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Standardization of eco-friendly technique for extraction of pectin from apple pomace

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Apple pomace, an industrial by-product generated from apple juice processing industries, is a good source of pectin. The method for pectin extraction has been standardized by autoclaving apple pomace flour for time intervals of 15, 30, 45 and 60 min at 121 °C. The extraction of pectin by using autoclave at 121°C for 60 min followed by precipitation with 95 % ethanol was found optimum with pectin yield of 13.01 % on dry weight basis and anhydrogalacturonic acid content of 43.21 %. The pectin extracted by using eco-friendly method is characterized by equivalent weight (942.04), methoxyl content (4.32 %), anhydrogalacturonic acid (43.21 %) and degree of esterification (56.76 %). However, autoclaving at 121 °C for 45 min also resulted in 12.97 % pectin, but on the basis of anhydrogalacturonic acid (%) contents, the autoclaving of pomace for 60 min was optimized. The standardized method can be used to replace the chemical extraction procedure for commercial extraction of pectin from apple pomace.

Keywords: Apple pomace, Autoclaving, By-product, Eco-friendly, Esterification, Methoxyl content, Pectin.

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Introduction

Apple is an important fruit crop, with an annual world production of 76.38 million tonnes from an area of 4.84 million hectares, out of which India produces 2.20 million tonnes from 0.32 million hectare area[1]. Even though apple is marketed mainly in the fresh form, a substantial quantity is used for the preparation of juice, pulp, concentrate and other processed products. During processing of apple fruits for juice or cider preparation, large amount of solid residues (peel, core, seed, calyx, stem and soft tissue) is generated[2]. The conventional juice extraction process removes 75 % of fresh weight as juice and 25 % remaining portion is called as pomace[3]. Apple pomace is the by-product left after extraction of apple juice, which is a major processing waste in the apple processing industry. It is a rich source of sugars (17.35 %), pectin (16.95 %) and crude fibre (16.16 %)[4] with very high biological oxygen demand (240-19000 mg/L)[5,6]. According to Sharma ***et al***[7], about 1000 tonnes of apple pomace goes waste in India. Although, efforts have been made to utilize apple pomace for making edible products but due to non-availability of proper infrastructure it is causing environmental pollution and economic loss, which needs to be addressed immediately. Apple pomace is reported to contain about 18-19 % pectin on moisture free basis[8] and 13.3 % on dry weight basis[7]. Pectin is a complex heterogeneous polysaccharide which mainly consists of 1, 4- linked α-D-galacturonic acid units partly methyl esterified[9]. It is a plant polysaccharide present in middle lamella of plant primary cell wall including apple. Pectin is known for being the traditional gelling agent in jams and jellies[10] and serves as a natural food function factor which plays an effective role as stabilizing agent, emulsifier and better thickener[11,12].

Various studies have been conducted to extract pectin from apple pomace by using chemical methods[8,13-17]. The pectin extraction methods used were quite generalized for direct application in apple pomace utilization, but literature on use of eco-friendly techniques is lacking. Using apple pomace as animal feed, compost and pulp preparation have been tested and employed to effectively utilize apple pomace, from which pectin extraction has been recognized as an economically feasible process.

Pectins are industrially obtained from apple pomace and citrus peels in a chemical way with

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strong acids such as oxalic acid[11], sulphuric acid[13], hydrochloric acid[15] and nitric acid[18], which are regarded as conventional acid extraction methods[19]. Even though chemical procedures are efficient and economic, they may cause environmental problems by producing hazardous contaminants. The chemicals used in the conventional pectin extraction are strong acids and are unsafe to handle. On the other hand, some of enzymatic extractions like polygalacturonase[20], hemi-cellulase and protease[21], microbial mixed enzymes[22], autoclave[23], microwave[24], etc. is also used, but these methods are costly. Thus, present study was designed to develop economical process for the commercial production of pectin from apple pomace in eco-friendly way.

Materials and Methods

Raw material

Fresh apple pomace left after extraction of juice from processable grade fruits was procured from Himachal Pradesh Horticultural Produce Marketing and Processing Corporation Ltd, Fruit Processing Plant, Parwanoo, District Solan, Himachal Pradesh. The pomace was dried in the dehydrator (55±2 °C) to remove extra moisture for preparation of apple pomace flour, which was further utilized as a substrate for pectin extraction. Apple pomace flour was packed in air-tight bags with vacuum packaging machine to avoid absorption of moisture.

Extraction procedure

The dehydrated flour was rehydrated with water in ratio of 1:3. The pectin was extracted using different methods as boiling at 95 °C at atmospheric pressure for 1 h (control), boiling in autoclave at 121 °C for 15, 30, 45 and 60 min. The apple pomace flour after autoclaving as per the treatment was filtered to separate extract and residue. The extract was utilized for extraction of pectin by precipitating it with ethanol. The detailed procedure for pectin extraction followed in the present study is depicted in Fig. 1.

Ethanol precipitation

Ethanol (95 %) was added to supernatant in 1:2 ratio to achieve 80 % v/v ethanol. Monosaccharide and oligosaccharides are reported to be soluble in 80 % ethanol whereas polysaccharides are reported to be insoluble[25]. Precipitation of the extract helps in filtration and extraction of pectin.

Analysis

Chemical analysis of apple pomace

The total soluble solids (TSS) of pomace was determined with the help of hand refractrometer and expressed as ^{o}Brix[26]. Reducing and total sugars in percent were estimated by using Lane and Eynon method[27]. The titratable acidity, ascorbic acid and crude protein were estimated by following the AOAC method[28]. Moisture content was estimated by drying the weighed sample up to a constant weight in hot air oven at 70±2 °C and expressed in terms of percentage. Crude fibre in percent (w/w) was calculated by following standard method described in AOAC[28]. Calcium pectate content in the apple pomace was determined using method described by Ranganna[26].

Chemical analysis of pectin

Pectin was analyzed for various chemical characteristics like equivalent weight, methoxyl content, anhydrogalacturonic acid[29], ash content and degree of esterification. Equivalent weight was estimated by weighing 0.5 g pectin (moisture free) in a 250 mL conical flask, moistened with 5 mL ethanol and added 1 g NaCl to sharpen the end point. Then 100 mL of CO_2 free distilled water was added followed by adding 6 drops of phenol red indicator. The pectin substances were stirred rapidly to dissolve, then titrated slowly with 0.1 N NaOH until the colour of the indicator changed (pH 7.5) and persisted for at least 30 sec. Methoxyl content was determined by adding 25 mL of 0.25 N NaOH to the neutral solution,

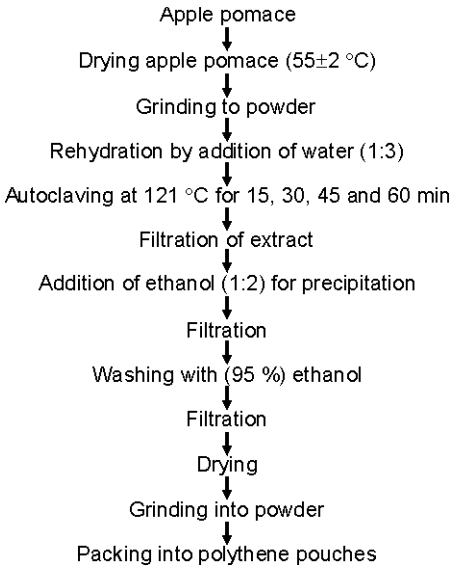

Fig. 1—Process flow chart for extraction of pectin from apple pomace

mixing thoroughly and allowed to stand for 30 min at room temperature in a stopper flask. 25 mL of 0.25 N HCl was then added and titrated with 0.1 N NaOH to the same end point. The degree of esterification of the extracted pectin was calculated from the methoxyl content and anhydrogalacturonic acid content as described by Owens ***et al*** [29] in Ranganna[26] by using the expression.

$$\text{Degree of esterification (\%)} = \frac{176 \times \text{\% methoxyl content} \times 100}{31 \times \text{\% anhydrogalacturonic acid}}$$

Statistical analysis

Data pertaining to the analysis and characterization of pectin were analyzed by using completely randomized design[30].

Results and Discussion

Physico-chemical composition of apple pomace

Table 1 shows that the pomace contains 8.65 °B TSS, 6.25 % total sugars, 0.27 % titratable acidity (as malic acid) and ascorbic acid 3.40 mg/100 g with 81.35 % moisture content (on fresh weight basis), while insoluble material constituted about 2.33 % pectin (as calcium pectate) and 5.41 % crude fibre. The crude protein on fresh weight basis was 2.70 %, while mineral content represented by total ash content was 0.85 %. On dry weight basis the apple pomace consisted of total sugars (38.05 %), acid (as malic acid) (1.81 %) and ascorbic acid (19.30 mg/100 g). The pectin content in pomace was recorded to be 14.66 % as calcium pectate, crude fibre 31.31 % and crude protein 6.12 % with 5.44 % ash contents. The data recorded during this study was found in accordance with reported findings in literature[31-33]. Thus, apple pomace was found to be a rich source of pectin, sugars, acid, minerals, but is deficient in crude proteins, most importantly pomace is a rich source of pectin and pectin production is an economical utilization of this by-product[34-36].

Table1—Physico-chemical composition of apple pomace

Attribute	Mean ± S.E.	
	Fwb*	Dwb**
Moisture (%)	81.35 ± 1.28	-
TSS (°Brix)	8.65 ± 0.16	-
Titratable acidity (% malic acid)	0.27 ± 0.01	1.81 ± 0.10
Total sugars (%)	6.25 ± 0.25	38.05 ± 2.43
Reducing sugars (%)	2.17 ± 0.05	14.30 ± 0.34
Ascorbic acid (mg/100 g)	3.40 ± 0.20	19.30 ± 1.03
Crude fibre (%)	5.41 ± 0.23	31.31 ± 1.52
Crude protein (%)	2.70 ± 0.22	6.12 ± 0.84
Ash content (%)	0.85 ± 0.04	5.44 ± 0.12
Pectin (% as calcium pectate)	2.33 ± 0.19	14.66 ±1.21

* Fresh weight basis, ** Dry weight basis

Extraction of pectin

The boiling and autoclaving of apple pomace at 95 and 121 °C, respectively influenced the pectin yield. The yield expressed as dry weight of extract varied from 9.28 to 13.01 % depending on the extraction conditions used. Boiling at 95 °C for 1 h was treated as control which resulted in lowest pectin yield (9.28 %) with less quality characteristics, whereas the highest percentage of pectin (13.01 %) was obtained on extraction by heating in autoclave at 121 °C for 60 min and lowest yield was recorded during autoclaving at 121 °C for 15 min (10.60 % pectin) (Table 2). The yield might be higher due to availability of more protopectin in small particles. The results were in conformity with pectin yield reported in passion fruit peel[37] and red dragon fruit (***Hylocereus polyrhizus***)[38]. Further, Yujaroen ***et al***[39] reported that the longer the extraction time, the higher the percentage of pectin derived. Canteri-Schemin ***et al***[40] also reported that the use of pomace flour as raw material yields higher pectin.

Chemical characteristics of pectin

The chemical characteristics of pectin extracted from apple pomace using autoclave for different time intervals are shown in Table 2. The autoclave extraction for 60 min gave lower equivalent weight (942.04) when compared to autoclaving for 15 min (1197.32). The equivalent weight was observed to

Table 2—Standardization of heating method for extraction of pectin

Method	Pectin yield (%)	Ash (%)	Equivalent weight	Methoxyl content (%)	Anhydroglacturonic acid (%)	Degree of esterification (%)
Boiling at 95° for 1 hour (control)	9.28	1.07	1223.35	3.88	36.42	60.49
Autoclave at 121 ° C for 15 min	10.60	1.26	1197.32	4.55	40.53	63.73
Autoclave at 121 °C for 30 min	12.12	1.32	1120.68	4.49	41.20	61.88
Autoclave at 121 °C for 45 min	12.97	1.44	1035.77	4.41	42.03	59.57
Autoclave at 121 °C for 60 min	13.01	1.56	942.04	4.32	43.21	56.76
$CD_{0.05}$	0.22	0.09	7.29	0.04	1.35	1.26

decrease with the increase in autoclaving time, whereas higher equivalent weight of 1223.35 was recorded in case of control (Boiling at 95 °C for 1 h). The increase or decrease of the equivalent weight might depend on the amount of free acid[41]. Similar results have also been reported with increase in the extraction time[38,40]. Long extraction time causes de-polymerization reaction and de-esterification and the pectin become pectic acid[42].

The methoxyl content of pectin varied between 3.88 to 4.55 %. The results showed that the methoxyl content decreased with increase in extraction time with the highest methoxyl content (4.55 %) recorded during autoclaving at 121 °C for 15 min and lowest (4.32 %) during autoclaving at 121 °C for 60 min. Similar results were reported on the decrease in methoxyl content in pectin extraction from passion fruit peel[37]. Further, slight increase in anhydroglacturonic acid (%) of pectin was recorded with the increase in time. The anhydrogalacturonic acid content of apple pomace pectin varied between 36.42 to 43.21 % with the highest anhydroglacturonic acid observed during autoclaving at 121°C for 60 min, which was statistically at par with autoclaving for 45 min. Therefore, on the basis of anhydrogalacturonic acid contents, the treatment consisting of autoclaving at 121 °C for 60 min was optimized. The anhydrogalacturonic content will increase with increasing time of extraction, which might be due to the hydrolysis reaction of protopectin which results in D-anhydrogalacturonic acid, a basic component of pectin[41]. Slightly lower level of anhydrogalacturonic acid content found in pectin might be attributed to the inherent quality characteristics of pectin within the apple pomace.

Further, autoclaving at 121 °C for 15 min led to the highest degree of esterification, i.e. 63.73 %, which gradually decreased to 56.76 % (autoclave at 121 °C for 60 min). The result is in conformity with the results reported for cocoa husk[41]. Canteri-Scheimen ***et al***[40] also reported 60 % degree of esterification for apple pomace pectin. Longer extraction time might cause degradation of methyl ester groups in pectin into carboxyl acid. The ash content of pectin ranged between 1.26 to 1.56 % on dry weight basis. Highest ash content (1.56 %) was observed during autoclaving at 121 °C for 60 min while lowest (1.26 %) was recorded during autoclaving at 121 °C for 15 min. Ramli and Asmawati found that the highest percentage of ash content (12.93 %) was obtained at extraction time of 120 min at low pH as compared to 60 min (8.15 %) in cocoa husk pectin, indicating that ash content increased with time[41].

Conclusion

On the basis of chemical characteristics of extracted pectin i.e. anhydrogalacturonic acid (43.21 %) and methoxyl content (4.32 %), the treatment consisting of autoclaving at 121 °C for 60 min was found optimum and thus standardized for pectin extraction. The extraction conditions had major impact on the extraction yield of pectin but did not have significant difference in other physico-chemical properties. Extraction of pectin was found highest (13.01 %) by heating in autoclave at 121 °C for 60 min and thus optimized for pectin extraction. The developed eco-friendly method will not only utilize apple pomace for commercial pectin production but also solve the problem of waste disposal and meet the requirement of pectin in the market to some extent.

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