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# FUNCTIONAL PROPERTIES OF CACTUS PEAR MUCILAGE: GEL FORMATION, EDIBLE COATINGS, FILMS AND SPHERIFICATION.

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By

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Submitted in fulfilment of the requirements for the degree of

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## Declaration

I declare that the dissertation hereby handed in for the qualification of M.Sc. Food 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.

I hereby concede copyright of this dissertation to the University of the Free State.

A handwritten signature in black ink, appearing to read 'ADD', is written over a horizontal line.

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## Meaning of abbreviations or symbols

% - Percentage

°C - Degree Celsius

°F- Degree Fahrenheit

a\*- Redness (+) or Greenish (-)

AMC - Algerian mucilage coating

ANOVA - Analysis of Variance

b\*- Yellowish (+) or Bluish (-)

C\*- Chromo

C.C- Cubic centimetre

CAM - Crassulacean Acid Metabolism

cm - Centimetres

cP – Centipose

EC - Edible coating

FD - Freeze-dried

FIMC - Ficus-indica mucilage coating

g - Grams

g / cm<sup>3</sup>- Grams per cubic centimetre

g/l - grams per litre

g/mol - Grams per mole

GPS - Global Positioning System

h° - Hue

HAD - Hot-air dried

HCl - Hydrochloric acid

kDa- Kilodalton

L\*- White (100) or Black (0)

MA - Mucilage and Agar

MG - Mucilage and Guar gum

MGA - Mucilage, Guar gum, and Agar

MGX - Mucilage, Guar gum, and Xanthan

MJ - Megajoules

ml - Millilitre

Mm - Millimetres

mm/s - millimetre per second

MX - Mucilage and Xanthan

MXA - Mucilage, Xanthan, and Agar

NaNO<sub>3</sub> - Sodium nitrate

NaOH - Sodium hydroxide

NMC - Nepgen mucilage coating

OHC - Oil Holding Capacity

RMC - Robusta mucilage coating

WHC - Water Holding Capacity

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## Abstract

Mucilage is a hydrocolloid, a gelatinous slimy substance that contains polysaccharides and proteins. Due to mucilage's ability to absorb large amounts of water, which result in modified viscosity, it can be used as a food additive in the food industry as a hydrocolloid to modify food texture. It is used as an emulsifier, to form gels and as a natural edible coating.

The aim of the study was to investigate the functional properties of mucilage and to potentially develop mucilage into a commercially viable food ingredient (hydrocolloid). Cladodes from four cultivars (and two species) were investigated. These include three cultivars from *Opuntia Ficus-indica*, namely *Nepgen*, *Algerian* and *Ficus-Indice* as well as one cultivar from the *O.robusta* spp. namely *Robusta*. Mucilage was extracted, and the yield was determined in percentage, with *Algerian* at 53.70%, *Nepgen* at 50.96%, *Robusta* at 48.40% and *Ficus-indice* at 37.75%. The mucilage was dried in two ways, namely, freeze-drying, and hot-air dehydration and colour difference was investigated. The drying impacted the colour of mucilage powder, with freeze-dried remaining green and the hot-air dried turned brown, colour difference was observed between the freeze-dried and hot-air dried mucilage  $a^*$  value.  $a^*$  value represents the scale from green (negative value) to red (positive value). It is evident that the freeze-dried samples had a green colour (negative values) while the hot air-dried samples had a reddish colour seen by a positive  $a^*$  value. For example, freeze-dried *Robusta* had a value of -7.87 as compared to that of hot-air dried at 1.

The viscosity of both native liquid and dried mucilage were investigated. *Robusta* and *Nepgen* native liquid's mucilage is viscous more than that of *Algerian* and *Ficus-indice*. The reconstituted mucilage powder is thicker than the native liquid powder. Gelling capacity was tested. For spherification or bead formation, mucilage was used to replace the usual gelling agents in both direct and reversed spherification.

During gelling ability tests, mucilage from *Robusta* showed gel-like ability as compared to the other three cultivars. However, it was concluded that mucilage does not form gels on

its own, but rather improves gel formation with other hydrocolloids. When mucilage was used to replace the sodium alginate in spherification, it did not form true spheres, but formed a temporary gel-like membrane when incorporated with xanthan and agar. Ultimately, mucilage may be a replacement for food hydrocolloids. It can be used as an emulsifying agent and an edible coating, although different cultivars will give different results.

**Keywords:** cultivar, functional, hydrocolloid, mucilage, *Opuntia Ficus-indica*, *Opuntia Robusta*, spherification

# Functional properties of cactus pears

## CHAPTER 1: INTRODUCTION

Cactus pear, also known as *Opuntia* nopal or prickly pear, is a dicotyledonous angiosperm flowering plant from Mexico belonging to the Cactaceae family (Saenz *et al.*, 2004; El-Mostafa *et al.*, 2014). The cactus pear is native to North America, South America, and the West Indies. It grows in the United States and Morocco in areas where only limited vegetation grows without extensive soil treatments such as irrigation and fertilisation. Furthermore, it flourishes in Africa, Australia, and Mediterranean countries (Felkai-Haddache *et al.*, 2016). The plant tolerates a wide range of temperatures, from cold temperatures as low as -6°C, to hot temperatures (it can survive for one hour at 60°C) and different moisture levels (Drennan and Nobel 2002). It also grows well in sunny deserts, poor soil and drought-like conditions (high temperatures) (Kumar *et al.*, 2018).

It is an edible succulent plant that is even-green coloured and has round pads that look like big leaves, which are known as cladodes or platyclades. The cladodes are the stems of the plant that expand into moist greenish structures that resemble leaves which comprise of chlorophyll and are vital for the development of the plant as seen in Figure 1.1A (Kumar *et al.*, 2018). The cladodes are equipped with two kinds of spines, the prominent glassy fixed spines and small hair-like thorns called glochids that pass through the skin and disassociate from the plant (Shetty *et al.*, 2012). Some cladodes are spineless (Figure 1.1B)



Figure 1.1 Spiny cactus pears plant (A) and Spineless cactus pears plant (B) (Kumar *et al.*, 2018)

In the past, sap from the cactus pear cladodes was widely applied as a natural medicine for burn wounds, edema and taken as a remedy for gastric problems. Cactus pear cladodes contain nutritionally essential components for human nutrition, including amino acids, vitamin C, organic acids, sugars, minerals, fiber, calcium, and antioxidants. They are low in calories, saturated fat and cholesterol-free (Stintzing *et al.*, 2001).

Cactus pear plants survive extremely dry conditions like drought because they use an alternative form of photosynthesis known as crassulacean acid metabolism (CAM). CAM is a carbon fixation pathway that evolved in some plants as an adjustment to the desert environment (Winter, 1996; Kumar *et al.*, 2018). The stomata in the cladodes do not open throughout the morning and afternoon to depreciate evapotranspiration. They commence respiration during the evening to assemble or accumulate carbon dioxide, when water loss is minimised because of low temperatures and high humidity (Stintzing and Carle, 2005).

In CAM plants, carbon dioxide is fixated at night, and oxaloacetate and malate assemble in the cytosol to be later moved and reserved in chlorenchyma cells which are eventually stored in the vacuoles as malic acid (Kumar *et al.*, 2018). During the day, the carbon

dioxide is remobilised and absorbed in the Calvin cycle (Winter, 1996). In younger cladodes, the water movement happens in the phloem, while xylematic water uptake begins after thirty days of growth.

The water potential is more significant in the developing cactus pear cladodes as compared to the developed cactus pear cladodes. In optimal conditions (mild winters, hot summers, and low humidity), cactus pear plants sequester water into their parenchyma tissue which makes it thicker. In contrast, during dry conditions, the parenchyma narrows down until it almost looks like the chlorenchyma surrounding the parenchyma. Since the cactus pear plant is inherently drought tolerant, it could be developed into a sustainable crop that could offer a solution for sustainable agriculture in less-than-ideal climates (Kumar *et al.*, 2018).

In Mexico, the young cactus pear cladodes (known as nopales) that are approximately three to four weeks old are consumed as a vegetable, either as a main dish or a side dish. The sliced nopales are called nopalitos. Nopalitos can be served and sold fresh or canned, bottled, and sometimes dried (Bowman, 2018).

The *Opuntia Ficus-indice* and *Opuntia robusta* are captivating plants due to their adaptation to withstand extreme heat, drought, and poor soil conditions (Shetty *et al.*, 2012). These plants produce a slimy, sticky, thick fluid substance known as mucilage which is distributed in the cladodes and fruit peel. Mucilage is a complex carbohydrate or hydrocolloid with a high molecular weight. It can be used in the food industry as a functional food or ingredient or even as a hydrocolloid (Du Toit, 2018).

Mucilage may be dried in different ways including heating through hot-air drying or spray-drying or cold air through freeze-drying. Freeze-drying (FD), also referred to as lyophilisation, is a low-temperature dehydration process. The product is frozen rapidly, then subjected to high vacuum where the ice is removed by sublimation (Bhambere *et al.*, 2015). On the other hand, hot-air drying uses heated air equipment to transfer heat to the wet product where the heated product absorbs heat and then moisture on the product is evaporated continuously until no vapour remains or it reaches a desired dried state (Zhao, 2016).

The cactus pear plant has been ignored in most parts of the world; however, it can potentially reform the agricultural landscapes in countries such as South Africa because it could offer a good measure of food security in semi-arid and arid regions (ref?). It can be developed into a useful and profitable crop due to its multifaceted nature.

### **Problem statement**

The cactus pear plant seems to be the possible solution as the world is going through immense climate change which has led to drought and made it difficult for crops to flourish. The ability to grow in drought can be attributed to its photosynthetic pathway, which is also known as CAM, which allows cactus pear plants to grow in extremely high temperatures. Cactus pear mucilage is of great interest to the food industry because of its many benefits. Apart from it being extracted from a plant that can survive climate change, it can serve as a hydrocolloid as well as soluble fiber with potential application in the food industry. These should be investigated in order to establish cactus pear as a multi-purpose crop.

### **Aim**

The aim of this study was:

1. To extract and dry mucilage from selected cultivars namely *Robusta*, *Nepgen*, *Algerian* and *Ficus-indice*.
2. Cultivars with high mucilage yields and viscosities were freeze-dried and hot-air dried and compared regarding colour, viscosity, and emulsifying ability.
3. To analyse the extracted mucilage for its functional properties including possible gelling ability, emulsifying ability, and thickening ability.
4. To apply the mucilage as a functional ingredient in edible coatings and films and spherification.

## **Project layout**

The project layout is demonstrated in Figure 1.2. The mucilage was extracted from the cladodes of four cactus pear cultivars and dried in two different ways. Gelling and emulsification capacity as well as viscosity was determined for each cultivar. In the last stage, mucilage was tested for its ability to be applied as an edible coating and to be used in spherification.

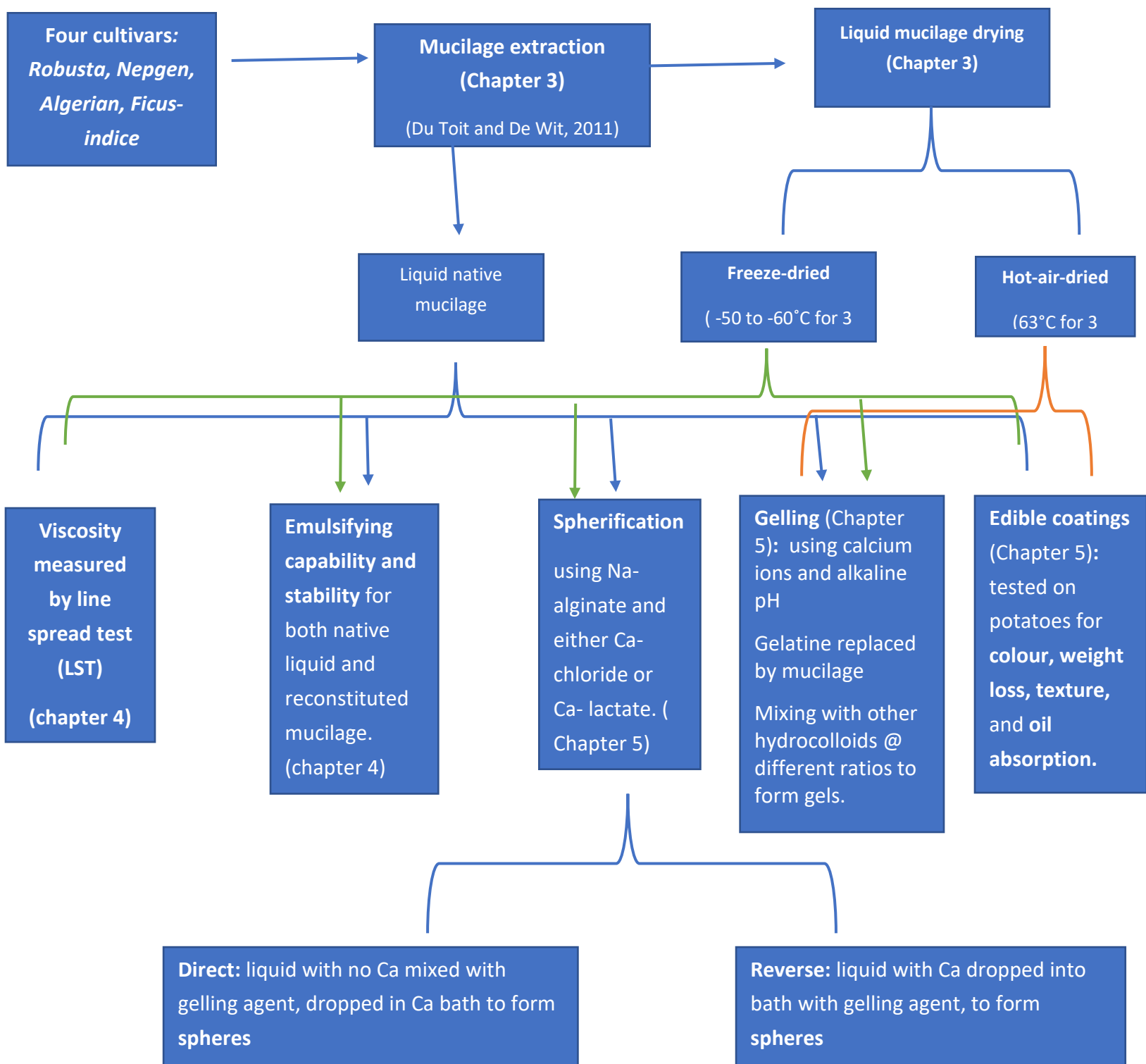


Figure 1. 2 Project layout

## Chapter 2: Literature review

Cactus pears are one of the many plants included in the genetic diversity of Cactaceae. This diversity has different species of approximately 1500 or more, which includes *Opuntia Ficus-indice*, *Opuntia robusta*, *Opuntia phaeacantha*, *Opuntia humifusa* and others. *Opuntia Ficus-indice* is the most common and important and has been domesticated as a plant crop adapted to semi-dry and dry lands (Shetty *et al.*, 2012).

### 2.1. The cactus pear flowers and fruits

Cacti are often used as ornamental plants; however, many are also cultivated as a crop. Over the other cacti, the cactus pear has been given more attention due to its variety of uses as food and agricultural products such as fruits, fodder, and freshly cut vegetables (Scalisi *et al.*, 2016). Cactus pear flowers blossom at night as they are pollinated by nocturnal insects and tiny animals, particularly moths and bats. This allows them to conserve water during the hot season. *Opuntia* produces flowers that give rise to elliptical or egg-shaped fruits known as tuna in Mexico (Figure 2.1).

Cactus pear fruits exist in a wide range of bodies and proportions (Shetty *et al.*, 2012). The fruit colour ranges from white to green, yellow to orange and red to purple, with a sweet acidic taste (Figure 2.1). Cactus pear fruits are stretched roundish berries with a thick skin and a juicy pulp, with weights ranging from 67 g to 216 g at the highest ripening stage (Saenz and Yahia, 2011). The fruit weight depends on the origin and the cultivar with an average weight of 100 g to 160 g. The fruits consist of 33% to 55% peel and 45% to 67% pulp. The pulp consists of 2% to 10% seeds and 44-45% strained pulp which is the basis of fruit (Kumar *et al.*, 2018). The pulp is the edible part of the fruit, comprised of approximately 84% to 90% water and 10-15% reducing sugars of degrees Brix ranging from 10° to 17° Brix (Saroj *et al.*, 2017). The pH varies from as high as 5.3-7 to a very low acidity of 0.05-0.18% in citric acid. When compared to other fruits such as apples, grapes and bananas, cactus pear fruit is high in vitamin C, with trace elements of other vitamins

such as thiamine, niacin, and carotenoids (Sepulveda and Saenz, 1999, Kumar *et al.*, 2018). Cactus pear fruits are rich in polyphenols and betalains (Piga, 2004; Hugo Cota-Sánchez, 2016). The consumption of the fruit has shown a positive effect on the body's redox balance and decreases oxidation damage in lipids (Tesoriere *et al.*, 2004).



Figure 2.1 Prickly pear fruit (Kumar *et al.*, 2018)

## 2.2. The cladodes

The *Opuntia* genus is extremely efficient in its ability to acquire and store water; thus, it grows mainly in arid and semi-arid regions. It is characterised by its fleshy, flat rounded leaf-like pads known as cladodes (Scalisi *et al.*, 2016). Cladodes are succulent, look like a tennis racket of approximately 60 to 70 cm in height, with a diameter of approximately 2-3 cm. The diameter depends on the age of the plant, the cultivar, and the water and nutrients received during growth (Sepulveda *et al.*, 2007).

The epidermis of the cactus pear cladodes is made up of the green and white cells known as chlorenchyma and parenchyma, respectively, as shown in Figure 2.2.

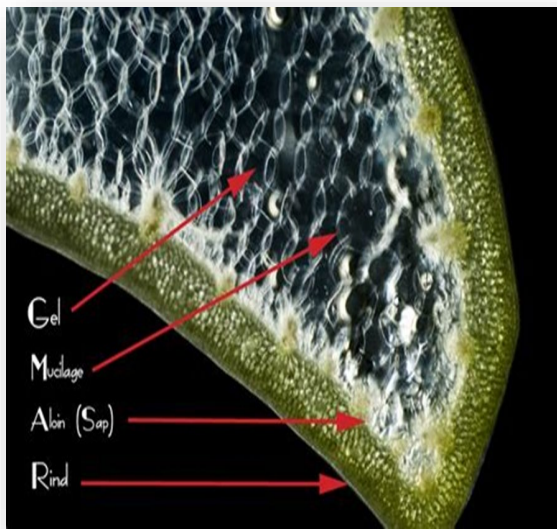


Figure 2.2. Layers inside the cladodes

Freshly harvested young cladodes that are three to four weeks of age are regarded as vegetables, also known as nopales or nopalitos (Stintzing *et al.*, 2001; Sepulveda *et al.*, 2007; Kumar *et al.*, 2018). Cladodes have been reported to be rich in pectin, mucilage, amino acids, polyphenols, vitamins, and minerals (Habibi *et al.*, 2004, Kumar *et al.*, 2018). They contain a high-water content (88-95%) and a small amount of protein (Sepulveda *et al.*, 2007).

Furthermore, they are a potential source of fiber, specifically soluble fiber. They contain both soluble and insoluble fiber (Glicksman, 1983). The soluble fiber is found in the mucilage and pectin inside the cladodes, while the insoluble fiber is found in the cellulose of the cell wall (Saenz *et al.*, 2013). The fiber content increases as the cladodes mature (Saenz, 1997; Saenz *et al.*, 2013).

Dietary fiber is correlated with several advantageous physiological effects on the human digestive system. It is known to decrease cholesterol levels in the blood; it is also widely believed that the effects of dietary fiber on plasma cholesterol concentrations may be

mediated primarily by increased fecal excretion of bile acids (Mumford *et al.*, 2011). Dietary fiber is also linked with the control of glucose, because the body is unable to absorb and break down fiber and, therefore, could potentially aid with diabetes (Mumford *et al.*, 2011). An intake of food with high fiber content is associated with reduced calorie intake, therefore leading to a decrease in the chances of cardiovascular diseases and cholesterol (Mumford *et al.*, 2011).

The younger cladodes contain more carbohydrates, protein, and water content. Young cladodes are situated more at the top of the plant and are richer in water, ash, and crude protein but poorer in fiber; the opposite is observed for cladodes located at the base of the plant (Santo *et al.*, 2013). The higher protein content in younger cladodes compared to matured cladodes might be related to the increase in metabolic activity that occurs at the early stage of maturation (Perucini-Avenidaño *et al.*, 2021). The juice from cladodes typically has a pH of 4.6 with 0.45% titratable acid and 6.9 g/100 g dry matter. The cladodes are characterised by a high malic acid content due to CAM (Abidi *et al.*, 2009). In 100 g dry matter of cladodes, with no glochids or spines, there are 19.6 g ash, 7.2 g lipids and waxes, 3.6 g lignin, 46 g cellulose, and 48 g of other polysaccharides (Stintzing and Carle, 2005). The young cladode's chemical composition is comparable in value to that of spinach, with 91 g of water, 1.5 g protein, 0.2 g fats, 4.5 g total carbohydrates, and 1.3 g ash (Saenz *et al.*, 2013)

The manufacturing of mucilage in Cactaceae is ascribed to the water-holding capacity of fleshy tissues (Stintzing and Carle, 2005) Within the two layers of the epidermis lies the mucilaginous cells that store nopal drip, known as mucilage, which is excreted into the apoplast where it aids in balancing the plasmic water load throughout dehydration (Figure 2.2) (Felkai-Haddache *et al.*, 2016). Mucilage is found in the idioblast cells that are scattered throughout the medullary and cortical of the parenchyma.

### 2.3. Different hydrocolloids used in the food industry.

Hydrocolloids are a heterogenous group made of protein and polysaccharides. They are composed of a considerable amount of hydroxyl groups which enhances the ability to

bind water molecules, making them hydrophilic substances (Li and Nie, 2016). Some hydrocolloids can be dispersed in water, form a colloidal solution, or swell in water, whereas some are soluble in water. They can form gels, viscous solutions, or pseudoplastic gels (Saha and Bhattacharya, 2010). Food hydrocolloids are a diverse group of long-chain polymers characterised by a diverse range of functional properties including gelling, thickening, coating, stabilisation, and emulsification (Cantwell *et al.*, 1992). Food hydrocolloids are useful in the food industry because of their potential to mimic or influence viscosity and texture (Saha and Bhattacharya, 2010). These may be extracts from plants, seaweeds, agar, microbial excretion like (xanthan) or from animals, like gelatine (Nishinari *et al.*, 2017). In this study, we will introduce hydrocolloids such as agar, gelatine, guar gum, xanthan, carrageenan, and alginate with a more in-depth focus on cactus pear mucilage.

### 2.3.1. Agar

Agar is a linear polysaccharide known as agarose and a heterogeneous mixture of small molecules known as agarpectin (Song *et al.*, 2012). It is a jelly-like substance that is extracted from red algae cell walls. Furthermore, it is composed of D-galactose and 3,6-anhydro-6-galactose (Figure 2.3). The agarose is the component responsible for strong gelling abilities. Agar gelation is dependent on cations. It is insoluble in water, and its dissolution occurs at above 80°C. It forms a thermo-reversible gel that has a firm texture upon cooling. The gel is heat tolerant and stable in acidic conditions (Cantwell *et al.*, 1992, Sousa *et al.*, 2021). Typical contents and properties of agar include 18% moisture content, maximum water absorption of 75 cubic centimeter (c.c.), 0.5% acidic insoluble ash, 6.5% total ash, pH 6.8 to 7, gel strength (1.5% sol at 20°C) of 700 to 1 000 g/cm<sup>3</sup> and viscosity (1.5% sol at 60°C) of 10 to 100 centipoise (cP) (Armisen and Galatas, 1987).

Acids such as tannic and proton scavengers such as potassium iodide inhibit the gelling process of agar. These effects can be prevented by adding a small amount of glycerol. Agar has been used in the food industries as a thickener and gelling agent in canned meat, fish, and poultry. It can be used as a texture stabiliser in ice cream and can be used

in the making of artificial caviar. Furthermore, it has been used in microbiology laboratories to formulate culture media (Imeson, 2010).

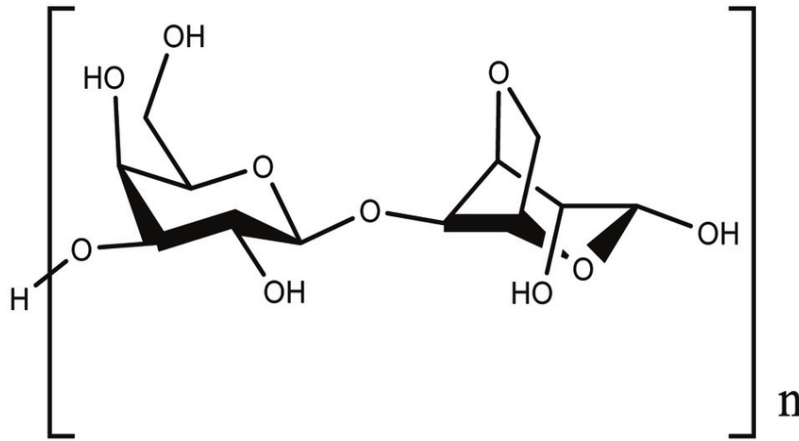


Figure 2. 3 Agar chemical structure (Shahidi and Rahman 2018).

### 2.3.2. Xanthan gum

Xanthan gum is a long-chain polysaccharide that is made by anaerobic fermentation of glucose or sucrose solution with a pure culture of *Xanthomonas campestris* or *phaseoli* (Hublik, 2012). It has a high molecular weight above 2000 kDa. It is made up of D-glucose, D-mannose, and D-glucuronic acid in a ratio of 3:3:2 (Scherz, 1996). The primary structure is made up of D-glucose molecules that are linked together by beta-1-4 bond substituted on a substitute glucose residue with a trisaccharide chain, which is composed of two mannose units separated by glucuronic acid (Scherz, 1996; Imeson, 2010) (Figure 2.4).

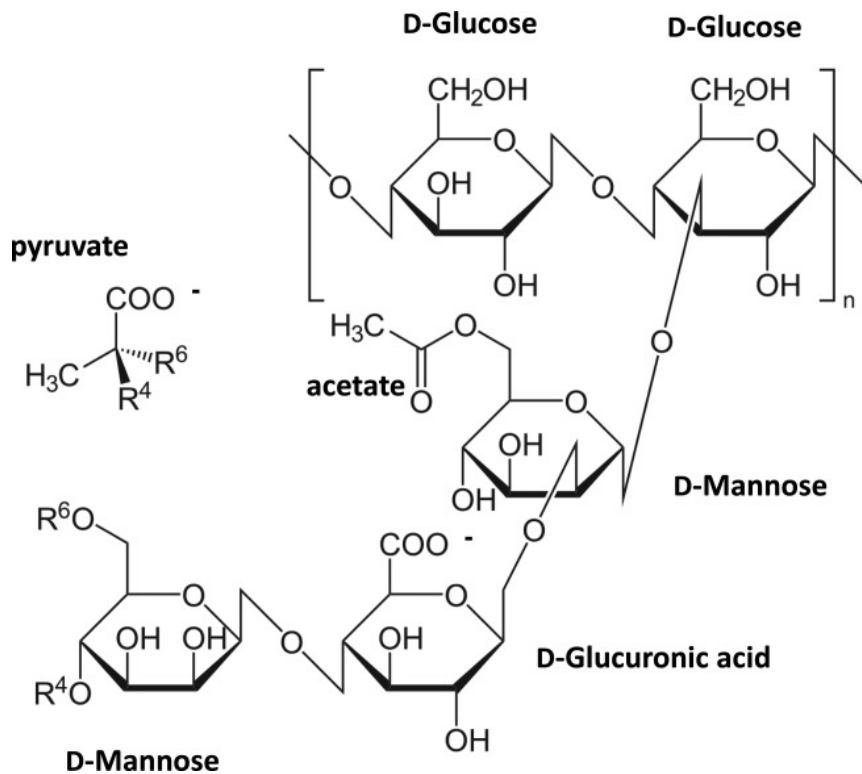


Figure 2. 4 Xanthan chemical structure (Petri, 2015).

Xanthan is a water-soluble hydrocolloid that melts at room temperature. It can form a high viscosity at low concentrations and form the viscoelastic property of weak gels in solution (Gowthaman *et al.*, 1999; BeMiller, 2008). The viscosity decreases as the temperature increases but recovers during cooling. It is compatible with organic acids, and its viscosity is not affected by pH values between 1 and 13. Xanthan gum properties include 8 - 15% moisture, 7 - 12% ash, 0.3 - 1% N, 1.9 - 6.0% acetate content, 1.0 - 5.7% pyruvate content, 3.6 - 14.3 g/l monovalent salts, 0.085 – 0.17 g/l divalent salts and 13 - 35 cP viscosity (García-Ochoa *et al.*, 2000). Xanthan has been used in the food industry in the making of sauces to retard staling as well as to prolong freshness in cakes containing fruits. Moreover, it has been utilised to improve the gel firmness of other hydrocolloids and reduce syneresis.

### 2.3.3. Alginate

Alginate is an unbranched co-polymer of mannuronic acid and alpha-L-guluronic acid residues that occur in the cell wall and intercellular spaces of brown algae produced by photosynthesis (Sachan *et al.*, 2009). Alginates are salts of alginic acid with cations of

Na<sup>+</sup>, NH<sub>3</sub><sup>-</sup>, Ca<sup>2+</sup> or K<sup>+</sup>. In between the alternating polymers of guluronic acid and mannuronic acid (M-G-M-G) of alginate exists a macromolecule that serves as the block of polymers. The block of polymers can be either guluronic acid (G-G-G-G) or mannuronic acid (M-M-M-M) which in turn forms a folded structure (zigzag) that is essential for gelation (Figure 2.5). Alginic acid and calcium alginate are insoluble in water, while the alkali metal and ammonium salts such as sodium and potassium are highly soluble (Scherz, 1996; Imeson, 2010).

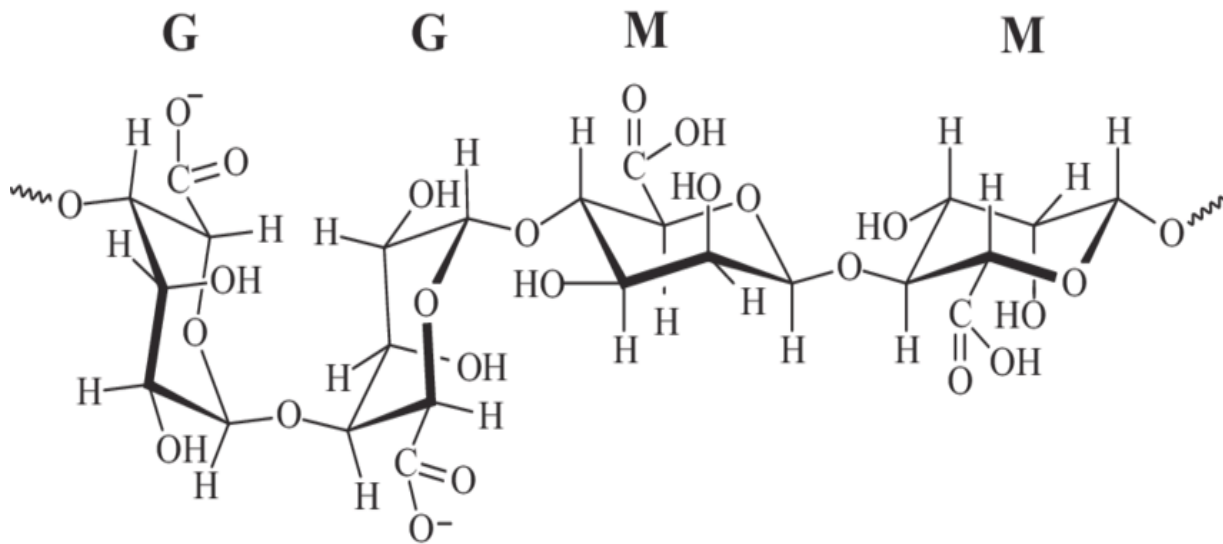


Figure 2. 5 Alginate molecule's chemical structure (Lwaki *et al.*, 2012)

The viscosity of alginate is influenced by ions, sugar, polyols, and alcohols. The aqueous solution has a high viscosity even at low concentration and is very stable when heated in a neutral pH range. The viscosity decreases in acidic conditions of pH less than 5. The calcium ion increases viscosity and results in gelation by incorporating the zigzag structure of the G-G block. The alginate forms a heat-stable gel in the presence of acid and cations. Furthermore, both acid and calcium form thermo-reversible gels. Alginates are used in the food industry as emulsifiers, gelling agents, coating agents and as thickeners. They can also be used to encapsulate prebiotics and functional oils (Scherz, 1996; Imeson, 2010).

### 2.3.4. Carrageenan

Carrageenan is a high molecular weight linear polysaccharide extracted from seaweed. It is composed of repeating units of galactose and 3,6-anhydro-galactose with both sulfate and non-sulfate groups joined by alpha-1-3 and beta-1-4 glycosidic links (Oliviera and Reis, 2008). Carrageenan can be divided into three fractions namely, iota, lambda, and kappa. These fractions differ in the number and position of sulfate ester groups and the content of 3,6-anhydro-galactose (Figure 2.6). Kappa carrageenan is the most commonly used as food additive. The carrageenan solubility depends on their structure, temperature, and presence of cations (Scherz, 1996; Imeson, 2010).

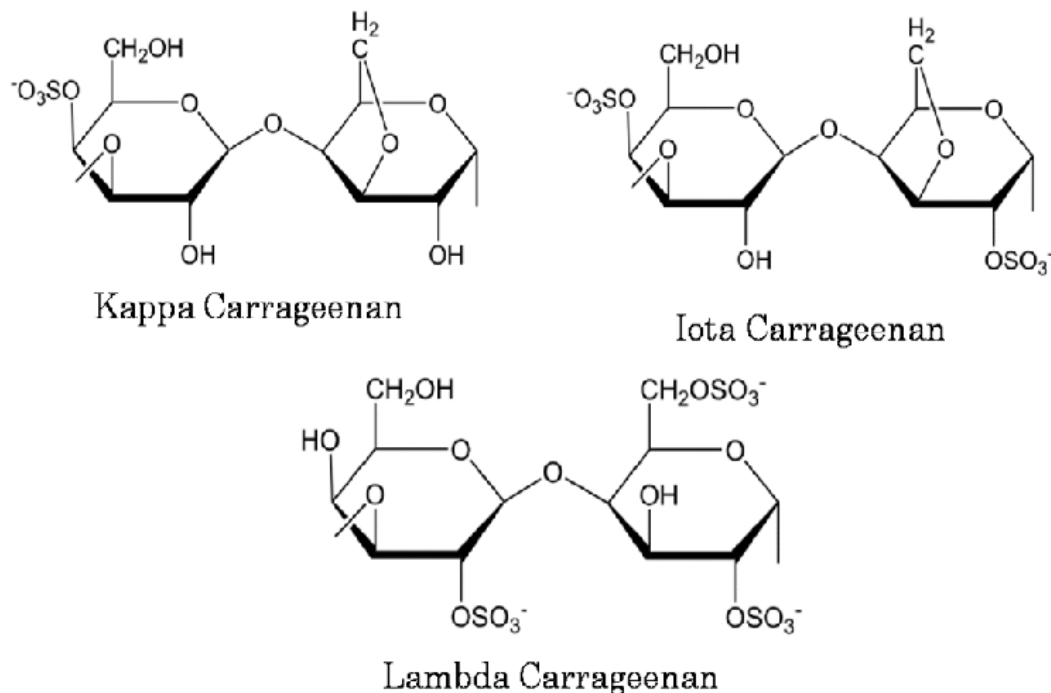


Figure 2. 6 Structure of kappa, iota, and lambda carrageenan (Pandey *et al.*, 2020)

Two of the three different fractions are soluble in hot water, apart from lambda. All three fractions are soluble in hot milk. This is due to electrostatic attraction forces between the negatively charged hydrocolloids with positively charged regions of k-casein. The swelling of the hydrocolloid molecules in milk occurs at a temperature above 40°C to 60°C as the particles hydrate and the viscosity increases. Acids that lower the pH below 4.5 lead to the hydrolysis of carrageenan during heating (Imeson, 2010).

Kappa carrageenan forms a firm, delicate gel in the presence of potassium. Lambda carrageenan forms a soft, syneresis-free elastic gel. With the addition of calcium, both gels are stable at room temperature, but they can be liquidised by heating to temperatures between 5 °C and 30 °C above the gelling temperature. Gelation of both gels is due to the relatively high content of hydrophobic anhydro-galactose and the low to medium contents of sulfate ester groups. Both gels are used as gelling and thickening agents for water-based jellies and dairy products (Scherz, 1996; Imeson, 2010).

#### 2.3.5. Guar gum

Guar gum is a highly branched neutral polysaccharide made of mannose and galactose linked together by alpha-1-6-glycosidic bonds in a ratio of 1.6:1 with an average molecular weight of 2000 kDa (Figure 2.7) (Krstonošić *et al.*, 2021). The mannose to galactose unit's ratio is approximately 1.6:1 to 1.8:1 (Williams and Philips, 2009; Pathak, 2015). The highly branched structure of guar molecules makes it very easy for hydration properties and hydrogen bonding activity (Kuravadi *et al.*, 2013). It has good solubility in cold water and can form high viscosity at low concentrations. When guar gum is heated above 90°C at a neutral pH or moderate heat at acidic pH of less than 5.5, the viscosity decreases. The loss of viscosity is due to decreasing of molecular weight caused by thermal degradation (Negla and Abubakr, 2015). The viscosity is stable in cold solutions over a pH scale of 2 to 10. Guar gum increases the strength of gels formed with other polysaccharides such as agar. Guar gum is used as a binder and thickener to control texture, to prevent syneresis and phase separation (Elkhalifa *et al.*, 2008). When fully hydrated, a process known as thixotropy occurs where the viscous colloidal dispersion can form a gel and return to the sol state and gel again (Mudgil *et al.*, 2011).

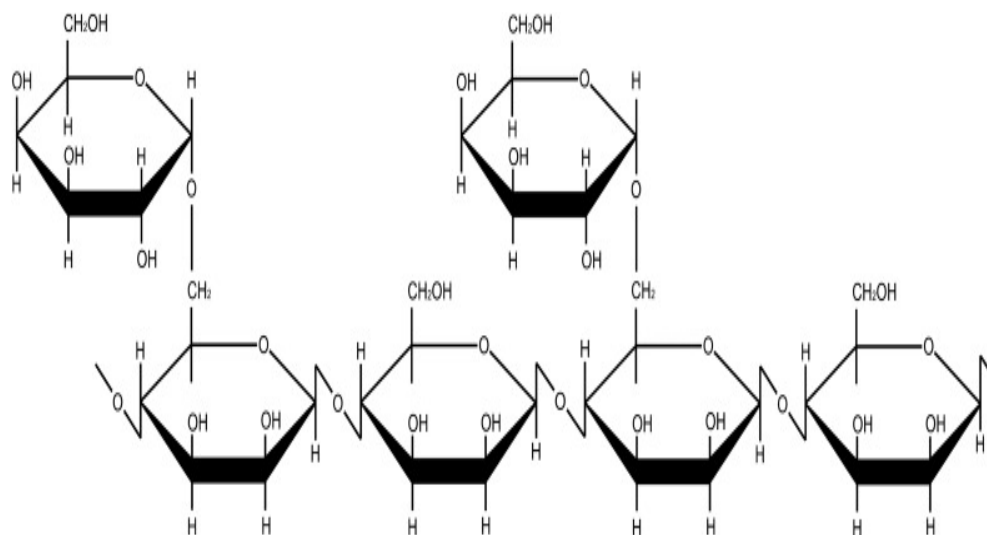


Figure 2. 7 Guar gum chemical structure (Veljko *et al.*,2021).

### 2.3.6. Gelatine

Gelatine is a colourless food ingredient derived from collagen extracted from animal body parts. It is a blend of peptides and proteins produced by partial hydrolysis of collagen extracted from bone, skin, and connective tissue. It is composed of proline, hydroxyproline, and glycine (Stevens, 2010; Chaplin, 2017) (Figure 2.8). The amino acid profile of the polymer determines the hydrogen formation and reactions via side chains such as amine, imidazole, alcohol, amide, and carboxylic acid (Stevens, 2010; Milani and Maleki, 2012). Gelatine contains 8-13% moisture and has a relative density of 1.3 g/cm<sup>3</sup> to 1.4 g/cm<sup>3</sup> (Chanchal *et al.*, 2014).

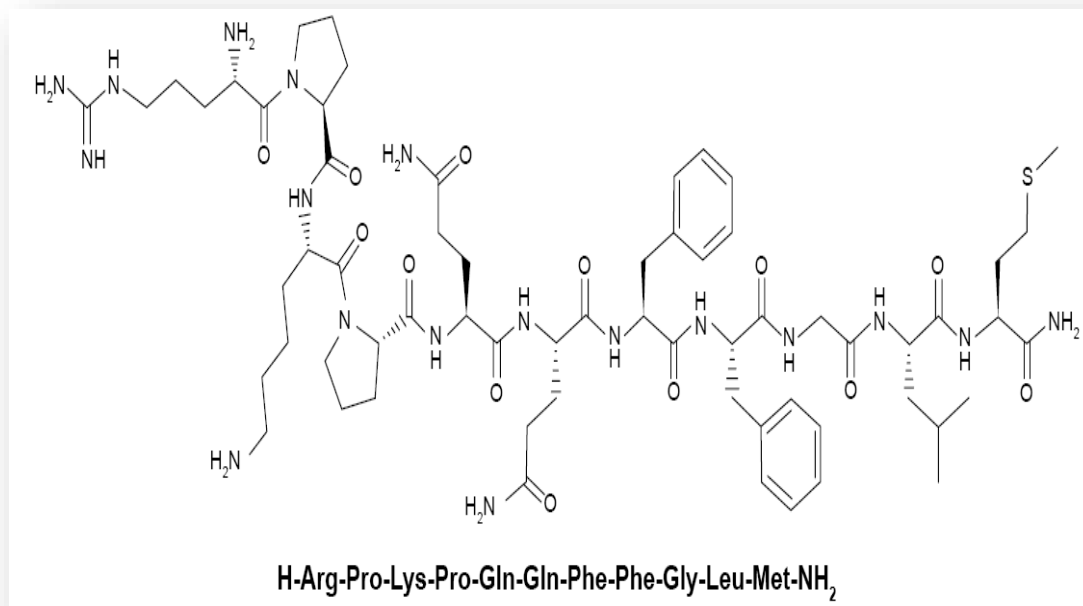


Figure 2. 8 Gelatine chemical structure (Chaplin, 2017).

Glycine is responsible for a close packing structure. Proline is responsible for conformation restriction, and hydroxyproline and proline play key roles in collagen stability. They permit the sharp twisting of the collagen helix. This is important for the gelation properties of gelatine (Scherz, 1996). The strength of the gel depends on the gelatine concentration, the intrinsic strength of the gelatine, pH, temperature, and the presence of additives such as sugar, ions, and salts (Banerjee and Bhattacharya, 2011). It dissolves readily in hot water and forms a gel on cooling. It does not dissolve well in cold water.

### 2.3.7. Mucilage

Mucilage is a complex hetero-polysaccharide (Madera-Santana *et al.*, 2018). It contains dietary fiber, which forms gelatinous colloids. It is a viscous, gelatinous, and sticky hydrocolloid found in various plants that contain polysaccharides and proteins. Mucilage is similar to gums. It is hydrophilic, inert, and indigestible.

*Opuntia* mucilage is comprised of L-galactose, L-arabinose, D-xylose, L-rhamnose, and galacturonic acid and are polymers with high molecular weights ranging from  $15.3$  to  $15.7 \times 10^5$  g/mol (Saenz *et al.*, 2004). It contains minerals such as calcium, magnesium, potassium, phosphorous and trace amounts of iron (Cardenas *et al.*, 1998). The primary structure of mucilage is described as a molecule with linear duplicating core chains of 1-4 linked beta-D-galacturonic acid and alpha 1-2 linked L-rhamnose with trisaccharide side chains of beta 1-6 linked D-galactose attached at O (4) of the L-rhamnose residue. The galactose side sediments may have a further cleft in either O (3) or both O (3) and O (4) locations, as shown in Figure 2.9. It is a complex acid-labile peripheral chain, with about 20 different types of oligosaccharides, as shown in Figure 2.9. The levels of rhamnose and uronic acid determines the hydrophobic and hydrophilic properties of mucilage (Sepulveda *et al.*, 2007).

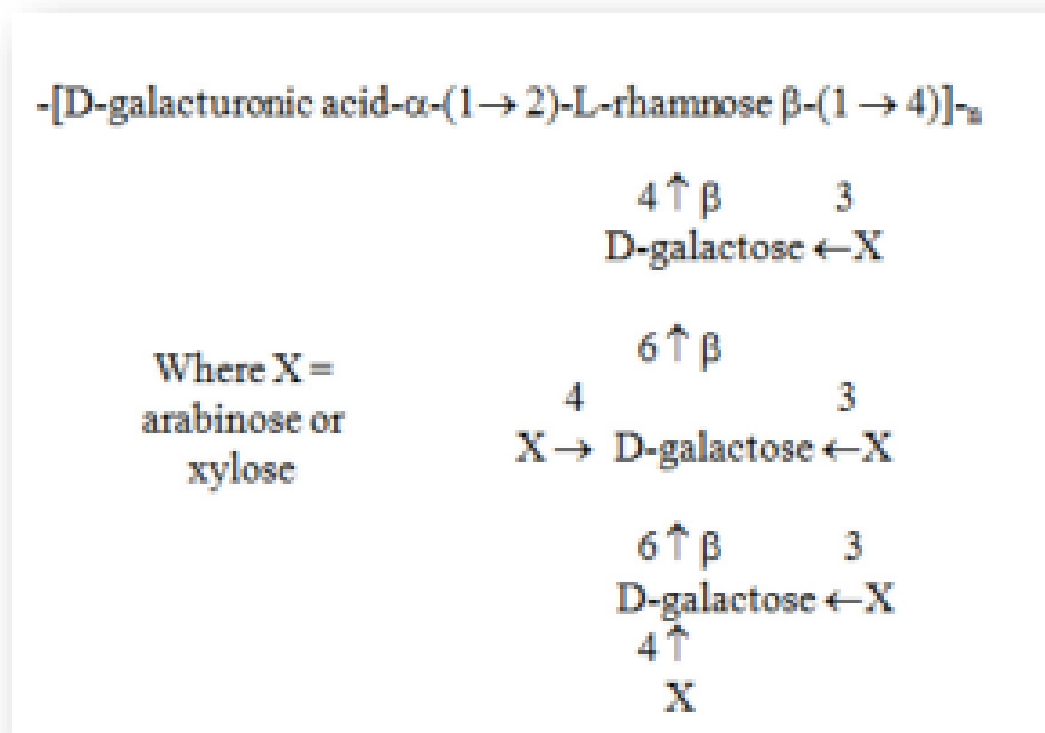


Figure 2. 9 Chemical structure of mucilage from *Opuntia Ficus-indice* cladodes (Ciriminna *et al.*, 2019)

The mucilage structure is made up of two discriminating water-soluble fractions, namely, the pectin fraction with gelling properties, which are prompted by the presence of calcium ions and the non-gelling fraction (Nurudeen *et al.*, 2017). The water-soluble fraction also contains approximately 10% of a fraction with thickening properties which is the mucilage (Sepulveda *et al.*, 2007). Mucilage from *O. Ficus-indice* is negatively charged. The intermolecular repulsion of negatively charged side chains with concomitant enlargement and prompted hardness of the molecule influences its water-holding capacity. The hydrodynamic properties of mucilage are influenced by pH and calcium content. The viscosity is determined by the changes in mucilage conformation and molecular form (Stintzing and Carle, 2005). Sepulveda (2007) stated that at pH ranging from 1.9 to 7 when charges are neutralised, viscosity increases as the molecular structure collapses and precipitates. Calcium partially binds to the negatively charged sidechains, keeping the rigidity and dimensional orientation.

The amount of mucilage that is found in cladodes is influenced by the management of the crop. It also depends on temperature, irrigation, and rain (Shetty *et al.*, 2012). Albeit the mucilage is not influenced by the rainfall or cladodes' weight, it is, however, positively correlated to the abundance of electrolytes in the cladodes during the warmer weather (Du Toit *et al.*, 2020). Mucilage is extracted from cladodes in different ways, which may include chemicals and heat treatment. The polysaccharide has become an ingredient of interest in the food industry because of its functional properties (Du Toit *et al.*, 2018). Mucilage can be used in the food industry as a food additive, a thickener, an emulsifier, and a stabiliser. Furthermore, it may be used to control crystallisation, facilitate foaming, inhibit syneresis, form gels, and act as an edible coating to increase the shelf-life of food products. Except for being a food additive, it can also be used in pharmaceutical and cosmetic industries because of its capability to change the functional properties of a system, with the added advantage of increased dietary fiber (Shetty *et al.*, 2012).

Hydrocolloids are used to alter the textural properties of food products when food ingredients such as fat, egg, dairy products, and gluten are not added to food products,

usually to increase the health properties of these products which are low-kilojoule and allergy-free (Du Toit *et al.*, 2018).

The pH is a critical factor in the gelation, aggregation, and coagulation processes. The pH should be between 5 and 7.5 for the flocculation of hydrocolloids. The pH of mucilage is 5.5-6 in contrast to that of gelatine (pH 8-9), xanthan (pH 6-8), agar (pH 6.5-7.5), and guar (pH 5.7). Mucilage contains higher amounts of crude protein (3.66-8%) (Trachtenberg and Mayer, 1982 b; Majdoub *et al.*, 2001) as compared to xanthan (1-2%) and agar (1%) but similar to that of guar gum (5-6%) (Iturriaga *et al.*, 2009a)

## 2.4. Gelation

Food gels are viscoelastic materials that are used in many food product applications. In food gels, the polymer fragments are held or cross-linked by mixtures of weak intramolecular forces such as hydrogen bonds, electrostatic forces, Van der Waals forces, hydrophobic interactions and sometimes disulphide bonds in proteins (Saha and Saha, 2010).

The mechanism or process of gelation is dependent on gelling agents and conditions of gel formation, such as temperature, pH, the existence of ions, and concentration of gelling agents. The characteristic physical properties of food gels result from the capability of specific proteins and polysaccharides to make a continuous three-dimensional fragments network (Banerjee and Bhattacharya, 2012). A gelling agent is a substrate added to food products to provide a texture of a gel. They are based on polysaccharides and proteins. Common gelling agents include natural gums, pectin, agar-agar, and gelatine (Oakenfull, 1987)

### 2.4.1. Gelation of hydrocolloids

Polysaccharides such as mucilage, starch and gums are utilised in the food industry as hydrocolloids because they can alter the textural properties of food systems, thus making the food products more useful (Medina-Torres *et al.*, 2000). Hydrocolloid molecules are

heterogeneous groups of long chains of polymers that can form viscous dispersions and to solidify when dissolved in water (Gawai *et al.*, 2017). Their large number of hydroxyl groups causes these hydrophilic properties. Hydrocolloids' useful properties in food include thickening, gelling, emulsifying, stabilisation, and controlling of crystal growth of ice and sugar (Milani and Maleki, 2012).

The hydrocolloids may be used in their pure or single form or in combination with other hydrocolloids to broaden the textural and rheological properties (Medina-Torres *et al.*, 2013).

Gelling of hydrocolloids is a process that involves a connection between polymer chains by cross-links and tangles through hydrogen bonding, resulting in a three-dimensional network that entraps the water within the molecular structure to form a solid structure (Saha, 2010). Another way of gelation is by branched polymers which can form links between chains resulting in larger branched polymers that form a single macroscopic molecule. The system then solidifies, and viscosity increases (Saha and Saha, 2010).

#### 2.4.2. Gelation of mucilage

According to one study by Cardenas *et al.*, 2008, mucilage has no gelling capacity, instead, only the pectin extracted from the cell wall has the ability to solidify into a gel in the presence of calcium ions. It was reported that although cactus mucilage is a non-gelling polysaccharide, it is elastic and has a high capacity to modify the viscosity and improve gels formed with other hydrocolloids (Medina-Torres *et al.*, 2003). For example, Medina-Torres and co-workers (2003) showed that mucilage does not form a gel on its own, but gelation is possible when combined with the kappa-carrageenan. Saenz (2004) corroborated the aforementioned report when observed that cactus mucilage stabilises syneresis in gels formed with kappa-carrageenan alone. Du Toit *et al.*, (2018) showed that mucilage does not form a gel. Still, it has emulsifying capabilities because it can reduce surface and interfacial tension, stabilise the oil-in-water emulsion, form small droplets and adsorb to the oil-water surface. This property is similar to that exhibited by xanthan and guar gum. Mucilage contains a higher amount of crude protein (3.66-8%) as compared to xanthan (1-2%) and agar (1%) but is similar to that of guar gum (5-6%) (Du Toit, 2017). Mucilage does not form a true gel even when mixed with xanthan. The long

negatively charged molecules of mucilage repels itself, uncoil and stretch out, resulting in an increased viscosity (Du Toit *et al.*, 2018)

Cactus mucilage is a soluble fiber and long-chain polymer that dissolves in water, causing thickening and viscosity-producing effects. The non-Newtonian viscous colloids that form when mucilage is dissolved in water come from its ability to absorb and store vast amounts of water in its structure. Because mucilage contains monosaccharides with varying carbonyl and carboxylic acid groups, it is electrically charged. As soon as the mucilage molecule comes in contact with water, the hydrogen atoms split off and leave the negatively charged group open along the chain. As a result, the long molecule repels itself causing it to uncoil and stretch out leading to an increased viscosity of fluid which can be decreased by neutralising with the addition of sodium or calcium ions (Du Toit, 2016).

#### 2.4.3. Edible coating (EC) and edible films (EF)

Edible coatings and films can be defined as a primary packaging produced with edible substances (Dhaka and Upadhyay, 2018). Edible films and coatings may also be explained as a thin sheet of palatable substance made around the outside layer of food as a coating or placed in-between food components. Any type of substance used for coating and covering a food product, to lengthen the shelf life and may be consumed together, with or without further removing it, is considered edible film or coating (Betoret *al.*, 2011).

Edible films and coatings offer several benefits over conventional non-edible polymeric packaging and decrease environmental pollution by virtue of their decomposable attributes. Furthermore, they play a role in preservation, distribution, and marketing. Their function is to shield the food product from mechanical damage and physical, chemical, and microbiological deterioration (Del-Valle *et al.*, 2005, Nussinovitch, 2013).

The use of edible films and coatings has gained more interest due to their multiple benefits in extending shelf life and as a carrier of several food additives. Edible coatings may be used to minimise moisture and solute migration, gases exchange, respiration, and oxidative reactions, physiological damage and reduce risks of pathogen growth on the

food surface, therefore extending the shelf life and maintaining the integrity of the products (Baldwin *et al.*, 1995; Blancas-Benitez *et al.*, 2022). Edible films can act both as barriers, by inhibiting or slowing down moisture migration, and as carriers of food-active ingredients such as antioxidants, flavours, nutrients, antimicrobials, colourants, spices, and anti-browning agents (Allegra *et al.*, 2017).

The properties of films or coatings can be classified into three categories, according to Pascall Lin (2013). They can be grouped as;

1. Barrier: because they protect the product from exposure to the environment. They also act as a barrier to oxygen, moisture, and other gases.
2. Carrier: active ingredients may be blended and added to the film or coating. The active ingredients may be antimicrobial agents, antioxidants, pigments, flavourings, and nutrients.
3. Enhancement: they improve the quality and mechanical properties of some fragile products.

Edible films can be differentiated from edible coatings by their applications to food and the way they are manufactured. Films are dried preformed thin material and used on or between layers of food. Their size usually ranges between 50 to 250 micrometres in thickness and can be used as wraps, whereas on the other hand, coatings are applied as liquids varying in viscosity to the outer surface of products, either by dipping, spraying, or brushing and then allowing to dry on the product (Allegra *et al.*, 2017).

The primary materials used to formulate or as a base of edible films or coatings are proteins, lipids, and polysaccharides. Resins may also be used and sometimes a combination is used (Espino-Diaz *et al.*, 2010; Allegra *et al.*, 2017). The polysaccharides and proteins-based films or coatings are hydrophilic, which in turn means that they are poor moisture barriers due to the ability of adjacent chains in the polymer to cross-link (Han and Gennadios, 2005; Pérez-Gago and Rhim, 2014). Polysaccharide-based films and coatings have strong hydrogen bonding that can be applied to bind useful additives. On the other hand, protein-based films and coatings have better mechanical strength and

can be applied to lower fruits injuries during distribution. Lipid-based films and coatings are the best moisture barrier because of their hydrophobic nature but exhibit low textural-mechanical properties such as low tensile strength and easy breaking at elongation (Pascall Lin, 2013).

The use of combined biopolymers in making edible films or coatings is recommended as it minimises the disadvantages of one polymer while improving functionality. It is common to use combinations of both hydrophilic and lipophilic ingredients as the combination prevents dehydration. It is used successfully in modified atmosphere packaging applications. Plasticisers, such as glycerol, are used to improve the appearance, mechanical, and the permeation properties of films by reducing intermolecular forces between polymeric chains (Lira-Vargas *et al.*, 2014).

#### 2.4.3.1 Mucilage as edible coating or film

Mucilage is a polysaccharide with a highly branched structure. When dried, it is made up of an average of 5.6% moisture, 37.3% ash, 1.14% nitrogen, 9.86% calcium, 7.3% protein, and 1.55% potassium (Allegra *et al.*, 2017). The cactus mucilage forms a large aggregate, and its viscosity distribution depends on pH and calcium ion concentration. As the ionic strength of calcium increases, the viscosity decreases, but the viscosity increases as the pH increases from acid to alkaline (Espino-Diaz *et al.*, 2010).

Due to the mucilage's hydrophilic character, it can play a role in or preventing water migration, holding back moisture, and extending the freshness of the fruit. Edible coatings made from mucilage have proved to be effective in extending the shelf life of fruits such as strawberries, mango, melon, grape, banana, and papaya (Khatodiya and Malik, 2022). Mucilage coatings have also been checked for their ability to hinder microbiological deterioration. Although mucilage did not inhibit undesirable microbial growth in one study of coated figs, it significantly limited spoilage as the mucilage-coated figs had lower microbial density than the control group during storage (Allegra *et al.*, 2017).

According to Allegra *et al.*, 2017 *Opuntia ficus indica* mucilage lowers the respiration rate but causes weight loss, firmness, reduction of colour and fungal infection. Del-valle *et al.*, (2006) showed that when mucilage is used as a coating, it increases the firmness of

strawberries, therefore, increasing its shelf-life and maintaining the physical and sensory properties.

The edible films can be made through two procedures, which are a wet and a dry process. In the wet process, biopolymers are dissolved in a film-forming solution followed by drying or removing the solvent (Suput *et al.*, 2017). The dry process relies on the thermoplastic behaviour displayed by the biopolymer at a low moisture level when compacting, moulding, and extrusion (Betoret *et al.*, 2011).

The coating method: After mucilage extraction from cladodes, the produced liquid mucilage is boiled to reduce its original volume to half. A 99% ethanol concentration is added in a 1:2 ratio to the mucilage. This mixture is then stored at 4°C to obtain the best aggregation of mucilage. The extra liquid that floats on top of the aggregate (supernatant) is eliminated, and the pure mucilage is stored. The pure mucilage is then used to prepare the coating treatment, by mixing 30 g of pure mucilage with 500 ml of water and 50 ml glycerol as a plasticiser. After that, the product must be soaked in the mucilage treatment for 60 seconds, removed, and dried (Allegra *et al.*, 2017).

Film treatment: 30 g of pure mucilage must be mixed with 500 ml of distilled water and 50 ml of glycerol. The content can be poured into a glass petri dish coated with Teflon and dried for 24 hours.

#### 2.4.4. Spherification using cactus mucilage.

Spherification is a technique of molecular gastronomy. It is a gastronomical process that uses sodium alginate and either calcium chloride or calcium lactate to mould the liquid into small, rounded bead-like pearls, which usually structurally and texturally resembles roe (fully ripe internal egg masses in the ovaries or the released egg masses of the fish or certain marine animals such as scallops and shrimps). The liquids are transformed into a spherical semi-gel, using hydrocolloids gum or others. The liquid content gushes out when the gel-enclosed balls are broken (Rogers and Lee, 2012).

Gelation on the surface is made possible by the interaction between the calcium ions and alginate. The calcium ion, which contains two positive charges per atom, enables each ion to link with the two alginates molecules. Alginate molecules are long and negatively

charged. When it is mixed with liquid, it floats freely, then elongates to form a thick jelly-like consistency.

Spherification can be done through two main methods depending on the calcium content in the liquid products to be shaped. The method can either be direct spherification or reverse spherification which can also be done in two ways (Rogers and Lee, 2012).

#### 2.4.4.1. Direct spherification

It is used for flavoured liquids that do not contain calcium. The liquid is combined directly with a gelling solution such as sodium alginate (Figure 2.10). It is then dripped or submerged in a cold solution of calcium chloride or other soluble calcium salts. Each drop of alginate liquid forms into a tiny sphere, with the calcium solution causing an outer layer to result in a thin, flexible skin. Calcium ions react rapidly with alginate, producing salt bridges and gels. For this reason, the spheres made from direct spherification cannot be kept for long because the gel-forming process does not stop when the liquid is removed from the calcium bath (Rogers and Lee, 2012).

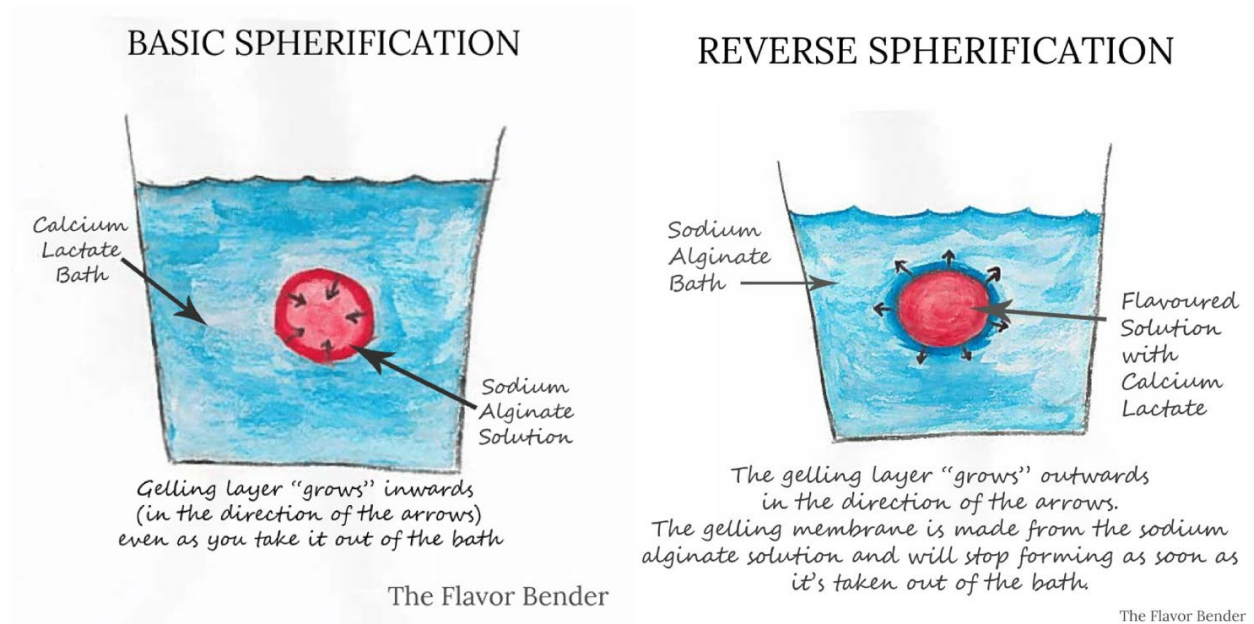


Figure 2. 10 Spherification technique (Xiang, 2018)

#### 2.4.4.2. Reverse spherification

It is a method for molecular gastronomy, used to enclose liquid containing alcohol or calcium, for example, milk, yogurt, and cream. In the reverse process, the substance containing calcium, or a high content of acid (calcium lactate) is dripped into an alginate bath (Figure 2.10). Cross-linking of calcium ions and alginate polymer strands causes it to form a spherical shape encapsulated in a gel-like membrane (Rogers and Lee, 2012). When liquid calcium is insufficient for the reaction, calcium lactate gluconate could be added to a liquid to produce a 2% concentration. Calcium chloride is not preferred in reverse spherification because it imparts a bitter taste to the food. Reverse spherification is also used to encapsulate alcoholic liquids. The gelling agent is in the bath, so once the spheres are removed, gelation immediately stops (D'Angelo *et al.*, 2016) (Figure 2.11).

A more recent method is the frozen reverse method wherein the spheres containing calcium lactate gluconate are pre-frozen, then submerged in a sodium alginate bath. Freezing allows greater precision in the formation or shaping and overcomes many limitations. Like in reverse spherification, the frozen-shaped liquids are immersed in a sodium alginate bath and rinsed. All these methods result in gelled spheres with a liquid inside (D'Angelo *et al.*, 2016).



Figure 2. 11 Olive spheres made with reverse spherification (D'Angelo *et al.*, 2016)

## 2.5. Conclusion

Mucilage from cactus pears is an interesting hydrocolloid that would benefit most industries in South Africa, including the food, pharmaceutical and water industry. Mucilage is a hydrocolloid rich in fiber and other nutrients such as vitamins, amino acids, and minerals (Iman *et al.*, 2021). The use of this hydrocolloid in the food industry as an ingredient and functional agent may not only improve the food systems functionality but also human nutrition and, therefore, human health (Tosif *et al.*, 2021).

Mucilage can be used in the food industry in the making of gel-food products because of its thickening ability and its viscosity modifying properties, that may be controlled by the existence of ions and pH range (Soukoulis *et al.*, 2018, Cakmak *et al.*, 2023)

# Chapter 3: Extraction and drying of cladodes mucilage

## 3.1 Introduction

Mucilage is a complex polymer with a highly branched structure composed of varying proportions of sugars such as arabinose, galactose, rhamnose, xylose and a varying amount of galacturonic acid (Sepulveda *et al.*, 2007). It is found in the mucilaginous cells lying between the green chlorenchyma and the white parenchyma cells. Mucilage can be extracted from the cladodes in a few ways, which may include the use of solvents such as ethanol (Goycoolea and Cardenas 2003), or acetone for precipitation of mucilage, 2-propanol to wash the mucilage out (Medina-Torres *et al.*, 2000) and petroleum ether to purify and degrease the mucilage after centrifugation (Medina-Torres *et al.*, 2000). Other mucilage extraction methods are heat-assisted, for example, the method used by Goycoolea and Cardenas (2003), where cladodes were heated to 85°C for 20 minutes before liquidising to a pulp and filtrating to obtain the mucilage.

Due to mucilage's high-water activity of >0.8 and its sugars and protein composition in fresh or native form (Figure 3.1 a), it is highly susceptible to spoilage and microbial attack, which leads to a short shelf life. For the above reason of concern, mucilage needs to be dried into a powder (Figure 3.1 b) to extend the shelf life.



Figure 3.1 Native liquid mucilage (du Toit 2016) and (B) dried mucilage powder (21food.com)

Mucilage drying can be complex or challenging, with mucilage being the cladode component that binds and holds water (Du Toit, 2016). Although drying improves mucilage shelf-life, it also impacts the properties of mucilage. Drying has an effect on the rheological properties of mucilage. The effects may be due to temperature, type of dryer and nozzle. Leon-Martinez *et al.*, (2011) reported that mucilage dried with a freeze-dryer had more pronounced shear-thinning behaviour than the spray-dried mucilage powder. In the same paper, the authors reported a low viscosity property but a stable powder for spray-dried mucilage compared to freeze-dried mucilage powder. The low viscosity is due to the thermal degradation of the mucilage polysaccharides. Another reason is that high temperature applied on the acidic mucilage, pH < 6, causes hydrolysis of long chain polymers resulting in shorter polymers.

### 3.2 Material and Methods

#### Cultivars

The four cultivars chosen were *Robusta*, *Ficus-indice*, *Nepgen* and *Algerian*. The four cultivars were selected based on the amount of mucilage (yield) and viscosity found in a previous study (Du Toit *et al.*, 2016). *Ficus-indice* and *Algerian* produced a high mucilage yield and a lower viscosity, while *Robusta* produced a medium yield of mucilage with a high viscosity, and lastly *Nepgen* mucilage yield was low but with a medium viscosity (Du Toit, 2017).

Four cladodes of each cultivar were harvested from the University of the Free State's west campus experimental orchard, GPS coordinates: 29° 10'53" S; 25° 58'38" E. For this project, mucilage was extracted from the cactus pear cladodes using the patented method developed by du Toit and de Wit (2011), which is a heat-assisted (microwave) method involving no use of chemicals.

#### 3.2.1. Extraction of Mucilage

Extraction of mucilage from the cactus pear cladodes was done according to a patented method (Du Toit and De Wit, 2011). The cactus pear cladodes were washed using distilled water to remove dust and dirt. The buds and thorns were then removed with a knife. The

whole cladode was diced with a knife into cubes of about 3 cm x 5 cm, placed into a microwave-safe container, and microwave for 4 minutes at high power (100% power in a 900-Watt microwave oven). It was then allowed to cool before putting the above into the blender. The cooled cladodes were macerated in a kitchen-scale blender (Moulinex 4-in-1 multipurpose juice machine (model MMJ004)) at high speed as shown in Figure 3.2a.



Figure 3.2A. Blended cladodes pulp in a blender.

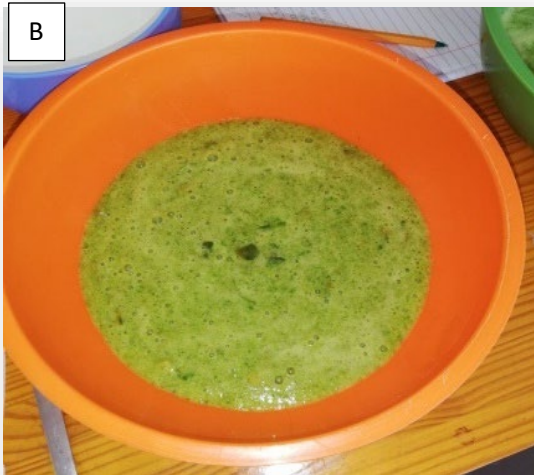


Figure 3.2 B Cladodes pulp containing mucilage and (C) centrifuged mucilage

The slimy resulting pulp, containing mucilage shown in Figure 3.2B, was then added into centrifuge bottles (Figure 3.2C).

It was centrifuged for 30 minutes at 8000 rpm speed at 4°C. After centrifugation, the resulting supernatant liquid was decanted from the sediments into plastic containers, and stored in the freezer at -20°C. Each container was filled with between 50 to 100 ml of mucilage.

The supernatant was weighed using a Radwag PS 750/C/2 scale to obtain the yield (g) of native mucilage in percentage. The following formula was used from (Du Toit, 2017):

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

### 3.2.1 pH determination

The pH of different cultivars' mucilages was measured using a pH meter (Eutech instruments pH 2700). The pH meter probe was removed from its covering and inserted into a jar with mucilage until a reading appeared on the pH meter screen. The reading was taken 4 times to calculate each cultivar's average pH value.



Figure 3.3 pH meter

### 3.2.3. Drying

The extracted native mucilage was dried in two ways: freeze-drying (FD) and hot-air drying (HAD).

#### 3.2.3.1 Freeze-drying (FD)

After a few days of freezing, the mucilage was dried. The freeze-drying procedure requires the mucilage in a solidly frozen form. The frozen mucilage, about 50 ml to 100 ml was placed in a freeze-dryer (Labconco FreeZone Cascade Benchtop Freeze Dry system) at a temperature of  $-50^{\circ}\text{C}$  to  $-60^{\circ}\text{C}$  for three days resulting in a powder (showed in Figure 3.4).



Figure 3.4 The inside of the freeze dryer with dried mucilage

The dried mucilage was removed from the containers and transferred into vacuum bags. The bag was immediately vacuum-sealed (Figure 3.5) to avoid moisture re-absorption that might result in lumps and caking of mucilage. Thereafter, the FD powder was stored in the freezer at  $-20^{\circ}\text{C}$ .



Figure 3.5 Vacuum-sealed mucilage

### 3.2.3.2. Hot-air drying (HAD)

During hot-air drying, mucilage is required in a liquid form. In this case, the frozen mucilage was thawed and placed into the food dehydrator plates, shown in Figure 3.6.

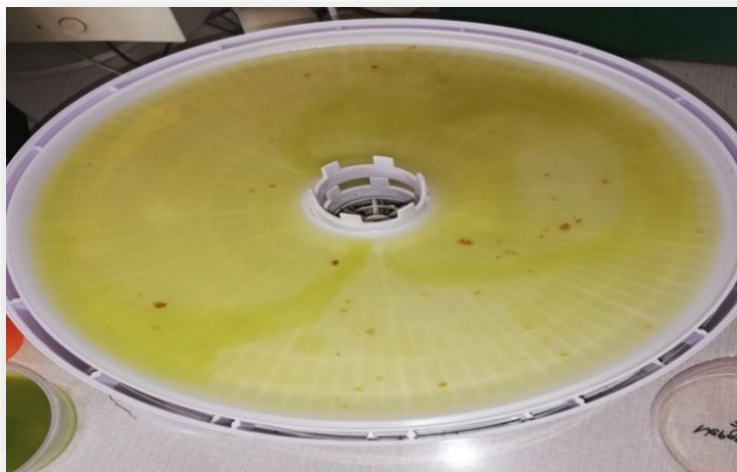


Figure 3.6 Mucilage in the food dehydrator

The dehydrator was set to the highest temperature (63°C) and allowed to dry for three days. The resulting lump-like layer was ground into a powder in a coffee grinder (model CGM16.000BK), as shown in Figure 3.7.



Figure 3.7 Dried mucilage ground in a coffee grinder

The dried mucilage was ground into powder, vacuum packed and sealed as shown in Figure 3.8 and stored in the freezer at  $-20^{\circ}\text{C}$ . The dried mucilage powder needs to be vacuumed and sealed to avoid moisture re-absorption.



Figure 3.8 Hot-air dried mucilage in vacuum pack.

### 3.2.4. Colour analysis for reconstituted dried mucilage

The FD and HAD mucilage powders were reconstituted into a liquid by mixing 5 g of powdered mucilage with 15 ml of distilled water and hydrated overnight. The colour was measured using a Konica Minolta colourimeter. The measurement readings included the  $L^*$ , which indicates the lightness.  $L^*=0$  shows a black colour and  $L^*=100$  represents white. The  $a^*$  indicates red and green, where the positive value shows a red colour, and a negative value represents green. The  $b^*$  of CIELAB, which measures the yellow and blue, where a negative value represents blue colour and positive value represents a yellow colour. The other values calculated from  $L^*$ ,  $a^*$  and  $b^*$  values include  $C^*$ (Chroma) and the  $h^\circ$  (hue).  $C^*$  is a value that indicates saturation of the colour, with 0 representing an unsaturated colour and 100 meaning brightness of colour. The  $h^\circ$  value indicates the location of colour degrees on a colour wheel with  $h^\circ=0^\circ$  showing red,  $h^\circ=90^\circ$  showing yellow,  $h^\circ=180^\circ$  showing green and  $h^\circ=270^\circ$  showing blue.  $A^*$  and  $b^*$  are used to calculate the chroma and hue angle with the help of CIELAB shown in Figure 3.9.

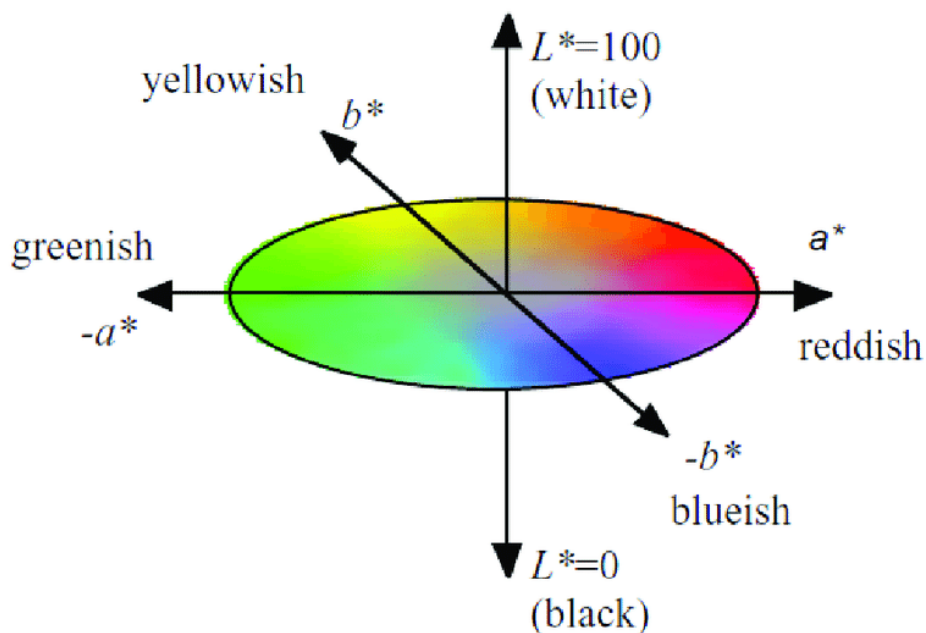


Figure 3.9 The Hunter colour scale indicating CIELAB and CIELCH colour values (Anderson, 2013)

### 3.3 Results and Discussion.

#### 3.3.1. Mucilage yield

Table 3.1 Native liquid mucilage yield from cladodes

Cladodes	<i>Nepgen</i>	<i>Robusta</i>	<i>Algerian</i>	<i>Ficus-indice</i>	Sign. Level
Cladodes weight	884.50 ± 228.14	1170.00 ± 550.75	1024.00 ± 478.10	768.75 ± 167.58	p = 0.523
Weight after cooking	874.50 ± 231.89	1168.50 ± 551.37	1046.00 ± 440.65	760.25 ± 166.02	p = 0.468
pH	4.46 <sup>a</sup> ± 0.01	4.49 <sup>ab</sup> ± 0.03	4.48 <sup>ab</sup> ± 0.01	4.51 <sup>b</sup> ± 0.01	p = 0.013
Minced weight	688.75 ± 300.17	748.50 ± 86.63	908.50 ± 408.47	621.50 ± 172.86	p = 0.509
Mucilage weight	428.75 ± 98.38	573.67 ± 141.96	526.75 ± 200.82	287.75 ± 92.83	p = 0.060
Yield mucilage%	50.96 ± 15.25	48.40 ± 9.59	53.70 ± 7.57	37.75 ± 10.51	p = 0.245

Mucilage extraction was done from the four cladodes of each of the four cultivars. The different cultivars produced different amounts of mucilage (Table 3.1). The amount of mucilage extracted from each cladode (leaf) showed that the weight or quantity of mucilage extracted is not influenced by the size of the cladodes as demonstrated in Figure 3.10. This means that a bigger cladode would not necessarily produce more mucilage than a smaller one. This conclusion was in agreement with Du Toit *et al.* (2019).

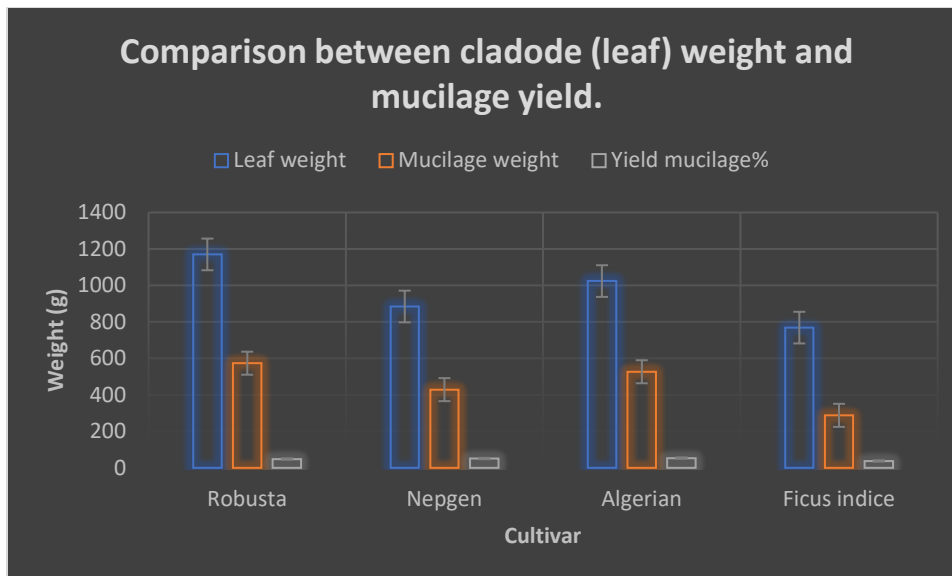


Figure 3.10 Comparison between mucilage yield and leaf weight.

From Table 3.1 it can be seen that *Robusta* had the heaviest cladodes (1170 g), followed closely by *Algerian* (1024 g). In contrast, *Ficus-indice* (768.75 g) had the lightest cladodes and *Nepgen*, (884.50 g) with a slightly higher value than *Ficus-indice*. The same trend was observed after cooking the cladodes during extraction and with the weight of extracted mucilage. Interestingly this trend was not observed for the final % mucilage yield. Although not significantly different, *Algerian* had the highest yield (53.70%), followed by *Nepgen* (50.96%) and *Robusta* (48.40%) whereas, *Ficus-indice* had the lowest yield of 37.75%. The relationship between the yield %, cladode mass and mucilage weight is further explained in Figure 3.10.

From Figure 3.10 it is clear that mucilage yield does not depend on cladode weight. To illustrate: *Robusta* had the heaviest cladodes and produced the second lowest mucilage yield, while *Algerian* (second heaviest cladodes) yielded the most mucilage. *Ficus-indice* had the lightest cladodes and also had the least % mucilage. This observation was also reported by Du Toit *et al.* (2019) that the size of the cladodes had no significant correlation to mucilage % yield. The relationship is usually influenced by the environmental temperatures (du Toit *et al.*, 2019). Therefore, the mucilage yield is influenced by the environment it is grown on.

The pH of the mucilage differed significantly between the cultivars, with *Nepgen* having the lowest (4.46) and *Ficus-indice* having the highest pH (4.51) (Table 3.1).

### 3.3.2. Reconstituted dried mucilage colour

Regarding the colour properties of the mucilage dried in different ways (FD and HAD) indicated in Tables 3.2a and b, a clear difference could be seen between the cultivars and drying methods. The freeze-dried samples were generally a lighter colour than the hot air-dried samples represented by  $L^*$  value in Tables 3.2 a and b and Figure 3.11. Freeze-dried *Robusta* had an  $L^*$  of 49.06 as compared to a lower  $L^*$  value of hot air-dried *Robusta* (37.85). The same pattern was observed for *Nepgen*, *Algerian* and *Ficus-Indice* freeze-dried  $L^*$  value of 34.22, 39.97, 42.26 and hot air-dried  $L^*$  value 31.29, 35.31 and 43.91, respectively. The biggest difference was observed for *Robusta*.  $a^*$  value represents the scale from green (negative value) to red (positive value). It is evident that the freeze-dried samples had a green colour (negative values) while the hot air-dried samples had a reddish colour seen by a positive  $a^*$  value.



Figure 3.11 Difference in HAD rehydrated *Robusta* mucilage (left) and FD rehydrated mucilage (right)

b\* values represent the scale from blue (negative value) to yellow (positive value); again, the biggest difference between drying methods was observed for *Robusta*. It is also necessary to consider that *Robusta* is from a different species, namely *O. robusta*, known for its bluish-green coloured cladodes. In general, the hue values of the hot air-dried samples were higher than the freeze-dried samples. Therefore, a darker (intensity) shade of colour. The brown colour of the hot air-dried mucilage can be ascribed to the prolonged high temperature during drying which can result in degradation due to enzymatic browning or non-enzymatic browning reactions, thus affecting polysaccharide properties (Qian *et al.* 2012). Therefore, freeze-drying will be used in subsequent experiments.

Table 3.2A Freeze-dried mucilage colour results

Freeze-dried powder	L*	a*	b*	hue°
<i>Robusta</i>	49.06	-7.87	23.22	-71.2768
<i>Ficus-indice</i>	42.26	-5.91	11.88	-63.5508
<i>Nepgen</i>	34.22	-2.39	6.25	-69.0731
<i>Algerian</i>	39.97	-6.17	10.57	-59.7267

Table 3.2B Hot air-dried mucilage colour results

Hot-air dried powder	L*	a*	b*	hue°
<i>Robusta</i>	37.85	1	16.96	86.62562
<i>Ficus-indice</i>	43.91	2.62	16.88	81.17733
<i>Nepgen</i>	31.29	4.69	11.53	67.86523
<i>Algerian</i>	35.31	3.13	14.13	77.50984

### 3.4. Conclusions

The mucilage yield produced had no correlation with the cactus pear cladodes weight from which it was extracted from. The cultivar with the highest mucilage yield % was

*Algerian* (53.70%) while *Ficus-indice* produced the least mucilage yield % (37.75). The drying method impacted the colour of dried mucilage powder, with the hot air-dried mucilage powder losing its greenness and turning brownish. Therefore, FD mucilage was used in further experiments.

## Chapter 4: Functional properties of freeze-dried mucilage

### 4.1. Introduction

Functional properties describe how ingredients behave during cooking and preparation and how they affect the texture, appearance and taste of the final food product (Chinaza *et al.*, 2019). Functional properties include caramelisation, coagulation, dextrinisation, flavour alterations, preservation, binding properties, solubility, gelation, denaturation, and emulsification (Chinaza *et al.*, 2019). Constituents such as polysaccharides, proteins and fats play a role in the functional properties of a molecule when added to food as a food additive or ingredient (Sikorski, 2006; Kinsella and Melachouris, 1976).

A food additive is a substance added to food to preserve flavour or enhance taste, colour, appearance, and other qualities (Tomaska and Brooke-Taylor, 2014). Mucilage is a hydrocolloid that can be added to food as a food additive for emulsification as an emulsion stabiliser (to prevent the liquid from coalescence) (du Toit *et al.*, 2019). An emulsion is a mixture of two or more liquids that are typically immiscible, usually oil and water, through the process known as emulsification (Speight, 2017). Mucilage has become a hydrocolloid of interest because of its functional properties and capability to absorb and hold an enormous amount of water (Glicksman, 1983). It can be used in the food industry as a food additive or ingredient to thicken or form gels, to control crystallization, and as an edible coating to increase shelf-life (De J Cano-Barrita and Leon-Martinez 2016). Mucilage also enhances the nutritional content of food (Coria Cayupan *et al.*, 2011).

The functional properties of cactus pear cladodes mucilage can be affected by the chemical composition thereof. Mucilage contains electrolytes which is valuable in the flocculation of a suspension (Gebresamuel and Gebre-Mariam, 2012). Du Toit (2017) showed that mucilage presents better water-holding capacity (WHC) than oil-holding capacity (OHC). This trait influences the ability to form viscous solutions that may play a role in industrial applications. Although not as good as WHC, mucilage presents a good OHC, which suggests that mucilage could improve texture (Monrroy *et al.*, 2017). The sugar composition of mucilage makes it a negatively charged polyelectrolyte molecule, which gives it the ability to attract and retain positively charged substances such as sugar

and fats in its matrix (Pichler *et al.*, 2012; Saenz *et al.*, 2004). Mucilage contains a low molecular weight compound, namely protein (Kumar *et al.*, 2018). Proteins can lower the oil-in-water interfacial tension and create emulsifying capability (Lam and Nickerson, 2013). Mucilage stabilises oil-in-water emulsions by reducing surface interfacial tension and forming small droplets and adsorb onto oil-water under-surface. Therefore, it has an emulsifying capacity similar to those of xanthan and guar gum (Bensadon *et al.*, 2010).

Mucilage exhibits rheological properties because of its high viscosity (mucilage solution viscosity of 1 and 4% are 1.6cP and 4.6cP, respectively) (Prasad *et al.*, 2005). The properties of high WHC and high OHC open an opportunity for mucilage to be used in food applications as a thickener, stabiliser, and emulsifier (Majdoub *et al.*, 2001). The mucilage's ability to form an emulsion allows it to be used in making edible coatings, a thin layer of edible material applied on the product's surface to provide a barrier to the solute, moisture, and gaseous movement (Dhall, 2012).

The research question investigated in this chapter is as follows: due to mucilage's ability to hold a huge amount of water, which causes increased viscosity, can it function as a thickening or gelling agent?

## 4.2. Materials and Methods

### 4.2.1 Mucilage extraction

Mucilage was extracted from four different cultivars namely *Robusta*, *Ficus-indice*, *Nepgen*, and *Algerian*, as discussed in section 3.2.1. For this chapter, native mucilage and dried mucilage powders, both freeze-dried (FD) and hot air-dried (HAD), will be used. This will further demonstrate any effects of the drying procedure on mucilage properties.

### 4.2.2. Viscosity

Viscosity is a state of a substance being thick, sticky, and semi-fluid in consistency due to an internal friction. Viscosity is the measure of a fluid's resistance to flow (Gresham, 2008). The viscosity of the different cultivar's mucilage was measured using the line spread method. The line spread test is used to compare the viscosity of viscous liquids such as gels, thickened liquids, and hydrocolloids. It is reliable and cheap (Kim *et al.*, 2014). The line spread test consists of a sheet with concentric circles (0.5 cm apart) with

quartered regions covered in glass. To measure, a small opened-end metal cylinder is placed at the centre circle of the lined spreadsheet. The liquid mucilage (5 ml) was used to fill the cylinder to the same level. The cylinder was then lifted from the sheet surface to allow the mucilage to flow or spread freely for the designated time (30 seconds), after which the readings were taken on limits at four points, as demonstrated in Figure 4.1. The dried powdered (FD and HAD) mucilage was rehydrated at a ratio of 1:3 by adding dried mucilage powder to water. After rehydration FD and HAD consistency was evaluated with the same procedure used for native liquid mucilage. The higher the line spread test value, the lower the viscosity of the mucilage as it spreads further (Kim *et al.* 2014). The viscosity was calculated using the formula:

$$\text{Viscosity} = (p1+p2+p3+p4)/4$$

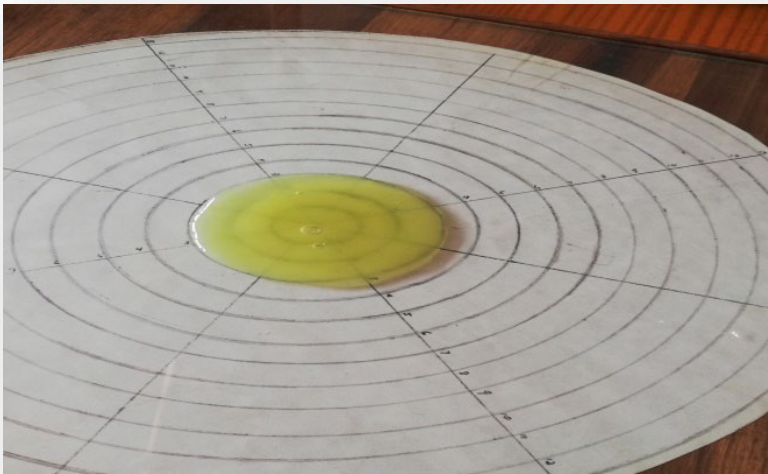


Figure 4.1 Line-spread sheet test

#### 4.2.3. Gel formation

To test for mucilage gelling ability, five different methods were used. Each method had five (5) replicates of each cultivar and drying method. These tests involved using mucilage in combination with either ethanol, or sugar, controlling the pH by adding acids or bases, different hydrocolloids, and various salts (Table 4.1)

Firstly, 60 ml of mucilage (both dried and reconstituted, as well as and native) was boiled to half its original weight. Ninety percent ethanol was added to evaluate the gelation ability.

Secondly, sucrose was added in different concentrations of 50 g, 100 g, and 150 g, respectively, to the 60 ml mucilage, allowing it to boil and cook in a procedure similar to making jelly with gelatine.

During the third type of test, the mucilage was treated to different pH levels by either adding HCl, to lower the pH or NaOH, to increase the pH.

Adding salts would improve gelation because it lowers the least gelation concentration (LGC) (forth method). Twenty grams of mucilage powder was added to 100 ml of water, mixed with 1 g of  $\text{NaNO}_3$  and NaOH to adjust the pH to 7, then heated for 1 hour, rapidly cooled under cold water, and allowed to cool to 4°C for two hours (Coffman and Garcia 1977).

The fifth test was to mix mucilage with other hydrocolloids such as guar (G), xanthan (X), and agar (A) in different ratios to observe the gelation effects thereof. The ratios were M:X (3:1), M:G (3:1), M:A (3:1), M:G:X (3:1:1), M:G:A (3:1:1), and M:X:A (3:1:1)

Table 4.1 Mucilage gelation tests.

		Gelation tests conducted in all four cultivars using dried reconstituted (both FAD and HAD) and native liquid			
Cultivar	Mucilage used	90% ethanol	Sugar	pH + NaNO <sub>3</sub>	Hydrocolloids
<i>Robusta</i> , <i>Nepgen</i> , <i>Algerian</i> , <i>Ficus-indice</i>	FAD (5 replicates of each cultivar), HAD (5 replicates of each cultivar), Native liquid (5 replicates of each cultivar),	10 mL was added to the mucilage of each cultivar	50 g, 100 g and 150 g were used in the preparation of the jellies	HCl was used to lower pH, and NaOH was used to increase pH and to test least gelation concentration	Guar (G), Xanthan (X), and Agar (A) were added to mucilage (M) separately and in a combination MGX, MXA, and MGA

Five replicate of each cultivar and the drying method, as well as the native mucilage were used for each gelling method

#### 4.2.4 Emulsification

To analyse the emulsifying ability of the liquid mucilage, the method used by Iturriaga *et al.* (2009a) was used. This method was adapted by adding 20 ml of mucilage in a tarred cylinder. The 10 ml sunflower oil was added in two stages by adding 5 ml during the first 30 s of homogenisation and adding the remaining 5 ml during the second 30 s of homogenisation. The volume of emulsion formed at the top (or creamed layer) was read after 20 seconds and repeated after 1 min, 2, 5, 10, 20, 60 minutes, and 24 hours to calculate % creaming using the formula below.

$$\text{Creaming}(\%) = \frac{\text{volume of the cream layer}(ml)}{\text{total volume of the emulsion}(ml)} \times 100$$

To test the emulsifying ability of the dried mucilage powder, 5 g of mucilage powder was mixed with 20 ml of distilled water to make a paste and allowed to hydrate. It was then mixed with 5 ml of sunflower oil for 30 seconds with a stick blender to homogenise, then another 5 ml of oil was added and homogenised. The resulting creaming layer was recorded at different time intervals. The same procedure was used for the liquid mucilage. This method was carried out for all four cultivars in dried (HAD and FD) reconstituted and native forms and their duplicates.

#### 4.2.4 Statistical analysis

Analysis of variance (ANOVA) was used to determine if differences exist between dried and native mucilage functional properties (NCSS 11 Statistical Software, 2016). The Tukey-Kramer multiple comparison test ( $\alpha = 0.05$ ) was applied to determine the direction of the differences between treatment means (NCSS 11 Statistical Software, 2016).

### 4.3. Results and Discussion

#### 4.3.1 Viscosity

The evaluation of mucilage flow over a period of 30 seconds was recorded for each cultivar of extracted mucilage. The various flow measurements can be seen in Table 4.2

Table 4.2 Native mucilage viscosity average

Cultivar	<i>Ficus-indice</i>	<i>Algerian</i>	<i>Nepgen</i>	<i>Robusta</i>	Sign level
Viscosity	5.03 <sup>b</sup> ± 0.51	5.08 <sup>b</sup> ± 0.68	3.75 <sup>a</sup> ± 0.44	3.28 <sup>a</sup> ± 0.22	p < 0.001

According to the results obtained *Robusta* had the highest viscosity (shortest flow distance of 3.28 cm) followed by *Nepgen* (3.75 cm), *Algerian* (5.08 cm), and *Ficus-indice* (5.03 cm) which had the lowest viscosity. These last two cultivars (*Algerian* and *Ficus-indice*) did not differ significantly from each other however, differed significantly from *Nepgen* and *Robusta*, which also did not differ significantly from each other. This result was also noticeable after extraction, with *Robusta* mucilage having an almost gel-like consistency, as was observed for *Nepgen*. Both *Algerian* and *Ficus-indice* had a watery consistency.

A similar trend was reported by Du Toit (2017), although few cultivars were reviewed for viscosity. *Nepgen* (14.88 cm) and *Robusta* (17.94 cm) had a higher viscosity as compared to *Algerian* (32.38 cm) and *Ficus-indice* (32 cm), which had the lowest viscosity demonstrated by the distance each cultivar spread. Du Toit (2017) also grouped these cultivars in terms of their viscosity, with *Robusta* ranking the highest, followed by *Nepgen* and *Algerian*, and *Ficus-indice* ranked as the cultivar with the least or lowest viscosity. This is the same trend that was observed in Table 4.2. The viscosity differed greatly between the native liquid mucilage and the reconstituted dried powders (Figure 4.2). The p value (p < 0.001) also shows that the difference in cultivars viscosities is highly significant.

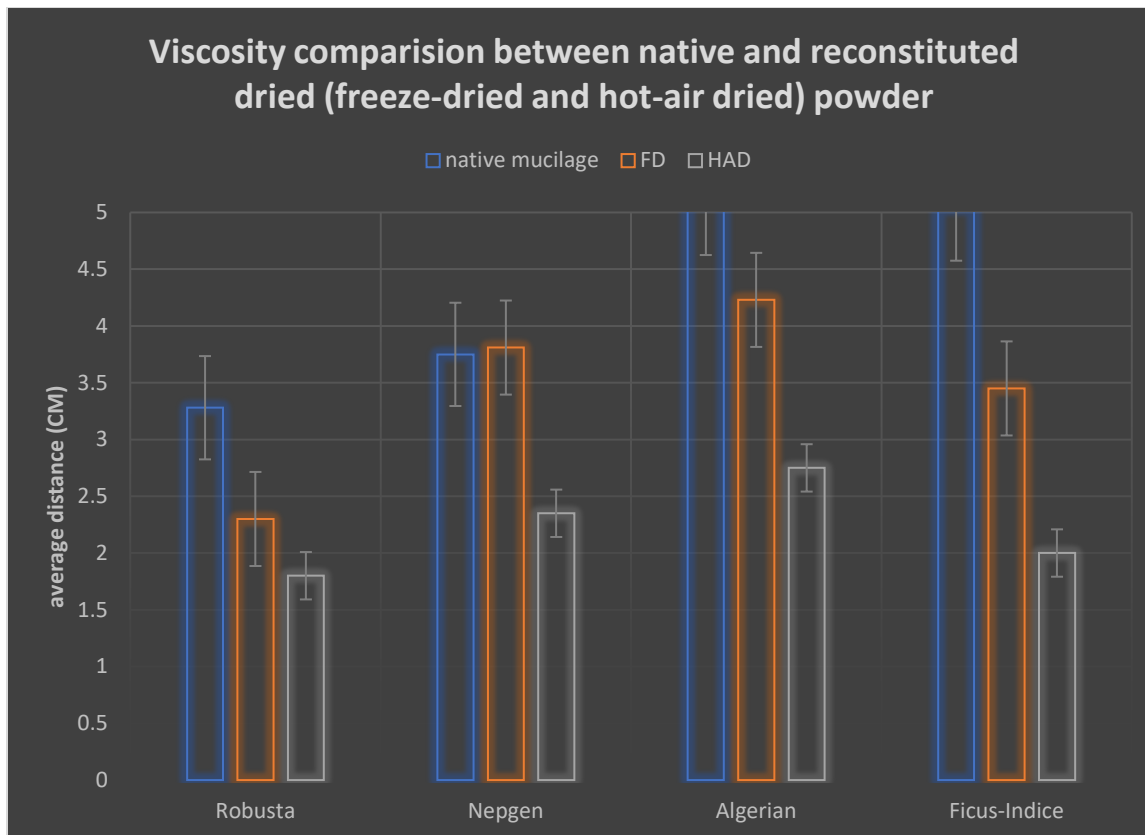


Figure 4.2. Viscosity comparison between native and reconstituted freeze-dried and hot air-dried mucilage

The dried mucilage became very thick when rehydrated. The line spread test results showed a decrease in the distance flowed by reconstituted mucilage powders for both drying methods. The results obtained from the line spread test of freeze-dried (FD) mucilage were slightly less than that of native liquid. *Robusta* viscosity increased for each drying method, and this was demonstrated by the least distance on the line spread when FD reconstituted mucilage was used (2.3 cm) and HAD reconstituted mucilage (1.8 cm) as compared to the native mucilage (3.28 cm). This shows that the viscosity increases when mucilage is dried and reconstituted, and also that the drying method had an impact on the mucilage properties. The same trend was observed for all three other cultivars. Du Toit (2017) reported the same trend for *Robusta* mucilage, with an increase in viscosity after freeze-drying treatment, although this was not observed for other cultivars.

There was a huge difference in the results obtained from the hot air-drying (HAD) mucilage compared to the native liquid mucilage shown in Figure 4.2. The line spread

values for hot air-dried (HAD) mucilage decreased; therefore, the viscosity increased, and a possible reason for this occurrence might be due to denaturation of the protein while heat-drying. The application of heat increases the kinetic energy which causes the molecules to vibrate. The expedite molecule vibration may result in disruption or breaking of protein molecule's hydrogen bonds which causes the unfolding of 3D structure and helix. The broken hydrogen bond increase solubility which also plays a role on viscosity. Youssef et al. (2009) reported a similar trend for fenugreek viscosity. It was reported that the viscosity increased when molecular weight of fenugreek was increased by removing attached protein. When visually observing these three types of mucilage, it was clear that the texture differed. The freeze-dried reconstituted mucilage still resembled the liquid native mucilage, whereas the hot air-dried mucilage became more viscous with a crumbly-like, sandy texture or consistency.

#### 4.3.1. Possible gelling properties

Cactus pear mucilage does not form a true gel. It can only thicken or increase the viscosity of the product being added to, as shown in Table 4.3. The same results were reported by Du Toit (2017).

Table 4.3 Gelation test results

Cultivar		Gelation test											
		90% ethanol	sugar			Hcl + NaNO <sub>2</sub>	NaOH + NaNO <sub>2</sub>	MX	MG	MA	MGX	MXA	MGA
			50 g	100 g	150 g								
<i>Robusta</i>	Native	-	-	-	-	+	-	-	++	+	+	++	
	FD	-	-	-	-	+	-	-	++	+	+	++	
	HAD	-	-	-	-	+	-	-	++	+	+	++	
<i>Nepgen</i>	Native	-	-	-	-	+	-	-	++	+	+	++	
	FD	-	-	-	-	+	-	-	++	+	+	++	
	HAD	-	-	-	-	+	-	-	++	+	+	++	
<i>Algerian</i>	Native	-	-	-	-	+	-	-	++	+	+	++	
	FD	-	-	-	-	+	-	-	++	+	+	++	
	HAD	-	-	-	-	+	-	-	++	+	+	++	
<i>Ficus-indice</i>	Native	-	-	-	-	+	-	-	++	+	+	++	
	FD	-	-	-	-	+	-	-	++	+	+	++	
	HAD	-	-	-	-	+	-	-	++	+	+	++	

= no gelling observed ++ = a gel-like film is form + = solution thickens.

MX= mucilage and xanthan mixture, MG= mucilage and guar gum, MA= mucilage and agar mixture, MGX= mucilage, guar gum and xanthan, MGA= mucilage, guar gum and agar mixture, MXA= mucilage, xanthan and agar mixture.

When cactus pear mucilage (powdered and reconstituted or native liquid) from all four cultivars was treated with 90% ethanol, no gel was formed. Cooking each cultivar of mucilage with sugar at different concentrations did not result in gel formation like expected when using gelatin in making of jelly.

When powdered and liquid mucilage was treated with NaOH to increase pH to 5.5-6, the viscosity increased with a gel-like membrane, but not a true gel. This proved that coagulation of hydrocolloids occurred at a pH of 5 to 7.5, as reported by Contreras-Padilla *et al.*, 2016 (Figure 4.3 A).

When the least gelation concentration was tested, no coagulum was formed either by using NaOH or HCl in all four cultivars of different mucilage (native, FAD, and HAD).

When the mucilage of each cultivar was mixed with different hydrocolloids, as shown in Table 4.3 C, no true gel was formed. Mixing mucilage either FD, HAD, or native, with xanthan or guar gum resulted in no gel being formed, but when combined with agar, a thin gel-like membrane and thickening occurred. This was concluded to be due to the capability of agar to form a gel. When mucilage was mixed with other hydrocolloids, i.e.,

xanthan, guar, and agar, the viscosity increased but did not form a rigid gel, as shown in Figure 4.3 B.

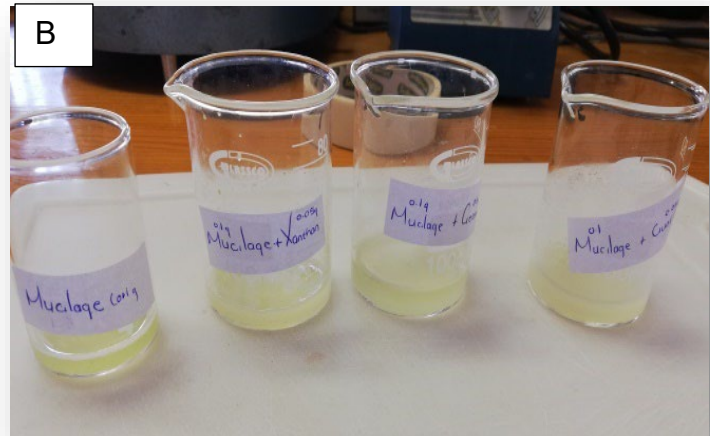
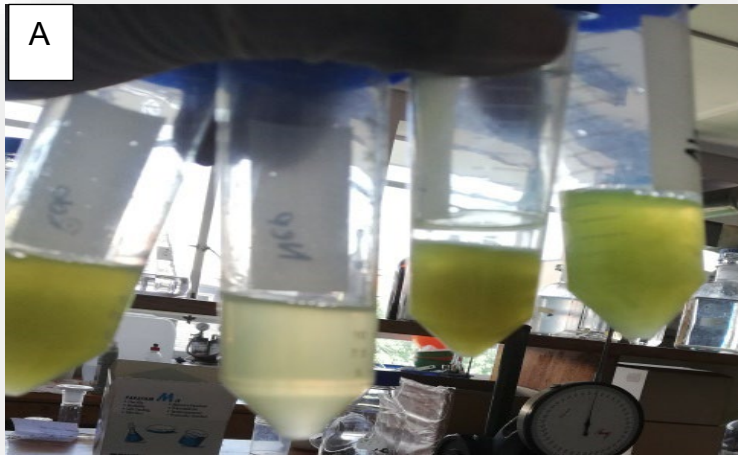


Figure 4.3 Viscous mixture formed with pH manipulation by adding NaOH and HCl (A), a mixture of mucilage with single hydrocolloids (xanthan, guar, and agar) (B) and mixture of mucilage with two hydrocolloids such as (xanthan, guar with mucilage or xanthan, agar with mucilage) (C)

For a hydrocolloid or a solution to form a gel, its polymer must cross-link to create a three-dimensional network, which locks in or immobilises water inside, forming a solid structure that cannot flow (Saha and Bhattacharya, 2010). Mucilage does not form a true gel; instead, the long polymer of mucilage repels itself. It uncoils and stretches, resulting in an increased viscosity (du Toit, 2018). Mucilage can be used as thickening agents and cannot be used on its own as a gelling agent.

### 4.3.3 Emulsification

The creaming layer that formed after the blending of mucilage and oil, was evaluated over time for emulsion stability. All four cultivars showed the ability to form an emulsion when mixed with oil and water, proved by the creamed layer produced in each blend. This is referred to as emulsion capability in Figure 4.4



Figure 4.4 Native mucilage emulsifying ability of *Robusta*, *Nepgen*, *Algerian* and *Ficus-indice* (from left to right)

Twenty seconds after mixing, *Algerian* (100%), *Ficus-indice* (100%), and *Robusta* (100%) showed high emulsifying capacity, with no decrease in the creaming layer when native liquid mucilage was used. The creaming started to show a slight decrease after two minutes with *Algerian* (90%), *Ficus-indice* (91%), and *Robusta* (90%). *Nepgen* had a lower ability to emulsify. The ability of *Nepgen* to form an emulsion was below that of the other cultivars, and it differed significantly, as shown in Table 4.4 at 20 seconds (83%). The creaming layer formed from *Nepgen*, increased after the first 20 seconds to 91% creaming layer after 2 minutes, which matched that of the other cultivars, and the reason for this is unknown, although the viscosity was similar to that of *Robusta*. After an hour, the emulsion or creaming layer for all four cultivars was still above 50%, which indicated a stable emulsion. This creamed layer remained above 50% for 24 hours (1 day).

Table 4.4 Creaming % for liquid mucilage

<b>Cultivar</b>	<b><i>Nepgen</i></b>	<b><i>Algerian</i></b>	<b><i>Ficus-indice</i></b>	<b><i>Robusta</i></b>	<b>Sign level</b>
<b>20 Seconds</b>	83.33 <sup>a</sup> ± 0.01	100.00 <sup>b</sup> ± 0.01	100.00 <sup>b</sup> ± 0.01	100.00 <sup>b</sup> ± 0.01	p < 0.001
<b>1 Minute</b>	95.00 ± 7.93	93.33 ± 9.43	95.00 ± 7.93	93.33 ± 9.43	p = 0.985
<b>2 Minutes</b>	91.25 ± 9.94	90.83 ± 9.57	91.25 ± 9.94	90.83 ± 9.57	p = 0.999
<b>5 Minutes</b>	88.75 ± 8.32	87.50 ± 9.57	88.75 ± 8.32	87.50 ± 9.57	p = 0.994
<b>10 Minutes</b>	84.17 ± 9.95	84.17 ± 11.98	84.17 ± 9.95	84.17 ± 11.98	p = 0.999
<b>20 Minutes</b>	78.33 ± 12.91	76.67 ± 11.86	78.33 ± 12.91	76.67 ± 11.86	p = 0.995
<b>60 Minutes</b>	67.50 ± 12.29	66.67 ± 10.89	67.50 ± 12.29	66.67 ± 10.89	p = 0.999
<b>24 Hours</b>	50.00 ± 2.72	49.17 ± 3.19	50.00 ± 2.72	49.17 ± 3.19	p = 0.955

Means with different superscripts in the same row differ significantly.

After 24 hours, all four cultivars still had about 50% of the creaming layer left, and there were no significant differences between the creaming layers of different cultivars. This may be interpreted as a stable emulsion.

Drying methods had an impact on the emulsifying capability. This was seen by the decrease in creaming layer formed after mixing (20 sec) or agitation when dried mucilage was used, compared to the native liquid mucilage. About a 25% decrease in the creaming layer of all four cultivars was observed when FD mucilage (Table 4.5a) was used, and around 30% was observed when HAD mucilage (Table 4.5b) was used. This may indicate that mucilage properties were sensitive to drying methods and more sensitive or prone to change during hot-air drying than freeze-drying.

The creaming layer decreased over time in all cultivars' liquid and was reconstituted mucilage. There was a significant decrease after 24 hours of standing time, although all four cultivars still had a creaming layer above 40%. *Algerian* and *Ficus-indice* seemed to withstand the drying treatment better than *Nepgen* and *Robusta*. When freeze-dried powders were used, *Algerian* had the most prominent creaming layer, and therefore, a more stable emulsion, significantly higher than that of the other three cultivars after 24 hours of standing time.

When hot air-dried powders were used, the emulsion stability of *Algerian* and *Ficus-indice* was similar but significantly higher when compared to *Nepgen* and *Robusta*. *Algerian* showed emulsion stability in both drying and liquid treatments from after a day of standing time (Table 4.5a and 4.5b). Mucilage proved to have an emulsification capacity and effective in emulsifying fat-containing products (Du Toit *et al.*, 2017).

Table 4.5A Creaming % for freeze-dried mucilage

<b>Cultivar</b>	<b><i>Nepgen</i></b>	<b><i>Algerian</i></b>	<b><i>Ficus-indice</i></b>	<b><i>Robusta</i></b>	<b>Sign. level</b>
<b>20 Seconds</b>	68.33 <sup>a</sup> ± 1.92	70.00 <sup>a</sup> ± 0.01	75.00 <sup>b</sup> ± 1.92	75.00 <sup>b</sup> ± 1.92	p < 0.001
<b>1 Minute</b>	66.67 <sup>a</sup> ± 0.01	68.33 <sup>a</sup> ± 1.92	73.33 <sup>b</sup> ± 0.01	75.00 <sup>b</sup> ± 1.92	p < 0.001
<b>2 Minutes</b>	66.67 <sup>a</sup> ± 0.01	66.67 <sup>a</sup> ± 0.01	73.33 <sup>b</sup> ± 0.01	66.67 <sup>a</sup> ± 0.01	p < 0.001
<b>5 Minutes</b>	61.67 <sup>a</sup> ± 1.92	66.67 <sup>b</sup> ± 0.01	68.33 <sup>b</sup> ± 1.92	66.67 <sup>b</sup> ± 0.01	p < 0.001
<b>10 Minutes</b>	56.67 <sup>a</sup> ± 0.01	65.00 <sup>b</sup> ± 1.92	66.67 <sup>b</sup> ± 0.01	66.67 <sup>b</sup> ± 0.01	p < 0.001
<b>20 Minutes</b>	56.67 <sup>a</sup> ± 0.01	63.33 <sup>b</sup> ± 0.01	66.67 <sup>c</sup> ± 0.01	61.67 <sup>b</sup> ± 1.92	p < 0.001
<b>60 Minutes</b>	53.33 <sup>b</sup> ± 0.01	63.33 <sup>c</sup> ± 0.01	61.67 <sup>c</sup> ± 1.92	46.67 <sup>a</sup> ± 0.01	p < 0.001
<b>24 Hours</b>	46.67 <sup>b</sup> ± 0.01	61.67 <sup>d</sup> ± 1.92	55.00 <sup>c</sup> ± 1.92	40.00 <sup>a</sup> ± 0.01	p < 0.001

Means with different superscripts in the same row differ significantly.

Table 4.5B Creaming % of hot air-dried mucilage

<b>Cultivar</b>	<b><i>Nepgen</i></b>	<b><i>Algerian</i></b>	<b><i>Ficus-indice</i></b>	<b><i>Robusta</i></b>	<b>Sign. Level</b>
<b>20 Seconds</b>	66.67 <sup>a</sup> ± 0.01	67.50 <sup>a</sup> ± 0.96	66.67 <sup>a</sup> ± 0.01	71.67 <sup>b</sup> ± 1.92	p < 0.001
<b>1 Minute</b>	68.33 <sup>ab</sup> ± 1.92	66.67 <sup>a</sup> ± 0.01	68.33 <sup>ab</sup> ± 1.92	71.67 <sup>b</sup> ± 1.92	p = 0.008
<b>2 Minutes</b>	66.67 ± 3.85	65.00 ± 1.92	68.33 ± 1.92	66.67 ± 0.01	p = 0.310
<b>5 Minutes</b>	61.67 <sup>a</sup> ± 1.92	63.33 <sup>ab</sup> ± 0.01	65.00 <sup>bc</sup> ± 1.92	66.67 <sup>c</sup> ± 0.01	p < 0.001
<b>10 Minutes</b>	58.33 <sup>a</sup> ± 1.92	63.33 <sup>b</sup> ± 0.01	63.33 <sup>b</sup> ± 0.01	61.67 <sup>b</sup> ± 1.92	p < 0.001
<b>20 Minutes</b>	56.67 <sup>a</sup> ± 3.85	63.33 <sup>c</sup> ± 0.01	61.67 <sup>bc</sup> ± 1.92	58.33 <sup>ab</sup> ± 1.92	p = 0.007
<b>60 Minutes</b>	48.33 <sup>b</sup> ± 1.92	60.00 <sup>d</sup> ± 0.01	53.33 <sup>c</sup> ± 0.01	43.33 <sup>a</sup> ± 3.85	p < 0.001
<b>24 Hours</b>	47.50 <sup>a</sup> ± 1.67	59.17 <sup>b</sup> ± 1.67	54.17 <sup>b</sup> ± 1.67	43.33 <sup>a</sup> ± 3.85	p < 0.001

Means with different superscripts in the same row differ significantly.

#### 4.4. Conclusion

It was found that differences exist between the mucilage's functional properties from four different cultivars. Differences such as emulsion capability and stability and viscosity were also observed between the native liquid and rehydrated dried mucilage. The processes of drying also had an impact on the properties of the mucilage. It was found that *Robusta* had the highest viscosity measured by the distance on linespread (3.28cm), and Algerian had the lowest (5.08cm). The hot air-dried mucilage all had lower viscosities than the freeze-dried mucilage. This shows that freeze-drying has less impact on the rheological properties of mucilage. Freeze-drying may be used to preserve mucilage in the future because of the minimal impact on properties of mucilage as compared to hot air-drying. The freeze-dried mucilage may be used as an emulsifying and a thickening agent.

No gel-forming ability was observed for any of the four cultivar's mucilage. Only an increase in consistency or viscosity was observed during different treatments. Regarding the emulsification properties, all four cultivars had emulsification abilities, with Algerian showing to be more stable in terms of retaining the foam in both types of dried mucilage. Both drying methods affected the emulsifying capacity of dried reconstituted mucilage, demonstrated by the decrease in creaming layer formed when dried mucilage was used compared to that formed when using native liquid mucilage. When freeze-dried and hot air-dried mucilage powder were used, all four cultivars showed significant decreases in emulsion stability with *Robusta* being the least stable. For the use in industry the freeze-drying should be used because it has less impact to the rheological properties of mucilage as compared to the hot-air drying.

# Chapter 5: Application of mucilage in food as functional ingredient or additive.

## 5.1 Introduction

Because of the high amount of water fruits and vegetables contain, they are highly spoilable and decayable. The short shelf life after harvest is due to the changes in gaseous exchange and the fact that the cells are not renewed (Dhall, 2013). The respiration of fruits and vegetables continues after their harvest, using up the stored starch or sugar until it is depleted, leading to aging or ripening.

Water is also lost in the process, causing shrinkage and weight loss. The use of edible coatings improves or increases the shelf-life because, on an applied surface of the product, it acts as a barrier for moisture, solute, and gaseous movement, therefore, increasing shelf-life by retarding respiration and holding of water (Del-Velle *et al.*, 2005). When the substance used for coating and covering the food product to lengthen its shelf-life is consumed together with or without further removal, it is considered an edible film or coating (Betoret *et al.*, 2011).

Mucilage can be used as an edible coating due to its functional properties, because it can form an emulsion. In this chapter, freeze-dried mucilage will be used (because hot-air drying had an effect on the properties as observed in Chapter 4) to formulate edible coatings, which will be applied on products (apples, tomatoes, and potatoes) to evaluate whether mucilage, as an edible coating, will prevent moisture loss (minimise weight loss), prevents loss of colour and also prevent or slow down the loss of firmness (texture).

Cactus pear mucilage is an inexpensive hydrocolloid that can add texture to functional food products and improve viscosity (Du Toit *et al.*, 2019). As a functional food, mucilage was used as a gelling agent in the spherification process, to further investigate the mucilage gelling properties. Spherification is a cooking procedure wherein the liquid is dropped into a solution to create a thin gel coat around the liquid, the whole technique is based on a chemical reaction (Bates, 2023).

The primary purpose of spherification is to form a thin gel layer on the outside of the liquid to hold it in a spherical shape. This technique may be carried out in two ways. The basic way uses sodium alginate and calcium lactate. The two substances, when in solution and contact with each other, alginate, link its polymer together to form a gel. The reverse way, which is used for products containing high calcium lactate level, adds calcium gluconate and sodium alginate (Bates, 2023). The intertwined alginate chains formed in an aqueous solution with calcium ions result in a thin membrane encapsulating the liquid into a sphere shape (Fu *et al.*, 2014).

The main objectives for this chapter were:

- To evaluate the effectiveness of cactus pear mucilage as an edible coating in preventing moisture loss, preserving the colour and texture, and therefore extending shelf-life of apples, tomatoes and potatoes and testing if edible mucilage coating will prevent the coated potato chips from absorbing the oil during drying
- To further test for mucilage gelling ability through the process of spherification.

## 5.2 Materials and Methods

### 5.2.1 Sample preparation

Mucilage was extracted from four different cultivars, namely *Robusta*, *Ficus-indice*, *Nepgen*, and *Algerian*, as previously discussed in section 3.2.1. The freeze-dried (FAD) mucilage from the four cultivars was used to make edible coatings. Freeze-dried mucilage powder was preferred over air-dried methods because it demonstrated more stable emulsifying abilities, a green colour and properties similar to the native mucilage. Another reason why freeze-dried mucilage was decided on for edible coating was because of the long shelf-life that comes with the powdered mucilage as compared to the native liquid that will have to be frozen and then thawed, which makes it prone to bacterial, fungal and yeast spoilage, therefore, shortening the shelf-life. Thawing will lead to the leaching of moisture and a change in the integrity properties of the mucilage. English tomatoes ((Pick 'n Pay (PNP brand) become red as they ripen), potatoes (PNP all-purpose), and store bought washed apples (Granny Smith apples, which are green in colour, and turn yellow

and lighter green as they mature) were selected to be coated with the freeze-dried mucilage to determine whether it will perform as a coating to improve the shelf-life, minimise weight loss and prevent the loss of colour and texture.

For spherification:

Rooibos tea ( it was chosen because it is alkaline, spherification does not work for too acidic liquids) was prepared by mixing four tea bags of rooibos with 1000 g of water. Sodium alginate and calcium lactate were included in a spherification kit. Yogurt (full cream (5.3g per 100g) strawberry flavoured, Nutriday), gelatin, vinegar, sunflower, and olive oil were purchased from Pick 'n Pay. Glycerol, xanthan gum, and agar were provided by the laboratory.

## 5.2.2 Edible coating

### 5.2.2.1 Edible coating preparation

Edible coatings were prepared by mixing 10 g mucilage in 50 ml of distilled water, and then 1.5 ml of glycerol was added as a plasticizer (Zegbe *et al.* 2015; Allegra *et al.* 2017). Treatments included coating prepared from the FD mucilage from four different cultivars and for each product (apples, tomatoes and potatoes) there was an uncoated control sample that was only brushed with distilled water.

After dip coating, the products were stored for seven days at room temperature, then in the fridge for 16 days at 4°C. The CT3 texture analyser was used for texture determination on different days, while the colour determination was performed using a colourimeter (Konika Minolta). The differences between the final weight and initial weight were measured for weight loss.

Table 5.1: Mucilage treatments for coating

Treatment (edible coating)	Ingredients
<b>Robusta mucilage coating (RMC)</b>	10 ml mucilage + 50 ml dwater+ 1.5 ml glycerol
<b>Ficus-indice mucilage coating (FMC)</b>	10 ml mucilage + 50 ml dwater+ 1.5 ml glycerol
<b>Nepgen mucilage coating (NMC)</b>	10 ml mucilage + 50 ml dwater+ 1.5 ml glycerol
<b>Algerian mucilage coating (AMC)</b>	10 ml mucilage + 50 ml dwater+ 1.5 ml glycerol

#### 5.2.2.2 Edible coating application

Three products, namely apples, tomatoes, and potatoes, were bought intact at a local supermarket. The apples, tomatoes, and potatoes with their skin were washed to remove any dirt or waxed used previously. Edible mucilage coatings were applied to each product by dipping product in treatment and allowed to dry. The aim of coating these three products was to potentially prevent weight loss, maintain colour, prevention of browning, prevent softening, and therefore extend shelf life. In the case of potatoes chips, the effect of edible coating on oil absorption during deep fat frying of chips (using peeled potatoes) were observed.

#### 5.2.2.3 Weight loss analysis

Whole apples, tomatoes, and potatoes were coated with different edible mucilage coating treatments and applied on the surface by dipping the samples into the coating solution (Jose *et al.*, 2020), allow it to dwell for 60 seconds, removing them from the solution, and allowing them to dry at a room temperature. The product was weighed just after coating, then weighed during and after the storage period of 23 days. The final weight was used to determine weight loss, which was presented as a percentage by comparing it to the weight before storage, using the following formula :

$$Weight\ loss(\%) = \frac{initial\ weight(g) - final\ weight(g)}{initial\ weight(g)} \times 100$$

#### 5.2.2.4 Colour analysis

Whole tomatoes, apples and potatoes, and sliced potato chips were coated, and colour readings were done using a Konica Minolta CR-400 before and after coating. These

readings were repeated after the storage of 23 days as an indication of colour deterioration. From the results,  $L^*$  indicates the lightness (100) or the darkness (0) of the products.  $a^*$  describe the redness (+) or greenness of the product and  $b^*$  indicates whether the product is yellow (+) or blue (-).  $C^*$  illustrates the level of saturation of the colour.  $h^\circ$  indicates the position colour angle/degree, with  $h^\circ=0^\circ$ , (red)  $h^\circ=90^\circ$ , (yellow),  $h^\circ=180^\circ$  (green), and  $h^\circ=270^\circ$  (blue) (Figure 3.9)

#### 5.2.2.5 Texture analysis

A penetration test was performed from the outside into the inside of the whole apple, tomato, and potato at room temperature using a CT3 texture analyser with a 1 mm diameter and 43 mm long needle probe to measure the force required to penetrate the product. To measure the firmness, the samples were penetrated to the depth of 15 mm (potatoes and apples) and 6 mm (tomatoes) at a speed of 2mm/s. The products were penetrated at their equatorial zone in three different spots. The coated tomatoes were stored at room temperature for seven days. After seven days of storage, the penetration test was repeated to measure the force required after initial storage. The tomatoes were stored in a fridge at 4°C for 16 days, after which the force was measured again. The apples were stored at room temperature for seven days, after which the texture was analysed by penetrating the probe at an equatorial zone for 15 mm depth at a speed of 2 mm/s to measure the force required. The same test was performed after 16 days of storage in the fridge (4°C). The potatoes were also stored at room temperature for seven days, after which the texture was analysed by penetrating the probe at an equatorial zone for 15 mm depth at a speed of 2 mm/s to measure the force.

Four coated tomatoes and two controls (uncoated) were penetrated by a 1 mm probe for 6 mm depth at a speed of 2 mm/s to measure the force required to penetrate.

Four coated apples and two uncoated control samples were penetrated by a 1 mm probe for 15 mm depth at a speed of 2 mm/s to measure the force required to penetrate the apples (method adopted from García-Nava et al., 2015.)

Four coated potatoes and two uncoated controls were penetrated by a 1 mm probe for 15 mm depth at a speed of 2 mm/s to measure the force required to penetrate the

potatoes. The same test was performed after 16 days of storage in the fridge (4°C) for all products.

After seven days at room temperature, the products were held in the refrigerator to minimize the deterioration and moisture loss after punching a few holes in the product (during the previous texture analysis puncture).

The maximum force needed to penetrate the sample over the target distance is measured as “hardness (g).” The higher the value obtained, the harder (firmer) the sample. The “total work” measures the energy required to overcome the strength of the internal bonds within the sample. A higher value indicated that more energy was required to fracture the sample (Mpemba, 2020)

#### 5.2.2.6 Oil absorption

Oil absorption for mucilage-coated potato chips was tested by frying coated potato chips in sunflower oil at a temperature of 180°C and then comparing the weight of chips before and after frying.

Potatoes were washed and peeled and then cut into chips. It was blanched in boiling water for 3 minutes to stop the enzyme activity. The chips were allowed to cool and then separated into ten bowls. Two bowls served as controls, and the remaining eight were coated with different treatments (two bowls of chips per treatment). The coatings were allowed to dry before frying. The initial weight of each batch was noted down. Each batch was deep-fried for 3 minutes, cooled, and weighed for the final weight measurement. The weight loss was calculated using the formula below:

$$\text{Weight loss (\%)} = \frac{\text{initial weight(g)} - \text{final weight(g)}}{\text{Initial weight (g)}} \times 100$$

#### 5.2.3 Spherification

An attempt was made to see if mucilage could be used to make beads or spheres by replacing the generally (original) used gelling agent, sodium alginate, in spherification, a

culinary technique that employs sodium alginate and either calcium chloride or calcium gluconate lactate.

#### 5.2.3.1 Basic or direct spherification

The basic spherification process is used for flavoured liquids containing no calcium. The liquid is combined directly with a gelling solution such as sodium alginate at a concentration of 0.5%. It was then dipped for 90 seconds submerged in a room temperature solution (calcium bath) of calcium chloride at concentration of 1% or any other soluble calcium salts e.g. gluconate or lactate. Each drop of alginate liquids forms into a small sphere, with the calcium solution causing an outer layer to form a thin, flexible skin. Calcium ions react rapidly with alginate, producing salt bridges and gels. For this reason, the spheres made from direct spherification cannot be kept for long because the gel-forming process does not stop when the liquid is removed from the calcium bath (Lee and Rogers, 2012 ; Gaikwad et al., 2019).

#### 5.2.3.2 Reverse and frozen-reverse spherification

It is a method for molecular gastronomy used to enclose a liquid containing alcohol or calcium, for example, milk, yogurt, and cream. For this study, in the reverse spherification process, the substance containing calcium (yogurt) was dripped into an alginate bath. Cross-linking of calcium ions and alginate polymer strands cause it to form a spherical shape encapsulated in a gel-like membrane. The gelling agent is in the bath. Therefore, gelation stops once the spheres are removed from the tub (D'Angelo *et al.*, 2016). This was used as a reference to compare with the results when the gelling agent is replaced with mucilage.

A more recent method is the frozen reverse method, wherein the spheres containing calcium lactate gluconate are pre-frozen and then submerged in a sodium alginate bath. Freezing allows greater precision in the formation or shaping and overcomes many limitations. Like in reverse spherification, the frozen-shaped liquids are immersed in a sodium alginate bath and rinsed (D'Angelo *et al.*, 2016).

#### 5.2.3.3. Basic spherification (mucilage replaces sodium alginate in the product)

Firstly, a calcium bath was prepared by mixing 5 g of calcium lactate with 1000 g of water, which was allowed to rest to collapse the bubbles formed. It was left covered to remove

bubbles. Using a liquid (Rooibos tea) containing 5 g of sodium alginate, drops were made by adding drops of tea with a syringe into the sodium-calcium bath for 1 minute. The spheres were removed from the bath and rinsed with clean water as a control (Figure 5.1A).

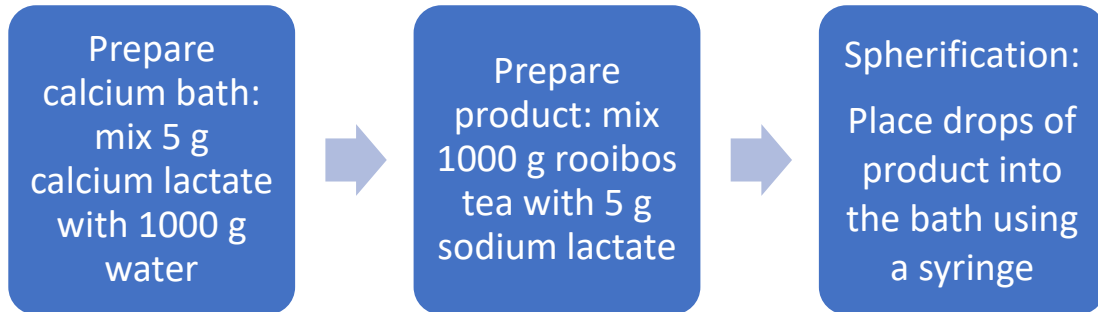


Figure 5.1A Direct spherification with sodium lactate as a gelling agent for reference.

Secondly, for this project, the 5 g sodium alginate was replaced with 5 g mucilage powder (5 g increments increased the concentration until it reached 20 g). The process started by preparing a calcium bath by mixing 5 g calcium lactate with 1000 g of water, which was allowed to rest to collapse the bubbles formed. Five grams of mucilage was mixed with tea (Rooibos tea brewed in 1000 g of water and allowed to cool). A spoonful of mucilage mixture was added to the calcium bath. The formed spheres were removed and rinsed (Figure 5.1B).

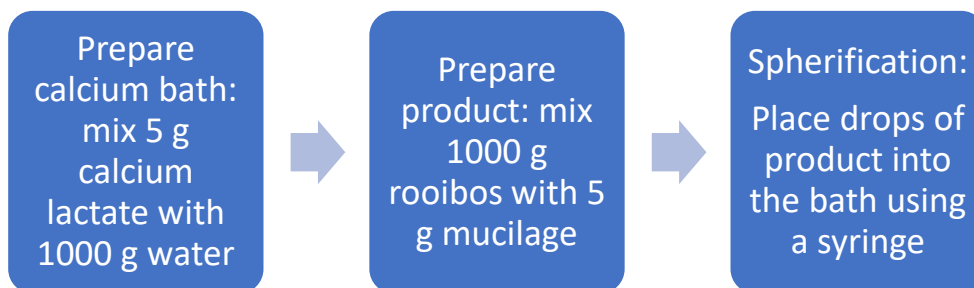


Figure 5.1B Direct spherification with mucilage as a gelling agent.

#### 5.2.3.4. Reverse and frozen-reverse spherification (mucilage replaces sodium alginate in the bath)

A sodium alginate bath was prepared by mixing 1000 g of distilled water with 5 g of sodium alginate and then blended. It was left covered to remove bubbles. A liquid containing calcium (in this project, yogurt was used) was mixed with 5 g of calcium lactate. Drops were made by adding a spoonful of the yogurt into the sodium alginate bath for 1 minute. The spheres were removed from the bath, and washed out with clean water as a control (Figure 5.1C).

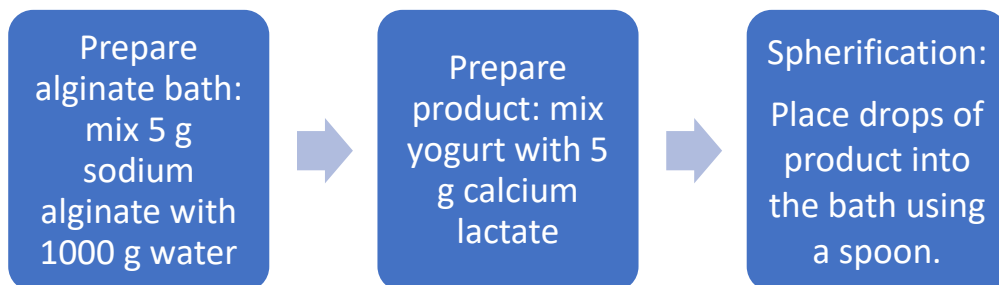


Figure 5.1C Reverse spherification with sodium lactate as a bath.

For this project, sodium alginate was replaced by mucilage to make a mucilage bath by mixing 5 g mucilage (5 g increments increased the concentration until it reached 20 g) with 1000 g of water. Yogurt was mixed with 5 g calcium lactate; drops were made by adding spoonful of the yogurt into the mucilage bath for 1 minute (Figure 5.1D).

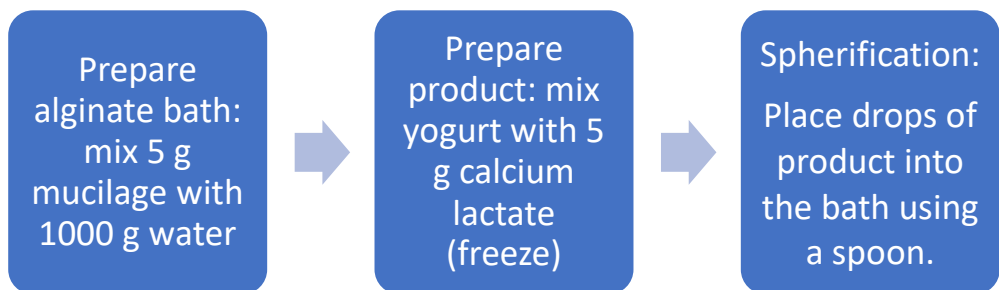


Figure 5.1D Reverse spherification with mucilage as a bath.

In the frozen-reverse spherification, the prepared liquid with enough calcium (yogurt from 5.2.3.4) (to cause a reaction) was poured into desired ice cube trays and then frozen. Once frozen, the cubes were submerged in the sodium alginate or mucilage bath. The formed spheres were removed and rinsed.

#### 5.2.3.5 Caviar-like beads

Materials used included extra virgin olive, Santa Bianca brand, seaweed gelatin and/or mucilage, and balsamic vinegar. It was made using basic cold oil spherification.

An oil bath was prepared by placing the olive oil in a deep bowl or a large glass bowl (depth allowed the droplets to cool and gel before reaching the bottom). The oil was refrigerated for at least 2 to 5 minutes. The balsamic vinegar agar/mucilage/gelatin was then prepared by adding balsamic vinegar and bringing it to a boil in a saucepan, then removed from heat and skimmed to eliminate impurities. Agar was added to hot balsamic vinegar to allow it to dissolve. The blend was allowed to cool to a temperature of 50 °C – 55 °C then mucilage and gelatin were added. A syringe was filled with a mixture of balsamic vinegar agar/mucilage/gelatin, and drops were expelled into the cold oil. The beads were then removed using a slotted spoon and rinsed with water.

#### 5.2.3.6 Statistical analysis

Analysis of variance (ANOVA) was used to determine if differences existed between control products and products treated with edible coatings of different mucilage preparations (NCSS 11 Statistical Software, 2016). The Tukey-Kramer multiple comparison test ( $\alpha = 0.05$ ) was applied to determine the direction of the differences between treatment means (NCSS 11 Statistical Software, 2016).

### 5.3. Results and Discussion

The efficiency of mucilage as an edible coating to extend shelf-life included measuring moisture loss and subsequent weight loss, texture loss and colour loss.

#### 5.3.1.1 Tomatoes

The progression of weight loss in tomatoes is indicated in Table 5.2

Table 5.2: The progression of weight loss for coated tomatoes after one, three, seven, fourteen, and twenty-three days (freeze-dried mucilage used as an edible coating)

Day	Cultivar	Weight (average weight change in grams of each product during storage)	P-value
<b>0</b>	Control	100.88 ± 9.06	p = 0.932
	<i>Robusta</i>	98.43 ± 1.69	
	<i>Nepgen</i>	98.68 ± 3.28	
	<i>Ficus-indice</i>	99.57 ± 7.54	
	<i>Algerian</i>	97.12 ± 6.12	
<b>1</b>	Control	98.99 ± 8.56	p = 0.921
	<i>Robusta</i>	96.88 ± 1.41	
	<i>Nepgen</i>	97.39 ± 3.47	
	<i>Ficus-indice</i>	98.57 ± 7.54	
	<i>Algerian</i>	96.07 ± 6.15	
<b>3</b>	Control	96.34 ± 7.86	p = 0.848
	<i>Robusta</i>	94.08 ± 1.06	
	<i>Nepgen</i>	95.02 ± 3.55	
	<i>Ficus-indice</i>	96.77 ± 7.43	
	<i>Algerian</i>	94.09 ± 6.15	
<b>7</b>	Control	93.58 ± 7.57	p = 0.754
	<i>Robusta</i>	91.20 ± 0.43	
	<i>Nepgen</i>	92.40 ± 3.79	
	<i>Ficus-indice</i>	94.66 ± 7.34	
	<i>Algerian</i>	91.66 ± 6.30	
<b>14</b>	Control	91.60 ± 7.06	p = 0.823
	<i>Robusta</i>	90.22 ± 0.53	
	<i>Nepgen</i>	91.90 ± 3.60	
	<i>Ficus-indice</i>	93.91 ± 7.56	
	<i>Algerian</i>	90.64 ± 6.57	
<b>23</b>	Control	90.78 ± 6.32	p = 0.439
	<i>Robusta</i>	85.33 ± 0.25	
	<i>Nepgen</i>	88.67 ± 4.45	
	<i>Ficus-indice</i>	91.68 ± 7.68	
	<i>Algerian</i>	88.27 ± 6.76	

Means with different superscripts in the same column differ significantly.

In general, samples from the control groups and those treated with mucilage (coated) products showed weight loss throughout the storage period (Table 5.2). This was expected because of water loss (transpiration) after harvest. There was no significant difference in the weight loss of both treated and control samples. The difference in weight loss of both groups from day 1 to 7 was the same; approximately 4 g for all groups and cultivars and approximately 5 g for *Algerian*. This indicated the ineffectiveness of moisture retention of the edible coating.

The p-value of both groups, treated and uncoated, showed no significant differences between the two groups. The treated group lost weight in the same manner as the uncoated group. There was no significant difference between treatments from day 1 to day 23. The p values note this on different days ( $p=0.932$ ,  $p= 0.921$ ,  $p= 0.848$ ,  $p= 0.754$ ,  $p= 0.823$ , and  $p= 0.439$ ).

Mucilage treatment was ineffective as a coating on tomatoes because there was no significant difference between the treated (coated) and untreated groups. The reason for ineffectiveness would require further investigation.

An interesting observation was made for *Ficus-indice*. All the weight loss values at the different intervals were higher (that is, closer to the control sample values) than the other cultivars. At the end of the storage, the treated groups had weights lower than the control groups. This result is not what was expected since we expected the mucilage to hold back water as a semi-permeable membrane or barrier against moisture loss, solute migration, and gases such as carbon dioxide and oxygen (Saha *et al.*, 2014).

This result can be ascribed to the difference in mucilage composition between the cultivars. The differences between mucilage from different cultivars in terms of its composition were reported by Miya *et al.* (2022). Not only did the protein contents and its compositions differ but also the sugar content and its compositions. This phenomenon may explain the different behaviour or effectiveness of *Ficus-indice* mucilage coating. Generally, tomatoes' shelf-life is short because of rapid water loss from transpiration and loss of carbon reserves resulting from respiration which results in weight loss (Duguma, 2021). Razali *et al.* (2021) also observed that mucilage could not improve tomato's shelf-

life on its own during a prolonged shelf-life but it can be used in combination with irradiation using UV- light to improve shelf life.

### 5.3.1.2 Potatoes

The progression in weight loss of potatoes is shown in Table 5.3.

Table 5.3: The progression of weight loss for whole potatoes after one, three, seven, fourteen and twenty-three days (freeze-dried mucilage used as an edible coating)

Days	Cultivars	Weight (Average weight change in grams of each product during storage)	P-value
0	<b>Control</b>	281.30 <sup>bc</sup> ± 0.02	p < 0.001
	<b>Robusta</b>	257.32 <sup>a</sup> ± 15.62	
	<b>Nepgen</b>	270.98 <sup>ab</sup> ± 0.08	
	<b>Ficus- indice</b>	312.14 <sup>d</sup> ± 0.08	
	<b>Algerian</b>	289.19 <sup>c</sup> ± 0.32	
1	<b>Control</b>	279.86 <sup>bc</sup> ± 0.24	p < 0.001
	<b>Robusta</b>	255.56 <sup>a</sup> ± 15.57	
	<b>Nepgen</b>	268.33 <sup>ab</sup> ± 0.40	
	<b>Ficus- indice</b>	311.05 <sup>d</sup> ± 0.13	
	<b>Algerian</b>	287.75 <sup>c</sup> ± 0.39	
3	<b>Control</b>	279.04 <sup>bc</sup> ± 0.27	p < 0.001
	<b>Robusta</b>	252.54 <sup>a</sup> ± 14.77	
	<b>Nepgen</b>	265.11 <sup>ab</sup> ± 0.30	
	<b>Ficus- indice</b>	309.65 <sup>d</sup> ± 0.94	
	<b>Algerian</b>	284.60 <sup>c</sup> ± 0.84	
7	<b>Control</b>	276.61 <sup>bc</sup> ± 0.32	p < 0.001
	<b>Robusta</b>	249.86 <sup>a±</sup> 14.52	
	<b>Nepgen</b>	262.46 <sup>ab±</sup> 0.36	
	<b>Ficus- indice</b>	306.88 <sup>d</sup> ± 0.65	
	<b>Algerian</b>	283.20 <sup>c</sup> ± 0.01	
14	<b>Control</b>	275.30 <sup>b</sup> ± 0.30	p < 0.001
	<b>Robusta</b>	245.83 <sup>a</sup> ± 14.11	
	<b>Nepgen</b>	258.09 <sup>a</sup> ± 0.40	

	<b><i>Ficus- indice Algerian</i></b>	304.53 <sup>c</sup> ± 1.32 281.68 <sup>b</sup> ± 0.05	
<b>23</b>	<b><i>control Robusta Nepgen Ficus- indice Algerian</i></b>	274.56 <sup>b</sup> ± 0.36 244.31 <sup>a</sup> ± 13.74 279.61 <sup>b</sup> ± 27.04 302.65 <sup>b</sup> ± 1.06 280.02 <sup>b</sup> ± 0.01	p < 0.001

Means with different superscripts in the same column differ significantly.

Generally, both control (untreated) and coated (treated) groups lost moisture and, therefore, weight during the storage period. For potatoes coated with mucilage, results showed in Table 5.3, all three treatments, namely RMC, NMC, and AMC, had the least weight loss (lower than the control and FIMC), and the weight loss was not significant during the first 14 days as compared to the control group.

There were no significant changes observed in all treatment groups during the first 14 days. Weight loss values were, in general, higher for the *Algerian* and *Ficus-indice* treatments.

From day 14, results started showing different results between the four treatments. All four treatment products continued to lose weight, but the RMC continued to have the lowest weight loss, and the weight loss was not significant (noted by the means with the same superscripts), 245.83 g on day 14 and 244,31 g on day 23. The products coated with NMC, FIMC, and AMC lost significantly more weight until day 23. Again, the highest weight loss was observed for FIMC. The mucilage treatments were more effective in slowing down moisture loss than and weight loss in tomatoes. RMC was the most effective treatment (Figure 5.2A – 5.2D).

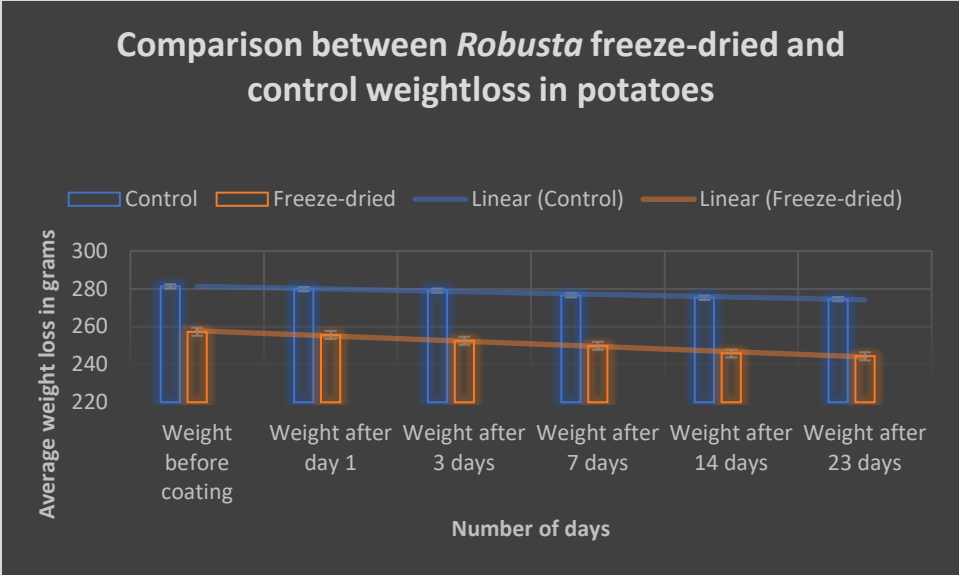


Figure 5.2A Comparison of weight loss between RMC potatoes and control. There were significant differences between FD mucilage EC and control ( $p < 0.001$ ) for all treatments at days 0, 1, 3, 7, 14, and 23 (Figure 5.2A – 5.2D).

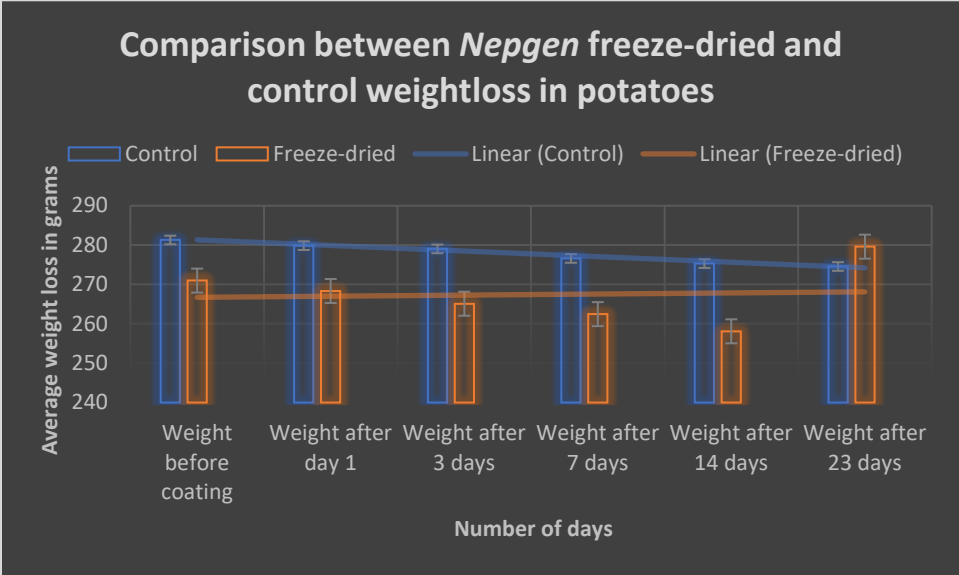


Figure 5.2B Comparison of weight loss between NMC potatoes and control. Differences existed between freeze-dried NMC mucilage and control ( $p < 0.001$ ) (Figure 5.2B). During the first three days, NMC-coated potatoes lost less weight compared to the remaining storage time. Between days 7 to 14, weight loss was significant ( $p < 0.001$ ) in coated groups, and the weight loss was more than that of the control group.

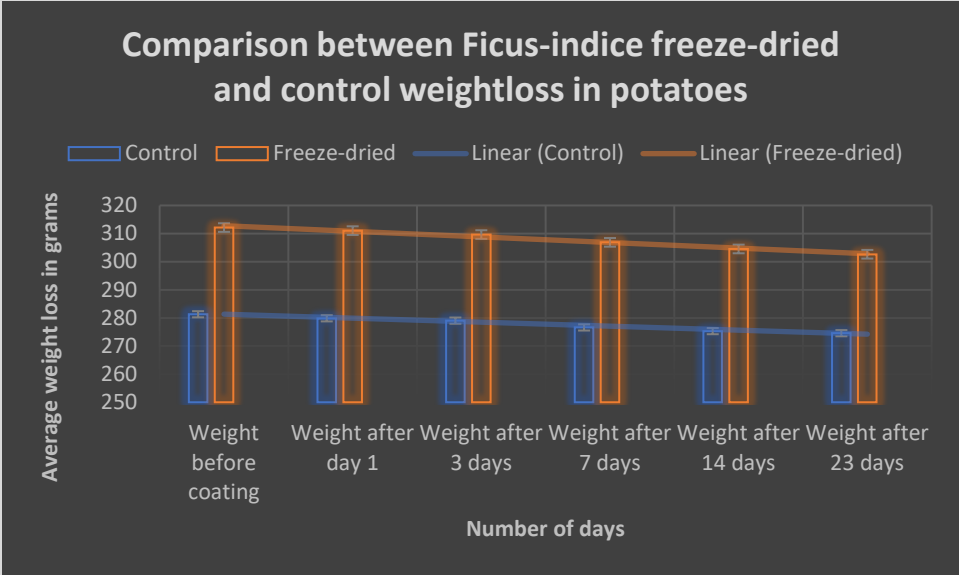


Figure 5.2C Comparison of weight loss between FIMC potatoes and control. The same trend that was observed for RMC (Figure 5.2A) was observed for FIMC (Figure 5.2C). The potatoes continued to lose weight over the storage period, although, during the first three days, FIMC was able to hold back moisture loss; therefore, weight loss was more similar in contrast to the control. At the end of the storage period from day 14 to 23, coated groups lost more weight than the control group.

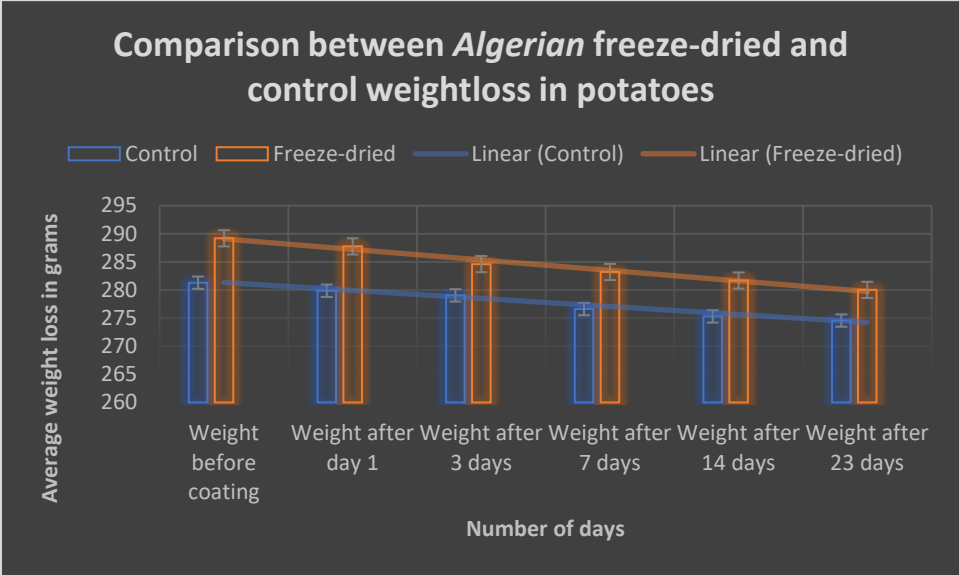


Figure 5.2D Comparison of weight loss between AMC potatoes and control

There was no significant difference between the AMC-coated potatoes and the control group (Figure 5.2). The weight loss percentage during storage for both groups was very similar. The weight loss might be due to respiration and transpiration that continues after harvest (Saha *et al.*, 2014; Khalid *et al.*, 2022).

In general, potatoes lost less weight as compared to the weight loss of tomatoes. This may be ascribed to the water content of the two products. Potatoes are made up of 77-80% water and 1.8g fibre, (Decker and Ferruzzi, 2013) while on the other hand tomatoes are 90-95% water and 1.2 g fibre (Hou *et al.*, 2020)

#### 5.3.1.3 Apples

The progression in weight loss of apples is shown in Table 5.4

Table 5.4: The progression of weight loss for coated apples after one, three, seven, fourteen, and twenty-three days (freeze-dried mucilage used as an edible coating)

Days	Cultivars	Weight  (Average weight change in grams of each product during storage)	P-value
<b>0</b>	Control	245.04 <sup>e</sup> ± 0.01	p < 0.001
	<i>Robusta</i>	232.50 <sup>d</sup> ± 0.01	
	<i>Nepgen</i>	192.94 <sup>a</sup> ± 0.08	
	<i>Ficus-indice</i>	194.75 <sup>b</sup> ± 0.32	
	<i>Algerian</i>	223.12 <sup>c</sup> ± 0.54	
<b>1</b>	Control	243.93 <sup>e</sup> ± 0.04	p < 0.001
	<i>Robusta</i>	230.33 <sup>d</sup> ± 0.01	
	<i>Nepgen</i>	191.43 <sup>a</sup> ± 0.01	
	<i>Ficus-indice</i>	194.19 <sup>b</sup> ± 0.05	
	<i>Algerian</i>	221.91 <sup>c</sup> ± 0.15	
<b>3</b>	Control	241.32 <sup>e</sup> ± 0.13	p < 0.001
	<i>Robusta</i>	229.03 <sup>d</sup> ± 0.01	
	<i>Nepgen</i>	190.16 <sup>a</sup> ± 0.01	
	<i>Ficus-indice</i>	193.31 <sup>b</sup> ± 0.01	
	<i>Algerian</i>	220.40 <sup>c</sup> ± 0.42	
<b>7</b>	Control	239.92 <sup>e</sup> ± 0.47	p < 0.001
	<i>Robusta</i>	225.27 <sup>d</sup> ± 0.22	
	<i>Nepgen</i>	188.13 <sup>a</sup> ± 0.01	
	<i>Ficus-indice</i>	191.89 <sup>b</sup> ± 0.10	
	<i>Algerian</i>	217.02 <sup>c</sup> ± 0.08	
<b>14</b>	Control	237.33 <sup>e</sup> ± 0.01	p < 0.001
	<i>Robusta</i>	223.99 <sup>d</sup> ± 0.03	
	<i>Nepgen</i>	187.67 <sup>a</sup> ± 0.02	
	<i>Ficus-indice</i>	190.34 <sup>b</sup> ± 0.01	
	<i>Algerian</i>	215.00 <sup>c</sup> ± 0.24	
<b>23</b>	Control	236.46 <sup>e</sup> ± 0.09	p < 0.001
	<i>Robusta</i>	220.82 <sup>d</sup> ± 0.09	
	<i>Nepgen</i>	185.60 <sup>a</sup> ± 0.01	
	<i>Ficus-indice</i>	189.96 <sup>b</sup> ± 0.09	
	<i>Algerian</i>	213.80 <sup>c</sup> ± 0.60	

Means with different superscripts in the same column differ significantly.

There was a similar trend in weight loss observed for apples and potatoes. The weight decreased throughout the storage period, but the decrease was not significant, and this was noted by means of different days (with the same superscripts). All products gradually lost weight during the storage time, including the control.

There was a significant difference ( $p < 0.001$ ) between the control and treated groups. In the case of the apples, it seems as if the RMC and AMC-treated apples lost more weight (water), and it appears that the NMC and FIMC treatments were more effective in preventing weight loss. This was a different pattern than what was observed for tomatoes and potatoes.

The expectation was that the coated samples would have less weight loss or maintain the weight due to the hydrophilic properties of mucilage that retard moisture loss (Allegra *et al.*, 2016). This characteristic was noted in NMC (Figure 5.3B) and FIMC (Figure 5.3C) coated groups with a minimum weight loss during the 14 days. RMC (Figure 5.3A) and AMC (Figure 5.3D) results didn't differ significantly from those of the control group.

During day 23 (9 days storage in the refrigerator), there was an increase in weight (weight gain) observed in apples. This is thought to be due to moisture re-absorption by mucilage, but this requires further investigation, as the control samples also gained weight. Another reason for this weight gain might be due to starch retrogradation since potatoes are high in starch compared to apples and tomatoes, and these two products did not gain weight when refrigerated.

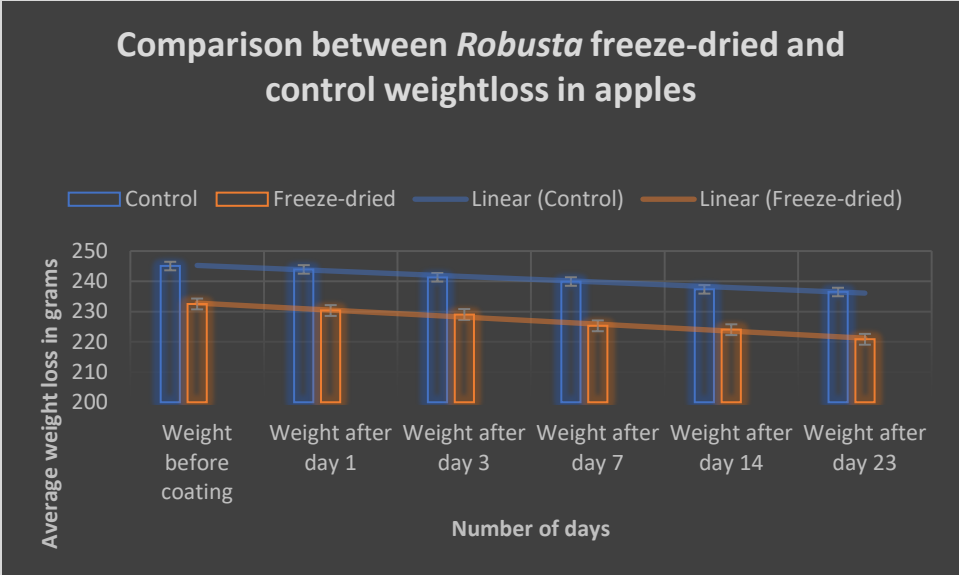


Figure 5.3A Comparison of weight loss between RMC apple and control *Robusta*-coated products were able to maintain their weight with a weight loss percentage below 1% over the first three days compared to the control group, which was above 1% (Figure 5.3A). Both groups continued losing weight over the storage period, but apples coated with FD had a lower weight loss percentage (0.57%) compared to the control (1.08%).

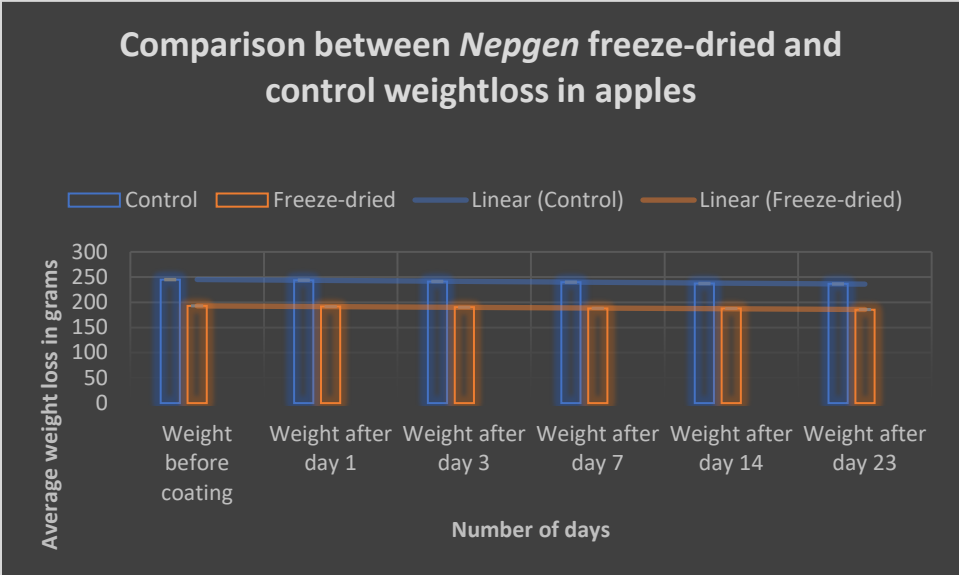


Figure 5.3B Comparison of weight loss between NMC apple and control

There was no significant difference between the NMC freeze-dried mucilage and the control group (Figure 5.3B). Both groups lost weight during the storage period.

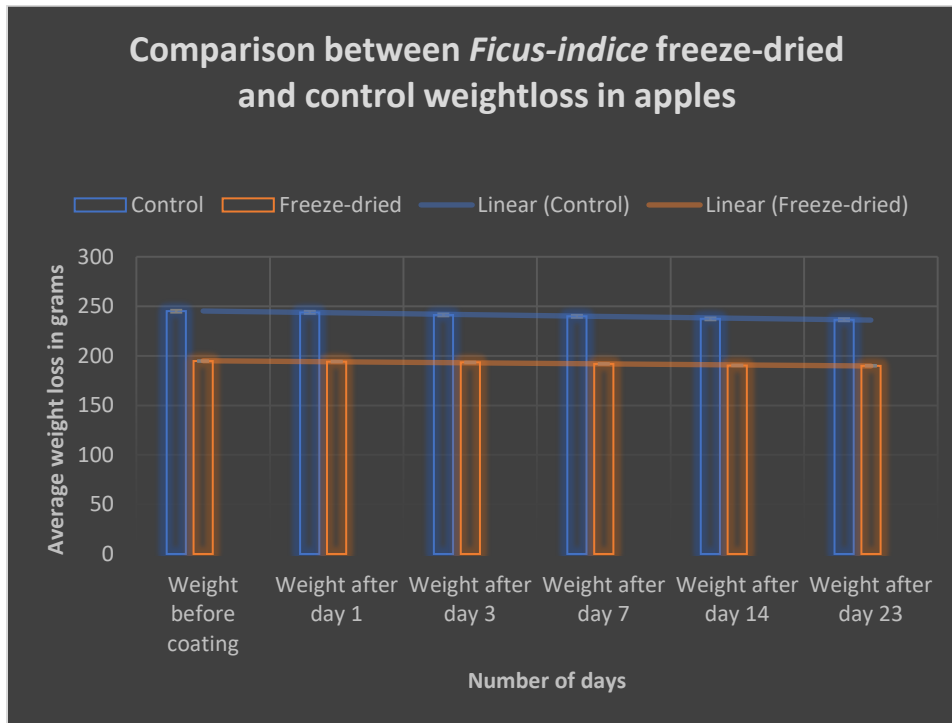


Figure 5.3C Comparison of weight loss between FIMC apple and control.

The *Ficus-indice* coated apples lost weight during the storage period (Figure 5.3C). The weight loss for both groups during the first three days was not significant ( $p < 0.001$ ). The coated group had lost about 0.45% of weight during these days as compared to the 1.08% of the control group. The weight continued to decrease for both groups throughout the storage.

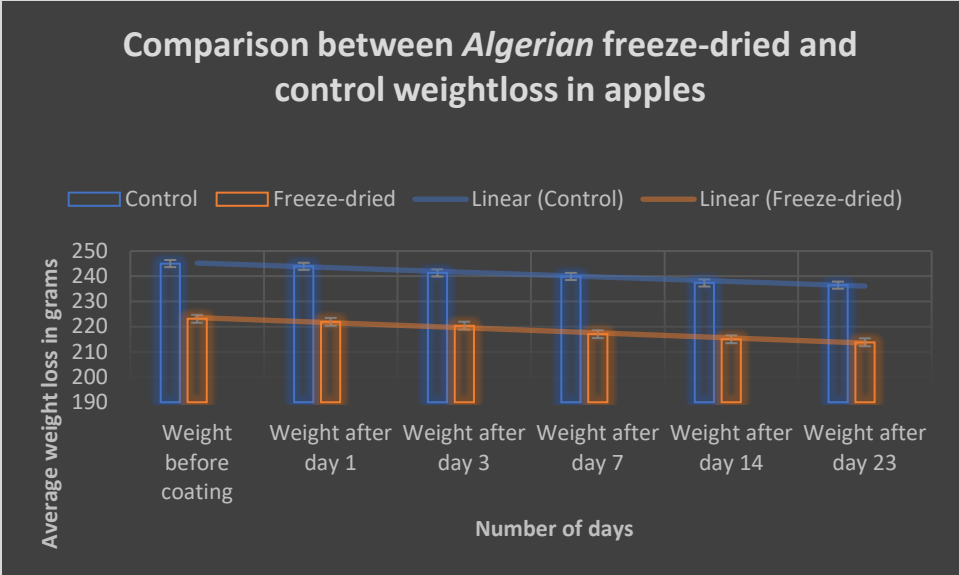


Figure 5.3D Comparison of weight loss between AMC apple and control

During the first three days, the *Algerian* coated group (AMC) had a weight loss percentage below 1% and below that of the control group (Figure 5.3D). On the last day of storage, the coated group (FD 0.56%) had a weight loss percentage above that of the control (0.36%), and this was proof that mucilage did not prevent moisture loss.

### 5.3.2 Colour analysis

#### 5.3.2.1 Tomatoes

Table 5.5: Results in colour changes of tomatoes coated with freeze-dried mucilage during the storage period.

Day	Cultivar treatments	L*value	a* value	b* value	Chroma* (Saturation Index)	Hue° Angle
<b>Before coating</b>	Control	53.10 <sup>fg</sup> ± 0.08	30.27 <sup>abc</sup> ± 3.49	31.65 <sup>efg</sup> ± 0.08	43.85 <sup>bcde</sup> ± 2.41	46.42 <sup>cd</sup> ± 3.31
	<i>Robusta</i>	53.03 <sup>fg</sup> ± 0.95	29.88 <sup>abc</sup> ± 0.06	31.22 <sup>defg</sup> ± 1.02	43.21 <sup>bcde</sup> ± 0.69	46.24 <sup>bcd</sup> ± 1.00
	<i>Ficus-indice</i>	51.86 <sup>defg</sup> ± 1.21	30.00 <sup>abc</sup> ± 0.09	30.22 <sup>cdefg</sup> ± 1.86	42.60 <sup>abcde</sup> ± 1.26	45.16 <sup>abcd</sup> ± 1.86
	<i>Nepgen</i>	51.76 <sup>defg</sup> ± 0.35	27.08 <sup>a</sup> ± 1.09	29.94 <sup>cdef</sup> ± 0.42	40.37 <sup>ab</sup> ± 1.04	47.89 <sup>d</sup> ± 0.75
	<i>Algerian</i>	50.18 <sup>abcdef</sup> ± 1.59	28.92 <sup>ab</sup> ± 0.08	27.60 <sup>abcd</sup> ± 2.23	40.00 <sup>ab</sup> ± 1.54	43.59 <sup>abcd</sup> ± 2.32
<b>Day1</b>	Control	51.33 <sup>cdefg</sup> ± 0.58	31.25 <sup>abc</sup> ± 1.96	30.10 <sup>cdefg</sup> ± 0.09	43.40 <sup>bcde</sup> ± 1.34	43.97 <sup>abcd</sup> ± 1.88
	<i>Robusta</i>	50.02 <sup>abcdef</sup> ± 0.99	32.33 <sup>bc</sup> ± 0.39	30.43 <sup>cdefg</sup> ± 0.80	44.40 <sup>bcde</sup> ± 0.84	43.26 <sup>abcd</sup> ± 0.41
	<i>Ficus-indice</i>	49.87 <sup>abcde</sup> ± 0.19	30.67 <sup>abc</sup> ± 0.86	28.79 <sup>abcdef</sup> ± 1.21	42.08 <sup>abcde</sup> ± 0.20	43.19 <sup>abcd</sup> ± 2.01
	<i>Nepgen</i>	49.06 <sup>abcde</sup> ± 0.58	33.22 <sup>bc</sup> ± 3.75	31.41 <sup>defg</sup> ± 2.44	45.72 <sup>de</sup> ± 4.40	43.47 <sup>abcd</sup> ± 1.01
	<i>Algerian</i>	48.12 <sup>ab</sup> ± 1.48	31.16 <sup>abc</sup> ± 2.77	28.58 <sup>abcdef</sup> ± 0.55	42.30 <sup>abcde</sup> ± 2.42	42.61 <sup>abcd</sup> ± 1.99
<b>Day3</b>	Control	50.93 <sup>bcdefg</sup> ± 0.07	32.59 <sup>bc</sup> ± 1.92	30.32 <sup>cdefg</sup> ± 0.51	44.52 <sup>bcde</sup> ± 1.75	42.97 <sup>abcd</sup> ± 1.21
	<i>Robusta</i>	50.15 <sup>abcdef</sup> ± 0.22	32.76 <sup>bc</sup> ± 0.02	28.10 <sup>abcde</sup> ± 0.78	43.16 <sup>bcde</sup> ± 0.49	40.61 <sup>a</sup> ± 0.81
	<i>Ficus-indice</i>	48.53 <sup>abc</sup> ± 2.29	32.05 <sup>abc</sup> ± 0.45	28.05 <sup>abcde</sup> ± 1.15	42.59 <sup>abcde</sup> ± 1.10	41.18 <sup>abc</sup> ± 0.77
	<i>Nepgen</i>	48.47 <sup>abc</sup> ± 0.68	30.62 <sup>abc</sup> ± 0.99	26.80 <sup>abc</sup> ± 0.16	40.70 <sup>abc</sup> ± 0.64	41.21 <sup>abc</sup> ± 1.09
	<i>Algerian</i>	47.49 <sup>a</sup> ± 0.08	28.92 <sup>ab</sup> ± 1.11	25.05 <sup>a</sup> ± 0.73	38.25 <sup>a</sup> ± 1.32	40.90 <sup>ab</sup> ± 0.26
<b>Day7</b>	Control	53.40 <sup>g</sup> ± 1.78	30.84 <sup>abc</sup> ± 4.87	33.87 <sup>g</sup> ± 2.04	46.01 <sup>de</sup> ± 1.76	47.86 <sup>d</sup> ± 6.22
	<i>Robusta</i>	49.61 <sup>abcde</sup> ± 2.21	29.50 <sup>abc</sup> ± 2.03	28.76 <sup>abcdef</sup> ± 0.53	41.21 <sup>abcd</sup> ± 1.82	44.32 <sup>abcd</sup> ± 1.45
	<i>Ficus-indice</i>	50.09 <sup>abcdef</sup> ± 1.28	33.40 <sup>bc</sup> ± 1.58	30.84 <sup>defg</sup> ± 0.28	45.47 <sup>cde</sup> ± 1.35	42.74 <sup>abcd</sup> ± 1.10
	<i>Nepgen</i>	50.63 <sup>bcdefg</sup> ± 0.94	34.28 <sup>c</sup> ± 1.69	31.78 <sup>efg</sup> ± 0.36	46.75 <sup>e</sup> ± 1.48	42.85 <sup>abcd</sup> ± 1.08
	<i>Algerian</i>	48.74 <sup>abcd</sup> ± 0.12	31.50 <sup>abc</sup> ± 2.49	28.56 <sup>abcdef</sup> ± 3.46	42.52 <sup>abcde</sup> ± 4.17	42.11 <sup>abc</sup> ± 1.21
<b>Day23</b>	Control	52.05 <sup>efg</sup> ± 2.12	31.94 <sup>abc</sup> ± 1.44	32.13 <sup>fg</sup> ± 3.20	45.39 <sup>cde</sup> ± 1.26	45.08 <sup>abcd</sup> ± 4.14
	<i>Robusta</i>	48.53 <sup>abc</sup> ± 1.45	32.86 <sup>bc</sup> ± 1.20	28.03 <sup>abcde</sup> ± 0.57	43.20 <sup>bcde</sup> ± 0.54	40.48 <sup>a</sup> ± 1.60
	<i>Ficus-indice</i>	49.41 <sup>abcde</sup> ± 1.05	32.80 <sup>bc</sup> ± 0.66	29.22 <sup>bcdef</sup> ± 1.00	43.93 <sup>bcde</sup> ± 1.16	41.69 <sup>abc</sup> ± 0.41
	<i>Nepgen</i>	49.89 <sup>abcde</sup> ± 0.33	32.00 <sup>abc</sup> ± 1.20	29.04 <sup>bcdef</sup> ± 0.30	43.21 <sup>bcde</sup> ± 1.09	42.24 <sup>abc</sup> ± 0.77
	<i>Algerian</i>	48.19 <sup>ab</sup> ± 0.84	30.05 <sup>abc</sup> ± 1.48	25.93 <sup>ab</sup> ± 1.29	39.69 <sup>ab</sup> ± 1.97	40.79 <sup>ab</sup> ± 0.01
<b>P-value</b>		p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001

The edible mucilage coating was expected to hinder or slow down the process of tomatoes ripening and, therefore, there should be change in colour. The results obtained showed no great difference between the control and coated tomatoes. The h° of tomatoes

was between 0° and 47°, which shows a red colour range. The redness of the tomatoes will be evaluated by discussing the  $a^*$ -value; positive values indicate red, while negative values indicate green. An increasing value shows an increase in redness, therefore ripening. The tomatoes showed an increase in redness after being coated, which is assumed to be due to the colour of the edible coating (because of the addition of the layer that might intensify the colour). Both coated and uncoated tomatoes had a slight increase in their redness, which is a characteristic of ripening. Although the increase in redness was insignificant, there is no evidence that the edible coating slows down or prevents the quick ripening of tomatoes because the same trend was observed for uncoated tomatoes.

In coated products, the lightness decreased during storage. This might be because mucilage provides a thick barrier against gas exchange and ethylene produced, slowing down the ripening process. Similar results and trends were reported by Ali *et al.* 2010 when they coated tomatoes with Arabic gum.

During the first three days, the control group had an increase in redness of about 4.30%. The control group's redness increased until the last day of storage. The results obtained for FIMC was similar to the control with a 5.87% significant increase (the percentage is the difference in  $a^*$  value between day 1 and 3). There was also an increase in the RMC tomatoes, although it was insignificant compared with the control and FIMC.

The NMC and AMC redness decreased significantly during the first three days. The NMC and AMC redness only increased on day 7; this colour increase slowed down after refrigeration. A reason for this might be that low temperature retards or slow down the ripening process.

The colour saturation of the untreated group increased significantly from day 1 to 7. The same trend was observed for tomatoes treated with FIMC. This observation was expected for the coated products because this would mean the mucilage was able to prevent, to some point, oxidation of lycopene, hence the red colour saturation (Le Maguer and Shi 2000). For the other three treatments, RMC, NMC, and AMC, the colour saturation ( $C^\circ$ ) decreased at room temperature. This shows the decrease in the red colour, and that mucilage did not prevent the oxidation of lycopene. The tomatoes were becoming dull or bluish instead of yellowish or bright red as expected. This can be further explained by

looking at the  $b^*$  value results. The  $b^*$  value increased for FIMC during the first day, which shows the colour was becoming more yellow than blue, whereas, with RMC, NMC, and AMC, the  $b^*$  value was decreasing.

The  $h^\circ$  of all cultivar samples results showed that the colour remained red, although with some changes in lightness and yellowness. The  $h^\circ$  was above 0 not more than 90 (yellow). The exact values from the results were between 40 and 47.

From the results obtained between days 1 and 3, it can be said that mucilage from *Nepgen* and *Algerian* was effective in maintaining the colour. We also noted a significant ( $p < 0.001$ ) difference in how different cultivars (treatments) can slow down the ripening.

#### 5.3.2.2 Potatoes

When it comes to potatoes, colour is also a quality measure. Consumers are more alert to the green colour that develops over the storage time. This colour change is caused by increased chlorophyll pigments when potatoes are exposed to light, but consumers also associate it with toxins. The same process is associated with the production of solanine, a mild natural toxin causing nausea and intestinal upsets, and neurological problems at high concentrations. The concentration of solanine is higher at the eye of a potato (Dalvi and Bowie 1983).

Table 5.6: Results of colour changes in potatoes coated with freeze-dried mucilage during the storage period.

Day	Cultivar treatments	L*value	a* value	b* value	Chroma* (Saturation Index)	Hue° Angle
<b>Before coating</b>	Control	81.07 <sup>cdef</sup> ± 0.18	-1.63 <sup>cdefg</sup> ± 0.23	22.51 <sup>a</sup> ± 0.01	22.56 <sup>a</sup> ± 0.01	-85.78 <sup>hijk</sup> ± 0.67
	<i>Robusta</i>	80.70 <sup>cdef</sup> ± 0.12	-0.22 <sup>j</sup> ± 0.02	25.17 <sup>bc</sup> ± 1.20	25.17 <sup>bc</sup> ± 1.20	-89.51 <sup>a</sup> ± 0.06
	<i>Ficus-indice</i>	82.60 <sup>defg</sup> ± 0.50	-0.27 <sup>j</sup> ± 0.06	26.25 <sup>cde</sup> ± 0.48	26.25 <sup>cde</sup> ± 0.49	-89.42 <sup>ab</sup> ± 0.13
	<i>Nepgen</i>	82.31 <sup>defg</sup> ± 0.02	-0.71 <sup>ij</sup> ± 0.02	25.91 <sup>cd</sup> ± 0.13	25.92 <sup>cd</sup> ± 0.13	-88.44 <sup>abcd</sup> ± 0.03
	<i>Algerian</i>	86.95 <sup>g</sup> ± 0.50	-0.75 <sup>ij</sup> ± 0.11	25.15 <sup>bc</sup> ± 0.57	25.16 <sup>bc</sup> ± 0.56	-88.30 <sup>abcde</sup> ± 0.29
<b>Day1</b>	Control	78.38 <sup>bcd</sup> ± 0.36	-1.19 <sup>fghi</sup> ± 0.11	27.14 <sup>cdefg</sup> ± 0.80	27.17 <sup>cdefg</sup> ± 0.80	-87.50 <sup>cdefg</sup> ± 0.16
	<i>Robusta</i>	81.48 <sup>cdef</sup> ± 4.17	-1.56 <sup>cdefgh</sup> ± 0.29	27.67 <sup>defgh</sup> ± 1.25	27.71 <sup>defgh</sup> ± 1.26	-86.79 <sup>efghij</sup> ± 0.45
	<i>Ficus-indice</i>	82.94 <sup>defg</sup> ± 3.87	-1.06 <sup>ghi</sup> ± 1.02	30.08 <sup>ij</sup> ± 0.87	30.11 <sup>ij</sup> ± 0.84	-87.94 <sup>bcdef</sup> ± 1.99
	<i>Nepgen</i>	84.65 <sup>fg</sup> ± 5.85	-0.76 <sup>ij</sup> ± 0.16	31.68 <sup>jk</sup> ± 0.89	31.69 <sup>jk</sup> ± 0.89	-88.63 <sup>abc</sup> ± 0.32
	<i>Algerian</i>	71.01 <sup>a</sup> ± 0.90	-1.10 <sup>ghi</sup> ± 0.03	29.11 <sup>ghi</sup> ± 0.65	29.13 <sup>ghi</sup> ± 0.65	-87.83 <sup>cdef</sup> ± 0.12
<b>Day3</b>	Control	81.07 <sup>cdef</sup> ± 0.46	-1.90 <sup>cdef</sup> ± 0.07	23.25 <sup>ab</sup> ± 0.14	23.33 <sup>ab</sup> ± 0.14	-85.33 <sup>jk</sup> ± 0.14
	<i>Robusta</i>	78.16 <sup>bcd</sup> ± 2.49	-2.16 <sup>bc</sup> ± 0.17	29.00 <sup>ghi</sup> ± 1.17	29.08 <sup>ghi</sup> ± 1.18	-85.74 <sup>hijk</sup> ± 0.17
	<i>Ficus-indice</i>	79.68 <sup>cdef</sup> ± 3.09	-2.07 <sup>cd</sup> ± 0.06	28.86 <sup>fghi</sup> ± 0.94	28.93 <sup>fghi</sup> ± 0.94	-85.91 <sup>hij</sup> ± 0.01
	<i>Nepgen</i>	80.80 <sup>cdef</sup> ± 1.91	-1.39 <sup>defghi</sup> ± 0.74	30.05 <sup>ij</sup> ± 2.37	30.09 <sup>ij</sup> ± 2.34	-87.26 <sup>cdefgh</sup> ± 1.62
	<i>Algerian</i>	86.95 <sup>g</sup> ± 0.62	-1.92 <sup>cdef</sup> ± 0.07	30.57 <sup>ijk</sup> ± 0.35	30.63 <sup>ijk</sup> ± 0.34	-86.40 <sup>fghij</sup> ± 0.17
<b>Day7</b>	Control	87.40 <sup>g</sup> ± 0.23	-2.02 <sup>cde</sup> ± 0.01	26.64 <sup>cdef</sup> ± 0.13	26.71 <sup>cdef</sup> ± 0.13	-85.67 <sup>ijk</sup> ± 0.03
	<i>Robusta</i>	76.85 <sup>bc</sup> ± 0.52	-2.15 <sup>bc</sup> ± 0.10	29.87 <sup>hij</sup> ± 0.27	29.94 <sup>hij</sup> ± 0.26	-85.88 <sup>hij</sup> ± 0.24
	<i>Ficus-indice</i>	80.30 <sup>cdef</sup> ± 0.62	-1.30 <sup>efghi</sup> ± 0.06	30.51 <sup>ijk</sup> ± 0.54	30.54 <sup>ij</sup> ± 0.54	-87.57 <sup>cdefg</sup> ± 0.08
	<i>Nepgen</i>	80.65 <sup>cdef</sup> ± 0.25	-1.95 <sup>cde</sup> ± 0.03	29.79 <sup>hij</sup> ± 0.18	29.85 <sup>hij</sup> ± 0.19	-86.26 <sup>ghij</sup> ± 0.04
	<i>Algerian</i>	84.65 <sup>fg</sup> ± 0.52	-2.84 <sup>ab</sup> ± 0.18	28.34 <sup>efghi</sup> ± 0.63	28.48 <sup>efghi</sup> ± 0.64	-84.28 <sup>kl</sup> ± 0.24
<b>Day23</b>	Control	70.58 <sup>cdef</sup> ± 0.11	-2.22 <sup>bc</sup> ± 0.05	26.91 <sup>cdefg</sup> ± 0.59	27.00 <sup>cdefg</sup> ± 0.59	-85.28 <sup>jk</sup> ± 0.20
	<i>Robusta</i>	73.96 <sup>ab</sup> ± 0.20	-1.74 <sup>cdefg</sup> ± 0.15	32.81 <sup>k</sup> ± 0.21	32.86 <sup>k</sup> ± 0.20	-86.96 <sup>defghi</sup> ± 0.28
	<i>Ficus-indice</i>	83.19 <sup>defg</sup> ± 0.17	-2.14 <sup>bcd</sup> ± 0.21	29.87 <sup>hij</sup> ± 0.76	29.94 <sup>hij</sup> ± 0.74	-85.89 <sup>hij</sup> ± 0.50
	<i>Nepgen</i>	79.47 <sup>cde</sup> ± 0.85	-0.83 <sup>hij</sup> ± 0.15	32.07 <sup>jk</sup> ± 1.64	32.08 <sup>jk</sup> ± 1.64	-88.50 <sup>abc</sup> ± 0.34
	<i>Algerian</i>	84.56 <sup>efg</sup> ± 0.32	-3.48 <sup>a</sup> ± 0.05	31.64 <sup>jk</sup> ± 0.09	31.83 <sup>jk</sup> ± 0.10	-83.73 <sup>l</sup> ± 0.07
<b>P-value</b>	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.

PNP all-purpose potatoes were used. From the results obtained in Table 5.6, L\*value was evaluated to monitor if the coating had preserved the lightness of the potatoes over the storage period. The application of the coating improved the shiny appearance of the potatoes due to the glycerol added as a plasticizer. The lightness of the product decreased during storage due to the moisture loss and depletion of energy in the cell wall. This might also be due to enzymatic browning due to the oxidation of phenols to orthoquinone polymerization to form melanin, a light-absorbing compound reflecting brown colour (Zeeze, 2020).

The control potatoes and potatoes coated with RMC, NMC, and FIMC lightness decreased significantly from day 1. The products coated with *Algerian* did not lose lightness and remained constant for the first three days. On the last day of the storage period, the coated group had lightness higher than the control group; the coated group had a good shiny appearance, while the control group had some wrinkled appearance. This was expected because edible coatings should be a partial barrier to moisture loss. A similar trend was observed by Saha *et al.* (2014). The lightness saturation (C°) decreased significantly for both uncoated and FD-coated products seen by means with different superscripts during the storage period. The a\* value results for both treated and control groups showed an increase in the greenness of the product, which is expected in harvested potatoes over a storage period.

### 5.3.2.3 Apples

The apples used for this project were Granny Smith apples, which are green in colour and turn yellow and lighter green as they ripen. To evaluate whether the mucilage coating effectively maintains the colour over the storage period, the a\* value will be evaluated, because it illustrates the greenness (-) and redness (+).

Table 5.7: Results of colour changes in apples coated with freeze-dried mucilage during the storage period.

Day	Cultivar	L*value	a* value	b* value	Chroma* (Saturation Index)	Hue° Angle
<b>Before coating</b>	Control	85.03 <sup>de</sup> ± 0.84	-20.22 <sup>c</sup> ± 0.12	59.19 <sup>de</sup> ± 0.02	62.55 <sup>ef</sup> ± 0.06	-71.14 <sup>lm</sup> ± 0.10
	<i>Robusta</i>	91.15 <sup>hij</sup> ± 0.33	-20.25 <sup>c</sup> ± 0.06	59.07 <sup>cd</sup> ± 0.21	62.44 <sup>ef</sup> ± 0.22	-71.08 <sup>lm</sup> ± 0.01
	<i>Ficus-indice</i>	85.58 <sup>e</sup> ± 0.30	-18.39 <sup>g</sup> ± 0.08	59.06 <sup>cd</sup> ± 0.12	61.86 <sup>de</sup> ± 0.09	-72.71 <sup>g</sup> ± 0.10
	<i>Nepgen</i>	84.19 <sup>d</sup> ± 0.24	-21.10 <sup>b</sup> ± 0.14	64.51 <sup>k</sup> ± 0.98	67.87 <sup>k</sup> ± 0.97	-71.89 <sup>ij</sup> ± 0.14
	<i>Algerian</i>	90.35 <sup>ghi</sup> ± 0.68	-21.12 <sup>b</sup> ± 0.01	58.72 <sup>cd</sup> ± 0.70	62.40 <sup>ef</sup> ± 0.65	-70.21 <sup>n</sup> ± 0.23
<b>Day1</b>	Control	92.01 <sup>j</sup> ± 0.02	-19.02 <sup>ef</sup> ± 0.23	60.36 <sup>efg</sup> ± 0.36	63.28 <sup>f</sup> ± 0.41	-72.51 <sup>gh</sup> ± 0.10
	<i>Robusta</i>	89.92 <sup>g</sup> ± 0.36	-19.46 <sup>de</sup> ± 0.37	60.40 <sup>fg</sup> ± 0.18	63.46 <sup>f</sup> ± 0.29	-72.14 <sup>hi</sup> ± 0.27
	<i>Ficus-indice</i>	81.00 <sup>b</sup> ± 0.46	-19.43 <sup>de</sup> ± 0.12	60.47 <sup>gh</sup> ± 0.12	63.51 <sup>f</sup> ± 0.15	-72.19 <sup>hi</sup> ± 0.07
	<i>Nepgen</i>	87.52 <sup>f</sup> ± 0.59	-18.47 <sup>fg</sup> ± 0.10	63.55 <sup>jk</sup> ± 0.55	66.17 <sup>hij</sup> ± 0.56	-73.79 <sup>f</sup> ± 0.05
	<i>Algerian</i>	85.91 <sup>e</sup> ± 0.70	-28.32 <sup>a</sup> ± 0.13	63.69 <sup>jk</sup> ± 0.10	69.70 <sup>l</sup> ± 0.15	-66.03 <sup>p</sup> ± 0.07
<b>Day3</b>	Control	91.43 <sup>ij</sup> ± 0.13	-15.57 <sup>j</sup> ± 0.12	64.64 <sup>k</sup> ± 0.10	66.49 <sup>ij</sup> ± 0.13	-76.46 <sup>c</sup> ± 0.08
	<i>Robusta</i>	90.18 <sup>gh</sup> ± 0.20	-17.76 <sup>h</sup> ± 0.13	63.89 <sup>jk</sup> ± 0.72	66.31 <sup>ij</sup> ± 0.66	-74.46 <sup>e</sup> ± 0.27
	<i>Ficus-indice</i>	79.58 <sup>a</sup> ± 0.88	-19.00 <sup>ef</sup> ± 0.25	57.90 <sup>c</sup> ± 0.49	60.93 <sup>cd</sup> ± 0.54	-71.84 <sup>ij</sup> ± 0.08
	<i>Nepgen</i>	79.91 <sup>ab</sup> ± 0.12	-28.64 <sup>a</sup> ± 0.24	60.80 <sup>gh</sup> ± 0.50	67.20 <sup>jk</sup> ± 0.35	-64.78 <sup>q</sup> ± 0.37
	<i>Algerian</i>	78.73 <sup>a</sup> ± 0.73	-20.59 <sup>bc</sup> ± 0.18	55.67 <sup>b</sup> ± 0.25	59.35 <sup>b</sup> ± 0.18	-69.71 <sup>o</sup> ± 0.25
<b>Day7</b>	Control	94.65 <sup>k</sup> ± 0.15	-19.61 <sup>d</sup> ± 0.17	56.00 <sup>b</sup> ± 0.65	59.33 <sup>b</sup> ± 0.67	-70.70 <sup>mn</sup> ± 0.05
	<i>Robusta</i>	89.79 <sup>g</sup> ± 0.29	-20.26 <sup>c</sup> ± 0.27	61.63 <sup>hi</sup> ± 0.75	64.87 <sup>g</sup> ± 0.80	-71.80 <sup>ijk</sup> ± 0.02
	<i>Ficus-indice</i>	84.84 <sup>de</sup> ± 0.21	-18.96 <sup>ef</sup> ± 0.08	56.14 <sup>b</sup> ± 0.54	59.26 <sup>b</sup> ± 0.54	-71.34 <sup>kl</sup> ± 0.09
	<i>Nepgen</i>	85.34 <sup>de</sup> ± 0.35	-19.53 <sup>de</sup> ± 0.22	62.91 <sup>j</sup> ± 0.12	65.87 <sup>ghi</sup> ± 0.05	-72.75 <sup>g</sup> ± 0.21
	<i>Algerian</i>	89.91 <sup>g</sup> ± 0.12	-18.56 <sup>fg</sup> ± 0.26	62.73 <sup>ij</sup> ± 0.53	65.41 <sup>ghi</sup> ± 0.58	-73.52 <sup>f</sup> ± 0.09
<b>Day23</b>	Control	97.89 <sup>l</sup> ± 0.23	-12.35 <sup>l</sup> ± 0.37	58.88 <sup>cd</sup> ± 0.01	60.16 <sup>bc</sup> ± 0.08	-78.15 <sup>b</sup> ± 0.34
	<i>Robusta</i>	88.35 <sup>f</sup> ± 0.29	-11.26 <sup>m</sup> ± 0.31	59.21 <sup>def</sup> ± 0.24	60.27 <sup>bc</sup> ± 0.30	-79.24 <sup>a</sup> ± 0.24
	<i>Ficus-indice</i>	80.86 <sup>b</sup> ± 0.44	-13.69 <sup>k</sup> ± 0.33	50.16 <sup>a</sup> ± 0.21	51.99 <sup>a</sup> ± 0.29	-74.73 <sup>de</sup> ± 0.29
	<i>Nepgen</i>	82.93 <sup>c</sup> ± 0.33	-16.73 <sup>i</sup> ± 0.35	62.89 <sup>j</sup> ± 0.49	65.07 <sup>gh</sup> ± 0.56	-75.11 <sup>d</sup> ± 0.19
	<i>Algerian</i>	80.82 <sup>b</sup> ± 0.61	-20.59 <sup>bc</sup> ± 0.01	61.56 <sup>ghi</sup> ± 0.12	64.91 <sup>g</sup> ± 0.12	-71.51 <sup>kl</sup> ± 0.03
<b>Sign. level</b>	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

After coating with freeze-dried mucilage (Table 5.7), the greenness decreased insignificantly for all cultivars, and it was due to the mucilage's colour. The freeze-dried mucilage colour is a lighter green. The control group's greenness decreased significantly over the storing period from day 1 (-19.02) to day 3 (-15.57). The coated apples also

showed a decrease in greenness, although the decrease was insignificant from day 1 to 3, except for the RMC products, with a significant decrease during this period. This might be due to the colour of Robusta mucilage which is blue-green, compared to the other cultivars (Robusta is from a different species, namely *O. robusta*).

At the end of the storage time, there was a notable significant difference between the treatments,  $p < 0.01$ . At the end of the storage period. Coated apples had greenness above that of the control, which showed its effectiveness.

The lightness ( $L^*$  value) of apples coated with FIMC, NMC, AMC, and control decreased from day 1 to 3. This was expected for the apples to retain their colour or for the mucilage to slow down the ripening process, therefore also colour change. Those coated with RMC had an increase in lightness. The  $b^*$  value for the RMC also increased, which showed a yellowish tint as compared to a blue tint during days 1 to 3, with increased colour saturation ( $C^\circ$ ).

The  $b^*$  value for FIMC, AMC, and NMC also decreased during that period which further confirmed that the product was not ripening fast, or the colour change was further from the yellow spectrum (bluish) and the green was still saturated. This showed that the mucilage was effective in slowing down the colour change. From day 7 to 23 the treated product's lightness continued to decrease, while the control group's lightness increased.

### 5.3.3 Texture

The texture is one of the parameters consumers use to measure quality. It can be measured by chewing or compressing the product (Borrett *et al.*, 2010). The firmness was assessed by measuring the hardness of the products (the resistance with which the product opposes penetration), the hardness work (energy required to penetrate the product) and deformation on target (distance). These all showed a decrease for both the control and treated groups. The softening of the products is due to the cell deterioration and cell composition involving hydrolysis of pectin and starch associated with ripening, then deterioration (Saha *et al.*, 2014).

From the CT3 texture analysis, a higher penetration value indicates a softer sample, as the probe will easily puncture deeper into a soft sample than a hard sample (du Toit, 2017).

### 5.3.3.1 Tomatoes

The firmness of both coated and control groups reduced during the storage period, as shown in Table 5.8.

Table 5.8: Results of texture changes in tomatoes coated with freeze-dried mucilage during the storage period.

Day	Cultivar	Hardness: Toughness (g)	Hardness: Work (mJ)	Deformation on Target (mm)
0	Control	86.00 <sup>c</sup> ± 4.62	2.00 <sup>d</sup> ± 0.01	5.94 <sup>a</sup> ± 0.01
7	Control	65.50 <sup>bc</sup> ± 5.20	1.80 <sup>cd</sup> ± 0.01	5.98 <sup>bc</sup> ± 0.01
	<i>Algerian</i>	56.50 <sup>ab</sup> ± 1.73	1.55 <sup>bcd</sup> ± 0.06	6.00 <sup>c</sup> ± 0.01
	<i>Robusta</i>	65.00 <sup>bc</sup> ± 18.48	1.70 <sup>bcd</sup> ± 0.35	5.97 <sup>ab</sup> ± 0.03
	<i>Nepgen</i>	36.50 <sup>a</sup> ± 5.20	1.05 <sup>a</sup> ± 0.06	5.96 <sup>ab</sup> ± 0.02
	<i>Ficus-indice</i>	47.00 <sup>ab</sup> ± 3.46	1.30 <sup>ab</sup> ± 0.01	5.94 <sup>a</sup> ± 0.01
23	Control	60.50 <sup>abc</sup> ± 9.81	1.50 <sup>abc</sup> ± 0.35	5.99 <sup>bc</sup> ± 0.01
	<i>Algerian</i>	51.50 <sup>ab</sup> ± 8.66	1.35 <sup>abc</sup> ± 0.29	5.94 <sup>a</sup> ± 0.01
	<i>Robusta</i>	57.50 <sup>ab</sup> ± 4.04	1.35 <sup>abc</sup> ± 0.06	5.94 <sup>a</sup> ± 0.01
	<i>Nepgen</i>	60.50 <sup>ab</sup> ± 25.98	1.40 <sup>abc</sup> ± 0.35	5.94 <sup>a</sup> ± 0.01
	<i>Ficus-indice</i>	52.00 <sup>ab</sup> ± 4.62	1.35 <sup>abc</sup> ± 0.06	5.94 <sup>a</sup> ± 0.01
P-value		p < 0.001	p < 0.001	p < 0.001

Means with different superscripts in the same column differ significantly.

This was expected due to the acid degradation of pectin (Mwaurah *et al.*, 2020). The deformation on the target of the control group increased significantly ( $p < 0.001$ ) over the storage period. This means that the control group was softening over time. Deformation on target results showed that the treated tomatoes' texture remained hard throughout the storage. This is seen by a decreasing penetration value compared to an increasing value of the control group. For example, looking at *Algerian* coated tomatoes, the deformation on target or penetration distance was 6 mm at seven days while the distance at day 23 was 5.94 mm, which is a decrease. This was expected for the coated products as the

edible coating should be able to inhibit enzyme activities responsible for tissue softening (Duguma, 2021).

The energy required to fracture the tomatoes decreased for both groups, but the decrease was not significant. This decrease was expected due to the ripening process and the remaining energy after harvest. The decrease in hardness continued over the storage period, e.g., *Algerian* from 56.50 g decreased to 48.50 g, and *Robusta* from 65.00 g decreased to 57.50 g (Table 5.8.), which was a significant decrease. The coated group had the lowest decrease compared with the control, from 86 g to 60.50 g. A comparable trend was noted by Bernardino-Nicanor *et al.* (2018). From this observation, one can conclude that *Algerian* and *Robusta* edible coating treatments did maintain or improve texture.

### 5.3.3.2 Potatoes

Firmness decreased for both groups over the storage period, indicated by both toughness of the product and the energy required to penetrate the product.

Table 5.9: Results of texture changes in potatoes coated with freeze-dried mucilage during the storage period.

Day	Cultivar	Hardness: Toughness	Hardness: Work	Deformation on Target
0	Control	346.00 <sup>g</sup> ± 0.01	22.20 <sup>b</sup> ± 13.00	14.97 <sup>b</sup> ± 0.01
7	Control	293.00 <sup>de</sup> ± 3.00	28.70 <sup>b</sup> ± 0.30	14.97 <sup>b</sup> ± 0.01
	<i>Algerian</i>	279.00 <sup>bc</sup> ± 1.00	28.95 <sup>b</sup> ± 0.05	14.94 <sup>ab</sup> ± 0.02
	<i>Robusta</i>	274.50 <sup>bc</sup> ± 1.50	28.15 <sup>b</sup> ± 0.15	14.91 <sup>a</sup> ± 0.01
	<i>Nepgen</i>	260.50 <sup>a</sup> ± 1.50	25.65 <sup>b</sup> ± 0.35	14.92 <sup>a</sup> ± 0.01
	<i>Ficus-indice</i>	286.00 <sup>cd</sup> ± 4.00	29.70 <sup>b</sup> ± 0.10	14.98 <sup>b</sup> ± 0.01
23	Control	265.00 <sup>f</sup> ± 15.00	26.75 <sup>a</sup> ± 0.75	14.97 <sup>b</sup> ± 0.01
	<i>Algerian</i>	277.50 <sup>bc</sup> ± 2.50	26.75 <sup>b</sup> ± 0.25	14.98 <sup>b</sup> ± 0.01
	<i>Robusta</i>	269.00 <sup>ab</sup> ± 1.00	9.00 <sup>a</sup> ± 0.01	14.98 <sup>b</sup> ± 0.01
	<i>Nepgen</i>	257.50 <sup>a</sup> ± 2.50	26.55 <sup>b</sup> ± 0.45	14.95 <sup>ab</sup> ± 0.03
	<i>Ficus-indice</i>	300.50 <sup>e</sup> ± 0.50	30.60 <sup>b</sup> ± 0.60	14.94 <sup>ab</sup> ± 0.04
P-value		p < 0.001	p < 0.001	p < 0.001

Means with different superscripts in the same column differ significantly.

The different cultivars' mucilage's cultivar effectiveness differs significantly (p<0.001) between day 7 to day 23. The control group lost hardness significantly (293 g to 265 g)

as compared to the group coated with *Algerian* (279 g to 277.50 g), *Robusta* (274.50 g to 269 g), and *Nepgen* (260.50 g to 257.50 g), as shown in Table 5.9. For *Ficus-indice*, the hardness in terms of both energies required to fracture as well as the toughness increased. However, the reason for this requires more investigation, i.e., to verify whether the mechanical properties of *Ficus-indice* mucilage are better than that of *Robusta*, *Algerian*, and *Nepgen*.

The energy required to penetrate the products was significantly different between cultivars ( $p < 0.01$ ). The energy required to penetrate potatoes decreased between days 7 to 23, and the decrease was significantly different for each cultivar,  $p < 0.001$ . There was no significant difference between energy required on days 7 and 23 for AMC, NMC, and FIMC, seen by the means with the same superscripts in the same column on different days (Table 5.9).

The energy required for RMC potatoes and the control decreased significantly. FD mucilage showed that FIMC, AMC, and NMC were effective compared to RMC and control. This result requires further investigation to evaluate how the mechanical properties of mucilage from different cultivars differ because, based on viscosity, one would expect RMC and NMC to behave similarly because their viscosities do not differ significantly, as shown in Table 4.2 results.

#### 5.3.3.3 Apples

From Table 5.10, both the control and treated products showed a decrease in texture hardness during the storage period. There was a significant difference between cultivars ( $p < 0.001$ ). There was no significant difference in toughness between days 7 and 23 for three cultivars (*Algerian*, *Nepgen*, and *Ficus-indice* and the control group. Only *Robusta*-coated apples lost toughness during this period.

Table 5.10: Results of texture changes in apples coated with freeze-dried mucilage during storage.

Day	Cultivar	Hardness: Toughness	Hardness: Work	Deformation on Target
0	Control	222.50 <sup>c</sup> ± 25.98	21.05 <sup>d</sup> ± 1.79	14.98 <sup>b</sup> ± 0.01
7	Control	130.50 <sup>b</sup> ± 0.58	9.75 <sup>ab</sup> ± 0.06	14.98 <sup>b</sup> ± 0.01
	<i>Algerian</i>	163.00 <sup>b</sup> ± 2.31	13.80 <sup>c</sup> ± 0.23	14.98 <sup>b</sup> ± 0.01
	<i>Robusta</i>	158.50 <sup>b</sup> ± 1.73	13.30 <sup>c</sup> ± 0.35	14.98 <sup>b</sup> ± 0.01
	<i>Nepgen</i>	441.00 <sup>e</sup> ± 1.15	37.20 <sup>g</sup> ± 0.23	14.92 <sup>a</sup> ± 0.01
	<i>Ficus-indice</i>	375.00 <sup>d</sup> ± 1.15	33.50 <sup>e</sup> ± 1.04	14.98 <sup>b</sup> ± 0.01
23	Control	112.00 <sup>b</sup> ± 4.62	11.20 <sup>b</sup> ± 0.23	14.98 <sup>b</sup> ± 0.01
	<i>Algerian</i>	129.00 <sup>b</sup> ± 1.15	9.00 <sup>a</sup> ± 0.35	14.97 <sup>b</sup> ± 0.01
	<i>Robusta</i>	71.50 <sup>a</sup> ± 67.55	8.40 <sup>a</sup> ± 0.12	14.97 <sup>b</sup> ± 0.01
	<i>Nepgen</i>	422.00 <sup>de</sup> ± 9.24	35.25 <sup>f</sup> ± 0.87	14.98 <sup>b</sup> ± 0.01
	<i>Ficus-indice</i>	387.00 <sup>d</sup> ± 1.15	32.15 <sup>e</sup> ± 0.17	14.98 <sup>b</sup> ± 0.01
P-value		p < 0.001	p < 0.001	p < 0.001

Means with different superscripts in the same column differ significantly

The energy required to penetrate the product decreased over the storage period and differed significantly between treatments. All four cultivars and the control had a significant decrease ( $p < 0.001$ ) in the energy required, which showed that the cells had softened; hence less energy was required to penetrate. The edible coating was, however, not effective in preventing texture loss in apples.

#### 5.3.4 Oil absorption of potato chips

The potato chips were deep-fried in oil for 3 minutes to evaluate whether the mucilage coating would influence oil absorption. Higher weight loss values in fries indicated no oil absorption, and lower weight loss values indicated oil absorption. Results in Table 5.11 show that the edible coatings had an impact on the chip's oil absorption. Both groups (treated and control) lost weight after frying, which was expected because frying causes dehydration or moisture loss. Coated potato chips were expected to gain more weight than uncoated potato chips as mucilage is said to be an oil absorbent (Alcantar *et al.*, 2012) and increases water-holding capacity (Lin and Mei, 2000).

Table 5:11: Oil Absorption % measured by weight differences after deep frying

Cultivar	Control	<i>Robusta</i>	<i>Ficus-indice</i>	<i>Nepgen</i>	<i>Algerian</i>	P-value
<b>Native liquid mucilage</b>	25.43 <sup>a</sup> ± 2.46	35.63 <sup>b</sup> ± 1.39	36.32 <sup>b</sup> ± 1.98	38.10 <sup>b</sup> ± 1.41	35.22 <sup>b</sup> ± 0.17	p < 0.001
<b>Freeze dried mucilage</b>	21.14 <sup>a</sup> ± 0.71	35.63 <sup>c</sup> ± 0.70	32.28 <sup>bc</sup> ± 2.83	41.62 <sup>d</sup> ± 0.97	31.91 <sup>b</sup> ± 1.37	p < 0.001

Means with different superscripts in the same row differ significantly.

The absorption was calculated by subtracting the weight average after frying from the initial weight average using the formula below:

$$\text{Weight loss (\%)} = \frac{\text{initial weight(g)} - \text{final weight(g)}}{\text{Initial weight (g)}} * 100$$

Coated chips showed less weight loss, therefore, higher oil absorption as compared to the uncoated chips. The mucilage edible coating did not prevent the chips from absorbing the oil because the weight increased. This might be because mucilage is an oil absorbent, and it also increases the water-holding capacity of the product, meaning the dehydration process during frying was slow compared to the uncoated products. AMC and RMC were the most effective, followed by FIMC. During the frying, it was observed that the coated chips developed a brown colour faster in contrast to the uncoated potato chips (Figure 5.5A to B). This browning was ascribed to and due to the Maillard reaction and

caramelisation due to sugars present in the mucilage, as was already explained earlier, reported by Miya *et al.* (2022).



Figure 5.5A: AMC chips (right) compared to control chips (left) (A) and FIMC chips (right) compared to control chips (left) (B)



Figure 5.5B NMC chips (right) compared to control chips (left) (A) and RMC chips (right) compared to control chips (left) (B)

### 5.3.5 Spherification

#### 5.3.5.1 Direct or basic spherification

In a trial to improve the gelling capability of mucilage, Xanthan gum and agar were added to the mucilage and tea mixture. When this mixture was added to the calcium bath, a flat

round-like structure with a gel-like membrane on the outside was formed but could not hold the shape and texture long enough.

However, this was not as globular or spherical as the spheres formed when sodium alginate was used (Figure 5.6A). This was further proof that mucilage does not gel due to its structure (du Toit, 2018).



Figure 5.6A Rooibos tea mucilage mixture drops added into calcium bath with a syringe (left) and basic spherification spheres (right)

#### 5.3.5.2 Reverse and frozen spherification

In a reverse method, mucilage was used to make a bath, and yogurt as a calcium-containing product was added. It did not form a sphere with a gel membrane. The same was observed when the yogurt was not frozen and in the frozen reverse process. Instead, the frozen yogurt thawed slowly with no membrane formed. Because mucilage is not a true gelling agent, the interaction between the calcium ions and mucilage polymers do not cross link to form a gel membrane.

#### 5.3.5.3 Caviar-like beads (Cold oil spherification)

In this trial and error, it was learned that some hydrocolloids could form a gel in extreme cold temperatures at acidic pH conditions, i.e., agar-agar. Therefore, we evaluated how the acidic pH of vinegar (balsamic) and cold olive oil would impact the gelling of mucilage.

It was observed that mucilage still didn't form a gel but formed a membrane that only held the vinegar-mucilage mixture for a few seconds before it broke (Figure 5.6 B).

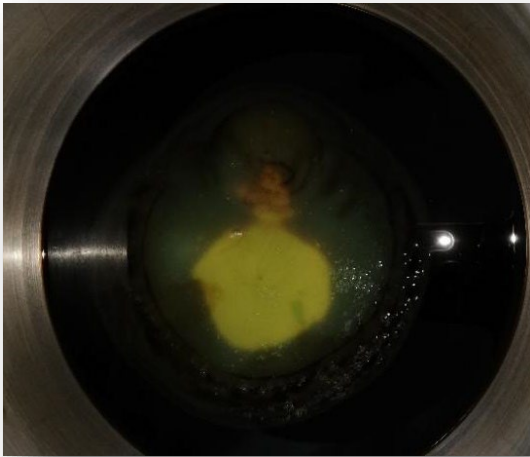


Figure 5.6B Balsamic vinegar with mucilage, cold oil spherification.

When agar was used to make caviar-like beads by dropping a mixture of vinegar and agar in cold oil, it resulted in a small bead with a gel membrane that enclosed the agar-vinegar mixture (Figure 5.6C (left)). When mucilage was used in the same process, where it was used to replace agar and was mixed with vinegar, the addition of the drops of this mixture in cold oil, a membrane was formed that entrapped the mixture, but this membrane broke shortly after addition (Figure 5.6 C (right)) and could not be removed from the oil without breaking the beads.



Figure 5.6C Caviar beads made from agar (left) and Caviar beads made from mucilage (right)

### 5.3.6. Conclusion

To assess the effectiveness of mucilage as an edible coating in preventing moisture loss, colour, and texture and therefore extending shelf-life: Edible coatings are effective in slowing down moisture loss or maintaining moisture loss, although effectiveness depends on the cultivars. Different cultivars perform differently when used as an edible coating, resulting in different results for each product. Mucilage edible coating was effective in preserving moisture loss of apples and potatoes. For apples the NMC and FIMC treatment were more effective in preserving moisture. NMC, RMC and NMC were all effective in preventing moisture loss for potatoes. Mucilage edible coating was effective in preserving texture of potatoes and colour of coated tomatoes and potatoes. The effectiveness of mucilage edible coatings in preserving texture and colour differed from cultivar to cultivar. FIMC was effective on preserving the texture of tomatoes. RMC was the least effective in preserving the texture of potatoes. The mucilage-coated chips absorbed more oil resulting in the chips being heavier due to oil absorption. This could be beneficial for the potato deep fried chips industry in term of weight and total cost.

To further test mucilage gelling ability, no spheres (beads) were formed with either basic or reverse spherification processes. In a trial to improve the gelling capability of mucilage, Xanthan and agar were added to the mucilage and tea mixture. When this mixture was added to the calcium bath, a flat roundish structure with a gel-like membrane on the outside was formed. But this was not as globular or spherical as the spheres formed when sodium alginate was used. This was further proof that mucilage does not gel due to its structure (du Toit, 2018). During the reverse method, mucilage was used to make a bath, while yogurt was added as the calcium-containing product. It did not form a sphere with a gel membrane. The same trend was observed when the yogurt was frozen in the frozen reverse process; instead, the frozen yogurt thawed slowly with no membrane formed. Cactus pear mucilage does not form a true gel and, therefore, could not be used in the making of caviar-like beads or spherification as a gelling agent.

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