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Optimization of alcoholic fermentation parameters for plum wine production

Optimierung der Alkoholgärungsparameter für Pflaumenweinproduktion

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Summary

The plum (*Prunus domestica* L.) is the most common fruit crop in Serbia, with chemical composition that renders it an attractive substrate for wine fermentation. In order to ensure the best quality characteristics of plum wine it is necessary to investigate and optimize fermentation parameters which mainly influence vinification process. Hence, this study aims to investigate wine production from plum pomace by optimizing fermentation temperature, pH and dose of pectinase applying response surface methodology, Box-Behnken experimental design, as well as desirability function approach. According to the high coefficients of determination (R^2 >90%) and statistical significance (*p* <0.05), all the models were adequate and suitable. Quality parameters used for assessing the efficiency of optimization were wine yield and the contents of ethanol, methanol and glycerol. Sensory analysis indicated that plum wine produced under the optimal conditions was good in terms of overall acceptability.

Keywords: essential oils, antibacterial activity, chemical composition

Zusammenfassung

Die Pflaume (*Prunus domestica* L.) ist die häufigste Obstpflanze in Serbien. Ihre chemische Zusammensetzung macht sie zu einem attraktiven Substrat für die Weingärung. Um die besten Qualitätsmerkmale des Pflaumenweins zu gewährleisten, müssen die Gärparameter untersucht und optimiert werden, die hauptsächlich den Weinbereitungsprozess beeinflussen. Daher zielt diese Studie darauf ab, die Weinproduktion aus Pflaumentrester zu untersuchen, indem die Fermentationstemperatur, der pH-Wert und die Dosis der Pektinase unter Anwendung der Antwortoberflächenmethode, des experimentellen Box-Behnken-Designs sowie des Ansatzes der Erwünschtheitsfunktion optimiert werden. Aufgrund der hohen Bestimmungskoeffizienten (R^2 >90%) und der statistischen Signifikanz (*p* <0,05) waren alle Modelle adäquat und geeignet. Qualitätsparameter, die zur Beurteilung der Effizienz der Optimierung herangezogen wurden, waren die Weinausbeute und die Gehalte an Ethanol, Methanol und Glycerin. Die sensorische Analyse ergab, dass der unter optimalen Bedingungen hergestellte Pflaumenwein eine gute Akzeptanz aufweist.

Schlüsselwörter: Pflaume, Wein, Fermentationsparameter, pektolytisches Enzym, Optimierung

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Introduction

Wine, one of the oldest alcoholic beverages, is a product of alcoholic fermentation of the grape juice or any fruit juice with a good proportion of sugar and acids. Most commercially produced wines are usually made from fermented grapes. However, fruit wines have recently been gaining consumer's interest and their production has been steadily growing. Apples, pears and oranges have been widely used, but several other fruits have the potential for use in wine production and many research groups have investigated the suitability of different fruits like banana (Idies & Odum, 2011), mango (Reddy & Reddy, 2011), cocoa (Dias et al., 2007), pineapple (Dellacassa et al., 2017), kiwi (Soufleros et al., 2001), gabiroba (Duarte et al., 2009), etc. The plum (*Prunus domestica* L.) is the most common fruit crop in Serbia. Besides the direct consumption, plums were used also for drying, freezing, processing and production of distillates (Nenadović-Mratinić et al., 2007). The previous researches and reported analysis of chemical composition of plum wine produced from three Serbian varieties prove that the plum (*Prunus domestica* L.) is a suitable fruit for the production of good quality wine (Miljić & Puškaš, 2015; Miljić et al., 2017a) Also, the antioxidative, antimicrobial and antiproliferative activities of plum wines have been emphasized (Miljić et al., 2015; Miljić et al., 2017b).

Generally, it is well known that the different factors affect the alcoholic fermentation and final composition of wine. The temperature is a variable that directly affect the yield of ethanol and fermentation byproducts, sensitivity of yeasts to alcohol concentration, growth rate, rate of fermentation, viability, length of lag phase, enzyme and membrane function, etc. (Morata et al., 2006; Beltran et al., 2008) Another significant variable, the must pH, ranging from 2.80 to 4.25, is also considered an important factor for the yeast growth and ethanol production (Walker, 1998). On other hand, the fruit wine technology is characterized by the specificity of the raw materials used. Differences in physicochemical characteristics consequently require the modifications of processing conditions prior and during the alcoholic fermentation. Numerous problems such as low juice yield, difficulties with pressing, extraction, maceration, slow juice clarification, and color changes in the final product are affecting the production of fruit wine (Jagtap & Bapat, 2015; Claus & Mojsov, 2018). The most effective solution to these technological problems is probably pre-treatment of fruit pomace with various enzymes in order to degrade pectin substances and increase the extraction of various biologically and nutritionally important components in the juice (Chauhan et al., 2001; Sandri et al., 2011). Hence, temperature, pH of pomace and dose of pectolytic enzyme must be studied with more detail, especially their influence on vinification and quality of wine produced.

Response surface methodology (RSM) is a collection of statistical techniques for experiment designing, model developing, factors evaluating and optimum conditions searching based on the minimum number of experiments. It has been widely applied to many biotechnological areas (Steinberg & Bursztyn, 2010; Kumar et al., 2009). The aim of the present study was to optimize the fermentation process parameters, temperature, pH and pectinase dose, to achieve the best quality of plum wine by employing RSM statistical approach and Box-Behnken experimental design. Furthermore, the effect of independent variables and their interaction on the selected wine quality parameters, such as yield and the contents of ethanol, methanol and glycerol, was evaluated.

Experimental

Plum pomace preparation and fermentation

Plum variety Čačanska lepotica (*P. domestica* L.) was used as raw material for fruit wine production in this study. Čačanska lepotica is a mid-early ripening variety. It represents one of the best and most abundant plum varieties grown in Serbia. The plums for this research were procured at commercial maturity in early August 2013 from the local market in Novi Sad, Serbia. After the pits were manually removed plums were subjected to crushing. Obtained pomace was treated with $K_2S_2O_5$ (SO_2 level was set to 50 mg SO_2/kg pomace), to prevent contamination and oxidation processes. In agreement with the defined aim of study and the applied experimental design doses of commercial pectinase Lallzyme-oe (Lallemand S.A., St. Simon, France) were varied. Selected doses of pectolytic enzyme were in the range recommended by the manufacturer's instructions. The plum juice sample was extracted by passing through cheesecloth and then subjected to analysis of total and reducing sugars, total acidity, pH and fermentable nitrogen, as described by Miljić et al. (2014).

The microvinification was carried out with 1 kg of plum pomace placed in the glass jar fitted with a fermentation bung for CO_2 release. Inoculation was performed with 0.25 g/kg of previously rehydrated commercial wine yeast *Saccharomyces cerevisiae* (Anchor WE372, South Africa). In order to determine the optimum pH and temperature, the experiments were carried out according the Box-Benkhen experimental plan by incubating the appropriate number of inoculated jars (prepared as above) at different pH values and temperatures. The adjustment of pH was carried out by means of mixture solution of malic, citric and tartaric acid (1:1:0.5, respectively) and calcium carbonate. Wine was passed through the cheesecloth when the fermentation was finished. SO_2 level was adjusted to 50 mg/L and the wine was poured into 500 mL bottles, closed with screw caps and kept at 12–13 °C in the absence of light. Wine yield and the contents of ethanol, methanol and glycerol were determined in obtained samples. Moreover, after two months, during which clarification and stabilization processes took place, young plum wines were subjected to sensory analysis.

Analytical methods

Ethanol and methanol content in wine samples were determined by gas chromatography, using an HP 5890 Series II GC (Agilent Technologies Inc, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and Carbowax 20 M column. Chromatography conditions were set according to the previously described procedure (Miljić & Puškaš, 2014). Glycerol was estimated by the enzymatic method (Wieland, 1988), using commercially available glycerol assay kit (Megazyme, Ireland).

Experimental design

The response surface methodology based on the Box-Behnken experimental design was used for the optimization of fermentation conditions (temperature and pH value) and dose of pectinase for plum wine production. The independent variables and their varied levels were: X_1 – temperature (16, 23, 30°C), X_2 – pH of pomace (3.5, 4.0, 4.5) and X_3 – dose of pectinase (0, 1, 2 g/100kg). In this regard, according to the Box-Behnken experimental design with three factors at three levels and five repetitions in the central point (n_0=5) a set of 17 experiments was carried out. All the experiments were done in triplicate. The relations between the independent variables and the responses Y (ethanol content (vol. %), methanol content (mg/L), glycerol content (g/L) and wine yield (%)) were determined by the second-order polynomial equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \quad (1)$$

where Y is the predicted response, X_1, X_2 and X_3 correspond to the independent variables, b_0 is intercept, b_1, b_2 and b_3 are linear effects, b_{11}, b_{22} and b_{33} are squared effects and b_{12}, b_{23} and b_{13} are interaction effects of the factors. The adequacy of the model was evaluated by coefficient of determination (R^2) and model *p*-value. The significance of regression coefficients was assessed by p-values at the 0.05 significance level. The statistical software package STATISTICA 10.0 (StatSoft, SAD) was used for the regression analysis of the experimental data, and also to generate the response surface graphs. The method of desirability function was applied for the determination of optimal values of examined variables (Design-Expert 8.1). In order to fulfill date for the optimization of plum wine production a descriptive sensory analysis of produced wines was carried out after the end of alcoholic fermentation.

Results and Discussion

Characteristics of plum pomace

For the purpose of this research the physicochemical characteristics of the base plum pomace were analyzed in terms of the total sugar, total acidity and fermentable nitrogen concentration and obtained values were 152.2 g/kg, 7.8 g/L and 380.0 mg/L, respectively. The initial pH of plum pomace was 3.45.

Mathematical models

In order to ensure the best quality characteristics of fruit wine it is necessary to investigate and optimize fermentation parameters which have the greatest influence on the production process. Temperature and pH are critical parameters that affect the production of wine in general. Enzymes are commonly used in winemaking, especially for the pomace preparation when fruit wine are produced. The main application of enzyme use in winemaking is to reduce the impact of the long chain compounds (polysaccharides – pectins) on different production stages. So, in this study the influence of temperature (X_1), pH (X_2) and dose of pectinase (X_3) on selected responses, ethanol content (Y_1), methanol content (Y_2), glycerol content (Y_3) and wine yield (Y_4) were investigated using response surface methodology (RSM) and Box-Behnken experimental design.

The Box-Behnken experimental design was chosen for the optimization not only because of its reduced number of experiments, but also because in BBD all design points fall within the safe operating zone. Also, Box-Behnken design has the maximum efficiency for an RSM problem involving three factors and three levels. The results are summarized in Table 1.

Based on the results of experiments formulated by the Box-Behnken design and regression analysis, quadratic polynomial equations were established to identify the relation between the selected responses and examined factors (Table 2). The mode of interactions between the examined factors is indicated by the regression equations coefficients.

A positive sign for the values of coefficients of interaction indicates a synergistic effect, while a negative sign represents an antagonistic effect of the factors on the selected response. The fitness of the model was checked and confirmed by the coefficient of determination (R^2). The R^2 value closer to 1 denotes better correlation between the observed and predicted values. The relatively high values of the determination coefficient (R^2>0.95) obtained for all responses (Table 2) indicate a good fit of the experimental data to second-order polynomial equation.

The adequacy and significance of the quadratic model was checked using the analysis of variance (ANOVA) which was tested using Fisher's statistical analysis. The *p*-values serve as a tool for checking the significance of the obtained model or each coefficient of the regression

TABLE 1: *Box-Behnken experimental design and responses.*

Temperature (°C) X_1	pH X_2	Pectinase (g/100 kg) X_3	Ethanol content (% v/v) Y_1	Methanol content (mg/L) Y^2	Glycerol content (g/L) Y_3	Wine yield (%) Y_4
16	3.5	1	7.1	705	3.5	46
16	4.5	1	7.12	745	4.8	49
16	4.0	2	7.41	850	4.37	53
16	4.0	0	6.82	299	4.51	37
23	4.0	1	7.56	912	5.4	51
23	4.0	1	7.52	903	5.62	48
23	4.5	0	7.3	499	5.74	42
23	4.5	2	7.8	1042	5.99	59
23	4.0	1	7.67	900	5.57	53
23	4.0	1	7.54	907	5.54	51
23	4.0	1	7.48	887	5.31	52
23	3.5	0	7.3	520	4.8	43
23	3.5	2	7.85	970	5.17	54
30	3.5	1	7.62	865	5.35	50
30	4.5	1	7.7	955	6.67	52
30	4.0	0	7.35	409	5.8	42
30	4.0	2	8.0	1098	6.06	57

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TABLE 2: *Second-order polynomial models for investigated responses (Y_{1-4}); X1: temperature (°C); X_2: pH; X_3: pectinase (g/100 kg); Y_1: ethanol content (vol. %); Y_2: methanol content (mg/L); Y_3: glycerol content (g/L); Y_4: wine yield (%); R^2: determination coefficient.*

Responses	Model	R^2
Ethanol	$Y_1 = + 5,244 + 0,1792X_1 - 0,2628X_2 + 0,3031X_3 + 0,0071X_1X_2 + 0,0036X_1X_3 - 0,025X_2X_3 - 0,0037X_1^2 + 0,17X_2^2 + 0,0043X_3^2$	0,979
Methanol	$Y_2 = - 36,8781 + 77,3602X_1 - 234,593X_2 + 277,318X_3 + 3,571X_1X_2 + 4,929X_1X_3 + 46,5X_2X_3 - 1,8168X_1^2 + 18,9X_2^2 - 148,775X_3^2$	0,989
Glycerol	$Y_3 = - 10,3692 + 0,4038X_1 + 3,8101X_2 - 0,0381X_3 + 0,0014X_1X_2 + 0,0143X_1X_3 - 0,06X_2X_3 - 0,0066X_1^2 - 0,336X_2^2 + 0,021X_3^2$	0,971
Wine yield	$Y_4 = + 25,319 + 2,4847X_1 - 7,107X_2 - 0,3036X_3 - 0,0714X_1X_2 - 0,0357X_1X_3 + 3X_2X_3 - 0,0408X_1^2 + X_2^2 - 1,75$	0,970

TABLE 3: *Analysis of variance (ANOVA) for the experimental results; X_1: temperature (°C); X2: pH; X_3: pectinase (g/100kg); Y_1: ethanol content (vol. %); Y_2: methanol content (mg/L); Y_3: glycerol content (g/L); Y_4: wine yield (%); *significant at p < 0.05; **not significant.*

	F-value				*p*-value			
	Y_1	Y_2	Y_3	Y_4	Y_1	Y_2	Y_3	Y_4
Model	36.26	73.91	25.90	25.59	< 0.0001*	< 0.0001*	0.0001*	0.0002*
X1	136.86	52.82	151.99	14.22	< 0.0001*	0.0002*	< 0.0001*	0.0070*
X2	0.071	3.27	64.96	4.50	0,798**	0.1137**	< 0.0001*	0.0716**
X3	156.15	496.98	1.85	193.39	< 0.0001*	< 0.0001*	0.2155**	< 0.0001*
X1X2	0.57	0.50	0.0027	0.11	0.4756**	0.5030**	0.9599**	0.7486**
X1X3	0.57	3.80	1.08	0.11	0.4756**	0.0924**	0.3325**	0.7486**
X2X3	0.11	1.72	0.098	4.0	0.718**	0.2306**	0.7639**	0.0856**
X_1^2	31.98	26.61	11.97	7.49	0.0008*	0.0013*	0.0105*	0.0291*
X_2^2	0.017	0.075	0.80	0.12	0.8993**	0.7922**	0.3995**	0.7424**
X_3^2	0.017	74.31	0.050	5.73	0.8993**	< 0.0001*	0.8290**	0.0479*

equations. The models with *p*-values lower than 0.05 is significant at a confidence level of 95%. The F- and *p*-values of the models shown in Table 3, suggest that the models were statistically significant at the confidence level higher than 97% which implies that at least 97% of the variability in the response could be explained by the second-order model equations.

Regression coefficients (b_0, b_1, b_2… b_{13}) were used to generate response surface plots in order to investigate the interaction among the temperature, pH and pectinase dose and to illustrate the effects of each factor and their interaction on the selected responses. In the response surface plots, two factors varied when the third factor was kept at a fixed level (zero level).

Effects of fermentation temperature, pH and dose of pectinase

The influence of temperature, pH values and the dose of pectolytic enzyme on ethanol content in plum wine of the Čačanska lepotica variety is shown in Figure 1 a–c. As it can be seen from the response surface plot, the increase of the fermentation temperature resulted in the increase of ethanol content in whole investigated range of pH value. Ribéreau-Gayon et al. (2006) pointed out that an increase in the fermentation temperature (25–30°C) leads to a reduction in the amount of energy required for yeast growth, so a higher amount of fermentable sugar remains available for ethanol production (Ribéreau-Gayon et al., 2006). Also, the content of ethanol increased with the increase of the pectinase concentration. These results could be considered as a consequence of enzymatic activity, which led to the pectin hydrolysis and increase in reducing sugars (Joshi et al., 1991). Fermentation of plum pomace at different pH values (3.5–4.5) did not show significant differences in the ethanol content in the produced wines. However, by lowering the pH of the pomace, the duration of the fermentation was prolonged. The obtained results could be explained by the fact that growth and fermentative activity of yeast are less pronounced at lower pH. This is in line with the literature data that point out the most optimal pH for the yeasts growth is in the range of 4.5–6.5 (Walker, 1998). In the acidic environment (pH<4.0), yeast cells were exposed to chemical stress and required more time to adapt to environmental conditions and therefore the beginning of ethanol synthesis was slower. According to the predictions of the model, the ethanol content was maximum (about 8.0 vol. %) at maximum values of temperature (30°C) and maximum dose of pectolytic enzyme (2 g/100kg), regardless of the pH of pomace. The high R^2 value (0.979) indicated that the data were close to the predicted values from the model (Y_1). The analysis of the variance confirmed the adequacy and significance of the Y_1 model (p<0.05). The model terms X_1, X_3 and X_{12} are significant at 0.05 level (p<0.05), while other terms, including pH, in the equation do not have a significant effect on ethanol content (Table 3). The regression coefficients of the statistically significant terms of the model (Table 2) indicate that the ethanol content was positively affected by the temperature and dose of pectinase, and negatively by the quadratic terms of temperature.

Figure 2 a–c illustrates the effects of examined factors on the methanol content in plum wine. The obtained plot shows that the concentration of methanol in wine significantly increased with the increase of fermentation temperature, while the application of different pH values from the experimental plan of the investigated range did not have a significant effect on the formation of this wine component. Also, it is noticed that the synergistic effect of plum's pectin methyl esterase and applied commercial enzyme is not reduced by reducing the pH value, despite the fact that the optimum value of this parameter for the activity of pectin methyl esterase is about 4.5 (Lozano, 2006). Since the optimal temperature for the activity of pectin methyl esterase is in the range of 45–55°C (Remize et al., 2000), the lowest concentration of methanol was in wine that fermented at the lowest temperature (16°C). By increasing the fermentation temperature at 30°C, its concentration increased up to 40%. The use of pectolytic enzyme led to a significant increase in methanol concentration due to more intense pectin hydrolysis and the release of methoxy groups. The application of pectinase in the amount of 2 g/100 kg resulted in the three times more methanol formation in produced wine compared to a fermentation which did not include enzymatic treatment of pomace. From the regression model (Y_2) of methanol concentration, the obtained R^2 value of 0.989 indicates that even 98.9% of the variability in the response could be explained by the model. The resulting model also proved to be adequate and reliable (p<0.05). The results of the ANOVA test (Table 3) show that in the fermentation of plum wine, the linear and quadratic terms of the temperature and dose of pectinase (X_1, X_3, X_{12} and X_{32}) had a statistically significant (p<0.05) influence on the formation of methanol predicted by the Y_2 model. On the other hand, linear and quadratic terms of pH value, as well the interactive model term of temperature and pH did not depict significant impacts on the methanol production. The largest, both the linear and the quadratic effect on this response had a dose of pectolytic enzyme (the resulting p-value is less than 0.0001). A smaller, but also statistically significant effect on the concentration of methanol had a fermentation temperature (p=0.0002). According to the regression coefficients of statistically significant terms of the fitted model (Table 2) production of methanol was positively affected by the linear effects of temperature and dose of pectinase, while quadratic terms of these two factors had a negative influence.

The effects of temperature, pH value and pectinase dose on glycerol content in plum wine are presented in the Figure 3 a–c. It can be noticed (Figure 3) that glycerol content increased with the increase of fermentation temperature and pH of pomace. These observations are also confirmed by the results of the preliminary screening of the effect of fermentation conditions on the quality of plum wines (Miljić & Puškaš, 2014), as well as numerous scientific studies (Remize et al., 2000; Kumar et al., 2009; Reddy & Reddy, 2011). Plum wines whose fermentation was kept at pH 4.5 contain 25–35% more glycerol compared to samples fermented at a lower pH (3.5). The production of this compound was about 30% higher at a temperature of 30°C compared to temperature of 16°C. The optimal temperature for the glycerol production during wine fermentation is 25–30°C. Also, it is reported that fermentation at pH value of 6.0–6.5 results in the production of wines with 40–50% more glycerol compared to fermentation at pH 3.5–4.0 (Yalcin & Yesim Ozbas, 2008). The use of different doses of pectolytic enzyme (0–2g/100 kg) did not significantly affect the content of

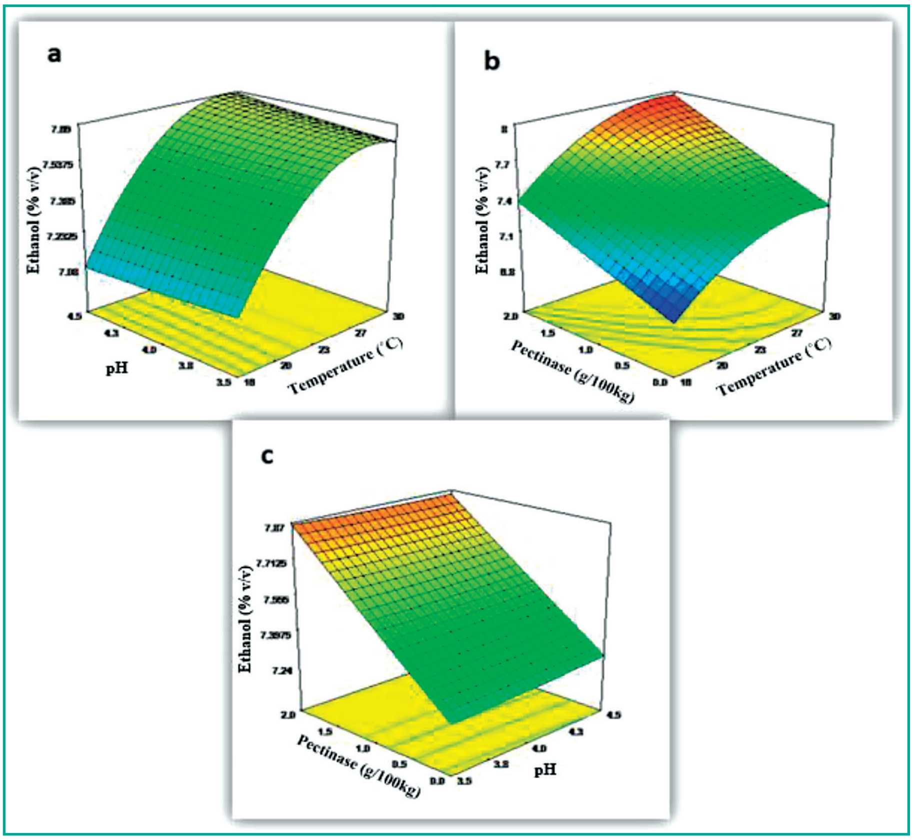

FIGURE 1: *Response surface plots of the interaction of a) pH-temperature (pectinase = 1.0 g/100kg), b) pectinase-temperature (pH = 4) and c) pectinase-pH (temperature = 23 °C), and their influence on ethanol content.*

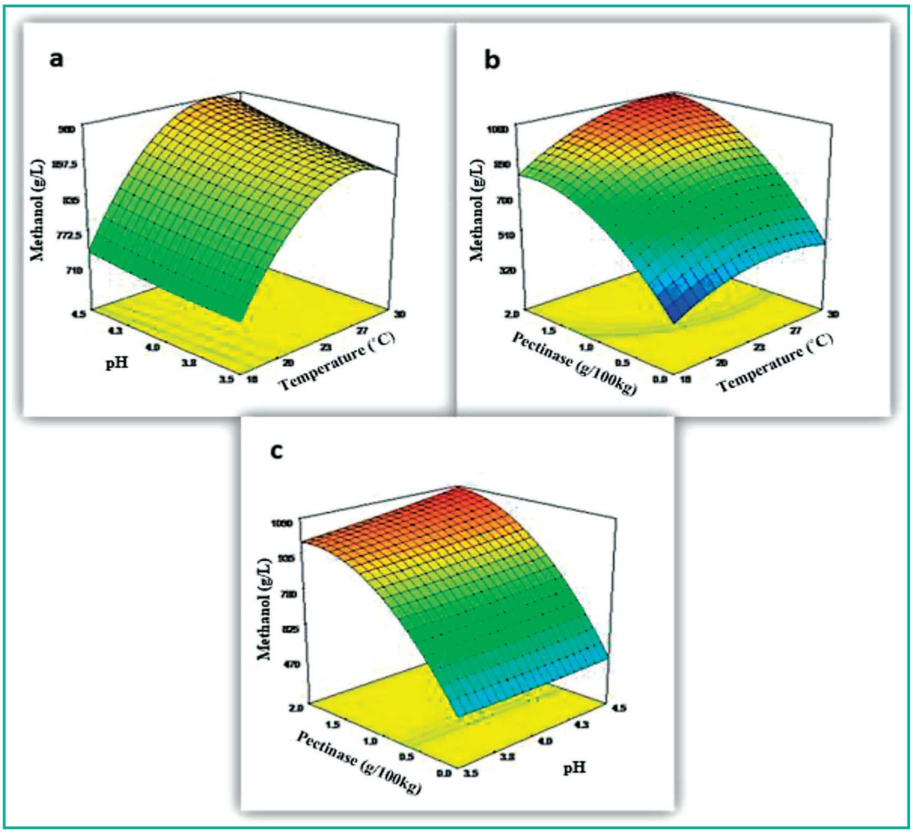

FIGURE 2: *Response surface plots of the interaction of a) pH-temperature (pectinase = 1.0 g/100kg), b) pectinase-temperature (pH = 4) and c) pectinase-pH (temperature = 23 °C), and their influence on methanol content.*

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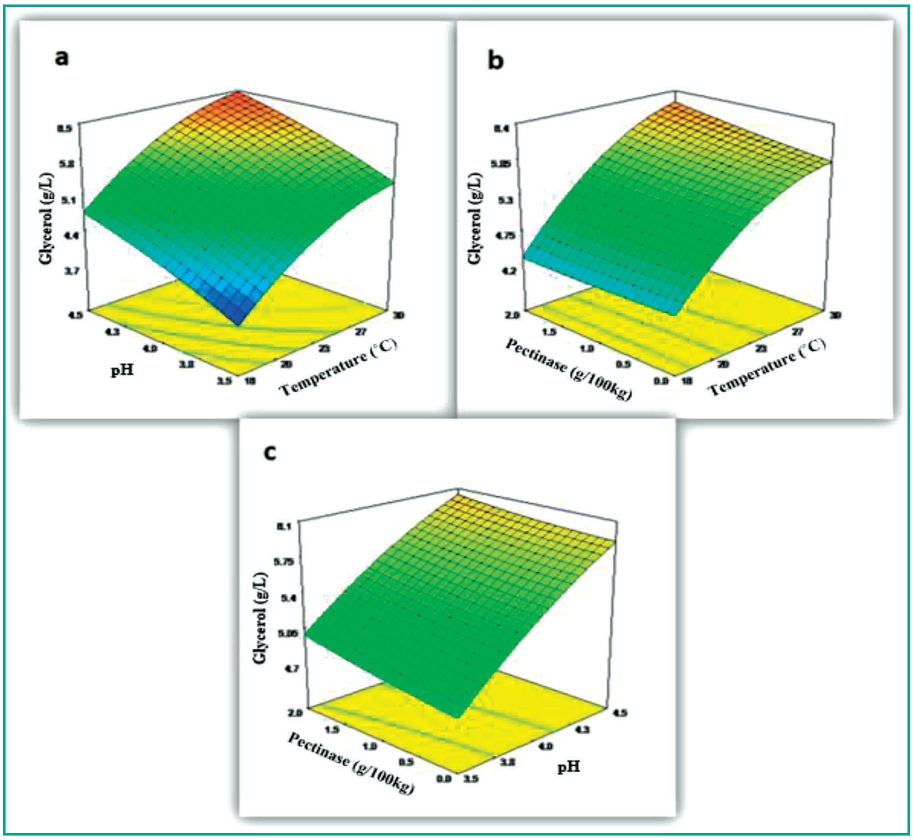

FIGURE 3: *Response surface plots of the interaction of a) pH-temperature (pectinase = 1.0 g/100kg), b) pectinase-temperature (pH = 4) and c) pectinase-pH (temperature = 23 °C), and their influence on glycerol content.*

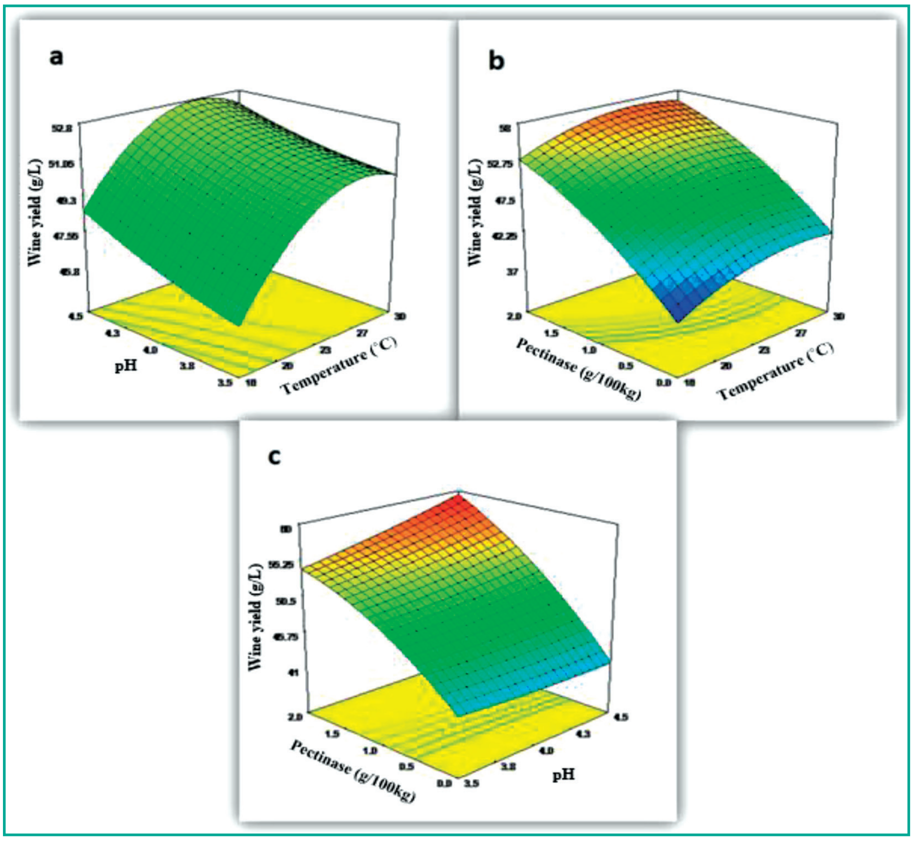

FIGURE 4: *Response surface plots of the interaction of a) pH-temperature (pectinase = 1.0 g/100kg), b) pectinase-temperature (pH = 4) and c) pectinase-pH (temperature = 23 °C), and their influence on wine yield.*

this component in plum wines. The obtained model (Y_2) for glycerol content, with a determination coefficient R^2=0.971 is proved as significant (*p*=0.0001), with only 2.9% of the total variations not explained by the model. Among the model terms, linear and quadratic effects of temperature and linear effect of pH (X_1, X_2, X_{12}) are significant with the probability of 95% (p<0.05), while other terms of the fitted second order equation for the glycerol content were not statistically significant (p>0.05). The linear effects of temperature and pH (p<0.0001) had the most significant influence on this response. The regression coefficients of statistically significant terms of the obtained model for glycerol content (Table 2) indicated that the linear effects of temperature and pH contributed positively to glycerol content. On the other hand, the quadratic effect of temperature negatively affected the production of this compound.

The response surfaces (Figure 4 a–c) show the influence of the temperature, pH value and the pectinase concentration on the plum wine yield. It can be noticed, from the obtained plots, that the yield of wine increased with the increase of fermentation temperature and pectinase concentration, while influence of pH values from investigated range did not show significant changes in the wine yield. As already emphasized, the activity of pectinase increased at higher temperatures of fermentation (25–30°C), which led to the more intense pectin hydrolysis and increase in the yield of plum wine. The application of 2 g of pectinase/100 kg of pomace, during fermentation at different temperatures (16–30°C) resulted in an increase in wine yield for 35–40% compared to a fermentation without enzymatic pomace treatment. The value of the determination coefficient (R^2=0.97) showed that 97.0% of the response variation was related to the variation of the independent variables and that the model Y_3 was highly significant and suitable (p<0.05) for representation of the real relationship between these variables. The effects of linear and quadratic terms of temperature and pectinase dose (X_1, X_3, X_{12} and X_{32}) were significant (p<0.05). The influence of quadratic term of the temperature and pectinase concentration was less pronounced (p-values 0.0291 and 0.0479, respectively).

The yield of plum wine was positively affected by the linear effects of temperature, while quadratic term of the temperature and linear and quadratic terms of the pectinase concentration had a negative influence (Table 2).

Optimization

In this study, the optimization of the fermentation parameters (temperature and pH) and dose of pectinase for the plum wine production was accomplished using a multiresponse method called desirability function. This method involves the transformation of each response variable (Yi) to an individual function of desirability (di). The values of *di* vary in the interval from 0 to 1, increasing as the desirability of the corresponding response increases. The individual desirability values are combined using the geometric mean to give an overall desirability, D. The factor settings with maximum overall desirability are considered to be the optimal parameter conditions (Derringer & Suich, 1980; Popov et al., 2010). According to the general winemaking practices the goals of the optimization set were to maximize ethanol content, glycerol content and wine yield and to minimize methanol content. The results of conducted wine sensory analysis were used to additionally define the investigated range of the examined fermentation parameters in the optimization process. The best sensory characteristics possessed the wines produced at the lowest pH (3.5) from the investigated range, which is also the typical pH of the Čačanska lepotica variety.

Fermentation at higher pH (pH>4) had a negative effect on the sensory characteristics of the obtained wines and caused microbial spoilage of a large number of samples. On the other hand, fermentation at temperatures higher than 25°C resulted in the production of wines with negative aromatic characteristics. Therefore, a higher degree of significance was assigned to fermentation at lower pH values (pH <4.0) and temperature (lower than 26°C) in the optimization procedure.

According to the model predictions for the highest value of desirability function (0.823) the optimal values of the fermentation temperature, pH and pectinase dose were 25˚C, 3.5 and 0.5 g/100kg, respectively. By applying such process condition during the fermentation of plum pomace, the model ensure production of 7.5 vol. % of ethanol, 710.0 mg/L of methanol, 5.0 g/L of glycerol and 48.0% of plum wine yield. In order to check and validate the obtained models a new series of repeated vinifications (triplicate set) were carried out with the optimum fermentation conditions. On average, the experimentally obtained values were: 7.7% v/v of ethanol, 4.67 g/L of glycerol, 683 mg/L of methanol and wine yield of 48%. These results show that the experimentally determined values were in good agreement with the statistically predicted values for all modeled responses, which confirmed the adequacy of the model.

Conclusion

In this study statistical methodology was employed to define the optimal fermentation parameters for wine production from plum variety Čačanska lepotica. Mathematical models and optimal conditions for plum wine production were determined leading to maximal wine yield, ethanol and glycerol concentration and minimal methanol concentration. It was shown that changes in ethanol, methanol, glycerol and wine yield during fermentation are well described by the obtained second order equations, according to the high coefficients of determination (R^2>90%) and statistical significance (p<0.05). From the obtained results it can be concluded that the fermentation temperature had the most significant influence on the observed responses as well on the quality of produced wine. It is also confirmed that the optimum pH and dose of pectinase are very important to produce good quality plum wines. The developed model predicts getting wine yield of 48%, 7.5 vol. % of ethanol, 710.0 mg/L of methanol and 5.0 g/L of glycerol in plum wine produced under the following optimized condition: fermentation temperature of 25˚C, pH of 3.5 and pectinase dose of 0.5 g/100 kg. The wine produced under the optimized fermentation conditions was found to be maximum score of overall acceptance in term of aroma, taste and appearance.

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Conflict of interest

The authors declare that they have no conflict of interest.

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