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Extraction and characterization of pectin from grapefruit (*Duncan cultivar*) and its utilization as gelling agent

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Abstract

Globally, agro-industrial by products such as fruit waste has attained immense recognition to be used for the extraction of valuable functional ingredients. Pectin is naturally occurring biopolymer that is widely recognized in food industry as well as in biotechnology. Keeping in view, current research was conducted for extraction, characterization and utilization of grapefruit peel pectin from Duncan cultivar. The extracted pectin was characterized for different parameters that explored its role in value added products. Acid extraction was carried out and then pectin was characterized for equivalent weight, ash content and methoxyl content. With the addition of extracted pectin, jam was prepared and analysed for physicochemical analysis and sensory attributes. The maximum extraction (22.55%) was done from grapefruit peel at temperature-120°C with pH-1.5, while minimum extraction (0.41%) was obtained at temperature-120°C with pH-2. Moreover, adding pectin in jam formulation resulted in significant effect on texture of the final product. Conclusively, pectin holds a great potential to be extracted and utilized in fruit based products for best quality and value addition.

Introduction

Citrus is among the most widely consumed fruits in the world and holds a greater value in human diet. Approximately one third of the total citrus produced is utilized for processing. Being a broad family, it comprises of kinnow, sweet orange, lemon, Grapefruit etc. Grapefruit (*Citrus paradisi*) is one of important member of family *Rutaceae* (citrus family). Citrus peel is rich source of bioactive molecules and its albedo layer is a good source of pectin (González-Molina *et al*., 2010).

The term pectin is a class of complex hydrophilic colloidal hetero-polysaccharide, consisting chiefly or partially methylated polygalacturonic acid units (a linear galacturonoglycan of (1-4) linked galactopyranosyluronic acid) with molecular weight ranging from 50,000 to 150,000 Daltons (Mohnen, 2008). Chemically pectin is a linear polysaccharide consisting of about 300 to 1000 monosaccharide units present in the cell wall of fruits and have important nutritional and technological properties, mainly because of their ability to form gels. Pectin also functions as a hydrating agent and cementing material for the cellulose network (Habrant *et al.*, 2009). Industrially prepared pectin is extracted from citrus peel (lemon, lime, orange, and grapefruit) and apple pomace using water acidified with a strong mineral acid, notably, nitric, hydrochloric or sulphuric acid under different pH, temperature, and duration conditions most often in the order of 1.4–3, 60–100°C, and 20–360 min, respectively (Abid *et al.*, 2009). The recovery of pectin can be enhanced using alcohols involving multiple-stage physical-chemical process (Shakir *et al.*, 2009; Cui *et al*., 2011).

The utilization of citrus peel for the extraction of pectin has many benefits as citrus peel contains higher amount of pectin that are in the range of 25-30% of the dried peel mass (Srivastava and Malviya, 2011). The extraction methods and conditions employed are also important in determining the viscosity of pectin that is also affected as a function of molecular weight, degree of methylation, pH, presence of counter-ions and sucrose (Rehman *et al*., 2004). The major use of pectin is as a gelling agent in jams, jellies and preserves. Pectin is important emulsifying, stabilizing and thickening agent used in the preparation of numerous food products such as ice cream, beverages, soft drinks, fish, meat and milk products (Barrera *et al.*, 2002; Wicker *et al*., 2003). Keeping in view all of above facts, the present study was designed to explore new horizons in agro-industrial waste management through extraction of pectin from grapefruit peel. Among different cultivars of grapefruit grown in Pakistan, Duncan cultivar has been reported previously to contain higher amounts of peel i.e. 33.60% (Ishfaq *et al*., 2007) thus the same cultivar was selected for the extraction of grapefruit

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pectin. The present research has practical implications as extracted pectin can be utilized to replace to commercial pectin as gelling agents in different food products.

Materials and Methods

Peel from fresh grapefruit was collected from fruit shops in the premises of University of Agriculture, Faisalabad, Pakistan followed by sun drying. After drying they were grinded into small pieces and packed in polythene bags for the extraction of pectin.

Extraction and characterization of pectin

Pectin from grapefruit peel was extracted by the method discussed by Liu *et al*. (2001). In which dried peel was powdered and mixed with deioinized water and concentrated HC1 is added to give a pH of 1.6 ± 0.5. The mixture was continuously stirred and maintained at 95°C for 30 minutes. Then iso-propanol and ethanol were applied to precipitate and purified the final pectin and the treatments were made as followed. Pectin was characterized by ascertaining the equivalent weight and Methoxyl contents as per the methods of Masmoudi *et al*. (2010). Moreover, ash content was also determined (AOAC, 2000). Later, the apple Jam was prepared following the method described by Awan and Rehman (1999).

Physiochemical attributes of apple jam

Various physicochemical attributes as pH, Brix, acidity of apple jam were recorded using their respective procedures. The pH of each sample was determined with the help of digital pH meter following the procedures of AOAC (2006). Purposely, 50 mL of fruit juice was taken in 100 mL beaker and pH was recorded by the pH meter (Ino lab pH 720, Germany). Total soluble solids of the coated samples were directly recorded by using Digital Refractometer (RA-600 refractometer, Kyoto Electronics Manufacturing Co., Ltd., Japan) following the standard procedure of AOAC (2006). A drop of fruit juice was placed on the prism of refractometer and reading was noted and the results were expressed as °Brix. The acidity of each sample was determined using digital fruit acidity meter (QA Supplies GMK-835, USA). Accordingly, 300 µl of the juice was poured in dilution bottle and mixed well by shaking. Afterwards, diluted fruit juice was placed on the sample well to determine the value for titratable acidity appeared on screen within 10 seconds.

Sensory evaluation of apple jam

The sensory parameters like colour, texture, flavour, taste and overall acceptability were compared and recorded following 9-point hedonic scale given by Meilgaard *et al*. (2007).

Statistical analysis

Data obtained from different parameters was analyzed statistically by respective method of Steel *et al.* (1997).

Results and Discussion

Optimization of pectin extracted from grapefruit peel

The results regarding the effects of temperature and pH on the recovery of pectin from the grapefruit peel (*Citrus paradisi*) are elaborated in (Table 1). Results gave conclusion that temperature and pH have positive effect on the extraction of pectin. The maximum extraction of pectin from grapefruit peel was at temperature-120°C with pH-1.5 (22.55%), followed by 17.02% at temperature-100°C and pH-1.5, while minimum extraction of pectin (0.41%) was obtained at temperature-120°C with combination of pH-2. It is evident from the mean table that best temperature range was at 120°C which gave average maximum extraction of pectin from grapefruit peel valued 11.17% and pH-1.5 showed greatest result and score 18.35% as selected best range among pH.

The results obtained from this study were supported by Arslan and Toğrul (1996) who concluded that the amount of pectin extracted from grapefruit peel was 21.1%. The variation in amount of recovery of pectin from peel depends on the maturity stage and composition of cell walls of the fruits. It is deduced from the experiments that as the temperature raised, recovery of pectin extracted from grapefruit peel was also increased and the pH has significant effect on the extraction of pectin from peel. It is clear from the results that as pH increased the recovery of pectin from grapefruit peel turn toward decline. The results of this work are supported by Liu *et al.* (2006) who concluded that pectin recovery reduced as the pH of the extracted medium increased and pectin extraction increased as the temperature raised.

Characterization of pectin extracted from grapefruit peel

The extracted pectin was analysed for their methoxyl contents, equivalent weight and ash contents.

Methoxyl content

Methoxyl content is an important molecular index for pectin classification that describes the extent to which carboxylic groups in pectin molecules exist as the methyl ester in ratio to all esterified groups. The

Table 1. Effect of temperature and pH on the extraction of pectin from grapefruit peel

Parameters	Temperature	pH of extraction			Pooled mean
		1.0	1.5	2.0	
Extraction yield of pectin	80°C	10.10	15.49	0.98	**8.85^b**
	100°C	10.22	17.02	1.63	**9.62^b**
	120°C	10.55	22.55	0.41	**11.17^a**
	Means	**10.28^b**	**18.35^a**	**1.00^c**	
Methoxyl contents	80°C	12.12	11.78	11.20	**11.69**
	100°C	12.20	12.50	11.50	**12.06**
	120°C	11.76	11.06	10.86	**11.22**
	Means	**12.02**	**11.77**	**11.18**	
Equivalent weight	80°C	962.50	1012.50	975.00	**983.33**
	100°C	1063.63	963.75	972.48	**999.95**
	120°C	1025.38	1000.50	937.50	**987.79**
	Means	**1017.17**	**992.25**	**961.66**	

Table 2. Effect of commercial and grapefruit extracted pectin on jam brix°

Storage intervals (days)	Sources of pectin		Pooled means
	Grapefruit extracted pectin	Commercial pectin	
0	66.330±1.964	67.000±2.368	66.665±0.474
7	66.660±2.466	67.330±0.728	66.995±0.474
14	67.000±0.614	67.330±1.133	67.165±0.233
21	67.000±1.418	67.660±3.079	67.330±0.467
28	67.330±3.674	68.000±2.902	67.665±0.474
35	67.660±2.101	68.330±1.330	67.995±0.474
Means	**66.997±0.471**	**67.608±0.490**	

Table 3. Effect of commercial and grapefruit extracted pectin on pH of jam

Storage intervals (days)	Sources of pectin		Pooled means
	Grapefruit extracted pectin	Commercial pectin	
0	3.460±0.225	3.390±0.151	3.425±0.049a
7	3.410±0.026	3.370±0.076	3.390±0.028ab
14	3.380±0.117	3.340±0.039	3.360±0.028b
21	3.370±0.079	3.320±0.021	3.345±0.035b
28	3.320±0.069	3.250±0.031	3.285±0.049c
35	3.290±0.051	3.240±0.065	3.265±0.035c
Means	**3.372±0.061a**	**3.318±0.062b**	

maximum value of methoxyl contents were 12.06% at temperature 100°C and pH gave maximum value at pH-1 (Table 1). The findings of the current study are in harmony with a similar work by Bagherian *et al.* (2011) involving comparative evaluation of different extraction techniques to extract carbohydrates, polysaccharides and other functional compounds from vegetable sources. It was inferred that highest total amount of pectin yield was found to be 27.81% (w/w) for 6 min of extraction at 900 W using microwave extraction technique. Moreover, yield, galacturonic acid content and degree of esterification increased with an increase in heating time. More recently, Assoi *et al.* (2014) assessed Palmyra palm (Borassus aethiopum Mart) for pectin content. In their study, yield and galacturonic acid content of soluble solids were investigated at various pH (natural pH of 5.2-5.5), time (30-120 min) and temperature (70°C, 80°C, 90°C). It was deduced that yield and galacturonic acid content of extracted soluble solids were dependent on temperature, time and pH of extraction. Moreover, a high galacturonic acid content was obtained at 70°C and 90°C under natural pH; at 70°C pH 7 and pH 2.5 at all temperatures studied. Analysis of pectin extracted at natural pH (30 min, 90°C) revealed highly esterified pectin with good gelling and emulsifying properties.

Equivalent weight

The maximum value of equivalent weight is 999.95 at temperature-100 and pH gave maximum value 1017.17 at pH-1 (Table 1). In a similar attempt, Jindal *et al.* (2013) explored bael fruit (Aegle marmelos) for pectin extraction. They observed that the process yielded 15% (w/w) pure pectin. Moreover, galacturonic acid content of 87.8%, degree of esterification of 47.2%, 17.3% methoxy groups, 0.29% acetyl groups and equivalent weight of 1209.5, indicate it to be a good gelling agent and easily amenable to derivatization. The results of this study are supported by Kumar and Chauhan (2010) who concluded that the equivalent weight of grapefruit peel pectin was 953.

Physicochemical attributes of product (jam)

Evaluation of extracted pectin was carried out by applying it in some eatable items. For this intention apple jam was developed by using extracted pectin and compared with jam prepared by using commercially available pectin.

Brix

The results regarding degree brix for Jam are presented in the (Table 2). Data regarding mean revealed that maximum brix was found at 35 days of storage followed by 28, 21, 14 and 7 days, whereas, lowest brix; was found at 0 days of storage. From the results it is indicated that the self prepared pectin showed increased in degree brix i.e. 1.33 percent during storage, whereas, same increase in degree brix was observed in case of commercial pectin. In a similar trial involving apricot jam, Touati *et al.* (2014) evaluated storage conditions and their effect on the overall quality of jam. They observed that total solids were considerably increased total solids during storage. Moreover, interaction time-temperature factor had significant effects on total solids along with other allied physicochemical parameters and sensorial profile. The result obtained in this study could be related to the findings of the Singh *et al.* (2009) who reported that brix increased during storage due to the formation of water soluble pectin from the protopectin.

pH and acidity

The results regarding pH of Jam are presented in the (Table 3). Analysis of variance for pH showed that results varied highly significantly among the storage time, while the effect of treatments and the interaction of treatments and storage intervals remained non significant on the pH of jam. Mean values for pH of jam during storage indicated the decline in pH during storage and the maximum pH was found at 0 days of storage, while the lowest pH was found at 35 days of storage. However, mean values of commercial pectin showed lower pH value (3.318±0.062) as compared

Table 4. Effect of commercial and grapefruit extracted pectin on acidity of jam

Storage intervals (days)	Sources of pectin		Pooled means
	Grapefruit extracted pectin	Commercial pectin	
0	0.680±0.015	0.700±0.035	0.690±0.014
7	0.700±0.026	0.720±0.017	0.710±0.014
14	0.720±0.021	0.730±0.031	0.725±0.007
21	0.720±0.027	0.740±0.028	0.730±0.014
28	0.740±0.013	0.760±0.028	0.750±0.014
35	0.750±0.047	0.760±0.029	0.755±0.007
Means	**0.718±0.026b**	**0.735±0.023a**	

Table 5. Effect of commercial and grapefruit extracted pectin on overall acceptability of jam

Storage intervals (days)	Sources of pectin		Pooled means
	Grapefruit extracted pectin	Commercial pectin	
0	6.600±0.115	7.400±0.061	7.000±0.566
7	6.600±0.188	7.400±0.122	7.000±0.566
14	6.400±0.119	7.200±0.305	6.800±0.566
21	6.600±0.252	7.000±0.232	6.800±0.283
28	6.400±0.206	7.000±0.340	6.700±0.424
35	6.200±0.155	6.800±0.114	6.500±0.424
Means	**6.467±0.163b**	**7.133±0.242a**	

to the local pectin (3.372±0.061).

The results obtained during this study were similar to the findings of Lindroth (1980) who reported the decreasing tendency for pH during storage. The decrease in pH might be attributed to the formation of acidic compounds during storage. Likewise, the results regarding acidity of jam are presented in the (Table 4). Mean values for acidity of jam during storage indicated the increasing tendency during storage and the maximum acidity was found at 35 days, whereas the lowest acidity was found at 0 days. However, mean values of commercial pectin showed more value for acidity (0.735±0.023) as compared to the local pectin (0.718±0.026).

Instant findings are in harmony with the work of Caro *et al.* (2004) who processed different varieties of citrus and kept juices for up to 12 days. In their research, it was expounded that ascorbic acid decreased significantly ranging from 1.63 to 5.10 mg per gram of dry matter, moreover, a significant increase in total flavonoids (mainly hesperidin) was found in the segments, while the juices showed a diminution in flavonoid content. The results regarding this study could be related to early findings of Muhammad *et al.* (2008) who concluded that acidity increases during storage that might be the result of formation of acidic compounds by the degradation or oxidation of the carbonyl compounds.

Sensory evaluation and overall acceptability

The results regarding overall acceptability of jam were presented in (Table 5). Analysis of variance showed that results varied highly significantly among the treatments, although the effect of storage intervals and interaction of treatments and storage intervals remained non significant on the overall acceptability of jam. Mean values for overall acceptability of jam during storage showed that maximum overall acceptability was found at 7 days of storage followed by 0, 14, 21 and 35 days, whereas lowest overall acceptability was found at 35 days of storage. However, mean values of commercial pectin showed higher value for overall acceptability (7.133±0.242) as compared to the local pectin (6.467±0.163). The similar findings were observed according to Shakir *et al.* (2009) and Cui *et al.* (2011) who reported that overall acceptability remained unchanged during storage period.

Conclusion

In the nutshell, the present research project was a successful attempt to optimize the extraction of pectin from grapefruit peel. The optimization resulted in two fold increase in the extraction rate that was limelight of the present project. The extracted pectin owing to its high methoxyl content can be used as stabilizer/ thickening agent in various foods. However, the extracted pectin can be improved for its quality and impurities present in extracted fractions should be removed to improve the quality of the final product.

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