Production of Blackberry Wine by Microfermentation using Commercial Yeasts Fermol Rouge® and Fermol Mediterranée®

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Summary

The aim of this paper was to determine the enological traits of two commercial yeasts (Fermol Rouge® and Fermol Mediterranée®) in a small scale and to evaluate the possibility of their application in commercial production of blackberry wine. Fermentation activity was monitored by measuring CO_2 evolution and CO_2 production rate during microfermentation of blackberry juice performed at 23°C. Blackberry wines produced by two different yeasts were analyzed in order to compare their composition differences. Fermentations were carried on to complete sugar consumption by both yeast strains. Levels of volatile acids formed by the two yeasts were significantly different and differences in concentrations of residual sugars, malic acid, lactic acid and pH-value were highly significant. There were no significant differences between concentrations of ethanol, total acids and glycerol in blackberry wines produced by both yeasts. Chemical composition of the produced blackberry wines was in accordance with the Croatian fruit wine legislation. Good fermentative properties and low potential of H_2S production of both commercial yeasts could be beneficial for blackberry wine production.

Key words

blackberry wine, fermentation, wine yeast, yeast selection

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Introduction

Blackberry wine is a popular fruit wine and traditional product in the continental part of Croatia. It is usually served in moderate quantities as a dessert wine and recognized as a natural source of essential minerals and many bioactive phytochemicals that can play an important role in health promotion and disease prevention. Traditionally, blackberry wine has been used as a popular medicine for anemia and iron deficiency, as well as for alleviating sleep disorders. Daily consumption of about 250 mL of blackberry wine is recommended as a source of essential minerals (Amidžić, 2011; Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2011b).

Previous research on blackberry wine is somewhat scarce. So far the published research conducted by different authors from different geographic regions have dealt with wine composition (Orser and Yang, 1966; Martin et al., 1971; Wrolstad et al., 1980; Pinhero and Paliyath, 2001; Yulin et al., 2007; Joh, 2010; Arozarena et al., 2012; Johnson and Gonzalez de Mejia, 2012), color and pigments (Wrolstad, 1976; Kalkan Yildirim, 2006; Arozarena et al., 2012) antioxidant activities (Heinonen et al., 1998; Pinhero and Paliyath, 2001; Kalkan Yildirim, 2006; Joh, 2010; Arozarena et al., 2012; Johnson and Gonzalez de Mejia, 2012), calmodulin-inhibitory effects (Pinhero and Paliyath, 2001), as well as effects of processing and storage on anthocyanin composition, color and appearance (Rommel, 1988; Rommel et al., 1992). Bish (2011) investigated inactivation of foodborne pathogens by blackberry wines. Influence of fermentation process on aroma composition (Wang et al., 2012), phenolics, antioxidant capacity, and volatiles in blackberry wines (Gao et al., 2012), as well as effects of yeast strains, with different fermentation properties, on volatile compounds in blackberry wine (Orser and Yang, 1966) were also investigated.

The Croatian blackberry wines are explored only recently and the literature published is very scarce. Previous research mostly covers different compounds present in blackberry wines such as minerals and heavy metals, methanol and polyphenols causing antioxidant activity and vasodilation effects (Amidžić, 2011; Amidžić Klarić et al., 2011a; Amidžić Klarić et al., 2011b; Mornar et al., 2011; Mudnić et al., 2012).

Selection of a good yeast strains having desirable properties, especially fermentative ones, is prerequisite for quality wine production. One of the major discoveries in wine production is better fermentation control using selected yeast strains (Degree, 1993; Zironi et al., 1993; Ciani and Picciotti, 1995; Gil et al., 1996; Romano 1997; Raineri and Pretorius, 2000; Petravić Tominac et al., 2005a; Petravić Tominac et al., 2005b; Fugelsang and Edwards, 2007; Petravić Tominac et al., 2008, Krieger-Weber, 2009; González et al, 2011). Enological traits of wine yeasts have been divided in two groups, i.e. technological and qualitative. Both groups of traits have to be considered when selecting the wine yeasts (Raineri and Pretorius, 2000). Technological traits influence the fermentation efficiency, while qualitative traits determine the chemical composition and sensorial characteristics of wines. Determination of these characteristics is very important since different strains have various traits. Technological traits can be evaluated by observing the fermentation progression and quantitative traits by determining the chemical composition of wine after fermentation. Sensorial analyses are used to complete the characterization of the yeast strains.

Until now, the evaluation of yeast strains for blackberry wine production was not conducted in Croatia. Therefore, the aim of this paper was to determine enological traits of two commercial yeasts in a small scale and to evaluate the possibility of their application in commercial production of blackberry wine. Fermentation activity was monitored by measuring CO_2 evolution and CO_2 production rate during microfermentation of blackberry juice performed at 23°C. Blackberry wines produced by two different yeasts were analyzed to compare the composition differences. Potential of H_2S production of the two yeasts was also assessed.

Material and methods

Microorganisms

The two investigated strains were commercial wine yeasts Fermol Rouge® and Fermol Mediterranée® (denominated in text as FR and FM, respectively), manufactured by AEB s.p.a. Brescia, Italy.

Fermentation medium

Commercially available blackberry juice, ecologically produced in 2009, was pasteurized before distribution at the market (producer: OPG Završki Darda from Baranja, eastern Croatia) and used as a fermentation medium. The juice contained 71.63 g L^{-1} of reducing sugars, 0.10 % of ethanol, 8.65 g L^{-1} of total acids, 0.3 g L^{-1} of volatile acids and the pH was 3.36. The juice was supplemented by 130 g L^{-1} of sucrose and sulfited by 30 mg L^{-1} of potassium metabisulfite. Before fermentation the juice contained 179.37 g L^{-1} of total sugars i.e. 109.86 g L^{-1} of sucrose, 38.03 g L^{-1} of glucose and 31.48 g L^{-1} of fructose.

Fermentation tests

A volume of 330 mL of sulfited blackberry juice was transferred in 500-mL Erlenmeyer flasks and inoculated with 0.33 g of commercial dry yeasts. Flasks were closed with fermentation locks and left to ferment at average fermentation temperature of 23°C. All fermentations were carried out in triplicate. Fermentation progress was evaluated by the weight loss caused by CO_2 production and flasks were weighed at 24-hour intervals using digital scale GM1502 (Sartorius, Germany) (range 0.01 - 1500 g). Yeast fermentation profiles were monitored through CO_2 evolution and CO_2 production rates as described in literature (Dittrich, 1987; Bely et al., 1990; Castellari et al., 1995; Rainieri et al., 1998; Romano et al., 1998; Guerra et al., 1999; Petravić Tominac et al., 2005a; Petravić Tominac et al., 2005b; Petravić Tominac et al., 2008).

Additional Erlenmeyer flask, containing water, served as a control to correct evaporation loss that was included in mass balance. Total evaporation losses throughout the whole experiment were up to 3.44 %. At the end of fermentation (stable weight for four days), the centrifugation was performed (6000 rpm / 4°C / 10 min) using Model J-21B Centrifuge, Beckman, with rotor JA-10. The blackberry wine was kept in the refrigerator for two days and then analyzed. Wines produced by microfermentation using yeasts Fermol Rouge® and Fermol Mediterranée® were denominated as wine FR and wine FM, respectively.

Numerical calculation

CO_2 evolution was calculated according to equation 1:

$$m = m_1 - m_2 \quad (1)$$

where:
m = mass of CO_2 released [g];
m_1 = mass difference between two weighings of fermentation flasks [g];
m_2 = mass difference between two weighings of control flask (containing water) [g].

CO_2 production rate was calculated according to equation 2:

$$\frac{dCO_2}{dt} = \frac{\Delta m}{V \times \Delta t} \quad (2)$$

where:
dCO_2/dt = CO_2 production rate [g $L^{-1}day^{-1}$];
Δm = mass of CO_2 released during time interval Δt [g];
Δt = time interval between two measurings [day];
V = volume of medium [L].

Analytical methods

Reducing sugars, ethanol, total acids, volatile acids and pH were analyzed according to standard wine analyses as described in literature (Ough and Amerine, 1998). The concentrations of glucose, fructose, sucrose and ethanol were determined by high-performance liquid chromatography (HPLC) using Shimadzu Class-VP LC-10AVP system (Shimadzu, Kyoto, Japan) with Supelcogel H precolumn (5 cm × 4.6 mm, i.d. 9 mm; Sigma-Aldrich, USA), Supelcogel C-610H column (30 cm × 7.8 mm, i.d. 9 mm; Sigma-Aldrich, St. Louis, MO, USA) and a refractive index detector (RID-10A). Analyses were perfomed at a temperature of 30°C and the eluation was done using isocratic mobile phase (0.1% H_3PO_4) conditions at a flow rate of 0.5 mL min^{-1}. Total acids were expressed as tartaric acid, volatile acids as acetic acid and pH values were determined by Metrohm 744 pH-meter (Switzerland). For each flask, analyses were done in triplicate and standard deviations were calculated. Commercial test kits produced by Megazyme (Ireland) (Megazyme, 2011) were used to measure concentrations of glycerol, L-malic and L-lactic acid in the produced wines.

Statistical analyses

Statistical analyses of analytical data were provided using Statistica SixSigma Software by using the t-test for dependent samples. Links between compared wine chemical traits were the same blackberry cultivar, the same must producer and finally the same vegetation year. Treatments were two different yeast strains (FR and FM).

or calculation of linear correlations for fermentation activity and CO_2 production rate from the first to the seventh day all data for $\underline{x}$ and y values were transformed as $x = x_i - \underline{x}$ and also $y = y_i - \underline{y}$.

Assessment of H_2S production potential

Twenty-four hours old yeast cultures grown on malt agar slants were inoculated by striking on BiGGY Agar (Bismuth Sulfite Glucose Glycine Yeast Agar) plates and incubated at 28°C / 72 h. BiGGY agar is a medium that is accepted for assessing potential of wine yeasts to produce H_2S, based on the ability of bismuth sulfite reduction to brown-black colored bismuth sulfide, leading to different coloration of colonies grown on this medium (in a concentration dependent manner). Bismuth sulfite also prevents the growth of most bacteria, giving the medium high degree of selectivity (Jiranek et al., 1995).

Results and discussion

Fermentation properties of two commercial yeasts, FR and FM, were tested in a small scale by fermentation of blackberry juice. Their fermentation activities, as well as composition of the produced blackberry wines were compared. Additionally, a simple method was used to assess potential of H_2S production of the two yeasts..

Fermentation activity

The concentration of reducing sugars in blackberry juice was in agreement with the literature (Souci, Fachman and Kraut, 1991). Microbiological analysis of commercial blackberry juice did not result in colony growth (data not shown). Therefore, one could conclude that the initial low concentrations of ethanol in blackberry juice were formed during alcoholic fermentation that started during juice production and ended due to inactivation of native microflora during pasteurization of the commercial juice.

Fermentation can be monitored indirectly, by liberated CO_2, which is stoichiometrically related with consumed sugar and produced ethanol (Bely et al., 1990). In this study, the monitoring of fermentation profiles was conducted by measuring CO_2 gravimetrically, in the same way as it was done in our previous studies on fermentation of synthetic medium and grape must (Petravić Tominac et al., 2005a; Petravić Tominac et al., 2005b; Petravić Tominac et al., 2008). This indirect method of alcoholic fermentation monitoring was applied as described elsewhere (Dittrich 1987; Bely et al., 1990; Castellari et al., 1995; Raineri et al., 1998; Romano et al., 1998; Guerra et al., 1999).

The comparison of fermentation characteristics of both yeast strains is presented in Figures 1 and 2. The fermentation has already started during the first day using both yeasts; however, yeast FM started fermentation slightly faster than yeast FR (Figure 1). The maximum fermentation rate was achieved

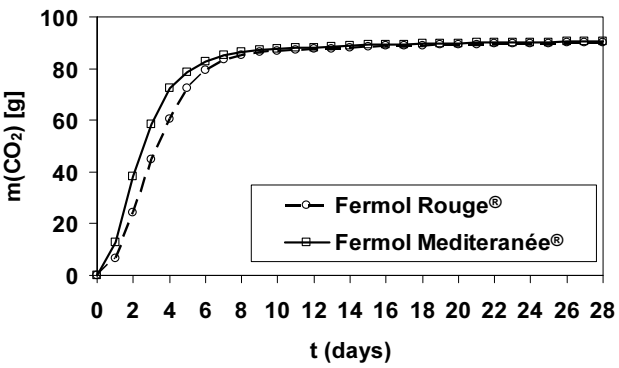

Figure 1. Fermentation activities of two commercial yeast strains during production of blackberry wine at 23°C

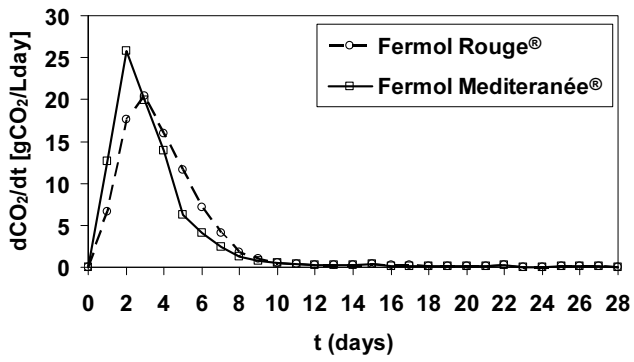

Figure 2. CO_2 production rate of two commercial yeast strains during production of blackberry wine at 23°C

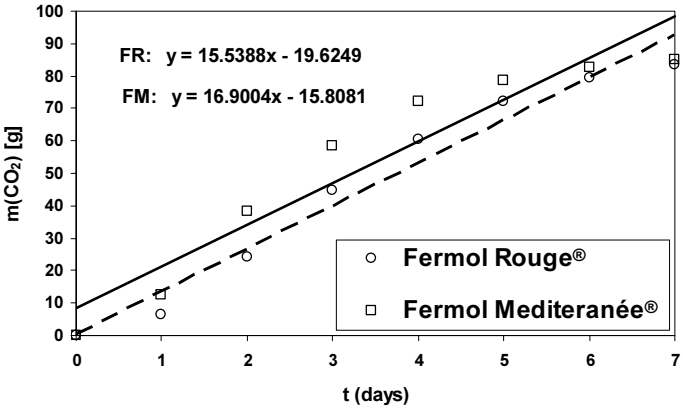

Figure 3. Results of calculation of linear correlations for fermentation activity in the tumultuous phase of fermentation

during the second and third day for yeasts FM and FR, respectively. It can be observed from the fermentation curves (Figure 1) and CO_2 production rate diagram (Figure 2) that the tumultuous phase of fermentation ended about a week from the beginning of fermentation, while silent fermentation lasted for the next three weeks. The total mass of CO_2 released at the end of fermentation by yeast FM was 90.52 g, whereas with yeast FR it was slightly lower and amounted to 90.10 g (Figure 1). Statistical analysis of the fermentation activity data showed that there were no analytical errors and curves achieved for parallel flasks were not significantly different.

Results of calculation of linear correlations for fermentation activity in the tumultuous phase of fermentation, i.e. from the beginning to the seventh day, are shown in Figure 3. During that phase of fermentation, mass of CO_2 liberated on daily basis by yeast FR was 15.54 g (b = 15.54). For yeast FM, CO_2 evolution for the same period was 16.90 g (b = 16.90). There was complete positive correlation between number of days during tumultuous phase and mass of CO_2 evolved (r_{FR} = 0.99, p = 0.00007; r_{FM} = 0.98, p = 0.003).

Maximum CO_2 production rate of 25.77 g CO_2 L^{-1} day^{-1}, that was achieved after three days by yeast FM, was slightly higher than that achieved by yeast FR after four days (20.37 g CO_2 L^{-1} day^{-1}) (Figure 2).

In spite of data transformation for small samples ($x = x_i - \bar{x}$), correlation between time of fermentation and CO_2 production rate was complete (r = 0.98) but not significant because p = 0.1296 (p > 0.05). Regression coefficient between time of fermentation and dCO_2/dt was also higher for yeast FM than for yeast FR (b = 8.27, ns). With a certain reserve, one can claim that dCO_2/dt was 8.27 g CO_2 L^{-1} day^{-1} for yeast FM and 5.39 g CO_2 L^{-1} day^{-1} for yeast FR (p = 0.032; p < 0.05).

Data on the chemical composition of two blackberry wines produced by microfermentations (Table 1) were consistent with data published for the wines produced from grapes (Jackson, 2000; Ribereau-Gayon et al., 2006a; Ribereau-Gayon et al., 2006b). There were no significant differences between concentrations of ethanol obtained by the two tested yeasts. Ethanol concentrations corresponded to the range published for other Croatian blackberry wines (Amidžić Klarić 2011a; Mudnić et al., 2012). The resulting concentrations of ethanol were also in accordance with the Croatian fruit wine regulation published in Official gazette of Republic of Croatia (Pravilnik o vinu, 1996; Pravilnik o voćnim vinima, 2004) that allows the addition of sugar to the extent that the actual alcohol content at the time of delivery to the consumer is up to 13% by volume. Fermentation efficiencies of both yeasts were very high and the produced wines

Table 1. Analysis of blackberry wines at the end of fermentation

	Mean ±σ		Difference	Std.Dev.Diff	t-value	p
	Wine FR	Wine FM				
γ (residual sugar) [g/L]	1.416 ± 0.039	1.296 ± 0.036	0.12**	0.071	4.124	0.009
φ (etanol) [% vol.]	12.188 ± 0.611	12.53 ± 0.069	0.343(ns)	0.559	1.061	0.399
γ (titratable acidity) [g/L]	10.821 ± 0.038	10.843 ± 0.034	0.021(ns)	0.052	1.249	0.247
γ (volatile acids) [g/L]	0.566 ± 0.06	0.486 ± 0.036	0.08*	0.09	2.666	0.028
pH	3.34 ± 0.007	3.331 ± 0.003	0.013**	0.007	5.656	0.0004
γ (glycerol) [g/L]	5.976 ± 0.647	4.491 ± 0.244	1.484(ns)	0.818	3.141	0.088
γ (malic acid) [g/L]	0.345 ± 0.043	0.621 ± 0.012	0.275**	0.032	14.681	0.0046
γ (lactic acid) [g/L]	0.645 ± 0.009	0.333 ± 0.011	0.312**	0.0012	354.64	0.001

* significant (p<0.05); ** highly significant (p<0.01); ns – not significant

contained low concentrations of unfermented sugars (Table 1). Concentrations difference of residual sugars was highly significant ($D = 0.12$; $p < 0.01$).

Concentrations of total acids obtained by both yeasts were not significantly different ($D = 0.021$; $p > 0.05$). Amidžić Klarić et al. (2011a) published that levels of total acids in Croatian blackberry wines ranged from 6.68 to 18.13 g L^{-1}. Total acids concentrations obtained during this experiment (Table 1) were within the specified range. This result is also in accordance with the Croatian fruit wine regulation (Pravilnik o voćnim vinima, 2004) that requires that the fruit wine on the market must contain at least 3.5 g L^{-1} of total acidity expressed as malic acid. The mentioned regulation also prescribes that fruit wine can contain maximum of 1.5 g L^{-1} volatile acids, expressed as acetic acid. The concentration of volatile acids in both wines obtained by microfermentation was about three times lower than the maximum concentration prescribed by the regulations.

Levels of volatile acids formed by the two yeasts were significantly different ($D = 0.08$; $p = 0.028$). Volatile acidity has increased during fermentation performed by both yeast strains but the increase was higher when yeast FR was used. Acetic acid, which predominates among the volatile acids, is one of the products of yeast metabolism, so different yeast strains may produce various levels of that compound (Bisson, 1993; Rainieri and Pretorius, 2000; Fleet, 2003; Ribéreau-Gayon et al., 2006a; Suárez-Lepe and Morata, 2012).

Difference of pH-values of the produced blackberry wines was highly significant ($D = 0.013$; $p < 0.01$) and pH-values of both wines were consistent with the literature. Generally, pH values of wines range from 2.8 to 4.0 (Ribéreau-Gayon et al., 2006b), while the range of pH-values for the blackberry wines produced in Croatia is 3.11 - 3.56 with an average value of 3.33 (Amidžić, 2011).

Malic acid is one of the most abundant acids in blackberries (Worobo and Splittstoesser, 2005), and its degradation by malolactic fermentation could reduce the acidity of blackberry wine. Therefore, concentrations of malic and lactic acid were determined. There were highly significant differences in concentrations of malic and lactic acids (D values were 0.275 and 0.312, respectively; $p < 0.01$) in tested wine samples. Malic acid level in wine FR was twice lower, while lactic acid concentration in the same wine was twice higher than in wine FM.

Glycerol contributes significantly to the sweetness, body and fullness of grape wines (Noble and Bursick, 1984). It is the major fermentation product of *Saccharomyces cerevisiae* after ethanol and carbon dioxide and can indirectly contribute to the sensory character of wine. Its production is one of the desirable features during grape must fermentation. Generally, there is a difference in the amount of glycerol formed by various yeast strains and therefore glycerol production should be considered in the selection of wine yeast strains (Zoecklein et al., 1995; Rainieri and Pretorius, 2000; Fleet, 2003; Šehović et al., 2004; Krieger-Weber, 2009; Suárez-Lepe and Morata, 2012). In the performed microfermentation experiments the differences between concentrations of glycerol in the produced blackberry wines were not significant (Table 1). The produced levels of glycerol were in accordance with the Croatian wine regulation (Pravilnik o vinu, 1996), as well as with concentrations present in grape wines (Šehović et al., 2004; Swiegers et al., 2005; Ribéreau-Gayon et al., 2006b). According to literature, ethanol levels in wine are usually 10-15 times higher than those of glycerol (Dittrich, 1987; Reed and Nagodawithana, 1991) and this is consistent with the presented data.

Potential of H_2S production

Variation in H_2S production should be investigated because low H_2S production is one of the key selection criteria for wine yeasts. The formation of H_2S on bismuth-containing indicator media indicates the intrinsic level of sulfite reductase activity of a strain and in turn its potential to produce H_2S in permissive conditions. Therefore, yeasts striking on BiGGY agar plates is valid as a rapid screening methodology and helps to determine the risk of H_2S production associated with the use of a given yeast strain (Jiranek et al., 1995). Cultivation on BiGGY agar showed that both yeasts had a small potential for H_2S production, because the individual colonies were light brown. The potential for H_2S production could be quantified more precisely in future investigations by using appropriate analytical methods.

Conclusions

It was shown that yeast FM started fermentation slightly faster than yeast FR. The maximum fermentation rates were obtained during the second and third day for yeasts FM and FR, respectively. Fermentations were carried on to complete sugar consumption by both yeast strains. Levels of volatile acids formed by the two yeasts were significantly different ($D = 0.08$; $p = 0.028$). Differences in concentrations of residual sugars, malic acid, lactic acid and pH-values were highly significant (D values were 0.12, 0.275, 0.312 and 0.013, respectively; $p < 0.01$). There were no significant differences between concentrations of ethanol, total acids and glycerol in blackberry wines produced by both yeasts. Chemical composition of the produced blackberry wines was in accordance with the Croatian fruit wine legislation. Good fermentative properties and low potential of H_2S production of yeasts FM and FR could be beneficial for blackberry wine production. To put this data into practice, additional tests are necessary, including characterization of yeast aroma profiles and sensorial analyses.

References

Amidžić D. (2011). Utjecaj ekološkog uzgoja kupine na udio nutritivnih i biološki aktivnih sastavnica kupinovog vina. Dissertation, University of Zagreb, Faculty of Pharmacy and Biochemistry.

Amidžić Klarić D., Klarić I., Velić D., Vedrina Dragojević I. (2011a). Evaluation of mineral and heavy metal contents in Croatian blackberry wines. Czech J. Food Sci. 29 (3): 260–267

Amidžić Klarić D.; Klarić I.; Mornar A. (2011b). Polyphenol content and antioxidant activity of commercial blackberry wines from Croatia: Application of multivariate analysis for geographic origin differentiation. J. Food Nutr. Res. 50 (4): 199-209

Arozarena Í., Ortiz J., Hermosín-Gutiérrez I., Urretavizcaya I., Salvatierra S., Córdova I., Remedios Marín-Arroyo M., José Noriega M., Navarro M. (2012). Color, ellagitannins, anthocyanins, and antioxidant activity of andean blackberry (*Rubus glaucus* Benth.) wines. J. Agric. Food Chem. 60: 7463–7473

Bely M., Sablayroles J., Barre P. (1990). Description of alcoholic fermentation kinetics: its variability and significance. Am. J. Enol. Viticult. 41 (4): 319–324

Bish T. J. (2011). Inactivation of foodborne pathogens by fruit wines. Master's thesis, Faculty of the Graduate School, University of Missouri.

Bisson L. (1993). Yeast – metabolism of sugars. In: Wine microbiology and biotechnology, Fleet G. H. (ed.) Harwood Academic Publishers, Chur. pp. 55-75

Castellari L., Magrini A., Passarelli P., Zambonelli C. (1995). Effect of must fermentation temperature on minor products formed by cryo and non-cryotolerant *Saccharomyces cerevisiae* strains. Ital. J. Food Sci. 7 (2): 125-132

Ciani M., Picciotti G. (1995). The growth kinetics and fermentation behaviour of some non-*Saccharomyces* yeasts associated with wine-making. Biotechnol. Lett. 17 (11): 1247-1250

Degree R. (1993). Killer yeasts. In: Wine microbiology and biotechnology, G.H. Fleet, ed., Harwood Academic Publishers, Chur 1993, pp. 243-264

Dittrich H. H. (1987). Mikrobiologie des Weines, 2. ed., Ulmer, Stuttgart.

Fleet GH. (2003). Yeast interactions and wine flavour. Int. J. Food Microbiol. 86 (1-2): 11-22

Fugelsang K. C., Edwards C. G. (2007). Wine microbiology: Practical applications and procedures, 2. ed., Springer Science and Business Media, LLC, New York.

Gao J., Xi Z., Zhang J., Guo Z., Chen T., Fang Y., Meng J., Zhang A., Li Y., Liu J. (2012). Influence of fermentation method on phenolics, antioxidant capacity, and volatiles in blackberry wines. Anal. Lett. 45 (17): 2603-2622

Gil J. V., Mateo J. J., Jiménez M., Pastor A., Huerta T. (1996). Aroma compounds in wine as influenced by apiculate yeasts. J. Food Sci. 61 (6): 1247-1250

González R., Munoz R., Carrascosa A. V. (2011). Production of wine starter cultures. In: Molecular wine microbiology, Carrascosa A. V., Munoz R., González R. (eds.) Elsevier, Amsterdam, Boston, pp. 279-302

Guerra E., Sordi G., Mannazzu I., Clementi F., Fatichenti F. (1999). Occurrence of wine yeasts on grapes subjected to different pesticide treatments. Ital. J. Food Sci. 3 (11): 221-230

Heinonen I. M., Lehtonen P. J., Hopia A. I. (1998). Antioxidant activity of berry and fruit wines and liquors. J. Agric. Food Chem., 46 (1): 25–31

Jackson R. S. (2000). Wine science: Principles, practice, perception, 2. Ed., Elsevier, Academic press, San Diego.

Jiranek V., Langridge P., Henschke P. A. (1995). Validation of bismuth-containing indicator media for predicting H_2S-producing potential of *Saccharomyces cerevisiae* wine yeasts under enological conditions. Am. J. Enol. Vitic. 46 (2): 269-273

Joh Y. J. (2010). Quantification of quality attributes, functional compounds, and antioxidant capacity of blackberry and blackberry wines. Master's thesis, Oklahoma State University.

Johnson M. H., Gonzalez de Mejia E. (2012). Comparison of chemical composition and antioxidant capacity of commercially available blueberry and blackberry wines in Illinois. J. Food Sci. 77 (1): C141-C148

Kalkan Yildirim H. (2006). Evaluation of colour parameters and antioxidant activities of fruit wines. Int. J. Food Sci. Nutr. 57 (1/2): 47-63

Krieger-Weber, S. (2009). Application of yeast and bacteria as starter cultures. In: Biology of microorganisms on grapes, in must and in wine (H. König, G. Unden and J. Fröhlich, eds.) Springer-Verlag, Berlin Heidelberg, 489- 511

Martin G. E., Sullo J. G., Schoenenian R. L. (1971). Determination of fixed acids in commercial wines by gas-liquid chromatography. J. Agric. Food Chem., 19 (5): 995-998

Megazyme (2011). Megazyme International Ltd, Wicklow, Ireland (http://www.megazyme.com).

Mornar A., Amidžić Klarić D., Nigović B. (2011). Kontrola kvalitete kupinovih vina primjenom HSS-GC-FID tehnike (Quality assessment of blackberry wines by HSS-GC-FID) Farmaceutski glasnik: glasilo Hrvatskog farmaceutskog društva 67: 741-746

Mudnić I., Budimir D., Modun D., Gunjača G., Generalić I., Skroza D., Katalinić V., Ljubenkov I., Boban M. (2012). Antioxidant and vasodilatory effects of blackberry and grape wines. J. Med. Food 15 (3): 315–321

Noble A. C., Bursick G. F. (1984). The contribution of glycerol to perceived viscosity and sweetness in white wine. Am. J. Enol. Vitic. 35 (2): 110–112

Orser R. E., Yang H. Y. (1966). Gas-liquid chromatographic analysis of blackberry wine extracts fermented by different yeast strains. Am. J. Enol. Vitic. 17 (2): 106-111

Ough C.S., Amerine M.A. (1998). Methods for analysis of musts and wines, John Wiley & Sons, Inc., New York.

Petravić Tominac V., Blagojević K., Novak S., Zechner-Krpan V., Marić V. (2005a). Fermentative properties of some *Saccharomyces cerevisiae* wine yeasts. Period. Biol. 107 (1): 59–65

Petravić Tominac V., Eke H., Šehović Đ., Zechner-Krpan V., Novak S., Marić V. (2005b). Fermentation of Graševina must with different strains of wine yeasts. Period. Biol. 107 (1): 51–58

Petravić Tominac V., Kovačević Ganić K., Komes D., Gracin L., Banović M., Marić V. (2008). Influence of media composition and temperature on volatile aroma production by various wine yeast strains. Czech J. Food Sci. 26 (5): 376–382

Pinhero R. G., Paliyath G. (2001). Antioxidant and calmodulin inhibitory activities of phenolic components in fruit wines and its biotechnological implications. Food Biotechnol. 15 (3): 179–192

Pravilnik o vinu (1996) Official Gazette of the Republic of Croatia 96, Zagreb (NN 96/03).

Pravilnik o voćnim vinima (2004) Official gazette of the Republic of Croatia 73, Zagreb (NN 73/04).

Rainieri S., Zambonelli C., Giudici P., Castellari L. (1998). Characterisation of thermotolerant *Saccharomyces cerevisiae* hybrids. Biotechnol. Lett. 20 (6): 543-547

Rainieri S., Pretorius I. S. (2000). Selection and improvement of wine yeasts. Ann. Microbiol., 50, 15-31

Reed G., Nagodawithana T. W., Wine yeasts. In: Yeast technology. Van Nostrand Reinhold, New York, 1991, pp. 186.

Ribereau-Gayon P., Glories Y., Maujean A., Dubourdieu D., Rychlewski C. (2006a). Handbook of enology: The chemistry of wine stabilization and treatments, 2. ed., John Wiley & Sons Ltd., Chichester, pp.64.

Ribereau-Gayon P., Dubourdieu D., Doneche B., Lonvaud A. (2006b). Handbook of enology: The microbiology of wine and vinifications, 2. ed., John Wiley & Sons Ltd., Chichester.

Romano P. (1997). Metabolic characteristics of wine strains during spontaneous and inoculated fermentation. Food Technol. Biotechnol. 35 (4): 255-260

Romano P., Monteleone E., Paraggio M., Marchese R., Caporale G., Carlucci A. (1998). A methodological approach to the selection of *Saccharomyces cerevisiae* strains. Food Technol. Biotechnol. 36 (1): 69-74

Rommel A. (1988). Red raspberry and blackberry juice and wine: the effect of processing and storage on color and appearance. Master's thesis, Oregon State University.

Rommel A., Wrolstad R. E., Heatherbell D. A. (1992). Blackberry juice and wine: processing and storage effects on anthocyanin composition, color and appearance. J. Food Sci. 57 (2): 385-391, 410.

Souci S. W., Fachmann W., Kraut H., Der kleine Souci/ Fachmann/ Kraut. Lebensmitteltabelle für die Praxis, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1991, pp. 353.

Suárez-Lepe J. A., Morata A. (2012). New trends in yeast selection for winemaking. Trends Food Sci. Tech. 23 (1): 39–50

Swiegers J. H., Bartowsky E. J., Henschke P. A., Pretorius I. S. (2005). Yeast and bacterial modulation of wine aroma and flavour. Aust. J. Grape Wine R. 11 (2), 139–173

Šehović Đ., Petravić V., Marić V. (2004). Glycerol and wine industry - glycerol determination in grape must and wine. Kem. Ind. 53 (11): 505-516

Wang Y., Li P., Kumar Singh N., Shen T., Hu H., Li Z., Zhao Y. (2012). Changes in aroma composition of blackberry wine during fermentation process. Afr. J. Biotechnol. 11 (99): 16504-16511

Worobo R. W., Splittstoesser D. F. (2005). Microbiology of fruit products. In: Processing fruits: Science and technology, 2. ed., Barrett D. M., Somogyi L., Ramaswamy H. (eds.) CRC Press, Boca Raton, pp. 262–284

Wrolstad R. E. (1976). Color and pigment analyses in fruit products. Oregon State University, Station Bulletin 624: 1-17

Wrolstad R. E., Culbertson J. D., Nagaki D. A., Madero C. F. (1980). Sugars and nonvolatile acids of blackberries. J. Agric. Food Chem. 28 (3): 553-558

Yulin F., Li Z., Jianqiang S., Shiren S., Chang X. F., Wang Hua W. (2007). Determination of volatile compounds of *Rubus idaeus* fruits and their fermented products. Scientia Silvae Sinicae 43: 133-138

Zironi R., Romano P., Suzzi G., Battistutta F., Comi G. (1993). Volatile metabolites produced in wine by mixed and sequential cultures of *Hanseniaspora guilliermondii* or *Kloeckera apiculata* and *Saccharomyces cerevisiae*. Biotechnol. Lett. 15 (3): 235-238

Zoecklein B., Fugelsang K. C., Gump B. H., Nury F. S., Wine Analysis and Production. Chapman & Hall, New York, 1995, pp. 99.

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