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Selecting optimal parameters for obtaining the extract of red grape pomace

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Selección de parámetros óptimos para la obtención de extracto de uva roja

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ABSTRACT

Keywords:
Anthocyanins
Antioxidant activity
Extraction parameters
Flavonoids

Due to the industrial processing of grapes, large amounts of by-products are produced. The main varieties of by-products are pomace, which is comprised of skins, seeds and any other solid remaining after pressing process and sediments. It is necessary to implement new effective ways of processing to minimize these residues. This problem is relevant for all the wine-producing countries, including Georgia. It is well-known fact that pomace is an important source of phenolic compounds, which are characterized by high antioxidant activity and possess healing-prophylactic properties. It is also worth mentioning that pomace is an easily spoiled product, and without the proper processing, it cannot be stored for a long time. Thus, this research aimed to obtain optimal parameters for extraction, preserving the antioxidant characteristics. The optimal range of the following parameters for extraction was determined: the temperature for drying 45-50 °C, grinding level 1.5 mm, diluent concentration 70% ethanol/water solvent, extraction module 1:20, extraction temperature 50-55 °C, and duration 2 h. This determination of technological parameters of extraction was done according to the best physical-chemical measures and antioxidant activity level. The physical-chemical tests were performed according to the European Union standards. These parameters can produce an extract with distinct antioxidant characteristics that can be used in the food industry as a natural antioxidant. The extract with distinct antioxidant properties was obtained, which can be used in the food industry as a natural antioxidant.

RESUMEN

Palabras clave:
Antocianinas
Actividad antioxidante
Parámetros de extracción
Flavonoides

Debido al procesamiento industrial de la uva, se producen grandes cantidades de subproductos. Las principales variedades de subproductos son el orujo, que se compone de cáscara, semillas y cualquier otro sólido que quede después del proceso de prensado y sedimentos. Es necesario implementar nuevas formas efectivas de procesamiento para minimizar estos residuos. Este problema es relevante para todos los países productores de vino, incluido Georgia. Es bien sabido que el orujo es una fuente importante de compuestos fenólicos, que se caracterizan por una alta actividad antioxidante y poseen propiedades curativas-profilácticas. También vale la pena mencionar que el orujo es un producto que se estropea fácilmente y, sin el procesamiento adecuado, no se puede almacenar durante mucho tiempo. Por lo tanto, esta investigación tuvo como objetivo obtener los parámetros óptimos de extracción, conservando las características antioxidantes. Se determinó el rango óptimo de los siguientes parámetros para la extracción: temperatura de secado 45-50 °C, nivel de molienda 1,5 mm, concentración de diluyente 70% etanol/agua solvente, módulo de extracción 1:20, temperatura de extracción 50-55 °C y duración 2 h. Esta determinación de los parámetros tecnológicos de extracción se realizó de acuerdo con las mejores medidas físico-químicas y nivel de actividad antioxidante. Las pruebas físico-químicas se realizaron de acuerdo con los estándares de la Unión Europea. Se obtuvo un extracto con altas propiedades antioxidantes, que puede ser utilizado en la industria alimentaria como antioxidante natural.

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By-products of winemaking might remain underutilized, causing the loss of large amounts of wholesome substances. Extracts from these residues can be used in food technology to increase the antioxidant levels of the product (García-Lomillo and González-San José, 2016). The chemical composition and antioxidant characteristics of those extracts depend on correctly choosing the extraction parameters (Pazir *et al*., 2020). In order to increase the shelf-life of products, antioxidants are used, which are usually synthetic due to their low price (Li *et al*., 2014). The impact of these kinds of antioxidants is not fully studied, but according to some research, synthetic antioxidants can not only harm the body but also provoke the development of serious illnesses e.g., allergic reactions induced by synthetic antioxidants in children are frequently. The risk group consists of people who previously had allergies to other substances. It can also trigger the development of oncological disease (Atta *et al*., 2017). Natural ingredients demand is continually increasing in the market, and the substitution of synthetic ingredients for natural analogs is a priority in the modern food industry (Mitterer-Daltoe *et al*., 2020).

The development of the food and the processing industry depends to a great extent on the rational use of natural resources of plant origin and the creation of new products (Knorr *et al*., 2020). When processing natural raw materials, maintaining the beneficial properties of the product is a crucial factor. This is closely linked to the optimization of technological processes and regimen of food processing (Jelley *et al*., 2016).

Interest in the by-products of wine production has increased tremendously. There are numerous studies about the usage of red grape processing by-products in the food industry, particularly pomace (Charalampia and Koutekidakis, 2016). This product has captured scientists' attention because of its high levels of biologically active compounds (Teixeira *et al*., 2014). Traditionally, pomace was used to produce food colorants and grape seed oil. Recent studies are directed to increase the beneficial characteristics and nutritional values by adding extracts of biologically active compounds in the foods (Iuga and Mironeasa, 2020). Pomace is usually utilized as an antioxidant, food coloring, and antimicrobial agent since it is rich in phenolic compounds (Luchian *et al*., 2019).

There are many extraction methods to produce an extract rich in new biologically active compounds from by-products; however, to maintain valuable components, an individual approach is needed since the preservation of the chemical content and the antioxidant characteristics depends on whether the extraction was carried out correctly or not. (Ju and Hovard, 2003).

In this context, this study aimed to determine optimal extraction parameters to produce an extract, which would be rich in antioxidants from pomace. In addition, this study shows an alternative to recycle wine waste and replace synthetic additives in food with natural ones, reducing environmental risk.

MATERIALS AND METHODS

Rare Georgian red grape varieties were used (Simonaseuli, Adreuli Shavi Sreluri, Gabasha, and also worldwide known Saferavi). The samples were taken from the Base of Permanent Crop Research Located in Jigaura, Georgia.

These samples are the secondary product of wine production. The temperature of the grape juice was 18 °C, alcoholic fermentation was carried out at 21-22 °C, in moisture content of 75-85%, the grape juice was stirred before and during the fermentation 4-5 times a day. Fermentation lasted 12 days and a maceration process of 5 days.

The pomace was removed after the completion of the alcoholic fermentation of the wine and pressed under a hydraulic press, resulting in moisture content of 37%. The grape pomace was stored at -20 °C. Before the experiment, it was defrosted at room temperature. The pomace samples used in the experiment are products obtained after alcoholic boiling. The determination of technological parameters of extraction was conducted according to the results obtained from the analysis conducted on the mixture of equal amounts of all the pomace samples (Luchian *et al.,* 2019).

Scientific research was conducted at Georgian Technical University, in the research laboratory of the Food Technology Department of Agrarian Sciences and Biosystems Engineering Faculty. Physical-chemical research was performed following the European Union Standards. The results given in the tables were obtained by calculating the average of the results attained from three test repeats. For the statistical processing of the test results was used MS Excel 2019.

One of the methods of preserving pomace is the drying process (Goula *et al*., 2016). In order to determine the optimal temperature, the drying process was conducted in the drying cabinet at different ranges of temperature: 45-50 °C, 95-100 °C, and 125-130 °C. After drying, chemical consistency (total phenols, flavonoids, anthocyanins, tannins) and antioxidant activity (ability to catch free radicals of DPPH 2.2'diphenil – 1 – picrylhydrazyl) of the pomace were observed (Kedare and Singh, 2011).

To determine the grinding level of pomace, the dried sample was ground in the laboratory mill to get the particle sizes of 0.5, 1, 1.5, 2 and 5 mm. Extraction was done via the retention method at 22 °C. The experiment was performed by using dried and ground raw material, which was placed in Erlenmeyer flasks, 20 g in each; 50% ethanol was added for the extraction and was stored for 24 h. After this process, the amount and antioxidant activity of extracted phenolic compounds were measured (Amendola *et al*., 2010).

To determine the extraction module, 50% ethanol with the ratios of 1:5; 1:10 and 1:20 were poured onto the pomace (raw material), which was ground into 1.5 mm particles and dried at 45-50 °C, and then, it was kept at 22 °C for 24 h. After this process, the amount and antioxidant activity of extracted phenolic compounds were measured.

One of the important factors in the extraction process is the nature of the solvent. The use of permissible extracts in the food industry creates the necessary conditions for the use of a solvent that is harmless to human health. There is a relationship between the nature of the extraction solvent and the antioxidant properties of grape pomace extracts (Yilmaz and Toledo, 2006). In order to identify the optimal diluent, a solvent with the ratio of 1:10 was poured onto the pomace (raw material), which was ground into 1.5 mm particles and dried at 45-50 °C. Usually, water, ethanol, and ethanol-water solutions at varying ratios were used as a solvent (water, ethanol, and 30%, 50%, 70% ethanol-water solutions). The mixture was kept at 22 °C for 24 h while being periodically stirred. After the extract was separated from raw material, the quantity and antioxidant activity of total phenols, flavonoids, anthocyanins and tannins were measured.

To determine the optimal extraction temperature, three variations of temperature were observed: 35-40 °C, 50-55 °C, 75-80 °C. As a diluent, 70% ethanol: water solution was used. The total amount of phenols, flavonoids, anthocyanins and tannins was measured in equal circumstances after retaining the mixture for 1 h.

To determine the extraction time, 70% ethanol was used. Extraction was conducted at the optimal temperature of 50-55 °C for 1, 2, 3, and 4 h. As in the previous procedures, the amount of extract obtained and antioxidant activity were measured.

RESULTS AND DISCUSSION

Khanal *et al.,* 2010 studied the effect of heating on the stability of grape and blueberry pomace phenolic compounds. Their study showed that heating at temperatures higher than 125 °C, the phenolic compounds suffer a considerable loss.

Lin and Chou (2008) studied effect of the heat treatment on total phenolic and anthocyanin contents of fermented black soybeans. The results of the study showed that 40-100 °C heating reduced the total phenolic and anthocyanin content. According to the results of the present study, it is posible to conclude that an increase in temperature has a negative effect on the content of phenolic compounds.

The chemical composition of the product was determined after drying (Table 1). Thermal processing does affect the chemical composition and oxidative activity of pomace. It also significantly increases the concentration of phenols, flavonoids, anthocyanins and tannins (Carmona-Jiménez *et al.,* 2018). This can be due to several factors: 1. The plant cells break down under the influence of high temperature, which makes easier the extraction of phenolic compounds and flavonoids. 2. As usual, phenolic compounds in plant cells are connected to sugars in form of glycosides. After affecting the cell with a high temperature, the glycoside connections are split, and the phenols are released from the cell. The phenols and flavonoids freed in the above-explained process maintain high antioxidant activity.

By thermal processing, pomace antioxidant activity was decreased compared to the raw pomace, which can be explained by the factor that, in addition to phenols and

Table 1. Phenolic compounds regarding the temperature of drying process of pomace.

Drying °C	Total Phenols g 100 g^{-1} dry material	Total Flavonoids g 100 g^{-1} dry material	Total Anthocyanins mg 100 g^{-1} productivity	Tannins mg 100 g^{-1} productivity	Antioxidant activity %
Raw Pomace	2.79±0.02	2.32±0.04	737.0±0.9	10.51±0.8	79±0.9
45-50 °C	3.31±0.18	3.01±0.03	682.0±1.3	82.40±1.3	64±1.1
95-100 °C	2.85±0.03	2.98±0.06	101.7±1.2	72.20±1.5	61±1.3
125-130 °C	2.29±0.02	2.11±0.04	223.2±0.8	49.20±0.8	51±1.3

flavonoids, plant cells contain vitamins C, A, E, as well as the fermentation system of the cell, which also represents an antioxidant. When affected by heat, these substances have degenerated, and only the antioxidant activity of phenolic compounds is held intact. Kurozawa *et al.,* 2014 studied the degradation of vitamin C in papaya fruits at different drying temperatures and found that increasing the temperature significantly reduced the amount of vitamin C.

At the temperature of 45-50 °C dried pomace contains a larger amount of phenols, flavonoids, anthocyanins and tannins. Apart from that, the mentioned temperature also results in the best antioxidant activity. Based on the results obtained in this experiment, 45-50 °C was chosen as the optimal temperature range for drying the grape processing by-products for 24 h. Below 45 °C and above 130 °C, the extraction of phenolic compounds was minimal, regarding the duration, a decrease in phenolic compounds was also observed when drying for more than 24 h.

The results of the physical-chemical analysis were performed to determine the optimal grinding level of pomace (Table 2).

Table 2. Phenolic compounds after grinding process of dried pomace.

Grinding Level mm	Total Phenols g 100 g^{-1} dry material	Total Flavonoids g 100 g^{-1} dry material	Total Anthocyanins mg 100 g^{-1} productivity	Tannins mg 100 g^{-1} productivity	Antioxidant activity %
0.5	2.01±0.01	2.11±0.02	625.1±1.2	9.61±1.4	31±0.9
1	3.15±0.03	2.84±0.04	668.7±1.4	71.98±1.2	60±1.1
1.5	3.62±0.04	3.19±0.06	912.9±0.7	68.41±1.3	64±1.2
2	2.19±0.03	2.03±0.03	216.8±0.9	48.0±0.9	30±0.9

The maximum productivity of extraction was reached when the grinding level was 1.5 mm, due to the maximum surface contact between solid/solvent. An increase in the grinding levels caused a decrease in the solvent adsorption capacity and, consequently, a decrease in the extraction quality according to the number of phenolic compounds and antioxidant activity. Brewer *et al.*, 2013 studied the wheat bran particle size influence on phytochemical extractability and antioxidant properties and showed that the coarse treatment exhibited significantly higher antioxidant properties than the fine treatment.

Physical-chemical analysis of the produced extract was performed to determine the extraction module using three variations of pomace/diluent ratios (Table 3). Physical-chemical analysis of the produced extract was performed to determine the optimal diluent concentration using diluents with three different concentrations (Table 4).

Dimcheva *et al.,* 2018 studied the effect of the solid/solvent ratio on the total flavonoid, polyphenol, anthocyanin contents, and TEAC of ethanolic extracts from the grape seeds and pomace. They found that the maximum yield of phenolic

Table 3. Impact of pomace extraction module on phenolic compounds.

Extraction Module	Total Phenols g 100 g^{-1} dry material	Total Flavonoids g 100 g^{-1} dry material	Total Anthocyanins mg 100 g^{-1} productivity	Tannins mg 100 g^{-1} productivity	Antioxidant activity %
1:5	2.12±0.17	2.23±0.04	658.8±0.8	35.27±1.2	41±1.2
1:10	3.62±0.25	3.19±0.03	912.9±0.9	68.41±1.1	64±1.1
1:20	3.63±0.03	3.20±0.02	913.1±1.3	68.42±1.3	64.2±1.3

Table 4. Impact of diluent concentration on the pomace extraction productivity.

Diluent	Total Phenols g 100 g^{-1} dry material	Total Flavonoids g 100 g^{-1} dry material	Total Anthocyanins mg 100 g^{-1} productivity	Tannins mg 100 g^{-1} productivity	Antioxidant Activity %
H_2O	2.11±0.01	1.30±0.04	339.9±1.4	14.36±1.3	32±0.9
30% C_2H_5OH	3.24±0.04	3.12±0.06	671.8±1.1	42.89±1.4	47±1.1
50% C_2H_5OH	3.62±0.02	3.19±0.02	912.9±1.5	68.41±1.4	51±0.8
70% C_2H_5OH	4.98±0.02	3.35±0.03	1167.1±0.7	72.90±1.6	68±1.3
C_2H_5OH	4.15±0.01	3.05±0.05	610.1±0.9	69.49±1.1	56±1.2

compounds was reached at 1:20 of the grape pomace and solvent ratio. Similarly, the same ratio of 1:20 was found to be optimal for the present study. There was a difference when using the solvent concentration. Dimcheva *et al.* (2018) used a 50% ethanol /water mixture as a solvent. The present research showed that the best extraction rate was 70% ethanol/water.

As a result of the experiment, the best performance of phenols, flavonoids, anthocyanins and tannins extraction from pomace was when 70% ethanol: water solution was used as a diluent.

Extraction temperature has an important influence on the extraction process. It is known that according to the temperature, the state of procyanidin complexes in the pomace can be changed.

To determine the optimal extraction temperature, three temperature ranges were investigated: 35-40 °C, 50-55 °C, 75-80 °C. Solution of 70% alcohol/water was used as the solvent. Total phenols, flavonoids, tannins and anthocyanins were determined under the same conditions after 1 h.

Table 5. Correlation between chemical composition and extraction temperature.

Extraction temperature °C	Total Phenols g 100 g^{-1}	Flavonoids g 100 g^{-1}	Anthocyanins mg 100 g^{-1}	Tannins mg 100 g^{-1}	Antioxidant activity %
35-40	3.47±0.05	2.88±0.03	986.9±1.3	72.49±1.4	47±1.2
50-55	3.79±0.02	3.29±0.05	813.3±1.1	75.27±1.3	64±1.1
75-80	3.71±0.03	3.25±0.01	596.9±1.1	69.86±1.2	51±0.9

Based on the results, it can be concluded that the optimal temperature of pomace extraction is 50-55 °C; however, a lower temperature is more convenient for anthocyanins, given that by increasing the temperature, the amount of anthocyanins decreases in response. A further increase in temperature does not increase the extraction integrity and destroys anthocyanins. Therefore, in this case, increasing the temperature is inexpedient. A similar experiment was carried out by Khanal *et al.* (2010), who found that a increase in the temperature causes a decline in the amount of anthocyanins.

In conclusion, the best pomace extraction temperature is 50-55 °C. Following the increase in temperature does not contribute to producing the best version of the extract; it only decomposes its chemically active substances such as polyphenols and vitamins. Consequently, no further increase in temperature is recommended in this case. Furthermore, 50-55 °C was the best temperature range to maintain the antioxidant activity as well.

Table 6 shows the extraction of total phenols, flavonoids, anthocyanins and tannins; the highest antioxidant activity was achieved during the extractions with a duration of 2 h. The analysis showed that the technologically justified duration of extraction for grape extractions is 2 h, a longer extraction leads to the destruction of phenolic substances. The optimal parameters to produce the extract are summarized in Table 7.

Table 6. Chemical composition of the extract concerning the extraction time.

Extraction duration (h)	Phenolic substances g 100 g^{-1}	Flavonoids g 100 g^{-1}	Anthocyanins mg 100 g^{-1}	Tannins mg 100 g^{-1}	Antioxidant activity %
1	3.79±0.02	3.29±0.05	0.8133± 0.9	75.27± 1.5	64±0.9
2	4.01±0.03	3.51±0.07	0.8251±1.1	79.40±1.4	68±0.7
3	3.91±0.02	3.45±0.06	0.8217±1.4	78.03±1.3	42±0.5
4	3.80±0.04	3.35±0.02	0.8191±1.5	76.26±1.5	38±0.7

To sum up, the extracts produced were distinguished by a high concentration of phenolic compounds, flavonoids and tannic substances and were characterized by high antioxidant activity. The data obtained from the study are not inferior to those conducted by other authors as Raiba *et al.* (2014), who recommend its use in the food industry.

Table 7. Chemical composition of the extract produced.

Dry substance %	Tartaric acid %	Total Phenols %	Flavonoids mg 100 g^{-1}	Anthocyanins mg 100 g^{-1}	Tannins mg 100 g^{-1}	Antioxidant activity %
17.5±0.12	8.0±0.01	4.73±0.01	4.0±0.06	437±0.08	87±1.2	80±1.3

CONCLUSION

The technology regimen of experimental pomace extraction was determined. This regime was selected for raw material: drying temperature 45-50 °C for 24 h, extraction temperature 50-55 °C, extraction duration 2 h. Concentration was done via vacuum. It was identified that by following the mentioned regimen, the extract stood out beacuase of its high concentration of phenolic compounds, flavonoids, and tannic substances. Due to this, it can be used in food technology as an antioxidant. Also, according to the performed tests, it can be concluded that the produced extract can be utilized as a natural food coloring due to its high concentration of anthocyanins. Further studies should be performed to determine in which foods can maintain the given color its stability, also what would be the advisable amount of it that can be used to replace synthetic food dye with the natural analog.

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