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Red wine concentrates as inhibitors of lipid oxidation in compound butter

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Abstract

Compound butter, a butter enriched with different ingredients, such as herbs and spices, has gained popularity in recent years. The main purpose of additional ingredients is to improve butter flavour, but these compounds can also affect the butter's oxidative stability (shelf life). In search for novel products, potential of 12 wine concentrates (Bermet, Cabernet Sauvignon and Merlot) to inhibit lipid oxidation that may occur in the butter was tested, butter mixed with concentrates of different wines (wine butter) was prepared, and preliminary evaluation of consumers acceptance of selected wine butters was done. Also, the content of total polyphenols, flavonoids, anthocyanins and tannins in wine concentrates was determined by spectrophotometric methods. Eleven of twelve wine concentrates showed ability to inhibit lipid oxidation comparable with activity of standard antioxidant trolox. The best capacity had Cabernet Sauvignon, with CS-4 as the most dominant one. In general, Bermet (B-4) was among the wines with the highest overall contents of total monomeric anthocyanins, polyphenols, tannins and flavonoids, but, the similar trend was not noticed regarding concentrates. Correlation coefficients implicate that there is no firm relationship between particular polyphenol group and expressed activity. However, the result of the sensory analysis survey pointed out compound butter with Bermet concentrate. In general, obtained results showed that wine concentrates could be used as compounds which could improve flavour, increase the oxidative stability of butter and possibly extend its shelf-life.

Key words*: compound butter; wine concentrate; Cabernet Sauvignon; Merlot; Bermet; lipid oxidation; sensory analysis.*

INTRODUCTION

Butter is a dairy product, produced by churning fresh or fermented cream or milk and is defined as a water-in-oil emulsion (W/O) with minimum of 82% fat [1]. It is widely used as a spread, as well as for cooking and baking. Like other dairy products, butter also has limited shelf-life. Butter stability is influenced by numerous factors, such as quality of original milk, production process, packaging material, storage conditions (temperature, light), etc [2-5]. Lipid oxidation, which results in rancidity, is one of the main processes that affect butter quality, generally causing changes in color, texture, aroma and nutritional value [3,6]. To increase oxidative stability and consequently food safety, antioxidants could be added to butter [3].

Synthetic antioxidants, such as *t*-butyl-4-hydroxyanisole (BHA) and 2,6-di-*t*-butyl-*p*-hydroxytoluene (BHT) may prevent lipid oxidation in butter, but, nowadays, intensive search for safe, natural food preservatives is carried out [3]. Ages ago, herbs and spices are used to enhance the organoleptic properties of food, besides providing beneficial health effects, including antioxidant activity. Today, compound butter is commonly prepared by adding different herbs and spices to the butter. There are numerous receipts and the leading idea is that additional compounds will change and improve the flavor of the butter. But, some studies testify that addition of different spices and herbs or their extracts could improve storage stability of butter. Sage [5,7], rosemary [5,7], oregano [7], hazelnut [8], cinnamon [9], green tea [10], garlic [11], *Satureja cilicica* essential oil [12], black cumin essential oil [13] could extend butter stability and shelf-life due to antioxidative or antimicrobial activity. Polyphenolic compounds found in these plants significantly contribute to their protective activity.

Red wines are rich sources of polyphenols, natural products with prominent biological activity linked to health benefits of moderate red wine consumption [14]. Having in mind that wine could be also used for cooking, as well as antioxidant activity of wine polyphenols, and intensive needs for natural antioxidants which could prevent oxidation of butter, we assumed that red wine concentrate added to butter could influence oxidative stability and that prepared wine butter could be interesting, novel product on Serbian market. Therefore, in this study, we examined the potential of twelve wine concentrates (four Bermet, four Cabernet Sauvignon and four Merlot wines from Fruska Gora region) to inhibit Fe^{2+}/ascorbate induced lipid peroxidation of butter. Also, the content of total polyphenols, flavonoids, anthocyanins and tannins in wine concentrates was determined by spectrophotometric methods and correlated with examined bioactivity. Preliminary evaluation of consumers' acceptance of selected wine butters was done.

MATERIAL AND METHODS

Wine samples and reagents

In this study, commercially available Bermet (B1-B4), Cabernet Sauvignon (CS1-CS4) and Merlot (M1-M4) wines from the area of Fruska Gora (Serbia) were used. Same procedure was applied for preparation of all concentrates: wine aliquots were evaporated on a rotary vacuum evaporator. For chemical analysis and testing potential to inhibit lipid oxidation, concentrates were dissolved in dimethylsulfoxide (300 mg/mL Cabernet Sauvignon and Merlot; 500 mg/mL Bermet wines). All samples were stored at -20 °C until use.

Butter was generously provided by local producer of butter and compound butter, Mrs. Popovic, from company „Nebesa", Novi Sad, Serbia. All reagents used in this study were of analytical grade.

Analysis of polyphenolics content

Analysis of total monomeric anthocyanins, polyphenols, tannins and flavonoids content was done according to previously published procedure [15,16].

Lipid oxidation assay

The ability of tested wine concentrates to inhibit lipid oxidation was determined by the thiobarbituric acid (TBA) method, where fatty acids from butter were used as a substrate for lipid oxidation, caused by Fe^{2+} ions in synergism with ascorbic acid. Fatty acid solution (2%) was prepared by adding 4 g of butter and 2 mL of Tween-80 in 198 mL of phosphate buffer (pH 7.4). This suspension (1 mL) was mixed with 70 µL of $FeSO_4$ (4.58 mmol/L), 70 µL of ascorbic acid (0.087 mmol/L) and 20 µL of the wine concentrate (final concentration 30 mg/mL) or trolox (range of final concentrations 0.016 – 3 mg/mL). In control, analyzed samples were substituted with the solvent. Prepared solutions were incubated for 1 hour at 37 °C. After the addition of 3.72% EDTA (70 µL) and 700 µL of TBA reagents (TBA (3.75 mg/mL), $HClO_4$ (1.3%) and trichloroacetic acid (0.15 g/mL)), the mixture was heated for 15 min at 100 °C, then cooled to room temperature and centrifuged for 15 min at 3500 rpm. The absorbance of the solution was measured by spectrophotometry at 532 nm. All probes and controls were done in three repetitions.

The inhibition of lipid oxidation of wine concentrates was calculated based on the following equation: $I\ (\%) = (A_0 - A)/A_0 \times 100$, where A_0 was the absorbance of the control sample and A was the absorbance of the samples examined, corrected for the value of blank probe. Percentage of inhibition achieved by a different concentration of trolox were calculated and corresponding inhibition–concentration curves were drawn using the Origin software, version 8.0. Based on the wine concentrates I (%) value, by comparison with the standard calibration curve of trolox, the inhibition values were converted to micrograms of trolox equivalents, and the result was expressed as the mean value of three measurements ± standard deviation (micrograms of trolox equivalents per milligram of concentrate).

Consumer acceptance survey

Concentrates of Bermet, Cabernet Sauvignon and Merlot wines produced in the same winery were prepared by evaporation on a rotary vacuum evaporator. Compound butters were prepared by adding 3 g of wine concentrate to 100 g butter. Prepared compound butters were subjected to consumer acceptance survey on food festival "Calabrc Fest", taking place in Novi Sad on 19.9.2021. Consumers (148) were asked to try three different wine butters, prepared with Bermet, Cabernet Sauvignon and Merlot concentrates and to state which one they prefer.

Statistical analysis

All analyses were performed in triplicate and results were expressed as mean values ± SD of three different trials. The differences between the samples were evaluated using one-way analysis of variance (ANOVA) followed by comparison of the means by Tukey HSD test ($p \leq 0.05$) using the STATISTICA version 14.0. (TIBCO Software Inc.). Correlations between lipid oxidation inhibitory potential and total polyphenols, total tannins, total flavonoids and total monomeric anthocyanins content were determined using the Pearson correlation test.

Table 1. Content[1] of total monomeric anthocyanins[2], polyphenols, flavonoids and tannins in analyzed concentrates and wines.

	Total monomeric anthocyanins		Total polyphenols		Total tannins		Total flavonoids	
	µg eq M-3-G /mg concentrate	µg eq M-3-G /mL	µg eq gallic acid /mg concentrate	µg eq gallic acid /mL	µg eq catechin / mg concentrate	µg eq catechin /mL	µg eq quercetin / mg concentrate	µg eq quercetin /mL
B-1	0.013 ± 0.002^{g}	1.582 ± 0.203^{g}	19.74 ± 1.864^{d}	2456 ± 231.9^{a,b}	8.941 ± 0.669^{f}	1112 ± 83.24^{b}	0.043 ± 0.010^{f}	6.002 ± 0.018^{e}
B-2	0.025 ± 0.003^{g}	3.080 ± 0.400^{g}	15.25 ± 1.114^{d}	1852 ± 135.3^{d,e}	4.663 ± 0.228^{d}	566.1 ± 27.64^{d}	0.073 ± 0.005^{f}	8.867 ± 0.641^{f,g}
B-3	0.029 ± 0.001^{g}	3.463 ± 0.160^{g}	12.58 ± 0.775^{d}	1478 ± 91.11^{e}	3.468 ± 0.308^{d}	407.4 ± 36.20^{e,f}	0.075 ± 0.002^{f}	8.784 ± 0.242^{f,g}
B-4	0.375 ± 0.009^{d,e}	51.19 ± 1.270^{a,b}	18.19 ± 1.083^{d}	2484 ± 147.9^{a,b}	10.26 ± 0.260^{f}	1401 ± 35.54^{a}	0.179 ± 0.015^{e}	24.41 ± 2.004^{a}
CS-1	0.799 ± 0.005^{c}	27.00 ± 0.159^{d}	73.87 ± 5.328^{a}	2495 ± 180.0^{a,b}	27.48 ± 0.065^{b}	928.3 ± 2.21^{c}	0.658 ± 0.015^{a}	22.22 ± 0.502^{a,b}
CS-2	0.531 ± 0.066	17.67 ± 0.132^{e}	79.69 ± 1.424^{a}	2473 ± 44.20^{a,b}	26.52 ± 0.792^{b,c}	822.9 ± 24.59^{c}	0.578 ± 0.042^{b}	17.93 ± 1.289^{c}
CS-3	1.556 ± 0.121^{a}	51.77 ± 4.018^{a}	82.39 ± 5.235^{a}	2742 ± 174.2^{a}	24.67 ± 0.787^{c}	821.0 ± 26.20^{c}	0.547 ± 0.044^{b}	18.21 ± 1.466^{c}
CS-4	1.475 ± 0.061^{a,b}	44.11 ± 1.834^{c}	70.81 ± 7.152^{a,b}	2117 ± 213.8^{b,c,d}	27.79 ± 1.864^{b}	830.9 ± 55.72^{c}	0.706 ± 0.040^{a}	21.11 ± 1.189^{b}
M-1	0.790 ± 0.090^{c}	23.43 ± 0.171^{d}	61.20 ± 2.886^{b,c}	1942 ± 91.56^{c,d}	13.25 ± 1.351^{e}	420.4 ± 42.86^{e}	0.235 ± 0.008^{d,e}	7.467 ± 0.241^{g,e}
M-2	1.390 ± 0.096^{b}	45.71 ± 3.142^{c,d}	60.34 ± 2.171^{b,c}	1984 ± 71.39^{c,d}	31.83 ± 2.715^{a}	1047 ± 89.26^{b}	0.423 ± 0.037^{c}	13.90 ± 1.210^{d}
M-3	0.335 ± 0.038^{e,f}	10.34 ± 1.182^{f}	58.81 ± 4.652^{c}	1816 ± 143.6^{d,e}	18.72 ± 0.389^{d}	577.8 ± 12.01^{d}	0.413 ± 0.023^{c}	12.74 ± 0.702^{d,e}
M-4	0.191 ± 0.003^{f}	7.840 ± 0.139^{f,g}	54.33 ± 2.286^{c}	2279 ± 102.0^{b,c}	9.713 ± 0.008^{f}	399.4 ± 0.316^{f}	0.266 ± 0.019^{d}	10.94 ± 0.555^{e,f}

[1]values are means ± SD of three measurements.

[2]Means within each column with different letters (a–g) differ significantly ($p \leq 0.05$).

Abbreviations: M-3-G: malvidin 3-*O*-glucoside; B-1 to B-4: Bermet concentrate; CS-1 to CS-4: Cabernet Sauvignon concentrate; M-1 to M-4: Merlot concentrate.

RESULTS

Content of total monomeric anthocyanins, polyphenols, flavonoids and tannins in analyzed wine concentrates and corresponding wines is shown in **Table 1.**

Total monomeric anthocyanin content is expressed as microgram equivalents of malvidin-3-*O*-glucoside (µg eq M-3-G), the dominant anthocyanin in red wines. Having in mind that each wine sample has different concentrations of active and other compounds, all determined contents can be expressed in two ways: as µg eq M-3-G per mg of wine concentrate (µg eq M-3-G/mg concentrate), or as µg eq M-3-G per mL of wine (µg eq M-3-G/mL wine), which gives a better projection of composition of original wine. The highest amounts of monomeric anthocyanins were found in concentrates C-3, C-4 and M-2 (1.556, 1.475 and 1.390 µg eq M-3-G/mg concentrate). On the other hand, monomeric anthocyanin is present from 1.582 to 51.19; 17.67 to 51.77 and 7.84 to 45.71 µg eq M-3-G/mL in Bermet, Cabernet Sauvignon and Merlot wines, respectively.

The content of total polyphenols was determined by the spectrophotometric method using the Folin-Ciocalteu (FC) reagent. Content of total polyphenols in concentrates ranged from 12.58 – 19.74 (Bermet), 70.81 – 82.39 (Cabernet Sauvignon) and 54.33 – 61.2 µg eq gallic acid/mg concentrate (Merlot), with dominant content in sample CS-2 and CS-3. The smallest amount of total polyphenols was found in B-3 sample (1487 µg eq gallic acid/ mL), being almost two folds lower than in CS-3 (2742 µg eq gallic acid/mL).

After determining the phenol content, tannins were precipitated by adding polyvinylpolypyrrolidone. The tannin content in the samples represents the difference in the content of total polyphenols before and after tannin deposition. In the concentrates, the lowest amount was detected in B-3 (3.468 µg eq catechin/mg concentrate), while the highest amount was determined in M-2 (31.83 µg eq catechin/mg concentrate). On contrary, in wines, the lowest amount was detected in M-4 (399.4 µg eq catechin/mL), while the highest amount was determined in B-4 (1401 µg eq catechin/mL).

Regarding total flavonoid content, concentrate CS-4 was dominant (0.706 µg eq quercetin/mg concentrate). Interestingly, Bermet wines were the samples with the lowest (B-1, 6.002 µg eq quercetin/mL) and the highest flavonoid content (B-4, 24.41 µg eq quercetin/mL).

The ability of the tested wine concentrates to inhibit lipid oxidation of butter is presented in **Figure 1.**

Lipid oxidation inhibitory activity was examined in model system where the same ratio of wine concentrate and butter was used as in prepared wine butters (3%). Only one concentrate of Bermet wine (B-2) did not show activity comparable to trolox (activity was out of range of trolox calibration curve). Generally, Bermet and Merlot concentrates had obviously lower inhibitory potential (0.728–1.719 µg eq trolox/mg concentrate) than Cabernet Sauvignon samples (2.9 – 23.59 µg eq trolox/mg concentrate). Anyway, sample CS-4 was singled out as the most active one.

Correlation coefficients between potential to inhibit lipid oxidation and total monomeric anthocyanins, polyphenols, tannins and flavonoids content in examined concentrates (**Table 2**) implicate that there is no strong correlation and that no firm relationship could be established between particular polyphenols' group and expressed activity.

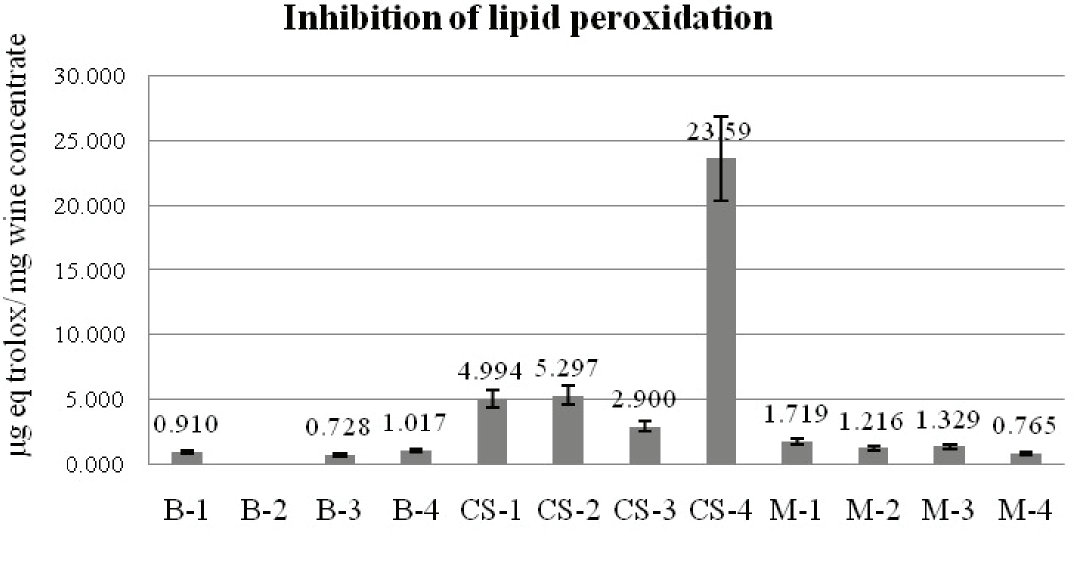
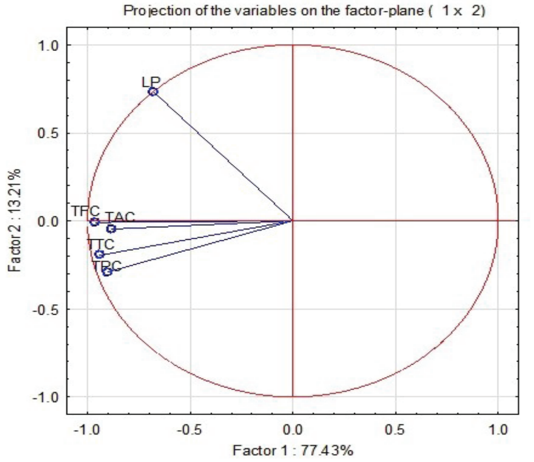


Figure 1. Inhibition of lipid peroxidation. Abbreviations: B-1 to B-4: Bermet concentrate; CS-1 to CS-4: Cabernet Sauvignon concentrate; M-1 to M-4: Merlot concentrate.

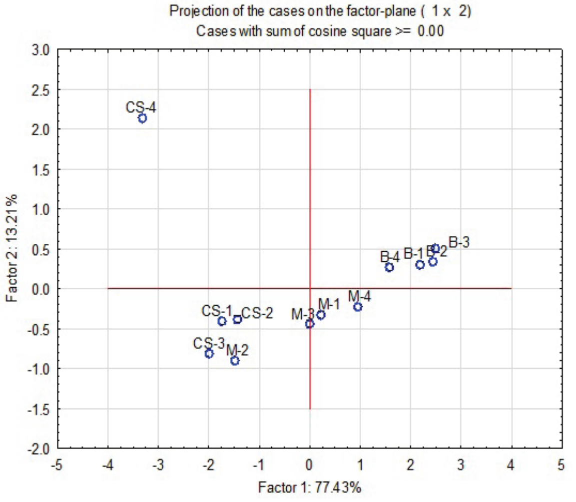
Table 2. Pearson's linear correlation coefficients between investigated chemical composition and potential to inhibit lipid oxidation.

	Lipid oxidation
Total anthocyanins content	0.5206
Total polyphenols content	0.3841
Total tannins content	0.4641
Total flavonoids content	0.6383

In the end, in order to get better insight into composition and activity differences between examined concentrates, PCA was applied and results are presented in **Figure 2.**



a)



b)

Figure 2. Principal component analysis of total polyphenols, tannins, flavonoids and monomeric anthocyanins content and expressed potential to inhibit lipid oxidation. (a) distribution of variables on loadings plot; (b) distribution of wine samples on scores plot.
Abbreviations: (a) TFC: total flavonoids content; TMA: total monomeric anthocyanins content; TPC: total polyphenols content; TTC: total tannins content; (b) B-1 to B-4: Bermet concentrate; CS-1 to CS-4: Cabernet Sauvignon concentrate; M-1 to M-4: Merlot concentrate.

The first and the second principal components (Factor 1 and 2) explained 77.43 and 13.21% of the total variance, respectively. Obvious separation of CS-4 sample and certain grouping of Cabernet Sauvignon and one Merlot concentrates (CS-1, CS-2, CS-3 and M-2), as well as grouping of other Merlot and Bermet concentrates could be noticed.

Regarding consumers' acceptance of prepared wine butters, 148 people took part in survey at the food festival in Novi Sad. About 50% of consumers (73 persons) stated that compound butter containing Bermet wine concentrate has the best taste, 40 consumers preferred Cabernet Sauvignon butter, while 35 designated Merlot butter as the best one.

DISCUSSION

According to the World Health Organization, intake of saturated fatty acids, present in fatty meat, butter, palm and coconut oil, cream, cheese, etc., is less preferable than intake of unsaturated fatty acids, found, for example, in fish, avocado, nuts and olive oil [17]. However, systematic review and meta-analysis performed by Pimpin et al. (2016) showed that there is small or neutral relation of butter with mortality, cardiovascular disease, and diabetes [18]. Addition of natural, biologically active and health-promoting compounds, such as polyphenols, to butter could contribute, at least partially, to increased functional value of butter as well as its oxidative stability.

Previously, we evaluated chemical composition and biological activities of several wines from Fruska Gora region (Serbia), including Cabernet Sauvignon and Merlot [16,19]. But, to the best of our knowledge, there is only one paper [20] about total polyphenols content in Bermet wine and this is the first report on the content of total monomeric anthocyanins, flavonoids and tannins in Bermet wines. Bermet wine is dessert wine characteristic for Fruska Gora region, prepared by adding different medicinal plants, such as wormwood, yellow gentian, anise, cinnamon, bitter bloom, black mustard, coriander, cloves, carob and liquorices [21]. Considering content of additional compounds in Bermet wine, it is obvious that the same amount of Bermet and pure wine concentrates will not have proportional amounts of wine polyphenols and that Bermet polyphenolic profile will be also influenced by added plant polyphenolics. In general, B-4 was among the wines with the highest overall contents of total monomeric anthocyanins, polyphenols, tannins and flavonoids, but, the similar trend was not noticed regarding concentrates. Namely, although concentrate B-4 had the highest content of analyzed polyphenols groups among Bermet wines, significantly higher concentrations were detected in Cabernet Sauvignon and Merlot concentrates (**Table 1**). Content of analyzed polyphenol classes depends on various factors, including grape variety and origin, as well as vinification process [22]. Since all examined wines originated from Fruska Gora region, it could be supposed that wine-making technique or wine age contribute to considerable differences among the same wines.

Eleven of twelve wine concentrates showed ability to inhibit lipid peroxidation comparable with activity of standard antioxidant trolox (**Figure 1**). The best capacity had Cabernet Sauvignon, with CS-4 as the most dominant one. These results confirm that addition of wine concentrate can impact oxidative stability and that it may be potentially used to prolong shelf-life of butter. To the best of our knowledge, wine samples have not been tested as butter preservatives until now, but it was previously found that dominant phenolic acid found in wines, gallic acid, has better activity regarding inhibition of butter oxidation than BHA [23]. However, correlation coefficients (**Table 2**) showed that there was no direct dependence between composition of wine concentrates and expressed potential to inhibit lipid oxidation of butter, implicating synergistic activity of compounds belonging to different groups.

Principal component analysis (PCA) revealed grouping among analyzed concentrates implies that samples show specificity regarding the polyphenolic content and can be clearly separated based on wine variety used for concentrate preparation. Absolute dominance in potential to inhibit lipid peroxidation influenced separation of CS-4 sample. Obvious grouping of other Cabernet Sauvignon and one Merlot concentrates (CS-1, CS-2, CS-3 and M-2) in the left lower part can be attributed to the higher polyphenolic content. Other Merlot concentrates are located in the central area of the plot due to moderate content of all analyzed classes. Bermet concentrates appeared in a compact group right upper part, according to the low content of polyphenolic compounds.

Although performed analysis pointed out Cabernet Sauvignon concentrates (particularly CS-4 sample) as the most suitable ingredient for improved and possibly more stable compound butter, consumers' opinion was different: hedonic tests to assess the overall liking pointed out butter enriched with Bermet concentrate, most probably due to Bermet`s complex composition, characterized by distinguishing, sweet taste.

CONCLUSION

Results obtained in this study support main idea that wine concentrates could be used as ingredients which could improve flavour and increase the oxidative stability of butter. Anyway, these preliminary results implicate further steps in new products development: finding (Bermet) wines with higher biological activity and further testing of parameters of butter stability (microbial, peroxide number, fatty acid content). However, there is certain interest in the market for Bermet compound butters, while further development of this product could help Bermet to gain more visibility.

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Koncentrati crvenog vina kao inhibitori lipidne oksidacije u maslacu

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Kratak sadržaj

Maslac (puter) obogaćen različitim sastojcima, kao što su bilje i začini, stekao je popularnost poslednjih godina. Glavna uloga dodatnih sastojaka je da poboljšaju ukus putera, ali ovi dodaci mogu uticati i na oksidativnu stabilnost putera (rok trajanja). U potrazi za novim proizvodima, ispitan je potencijal 12 koncentrata vina (Bermet, Cabernet Sauvignon i Merlot) da inhibiraju oksidaciju lipida, koja može da se javi u maslacu, pripremljen je maslac sa koncentratima različitih vina (maslac sa vinom) i urađeno je preliminarno ispitivanje o mišljenju potrošača o odabranim maslacima sa vinom. Takođe, određen je sadržaj ukupnih fenola, flavonoida, antocijana i tanina u vinskim koncentratima (spektrofotometrijska metoda). Jedanaest od dvanaest koncentrata vina pokazalo je sposobnost da inhibira oksidaciju lipida uporedivu sa aktivnošću standardnog antioksidantnog jedinjenja troloksa. Najbolji kapacitet ispoljio je Cabernet Sauvignon, sa dominantnim CS-4 uzorkom. Uopšteno, Bermet (B-4) je bio među vinima sa najvećim ukupnim sadržajem monomernih antocijana, polifenola, tanina i flavonoida, ali kod koncentrata sličan trend nije primećen. Međutim, koeficijenti korelacije impliciraju da ne postoji čvrsta veza između određene grupe polifenola i izražene aktivnosti. Po mišljenju korisnika, maslac sa koncentratom Bermeta ima najbolji ukus. Uopšteno, dobijeni rezultati su pokazali da se koncentrati vina mogu koristiti kao dodatni sastojci koji mogu poboljšati ukus, povećati oksidativnu stabilnost putera i eventualno produžiti njegov rok trajanja.

Ključne reči: *puteri sa dodacima; koncentrat vina; Cabernet Sauvignon; Merlot; Bermet; lipidna peroksidacija; senzorna analiza.*