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EFFECT OF ACID TYPE AND PARTICLE SIZE ON THE YIELD AND PURITY OF APPLE (*MALUS DOMESTICA* 'FĂLTICENI') POMACE PECTIN

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Abstract: *This work proposes the use of Malus domestica 'Fălticeni' pomace, resulted from the processing of apples into juice in the geographical area of Fălticeni, Suceava (Romania), as a source for pectin extraction. Pectin was extracted from this plant source by using two extractants - hydrochloric acid and citric acid, separately at pH 2, solid-to-liquid ratio of 1:20, temperature of 90°C, and extraction time of 120 min. Together with acid type, another variable that was varied was the particle size: 200-300 µm, 125-200 µm, and <125 µm. To study the influence of acid type and particle size, pectin was characterized in terms of yield and purity, expressed as uronic acid content. The results showed that acid type and particle size had significant effects on the pectin yield and its uronic acid content. A strong influence of acid type on the extraction yield was recorded by particle sizes of 200-300 µm and 125-200 µm, respectively. Regarding the uronic acid content, it was observed that particle sizes of 200-300 µm determined a good purity of the extracted pectin, independent of acid type, while for particle sizes of 125-200 µm and <125 µm, the uronic acid content of pectin was strongly affected by the acid used. The highest pectin yield (21.24%) and uronic acid content (93.90 g/100g) were obtained for citric acid extraction and particle sizes between 125 and 200 µm.*

Keywords: *hydrochloric acid, citric acid, pectin recovery, composition.*

1. Introduction

As important components of the plant cell walls, polysaccharides of fruits and vegetables have drawn the interest of researches, manufacturers, and also consumers due of their physical properties and health promoting and disease-preventing potentials [1], which supported their application in food, pharmaceutical, and cosmetic industries. Of the different types of plant polysaccharides, pectin is mostly abundant in the middle lamella layers and the primary cell walls of plants [2,3]. Pectin is a complex polymer composed of at least 17 different monosaccharides, with galacturonic acid units linked by α(1→4) linkages (*D*-GalA) being the main constituents, followed by *L*-arabinose, *D*-galactose, *L*-rhamnose and others [4,5]. Structurally, 3 major polysaccharide domains are recognized in the pectic network, i.e. homogalacturonan (HG), which is a linear polymer of *D*-GalA, rhamnogalacturonan I (RG-I), described as a repeating disaccharide of *D*-GalA and *L*-rhamnose, and rhamnogalacturonan II (RG-II), which presents a homogalacturonic backbone with numerous complex side chains containing rhamnose and other neutral sugars [6]. Within the *D*-GalA units, the carboxylic or hydroxyl groups may be methyl-esterified and/or *O*-acetyl-esterified [7].

Commercial pectin is extracted from 3 main sources: apple pomace [8], citrus peel [9], and sugar beet pulp [10]. In the last few years several non-conventional sources of

pectin, such as carrot pomace, watermelon rinds, cocoa husks, mulberry branch bark, faba bean hulls, sisal waste, and pumpkin waste have been studied [6]. Advances have also been made regarding the extraction of pectin, through the development of some innovative approaches aimed to improve the overall extraction process and the quality of the final product. The innovative techniques applied include subcritical water extraction, ultrasound-, microwave-, and enzyme-assisted extraction [11].

An important tendency observed in the research on the extraction and characterization of pectin is the capitalization of waste streams resulted from processing fruit and vegetables locally. Based on this observation, we propose in this study the use of *Malus domestica* 'Fălticeni' pomace, obtained from processing apples into juice in the geographical area of Fălticeni, Suceava (Romania), as a source for pectin extraction. *Malus domestica* 'Fălticeni' is an apple hybrid developed through free pollination of the Jonathan variety at S.C.D.P. Fălticeni in the year 1962. This apple variety was selected as an elite cultivar in 1971; it was approved as a new variety in 1979 and registered in the Romanian Official Catalogue of the Varieties of Plant Species [12]. The enlisting of the variety in the Romanian Official Catalogue was renewed at the end of 2015 [13]. *Malus domestica* 'Fălticeni' is an autumn variety that is resistant against disease, drought, frost, and other stress factors. According to S.C.D.P. Fălticeni, the average production is estimated to 28 t/ha, however, a later study reported a lower average production (18.6 t/ha) [14]. Regarding the main characteristics of the fruits, apples of the Fălticeni variety are medium sized, of spherical shape, flattened, and have a weight of about 148 g. The best quality of the fruit was recorded from the middle of September until December [12].

This study aims at investigating the potential for capitalization of *Malus domestica* 'Fălticeni' pomace as a source of pectin, which provides a mean to increase the economic viability of this apple variety. Pectin extraction from apple pomace was conducted on a laboratory scale with the purpose of studying the influence of the type of acid (mineral vs. organic) and particle size on the overall process. The extracted pectin was characterized in terms of yield and purity, expressed by the content of uronic acid.

2. Materials and methods

2.1. Materials

Apple pomace was collected by processing *Malus domestica 'Fălticeni'* apples from 2016 harvest, cultivated in the Fălticeni area of Suceava, Romania. The pomace was dried in an oven with air circulation at 60°C until constant weight was achieved and then powdered using a food processor. The obtained powder was passed through an analytical sieve shaker Retsch AS 200 (Retsch GmbH, Germany) to separate it on the following particle size intervals: 200-300 μm, 125-200 μm, and <125 μm.

Hydrochloric acid, citric acid, ethyl alcohol, sulfamic acid, potassium hydroxide, sulfuric acid, sodium tetraborate, sodium hydroxide, *D*-galacturonic acid, and *m*-hydroxydiphenyl were purchased from Sigma-Aldrich (Munich, Germany).

2.2. Extraction and purification procedure

The extraction mixture was prepared by mixing 5 g of apple pomace powder with 100 ml of extraction solvent (solid-to-liquid ratio of 1:20) obtained by adding hydrochloric acid and, respectively, citric acid to distilled water until a pH value of 2 was reached. For each type of acid, 3 separate mixtures, according to the particle size intervals mentioned above, were prepared. To extract pectin, the mixture was

kept in a water bath at the temperature of 90°C for 120 min.
After the extraction was completed, the mixture was cooled to room temperature prior to the precipitation and purification steps. Pectin was first separated from the remaining solid material by centrifugation at 4000 rpm for 40 min; the resulting supernatant was passed through clean cheesecloth folded 6 times, fitted into the neck of a laboratory bottle with screw cap, and then mixed with ethyl alcohol (>96%, v/v) in a 1:1 ratio (v/v). The content of the bottle was thoroughly mixed and kept at 4-6°C for 12 h to complete the precipitation. Next, the centrifuge was used again to separate the precipitated pectin from the liquid (4000 rpm, 30 min). The wet pectin was washed by ethyl alcohol 3 times and dried at 50°C to a constant weight.

2.3. Determination of pectin yield

Pectin yield was calculated using Eq. (1):

$$Yield\,(\%) = \frac{m_0}{m} \times 100 \qquad (1)$$

Where: m_0 is the weight of dried pectin (g) and m is the weight of dried apple pomace powder (g). [15]

2.4. Determination of uronic acid content

The uronic acid content of pectin was estimated using the sulfamate/*m*-hydroxydiphenyl method developed by Filisetti-Cozzi and Carpita [16]. For each batch of samples was prepared a standard curve of *D*-galacturonic acid. Sample preparation for the analysis was made according to [17]. Briefly, 20 mg of dry pectin were added to 50 mL distilled water (at 40ºC) and mixed using a magnetic stirrer until the sample was completely dispersed. The volume was finally adjusted to 100 mL with distilled water at 40ºC.
Aliquots of 400 µL from the pectin solutions were placed in glass tubes. To the pectin solution were added 40 µL of 4 M sulfamic acid solution (adjusted to pH 1.6 with saturated solution of potassium hydroxide), followed by 2.4 mL of sulfuric acid containing 75 mM of sodium tetraborate, vigorously vortexing for at least 5 s the content of the tubes in between the addition of the solutions. The samples were place in a 100ºC water bath (boiling) for 20 minutes, and then cooled in an ice bath for 10 minutes. After cooling, 80 µl of *m*-hydroxydiphenyl solution in 0.5% (w/v) sodium hydroxide were added and vortex mixed. Between 10 min and 30 min after complete mixture the absorbance was read at 525 nm against the reagent control using a UV-Vis-NIR spectrophotometer (Shimadzu Corporation, Japan).

3. Results and discussion

An important observation must be first made regarding the pectin yield achieved through the use of pomace from *Malus domestica* 'Fălticeni' apples. As Fig. 1 shows, the maximum pectin yield was 21.24%. As *Malus domestica* 'Fălticeni' is an autumn variety (late season), the content of pectin is expected to be high, however, the maximum yield determined for this apple variety exceeds the values reported for a citric acid extraction of pectin from the late season Royal (16.65%) and Golden (18.79%) apple varieties [18].

3.1. Influence of acid type on pectin yield and purity

According to the results presented in Fig. 1, pectin yield was significantly influenced by the type of acid used in the preparation of the extraction mixture. As it can be observed, pectin yield varied between 10.41% and 13.51% for the extraction with water acidified with hydrochloric acid and between 15.83% and 21.24% when citric acid was used. The fact that citric acid is

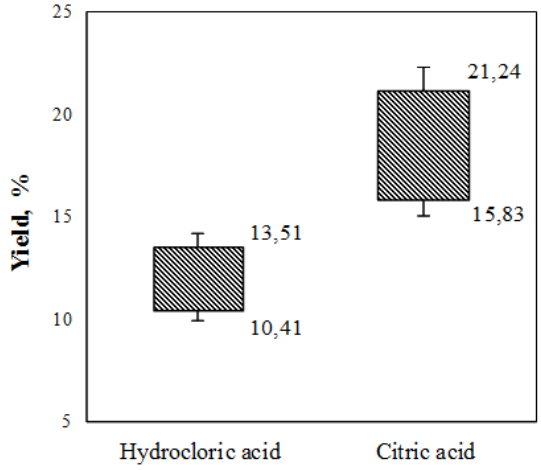

Fig. 1 Influence of the type of acid on pectin yield

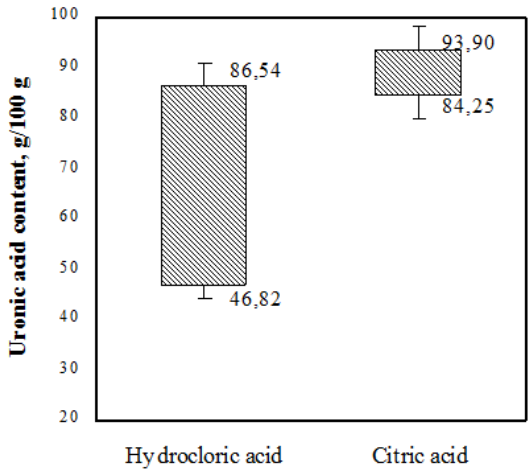

Fig. 2 Influence of the type of acid on the content of uronic acid

more effective in the extraction of pectin was also confirmed by Virk and Sogi [19], who studied the extraction of pectin from apple (*Malus pumila* cv. Amri) peel waste with 0.02M hydrochloric acid, 0.05M hydrochloric acid, and 1% citric acid. Another pectin source for which a high extraction efficiency of citric acid was reported is passion fruit peel (*Passiflora edulis* flavicarpa) [20]. In this study, a comparison between the extraction of pectin with three different acids (citric, hydrochloric, and nitric acid) reached the conclusion that although a low pH is necessary to improve pectin yield, strong acid solutions could lead to highly soluble smaller pectin molecules due to partial hydrolysis. These pectin molecules may not be precipitated by the addition of alcohol [21], explaining why in this case the extraction using hydrochloric acid gave a pectin yield almost 2 times lower than that obtained with citric acid.

The effect of acid type on the uronic acid content of pectin is shown in Fig. 2. It can be observed that the use of citric acid determined a higher uronic acid content (84.25-93.90 g/100 g). On the other side,

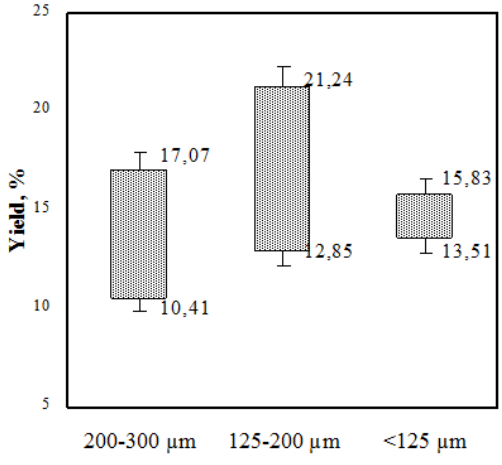

Fig. 3 Influence of particle size on pectin yield

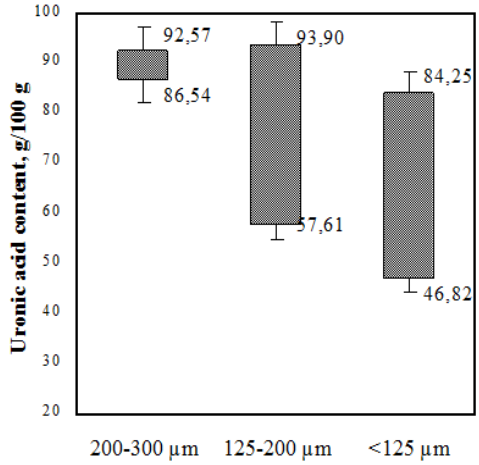

Fig. 4 Influence of particle size on the content of uronic acid

hydrochloric acid produced a larger variation of this chemical parameter, which ranged between 46.82 g/100 g and 86.54 g/100 g. Regarding the influence of acid type on the content of uronic acid, it was previously showed that the impact of a strong acid at high temperature seems to combine two simultaneous phenomena: firstly, the release of sugars as product of pectin hydrolysis, and secondly, their degradation under the action of both the acid and the heat [22]. Thus, citric acid most likely caused less degradation to the structure of pectin, contributing to a higher uronic acid content.

3.2. Effect of the particle size on pectin yield and purity

Properties of the plant material such as particle size, particle size distribution, and chemical composition have been extensively studied and are known to influence pectin extraction. Fig. 3 shows the influence of particle size on the extraction yield, and Fig. 4 indicates its impact on the uronic acid content of pectin. While both graphics depict a large influence of the particle size of apple pomace on pectin recovery, the most pronounced variation was observed for pectin yield. For all particle size intervals studied, the lower values (10.41%, 12.85%, and 13.51%) were obtained using hydrochloric acid. Citric acid extraction of pectin gave the highest yield (21.24%) when particle size varied between 125 and 200 μm. A similar conclusion was made in a previous study [23], where it was found that the highest average yields of pectin from apple pomace were obtained when the particles was larger than 106 μm and smaller than 250 μm.

In a similar manner to the variation of pectin yield, for all particle size intervals, the lower uronic acid contents (86.54 g/100 g, 57.61 g/100 g, and 46.82 g/100 g) resulted by using hydrochloric acid. Particle sizes of 200-300 μm determined a good purity of the extracted pectin (uronic acid content above 86 g/100 g), independent of acid type; in the case of particle size intervals of 125-200 μm and <125 μm, the purity of pectin was strongly effected by acid type. Whereas citric acid extraction gave good results in terms of pectin purity, the uronic acid content of pectin extracted with hydrochloric acid decreased with the reduction of particle size. The lower purity and yields may be attributed to acid strength and also to the fact that particles that are too small can hinder solvent flow through the matrix due to surface tension of the solvent employed [24].

4. Conclusion

Considering the high yield of pectin extracted from *Malus domestica* 'Fălticeni' pomace and its quality, i.e. the high uronic acid content, it can be concluded that this apple variety is a viable source of pectin. The best results for pectin recovery (extraction yield of 21.24% and uronic acid content of 93.90 g/100g) were obtained using citric and apple pomace with particle sizes of 125-200 μm. This statement is in agreement with previous studies in which it was proved that citric acid is a good alternative to the mineral acids usually used in pectin extraction. Further research on this pectin source could be focused on the influence of other extraction parameters, the application of non-conventional extraction techniques and the physico-chemical properties of pectin.

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