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OPTIMIZATION OF PHENOL EXTRACTION FROM GRAPE POMACE USING ECO-FRIENDLY SOLVENTS AND DIFFERENTIATED THERMAL TREATMENTS

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ABSTRACT

This study examines the effects of solvent type and temperature on the extraction efficiency of phenolic compounds from grape pomace. Four solvents were tested: 50 % hydroalcoholic solution, 3 % and 5 % citric acid solutions, and pectinase, with extractions conducted at 20 °C, 40 °C, and 60 °C. The results indicate that the hydroalcoholic solvent at 20 °C yielded the highest extraction of total polyphenols, anthocyanins, and catechins. Increasing the temperature caused significant degradation of thermally sensitive phenolics, particularly anthocyanins, which was also evidenced by reduced color intensity of the extracts. The Folin-Ciocalteu index, serving as an indicator of total antioxidant capacity, correlated strongly with total polyphenol content, validating the analytical approach. While citric acid- and pectinase-based solvents showed lower extraction efficiency, they maintained better compound stability at higher temperatures. These findings suggest that hydroalcoholic extraction at low temperatures optimizes phenolic yield from grape pomace by minimizing thermal degradation and preserving antioxidant activity.

INTRODUCTION

Phenolics represent an important class of bioactive compounds, known for their multiple health benefits, including antioxidant, anti-inflammatory, and antimicrobial properties (Drewnowski & Gomez-Carneros, 2000; Scalbert et al., 2005). These compounds are found in significant amounts in grape pomace obtained after the winemaking process, an agro-industrial residue with high valorization potential. Efficient extraction of phenolics from grape pomace can lead to obtaining valuable ingredients for the food, pharmaceutical, and cosmetic industries (Ribéreau-Gayon et al., 2006).

Traditional extraction methods often use toxic organic solvents or harsh conditions that can affect the quality of the extracted compounds as well as the environment. In the context of increasing concerns about sustainability and product safety, the focus is on the use of green solvents, such as moderate concentration hydroalcoholic solutions, citric acid, or natural enzymes like pectinase, which enable extraction under milder and more environmentally friendly conditions (Herrero et al., 2010). Besides the solvent type, thermal treatments applied to the raw materials significantly influence the yield and composition of the extracted phenolics. Drying at high temperatures can induce degradation of phenolic compounds, reducing their biological activity (Spigno et al., 2007), whereas moderate temperatures and optimal

drying times can promote the release of phenolics without major losses. This study aims to optimize the extraction process of phenolics from grape pomace using green solvents and varied thermal treatments, with the goal of identifying optimal drying and extraction conditions to maximize phenolic yield and valorize the bioactive potential of this by-product.

MATERIAL AND METHODS

For this study, grape pomace from two grape varieties, Fetească Neagră and Cabernet Sauvignon, obtained after the winemaking process, was used. The plant material was subjected to drying at three different temperatures: room temperature (20 °C) for 24 hours, oven drying at 40 °C for 72 hours, and at 60 °C for 24 hours. After drying, the pomace was finely ground to increase the contact surface area during the extraction process.

Phenolic compounds extraction was carried out by contacting the dried pomace with green solvents, namely a 50 % hydroalcoholic solution, citric acid solutions at concentrations of 3 % and 5 %, as well as an enzymatic solution containing pectinase at 10 U/ml. The extraction was performed at room temperature for 24 hours, under continuous stirring to homogenize the mixture and optimize the recovery of phenolic compounds.

The determination of phenolic compound content was conducted using specific spectrophotometric methods. Total polyphenols were quantified by the Folin-Ciocalteu method. Anthocyanins were determined by the pH-differential method, while phenolic acids were evaluated using standardized spectrophotometric assays. Color intensity and catechin content were also measured using UV-Vis spectrophotometry. The Folin-Ciocalteu Index and Total Polyphenol Index (TPI) were calculated to assess the overall antioxidant capacity of the extracts.

All experiments were performed in triplicate to ensure reproducibility of results, and data were expressed as means ± standard deviations. Statistical analysis was conducted using ANOVA, followed by Tukey's test to identify significant differences between treatments, with a significance level of $p < 0.05$.

RESULTS AND DISCUSSIONS

Extractions with 50 % hydroalcoholic solvent at room temperature (20 °C/24 h) yielded the highest total polyphenol content (e.g., Fetească Neagră: 25,067 ± 100 mg/100 g; Cabernet Sauvignon: 25,822 ± 120 mg/100 g), indicating the efficiency of this solvent in solubilizing phenolic compounds. The significant decrease in TPC at 40 °C and 60 °C is attributed to thermal degradation, as well as possible oxidation and polymerization reactions induced by temperature, according to the literature (Singleton et al., 1999).

Acidic solutions (3 % and 5 % citric acid) extracted lower amounts of polyphenols (e.g., Fetească Neagră, 3 % citric acid: 2,889 ± 30 mg/100 g), but the values remained relatively constant across treatments, suggesting a stabilizing effect of the phenolics in acidic medium, albeit with limited efficiency due to the solvent's polarity. Pectinase treatment (10 U/mL) at 20 °C led to significant increases in TPC (e.g., Fetească Neagră: 6,156 ± 50 mg/100 g) compared to thermal treatments, due to the release of bound phenolics from the cell walls. At higher temperatures, enzymatic efficiency decreased, highlighting the sensitivity of phenolic compounds to heat and the important role of enzymes in extraction (Rodriguez et al., 2012).

Anthocyanins, flavonoid pigments sensitive to pH and temperature, showed a similar behavior to TPC. Extraction at room temperature provided the best values, while the pronounced decrease at higher temperatures is associated with thermal degradation, which affects the color intensity of the extracts (Giusti & Wrolstad, 2001). Maintaining a low temperature is crucial to obtain extracts with high aesthetic and functional value.

Citric acid solutions, due to their moderate acidity, can help stabilize the anthocyanin structure by maintaining the characteristic red cationic form, although the extracted concentrations were lower.

The highest anthocyanin values were obtained in the hydroalcoholic extraction at 20°C: Fetească Neagră: 584 ± 10* mg/100 g and Cabernet Sauvignon: 718 ± 12* mg/100 g. Values decreased significantly at 40 °C and 60 °C (e.g., Fetească Neagră at 60 °C: 429 ± 9* mg/100 g), demonstrating thermal degradation of these sensitive pigments. Citric acid and pectinase solutions showed lower quantities but relatively stable values across treatments (Table 1).

Table 1

Mean content ± standard deviation of total polyphenols, anthocyanins, and phenolic acids depending on the solvent and thermal treatment applied for the Fetească Neagră variety

No.	Solvent/ Thermal treatment	Total polyphenols (mg/100g)	Anthocyanins (mg/100g)	Phenolic acids (mg/100g)
50 % hydroalcoholic solution				
1	Temperature= 20 °C/24 h	25067 ± 540 *	584 ± 20 *	735 ± 15 *
2	Hot air oven 40 °C / 72 h	21400 ± 500 *	437 ± 18 *	547 ± 10 *
3	Hot air oven 60 °C / 24 h	17311 ± 470 *	429 ± 16 *	505 ± 12 *
3 % citric acid solution				
4	Temperature= 20 °C/24 h	2889 ± 100 *	23 ± 3 *	66 ± 4 *
5	Hot air oven 40 °C / 72 h	2767 ± 90 *	15 ± 2 *	49 ± 3 *
6	Hot air oven 60 °C / 24 h	2333 ± 80 *	17 ± 2 *	48 ± 3 *
5 % citric acid solution				
7	Temperature= 20 °C/24 h	2944 ± 95 *	26 ± 3 *	65 ± 4 *
8	Hot air oven 40 °C / 72 h	2867 ± 85 *	17 ± 2 *	57 ± 3 *
9	Hot air oven 60 °C / 24 h	3700 ± 110 *	17 ± 3 *	52 ± 3 *
Pectinase solution 10 U/ml				
10	Temperature= 20 °C/24 h	6156 ± 120 *	23 ± 3 *	69 ± 5 *
11	Hot air oven 40 °C / 72 h	2922 ± 95 *	16 ± 2 *	51 ± 4 *
12	Hot air oven 60 °C / 24 h	3756 ± 110 *	19 ± 3 *	48 ± 3 *

* Significant difference compared to extraction at ambient temperature ($p < 0.05$)

Phenolic acids showed less variation compared to other phenolic compounds, indicating greater stability under thermal extraction conditions and different solvent types (Rice-Evans et al., 1997). These compounds are more resistant to thermal oxidation and more soluble in polar media, making them suitable as complementary markers for assessing extract quality.

Phenolic acids were more stable across different temperatures, with a

significant decrease observed only at 60 °C (e.g., Cabernet Sauvignon hydroalcoholic extract: 1017 ± 15* mg/100g at 20 °C vs. 799 ± 13* mg/100g at 60 °C). This confirms their relative resistance, while also indicating some sensitivity to high temperatures. Extraction using 50 % hydroalcoholic solvent at ambient temperature yielded the highest content of total polyphenols, anthocyanins, and phenolic acids, with a significant decrease at higher temperatures (40 °C and 60 °C), highlighting the thermal sensitivity of phenolic compounds. Citric acid-based eco-friendly solvents provided lower but consistent yields, while the pectinase solution gave intermediate results, with a positive effect on phenolic release at ambient temperature. Phenolic acids showed less variability compared to anthocyanins, suggesting better resistance to thermal treatments.

The values obtained for the Folin–Ciocalteu index followed the same trend as total polyphenol content (TPC), confirming the method's utility as a global indicator of antioxidant capacity. The highest value (23.23 ± 0.50) was recorded for the extraction with 50 % hydroalcoholic solution at ambient temperature, significantly higher than values from thermal treatments (Table 2).

Table 2

Average content ± standard deviation of the Folin-Ciocalteu index, color intensity, and catechin content for the Fetească Neagră grape variety

No.	Solvent/ Thermal treatment	Folin – Ciocalteu Index (abs)	Color intensity (abs)	Catechins (mg/100g)
50 % hydroalcoholic solution				
1	Temperature= 20 °C/24 h	23.23 ± 0.50 *	22.47 ± 0.60 *	4.84 ± 0.10 *
2	Hot air oven 40 °C / 72 h	20.08 ± 0.40 *	17.69 ± 0.50 *	1.74 ± 0.07 *
3	Hot air oven 60 °C / 24 h	16.44 ± 0.30 *	16.73 ± 0.40 *	1.98 ± 0.05 *
3 % citric acid solution				
4	Temperature= 20 °C/24 h	3.50 ± 0.10 *	1.20 ± 0.05 *	0.77 ± 0.02 *
5	Hot air oven 40 °C / 72 h	3.39 ± 0.10 *	0.95 ± 0.03 *	0.63 ± 0.01 *
6	Hot air oven 60 °C / 24 h	3.01 ± 0.10 *	1.02 ± 0.04 *	0.64 ± 0.02 *
5 % citric acid solution				
7	Temperature= 20 °C/24 h	3.55 ± 0.10 *	1.62 ± 0.05 *	0.81 ± 0.02 *
8	Hot air oven 40 °C / 72 h	3.49 ± 0.10 *	1.20 ± 0.04 *	0.65 ± 0.02 *
9	Hot air oven 60 °C / 24 h	4.24 ± 0.10 *	1.12 ± 0.04 *	0.70 ± 0.02 *
Pectinase solution 10U/ml				
10	Temperature= 20 °C/24 h	6.46 ± 0.20 *	0.71 ± 0.02 *	0.58 ± 0.01 *
11	Hot air oven 40 °C / 72 h	3.55 ± 0.10 *	0.51 ± 0.01 *	0.39 ± 0.01 *
12	Hot air oven 60 °C / 24 h	4.31 ± 0.10 *	0.61 ± 0.02 *	0.49 ± 0.01 *

* Significant difference compared to extraction at ambient temperature ($p < 0.05$)

At 40 °C and 60 °C, the values decreased (20.08 ± 0.40 and 16.44 ± 0.30), indicating thermal degradation of phenolic compounds. Citric acid solutions yielded lower but stable values (3.50–4.24), reflecting weaker yet consistent extraction. Pectinase increased the Folin-Ciocalteu index at 20 °C (6.46 ± 0.20), but its efficiency declined at higher temperatures, suggesting the thermal sensitivity of the extracted compounds.

Color intensity, which correlates with anthocyanin content and other phenolic pigments, reached its maximum in the hydroalcoholic extraction at 20 °C

(22.47 ± 0.60), decreasing significantly at 40 °C (17.69 ± 0.50) and 60 °C (16.73 ± 0.40), highlighting pigment degradation caused by elevated temperatures. Citric acid solutions resulted in low but thermally stable color intensity values (ranging from 0.95 to 1.62), while pectinase extraction yielded the lowest values (0.51–0.71), indicating poor efficiency in extracting colored pigments.

Catechins were most efficiently extracted with hydroalcoholic solvent at 20 °C (4.84 ± 0.10 mg/100 g), while higher temperatures significantly reduced their content (1.74–1.98 mg/100 g), reflecting their sensitivity to thermal treatment. Citric acid and pectinase extractions produced concentrations below 1 mg/100 g, suggesting low solubility in acidic media and limited enzymatic efficiency for these compounds.

The Folin-Ciocalteu index confirms a strong correlation with the total polyphenol content, indicating the reducing capacity of phenolic extracts. Color intensity, an indirect indicator of anthocyanin content, decreases significantly under thermal treatments, reflecting pigment degradation. Catechins, key flavonoid components, also show sensitivity to extraction temperature, with maximum values obtained at ambient conditions. Overall, these parameters support the conclusion that maintaining low temperatures is essential for optimal extraction of phenolic compounds.

CONCLUSIONS

Total polyphenol content (TPC) extracted with 50 % hydroalcoholic solvent at ambient temperature (20 °C) reached the highest values, being approximately 14–45 % higher compared to extractions at 40 °C and 60 °C (e.g., *Feteasсă Neagră*: 25067 mg/100 g at 20 °C vs. 21400 mg/100 g at 40 °C and 17311 mg/100 g at 60 °C). Raising the extraction temperature to 40 °C and 60 °C significantly decreased polyphenol, anthocyanin, and catechin contents, with reductions of up to 31 % for TPC and up to 26 % for anthocyanins (e.g., Feteasсă Neagră anthocyanins decreased from 584 mg/100 g at 20 °C to 429 mg/100 g at 60 °C).

Citric acid-based solutions (3 % and 5 %) extracted considerably lower amounts of total polyphenols, with values approximately 88–90 % lower than those obtained with the hydroalcoholic solvent at 20 °C. However, these values remained relatively constant across thermal treatments, suggesting a protective effect on phenolic compounds in acidic media.

Enzymatic treatment with pectinase at 20 °C increased total polyphenol content by approximately 145 % compared to citric acid extraction and by around 75 % compared to pectinase extractions at higher temperatures, due to the release of bound phenolics from cell walls.

Phenolic acids were the most stable compounds, with only a 15–20 % decrease observed at 60 °C. The Folin–Ciocalteu index, which correlates with total polyphenol content, dropped by up to 41 % at elevated temperatures. Both color intensity and catechin content were also negatively affected, decreasing by over 25 % and 60 %, respectively, confirming the thermal sensitivity of phenolic pigments.

In conclusion, phenolic compound extraction from grape pomace is optimized by using 50 % hydroalcoholic solvent at low temperatures, avoiding thermal degradation and maximizing the bioactive potential of the extract. Alternative solvents such as citric acid and pectinase provide more thermally stable extracts but with lower extraction efficiency.

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