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BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF WINES FROM DIFFERENT CURRANT CULTIVARS

BIOAKTIVNI SASTAV I ANTOKSIDANTNA AKTIVNOST VINA OD RAZLIČITIH VRSTI RIBIZLE

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ABSTRACT

This work was aimed to investigate the bioactive compounds and antioxidant activity of wines from different currant cultivars. Currant fruits, black currant (Ribesnigrum Moravia CV.), red currant (R. rubrumLosan CV.) and white currant (R. rubrum Primus CV.), were collected from a fruit nursery in Modrice, Czech Republic in the year July 2015 and frozen at -18^{o}C until used for further experiment.Black currant was crushed and the whole fruit was directly used for wine making, whereas only the juice of the red and white currents was used for wine making. Total soluble solid (TSS), titratable acidity (TA), and pH of the juice were determined. The TSS was adjusted to 20 °Brix, and the TA of the juice was adjusted to 7.5 g/L. Then, the juice was transferred to fermentation bottles. After 12 days-fermentation, chemical composition (TSS, TA, pH, total SO_2, bioactive compounds (total phenolic compounds (TPC) and total monomeric anthocyanin (TMA)) and antioxidant activity (DPPH and FRAP assays) were determined. The results showed that the black currant wine had the higher total soluble solid (15.80 Brix) followed by white currant wine (12.03 °Brix) and red currant wine (8.40 °Brix), respectively.The white currant wine exhibited the highest titratable acidity (9.32 g anhydrous citric acid/ mL), whereas there was no significant difference in pH found for all currant wines (P>0.05). The optimum TSS and TA of white currant contributed to the highest alcohol content in white wine (11.6 %). However, the black currant wine provided the highest TPC, TMA and antioxidant activity (DPPH and FRAY assays). The wine with the higher TPC exhibited the higher antioxidant activity, indicating that the TPC is responsible for the antioxidant activity of the wine.

Keywords: *Ribes*spp., black currant, red currant, white currant, anthocyanin

REZIME

Cilj ovog rada bio je istraživanje bioaktivnih jedinjenja i antioksidativna aktivnost vina od različitih vrsti ribizle. Plodovi ribizle, crna (Ribesnigrum Moravia CV.), crvena (R. rubrumLosan CV.) i bela (R. rubrum Primus CV.), prikupljeni su iz većnjaka u Modricu u Češkoj Republici u julu 2015. godine i zamrznuti na -18oC sve do početka eksperimenta. Crna ribizla je samlevena i celi plodovi direktno su korišćeni za proizvodnju vina, dok je od crvene i bele ribizle korišten samo sok. Izmeren je sadržaj ukupno rastvorljivih materija (TSS), titratibilna kiselost (TA) i pH sokova. TSS je podešen na 20°Brix, a TA soka je podešen na 7,5 g/L. Zatim je sok premešten u fermentacione posude. Nakon 12 dana fermentacije, utvrđeni su hemijski sastav (TSS, TA, pH, ukupni SO2, bioaktivna jedinjenja (ukupna fenolna jedinjenja (TPC) i ukupni monomermi antocijan (TMA)) i antioksidativna aktivnost (DPPH i FRAP analiza). Rezultati su pokazali da je crno vino imalo najviše ukupno rastvorljivih materija (15,80oBrix), a zatim belo vino (12,03°Brix) i crveno (8,40oBrix). Belo vino pokazalo je najvišu titratibilnu kiselost (9,32 g/mL). Značajna razlika u pH vrednostima između vina nije ustanovljena (P>0,05). Optimalne vrednosti TSS i TA kod belog vina doprinle su najvišem sadržaju alkohola u belom vinu (11,6%). Crno vino imalo je najvišu TPC , TMA i antioksidantnu aktivnost (DPPH i FRAI analize). Vino sa višim TPC pokazalo je veću antioksidativnu aktivnost, što ukazuje da je TPC odgovoran za antioksidantnu aktivnost vina.

Ključne reči: *Ribesspp., Crna ribizla, crvena ribizla, bela ribizla, antocijan.*

INTRODUCTION

Currant (*Ribes* spp.) has a short harvest season and it has fragile fruit that is prone to spoilage during storage and transportation. It can be consumed fresh or processed to various products, such as juice, jam, dried fruit, etc. There are many works reported that the currant exhibits numerous biological activities, for instance, anti-inflammatory, anticancer, antioxidant activities, etc. (Konić-Ristić et al., 2011; Lipińska, Klewicka, & Sójka, 2014). The main bioactive compounds in red and black currants are anthocyanins, flavonols, procyanidins, phenolic acids, ascorbic acid and flavonoids (Bakowska-Barczak & Kolodziejczyk, 2011; Zdunić, Šavikin, Pljevljakušić, & Djordjević, 2016), whereas proanthocyanidins, hydroxybenzoic acid, quercetin and hydroxycinnamic acid derivatives such as caffeic acid and ferulic acid and phenolic acids are the main bioactive compounds in white currant (Määttä, Kamal-Eldin, & Törrönen, 2004; Rajakangas, Misikangas, Pa¨iva¨rinta, & M., 2008; Tian et al., 2017; Vuorinen, Määttä, & Törrönen, 2000). Fermentation has known to extend the shelf-life and could enhance nutritional, organoleptic as well as functional qualities of food (Terefe, 2016). Fruit wine, made from a variety of fruits in which are very rich in fermentable sugars with additional specific flavors and aromas, is becoming popular among the world health concern consumers. Wine production from currants increasingly interests people particularly in European countries as a result of its health benefits perception from the fruit itself and the compounds generated from the fermentation process.

Currant biochemical reactions occurring during fermentation have been reported to both reduce and enhance the antioxidant activity depending on various factors (Wang et al., 2015; Wen, Yan, & Chen, 2013; Xiao et al., 2015).Various works have reported on the bioactive compounds and biological activities of grape wines from different grape cultivars and their by-products *(Bajić et al., 2015; Durak et al., 1999; Radovanović, Đekić, Radovanović, 2011).* In addition, recent research of currant wine brewing has intensively investigated on optimizing the fermentation process. However, report on chemical composition,

bioactive compounds and antioxidant activity of the wine produced from different currant cultivar is limited. This work, therefore, was aimed to investigate of chemical properties (total soluble solid (TSS), titratable acidity (TA), pH, total sulfur dioxide and alcohol content), bioactive compounds (total phenolic compounds (TPC) and anthocyanin (TMA) and antioxidant activity (DPPH and FRAP assays) of different currant wines from different currant cultivars to provide some information for currant wine processing.

MATERIALS AND METHODS

Reagents

All reagents were analytical grade. Folin-Ciocalteu's phenol reagent, gallic acid, 2-2-diphenyl-1-picrryl-hydrazyl (DPPH), trolox (±)-6-Hydroxy-2, 5, 7, 8 - tetramethylchromane-2-carboxylic acid and ascorbic acid were purchased from Sigma-Aldrich Corp. (USA).

Currant fruits

Currant fruits, black currant (*Ribesnigrum*Moravia CV.), red currant (*R. rubrum*Losan CV.) and white currant (*R. rubrum* Primus CV.), were collected from a fruit nursery in Modrice, Czech Republic in the year July 2015 and frozen at -18 C until used for further experiment.

Microorganisms

Commercial wine yeast, *Saccharomyces cerevisiae* was purchased from a commercial wine yeast merchandize in Lednice, Czech Republic.

Fruit Juice Preparation

Black currant was crushed by hands and the whole fruit underwent directly for wine making, whereas only the juice of the red and white currents was used for wine making. Titratable acidity (TA), pH and total soluble solid (TSS) of the juice were determined. Then, TSS was adjusted to 20 Brix by sugar and TA of the juice was adjusted to 7.5 g/L by water and the juices (2.0 kg) were transferred to fermentation bottleswith air lock assembled.

Fruit Wine Fermentation

Potassium metabisulfite was added into the fermentation bottles in order to obtain 30 mg active sulfur dioxide /kg juice and left overnight at ambient temperature. The wine yeast (0.4 g/L) was inoculated into the fermenters. The fermenters were left at ambient temperature for 12 days. Sampling was conducted at the end of fermentation and the samples were stored at the temperature of −18 °C for titratable acidity (TA), pH, total soluble solid (TSS), free sulfur dioxide, alcohol content, bioactive compounds (total phenolic content, anthocyanin), and antioxidant activity (DPPH and FRAP assays) determination. Residue sulfur dioxide was determined at 12-day fermentation.

Chemical properties determination

Titratable acidity (TA) was determined according to AOAC (2005) method and expressed as g anhydrous citric acid (ACA) per mL sample.Total soluble solid (TSS) and pH were measured by a refractometer and pH meter, respectively.

Total sulfur dioxide determination

The total sulfur dioxide was determined by Ripper titration method (Iland, 2000) with some modifications. Briefly, the sample (50 mL), 16 % sulfuric acid (10 mL) and starch solution (5 mL) were pipetted to an Erlenmeyer flask and titrated with 0.02 M iodine solution until blue color was obtained (30 sec. stable). The total sulfur dioxide was calculated and expressed as milligram free sulfur dioxide per liter of the sample.

Alcohol content determination

The alcoholic content in the wine was determined by distillation and relative specific gravity measurement by hydrometer (Amerine & Ough, 1974). Briefly, a known volume of sample (150 mL) with an antifoaming agent were filled into the distillation chamber and distilled in a distillation unit to obtain the distillate with the same volume of the original sample. The specific gravity of the distillate and the temperature were determined. Then, the specific gravity was converted to % alcohol by volume using the table of specific gravity of aqueous ethanol solutions as a function of % alcohol (v/v).

Bioactive Compounds Determination

Total phenol content (TPC)

The TPC was determined as described by Singleton and Rossi (Singleton & Rossi, 1965) with some modifications. Gallic acid (10, 20, 40, 60, 80 and 100 µg/mL) was used as a standard. Five milliliters of 10 % v/v Folin-Ciocalteu's reagent and 10 mL of 7.5 % sodium carbonate were added to one milliliter of the diluted sample or standard and vortexed. Then, the solution was left at room temperature for 60 min before the absorbance was determined at 765 nm. The total phenolic content of the sample was expressed as gram gallic acid equivalent (GAE) per liter. All samples were analyzed in triplicates.

Total monomeric anthocyanin (TMA)

The TMA was determined using pH-differential method (AOAC, 2005). Equal portions of the extract were mixed with pH 1.0 buffer or pH 4.5 buffer, then the absorbance of both solutions was determined at the wavelength of 520 and 700 nm, respectively, using distilled water as a blank. The TMA was calculated and expressed as milligram malvidin-3-glucoside (M-3-G) equivalent per liter.

Antioxidant activity determination

Free radical scavenging activity (DPPH) assay

The antioxidant activity of all sample was evaluated through the free radical scavenging effect on DPPH radical (Brand-Williams et al., 1995). Trolox (0, 200, 400, 600, 800 and 1000 µM) was used as a standard. The sample or the standard (50 µL) and 60 mM DPPH solution (2,000 µL) were transferred to a test tube and thoroughly mixed and stored in the dark for 10 min. The absorbance was determined at the wavelength of 517 nm using methanol as a blank. DPPH scavenging activity was expressed as mmoltrolox equivalent (TE) per liter.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was conducted according to Benzie and Strain (Benzie & Strain, 1996) with some modifications. Potassium hexacyanoferrate solution (1 % w/v; 2.5 mL) and phosphate buffer (pH 6.6) were added into tubes that contained one milliliter of the sample or the standard solution. The tubes were then placed in a water bath at 50 ^{o}C for 30 min. Then, trichloroacetic acid (10 % w/v; 0.5 mL) was added and the absorbance was determined at 700 nm. FRAP reducing power was expressed as mmoltrolox (TE) per liter.

Statistical analysis

All tests was conducted in triplicates for verification of the results and subjected to analysis of variance (ANOVA). A mean composition was carried out by Duncan's Multiple Range Tests. Statistical significance was set at p-value ≤ 0.05. All data analyses were performed using SPSS package (V16).

RESULTS AND DISCUSSION

Black currant wine had the higher total soluble solid (15.80 °Brix) followed by white currant (12.03 °Brix) and red currant (8.40 °Brix), respectively, as shown in Table 1. White currant wine exhibited the highest titratable acidity (9.32 g anhydrous citric acid/ mL), whereas there was no significant difference in pH was found for all currant wines (P>0.05). Alcohol content in red (9.76 %) and black currant (9.58 %) wines were similar, however these were higher than that of the which currant wine (11.6 %). This higher alcoholic content in white currant wine may due to the influence of the preferable acidity and the TSS of the white currant juice. In addition, black and red currants

generally contain anthocyanin in which undetectable in white currant and contains higher flavonoids compared to the white currant. These compounds have been known to have antimicrobial activity, therefore, may affect the starter culture activity resulting in the lower alcohol content in the wine (Mattila, Hellström, Karhu, Pihlava, & Veteläinen, 2016). Total sulfur dioxide present in wine has to be limited to a certain amount in order to comply with the legislation (< 150-200 mg/L) and not to provide undesirable tastes and aromas to the wine and adverse health effect to the human health. From the results it was found that total sulfur dioxide in all currant wines was lower than the maximum permitted level (Table 1).

Table 1. Chemical properties of different currant wines

Currant cultivars	Chemical properties				
	TSS (°Brix)	TA (g ACA /mL)	pH ^{ns}	Total SO_2 (mg/L)	Alcohol content (%)
White currant	12.03 ± 1.46 ^{b}	9.32 ± 0.94 ^{a}	3.14 ± 0.12	4.80	11.6
Red currant	8.40 ± 0.85 ^{c}	6.34 ± 0.94 ^{b}	2.93 ± 0.01	5.30	9.76
Black currant	15.80 ± 1.10 ^{a}	6.17 ± 1.19 ^{b}	2.95 ± 0.07	7.12	9.58

**Values are the mean ± SD. Means with different superscript within a column are significantly different (P≤0.05), TSS: total soluble solid, TA: titratable acidity, ACA: anhydrous citric acid, ns: no significant different (P> 0.05)*

Bioactive compounds and antioxidant activity

The TPC and TMA in the wine from different currant cultivars are shown in Table 2. The TPC in the black current wine (1457.54 mg GAE/L) was higher than that of the white (1077.92 mg GAE/L) and the red currant (501.18 mg GAE/L) wines respectively. These results are consistent with Vuorinen et al.(2000) who reported that white currant wine contained the highest TPC (520-1820 mg GAE/L) compared to that of the red currant wine (335-1250 mg GAE/L). However, the white currant wine in this work had the higher TPC compared to the previous report one (250-270 mg GAE/L) which may due to a variety of factors, including the variety of the currant, fermentation conditions, etc *(Yang, Zheng, Laaksonen, Tahvonen, & Kallio, 2013).*

Table 2. Bioactive compounds and antioxidant activity of different currant wines

Currant cultivars	Bioactive compounds		Antioxidant activity	
	TPC (mg GAE / L)	TMA (mg M-3-G/ L)	DPPH assay (mM TE)	FRAP assay (mM TE)
White currant	1077.92 ± 39.71 ^{b}	ND	7.49 ± 0.89 ^{a}	7.52 ± 1.07 ^{b}
Red currant	501.18 ± 58.08 ^{c}	81.00 ± 6.56 ^{b}	3.11 ± 0.61 ^{b}	5.03 ± 0.57 ^{c}
Black currant	1457.54 ± 33.40 ^{a}	382.50 ± 29.86 ^{a}	6.91 ± 0.46 ^{a}	10.37 ± 0.46 ^{a}

**Values are the mean ± SD. Means with different superscript within a column are significantly different (P≤0.05) ND: not detected. TPC: total phenolic content, TMA: total monomeric anthocyanin, GAE: galic acid equivalent, M-3-G: mulvidin-3-glucoside, ND: not detected, TE: trolox equivalent*

The black currant wine exhibited five times higher in TMA (382.50 mg M-3-G/L) compared to the red currant one (81 mg M-3-G/L), while TMA was not detected in the white currant one. The TPC and the TMA in the black and red currants wines showed the same trend, in which the higher TPC and TMA in the one compared to those of the red one. These are consistent with those of the fresh currants fruits as reported byMattila et al. (2016). TMA is normally found in the peel of the black and red currants contributing to their dark peel color and also the main phenolics in the black and red currants, whereas, phenolic acids and proanthocyanin are the main total phenolic compounds in white currant (Maatta, Kamal-Eldin, & Torronen, 2004).

Table 2 shows the antioxidant activity of the wines from different currant cultivars. The black currant wine exhibited the highest antioxidant activity, both in DPPH assay (6.91 mM TE) and FRAP assay (10.37 mM TE) which is consistent to previous work reported that the black currant juice exhibited the higher antioxidant activity compared to the red and white currants (Mendelová et al., 2016). The currant wine with the higher TPC tends to provide the higher antioxidant activity indicating that the TPC is responsible for the antioxidant activity in the wine. In addition, the red currant showed the lowest antioxidant activity compared to the white currant and black currant wine, respectively. These results also imply that the TMA, even though the main phenolic in the currants does not contribute to the antioxidant activity of the currant wine. This may due to the TMA is prone to degradation (Hager, Howard, & Prior, 2008; Mgaya-Kilima, Remberg, Chove, & Wicklund, 2015) depending on various factors including, pH, temperature, light, water activity, and presence of oxygen (Contreras-Lopez, Casta?eda-Ovando, Gonz¨¢lez-Olivares, A?orve-Morga, & Jaimez-Ordaz, 2014). Upon the TMA depletion, TMA may be conversed by inherit enzymes or acids in the fruit to other stable compounds through polymerization and hydrolysis reactions. These reactions would consequently result in an increasing of the overall polyphenols in the black currant wine (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Bimpilas, Tsimogiannis, Balta-Brouma, Lymperopoulou, & Oreopoulou, 2015) Whereas, the main phenolic derivatives including, caffeic acid-hexosides, coumaroylquinic acid, and vanillic acid, made up to 47 % of the white currant TPC (Tian et al., 2017). These compounds are much more stable compared to the TMA in the other currants and could contribute to the antioxidant activity of the white wine.

CONCLUSION

In conclusion, the black currant wine exhibited higher bioactive compounds and antioxidant activity compared to the white and red currant wine, respectively. In addition, the compound responsible for the antioxidant activity in the black currant wine was mainly phenolic compound.

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