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SUPPLEMENTARY MATERIAL

The natural product content of the selected Cabernet Franc wine samples originating from Serbia: a case study of phenolics

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Abstract

This work aimed to evaluate the content of selected phenolic natural products in the wine samples made of three new Serbian Cabernet Franc clones (Nos. 02, 010 and 012, respectively) and mother vine (used as the relevant standard) during the period 2008-2012. Compared with all other wine samples, the Cabernet Franc wine of the clone No. 010 was found to have the highest total content of polyphenolics (1.85 ± 0.02 g/L) and anthocyanins (178.55 ± 3.75 mg/L). In addition, its Folin-Ciocalteu index (36.86 ± 0.12) stood out among the examined samples. Finally, the same wine was enriched with ellagic and gallic acids (3.44 ± 0.29 and 27.46 ± 0.21 mg/L, respectively), catechin (135.16 ± 6.47 mg/L) and epicatechin (51.33 ± 2.33 mg/L), the natural products known to exert significant lipid-lowering effects. Taken all together, the clone No. 010 developed in Serbia may offer new Cabernet Franc wine with geographical indication.

Keywords: clonal selection, Cabernet Franc, wine chemistry, ellagic acid, gallic acid, catechin, epicatechin

Experimental

Biological material

In the third phase of individual clonal selection process, the experimental site was planted with Cabernet Franc variety population and three new clones (Nos. 02, 010 and 012, respectively) on Radmilovac locality (Grocka vineyards, Belgrade, Serbia) which belongs to the Faculty of Agriculture of the University of Belgrade. The site is located at 44º45'24.66' (north latitude) and 20°34'54.50' (east longitude), at an altitude of 153 m. The aforementioned clones and standard were grown under same conditions.

Microvinification

Immediately after harvest, the grape was processed in laboratory conditions, using microvinification technique. For the purpose of microvinification, 20 kg of grapes, both for the standard and clones, were used. Crushing was done manually using grape crusher with rollers and supplement for separating the stems. In the stum 100 mg/L potassium metabisulfite was added. Alcoholic fermentation was performed by *Saccharomyces cerevisiae* K1-V1116 (Lallemand, Montréal, Canada) which was rehydrated and inoculated into pomace in the amount of 0.2 g/kg. The fermentation took place in the glass of a capacity 10 L at a temperature of 20-25 °C. During maceration, must mixing was done twice a day. After maceration, the must was leached while the obtained wine was separated into glass balloons sealed with a cork stopper, that prevented the penetration of air into the space above the surface of the wine. When the phase of quiet fermentation and sedimentation was finished, the wine was decanted. First decanting was done after a month. On the occasion of decanting, the wine was tested for SO_2 and the necessary corrections were done to make the total and free content of SO_2 around 80 mg/L and 20-25 mg/L, respectively. Upon the completion of the fermentation, the wine was decanted from the litter, bottled and stored at 10-12 °C, until chemical analyses. After two months aging in a bottle, the selected parameters of the wines (clones and standard) were determined. Each measurement was done in five replications.

Chemical analyses

Anthocyanins and polyphenolics

The total contents of anthocyanins and polyphenolics were determined by spectrophotometric procedure (using pH differential method) and the Riberau-Gayon-Maurié procedure, respectively (Danicic 1988; Lee et al. 2005). In addition, Folin-Ciocalteu (FC) index was determined with a standard procedure (Danicic 1988).

HPLC/MS/MS analysis

The content of selected phenolics was determined using UPLC/MS chromatography with TQ analyser as previously reported (Vujovic et al., 2015). The analysis of phenolics in the relevant wine samples was carried out using a Waters Acquity UPLC H-Class (WAT-176015007) equipped with quaternary pump (Waters Quaternary Solvent Menager), injector (Waters Sample Menager-FTN /Flow Through Needle/), column compartment with ZORBAX Eclipse XDB C18 column (150×4.6 mm; 5µm), Waters 2998 PDA (Photodiode Array) detector and mass detector (Waters TQ /Tandem Quadrupole, WAT-176001263/). For acquisition and processing data, MassLynx V4.1 software was used. Quantitative determinations were performed using the external standard method with commercial standards. The calibration curves were obtained by injection of standard solutions, under the same conditions as for the analysed samples, over the range of concentrations observed. Based in the area under peaks of each extracted transition of ions from certain compounds of standard solutions in ESI-mode, standard curves for each standard solution compounds were plotted. Identification of individual compounds relied on the retention times of original standards and spectral data.

<u>Sample preparation</u>

10 µL of wine were directly injected in Waters ACQUITY UPLC/MS system. Based in the area under peaks of each extracted transition of ions from certain compounds of the sample in ESI mode, in relation to calibration curves of each compounds, concentration of certain compounds of wine sample was calculated and expressed in mg/L of wine.

Recording conditions at Waters ACQUITY UPLC/MS system

The mobile phase consisted of 0.2% (v/v) formic acid in deionised water (solvent A) and acetonitril (solvent B), starting from 95% to 84% solvent A (20 min), from 84% to 60% solvent A (28 min), from 60% to 30% solvent A (32 min), from 30% to 2% solvent A (36 min), constant at 2% solvent A (45 min), from 2% to 95% solvent A (46 min), and then constant at 95% solvent A (55 min) for reconditioning of the column. Temperature of column was 25ºC. Flow rate of mobile phase was 0.7 mL/min.

Recording conditions at MS

Recording was performed in ESI^- and ESI^+ mode, capillary voltage was 3.5 kV, cone voltage was n range between 20-60 V, volues of collision energy varied from 10 to 56 eV, depending on the tested component. Source temperature was 150 °C and desolvation temperature was 450 °C. Flow rate of desolvation gas was 700 L/h.

Statistical analysis

The analysis of the acquired experimental data was performed using the statistical package IBM SPSS Statistics 20. First of all, indicators of descriptive statistics were calculated for all the observed properties: mean and standard error of the mean ($S\ x$). In order to reach more objective conclusions, ANOVA and LSD test ($p<0.05$) were applied.

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