EXAMINATION OF CERTAIN TECHNOLOGICAL PROCEDURES DURING THE ALCOHOLIC FERMENTATION OF PLUM WINE

ISPITIVANJE POJEDINIH TEHNOLOŠKIH POSTUPAKA U TOKU ALKOHOLNE FERMENTACIJE VINA OD ŠLJIVE

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ABSTRACT

The purpose of this paper is to examine the effect of certain technological parameters of alcoholic fermentation on the quality of plum wines produced. As part of this research, the application of various commercial pectolytic enzymes to the treatment of plum pomace was examined, as well as the frequency of pomace stirring and the influence of different quantities of plum pit additions to the pomace. The domestic plum cultivar 'Čačanska lepotica' was used as a raw material. The present study gave an insight into the efficiency and justification of the pectolytic enzyme application examined. The once-per-day method of pomace stirring proved to be optimal, whereas the presence of pits during fermentation led to a significant increase in the hydrocyanic acid and benzaldehyde contents of the plum wines analysed. However, the hydrocyanic acid and benzaldehyde contents obtained did not exceed the maximum content allowed.

Key words: plum, alcoholic fermentation, fruit wine, pit, pectolytic enzymes.

REZIME

Cilj ovog rada je da se oceni efekat pojedinih tehnoloških postupaka u toku alkoholne fermentacije na kvalitet proizvedenih vina od sorti domaće šljive. U sklopu ovog istraživanja ispitivana je primena različitih komercijalnih pektolitičkih enzima u tretmanu kljuka, učestalost i tehnika mešanja kljuka kao i uticaj dodatka različitih količina koštica šljive u kljuk. U istraživanju je korištena sorte domaće šljive Čačanska lepotica. Primena pet različitih komercijalnih pektolitičkih enzima je u ovom istraživanju bila usmerena ka ispitivanju efekata na prinos vina, ekstarkciju fenolnih materija, produkciju etanola, glicerola i metanola. Mešanje kljuka je obavljano klasičnim mehaničkim potapanjem tj. potiskivanjem klobuka, na tri načina koje karakteriše različita frekventnost (jedno mešanje kljuka nakon 48 h od početka fermentacije, jednom i dva puta dnevno). Koštice šljive, odvojene tokom faze primarne prerade, dodavane su u različitim udelima (25%, 50% i 100 %) u kljuk neposredno pred početak fermentacije, a kvalitet i prihvatljivost proizvedenih vina ocenjivan je određivanjem sadržaja etanola, metanola, cijanovodonične kiseline i benzaldehida. Istraživanja su dala uvid u efikasnost i opravdanost primemene ispitivanih pektolitičkih enzima, postupak mešanja kljuka jedan put na dan se pokazao optimalnim, dok prisustvo koštica tokom fermentacije, dovodi do značajnog povećanja sadržaja cijanovodonične kiseline i benzaldehida u vinu od šljive, ali su i utvrđene vrednosti ovih parametara značajno niže od maksimalno dozvoljenih.

Ključne reči: šljiva, alkoholna fermentacija, voćno vino, koštica, pektolitički enzimi.

INTRODUCTION

Plum fruits are very suitable raw materials for the production of fruit wines (Miljić and Puškaš, 2015). Plum wine is an alcoholic beverage produced by the complete or partial alcoholic fermentation of plum juice or pomace obtained from fresh and technologically mature plum fruits. The technology of fruit wines is similar to the technology of conventional wines, accompanied by a number of procedure modifications which mostly arise from differences in the raw materials used. Therefore, a detailed study on the enzymatic treatment of pomace (mostly with pectinases) and fermentation cap management, as well as the influence of plum pits on the wine quality, is required.

The activity of pectolytic enzymes during fruit processing for wine production is associated with the hydrolysis of pectic substances (which leads to the liquefaction of the pomace, yield increase, clarification and filtration) and the increased efficiency of colour and flavour component extraction (Byarugaba-Bazirake, 2008; Bayindirli, 2010). Commercial preparations of pectolytic enzymes are a mixture of polygalacturonase, pectin lyase, and pectin methyl esterase (PME) (Dietrich et al., 1991). The optimum pH for the activity of polygalacturonase and pectin methyl esterase is about 4.5 and with a pH increase of above 5.0 their activity decreases significantly. Pectin lyase shows the

optimal activity in a much wider pH range (5.0-6.0) (Lozano, 2006). The activity of polygalacturonase is best in the temperature range of 30-50 °C, whereas it begins to decrease significantly with an increase in temperature over a short period of time. The similar temperature dependence is characteristic of the pectin lyase activity (Bayindirli, 2010). The optimal temperature for the activity of pectin methyl esterase enzymes is in the range of 45-55 °C, depending on the origin of the enzymes and the external environmental factors. The activity of the pectolytic enzymes involved in the wine production is lower, given the lower pH of the fruit pomace (3.0-4.0) and the lower temperature during fruit processing and fermentation (10-30 °C) (Ducasse et al., 2011).

The enzymatic degradation of pectic substances during the pre-fermentation phase of wine production from different fruits leads to a significant increase in the first run volume and a reduction of the time of juice pressing (Pilnik, 1996). Accordingly, the maceration of grapes using pectolytic enzymes can lead to an increase in the free run yield by approximately 10 % (Laurentiu Itu et al., 2011). Furthermore, the yield of clear juice in banana wine production, which did not include the addition of commercial pectolytic enzymes, was approximately 38 %, whereas the yield was significantly higher (60-65%) with the use of the enzymes (Byarugaba-Bazirake, 2008). The extraction of anthocyanins and other phenolic compounds during

maceration requires the degradation of cell wall structures. The addition of pectinases during the maceration of different fruits leads to a significant increase in the content of extracted anthocyanins, increases the intensity and stability of colour over a longer period of time (Kelebek *et al.*, 2007; Romero-Cascales *et al.*, 2008; Mieszczakowska-Frac *et al.*, 2012; Punbusayakul, 2018). The addition of pectolytic enzymes during fruit pomace processing also has an impact on the content of methanol, a compound which is a natural ingredient of alcoholic beverages and soft drinks. However, the products of metabolic transformations of methanol (formaldehyde and formic acid) are toxic to humans. Extensive research on the methanol formation and content in plum wines was argued in our previous studies (Miljić *et al.*, 2014; Miljić *et al.*, 2016).

The effectiveness of extracting soluble substances from raw materials for wine production is very much dependent on the frequency and technique of pomace stirring. Modern cap management systems include the following components: punch-down devices (manual or mechanical), pump-over devices, roto tanks, sprinklers, thermovinification, gas systems such as pulsed air, etc.

Plum pits contain a significant amount of amygdalin, a glycoside that is degraded into two molecules of glucose, benzaldehyde and hydrocyanic acid (HCN) in the acidic environment and under the action of beta-glucosidase enzymes. The enzymatic degradation of amygdalin involves the splitting of amygdalin to prunasin and glucose by the enzyme amygdalin lyase, which is followed by the hydrolysis of prunasin to mandelonitrile and glucose by the enzyme prunasin lyase. The final stage of the hydrolysis is the breaking down of mandelonitrile to benzaldehyde and hydrogen cyanide (HCN) by hydroxynitrile lyase. The optimal temperature for the activity of enzymes is in the range 20-40 °C as they can be deactivated at higher temperatures (Bolarinwa *et al.*, 2014). The inability of technologically efficient pit separation during primary fruits processing can significantly affect the chemical composition of the produced wine. Therefore, special attention needs to be devoted to the monitoring of benzaldehyde and hydrocyanic acid contents, which exert a potentially toxic effect on human health.

The objective of this research was to assess the impact of various commercial pectolytic enzymes, used in the plum pomace treatment, the frequency and technique of pomace stirring and the plum pit addition to the pomace during fermentation, on the yield and quality of wine.

MATERIAL AND METHOD

The plum cultivar 'Čačanska leptotica' was procured from registered fruit producers in the Fruška gora region. Plums were harvested in the middle of August at the stage of technological maturity. Plum fruits were pitted, crushed and homogenized. A total of 3 kg of plum pomace was placed in each plastic vessel for the microvinification process according to the experimental plan. The pomace samples obtained were treated with K₂S₂O₅ (SO₂ level was set to 50 mg SO₂/kg pomace). The effect of five different commercial pectolytic enzymes on the yield and quality of wine was evaluated in the first part of the study (Table 1). The pectolytic enzyme Lallzyme EX-V (Lallemand S.A., St. Simon, France, 0.02 g/kg of pomace) was used in the experiments for assessing the fermentation cap management and plum pit addition to the pomace during fermentation.

Under laboratory conditions, the pomace stirring was performed using the conventional mechanical immersion, i.e. the punch-down technique, whereas the effects of stirring frequency on the yield and quality parameters of wine were examined.

Table 1 Pectolytic enzymes used for the plum pomace treatment

	Enzyme	Dose	Producer
E ₁	Lallzyme EX-V	0.02 g/kg	Lallemand, St. Simon, France
E ₂	Lallzyme Cuvee Blanc	0.02 g/kg	Lallemand, St. Simon, France
E ₃	Trenolin Color DF	0.02 g/kg	Erbslöh, Geisenheim, Germany
E ₄	Trenolin Frio DF	20 µL/kg	Erbslöh, Geisenheim, Germany
E ₅	Enartis zym balance	0.02 g/kg	Enartis, Novara, Italy

The stirring of the plum pomace was performed in three manners characterized by different frequencies: 48 h after the start of fermentation, once a day and twice a day. The effect of pomace stirring was tested in two series of microvinification: without (V₁₋₃) and with (V₄₋₆) the addition of pectolytic enzymes.

Examination of the effect of various quantities of plum pit in the pomace during fermentation on the quality and acceptability of the produced wines implied a series of microvinifications (K₀₋₃, Table 2). The pits were added in partially crushed form.

Table 2 The amount of pits added to the pomace during fermentation

Microvinification	Pits amount (%) [*]
K ₀	0
K ₁	25
K ₂	50
K ₃	100

^{*} quantity of added pits represents the share of the total mass of pits separated for obtaining 3 kg of plum pomace

The fermentation in all the experiments conducted (E₁₋₅, V₁₋₆, K₁₋₃) was performed at a temperature of 25 °C and a pH value of 3.5, using 0.25 g/kg of the commercial wine yeast *Saccharomyces cerevisiae* (Spiriferm, Erbslöh, Geisenheim, Germany). Upon fermentation, the plum wine obtained was passed through the cheesecloth and kept in 1.5 L glass bottles at 10 °C in order to remove coarse precipitates. After three days, the wine was racked off (the free SO₂ level was adjusted to 30 mg/L), poured into 0.5 L bottles, closed with screw caps and kept at 10 °C in the absence of light until analyses.

The plum pomace samples were analysed for ethanol, methanol and total phenolic compounds using the standard OIV method (OIV, 2013). The content of glycerol was determined using the enzymatic methods (OIV, 2013) and commercial enzyme tests (Megazyme, CO, Wicklow, Republic of Ireland). The determination of hydrocyanic acid and benzaldehyde contents in the plum wine samples was carried out using the colorimetric methods defined in the Ordinance on Methods for Sampling and Performing Physical and Chemical Analyses of Alcoholic Beverages (Official Gazette of SFRY, 70/1987). The methods applied include the distillation of wine samples and the determination of hydrocyanic acid and benzaldehyde contents in the distillates obtained.

The STATISTICA 12.0 (Statsoft) program was used for statistical analysis in the present study. All the experiments (E₁₋₅, V₁₋₆ and K₁₋₃) were conducted in triplicate. The values shown in the tables represent the average values of 3 independently set fermentations. The statistical difference between the mean values of the parameters examined was based on the analysis of

variance (ANOVA) at the 95 % confidence level. The values recorded as significantly different using the Duncan's test were marked with different letters (a, b, c ...).

RESULTS AND DISCUSSION

The activity of pectolytic enzymes during fruit processing is important for the production of wine, as already stated above. The effects of five different commercial pectolytic enzymes on the wine yield, extraction of phenolic substances and production of ethanol, glycerol and methanol were assessed (Table 3).

The addition of pectolytic enzymes to the pomace significantly influenced the yield and chemical composition of the plum wines produced. The results shown in Table 3 indicate a significant ($P < 0.05$) increase in the wine yield, as well as in the content of ethanol, methanol and total phenolic compounds, compared to the control sample (K) produced without the use of enzymes.

Table 3 Influence of pectolytic enzyme addition on the yield and quality parameters of the plum wines

	Ethanol (% v/v)	Methanol (mg/L)	Glycerol (g/L)	Wine yield* (%)	Total phenols (g/L)
K	7.38 ± 0.12 ^a	480 ± 17 ^a	5.78 ± 0.09 ^a	48 ± 4 ^a	2.01 ± 0.08 ^a
E ₁	7.71 ± 0.10 ^b	1109 ± 13 ^b	6.05 ± 0.12 ^{ab}	67 ± 1 ^c	2.21 ± 0.04 ^b
E ₂	7.79 ± 0.27 ^b	1150 ± 24 ^b	6.03 ± 0.09 ^{ab}	60 ± 8 ^b	1.96 ± 0.10 ^a
E ₃	7.58 ± 0.15 ^{ab}	1232 ± 5 ^c	6.19 ± 0.25 ^{ab}	58 ± 3 ^b	2.19 ± 0.03 ^b
E ₄	7.89 ± 0.02 ^b	1310 ± 18 ^d	6.06 ± 0.17 ^{ab}	72 ± 3 ^c	2.29 ± 0.06 ^{bc}
E ₅	7.66 ± 0.22 ^b	1121 ± 29 ^b	5.8 ± 0.31 ^a	58 ± 5 ^b	2.07 ± 0.05 ^{ab}

* wine yield in percent or expressed as ml of wine per 100 g of pomace

^{a, b, c} different letters within the same column indicate statistically significant differences between the values ($P < 0.05$).

The use of different commercial pectolytic enzymes did not cause significant differences in the ethanol concentration of the produced wines. Conversely, the content of methanol in the E₁₋₅ wines differed significantly, depending on the enzyme used (1109-1310 mg/L). The enzymatic treatment of the pomace samples resulted in the formation of approximately threefold higher concentrations of methanol in the wine samples compared to that recorded in the control sample. As commercial pectinase is a mixture of polygalacturonase, pectin lyase and pectin methyl esterase (PME) (Dietrich et al., 1991), the concentration of methanol in wine largely depends on the specific activity of PME in the particular enzyme preparation. The highest concentration of methanol (1310 mg/L) was determined in the plum wine in which the pomace was treated with the Trenolin Frio DF (E₄) enzyme. The use of pectolytic enzymes during vinification did not lead to significant changes in the glycerol content. Conversely, the control wine yield was significantly lower than those recorded in the wines produced from the enzymatically treated pomace. The application of pectolytic enzymes caused an increase in the wine yield up to 50 %. The wine yield increase was the most pronounced in the case of Trenolin Frio DF and Lallzyme EX-V use. The enzymatic treatment of the plum pomace also led to an increase in the efficiency of phenolic compound extraction. The content of total phenolic compounds in the wine E₄ increased by 15 % compared to the control wine due to the activity of Trenolin Frio DF enzyme. A significant increase in the total phenolic content was also recorded in the wines E₁ and E₃. The results obtained indicate that the

best technological characteristics of plum wines were obtained with the use of Lallzyme EX-V enzyme, which is manifested in high contents of ethanol, glycerol and total phenolic compounds, as well as high wine yields, and the lowest concentration of methanol. The efficiency of the extraction of soluble matter from the raw material for wine production is greatly dependent on the fermentation cap management (Table 4). Differences in the frequency of pomace stirring did not significantly affect the content of ethanol, methanol and glycerol in the wines produced both with and without enzyme addition. The wine yield in vinifications without the use of pectolytic enzyme was significantly higher when the pomace was stirred more frequently (V₂ and V₃), compared to the V₁ regime. A similar trend was also noted in the case of enzyme-treated pomace (V₅ and V₆ compared to V₄). Conversely, there were no significant differences in the wine yield in the case of pomace stirring once and twice a day. A more pronounced extraction of the phenolic compounds was achieved by applying more frequent stirring regimes. It was observed that one stirring of the pomace 48 h after the start of fermentation does not ensure the production of wines of adequate phenolic content. The stirring of pomace once and twice a day led to an increase in the total phenolic content by 20-25 %, and the obtained values were higher for the enzyme-treated pomace samples. The application of pectolytic enzyme during fermentation exerted a stronger impact on the wine yield than the frequency of pomace stirring. However, the effect of pomace stirring on the content of total phenols was more significant. Ultimately, the effective extraction of plum polyphenols was achieved by pomace stirring once a day, whereas further increase in the frequency of this operation did not have a significant effect ($P < 0.05$).

The contents of benzaldehyde and hydrocyanic acid are not defined by the legal acts regulating the quality of wine in Serbia but by the appropriate national technical regulation on the quality of alcoholic beverages (Regulation on categories, quality and declaration of spirits and other alcoholic beverages, 2010), where the maximum permitted content of benzaldehyde and hydrocyanic acid in plum brandy is 100 mg/L and 70 mg/L respectively (expressed per liter of absolute alcohol). This is equivalent to 7 mg/L and 5 mg/L for alcoholic beverages with an ethanol content of 7% v/v (such is the plum wine produced).

Plum pits, separated during the primary processing of fruits, were added in different portions (25 %, 50 % and 100 %) to the pomace before the start of fermentation, and the quality and acceptability of the wines produced were assessed by determining the content of ethanol, methanol, hydrocyanic acid and benzaldehyde (Table 5).

Table 4. Effect of the pomace stirring frequency on the yield and quality parameters of the plum wines

	Ethanol (% v/v)	Methanol (mg/L)	Glycerol (g/L)	Wine yield* (%)	Total phenols (g/L)
V ₁	7.51 ± 0.12 ^a	460 ± 11 ^a	5.71 ± 0.11 ^a	44 ± 3 ^a	1.68 ± 0.10 ^a
V ₂	7.34 ± 0.08 ^a	496 ± 17 ^a	5.81 ± 0.16 ^a	50 ± 2 ^b	1.95 ± 0.03 ^b
V ₃	7.44 ± 0.15 ^a	482 ± 22 ^a	5.94 ± 0.08 ^{ab}	47 ± 2 ^{ab}	2.05 ± 0.07 ^b
V ₄	7.62 ± 0.22 ^{ab}	1088 ± 27 ^b	6.1 ± 0.17 ^b	61 ± 1 ^c	1.75 ± 0.03 ^a
V ₅	7.84 ± 0.12 ^b	1121 ± 19 ^b	5.98 ± 0.12 ^{ab}	65 ± 1 ^{cd}	2.22 ± 0.05 ^c
V ₆	7.63 ± 0.04 ^{ab}	1125 ± 18 ^b	6.12 ± 0.09 ^b	66 ± 3 ^d	2.16 ± 0.09 ^c

* wine yield in percent or expressed as ml of wine per 100 g of pomace.

^{a, b, c} different letters within the same column indicate statistically significant differences between values ($P < 0.05$)

Table 5 Indicators of the impact of pit addition on the chemical composition of the plum wines

	Ethanol (% v/v)	Methanol (mg/L)	HCN (mg/L)	Benzaldehyde (mg/L)
K ₀	7.43 ± 0.05 ^a	703 ± 5 ^a	0.02 ± 0.01 ^a	0.81 ± 0.07 ^a
K ₁	7.39 ± 0.11 ^a	722 ± 18 ^a	0.23 ± 0.02 ^b	1.75 ± 0.11 ^b
K ₂	7.52 ± 0.05 ^a	691 ± 6 ^a	0.5 ± 0.04 ^c	2.53 ± 0.04 ^c
K ₃	7.64 ± 0.13 ^a	705 ± 5 ^a	0.75 ± 0.1 ^d	3.74 ± 0.13 ^d

^{a, b, c} different letters within the same column indicate statistically significant differences between the values ($P <$

The concentration of ethanol in the experimental wines (K₀₋₃) did not significantly ($P < 0.05$) change with an increase (25-100 %) in the amount of pits added. The results obtained indicate that the amount of ethanol, produced by the fermentation of glucose resulting from the enzymatic degradation of amygdalin, was statistically negligible. The addition of pits also exerted no effects on the concentration of methanol. However, an increase in the amount of pits added to the fermentation of the plum pomace significantly affected the contents of HCN and benzaldehyde in the wines obtained. With an increase in the proportion of pits added, the content of these compounds increased significantly. The maximum hydrocyanic acid content was determined in the K₃ wine (0.75 mg/L or 10 mg/L of absolute alcohol). The highest content of benzaldehyde (3.74 mg/L or 50 mg/L of absolute alcohol) was measured in the same experimental wine (K₃). However, in addition to an increase in the amounts of these compounds, resulting from the management of fermentation with pits added, the contents obtained are significantly lower than the maximum permitted. The increased share of pits in the fermentation also affected the sensory properties of the wines produced. The characteristic almond flavor, due to the increased content of benzaldehyde, was particularly pronounced in the K₃ wine.

CONCLUSION

The present study gave an insight into the efficiency and justification of the pectolytic enzyme application examined. The once-per-day method of pomace stirring proved to be optimal, whereas the presence of pits during fermentation led to a significant increase in the hydrocyanic acid and benzaldehyde contents of the plum wines analysed. However, the hydrocyanic acid and benzaldehyde contents obtained did not exceed the maximum content allowed.

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REFERENCES

- Bayindirli, A. (2010). Enzymes in Fruit and Vegetable Processing: Chemistry and Engineering Applications. CRC Press, Florida, USA.
- Bolarinwa, I.F., Orfila, C., Morgan, M.R.A. (2014). Amygdalin content of seeds, kernels and food products commercially-available in the UK. Food Chemistry, 152, 133-139.
- Byarugaba-Bazirake, G.W. (2008). The effect of enzymatic processing on banana juice and wine. PhD dissertation. Stellenbosch University, Institute for Wine Biotechnology, South Africa.
- Dietrich, H., Patz, C., Schöpplain, F., Will, F. (1991). Problems in evaluation and standardization of enzyme preparations. Fruit Processing, 1, 131-134.
- Ducasse, M.A., Williams, P., Canal-Llauveres, R.M., Mazerolles, G., Cheynier, V., Doco, T. (2011). Effect of macerating enzymes on the oligosaccharide profiles of Merlot red wines. Journal of Agricultural and Food Chemistry, 59, 6558-6567.
- Kelebek, H., Canbas, A., Cabaroglu, T., Selli, S. (2007). Improvement of anthocyanin content in the cv. Öküzgözü wines by using pectolytic enzymes. Food Chemistry, 105, 334-339.
- Laurentiu Itu, N., Rapeanu, G., Hopulele, T. (2011). Effect of maceration enzymes addition on the aromatic white winemaking. The Annals of the University Dunarea de Jos of Galati, 35 (1), 77-91.
- Lozano, J.E. (2006). Fruit Manufacturing: Scientific Basis, Engineering Properties and Deteriorative Reactions of Technological Importance. Springer, USA, 21-54.
- Mieszczakowska-Frac, M., Markowski, J., Zbrzezniak, M., Plochanski, W. (2012). Impact of enzyme on quality of blackcurrant and plum juices. Journal of Food Science and Technology, 49, 251-256.
- Milić, D., Galić, D., Vukoje, V. (2011). Mogućnosti unapređenja voćarske proizvodnje u Srbiji. Journal of Processing and Energy in Agriculture, 15 (1), 27-30.
- Miljić, U., Puškaš, V. (2014). Influence of fermentation conditions on production of plum (*Prunus domestica* L.) wine: A response surface methodology approach. Hemijska Industrija, 68 (2), 199-206.
- Miljić, U., Puškaš, V. (2015). Suitability of chosen plum cultivars (*Prunus domestica* L.) for fruit wine production. Journal on Processing and Energy in Agriculture, 19 (2), 95-97.
- Miljić, U., Puškaš, V., Vučurović, V. (2016). Investigation of technological approaches for reduction of methanol formation in plum wines. Journal of the Institute of Brewing, 122 (4), 635-643.
- OIV - Office International del la Vigne et du Vin (2013). Compendium of International Methods of Wine and Must Analysis. Organisation Internationale de la Vigne et du Vin. Volume 1 and 2, Paris, France.
- Pilnik, W. (1996). From traditional ideas to modern fruit juice wining processes. Agricultural University, Department of Food Science, Wageningen, The Netherlands, 159-179.
- Pravilnik o kategorijama, kvalitetu i deklarisanju rakije i drugih alkoholna pića (2010). Službeni glasnik Republike Srbije, 74/2010.
- Punbusayakul, N. (2018). Bioactive compounds and antioxidant activity of wines from different currant cultivars. Journal of Processing and Energy in Agriculture, 22 (1), 27-30.
- Romero-Cascales, I., Fernández-Fernández, J.I., Ros-Garcia, J.M., López-Roca, J.M., Gómez-Plaza, E. (2008). Characterisation of the main enzymatic activities present in six commercial macerating enzymes and their effects on extracting colour during winemaking of Monastrell grapes. International Journal of Food Science and Technology, 43, 1295-1305.

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