Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XX, Issue 4 - 2021, pag. 314 - 321

FOOD AND ENVIRONMENT SAFETY

EXTRACTION TEMPERATURE AND pH AS DECISIVE FACTORS FOR THE YIELD AND PURITY OF GRAPE POMACE PECTIN

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Received 15th October 2021, accepted 16th December 2021

Abstract: *The purpose of this paper is the use of grape pomaces, obtained by processing two different Vitis vinifera varieties (Rară Neagră and Fetească Neagră) from 2019 harvest, cultivated in the Bugeac area, Republic of Moldova, as a source of unconventional pectin. Pectin was extracted under the following conditions, citric acid used as extractant for 3 h of extraction, 125-200 µm of grape pomace particle size and solid to liquid ratio 1:10. The variables which were varied were temperature (70, 80 and 90 °C) and pH (1, 2 and 3). So, pectin yield and galacturonic acid content were determined under the influence of extraction temperature and pH. The obtained results demonstrated that the extraction conditions (temperature and pH) have a significant impact on the yield and purity of pectin from grape pomace. The highest influence on pectin yield was determined by pH 1 (15.54% and 9.96% for Fetească and Rară Neagră grape pomace pectin, respectively) and temperature of 90 °C (7.34% and 7.56% for Fetească and Rară Neagră grape pomace pectin, respectively). The same tendency is observed for galacturonic acid content.*

Keywords: *grape pomace, galacturonic acid, pectin recovery, citric acid extraction.*

1. Introduction

Pectin is the third group of complex polysaccharides which constitute a part of higher plant wall characterized by relatively extractability using different type of acids (mineral or organic) or chelators (ammonium oxalate, sodium citrate etc.) and a high content of galacturonic acid [1–3]. Pectin is found in the middle lamella of the cell wall (CW), with a gradual reduction from the primary CW to the plasma membrane [4]. The structure and firmness of the plant CW depend on the mechanical properties, orientation and link between cellulose and pectic substances [5]. Although, some pectin molecules are linked to xyloglucan chains of the CW [6]. Pectin accomplishes two different functions, as a thickening element on the CW and as a „cementing" component in the middle lamella of plant CW [7]. Pectin is extracted from fruits, vegetables and their processing by-products (peel, pomace or seeds) [8,9]. Presently, the main sources of commercially pectin are citrus peels and apple pomace which are suitable for specific applications in food industry [4,10,11]. Due to source of pectin, extraction conditions (time, pH, temperature, solid to liquid ratio, acid type etc.) and technique (conventional and unconventional extraction), the pectin has different ability to form gels [8]. Chen et al. [12] established that extraction parameters influence the pectin structure and properties. Moreover, the temperature is a decisive factor [12]. The low temperature kept the pectin structure more intact, remaining more neutral sugars (arabinose, fucose, glucose, galactose, mannose etc.) and possibly more close to the initial molecule in the CW [12]. The pH is considered as one of the most sensitive parameter to note while extracting pectin [13,14]. Commonly, low

DOI: https://doi.org/10.4316/fens.2021.034

acidic pH is essential for the hydrolysis of protopectin [14,15]. A compromise is often made between having a poor quality of pectin, but with higher yield at a lower pH and having a better quality of extracted pectin, but with low yield at a high pH; this trade-off is made between temperature and extraction time [14]. Mainly, the reported values for pH range from 1 to 3, but optimal pH value used for pectin extraction is 2 [4].

Due to the lack of information about how extraction factors influence grape pomace pectin yield and structure, we aimed to study the impact of pH and temperature on the yield and purity of pectin.

2. Materials and methods

2.1. Materials

Grape pomace was obtained by processing two different *Vitis vinifera* varieties (Rară Neagră and Fetească Neagră) from 2019 harvest, cultivated in the Bugeac area, Republic of Moldova. The grape pomace was dried at 50 °C until constant weight was achieved. The moisture content of dried grape pomace varied between 0.11% and 0.35%. Then, dried pomace was powdered and separated it on the particle size interval of 125-200 µm using an analytical sieve shaker Retsch AS 200 Basic (Retsch GmbH, Haan, Germany).

2.2. Extraction and purification of pectin

Initially, a sample of 10 g grape pomace powder was mixed with 100 mL of solvent (solid-liquid ratio of 1:10, w/v) acquired by adding citric acid to ultrapure (Milli-Q) water until a pH 1, 2 and 3 were achieved. Then, the mixtures were kept in a water bath Precisdig (JP Selecta, Barcelona, Spain) at the different temperature (70, 80 and 90 °C) for 3 h.

After extraction, the mixtures were cooled to room temperature, around 20-22 °C. Firstly, the pectin was segregated by centrifugation at 3500 rpm for 35 min. Then, the obtained supernatants were got through clean strainer, plased into the neck of a Duran® laboratory glass bottle with pouring ring and screw cap. Afterwards, ethyl alcohol (>96%, v/v) was added to supernatants in order to achieve 1:1 ratio (v/v). The mixtures were kept at 4-6 °C for 12 h to accomplish the precipitation. The precipitated pectin was separated by centrifugation at 4000 rpm for 30 min. The pectin was washed 3 times with ethyl alcohol (>96%, v/v) and dried in an oven with air circulation Zhicheng ZRD-A5055 (Zhicheng, Shanghai, China) at 50 °C until constant weight was achieved.

2.3. Pectin yield

Pectin yield was calculated using Eq. (1):

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

where: m_0 – weight of dried pectin (g), m – weight of dried grape pomace powder (g) [16,17].

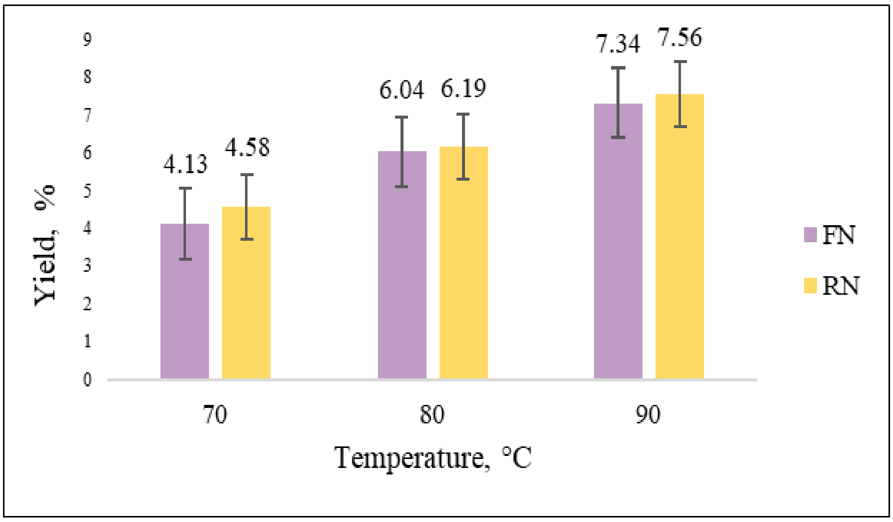
2.4. Galacturonic acid content

The galacturonic acid content (GalA) of pectin was estimated using the sulfamate/m-hydroxydiphenyl method developed by Filisetti-Cozzi & Carpita [18,19]. Sample preparation was made according to Miceli-Garcia [20].

3. Results and discussion

3.1. Effect of temperature on the pectin yield and galacturonic acid content

In agreement with the results in Fig. 1, yield of pectin was significantly influenced by extraction temperature. It is possible to



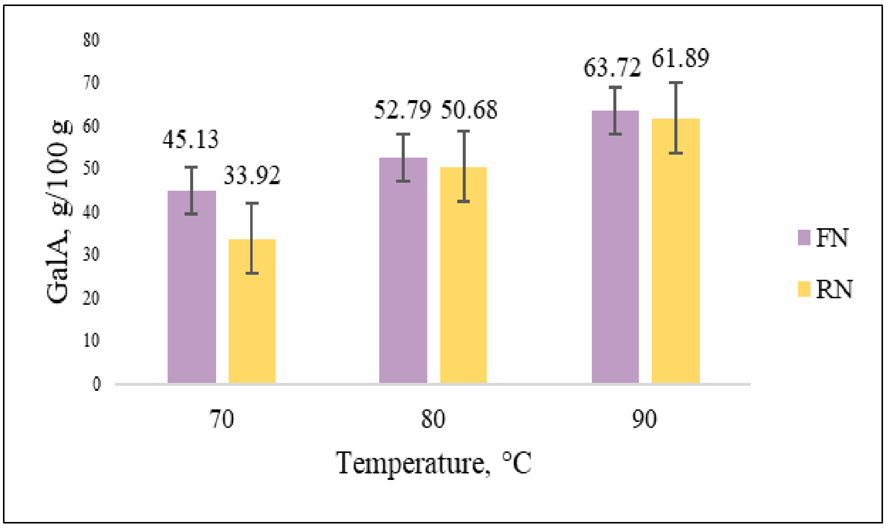
* FN – Fetească Neagră, RN – Rară Neagră. Extraction conditions – pH 2, extraction time of 3 h and 125-200 µm of particle size.

Fig. 1 Effect of the temperature on the pectin yield

observe that yield varied between 4.13% and 7.34% for Fetească Neagră (FN) grape pomace pectin and between 4.58% and 7.56% for Rară Neagră (RN) grape pomace pectin depending on the extraction temperature. The obtained results showed that temperature has a major impact on the yield of pectin. A rising temperature from 50 to 95 °C utilizing citric acid at pH 2.5 or 4.0 significantly enhanced the yield of cocoa husks pectin, from 3.58% to 5.66% (extraction temperature of 50 °C and 95 °C, respectively at pH 2.5) and from 3.72% to (extraction temperature of 50 °C and 95 °C, respectively at pH 4.0) [21]. Also, Vriesmann et al. [22], Méndez et al. [23] and Gutöhrlein [24] demonstrated that increasing temperature significantly enhanced the pectin yield from cocoa husks by citric acid, watermelon rind waste by hydrochloric acid and pea hulls by citric acid, respectively. The heated acid solution facilitated to solubilize the pectin and other pectic compounds retained in the CW of plants (protopectin), thus increased of the pectin yield [21,25]. Furthermore, a low value of temperature may be inadequate to allow the protopectin hydrolysis (water-insoluble pectic substance) by different acids, thereby achieving a lower pectin yield [21].

The influence of the temperature on the galacturonic acid content (GalA) is presented in Fig. 2. Therefore, temperature influenced the GalA of grape pomace pectin from both grape pomace varities (FN and RN). The GalA varied from 45.13 g/100 g to 63.72 g/100 g and from 33.92 g/100 g to 61.89 g/100 g for pectin of FN and RN grape pomace, respectively. This is in accordance with the research of Chan & Choo [21] who stated that the uronic acid content of cocoa husks pectin where higher at 95 °C than at 50 °C when was extracted with citric acid at different pH (2.5 or 4.0). Also, Garna et al. [26] demonstrated that 90 °C contributes to a higher GalA obtained from apple pectin then 80 °C, with a sulphuric acid solution at pH 1.5 or 2.0. These results may be ascribed to the increased chemical hydrolysis of pectin sugars when temperature significantly enhances [27–29]. Chaharbaghi et al. [30], who analyzed the optimization of pectin extraction from pistachio hull, noted that the pectin yield

was enhanced with an increase in extraction temperature.
Thus, they obtained the highest yield of pectin from pistachio hull (23.42%), under the following conditions: pH 0.5, extraction time of 30 min, temperature of 90 °C and a solid to liquid ratio of 50 v/w [30].



* FN – Fetească Neagră, RN – Rară Neagră, GalA – galacturonic acid content. Extraction conditions – pH 2, extraction time of 3 h and 125-200 µm of particle size.

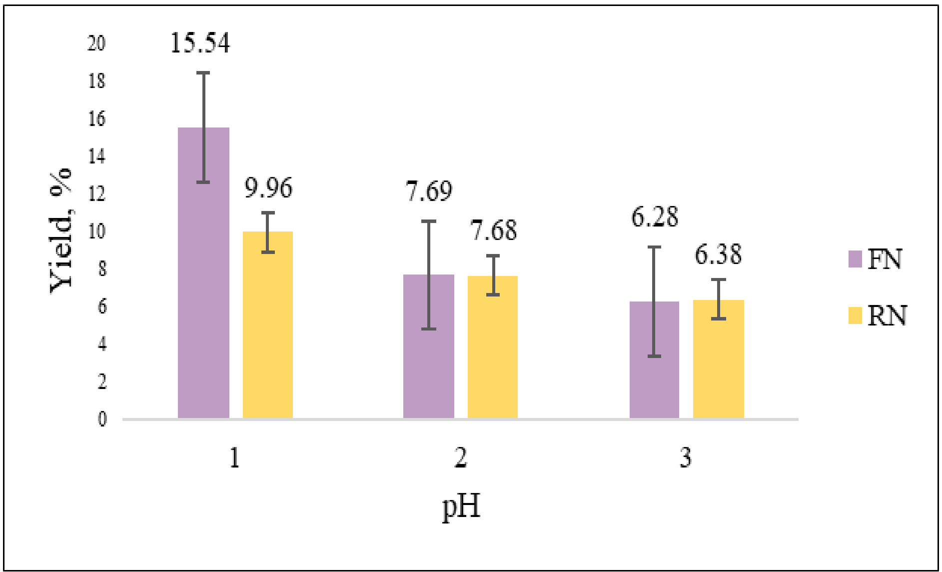
Fig. 2 Effect of the temperature on the galacturonic acid content

A higher temperature increased the yield of pectin due to diffusion of solvent into the structure of CW and so, this movement caused the increase of the mass of polysaccharides releasing from the CW's particles into the acid solution [30]. Further, these conclusions are related to the research data obtained from waste durian rinds and sour orange peel pectin by Maran [31] and Hosseini et al. [32], respectively.

3.2. Effect of pH on the pectin yield and galacturonic acid content

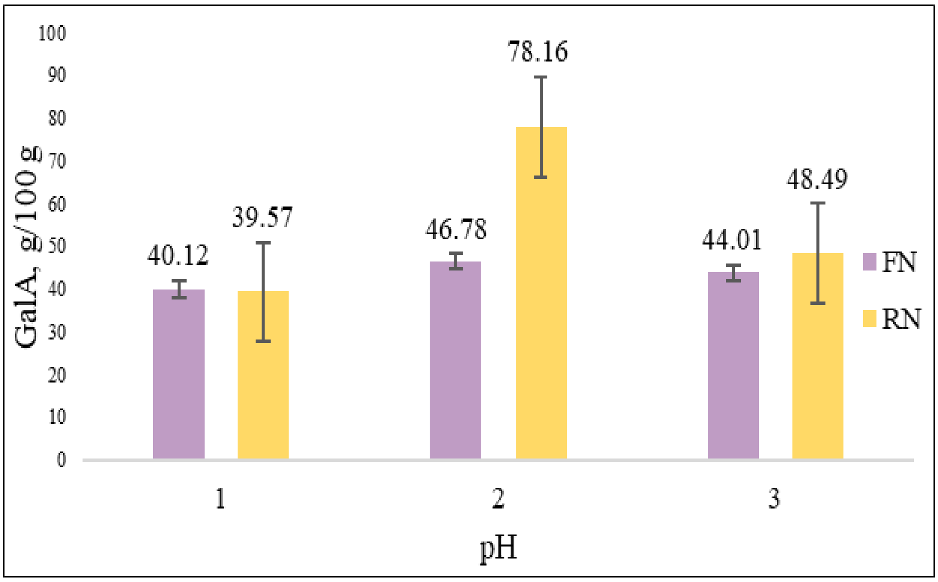
Stronger acid conditions (pH of about 2) play an essential role in influencing the structure and properties of pectin [33]. Fig. 3 presents the influence of pH on the pectin yield, and Fig. 4 illustrates its impact on the galacturonic acid content of pectin. The yield varied between 6.28% and 15.54% for FN grape pomace pectin and between 6.38% and 9.96% for RN grape pomace pectin. Thus, the highest influence on pectin yield was determined by pH 1 (15.54% and 9.96% for FN and RN grape pomace pectin, respectively). These results also are in line with the research of Chaharbaghi et al. [30], who studied the extraction optimization of pectin from pistachio hull and obtained the highest pectin yield (23.42%) at pH 0.5. Furthermore, Ma et al. [34] examined the influence of extraction factors on the sugar beet pulp pectin, and noted that decrease in pH increased the pectin yield. This is possibly as a result of CW's lysis in strong acidic solutions and thus, dissolve and release the pectin in acid extract [34]. Also, Raji et al. [35] obtained the maximum extraction yield of pectin from melon peel (29.48%) under pH 1, temperature of 90 °C after 200 min of citric acid extraction.
The GalA is a great indicator of pectin purity, thus that a direct relation between

this factor and purity of extracted pectin was occured [30,36]. Moreover, according to FAO/WHO the GalA of pectin should not be less than 65% [37]. In current study, the data achieved from used citric acid extraction of pectin showed a GalA range of 40.12-46.78 g/100 g and 39.57-78.16 g/100 g for FN and RN grape pomace pectin, respectively (Fig. 4). The highest value of GalA (78.16 g/100 g) was achieved for RN grape pomace pectin under the following conditions: pH 2, 90 °C, extraction time of 3 h and 125-200 µm of particle size. Also, this result is nearly related to data acquired from sugar beet pulp (78.8 g/100 g) at pH 2 of citric acid solution, for 2 h of extraction by Ma et al. [34].



* FN – Fetească Neagră, RN – Rară Neagră. Extraction conditions – temperature of 90 °C, extraction time of 3 h and 125-200 µm of particle size.

Fig. 3 Effect of the pH on the pectin yield



* FN – Fetească Neagră, RN – Rară Neagră, GalA – galacturonic acid content. Extraction conditions – temperature of 90 °C, extraction time of 3 h and 125-200 µm of particle size.

Fig. 4 Effect of the pH on the galacturonic acid content

Furthermore, Yapo [38] presented similar results for GalA (70.6%, w/w) extracted from pectin of yellow passion fruit rind under citric acid extraction at pH 1.8, for 60 min at temperature of 75 °C.

The obtained data confirmed that pH as well as temperature can influence the yield and GalA of extracted pectin from grape pomace.

4. Conclusion

The extraction conditions (temperature and pH) have a significant impact on the yield and purity of pectin from grape pomace. Considering the high yield of pectin and its purity, it can be assured that grape pomace is an unconventional source of pectin.
The highest influence on pectin yield was determined by pH 1 (15.54% and 9.96% for FN and RN grape pomace pectin, respectively) and temperature of 90 °C (7.34% and 7.56% for FN and RN grape pomace pectin, respectively). Moreover, these results are in accordance with previous studies based on unconventional sources of pectin extraction. Additional study about grape pomace pectin should be conducted to the influence of other extraction conditions with application of different extraction techniques.

5. Acknowledgments

This paper has been financially supported within the project entitled "DECIDE – Development through entrepreneurial education and innovative doctoral and postdoctoral research, project code POCU/380/6/13/125031, supported by project co-financed from the European Social Fund through the 2014-2020 Operational Program Human Capital".

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