) increased to 88.92 % (21 cm) and no significant differences between the two seasons were observed. The differences in cladode moisture content can be reflected by observing the changes in the thickness of cladodes (Lerdau *et al.*, 1992). Boutakiout *et al*. (2018) stated that cladodes harvested in March had higher moisture content (95.68 g/100 g^{-1}) than those harvested in August which were reported with the lowest water content (91.00 g/100 g^{-1}). Mucilage yield of nopalitos harvested in autumn at 9 cm (16.09 %) was significantly (p = 0.012) lower than nopalitos at 24 cm (52.79 %). There were significant (p = 0.012) differences in the mucilage yield between nopalitos at 9 cm, 12 cm, 18 cm and 24 cm harvested in autumn. Mucilage yield of nopalitos harvested in spring at 9 cm (18.56 %) was significantly (p = 0.012) lower than nopalitos at 24 cm (45.06 %). There were significant (p = 0.012) differences in the mucilage yield between nopalitos at 9 cm, 15 cm and 21 cm. No significant differences were observed in the mucilage yield of nopalitos harvested over the two seasons. In contrast, Boutakiout *et al.* (2018) found that mucilage of cladodes harvested in March had higher yields (51.96 %) than those harvested in August reported with the lowest yields (20.83 %). The viscosity of nopalitos harvested in autumn ranged from 21.90 cP (9 cm) to 61.90 cP (18 cm) and no significant differences were observed between growth sizes. The viscosity of nopalitos harvested in spring at 9 cm (121.64 cP) was significantly (p < 0.001) lower than nopalitos at 21 cm (317.63 cP). The viscosities of nopalitos harvested in spring were significantly (p < 0.001) higher than those harvested in autumn in all growth sizes excluding at 9 cm. The substantial difference between the seasons could be due to the differences in the climate between 2017 and 2018 as explained in section 3.2.1 that the year 2017 was the driest year and especially autumn and winter seasons were determined to be the driest compared to other years. This could mean that the crops harvested during drier season may not be in a same standard compared to those harvested in less dry season.

3.4.6.3 Interaction between size and year

In Table 3.19, the moisture content of nopalitos harvested in 2017 remained high and there were no significant differences between the growth sizes. The moisture content of nopalitos harvested in 2018 at 9 cm (87.29 %) was significantly ($p = 0.001$) lower than nopalitos at

15 cm (88.70 %). The moisture content of nopalitos harvested in 2017 was significantly ($p = 0.001$) high compared to those harvested in 2018, only at 21 cm. The mucilage yield of nopalitos harvested in 2017 at 9 cm (17.95 %) was significantly ($p < 0.001$) lower than nopalitos at 24 cm (54.80 %). There were significant ($p < 0.001$) differences in the mucilage yield of nopalitos between 9 cm, 12 cm and 18 cm. The mucilage yield of nopalitos harvested in 2018 at 9 cm (16.69 %) was significantly ($p < 0.001$) lower than nopalitos at 24 cm (43.06 %). There were significant ($p < 0.001$) differences in the mucilage yield of nopalitos between 9 cm, 15 cm and 21 cm. The mucilage yield of nopalitos harvested in 2017 was significantly ($p < 0.001$) higher than the mucilage yield in 2018, excluding nopalitos at 9 cm. The viscosity of nopalitos harvested in 2017, ranged from 18.65 cP (18 cm) to 31.07 cP (21 cm) and there were no significant differences. The viscosity of nopalitos harvested in 2018 at 9 cm (117.33 cP) was significantly ($p < 0.001$) lower than nopalitos at 21 cm (310.06 cP). The viscosity of nopalitos harvested in 2017 was significantly ($p < 0.001$) lower than in 2018, excluding mucilage at 9 cm. Thus, environmental factors such as precipitation and temperature may influence moisture content and mucilage yield and viscosity.

Table 3.17: Analysis of variance (ANOVA) for the interaction between size and cultivar on the characteristics of nopalitos

Size	**9 cm**		**12 cm**		**15cm**		**18cm**		**21cm**		**24cm**		**Sign.**
Cultivar	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**level**
Moisture content (%)	87.70	87.56	88.83	87.47	88.80	87.95	88.37	87.877	88.85	87.84	88.20	87.63	p = 0.323
Mucilage yield (%)	19.80	14.8	34.33	24.61	39.04	31.89	44.34	38.95	46.14	44.78	51.295	46.55	p = 0.092
Viscosity (cP)	62.99^{a}	80.56^{a}	149.57^{a}	154.58^{a}	114.90^{a}	172.77^{a}	167.92^{a}	139.55^{a}	148.37^{a}	192.75^{a}	129.238^{a}	196.22^{a}	p = 0.047

Means with different superscripts in the same row differ significantly

Table 3.18: Analysis of variance (ANOVA) for the interaction between size and season on the characteristics of nopalitos

Size	**9 cm**		**12 cm**		**15cm**		**18cm**		**21cm**		**24cm**		**Sign.**
Season	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**level**
Moisture content (%)	87.41^{a}	87.85^{a}	88.47^{a}	87.83^{a}	88.40^{a}	88.45^{a}	87.78^{a}	88.41^{a}	88.92^{a}	87.77^{a}	87.97^{a}	87.86^{a}	p = 0.020
Mucilage yield (%)	16.09^{a}	18.56^{ab}	31.52^{cd}	27.41^{bc}	37.77^{def}	33.18^{cde}	42.61^{ef}	40.07^{def}	45.88^{fg}	45.04^{fg}	52.79^{g}	45.06^{fg}	p = 0.012
Viscosity (cP)	21.90^{a}	121.64^{abc}	42.62^{a}	261.52^{cd}	37.28^{a}	239.14^{bcd}	61.90^{ab}	272.62^{cd}	23.49^{a}	317.63^{d}	30.04^{a}	295.42^{cd}	p < 0.001

Means with different superscripts in the same row differ significantly

Table 3.19: Analysis of variance (ANOVA) for the interaction between size and year on the characteristics of nopalitos

Size	**9 cm**		**12 cm**		**15 cm**		**18 cm**		**21 cm**		**24 cm**		**Sign.**
Year	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**level**
Moisture content (%)	87.97^{ab}	87.29^{a}	88.28^{ab}	88.02^{ab}	88.05^{ab}	88.70^{ab}	88.39^{ab}	87.86^{ab}	89.30^{b}	87.40^{a}	88.39^{ab}	87.44^{a}	p = 0.001
Mucilage yield (%)	17.95^{a}	16.69^{a}	35.20^{cd}	23.74^{ab}	43.24^{def}	27.68^{bc}	47.18^{efg}	36.12^{cd}	51.27^{fg}	39.65^{de}	54.80^{g}	43.06^{def}	p < 0.001
Viscosity (cP)	26.20^{a}	117.33^{ab}	23.40^{a}	280.74^{bc}	27.89^{a}	259.78^{bc}	18.65^{a}	288.83^{bc}	31.07^{a}	310.06^{c}	25.73^{a}	299.73^{c}	p < 0.001

Means with different superscripts in the same row differ significantly

3.4.7 Gustatory properties (acidity and TSS)

The nopalito acidity affects the taste of nopalitos leading to consumer preference and acceptability, as the acceptability decreases with increased acidity. Titratable acidity has an impact on the overall sensory quality of the fresh produce as it contributes to the taste of the vegetables (Mattheis & Fellman, 1999). Therefore, titratable acidity of nopalitos was determined to distinguish which growth length will indicate an acceptable acidity level. The acidic taste of nopalitos is one of the most important traits for consumers, it should be a pleasantly sour taste, if the pH is too low, the sourness will become too intense and will be too sour to enjoy when eaten. Therefore, the pH as an indication of the level of sourness of nopalitos, and was determined in order to establish the consumer acceptable pH range in which nopalitos have optimal eating quality. The TSS content was measured to determine the amount of sugar in the mucilage of nopalitos. TSS content may describe how the product taste and not how nutritious it may be. Taste of nopalitos depend on the soluble solids and sugars are the soluble solids that are found in fruits and vegetables (Kleinhenz & Bumgarner, 2013). Thus, the taste is influenced by the composition of the malic acid and sugar in plant tissue (Muñoz-Robredo *et al*., 2011). A record of °Brix values may be helpful to growers to be able to compare varieties, production practices and harvest times with other growers for better quality products (Kleinhenz & Bumgarner, 2013).

3.4.7.1 Interaction between size and cultivar

The titratable acidity (TA) of Fusicaulis nopalitos at 9 cm (0.31 %) was significantly ($p = 0.028$) lower than nopalitos at 21 cm (0.46 %) illustrated in Table 3.20. The TA value of 0.46 % can be comparable to that of lemons. There were significant ($p = 0.028$) differences between 12cm and 24 cm. The TA of Morado nopalitos at 9 cm (0.29 %) was significantly ($p = 0.028$) lower than nopalitos at 24 cm (0.46 %) (24 cm). There were significant ($p = 0.028$) differences observed between 12 cm and 21 cm. No significant differences in the titratable acidity were observed between the two cultivars. The acidity values reported by Corrales- García *et al*. (2004) varied from 0.28 % to 0.76 % and Calvo-Arriaga *et al*. (2010) reported values within the interval from 0.41 % to 0.55 %. Betancourt-Dominguez *et al*. (2006); Rodriguez-Felix & Cantwell (1988), studied acidity values that ranged from 0.28 % to 0.95 % of nopalitos harvested in the morning. Furthermore, Stintzing & Carle (2005), stated that nopalitos harvested in the morning have an acidity that ranges from 0.94 % to 0.47 % in the afternoon.

The values observed in this study were within the ranges reported in the above-mentioned studies.

The pH of Fusicaulis nopalitos at 9 cm (4.35) was significantly ($p < 0.001$) higher than nopalitos at 24 cm (4.1). There were significant ($p < 0.001$) differences observed between 9 cm and 21 cm. The pH of Morado nopalitos at 9 cm (4.52) was significantly ($p < 0.001$) higher than nopalitos at 24 cm (4.00). There were significant ($p < 0.001$) differences observed between 12 cm and 21 cm. Nopalitos with the lowest pH value were perceived with more intense sourness. In other words, nopalitos with growth length of 9 cm were significantly less sour than nopalitos with growth length of 24 cm. The low pH content will contribute to the shelve-life as high acidity causes natural preservation action.

According to Rodriguez-Felix & Cantwell (1988), the sour taste can be caused by an increase in acid content during development. The authors observed a decrease of pH from an average of 5 to 4.3 with development, the data of this study followed a similar trend however, the pH values were lower. It was observed from the data that the matured cladodes were more sour than the young cladodes. In López-Palacios *et al*. (2010), the pH values of *O. ficus-indica* decreased from 4.68 to 4.22 and then increased from 4.22 to 4.42 with development. A similar pattern was reported by Betancourt-Dominguez *et al*. (2006), where the pH decreased from 4.48 to 4.05 and then increased from 4.05 to 4.25 throughout development. In contrast, Rodríguez-Garcia *et al*. (2007) observed an increase in pH that ranged from 4 to 4.5 with development. Rodriquez-Felix & Cantwell (1988), further stated that a decrease in pH during the development of nopalitos may cause an increase in acidity and a similar pattern was observed in this study.

The TSS content of Fusicaulis mucilage ranged from the lowest °Brix value of 5.73 (24 cm) to the highest value of 6.43 °Brix (9 cm) and Morado mucilage ranged from 5.36 °Brix (21 cm) to 6.01 °Brix (9 cm). No significant differences in the TSS content were observed between the two cultivars. A similar pattern was reported by Betancourt-Dominguez *et al*. (2006), however, the values were higher (7.4 to 4 °Brix) than the range observed in this study. García-Nava *et al*. (2015) reported on cladodes harvested at different times of the day and detected, *O. ficus-indica* had the highest value of 6.8 ° Brix when harvested very early in the day at 05:00, 6.4 ° Brix at midday at 13:00 and 2.64-3.28 ° Brix in the evening 19:00-20:00. As a result, the ° Brix index was found to be different due to the time of the day that the harvest took place.

3.4.7.2 Interaction between size and season

In Table 3.21, the titratable acidity of nopalitos harvested in at 9 cm (0.28 %) was significantly ($p < 0.001$) lower than nopalitos at 21 cm (0.48 %). There were significant ($p < 0.001$) differences observed between nopalitos at 9 cm, 15 cm and 24 cm. The titratable acidity of nopalitos harvested in spring harvest at 12 cm (0.30 %) was significantly ($p < 0.001$) lower than nopalitos at 24 cm (0.41 %) illustrated in Table 25. There were significant ($p < 0.001$) differences observed between nopalitos at 9 cm and 24 cm. No significant differences in the titratable acidity were observed over the two seasons, excluding nopalitos at 21 cm. Mucilage of 21 cm nopalitos harvested in autumn had a significantly ($p < 0.001$) higher acidity than in spring harvest. Boutakiout *et al*. (2018) stated that mucilage of cladodes harvested in March had lower TA (1.45 g/L^{-1}) than those harvested in August that had the highest TA (1.88 g/L^{-1}). Lerdau *et al*. (1992) suggested that the variations in titratable acidity of the interaction between size and season were as a result of the bigger cladodes which were able to utilise the stored water in their stems and main branches. The pH of nopalitos harvested in autumn at 9 cm (4.38) was significantly ($p < 0.001$) higher than nopalitos at 24 cm (4.03). There were significant ($p < 0.001$) differences observed between nopalitos at 12 cm and 21 cm. The pH of nopalitos harvested in spring at 9 cm (4.49) was significantly ($p < 0.001$) higher than nopalitos at 24 cm (4.09). The pH of nopalitos at 9 cm had a significantly ($p < 0.001$) higher pH (less sour) than nopalitos at 24 cm in both seasons and no significant differences were observed over the two seasons. In contrast, Boutakiout *et al*. (2018) reported that cladodes harvested in March had lower pH 4.68 compared to those harvested in August that had higher pH 4.77. There was no significant difference ($p = 0.142$) in the TSS content observed over the two seasons. However, Boutakiout *et al*. (2018) observed that cladodes harvested in March had higher TSS (55 °Brix) compared to those harvested in August that had lower TSS (11 °Brix).

3.4.7.3 Interaction between size and year

The titratable acidity of nopalitos harvested in 2017 at 9 cm (0.32 %) was significantly ($p = 0.007$) lower than nopalitos at 24 cm (0.48 %) illustrated in Table 3.22. There were significant ($p = 0.007$) differences between nopalitos at 12 cm and 21 cm. The titratable acidity of nopalitos harvested in 2018 at 12 cm (0.26 %) was significantly ($p = 0.007$) lower than nopalitos at 24 cm (0.40 %). There were significant ($p = 0.007$) differences observed between nopalitos at 9 cm and 21 cm. The titratable acidity of nopalitos harvested in 2017 was significantly ($p = 0.007$) higher than those harvested in 2018, excluding mucilage at 9 cm and 24 cm. No significant differences ($p = 0.564$) were observed in the pH of nopalitos over the two years. The TSS content of nopalitos harvested in 2017 ranged from 5.15 °Brix (21 cm) to 5.71 °Brix (9 cm) and no significant differences were observed between growth sizes. The TSS content of nopalitos harvested in 2018 ranged from 6.07 °Brix (12 cm) to 6.73 °Brix (9 cm) and no significant differences were observed between growth sizes. Nopalitos harvested in 2017 had a significantly ($p = 0.024$) lower TSS content than those harvested 2018, excluding mucilage of 12 cm nopalitos. It was determined that nopalitos contained less sugar content in drier season.

Table 3.20: Analysis of variance (ANOVA) for the interaction between size and cultivar on the gustatory properties of nopalitos

Size	9 cm		12 cm		15cm		18cm		21cm		24cm		Sign.
Cultivar	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**Fusicaulis**	**Morado**	**level**
TA as citric acid (%)	0.31^{abc}	0.29^{a}	0.32^{abc}	0.31^{ab}	0.3^{abcd}	0.35^{abcd}	0.40^{cde}	0.39^{bcde}	0.46^{e}	0.41^{de}	0.42 ^{de}	0.46^{e}	$p = 0.028$
pH	4.35^{de}	4.52^{e}	4.27^{cd}	4.26^{cd}	4.21^{bcd}	4.15^{abc}	4.19^{bcd}	4.0^{ab}	4.107^{abc}	4.07^{ab}	4.10^{abc}	4.01^{a}	$p < 0.001$
TSS (°Brix)	6.43^{b}	6.01^{ab}	5.98^{ab}	5.80^{ab}	5.87^{ab}	5.98^{ab}	5.83^{ab}	5.81^{ab}	5.90^{ab}	5.36^{ab}	5.73^{ab}	5.81^{ab}	$p = 0.020$

Means with different superscripts in the same row differ significantly

Table 3.21: Analysis of variance (ANOVA) for the interaction between size and season on the gustatory properties of nopalitos

Size	9 cm		12 cm		15cm		18cm		21cm		24cm		Sign.
Season	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**level**
TA as citric acid (%)	0.28^{a}	0.31^{abc}	0.33^{abcd}	0.30^{ab}	0.38^{bcd}	0.35^{abcd}	0.41^{def}	0.37^{bcd}	0.48^{f}	0.39^{cde}	0.47^{ef}	0.41^{def}	$p < 0.001$
pH	4.38^{de}	4.49^{e}	4.28^{cd}	4.25^{bcd}	4.25^{bcd}	4.11^{abc}	4.13^{abc}	4.12^{abc}	4.08^{ab}	4.09^{ab}	4.03^{a}	4.08^{ab}	$p < 0.001$
TSS (°Brix)	5.90	6.54	5.43	6.35	5.57	6.31	5.59	6.09	5.43	5.82	5.31	6.23	$p = 0.142$

Means with different superscripts in the same row differ significantly

Table 3.22: Analysis of variance (ANOVA) for the interaction between size and year on the gustatory properties of nopalitos

Size	9 cm		12 cm		15 cm		18 cm		21 cm		24 cm		Sign.
Year	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**level**
TA as citric acid (%)	0.32^{abc}	0.28^{ab}	0.37^{cde}	0.26^{a}	0.41^{def}	0.31^{abc}	0.44^{ef}	0.34^{bcd}	0.47^{f}	0.40^{de}	0.48^{f}	0.40^{def}	$p = 0.007$
pH	4.37	4.50	4.23	4.30	4.14	4.22	4.11	4.15	4.05	4.12	4.03	4.08	$p = 0.564$
TSS (°Brix)	5.71^{abc}	6.73^{d}	5.70^{abc}	6.07^{bcd}	5.48^{ab}	6.37^{cd}	5.36^{a}	6.28^{cd}	5.15^{a}	6.10^{bcd}	5.20^{a}	6.34^{cd}	$p < 0.024$

Means with different superscripts in the same row differ significantly

3.4.8 Principal component analysis (PCA)

The aim of PCA was to identify characteristics that are associated with specific nopalitos sizes (growth length). This statistical technique is based on the associations which allow us to know which nopalito size has strong associations with the characteristics analysed. The biplot showed characteristics that are clustered closely to different nopalitos sizes, which may explain the association between the two (characteristics and sizes). In the PCA, factor 1 (F1) and 2 (F2) explained 91.73 % of the variation (Figure 3.14).

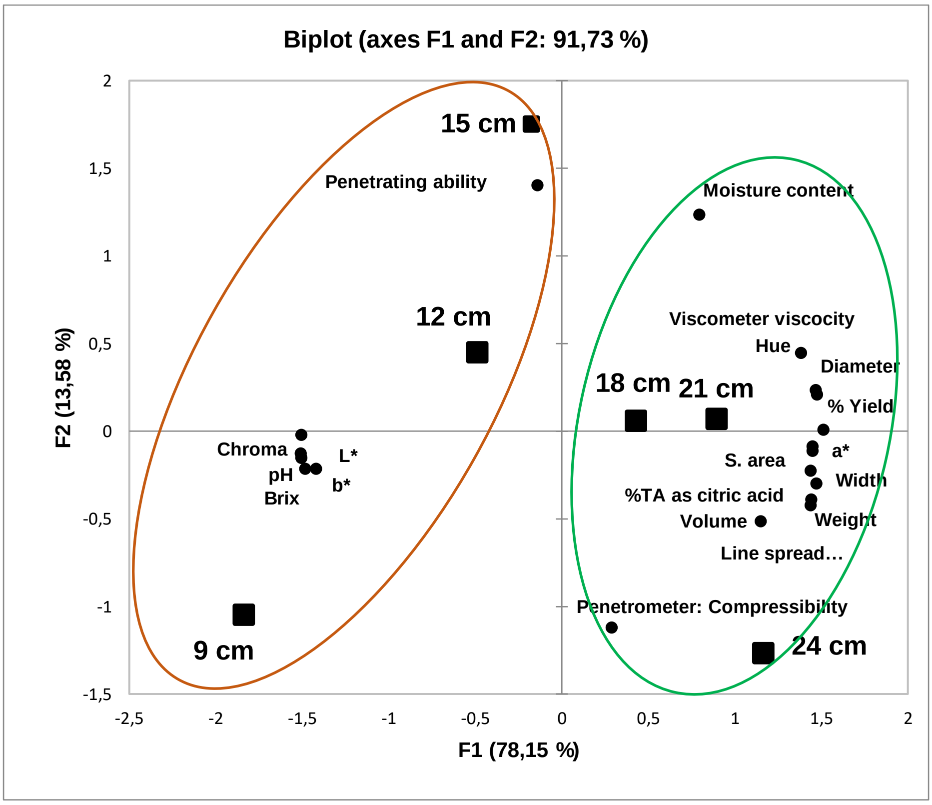


Figure 3.14: Principle Component Analysis of properties of nopalitos as influenced by nopalito size

F1, which explained 78.15 % of the total variance, was mainly characterized by most of the nopalito characteristics which were closely linked to nopalitos at 18 cm and 21 cm. These included high values for viscometer viscosity, diameter, hue, a*, % TA as citric acid, width, weight, surface area, volume, mucilage yield and line-spread viscosity. These high values are associated with negative quality characteristics. There were also an association between nopalitos at 24 cm and the negative quality characteristics of high compressibility. Moisture content was located at a distance (top right of the diagram), indicating a weak association with specific nopalito sizes.

On the other hand, F2 explained 13.58 % of the total variance. Nopalitos at size 12 cm and 15 cm were associated with penetrating ability (softness of texture). Nopalitos at 12 and 15 cm presented nopalitos with highest values of penetrating ability providing nopalitos with a softer texture. The nopalito characteristics halfway on the graph between 9 cm and 12 cm, indicated that the TSS content was higher, the pH was higher (less sour taste) and the colour a brighter green tone. It was observed that smaller size nopalitos between 9 cm and 15 cm were associated with positive and important characteristics such as texture, colour and taste. On the other hand, nopalitos between 18 cm and 24 cm were associated with negative characteristics namely, the morphology (weight, width, diameter, volume and surface area) mucilage yield and viscosity of nopalitos.

3.4.9 Pearson correlation analysis

The aim of Pearson correlation analysis between the characteristics of nopalitos was done to determine the relationship between these characteristics. Strong correlations are described by values > 0.5 (correlations that are discussed are indicated bold). The PCA table was broken into three tables in order to discuss the results according to morphology (Table 3.23), colour (Table 3.24) and eating quality (Table 3.25).

The results in Table 3.23 showed a significantly ($p < 0.001$) strong positive correlation (0.8441) between length and weight, which indicated that as the length of nopalitos increased, the weight also increased. A significantly ($p < 0.001$) strong positive correlation (0.8927) was observed between length and width of nopalitos; the width increased as the nopalitos grew longer. There was a significantly ($p < 0.001$) strong positive correlation (0.7818) between weight and width which implied that an increase in nopalito weight resulted in an increase in width. A significantly ($p < 0.001$) positive correlation (0.5732) was observed between length and volume; it was deduced that as the length of nopalitos increases, so does the volume. There was a significantly ($p < 0.001$) positive correlation (0.5157) between weight and volume and between width and volume (0.5972), which implied that as the weight and width of nopalitos increased, the volume increased. A significantly ($p < 0.001$) positive correlation (0.6869) was observed between volume and surface area, indicating that higher volume nopalitos also have higher surface areas. It was interesting to observe from Table 3.23 that there was no relationship between firmness and compressibility, neither were there correlations between both firmness and compressibility and any of the morphological characteristics.

Table 3.23: Pearson correlation analysis between morphological properties

	Length	Weight	Width	Diameter	Volume	Surface area	Firm.	Comp.
Length (cm)	1							
Weight (g)	**0.8441****	1						
Width (cm)	**0.8927***	**0.7818***	1					
Diameter (cm)	0.2749***	0.4865***	0.1682**	1				
Volume (cm^3)	**0.5732***	**0.5157***	**0.5972***	0.2235***	1			
Surface area (cm^2)	0.4315***	0.2476***	0.4765***	0.0711NS	**0.6869***	1		
Firmness (10ths m/m)	-0.1256*	-0.3320***	-0.0741NS	-0.3145***	-0.1826**	0.0824NS	1	
Compressibility (10ths m/m)	0.0429NS	0.0805NS	0.0276NS	-0.1634**	-0.0368NS	-0.0541NS	-0.0171NS	1

NS = no significant differences, * = p < 0.05, ** = p < 0.01, *** = p < 0.001

In Table 3.24, Lightness of colour (L*) had a significantly (p < 0.001) strong positive correlation to yellow tone (b*) (0.9223) and to brightness (C*) (0.8988), implying that as the lightness of colour increased, so did the brighter yellow colour of nopalitos. Lightness of colour had a significantly (p < 0.001) negative correlation to green tone (a*) (-0.8810) and to yellow-green tone (h°) (-0.6473), which implied that as green and yellow-green tone increases, lightness will decrease. The green tone (a*) had a significantly (p < 0.001) moderate negative correlation to yellow tone (*0.8887) and to brightness (-0.8898). Yellow-green tone (h°) had a significantly (p < 0.001) negative correlation to b* (-0.7657) and to C* (-0.7430). A significantly (p < 0.001) strong positive correlation (0.9610) was observed between b* and C*, implying that there was an increase in brightness and yellow tone of nopalitos.

Table 3.24: Pearson correlation analysis between colour properties of nopalitos

	L*	a*	b*	C*	h°
L*	1				
a*	**-0.8810*****	1			
b*	**0.9223*****	**-0.8887*****	1		
C*	**0.8988*****	**-0.8898*****	**0.9610*****	1	
h°	**-0.6473*****	0.4550***	**-0.7657*****	**-0.7430*****	1

NS = no significant differences, * = p < 0.05, ** = p < 0.01, *** = p < 0.001

In Table 3.25, it was observed that there was a significantly (p < 0.001) negative correlation (-0.5155) between pH and mucilage yield, indicating that a decrease in pH may cause an increase in mucilage yield, and vice versa. A significantly (p < 0.001) positive correlation (0.5375) was observed between yield and TA percentage, implying that as titratable acidity increased, the mucilage yield increased. Thus, it was observed from the data that nopalito acidity and mucilage yield were interconnected. No other characteristics were strongly correlated in Table 3.25.

Table 3.25: Person correlation analysis between properties of nopalitos

	pH	% Yield	% Moisture	Viscometer	% TA	°Brix
pH	1					
% Mucilage yield	**-0.5155*****	1				
% Moisture	-0.0005NS	0.1877**	1			
Viscosity (cP)	-0.2666***	-0.1531**	-0.1091NS	1		
% TA	-0.3939***	**0.5375*****	-0.0239NS	-0.3545***	1	
TSS (°Brix)	0.1946***	-0.4704***	-0.3528***	0.3974***	-0.1350*	1

NS = no significant differences, * = p < 0.05, ** = p < 0.01, *** = p < 0.001

3.4.10 SEM

Calcium oxalate crystals were identified in both cultivars and they differed in the number of crystals present at different growth lengths. Fusicaulis nopalitos at 9 cm had 58 crystals, at 15 cm had 34 crystal and at 24 cm had 11 crystals. Morado nopalitos at 9 cm had 24 crystals, at 15 cm had 31 crystals and at 24 cm had 32 crystals as illustrated in Table 30. It was interesting to observe that Fusicaulis nopalitos had more crystals at 9 cm and fewer crystals at 24 cm however, Morado nopalitos had fewer crystals at 9 cm and more crystals at 24 cm. Du Toit (2017) reported that Morado cladodes (1 year old) had large and abundant crystals, which can correspond with an observation from this study that at later growth length crystals were found in abundance. More than one type of crystal may exist in the same plant, based on chemical composition, and those crystals may not necessarily be calcium oxalate or calcium carbonate (He, 2012; Horner & Wagner, 1992).

Table 3.26: The number of crystals present in Fusicaulis and Morado samples at 9 cm, 15 cm and 24 cm

	9 cm	15 cm	24 cm
Fusicaulis	58	34	11
Morado	24	31	32

The crystals observed were distributed throughout the cell structure, crystals which did not have sharp points known as wheddellite (conglomeration of tetragonal crystals) (Du Toit, 2017; Rojas-Molina *et al*. 2015) were frequently observed to be located below the epidermis (Figure 3.15). Most crystals with sharp points (whewellite) were found in the mesophyll. The different structures and occurrence of crystals were noticeably observed in Fusicaulis nopalitos (Figure 3.16) and interestingly these crystals were found in vacuoles.

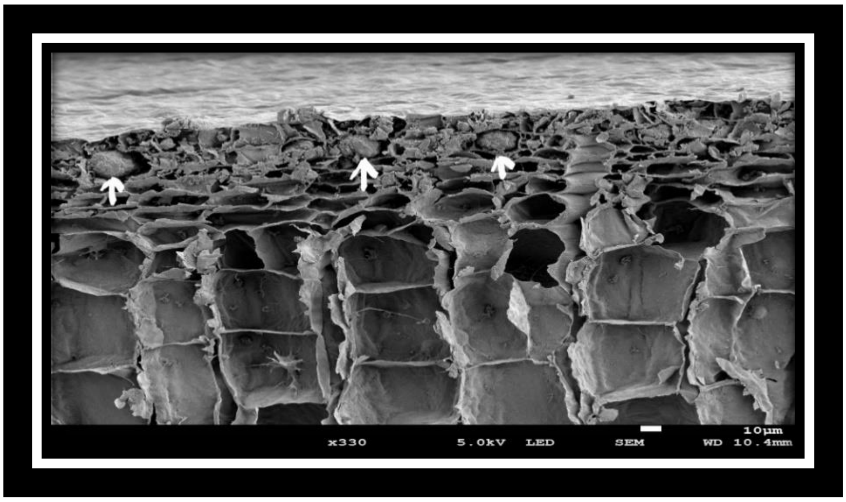
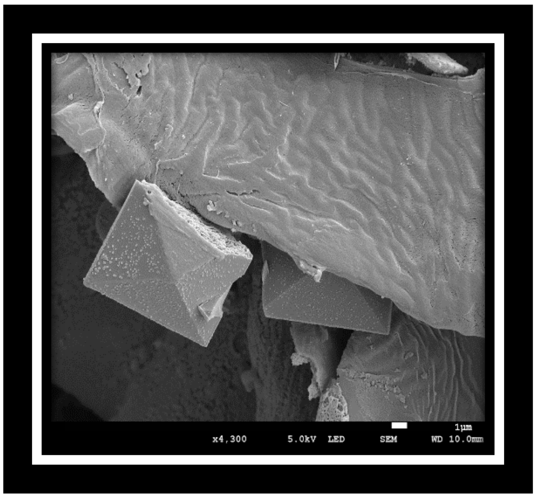
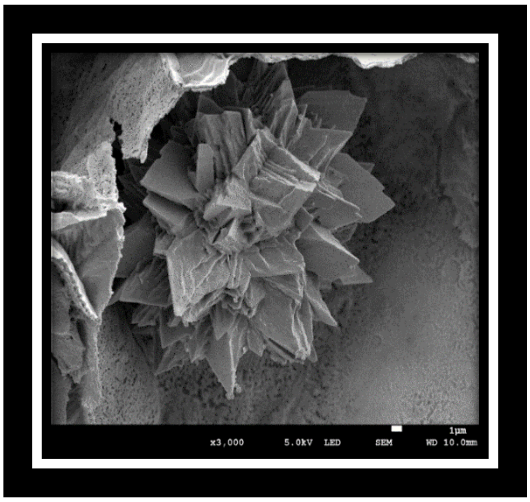


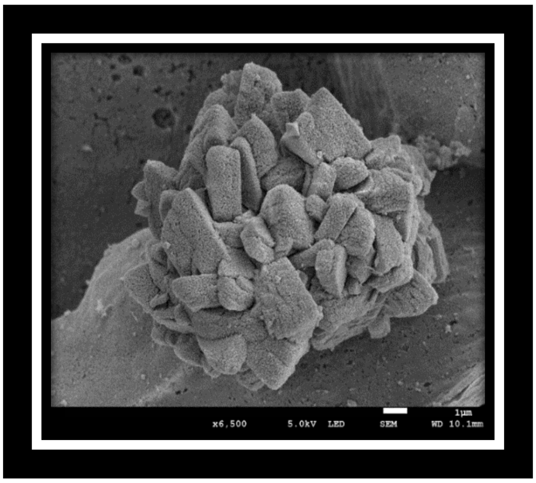
Figure 3.15: Calcium oxalate crystals located below the epidermis



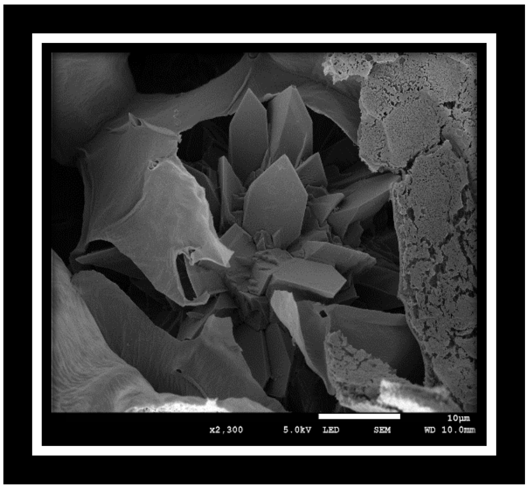
Whedellite



Whewellite



Whedellite



Whewellite

Figure 3.16: Different structures of crystals observed in Fusicaulis nopalitos

3.5 Conclusion

Nopalito characteristics were highly influenced by size and season, as opposed to cultivar and year. This therefore highlighted the importance of understanding the time of harvest and size of nopalitos in order to obtain a good quality product. *Opuntia ficus-indica* cultivars have proven to be both suitable for nopalito production, however, Morado had better quality attributes. The variations observed over the two years could be explained by weather which were experienced in 2017 and 2018. Thus, harvest quality is influenced by weather conditions and may vary over years.

Nopalito size had a significant effect on the morphological characteristics, thus, nopalitos between 15 and 18 cm were found to have optimal results which could be described as high quality and ready to be harvested. In terms of texture, both cultivars reached their highest value at 15 cm which is associated with less firm texture and can be characterized as an ideal length of harvest as both cultivars became more tender at this size. Nopalitos at 9 cm had a lighter, brighter and greener colour than nopalitos at 24 cm. Morado was identified with colour parameters associated with higher quality than Fusicaulis. Similar to other studies, moisture content of nopalitos remained high for both cultivars throughout development. In terms of taste, nopalitos with growth length of 9 cm were significantly less sour (associated with high quality) than nopalitos with growth length of 24 cm.

The PCA analysis showed nopalitos between 9 cm and 15 cm were associated with good texture, colour and taste characteristics. Pearson correlation analysis showed a negative correlation between mucilage yield and pH, moreover, titratable acidity and mucilage yield had a positive correlation, indicating that nopalito acidity and mucilage yield were interconnected.

SEM results indicated that Fusicaulis nopalitos at early growth length (9 cm) had more crystals than at the later growth length (24 cm). However, Morado nopalitos had more crystals at later growth length (24 cm). Interestingly both cultivars had the same number of crystals at 15 cm.

It was concluded from the data presented in this chapter that Morado nopalitos, harvested in spring at 15 cm showed the highest eating quality characteristic.

3.6 Recommendations

Both *Opuntia ficus-indica* cultivars showed quality characteristics which are acceptable for human consumption. It can be recommended that Morado nopalitos to be investigated further since the eating quality was higher than that of Fusicaulis.

Chapter 4

4 Comparison of the characteristics of selected popular vegetables to two nopalito cultivars

4.1 Introduction

Nopalitos are young cactus pear cladodes which have been an important food source in Mexico and consumed raw and cooked (Bensadón *et al*., 2010; López-Palacios *et al*., 2010; Betancourt-Dominguez *et al*., 2006; Feugang *et al*., 2006; Graffith, 2004;). They can be cooked by applying moist and dry heat methods such as boiling, grilling, microwaving and frying (De Santiago *et al*., 2018). The cactus pear plant has been considered a potential alternative food crop due to its nutritive composition and drought-resistant nature in dry regions. The two highly consumed cultivars of *Opuntia ficus indica* L. Miller cladodes in Mexico are Milpa Alta and Atlixco (Ramírez-Moreno *et al*., 2013). There has been an increasing demand for vegetables on the market due to consumers acquiring knowledge of its nutritional benefits (Vandresen *et al*., 2009). Vegetables are essential in the diet and should be made part of every meal, together with the protein and carbohydrate-rich dishes, to ensure balanced diets. It is the cheapest food source that is readily available and it contributes to the nutrition and health of a human being (Satter *et al*., 2016). The consumption of vegetables plays an important role in lowering the risk of chronic diseases and prevention of diseases (De Santiago *et al*., 2018).

Inclusion of nopalitos in the South African diet could improve the food security status of poor households in South Africa due to the plant's adaptability to undesirable climate change (Patel, 2013; Fouché, 2010; Betancourt-Domínguez *et al*., 2006; Stintzing & Carle, 2005). Cladodes have only been used for animal feed in South Africa (Betancourt-Dominguez *et al*., 2006; Middleton *et al*., 2005). An increase in the price of maize has been one of the reasons that caused food insecurity due to the production process that require huge amounts of water for irrigation (Altman *et al*., 2009). South Africa has been considered a dry country and farmers might not be able to produce maize in the future due to climate change (high temperatures and lack of rainfall). Therefore nopalitos can be introduced as a sustainable form of vegetable due to its adaptability to climate change. The first question consumers ask when introduced to a new food item is: "How does it taste"? The aim of this chapter was to compare the characteristics of nopalitos from the two nopalito cultivars (Fusicaulis and

Morado) with those of well-known vegetables in order to describe the texture, taste and turgidity of nopalitos.

4.2 Materials and Methods

4.2.1 Collection and preparation of vegetables

Eight different fresh vegetables were bought at a local supermarket in Bloemfontein. The vegetables were prepared and analysed the same day that they were bought. Of each vegetable, six samples were analysed. The following vegetables were used:

1. Baby marrow (cut into half lengthwise)
2. Carrot (cut into half lengthwise)
3. Celery
4. Cucumber (peeled and halved lengthwise)
5. Green beans
6. Green pepper (pips were removed)
7. Onion (a single layer was used)
8. Tomato (seeds were removed)

The nopalitos were compared to different vegetables in order to observe the differences and similarities of nopalitos compared to other popular vegetables. Nopalitos of only one length, (15 cm) were used in this comparison test. The vegetables were compared to nopalitos harvested in October 2018 (spring harvest) due to nopalitos showing best quality characteristics compared to other harvesting times.

4.2.2 Methods

4.2.2.1 Texture (firmness and compressibility)

Firmness (the ability to penetrate) of vegetables was measured using the test as discussed in Chapter 3 section 3.2.1.6 and compressibility in section 3.2.1.7.

4.2.2.2 Turgidity and sliminess (moisture and viscosity content)

The moisture content of vegetables was measured using the test as discussed in Chapter 3 section 3.2.1.10 and viscosity in section 3.2.1.11.

4.2.2.3 Gustatory properties (TA, pH and TSS)

Titratable acidity was measured using the test as discussed in Chapter 3 section 3.2.1.8.1, the pH in section 3.2.1.8.2 and Total Soluble Solids in section 3.2.1.8.3.

4.3 Statistical analysis

One-way Analysis of Variance (ANOVA) was performed to determine significant differences of functional attributes between two cultivars of nopalitos and eight other vegetables (NCSS 11 Statistical Software, 2016). The Tukey-Kramer multiple comparison test ($\propto = 0.05$) was carried out to determine significant differences between the treatment means (NCSS 11 Statistical Software, 2016)

4.4 Results and discussion

4.4.1 Texture (firmness and compressibility)

Texture is an important factor to identify ripeness of most vegetables. It can be described as a "make or break" factor. Consumers may have a perception of food and if, while chewing the food, the texture does not feel right they may not try it again (Johnson, 2018).

In Table 4.1, the firmness of carrots (23.58 10ths m/m), celery (25.94 10ths m/m), green beans (24.94 10ths m/m) and onion (30.94 10ths m/m) was significantly ($p < 0.001$) lower (harder) than all other tested vegetables. The values obtained for Fusicaulis nopalitos (78.61 10ths m/m) and Morado nopalitos (77.31 10ths m/m) indicated that they were significantly ($p < 0.001$) softer than all other tested vegetables. The compressibility of carrots (11.00 10ths m/m) was significantly ($p < 0.001$) lower than tomatoes (61.75 10ths m/m), baby marrow (25.67 10ths m/m), onion (41.33 10ths m/m), Fusicaulis (25.00 10ths m/m) and Morado (29.00 10ths m/m) which demonstrated to be easier to compress than carrots. No significant differences were observed in the compressibility between the two nopalito cultivars (Fusicaulis 25.00 10ths m/m and Morado 29.00 10ths m/m), green pepper (21.67 10ths m/m), celery (20.50 10ths m/m), baby marrow (25.67 10ths m/m), cucumber (21.67 10ths m/m) and green beans (18.67 10ths m/m). The data thus indicated that the compressibility of nopalitos was comparable to other popular vegetables, but the firmness was higher (softer) than the other vegetables.

Table 4.1: Analysis of variance (ANOVA) for the comparison of nopalitos with other vegetables on functional properties

Cultivar	Firmness (10ths m/m)	Compressibility (10ths m/m)	% Moisture	Viscosity (cP)	% TA as citric acid	pH	TSS (°Brix)
Green pepper	$48.47^{b} \pm 2.17$	$21.67^{ab} \pm 11.47$	$89.96^{bc} \pm 4.39$	$3.27^{a} \pm 1.32$	$0.09^{bc} \pm 0.06$	$5.36^{b} \pm 0.28$	$5.63^{cd} \pm 1.11$
Celery	$25.94^{a} \pm 6.57$	$20.50^{ab} \pm 10.13$	$85.89^{ab} \pm 1.87$	$2.20^{a} \pm 0.00$	$0.05^{ab} \pm 0.00$	$6.19^{d} \pm 015$	$7.20^{e} \pm 0.00$
Baby marrow	$48.00^{b} \pm 10.35$	$25.67^{b} \pm 4.63$	$88.65^{ab} \pm 3.73$	$1.32^{a} \pm 0.18$	$0.07^{ab} \pm 0.00$	$6.22^{d} \pm 0.25$	$4.82^{bc} \pm 0.35$
Cucumber	$61.08^{c} \pm 5.26$	$21.67^{ab} \pm 2.94$	$93.41^{c} \pm 1.00$	$1.70^{a} \pm 0.24$	$0.02^{a} \pm 0.00$	$7.07^{e} \pm 0.23$	$3.08^{a} \pm 0.41$
Green beans	$24.94^{a} \pm 4.23$	$18.67^{ab} \pm 5.05$	$85.16^{a} \pm 1.37$	$3.27^{a} \pm 3.40$	$0.08^{b} \pm 0.01$	$5.80^{c} \pm 0.14$	$6.87^{e} \pm 0.10$
Carrot	$23.58^{a} \pm 3.11$	$11.00^{a} \pm 1.10$	$85.93^{ab} \pm 0.94$	$1.93^{a} \pm 0.10$	$0.04^{ab} \pm 0.00$	$6.22^{d} \pm 0.16$	$9.33^{f} \pm 0.26$
Tomato	$46.97^{b} \pm 8.77$	$61.75^{d} \pm 4.02$	$93.27^{c} \pm 0.79$	$5.77^{a} \pm 1.44$	$0.15^{d} \pm 0.02$	$4.17^{a} \pm 0.35$	$4.63^{b} \pm 0.42$
Onion	$30.94^{a} \pm 4.23$	$41.33^{c} \pm 7.26$	$85.37^{a} \pm 3.25$	$9.87^{a} \pm 5.93$	$0.13^{cd} \pm 0.02$	$5.77^{c} \pm 0.14$	$12.83^{g} \pm 0.48$
Fusicaulis	$78.61^{d} \pm 4.25$	$25.00^{b} \pm 3.69$	$89.15^{abc} \pm 0.79$	$563.17^{b} \pm 214.97$	$0.29^{e} \pm 0.02$	$4.07^{a} \pm 0.06$	$6.50^{de} \pm 0.35$
Morado	$77.31^{d} \pm 6.34$	$29.00^{b} \pm 4.10$	$87.18^{ab} \pm 0.83$	$649.20^{b} \pm 41.19$	$0.28^{e} \pm 0.05$	$3.88^{a} \pm 0.15$	$6.88^{e} \pm 0.64$
Sign. Level	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

4.4.2 Turgidity and sliminess (moisture and viscosity)

The moisture content available in food has an effect on the taste, texture, weight, appearance and storage of food (Appoldt & Raihani, 2017). Viscosity of juices has an influence on the appearance and consistency of food which is due to changes in the flow (PCE Instruments, 2016).

It was observed in Table 4.1, that the moisture content of green beans (85.16 %) was the lowest (significantly at $p < 0.001$) and cucumber (93.41 %) the highest. Fusicaulis (89.15 %) and Morado nopalitos (87.18 %) were in the middle of this range. No significant differences were observed for moisture content between the two nopalito cultivars, green pepper (89.96 %), celery (85.89 %), baby marrow (88.68 %), green beans (85.16 %), carrot (85.93 %) and onion (85.37 %). The moisture content values were high for all tested vegetables. The moisture content for vegetables used in this study was reported in a list of 200 vegetables published by Whitbread (2019). Nopales was reported to contain 94.1 % water, and the water content for vegetables such as celery (95, 4 %), cucumber (95.2 %), green pepper (93.9 %), onion (89.11 %) and carrot (88.3 %) was reported (Whitbread, 2019). According to the study, the moisture content of vegetables may be influenced by the weather.

The viscosity of extracted juices ranged from the low viscosity (1.32 cP) of baby marrow to the higher viscosity (9.87 cP) of onion (not significantly different) which was significantly ($p < 0.001$) lower than the much higher viscosity of Fusicaulis mucilage (563.17 cP) and Morado mucilage (649.20 cP). Nopalito mucilage can be described as a non-Newtonian as it is thick and slimy (De Wit *et al.*, 2017; Ramírez-Moreno *et al.*, 2013; Medina-Torres *et al.*, 2000; Sáenz, 2000), while vegetable juices are described as Newtonian (behaves water-like). Thus, the data showed that the moisture content of nopalitos was comparable with other vegetables but that the viscosity of the juices was higher. Some cultures including Chinese and Japanese are fond of foods that are associated with a slippery and slimy mouthful. These foods, comparable to nopalitos due to sliminess, are associated with nutritional benefits. Okra which is produced in Africa, South USA and India (Orabane, 2015), Chinese yam and *nattō* (fermented soy beans) found in Japanese cuisines (Itoh, 2016) and lotus root which is enjoyed in cuisines of cultures such as Chinese, Japanese and Indians (Oskay, 2008).

4.4.3 Gustatory properties (acidity and TSS)

It is important to understand the acidity of food particularly for home canning. The amount of acidity (pH value) the vegetables contain has an impact on heat processing to ensure a safe final product (McGlynn, 2016; Cunningham, 2009). The TSS content influence eating quality, how consumers react after eating the product or how sweet it may taste (Kleinhenz & Bumgarner, 2013).

The % TA ranged from the low acidity of cucumber (0.02 %), carrot (0.04 %), celery (0.05 %), baby marrow (0.07 %) and green beans (0.08 %) to the significantly ($p < 0.001$) higher acidity of onions (0.13 %) and tomatoes (0.15 %). The titratable acidity of the two nopalito cultivars, Fusicaulis nopalitos (0.29 %) and Morado nopalitos (0.28 %) was significantly ($p < 0.001$) higher compared to all tested vegetables illustrated in Table 4.1. The acidity of nopalitos was higher than tomatoes, which had the closest acidity values to nopalitos.

The pH of the two nopalito cultivars (Fusicaulis 4.07 and Morado 3.88) was not statistically different from tomatoes (4.17), but was significantly ($p < 0.001$) lower than all other tested vegetables. Green pepper (5.36) was significantly ($p < 0.001$) lower than green beans (5.80) and onions (5.77) which was significantly lower than celery (6.19), baby marrow and carrots (6.22). Cucumber (7.07) had the highest pH than all tested vegetables (statistically significant). The pH of tomatoes may vary from 4.6 due to seasonal changes and variety (McGlynn, 2016). Bourassa (2019) listed the following vegetables, green pepper, onions, celery and cucumber in the list of high alkaline vegetables. In fact, these vegetables are characterized as low acid foods. McGlynn (2016) defines a low acid food as food with a pH higher than 4.6, which correlate with the data observed in this study. While, high acid food is defined as food with pH 4.6 or lower, indicating that nopalito cultivars may be identified as high acid foods. It is mostly fruits such as peaches, pears, oranges (to name a few) and pickled vegetables which are known as high acid foods. Pickled vegetables are mostly eaten with savoury dishes such as egg-fried rice and ham hock soup (Spoler & Pellegrino, 2015). Nopalitos cannot be compared to lemons because lemons are extremely acidic with a pH that ranges between 2.0 and 3.0 (Helmenstine, 2019).

The TSS content ranged from the lowest sugar content of cucumber (3.08 °Brix) to the highest of onions (12.83 °Brix). Fusicaulis (6.50 °Brix) and Morado (6.88 °Brix) were in the middle of this range and there were no significant differences between the two nopalito cultivars

Fusicaulis had a similar (statistically significant) sugar content to that of green pepper (5.63 °Brix), celery (7.20 °Brix) and green beans (6.87 °Brix), while Morado had a similar (statistically significant) sugar content to celery and green beans. The TSS content of nopalito cultivars may be comparable to celery, green pepper and green beans, which is known to influence the consumer acceptability of the product. This can be helpful for consumers to understand how nopalitos may taste like.

4.5 Conclusion

Nopalitos had similar turgidity compared to other vegetables however, the nopalito juices had higher viscosity compared to other tested vegetables. Thus consumers are likely to experience nopalitos as being slimy. The texture of nopalitos could be roughly compared to cucumber (penetrating ability) and baby marrow (compressibility). The taste of nopalitos could be described as being more sour than tomatoes, and as having comparable sugar content to celery, green pepper and green beans. The two nopalitos cultivars had comparable turgidity, texture and taste characteristics compared to popular vegetables. Nopalitos have potential as a source of vegetables due to their comparable characteristics to well- known vegetables in South Africa.

4.6 Recommendations

Further study should be done on cooked vegetables which have similar turgidity, taste and texture to nopalito cultivars and be compared to cooked nopalitos in regards to sensory analysis.

Chapter 5

5 The effect of heat on nopalito properties compared to green pepper

5.1 Introduction

Nopalitos have been cooked in Mexico for decades however, due to the different customs and eating habits, Mexican recipes are not compatible with South African eating habits. South African consumers don't have the knowledge necessary to prepare and cook nopalitos. Getting the interest of the people to try nopalitos can be promoted by the use of cooking instructions and recipes. Recipes can only be developed with the knowledge of different cooking techniques. It is important for consumer and food scientists to develop recipes based on South African customs and culture.

The aim of this chapter was to determine post-harvest handling, pre-cooking and preferred cooking methods and optimal cooking times to use in newly developed South African recipes. It is important to understand the preparation techniques in order for the South African population to become familiar with the handling of the new product.

Nopalitos are similar to green pepper in the sense that both can be eaten raw and cooked using a variety of different cooking methods, and in different types of dishes. Therefore nopalitos and green pepper samples were cooked using different cooking methods and different periods to determine the optimal cooking instructions for nopalitos. Heat application could lead to nutrient loss, thus the right balance must be found between cooking and optimal taste and texture (De Santiago *et al*., 2018).

5.2 Materials and Methods

5.2.1 Pre-cooking handling of nopalitos

It is important to understand the handling of nopalitos before cooking in order to be able to prepare tasty and nutritious meals and dishes containing nopalitos.

5.2.1.1 Sample preparation

Morado nopalitos between 15 and 18 cm were used in this study. The spines of nopalitos were removed manually using a sharp blade. Nopalitos were washed by rinsing in potable water. The nopalitos were placed on a chopping board and roughly cut into small pieces.

5.2.1.2 Pre-cooking preparation tests

Mucilage content is undesirable in nopalitos as it is slimy and therefore may be assumed that consumers would prefer to eat nopalitos with lower mucilage content (Du Toit, 2017). Thus, different pre-cooking preparation methods were tested for the purpose of reducing the mucilage content. Three pre-cooking methods were applied:

Method 1

The nopalito samples were covered in salt and allowed to stand for ten minutes (Figure 5.1), and rinsed until no visible mucilage was observed (Shaw, 2019; Wang, 2014).

Figure 5.1: The cut pieces of nopalitos covered in salt

Method 2

The nopalito samples were covered in salt and allowed to stand for ten minutes and rinsed. After rinsing, the nopalitos were soaked in clean water for thirty minutes and rinsed until no visible mucilage was observed (Figure 5.2).

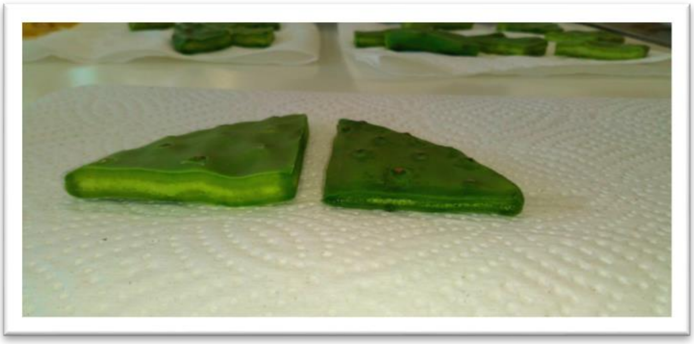


Figure 5.2: Sample soaked in clean water (left) vs sample soaked in saltwater (right)

Method 3

The nopalito samples were covered in salt and allowed to stand for ten minutes and rinsed. After being rinsed, the nopalitos were soaked in saltwater (500 ml of water with 20 g of salt) for thirty minutes and rinsed until no visible mucilage was observed (Figure 5.2).

5.2.2 Cooking methods

5.2.2.1 Sample preparation

Twenty-four nopalitos from the cultivar Morado of sizes between 15 cm and 18 cm were harvested and prepared for analysis. The spines were removed and nopalitos were rinsed in water. Each nopalito, was divided crosswise into three pieces. All the samples were cut to the same length of 20 mm.

Ten green peppers were bought at the local market. The green peppers were cut lengthwise into the same number and size as the nopalito samples. For nopalitos and green pepper, each test was repeated six times using six different samples.

Nopalitos may be eaten raw however, nopalitos would be required to be cooked in many recipes. The influence of cooking on the texture of nopalitos and green peppers was compared. Therefore the effect of different cooking methods and different time increments was determined for nopalitos and green pepper. Four cooking methods were used for tests namely roasting, boiling, microwave cooking and shallow frying. These methods were chosen because they are the common cooking methods that are used to cook vegetables (Brown, 2018).

5.2.2.1.1 Roasting

The oven was preheated at 180 °C and the roasting tray was greased with cooking oil spray. The nopalitos and green peppers were placed in the roasting tray and baked for 10 minutes and 15 minutes. It was removed from the oven and left to cool down for observations (Figure 5.3).

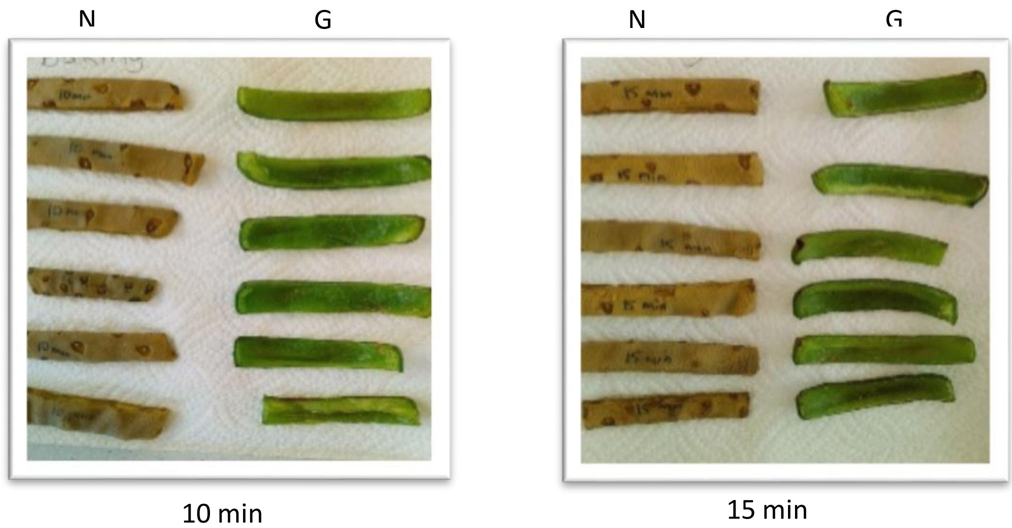
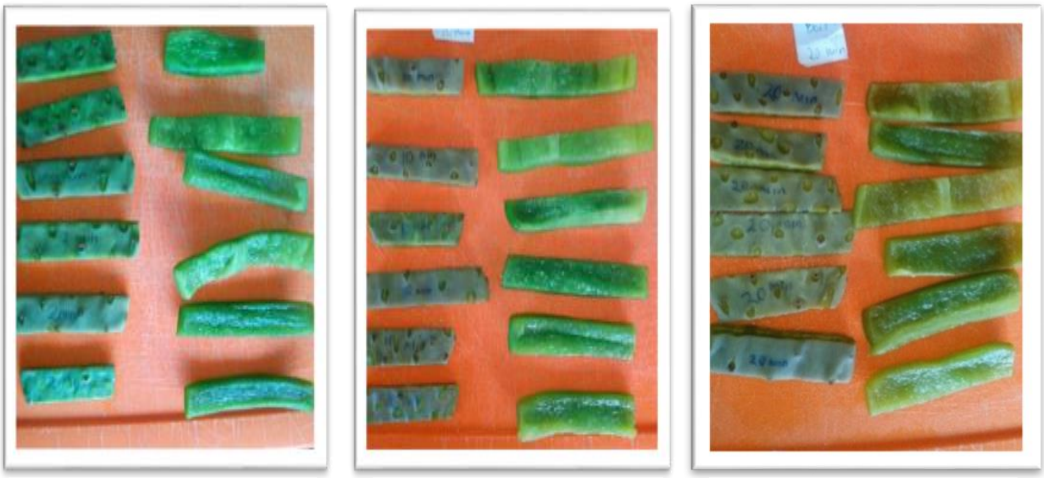


Figure 5.3: Roasted samples of nopalitos (N) and green pepper (G) for 10 min and 15 min

5.2.2.1.2 Boiling

The nopalitos and green peppers were boiled under controlled temperature in a water bath at 85 °C to maintain the temperature it required to boil the samples (Figure 5.4). The nopalitos and green peppers were boiled at three different time increments namely 2 minutes, 10 minutes and 20 minutes.

N G N G N G



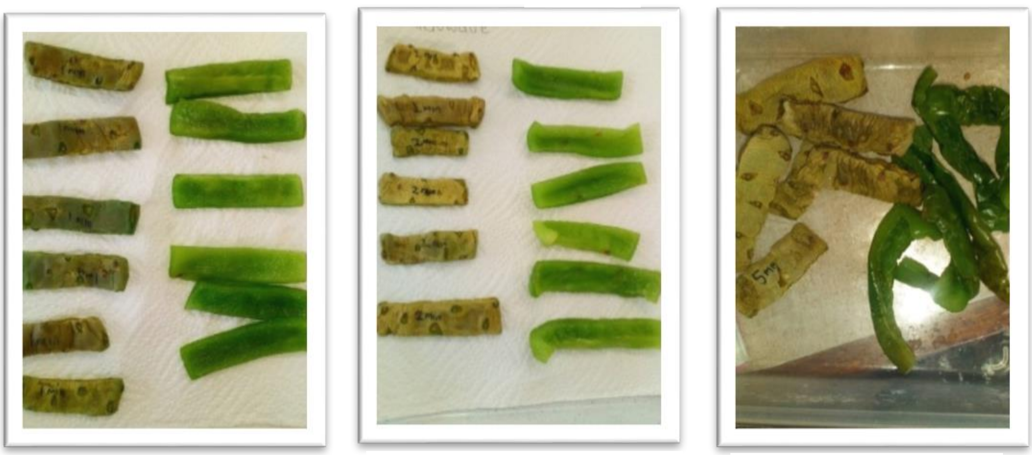
2 min 10 min 20 min

Figure 5.4: Samples of nopalitos (N) and green pepper (G) boiled for 2, 10 and 20 min

5.2.2.1.3 Microwave cooking

Microwave safe and heat resistant glass bowls were used to microwave cook (Russell Hobbs manual microwave, 700 W) nopalitos and green peppers at different time periods of 1 minute, 2 minutes and 5 minutes, without the addition of water (Figure 5.5).

N G N G N G



1 min 2 min 5 min

Figure 5.5: Nopalitos (N) and green pepper (G) microwave cooked for 1, 2 and 5 min

5.2.2.1.4 Shallow Frying

A Pineware square electric frying pan was used and set to a medium-low heat (150 °C) and preheated. The nopalitos and green peppers were fried in 10 ml canola oil for 2 minutes and 5 minutes. The nopalitos were constantly turned to avoid burning. Samples were placed on a paper towel to cool down before observations were made (Figure 5.6).

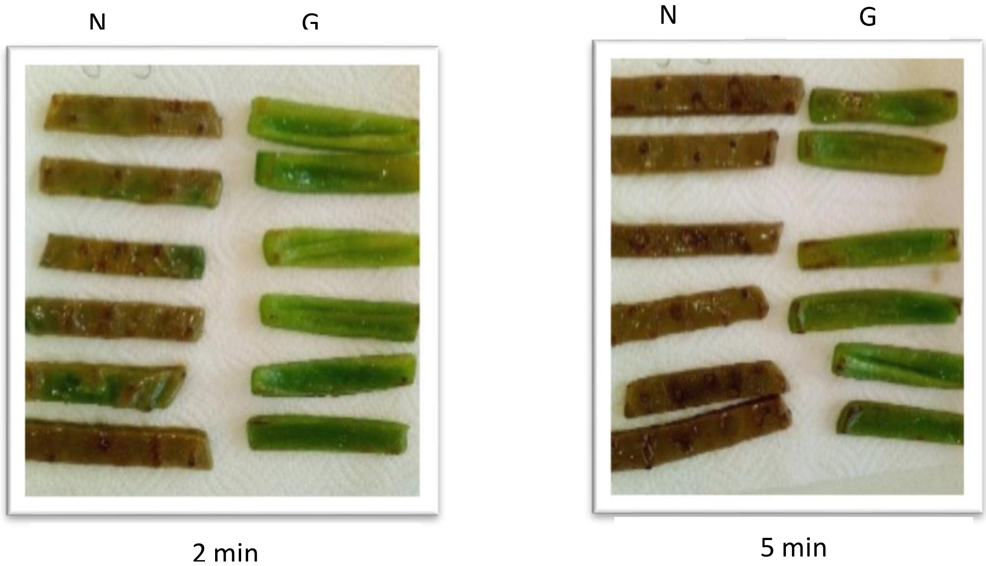


Figure 5.6: Nopalitos (N) and green pepper (G) shallow fried for 2 and 5 minutes

5.2.2.2 Texture analysis: Penetration testing

The texture of nopalitos and green pepper were measured using a Brookfield CT3 Texture analyser. The test type was a compression test with a pre-test speed of 2.00 mm/s, test speed of 0.50 mm/s and post-test speed of 0.5 mm/s. The load cell was 10 000 g, trigger load was 7 g and the target distance at 7 mm. The results were observed from an installed Texture Pro CT software, the force was measured in grams, deformation in millimetre (mm) and work done in millijoule (mJ). Six different nopalito samples were tested for each cooking method and time. In each sample (nopalito strip), three locations (top, middle and bottom) were tested.

Samples were placed on the fixture base table TA-CJ below the needle probe TA9 (stainless steel, 1.0 mm diameter and 43 mm long) shown in Figure 5.7. The test was started and the probe penetrated and fractured the sample over a target distance of 7 mm. As the probe made contact to the sample, the force necessary to fracture the sample increased.

Figure 5.7: A sample placed on the fixture base table of a texture analyser

Hardness (g)

The maximum force value to penetrate the sample, indicated over the target distance is a measure of hardness (g) which can be explained as, the higher the maximum force value, the harder the sample.

Hardness work (mJ)

The area under the curve indicated in Figure 5.8 is the energy required to overcome the strength of the internal bonds within the sample when fracturing the sample and is a measure of the hardness work (mJ), thus, the higher the value, the more energy required to fracture the sample.

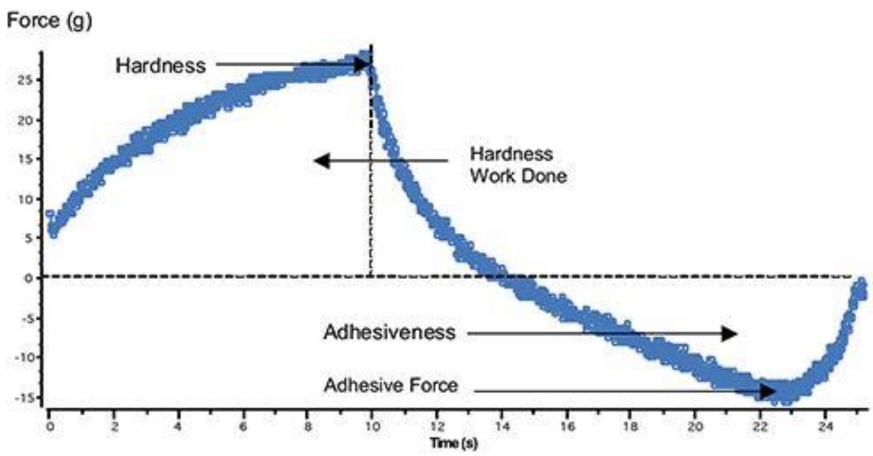


Figure 5.8: Hardness work done of fromage frais. Brookfield CT3 Texture Analyser

Deformation at target (mm)

The distance that the probe travel to fracture the sample is a measure of deformation at hardness (mm), the shorter the distance, the harder the sample.

5.2.2.3 Tension testing

The texture of nopalitos and green pepper were measured using a Brookfield CT3 Texture Analyser. The test type was a tension test for a target distance of 30 mm. Six samples were tested for each cooking method and time.

The samples were cut into strips and fixed to two grips, the platform (bottom) and the traveling grip (top) of a roller cam accessory grips (TA-RCA) to measure the tensile strength. When the test is started, the top travelling grip moves upwards at a speed of 0.50 mm/s, thus pulling the sample apart at a target distance of 30 mm illustrated in Figure 5.9.

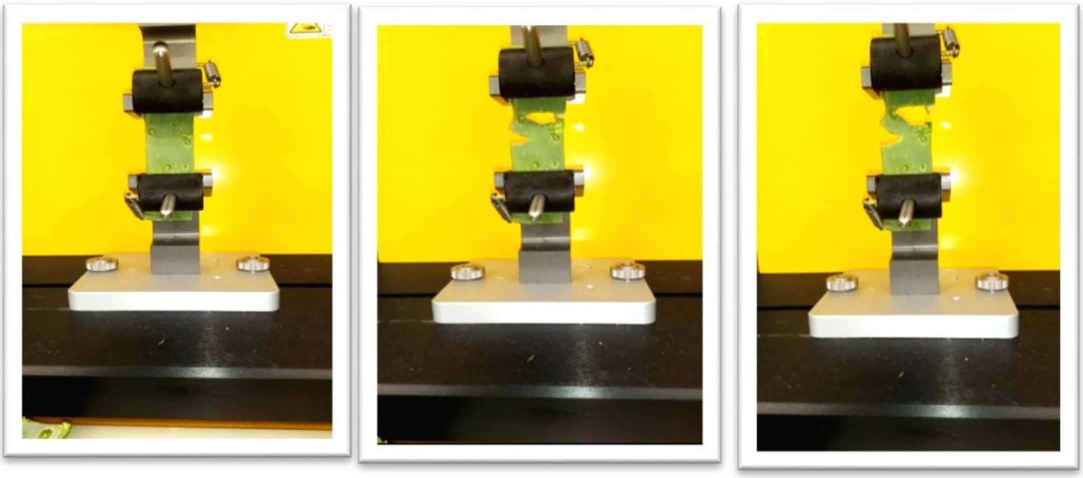


Figure 5.9: Nopalito strips tear as the travelling grip slowly move upward

Peak load (g)

The maximum force value necessary to tear the sample is measured as peak load (g), the higher the value, the more force was needed to pull the sample to a point that it tears apart.

5.3 Statistical analysis

Attributes from Morado and Green pepper were compared with a two sample t-test (NCSS 11 Statistical Software, 2016). The interaction between Morado and Green Pepper were analysed with a one-way Analysis of Variance procedure (NCSS 11 Statistical Software, 2016).

5.4 Results and discussion

5.4.1 Pre-cooking

The process of osmosis can be described as the flow of water from a less concentrated solution to a more concentrated solution, through a semi-permeable membrane (skin of a plant) (Brown, 2018). Salt was used to extract moisture (mucilage) from nopalitos through the process of osmosis.

Three different results were observed with three pre-cooking preparation methods. When nopalito pieces were soaked in dry salt, the mucilage was visually observed as the salt absorbed the mucilage. As a result, the salt became wet as the mucilage was visible on the surface of nopalitos within ten minutes. However, it showed with minimum impact, as less mucilage was observed compared to the other two methods.

The second method included soaking in clean water after being covered with dry salt, the results were similar to the first method. It was interesting to observe that, clean water stopped the mucilage from coming into the surface after being absorbed by salt. Therefore, this method could be recommended to stop the removal process of mucilage.

The method that showed the best results as there was a huge amount of mucilage that was removed from the nopalito pieces compared to other methods was the third method. The nopalitos were soaked in salt water after being covered with dry salt. The dry salt absorbed the mucilage and when soaked in salt water that process continued. The difference was shown in Figure 5.2. As a result, the third method could be recommended for the removal of mucilage in nopalitos.

5.4.2 Comparison of the effect of cooking methods on attributes of Morado nopalitos and green pepper

The measurements of attributes for the effect of cooking methods (roasting, boiling, microwave cooking and shallow frying) on the penetration and tension testing were observed and illustrated in Table 5.1.

5.4.2.1 Hardness (g)

Roasting

The hardness (g) of raw nopalitos (503.67 g) was significantly ($p < 0.001$) lower than raw green peppers (1137.61 g). This implies that raw nopalitos have a softer texture than raw green peppers. Nopalitos roasted for 10 min (363.18 g) had a significantly ($p < 0.001$) lower hardness value than roasted green peppers (847.78 g), indicating that nopalitos had a softer texture than green peppers after roasting for 10 min. No significant differences on hardness were observed between nopalitos and green peppers roasted for 15 min. This may indicate that a similar softness was observed between nopalitos and green peppers when roasted for 15 min. It was observed that there were no significant differences in hardness between raw nopalitos and roasted nopalitos (10 and 15 min), implying that roasted nopalitos can be enjoyed in salads and can perceive a comparable texture to raw nopalitos. There were no significant differences on hardness between raw and roasted green peppers (10 min), however, raw green peppers had a significantly ($p < 0.001$) higher hardness value than green peppers roasted for 15 min, indicating that a longer roasting period, caused the green peppers to be significantly softer, while nopalitos were not significantly softer at longer roasting periods.

Boiling

Nopalitos boiled for 2 min (506.89 g) had a significantly ($p < 0.001$) lower hardness value than green peppers (1065.94 g). No significant differences in hardness were observed between nopalitos and green peppers boiled for 10 and 20 min. There were no significant differences in hardness observed for raw nopalitos and boiled for 2, 10 and 20 min. As a result nopalitos can be boiled for up to 20 min making the nopalitos softer. It can be recommended not to boil nopalitos for a longer time to avoid losing nutrients. Significant differences in green peppers were observed between the three boiling times, indicating that boiling green peppers for a longer time softens the texture as raw green peppers had a significantly ($p < 0.001$) higher hardness value than those boiled for 20 min.

Microwave

There were no significant differences in hardness between nopalitos and green peppers that were microwave cooked for 1 and 2 min. Nopalitos microwave cooked for 5 min (596.17 g) had a significantly ($p < 0.001$) higher hardness value than green peppers (185.83 g). It was deduced that green peppers cooked for a longer period had a softer texture than nopalitos. No significant differences were observed between raw and 1 min microwave cooked nopalitos. Nopalitos microwave cooked for 1 min (297.56 g) had a significantly ($p < 0.001$) lower hardness value than those cooked for 5 min (596.17 g). Nopalitos reached maximum hardness value because the heat inside the microwave can remove moisture from the sample causing it to dry up and become firm (Gonzalez, 2016), especially when the sample was cooked for a longer period. As a result nopalitos should not be cooked in the microwave for longer than 1 minute. The hardness value for raw green peppers was significantly ($p < 0.001$) higher than those microwave cooked for 1, 2 and 5 min. Thus, microwave cooking softened the texture of green peppers while it hardened the texture of nopalitos.

Table 5.1: Analysis of variance (ANOVA) on the effect of cooking method on penetrometer and tension measurement of attributes of Morado nopalitos and green pepper

Cultivar	Processing method	Processing time (min)	Hardness (g)	Hardness work (mJ)	Deformation at target (mm)	Peak load (g)
Morado	Raw		503.67[bcd] ± 61.36	8.43[abcde] ± 0.93	3.97[a] ± 0.01	2684.17[ij] ± 390.81
Green pepper			1137.61[f] ± 76.78	20.87[g] ± 1.83	3.95[a] ± 0.01	2708.17[j] ± 392.13
Morado	Roasting	10	363.18[abc] ± 59.29	9.25[abcdef] ± 0.54	6.99[c] ± 0.01	1584.83[defg] ± 348.25
Green pepper			847.78[ef] ± 218.26	12.13[def] ± 2.52	3.96[a] ± 0.02	1699.50[efg] ± 369.66
Morado	Roasting	15	308.79[abc] ± 54.05	7.64[abcd] ± 1.43	6.98[c] ± 0.02	1395.50[cdefg] ± 278.59
Green pepper			502.22[bcd] ± 190.82	7.23[abcd] ± 2.27	3.96[a] ± 0.01	989.50[abcde] ± 305.42
Morado	Boiling	2	506.89[bcd] ± 29.61	11.91[def] ± 0.83	6.98[c] ± 0.01	1944.67[fghi] ± 186.58
Green pepper			1065.94[f] ± 226.06	14.96[efg] ± 2.20	3.95[a] ± 0.02	2473.83[hij] ± 550.19
Morado	Boiling	10	474.50[abcd] ± 50.19	11.41[cdef] ± 1.46	6.98[c] ± 0.01	1946.33[fghi] ± 386.23
Green pepper			707.28[de] ± 197.77	11.02[bcdef] ± 3.02	3.95[a] ± 0.01	2066.33[ghij] ± 384.16
Morado	Boiling	20	377.78[abc] ± 41.08	4.43[ab] ± 4.93	6.99[c] ± 0.01	1420.67[defg] ± 291.86
Green pepper			352.67[abc] ± 82.32	5.12[abc] ± 1.50	3.96[a] ± 0.01	857.67[abcd] ± 282.24
Morado	Microwave	1	297.56[ab] ± 181.10	6.44[abcd] ± 4.93	5.48[b] ± 1.65	1250.67[bcdef] ± 336.89
Green pepper			443.50[abcd] ± 125.77	6.27[abcd] ± 1.70	3.97[a] ± 0.01	922.67[abcd] ± 309.14
Morado	Microwave	2	415.06[abc] ± 142.47	9.37[abcdef] ± 3.88	6.49[c] ± 1.23	1372.33[cdefg] ± 430.79
Green pepper			337.89[abc] ± 127.06	4.81[abc] ± 1.74	3.97[a] ± 0.01	656.67[abc] ± 181.85
Morado	Microwave	5	596.17[cde] ± 303.09	15.72[fg] ± 10.26	6.98[c] ± 0.01	1742.67[efgh] ± 385.26
Green pepper			185.83[a] ± 30.79	2.97[a] ± 0.64	3.96[a] ± 0.01	287.33[a] ± 128.17
Morado	Shallow fry	2	386.94[abc] ± 81.92	9.10[abcdef] ± 1.50	6.98[c] ± 0.02	1495.83[defg] ± 357.66
Green pepper			500.28[bcd] ± 155.78	8.73[abcde] ± 2.87	3.95[a] ± 0.01	1276.33[bcdef] ± 642.47
Morado	Shallow fry	5	329.67[abc] ± 36.58	8.01[abcd] ± 0.62	6.97[c] ± 0.01	1553.17[defg] ± 253.88
Green pepper			270.50[ab] ± 40.04	4.33[ab] ± 0.62	3.97[a] ± 0.01	558.50[ab] ± 217.73
Significance level			$p< 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly.

Shallow fry

No significant differences in hardness were observed between nopalitos and green peppers that were shallow fried (2 and 5 min). This implied that the texture of nopalitos was comparable to that of green peppers when shallow fried. There were no significant differences in hardness between raw and shallow fried nopalitos. The hardness value of raw green peppers was significantly ($p < 0.001$) higher than shallow fried nopalitos.

There were no significant differences in the hardness value of raw nopalitos compared to nopalitos cooked for each cooking method and period. As a result, samples had similar texture characteristics. The shortest cooking period for each cooking method can be considered as potential cooking period to obtain optimal texture of cooked nopalitos. Suggesting that nopalitos can be briefly shallow fried to obtain a similar soft texture to raw nopalitos when added in salads.

5.4.2.2 Hardness work (mJ)

Roasting

The hardness work value of raw nopalitos (8.43 mJ) was significantly ($p < 0.001$) lower than raw green peppers (20.87 mJ), indicating that the probe required less energy to fracture nopalitos than green peppers, thus nopalitos were softer than green peppers. No significant differences in hardness work were observed between nopalitos and green peppers roasted for 10 and 15 min thus, the probe used the same energy to break both nopalitos and green peppers. No significant differences in hardness work were observed between raw and roasted nopalitos. The hardness work value of raw green peppers was significantly ($p < 0.001$) higher than roasted green peppers thus, roasted peppers were softer as the probe of roasted green peppers required less energy to break the sample than in raw green peppers.

Boiling

There were no significant differences in hardness work between nopalitos and green peppers boiled over 2, 10 and 20 min. No significant differences in hardness work values were observed between raw and boiled nopalitos. Raw green peppers had a significantly ($p < 0.001$) higher hardness work value compared to green peppers boiled for 10 and 20 min, meaning that boiled green peppers were softer than when raw.

Microwave

No significant differences in hardness work values were observed between nopalitos and green peppers microwave cooked for 1 min and 2 min. Nopalitos had significantly ($p < 0.001$) higher hardness work values compared to green peppers microwave cooked for 5 min thus, nopalitos were harder. There were no significant differences between raw nopalitos and those microwave cooked for 1 and 2 min. Raw nopalitos had a significantly ($p < 0.001$) lower hardness work value (softer) compared to nopalitos microwave cooked for 5 min (harder). Raw green peppers had a significantly ($p < 0.001$) higher hardness work value (harder) than microwave cooked green peppers (softer).

Shallow fry

There were no significant differences in hardness work values observed between nopalitos and green peppers shallow fried for 2 and 5 min, meaning that shallow fried nopalitos and green peppers had a similar softness. No significant differences in hardness work values were observed between raw nopalitos and shallow fried nopalitos and as a result, shallow fried nopalitos were as soft as raw nopalitos. Raw green peppers had a significantly ($p < 0.001$) higher hardness work value (harder) compared to shallow fried green peppers (softer).

Overall, the hardness work value of raw nopalitos (8.43 mJ) was significantly ($p < 0.001$) lower (softer) than raw green peppers (20.87 mJ) (harder).

5.4.2.3 Deformation (mm)

Roasting

No significant differences in deformation values were observed between raw nopalitos and green peppers. Nopalitos roasted for 10 and 15 min (6.99 mm) had a significantly ($p < 0.001$) higher deformation value (softer) than green peppers (3.96 mm) roasted for 10 and 15 min (harder). Raw nopalitos had a significantly ($p < 0.001$) lower deformation value (harder) than roasted nopalitos (softer), thus roasting significantly softened nopalitos. No significant differences in deformation value were observed between raw and roasted green peppers.

Boiling

Nopalitos boiled for 2, 10 and 20 min had a significantly ($p < 0.001$) higher deformation value (softer) compared to green peppers boiled for 2, 10 and 20 min (harder), meaning boiling softened nopalitos more compared to green peppers. Raw nopalitos had a significantly ($p < 0.001$) lower deformation value (harder) compared to boiled nopalitos (softer) thus, boiling significantly softened nopalitos. No significant differences were observed in deformation values between raw and boiled green peppers.

Microwave

Nopalitos microwave cooked for 1 min (5.48 mm) had a significantly ($p < 0.001$) higher deformation value (softer) than green peppers (3.97 mm) microwave cooked for 1 min (harder). Nopalitos microwave cooked for 2 min (6.49 mm) had a significantly ($p < 0.001$) higher deformation value (softer) compared to green peppers (3.79 mm) microwave cooked for 2 min (harder). Nopalitos microwave cooked for 5 min (6.98 mm) had a significantly ($p < 0.001$) higher deformation value (softer) than green peppers (3.96 mm) microwave cooked for 5 min (harder). Thus, microwave cooking softened nopalitos more compared to green peppers. Raw nopalitos had a significantly ($p < 0.001$) lower deformation value (harder) compared to microwave cooked nopalitos, meaning microwave cooking softened nopalitos. No significant differences in deformation values were observed between raw and microwave cooked green peppers.

Shallow fry

Nopalitos shallow fried for 2 min (6.98 mm) had a significantly ($p < 0.001$) higher deformation value (softer) than green peppers (3.95 mm) shallow fried for 2 min (harder). Nopalitos shallow fried for 5 min (6.97 mm) had a significantly ($p < 0.001$) higher deformation value (softer) than green peppers (3.97 mm) shallow fried for 5 min (harder). This indicated shallow frying softened nopalitos more than green peppers. Raw nopalitos had a significantly ($p < 0.001$) lower deformation value (harder) compared to shallow fried nopalitos (softer). No significant differences in deformation values were observed between raw and shallow fried green peppers.

Overall, nopalitos were significantly softer compared to green peppers in each cooking method and period, while raw nopalitos were significantly harder compared to cooked nopalitos for each cooking method and period.

5.4.2.4 Peak load (g)

Roasting

There were no significant differences observed in peak load values between raw nopalitos and green peppers. No significant differences in peak load values were observed between nopalitos and green peppers roasted for 10 and 15 min. This indicated that the force required to pull apart and tear nopalitos was comparable to green peppers. Raw nopalitos had a significantly ($p < 0.001$) higher peak load value compared to roasted nopalitos, meaning roasting softened nopalitos and less force was needed to tear it apart. Raw green peppers had a significantly ($p < 0.001$) higher peak load value (harder) than roasted green peppers (softer). Thus, roasting significantly softened green peppers.

Boiling

There were no significant differences in peak load values between nopalitos and green peppers boiled for 2, 10 and 20 min. No significant differences in peak load values were observed between raw and boiled nopalitos for 2 and 10 min, meaning raw and boiled nopalitos had a similar softness. Raw nopalitos boiled for 20 min (2684.17 g) had a significantly ($p < 0.001$) higher peak load value (harder) than nopalitos (1420.67 g) boiled for 20 min (softer), meaning that boiling nopalitos for 20 min softened them. No significant differences in peak load values were observed between raw and boiled green peppers over 2 and 10 min. Raw green peppers boiled for 20 min (2708.17 g) had a significantly ($p < 0.001$) higher peak load value (harder) than green peppers (857.67 g) boiled for 20 min (softer) as a result, boiling for 20 min had an effect on green peppers as the texture softened.

Microwave

No significant differences in peak load values were observed between nopalitos and green peppers microwave cooked for 1 and 2 min. Nopalitos microwave cooked for 5 min (1742.67 g) had a significantly ($p < 0.001$) higher peak load value (harder) compared to green peppers (287.33 g) microwave cooked for 5 min. This indicated that microwave cooking for 5 min hardened nopalitos. Raw nopalitos had a significantly ($p < 0.001$) higher peak load value (harder) compared to microwave cooked nopalitos (softer). Raw green peppers had a significantly ($p < 0.001$) higher peak load value (harder) than boiled green peppers (softer).

Shallow fry

There were no significant differences in peak load values observed between nopalitos and green peppers shallow fried over 2 min. Nopalitos shallow fried for 5 min (1553.17 g) had a significantly ($p < 0.001$) higher peak load value compared to green peppers (558.50 g) shallow fried over 5 min. This indicated that shallow frying for 5 min softened green peppers more compared to nopalitos. Raw nopalitos had a significantly ($p < 0.001$) higher peak load value (harder) than nopalitos shallow fried over 2 and 5 min (softer) meaning, shallow frying significantly softened nopalitos. Raw green peppers had a significantly ($p < 0.001$) higher peak load value compared to green peppers shallow fried over 5 min as a result, shallow frying substantially softened green peppers.

Samples with higher peak load values indicated a firmer sample as it required more force to tear it apart. Raw nopalitos had the highest peak load values and were the hardest. On the other hand, nopalitos that had undergone heat treatment, in terms of the peak load measurements, the texture was classified into three categories namely, soft texture (1944.67 to 1946.33 g), softer texture (1420.67 to 1584.83 g) and softest texture (1250.67 to 1395.50 g). Nopalitos that had a soft texture were boiled (2 and 10 min), softer texture were boiled (20 min), shallow fried (2 and 5 min) and roasted (10 min) and softest texture were microwave cooked (1 and 2 min) and roasted (15 min).

5.5 Conclusion

The hardness value of nopalitos that were cooked using roasting and boiling was comparable to those that undergone microwave cooking and shallow frying. Interestingly, nopalitos cooked for a shorter time were comparable to those cooked for a longer time. It can be concluded that nopalitos don’t require a longer cooking time for a softer texture. This may imply that nopalitos could be recommended to be cooked for a shorter time in order to retain nutrients, while longer cooking time could be recommended for matured or tougher nopalitos. As observed, raw nopalitos had a soft texture as there were no significant differences in hardness between raw and cooked nopalitos. Nopalitos could be cooked using the following methods roasting, boiling and shallow frying over the shorter cooking time. Microwave cooking is not recommended as it causes nopalitos to become hard and firm.

Interestingly, green peppers required longer cooking time to obtain a softer texture of which that was not the case with nopalitos.

Chapter 6

General discussion and conclusion

Nopalitos have been considered a valuable food source and enjoyed in cultural cuisine mostly in Mexico. The nopalito production has been thriving and sustaining people in many countries for many years. According to the research it was identified as a vegetable with nutritious benefits and can be used for medicinal purposes. This study evaluated the quality characteristics in terms of morphology, colour, texture, turgidity, sliminess and gustatory properties of nopalitos from South African cactus pears. The quality characteristics observed in this study provided information on mostly consumed known vegetables comparable to *Opuntia* nopalitos. The two nopalito cultivars (Fusicaulis and Morado) showed good quality characteristics in terms of consumer preferences.

The size of nopalitos had an influence on the morphological characteristics, resulting in nopalitos between 15 and 18 cm having a thinner diameter compared to other nopalitos sizes. The two nopalito cultivars were observed with highest firmness values at

15 cm, nopalitos harvested at 15 cm were softer and more tender compared to other nopalito sizes. Colour was influenced by nopalito size, nopalitos (15 cm) had a brighter and greener colour compared to nopalitos at 24 cm. This study showed that middle size (15-18 cm) harvest of Morado nopalitos had a brighter greener colour compared to Fusicaulis nopalitos. The observation of moisture content was similar to other studies, which remained high for both cultivars throughout development. Hence the two cultivars nopalitos are considered fresh and ready to eat. In terms of taste, nopalitos at 9 cm were less sour (associated with high quality) than nopalitos at 24 cm. It can be stated that the middle size growth can produce nopalitos that are acceptably sour nopalitos. The PCA analysis showed nopalitos between 9 cm and 15 cm were associated with good texture, colour and taste characteristics.

SEM results indicated that Fusicaulis nopalitos at 9 cm had the most number of crystals compared to at 24 cm however, Morado nopalitos had the most number of crystals at 24 cm. The number of crystals observed for both nopalito cultivars was comparable at 15 cm. In this study, results showed that sizes between 15 and 18 cm were ideal sizes for a higher quality nopalito production. Both *Opuntia* cultivars could be associated with good quality nopalitos to be enjoyed in South African dishes.

Nopalitos were found to have the same "fresh" properties as other vegetables, similar turgidity was observed between tested vegetables and nopalitos. On the other hand, nopalitos were found to have non-Newtonian behaviour (juices were thick and slimy) and vegetables demonstrated Newtonian behaviour (thin and runny). Nopalitos can be described as slimy food, in fact, some cultures such as the Chinese are fond of slimy foods. Nopalitos demonstrated a texture that could be roughly compared to cucumber according to penetrating ability, while it can also be compared to baby marrow in terms of compressibility. The taste observed for nopalitos could give South Africans an insight of what to expect in terms of sourness. Nopalitos could be described to be more sour than tomatoes yet, less sour than lemons. The sugar content of nopalitos proved to be similar to celery, green pepper and green beans. Therefore, the two nopalito cultivars had comparable turgidity, texture and taste characteristics compared to known vegetables. As observed, nopalitos are a valuable source of vegetable due to their comparable characteristics to well-known vegetables in South Africa.

Cooked nopalitos presented similar softness texture for each cooked method. Roasting and boiling were comparable to microwave cooking and shallow frying. The time of cooking did not influence the texture of nopalitos as, nopalitos cooked for a shorter time were comparable to those cooked for a longer time. Thus nopalitos should be simply cooked for a shorter period while, longer cooking period could only be recommended for matured or tougher nopalitos. Nopalitos can be cooked using the following methods roasting, boiling and shallow frying over a shorter cooking period.

Recommendations for further research

Future research may include the evaluation of nutritional information and a sensory analysis of all available cultivars of raw and cooked nopalitos, thus, a study on sensory analysis and detailed nutritional information of cooked nopalitos.

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