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Extraction, characterization and comparison of pectin from purple (*Passiflora edulis*) and yellow (*Passiflora edulis f. flavicarpa*) varieties of passion fruit peel by ultrasonic extraction method under two pretreatment conditions

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Abstract

Passion fruit contains 50–55 g of peel for every 100 g of fresh fruit, and this peel is discarded as waste in the production of fruit juice and other processed fruits. The utilization of waste passion fruit peel in pectin extraction is studied and the main objective of this study is to compare the purple passion fruit variety (*Passiflora edulis*) against the yellow variety (*Passiflora edulis f. flavicarpa*). Also, pectin was extracted, isolated, and compared by characterization under two pretreatment conditions: blanched and non-blanched, and the physio-chemical properties were compared to those of commercial pectin. The results indicate that blanching, as a pretreatment, plays a significant role. Thus, the blanched samples have given favorably higher values than non-blanched samples in both varieties in terms of tested characteristics and yields. The extraction was done at 60°C of temperature, 20 kHz of frequency, and under four power intensities; 150, 370, 660, and 890W. Optimum results were obtained at 660W and it was noticed that the rising power intensity negatively affects DE, equivalent weight, methoxyl content, and anhydrouronic acid content. The yield, DE, equivalent weight, methoxyl content (per 100g of pectin) and anhydrouronic acid content (per 100g of pectin) at 660w from blanched purple passion fruit peel are 13.80%, 82.99%, 1152.68, 8.26g, 61.95% respectively and from blanched yellow passion fruit peel are 13.38%, 78.61%, 696.51, 7.05g, 65.38% respectively. Purple passion fruit peel is a better source of pectin than the yellow variety under the studied physio-chemical properties and is a good match to commercial citrus pectin, thus an effective utilization of fruit processing waste.

Keywords: yellow passion fruit, purple passion fruit, pectin, ultrasonic extraction, yield

Introduction

Passion fruit, which belongs to the family Passifloraceae, is an exotic fruit with a distinctive flavor and the two most widely grown varieties are yellow (*Passiflora edulis f. flavicarpa*) and purple (*Passiflora edulis*). It is a fruit with significant commercial value that can be effectively grown in the majority of tropical and subtropical parts of the world, as well as in the South Asian region. Passion fruit juice and juice concentrates are popular processed fruit products in the international market. According to Kulkarni and Vijayanand (2010) [18], peel and seed make up the majority of the waste produced during the processing of passion fruit. Because peel accounts for about half of the fruit mass, it is a significant waste with disposal issues. Consequently, it's important to convert the peel into beneficial byproducts. It is reported that the peel of a passion fruit contains a significant amount of pectin (Kulkarni and Vijayanand, 2010) [18], and with additional advancements and alterations, it might be employed commercially. Commercial pectin extraction uses waste produced by the citrus and apple processing industries since they are said to be rich sources of pectin. In both situations, the leftover pulp from juice pressing is used as the primary ingredient in the production of pectin. Pectin content in apple pomace ranges from 10 to 15% on a dry matter basis, however it is significantly greater in citrus peel at 20 to 30%. Citrus and apple pectins are generally equal in terms of functionality.

All dicotyledonous plants contain pectin, a structural polysaccharide, in their cell walls. A linear 1, 4 α linked D-Galacturonic acid chain with various levels of methylation is the main structural component of pectin (Sriamornsak, 2003) [33]. Galacturonic acid is a sugar acid that forms α-(1-4) glycosidic linkages between the pyranose rings of D-galacturonic acid units. These links allow galacturonic acid to be connected in chains.

Pectin is largely used throughout most of the food processing industries to get the desired textural characteristics of food by altering the rheological properties of food ingredients. Pectin is been a well-known gelling agent, thickener, and a stabilizer. The conversion of passion fruit peel into pectin offers great scope for utilization of it. Numerous research have discussed the extraction of pectin using various extraction conditions from conventional sources such as apple (Fertonani *et al*., 2006) [10], citrus (Tamaki *et al*., 2008) [35] and sugar beet (Yapo *et al*., 2007) [40], and also from other alternative raw materials such as peach pomace (Faravash and Ashtiani, 2008) [9], ambarella (Koubala *et al*., 2008) [16, 17], banana (Emaga *et al*., 2008) [8], and mango (Koubala *et al*., 2008) [16, 17].

The extraction of target compounds from various plant sources has been suggested using a number of modern alternative techniques. Ultrasound (de Oliveira *et al*., 2016; Xu *et al*., 2014) [25, 39], moderate electric field (de Oliveira *et al*., 2015) [24], pulsed electric field (Medina and Barbosa, 2015) [22], microwave (Seixas *et al*., 2014; Dang *et al*., 2014) [31, 7] and enzymatic (Liew *et al*., 2016) [20] method are few of them. Through the use of these novel approaches, it may be possible to increase the extraction yield while also increasing the quality of the extracted component and process efficiency.

The ultrasound technique is a novel extraction method and acoustic energy is used in this technique to extract the compounds from different plant materials. One of the key benefits of this extraction method is the increased mass transfer caused by acoustic cavitation in a liquid medium (Wang *et al*., 2015) [37]. Increased cavitation and cell breakdown driven by the ultrasonic waves results in more pectin being extracted in a soluble solution (de Oliveira *et al*., 2016) [25].

The objective of this study is to compare the purple passion fruit peel against the yellow passion fruit peel and to compare the characterization properties between two passion fruit peel varieties and also between blanched and non-blanched conditions while the ultrasonication power intensity is kept as a variable.

Materials and methods

Fully mature, undamaged passion fruit peels at the same stage of ripeness, and similar peel colors with the best quality of yellow variety (*Passiflora edulis s. flavicarpa*) and purple variety (*Passiflora edulis*) were collected from the processing yard of a leading passion fruit processing factory in Sri Lanka. These two varieties were further confirmed by the Fruit Crop Research and Development Center, Department of Agriculture, Horana, Sri Lanka. The commercial citrus pectin which is used for the comparative study with passion fruit peel pectin was procured from Glorchem Enterprise, Colombo, Sri Lanka. All chemical reagents were of analytical grade.

1. Preparation of Passion Fruit Peel Powder

Collected samples of passion fruit peels were immediately washed and cleaned with running water to remove the pulp and seed residuals. One set of samples was kept separately from both varieties. Another set of samples from both varieties were subjected to blanching. For that, the two sets of peels were immersed in water at 100 ºC separately, in two separate stainless steel vessels, for 3 minutes followed by cooling in an ice bath. The non-blanched samples were grounded in a food crusher and the blanched samples were sliced using stainless steel knives. Then the samples were dried in a drier separately, with air circulation at 55 ºC for 72 hours until a constant weight was achieved. The dried peels were then milled and passed through a 60 size sieve.

The obtained passion fruit peel powder from both varieties were sealed and packed in airtight containers and stored at 6 -10 ºC until taken for further analysis and pectin extraction.

2. Extraction and Purification of Pectin

Pectin was extracted according to the method described by Oliveira *et al*. (2016) [25] with slight modifications.

Passion fruit peel powder was weighed (6 g) on an analytical balance into a beaker and distilled water was added at 1: 30 (g/ mL) ratio to the powder. The mixture was then stirred using a glass rod until the fruit peel powder was evenly moistened by distilled water in a homogeneous form. Then the pH of the mixture was measured and adjusted to pH 2.0 using 1.0 M nitric acid. The prepared solution was heated to 60ºC in a water bath. Then it was placed in the ultra-sonication machine (Ningbo Yinzhou Sjia Lab Equipment, SJIA-1500W). Samples were subjected to four different power intensities; 150, 370, 660, and 890W under a constant temperature of 60ºC. The frequency of the equipment was 20 kHz. Ultrasound waves were given for 1.5 seconds and the resting time was 2.5 seconds. This was practiced for 10 minutes and the ultrasound probe used was 15 mm. The processed sample was vacuum filtered. The retentate was discarded and the filtrate was stored in a refrigerator at 4ºC for 30 minutes. Extracted pectin was precipitated using 95% ethanol at 4ºC for 30 minutes. The precipitated pectin was separated by vacuum filtration and then immersed in a solution of 70% ethanol for 16 hours to remove the impurities. The obtained pectin was washed with acetone and immediately dried in an air-circulated oven at 40ºC until a constant weight was obtained. The resulting material was milled to dry powdered pectin.

2. 1 Determination of the Yield of Pectin

The extraction yields of pectin (dry matter basis) were calculated as described by Kulkarni and Vijayanand (2010) [18] according to the following equation

$$\text{Pectin yield (\%)} = \frac{\text{Weight of dried pectin (g)} \times 100}{\text{Weight of the dried peel taken for extraction (g)}}$$

Characterization of Pectin

1. Determination of the Degree of Esterification Value

Degree of esterification was determined according to the titrimetric method described by Bochek *et al*. (2001) [3]. Initially, 0.2g of dried pectin was weighed into a 100ml titration flask. Then it was made wet with 5ml of 95% ethanol. The pectin sample was then dissolved in 20 ml of distilled water and it was placed in a water bath at 45ºC until pectin is dissolved completely. Next, 3 drops of phenolphthalein was added to it and titrated with 0.1N sodium hydroxide solution and the result was recorded as initial titration volume once slight pink color

appeared. Then, 10ml of 0.1N sodium hydroxide was added. The sample was plugged with a stopper and shaken vigorously, then allowed to stand at room temperature for 2 hours. Then, 10ml of 0.1N hydrochloric acid was added and the sample was shaken until the pink color disappeared. Three drops of phenolphthalein were added into the sample and was titrated with 0.1N sodium hydroxide. The volume of titration was recorded as the final titration volume, once a slight pink color appeared. DE value was calculated according to the following equation;

$$\text{DE (\%)} = \frac{\text{Final titer (mL)} \times 100}{\text{Initial titer (mL)} + \text{Final titer (mL)}}$$

2. Determination of the Equivalent Weight Value

Equivalent weight was determined according to the method explained by Ranganna (1995) [28]. Initially, 0.5g of sample was weighed into a 250 mL conical flask and then the sample was moistened with 5mL of 95% ethanol. 1g of sodium chloride and 100 mL of carbon dioxide free distilled water were added to it and pectin was dissolved well in it. Finally, 6 drops of phenol red indicator were added to it and it was titrated with 0.1N sodium hydroxide until the endpoint indicated the orange red color and this color should persist for at least 30 seconds. This neutralized solution was used for the determination of methoxyl content. Equivalent weight was calculated according to the following equation;

$$\text{Equivalent weight} = \frac{\text{Weight of the sample (g)} \times 1000}{\text{mL of alkali} \times \text{Normality of alkali}}$$

3. Determination of Methoxyl Content

Methoxyl content was determined according to the method explained by Ranganna (1995) [28]. Initially, 25 mL of 0.25 N sodium hydroxide was added to the neutral solution obtained from equivalent weight analysis, containing 0.5g of pectic substance. Then it was shaken thoroughly and allowed to stand for 30 minutes at room temperature in a stoppered flask. Next, 25 mL of 0.25 N hydrochloric acid was added and titrated against 0.1N sodium hydroxide to the same endpoint as earlier with phenol red indicator. Methoxyl content was calculated according to the following equation;

$$\text{Methoxyl content (\%)} = \frac{\text{mL of alkali} \times \text{Normality of alkali} \times 31 \times 100}{\text{Weight of the sample (g)} \times 1000}$$

4. Determination of Total Anhydrouronic Acid Content

Total anhydrouronic acid content was determined by the following formula explained by (Mohamed and Hasan, 1995) [23].

$$\text{Total anhydrouronic acid content (\%)} = \frac{176 \times 0.1z \times 100}{w \times 1000} + \frac{176 \times 0.1y \times 100}{w \times 1000}$$

When, molecular weight of anhydrouronic acid (1 unit) = 176g
Where,
z = mL (titer) of sodium hydroxide from equivalent weight determination
y = mL (titer) of sodium hydroxide from methoxyl content determination
w = weight of pectin sample

5. Determination of Functional Groups of Extracted Pectin by Fourier Transform Infrared (FTIR) Spectroscopic Analysis

A blank disc was prepared by using potassium bromide and then, 3 mg of finely ground pectin powder was weighed using an analytical balance. It was well-mixed with 300 mg potassium bromide and made into discs with a laboratory hand press. IR absorbance data were obtained spectrometrically by FTIR spectrophotometer (Thermo Scientific, NICOLET iS10) at wavenumbers in the range of 500 to 4000 cm^{-1}. The absorbance data were analyzed by using OMNIC software.

6. Determination of Morphological Differences of Extracted Pectin by Scanning Electron Microscopy (SEM)

Morphology of wet pectin was observed by scanning electron microscopy according to the method described by Seixas *et al*. (2014) [31] and Liew *et al*. (2016) [20]. All pectin samples were air dried and mounted on aluminum studs with sticky double-side conductive carbon tapes and coated with gold in a vacuum before being observed. Then the samples were placed in the scanning electron microscope (Zeiss, EVO LS15) and observed under a magnification of 600.

7. Statistical Analysis

All reported values represent the average value of the analysis of four replicates. One way ANOVA was performed followed by Tukey's test for mean comparison. The statistical analysis was conducted at a 95% confidence interval. Minitab 17 statistical software was employed for the statistical data analysis.

Results and Discussion

1. Extraction of pectin

Table 1: Yields of pectin (%) on dry matter basis and the color of pectin from yellow and purple passion fruit peels by ultrasonic extraction method

Power intensity (W)	Yellow variety		Purple variety	
	Blanched	Non-blanched	Blanched	Non-blanched
150	$8.36^{a} \pm 0.27$	$6.29^{b} \pm 0.60$	$9.16^{a} \pm 0.44$	$9.11^{a} \pm 0.17$
370	$9.25^{a} \pm 0.14$	$8.02^{b} \pm 0.63$	$9.88^{a} \pm 0.13$	$9.32^{a} \pm 0.17$
660	$13.38^{a} \pm 0.99$	$9.86^{c} \pm 0.34$	$13.80^{a} \pm 0.14$	$11.80^{b} \pm 0.13$
890	$11.57^{ab} \pm 0.40$	$9.42^{c} \pm 0.19$	$12.05^{a} \pm 0.18$	$11.21^{b} \pm 0.24$
Color of pectin	Pale yellow	Pale yellow	Pale pink	Pale pink

Data represented as mean (n=3) ± standard deviation. Mean values in a row superscripted by different letters are significantly different.

According to the data obtained from the yields of two varieties, it is clear that the blanched samples of both yellow and purple varieties have given higher yields of pectin than that of non-blanched samples. Further, higher values are obtained from the purple variety than from the yellow variety under the same extraction conditions. When considering the yield with respect to the ultrasonication power intensity, it is revealed that the optimum yield has been obtained under the power intensity of 660W. This significant characteristic was observed in both purple and yellow varieties. The highest yield; 13.80%, was recorded for the purple blanched sample. In contrast, here the power intensity of 890W was also studied and it was noticed that the yield goes down when the power intensity is increased above the optimum condition.

According to earlier studies, blanching of passion fruit peels inactivates pectin degrading enzymes such as pectin methyl esterase enzyme activity (Prasad, 1980) [26]. Sudhakar and Maini (1999) [34] and Chaliha *et al*. (1963) [4] have reported that blanching and drying yielded good quality jelly grade pectin from citrus peels and mango peels. Thus, blanching of passion fruit peels is taken into consideration as a desirable pretreatment and it is being proven with this present study.

In this study, a series of ultrasonic power intensities were used and came to know that the optimum intensity in terms of the yield is 660W. Waves were given for 1.5 seconds and the resting time was 2.5 seconds in order to get the maximum efficiency of the instrument. Bagherian *et al*. (2011) [2] reported that the intermittent sonication has given better results compared to continuous sonication during the extraction of pectin from grapefruit.

Oliveira (2016) [25] has reported that the sonication time of 10 minutes was enough to attain the equilibrium of mass transfer during ultrasound extraction under the conditions used. Xu *et al*., (2014) [39] reported the degradation of pectin when exposed to ultrasound for a long time. The yield from this study; 13.38% from yellow passion fruit peel has a slight increment from the value of 12.67% reported by Oliveira (2016) [25] under the optimum conditions. Under the optimum condition, the purple variety has given an even higher yield of 13.80% in the present study.

In order to enhance the extraction of specific components from plant sources, the ultrasonic approach has been researched (Guaman-Balcazar *et al*., 2015) [14]. The increased effectiveness of ultrasound-assisted bioactive compound extraction can be attributed to an intensification of mass transfer caused by the cavitation bubble collapse phenomenon caused by sonication, which makes it easier for solvent to move through plant tissue and thus increases extraction efficiency (Chemat *et al*., 2011) [5]. The features (frequency and intensity), the qualities (viscosity and surface tension), and the environmental variables all affect how cavitation is caused by ultrasound (temperature and pressure). According to Toma *et al*. (2001) [36], the hydration of pectin material from the middle lamella, which results in the destruction of vegetal tissue during sonication, promotes the swelling and softening process of the cell walls during ultrasonic treatment. Hence, it is evident that sonication is a key factor in dissolving the vegetal tissue and increasing extraction yields.

The non-conventional ultrasonication procedure has indicated several advantages, principally, greater cost-effective operation at less energy input, better quality pectin due to less degradation, and improved yield at lesser processing time.

Both the yellow and purple varieties of passion fruit peel can have their skin separated from the endocarp much more easily after being blanched. The endocarp is easily detached from the skin, but here the study was done with the skin of passion fruit to identify the yields if it is done with the skin in commercial production. Additionally, it was noted that the purple variety of passion fruit had a substantially thicker endocarp than the yellow variety. Pectin yield typically depends on the source of the pectin and the conditions of the extraction (Rha *et al*., 2011) [29].

Table 2: Characterization values of commercial citrus pectin

Degree of esterification (%)	Equivalent weight	Methoxyl content (g per 100g of pectin)	Total anhydrouronic acid content (g per 100g of pectin)
74.53 ± 0.39	1225.37 ± 4.22	8.95 ± 2.05	65.17 ± 6.13

Data represented as mean (n=6) ± standard deviation.

2. DE Value

Degree of esterification (DE) represents the carboxyl groups of the main chain of galacturonic acid that are esterified with methyl or acetyl groups. In this study, DE was calculated for both yellow and purple varieties under two pretreatment conditions and for the commercial citrus pectin and this is expressed as a % value.

Table 3: Degree of esterification (%) of pectin from yellow and purple passion fruit peels by ultrasonic extraction method

Power intensity	Yellow variety		Purple variety	
(W)	Blanched	Non-blanched	Blanched	Non-blanched
150	$79.17^{b} \pm 0.26$	$70.37^{c} \pm 1.70$	$85.14^{a} \pm 1.30$	$78.23^{b} \pm 1.32$
370	$78.30^{b} \pm 1.61$	$66.25^{c} \pm 2.40$	$85.09^{a} \pm 1.79$	$76.36^{b} \pm 0.56$
660	$78.61^{b} \pm 0.42$	$64.73^{d} \pm 0.35$	$82.99^{a} \pm 2.03$	$70.51^{c} \pm 1.37$
890	$69.08^{b} \pm 0.98$	$60.69^{c} \pm 1.28$	$78.16^{a} \pm 1.36$	$66.94^{b} \pm 3.08$

Data represented as mean (n=4) ± standard deviation. Mean values in a row superscripted by different letters are significantly different.

The values for the DE for pectin obtained from the ultrasonic extraction method from purple and yellow passion fruit varieties showed values higher than 50% and usually pectin with DE higher than 50% is named high methoxyl pectin and it forms a gel in the presence of a high amount of sugar (65% or more) and low pH condition around 3.2. High methoxyl pectins are used for jellies, jams, and products with high sugar concentrations. The DE values obtained from both varieties are competitive compared to the value obtained from the commercial citrus pectin which is 74.53%.

Both varieties have shown higher DE values for the blanched samples than for the non-blanched samples and high DE values are obtained for lower power intensities. The lowest intensity, 150W has given the highest DE values. Similar results were obtained by de Oliveira *et al*. (2016) [25]. DE value has decreased with rising power intensities, thus the lowest DE was reported at the highest power intensity, which are 60.69% and 890W respectively. This characteristic feature was observed in both varieties and in both pretreatment conditions.

In the present study, ripen, mature passion fruits were selected from both varieties to extract pectin because, most passion fruits are in the ripen stage when they are used to extract juice since they have better organoleptic properties than immature ones. According to the study with lemon pomace, Azad *et al*. (2014) [1] reported that the degree of esterification decreased with the increase of maturity.

It is important to emphasize that, depending on the source and mainly, on the experimental conditions applied during the extraction process, the pectin will have different characteristics. This statement is clear according to the results obtained from this study. The DE values have differences between the pretreatments given prior to the extraction and the variety of the passion fruit.

3. Equivalent weight

Table 4: Equivalent weight of pectin from yellow and purple passion fruit peels by ultrasonic extraction method

Power intensity (W)	Yellow variety		Purple variety	
	Blanched	Non-blanched	Blanched	Non-blanched
150	$680.38^{b} \pm 4.76$	$550.29^{d} \pm 2.56$	$1171.94^{a} \pm 2.45$	$583.39^{c} \pm 0.89$
370	$670.59^{b} \pm 3.12$	$510.84^{d} \pm 1.82$	$1160.25^{a} \pm 8.50$	$576.49^{c} \pm 6.41$
660	$696.51^{b} \pm 1.54$	$487.38^{d} \pm 7.29$	$1152.68^{a} \pm 2.18$	$565.28^{c} \pm 4.16$
890	$671.73^{b} \pm 1.71$	$471.53^{d} \pm 4.36$	$1128.37^{a} \pm 2.43$	$542.83^{c} \pm 2.51$

Data represented as mean (n=4) ± standard deviation. Mean values in a row superscripted by different letters are significantly different.

As shown in the table, higher equivalent weight values were reported in blanched samples of both varieties than that of non-blanched samples but all the above obtained values are lower than the value obtained from the commercial citrus pectin sample which is 1225.37 (table 2). According to Salam *et al*. (2012) [30], the commercial importance of this is, that higher equivalent weight values would have a higher gel-forming effect. The highest value, 1171.94 was reported from blanched purple peel pectin under 150 W intensity. The results indicate that the increase of the power intensity negatively affects the value of the equivalent weight. The lower equivalent weight could be due to the higher partial degradation of pectin. It can also be said that the

pretreatment condition is more effective for the equivalent weight thus the blanched samples have given higher values than non-blanched samples in both varieties.
The value ranges for yellow-blanched, yellow-non blanched, purple-blanched, purple-non blanched are 670.59 to 696.51, 471.53 to 550.29, 1128.37 to 1171.94, and 542.83 to 583.39 respectively. Studies show that the apple pomace pectin range is 833.33 to 1666.30 (Kumar and Chauhan, 2010) [19] and the cocoa husk pectin range is 510.68 to 645.19 (Ramli and Asmawati, 2011) [27].
However, the purple variety exhibits a greater variation in equivalent weights on pretreatment than the yellow variety according to the values obtained from this study.

Methoxyl Content
According to the results shown in table 5, higher methoxyl contents were obtained in blanched samples than in non-blanched ones for both yellow and purple varieties.

Table 5: Methoxyl content (g per 100g of pectin) of pectin from yellow and purple passion fruit peels by ultrasonic extraction method

Power intensity (W)	Yellow variety		Purple variety	
	Blanched	Non-blanched	Blanched	Non-blanched
150	$7.89^{b} \pm 0.48$	$7.15^{c} \pm 0.03$	$8.96^{a} \pm 0.19$	$5.97^{d} \pm 0.22$
370	$7.31^{b} \pm 0.24$	$7.09^{b} \pm 0.31$	$8.78^{a} \pm 0.15$	$5.06^{c} \pm 0.12$
660	$7.05^{b} \pm 0.35$	$6.83^{b} \pm 0.07$	$8.26^{a} \pm 0.21$	$5.27^{c} \pm 0.80$
890	$7.02^{b} \pm 0.16$	$6.35^{c} \pm 0.21$	$7.83^{a} \pm 0.40$	$4.31^{d} \pm 0.33$

Data represented as mean (n=4) ± standard deviation. Mean values in a row superscripted by different letters are significantly different.

Values obtained from the blanched samples give comparatively higher values than from the non-blanched samples in both yellow and purple varieties. The value obtained from the blanched purple pectin sample under 150W; 8.96g, is almost similar to the value obtained from the commercial citrus pectin which is 8.95g. According to the results obtained from this study, the lower power intensities have given a much higher methoxyl content and the rising power intensities have caused the reduction of the methoxyl content. This could be because of the partial degradation of pectin. Further, purple variety has shown higher values for methoxyl content than the yellow variety.
Methoxyl content plays a pivotal role in controlling the setting time of pectin (Constenla and Lozano, 2003) [6]. Madhav and Pushpalatha (2002) [21] reported that the sugars such as arabinose, galactose, galacturonic acid and rhamnose are the structural components of pectin from fruit peel, which have free hydroxyl group (-OH) that can be methylated to methoxyl groups ($-OCH_3$) and the methoxyl content of pectin can vary with the source of raw material used for the extraction of pectin. Moreover, sugar binding capacity and spreading quality of pectin are increased with increased methoxyl content levels. Whistler and BeMiller (1997) [38] reported that methylation increases the capacity to form gels.

Total Anhydrouronic Acid Content

Table 6: Total anhydrouronic acid content (%) of pectin from yellow and purple passion fruit peels by ultrasonic extraction method

Power intensity (W)	Yellow variety		Purple variety	
	Blanched	Non-blanched	Blanched	Non-blanched
150	$70.49^{a} \pm 2.88$	$72.34^{a} \pm 0.35$	$65.65^{b} \pm 1.09$	$63.97^{b} \pm 0.97$
370	$67.75^{b} \pm 1.36$	$73.92^{a} \pm 1.25$	$64.94^{c} \pm 0.88$	$59.22^{d} \pm 1.05$
660	$65.38^{b} \pm 1.99$	$74.80^{a} \pm 0.73$	$61.95^{b} \pm 1.19$	$60.81^{b} \pm 4.56$
890	$65.91^{b} \pm 0.92$	$73.30^{a} \pm 0.93$	$59.93^{c} \pm 2.27$	$56.67^{c} \pm 1.92$

Data represented as mean (n=4) ± standard deviation. Mean values in a row superscripted by different letters are significantly different.

The purity of the extracted pectin is determined by the amount of anhydrouronic acid present and this is an important criteria which indicates the suitability of pectin for its use in products such as jams and jellies. This value should not be less than 65% according to the standards (Food Chemical Codex, 1996) [11]. In this study, the highest; 74.80% and the lowest; 56.67% values were obtained from the yellow non-blanched and purple non-blanched passion fruit peels respectively. Certain samples didn't fulfill the standard criteria which should not be less than 65%. As a result of the potential presence of proteins, sugars, and starch in the extracted pectins, the results suggest that the extracted pectin from the current investigation may not be adequately pure. Ismail *et al*. (2012) [15] said that the low value of anhydrouronic acid content implies that the extracted pectin might contain a high amount of protein.

FTIR Analysis

The Fourier Transform Infrared (FTIR) spectra show the functional groups and provides structural information about the extracted passion fruit peel pectin obtained from the ultrasonic extraction method in the wavelengths between 500 and 4,000 cm^{-1} (Figure 1). Here, the pectins obtained under the power intensities of 370W and 660W were subjected to FTIR as these power intensities have given the comparatively optimum results in terms of yield and other characterizations. The extracted pectin samples under 370W and 660W were compared with standard 150 grade commercial citrus pectin.

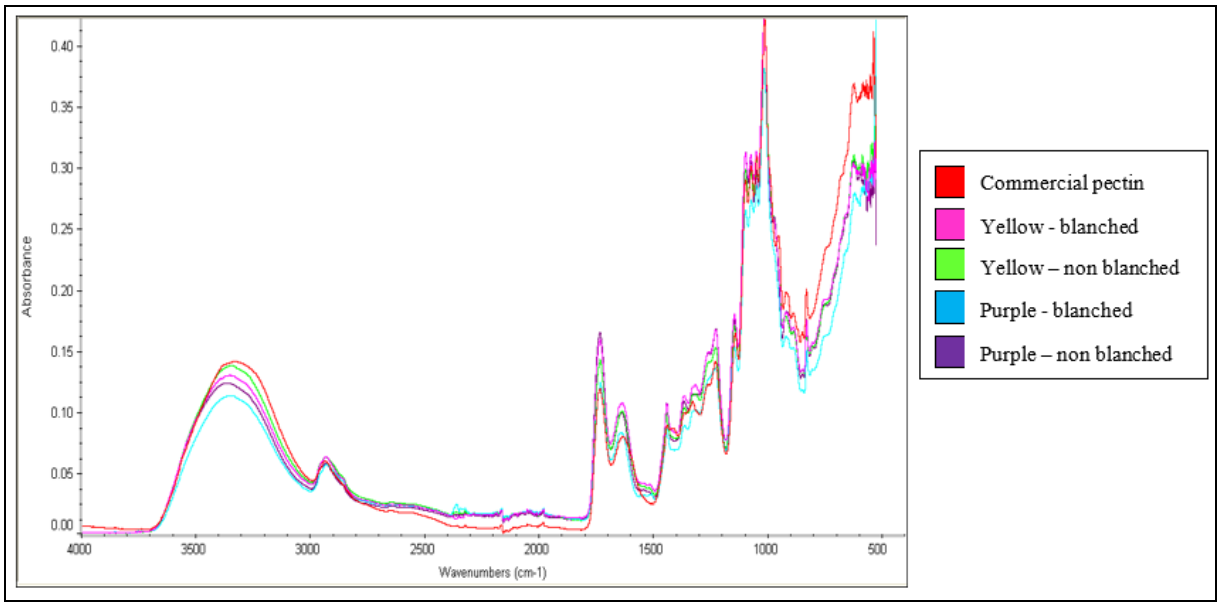

Fig 1: FTIR spectra for the pectins extracted from ultrasonic method at 370W

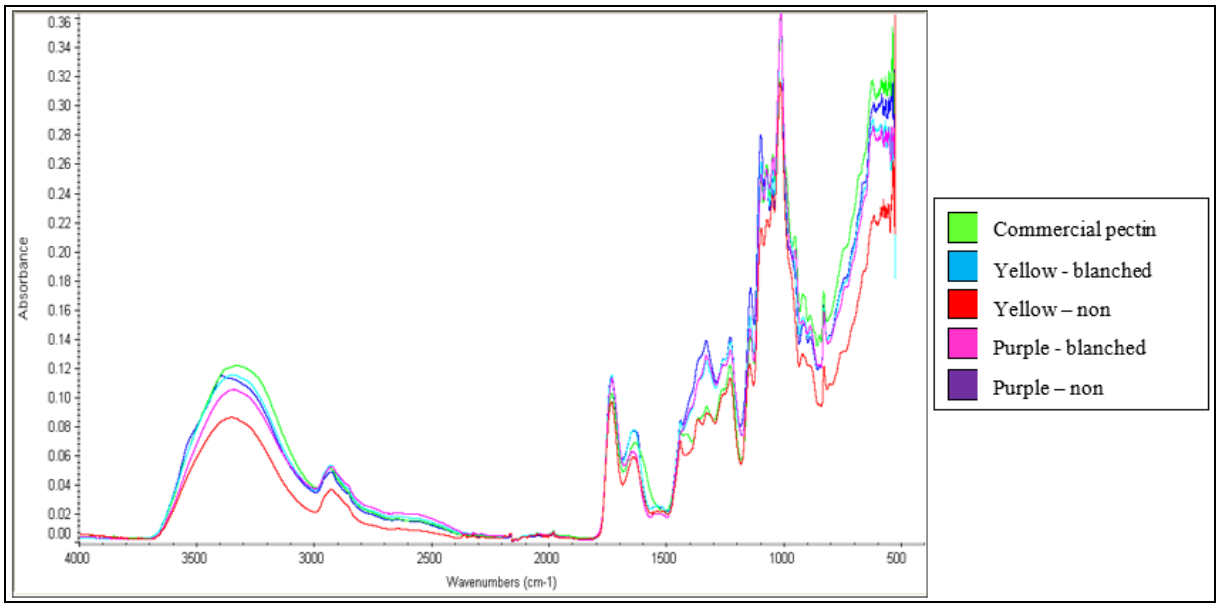

Fig 2: FTIR spectra for the pectins extracted from ultrasonic method at 660W

According to the spectra obtained from the FTIR, it is clear that the passion fruit peel pectin from both yellow and purple varieties are much similar to the commercial pectin. The peaks given at different wave numbers are similar and there are slight differences in the absorbance of pectin samples from two varieties. Further, the spectra obtained from the samples of two power intensities are also much similar.

Due to the existence of -OH stretching of the intermolecular or intramolecular hydrogen bonding of the backbone of galacturonic acid, all spectra contain an abundance of similarity of characteristic broad bands between 3000 and 3500 cm-1 (Singthong *et al*., 2004) [32]. This suggests that the pectin molecule or other pectin molecules contain a lot of -OH groups. A clear intermolecular hydrogen bond was present, as demonstrated by the presence of the peak closer to 3450 cm-1.

An O-CH3 stretching band between 2950 and 2750 cm^{-1} typically refers to C-H adsorption, which incorporates C-H, C-H_2 and C-H_3 stretching, and vibration bands; and specifically referring to the band at 2939 corresponding to the presence of methyl ester group of galacturonic acid (Singthong *et al*., 2004; Kumar and Chauhan, 2010)

[32, 19]. Due to an increase in the intensities and band spaces of the esterified carboxyl groups, the wavelength range between 950 and 1200 cm-1 is the "fingerprint" region for carbohydrates. This might be used to compare the different pectin varieties (Ismail *et al*., 2012) [15].

The carbonyl bands at the ranges of 1630-1650 cm^{-1} and 1740-1760 cm^{-1} indicate the free and esterified carboxyl groups, respectively (Gnanasambandan and Proctor, 1999) [12]. The absorbance is higher at 1750 cm^{-1} than at 1650 cm^{-1}, which is a unique characteristic of a high methoxyl pectin (Gnanasambandan and Proctor, 2000) [13]. The uneven C-O-C stretching vibration was attributed to the weak band at the range of 1150–1060 cm-1, which denotes the presence of -O–CH3. The ether and hydroxyl absorption peaks of the pyranose sugar ring were thought to be responsible for the absorption peaks at 1103 and 1015 cm-1 and at 1200-1000 cm-1. The distinctive D-pyranose glucose absorption peak was attributed to the absorption peak near 910 cm-1. (Yu and Sun, 2013) [41].

The extracted passion fruit peel spectra exhibited a good match with the spectrum of the commercial pectin, as evidenced by the peaks of functional groups with particular band stretching.

Pectin Morphology by Scanning Electron Microscope (SEM)

SEM images were used to examine the morphological properties and structure of wet pectin. SEM analysis was done for the blanched yellow and purple pectin samples extracted under 370W and 660W. The morphologies are compared under the magnification of 600.

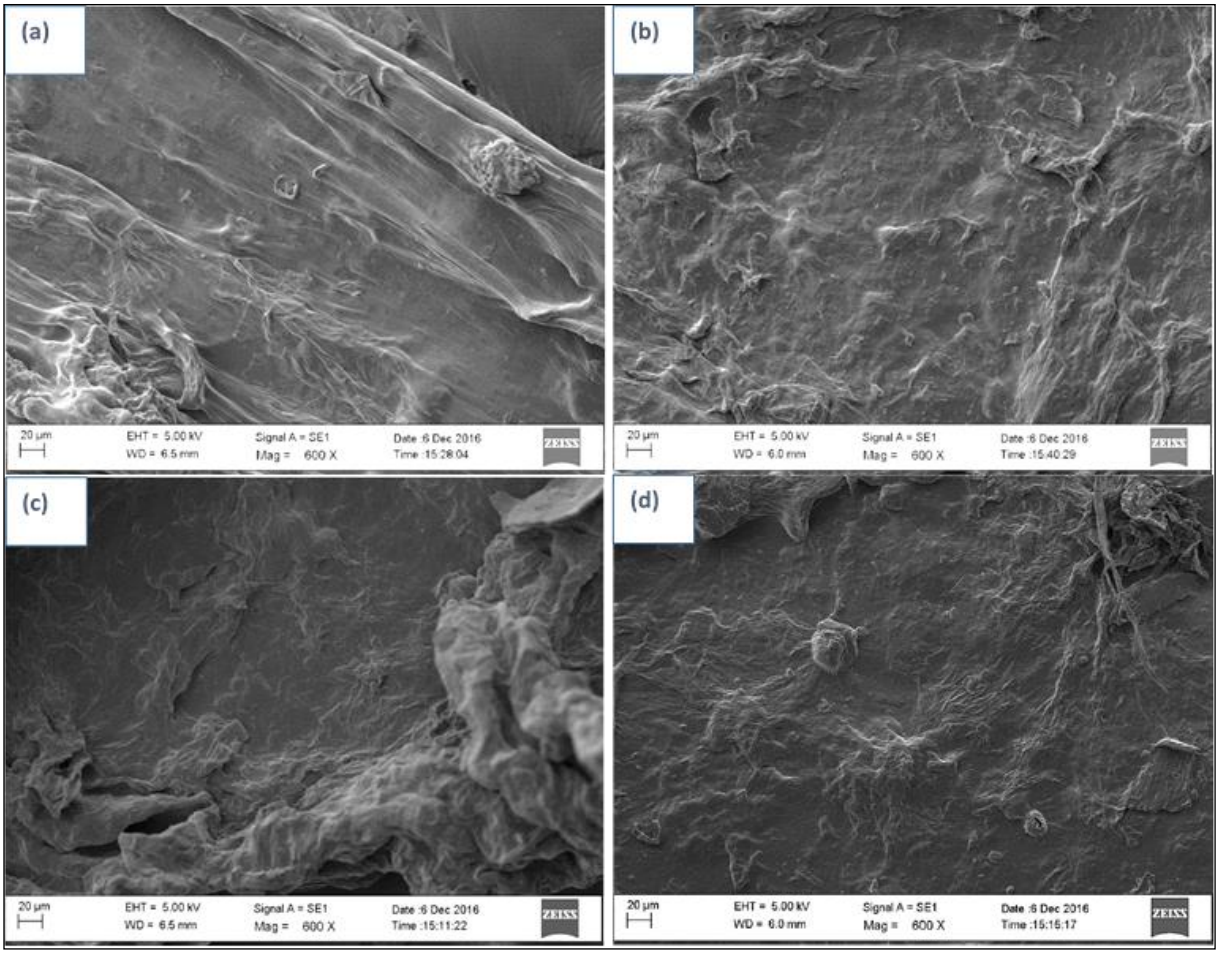

Fig 3: Scanning electron micrograph of ultrasonic extracted wet pectin (a) yellow variety – blanched, 370W (b) purple variety – blanched, 370W (c) yellow variety – blanched, 660W (d) purple variety – blanched, 660W

The wet pectin of the blanched yellow type has a nanostructure that is smooth and compact with few surface wrinkles, as seen by the SEM images in figure 3(a) and 3(c). For yellow blanched wet pectin, there is also a slight difference between the SEM pictures under the two power intensities. The wet pectin morphology of the purple variety is different, as evidenced by Figures 3(b) and 3(d), which indicate that they are less smooth on the surface and have more wrinkles.

Conclusion

According to this study, passion fruit peel found to be considerably a good source of pectin and purple variety is better than the yellow variety under the tested parameters.00. Results revealed that the highest extraction yield; 13.8%, obtained from the power intensity of 660Wand that is from blanched purple –passion fruit peel. Yield from the blanched yellow peel under 6660W is 13.38%. 660W, 60ºC and 20 kHz combination can be concluded

as the optimum condition in terms of yield and other tested characteristic properties. Rising ultrasonic power intensity negatively affected the DE, equivalent weight, methoxyl content and anhydrouronic acid. Blanching is a favorable pretreatment and purple variety has given a better pectin output than the yellow variety. Pectin obtained from purple and yellow passion fruit peels have given high DE values; 66.94% - 85.14% and 60.69% - 79.17% respectively. However, the extracted pectin from purple variety had a lower anhydrouronic content than the standard level while the yellow variety fulfilled the requirement. Future studies can be done on the energy aspect and to improve the purity of the extracted pectin, as blanched purple and yellow passion fruit peels can be concluded as good sources of pectin.

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