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Extraction and characterisation of pectin from two apple juice concentrate processing plants

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Abstract

Pectin was extracted from apple pomace procured from two different sources (S1-FIL industry and S2- HPMC sopore) of Kashmir valley by hot acid extraction method using three different acids Hydrochloric acid (HCl), sulphuric acid (H_2SO_4) and citric acid. The pomace was sieved through three different mesh sizes (m1=30, m2=72, and m3=120) and all these meshes were subjected to extraction under pH 1.0 and temperature 70^{O}C. The comparison of the pectin extracted was made on the basis of yield of pectin and physiochemical features between sources as well as between the acids and mesh sizes. Apple Pomace from source S_1 showed a higher percent yield of pectin (52.6%) whereas between acids and mesh sizes citric acid and mesh m1 showed the highest percent yield of 52%. The physiochemical characteristics of the pectin extracted varied significantly. Citric acid extraction produced pectin with a higher quantity of methoxyl content (10.39%), anhydrouronic acid (65%), and degree of esterification (49.35%). Overall the pectin extracted from both the sources had a lower degree of esterification (50%) that reveals its low methoxyl content.

Introduction

Pectin belongs to a family of polysaccharides which are largely comprised of linked *D – galacturonic acid* units. They are generally described as an alternation of smooth *homogalacturanan* (HG) and hairy type 1 and type 2 *rhamnogalacturonan units* (RG1, RG 2) (Ridley *et al*., 2001). Most of the units are esterified with methyl alcohol at the carboxylic acid end, with a few L- rhamnose residues linked to neutral arabinogalactan side chains. Pectin is a natural polymer which widely exists in green plants and plays a role in combining plant structures (Hoff, 1969). Pectins can have diverse functional properties, but the one clearly known and common to various sources since the earliest studies (Vanquelin, 1790; Braccanot, 1825) is the gel formation under specified conditions. The characterization and the gelling properties which includes the functional properties of pectin depends upon the source, methods of extraction, the extraction medium and the purification steps involved prior to analysis (Pilnik *et al.*, 1980). Citrus peels followed by apple pomace serve as the major source for extraction of pectin. Apple pomace is a by product obtained from the fruit juice, cider or syrup manufacture (Pilnik *et al.*, 1980). Hence pectin is still considered as one of the most reasonable way for apple pomace utilization both from an economical and from an ecological point of view (Sheiber *et al*., 2001). These polymers can be used for different functions like thickening, gelling and stabilising of jams, jellies, sweets confectionery and for the manufacture of drugs (May, 1990).

The extraction step is the most important operation for obtaining the pectin from different sources. The extraction can be carried in hot acid solutions so called conventional extraction. The acids employed can be strong acids like mineral acids (hydrochloric acid, sulphuric acid, nitric acid) or weak food grade acids (citric acid). The extraction is a multistage physicochemical phenomenon which involves the acid solubilisation of the pectic materials under different pH, temperature and duration conditions generally in the range of 1.3-3.0, 60-100^{O}C and 20-360 minutes respectively (Yapo, 2009). Hot dilute acid solutions are capable of releasing the cell wall materials which include pectins by cleaving the linkages between the protopectins and the plant materials. By using citric acid, sulphuric acid or hydrochloric acid extractants, it has been recently shown that the type of acid strongly influences the macromolecular state and gelling properties of pectins (Yapo, 2009) with citric acid being the least pectin degrading and de polymerizing and deesterifying extracting agent and therefore leading to pectin isolates with best gelling properties. The effect of extraction conditions on the yield and purity of the apple pomace has been reported (Nikolic and Mojovic, 2007).

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Materials and Methods

Procurement of raw material

Wet apple pomace was procured from two apple juice concentrate producing plants of Kashmir valley, Fungicide India limited Srinagar (FIL) and Horticulture Produce and Marketing Corporation Doabgah Sopore (HPMC) in the month of August, 2013. The wet apple pomace was subsequently dried in a conventional hot air oven at 50^{O}C to a moisture content of 9% on wet basis. The dried apple pomace was then subjected to the removal of seeds stalks and other extraneous matter which was carried out manually. Apple pomace was then ground in a mixer grinder (model: Sujata J077921) and was sieved through three different mesh sizes 30 (87microns), 72 (210 microns), 120 (125 microns) denoted by m1, m2 and m3 respectively (model: MSW325). Fine pomace powder was stored in plastic bags.

Extraction of pectin

Extraction of pectin was carried out in three different acids (hydrochloric acid, sulphuric acid and citric acid). The pH of the extraction medium was set at 1.0 and the temperature was kept at 70^{O}C. Pomace powder of all mesh sizes of both the industries were subjected to same treatments of acid, pH and temperature. 30 grams of the fine apple pomace powder were taken in the medium (400ml) containing different acids of pH=1 and distilled water. The mixture was then subjected to extraction of pectin at a temperature of 70^{O}C for 1 hour and 30 minutes. The mixture was then filtered through a cheese cloth and the supernatant discarded. The filtrate containing the solubilised pectin was treated with equal volumes of 95% ethanol. The pectic substances precipitated in the medium and were later on centrifuged at 10000 rpm. The pellets were recovered after washing by using 70% ethanol on petriplates and dried in hot air oven at 50^{O}C. Dried pectin was then ground to fine powder and stored in airtight pouches (high density polyethene).

Pectin yield

The percentage yield was calculated for each batch of extraction. The percentage was calculated by the following formula.

$$y\% = \frac{x}{w}(100) \quad (1)$$

y = Pectin yield; x = Weight of dried pectin extracted; w= Weight of fine apple pomace taken for extraction.

Moisture content

Moisture content of pectin was calculated gravimetrically by (Johnson, 1945). One gram of pectin sample was oven dried at 100^{O}C in pre weighed petridishes till constant weight was attained. After drying petriplates were covered with a lid and cooled in dessicater. The moisture content of the sample was calculated using the following formula.

$$Mc = \frac{Wi - Wf}{Wi} \quad (2)$$

Mc = moisture content, *Wi*= initial weight, *Wf*=final weight

Ash content

Total ash content was determined according to method by (Owens *et al*., 1952). Direct analysis carried out on the greyish white residue remaining after the pectin sample was charred on a hot plate in a crucible and later incinerated in a muffle furnace at 550^{O}C for 6 hours and the residue was weighed.

Alkalinity of ash

Alkalinity of ash was determined according to method by (Owens *et al.*, 1952). The ash from the complete ashing of the pectin sample was taken in a titration flask and dissolved in 25 ml of 0.1N hydrochloric acid. The solution was heated gently to boiling and then cooled to room temperature followed by slow titration with 0.1N sodium hydroxide using phenolphthalein as an indicator. The percentage alkalinity of ash was calculated using formula.

$$\%\text{Alkalinity of ash} = \frac{\text{Titer value X Normality of NaOH X 60}}{\text{weight of sample X 1000}} \text{X100} \quad (3)$$

Methoxyl content (MC)

Methoxyl content was determined by method given by (Owens *et al*., 1952; Gee *et al*., 1958). To the neutralized solution [which was obtained from the equivalent weight determination] 25ml of 0.25N sodium hydroxide was added and the mixture was shaked thoroughly and allowed to stand for 30 minutes at room temperature in a stoppered flask. After 30 minutes of standing 25 ml of 0.25N hydrochloric acid was added and the solution was titrated with 0.1N NaOH to the same end point as before. Methoxyl content was calculated using the following formula

$$\textit{Methoxyl content} = \frac{\textit{ml of alkali X normality of alkali X 3.1}}{\textit{weight of sample}} \quad (4)$$

Anhydrouronic acid (AUA)

Anhydrouronic acid was determined by method given by (Owens *et al.*, 1952). Anhydrouronic acid content was calculated directly by the formula as shown below.

$$AuA = \frac{176\ (\text{ml of alkali for free acid} + \text{ml of alkali for saponification} + \text{ml of titrable ash})}{\text{weight of Sample}} X100 \quad (5)$$

Degree of esterification (DE)

The degree of esterification (DE) was assessed by a direct titrimetric method described by (Pinheiro *et al*., 2008). Briefly, pectins (200 milligrams) in 50 ml conical flasks were moistened with ethanol and dissolved in 20 ml of deionized water at 40^{O}C for 2 hours. After the pectins were completely dissolved, one drop of phenolphthalein was added. The solutions were then titrated with 0.1 M sodium hydroxide and the results were recorded as . Then, 10 ml of 0.1 M sodium hydroxide was added, with the conical flasks covered with glass stopples, the solutions were stirred at room temperature for two hours. Another 10 ml of 0.1 M hydrochloric acid was added and the Solutions were shaken until the pink colour disappeared. The solutions were titrated with 0.1 M sodium hydroxide again, and the final results were recorded as . The DE was calculated according to the following formula:

$$DE(\%) = \frac{V2}{V1} + V2\ x\ 100 \quad (6)$$

Results and Discussions

The results were analyzed statistically by ANOVA. To visualize the relations between the different treatments the two dimensional plots were generated for the models in functions of mesh size and acid treatment on the pectin yield.

Effect of acid on pectin yield

The results regarding the effect of acids on the pectin yield is presented in Figure 1. The yield of the pectin was found highest (56.6%) when citric acid was used as an extracting medium followed by sulphuric acid (12.6%) and hydrochloric acid (12%). The significant difference in the yield of pectin between the media containing citric acid and the mineral acids can be attributed to the fact that citric acid being a weak acid has less effect on the chain degradation of the pectin molecule while as mineral acids being strong acid cause an extensive chain degradation predominantly on the neutral sugar linkages giving considerably a lower yield (Garna *et al*., 2007). In this study the increase in the yield was probably due to low pH. The hot acid extraction at low pH leads to the cleavage between the neutral sugar linkages by way of which some non pectinous substances are also incorporated in the filtrate giving a higher yield of precipitate however the final purity of pectin depends upon the purification procedure implied (Hwang *et al.*, 1992). The highest content of pectin was obtained when citric acid was used as an extraction medium (Contreras *et al*., 1997). Similar results were reported by (Pagan and Ibraz, 2001). Between the sources the yield of pectin was higher from FIL Industries (S_2) as compared to HPMC Sopore (S_1). This difference in yield can be hypothesized to the differences in the technology used by the two sources and the level of maturity of the raw material at the time of juice extraction and extent of microbial action on the pomace before drying.

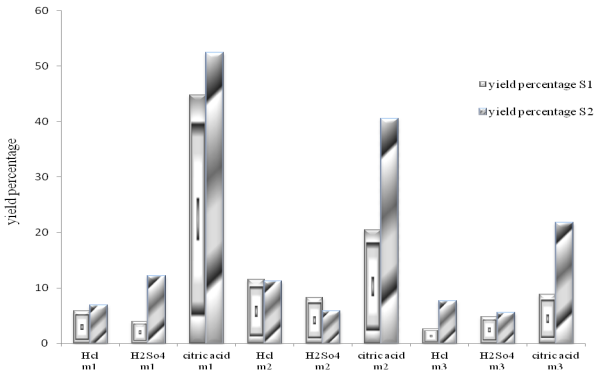

Figure1. Percentage yield of pectin from apple pomace (S_1 and S_2)

In pectin yield the two mineral acids differ significantly in terms of pectin from their organic counterparts (the mean difference between citric acid and mineral acids is 18.03), the results were found to be in agreement with the results obtained for extraction of peach pomace. (Pagan and Ibraz, 2001; Kalapathy and Procter, 2001).

Effect of mesh size on the pectin yield

The results regarding the effect of mesh size on the pectin yield are presented in Figure 1. The mesh size had a notable influence on the pectin yield with mesh m1 leading to higher yield than the other two meshes (m2 and m3). Amongst the two sources, m1 of S_2 showed the highest percentage of yield (56%). Pomace of varying mesh size (m1, m2, and m3) differed significantly wherein the superfine pomace flour of mesh 120 (i.e. m3) recorded a low percentage yield, the probable reasons for this can be retention of filtrate containing pectic polysaccharides as the pomace with smaller particle size imbibes more of the solution and forms jelly like mass than the pomace with larger particle size.

Effect of acids on characteristics of pectin

The results regarding the effect of acids on the characterization of pectin are presented in Figure 2 and 3 obtained from apple pomace of HPMC and FIL respectively. The methoxyl content was found

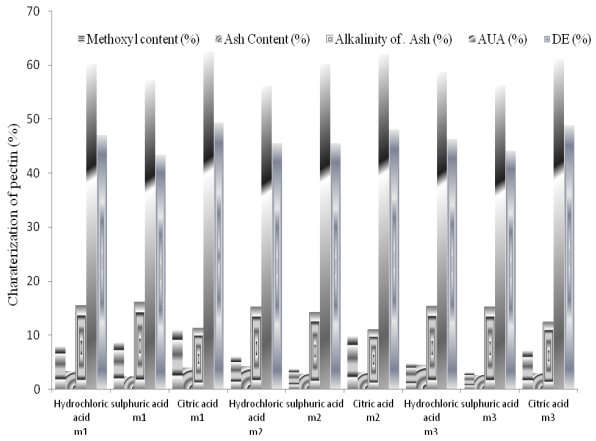

Figure 2. Characterisation of pectin from apple pomace S_2 (HPMC)

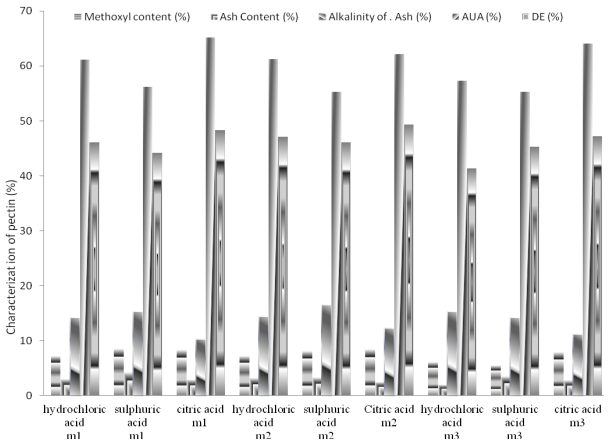

Figure 3: Characterisation of pectin from apple pomace S_1 (FIL industry)

to be highest for the pectin extracted in citric acid (10.93%). Same values of methoxyl content were observed by (Kumar *et al*., 2010) while extracting pectin from Spondias dulcis pomace using differential pH. The samples extracted from S_1 showed a slightly higher value of methoxyl content than S_2 (S1-10%, S2-8.6%). Ash content was comparatively found higher in samples of S1 treated in hydrochloric acid. The increase in ash content of the samples treated in mineral acids could be possibly due to extreme acidic conditions in which some non pectinous materials also get extracted (Kalapathy and Procter, 2001) and inadequate ethanol washing which aims at removing salts, free sugars and other alcohol soluble compounds converting the pectin in its free acid form.

The citric acid treatment produced pectin with higher anhydrouronic acid value of 65% and degree of esterification (DE) value of 49.39%, there by confirming the pectin extracted is of low methoxyl pectin (DE 50%) which is in coincidence with the observations of (Pinheiro *et al*., 2008). It was also observed that DE value of pectin extracted from mineral acids was low than those extracted in citric acid which might be ascribed to the less deesterifying nature of citric acid on pectin solubilisation (Jiang *et al*., 2012). It has been recently revealed that the acid type strongly influences the pectin properties with citric acid being the least pectin deesterifying extracting agent (Yapo, 2009). Samples from S2 showed higher value for anhydrouronic acid (AUA) content whereas negligible difference was found in the degree of esterification (DE) content between the two sources.

The observations revealed that citric acid as an extracting agent was found to be superior to the mineral acids in terms of the pectin properties like yield percentage, anhydrouronic acid (AUA), methoxyl content (MC), degree of esterification (DE) and ash. Similar results were reported by (Wang and Lui, 2014). Though the pectin yield was higher in citric acid extraction but the degradation of polymer chains was also more severe due to low pH and longer duration of extraction. The degree of esterification decreases significantly on decreasing the pH of the medium (Pagan and Ibraz, 2001) and in this case it was lower than the commercial pectin and also lower than that reported by earlier researches (Min *et al.*, 2011). However this low degree of esterification observed in the pectin extracted in mineral acid could possibly be because of the degradation brought about by hot acid extraction. It is therefore observed in this study that quality parameters of pectin extracted were lower than the corresponding values in commercial pectins (Jain *et al*., 1984; Sharma *et al*., 1985). The difference therefore necessitates the need to modify the extraction technique to bring the aforesaid values at par with commercial pectins. The pectin extracted in this study had lower methoxyl content and consequently a lower tendency to form gels as the gelling capability depends upon the methoxyl content of the pectin (Fraeye *et al*., 2009).

Effect of mesh size on characteristics of pectin

The results regarding the effect of mesh size on the characterization of pectin are presented in Figure 2 and 3 obtained from apple pomace of HPMC and FIL respectively. On an average the methoxyl content was moderately higher for source S_2 and among the mesh size it was highest for m1 whereas the ash content showed similar occurrences in all the meshes though the percentage was seen to occur in increased amounts in samples of source S_1. Similar trend was seen for the percent alkalinity of ash.

Pectin extracted from larger pomace particles of mesh m1 depicted enhanced values of anhydrouronic

acid wherein the extracted samples belonged to S_2, also the degree of esterification was in harmony with the values of AUA as for as the mesh and source were concerned. All the three mesh sizes differed significantly from each other, the prominent mean difference was found among mesh m1 and mesh m3 wherein the properties of the pectin are concerned.

Conclusion

This study investigated the potential to use apple pomace generated from the two major fruit juice concentrate plants of Kashmir as a source for the extraction of pectin. In particular the pectin extracted was chemically characterized. Cell wall materials of pomace from both the industries appeared to be rich source of pectin. Under the different extraction conditions used in this study the pectin recovered presented variable characterized features which varied with the type of acid and mesh size. Overall a superior quality of pectin was recovered with citric acid as a medium which explores a valuable idea to use this type of the acid in the extraction procedures. The pectin extracted from both the industries showed a low degree of esterification values suggesting it to be low methoxyl pectin, therefore the pectin recovered can be effectively used in forming rigid gels for emulsification and in various food formulations. However sophisticated extraction procedures have to be inculcated to obtain pectin from the apple pomace which serves as a suitable raw material for the industrial pectin production.

References

Braconnot, H. 1825. Recherches sur un nouvel acide universellement répandu dans tous les végétaux. Annales de Chimie et de Physiques 28(2): 173–178.

Contreras-Esquivel, J. C., Hours, R. A., Aguilar, C. N., Reyes Vega, M. L. and Romero, J. 1997. Microbial and enzymatic extraction of pectin: A review. Archivos Latinoamericanosde Nutricon 47(3): 208-216.

Fraeye, I., Doungla, E., Duvetter, T., Moldenaers, P., Loey, A.V. and Hendricks, M. 2009. Influence of intrinsic and extrinsic factors on rheology of pectin calcium gels. Food Hydrocolloids 23(8): 2069-2077.

Garna, H., Mabon, N., Robert, C., Cornet, C., Nott, K. and Legros, H. 2007. Effect of extraction conditions on the yield and purity of apple pomace pectin precipitated but not washed by alcohol. Journal of Food Science 72(1): C1–C9.

Gee, M., McComb, E.A. and McCready, R.M. 1958. A method for the characterization of pectic substances in some fruit and sugar-beet marcs. Food Research international 23(1): 72- 75.

Hoff, J. E. and Castro, M. D. 1969. Chemical composition of potato cell wall. Journal of Agriculture and Food Chemistry 17(6): 1328- 1331.

Hwang, J. K., Roshdy, T. H., Kontominas, M. and Kokini, J. L. 1992. Comparison of dialysis and metal precipitation effects on apple pectins. Journal of Food Science 57(5): 1180-1184.

Jain, R. K., Ghankrokta, S. S. and Agrawal, J. D. 1984. Isolation and characterization of pectin from apple pomace. Indian Food Packer 38(6): 65-70.

Jiang, Y., Yanxue, D., Xuemei, Z., Xiong, H., Meng, W. W. and Juwu, H. 2012. Physicochemical and comparative properties of pectins extracted from *Akebiatrifoliata* var. australis peel. Carbohydrate polymers 87(2): 1663-1669.

Johnson, C. M. 1945. Determination of Water in Dry Food Materials Application of the Fischer Volumetric Method. Industrial Engineering Chemistry17(5): 312-316.

Kalapathy, U. and Proctor, A. 2001. Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin. Food Chemistry 73(1): 393-396.

Kulkarni, S. G. and Vijayanand, P. 2001. Effect of extraction conditions on the quality characteristics of pectin from passion fruit peels (*Passiflora edulis* f. flavicarpa L.). Food Science and Technology 43(7): 1026–1031.

Kumar, A., Ghanshaym, S. and Chauhan, 2010. Extraction and characterization of pectin from apple pomace and its elevation as lipase steapsin inhibitor. Carbohydrate Polymers 82(2): 454-459.

May, C. D. 2010. Industrial pectins, sources, production and application. Carbohydrate Polymers 12(1): 79-99.

Min, B., Lim, J., Ko, S., Lee, K. and Lee, S. H. 2011. Environmentally friendly preparation of pectins from agricultural by-products and their structural/ rheological characterization. Bioresource Technology 102 (4): 3855-3860.

Nikolic, M. V. and Mojovic, L. 2007. Hydrolysis of apple pectin by the coordinated activity of pectic enzymes. Food Chemistry 101(1): 1-9.

Owens, H. S., McCready, R. M., Shepherd, A. D., Schultz, S. H., pippen, E. I., Swenso, H. A., Miers, J. C., Erlandsen, R. F. and Maclay, W. D. 1952. Methods used at western regional research Laboratory for extraction and Analysis of pectic materials. Western regional Research Laboratory, Albania calfornia AIC-340.

Pagan, J., Ibraz, M., Loria, A., Pagan, A. and Barbosa–Canovas, G.V. 2001. Extraction and characterization of pectin from stored peach pomace. Food Research International 34: 605–612.

Pinheiro, E. R., Silva, I. M. D. A., Gonzaga, L. V., Amante, E. R., Tefilo, R. F. and Ferreira, M. M.C. 2008. Optimization of extraction of high-ester pectin from passion fruit peel (*Passiflora edulis* flavicarpa) with citric acid by using response surface methodology. Bioresource Technology 99: 5561–5566.

Ridley, B. L., O'Neill, M.A. and Mohnen, D. A.

2001. Pectins: Structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry 57(6): 929–967.

Sharma, T. R., Lal, B. B., Kumar, S., Goswami, P. 1985. Pectin from different varieties of Himachal Pradesh apples. Indian Food Packer 39(4): 53–57.

Schieber, A., Stintzing, F.C. and Carle, R. 2001. By products of plant food processing as a source of functional compounds recent developments. Trends in Food Science and Technology 12(11): 401-413

Vauquelin, M. 1790, Analyse du tamarin. Annales de Chimie. 5: 92–106.

Wang, X. and Lü, X. 2014. Characterization of pectic polysaccharides extracted from apple pomace by hot-compressed water. Carbohydrate polymer 102(1): 174-184.

Yapo, B. M. 2009. Biochemical characteristics and gelling capacity of pectin from yellow passion fruit as effected by extract nature. Journal of Agriculture and Food Chemistry 57(4): 1572-1578.