Fresh Produce ©2011 Global Science Books

Extraction of Pectin from Pineapple (*Ananas comosus*) Peel using Inorganic/Organic Acids and Aluminum Chloride

Luke N. Ukiwe* • **Jude I. Alinnor**

Department of Chemistry, Federal University of Technology, P.M.B 1526, Owerri, Nigeria

Corresponding author: * luggil2002@yahoo.com

ABSTRACT

The ability of inorganic acids (HCL, H_2SO_4, HNO_3) and organic acids (citric, acetic, oxalic) to extract pectin from pineapple (*Ananas comosus*) cv. 'Agege Parson Brown' peel was investigated. Results obtained using ethanol precipitation showed that HNO_3 was the best inorganic acid for pectin extraction (1.6/200 g sample), while acetic acid was also observed as the best organic acid for pectin extraction (0.6/200 g sample). However, $AlCl_3$ extracted the highest amount of pectin overall (4.8/200 g). The degree of methoxylation ranged from 2.40 to 5.60% for the inorganic acids, while for the organic acids and $AlCl_3$, the range was 2.80 to 3.89%. $AlCl_3$ was the best substance for pectin extraction.

Keywords: esterification, fruit, galacturonic, jam, methoxyl, precipitation, spectrophotometer

INTRODUCTION

Pectin is a heterogeneous grouping of acidic structural polysaccharide found in fruit and vegetables and mainly prepared from waste citrus peel and apple pomace. It makes up between about 2 and 35% of plant cell walls (Madlav and Pushpalatha 2002) and it is important for plant growth, regulation of ion and water exchange, and development and defense of plant. It was first isolated and described in 1825 by Henri Braconnot, though the action of pectin to make jams and marmalades was known long before (Sharma *et al*. 2006). It has a complex structure with units of galacturonic acid as the main chain (Mohamadzadeh *et al.* 2010). In this main chain, α-L-rhamnose units are occasionally inserted through glycosidic linkages and the carboxyl groups are partially esterified by methyl alcohol. These molecules have been isolated and extensively studied from various plant tissues such as apples (Garna *et al.* 2007), chicory roots (Robert *et al.* 2006), and other materials (Polle *et al.* 2002). Pectin also contain non-sugar substituents, essentially, methanol, acetic acid, phenolic acids and, occasionally, amide groups. Normal extracted pectin is a multiple-stage physico-chemical process in which hydrolysis and extraction of pectin macromolecules from plant tissue take place under the influence of different factors, namely, temperature, pH, acid (inorganic and organic) or chelating agents (EDTA, ammonium oxalate or sodium hexametaphosphate) (Boonrad *et al.* 2006; Kliemann *et al*. 2009). This type of pectin has more than 50% of the carboxylic acid groups esterified, and is classified as "high methyl ester" pectin according to their gelling temperature. Modification of the extraction process or continued acid treatment yield "low methyl ester" pectin with less than 50% methyl ester groups. Some pectins are treated during manufacture with ammonia to produce amidated pectins. The structure of the pectin molecule is the key to the properties of pectin, and their use in different applications. High methoxyl pectin gel by the formation of hydrogen bonding and hydrophobic interaction in the presence of acids (pH 3.0) (to reduce electrostatic repulsions) and sugars (to reduce polymer-water interactions). Low methoxyl pectins in the absence of added cations, gel by the formation of cooperative associations at low temperatures (Constenla *et al.* 2003). The rheological properties of low methoxyl pectins are highly dependent on the salt cation, salt concentration, and pH (Pagán and Ibarz 1999). The non-esterified galacturonic acid units can be either free acid or salts with sodium, potassium or calcium. The salts of partially esterified pectins are called pectinates (Fertonani *et al.* 2009).

One of the materials used for production of pectin is sunflower head-residue which is a potential natural source of low methoxyl pectin (Mohamadzadeh *et al.* 2010). Sunflower head-residue of five cutivars ('Golshid', 'Record', 'Gabor', 'Azargol' and 'Progress') grown in Golestan Province of Iran were evaluated for pectin extraction using 0.75% sodium hexametaphosphate at pH 5.0 and at 85°C for 20 min. Result obtained showed that the pectin extracted from 'Golshid' and 'Record' varieties had higher yields compared to other cultivars with a galaturonic acid content of 80.4 and 81.8%, respectively. The degree of esterification of pectin varied from 33.2 to 35.1%. However, there was no significant difference between degree of esterification in pectin extracted from different cultivars. Another study on extraction model of low methoxyl pectin from apple pomace: Effects of acid concentration and time on the process and product revealed that to extract pectin from pomace, factors affecting process and product such as extracting medium and time of reaction should be optimized (Fertonani *et al.* 2009). A model to produce low methoxyl pectin directly from dried apple pomace was established observing the effects of HNO_3 concentration and the time of reaction at 97°C. Gravimetric yield predicted the highest value of 20.07/100 g of apple pomace sample of pectin with a degree of esterification of 48.49% and 16.77/100 g of apple pomace sample with a 43.73% degree of esterification as the lowest value. The result demonstrated that a practical model for acid extraction of pectin in which the main effects are from HNO_3 concentration, would be a linear and quadratic model form, though time influence was seen as equally relevant.

Pectin extraction has been studied by several authors. Pagán and Ibarz (1999) and Pagán *et al.* (2001) studied the extraction and the rheological properties of pectin from peach pomace where the maximum yield was obtained using

Received: 27 January, 2011. Accepted: 7 March, 2011.

Research Note

70% HNO_3 at 80°C at pH 1.2 and 60 min. Virk and Sogi (2004) studied pectin extraction and characterization from apple peel waste and revealed that citric acid was more effective than HCL. Rehmann *et al.* (2004) extracted pectin from mango peels with sulphuric acid and the maximum yield was obtained at 80°C and pH 2.5 at extraction time of 120 min. Schemin *et al*. (2005) also experimented on practical follow-up to pectin extraction from apple pomace and observed that pectin yield was higher with 6.2/100 ml of citric acid at 150 min. However, Wosiacki and Nogueira (2001) investigated the production of pectin from apple varieties growing in subtropical areas of Paraná State, Brazil. Determination of the effects of extraction time and pH variation on the yield of pectin isolated from pomace peaches was studied by Faravash and Ashtiani (2007). Their study suggested that maximum yield of pectin was obtained at initial pH 2.5 and 120 min contact washing time. Kliemann *et al.* (2009) demonstrated that optimal conditions for maximization of pectin yield was obtained using citric acid at pH 1.0 and 80°C with an extraction time of 10 min using passion fruit peel (*Passiflora edulis*).

The main use for pectin is as a gelling and thickening agent as well as stabilizer in food. The classical application of pectin is in given the jelly like consistency to jams or marmalades, which would otherwise be sweet juices. For household use, pectin is an ingredient in jelling sugar where it is diluted to the appropriate concentration with sugar and citric acid to adjust pH. The substance is also use against constipation and diarrhea since it increases the viscosity and volume of stool. In cosmetics products, the substance has application as a stabilizer as well as in ruminant nutrition, depending on the extent of lignification of the cell walls of the plant used for the preparation (Schemin *et al.* 2005; Boonrad *et al.* 2006). Pectin has also been shown to be useful in the production of cranberry juice and clarification of apple juice in the food industry (Semenova *et al.* 2006). Alborzi *et al*. (2010) reported that k-carrageenan protects skim milk from destabilization induced by high methyl pectin. The use of pectin and alginate in forming electrostatic complexation to improve pH-sensitivity drug release properties was studied by Yu *et al*. (2009). Result obtained from the study clearly suggested that microparticles formed based on complexation of pectin with alginate had potential for site-specific protein drug delivery through oral administration. Other aspects of pectin usage in the industry have been studied by Sperber *et al.* (2009), and Mirhosseini *et al.* (2008).

The purpose of the present study was to investigate the extractability of pectin from pineapple (*Ananas comosus*) peels using inorganic acids (HCL, H_2SO_4, HNO_3) and organic acids (citric, acetic, oxalic) as well as $AlCl_3$. Acid extraction of pectin appears to be the most widely used method of extraction due to high yield of extraction of pectin is achieved (Boonrad *et al*. 2006).

MATERIALS AND METHODS

The 'Agege Parson Brown' cultivar (ICS-Nigeria 2011) pineapple fruits used in this experiment were of the same stage of ripeness and were bought from the Eke-Onunwa market in Owerri, Southeastern, Nigeria. The fruits were harvested at the light-orange stage of ripeness. Maturity of pineapple is achieved when the fruit gradually changes color from dark-green to light-green-yellow with onset of maturity (Wijesinghe and Sarananda 2002). About 60 fruits were obtained for the analysis but three fruits were used for each treatment. The fruits were first washed thoroughly with distilled water, then skinned and the peels further washed with distilled water and chopped to bits using a knife. The peels were then dried in an AKSON Scientific Oven (AK 21) at 80°C until constant weight. The dried peels were then milled to a dry powder using a blender (SAISHO S-T4PN). About 200 g of the ground powder was further placed in a 500 ml beaker and 50 ml of a 0.5 M HNO_3 was added to the beaker. The pH of the mixture was adjusted to 4.0 using 10 M NaOH added as appropriate. The beaker was placed in a water bath at 100°C for 90 min. After 90 min, the mixture was cooled to 20°C and strained using clean Cheese cloth previously rinsed in distilled water. The resultant extract was filtered at 10°C through Whatman No. 42 filter papers. The filtered extract was coagulated using 96% ethanol added at intervals with constant stirring until precipitation was complete. The precipitate was set aside and filtered through Whatman No 42 filter papers. The resultant residue was collected in an evaporated dish, allowed to dry on a water bath (Sims Nig. Ltd. P6CE) at 50°C and placed in a desiccator to cool and congeal. The coagulated pectin was further washed once with 70% ethanol and twice with 96% ethanol. The resulting material was placed in an oven (AKSON Scientific AK 21) for 24 h at 50°C and in a desiccator for 12 h. The pectin yield was calculated using the following equation:

$$\omega_{pec}\ (\%) = {}^{xy}/yg\ (100)$$

where ω_{pec} = percentage pectin yield; xg = weight of extract obtained; yg = weight of sample (200 g).

Three treatments were determined for 0.5 M HNO_3 and the mean value of these treatments were obtained. This procedure was repeated using 0.5 M HCL, H_2SO_4, acetic acid, citric acid, and oxalic acids respectively. The procedure was also repeated for 0.5 (w/v) $AlCl_3$ solution.

SPSS software (version 14) was applied on datasets for statistical studies. One-way analysis of variance (ANOVA) was used to define optimum extraction of pectin using the various extractants, while the generalized *t*-test was used to test for significance in extractant types of the quantity of pectin extracted. Values are reported as arithmetic mean and standard deviation.

Moisture content

Moisture content was determined by oven method using AKSON Scientific (AK 21) using the equation:

$$\text{Moisture (\%)} = \frac{\text{loss in weight on drying (100)}}{\text{Initial sample weight}}$$

Degree of methoxylation

About 100 ml of a 0.2% (w/v) purified pectin from HNO_3 extract was neutralized with 0.5 M NaOH using phenolphthalein indicator. An excess of 16 ml of NaOH was added and the mixture allowed standing for 24 h at 26°C in a stoppered conical flask. The excess alkali was then determined by titration with 0.5 M Na_2SO_4. The amount of NaOH required for saponification of the pectin was determined and the percentage methoxyl content calculated using the equation:

$$\text{Methoxyl content (\%)} = \frac{\text{ml of NaOH} \times \text{M of NaOH (100)}}{\text{weight of sample}}$$

ml of NaOH = NaOH used for neutralization pectin (BT) – NaOH used for saponification of pectin (AT)

Hence, the equation was modified to

$$\text{Methoxyl content (\%)} = \frac{\text{(BT-AT)} \times \text{M of NaOH}}{\text{weight of sample}}\ (100)$$

Three treatments were determined and the mean value obtained. This procedure was repeated for the pectin extract of HCL, H_2SO_4, acetic, citric, oxalic acids and $AICI_3$ respectively.

About 2 g of the $AICI_3$ pectin extract was weighed and analyzed using an Infra-red (IR) spectrophotometer (Unicam 1252-98). The various functional groups present in the pectin extract are shown in **Table 1**.

RESULTS AND DISCUSSION

Table 2 show values of pectin yield extracted using HNO_3, HCL, H_2SO_4, acetic, citric, oxalic acids and $AlCl_3$ respectively. For the inorganic acids, HNO_3 was the best acid for pectin extraction yielding 0.8%/200 g of the sample. This result agrees with the study of Kliemann *et al.* (2009) who compared the optimization of pectin acid extraction from

Table 1 Peak positions and wavelength intensity of $AICl_3$ pectin extract.

Peak position (cm^{-1})	Wavelength intensity
536.38	8.065
1078.63	17.520
1124.13	17.110
1386.92	25.490
1399.80	26.990
1419.99	10.644
1718.11	11.258
3436.77	3.212

Table 2 Mean values of pectin yield using inorganic/organic acids and $AICl_3$.

Extraction medium	Pectin content (%) ± SD
HNO_3	0.8 ± 0.2
HCL	0.4 ± 0.0
H_2SO_4	0.1 ± 0.0
Acetic acid	0.3 ± 0.0
Citric acid	0.1 ± 0.0
Oxalic acid	0.2 ± 0.0
$AICl_3$	2.4 ± 0.6

Sample size = 200 g; *t*-test = 3.34

Table 3 Mean values of moisture and methoxyl contents of pectin extract using inorganic/organic acids and $AICl_3$.

Extraction medium	Moisture content (%) ± SD	Methoxyl content (%) ± SD
HNO_3	2.9 ± 0.5	5.6 ± 0.6
HCL	1.7 ± 0.5	4.2 ± 0.1
H_2SO_4	1.1 ± 0.3	2.4 ± 0.1
Acetic acid	2.0 ± 0.2	3.9 ± 0.4
Citric acid	3.0 ± 0.7	3.5 ± 0.1
Oxalic acid	3.0 ± 0.3	3.7 ± 0.2
$AICl_3$	4.8 ± 0.2	2.8 ± 0.3

Sample size = 200 g; *t*-test = 1.19

passion fruit (*Passiflora edulis*) peel. Acetic acid was also observed to have extracted the highest quantity of pectin using the organic acids (0.3%/200 g of the sample). Citric acid extracted the least amount of pectin (0.1%/200 g of the sample).This result is in contrast with the observation of Virk and Sogi (2004) and Schemin *et al*. (2005), who both compared the yields of pectin extracted from apple pomace with different acids and noted that citric acid was the best acid for extraction of pectin. Between the two types of acids (inorganic and organic), the present study noted that there was no significant difference in pectin yield since $P < 0.01$, when the generalized *t*-test = 3.34 (df = 4). However, $AlCl_3$, was observed to have extracted the highest amount of pectin overall totaling 2.4%/200 g of the sample.

Pectin consists of a group of macromolecules (acidic polysaccharides) that constitute a large part of the cell wall of plants (Scheller *et al*. 2007). To examine the purification procedure effect on pectin amount, purity, macromolecular characteristics, and gelling ability, three pectin isolates obtained from the following purification methods [alcohol-precipitation (APP), dialysis (DPP), and metal ion-binding precipitation (MPP)] were used. Results obtained showed that the metal ion pectin isolates, gelled rapidly with higher strength than the other two isolates. Hence, it was concluded that the pectin amount, composition, and physicochemical properties could be considerably affected by the purification method (Yapo 2009).

Pectin has been reportedly prepared from raw papaya peel by ethanol and $AlCl_3$ precipitation method with yields of 2.33 and 5.84% on a basis of peel fresh weight respectively (Boonrad *et al.* 2006).

The degree of esterification of 46 and 51%, respectively was reported for ethanol and $AICl_3$ precipitated pectins from the papaya fruit. In the present study, **Table 3** show values of degree of methoxylation in the various pectin extracts. With the inorganic acids 5.60, 4.20 and 2.40% methoxyl content was obtained for HNO_3, HCL, and H_2SO_4 respectively, while 3.89. 3.50 and 3.70%, respectively was obtained for acetic, citric, and oxalic acids. However, 2.80% degree of methoxylation was obtained for the $AlCl_3$ extract. The generalized *t*-test of degree of methoxylation between inorganic and organic acids is 1.19. This value show no significance, $P < 0.01$ (df = 4).

Table 1 show a list of peaks positions of IR spectrum of the pectin extract ($AlCl_3$). The absorption peak at 536.38 cm^{-1} appears to have strong absorption intensity due to the presence of aromatic ring structure in the compound extracted. The peak at 1124 cm^{-1} (C – O), stretch is characteristics of the methoxyl group. Hydroxyl (–OH) stretching vibration is typically observed at 3436.77 cm^{-1}, while the peak at 1718 cm^{-1} show medium strength absorption characteristics of carbonyl stretching vibration. Also, the intensity of aromatic (1419.99 and 1560.07 cm^{-1}) C–C multiple bond stretching vibration appear most pronounced.

The present study advice on more research on pectin expanding beyond its associated areas of usage since properties of pectin such as ash content, melting point, solubility etc, which are factors that influence the substance's potentials for novel commercial use and application have been limitedly researched.

REFERENCES

Alborzi S, Lim L, Kakuda Y (2010) Electrospinning of sodium alginate-pectin ultrafine fibers. *Journal of Food Science* **75**, 100-107

Boonrad D, Reanma K, Nlamsup H (2006) Extraction and physicochemical characteristics of acid-soluble pectin from raw papaya (*Carica papaya*) peel. *Chiang Mai Journal of Science* **33**, 129-135

Constenla D, Ponce AG, Lozano JE (2003) Kinetic model of pectin demethylation. *Latin American Applied Research* **33**, 91-96

Faravash RS, Ashtiani FZ (2007) The effect of pH, ethanol volume and acid washing time on the yield of pectin extraction from peach pomace. *International Journal of Food Science and Technology* **42**, 1177-1187

Fertonani HCR, Scabio A, Carneiro EBB, Schemim MHC, Nogueira A, Wosiacki G (2009) Extraction model of low methoxyl pectin from apple pomace effects of acid concentration and time on the process and product. *Brazilian Archives of Biology and Technology* **52**, 177-185

Garna H, Mabon N, Robert C, Cornet C, Nott K, 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**, 1-9

ICS-Nigeria (2011) Growing citrus in Nigeria. Available online: http://www.ics-nigeria.org

Kliemann E, Numes SK, Amante ER, Prudêncio ES, Teófilo RF, Ferreira MC, Amboni RDM (2009) Optimization of pectin acid extraction from passion fruit peel (*Passiflora edulis*) using response surface methodology. *International Journal of Food Science and Technology* **44**, 476-483

Lopex AA, Alexander M, Corredig M (2010) Diffusing wave spectroscopy and rheological studies of rennet-induced gelation of skim milk in the presence of pectin and k-carrageenan. *International Dairy Journal* **20**, 328-335

Madlav A, Pushpalatha PB (2002) Quality upgradation of jellies prepared using pectin extracted from fruit wastes. *Journal of Tropical Agriculture* **40**, 31-34

Mirhosseini H, Tan CP, Aghlara A, Chena BH (2008) Influence of pectin and CMC on physical stability, turbidity, loss rate, cloudiness and flavor release of orange beverage emulsion during storage. *Carbohydrate Polymers* **73**, 83-91

Mohamadzadeh J, Mahoonak AR, Yaghbani M, Aalami M (2010) Extraction of pectin from sunflower head residues of selected Iranian cultivars. *World Applied Sciences Journal* **8**, 21-24

Pagán J, Ibarz A (1999) Extraction and rheological properties of pectin from fresh peach pomace. *Journal of Food Engineering* **39**, 193-201

Pagán J, Ibarz A, Llorca M, Pagán A, Barbosa-Cànovas GV (2001) Extraction and characterization of pectin from stored peach pomace. *Food Research International* **34**, 605-612

Polle AY, Ovodova RG, Shashkov AS, Yu SO (2002) Some structural features of pectic polysaccharide from tansy (*Tanacetum vulgare*). *Carbohydrate Polymers* **49**, 337-344

Rehmann ZU, Salatiya AM, Shah WH (2004) Utilization of mango peels as a source of pectin. *Journal of the Chemical Society of Pakistan* **26**, 73-76

Robert C, Devillers T, Wathelet B, Herck VJC, Paquot M (2006) Use of a placket-Burman experimentation design to examine the impact of extraction parameters on yield and compositions of pectins extracted from chicory roots (*Chicorium intybus*). *Journal of Agricultural and Food Chemistry* **54**, 7167-7174

Scheller HV, Jensen JK, Sorensen SO, Harholt J, Geshi N (2007) Biosynthesis of pectin. *Physiologia Plantarum* **129**, 283-295

Schemim MHC, Fertonani HCR, Waszczynskyj N, Wosiacki G (2005)

Extraction of pectin from apple pomace. *Brazilian Archives of Biology and Technology* **48**, 259-266

Semenova M, Sinitsyna O, Morozova V, Fedorova E, Gusakov A, Okunev O, Sokolova L, Koshelev A, Bubnova T, Vinetskii Y, Sinitsyn A (2006) Use of a preparation from fungal pectin lyase in the food industry. *Applied Biochemistry and Microbiology* **42**, 598-602

Sharma BR, Naresh L, Dhuldhoya NC, Merchant SU, Merchant UC (2006) An overview on pectins. *Times Food Processing Journal* **6**, 44-51

Sperber B, Schols H, Cohenstuart M, Norde W, Voragen A (2009) Influence of the overall charge and local charge density of pectin on the complex formation between pectin and β–lactoglobulin. *Food Hydrocolloids* **23**, 765-772

Virk BS, Sogi DS (2004) Extraction and characterization of pectin from apple pomace (*Malus pumila*) peel waste. *International Journal of Food Properties* **7**, 1-11

Wijesinghe WA, Sarananda KH (2002) Post-harvest quality of Mauritus pineapple and reasons for reduced quality. *Tropical Agricultural Research and Extension* **5**, 53-57

Wosiacki G, Nogueira A (2001) Apple varieties growing in subtropical areas. The situation in Paraná State. *Fruit Processing* **11**, 177-182

Yapo BM (2009) Pectin quality, composition and physicochemical behaviour as influenced by the purification process. *Food Research International* **42**, 1197-1202

Yu CY, Yin BC, Zhang W, Cheng SX, Zhang XZ, Zhou RX (2009) Composite microparticle drug delivery system based on chitosan, alginate and pectin with improved pH-sensitive drug release property. *Colloids and Surfaces B: Biointerfaces* **68**, 245-249