

Research Article

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Physicochemical properties of South African prickly pear fruit and peel: Extraction and characterisation of pectin from the peel

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Abstract: The aim of this study was to determine the physicochemical properties, extract and characterise pectin from green (*Morado*), purple (*Algerian*), and orange (*Gymno carpo*) varieties of South African prickly pear (PP) (*Opuntia ficus-indica*) fruit peel. Pectin was extracted using sulphuric acid at four different pH levels (1, 2, 3, and 4) and three different microwave power levels (low 300 W, medium 400 W, and high 500 W) at a fixed time of 10 min. 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 (PPP). Maximum yield of 13.8% on purple, 10.0% on green, and 9.8% on orange was obtained at pH 1.0 and medium power level. Ash content of the extracted pectin was significantly high at 25.16, 34.26, and 36.30% for green, orange and purple PPP, respectively. However, pectin showed lower moisture content and equivalent weight. The methoxyl contents were 2.28, 2.38, and 3.86%, for anhydrouronic acid contents were 25.58, 25.93, and 38.84%, and degree of esterification was 49.87, 50.63, and 56.39% across the orange, purple, and green varieties. The PPP pectin spectra exhibited similarities in their absorption pattern to that of commercial citrus pectin.

Keywords: prickly pear, pectin, extraction, yield, functional group

1 Introduction

Fruits processing industries generate solid wastes such as peel, core, unripe, and over-ripe fruits, as high as 50% of raw materials (Virk and Sogi 2004). These solid wastes are cheap sources of raw materials for animal feed; moreover, the peel also contains high amounts of pectin. Therefore, the utilisation of prickly pear peels (PPP) will be beneficial in both decreasing the amount of solid wastes and adding the value of agricultural by-products (Padam et al. 2014). Prickly pear (PP) (*Opuntia* spp.) belongs to the family of Cactaceae. PP cultivars can grow in harsh, rocky, and dry environmental conditions. It has gained the attention of consumers because of its high nutritional value that has a positive health benefit (López-Palacios et al. 2012). PP fruit is oval shaped with thick, waxy, and thorny peel and is available in different colours ranging from green, yellow, purple, red, and orange (Gengatharan et al. 2015; Khatabi et al. 2016). 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). 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 2–4 weeks (Yahia 2012). PP fruit components and extracts are being used in the treatment of diabetes, cholesterol, and immune system health (Ncibi et al. 2008; Patel 2015). The pulp and seeds are the most edible parts of the PP fruit that constitute about 40–60% of the fruit (Ncibi et al. 2008). During the production of pulp, wine, and juice, the PP fruit peel is regarded as a by-product. However, the peel contains high antioxidants and pectin that can be used as an alternative source of pectin in the production of jam (Chan and Choo 2013).

Pectin is a linear polysaccharide which is made up of 1,4 linked α -D-galacturonic acid (Hosseini et al. 2016). Pectin is naturally present in fruits and is found between adjacent plant cells in the layers of middle

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lamella (Lefsih et al. 2016). The functional properties of pectin depend on the degree of esterification (DE), and these are characterised as low methoxyl pectin with DE <50% and high methoxyl pectin with DE >50% (Jafari et al. 2016). Low and high methoxyl pectin have different DE and therefore different physicochemical properties and application (Ngouémazong et al. 2012). The important process in pectin production is extraction which is usually achieved by hot acid extraction (Guo et al. 2012). Pectin is extracted from fruit peel which is acidified with organic or mineral acids such as sulphuric, nitric, and hydrochloric acids, which are the commonly used extractants (Oliveira et al. 2016; Pereira et al. 2016).

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). The yield and quality of pectin depend mainly on the source as well as the method used for the extraction of pectin. In recent years, substantial research efforts have been conducted to search for high-quality pectin from peels of plant materials, such as peels of passion fruit, pomelo, and mango, to be used as a substitute for commercial pectin (de Oliveira et al. 2016). However, the utilisation of pectin from these sources remains limited in food industry. Indigenous fruits and their by-products have the potential to be used in commercial pectin production, especially in developing countries.

Extraction and characterisation of pectin have been studied for many plant materials. However, extraction of pectin using conventional methods at acidic pH and high temperature beyond 80°C decreases the quality of pectin extracted (Pagan et al. 2001). Therefore, there is a need for researchers to use other extraction methods such as microwave to prevent low quality pectin. When compared to conventional extraction techniques, microwave is reliable because it decreases the consumption of energy and solvent as well as reducing extraction time (Wang et al. 2007). The objective of this study was to determine the physicochemical properties of PP fruit, extraction of pectin from the peel, and to determine its yield, physicochemical, and structural properties.

2 Materials and methods

2.1 Raw materials

Fresh PP fruits of green (*Morado*), purple (*Algerian*), and orange (*Gymno carpo*) were harvested from Bothlokwa Mphakane village in Limpopo province, South Africa. All the selected fruits were at physiological maturity

stage and were assessed by the external colour index. All fruits were cleaned with tap water and stored in a cold room for 10 days at 5°C for further processing.

2.2 Prickly pear peel (PPP) powder preparation

PP fruits were thoroughly cleaned with distilled water and peeled with knife to separate the peels from the pulp. The peels were then sliced (1–2 cm thickness) and placed on trays divided into three batches according to the variety. The three batches of PP peels were immediately dried using oven (EcoTherm 278 Digital oven, South Africa) at 60°C for 72 h. After drying, the samples were cooled in a desiccator, milled using a milling machine (Polymix PX-MFC 90 D, Kinematic AG, Switzerland), and sieved through a 500 µm sized aperture sieve, and the powder was stored in polyethylene bags in a desiccator until further analysis (Castillo-Israel et al. 2015).

2.3 Extraction process of pectin from PPP

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, and 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 for 10 min at low (300 W), medium (400 W), and high power (500 W) levels. The solution was then cooled and centrifuged (Universal 320 R, Herttich, Germany) at 3,000 rpm for 15 min. 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 using weighing balance BOECO Germany BPS 51, and the percentage yield was calculated using equation (1) (Altat et al. 2015):

$$\text{Pectin yield (\%)} = \frac{P}{Bi} \times 100, \quad (1)$$

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

2.4 Physical properties of PP fruits

The length, width of the fruit, and peel thickness were measured using a digital Vernier calliper (Zarei et al. 2011). The weight was measured using a weighing balance BOECO Germany BPS 51 plus.

2.5 Texture analysis of PP fruits

The hardness of PP fruits was measured using Texture Analyser (model TA.XT.Plus, from Stable Micro Systems, UK) with mode of measure force in compression using 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 = 16 mm, and trigger type = 100 g (force). Hardness was expressed in Newtons (N).

2.6 Colour analysis of PPP

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 prickly pear peel (PPP) and dried PPP powder. From the colour values, Chroma (C), hue angle (H°), and total colour change (ΔE) were calculated using equations (2–4) (Maskan 2001):

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

$$\text{Hue angle} = \tan^{-1} \left(\frac{b^*}{a^*} \right), \quad (3)$$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}, \quad (4)$$

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

2.7 pH value of PP fruits

The pH of fruit pulp was determined using a digital pH meter (Crison Basic 20) following AOAC (2002) standard procedure.

2.8 Total soluble solids of PP fruits

A digital refractometer (Atago SMART-1, Japan) was used to measure the total soluble solids (TSS), and the results were directly recorded following AOAC (2002) standard procedure. A drop of the fruit pulp was placed on the refractometer prism, and results expressed as °Brix and readings were recorded.

2.9 Titratable acidity of PP fruits

Titrateable acid (TA), as % citric acid, was determined using the direct titration method; 20 mL of fruit pulp was placed in a 100 mL conical flask and three drops of phenolphthalein indicator was added. Thereafter, the sample was titrated with 0.1 N NaOH to an endpoint where the colour changed to pink and the volume of NaOH used was recorded. TA was calculated using equation (5):

$$\begin{aligned} \text{\% Citric acid} \\ = \frac{(\text{mL of NaOH})(0.1\text{N NaOH})(0.064)(100)}{\text{mL of sample}}. \end{aligned} \quad (5)$$

2.10 Characterisation of PPP pectin

2.10.1 Moisture content

Moisture content was determined following the AOAC (2002) standard procedure. 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):

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

2.10.2 Ash content

Approximately 2 g of pectin sample was weighed, placed into tared crucibles and in a muffle furnace (Lasec model EMF 035) for 4 h at 550°C, cooled in a desiccator, and weighed. The ash content was determined according to AOAC (2002) by applying equation (7):

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

2.10.3 Equivalent weight

Pectin sample of 0.5 g was taken into a 250 mL conical flask, and 5 mL of ethanol (95%) was added. Approximately 1 g of NaCl and 100 mL of distilled water were added, and finally six drops of phenol red indicator

were added against 0.1 N NaOH. Titration point was indicated by purple colour. The neutralised solution was stored for further determination of methoxyl content. The equivalent weight was calculated by applying equation (8) (Ranganna 1995):

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

2.10.4 Methoxyl content

A solution of 25 mL of 0.25 N NaOH was added to the neutralised solution from the equivalent weight titration, and the solution was stirred and kept for 30 min 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) (López-Palacios et al. 2012):

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

2.10.5 Total anhydrouronic acid

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

$$\text{AUA(\%)} = \frac{176 \times 0.1z \times 100}{w \times 1,000} + \frac{176 \times 0.1y \times 100}{w \times 1,000}, \quad (10)$$

where z = mL of NaOH from equivalent weight, y = mL of NaOH from methoxyl content, and w = sample weight (g).

2.10.6 Degree of esterification

The pectin DE was determined according to equation (11) (Shaha et al. 2013):

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

where %MeC = methoxyl content and %AUA = anhydrouronic acid content.

2.11 Structural analysis of PPP pectin using Fourier transform infrared (FTIR) spectra

The Fourier transform infrared (FTIR) spectrum was used to acquire information on chemical structure of the extracted and commercial pectin. The pectin powder was encapsulated in KBr at 1:100 ratio. FTIR data were obtained using Spectrum 65 FT-IR (Bruker) in the range 400–4,000 cm^{-1} , scanning rate at 32 at resolution rate 4 cm^{-1} (Dehbi et al. 2014).

2.12 Statistical analysis

The experimental data for this study were captured in Microsoft excel, and all analyses were done in triplicate. The data obtained were analysed and interpreted by one-way analysis of variance using SPSS Statistics version 25. Values were expressed in mean standard deviation, and the significance level was set at $p < 0.05$.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Physicochemical properties of PP fruit and peel

The physicochemical properties of PP fruit are presented in Table 1. Fruit size is the ratio of weight, length, and width. Dehbi et al. (2014) reported that the fruit size is affected by the seed content and weight. The average PP fruit size varied from 153.58 to 222.58 g. The orange PP fruit had significantly ($p < 0.05$) larger size compared to that of purple and green PP; however, the orange PP had the lowest pectin yield. The fruit size differed significantly among varieties. There are many PP varieties, which 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 differ significantly among cultivars and genetic constitution. For this reason, it is necessary to correlate PP size with the yield of pectin. Moreover, PP variety with larger 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 contains 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 thicknesses were 0.40, 0.48, and 0.54 g. Green PPP thickness was significantly ($p < 0.05$) higher than purple PPP but it was not significantly ($p < 0.05$) higher than the orange PPP. 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 ($\text{pH} > 4.5$). The average pH values were 5.04, 5.58, and 6.04, and acidity values were 0.03, 0.03, and 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 ($p < 0.05$) in the pH values was observed; however, no significant difference ($p < 0.05$) was observed in 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 that has less pH or high acidity may yield highest pectin extract. Pectin extracted in acidic condition (low pH) results in high pectin yield. This might be because of the use of less acid in a fruit that already contains high acid which gives less damaging effect on the pectin extraction (Yapo and Koffi 2013). The high concentration of TSS may also mean high amount of pectin concentration in the fruit.

The average TSS concentrations obtained were 13.02, 14.45, and 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 was significantly ($p < 0.05$) higher than that of purple PP. The increased TSS on orange PP was because of the hydrolysis of starch into sugars during the maturation process. Zarei *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.

3.2 Texture of PP fruits

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

Table 1: Physical and chemical properties of prickly pear fruits

Properties	Prickly pear		
	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
Titrateable acid (as % citric acid)	0.06 ± 0.01 ^a	0.03 ± 0.01 ^a	0.03 ± 0.01 ^a

Values are mean ± standard error of mean. Means sharing the same letters in row are not significantly different from each other ($p < 0.05$).

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 presented in Figure 1. The average force was 128.07, 137.59, and 227.04 N on orange, purple, and green PP fruits. The green variety had significantly higher ($p < 0.05$) force as compared to those of purple and orange PP fruits as shown by the force required to break the surface of the green PP fruit. This may be possibly because of minimal degree of maturity changes in the cell wall structure (geometric properties) and chemical composition of plant cell wall and middle lamella during the development and ripening of the fruit (Nyorere and 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, and 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, and hence less firmness (force) as compared to green PP fruit. Moreover, loss of firmness is 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 are. However, the results showed that there was no relationship between firmness and pectin yield; the green PP had the

higher firmness but obtained lower pectin yield (10.0%) as compared to purple PP pectin yield of 13.8%.

3.3 Colour analysis of PPP and powder

The colour of PPP is shown in Table 2. 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 reddish-brown colour on purple variety, especially the lower a^* value. Colour is a quality parameter in any food product, which determines the consumer liking or not. The colour of PPP after drying still resembled the original colour of the fresh fruit. This could be because of betalains that are affected by heat during drying of PPP. The darker colour change could be attributed to the high temperature of drying which enhanced the isomerisation of betalains. The result obtained is in 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.

There are several factors affecting the stability of betalains, such as high temperature, high water activity, light, and oxygen, and pH above 7 or below 3 promotes degradation of betalains. However, in this study, it could not be because of pH as the pH of PP fruit 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 colours of PP were influenced by juice treatments. Light and bright colours are the result of betalain pigments that maintain colour stability throughout processing and also give the juice an appealing colour. Colour variations were caused by modifications in betalain pigments as well as furfural and hydroxyl-methylfurfural compounds' development. The sample of heat-treated juice was darker. This may be because of the Maillard reaction, as this reaction requires reducing sugar and amino acids. Therefore, the PP variety, size, and the level of maturity or ripeness showed the potential of PP fruit to be an alternate source for pectin production.

The total colour difference (ΔE^*) is a combination of L^* , a^* , and b^* values that characterise the colour variant in foods that occurs during processing. On average, the

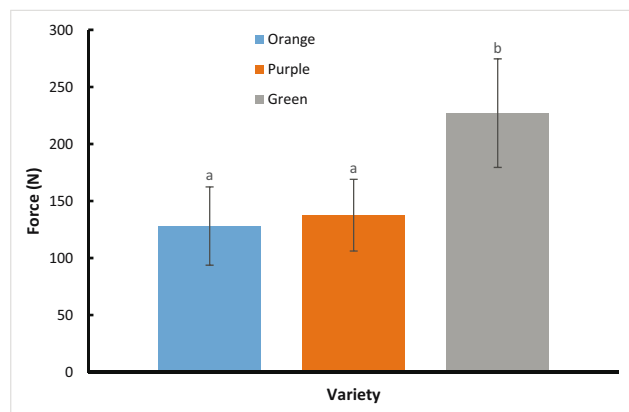


Figure 1: Firmness of prickly pear fruit. Different superscripts show significant different at $p < 0.05$.

Table 2: Colour quality of prickly pear peels and dried powder

Parameter	Orange			Purple			Green		
	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
ΔE			27.92 ± 2.87			16.42 ± 4.16			20.59 ± 2.29

Values are mean ± standard error of mean. Means sharing the same letters in row are not significantly different from each other ($p < 0.05$). L^* , lightness; a^* , red-green; b^* , yellow-blue (HunterLab values). O, outside, I, inside.

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 lower 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.

3.4 Extraction of PPP pectin

The results of extracted pectin yield from orange, purple, and green PPP are shown in Figure 2. 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 pectin yield was dependent on the microwave power levels, pH levels, and the interactions of both variables. The purple PPP showed maximum pectin yield (13.8%) at medium power (400 W) and pH 1, and 11.9% at high power (500 W) and pH 1. Similarly, orange (9.8%) and green (10.0%) PPP maximum yields were obtained at medium power and pH 1, 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 (300 W) and high power at pH levels of 2, 3, and 4. However, within each extraction condition, there were variations in the pectin yield. The difference in pectin yield could be because of the different amount 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 into 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 had 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 showed that it was necessary to consider medium power at pH 1 for better extraction condition for PPP. It was

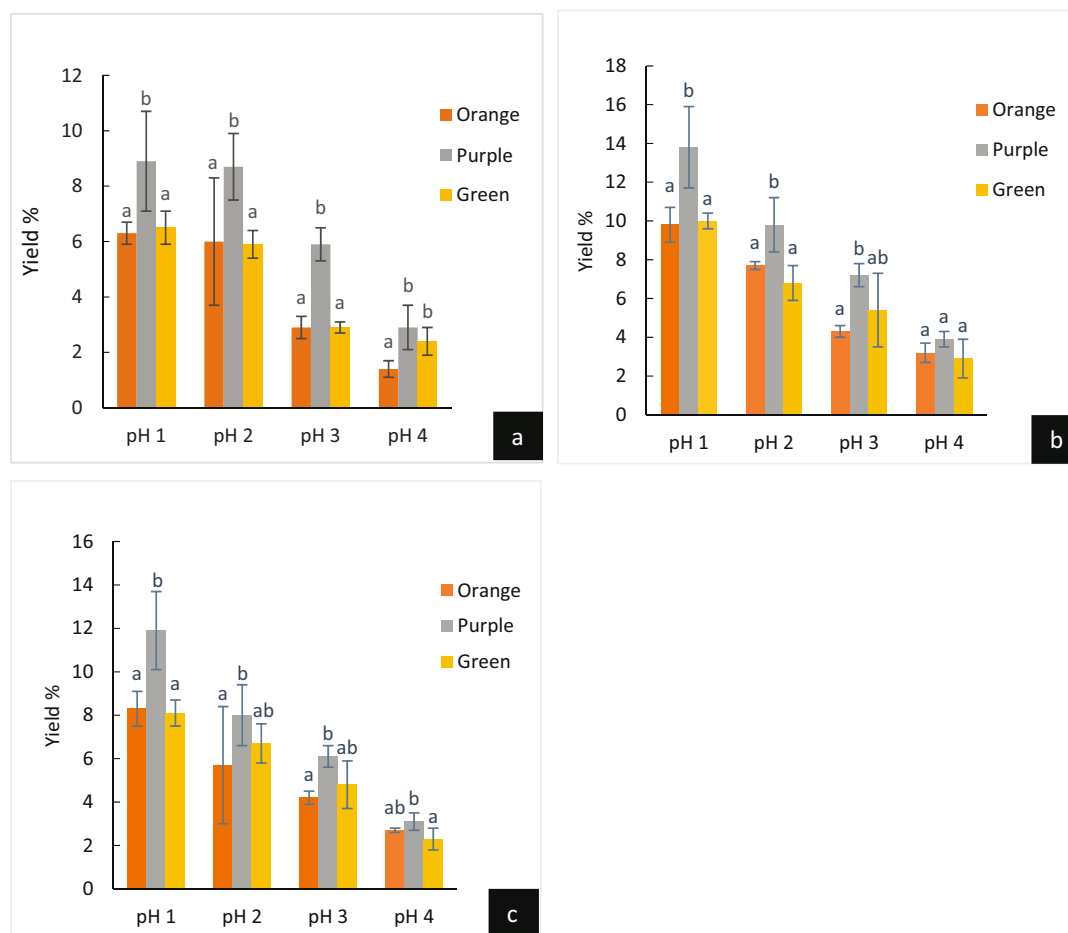


Figure 2: Pectin yield at different microwave levels: (a) low, (b) medium, and (c) high power levels at different pH levels on orange, purple, and green prickly pear peels (low = 300 W, medium = 400 W, high = 500 W). Different superscripts show significant different within same pH level at $p < 0.05$.

observed that the change in microwave power level and pH had a greater impact on the pectin extraction. The high yields were obtained when PPP were extracted under medium power level and at a very low pH of 1 or more acidic conditions. Pectin yield obtained at pH 1 was significantly higher ($p < 0.05$) than that of pH 2–4 at medium power.

The results also revealed that as the pH increased, the pectin yield decreased. At higher pH, there is an accumulation of pectin that 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. At the same time, removal of calcium and magnesium ions occurred resulting in protopectin being converted to soluble pectin (Liew et al. 2016). The 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 because of the acid-enhanced cell wall disruption and hence increases pectin release (Kirtchev et al. 1989). 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, according to

Liew *et al.* (2016), strong acids such as sulphuric acid are corrosive and potentially pose a danger to human health.

3.5 Characterisation of extracted PPP pectin

The physicochemical properties of PPP are shown in Table 3. The average moisture content of orange, purple, and green PPP varieties was 7.57, 7.70, and 8.87%. The 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 ranged from 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 a study by Perez-Martinez *et al.* (2013) who reported an average moisture content of 7.55% on *Opuntia* cladode flour pectin. The study was also supported by Islam *et al.* (2012) who reported an average moisture content of 11.3% on dragon fruit 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 because of the production of pectinase enzymes. The moisture content of PPP pectin was 7.57–8.87% and most of the 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 variety has a greater stability and is of good quality as compared with purple and orange PPP pectin.

Ash content measures the total amount of minerals present within a food. The higher the amount of minerals present in food, the higher the ash content. The average ash contents of pectin extracted from orange, purple, and

green PPP varieties were 25.16, 34.26, and 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 because of 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 to be considered as a functional additive.

High calcium content contributes to the majority of ash content. Islam *et al.* (2012) pointed out that ash content varies depending on the methodology and nature of fruits used for extraction. The result of this study was higher than that 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 Islam *et al.* (2012). However, the ash content from the orange, purple, and green PPP was generally higher and not in range with findings by Ranganna (1995), who stipulated that pectin of high ash content contains about 10.69% and low ash pectin contains about 0.76% ash from the gel formation viewpoint. Therefore, in viewpoint of gel formation, the pectin extracted from the green PPP is considered of good quality as compared to those of orange and green PPP.

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 weights of pectin extracted from orange, purple, and green PPP were 119.73, 153.35, and 155.00. 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 may be because the green PP fruit was less matured as compared to the orange and purple PP fruit. Wonago (2016) indicated that equivalent weight differs depending on the method, source, and maturity of

Table 3: Physicochemical properties of pectin extracted 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

Values are mean ± standard error of mean. Means sharing the same letters in row are not significantly different from each other ($p < 0.05$). TAUA, total anhydrouronic acid.

the fruit used for extraction. Nonetheless, the equivalent weight of pectin extracted from orange, purple, and green PPP was 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 the 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 (GalA); 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 2011). The pectin extracted from PPP varieties generally showed lower equivalent weight, and therefore, it will have a lower gel formation.

The average methoxyl contents obtained were 2.28, 2.38, and 3.86% for orange, purple, and green PPP. 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 to 4.34%. Salma 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 to 12% depending on the pectin source and extraction method. Kanmani et al. (2014) pointed out that pectins that have less than 7% methoxyl content are classified as low methoxyl pectins, and 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.

The total anhydrouronic (AUA) content determines the purity and DE. It also evaluates the physical characteristics of extracted pectin, and it should be not less than 65% as suggested (Food Chemicals Codex 2016). The average AUA content for pectin extracted from orange, purple, and green PPP was 25.58, 25.93, and 38.84%. The AUA content of green PPP was significantly ($p < 0.05$) higher than those 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 point out

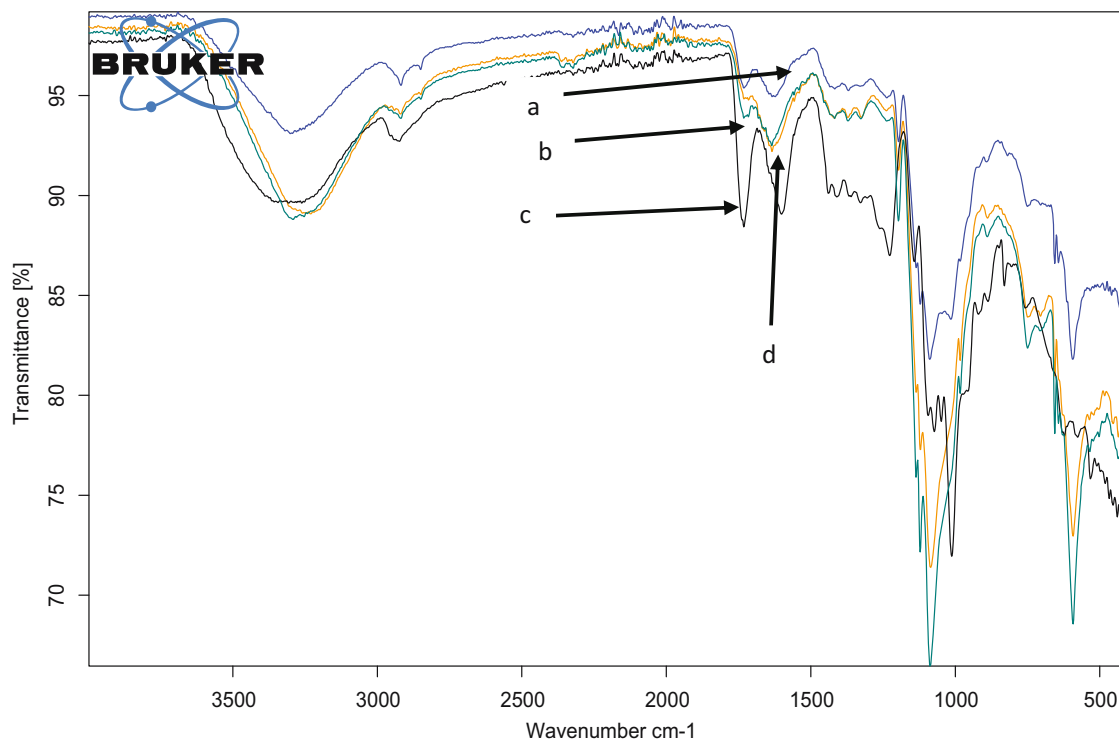


Figure 3: Fourier transform infrared spectra of (a) green, (b) purple, (c) commercial citrus, and (d) orange prickly pear pectin.

Table 4: Functional groups present in pectin from prickly pear peel varieties and commercial citrus

Functional groups	Bond	Frequency (cm ⁻¹)			
		Control	Purple PPP	Orange PPP	Green PPP
Alcohols	O–H stretch, H bonded	3,261	3,292	3,219	3,301
Alkane	C–H stretch	2,923	2,918	2,919	2,918
Ketone (carboxylic group)	C=O stretch	1,731	1,722	1,729	1,731
Pyranose cycle	C–O stretch	1,226	1,121	1,196	1,196

PPP, prickly pear peels.

that the pectin may not be adequately pure because of the existence of proteins, starch, and sugars in the extracted pectin. Furthermore, PPP pectin has high content of neutral sugars, consisting mainly of galactose, arabinose, and rhamnose. 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 45.3–52.2%.

DE is the proportion of esterified GalA groups to the total GalA groups present in pectin. It is a significant factor that determines the pectins gel formation (Lira-Ortiz *et al.* 2016). The DE of pectin from orange, purple, and green PPP varieties obtained was 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. 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 Islam *et al.* (2012) whereby the DE values of 31.05–46.96% from dragon fruit were obtained. Therefore, the pectin obtained from green and orange varieties can be classified as high methoxyl pectin because it has DE higher than 50%. The high methoxyl pectin form gels with high amounts of sugar as well as low pH value and is used as 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. Purple PPP pectin can be classified as low methoxyl pectin because the DE is less than 50%. They will form a thermo-irreversible gel that even when heated to higher

temperatures will remain gelled. Low methoxyl pectin is used in the production of low-sugar jams because it gels in the absence of sugar.

3.6 Characterisation of PPP pectin using FTIR spectroscopy

Figure 3 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 4. The FTIR spectra in the region between 400 and 1,500 cm⁻¹ are considered to be “finger print” 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 has similar spectra in the “finger print” region. The spectra are 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 1,722, 1,729, and 1,731.85 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). The spectra showed a broad peak at 3,292, 3,219, and 3,301 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 2,918, 2,919, and 2,918 cm⁻¹ for pectin extracted from purple, orange, and green PPP varieties. The bands at wavelengths 1,196, 1,121, and 1,196 cm⁻¹ in pectin extracted from orange, purple, and green varieties of PPP were as a result of pyranose cycle vibrations. These results show that PPP pectin is probably rich in carboxylic acids like GalA. FTIR showed 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.

4 Conclusions

Physicochemical properties of the PP fruit such as pH, titratable acidity, total soluble solids, texture, and peel thickness had effects on pectin yield and quality. Extraction of pectin using microwave medium power level and pH 1 produced a higher pectin yield than higher pH and higher microwave power levels and lower levels. 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 pectins were within the acceptable quality range in terms of moisture content, ash content, methoxyl content, equivalent weight, AUA, and DE. The FTIR spectroscopy confirmed the presence of different functional groups in extracted PPP pectin similar to those of commercial citrus pectin. 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. It has the potential application as citrus replacement for high-quality pectin and has potential for use in the processing of jams and jellies, and for use in the food and pharmaceutical industries.

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