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Full Length Research Paper

Comparative Study of Pectin Extracted from Wastes of Guava and Grapefruit and its Application in Strawberry Jam

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The research study was aimed at finding the influence of temperatures (80, 90 and 100ºC) and pH (1.5 and 3.0) on extraction of pectin from guava pomace and grapefruit peel as well as comparing the characteristics of two different pectin and to see which one is more suitable for industrial applications. First of all, guava pomace and grapefruit peel were chemically evaluated for their moisture, ash, protein, fat and fiber content. Pectin, which is a family of complex polysaccharides, was extracted using alcohol precipitation method from guava pomace and grapefruit peel after which it was characterized using both qualitative and quantitative analysis to determine and compare the color, each pectin solubility in cold and hot water, equivalent weight, methoxyl content, anhydrouronic acid content and degree of esterification. The result showed that the colors of the pectin from these 2 sources were brown and light yellow, they were all soluble in hot and cold water, the equivalent weight, the methoxyl content, anhydrouronic acid content and degree of esterification were all higher in the pectin extracted from peels of grapefruit with 4445.4g/mL, 1.55%, 67.35% and 89.23%, respectively. Strawberry jam samples were prepared with two different pectin and analyzed for physicochemical and organoleptic attributes. Physicochemical analysis of jam showed that brix and acidity increased for all treatments during one month storage. The pH reduced for T_1 (from 3.01 to 2.86) and for T_2 (from 2.97 to 2.83). T2 (grapefruit pectin) showed maximum scores for organoleptic characteristics. The overall results showed that the pectin from grapefruit peel was more suitable for industrial use.

Keywords: Guava, Grapefruit, pomace, peel, pectin, characterization, jam, physicochemical, organoleptic

INTRODUCTION

Guava is also known as ‘‘apple of tropics’’ or ‘‘poor man’s fruit’’ with botanical name *Psidium guajava* L. and family Myrtaceae. It is a vital fruit crop of sub-tropical and tropical areas of the world. *Psidium* consists of five species, i.e. *P. chinensis*, *P. cattleianum*, *P. Friedrichsthalianum, Psidium guianense* and *P. guajava*. Guajava species include most of the cultivated guava (Hui, 2006).

Grapefruit (*Citrus paradisi*) is one of significant member of family Rutaceae (citrus family). It is subtropical in origin and well known for its bitter taste (initially cultivated in Jamaica around 18th century and now grown in all parts of the world). It has yellow-orange skin. It has been consumed as a traditional medicine in numerous countries as anti-fungal, antibacterial, antimicrobial, antiviral, preservative, anti-inflammatory, astringent and antioxidant.

Processing of fruit produces solid waste that is 50% of raw material. Usually the wastes contain immature fruit, pomace, peel, discarded fruit, core, mechanically spoiled fruit etc. The major waste is peel since whole fruit is processed into preserves after peeling. Currently peel is either thrown into rubbish or served to animals. However, alternative and useful method of exploiting the peel waste is the production of pectin. Pectin is one of the key products reacquired chiefly from citrus and apple wastes (Virk and Sogi, 2004).

For industrial purpose, the obtaining of pectin is mainly restricted to the peel of citric fruits, with approximately 25% of peptic substances and of apple husks, producing from 15 to 18% of pectin. Other sources of pectin include peels of mango, residues of sunflower and guava (Addosio *et al*., 2005).

Pectin is a heteropolysaccharide made up of hydrocolloids which are present naturally in higher plants and find applications in food industry extensively, due to their capability to make gels, to emulsify and stabilize (Winning *et al*., 2007).. Pectin finds its usage in processing of food i.e. marmalades, jam, sauces, juices, jellies, ketchups, syrups, concentrate and yoghurt etc. besides medicinal preparation like medical formulations to stabilize the suspensions (Canteri-Schemin *et al.,* 2005. The suitability of pectins for different purposes is determined by their character viz, anhydrouronic acid content, methoxyl content, degree of esterification and acetyl values. Hence, it is an unavoidable aspect that every pectin should be described properly for its biochemical characters. The pectin has commercial uses as agglutinates in blood therapy and also as thickening agent in medium used for canning meats (Aina *et al*., 2012).

Objectives of the research

- Effect of pH and temperature on extraction of pectin from guava pomace and grapefruit peel.
- Characterization of the extracted pectin to determine the color of the pectin, the solubility of dry pectin in cold and hot water, equivalent weight, methoxyl content, anhydrouronic acid content and degree of esterification.
- To compare the characteristics of two different pectin and see which one is more suitable for industrial application?

MATERIALS AND METHODS

Sample preparation

Pomace of guava and peel of grapefruit were collected from fruit shops in the premises of University of Agriculture, Faisalabad. Collected pomace of guava and peel of grapefruit were scrubbed to remove adhering and other mucilages through washing and dried in oven. They were then ground on complete drying and packed in polythene bags for extraction of pectin.

Proximate analysis

Dried pomace of guava and peel of grapefruit were evaluated for proximate composition i.e. moisture content, ash content, crude protein, crude fat and crude fiber according to their respective methods of AOAC, 2007.

Pectin extraction from the prepared sample

Pectin was extracted by using the method of Liu *et al.* (2001) with certain modification. The dried peel powder 15 g mixed with 100 mL distilled water and concentrated HCl was added till a pH of 1.5 and 3. The mixture was continuously stirred and maintained at 90º C ± 10º C for 30 minutes in water bath. After the heating period was over, the mixture was passed through two fold muslin cloth and was cooled to room temperature. Isolation of pectin was carried out using ethyl alcohol as precipitating agent. Following that, concentrated pectin extracts were precipitated in 95% ethanol. Continuous stirring was done for 15 min. Then the mixture was kept aside for 2hrs without stirring. Pectin was filtered through four layered muslin cloth. The precipitate was washed 2 to 3 times by ethyl alcohol, to further remove any remaining impurity. Finally, precipitate was kept for drying at 35ºC to 40ºC in hot air oven and percentage yield was calculated by following expression (Kulkarni and Vijayanand, 2010).

$$\%\ \text{Pectin Yield} = \frac{\text{Wt. of dried pectin}}{\text{Wt. of dried peel/pomace taken for extraction}} \times 100$$

Characterization of extracted pectin

The dried pectin obtained from the various peels of the three fruits was subjected to the following qualitative and quantitative test to characterize them.

Table 1. Chemical composition of guava pomace and grapefruit peel on dry weight basis

Parameters	Guava pomace	Grapefruit peel
Moisture	11.27 ± 1.81	12.7 ± 1.15
Ash	2.67 ± 0.58	3.33 ± 0.58
Crude protein	10.67 ± 0.98	6.82 ± 0.68
Crude fat	10 ± 0.5	5 ± 0.5
Crude fiber	30.64 ± 0.67	12.62 ± 0.87

Values presented are mean ± SD

Table 2. Effect of pH and temperature of extraction on pectin yield (%)

	pH 1.5			pH 3.0		
	80ºC	**90ºC**	**100ºC**	**80ºC**	**90ºC**	**100ºC**
Guava	10.32 ± 1.15	11.25 ± 0.80	9.12 ± 0.44	5.33 ± 0.88	5.78 ± 1.26	2.66 ± 1.35
Grapefruit	11.11 ± 2.33	15.55 ± 3.67	18.22 ± 0.77	2.15 ± 0.17	2.33 ± 0.34	4 ± 0.67

Mean Values ± S.D. Triplicate determinations

Table 3. Results of qualitative tests of pectin

Parameters	Guava	Grapefruit
Color	Brown	Light yellow
Solubility in cold water	Insoluble, form suspension after vigorous shaking	Insoluble, form suspension after vigorous shaking
Solubility at 85-90ºC for 15 minutes	Pectin dissolves	Pectin dissolves

Qualitative Tests

• **Color: This was done by visual observation**

• **Solubility of dry pectin in cold and hot water:** (0.25%) of the pectin samples were separately placed in a conical flask with 10 mL of 95% ethanol added followed by 50 mL distilled water. The mixture was shaken vigorously to form a suspension which was then heated at 85-95°C for 15 min (Fishman *et al.*, 1984).

QUANTITATIVE TEST

• **Equivalent weight determination**

Pectin sample (0.5 g) was weighed into a 250 mL conical flask and moistened with 5 mL ethanol, 1.0 g sodium chloride was added to the mixture followed by 100 mL distilled water and few drops of phenol red indicator. Care was taken at this point to ensure that all the pectin had dissolved and that no clumping occurred at the sides of the flask before the solution was then slowly titrated (to avoid possible de-esterification) with 0.1 M NaOH to a pink color at the endpoint.

Equivalent weight was calculated using the equation below:

$$\text{Equivalent weight} = \frac{\text{Wt. of sample (g)} \times 1000}{\text{Vol. of alkali (mL)} \times \text{Normality of alkali}}$$

• **Methoxyl Content**

To the neutral solution titrated for equivalent weight containing 0.5 g of pectic substance, added 25 mL of 0.25M NaOH, shaken thoroughly and allowed to stand for 30 min at room temperature in a stoppered flask. HCl (25 mL, 0.25 M) was added and titrated with 0.1M NaOH to the same end point as before.

Table 4. Showing the characterized parameters for two sources

Parameters	Guava	Grapefruit
Equivalent weight (g/mL)	818.8 b	1088.9 a
Methoxyl content (%)	8.37 b	10.55 a
Anhydrouronic acid content (%)	56.83 b	67.35 a
Degree of esterification (%)	83.75 b	89.23 a

Values with different letter in each row are significantly different

Table 5. Different jam formulations

Treatments	Pectin
T_1	Guava pectin
T_2	Grapefruit pectin

Table 6. Effect of storage period and treatments on brix (ºB) of strawberry jam samples

Treatments	Storage (Days)			
	0	**15**	**30**	**Mean**
T_1	68	68.67	69.67	**68.78 a**
T_2	68	68	68.67	**68.22 b**
Mean	**68.00 b**	**68.33 b**	**69.17 a**	

Mean carrying same letter are statistically non-significant

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

• **Total Anhydrouronic Acid Content**

By using the values of the equivalent weight and the methoxyl content, total anhydrouronic acid content (Azad *et al*., 2014) was calculated by using expression given below:

$$\%\ \text{AUA} = \frac{176 \times 0.1\ z \times 100}{\text{Wt.} \times 1000} + \frac{176 \times 0.1\ y \times 100}{\text{Wt.} \times 1000}$$

When molecular unit of AUA (1 unit) = 176 g

Where,

z = ml (titre) of NaOH from equivalent weight determination

y = ml (titre) of NaOH from methoxyl content determination

w = weight of sample

• **Degree of Esterification**

The DE of pectin was measured on the basis methoxyl and AUA content (Azad *et al*., 2014) and calculated by following formula.

$$\%\ \text{DE} = \frac{176 \times \%\ \text{MeO}}{31 \times \%\ \text{AUA}} \times 100$$

Preparation of jam samples

Strawberry jam was prepared following the method given in Food Preservation Manual (Awan and Rehman, 2011) with certain modification.

Fresh strawberries were washed with water and put into a steam pan. Strawberries were cooked for 15 min till became soft. These were crushed for pulp extraction and passed through the fine pulper to remove the impurities. Strawberries puree and sugar were taken and heated till 65^{o}brix. Pectin which was previously dissolved in 200 mL of water was added in it and heated for some time till 68^{o}brix. Drop test or sheet test was done to check the

Table 7. Effect of storage period and treatments on pH of strawberry jam samples

Treatments	Storage (Days)			
	0	15	30	Mean
T_1	3.01	2.9	2.86	**2.92 a**
T_2	2.97	2.87	2.83	**2.89 b**
Mean	**2.99 a**	**2.89 b**	**2.85 c**	

Mean carrying same letter are statistically non-significant

Table 8. Effect of storage period and treatments on acidity (%) of strawberry jam samples

Treatments	Storage (Days)			
	0	15	30	Mean
T_1	1.12	1.15	1.18	**1.15 b**
T_2	1.22	1.23	1.25	**1.23 a**
Mean	**1.17 c**	**1.19 b**	**1.22 a**	

Mean carrying same letter are statistically non-significant

Table 9. Sensory evaluation of jam with different pectin

Parameters	Guava pectin	Grapefruit pectin
Color	7.33 b	8.00 a
Flavor	7.13 b	8.33 a
Taste	6.13 b	7.80 a
Texture	6.20 b	8.33 a
Spread ability	4.20 b	8.47 a
Overall acceptability	5.20 b	7.93 a

Values with different letter in each row are significantly different

total solids or brix and oven was switched off. Citric acid, flavor and color were added in it and cooled. Jam was poured in glass jars.

Physicochemical analysis of jam

Brix, pH and acidity of jam samples were analyzed by the standard method of AOAC, 2007.

Sensory evaluation of jam

Strawberry jam was evaluated by a penal of judges for sensory parameters like color, flavor, taste, texture, spread ability and overall acceptability according to 9-point hedonic scale given by Meilgaard *et al*. (2007).

Statistical analysis

The data obtained for different parameters was subjected to statistical analysis (2 Factorial) according to Steel *et al*. (1997). Significant ranges were further postulated by LSD mean comparison test using statistics 8.1 software.

RESULTS AND DISCUSSION

Chemical composition of guava pomace and grapefruit peel

Table 1 showed that the moisture content of guava pomace and grapefruit peel was found to be 11.27% and 12.7% respectively. The results obtained were closely related to the findings of Nassar *et al*. (2008) who reported that orange peel consists of 9.46% moisture content. The results were also supported by Ocen and Xu (2013) who concluded that orange by-product powder contains 9.25% moisture. The ash content of guava pomace and grapefruit peel was found to be 2.67% and 3.33% respectively. The results of ash obtained for guava pomace were in accordance with the findings of Bernardino-Nicanor *et al*. (2006) who concluded that guava seed meal contains 1.50% ash. The results were also in close association with Nassar *et al*. (2008) who concluded that orange peel contains 2.61% ash. The results of ash content obtained for grapefruit peel were supported by Kohajdova (2013) who reported that grapefruit peel comprises 3.55% ash. Furthermore, the results were related to the findings of Annon (2010) who concluded that grapefruit peel contains 3.7% ash. Similarly, Ocen and Xu (2013) stated that orange by-product powder consists of 3.43% ash. Crude protein content of guava pomace and grapefruit peel was found to be 10.67% and 6.82% respectively. Results of crude protein obtained for guava pomace were associated with the results of Shams El-Din and Yassen (1997) who concluded that guava seed meal contains 9.6% crude protein. The results of crude protein obtained for grapefruit peel were related to the findings of Annon (2010) who reported that grapefruit peel contains 6.8% crude protein. Moreover, the results found were supported by Kohajdova (2013) who described that grapefruit peel comprises 6.65% crude protein. Crude fat content of guava pomace and grapefruit peel was found to be 10% and 5% respectively. Results of crude fat for guava pomace were associated with the results of Shams El-Din and Yassen (1997) who concluded that guava seed meal contains 10.5% crude fat. The results of crude fat obtained for grapefruit peel were in accordance to the findings of Annon (2010) who reported that grapefruit peel contains 4.9% crude fat. Moreover, the results acquired were supported by Nassar *et al*. (2008) who described that orange peel consists of 4.53% crude fat. The fiber content of guava pomace and grapefruit peel was found to be 30.64% and 12.62% respectively. Results of crude fiber obtained for guava pomace were compared with the results of Shams El-Din and Yassen (1997) who concluded that guava seed meal contains 53.6% fiber. The results obtained were also supported by Annon (2010) who reported that grapefruit peel contains 10.5% crude fiber. Although there is little variation in all these results, the reason could be attributed due to type of soil, varieties, season, maturity and environmental changes.

Pectin extraction from prepared samples

Data presented in Table 2 showed that pH played a great role in the extraction of pectin from guava pomace and grapefruit peel. At pH 1.5 and 3.0, pectin yield was 11.25% and 5.78% from guava pomace at 90ºC and 18.22% and 4% from grapefruit peel at 100ºC respectively. It is apparent from the results that maximum pectin yield was obtained at pH 1.5. However, decline in pectin yield was observed at pH 3.0 at all temperatures 80ºC-100ºC after 30 minutes extraction.

The results were in accordance with the findings of Emaga *et al*. (2008) who reported that pectin yields from various extractions at pH 1.5 were higher than those at pH 2.0 from banana peel. Yapo *et al*. (2007) and Levigne *et al*. (2002) observed the same trends on pectin extracted from sugar beet, unlike with soy hull pectin where the yields decreased with increasing acid strength (Kalapathy & Proctor, 2001).

Extraction of pectin was also affected by changing the temperature ranging from 80ºC-100ºC (Table 2). Maximum pectin yield (11.25%) was obtained at 90ºC from guava pomace at pH 1.5 for 30 minutes. On increasing the temperature from 80ºC-90ºC increase in the yield of pectin was observed and further increase in temperature from 90ºC-100ºC resulted in reduction in the yield of pectin. Decrease in pectin yield at higher temperature (90ºC) could be attributed to break down of pectin molecules. From grapefruit peel, maximum pectin yield (18.22%) was obtained at 100ºC for 30 minutes at pH 1.5. On increasing the temperature from 80ºC-100ºC increase in the yield of pectin was observed.

Characterization of extracted pectin

Qualitative analysis

From Table 3, the characteristic color of pectin obtained from guava pomace and grapefruit peel was brown and light yellow respectively while according to IPPA (2009), pectin are usually light in color, factors such as surface contamination, environmental factors, types of fruits used and human error might have contributed to the discrepancy in color, this could be due to the amount of alcohol used for precipitation and purification during the experiment not been enough.

Quantitative analysis

From Table 4, equivalent weight was found significantly higher (1088.9 g/mL) in grapefruit peel than that recorded for guava pomace (818.8 g/mL). The results were higher than cocoa husk pectin (510.68 mg/mLto 645.19 mg/mL) (Ramli and Asmawati, 2011). The results were supported by Kumar & Chauhan, 2010 who reported that equivalent weight of apple pomace pectin ranged from 833.33 to 1666.30 mg/mL.Methoxyl content was found significantly higher (10.55%) in grapefruit peel than that recorded for guava pomace (8.37%). The results obtained were supported by Sudhakar and Maini (2000) who reported

8.62% methoxyl content in mango peels. The results were also in accordance with Madhav and Pushpalatha (2002) who described 7.33% methoxyl content in peel of mango, banana (7.03%), pomelo peel (8.57%), Lime (9.92%), Kalamanzi (musk lime) (10.3%) and pineapple skin (10.2%) but higher than dragon fruit pectin (2.98% to 4.34%) (Ismail *et al*., 2012). Moreover they studied that spreading quality and sugar binding capacity of pectin also increased with increase in methoxyl content (Madhav and Pushpalatha, 2002). It is obvious from the results that grapefruit peel pectin showed more spreading quality and sugar binding capacity as compared to guava pomace pectin.

Anhydrouronic acid content was found significantly higher (67.35%) in grapefruit peel than that recorded for guava pomace (56.83%). The results were supported by Nazaruddin *et al*., (2013) who observed 68.05% AUA in HCI extraction of roselle calyces. Resembled values were also found in apple pomace pectin, commercial apple pectin and dragon fruit pectin which was 59.52 to 70.50%, (Kumar & Chauhan, 2010), 61.72% and 45.25 to 52.45% (Ismail *et al*., 2012) respectively. Low value of AUA means that the extracted pectin might have a high amount of protein (Ismail *et al*., 2012). Degree of esterification was found significantly higher (89.23%) in grapefruit peel than that recorded for guava pomace (83.75%). These results were consistent with previous measurement of 76.30 % DE in *Citrus maxima* (Sotanaphun, *et al*., 2012) and 79.51% DE in premature lemon pomce (Azad *et al*., 2014).

Physicochemical analysis of jam samples

Jam is product of sugar and pectin contained fruits. It has characteristics of texture, color and taste. It should be capable of storage for reasonable period after opening of bottle without risk of spoilage. Strawberry jam samples prepared with grapefruit pectin gave better gel when compared to sample with guava pectin. The poor jelling property of guava pectin as anticipated may due to low level of pectin and solid matter present. All the prepared jam samples were further analyzed for brix, pH and acidity during an interval of 15 days up to one month. It was observed that the highest brix (68.78ºB) was observed for T_1 (guava pectin). However, the lowest brix (68.22ºB) was recorded in T_2 (grapefruit pectin). The mean value for brix in strawberry jam treated at 0 day was found 68ºB whereas it was increased with the passage of time and until unless the highest brix of 69.17ºB was recorded at 30th day of storage time. The increase in brix might be due to the formation of water soluble pectin from protopectin during storage. However, results obtained were in accordance with the findings of Garcia-Martinez (2002) who reported that in orange products, brix values range between 49ºB and 66ºB while studying jam/marmalade prepared from osmodehydrated fruit. The results were also supported by Egbekun *et al*., (1998) who concluded that brix of fluted pumpkin marmalade was 68.50ºB. The statistical analysis for brix revealed that effect of treatments and storage period on samples was significant (Table 6). The pH of jam is an important factor to obtain optimum gel condition. In the present study maximum pH (2.92) was recorded for T_1 (guava pectin) and minimum pH (2.89) recorded for T_2 (grapefruit pectin). The mean value for pH in strawberry jam treated at 0 day was found 2.99 whereas it was decreased with the passage of time and until unless the lowest pH of 2.85 was recorded at 30th day of storage time. This decrease in pH (more acidic) might be due to ascorbic acid degradation, hydrolysis of pectin (Sogi and Singh, 2001) and other acidic compounds (Shakir *et al*., 2007). The results were supported by Hussain and Shakir (2010) who reported that during storage intervals pH of mixed jam of apricot and apple decreased due to increase in acidity during storage. The results were in accordance with the findings of Mohamad *et al*. (2012) who studied that pH of the formulated jam (varied composition of watermelon rind and sugar) decreased gradually over the 90 days storage. The statistical analysis for pH revealed that effect of treatments and storage period on samples were significant (Table 7). Jam samples were also analyzed for acidity. The highest acidity (1.23%) was observed for T_2 (grapefruit pectin). However, the lowest acidity (1.15%) was recorded in T_1 (guava pectin). The mean value for acidity in strawberry jam treated at 0 day was recorded 1.17% whereas it was increased with the passage of time and until unless the highest acidity of 1.22% was recorded at 30th day of storage time. The increase in acidity might be the result of formation of acidic compounds by degradation or oxidation of carbonyl compounds during storage. Increase in acidity of fruit jams was reported earlier to be a result of ascorbic acid degradation or hydrolysis of pectin (Sogi and Singh, 2001). The results were in close association with the findings of Egbekun *et al*. (1998) who reported 1.29% acidity value for fluted pumpkin marmalade. The statistical analysis for acidity revealed that effect of treatments and storage period on samples were significant (Table 8).

Sensory evaluation of jam samples

Statistical analysis showed a significant difference in acceptability among different treatments (Table 9). Color is important sensor character on which consumer preferences dependent. Maximum mean value was recorded for grapefruit pectin (8) and minimum mean value for guava pectin (7.33). Flavor acceptability was highest for grapefruit pectin (8.33) while jam with guava pectin gave lowest (7.13) means value for flavor acceptability. For taste the maximum mean value was for grapefruit pectin (7.80) which is significantly different from guava pectin (6.13). The mean value of the jam texture was low for guava pectin. It was 6.20 for guava pectin while 8.33 was recorded for grapefruit pectin. For spread ability and overall acceptability, grapefruit pectin was most acceptable with mean values of 8.47 and 7.93 respectively.

CONCLUSIONS

Guava pomace and grapefruit peel are good sources of pectin which were extracted using alcohol precipitation. Grapefruit peel pectin is more suitable for industrial application.

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