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ENHANCING THE EXTRACTION EFFICIENCY OF CAFTARIC ACID FROM GRAPE PULP THROUGHT OPTIMIZATION PROCESS

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Abstract: This study explores the impact of operational extraction parameters, including ethanol concentration, extraction time, and temperature, on the extraction of caftaric acid from grape pulp. Optimal conditions for maximizing caftaric acid content were identified as follows: 20% ethanol concentration, 300 minutes extraction time, and a temperature of 35°C. The investigation employed a full factorial experimental plan (2^3) to assess the influence of these operational factors on caftaric acid content.

Keywords: extraction, experimental plan, operation factors

Introduction

Since the early 20th century, a plethora of studies has investigated the chemical composition of various organs of the grapevine. These studies, spanning from the 20th century to the present day, have consistently revealed the remarkable variability in the chemical makeup among different grape varieties and genotypes (Marais et al., 1991; Kennedy et al., 2001; Zhu et al., 2012). The components under scrutiny encompass a wide array, with polyphenolic compounds taking precedence, followed by organic acids, vitamins, enzymes, carbohydrates, organic nitrogen compounds, terpenoids, volatile compounds, waxes, lipids, polysaccharides, and gums.

The proportion of different components within a grape berry's total mass is subject to variation, primarily influenced by the grape variety and external environmental factors. Among the constituent parts of the ripe fruit, the mesocarp (pulp) constitutes the largest share, ranging from 75% to 90%. Following this, the skin accounts for 9% to 20%, while the seeds represent the smallest fraction, ranging from 2% to 6% (Vujović, 2013). Notably, the

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quantitative distribution of these berry components varies not only across different grape varieties but also within the same variety.

This study seeks to enhance the extraction method through experimental design, aiming to identify the optimal conditions for extracting caftaric acid from the pulp of three grape varieties: black Tamjanika, Smederevka, and Muscat Hamburg. The optimization phase employed a 2^3 experimental design, systematically investigating the impact of key factors—ethanol concentration, extraction time, and extraction temperature—on the caftaric acid content in the extracts obtained under various extraction conditions. Through thorough statistical analysis of the results, conclusions were drawn regarding the influence of the examined factors.

Materials and methods

Materials

For this study, the grape varieties chosen were Hamburg, Smederevka, and Black Tamjanika. In the experimental phase, the pulp extracted from the grape was utilized, having been finely chopped immediately prior to the experiment. The skin and seeds were manually removed and excluded from the study.

Extraction procedure

The extraction experiments were performed using different conditions of solvent concentration, extraction temperature and extraction time. The conditions used in each experiment were settled according to the 2^3 full factorial design (Table 1). After the filtration (filter paper with 6 μm pore size – Whatman, USA), the extracts were stored in the flask.

HPLC analysis

Quantification of individual phenolic compounds was determined using reversed-phase HPLC method. An Agilent-1200 series HPLC with UV-Vis DAD for multi-wavelength detection was used. The column was thermostated at 30 °C. After injecting 5 μL of the diluted plant extract, the separation was performed in an Agilent-Eclipse XDB C-18 4.6 × 150 mm column. Two solvents were used for the gradient elution: A (H2O+2% HCOOH) and B (80 % acetonitrile (ACN) +5 % HCOOH + H2O). The elution programme used was as follows: from 0 to 10 min 0 % B, from 10 to 28 min gradually increases 0–25 % B, from 28 to 30 min 25 % B, from 30 to 35 min gradually increases 25–50 % B, from 35 to 40 min gradually increases 50–80 % B, and finally for the last 5 min

gradually decreases 80–0 % B. Flow rate of the mobile phase was 0.8 mL min–1. Caftaric acid present in the extracts were identified by comparing their retention times and spectra with pure component.

Statistical analysis

A full factorial model 2^3 with replication was used to optimize the caftaric acid extraction. The significance of the factors and their combinations were evaluated by the ANOVA using a computer program. The linear first order regression equations were also developed to show the dependence of caftaric acid yield on factors and their interactions.

Results and discussion

In grape pulp, the predominant hydroxycinnamic acid is caftaric acid, in the trans-form. To extract caftaric acid from the pulp of three grape varieties—black Tamjanika, Smederevka, and Muscat Hamburg—maceration, a traditional extraction method, was employed. The study aimed to optimize experimental maceration conditions by investigating the effects of three distinct extraction parameters.

Preliminary tests were conducted to identify the two most influential levels for each tested factor, with significant impact on caftaric acid extraction, mirroring conditions encountered in the wine production process during maceration. These selected levels were subsequently incorporated into the factorial design matrix for the experiments, as showed in Table 1.

Table 1. Eksperimental design and caftaric acid content in grape pulp

	x_1	x_2	x_3	Etanol (%)	Vreme (min)	Tempera. (°C)	BT[1] (μg/g)	S[2] (μg/g)	MH[3] (μg/g)
1	-1	-1	-1	10	120	25	13,74	15,02	6,13
2	+1	-1	-1	20	120	25	15,39	16,91	6,83
3	-1	+1	-1	10	300	25	22,38	22,63	9,53
4	+1	+1	-1	20	300	25	23,67	26,27	10,49
5	-1	-1	+1	10	120	35	20,19	19,62	8,72
6	+1	-1	+1	20	120	35	22,16	21,50	9,53
7	-1	+1	+1	10	300	35	27,96	29,87	13,25
8	+1	+1	+1	20	300	35	30,51	33,52	14,58

[1]BT-Black Tamjanika, [2]S-Smederevka, [3]MH-Muskat Hamburg

The determined caftaric acid content in extract samples 1-8 exhibited a range of 13.75 μg/g to 30.51 μg/g in black Tamjanika pulp, 15.02 μg/g to 33.52 μg/g in Smederevka pulp, and 6.13 μg/g to 14.58 μg/g in Muscat Hamburg pulp. Extract No. 8, characterized by 20% ethanol, extraction time of 300 minutes, and a temperature of 35°C, demonstrated the highest caftaric acid content. In contrast, extract No. 1, with 10% ethanol, extraction time of 120 minutes, and a temperature of 25°C, displayed the lowest caftaric acid content.

The average caftaric acid contents in the pulp of black Tamjanika, Smederevka, and Muscat Hamburg were found to be 22.00 μg/g, 23.17 μg/g, and 9.88 μg/g, respectively.

Gutiérrez-Gamboa and Moreno-Simunovic (2018) determined in Caringan grapes from different regions of Chile that the content of trans-caftaric acid ranges from 24.82 to 57.87 μg/g, while the content of total hydroxycinnamic acids ranges from limits from 51.96 to 130.63 μg/g.

Pajović et al. (2014) determined the content of caftaric acid in the pulp of Vranac, Kratošija and Cabernet Sauvignon grapes. The content of caftaric acid in the pulp of the mentioned grape varieties is similar (16.0 μg/g, 28.4 μg/g, 30.5 μg/g of fresh pulp) to our results. Di Lecce et al. (2014) identified and quantified trans- and cis-caftaric acid in the pulp of Albarino grapes from Spain in the amount of 3.7 and 1.1 μg/g of fresh pulp, which is significantly less compared to our results.

The experimental data of the caftaric acid yield were analyzed with a linear first-order regression model. The statistical significance of all three factors and their possible two- and three-way interaction for the caftaric acid yield were evaluated for their *F*- and *p*-values. The value of $p < 0.05$ indicates the significance of the factors and their interaction. The *F*-values for x_1, x_2 and x_3 factors are in interval 71.858–9289.2, respectively. To simplify the linear regression model, all factors, and interactions, which were assessed to be statistically insignificant with the significance level of 0.05, were omitted; the simplified regression equations are given in Table 2. The most important factor was the extraction time (x_2), which was followed by the extraction temperature (x_3).

Our results showed that the R^2 ranges between 99.88 and 99.90%, indicate that the regression model was suitable for explaining the behavior. Our results showed also that the coefficients of variation were < 2% for all the responses, representing a better precision and reliability of the conducted experiments.

Table 2. Regression equations

BT[1]	$y = 22.00 + 0{,}932x_1 + 4.130x_2 + 3.205x_3 + 0.197x_1x_3 - 0.100x_2x_3 + 0{,}117x_1x_2x_3$
S[2]	$y = 23{,}169 + 1{,}381x_1 + 4{,}906x_2 + 2{,}959x_3 + 0{,}439x_1x_2 + 0.661x_2x_3$
MH[3]	$y = 9{,}882 + 0{,}475x_1 + 2{,}080x_2 + 1{,}637x_3 + 0.097x_1x_2 + 0.060x_1x_3 + 0{,}315x_2x_3$

[1]BT-Black Tamjanika, [2]S-Smederevka, [3]MH-Muskat Hamburg

Conclusion

A full factorial experiment 2^3 was used to determine the optimum parameters that gave a high extraction yield of caftaric acid from grape pulp. The analysis of variance showed that the effects of all variables (ethanol concentration, extraction temperature and extraction time) were extremely significant. The linear regression mathematical models had higher correlation and could be employed to optimize the caftaric acid extraction from grape pulp.

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