

Original article

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Comparison of different technologies using organic acid and mineral acid solvents on pectin extraction from apple pomace

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Abstract. Pectin is one of the most widely used functional polysaccharide, which is extensively used in food, biotechnological, chemical, cosmetic, and pharmaceutical industries. Pectin can be suitably extracted from apple pomace which is the main by-product of apple juice industry. In this study, pectin obtained from microwave assisted extraction (MAE) and conventional extraction (CE) were compared in terms of extraction yield, extraction time and extraction temperature. The type of acids namely organic acids and mineral acids were also compared, in which pectin from apple pomace was extracted using different concentration of organic acid (citric acid, 0.1 M and 0.05 M) and mineral acid (hydrochloric acid, 0.1 M and 0.05 M). The best extraction condition of the extraction process was found to be higher in yield by using citric acid at microwave power of 180 W, solid-to-liquid ratio of (1:25), concentration of citric acid of 0.1 M for 90 s. Under these conditions, the extraction yield of pectin reached $19.1 \pm 0.2\%$, methoxyl content (5.27%), anhydroglacturonic acid (43.38%) and degree of esterification (68.97%). The current work showed that citric acid is a 'green' and safe acidic solvent that could be used to extract pectin from apple pomace that was highly competitive and environmentally friendly process. The microwave Assisted extraction method is considered to be a potential alternative conventional (traditional) extraction method. The obtained data undertakes that apple pomace obtained by the green extraction has a great potential to be a valuable source of pectin, while preserving similar quality to conventional sources of pectin.

Keywords: food products; pectin; apple pomace; extraction; organic acids; microwave

Научная статья

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Сравнение различных технологий использования органических и неорганических кислот при извлечении пектина из яблочного жмыха

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Аннотация. Исследовали и сравнивали пектин из яблочного жмыха, полученный путем экстракции микроволнами и обычной экстракции, с точки зрения выхода экстрагируемого вещества (пектина), времени и температуры экстракции. Изучали типы кислот (органические и неорганические), используемые для экстракции пектина в различных концентрациях: лимонная кислота – 0,1 и 0,05 М и соляная кислота – 0,1 и 0,05 М. Установлено, что наилучшими условиями процесса экстракции по выходу пектина является использование лимонной кислоты в концентрации 0,1 М, воздействие микроволнами мощностью 180 Вт в течение 90 с и соотношение твердой и жидкой фаз 1:25. Выход пектина в этих условиях достигал $19,1 \pm 0,2\%$, содержание метоксила (5,27%), ангидрогалактуроновой кислоты (43,38%) и степень этерификации (68,97%). Показано, что применение органических кислот соответствует подходам «зеленой экстракции» и является альтернативой агрессивным химическим растворителям, которые могут широко использоваться для извлечения пектина из яблочного жмыха. Метод микроволновой экстракции может служить альтернативой общепринятому (традиционному) методу экстракции. Полученные данные свидетельствуют о том, что пектин, полученный из яблочного жмыха путем экстракции органическим растворителем с применением микроволнового воздействия, является ценным пищевым ингредиентом, не уступая по качеству пектину, полученному традиционным методом экстракции с использованием неорганических кислот.

Ключевые слова: пищевые продукты; пектин; яблочный жмых; экстракция; органические кислоты; микроволны

Introduction

Pectin's are complex polysaccharides that are commonly spread in the primary cell walls and the middle lamella of higher plants, having vital functions in their growth, and maturation [1]. Recently, there is an increase in the consumption of fruit derivatives, such as pulps and juices, for reasons of practicality and exploration of quality of life [2]. However, during the processing of passion fruit in food industry, large volume of byproducts and waste are produced, which can lead to a significant economic loss [3]. These waste parts have chemical compositions that allow their transformation into new materials, such as polysaccharides and oils [4, 5]. Pectin is extensively used in food additives, medical and health products. Indeed, around 85% of pectin production comes from the waste by product of juicy fruits [6]. Agricultural by-products are renewable sources for pectin, correspondingly they are considered for the world-wide supply of raw materials for its production. Apple pomace is a main byproduct of juice processing industries [7] it can serve as a key source of pectin products. Certainly, pectin is an important structural component which is widely used in various fields especially in the food industries as a gelling agent, thickener, texturizer, emulsifier, and stabilizer in some products such as jams, jellies and preserves agent due to its technological and functional properties [8, 9]. The joint FAO/WHO committee on food additives endorsed pectin as a safe additive with no limit on suitable daily intake except as dictated by good manufacturing practice [10].

Extraction is a vital technique for the isolation of biological active products from natural sources for use in food systems [11]. Chemical extraction approaches are extensively used, but they can lead to serious environmental problems as they produce acidic wastewater [12]. Commonly, conventional (traditional) heating extraction uses high temperature in which the hot water extends from 60°C to 100°C acidified with a strong mineral acids like hydrochloric, sulfuric or nitric acids [13]. Additionally, this process is time consuming and strongly influence on natural macromolecular structure that leads to pectin degradation. To overcome the mentioned serious problems (shortcomings), Tongkham et al., [14] reported recently that microwave-assisted extraction is an innovative method that was developed as an alternative to traditional methods, promoting low energy consumption, easy controllability, short processing time, low solvent necessities, low price, and high competence.

An imperative matter of pectin extraction research is to study the application of one extraction technique, together with conducting a comparative study between two or more techniques used to isolate pectic polysaccharides from natural sources. As we explored Recently published articles by Rodsamran et al., [13] and Karbuz et al., [15] conveyed a comparison between techniques used for pectin extraction from plant sources. However, these studies are limited to the techniques comparing microwave with conventional (traditional) extraction or with other extraction techniques of pectin from fruit wastes without considering the type of solvents. With the growth of the worldwide population, waste generation is growing, and environmental pollution has become main problem throughout the world. Therefore, the objective of this study was to investigate the effect of different concentrations of organic acids and mineral acids and apple pomace-to-extractant ratios under the potential microwave heating on the extraction yield of pectin from pink crisps apple pomace compared with conventional heating. This study reflects the use of organic acids (environmentally friendly) with advanced method, such as microwave-assisted extraction.

In brief, the present work represented a holistic approach to this subject since it aims at comparing different extraction techniques and solvents. To extract pectin from pink crisps apple pomace, non-conventional method, microwave-assisted (2450 MHz) extraction using organic acid (citric acid) and mineral acid (hydrochloric acid) were investigated in comparison with conventional mineral acid (hydrochloric acid) and organic acid (citric acid). Moreover, the highest pectin yields were subjected to chemical analysis of pectin obtained. Therefore, the results of this work will allow food waste to be recycled to produce a natural pectin polysaccharide, making an involvement to the national economy and environmental safety. Furthermore, the pectin extract will be an alternative biopolymer material for edible film and coating application in the future study.

Material and method

Fresh pink crisps apple (approximately 10 kg from several collections) was collected, cleaned with tap water and cut into small pieces. The small pieces of apples were processing into juice in laboratory. After juice extraction, apple pomace was dried at 70°C in an oven with air circulation to remove extra moisture for

preparation of apple pomace flour, which was further utilized as a substrate for pectin extraction. Apple pomace flour was filled in air-tight bags with vacuum packaging machine to keep away from absorption of moisture. All chemicals and reagents, including citric acid, ethyl alcohol, sodium hydroxide, hydrochloric acid, and were of analytical grade and were purchased from Chem Express (Russia).

Chemical analysis of apple pomace. Moisture content was estimated by drying the weighed sample up to a constant weight in hot air oven at $70 \pm 2^\circ\text{C}$ and expressed in terms of percentage. Crude fiber in percent (w/w) was calculated by following standard method described in AOAC [16]. Calcium pectate constituent in the apple pomace was explored using method designated by Ranganna [17].

Chemical analysis of pectin. Two highest pectin yield using conventional extraction (table 2) and microwave extraction (table 3), were selected for further chemical characteristics of pectin. Methoxyl component was analyzed by adding 25 mL of 0.25 N NaOH to the neutral solution, mixing carefully and permitted 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 identical end point. The degree of esterification of the extracted pectin was considered from the methoxyl component and anhydro galacturonic acid component as described by Owens [18], and Ranganna [17] using the following formula:

$$\text{Degree of esterification (\%)} = \frac{176 \cdot \% \text{ methoxyl content}}{31 \cdot \% \text{ anhydro galacturonic acid}} \cdot 100.$$

Pectin extraction. Optimal conditions of two different Extraction methods were selected to extract pectin from apple pomace, as presented in the following sections. Each extraction was made in triplicate.

➤ Conventional citric acid extraction (CE). The extraction mixture was prepared by mixing 1 g of apple pomace powder with 25 mL of organic acid (Citric acid at 0.1 M and 0.05 M concentration) and mineral acid (Hydrochloric acid at of 0.1 M and 0.05 M concentration). The extraction was heated at 80°C for 1 hour and 1½ hour in a shaker water bath.

➤ Microwave-assisted extraction (MAE). 1 g of apple pomace was mixed powder with 25 mL of organic acid (Citric acid at concentration of 0.1 M and 0.05 M) and mineral acid (Hydrochloric acid at concentration of 0.1 M and 0.05 M). The extraction was performed in an experimental microwave oven with frequency of 2450 MHz at a power of 180 W for 60 s and 90 s.

Englyst et al. [19] reported monosaccharide and oligosaccharides soluble in 80% ethanol whereas polysaccharides are insoluble. Therefore, precipitation of the extract helps in filtration and extraction of pectin. To isolate pectin from the plant material the same method was applied for all the extracts. These, the precipitation and purification steps were followed to Rodsamran et al., [13] method with slight modification. After each extraction, pectin was separated from the remaining solid material by centrifugation at 8000 rpm for 20 min, the supernatant was collected, filtered and transferred in a laboratory glass bottle where it was precipitated by adding 96% ethanol, thereafter the extract kept at 4°C for 24 h to complete the precipitation. The precipitated pectin was separated by centrifugation (8000 rpm, 20 min) and was washed 3 times by ethanol and finally dried in an oven with air circulation at 70°C to a constant weight.

The yield of pectin was calculated using the following equation

$$\text{Yield (\%)} = \frac{\text{Weight of pectin g}}{\text{Weight of apple pomace g}} \cdot 100.$$

Statistical analysis. The results were given as mean \pm standard deviation (SD). The analysis of variance (ANOVA) was used to demonstrate the differences in mean values. The data were statistically analyzed using the Microsoft Excel and GraphPad Prism 8.00, t test was carried out to detect any significant differences or was used to determine statistically significant differences ($P < 0.01$).

Results and discussion

Proximate composition of apple pomace. The result during this study was found in accordance with reported findings in literature [20]. Thus, apple pomace was found to be a rich source of pectin, sugars, and minerals. Most importantly pomace is a rich source of pectin and pectin production. This implies it is an economical utilization of agroindustry byproduct [21, 22].

Table 1. Proximate composition of apple pomace, values are mean of three replicates ± standard deviation, FW = Fresh weight DW = Dry weight

Parameters	Observed value (%) FW	Observed value (%) DW
moisture content	82.91 ± 1.28	–
crude fiber	5.47 ± 0.22	34.21 ± 0.91
total ash	0.91 ± 0.23	7.22 ± 0.84
total sugar	5.25 ± 0.05	39.51 ± 1.43
crude protein	3.80 ± 0.19	7.21 ± 0.84
pectin (% as calcium pectate)	3.41 ± 0.22	21.06 ± 0.19

Pectin yield. In this study the search criteria were to obtain the higher pectin yield from the dried apple pomace while considering environmental concern, efficiency, economization of energy and feasibility of experiment. The yield of apple pomace obtained in this study ranged from 12.6 to 19.1% table 2 and table 3. The heating method and acid type were the most influential factors on the pectin yield. Microwave heating using citric acid extraction proved slightly higher pectin yields to conventional (traditional) heating using hydrochloric acid (HCl).

Table 2. Pectin yield using conventional extraction

Acid type	Concentration (M)	Extraction temperature (°C)	Extraction time (hour)	Yield (%)
HCl	0.1	80	1	17.0 ± 0.32 ^a
	0.05			13.3 ± 0.53 ^c
Citric acid	0.1			15.9 ± 0.45 ^b
	0.05			12.7 ± 1.05 ^d
HCl	0.1		1½	18.5 ± 0.97 ^b
	0.05			15.3 ± 1.33 ^c
Citric acid	0.1			18.9 ± 0.09 ^a
	0.05			15.1 ± 1.23 ^d

Notes: Values are means ± SD, n = 3. Small letters in each column (a, b, c, d), indicates significant different (p < 0.01)

Table 3. Pectin yield using microwave assisted extraction

Acid type	Concentration (M)	Power (watt)	Extraction time, (min)	Yield (%)
HCl	0.1	180	1	15.4 ± 0.05 ^a
	0.05			12.6 ± 0.35 ^c
Citric acid	0.1			16.1 ± 0.61 ^b
	0.05			13.1 ± 1.22 ^d
HCl	0.1		1½	17.5 ± 0.52 ^b
	0.05			15.8 ± 0.99 ^c
Citric acid	0.1			19.1 ± 0.82 ^a
	0.05			15.1 ± 0.52 ^d

Notes: Values are means ± SD, n = 3. Small letters in each column (a, b, c, d) indicates significant different (p < 0.01)

When comparing different extraction approaches to get pectin from a plant material it is vital to reflect the maximum extraction yield attained with each method since this is prospective to have a major influence on its industrial feasibility. The mean yield obtained by means of each technique, for the conditions of pectin extraction detailed in section 3.2, is presented in table 2 and table 3.

As it can be observed, the lowest pectin recovery from apple pomace (12.6%) resulted when the MAE with HCl at 0.5 M while the highest yield (19.1%) was achieved when MAE was applied with citric acid at 0.1 M. Indeed, the conventional citric acid extraction (CE) also produced similarly a high pectin yield (18.9%).

In this study the highest pectin yield comparatively higher than the previous study using CE with HNO_3 15.04% [23] and MWAE with HCl 15.75% [24], However it is lower relatively than CE with HNO_3 25.3% [25] and when MAE with HCl (23.32%) was achieved [26]. Thus, Between CE and MAE using CA and HCl at 0.1 M was the similar pectin yield, however, the extraction time was significantly shorter for MAE (90 s) than CE (~150 min).

Chemical analysis of pectin. A major influence on pectin's methoxyl content, anhydroglacturonic acid and degree of esterification content exhibited by MAE (Figure). The result is in harmony with the results conveyed by Dance et al., [27] and Hosseini et al. [28]. In the microwave approaches, the heating happens speedily in the entire substance and, this deactivates the pectolytic enzymes more rapidly. Thus, the microwave treatment of the fruit material applied a great effect with respect to the quality of the pectic substances in the raw material. Moreover, the anhydrogalacturonic content was higher in MAE than CE, which might be due to the hydrolysis reaction of protopectin which results in D-anhydrogalacturonic acid, a basic component of pectin [29].

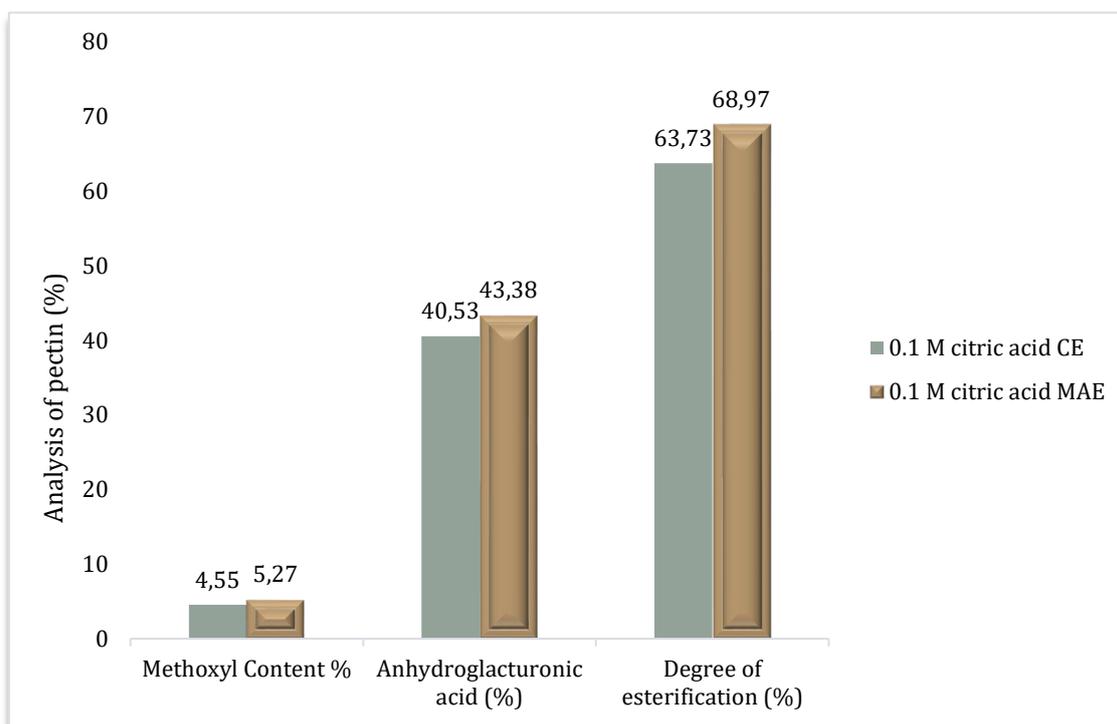


Figure. Chemical characterization of pectin obtained, CE = conventional extraction and MAE = microwave assisted extraction

Conclusion

Citric acid has an unexpected high efficiency in the extraction of pectin from apple residue, among which citric acid with a concentration of 0.1 M has the best effect in this study, while hydrochloric acid may be less ideal due to its volatilization under high temperature. In this study, in general, the longer the extraction time, the higher the extraction rate were observed, but due to the relatively small number of experimental groups, the optimal extraction time is still unknown. For the next stage of research, the concentration range and time range can be expanded upward to explore. Indeed, comprehensive comparison between extraction technique (conventional vs microwave heating) and acid type (organic acids vs mineral acids) were investigated. The results obtained presented microwave extraction (pectin yield and chemical characterizations of pectin obtained) was possibly a significant in the hydrolysis reaction and energy saving technique with a reduction in the extraction time, compared with conventional extraction. Moreover, the results of this study suggest that organic acids are truly capable of replacing mineral acid as an eco-friendly green processing technology for pectin extraction. High-quality pectin from apple pomace waste can be produced for application in the food industry through this processing technology that is capable of reducing and recycling food wastes. Therefore, pectin can be obtained that has potential be applied as a high-quality food ingredient. 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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