Optimization of Process Conditions for Pectin Extraction from Citrus Peel

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Abstract: Pectin is a complex polysaccharide and natural food additive extracted from citrus peel and apple pomace in the form of brownish powder. It is widely used as a jelling and thickening agent and the food industry especially in jams and jellies. It is also recommended for use as fat replacer and also lowers blood cholesterol by increasing viscosity in the intestinal tract thus lowering cholesterol absorption. Peel of sweet oranges was chopped to small pieces in a vegetable cutter and dried in an electrical drier to 8-10% moisture contents. The dried peel was ground and then subjected to pectin extraction using water as solvent. Three treatments with different levels i.e., pH (1, 2, 2.5, 3, 3.5, 4, 4.5 and 5), temperature of solvent (70, 85 and 95°C) and extraction time (0.5, 1, 2, 3, 4 and 5 h) were evaluated. Development of an economical processing method for pectin extraction from citrus peel, that offers energetic properties nearly identical to those prepared by other methods (soxhlet method and microwave) using other substrates, is reported. Dried citrus peel contains about 30% pectin. It has been found that 30 min heating at 70°C and pH level 2.5 resulted in 21% pectin extraction followed by 1 h heating at 85°C at pH level 3.0 and a similar pectin yield. Up to 17% yield was obtained for heating at 95°C at pH level 1 irrespective of the time of heating. It can be inferred from these experiments that pH range between 2.0-2.5 and heating at temperature of 70°C for 30 min seems to be the most suitable condition for pectin extraction as time and energy efficient process conditions.

Key words: Citrus peel, pectin, extraction, purification, characterization

INTRODUCTION

Pectins are complex polysaccharides mainly composed of 1-4 linked α -D-galacturonic acid (Fig. 1) and found in middle lamella of plant cell membrane (Garna *et al.*, 2004).

D-Galacturonic acid is a sugar acid, an oxidized form of D-galactose and is present as polygalacturonic acid in pectin as shown in Fig. 2. It has an aldehyde group at C1 and a carboxylic acid group at C6. Other oxidized forms of D-galactose are D-galactonic acid (carboxylic group at C1) and meso-galactaric acid (mucic acid) (carboxylic groups at C1 and C6). It is also a uronic acid or hexuronic acid.

The non-esterified galacturonic acid units can be either free acids (carboxyl groups) or salts with sodium, potassium or calcium. The salts of partially esterified pectins are called pectinates, if the degree of esterification is below 5% the salts are called pectates, the insoluble acid form, pectic acid. Some of its galacturonide units are esterified as methyl galacturonate. One of the most important properties of pectin is gelling. The Degree of Esterification (DE) affects the gelling properties of pectin. The structure shown in Fig. 3 has three methyl ester forms ($-\text{COOCH}_3$) for every two carboxyl groups ($-\text{COOH}$), hence it has a 60% degree of esterification, normally

called a DE-60 pectin. High Methoxy (HM) pectins have the ability to form gels with sugar and acid, so-called low water activity gels or sugar-acid- pectin gels.

Such a gel is considered as 2-dimensional network of pectin molecules in which the solvent (water) with the co-solutes sugar and acid are immobilized. This results in a system resisting deformation and showing a stress-strain relationship for small deformation. The buildup of the 3-dimensional network is based on the formation of junction zones in which there are chain associations stabilized by hydrogen bonding between undissociated carboxyl and secondary alcohol groups and by hydrophobic interaction between methyl esters. The gelation mechanism of pectin is mainly governed by their Degree of Esterification (DE). For the low methoxyl pectins, denoted LMP (DE<50%), gelation results from specific non-covalent ionic interactions between blocks of galacturonic acid residues of the pectin backbone and

Fig. 1: Structure of pectin

Fig. 2: Structure of polygalacturonic acid

Fig. 3: Pectin a polymer of α -galacturonic acid with a variable number of methyl ester groups

with divalent ions such as calcium. The affinity of pectin chains towards calcium is known to increase with decreasing degree of esterification or ionic strength and with increasing polymer concentration. Besides the influence of the charge density of the polygalacturonate chain, the distribution pattern of free and esterified carboxyl groups has an important effect on the strength of calcium binding. Due to this property, pectin has wide applications in a variety of food formulations as jellying and thickening agent. Pectin is a natural food additive used extensively in the food industry as a gelation agent. It is also being recommended for use as fat replacer. The world market demand for pectin is in excess of 30,000 t annually and is growing by about 4-5% per annum (Garna *et al.*, 2006). On account of its ever-increasing use and demand pectin has become an indispensable ingredient in food industry. Major sources of pectin are apple pomace and citrus peel. Dried citrus peel has about 30% pectin. The orange peels, if treated as waste materials, may create environmental problems, particularly water pollution, since the presence of biomaterials in orange peels such as peel oil, pectin, as well as sugar, stimulate aerobic bacteria to decompose the biodegradable organic matters into products such as carbon dioxide, nitrates, sulfates and phosphates in water. This problem could be turned into an opportunity, if potentially marketable by products such as pectin could be extracted from the peel. The peel, pectin after extraction, can be used as a high protein stock feed in dry form, increasing the potential return for the orange juice industry and reducing the pollution load to the environment.

The manufacture of pectin typically comprises extraction, purification/concentration and drying. The use of a suitable method for pectin extraction is significant in

order to maximize its yield and quality. The literature concerning most commonly used methods for the extraction of pectin includes direct boiling, microwave heating (Garna *et al.*, 2007; El-Nawawi and Shehata, 1987). Nevertheless, none of the methods extract all the pectin without causing some degradation. Microwave heating extraction takes no more than fifteen minutes to extract a satisfactory amount of pectin. However, its commercial application may not be realistic. Production of pectin by enzymes may take up to two days (Donaghy and McKay, 1994). The yield of pectin depends on extraction operation conditions such as temperature, extraction time, pH and the types of extraction peels (Fishman *et al.*, 1999; Liu *et al.*, 2006). The usual extraction procedure involves the use of water acidified to a pH between 1 and 4.5 with mineral acid, such as hydrochloric acid, sulphuric acid or nitric acid. The crushed citrus peels are added to this acidified water and then slightly stirred at a temperature between 60-95°C for time period ranging from 30 min to several hours. The solid water mixture is subsequently separated by centrifugation or filtration. In the filtrate, the pectin is precipitated with the addition of alcohol. The filtrate is concentrated through evaporation in order to minimize the amount of alcohol used. The isolated pectin is then washed again with alcohol, dried and ground.

Citrus processing industry generates large amount of peel. Major gains for the industry could be the use of the waste to produce value added product, boosting indigenous production of high grade pectin as import substitute and the use of depectinized byproduct in cattle feed. The present study was therefore, undertaken to develop a simple and economical method for pectin extraction.

MATERIALS AND METHODS

Chemicals: Sodium chloride, ethanol, phenol red and sodium hydroxide, magnesium sulphate and sulphuric acid, distilled water, acetone and 30% w/w glycerin in water.

Preparation of sample: About 400-500 crates of sweet oranges were purchased from the fruit market in Peshawar. After extraction of juice, the peel was chopped to small pieces in a vegetable cutter and dried in electrical drier to 8-10% moisture level. The dried peel was ground and then subjected to pectin extraction using water as solvent. Three treatments with different levels i.e., pH (1, 2, 2.5, 3, 3.5, 4, 4.5 and 5), temperature of solvent (70, 85 and 95°C) and extraction time (0.5, 1, 2, 3, 4 and 5 h) were evaluated.

Extraction and isolation of pectin from citrus peel: Twenty five gram of dried citrus peel was weighed into a stainless steel jug. It was blended in 1000 mL distilled water for 2-3 min. The homogenate was transferred to 1500 mL beaker and the pH of the mixture was adjusted (1.0-5.0). The flow diagram of the overall process is given in Fig. 4.

Characterization of prepared pectin: Prepared pectin can be characterized and compared with other pectin (commercial and reagent grade) by determination of:

- Methoxy contents
- Acetyl value
- Viscosity

Determination of methoxy contents: The Degree of Methylation (DM) is defined as the percentage of carbonyl groups esterified with methanol. Methoxy content is determined by the saponification of pectin followed by titration of the free carboxyl acid groups:

- 2.5 mL of ethyl alcohol and 0.5 g NaCl was added to 0.25 g dried pectin

A yellow solution was obtained after addition of 100 mL CO₂, free distilled water and 6 drops phenol red to the above reaction mixture. Ten milliliter of this solution was titrated with 0.1 M NaOH till appearance of pink color (V1). Then, 12.5 mL (0.25 M) NaOH was added to the reaction mixture, the solution was shaken, kept for 30 min at room temperature in stoppered flask, titrated against 0.25 M HCl till the color of solution changed to yellow and again titrated with 0.1 M NaOH till formation of pink coloration (V2). The volume obtained was used to calculate (%) methoxy content which is equal to the total mass of methoxy over the mass of the pectin sample multiplied by 100.

Fig. 4: Flow diagram of the pectin extraction process

Determination of acetyl value: 0.5 g pectin was weighed into a 250 mL conical flask and then 25 mL of 0.1 M NaOH solution was added. The content was shaken until the pectin was dissolved. It was kept for at least one hr followed by dilution to 50 mL with distilled water. From this solution, 20 mL was taken to distillation apparatus and 20 mL of magnesium sulphate-sulphuric acid solution was added. Steam distillate of 100 mL was collected and titrated against 0.05 M NaOH using phenol red as indicator. Acetyl value was calculated with following equation:

$$\text{Acetyl (\%)} = \frac{\text{Normality of NaOH} \times \text{mL of NaOH} \times 4.3}{\text{Wt of sample}}$$

Determination of viscosity: The measure of a fluid resistance to flow is termed as its “Viscosity”. Fluids can Newtonian (or simple flow) and non-Newtonian (complex flow). Newtonian flow represents an ideal situation, in which viscous liquids obey Newton’s law of viscous flow, according to which the flow rate is directly dependent on the applied shearing stress. These liquids are called Newtonian fluids and include most solvents such as water, alcohol, benzene, true solutions and very dilute colloidal solutions. Newtonian fluids exhibit a straight-line relationship

between shear rate (G) and shear stress (F) and are described in terms of absolute viscosity (η):

$$F = \eta G \quad (1)$$

Other fluids do not obey Newton's law of flow and are referred to as non-Newtonian fluids. These fluids do not exhibit a linear relationship between shear rate and shear stress and are usually described in terms of apparent viscosity (η_{app}).

The instruments used in measuring the flow properties of fluids are called "viscometers or rheometers". Single point instruments, such as a simple capillary viscometer (e.g., Ostwald viscometer) was used for determining viscosity. The Ostwald viscometer has the form of a U-tube. Its use is based on measuring the time required for a liquid to flow between two marks. The viscosity of the liquid in poise or in stokes is then determined using Eq. 2 or 3:

$$\eta = K t \rho \quad (2)$$

$$\text{Kinematic viscosity} = K t \quad (3)$$

where, K is an instrumental constant, t is the flow time and ρ is the density of the liquid.

Usually, a liquid of known viscosity is compared with another liquid of unknown viscosity, therefore,

eliminating the necessity for the determination of the instrumental constant as shown in Eq. 4.

$$\eta_1/\eta_2 = \rho_1 t_1/\rho_2 t_2 \quad (4)$$

RESULTS AND DISCUSSION

Data comprising pectin isolation using three levels of temperature (70, 85 and 95°C), three levels of heating time (30 min, 1, 2 and 3 h) and eight pH values (1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0) are summarized in Fig. 5a-c.

As is evident from Fig. 4, heating at 70°C for 30 min while maintaining the pH level at 2.0-2.5 was optimum to extract highest % age of pectin. At this temperature (70°C), no precipitate of pectin was obtained beyond pH 4.0 even though the heating time being increased to 3 h. It can be ascertained from the experiment that pH 1.0-2.5 can be the range (pH 2.0 being the best) where maximum precipitation of pectin was possible at a temperature of 70°C. With regards to pectin precipitation as a function of heating time, it can be said that 30 min to 1 h heating time is sufficient to isolate maximum percentage of pectin as on further increase in heating time did not result in any increase in pectin yield.

Effect of heating at 85°C, heating times and pH values on extraction of pectin is shown in Fig. 5a. At this temperature, pH 1.0-3.0 and 1 h heating time was

Fig. 5(a-c): Effect of heating, (a) 70°C, (b) 85°C and (c) 95°C, respectively times and pH values on extraction of pectin (%), each value represents average of three determinations

the optimum condition for maximum percentage extraction of pectin. Heating for one hour at pH 2.0 was the condition where highest percentage of pectin was extracted. It is interesting to note that increase in pH from 2.0-3.0 resulted in a gradual decrease in percent extraction of pectin at 85°C. Increasing further pH value (3.5-5.0) was followed by increase in pectin extraction. At pH level 5.0, 15.32% pectin was isolated which is comparable to the pectin values obtained with pH levels 2.5 and 3.0. It is worthy to mention here that the pH level of slurry obtained by blending citrus peel with distilled water ranged from 4.0-5.0. In this experiment, the best condition for pectin extraction was heating for 1 h at pH level 2.0.

Effect of heating (95°C) times and pH values on extraction of pectin (%) are depicted in (Fig. 5b). This is the boiling point of water at Peshawar valley. The highest percent pectin extraction was obtained at pH 1.0 and 5.0 (17.36%). As for the 85°C heating time, percent pectin extraction decreased with increase in pH level up to 3.5 and thereafter, increased with further increase in pH value. Percent extraction at pH level 5.0 was comparable to that of percent extraction at pH values 1.0-2.5. It is evident from the data that pH level 4.5-5.0 is the best pH range where maximum pectin can be isolated at the boiling temperature. Moreover, 1 h heating was enough to get maximum extraction.

Most scientific publications have studied the influence of different acid extraction conditions on the chemical characteristics of the extracts from various plant tissues using an experimental design (Levigne *et al.*, 2002; Pagan *et al.*, 2001; Phatak *et al.*, 1988; Robert *et al.*, 2006; Yapo *et al.*, 2007). This statistical approach has allowed the quantification of each parameter and their potential interactions on the extraction yield and chemical characteristics of pectin. In addition, the initial step in the extraction of pectin often involves the preparation of an acetone or alcohol insoluble residue, with the purpose of removing low molecular weight compounds, including any trace of free galacturonic acid. The aim of this step is to remove small molecules (Qi *et al.*, 2000).

Indeed, at constant pH and temperature, the yields of pectin obtained for 1 h of extraction were comparable to those for 3 h. Similarly, the pectin yields from various extractions at pH 1.0-2.5 were higher than those at pH 3.0-4.0. It was interesting to note that extraction yield at pH 4.5-5.0 was comparable to that of pH 1.0 to 2.5. Yapo *et al.* (2007) and Levigne *et al.* (2002) observed the same trends on pectins extracted from sugar beet, unlike with soy hull pectin where the yields decreased with increasing acid strength (Kalapathy and Proctor, 2001). The total extraction yield reflected the pectin yield but depending on the experimental conditions, some impurities or degraded pectin could have been obtained.

Moreover, Mohamed and Hasan (1995) found a pectin yield (120 mg g⁻¹) from banana peels using other experimental conditions (acetone-HCl, pH 4.0, 1 h and 75°C); this value being in the range of the present study. pH and time were the most significant interactive effect on the pectin yield. Wang *et al.* (2014) using subcritical water extracted 21.95 and 16.68% pectin from citrus peel and apple pomace, respectively.

The suitability of pectins for different purposes is determined by their characters viz., methoxyl content, degree of esterification and acetyl value. Hence, it is an unavoidable aspect that pectin should be described properly for its biochemical characters. If more than 50% of the carboxyl groups are methylated the pectins are called high-methoxy pectins (HM) and less than that degree of methylation are called Low Methoxy (LM) pectins. This same principal applies also to acetylation (Sharma *et al.*, 2006). High Methoxy (HM) pectins (with DE>50%) require a relatively high concentration of soluble solids and a low pH for gel formation (Garna *et al.*, 2004, 2006). Low Methoxy (LM) pectins (with DE<50%) form rigid gels by the action of calcium or multivalent cations, which cross-link the galacturonic acid chains (Garna *et al.*, 2004).

The Degree of Esterification (DE), which is expressed as a percentage of the esterified carboxyl groups, is an important means to classify pectins. Jams and preserves are of course the main use of industrially extracted pectins. High dissolved sugar and acid conditions ensure that chain-chain interactions dominate over chain-solvent interactions (Sharma *et al.*, 2006). Most chain-chain interactions in these systems are not based on electrostatic interactions and so the other hydrophobic and hydrogen bonding effects exert most influence. High sugar conditions create low water activities which can be mimicked by other solutes with the same resulting gels. This is reflected in the subdivision of HM pectins into rapid-set pectins of DM~77 to slow-set with DM~60. The larger hydrophobic element in HM pectins allows for rapid arrest of the systems.

The characteristics of the extracted pectins varied over a large range depending on the experimental conditions of extractions. The pH was the main significant factor on yield. Having a large range of DM, these pectins could probably gel with calcium or with high sugar concentrations in acidic condition. By considering the pectin yield, degree of methylation the acid extraction of citrus peels pectin at pH 2.0, for 1 h, at 95°C could be suitable. Methoxy content of pectin ranged between 50-70% depending upon the pH values employed and heating time. Methoxy content decreased with the increase in pH value and heating time. Percent acetyl value of pectin was 0.43. It is reported (Partos, 2005) that

Table 1: Characterization of pectin extracted from citrus peel

Samples	Methoxy content (%)	Acetyl value (%)	Viscosity
Standardized pectin	70 ^b	0.34 ^b	0.964 ^b
Commercial pectin	65 ^c	0.34 ^b	0.984 ^b
Reagent grade pectin	80 ^a	0.37 ^a	1.264 ^a

Each value represents average at three determinations, values in the same column with the same letter are not statistically significant ($p>5\%$)

Jams and preserves are of course the main use of industrially extracted pectins. High dissolved sugar and acid conditions ensure that chain-chain interactions dominate over chain-solvent interactions. Most chain-chain interactions in these systems are not based on electrostatic interactions and so the other hydrophobic and hydrogen bonding effects exert most influence. High sugar conditions create low water activities which can be mimicked by other solutes with the same resulting gels. This is reflected in the subdivision of HM pectins into rapid-set pectins of DM~77 to slow-set with DM~60. The larger hydrophobic element in HM pectins allows for rapid arrest of the systems. As shown in Table 1 the viscosity of our standardized pectin (protopectin) was 0.964 centipoise (cp) which was comparable to that of pectin obtained from SIGMA chemical Company, USA (0.984 cp). Kurita *et al.* (2008) reported that citric acid treatment of peel at neutral pH resulted in significant increase in viscosity. However, citric acid treatment at high temperature decreased viscosity of pectin.

CONCLUSION

It is concluded from this study that heating at 70°C, for 30 min and pH level of 2.0-2.5 were optimum conditions for getting maximum yield of pectin. At this temperature and pH>4.0, pectin could not be obtained. At 85°C temperature a higher range of pH is (1.0-3.0) was required for maximum yield. In this case one hr heating was sufficient to extract most of the pectin; however, heating for 30 min yielded comparable results.

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