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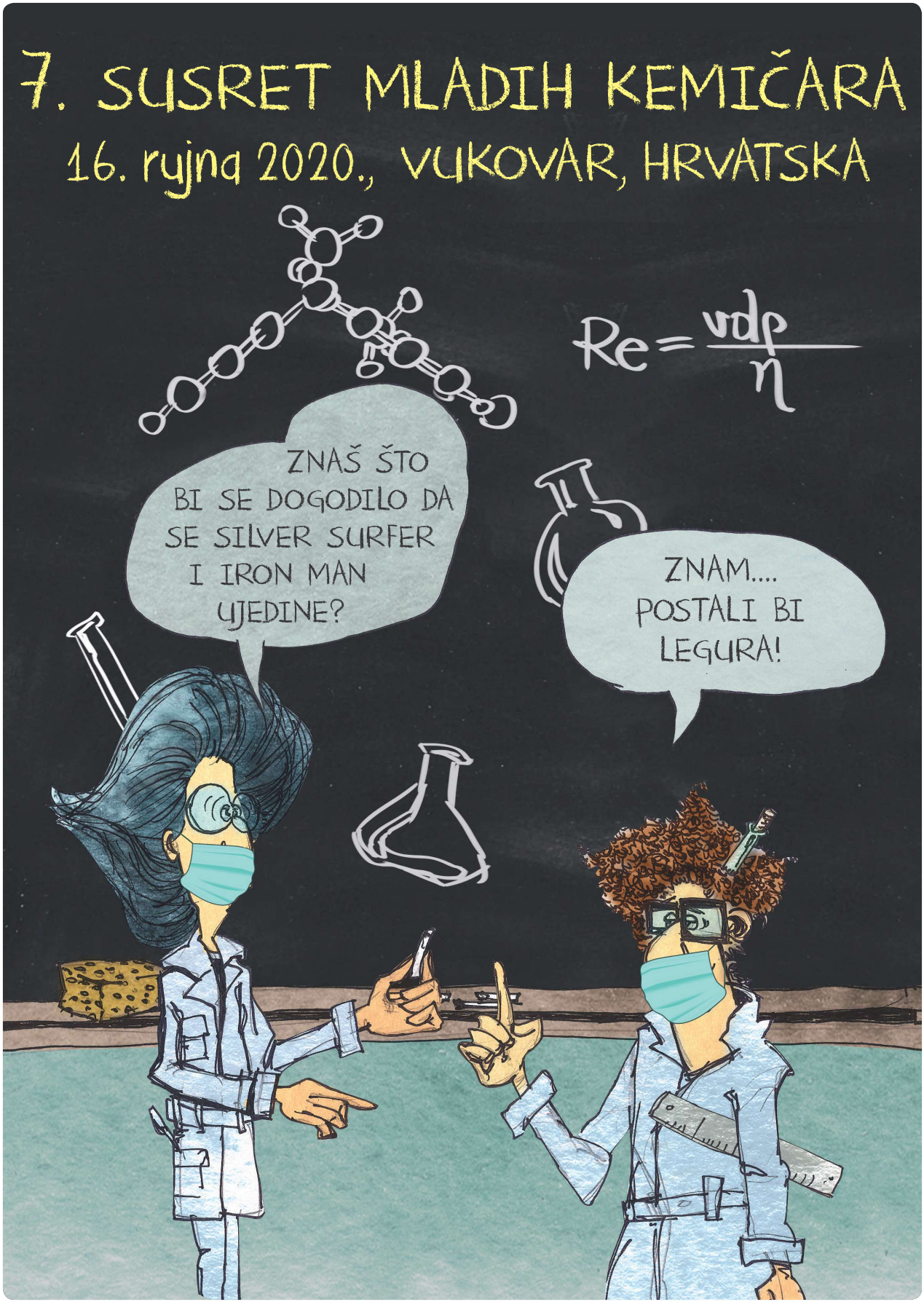
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Fermentation process optimisation and characterisation of pear fruit wine

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Summary

This study aimed to investigate the influence of two commercially available oenological yeasts (Uvaferm BDX and Cross Evolution) on fermentation kinetics, physicochemical properties, and total polyphenols content of organic pear juice with and without the addition of industrial pectolytic enzyme (Lallzym OE) and their respective wines. The alcoholic fermentation (AF) kinetics was monitored on a laboratory-scale (microfermentations), while the controlled fermentation (CF), as well as induced malolactic fermentation (MLF) of pear wine, was carried out on a pilot-scale custom made fermentation system. The pure culture of lactic acid bacteria (LAB) *Oenococcus oeni* was used for pear wine fermentation with the establishment of temperature optimum for selected LAB type. The results of the study showed that selected yeasts Uvaferm BDX and Cross Evolution could successfully ferment pear juice. The higher specific fermentation rate was achieved using Uvaferm BDX. In samples produced with the addition of the pectolytic enzyme Lallzym OE, a higher specific fermentation rate, as well as a higher total polyphenols content, were observed compared to other pear wine samples. The stimulated MLF led to decreased malic acid concentration, which usually leads to harmonisation of taste.

Keywords: fruit wine, pear wine, controlled fermentation, malolactic fermentation, total polyphenols

Introduction

Wines made from fruits other than grapes have recently been gaining wider acceptance at the market (Rivard, 2009), which may be connected to the enhanced consumers' interest in foods that are rich in bioactive compounds, *i.e.* have a beneficial effect on human health (Velić et al., 2018a). European regulations define fruit wines as beverages obtained by the fermentation of the juices of fruits other than grapes and with the permitted alcoholic strength between 1.2 % and 14 % by volume (Kosseva et al., 2017). Fruit varieties of different shape, colour, nutritive value have proven to be good raw materials for quality fruit wines production (Jagtap and Bapat, 2015; Velić et al., 2018b), *e.g.* apples, pears, berries, cherries, wild

apricots, plums, peaches, strawberries, currants, bananas, pineapples, kiwifruit, cashew nuts, pomegranates, oranges, lemons, tangerines, dates, and figs (Joshi et al., 2017). Various fruit wines have proved to be a good dietary source of phytonutrients (*e.g.* phenolic compounds), antioxidants and minerals (Rupasinghe et al. 2017). Pear wine is a fruit wine obtained by the alcoholic fermentation of juice or mash from fresh and technological suitable pears. In order to produce fruit wine of satisfactory quality, varieties containing more tannins such as Bartlett pear are used (Kosseva et al., 2017). Since alcoholic pear beverages, other than perry and brandy, are not widely present, pear wine production is both adding a value to pears and providing consumers with more choices when it comes to fruit wines (Yang et al., 2020). In a technological manner, perry is a drink similar to cider, but instead of apples, pears are used as the raw material. The difference between perry and pear (fruit) wine is that perry contains CO_2, and no selected yeasts are added during perry production (Kosseva et al., 2017). Pear wine has lower acidity and lower polyphenol content compared to other fruit wines. Lower acidity can reduce the overall taste, while lower polyphenol content is desirable because of the lower bitterness (associated with polyphenols). On the other hand, if polyphenols content is too low, the specific fruit taste of wine is lost. Therefore, it is necessary to achieve an appropriate balance between these compounds, so pear wine is often mixed with cider. Compared to other fruit wines, a lower antioxidant effect of pear fruit wine was found due to the lower content of polyphenolic compounds (Velić et al., 2018a). One of the main problems associated with the production and processing of pear wine is browning because it can cause irreversible defects in the quality (Yang et al., 2020). The fruit variety mostly determines the aroma and flavour of different fruit wines. However, wine yeast and fermentation conditions can also define the wine aroma and flavour, as well as the overall wine quality. Yeast strains with desirable (fermentative and other) properties are of utmost importance for quality fruit wine production. Apart from yeast, lactic acid bacteria also play a very significant role in carrying out a secondary process, known as malolactic fermentation (conversion of L-malic acid to L-lactic acid) that takes place during or at the end of primary alcoholic fermentation. Lactic acid has a softer, more mellow flavour that can create a beverage with a more desirable flavour profile (Herrero et al., 2005). Until now, the evaluation of yeast strains for pear fruit wine production was not conducted in Croatia. Therefore, the aim of this study was to investigate the influence of two commercially available oenological yeasts (Uvaferm BDX and Cross Evolution) on the fermentation kinetics, physico-chemical properties, and the total polyphenols content of organic pear juice and the respective wines produced with and without the addition of industrial pectolytic enzyme (Lallzym OE).

Materials and Methods

Commercially available organic pear juice (variety: *Abata Fetel*) was obtained from an organic producer in eastern Slavonia region and used as a fermentation medium.
The pear juice was supplemented by 108 g/L of sucrose and sulphited by 35 mg/L of potassium metabisulphite ($K_2S_2O_5$). Before fermentation, the pear juice contained 200 g/L of total sugars, 4.4 g/L of malic acid, 0.51 g/L of lactic acid and the pH was 3.68.

Microfermentations experiment

The alcoholic fermentation (AF) kinetics was monitored on a laboratory-scale (microfermentations) using Erlenmeyer flasks (2 L). A volume of 1.75 L of prepared pear juice was transferred to each flask. Flasks were then inoculated with 0.53 g (30 g/hL) of commercial dry yeasts (4 flasks by Uvaferm BDX and 4 by Cross Evolution). To investigate the influence of the pectolytic enzymes addition to the quality of the final product, enzyme Lallzyme POE was added (1.5 g/hL) to 2 flasks inoculated with each yeast (in total 4 flasks). Flasks were closed with fermentation airlocks and left to ferment at average fermentation temperature of 21˚C. All fermentations were carried out in duplicate. Additional Erlenmeyer flask, containing water, served as a control to correct evaporation loss, which was included in mass balance. The fermentation progress was evaluated by the weight loss caused by CO_2 production, and flasks were weighed at 24 hour-intervals using a digital scale (572, Kern, Germany). Fermentation activity was monitored by measuring CO_2 evolution and CO_2 production rate during microfermentation of organic pear juice.

Controlled fermentation (CF) – malolactic fermentation (MLF)

The controlled fermentation (CF), as well as induced malolactic fermentation (MLF) of pear juice, was carried out on a pilot-scale custom-made fermentation system. Identically prepared pear juice with enzyme Lallzyme POE addition was subjected to controlled fermentation in a fermentation system consisting of three double-wall fermenters (15 L × 3 pcs; AISI 304), compressor and cooling/heating medium preparation tank. The vertical fermenters with the conical bottom are equipped with valves, sight gauge glass, and fermentation airlock. The control panel enables the individual temperature control and regulation of each fermenter. The volume of pear juice per fermenter (2 fermenters) was 11 L, and each fermenter was inoculated with different investigated yeast. Upon completion of the alcoholic fermentation at 18 °C, a pure culture of lactic acid bacteria (LAB) *Oenococcus oeni* (LALVIN VP 41 MBR) was inoculated in both fermenters, with the establishment of the optimal temperature for the selected type of LAB (at 22 °C). After malolactic fermentation (MLF), the young wine was removed from the lees and poured into bottles for further ageing and maturation.

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 weighing of fermentation flasks [g];
m_2 = mass difference between two weighing of control flask (containing water) [g].

The CO_2 production rate was calculated according to equation 2:

$$\frac{d\mathrm{CO}_2}{dt} = \frac{\Delta m}{V \cdot \Delta t} \quad (2)$$

where:

$d\mathrm{CO_2}/dt$ = $\mathrm{CO_2}$ production rate [g/L day];
Δm = mass of $\mathrm{CO_2}$ released during time interval Δt [g];
Δt = time interval between two measuring [day];
V = volume of medium [L].

Analytical methods

Standard AOAC INTERNATIONAL and OIV (*Organisation Internationale de la vigne et du vin*) methods of analysis were used for pear wines analysis, namely total dry extract, ethanol, free $\mathrm{SO_2}$, total $\mathrm{SO_2}$, volatile acids, total acids, residual sugar, total sugar and pH. The total polyphenol concentration (TPC) of pear juice and pear wine were estimated by Folin-Ciocalteu colorimetric assay based on the procedure described by Waterhouse (2019). Reflectometric determination (RQflex plus 10) after enzymatic reaction with malate dehydrogenase was used for malic acid. Lactic acid (lactate) is oxidized by nicotinamide adenine dinucleotide (NAD) under the catalytic effect of lactate dehydrogenase to a pyruvate. In the presence of diaphorase, the NADH formed in the process reduces a tetrazolium salt to a blue formazan that is determined reflectometrically on the RQflex.

Results and Discussion

The monitoring of fermentation by released $\mathrm{CO_2}$ is based on the fact that $\mathrm{CO_2}$ is stoichiometrically related to both consumed sugar and produced ethanol (Bely et al., 1990). Fermentation profiles in this study were monitored by measuring $\mathrm{CO_2}$ gravimetrically, as described in our previous study dealing with microfermentation on blackberry must (Petravić Tominac et al., 2013). Figs. 1 and 2 show the fermentation characteristics comparison of both yeast strains. It can be seen that the fermentation has started during the first day regardless of the used yeast or the addition of enzymes. However, yeast K1 started fermentation slightly faster than yeast K2 (Fig. 1). In samples produced with the addition of the pectolytic enzyme Lallzym OE, a higher specific fermentation rate was observed than pear wine samples without the enzyme addition. The maximum fermentation rate was achieved during the second day for yeast K1 and the third day for yeast K2. The tumultuous phase of fermentation ended around the 17th day from the beginning of fermentation (as observed from Fig. 1 and Fig. 2), while silent fermentation lasted for the next 12 days. The total mass of $\mathrm{CO_2}$ released at the end of fermentation by yeast K1 was 95.81 g, whereas, with yeast K2, it was slightly lower and amounted to 95.42 g (Fig. 1). This is in accordance with the previous study of Petravić Tominac et al. (2013) that reported a similar trend in fermentation kinetics during microfermentation of blackberry must containing the same initial sugar level as well as a very similar total mass of $\mathrm{CO_2}$ released at the end of fermentation by the yeasts investigated.

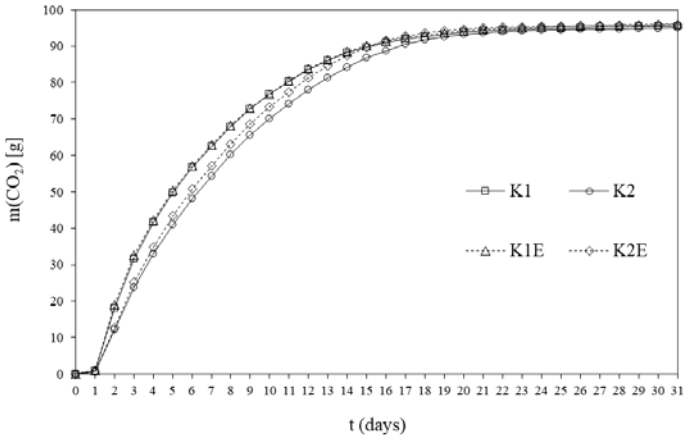

Figure 1. Fermentation activities of two commercial yeast strains during the production of pear wine at 21 °C (with and without the addition of industrial pectolytic enzyme); K1 - Uvaferm BDX, K2 - Cross Evolution, E - enzyme

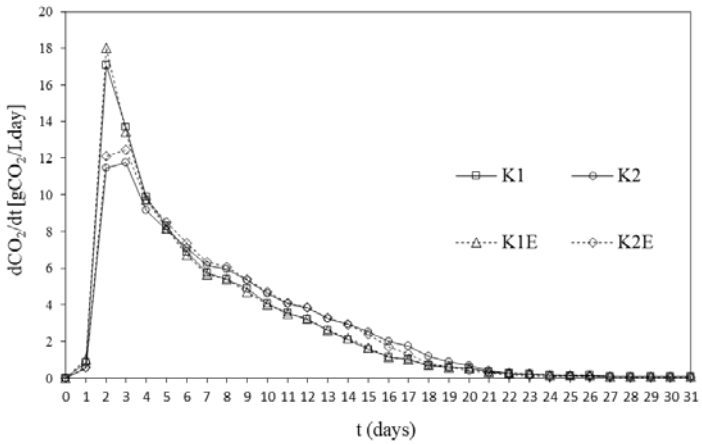

Figure 2. CO_2 production rate of two commercial yeast strains during the production of pear wine at 21 °C (with and without the addition of industrial pectolytic enzyme), K1 - Uvaferm BDX, K2 - Cross Evolution, E – enzyme

The total polyphenol concentration (TPC) determined by Folin-Ciocalteu method and expressed as mg/L gallic acid is presented in Table 1. It can be observed that a somewhat higher concentration of polyphenols is present in young pear wine obtained by yeast K1 (with and without enzyme) than by yeast K2 for the microfermentation experiments. The same trend can be observed in wine produced using the controlled fermentation system (CF). In samples produced with the addition of the pectolytic enzyme Lallzym OE a higher total polyphenols contents were observed compared to pear wine samples without the enzyme addition. The TPC is in accordance with the TPC of apple wine (451 mg GAE/L) and plum wine (555 mg GAE/L) reported by Rupasinghe and Clegg, (2007). However, TPC concentration is significantly lower than that of cherry wine (991 mg GAE/L) and almost four times lower than TPC of blueberry wines (676 mg GAE/L) reported by the same authors.

The higher polyphenol content of any fruit usually leads to oxidative browning which products strongly contribute to wine's final bouquet.

Table 1. The total polyphenol concentration (TPC) in samples expressed as mg/L gallic acid

Sample	TPC [mg_{GAE}/L]
Pear juice	514
Pear juice, sweetened	567
K1	551
K2	516
K1E	563
K2E	523
K1E - CF	563
K2E - CF	538

K1 – wine yeast (Uvaferm BDX), K2 - wine yeast (Cross Evolution), E– enzyme, CF – controlled fermentation, TPC - total polyphenols concentration

The results of the physico-chemical analysis of pear wine at the end of microfermentations are given in Table 2. The results indicate that the obtained pear wines meet the physico-chemical quality parameters prescribed by the Fruit Wine Ordinance (OG 73/06, 24/11,120/12, 59/13).

Table 2. Analyses of pear wines at the end of microfermentation

Samples	K1	K2	K1E	K2E
Total dry extract (g/L)	78.4	78.4	77.6	77.5
Ethanol (vol %)	11.59	11.53	11.58	11.55
Free SO_2 (mg/L)	6.4	20.48	7.04	8.96
Total SO_2 (mg/L)	32.00	99.20	41.6	53.12
Volatile acids (g/L)	0.53	0.65	0.57	0.58
Total acids (g/L)	5.74	6.15	5.89	6.41
pH	3.69	3.68	3.67	3.67
Residual sugar (g/L)	51.77	51.20	53.18	53.10
Total sugar (g/L)	52.44	51.43	54.67	53.26

The concentrations of malic and lactic acid of pear wine samples during malolactic fermentation are shown in Table 3. The addition of a pure culture of lactic acid bacteria (LAB) *Oenococcus oeni* (LALVIN VP 41 MBR) in both samples resulted in a significant reduction in malic acid content. Most of the degraded malic acid was observed after the first week of MLF implementation (between 72 and 80 %). Sun et al. (2017) studied the implementation of MLF on cherry fruit wine and reported the reduction of malic acid content from 2.66 g/L to 0.44 g/L at initial pH of 3.55, which is in accordance with the data obtained in this study for pear fruit wine.

Table 3. The concentration of malic and lactic acid in the pear fruit wine samples (CF)

	At the beginning of MLF		After one week of MLF		After one month of MLF	
Samples	**K_1E - CF**	**K_2E - CF**	**K_1E - CF**	**K_2E - CF**	**K_1E - CF**	**K_2E - CF**
Malic acid (g/L)	4.35	4.45	1.205	0.88	0.715	0.68
Lactic acid (g/L)	0.85	0.97	2.65	2.75	3.24	2.98

K1 – wine yeast (Uvaferm BDX), K2 - wine yeast (Cross Evolution), E – enzyme , CF – controlled fermentation, MLF –malolactic fermentation

Conclusions

The results of the study showed that selected yeasts Uvaferm BDX and Cross Evolution could successfully ferment pear juice. The higher specific fermentation rate was achieved using Uvaferm BDX. In samples produced with the addition of the pectolytic enzyme Lallzym OE, a higher specific fermentation rate, as well as a higher total polyphenols content, were observed compared to pear wine samples without the enzyme addition. The stimulated MLF led to a decrease of malic acid concentration, which usually results in improved sensory properties of pear wine.

References

Bely, M., Sablayroles, J., Barre, P. (1990): Description of alcoholic fermentation kinetics: its variability and significance, *Am. J. Enol. Viticult.* 40: 319–324.

Fruit Wine Ordinance, Ministry of Agriculture, Republic of Croatia, OG 73/06, 24/11, 120/12, 59/13.

Herrero, M., Noriega, E., García, L. A., Gennaro, M. (2005): Influence of a malolactic starter on the quality of the cider produced on an industrial scale, *Eur. Food Res. Technol.* 221 (1–2), 168–174.

Jagtap, U. B., Bapat, V. A. (2015): Wines from fruits other than grapes: Current status and future prospectus, *Food Biosci*. 9, 80–96.

Joshi, V. K, Sharma, S., Thakur, A. D. (2017): Wines: White, Red, Sparkling, Fortified, and Cider., In: Current Developments in Biotechnology and Bioengineering. Food Bever. Ind., Pandey et al. (ed.), Elsevier, pp. 353–406.

Kosseva, M. R., Joshi, V. K., Panesar, P. S. (2017): Science and technology of fruit wine production, Elsevier Inc., Academic Press.

Petravić-Tominac, V., Mesihović, A., Mujadžić, S., Lisičar, J., Oros, D., Velić, D., Velić, N., Srečec, S., Zechner-Krpan, V. (2013): Production of blackberry wine by microfermentation using commercial yeasts Fermol Rouge® and Fermol Mediterranée®, *Agri. Conspec. Sci.* 78 (1), 49–55.

Rivard, D. (2009): The Ultimate Fruit Winemaker's Guide, 2nd ed., Bacchus Enterprises Ltd.

Rupasinghe, HPV, Clegg, S. (2007): Total antioxidant capacity, total phenolic content, mineral elements, and histamine concentrations in wines of different fruit sources. *J. Food Comp. Anal.* 20 (2), 133–137.

Sun, S. Y., Chen, Z. X., Jin, C. W. (2017): Influence of lactic acid bacteria starter and pH after alcoholic fermentation, and interaction between those factors, on the induction of malolactic fermentation, and on chemical and sensory properties of cherry wines, *LWT - Food Sci. Tech.* 89, 449–456.

Rupasinghe, H. P. V, Joshi, V. K., Smith, A., Parmar, I. (2017): Chemistry of Fruit Wines. In: Science and Technology of Fruit Wine Production, Kosseva, M. R., Joshi, V. K., Panesar, P. S. (ed.), Elsevier Inc., Academic Press, pp. 105–176.

Velić, D., Amidžić Klarić, D., Velić, N., Klarić, I., Petravić Tominac, V., Mornar, A. (2018a): Chemical Constituents of Fruit Wines as Descriptors of their Nutritional, Sensorial and Health-Related Properties. In: *Descriptive Food Science*, Díaz V., García-Gimeno, A., María, R. (ed.). London, GB: IntechOpen, pp. 59–91.

Velić, D., Velić, N., Amidžić-Klarić, D., Klarić, I., Petravić Tominac, V., Košmerl, T., Vidrih R. (2018b): The production of fruit wines – a review, *Croat. J. Food Sci. Technol.* 10 (2), 279–290.

Waterhouse, A. (2019): Folin-Ciocalteu Micro Method for Total Phenol in Wine, Department of Viticulture & Enology, University of California, Davis.

Yang, H., Tian, T., Gu, H., Li, X., Cai, G., Sun, J., Wu, D., Lu, J. (2020): Analysis of factors related to browning of Dangshan pear (*Pyrus spp.*) wine. *Food Chem*. 5; 308: 125665.