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OPTIMIZATION OF SEQUENTIAL ULTRASOUND-ASSISTED EXTRACTION – HEATING TREATMENT TO OBTAIN PECTIN FROM *MALUS DOMESTICA* 'FĂLTICENI' POMACE

***Florina DRANCA[1], Mircea OROIAN [1]**
[1]*Faculty of Food Engineering, Stefan cel Mare University,*
Suceava, Romania, florina.dranca@usm.ro,
*Corresponding author
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Abstract: *In this study, a sequential ultrasound-assisted extraction – heating treatment (UAEH) was applied for pectin extraction from Malus domestica 'Fălticeni' pomace. The extraction procedure involved a sonication at 20 kHz and 100% amplitude for 30 min (solid-to liquid ratio of the apple pomace-water mixture of 1:10, pH of 1.8), followed by a heating treatment at different temperatures (70, 80 or 90 °C) and time (60, 120 or 180 min). It was observed that with the increase of temperature and time, pectin yield and the degree of esterification increased. At the same time, the increase of temperature determined a decrease of the galacturonic acid content of the extracted pectin. A Box-Behnken response surface design was used to optimize the effects of process variables on the pectin yield and physico-chemical properties. The optimum conditions to obtain a maximum yield of 6.85%, with a 69.694 g/100 g galacturonic acid content and 80.09% degree of esterification of the extracted pectin were the temperature of 85.71 ºC and 147 min heating time.*

Keywords: *ultrasound, heating, pectin yield, composition.*

1. Introduction

Pectin is a complex and heterogeneous polysaccharide molecule found in the middle lamella and primary cell walls of higher plants [1]. Structurally, pectin is a polymer composed of at least 17 different monosaccharides interconnected through more than 20 different linkages, having a backbone that consists primarily of a linear chain of α-(1→4)-*D*-galacturonic acid (GalA) units interrupted by occasional *L*-rhamnose (*L*-Rha) residues [2]. The major pectic substructures are homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). The HG substructure has a backbone formed of *D*-GalA units which may be methyl esterified at C-6 and/or O-acetylated at O-2 and/or O-3 [3]. RG-I, the second major substructure, is composed of a repeating [→2)-α-*L*-Rha-(1→4)-α-*D*-GalA-(1→] disaccharide in which the *L*-Rha residues can be substituted and may vary among plants [4]. RG-II, a very complex branched substructure occurring with much less frequency than RG-I, has highly conserved side chains containing different sugars and derivatives [5]. Pectin structure is described as consisting of a HG backbone that has RG-I and RG-II attachments, however, recent direct visualization of the molecular structure of the polysaccharide contributed to a new alternative structure characterized by the presence of HG as side chains of RG-I [6].

Commercially, pectin is extracted from two main sources, namely citrus peel and apple pomace. The sources for pectin extraction are the focus of numerous recent studies that investigated other agriculture by-products and wastes containing important

amounts of pectic polysaccharides. Among waste streams from fruits and vegetables processing, sugar beet pulp, berry pomace, potato pulp, cocoa husks, mulberry branch bark, faba bean hulls, pistachio green hulls, pomegranate peels, pumpkin, banana peel and mango peel were studied [7]. Apart from achieving waste valorization, these other sources can also provide pectins with diverse structures and functional properties.

The utilization of a suitable method for pectin extraction is necessary in order to maximize the extraction yield and obtain a good product quality. The methods applied to extract pectin from different plant sources can be distinguished by the approach taken: on one side, conventional techniques such as extraction with mineral acids offer the advantage of an increased pectin yield, and on the other side several innovative techniques bring advantages such as the shorter extraction time, lower energy consumption, and therefore reduced impact on the environment. The innovative extraction techniques are microwave-, ultrasound-, enzyme-assisted extraction, subcritical water extraction, and combined techniques such as ultrasound-microwave assisted extraction, ultrasound-assisted heating extraction and enzymatic-ultrasonic extraction.

This study aimed at evaluating the use of a sequential ultrasound-assisted extraction – heating treatment (UAEH) process to isolate pectin from apple (*Malus domestica* 'Fălticeni') pomace. The extraction of pectin from this plant source provides a mean to increase the economic viability of *Malus domestica* 'Fălticeni', an apple hybrid that is native to the geographical area of Fălticeni, Suceava County (Romania), where it is primarily cultivated and also processed into juice in small scale plants. The effects of the combined extraction technique were evaluated in terms of pectin yield and galacturonic acid content and degree of esterification of the extracted pectin.

2. Materials and methods

2.1. Materials

Apple pomace was obtained after the extraction of juice from *Malus domestica 'Fălticeni'* apples. The apples were from 2016 harvest, and were cultivated in the Fălticeni area of Suceava, Romania. The pomace was dried in an oven with air circulation at 60 °C to a constant weight then powdered in a food processor. The resulting powder was passed through an analytical sieve shaker Retsch AS 200 (Retsch GmbH, Germany), and pomace with 125-200 µm particle sizes was used in the extraction process.

Citric acid, ethyl alcohol, sulfamic acid, potassium hydroxide, sulfuric acid, sodium tetraborate, sodium hydroxide, hydrochloric acid, *D*-galacturonic acid, and *m*-hydroxydiphenyl were purchased from Merck KGaA (Germany).

2.2. Extraction and purification procedure

The extraction mixture was prepared by mixing 10 g of apple pomace powder with 100 ml of distilled water acidified to a pH of 1.8 with citric acid (solid-to-liquid ratio of 1:10, w/v). The ultrasound-assisted extraction (UAE) was performed with an ultrasonic device (Sonopuls HD 2070, Bandelin, Germany), operated at 20 kHz, and equipped with a flat tip probe (KE 76, Bandelin, Germany) that was submerged at 15 mm depth into the extraction mixture. Sonication was carried out for 30 min at 100% amplitude. Following the UAE, the samples were placed in a water bath and exposed to a heating treatment in the following conditions: temperature of 70, 80 or 90 °C and time of 60, 120 or 180 min (Table 1).

After the sequential ultrasound-assisted extraction – heating treatment (UAEH), the mixture was cooled to room temperature prior to precipitation and purification. The

extract was first separated from the remaining solid material by centrifugation at 4000 rpm for 40 min, after that it was passed through a 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) to precipitate pectin. The bottles were tightly closed, and then the content was thoroughly mixed and kept at 4-6 °C for 12 h to complete the precipitation. Next, pectin was separated from the liquid by centrifugation (4000 rpm, 30 min), was washed 3 times by ethyl alcohol, and dried in a hot air oven at 50 °C to a constant weight.

2.3. Determination of pectin yield

Pectin yield was calculated using Eq. (1):

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

Where: m_0 – weight of dried pectin (g) and m – weight of dried apple pomace powder (g) [8].

2.4. Determination of galacturonic acid content

The galacturonic acid content (GalA) of pectin was determined in triplicate by the sulfamate/*m*-hydroxydiphenyl method developed by Filisetti-Cozzi and Carpita [9]. Sample preparation was made according to [10], as follows: 20 mg of dry pectin were added to 50 ml of distilled water (at 40 ºC) and mixed using a magnetic stirrer until completely dispersed. After that, the volume was adjusted to 100 ml with distilled water at 40 ºC.

Aliquots of 400 µl from the pectin solutions were placed in glass tubes, to which 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. The mixture was hydrolyzed in a 100 ºC water bath 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 the content was 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). For each batch of samples, a calibration curve of *D*-galacturonic acid was performed.

2.5. Determination of degree of esterification

The degree of esterification (DE) of pectin was determined in triplicate by the titrimetric method described by Franchi et al. [11], as follows: 50 mg of pectin were dissolved in 10 ml of boiled distilled water, then the resulting solution was titrated with 0.1 N NaOH using phenolphthalein as indicator; the volume of sodium hydroxide used for titration, V_1, was recorded. After titration, 20 ml of 0.5 M NaOH were added and the solution was kept under continuous stirring at 400 rpm for 30 min to achieve saponification. Then, 20 ml of 0.5 M HCl were added to neutralize the solution and a final titration with NaOH was made (V_2). The degree of esterification was calculated using Eq. (2):

$$DE\ (\%) = \frac{V_2}{V_1 + V_2} \times 100 \quad (2)$$

Where: V_1 – volume of sodium hydroxide used for the first titration (ml) and V_2 – volume of sodium hydroxide used for the second titration (ml).

2.6. Experimental design and statistical analysis

A two factors, three levels Box-Behnken response surface experimental design (BBD), with two repetitions at the center points, was employed to investigate the individual and interactive effects of process variables – temperature (X_1) and time (X_2) – on pectin yield and its galacturonic acid content and degree of esterification. The independent variables and their coded

levels are presented in Table 1. Experimental data was analyzed using Design Expert 11 (trial version) (State-Ease Inc., Minneapolis, USA).

3. Results and discussion

3.1. Model fitting and statistical analysis

The experimental results of UAEH extraction of pectin from *Malus domestica* 'Fălticeni' apple pomace using two-factor-three level BBD are shown in Table 2. Analysis of variance (ANOVA) was used to examine the statistical significance of the model terms and the results are listed in Table 3. The model F-values and the associated low *P*-values ($P < 0.05$) implies the developed model was significant and indicates that most of the variation in the response can be explained by the regression equation. The high value of R^2 clearly stated that the quadratic model can explain and predict most of the variation of pectin yield (R^2=0.9591), galacturonic acid content (R^2=0.9317) and degree of esterification (R^2=0.9263). The square polynomial equations that describe the combined effect of temperature (X_1) and time (X_2) on the extraction yield (Eq. (3)), galacturonic acid content (Eq. (4)) and degree of esterification (Eq. (5)) are shown below:

$$Y\ (\%) = 4.073 + 2.445 \times X_1 + 1.134 \times X_2 + 0.886 \times X_1 \times X_2 + 2.008 \times X_1^2 + 0.018 \times X_2^2 \quad (3)$$

$$GalA\ (g/100\ g) = 71.218 - 4.6 \times X_1 + 2.178 \times X_2 + 5.547 \times X_1 \times X_2 - 0.925 \times X_1^2 - 4.9 \times X_2^2 \quad (4)$$

$$DE\ (\%) = 89.95 - 5.36 \times X_1 - 4.011 \times X_2 - 2.615 \times X_1 \times X_2 - 7.4 \times X_1^2 + 0.135 \times X_2^2 \quad (5)$$

Table 1. Independent variables and levels used for Box-Behnken design

Variables	Levels		
	-1	0	1
X_1: Temperature, ºC	70	80	90
X_2: Time, min	60	120	180

Table 2. Box-Behnken design with experimental and predicted values

Run	Independent variables		Measured response			Predicted response		
	X_1	X_2	Y (%)	GalA (g/100 g)	DE (%)	Y (%)	GalA (g/100 g)	DE (%)
1	80	120	3.49	70.55	90.65	4.07	71.22	89.95
2	80	180	5.87	68.88	88.56	5.23	68.50	86.07
3	70	180	3.54	65.09	85.49	3.90	66.63	86.65
4	70	60	3.10	71.55	91.47	3.41	73.37	89.44
5	70	120	4.31	78.26	87.04	3.64	74.90	87.91
6	90	60	6.31	53.93	85.81	6.52	53.06	83.95
7	80	60	3.48	65.08	90.21	2.96	64.14	94.10
8	80	120	3.49	70.55	90.65	4.07	71.22	89.95
9	90	180	10.30	69.66	69.37	10.57	68.51	70.70
10	90	120	9.01	70.55	76.66	8.53	71.22	77.19

X_1 – temperature, X_2 – time;
Y – pectin yield, *GalA* – galacturonic acid content, *DE* – degree of esterification.

Table 3. Analysis of variance (ANOVA) of quadratic model

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*P*-value
(A) Pectin yield, %					
Model	56.47	5	11.29	18.77	0.0070
Temperature	35.89	1	35.89	59.66	0.0015
Time	7.73	1	7.73	12.85	0.0231
Temperature × time	3.15	1	3.15	5.23	0.0842
R^2			0.9591		
Adjusted R^2			0.9080		
(B) Galacturonic acid content, g/100 g					
Model	342.27	5	68.45	10.92	0.0190
Temperature	127.39	1	127.39	20.32	0.0108
Time	28.47	1	28.47	4.54	0.1001
Temperature × time	123.08	1	123.08	19.63	0.0114
R^2			0.9317		
Adjusted R^2			0.8464		
(C) Degree of esterification, %					
Model	426.96	5	85.39	10.05	0.0220
Temperature	172.38	1	172.38	20.29	0.0108
Time	96.56	1	96.56	11.37	0.0280
Temperature × time	27.35	1	27.35	3.22	0.1472
R^2			0.9263		
Adjusted R^2			0.8341		

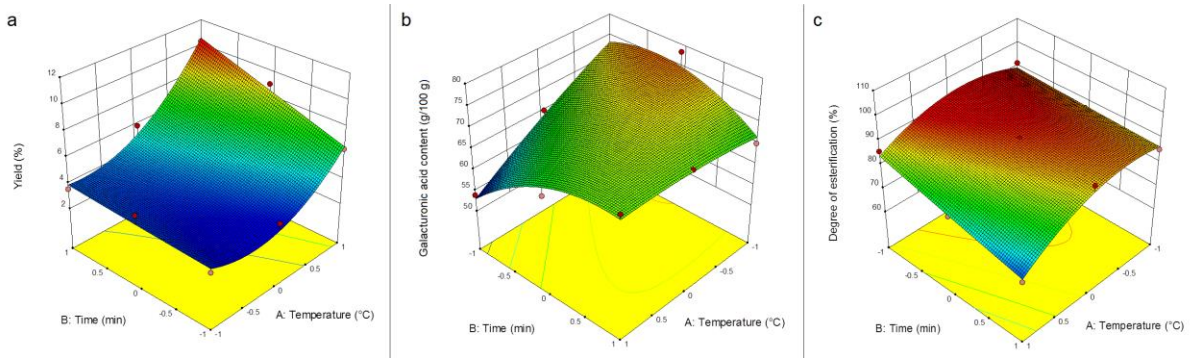

Fig. 1. Response surface plots showing the effect of process variables on pectin yield (a), galacturonic acid content (b) and degree of esterification (c)

3.2. Effect of process variables

According to the data presented in Table 2, pectin yield varied between a minimum value of 3.10% (temperature of 70 ºC, 60 min heating time) and a maximum of 10.30% (temperature of 90 ºC, 180 min heating time). Fig. 1a shows that an increase of temperature and time led to a substantial increase of pectin yield; this increase was more pronounced when the temperature was higher than 80 ºC. Both process variables had a significant influence on yield ($P < 0.05$), however, the interaction between them did not significantly influence this parameter ($P > 0.05$), as indicated by the results of the analysis of variance (Table 3). Some previous studies had a similar observation

regarding the positive effects of high temperatures and longer exposure to heating on pectin extraction yield from pomegranate peel [12], banana peel [13], apple pomace [14], and lemon by-product [15].

The galacturonic acid content (Fig. 1b) of the extracted pectin, which had values between 53.93 g/100 g (temperature of 90 ºC, 60 min heating time) and 78.26 g/100 g (temperature of 70 ºC, 120 min heating time), was significantly influenced by temperature and the temperature-time interaction ($P < 0.05$), as seen in Table 3. The evolution of this parameter was opposite to that reported by previous studies, where the increase of GalA at high temperatures was associated with a substantial hydrolysis of pectin neutral sugars found in the rhamnogalacturonic regions [12,16]. In the case of this study, the heating treatment applied after UAE might have determined some degradation of pectin structure, which was more pronounced at the highest temperature and the shortest heating time.

The evolution of the degree of esterification presented in Fig. 1c shows that higher extraction temperature and prolonged time led to a decrease of this parameter to 69.37% (temperature of 90 ºC, 180 min heating time, Table 2), while lower temperature and heating time was correlated with an increased DE (91.47% at a temperature of 70 ºC, 60 min heating time, Table 2). A similar observation was made regarding the DE of pectin extraction from pomegranate peel [12] and banana peel [13], and was ascribed to the contributing effect of harsher extraction conditions on the deesterification of the polygalacturonic acid chain [17]. However, it is possible that harsher treatments may also extract more strongly bound pectins, possibly of lower degree of esterification, as stated previously [18]. Overall, all extracted pectin samples were of high DE and this psychochemical parameter was significantly influenced ($P < 0.05$) by temperature and time, but not by the interaction of these two process variables ($P > 0.05$).

3.3. Optimization of extraction parameters

The objective of optimization was to find out the UAEH conditions which give the maximum extraction yield of pectin and the highest galacturonic acid content and degree of esterification. The optimum extraction conditions were a temperature of 85.71 ºC and time of 147 min, and the maximum yield of pectin achievable in these conditions was 6.85%, with a 69.694 g/100 g GalA and DE of 80.09%.

4. Conclusion

In this study, sequential ultrasound-assisted extraction – heating treatment was optimized for the extraction of pectin from *Malus domestica* 'Fălticeni' pomace. Two factors at three levels Box-Behnken response surface experimental design were successfully used to optimize and study the individual and interactive effect of process variables such as temperature and time on the extraction yield of pectin and its galacturonic acid content and degree of esterification. From the experimental results, the change of temperature was found to influence both the extraction yield and the physico-chemical properties of pectin, while the variation of time strongly affected pectin yield and its degree of esterification. For the optimum extraction conditions (85.71 ºC, 147 min) it was estimated a maximum pectin yield obtained by UAEH of 6.85%, with 69.694 g/100 g GalA and DE of 80.09%.

5. Acknowledgement

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6. References

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