UDC 547.565.2:663.222
ISSN 1330-9862
(FTB-1346)

original scientific paper

Study of Dynamics of Polyphenol Extraction During Traditional and Advanced Maceration Processes of the Babić Grape Variety

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Received: June 23, 2004
Revised version: November 4, 2004
Accepted: November 22, 2004

Summary

The influence of different maceration techniques on the dynamics of polyphenol extraction during the maceration of the autochthonous Croatian grape Babić has been investigated. The process of wine production by maceration in traditional procedure and by maceration with advanced technique has been compared. During maceration, the dynamics of extraction of total anthocyanins, total phenols, low-molecular proanthocyanidins and high-molecular proanthocyanidins was determined. Mathematical models are proposed for each above mentioned and determined parameter. The models present the values under observation depending on treatment – traditional or modern. Time expressed in days is the input variable for both monitored models. Presented models indicate a significant positive correlation and strongly sustain the concept that the duration and procedure of maceration have considerable influence on the measured variables (R=0.83–0.98).

Key words: Babić wine, maceration, polyphenols, spectrophotometric analysis, mathematical models

Introduction

Polyphenols are significant components responsible for colour, astringency and bitterness of red wine (*1*,*2*). The polyphenol content in wine is substantially influenced by maceration as the basic process, during which polyphenols turn into wine from solid parts of grapes. Flavonoids, which include anthocyanins, low-molecular (catechins) and high-molecular proanthocyanidins (condensed tannins, proanthocyanidins), are among the most important polyphenols. The polyphenol extraction intensity depends on maceration conditions, type and localization of polyphenols in grapes, alcohol concentration and SO_2 (*3–5*). Although the skin contains lower quantities of catechins and proanthocyanidins than the seeds and the stems, it is of primary significance in early stages of maceration, since the polyphenols are easiest to extract from it (*5*,*6*). With the increase in maceration duration, both the skin and the seeds turn into sources of polymeric proanthocyanidins in wine (*6*,*7*).

Among the most significant factors that influence the polyphenol content of wine, the duration of maceration is the one that affects the polyphenol extraction from grapes the most (*8*,*9*). The optimum maceration pe-

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riod depends on the type of wine one wishes to produce, *i.e.* on the character of the future wine (*10*). Extended maceration is known to cause an increase in catechin and proanthocyanidin content in wine. Thus, extended maceration has a positive impact on creation of anthocyanin-tannin complex, which is responsible for the definition and stability of the colour of red wine (*11*). Namely, soon after their release from grape skins, free anthocyanins take part in formation of polymer compounds with other polyphenols, mainly with catechins and proanthocyanidins (*12,13*). However, since catechins and proanthocyanidins are simultaneously responsible both for astringency and bitterness of wine, the wines made through extended maceration are more astringent and have a more stable colour than those produced through shorter maceration.

In the standard production of the red wine, traditional maceration process is applied, in which the punching of pomace is done several times a day, while in the advanced production vinification in horizontal or vertical rototanks or macerators is used.

The objective of the performed research was to determine the influence of the traditional and advanced maceration procedures on the dynamics of extraction of low-molecular and high-molecular proanthocyanidins, total phenols and total anthocyanins from the Babić variety grape. Spectrophotometric analysis methods were applied in research. The research findings were statistically processed by means of variance analysis and the LSD test. Matematical models are proposed for each measured parameter.

Materials and Methods

The vintage was made in the Primošten vineyard area on locations for production of the high-quality Babić wine on September 19, 2001. The grapes were harvested when technologically ripe, containing 21.1 % of sugar and 7.45 g/L of total acidity.

High-quality wine of the Babić variety from the littoral, karstic (distinctly rocky) region in southern Croatia (Dalmatia, the Primošten vineyard area) is produced on small-scale family farms by traditional maceration. In addition, the high-quality Babić wine is made by contemporary vinification technique for maceration of red wine on industrial scale.

Traditional maceration procedure

A representative sample of 150 kg of grapes was used for the traditional maceration test, selected by random sampling. The grapes were crushed and destemmed immediately after the harvest and SO_2 (100 mg/kg of grapes) was added. The sulphited pomace was homogenized and divided into three 70-L vessels, so that each vessel contained 50 kg of pomace. The maceration process was performed in a closed vessel at the temperatures from 19 to 29 °C. Fermentation went on spontaneously (with no selected yeasts added), while temperature was not controlled. The pomace was punched every seven hours during the course of the day, during which it was well mixed. Maceration lasted for 11 days. The samples for analysis were taken every other day, with parallel measurements of temperature and sugar content.

Advanced maceration procedure

Maceration was performed in a horizontal macerator (»vinimatic«), with 1500 kg capacity, in the winery of Vinoplod-Vinarija d.d. Šibenik. Each day the macerator was turned sideways two to three times, and then again to the other side. Samples for the analyses were taken from the macerator after its turning every twelve hours, with simultaneous measuring of temperature and sugar content. Maceration took 68 hours and was stopped when the sugar content reached the value of zero Babo (content of sugar in %).

Analysis of polyphenol compounds

Total phenols were determined with the standard AOAC method (*14*). Concentration of total phenols is expressed in mg/L of the gallic acid equivalent.

Total anthocyanins were determined according to the method of Rigo *et al.* (*15*). Concentration of total anthocyanins is expressed in relation to malvidin-3-monoglucoside chloride (mg/L). The RSD of the method is 1.39 %.

The vanillin index served to determine catechins and proanthocyanidins reacting with the vanillin, according to the optimized and controlled vanillin-HCL method (*16*), by following the optimized conditions as described by Rigo *et al.* (*15*). Concentration of compounds determined by the vanillin index was calculated using the calibration curve with the (+)-catechin and expressed in mg/L. The vanillin index is more sensitive to catechins (monomers) and oligomeric proanthocyanidins than to high-molecular proanthocyanidins. The RSD of the method is 3.54 %.

Proanthocyanidins were determined by the very specific Bate-Smith reaction of splitting the interflavanoid link of proanthocyanidins with catalyzed acids according to the method of Rigo *et al.* (*15*), and then measured as a part that transforms into cyanidin (*17*). This method is more sensitive to high-molecular proanthocyanidins than to flavan-3-ol monomers and oligomers.

Modeling and analysis

The experimental data obtained during monitoring of traditional and advanced maceration procedures served in preparation of mathematical models for the observed variables (total anthocyanins, vanillin index, proanthocyanidins, total phenols, and reducing sugar) according to Reynier *et al.* (*18*) and Hammer *et al.* (*19*).

Two models are proposed, the first based on the experimental data obtained by the traditional maceration method (traditional model; TM), and the second derived from the experimental data obtained by the advanced maceration method (advanced model; AM). Variable i, where i=1, has been used for the TM model, whereas i=2 serves for the AM model. In both models total anthocyanins, vanillin index, proanthocyanidins, total phenols and reducing sugar were observed as output (predictor) variable (y), while the input variable (regressor) denoted time expressed in days (x).

$$y_{in} = (a_{1i}x_{i1}^2) + (a_{2i}x_{i2}) + a_{3i} + e_{i1} \qquad /1/$$

y_{i1} = total anthocyanins$_i$
y_{i2} = vanillin index$_i$
y_{i3} = proanthocyanidins$_i$
y_{i4} = total phenols$_i$
y_{i5} = reducing sugar$_i$
n = number of output variables (n=5)
x = observed time (days)
e_i = error

The model parameters according to the Equation 1: a_1, a_2, a_3, are constants obtained by minimization of the variance between experimental data and the models (according to the least-squares method) for n=2, 3, 4. For the models where n=1 or 5, the proposed models are polynomials of degree 2 that can describe experimental data with minimal variation. The constants for the proposed equations of the models a_1, a_2, a_3 are given in Table 1, while the very equations are shown in captions of Figs. 1–5.

Results and Discussion

Traditional maceration procedure

The results of the dynamics of total phenol extraction, total anthocyanins, low-molecular and high-molecular proanthocyanidins during an 11-day traditional maceration treatment are shown in Table 2. The Fisher quotient and p-values of the analysed classes of polyphenols from three repeated processes are presented in Table 3, which shows no significant difference ($p>0.05$) in compound concentrations among fermentation tanks.

An increase of the vanillin index was observed during the eleven-day maceration. The highest increase in concentration of low-molecular proanthocyanidins was noted from the third (776 mg/L) to the fifth (1341 mg/L) day of maceration.

A significant variance in concentrations of high-molecular proanthocyanidins was determined from day five to day seven, from day seven to day nine, and from day nine to day eleven ($p<0.001$). High-molecular proanthocyanidins were present in significantly higher concentrations than low-molecular proanthocyanidins. There was an exceptional reversal to this on the fifth day of maceration, when the high-molecular proanthocyanidin concentration (1191 mg/L) was lower than that of low-molecular proanthocyanidins (1341 mg/L). This is probably a consequence of slower extraction of high-molecular proanthocyanidins from grape skin and seeds. Due to their presence mostly in seeds, the release of proanthocyanidins is slower, depending on migration speed of vacuoles toward cell walls, and is aided by the action of alcohol. Namely, it has been determined that alcohol has a significant effect on the membranes of living cell seeds (*20*). As the maceration period gets longer, seeds turn into a significant source of proanthocyanidins (*21*).

Table 1. Model constants for construction of mathematical models for the traditional maceration treatment (TM) and the advanced maceration treatment (AM) in the production of Babić wine

Model TM (for i=1)				
	$\mathbf{a_{in}}$ (mg/L/h^2)	$\mathbf{a_{in}}$ (mg/L/h)	$\mathbf{a_{in}}$ (mg/L)	**r**
y_{11} = total anthocyanins for x≤12 days	–15.51	225.71	209.02	0.8322
y_{12} = vanillin index	0	179.94	232.27	0.9855
y_{13} = proanthocyanidins	0	313.47	15.34	0.9622
y_{14} = total phenols	0	276.34	344.94	0.9854
y_{15} = reducing sugar	3.57	–66.63	294.74	0.9495
Model AM (for i=2)				
	$\mathbf{a_{in}}$ (mg/L/h^2)	$\mathbf{a_{in}}$ (mg/L/h)	$\mathbf{a_{in}}$ (mg/L)	**r**
y_{21} = total anthocyanins for x≤3 days	–176.29	791.38	–217.81	0.9292
y_{22} = vanillin index	0	511.80	–25.17	0.9662
y_{23} = proanthocyanidins	0	861.13	–119.61	0.9745
y_{24} = total phenols	0	775.80	670.56	0.9555
y_{25} = reducing sugar	24.52	–158.08	258.68	0.9754

Table 2. Concentrations (mg/L) of anthocyanins (AT), total phenols (TP), low-molecular (VAN) and high-molecular (PROC) proanthocyanidins during the traditional maceration treatment of the Babić grape variety

	t/(day/h)					
	1/24	3/72	5/120	7/168	9/216	11/264
AT	443±8.66	631±9.06	1096±54.40	1036±23.91	870±7.67	866±29.62
TP	639±16.70	923±33.40	1884±37.70	2504±45.10	2827±24.90	3242±106.60
VAN	290±13.08	776±38.70	1341±168.70	1521±121.40	1774±131.90	2174±43.80
PROC	–	–	1191±21.30	2219±60.70	3049±75.20	3460±44.30

Table 3. The Fisher quotients (F) and p-values obtained through analysis of variance in three repeats of maceration

	F	p
Total anthocyanins	0.01	0.96
Vanillin index	0.05	0.77
Proanthocyanidins	0.26	0.95
Total phenols	0.05	0.99

It has been indicated that flavan-3-ols and oligomeric proanthocyanidins are mainly released from grape skin, while the seeds and peduncle are significant sources of polymeric proanthocyanidins in wine (*6*).

A significant increase ($p<0.001$) in concentrations of anthocyanins was achieved on the fifth day of the maceration, when the measured concentration was highest (1096 mg/L). Increased temperature (29 °C), with concurrent action of alcohol, favourably influences the re-

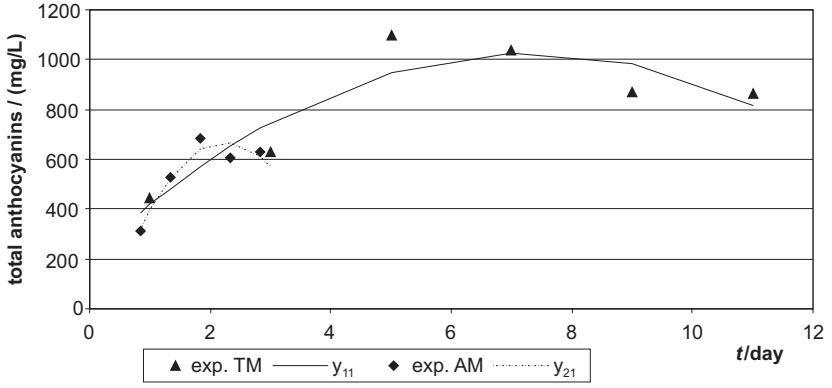

Fig. 1. Mathematical models for total anthocyanins, compared to experimental data for traditional (TM) and advanced (AM) maceration treatment. $y_{11} = -15.51x^2 + 225.71x + 209.02$; $y_{21} = -176.29x^2 + 791.38x - 217.81$

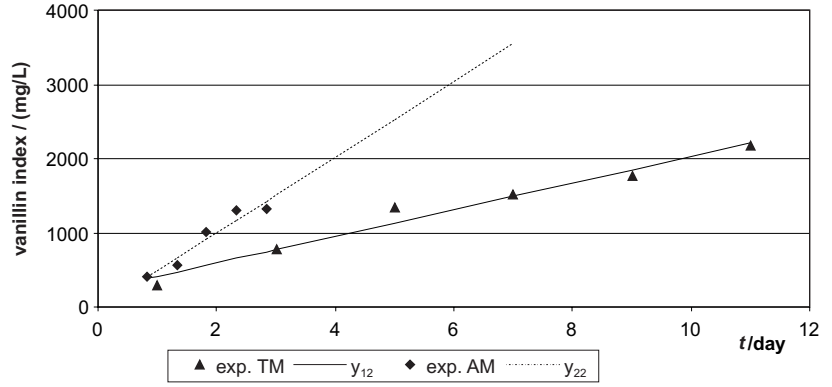

Fig. 2. Mathematical models for vanillin index, compared to experimental data for traditional (TM) and advanced (AM) maceration treatment. $y_{12} = 179.94x + 232.27$; $y_{22} = 511.80x - 25.17$

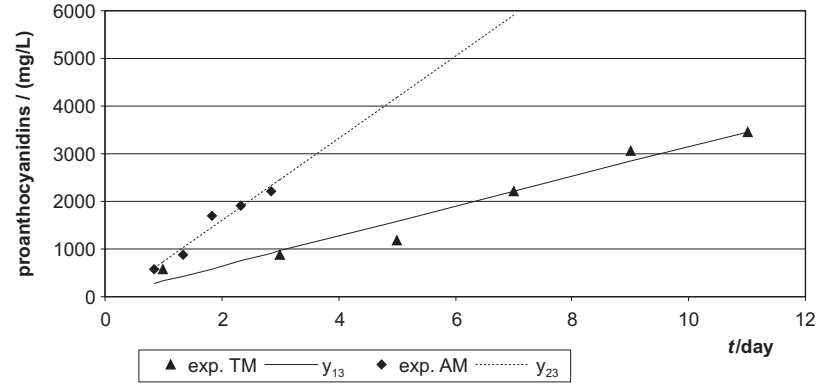

Fig. 3. Mathematical models for proanthocyanidins, compared to experimental data for traditional (TM) and advanced (AM) maceration treatment. $y_{13} = 313.47x + 15.34$; $y_{23} = 861.13x - 119.61$

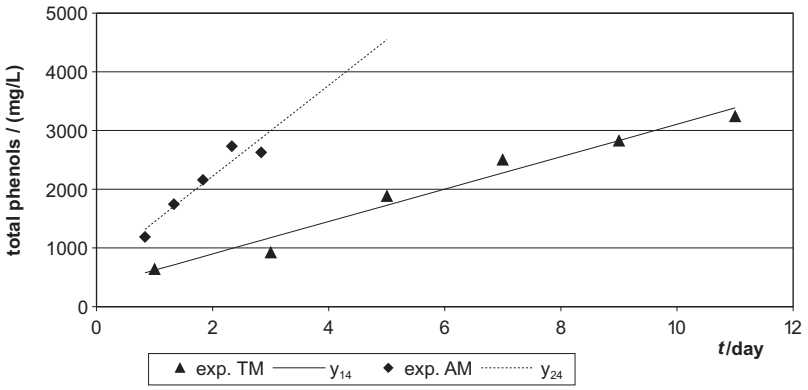

Fig. 4. Mathematical models for total phenols, compared to experimental data for traditional (TM) and advanced (AM) maceration treatment. $y_{14} = 276.34x + 344.94$; $y_{24} = 775.80x + 670.56$

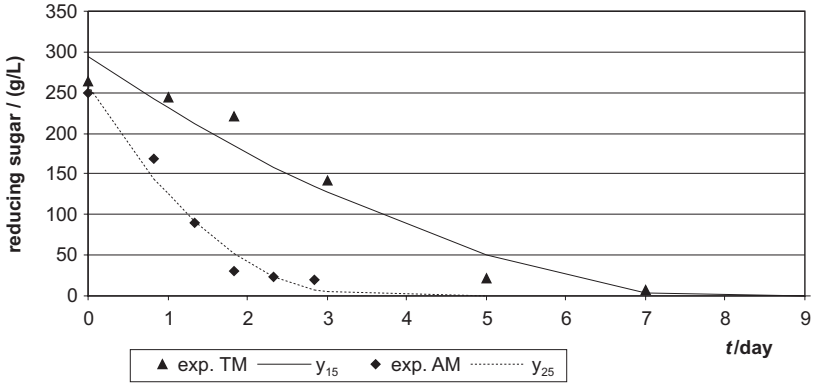

Fig. 5. Mathematical models for reducing sugar, compared to experimental data for traditional (TM) and the advanced (AM) maceration treatment. $y_{15} = 3.57x^2 - 66.63x + 294.74$; $y_{25} = 24.52x^2 - 158.08x + 258.68$

lease of anthocyanins from grape skin cells, which was the cause of a substantial increase in anthocyanin concentration compared to the release of anthocyanins on the third day of maceration. On the fifth day of maceration, the bulk of sugar had already fermented; there were 6 Babo degrees of unfermented sugar left in the pomace. After the fifth day of maceration, a gradual decline in anthocyanin concentration was noted, which was not significant ($p>0.05$). A more significant decrease of anthocyanin concentration occurred from day seven to day nine of the maceration (870 mg/L), while such decrease of anthocyanin concentration from day nine to day eleven was not significant ($p>0.05$). Ribéreau-Gayon (22) reported the decline of anthocyanin concentration after the sixth day of maceration for the Cabernet Sauvignon wines. It is probable that different physical and chemical conditions lead to the decrease of anthocyanins at the prolonged macerations. It has been shown that anthocyanins, due to their polarity, can absorb yeast through their hydrogen bonding. Another reason may lie in a destabilizing action of alcohol, effects of SO_2, and reaction between flavylium cations (A^+) and bisulfit ions leading to colourless anthocyanins (*23*), precipitation with tartaric acid, enzymatic hydrolysis and forming copolimerization products with other polyphenols (*24,25*). Namely, free anthocyanins extracted from grapes are responsible for the bright red colour of young red wine. Development of new, more stable polymeric pigments derived from the reactions between anthocyanins and other polyphenols, mainly proanthocyanidins, is responsible for the colour changes from a bright red of young red wines to a reddish-brown hue (*26*).

Advanced maceration procedure

The results of monitoring of extraction dynamics by advanced maceration procedure in a macerator (horizontal rototank) are shown in Table 4. The maximum concentration (684 mg/L) of anthocyanins, achieved in the forty-fourth hour (1.8 days) of the maceration, was approximately in the region of anthocyanin concentration observed on the third day of traditional maceration, but was lower than the maximum concentration in traditional maceration (1069 mg/L). At the time there were 30.28 g/L of unfermented sugar in the sample, while the temperature in the macerator was 27 °C. The increase of anthocyanins in the macerator was 220 %, while the increase in traditional maceration was 241 %. By the end of maceration, the anthocyanin concentration did not drop significantly; in the 68th hour (2.8 days) it was 632 mg/L (T=20 °C, 20 g/L), which is 7.6 % lower than the maximum concentration. Due to the limited duration of modern maceration treatment it was not possible to observe a significant drop of anthocyanins, because maceration was interrupted after 68 hours. Therefore, the

Table 4. Concentrations (mg/L) of anthocyanins, total phenols, low and high-molecular proanthocyanidins during the advanced maceration treatment of the Babić grape variety

	t/h				
	20	32	44	56	68
Anthocyanins	311±15.30	526±26.86	684±20.20	609±13.45	632±12.70
Total phenols	1188±34.80	1746±30.02	2160±87.59	2735±31.06	2633±23.67
Low-molecular proanthocyanidins	402±9.83	554±11.15	1004±14.84	1291±25.78	1313±10.11
High-molecular proanthocyanidins	583±13.05	874±11.01	1710±13.57	1911±9.82	2217±9.86

above-mentioned effects, responsible for the drop in anthocyanin concentrations, could not come forward.

Concentrations of low-molecular proanthocyanidins were from 402 to 1313 mg/L, with an increase of 327 %, while the rise of low-molecular proanthocyanidin concentration in traditional maceration was 268 %. This indicates that the concentration of low-molecular proanthocyanidins in the advanced maceration treatment was twice as high as that in the traditional maceration during the same period of time of maceration.

Concentrations of high-molecular proanthocyanidins in maceration were from 583 to 2217 mg/L. The increase of proanthocyanidin concentration was 379 %. The highest concentrations of low-molecular and high-molecular proanthocyanidins in the advanced maceration, achieved in 68 hours (2.8 days), correspond to 120 hours (day five) of the traditional maceration, while the maximum concentration of total phenols in the advanced maceration corresponds to the hour 168 (day seven) of the traditional maceration.

Mathematical models

Correlation between the parameters that define wine character and the duration and process of maceration were examined. Namely, a substantial influence of maceration procedure (traditional and advanced) on the concentration of polyphenols in wine was determined by the variance analysis (Table 3). It was possible to determine the behaviour of total anthocyanins, vanillin index, proanthocyanidins, total phenols and reducing sugar from the experimental data obtained under the traditional maceration treatment. The basic shape of the obtained curves was the guiding principle in the construction of models for the advanced maceration treatment. Thereafter, the modeling was to serve in estimation as to what could be expected during the maceration treatment if it were performed for a longer or shorter period of time. Even though the advantage of the advanced procedure is its shorter duration, the proposed models facilitate calculation of polyphenol concentration that can be expected if the advanced procedure lasts longer. However, a realistic period for the estimation of polyphenol concentration under the advanced maceration procedure (with respect to the maceration monitoring time) would be the maximum of 7 days, except for anthocyanins ($t_{max} \leq 3$ days), for which the restriction indicated in Table 1 must be applied, since anthocyanin content cannot drop down to zero.

Whether linear or non-linear models are concerned depends on the constants a_{in} for the 5 parameters examined in this paper. Simple models of linear and polynomial character are proposed and shown in Figs. 1–5, while the coefficients in Table 1 have been used. The figures indicate that the models are of the same shape, regardless of the maceration procedure, but at the same time it is obvious that the advanced procedure effects the same polyphenol concentration in a shorter time. The regression coefficient is considered as the criterion for the estimation of quality of models, since it evaluates the correlation of proposed models with experimental data. Standard errors of parameters, input variables and the Fisher coefficient were determined for all models. The average correlation coefficient for the models proposed for the traditional maceration treatment (TM) is 0.943, while in the models developed for the advanced maceration it is 0.964. The developed models can be used with great certainty in prediction of polyphenol concentration in advanced maceration within 7 days approximately (except for total anthocyanins, where the estimate in the model for modern treatment must be restricted to 3 days maximum).

Conclusions

Through monitoring of polyphenol extraction dynamics in the maceration of the Babić grape variety, it was determined that the maceration process significantly influenced the concentration of total anthocyanins, total phenols, low-molecular and high-molecular proanthocyanidins. In advanced maceration, the highest concentration of low-molecular and high-molecular proanthocyanidins was achieved in 68 hours (2.8 days), which corresponds to the hour 120 (day five) of traditional maceration. The highest concentration of total phenols in the advanced maceration corresponds to 168 hours (day seven) of traditional maceration. The highest anthocyanin concentration observed in the advanced maceration was lower than the highest concentration in traditional maceration. The developed matematical models can be used in the prediction of polyphenol concentration and reducing sugars in advanced maceration within 7 days. The mathematical model in the advanced treatment for total anthocyanins should be limited to three days, for the results obtained beyond that time period are not realistic.

Acknowledgements

The authors want to express their gratitude to Vinoplod-Vinarija, d.d. Šibenik for their help in this research. This work was supported by the Ministry of Science, Education and Sport of the Republic of Croatia.

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Ispitivanje dinamike ekstrakcije polifenola tijekom tradicionalnog i suvremenog procesa maceracije grožđa sorte Babić

Sažetak

Istraženi su utjecaji različitih tehnoloških postupaka maceracije na dinamiku ekstrakcije polifenola tijekom maceracije grožđa autohtone hrvatske sorte Babić. Uspoređen je proces proizvodnje vina maceracijom tradicionalnim i suvremenim tehnološkim postupkom. Određena je dinamika ekstrakcije ukupnih antocijanina, ukupnih fenola, niskomolekularnih proantocijanidina i visokomolekularnih proantocijanidina tijekom maceracije. Predloženi su matematički modeli za svaki eksperimentalno mjereni parametar. Modeli predstavljaju promatrane vrijednosti ovisno o tradicionalnom ili suvremenom postupku. Vrijeme, izraženo u danima, predstavlja ulaznu varijablu za oba promatrana modela. Predloženi modeli pokazuju signifikantnu pozitivnu korelaciju i time potvrđuju pretpostavku da vrijeme trajanja postupka te tehnološki postupak maceracije imaju značajan utjecaj na mjerene varijable (R=0,83–0,98).