# Extraction and characterisation of pectin from prickly pear *(Opuntia spp.)* peel

**BY**

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**Declaration**

I, Lekhuleni Isobel Lerato Gosh, Student No. 11533392, hereby declare that this dissertation for Master of Science in Food Science and Technology submitted by me to the Food Science and Technology Department at University of Venda, has not been previously submitted for a degree at this or any other University. This work is my design, implementation and that all reference material contained in this document has been acknowledged.

24 August 2020

Signature Date:

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## Dedication

It is my pleasure to dedicate this work to my parents Mishack and Mamsie Lekhuleni for their unconditional love. Their encouragement in my small achievements has always been a driving force for me. I am deeply grateful for my daughter Khanyisile Masilela and son Owethu Themba, who are the source of my inspiration. My appreciation to my sister Bridgette, my brothers Cambridge and Zakhele Lekhuleni for their continuous encouragement. They were with me when I needed their support. I am sure that I would not have been able to achieve this without their support and prayers.

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## Acronyms

| | |
|---|---|
| ANOVA | Analysis of variance |
| AOAC | Association of Official Analytical Chemists |
| AUA | Anhydrouronic acid |
| CBRD | Completely blocked random design |
| DE | Degree of esterification |
| FAO | Food and Agricultural Organisation |

| | |
|---|---|
| FTIR | Fourier-transform infrared spectroscopy |
| HG | Homogalcturonan |
| HMP | High methoxyl pectin |
| LMP | Low methoxyl pectin |
| PP | Prickly pear |
| PPP | Prickly pear peel |
| TSS | Total soluble solids |

## Abstract

The study aimed to determine the physicochemical properties, extract and characterise pectin from three varieties (green, purple and orange) of prickly pear (*Opuntia spp*) fruit peel. Research samples were collected from Bothlokwa Mphakane village in Limpopo province of South Africa. The experimental design for this study was randomized complete block design and each treatment was conducted in triplicate. Pectin was extracted using sulphuric acid at four different pH levels (1, 2, 3 & 4) and microwave power levels (low, medium & high). The characterisation of extracted pectin was done by calculating the ash, moisture, equivalent weight, anhydrouronic acid, methoxyl content, and degree of esterification. Functional groups present in extracted and commercial pectin were investigated for similarities using Fourier Transform Infrared Spectroscopy. The yield of pectin for purple ranged from 2.9% to 13.8%, orange from 1.4% to 9.8% and from 2.3% to 10.0% for green prickly pear peel. A decrease in pH resulted in an increased pectin yield and an increase in microwave power level increased the pectin yield. Maximum yields of 13.8% on purple, 10.0% on green and 9.8% on orange were obtained at pH 1.0 and medium power level. The best condition for extraction using sulphuric acid was at a medium power level and pH 1.0. The pectins obtained were compared against each other in terms of yield, physicochemical characteristics and chemical structure. The ash content of the extracted pectin was significantly higher at 25.16 ± 0.69, 34.26 ± 1.92 and 36.30 ± 1.07, however the pectin showed lower moisture content and equivalent weight. The methoxyl contents were 2.28 ± 0.26,

2.38 ± 0.21 and 3.86 ±0.31, anhydrouronic acid contents were 25.58 ± 2.03, 25.93 ± 2.35 and 38.84 ± 2.29, and degree of esterification were 49.87 ± 0.17, 50.63 ± 4.76 and 56.39 ± 1.60 across the orange, purple and green varieties, respectively. The prickly pear peel pectin spectra exhibited similarities in its absorption pattern to that of commercial citrus pectin. Therefore, the extraction of pectin from prickly pear peels is feasible in viewpoint of yield and quality, and that the pectin has potential for substitution of citrus pectin in the food processing industry.

***Keywords:*** Prickly pear, pectin, extraction, yield, functional group.

# CHAPTER 1: INTRODUCTION

## 1. Background and research justification

Prickly pear (*Opuntia spp*) (PP) is a family of Cactaceae. PP cultivars can grow in harsh, rocky and dry environmental conditions (Figure 1a) (Butera *et al*., 2002). PP has gained the attention of consumers due to its high nutritional value that has a positive health benefit (López *et al*., 2012; Piga, 2004). It has a high and unique composition of nutrients including B-family vitamins, magnesium, calcium, potassium, copper, dietary fibre, flavonoids, carotenoids, betalain, amino acids and lipids (Panda *et al*., 2016; Saenz, 2013; Morales *et al*., 2012; Alimi *et al*., 2010; Valente *et al*., 2010; Mannoubi *et al*., 2009; Kulger *et al*., 2006; Stintzing *et al*., 2005; Tesoriere *et al*., 2005, 2004; Piga, 2004; Galati *et al*., 2003). The PP fruit components and extracts are being used in the treatment of diabetes, cholesterol, and immune system health (Yahia, 2010; Ncibi *et al*., 2008). Polyphenols are antioxidants that trap the free radicals generated by human bodies which serve as protection against pathogenic bacteria (Gengatharan *et al*., 2015; Patel, 2015; Saenz, 2013). Antioxidants of carotenes and vitamin E improve the stability of fatty oils (Ramadan & Mörsel, 2003).

PP is oval-shaped with thick, waxy and thorny peel (Ochoa-Velasco & Guerrero-Beltrán, 2014) and is available in different colours ranging from green (Figure 1b), purple (Figure 1c) and orange (Figure 1d) (Khatabi *et al*., 2016; Gengatharan *et al*., 2015; Ochoa & Guerrero, 2014; Marran & Manikandan, 2012; Gandia-Herrero *et al*., 2010; Chavez-Santoscoy *et al*., 2009; Morales *et al*., 2008; Castellanos-Santiago *et al*., 2008). The fruit has a high level of sugar and low acidity which gives it the sweet acidic taste and has a short shelf life of approximately 2 to 4 weeks (Marran & Manikandan, 2012; Yahia, 2010; Feugang *et al*., 2006). The pulp and seeds are the most edible part of the PP fruit which and constitutes about 40 – 60 % of the fruit (Yahia, 2012; Feugang *et al*., 2006). During the production of pulp, wine and juice, the PP fruit peel is regarded as a byproduct. However, the peel contains high antioxidants and pectin (Patel, 2015; Morale *et al*., 2012; Tesoriere *et al*., 2005), and can be used as an alternative source of pectin in the production of jam.

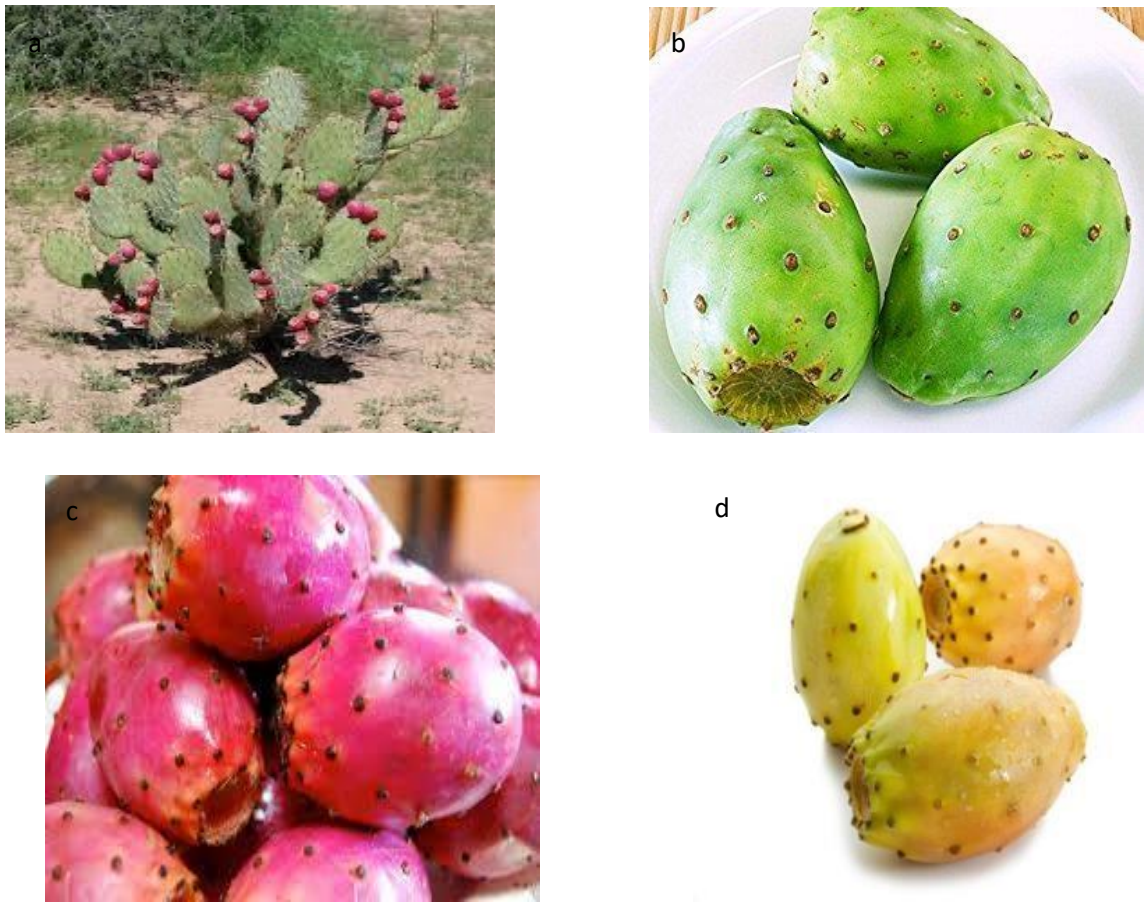



Figure 1: Prickly pear fruit, a. plant, b. green, c. purple and d. orange PP

Pectin is a polysaccharide made up by 1,4-α linked galacturonic acid (GalA) units (Chan *et al*., 2017; Vasco-Correa & Zapota Zapota, 2017; Hosseini *et al*., 2016a; Swamy & Muthukumarappan, 2016; Wang *et al*., 2015; Liew *et al*., 2014). The GalA units may both be methyl-esterified and acetylated (Chan *et al*., 2017; Vasco-Correa & Zapota Zapota, 2017; Kaya *et al*., 2014). Pectin is naturally present in fruits and is found between adjacent plant cells in the layers of middle lamella (Chan *et al*., 2017; Lefsih *et al*., 2016; Marran *et al*., 2015; Chan & Choo, 2013; Suárez *et al*., 2013). The functional properties of pectin depend on the degree of esterification (DE) and these are characterised as low methoxyl pectin (LMP) with DE < 50% and high methoxyl pectin (HMP) with DE > 50% (Jafari *et al*., 2016; Liu *et al*., 2010). The HMP and LMP have different DE and therefore different physiochemical properties and applications (Chan & Chao, 2013; Ngouémazong *et al*., 2012).

The important process in pectin production is extraction and is usually achieved by hot acid extraction (Guo *et al*., 2015; Ma *et al*., 2013). Pectin is extracted from fruit peel which is acidified with organic or mineral acid: sulphuric, nitric, and hydrochloric acid, are the commonly used

extractants (Andersen *et al*., 2017; Lefsih *et al*., 2016; Oliveira *et al*., 2016; Pereira *et al*., 2016; Raji *et al*., 2016; Denman & Morris, 2015; Li *et al*., 2015; Khan *et al*., 2014; Methacanon *et al*., 2014; Minjares-fuentes *et al*., 2014; Chan & Choo, 2013; Ma *et al*., 2013). Pectin is mainly used as a stabiliser, emulsifier, texturiser and thickener in the production of jams, jellies, confectionery products, beverages and acidified milk drinks (Petkowics *et al.*, 2017; Raji *et al*., 2016; Swamy & Muthukumarappan, 2016; Kermani *et al*., 2015; Yuliarti *et al*.,2015; Brouns *et al*.,2012).

### 1.2 Problem statement

The increasing human population in South Africa is causing growing concern about environmental pollution caused by industrial and agricultural waste. Although several studies have been conducted on clean technologies, resource efficiency and recycling, no study has been conducted on the utilisation of PP peel waste.

PP peel waste may cause environmental issues, especially water pollution. The peel waste contain biomaterials such as peel oil, pectin and sugar can cause aerobic bacteria to breakdown biodegradable organic matters into nitrates, phosphates, carbon dioxide and sulfates in water (Girma & Worku, 2016).

The main challenge is how to utilise PP waste material for a better purpose. The PP fruit peel is an underutilised waste that contains high antioxidants, pectin and other useful components (Patel, 2015). Moreover, the peels contain pectin which can be extracted and utilised in the production of different products such as jam and jellies. Therefore, this study explores the possible extraction of pectin from PP peels for potential commercial purposes in jam production.

### 1.3 Aim and objectives

#### 1.3.1 Aim

The study aims to extract and characterise pectin from orange, purple and green PP peels.

#### 1.3.2 Specific objectives

1. To extract and determine the pectin yield (%) from orange, purple and green PP peel
2. To characterise the PP peel pectin in terms of ash content, moisture content, equivalent weight, methoxyl content, anhydrouronic acid, degree of esterification and chemical structure using Fourier transform infrared (FTIR).

## 1.4 Hypothesis

### 1.4.1 Null hypothesis

a. The pectin yield extracted from orange, purple and green PP peel will not be similar.
b. The characteristics of the PP pectin extracted from three varieties in terms of ash, moisture, equivalent weight, methoxyl, anhydrouronic acid contents and degree of esterification and chemical structure will not be similar.

### 1.4.2 Alternative hypothesis

a. The pectin yield extracted from orange, purple and green PP peel will be similar.
b. The characteristics of the PP pectin extracted from three varieties in terms of ash, moisture, equivalent weight, methoxyl, anhydrouronic acid contents and degree of esterification and chemical structure will be similar.

# CHAPTER 2: LITERATURE REVIEW

In this study, the literature focuses on the background of the PP fruit, chemical and nutritional composition of the fruit. Furthermore, the literature discusses pectin and its sources, chemical structure, degree of esterification and general properties of pectin. It also outlines the different extraction methods, the extraction and isolation procedure and the factors affecting the extraction yield. Moreover, the physicochemical characteristics of the extracted pectin such as; moisture, ash, methoxyl, equivalent weight, anhydrouronic acid contents and degree of esterification are discussed in this chapter.

## 2.1 Prickly pear fruit

### 2.1.1 Background

Prickly pear (PP), scientifically referred to *Opuntia ficus-indica* is a dicotyledonous angiosperm plant that belongs to the *Cactaceae* family. It is characterised by its ability to grow in harsh, rocky and dry environmental conditions (Butera *et al*., 2002). It is oval-shaped with thick, waxy and thorny peel (Ochoa-Velasco & Guerrero-Beltrán, 2014) and is available in different colours ranging from green, yellow, purple-red and orange (Khatabi *et al*., 2016; Gengatharan *et al*., 2015; Ochoa & Guerrero, 2014; Marran & Manikandan, 2012; Gandia-Herrero *et al*., 2010; ChavezSantoscoy *et al*., 2009; Morales *et al*., 2008; Castellanos-Santiago *et al*., 2008). Prickly pear fruit is oval-shaped with thick, waxy and thorny peel (Ochoa-Velasco & Guerrero-Beltrán, 2014). The fruit generally contains 85% of water, a high level of sugar (15%) and low acidity which gives it the sweet acidic taste. However, it has gained considerable attention because of its nutritional value to have a positive health benefit (Yahia & Mondragon-Jacobo, 2011).

PP fruit is generally recognised as an important source of vitamins for local people where the fruit is naturally grown. The pulp is the edible part of the fruit, however the peels and seeds are considered the waste product of the fruit. The fruit has a thick peel covering a flavoured seedy pulp which constitutes about 10-15% of the edible pulp, moreover the thickness of the peel and amount of pulp varies depending on the variety and cultivation region of the fruit. It is reported that the peel is a good natural source of energy, nutritive components and antioxidants such as vitamin C, carotenoids and pectin (El-Said *et al*., 2011).

### 2.1.2 Chemical and nutritional composition of prickly pear fruit

PP fruit has a unique composition of nutrients, including high amounts of vitamins and minerals, and also contains considerable amounts of vitamin C, vitamin E, carotenoids, fibres, amino acids and antioxidant compounds (phenols, flavonoids, betaxanthin and betacyanin) (Figure 2) that provide positive health benefits such as hypoglycemic and hypolidemic action and antioxidant properties (Panda *et al*., 2016; Saenz, 2013; Morales *et al*., 2012). Moreover, PP is a valuable source of compounds such as antiulcerogenic, antioxidant, anticancer, neuroprotective, hepatoprotective and antiproliferative (Yahia, 2010; Ncibi *et al*., 2008).

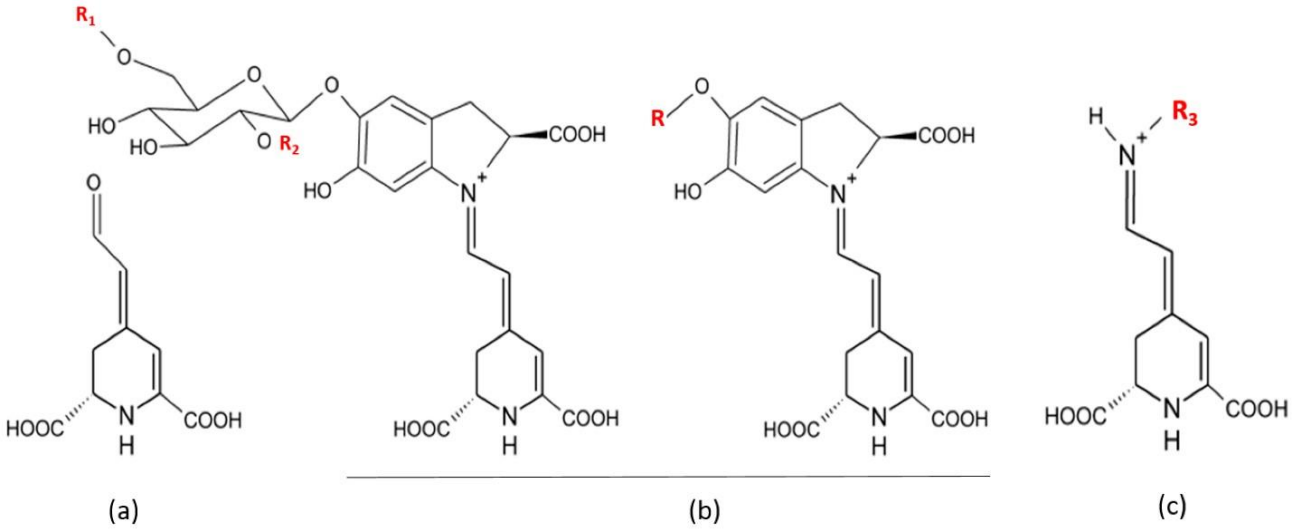



Figure 2. General structure of (a) betalamic acid, (b) betacyanins and (c) betaxanthins (Galati *et al.*, 2005)

The chemical composition of the fruit varies depending on several factors such as the variety of fruit, area of cultivation and ripening stage amongst others (Kamble *et al*., 2017). Several authors have conducted studies on the chemical composition of PP as shown in Table 1 (Kamble *et al*., 2017). The fruit contains 85% water, and it is the main important component of the fruit. The thick peel is rich in mucilage protects the water content of the fruit by strongly binding the water and prevents dehydration of the fruit (El-Mostafa *et al*., 2014). Very few fruits have proteins, the amount of proteins present in PP are comparable to those present in other common fruits (ElGharras *et al*., 2006). Fat is present in low levels, these low levels indicate that the fruit is not a good source of energy (El-Gharras *et al*., 2006). Total sugar content varies depending on the maturity stage, colour and origin of the fruit (El-Gharras *et al*., 2006).

Table 1: Chemical composition of prickly pear fruit.

| Parameter | A | B | C | D |
|---|---|---|---|---|
| Moisture (%) | 91 | 85-90 | 83.8 | 84.2 |
| Protein (%) | 0.6 | 1.4-1.6 | 0.82 | 0.99 |
| Fat (%) | 0.1 | 0.5 | 0.09 | 0.24 |
| Fibre (%) | 0.2 | 2.4 | 0.23 | 3.16 |
| Ash (%) | - | - | 0.44 | 0.51 |
| Total sugar (%) | 8.1 | 10-17 | 14.06 | 10.27 |
| Vit C (mg/100 g) | 22 | 4.6-44 | 20.33 | 22.56 |

*Authors*: (A) Munoz de Chaves *et al*., (1995); (B) Pimiental (1999); (C) Sepulveda & Saenz (1990); (D) Rodriguez *et al*., (1996). Source: Kamble *et al*., (2017)

Vitamins

PP fruits are recognised as an important source of vitamins. The high content of vitamin E is dominated by α-tocopherol amounting to 80% of the total vitamin E (Kamble *et al*., 2017). Vitamin E is known for its antioxidant property that improves the stability of fatty oils (Ramadan & Mörsel, 2003). Kamble *et al* (2017) reported total vitamin E content of 527.4 mg/100 g in the pulp. Ascorbic acid is one of the major vitamins present in PP, amounting to 20-25 mg/100 g as reported by Kamble *et al*., (2017), higher light intensity during the growing season yields higher vitamin C content. Vitamin B1, B2 and B3 are only present in trace amount reported by Stintzing *et al*., (2005).

Amino acid

PP fruits contain great amounts of amino acids, including eight essential amino acids. It contains high amounts of amino acids especially proline 46%, taurine 15.79 % and serine 6.68% (Kamble *et al*., 2017). The two predominant amino acids are proline and taurine, which represent about 46% and 15.78% of the total amino acids, respectively. Hence the PP fruit is considered an important source of amino acids and total proteins (13.62) (El-Said *et al*., 2011).

Mineral composition

PP fruit is considered a rich source of minerals. Minerals are elements that originate from the soil and cannot be created by plants. Yet plants, animals and humans need minerals to be healthy. The mineral content of plant sources may differ from place to place because the mineral content of the soil varies according to the location in which the plant was grown (El-Said *et al*., 2011). The mineral composition of PP fruit is shown in Table 2. Calcium is present in a high amount of 316.5 mg/100 g, followed by potassium and magnesium at 108.8 and 63.4 mg/100 g respectively, and the other minerals are present in trace amounts (Chiveta & Wairagu, 2013). Table 2: Mineral composition of prickly pear fruit

| **Minerals** | **Content (mg/100 g)** |
|---|---|
| Calcium | 316.5 |
| Magnesium | 63.4 |
| Sodium | 18.7 |
| Potassium | 108.8 |
| Iron | 25.9 |
| Phosphorus | 0.05 |
| Zinc | 12.6 |
| Manganese | 37.8 |

Source: Chiteva & Wairagu (2013), Feugang *et al* (2006), El-Said *et al*., (2011)

Phenolic compounds

Polyphenols are a family of organic molecules that are distributed in plant material. Their chemical structures are characterised by the presence of some phenolic groups that are linked with complex chemical groups of high molecular weight (El-Mostafa *et al*., 2014). PP fruit is well known for its high polyphenols content presenting antioxidant and anti-inflammatory properties (Ahmed *et al*., 2005). The fruit has a total phenol content of 218.8 mg/100 g (Fernandez-Lopez *et al*., 2010).

Nutritional composition

PP contains components of nutritional importance, free amino acid content, readily absorbable sugars, high contents of magnesium and calcium as well as technological interesting fibres make this fruit very distinct (El-Gharras *et al*., 2006). Moreover, the processing of the fruit represents a great technological challenge since it presents unique characteristics such as antioxidants and pectin than can be suitable to be a natural food additive (Patel, 2015; El-Gharras *et al*., 2006).

## 2.2 Chemistry of pectin

### 2.2.1 Pectin

Pectin is a polysaccharide that is naturally present in all plant tissues. It is available in different amounts in fruit cell walls and has important technological properties and nutritional value. Pectin serves as a cementing agent to the cellulose fibrils and is covalently linked to other polymers (Wonago, 2016; Srivastavva & Malviya, 2011). Pectin is the methylated ester of polygalacturonic acid, and consists of 300 to 1000 galacturonic acid units (Wonago, 2016). It is found in the plant cell walls located in the middle lamella, and primary, and secondary cell walls (Figure 3) (MüllerMaatsch *et al*., 2016; Sundar Raj *et al*., 2012). For example, pectin is found in the peels of most fruits, such as oranges, apple pomace, passion fruit, lemons and prickly pears. (Wonago, 2016).

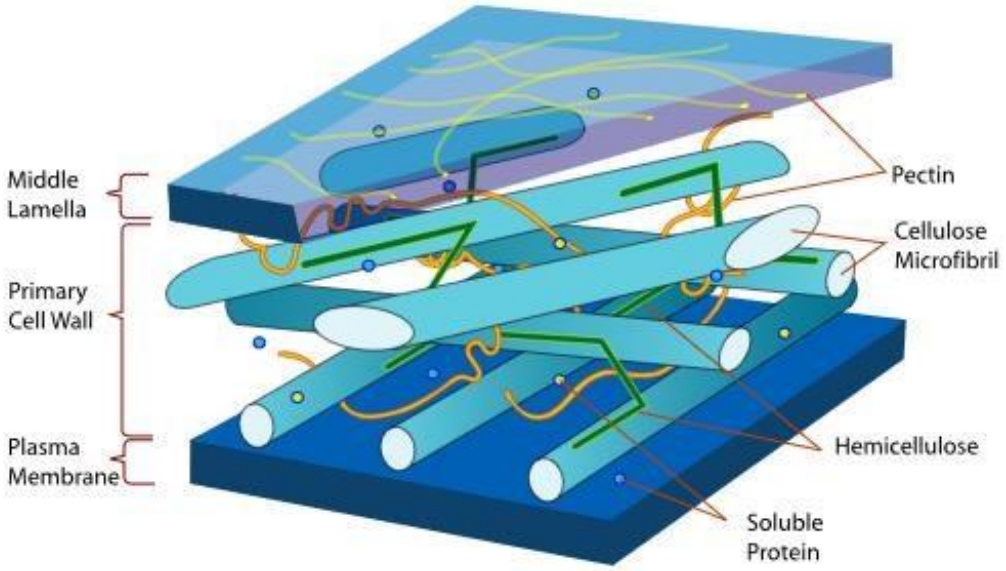



Figure 3. The structure of cell wall. Pectin in the plant cell walls located in the middle lamella, primary and secondary cell walls (Müller-Maatsch *et al.,* 2016).

### 2.2.2 Sources of pectin

Pectin is a complex mixture of polysaccharides that make up approximately one-third of the cell wall. The middle lamella contains high levels of pectin followed by the primary cell walls and in the secondary cell wall, the pectin is greatly decreased towards the plasma membrane of the plant cell (Chan *et al*., 2017; Willats *et al.*, 2001). Pectin commonly occurs in most plant tissues, however only limited plant sources may be used for commercial pectin production (Wonago, 2016). The ability of pectin to form gels depends on the degree of esterification (DE) and molecular

size, the pectin from different sources have different gelling abilities due to the parameters variation (Sundar Raj *et al*., 2012). Therefore, the detection of pectin in fruit in large amounts does not qualify the fruit alone as a source of commercial pectin. Commercially, pectins are derived mainly from citrus peel and apple pomace all over the world; nonetheless, efforts have been carried out to find alternative pectin sources (Min *et al*., 2011). Listed in Table 3 are some of the common fruits and their pectin content (Sundar Raj *et al*., 2012). Table 3. Pectin content of some fruits

| **Fruits** | **Pectic substances (% Wet Weight)** |
|---|---|
| Apple pomace | 1.5 - 2.5 |
| Banana | 0.7 - 1.2 |
| Granadilla | 0.4 |
| Guava | 0.77 - 0.99 |
| Lemon | 2.5 - 4.0 |
| Mango | 0.26 - 0.42 |
| Orange | 3.5 - 5.5 |
| Papaya | 0.66 - 1.0 |
| Passion fruit | 2.1 - 3.0 |
| Pineapple | 0.04 - 0.13 |
| Prickly pear | 1.2 |
| Strawberries | 0.6 - 0.7 |

Source: Wonago, (2016), Liew, Chin & Yusof (2014), Sundar Raj *et al*., (2012), Min *et al*., (2011).

### 2.2.3 Chemical structure of pectin

Pectin is a linear polysaccharide which is made up of 1,4 linked α-D-galacturonic acid (GalA)(Figure. 4) (Chan *et al*., 2017; Hosseini *et al*., 2016; Swamy & Muthukumarappan, 2016; Castillo-Israel *et al*., 2015; Wang *et al*., 2015; Liew *et al*., 2014). These uronic acids have carboxyl groups which are naturally present as methyl esters and free acid groups (Wonago, 2016). These uronic acids have carboxyl groups which are naturally present as methyl esters and free acid groups, pectin has three methyl esters forms (-$COOCH_3$) for every two carboxyl groups (-COOH) as shown in Figure 5 (Khan *et al*., 2015). Pectin is assembled by at least 17 different

monosaccharides, of which GalA is the most followed by L-arabinose, D- galactose, L- rhamnose and others (Chan *et al*., 2017; Kaya *et al*., 2014).

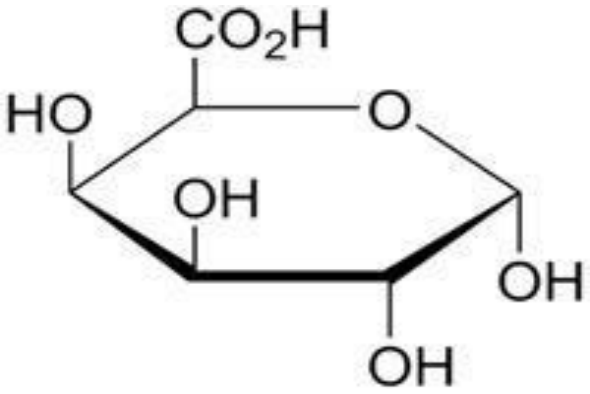



Figure 4: Pectin structure (Chan *et al*., 2017)

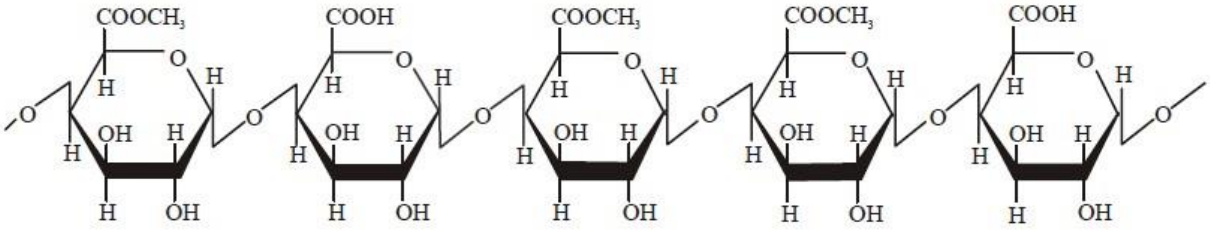



Figure 5: Pectin a polymer of α-galacturonic acid with a variable number of methyl ester groups (Khan *et al*., 2015)

### 2.2.3.1 Pectin polysaccharides containing galacturonic acid

#### I. Homogalacturonan (HGA)

Homogalacturonan (HGA) is a linear homopolymer of 1.4-linked α-D-GalA (Figure 6) residues with a variable degree of methyl esterification at carboxyl group. It could be O-acetylated at C2 or C3 depending on the source. The amounts of GalA units present in HG chains is estimated to be 100-200 units (Chan *et al*., 2017; Alba *et al*, 2015) and constitute about 65% of pectin molecule.

The GalA units may both be methyl-esterified and acetylated (Vasco-Correa & Zapota Zapota 2017).

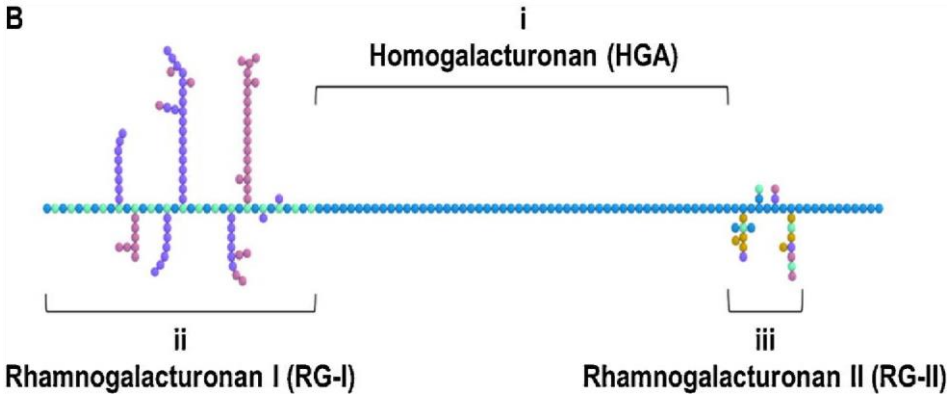



Figure 6: Pectin chain composing of covalently linked (i) homogalacturonan (HGA), (ii) rhamnogalacturonan I (RG-I) and (iii) rhamnogalacturonan II (RG-II). The diagram only illustrate some of the major domains found in most pectin rather than indicate definitive structures (Chan *et al*., 2017).

**II. Rhamnogalacturonan I**

It consists of repeating units of disaccharide (→4)-α-D-GalA-(1→2)-α-L-Rha-(1→) to which different glycan chains are attached to the GalA residues which can be O-acetylated at the C-2 or C-3, while 20-80% of the rhamnose residues can be substituted at C-4 or C-3 with neutral sugar side chains (Alba *et al*., 2015) and constitutes 20 – 35% and the remaining is comprised of galacturonans (Naqash *et al*., 2017).

**III. Rhamnogalacturonan II**

It has a backbone of homogalacturonan with complex side chains attached to the GalA residues including 2-keto-3-deoxy-D-anno-octulosonic acid, 3-deoxyD-lyxo-2-heptulosaric acid, apiose, and aceric acid (Alba *et al*., 2015). All neutral sugars are located as side chains in the rhamnogalacturonan I and rhamnogalacturonan II domains, and therefore often referred to “hairy regions” (Zhang *et al*., 2015; Koubala *et al*., 2014).

### 2.2.4 Degree of esterification

The polygalacturonic acid chain is partly esterified with methyl groups and the free acid groups may be partly or fully neutralised with sodium, potassium or ammonium ions (Sundar Raj *et al*., 2012). Pectin can be formed in a highly esterified form, undergoing de-esterification after they have been inserted into the cell wall or middle lamella (Sundar Raj *et al*., 2012). DE’s can be dependent on species, tissue, and maturity (Sundar Raj *et al*., 2012). The distribution of free carboxyl groups along the pectin chains is regular, and the free carboxyl groups are isolated

(Sundar Raj *et al*., 2012). Pectin is categorised into two major types of DE; low methoxyl pectin (LMP) with DE <50% and high methoxyl pectin (HMP) with DE >50% (Naqash *et al*., 2017; Patova *et al*., 2017; Jafari *et al*., 2016; Liu *et al*., 2010). HMP and LMP have different DE and thus different physiochemicals and applications (Chan & Choo 2013).

#### 2.2.4.1 High methoxyl pectin

They form gels with sugar and acid, low water activity gels or sugar-acid-pectin gels and contain dextrose as a dispersion agent to prevent humping (Castillo-Israel *et al*., 2015). HMP gels are hot water-soluble and pH-sensitive but stable at low pH levels (Castillo-Israel *et al*., 2015). It is considered to be a 2-dimensional network of pectin molecules in which co-solute (sugar) with the solvent (water) and acid are immobilised resulting in resisting deformation and showing a stressstrain relationship for small deformation (Sharma *et al*., 2006). The formation of the 3D network is based on the structure of junction zones, in which there are chain associations stabilised by hydrogen bonding between the un-dissociated carboxyl and secondary alcohol groups and by hydrophobic interaction between methyl esters (Ahmmed, 2013). The HMP gels are thermally reversible and commercial value ranges from 60-75% (Sundar Raj *et al*., 2012).

#### 2.2.4.2 Low methoxyl pectin

They independently produce gels of sugar content and require a controlled amount of $Ca^{2+}$ or divalent cations that will be present for gelation (Castillo-Israel *et al*., 2015; Ngouémazong *et al*., 2012). Gelation is caused by the formation of intermolecular junction zones between homogalacturonic regions of different chains (Ahmmed, 2013). The structure of a junction zone is known as “egg-box” model. The gelation ability of LM pectin increases with a decreasing degree of methylation. The presence of acetyl groups in pectin is very useful because it prevents gel formation with calcium ions and gives the pectin emulsion stabilising properties (Ahmmed, 2013). LMP is not pH sensitive and stable at low pH levels (Castillo-Israel *et al*., 2015) and the commercial value ranges from 20-40% (Sundar Raj *et al*., 2012).

### 2.2.5 General properties of pectin

Pectin is soluble in water, monovalent cation (alkali metal) salts of pectinic and pectic acids are usually soluble in water; di-and trivalent cations salts are weakly soluble or insoluble (Sundar Raj *et al*., 2012). Dry powdered pectin, when added to water, will hydrate rapidly and forms clumps (Sundar Raj et al., 2012). Viscosity, solubility, and gelation are related, factors that increase gel strength will increase the tendency to gel, decrease solubility, and increase viscosity, and vice

versa (Wonago 2016; Sundar Raj et al., 2012). As with solubility, the viscosity of a pectin solution is related to the molecular weight, degree of esterification, concentration of the preparation, and the pH and presence of counter ions in the solution (Sundar Raj *et al*., 2012). These properties of pectin are a function of their structure, which is that of a linear polyanion.

Pectins are not stable molecules in an aqueous environment; depending on the pH and temperature, pectin molecules may undergo several chemical reactions and modifications (Khan et al., 2015). Glycosidic bonds and methyl-ester linkages may undergo hydrolysis to different extents under acidic conditions. Hydrolysis of the more sensitive glycosidic linkages, like those involving the neutral sugar side chains, may lead to the increase of the galacturonic content and decrease of the neutral sugar content of the acid-treated pectins (Khan et al., 2015, Sundar Raj *et al*., 2012). During acid treatment at reduced temperature, the rate of glycosidic-bond hydrolysis is much slower than the rate of DE; therefore, preparations of LMP may be obtained by acid treatment without extensive main-chain breakdown and decreasing of the pectin molecular weight. Any increase of temperature increases the rate of the b elimination reaction more than that of the de-esterification (Khan *et al*., 2015). Under alkaline conditions, or even at a pH close to 7, especially at elevated temperatures, extensive and rapid degradation may occur, involving both de-esterification and cleavage of glycosidic bonds, the last occurring generally by a trans-belimination mechanism (Khan *et al*., 2015). The degradation of pectins under these conditions increases with temperature and with the DE (Wonago 2016).

### 2.2.6 Gelling properties of pectin

The most important role of pectin is based on its ability to form gels, gelling is the formation of a three-dimension (3D) network of polymer chains with solvent and solutes trapped within (Chan *et al*., 2017). Gel formation is caused by hydrogen bonding between free carboxyl groups on the pectin molecules and the hydroxyl groups of neighbouring molecules (Abid *et al*., 2017). Pectin Homogalacturonan is responsible for its gelling capacity and the pectin Rhamnogalacturonan (I) region role is to stabilise the gel (Koffi *et al*., 2013). The microstructure and rheology of pectin gels are affected by molecular weight, DE, sucrose content, pH, temperature and $Ca^{2+}$ ion concentration (Abid *et al*., 2017). The gel formation mechanism is by hydrophobic interactions and hydrogen bonds in acidic conditions (pH < 3.4) and low $a_w$ (typically 55% sugar content) (Javanmard *et al*., 2012). The rate at which gel formation takes place is affected by the DE. A higher DE causes a rapid setting. Rapid set pectins (pectin with DE of above 72%) gel at lower soluble solids and higher levels than slow set pectins (pectins with DE of (58-65%) (Sundar Raj *et al*., 2012). The HMP gels with sugar and acid and the LMP requires the presence of divalent

cations for proper gel formation (Sundar Raj *et al*., 2012). HMP and LMP have different gelation mechanisms, however their gelling characteristics are governed by the same macromolecular properties such as size, composition and polymer conformation (Chan *et al*., 2017). The pectin gelling ability depends on its viscosity and solubility, which are normally the measure of its molecular weight, the higher the molecular weight, the higher the viscosity (Abid *et al*., 2017; Chan *et al*., 2017).

### 2.3 Extraction of pectin

Extraction is the most important step in the isolation and recovery of pectin (Chan & Choo, 2013). Pectin is extracted from fruit peels especially citrus; during the extraction of pectin, extractants such as mineral acids, organic acids, enzymes as well as calcium ions are normally used (Guo *et al*., 2017; Lefsih *et al*., 2016). The physicochemical properties of pectin depend on the plant source and extraction conditions selected for isolation and purification of pectin (Chan & Choo, 2013). The different extraction conditions are the type of acid, extraction temperature, time, pH and solvent to sample ratio (Guo *et al*., 2017; Lefsih *et al*., 2017; Oliveira *et al*., 2016; Raji *et al.,* 2016; Denman & Morris, 2015; Li *et al*., 2015; Methacanon *et al*., 2014; Chan & Choo, 2013). However, there are several methods used in the extraction of pectin. The pectin yield increases when temperature increases and pH decreases (Andersen *et al*., 2017).

#### 2.3.1 Extraction methods

The quality of pectin depends on the plant source and extraction conditions (Guo *et al*., 2017). Several extraction methods are commonly used in the extraction of pectin as outlined in Table 4. Compared with other extraction methods, microwave heating extraction reduces the extraction period considerably (Bagherian *et al*., 2011). A fiveteen 1(5) minute microwave heating period is enough to extract almost the same amount of pectin as that obtained from water-based extraction with a three hour extraction period (Srivastava & Malviya, 2011).

Table 4: Different pectin extraction methods

| Extraction method | Functions | Advantages | Disadvantages | References |
|---|---|---|---|---|
| Acid extraction | Organic and mineral acids | Time-saving, reduced extraction time | Use strong acids which are toxic, corrosive and not environmentally friendly | |
| | | Use of organic acids which are natural and non -toxic | Requires high temperatures for extraction | Yu & Sun, 2013 |
| Enzymatic extraction | Protopectinase, Celluclast 1.5 L | Can be performed at mild temperatures (60°C) | Requires longer time for extraction | Vasco-Correa *et al*., 2017 |
| | | More environmentally friendly | | Yu & Sun, 2013 |
| | | Lower extraction temperatures | | Yu & Sun, 2013 |
| Microwave extraction | Microwave and acid or water | Reduced extraction time | | Bagherian et al., 2011 |
| | | Gives better quality of pectin | | Zouambia *et al*., 2017 |
| Direct boiling extraction | Boiling water | Non flammable | Increased extraction time | Bagherian *et al*., 2011 |
| | | Non-toxic | Thermal degradation of pectin | Liu *et al*.,2006 |
| | | Readily available<br>Cheap solvents used | Solvent consuming | Seixas *et al*., 2014 |
| Electromagnetic induction heating extraction | Magnetic field | Excellent protein yield | Cost is very high | Zouambia *et al*., 2017 |
| Soxhlet extraction | Conventional extraction | Can be performed at mild temperatures | Cost is very high | Liu *et al*.,2006 |
| | | | Increased extraction time<br>Thermal degradation of pectin | Yeoh *et al*., 2008 |

### 2.3.1.1 Conventional acid extraction

This is a conventional method of pectin extraction that is carried out at a high temperature of 85°C and prolonged extraction time of 120 min (2 hours) to obtain good pectin yield (Oliveira *et al*., 2016). High temperature accelerates the acidic hydrolysis of pectin side chains which further increase the pectin yield (Chan & Choo 2013). Longer extraction time (2 h) allows more reaction opportunity and carbohydrate polymer need heat to soften its structure for pectin extraction (Liew *et al*., 2015). Due to long extraction period, the pectin undergoes thermal degradation and therefore yield will be affected (Einhorn-Stoll *et al*., 2014). Acids are one of the strongest extracting agents that are used during the pectin extraction process. Acids enable the extraction of insoluble pectin that is tightly bound to the cell matrix of the plant material and result in higher yields (Maria *et a*l., 2015). Pectin is generally enriched in galacturonic acid. Studies have shown the effects of acid on the pectin yield and physicochemical characteristics. The most commonly used acids are citric, acetic, nitric, hydrochloric, phosphoric and sulphuric acids (Sandarani, 2017). An increased acid strength (low pH) plays an important role in increasing the galacturonic acid content. Moreover, acid type and concentration affect the yield, physicochemical and functional properties of pectin (Yapo & Koffi, 2013). During acid hydrolysis, protopectin is split up to produce soluble pectin and cellulose by removing water molecules and at the same time removal of calcium and magnesium ions occur resulting in protopectin being converted to soluble pectin (Devi *et al.*2014). At lower pH, the highly hydrated carboxylate groups are repressed in the larger hydrogen ion concentrations and converted into slightly hydrated carboxylic acid groups. The loss of charge can reduce the repulsion of the polysaccharide molecules which stimulate the gelation properties of pectin giving more precipitated pectin (Devi *et al.,* 2014).

### 2.3.1.2 Enzymatic extraction

The plant cell wall is composed of a network of different polysaccharides including pectin. Cell wall degrading enzymes with minimum pectinolytic activity are used to hydrolyse non-pectin plant cell wall components in enzymatic extraction of pectin (Puri *et al*., 2012). Enzymatic extraction is carried out at a low temperature of 60°C and a prolonged time of 120 minutes (Liew et *al.,* 2015). Enzymatic extraction of pectin is environmentally safe and more effective in terms of pectin yield. Different enzymes such as polygalacturonase, hemicellulose, protease and microbial mixed enzymes, cellulase, α-amylase, celluclast, alcalase and α-amylase and neutrase. Xylase, cellulase, B-glucosidase, endopolygalacturonase and pectinesterase are used in pectin extraction as enzymes can degrade pectin and modify the physicochemical properties of the pectin (Sandarani, 2017). Celluclast enzymes are protein that are thermally sensitive thus requires lower temperatures, high temperatures cause enzymes to lose their function or cause enzyme inactivation (Liew *et al*., 2015). Celluclast is commonly used to

promote the release of pectin substances during the extraction. This enzyme acts as a catalyst to breakdown the cellulose in cellwall and turns them into glucose, cellobiose and higher glucose polymers (Liew *et al*., 2015). Enzymes take time to interact with cellulose and galactan side chains of pectin, thus longer time is needed for celluclast to digest the cellulose in plant wall more completely for more pectin to be extracted (Liew *et al*., 2015).

#### 2.3.1.3 Microwave extraction

Microwave extraction involves dielectric heating of plant molecules through the exposure of microwaves. Dipolar rotation of water is taken place due to the absorbance of microwave energy, the high pressure then modifies the physical properties of the fruit peel tissues, improving the capillary-porous structure of the peel tissues (Oliveira *et al*., 2016). This allows for better penetration of the extracting solvents into tissues (Oliveira *et al*., 2016). Microwave extraction is carried out at a high temperature and a very short time of 15 min. During microwave extraction, there is inactivation of pectin esterase enzyme and destruction of plant skin cells due to rapid heat generation in microwave environment (Sandarani, 2017). Since the pectin esterase interacts with the pectic substances in the fruit peels and reduces their solubility, their inactivation improves the pectin extraction. Moreover, due to the disintegration of parenchyma cells, there is also an increase in specific surface area, which facilitates the water absorption capacity of the plant cell. It has been used to reduce extraction time and energy (Sandarani, 2017, Kratchanova *et al* 2004). During extraction, the addition of acid to the substrate and ethanol precipitation should be done in a short period to avoid acid from breaking down the glycoside and ester linkage since this could affect the molecular weight of pectin and its gelling properties (Hamidon & Abang Zardel, 2017). Moreover, high microwave power accelerates cell rupture by sudden temperature rise and internal pressure increase inside the cells of plant samples, which stimulates the destruction of sample surface and exudation of pectin within the plant cells into the surrounding solvents and increased the extraction yield. Prolonged extraction time can induce pectin digestion, making it difficult to be precipitated by ethanol which lowers the extraction yield (Sandarani, 2017, Maran *et al*., 2013).

#### 2.3.1.4 Soxhlet extraction

Soxhlet extraction is carried out at a low temperature below 60°C and prolonged time of 20 h. A decrease in pectin yield by increasing the extraction time may be due to thermal degradation of the extracted pectin. Galacturonal chain of pectin depolymerizes by a mechanism known as beta elimination, as a result of degradation, the pectin cannot be recovered by precipitation with alcohol (Liew *et al*., 2015).

#### 2.3.1.5 Electromagnetic induction heating

Extraction is carried out at a high voltage of 100 V and short time of 15 minutes (Oliveira *et al*., 2016). Direct induction heating is assisted by magnetic field, the application of an electromagnetic field destroys the cell membrane structure after exceeding a critical value of approximately 1 V of transmembrane potential, and repulsion occurs between the charge carrying molecules that form pores in weak areas of the membrane which cause a drastic increase in permeability (Oliveira *et al*., 2016). During extraction, boiling is homogenous throughout the liquid volume. The homogeneity allows greater heat transfer and thus extraction rate increases (Zouambia *et al*., 2017).

### 2.3.2 Factors affecting the pectin yield

Pectin extraction is a multiple-stage physical-chemical process in which the extraction and hydrolysis of pectin macromolecules from plant tissue and solubilisation take place under the influence of various extraction factors and these factors widely affect quality or yield of extracted pectin (Devi *et al*., 2014).

#### 2.3.2.1 Temperature

Temperature is an important parameter in the extraction of pectin, and is responsible for the expansion and loosening effects during sonication process (Bayer *et al*., 2017). It affects the extraction yield of pectin whereby the extraction yield is increased with the increase in temperature (Bayar *et al*., 2017; Girma & Worku, 2016). However, extreme high temperatures further decrease the extraction yield and due to the high temperatures the pectin will degrade (Hartati & Subekti, 2015; Devi *et al*., 2014). Extraction with maximum temperature induces the hydrolysis of pectin to short-chain sugars which cannot be precipitated using ethanol, thus decreasing the extraction yield (Hamidon & Abang Zardel., 2017). Low temperatures are insufficient in hydrolysis of the protopectin by acids, which will result in low pectin yield (Chan & Choo, 2013). The microwave power level is directly related to the sample quantity and extraction time. However, the power level provides localised heating in the sample, which acts as a driving force to destroy the plant matrix so that the solute can diffuse out and dissolve in the solvent (Maran *et al*., 2014). Therefore, an increase in power level will generally increase the extraction yield and result in shorter extraction time (Maran *et al*., 2014).

#### 2.3.2.2 Time

The most important factor to be considered in the microwave extraction of pectin is the extraction period. Extraction time in microwave extraction is very short and normally varies from a few minutes to 30 minutes (Hartati & Subekti, 2015; Khan *et al*., 2015; Yeoh *et al*.,

2008). There is a positive linear correlation between extraction yield and irradiation time; the longer exposures, the higher the extraction yield as the protopectin naturally present in cells take time to solubilize and go into solution (Girma & Worku, 2016; Hartati & Subekti, 2015). However, a further increase in time leads to a decrease in the extraction yield since the extraction yield only increases with time up to 15 minutes and thereafter extraction yield decreases. Prolonged extraction time can induce the pectin digestion making it difficult to be precipitated by ethanol which lowers the extraction yield. During extraction, the addition of acid to the substrate and ethanol precipitation should be done in a short period (15 minutes) to avoid acid from breaking down the glycoside and ester linkage which could affect the molecular weight of pectin and its gelling properties (Hamidon & Abang Zardel, 2017). The irradiation time is also influenced by the dielectric properties of the solvent and power level of the microwave.

#### 2.3.2.3 pH

pH highly influences the characterisation of the pectin extraction yield (Zaid *et al*., 2016). The extraction yield increases when the pH is low and reduces the molecular weight of pectin (Maran *et al*., 2014) and hydrolysis of the protopectin. An increase in the pH leads to a decrease in pectin extortion yield and can be due to some pectin molecules that can be partially solubilised from plant tissues without degradation in a weak solution (Girma & Worku, 2016). The protopectin is insoluble in water and can undergo acidic or enzymatic hydrolysis to be transformed into soluble pectin (Liew *et al*., 2016) and the presence of calcium and magnesium increases the insolubility of protopectin (Yeoh *et al*., 2008). The pH variables affect the chemical composition during pectin extraction.

#### 2.3.2.4 Acid and acid solution concentration

Different types of acid play an important role in the pectin yield. This may be due to different abilities of acids to penetrate the cell structure of the tissue and come in contact with the pectin substances present on or in between the cell walls and cannot be insoluble pectin substances into soluble pectin (Seggiani *et al*., 2009). The use of sulphuric acid showed the highest pectin yield followed by hydrochloric acid for inorganic acids and citric acid has the highest pectin yield for organic acids. However, the citric acid has better yield than hydrochloric acid and is better than the other acids from an economic as well as environmental point of view (Sayah *et al*., 2014). The organic and non-corrosive nature of citric acid makes it suitable for pectin extraction (Sayah *et al*., 2014).

There is a positive linear correlation between extraction yield and acid solution concentration; the higher the acid concentration, the higher the extraction yield; whereas a further increase

in acid concentration decreases the extraction yield (Hartati & Subekti, 2015). Extraction increases with acid concentration up to 0.5 M of sulphuric acid and thereafter begins to decrease (Hartati & Subekti, 2015). An increase in acid solution concentration volume does not significantly increase the extraction yield and an increase in acid strength decreases the extraction yield. However, the type of acid does not affect the extraction yield (Chan & Choo, 2013; Faravash *et al*., 2007).

#### 2.3.2.5 Solid-liquid ratio

The extraction yield increases with an increased solid liquid ratio up to 1:8 and thereafter the extraction yield starts to decrease (Hartati & Subekti, 2015). Increasing the ration of liquidsolid leads to higher pectin yield. The bigger solid-liquid ratio is implicated in the extraction contact area because the volume of the liquid is bigger. A higher extraction contact area leads to higher extraction efficiency (Hartati & Subekti, 2015). Moreover, further expanding the liquid-solid ratio leads to a decrease in extraction yield. This is due to the dielectric properties of the solvent or acid and water (Hartati & Subekti, 2015). Solvents with high dielectric are the best absorbers of microwave radiation, which heats up tremendously on a long exposure, thus risking the pectin and may lead to a decreasing extraction yield (Hartati & Subekti, 2015).

### 2.4 Physicochemical characterisation of pectin

Characterisation of pectin principally depends on the source as well as the extraction conditions. The physicochemical properties of pectin are influenced by chemical composition and molecular structure of the biopolymer. The moisture content, ash content, equivalent weight, methoxyl content, anhydrouronic acid and degree of esterification are further discussed.

#### 2.4.1 Moisture content

Moisture content measures the amount of water present in a given material (Ruckold *et al*., 2000). Low moisture content of pectin inhibits the growth of microorganisms that can affect the quality of the pectin and affect the storage due to the production of pectinase enzymes (Castillo-Israel *et al*., 2015; Muhmadzadeh *et al*., 2010). Pectin is very hygroscopic, and should be preserved in a closed dry atmosphere (Castillo-Israel *et al*., 2015). The moisture content of quality pectin should be less than 12% as stated by Food Chemicals Codex (2016).

#### 2.4.2 Ash content

Ash content measures the total amount of minerals present within a food (Akubor & Onimawo 2003), the higher the amount of minerals present in food, the higher the ash content. The inorganic impurities in pectin are indicated by the ash content (Girma & Worku, 2016). The

maximum limit of ash content for good quality pectin is considered to be 10% and the minimum limit of ash content is considered to be 0.76% from the view point of gel-formation, lower ash content indicates good quality of pectin (Devi *et al*., 2014). The ash content indicates the purity of pectin, the lower the ash content, the higher the purity of pectin (Castillo-Israel *et al*., 2015). The ash content differs depending on the methodology and nature of fruit used during extraction of pectin.

### 2.4.3 Equivalent weight

Equivalent weight is the total content of non-esterified galacturonic acid in the pectin molecular chains (Ranganna, 1995). It is used for calculating the anhydrouronic acid content and the degree of esterification. Equivalent weight differs depending on the method source and nature of the fruit used for extraction (Devi *et al*., 2014). The increased or decreased equivalent weight can also depend on the amount of free acid, lower equivalent weight is due to partial degradation of pectin, (Devi *et al*., 2014). Moreover, increased and decreased equivalent weight depends on the amount of free (non-esterified) galacturonic acid, higher equivalent weight will have a higher gelling effect (Devi *et al*., 2014).

### 2.4.4 Methoxyl content

Methoxyl content is the number of moles of methyl alcohol in 100 moles of galacturonic aid, it is an important factor in controlling the setting time of pectins, sensitivity to polyvalent cations and their usefulness in the preparation of low solid gels, fibres and film. It is determined by saponification of the pectin and titration of liberated carboxyl groups (Devi *et al*., 2014). Methoxyl content influences the dispersability of pectin in water, higher methoxyl is more dispersible in water than low methoxyl pectin (Castillo-Israel *et al*., 2015). Methoxyl content of extracted pectin varies from 0.2–12% depending on the source and method of extraction (Castillo-Israel *et al*., 2015; Aina *et al*., 2012). Methoxyl content of commercial pectin varies from 8–11% and can form high sugar gels (>65% sugar), whereas low methoxyl pectins (less than 7%) can form gels with lower sugar concentrations (Castillo-Israel *et al*., 2015).

### 2.4.5 Anhydrouronic acid

Anhydrouronic acid (AUA) determines the purity of pectin due to the presence of proteins, starch and sugars in the precipitated pectin and degree of esterification and also evaluates the physical properties of pectin (Castillo-Israel *et al*., 2015). The content of AUA of the extracted pectin is suggested to be not less than 65% (Food Chemicals Codex, 2016). Pectin which is partly esterified polygalacturonide contains 10% or more of organic materials composed of arabinose, galactose and other sugars. The higher the galacturonic acid, the lower the ash content, since both govern the purity of pectin (Castillo-Israel *et al*., 2015).

### 2.4.6 Degree of esterification

The degree of esterification (DE) is the ratio of esterified galacturonic acid groups to the total galacturonic acid groups present in pectin. It is an important factor that determines the gel formation of pectin (Sundar Raj *et al*., 2012). It is the sum of degree of methylation and degree of acetylation. DE of extracted pectin varies depending on the source, type of acid used and method of extraction. Pectins are classified as rapid set (DE >72%) and slow set (DE 58 – 65%). This describes the rate of gel formation (Saha *et al*., 2013). Commercial DE values for HMP range from 60-75% and LMP ranges from 20-40% (Leong *et al*., 2016).

## 2.5 Gelling properties and its applications

The most important role of pectin is based on its ability to form gels, gelling is the formation of a three-dimension (3D) network of polymer chains with solvent and solutes trapped within (Chan *et al*., 2017). Gel formation is caused by hydrogen bonding between free carboxyl groups on the pectin molecules and the hydroxyl groups of neighbouring molecules (Abid *et al*., 2017; Sundar Raj *et al*., 2012). Pectin homogalacturonan is responsible for its gelling capacity and the pectin Ramnogalacturonan (I) region role is to stabilise the gel (Koffi *et al*., 2013). The microstructure and rheology of pectin gels are affected by molecular weight, DE, sucrose content, pH, temperature and $Ca^{2+}$ ion concentration (Abid *et al*., 2017; Khan *et al*., 2014). The gel formation mechanism is by hydrophobic interactions and hydrogen bonds in acidic conditions (pH < 3.4) and low water activity (typically 55% sugar content) (Javanmard *et al*., 2012). The rate at which gel formation takes place is affected by the DE, a higher DE causes rapid setting. Rapid set pectins (pectin with DE of above 72%) also gel at lower soluble solids and higher levels than slow set pectins (pectins with DE of (58-65%) (Sundar Raj *et al*., 2012). The HMP gels with sugar and acid and the LMP requires the presence of divalent cations for proper gel formation (Sundar Raj *et al*., 2012). HMP and LMP have different gelation mechanisms. However, their gelling characteristics are governed by the same macromolecular properties such as size, composition and polymer conformation (Chan *et al*., 2017). The pectin gelling ability depends on its viscosity and solubility which are normally the measure of its molecular weight; the higher the molecular weight, the higher the viscosity (Abid *et al*., 2017; Chan *et al*., 2017).

## 2.6 Uses of pectin in food

Pectins have always been a natural constituent of human foods. It has been recommended by the FAO/WHO committee that pectin (Codex Alimentarius 2005) is a safe food additive with

no limit on acceptable daily intake (Chan *et a*l., 2017)). Pectin is widely used as a gelling agent, thickener, texturiser, emulsifier, and a stabiliser in some foods. The pectin molecules are polar and non-polar regions that enable them to be incorporated into different food systems (Tyagi *et al*., 2015). The function of pectin molecule is determined by some factors, including the degree of esterification and pectin grades. Pectin grades are based on the number of sugar parts that one part of pectin will gel to an acceptable firmness under standard conditions of pH 3.2 to 3.5, sugar 65 to 70% and pectin limit of 1.5 to 2.0% (Cancela *et al*., 2005).

### 2.6.1 Jam and Jellies

The major food items in which a large amount of pectins are used are jam and jelly. Jam preparation requires brief cooking of the fruit to liberate juice and pectin through conversion of proto pectin to soluble pectin. Depending upon the conditions, additional pectin may be added at any point during preparation. Pectin is added as a dry powder mixed with sugar in a solution (Tyagi *et al*., 2015).

### 2.6.2 Conserves

They are products that do not contain a sweetener but fruit juice or fruit concentrate that contains a sweetener. Their soluble solid contents are lower than products containing a sweetener. They are highly preferable by consumers because they do not contain any added sugar. The total soluble solid content of conserves is 55 to 62%. At the upper level, a rapidset HM pectin is used, while at the lower level, an LM pectin is added to give the desired mouthfeel and body of the products (Tyagi *et al*., 2015).

### 2.6.3 Bakers' jellies

Pectin is used to make jellies that are applied to prepare bakery products. HM pectins are thermally stable and are used to make jellies that are placed in the batter or dough and baked product. Fibre entanglements will further emphasise the gel structure if the fibre content is increased. LM pectin has a wide application in bakery jam and jelly production. The use of LM pectin requires a large amount of pectin in the formula, compared with HM pectin, to the exact firmness (Tyagi *et al*., 2015).

### 2.6.4 Confectionery products

Different flavored candies are produced using HM pectin. Artificial cherries can be made using pectin, where a synthetic medium is produced to control setting conditions. Pectin is also used

in edible coatings for inhibiting lipid migration in confectionery products (Tyagi *et al*., 2015; Tikhomirova *et al*., 2012).

### 2.6.5 Frozen barriers

Pectin is used in frozen foods to prevent crystal growth, syrup during thawing, and to improve shape (Tyagi *et al*., 2015). Ice-cream factories use pectin for ice-cream production. They use LM pectins to improve the texture and quality of ice creams. Pectin helps to improve the texture of frozen foods by controlling the ice crystal size in them (Tikhomirova *et al*., 2012. Pectin is also used in the different gelled pudding desserts, where there is the mixing of fruit syrup containing pectin with cold milk. This type of dessert can be prepared without refrigeration because of the use of pectin (Tyagi *et al*., 2015; Tikhomirova *et al*., 2012).

### 2.6.6 Beverages

Pectin can also be used as a beverage-clouding agent (Tyagi *et al*., 2015). A certain mouthfeel is not present in the conventional soft drinks, therefore the loss of mouthfeel can be restored by the addition of HM pectin 0.05 to 0.10% (Tyagi *et al*., 2015; Tikhomirova *et al*., 2012).

### 2.6.7 Barbecue sauce

Low methoxyl pectin is added to the barbecue sauces due to its flavor release attributes and texture. The LM pectin and calcium content in the mixture determines the product's final consistency and texture (Tikhomirova *et al*., 2012).

## 2.7 Pharmaceutical application of pectin

Pectin use in the pharmaceutical industry is growing, but smaller amounts are used as compared to food industry. It is mainly used as an excipient, thickener, stabiliser, film coating and binding agent. Pectin is used as a detoxifying agent because of its ability to bind positively charged heavy metal ions it is used to remove toxic metal ions, such as lead and mercury, from the gastrointestinal tract and respiratory organs of individuals who have been poisoned with heavy metals. Moreover, it is also used as hemostatic device to control hemorrhage or localized bleeding as it can delay blood clotting (Wonago, 2016).

Pectin is commonly used as a drug carrier, and as an ingredient in controlled and sustained drug release formulations (Wong *et al*., 2011). Delivery systems designed to release drugs in the colon need to protect the drug during transit through the stomach and small intestine. The resistance of pectin to digestion in the upper gastrointestinal tract has allowed its use in the development of colon-specific drug delivery systems (Liu *et al*., 2003). Tablets coated with

pectin film, pellets and micro particles made from pectin-based matrices are commonly used to successfully deliver drugs to the colon (Wonago, 2016; Wong *et al*., 2011).

### 2.8 Summary of literature findings

The high value of food-grade pectin has led to research into methods for extracting pectin that increase the yield and quality. Conventionally, pectin is extracted from citrus peels in heated water (85 °C) at a pH range of 1–3 for a time of less than 30 minutes. Manabe *et al*. (1988) used microwave energy for extracting pectin from mandarin orange pulp and reported that about 5% more pectin was extracted in 15 minutes using microwave energy than could be extracted by conventional methods in 60 minutes. Furthermore, after 10 minutes, when 95% of the pectin has been extracted, microwave-extracted pectin had higher relative viscosity, anhydrogalacturonic content and degree of esterification than pectin extracted by conventional heating. The reported results indicate that rapid heating with microwave energy has the potential to increase yield and quality of extracted pectin (Manabe *et al* 1988). Hartati *et al.* (2015) reported that microwave-assisted extraction of pectin from watermelon rind showed high extraction yields. The highest extraction yield of 11.25% was obtained at 15 minutes, 0.5 M of sulphuric acid solution, and the solid-liquid ratio of 1:8. Maran *et al.* (2014) reported that optimum microwave-assisted extraction conditions for the highest pectin yield from waste *C. lanatus* fruit rinds (25.79%) were obtained with a microwave power of 477 W, the irradiation time of 128 s, pH of 1.52, solid–liquid ratio of 1:20.3 g/ml. Yeoh *et al*. (2008) reported that the highest total amount of pectin yield was 5.27% at 15 minutes of microwave extraction, although the highest amount of material per unit time (%/minutes) was obtained after 5 minutes, which was the same amount as that extracted using Soxhlet extraction for 3 h.

## CHAPTER 3: MATERIALS AND METHODS

### 3.1 Site and sample collection

Fresh prickly pear (PP) fruits (orange, purple and green) as shown in Figure 7 were harvested at a physiological maturity stage from Bothlokwa Mphakane village in Limpopo Province. Fruits were brought to the Food Science and Technology Department, University of Venda, South Africa and stored in a cold room at 5°C.


Figure 7: Prickly pear fruits a. orange, b. purple and c. green coloured varieties

The prickly pear fruits were harvested from a population of fifty trees of which were divided into four clusters with two trees in each cluster and fifty fruits were picked randomly from each tree, a total of 200 fruits were harvested for the experiment. Thereafter, 10% of the 200 fruits were carefully selected according to a similar maturity ripening stage for laboratory samples and it was a single sampling. The harvesting of the fruits took place in the morning as it is the best time to harvest.

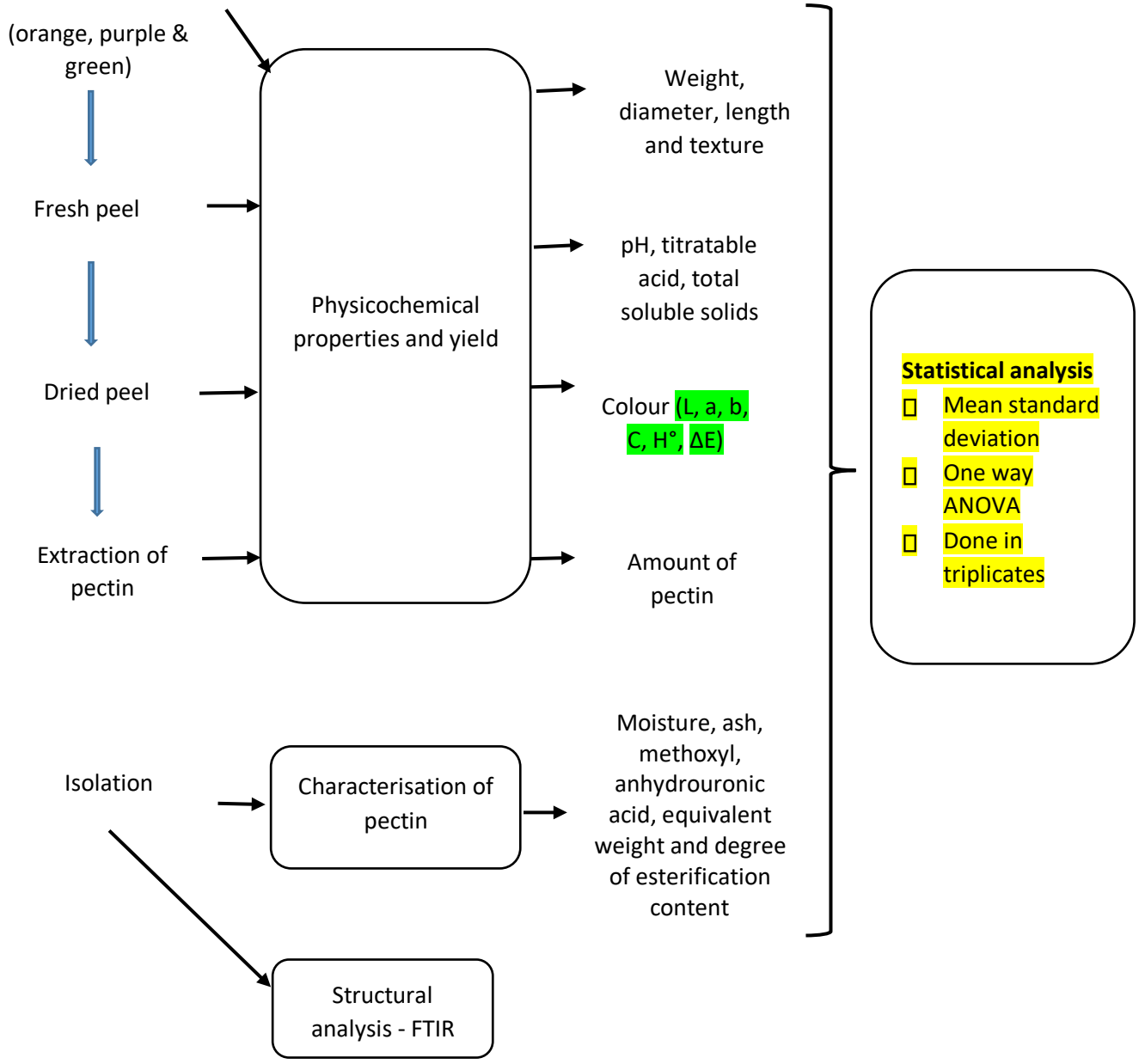
### 3.2 Experimental design

The experimental design selected for this study was a randomized complete block design (RCBD) with two independent treatments: microwave power levels and pH. All measurements were made in triplicate. The schematic representation of experimental flow is presented in Figure 8.

**Process flow**

Objectives

**Experimental parameters**




**Objectives**

Prickly pear fruits

Figure 8: Schematic illustration of the experimental flow

### 3.3. Experimental setup

The experimental setup summarises the preparation of the prickly pear peel powder and the extraction of pectin as shown in Figure 9.

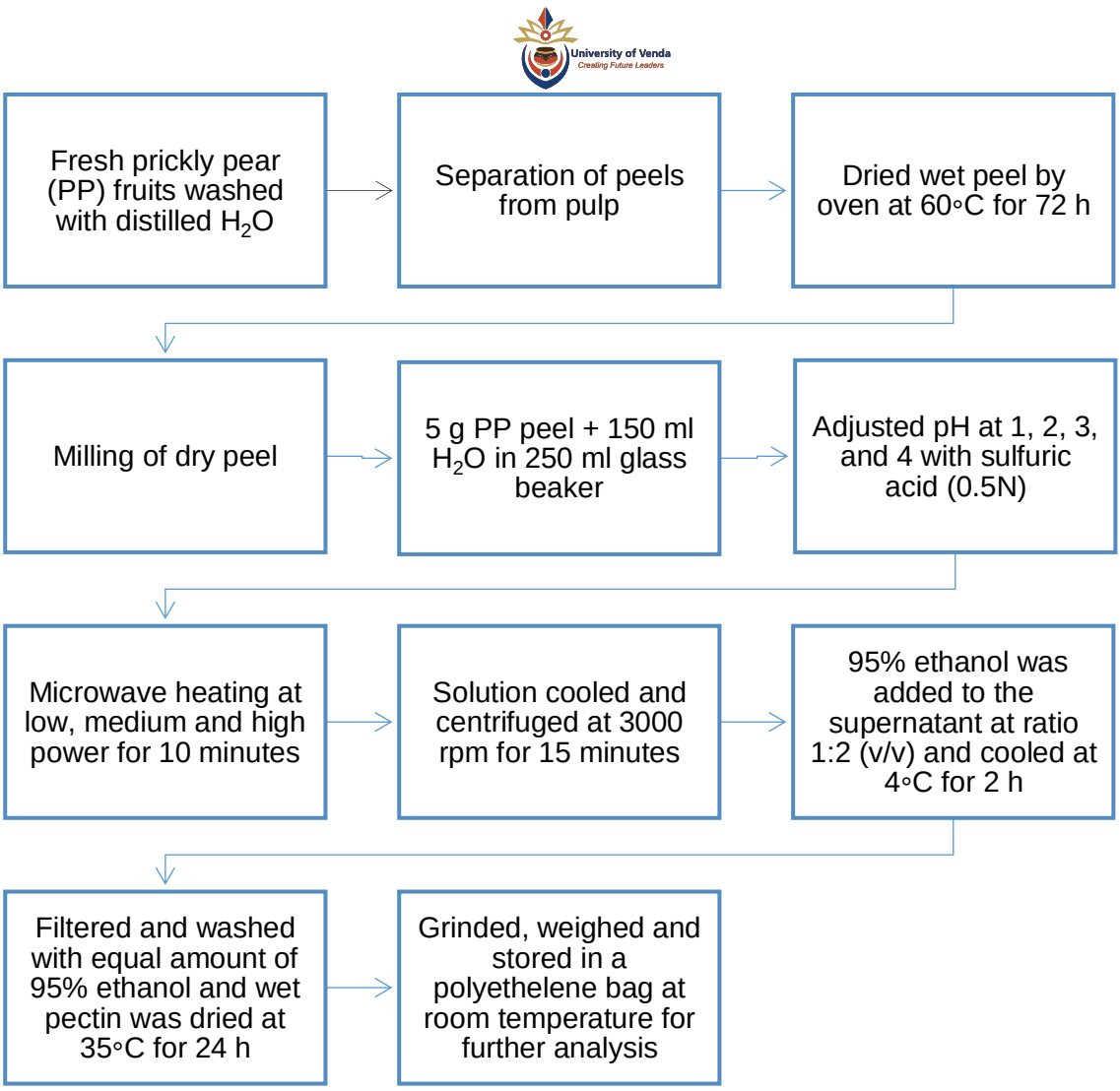



Figure 9: Schematic diagram of experimental setup (Wonago 2016; Castillo-Israel *et al*., 2015)

### 3.4 Physicochemical properties of prickly pear fruit and peel

#### 3.4.1 Physical properties

The length, width of the fruit and peel thickness were measured using a vernier caliper (Zarei *et al*., 2011)**.** The weight was measured using a weighing balance.

Texture

The textural property of hardness was measured using Texture Analyser (model TA.XT.Plus, from Stable Micro Systems, UK) with the mode of measure force in compression using the P/75 probe. The determination parameters were pre-test speed = 1.0 mm/s, test speed = 0.02 mm/s, post-test speed = 10.0 mm/s, target mode = distance, distance = 16 mm, trigger type = 100 g (force) (Kotwaliwale et al., 2007). Hardness was expressed in Newtons (N).

Colour

Colour parameters were measured using the colorimeter (HunterLab ColorFlex, USA) (L*, a*, b*) system. The instrument was calibrated with a standard white and black plate. The parameter L* represents the brightness, a* represents redness (+) and greenness (-) and b* represents yellowness (+) and blueness (-). Three measurements were made on the surface of fresh PP peels and dried PP peel powder. From the colour values, Chroma (C), hue angle (h) and total colour change (ΔE) were calculated using following equations (Maskan 2001):

$$\text{Chroma} = \sqrt{(a)^2 + (b)^2} \quad (1)$$

$$\text{Hue angle} = \tan^{-1}\left(\frac{b}{a}\right) \quad (2)$$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (3)$$

where: ΔL, Δa, Δb are the difference between fresh and dried values.

### 3.4.2 Chemical properties

pH

The pH of fruit pulp was determined by the use of a digital pH meter following the AOAC (2002) standard procedure.

Total Soluble Solids (TSS)

A digital refractometer (Atago, Japan) was used to measure the TSS and the results were directly recorded following AOAC (2002) standard procedure. The instrument was calibrated by putting a drop of distilled water on the refractometer prism. A drop of the fruit pulp was placed on the refractometer prism and results expressed as ºBrix and readings were recorded in triplicates.

Titratable Acid

Titratable acid (TA), as % citric acid, was determined by using the direct titration method, 20 ml of fruit pulp was placed in a 100 ml conical flask and added 3 drops of phenolphthalein indicator. Thereafter, the sample was titrated with 0.1N NaOH to an endpoint where the colour changed to pink and the volume of NaOH used was recorded. TA was calculated using equation 4:

$$\%\text{citric acid} = \frac{(\text{ml of NaOH})(0.1\text{N NaOH})(0.064)(100)}{\text{ml of sample}} \quad (4)$$

### 3.5 Prickly pear peel powder preparation

Prickly pear (PP) fruits were thoroughly cleaned with distilled water and peeled to separate the peels from the pulp. The peels were then sliced (4 – 5 cm thickness) and placed on trays divided into 3 batches according to the variety. The three batches of PP peels were immediately dried using the oven (Ecotherm, 240L digital oven, South Africa) at 60°C for 72 h. After drying, the samples were cooled in a desiccator and then the samples were milled using a milling machine( Polymix PX-MFC 90 D, Kinematic AG, Switzerland) and sieved through a 500 µm sized aperture sieve and stored in polyethylene bags in a desiccator until further analysis (Castillo-Israel *et al*., 2015).

### 3.6 Extraction process of pectin from prickly pear peels

Extraction was carried out using sulphuric acid. Sample of 5 g of PP peel powder and 150 ml of distilled water was added into a 500 ml glass beaker, 0.5 N sulphuric acid was added to adjust the pH levels of 1, 2, 3 and 4. The solution was placed and heated in a microwave (Logik 700 W) for 10 minutes at low (65°C), medium (110°C) and high power (190°C) levels respectively. The solution was cooled and centrifuged (Universal 320R, Herttich, Germany) at 3000 rpm for 15 minutes. Ethanol 95% was added to the supernatant at a ratio of 1:2 (v/v) and was allowed to cool at 4°C for 2 h. To separate the coagulated pectin, the sample was filtered and washed with an equal amount of 95% ethanol. The wet pectin was dried in an oven at 35°C for 24 h. After drying, the pectin was weighed and the percentage yield was calculated using equation 5 (Altaf et al., 2015).

$$\text{Pectin yield } (\%) = \frac{P}{Bi} \times 100 \quad (5)$$

Where: P is the amount (g) of the pectin extracted and Bi is the initial amount of PP dry peel (5 g).

### 3.7 Characterisation of prickly pear peel pectin

#### 3.7.1 Moisture content determination

Moisture content was determined following the AOAC (2002) standard. A dried clean metal dish was weighed and 2 g of pectin sample was placed on the dish. The sample was dried in an oven at 105°C for overnight, and cooled in a desiccator and weighed. The moisture content was determined using equation 6.

$$\text{Moisture content } (\%) = \frac{\text{weight of dried sample}}{\text{weight of pectin}} \times 100 \quad (6)$$

### 3.7.2 Ash content determination

Pectin sample of 2 g was weighed, placed into tared crucibles and in a muffle furnace for 4 h at 550°C, and cooled in a desiccator and weighed. The ash content was determined according to AOAC (2002) by applying equation 7.

$$\text{Ash content } (\%) = \frac{\text{weight of ash}}{\text{weight of pectin}} \times 100 \quad (7)$$

### 3.7.3 Equivalent weight

Pectin sample of 0.5 g was taken into a 250 ml conical flask and 5 ml of ethanol 95% was added. Sodium Chloride of 1 g was added in 100 ml of distilled water, lastly 6 drops of phenol red indicator were added against 0.1 N NaOH. Titration point was indicated by a purple colour. This neutralised solution was stored for further determination of methoxyl content. The equivalent weight was calculated by applying equation 8 (Rangannas 1995).

$$\text{Equivalent weight (EW)} = \frac{\text{weight of sample (g)}}{\text{mL of alkali} \times \text{N of alkali}} \times 100 \quad (8)$$

### 3.7.4 Methoxyl content

A solution of 25 ml of 0.25 N NaOH was added to the neutralised solution from equivalent weight titration, the solution was stirred and kept for 30 minutes at room temperature in a flask with a stopper. A solution of 25 ml of 0.25 N HCl was added and titrated with 0.1 N NaOH until the colour changed to purple (pH:7.5). The methoxyl content was calculated using equation 9 (Rangannas 1995):

$$\text{Methoxyl content } (\%) = \frac{\text{mL}}{\text{weight of sample}} \frac{\text{alkali} \times \text{N alkali} \times 3.1}{} \quad (9)$$

### 3.7.5 Total anhydrouronic acid

Estimating the content of anhydrouronic acid (AUA) is crucial for determining the purity, degree of esterification and the physical characteristics of the extracted pectin. AUA was calculated by making use of the equivalent weight and methoxyl content by applying equation 10 (Mohamed & Hasan 1995):

$$\text{AUA}(\%) = 176 \frac{}{} \frac{}{} \times 0.1z \times 100 + 176 \times 0.1y \times 100 \quad (10)$$

where z = ml of NaOH from equivalent weight

Y = ml of NaOH from Methoxyl content

W = sample weight (g)

### 3.7.6 Degree of esterification

The pectin degree of esterification (DE) was determined according to equation 11 (Shaha *et al*., 2013).

$$DE = \frac{176 \times MeC\ (\%)}{31 \times AUA(\%)} \times 100 \quad (11)$$

Where: % MeC = Methoxyl content, % AUA = Anhydrouronic acid content.

## 3.8 Structural analysis of prickly pear peel pectin using Fourier Transform Infrared (FTIR) spectra

The FTIR spectrum was used to acquire information on chemical structure of the extracted and commercial pectin. Fourier transform infrared data were obtained using Spectrum 65 FTIR (Bruker) in the range 400-4000 $cm^{-1}$, scanning rate at 32 at resolution rate 4 $cm^{-1}$ in the Department of Chemistry in the School of Life Sciences, University of Venda.

## 3.9 Statistical analysis

The experimental data for this study were captured in Microsoft excel and all analysis was done in triplicate. The data obtained was analysed and interpreted by one-way analysis of variance using SPSS Statistics version 24 (IBM, 2017). Values were expressed in mean ± standard deviation and significance level was set at $p < 0.05$.

# CHAPTER 4: RESULTS AND DISCUSSION

## 4.1 Physicochemical properties of prickly pear fruit and peel

In this study, the prickly pear (PP) physical and chemical characteristics were investigated in orange, purple and green fruit and peel. The physicochemical properties of fruits are represented in Table 5.

Table 5: Physical and chemical properties of orange, purple and green prickly pear fruit and peel

| | Prickly pear | | |
|---|---|---|---|
| Properties | Orange | Purple | Green |
| Weight (g) | 222.58 ± 26.02[b] | 154.59 ± 14.03[a] | 153.58 ± 12.63[a] |
| Length (cm) | 8.86 ± 0.39[b] | 7.62 ± 0.59[a] | 7.88 ± 0.14[a] |

| | | | |
|---|---|---|---|
| Width (cm) | 6.70 ± 0.37[b] | 5.90 ± 0.20[a] | 6.02 ± 0.36[a] |
| Peel thickness (cm) | 0.48 ± 0.08[ab] | 0.40 ± 0.10[a] | 0.54 ± 0.05[b] |
| Total soluble solids (°Brix) | 14.47 ± 0.02[b] | 13.02 ± 0.03[a] | 14.45 ± 0.13[b] |
| pH | 5.04 ± 0.38[a] | 5.48 ± 0.40[b] | 6.04 ± 0.21[c] |
| Titratable Acid (as % citric acid) | 0.06 ± 0.01[a] | 0.03 ± 0.01[a] | 0.03 ± 0.01[a] |

Mean ± SD. Different letters within a row indicate significantly different ($p < 0.05$)

Dehbi *et al*. (2014) reported that the fruit size is affected by the seed content and weight. The average PP fruit weight varied from 153.58 to 222.58 g as shown in Table 5. The orange PP fruit had significantly ($p < 0.05$) larger size compared to that of purple and green PP. The fruit size differed significantly among varieties. There are many PP varieties and they are identified through size, shape and colour. These results are in line with the study by Dehbi *et al*. (2014) who pointed out that size, weight and length of PP fruits differs significantly among cultivars and genetic constitution. For this reason, it is necessary to correlate PP size to the yield of pectin. Moreover, PP variety with higher fruit size had lower pectin yield whereas variety with lower fruit size had higher pectin yield, therefore there was no positive relationship between fruit size and pectin yield. Canteri-Schemin *et al*. (2005) pointed out that apple pomace pectin yield is higher (9.73%) when smaller flour particle size is used and lower (6.13%) when larger size is used. Therefore, the small fruit size contained high pectin content available in the middle lamella of the plant cell wall.

Sundar Raj *et al*. 2012 stated that pectin is mostly found in the peel of fruits, where it is available in high concentration in the middle lamella of plant cell walls. Furthermore, Lira-Ortiz *et al*. (2014) reported that the cell wall material from the peel of PP represents reasonable amounts of pectin substance. The average peel thickness ranged from 0.40 to 0.54 g (Table 5). Green PP peel thickness was significantly ($p < 0.05$) higher than purple PP peel, however, green PP peel thickness was not significantly ($p < 0.05$) higher than the orange PP peel. The variations in peel thickness may be attributed to metabolic changes during ripening and the variety of the fruit indicating that the green PP degree of maturity was low compared to those of purple and orange PP fruit. The purple PP variety with low peel thickness obtained high (13.8%) pectin yield, whereas green PP with high peel thickness obtained low (10.0%) pectin yield. The results obtained show that peel thickness determines the amount of pectin present. The peel thickness is of significance on the yield, the smaller the peel thickness the greater the pectin yield (Nunes *et al*., 2017). Therefore, there was a negative correlation between pectin yield and peel thickness.

PP fruits are generally considered as a low acid fruit (pH > 4.5) (Marran & Manikandan, 2012). The average pH and acidity values ranged from 5.04 to 6.04, 5.58 and 0.0.3 to 0.06% on orange, purple and green PP, respectively. The green PP had high pH and low acidity, whereas the orange had low pH and high acidity values. A significant difference ($p < 0.05$) on the pH values was observed, however no significant difference ($p < 0.05$) was observed on the acidity amongst the orange, purple and green PP fruits. Factors such as fruit variety and maturity stage contribute to the pH and acidity value variations. An inverse correlation between pH and acidity was observed, where the increased pH led to decreased acidity. The fruit which has less pH or high acidity may yield the highest of pectin extract. Pectin extracted in acidic conditions (low pH) result in high pectin yield. This might be due to the use of less acid in a fruit that already contains high acid which gives less damaging effect on the pectin extraction (Yapo, 2009).

The high concentration of total soluble solids may also mean a high amount of pectin concentration in the fruit. The average total soluble solids (TSS) concentration obtained ranged from 13.02 to 14.47°Brix on the three PP varieties. The TSS of the orange variety was higher than that of the green and purple varieties. The TSS of orange and green PP were significantly ($p < 0.05$) higher than that of purple PP. The increased TSS on orange PP was due to the hydrolysis of starch into sugars during the maturation process. Mehdi *et al.* (2011) reported that the concentration of TSS increases significantly during fruit ripening. The results showed that there is a negative correlation between the TSS content and the pectin yield, the lower the TSS content, the higher the pectin yield. The purple PP had low TSS (13.02°Brix) and high (13.8%) pectin yield, whereas orange PP had high TSS (14.47°Brix) and low pectin yield (9.8%). Therefore, the TSS content determines the pectin yield.

Pectins are water-soluble carbohydrates. Pectin is present in most fruits, but the amount and quality differ with the fruit type and its degree of maturity. The texture is one of the simplest methods to determine fruit ripeness. For this reason, it is necessary to correlate the texture with the amount of pectin yields. One of the important changes taking place during fruit ripening is the formation of pectin from protopectin, which contributes to the softening of fruit flesh. Furthermore, during ripening of fruit pectin is converted into pectic acid, and into other substances by enzymatic action that takes place. Van Buggenhout *et al*. (2009) pointed out that pectin changes play a significant role in textural characteristics of fruits. The textural property firmness of the orange, purple and green PP varieties is represented in Figure 10.

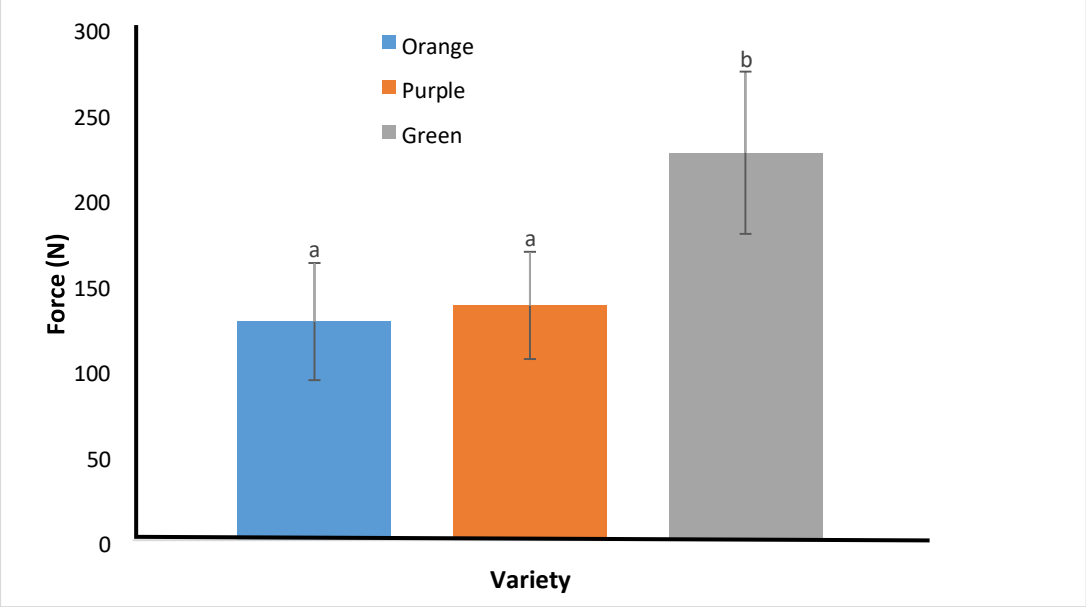



Figure 10: Firmness of prickly pear fruit.

The average force was 128.07, 137.59 and 227.04 N on orange, purple and green PP fruits. The green variety had significantly harder ($p < 0.05$) purple and orange PP fruits as shown by the force required to break the surface of the green PP fruit. This may be possibly due to minimal degree of maturity where changes in the cell wall structure (geometric properties) and chemical composition of the plant cell wall and middle lamella during the development and ripening of the fruit (Nyorere & Uguru, 2018). Van Buggenhout *et al.* (2009) further noted that fruit texture is determined by the cell wall mechanical characteristics in combination with the cells internal pressure and intracellular adhesion. Ying *et al*. (2011) reported that there is a positive correlation between ripening and firmness, the fruit firmness decreases as fruits become more mature and rapidly decreases as they ripen. The results obtained show that the orange PP was more mature, hence less firmness (force) as compared to green PP fruit. Moreover, loss of firmness is as a result of membrane disruption, solubilisation and depolymerisation of pectic polymers that are involved in cell wall adhesion. Therefore, the firmer the fruit, the higher the pectic polymers. However, the results showed that there was no relationship between firmness and pectin yield, the green PP had higher firmness but obtained lower pectin yield (10.0%) as compared to purple PP pectin yield of 13.8%.

Colour

Colour is considered as one of the significant quality components of fresh fruits. It is obtained from natural pigments, although many pigments may degrade as the plant proceeds through maturation, ripening and drying. For this reason, it was of importance to study the colour as it may influence the colour of pectin extracted from the peels. In this study, convective air drying method was used, as it is one of the traditional, economic and most controlled method. The

colour of prickly pear peel (PPP) is shown in Table 6. The colour of fresh PPP was lighter and tended to be darker in purple and green, but less in orange when compared to dried peels. A similar manner of significant differences ($p < 0.05$) was also seen on the colour values of green and orange varieties but to a greater extent, as shown by the higher a* and b* values. The main reason for the changes in the colour of both colours-containing samples is the reddishbrown colour on purple variety, especially the lower a* value.

Colour is a quality parameter in any food product, which determines the consumer's liking or not. The colour of PPP after drying still resembled the original colour of the fresh fruit. This could be due to betanins that are affected by heat during the drying of PPP. The darker colour change could be attributed to the high temperature of drying that enhanced improving the isomerisation of betanin. The result obtained is in close agreement with those reported by Herbach *et al*. (2004), who reported an increase in the isobetanin/betanin ratio from 0.25 of untreated to 0.28, 0.46, 0.52 and 0.57 after heating red beetroot juice at 85°C for 1, 3, 5, and 8 h respectively. Herbach *et al*. (2004) suggested that betanin isomerisation occurred by rearranging the carboxylic group (-COOH) and H at the C15 position. Betalains are betalamic acid immonium conjugates with either cyclo-dopa, producing violet-red or purple betacyanins, or amino acids and amines, producing yellow betaxanthins (Herbach *et al*., 2004). Of the major betalains in PPP, betanin and isobetanin (5-O-β-glucosides of betanidin and isobetanidin) are betacyanins, whereas vulgaxanthin I is a betaxanthin (Herbach *et al*. 2004). There are several factors affecting betalains stability, such as high temperature, high water activity, light, oxygen and pH above 7 or below 3 promotes degradation of betalain. However, in this study it could not be due to pH since the PP fruit pH ranged from 5.04 to 6.04. Kgatla *et al*. (2011) studied the effect of heat processing on PP juice and showed that the light and bright red-purple colour of PP was influenced by juice treatments. Light and bright colours are the result of betalian pigments that maintain colour stability throughout processing, and also give the juice an appealing colour. Colour variations were caused by modifications in betalian pigments as well as furfural and hydroxyl-methylfurfural compounds development (Kgatla *et al*., 2011). The sample of heat-treated juice was darker (low CDML* value).

The total colour difference (ΔE*) is a combination of L*, a* and b* values which characterise the colour variant in foods that occurs during processing. The ΔE* values for orange, purple and green PPP are shown in Figure 11.

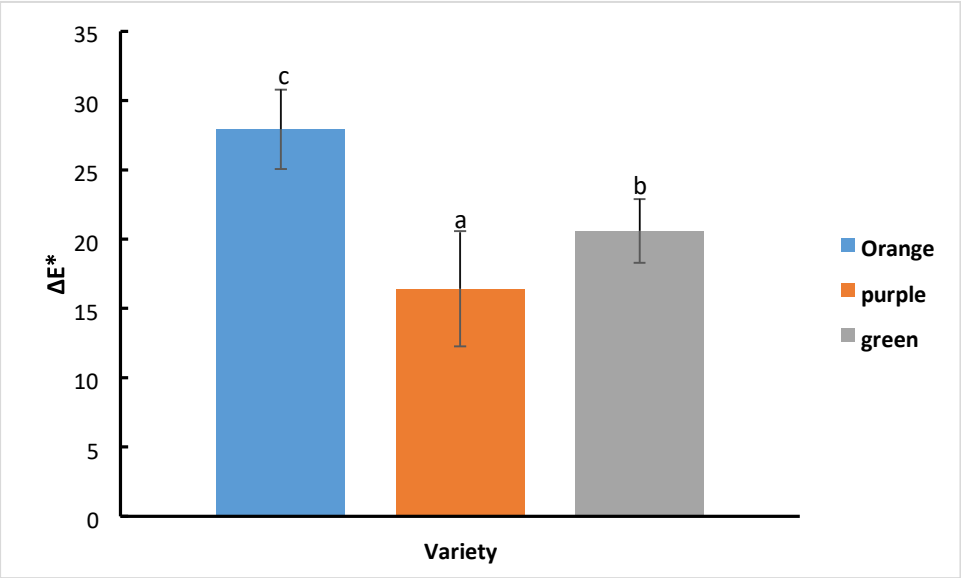



Figure 11: Total colour difference (ΔE*) for dried orange, purple and green prickly pear peel

On average, the ΔE* values were 16.42, 20.59 and 27.92 on orange, purple and green PPP. It can be observed that the purple variety showed a significantly low colour difference of 16.42, followed by green at 20.59. The orange PPP variety showed the highest colour difference at 27.92. The results showed that the overall colour change was affected by the non-enzymatic browning reaction, and also by the betalains pigment destruction on purple PP, chlorophyll pigments on green PP that are stable to heat, and carotenoid pigments on orange PP that are strongly affected by heat.

Table 6: Colour quality of orange, purple and green varieties of prickly pear peels and powder

| | Orange | | | Purple | | | Green | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Fresh (O)** | **Fresh (I)** | **Dried** | **Fresh (O)** | **Fresh (I)** | **Dried** | **Fresh (O)** | **Fresh (I)** | **Dried** |
| **L*** | 39.95 ± 2.26$^{b}$ | 31.19 ± 4.42$^{a}$ | 60.69 ± 2.23$^{c}$ | 33.56 ± 3.61$^{b}$ | 21.89 ± 2.14$^{a}$ | 47.82 ± 2.88$^{c}$ | 49.46 ± 1.53$^{a}$ | 54.60 ± 3.38$^{b}$ | 66.89 ± 2.56$^{c}$ |
| **a*** | 27.67 ± 3.76$^{b}$ | 33.12 ± 5.49$^{b}$ | 10.42 ± 1.86$^{a}$ | 23.12 ± 5.19$^{a}$ | 31.12 ± 3.94$^{b}$ | 19.64 ± 0.83$^{a}$ | -4.45 ± 4.45$^{a}$ | -6.98 ± 0.49$^{a}$ | 3.32 ± 1.67$^{b}$ |
| **b*** | 28.31 ± 2.27$^{a}$ | 30.86 ± 3.56$^{ab}$ | 33.56 ± 3.24$^{b}$ | 8.05 ± 2.13$^{a}$ | 9.21 ± 2.59$^{a}$ | 14.33 ± 1.98$^{b}$ | 41.71 ± 5.19$^{b}$ | 38.07 ± 2.79$^{ab}$ | 36.12 ± 0.32$^{a}$ |
| **Chroma** | 40.26 ± 5.23$^{ab}$ | 45.29 ± 6.36$^{b}$ | 35.15 ± 3.64$^{a}$ | 24.61 ± 4.82$^{a}$ | 32.48 ± 4.82$^{b}$ | 24.38 ± 0.54 | 42.16 ± 4.92$^{b}$ | 38.71 ± 2.71$^{ab}$ | 36.30 ± 0.35$^{a}$ |
| **Hue** | 46.71 ± 3.78$^{a}$ | 43.15 ± 2.02$^{a}$ | 72.84 ± 1.41$^{b}$ | 19.91 ± 6.69$^{a}$ | 16.26 ± 2.28$^{a}$ | 36.05 ± 4.92$^{b}$ | 82.38 ± 4.44$^{ab}$ | 79.54 ± 1.28$^{a}$ | 84.76 ± 3.59$^{b}$ |

Mean ± SD. Different letters within the same row of each variety indicate significantly different ($p < 0.05$). L* - Lightness, a* - red-green, b* - yellow-blue (HunterLab values). O – outside, I – inside.

### 4.2 Extraction of prickly pear peel pectin

Extraction is the separation of the desired compound from a mixture of compounds. The extraction of pectin is a multiple physicochemical processes where pectin molecules are hydrolysed and solubilised from the cell wall and middle lamella of the plant tissue (Devi *et al*, 2014). Pectin was extracted using microwave at three different power levels (low, medium & high) and sulphuric acid to adjust the pH levels (1, 2, 3 & 4) from the peel of the three varieties. The results for extracted pectin yield from orange, purple and green PPP are shown in Figure 12. The microwave heating extraction method exhibits large handling capacity, considerably reduced extraction period and good pectin purity as compared with conventional method. Oliveira *et al.* (2016) pointed out that significant pressure builds up inside a material during microwave heating, which then changes the physical properties by enhancing the capillary-porous structure of the fruit peel tissues and enabling the extracting solvents to penetrate better into the tissues. However, extended extraction time may induce pectin hydrolysis making it difficult for ethanol to precipitate, which lowers the extraction yield. The objective of the study was to extract pectin and compare the pectin yield from orange, purple and green PPP. The objective was achieved by using different parameters with different levels during the extraction process. The parameters were microwave power levels and pH, time was kept constant.

Figure 12 illustrates the significant difference at 95% confidence level of the microwave power levels and pH levels of sulphuric acid model for the determination of pectin. The average yield of pectin extracted from orange ranged from 1.4% to 9.8%, purple from 2.9% to 13.8% and green PPP from 2.3% to 10.0%. The results indicated that the pectin yield was dependent on the microwave power levels, pH levels and on the interactions of both variables. The experimental design indicated the suitable power level and pH level combination for better pectin extraction from varieties of PP based on their colours. The purple PPP showed maximum pectin yield (13.8%) at medium power and pH 1, and 11.9% at high power and pH 1 (Figure 13). Similarly, orange (9.8%) and green (10.0%) PPP maximum yields were obtained at medium power and pH 1 (Figure 13), which are similar to those yield of purple (9.8%) obtained at medium power and pH 2. The highest pectin yield was observed at pH 1 and medium power level, which was significantly ($p < 0.05$) different to the yield obtained at low and high power at pH levels of 2, 3, & 4. However, within each extraction condition there were variations in the pectin yield. The difference in pectin yield could be due to different amounts of polysaccharide content which is present in the middle lamella and the thickness size of the cell wall of the fruit. Wonago (2016) pointed out that pectin is a polysaccharide present in the middle lamella of plant tissues and is available in different

amounts depending on the maturity and cultivar of the fruit. Furthermore, the high yield was attributed to the extracting solvent that had greater chance to penetrate the finer tissue of the purple peels and come in contact with the pectic substances present in the cell wall, whereby the insoluble pectic substance was converted into soluble pectin (Liew *et al*., 2016). Purple PPP has a high concentration of soluble pectin and the pectin extracted from the fruit showed a pronounced variation that was observed by having statistical difference amongst the varieties. The results indicated that it was necessary to consider medium power at pH 1 for better extraction conditions for PPP.

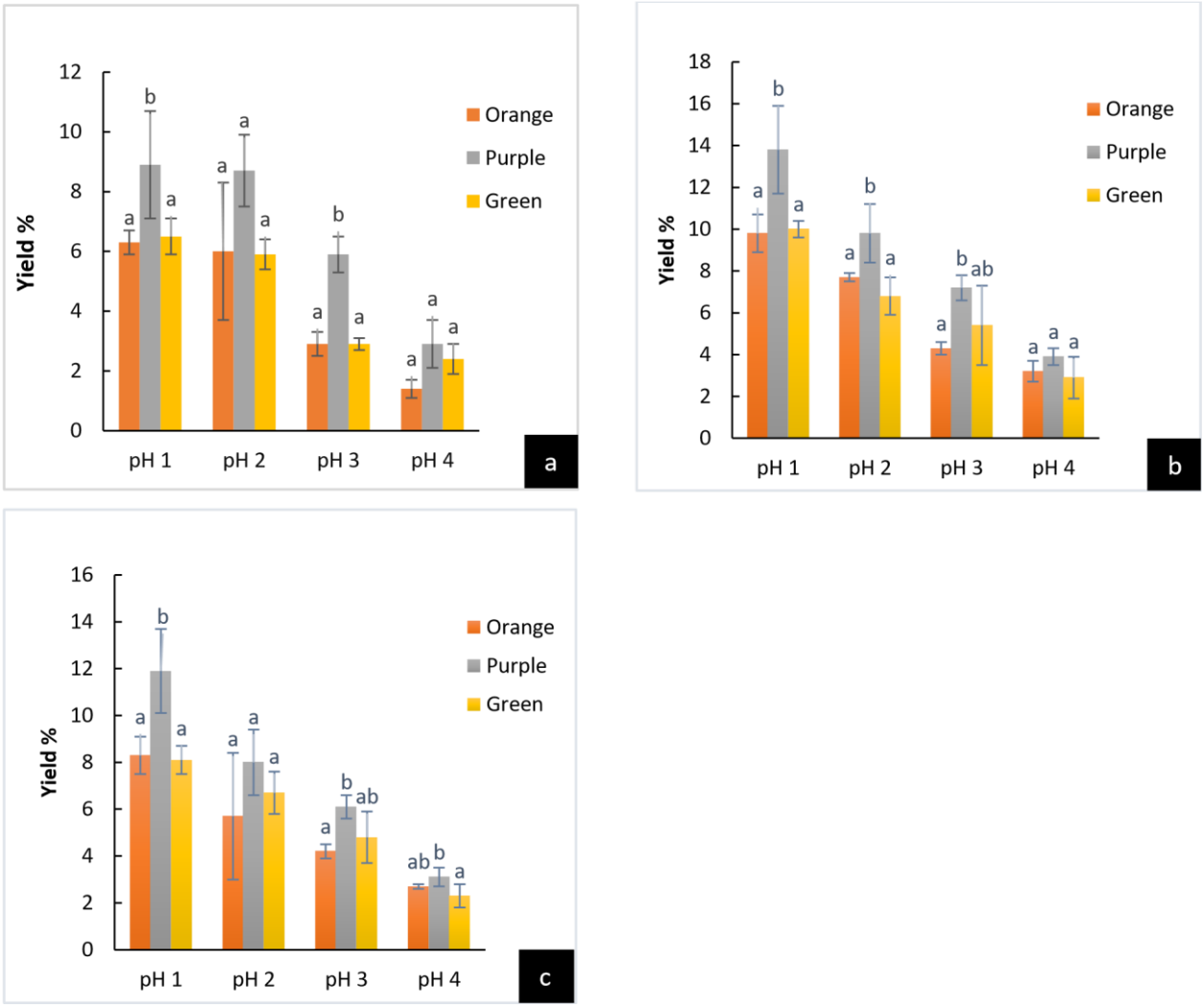



Figure 12: Pectin yield at different microwave levels (a) low, (b) medium and (c) high power levels at different pH levels on orange, purple and green prickly pear peels. (Low = 65°C, medium = 110°C, high = 190°C based on 700 W of power)

It was observed that the change in microwave power level and pH change had a greater impact on the pectin extraction. The high yields were obtained when PPP was extracted under medium power level and at a very low pH of 1 or more acidic conditions (Figure 13). Pectin yield obtained at pH 1 was significantly higher ($p < 0.05$) than that of pH 2 to 4 at medium power. It was revealed that as the pH increased, the pectin yield decreased. At higher pH, there is an accumulation of pectin which retards the release of pectin from the plant material. Devi *et al.* (2014) reported that protopectin is made by the combination of cellulose and pectin molecules. Therefore, during acid hydrolysis, protopectin is separated up producing soluble pectin and cellulose by the removal of water molecules. And at the same time, removal of calcium and magnesium ions occurred resulting in protopectin being converted to soluble pectin (Liew *et al*., 2016). The particularly hydrated carboxylate groups are however repressed at lower pH in the large hydrogen ion concentrations and transformed into slightly hydrated groups of carboxylic acids. The loss of charge reduces the repulsion of the polysaccharide molecules, which accelerates the pectin gelation characteristics, resulting in higher pectin precipitation at lower pH as observed in this study. Thus, the decrease in pH promotes the release of pectin molecules from the peel because the interaction of pectins to the hemicelluloses fractions is split. The results obtained are supported by Yeoh *et al.* (2008) who reported that orange peel pectin yield increased by 4.5% at pH 1 using microwave extraction. The increase in pectin yield was due to the acid-enhanced cell wall disruption and hence increases pectin release (Kirtchev *et al.,* 1989).

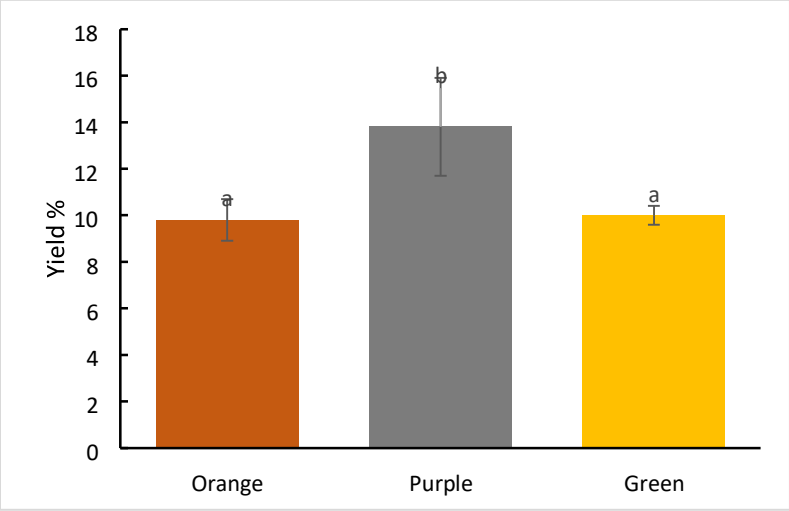



Figure 13: Maximum pectin yield obtained at medium power and pH 1 from orange, purple and green prickly pear peels.

A lower pH enhanced the release of glucose from starch hydrolysis and disintegrated the polysaccharides that could co-precipitate with pectin in ethanol. The large standard deviations

proposed differences in the PPP components from distinctive varieties and their extraction conditions. However, strong acids such as sulphuric acid are corrosive and potentially pose a danger to health (Liew *et al*., 2014). Also, the liquid waste produced by extraction procedures could result in a burden to the environment and high costs of handling the strong acidic waste could be incurred. Varying pH solutions of sulphuric acids might be replaced with edible acids such as citric acid for the pectin extraction, as it is better than other acids from an environmental and economic perspective. It would be necessary to carry further investigation on the effect of extraction of pectin using citric acid and sulphuric acid on the same microwave power level at medium, to determine if the yield would be similar.

It was observed that the pectin yield obtained at medium power showed to be significantly higher ($p < 0.05$) than those obtained at low and high power. The difference in pectin yield was attributed by the effect of microwave power levels. It was seen that the pectin yield of orange, purple and green PPP increased with an increase in microwave power from low to medium, however extended increase to high power decreased the pectin yield. At low power, the irradiation may be insufficient to allow the hydrolysis of protopectin by extracting solvents thus decreased pectin yield. The increased microwave power to medium level, increases the penetration of the solvent into the plant tissues, and effectively delivers to the plant material through molecular interaction with the electromagnetic field and provides a rapid transfer of energy to the solvent and tissues enabling the extraction of dissolution components (Sandarani, 2017). Moreover, Zhang *et al.* (2008) pointed out that microwave irradiation expedites cell rapture inside the sample plant cells, which then stimulates the destruction of sample surface therefore allowing better penetration of the extracting solvents into tissues and resulting in increased extraction yield. Furthermore, Hartati & Subekti (2015) pointed out that high power causes pectin breakages and degradation where the pectin disaggregates into its smaller parts. The degradation of pectin is caused by depolymerisation process of galacturonan chain of pectin known as beta-elimination. The results obtained from this study are supported by Maran *et al.* (2014) who reported that the pectin yield increased by increasing microwave power from 160 – 480 W, where maximum pectin yield of 25.79% was obtained at 477 W from watermelon (*Citrullus lonatus*) waste. Therefore, the study confirms that microwave power level influences the pectin yield.

The results revealed that the best condition for extraction of pectin from orange, purple and green PPP was medium power at pH 1 using sulphuric acid as an extracting solvent. Therefore, the results of the study support the alternative hypothesis that the pectin yield extracted from the orange, purple and green PPP was not the same. From the results obtained, the extraction of

pectin from PPP is feasible in viewpoint of yield, and that the pectin has a great potential for use in the processing of jam and jellies and the food processing and pharmaceutical industries.

### 4.3 Characterisation of extracted prickly pear peel pectin

Characterisation is the measuring of compounds (physical and chemical) properties and structure. Pectin characterisation principally depends on the source as well as the extraction conditions. The characterisation of pectin obtained from orange, purple and green PPP has been carried out for different parameters to evaluate the quality and stability. The objective of the study was to characterise and compare the physicochemical properties of pectin extracted from orange, purple and green PPP. The objective was achieved by characterising the pectin extracted at medium power and pH 1 in terms of ash, moisture, equivalent weight, anhydrouronic acid and methoxyl contents and degree of esterification. The average physicochemical properties of the extracted pectin are given in Table 7.

Table 7: Physicochemical properties of pectin extracted from orange, purple and green prickly pear peel varieties.

| Characteristics | Orange | Purple | Green |
|---|---|---|---|
| Moisture content | 8.87 ± 2.18[b] | 7.57 ± 0.21[a] | 7.70 ± 2.00[a] |
| Ash | 34.26 ± 1.92[b] | 36.30 ± 1.07[b] | 25.16 ± 0.69[a] |
| Equivalent weight | 155.00 ± 16.41[b] | 153.35 ± 10.63[b] | 119.73 ± 5.74[a] |
| Methoxyl content | 2.38 ± 0.21[a] | 2.28 ± 0.26[a] | 3.86 ± 0.31[b] |
| TAUA | 25.58 ± 2.03[a] | 25.93 ± 2.35[a] | 38.84 ± 2.29[b] |
| Degree of esterification | 50.63 ± 4.76[a] | 49.87 ± 0.17[a] | 56.39 ± 1.60[b] |

Mean ± SD. Different letters within the same row indicate a significant difference ($p < 0.05$). TAUA = Total Anhydrouronic Acid

#### 4.3.1 Moisture content

Moisture content is the amount of water contained in a material. The moisture content of pectin determines the quality and stability of the extracted pectin. As shown in Table 7, the average

moisture content of orange, purple and green PPP varieties ranged from 7.70 to 8.87%. It was observed that moisture content of orange PPP was significantly ($p < 0.05$) higher than that of purple and green PPP pectin. However, the moisture content of the isolated pectin obtained in this study was found to be within the range of 7.57 to 8.87%, which falls within the pectin quality standard range of less than 12% as reported by Food Chemicals Codex (2016). The moisture content of orange, purple and green PPP pectin was comparable to that reported by PerezMartinez *et al.* (2013) who reported an average moisture content of 7.55% on *Opuntia* cladode flour pectin. The study was also supported by Ismail *et al*. (2012) who reported an average moisture content of 11.3% on dragon fruit pectin and Mohammed (1999) who reported 7.88 – 8.96% on grapefruit pectin. The low moisture content on pectins indicates that the pectin has low water absorption capacity and also dependant on the drying method. Castillo-Israel *et al*. (2015) pointed out that low moisture content of pectin inhibits the development of microorganisms that may influence the pectin quality due to the production of pectinase enzymes. The moisture content of PPP pectin was 7.57 to 8.87% and most of commercial pectin range around 7.0%. Jain *et al*. (1984) reported moisture content of 8.80% from golden delicious apple pomace pectin. Therefore, the low moisture content obtained from green has greater stability and is of good quality as compared with purple and orange PPP pectin. Generally, it can be concluded that the extracted pectin from PPP is of good quality standard range and acceptable because of the low moisture content.

### 4.3.2 Ash content

Ash content measures the total amount of minerals present within a food (Akubor & Onimawo 2003). The higher the amount of minerals present in food, the higher the ash content. However, for this study ash content determines the quality in terms of purity of extracted pectin (CastilloIsrael *et al*., 2015). As shown in Table 7, the average ash content of pectin extracted from orange, purple and green PPP varieties ranged from 25.16 to 36.30% respectively. The ash content of the green variety was significantly ($p < 0.05$) lower than the ash content of orange and purple variety. The high ash content observed might be due to the acidic (low pH) extraction conditions where the pectin was partially esterified and the elevated negatively charged carboxylic group concentrations of pectin and the counter ions caused increased ash content. Hot acid extracted pectin has sufficient galacturonic acid (GalA) to be considered a functional additive. High calcium content contributes to the majority of ash content. Ismail *et al*. (2012) pointed out that ash content varies depending on the methodology and nature of fruits used for extraction. The results of this study were higher than those reported by Perez-Martinez *et al.* (2013) who reported an average ash content of 16.65% on cladode pectin. The ash content of dragon fruit pectin ranged from 6.9

to 11.6% as reported by Ismail *et al*. (2012). However, the ash content from the orange, purple and green PPP was generally higher and not in range with findings from Ranganna (1995), the findings stipulated that pectin of high ash content contains about 10.69% and low ash pectin contains about 0.76% ash from the gel formation view point. Therefore, in view point of gel formation, the pectin extracted from the green PPP is considered of good quality as compared to those of orange and green PPP.

### 4.3.3 Equivalent weight

Equivalent weight is the total content of non-esterified galacturonic acid in the pectin molecular chains (Ranganna, 1995). Equivalent weight determines the gel strength of pectin. The average equivalent weight of pectin extracted from orange, purple and green PPP varied from 119.73 to 153.35. The results showed that the equivalent weight of green PPP was significantly ($p < 0.05$) lower than those of orange and purple PPP. The low equivalent weight on green PPP may be because the green PP fruit was less matured as compared to the orange and purple PP fruit. Wonago (2016) pointed out that equivalent weight differs depending on the method, source and maturity of the fruit used for extraction. Nonetheless, the equivalent weight of pectin extracted from orange, purple and green PPP was observed to be generally lower than that reported by Wonago (2016), who reported that the average equivalent weight of lime and lemon was 326.79 and 396.82. The low equivalent weight obtained in this study may be caused by the use of sulphuric acid (strong acid) as an extractant that strongly influenced the pectins macromolecular and gelling characteristics by depolymerising of galacturonan chain and decreases the free acid content as reported by Devi *et al*. (2014). However, increased and decreased equivalent weight depends on the amount of free (non-esterified) galacturonic acid, furthermore high equivalent weight would have high gel formation, whereas low equivalent weight would have low gel formation because the pectin would be highly degraded (Ramli & Asmawati, 2011). The pectin extracted from PPP varieties generally showed lower equivalent weight, therefore it will have a lower gel formation.

### 4.3.4 Methoxyl content

Methoxyl content is the amount of moles of methyl alcohol in 100 mol of galacturonic aid. It is a significant factor in regulating the setting time, the gelling strength, metal ions sensitivity and to determine the functional properties of pectin solutions (Constenla & Lozano, 2003). Moreover, it influences the pectin dispensability in water, higher methoxyl content is more dispersible in water

than lower methoxyl content (Castillo-Israel *et al*., 2015). The average methoxyl content obtained was 2.28, 2.38 and 3.86% for orange, purple and green PPP respectively. The methoxyl content of green PPP variety was significantly ($p < 0.05$) higher than that of orange and purple PPP pectin. The low methoxyl content obtained may be attributed by the low pH and medium extraction power that depolymerised galacturonan chains into shorter polygalacturonic acid chains. The results are comparable to those reported by Islam *et al*. (2012) on dragon fruit pectin with methoxyl content ranging from 2.98 – 4.34% and Islam *et al*. (2012) reported methoxyl content of 1.56% on lemon peel pectin. According to Aina *et al*. (2012), the methoxyl content varies from 0.2 – 12% depending on the pectin source and extraction method. Kanmani *et al.,* (2014) pointed out that pectin that has less than 7% methoxyl content is classified as low methoxyl pectins, they form gels with lower sugar concentrations or in the absence of sugar. Generally, the methoxyl content of PPP was below 7%, therefore, the pectin is characterised as of low ester, indicating that they are desirable in terms of quality.

### 4.3.5 Total anhydrouronic acid

The total anhydrouronic acid (AUA) content determines the purity and degree of esterification. It also evaluates the physical characteristics of extracted pectin (Castillo-Israel *et al*., 2015), and not less than 65% is suggested (Food Chemicals Codex 2016). The average AUA content for pectin extracted from orange, purple and green PPP varieties were 25.58, 25.93 and 38.84% as shown in Table 7. It was observed that the AUA content of green PPP was significantly ($p < 0.05$) higher that of orange and green PPP pectin. The purity of pectin obtained from green variety is higher when compared with those from orange and purple varieties as shown by the lower ash content. Generally, the AUA content obtained was less than 65% which points out that the pectin may not be adequately pure due to the existence of proteins, starch and sugars in the extracted pectin. Furthermore, PPP pectin has a high content of neutral sugars, consisting mainly of galactose (Gal), arabinose (Ara), and rhamnose (Rha) (Hwang *et al*., 1992). However, the GalA and neutral sugar content in pectin rely on the conditions of extraction. Hot acid extracted pectin has sufficient GalA as a functional additive. Pectins that are partially esterified contain 10% or more organic materials composed of arabinose, galactose and other sugars. However, the AUA values obtained in this study are significantly different from those reported by Islam *et al*. (2012) from dragon fruit pectin of 45.3 to 52.2%. Therefore, the results indicate that green PPP pectin is purer as compared to those of purple and orange PPP varieties.

### 4.3.6 Degree of esterification

The degree of esterification (DE) is the proportion of esterified galacturonic acid groups to the total galacturonic acid groups present in pectin. It is a significant factor that determines the pectin's gel formation (Sundar Raj *et al*., 2012). The %DE of pectin from orange, purple and green PPP varieties obtained were 49.87, 50.63 and 56.39%. The DE of pectin from green variety was significantly ($p < 0.05$) higher than those of orange and purple PPP varieties (Table 7). The high DE values of green PPP may be attributed to the degree of maturity, source, tissues and method of extraction. Moreover, the galacturonan chains of green PPP pectin were probably less depolymerised into short polygalacturonic acid chains as compared to those of purple and orange PPP. The DE values obtained are comparable to those reported by Norazelina *et al*., (2012) whereby the DE values of 31.05-46.96% from dragon fruit were obtained. The variations may be attributed to the type of acid used during the extraction. Sulphuric acid was used as an extractant that strongly influenced the pectins macromolecular and gelling properties by depolymerising of galacturonan chain and decreases the free acid content and extraction method used (Hartati & Subekti, 2015). Therefore, the pectin obtained from green and orange varieties can be classified as high methoxyl pectin (HMP) pectin because it has DE higher than 50%. HMP form gels with high amounts of sugar as well as low pH value. HMP are thickeners for soft drinks. With these characteristics, pectin builds a comparable mouthfeel to that of fruit juices, and is therefore helpful in juice and low calorie or diet beverages. Whereas purple PPP pectin can be classified as low methoxyl pectin (LMP) because the DE is less than 50%. They will form a thermo-irreversible gel that even when heated to higher temperatures will remain gelled. LMP is used in the production of low-sugar jams because they gel in the absence of sugar.

## 4.4 Characterisation of prickly pear peel pectin using FTIR spectroscopy

Fourier-transform infrared spectroscopy (FTIR) is a rapid and convenient technique for investigation of polysaccharide functional groups. Functional groups contained in pectin absorb light at specific wavelengths. The spectrum indicates the functional groups and provides structural information about the pectin extracted from various PP varieties (orange, green and purple) and commercial citrus pectin (control) obtained at different wavelengths between 400 and 4000 $cm^{-1}$. Figure 14 shows the FTIR spectra for pectin extracted from three different varieties of PP and commercial citrus pectin and corresponding functional groups are given in Table 8. The FTIR spectra in the region between 400 and 1500 $cm^{-1}$ is considered to be 'fingerprint' region for carbohydrates enabling the identification of major functional groups specific to particular polysaccharides. It can be noted that the pectin extracted from PP peels have similar spectra in

the 'fingerprint' region. The spectra can be comparable to those of citrus peel as well as to those of pectin reported by Khamsucharit *et al*. (2018) and Muhammad *et al*. (2014) indicating that the extracted polysaccharides obtained in this study were pectin. Absorption bands observed at 1722, 1729, 1731.85 $cm^{-1}$ for the pectin extracted from the purple, orange and green varieties of PP, respectively, were attributed by the stretching vibration of ester carbonyl groups (C=O).

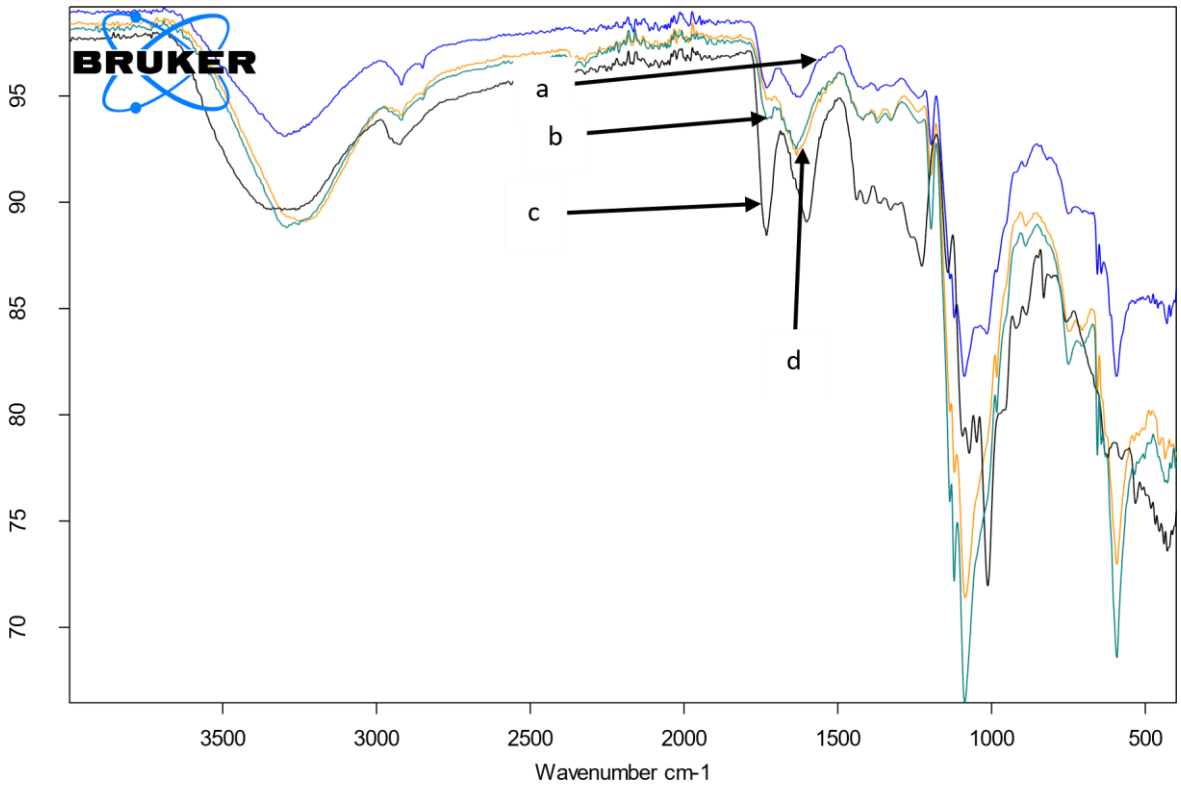



Figure 14: Fourier transform infrared spectra of a. green, b. purple, c. commercial citrus and d. orange prickly pear pectin.

The spectra showed a broad peak at 3292, 3219 and 3301$cm^{-1}$ for pectin extracted from the purple, orange and green varieties of PPP, respectively, which is a result of an O-H stretch of hydroxyl groups on the pectin structure. The C-H stretching bands were observed at wavelengths, 2918, 2919, 2918$cm^{-1}$ for pectin extracted from purple, orange and green PPP varieties, respectively. The bands at wavelengths 1196, 1121 and 1196 $cm^{-1}$ in pectin extracted from orange, purple and green varieties of PPP were contributed by pyranose cycle vibrations. These results show that PPP pectin is probably rich in carboxylic acids like galacturonic acid. FTIR indicated that there are no major structural differences in pectin that were extracted from orange, purple and green varieties of PPP. The PPP pectin spectra exhibited similarities in its absorption pattern to that of commercial pectin.

Table 8: Functional groups present in pectin from prickly pear peel varieties and commercial citrus

| Functional groups | Frequency ($cm^{-1}$) | | | |
|---|---|---|---|---|
| | Control | Purple PPP | Orange PPP | Green PPP |
| O-H | 3261 | 3292 | 3219 | 3301 |
| C-H | 2923 | 2918 | 2919 | 2918 |
| C=O | 1731 | 1722 | 1729 | 1731 |
| C-O | 1226 | 1121 | 1196 | 1196 |

PPP = prickly pear peel, control = commercial citrus pectin

## CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Conclusions

When the effects of physicochemical properties of the fruit such as pH, titratable acids, total soluble solids, texture and peel thickness were studied it was found that these properties have a positive effect on yields but not on the quality of pectin. These findings show the effective pectin extraction, offering potential financial and environmental benefits for industrial extraction of pectin. The results showed that it was necessary to produce high pectin yield as an intermediary step in the acid extraction of pectin from purple PPP variety. The purple pectin yield was 29% higher than orange and green PPP. However, the green PPP pectin was the highest in pectin quality. Pectin extraction from different varieties of PPP did not show any significant effect on pectin yield as compared with common fruit sources such as citrus peels and apple pomace. All PPP pectin were within the acceptable quality range in terms of moisture content, ash content, methoxyl content, equivalent weight, anhydrouronic acid and degree of esterification. The present research revealed that PPP is a good source of pectin and has the potential to be used in food processing industries as a significant raw material for pectin. The FTIR spectroscopy confirmed the presence of different functional groups in extracted PPP pectin similar to those of commercial citrus pectin. Pectin

obtained from PPP is viable and can be an alternative source of pectin production in terms of yield and quality. It has the potential application as a citrus replacement for high quality pectin and has excellent potential for use in the processing of jams, jellies and use in the food and pharmaceutical industry as a whole. Using PPP for pectin production will thus improve the economy of the country by saving rand reserves due to minimised pectin importers, and generating jobs through the development of new industry to help alleviate poverty. Moreover, by converting solid wastes into a valuable industrial product, it would have a very strong positive impact on the environment, thereby preserving the planet earth.

### 5.2 Recommendations

1. With the elevated import pectin costs, a nation such as South Africa with plenty of prickly pear (PP) fruits may consider it setting up factories to fulfill the national pectin demand. Apart from that, if all the projected accessible PP can be used for pectin manufacturing, the country can be a significant pectin exporter.
2. A pilot-scale PPP pectin production must be carried out to address the problems and concerns that will arise in commercial implementation.

3. Further research on the use of organic acids (citric and acetic acids) at different concentrations and different PP maturity stages as well as different extraction time is necessary.

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## APPENDICES

Appendix I: Correlation between physicochemical properties of prickly pear fruit and pectin yield extracted from orange, purple and green prickly pear peel.

**Correlations**

| | | Yield | Firmness | Size | PeelT | TSS | pH | TA |
|---|---|---|---|---|---|---|---|---|
| Yield | Pearson Correlation | 1 | -0,382 | -0,527 | -0,884 | -.999* | -0,025 | -0,538 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Sig. (2tailed) | | 0,750 | 0,647 | 0,309 | 0,021 | 0,984 | 0,638 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Firmness | Pearson Correlation | -0,382 | 1 | -0,584 | 0,769 | 0,412 | 0,933 | -0,574 |
| | Sig. (2tailed) | 0,750 | | 0,603 | 0,441 | 0,730 | 0,234 | 0,611 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Size | Pearson Correlation | -0,527 | -0,584 | 1 | 0,069 | 0,499 | -0,836 | 1.000** |
| | Sig. (2tailed) | 0,647 | 0,603 | | 0,956 | 0,667 | 0,369 | 0,008 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| PeelT | Pearson Correlation | -0,884 | 0,769 | 0,069 | 1 | 0,899 | 0,489 | 0,082 |
| | Sig. (2tailed) | 0,309 | 0,441 | 0,956 | | 0,289 | 0,675 | 0,948 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| TSS | Pearson Correlation | -.999* | 0,412 | 0,499 | 0,899 | 1 | 0,057 | 0,510 |
| | Sig. (2tailed) | 0,021 | 0,730 | 0,667 | 0,289 | | 0,964 | 0,659 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| pH | Pearson Correlation | -0,025 | 0,933 | -0,836 | 0,489 | 0,057 | 1 | -0,829 |
| | Sig. (2tailed) | 0,984 | 0,234 | 0,369 | 0,675 | 0,964 | | 0,377 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| TA | Pearson Correlation | -0,538 | -0,574 | 1.000** | 0,082 | 0,510 | -0,829 | 1 |
| | Sig. (2tailed) | 0,638 | 0,611 | 0,008 | 0,948 | 0,659 | 0,377 | |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix II: Extracted pectin yield (%) for orange, purple and green prickly pear peel varieties

| Microwave power level | pH | Orange | Purple | Green |
|---|---|---|---|---|
| | | | Yield % | |
| Low | 1 | 6,3 ± 0,4[a] | 8,9 ± 1,8[b] | 6,5 ± 0,6[a] |
| Medium | 1 | 9,8 ± 0,9[a] | 13,8 ± 2,1[b] | 10,0 ± 0,4[a] |
| High | 1 | 8,3 ± 0,8[a] | 11,9 ± 1,8[b] | 8,1 ± 0,6[a] |

| | | | | |
|---|---|---|---|---|
| Low | 2 | 6,0 ± 2,3[a] | 8,7 ± 1,2[a] | 5,9 ± 0,5a |
| Medium | 2 | 7,7 ± 0,2[a] | 9,8 ± 1,4[b] | 6,8 ± 0,9[a] |
| High | 2 | 5,7 ± 2,7[a] | 8,0 ± 1,4[a] | 6,7 ± 0,9[a] |
| Low | 3 | 2,9 ± 0,4[a] | 5,9 ± 0,6[b] | 2,9 ± 0,2[a] |
| Medium | 3 | 4,3 ± 0,3[a] | 7,2 ± 0,6[b] | 5,4 ± 1,9[ab] |
| High | 3 | 4,2 ± 0,3[a] | 6,1 ± 0,5[b] | 4,8 ± 1,1[ab] |
| Low | 4 | 1,4 ± 0,3[a] | 2,9 ± 0,8[a] | 2,4 ± 0,5[a] |
| Medium | 4 | 3,2 ± 0,5[a] | 3,9 ± 0,4[a] | 2,9 ± 1,0[a] |
| High | 4 | 2,7 ± 0,1[ab] | 3,1 ± 0,4[b] | 2,3 ± 0,5[a] |

Mean ± SD. Different letters in a row indicates significant difference ($p<0.05$).

**Appendix III: List of conferences attended**

1. BioAfrica Convention Date: 27 to 29 August 2018

Title: Potential microwave extraction of pectin from indigenous prickly pear fruit waste

Authors: Lerato Lekhuleni, Tsietsie Kgatla, Mpho Mashau, and Afam Jideani

Venue: Durban International Convention Centre, South Africa.

2. BioAfrica Convention Date: 26 to 28 August 2019

Title: Extraction and characterisation of pectin from prickly pear fruit waste.

Authors: Lerato Lekhuleni, Tsietsie Kgatla, Mpho Mashau, and Afam Jideani Venue: Durban International Convention Centre, South Africa.

**Appendix IV: Publications**

Manuscript type: Article

Title: Physicochemical properties of prickly pear fruit and peel; extraction and characterisation of pectin from the peel

Authors: Lerato Lekhuleni, Tsietsie Kgatla, Mpho Mashau, and Afam Jideani

Journal submitted to: Foods

Date of submission: 8 November 2019