The Balkans Scientific Center of the
Russian Academy of Natural Sciences

1st International Symposium:

Modern Trends in Agricultural Production and Environmental Protection

PROCEEDINGS

Tivat-Montenegro
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Publisher
The Balkans Scientific Center of the Russian
Academy of Natural Sciences

For publisher
Academician dr Mitar Lutovac

In cooperation
Faculty of Agriculture, Lesak
Faculty of Agriculture Cacak
Institute for Animal Husbandry, Belgrade, Zemun
Fruit Research Institute, Cacak
Faculty of Agriculture, East Sarajevo
Soil Science Institute, Belgrade
Faculty of Management, Sremski Karlovci
Pedagogical Club, Tivat

Editors
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Prof. dr Zoran Ž. Ilić

Technical editor
SaTCIP

ISBN
978-86-6042-008-6

Circulation
70 exemplars

Printed by
SaTCIP d.o.o. Vrnjačka Banja

2019.

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PRIMARY METABOLITES IN FRUIT OF CURRANTS FROM WESTERN SERBIA

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ABSTRACT

The objective of this study was to identify and quantify primary metabolites in berries of six black ('Ben Lomond', 'Ben Sarek', 'Titania', 'Čačanska Crna', 'Tisel', and 'Tiben'), one red ('Jonkheer van Tets') and two white currant cultivars ('Weisse aus Juteborg' and 'Primus'). High-performance liquid chromatography (HPLC) was used for the identification of sugars (glucose, fructose, sucrose) and organic acids (citric, malic, quinic, shikimic and fumaric acid). Significant differences in the tested parameters were detected among the cultivars. Fructose was the dominant sugar, followed by glucose and sucrose in all tested cultivars. 'Titania' was the cultivar with the highest content of all individual and total sugars, while the cultivars 'Ben Sarek', 'Tisel', and 'Tiben' had a low content of sugars.'Ben Lomond' was the cultivar with the highest content of all examined acids and total acids, except shikimic. The best sugar and acid ratio was recorded in the berries of 'Titania' and 'Čačanska Crna'. Wide variations in major taste compounds of the target analytes examined were observed, and thus, it is clear that the differences in currant cultivars play an important role in determining fruit composition. The most suitable currant cultivars for grown in Western Serbia proved to be 'Titania' and 'Čačanska Crna'.

Keywords: *black currant, red currant, white currant, sugars, organic acids*

INTRODUCTION

Small fruits are widely recognized for their nutritional quality and potential health benefits. Currants (*Ribes* sp.) are an important small fruit crop due to its good fruit colour and organoleptic properties. Its berries provide a rich source of sugars and organic acids as important primary metabolites. Fructose and glucose are the major sugars that contribute to the sweetness of current berries, while the major acid is malic acid, which causes a sour and bitter taste. Generally, sugars and organic acids play a key role in cell metabolism and reproduction (Hartmann, 2007). For this reason, Kampuss (2005) pointed to the significance and the need for more intensive exploitation of this species. Therefore, an important goal in currant breeding is to improve the chemical composition of the fruit i.e. its content of sugars, acids and colorants, and create new dessert genotypes intended for fresh use (Mišić and Nikolić, 2003). According to Kafkas et al. (2006) and Tosun et al. (2009), sugars and organic acids are main soluble constituents and have a major effect on taste and fruit ripeness, or even represent a suitable index of consumer's acceptability. Also, Bordonaba and Terry (2008) reported that sugar and acid content and sugar to the acid ratio in currants and other fruits are essential in flavor formation. In this ratio, the organic acid content as well as the composition of individual metabolites play a crucial role in fruit taste perception. It is often understood that the fruits that exhibit pleasant sensory characteristics have a high sugar content and a relatively low content of acids (Zheng et al., 2009).

Therefore, the objective of this study was to examine the effect of genotype on the content of primary metabolites in berries of six black, one red and two white currant cultivars using high-performance liquid chromatography (HPLC). On the basis of the obtained results, we could recommend cultivars suitable for growing in the area of Western Serbia, all with the aim of improving the existing technology of currant production and achieving satisfactory fruit quality.

Materials and methods

The research was conducted at the Fruit Research Institute, Čačak (Western Serbia) in 2018. A currant planting was established in the spring of 2011 using two-year-old plants. Currants were grown as bushes at a spacing of 3 m between rows and 1 m in the row. Fruits of six black ('Ben Lomond', 'Ben Sarek', 'Titania', 'Čačanska Crna', 'Tisel', and 'Tiben'), one red ('Jonkheer van Tets') and two white currant cultivars ('Weisse aus Juteborg' and 'Primus') were analyzed. Each cultivar was replicated three times in a randomized complete block design. Fruits were sampled at full ripeness in June, at the stage of full development. Berries were selected from the inner and outer range of the bush. A total of 100 g berries were sampled from 5 bushes per replication.

Analyses of sugars and organic acids

Plum (2.0 g) was homogenized in 10 ml double-distilled water. After 30 min shaking at room temperature, the extract was centrifuged 10 min. at 10,000 rpm (Eppendorf Centrifuge 5810 R), filtered through a 0.20 µm cellulose ester filter (Macherey-Nagel, Germany) and poured into a vial prior to the analysis on a high performance liquid chromatography system (HPLC; Thermo Scientific, Finnigan Spectra System, Waltham, MA, USA) as it was previously described by Jakopic et al. (2016). Briefly, sugar separations were carried out using a Rezex RCM-monosaccharide column from Phenomenex (Ca+ 2%) operated at 65 °C (300 mm x 7.8 mm). The mobile phase was double distilled water, the total run time was 30 min, and a refractive index (RI) detector was used. The injection volume was 20 µl, and the flow rate was maintained at 0.6 ml/min. Analyses of organic acids were performed on the same HPLC system, equipped with a UV detector set at 210 nm, using a Rezex ROA-organic acid (H+ (8%)) column from Phenomenex (300 mm x 7.8 mm). The column temperature was set at 65 °C, the elution solvent was 4 mM sulphuric acid in double distilled water and the flow rate 0.6 ml/min.

The results are presented in milligrams per gram fresh weight (mg/ g FW) for sugars and organic acids. The content of all analyzed sugars and acids were summed and presented as total sugars and total acids. The sugar to acid ratio is defined as the proportion of total sugars and the total organic acids in the currant samples.

Chemicals

The following standards were used for determination of sugars: sucrose, fructose, glucose, sorbitol, and organic acids: citric, malic, quinic, and fumaric acids were purchased from Fluka Chemie (Buchs, Switzerland) and shikimic acid from Sigma-Aldrich Chemicals (St. Louis, MO, USA).

Statistical analysis

The data obtained in the research was processed applying the one-way analyses of variance (ANOVA, F test) Hypotheses were rejected at $P<0.05$. The analyses were performed in four replications and the obtained values were expressed as the means ± standard error. Means were compared with the Duncan test at $P \leq 0.05$.

Results and discussion

Sugars and organic acids play an important role during maturation and senescence of the fruit, and therefore important parameters at harvest. The composition and contents of sugars and acids affect fruit organoleptic characteristics (Mikulič-Petkovšek et al., 2012). Glucose, fructose and sucrose are the major sugars, citric acid is a major organic acid, and malic acid is present in minor concentrations in the fruit of currant (Hummer and Barney, 2002; Rubinskiene et al., 2006; Milivojević et al., 2009). Individual sugars and organic acids in berry extracts were identified by the HPLC-DAD analysis, with the corresponding results presented in Tables 1 and 2.

Table 1. Content of individual and total sugars in the fruit of currant

	Sucrose (mg/g FW)	Glucose (mg/g FW)	Fructose (mg/g FW)	Total sugars (mg/g FW)
Black currant				
'Ben Lomond'	5.8±0.1 b	36.3±0.8 c	43.7±1.1 c	85.8±0.9 c
'Ben Sarek'	n.d.	12.4±1.0 e	23.8±1.8 e	36.2±2.7 e
'Titania'	8.6±0.2 a	43.9±1.1 a	55.2±0.9 a	107.8±2.2 a
'Čačanska Crna'	3.7±0.7 c	38.9±0.8 bc	50.0±0.9 b	92.6±1.8 b
'Tisel'	n.d.	10.9±0.5 e	25.5±1.3 e	36.5±1.1 e
'Tiben'	n.d.	2.4±0.1 f	24.0±1.4 e	26.4±1.5 f
Red currant				
'Jonkheer van Tets'	0.6±0.0 d	26.2±1.4 d	26.3±0.9 e	53.1±2.2 d
White currant				
'Weisse aus Juteborg'	8.6±0.6 a	41.0±0.6 ab	38.2±0.4 d	87.9±0.9 bc
'Primus'	4.6±0.6 bc	38.9±2.6 bc	42.7±1.5 c	86.2±3.0 c
ANOVA	*	*	*	*

Data represent the means of four replicates ± standard error. The different lower-case letters in the columns indicate statistically significant differences among the mean values relative to cultivars at $P \leq 0.05$ level (Duncan's test).

Sugars represent the basic components in the formation of fruit flavor. The level of sugars and organic acids in the fruits depending on the genotype and are also influenced by the environmental factors and agrotechnical practices carried out in the orchard (Colaric et al., 2005; Hudina and Štampar, 2009). The sugar content in our study was significantly affected by genotype. The dominant participation in the structure of total sugars had fructose and glucose, while sucrose is detected at low concentrations. 'Titania' was the cultivar with the highest content of all individual and total sugars, while the cultivars ‘Ben Sarek’, ‘Tisel’, and ‘Tiben’ had a low content of sugars. The content of fructose ranged from 23.8 in the fruit of ‘Ben Sarek’ to 55.2 mg/g in the fruit of ‘Titania’,while the content of glucose in the fruit of the cultivar ‛Jonkheer van Tets’ (41.0 mg/g) was not significantly different from cultivar ‛Titania’ (43.9 mg/g).

Acids represent another important component that is involved in the formation of fruit flavor. The nature and the concentration of organic acids are important factors influencing the organoleptic properties of fruit and fruit products (Silva et al., 2002). The sum of all quantified acids ranged from 28.12 in the fruit of ‛Jonkheer van Tets’ to 53.51 mg/g FW in the fruit of ‘Ben Lomond’. The most common acid in the fruits of the studied cultivars was malic, with the highest proportion in the total acidity, while the proportion of quinic, citric, shikimic and fumaric acids were significantly lower (Table 2).‘Ben Lomond’ was the cultivar with the highest content of all examined acids and total acids, except shikimic. As compared to the present results, under the environmental conditions of Serbia, Djordjević et al. (2015) reported similar values for content of sugars and acids, while Milivojević et al. (2009, 2013), Paunović et al. (2013) and Paunović and Mašković (2018) obtained higher values for individual sugars, but lower values for total and organic acid. The difference in the measured contents can be explained by strong variations in the synthesis and accumulation of chemical compounds under different climates.

Table 2. Content of organic acids in extracts berries of currants

	Citric acid (mg/g FW)	Malic acid (mg/g FW)	Quinic acid (mg/g FW)	Shikimic acid (mg/g FW)	Fumaric acid (μg/g FW)×10^{-3}	Total acids (mg/g FW)
Black currant						
‘Ben Lomond’	35.07±0.92 a	10.50±0.29 a	7.69±0.35 a	0.23±0.01 cd	19.13±0.89 a	53.51±1.50 a
‘Ben Sarek’	29.58±0.14 bc	6.76±0.09 b	2.70±0.68 c	0.05±0.00 f	8.98±0.14 cd	39.10±0.65 c
‘Titania’	26.10±1.91 d	4.00±0.31 c	5.63±0.50 ab	0.26±0.02 c	11.62±0.55 b	35.99±2.65 c
‘Čačanska Crna’	24.16±0.54 dc	3.68±0.11 c	6.26±0.31 ab	0.24±0.01 c	10.73±0.29 bc	34.35±0.79 de
‘Tisel’	20.57±0.16 e	5.84±0.50 b	4.29±0.58 abc	0.12±0.01 cf	6.01±0.74 e	30.83±2.55 c
‘Tiben’	23.04±0.12 de	6.92±0.43 b	5.92±0.22 ab	0.05±0.00 f	10.55±0.92 bc	35.94±2.35 cd
Red currant						
‛Jonkheer van Tets’	22.52±0.48 de	2.89±0.15 c	2.56±0.34 c	0.15±0.01 de	4.64±0.78 e	28.12±0.95 e
White currant						
‛Weisse aus Juteborg’	32.47±1.73 ab	5.83±0.81 b	7.67±1.06 a	0.54±0.06 a	12.21±0.55 b	46.52±0.79 b
‛Primus’	26.38±2.02 d	6.58±0.64 b	4.14±1.12 abc	0.39±0.06 b	8.59±0.59 cd	37.49±1.45 c
ANOVA	*	*	*	*	*	*

Data represent the means of four replicates ± standard error. The different lower-case letters in the columns indicate statistically significant differences among the mean values relative to cultivars at $P \leq 0.05$ level (Duncan's test).

Generally, the ratio of sugars and organic acids is an important indicator of perceived taste, maturity/ripeness and general quality, which may serve as an index of consumer acceptance (Bordonaba and Terry, 2008). Besides sugar, an important role in the formation of fruit taste has the sugar/acid ratio. Study of Colaric et al. (2005) shows that sugars/organic acids ratio and levels of citric acid and shikimic acid have significant impacts on the perception of sweetness. The best sugar and acid ratio was recorded in the cultivars 'Titania' and 'Čačanska Crna'(Figure 1).

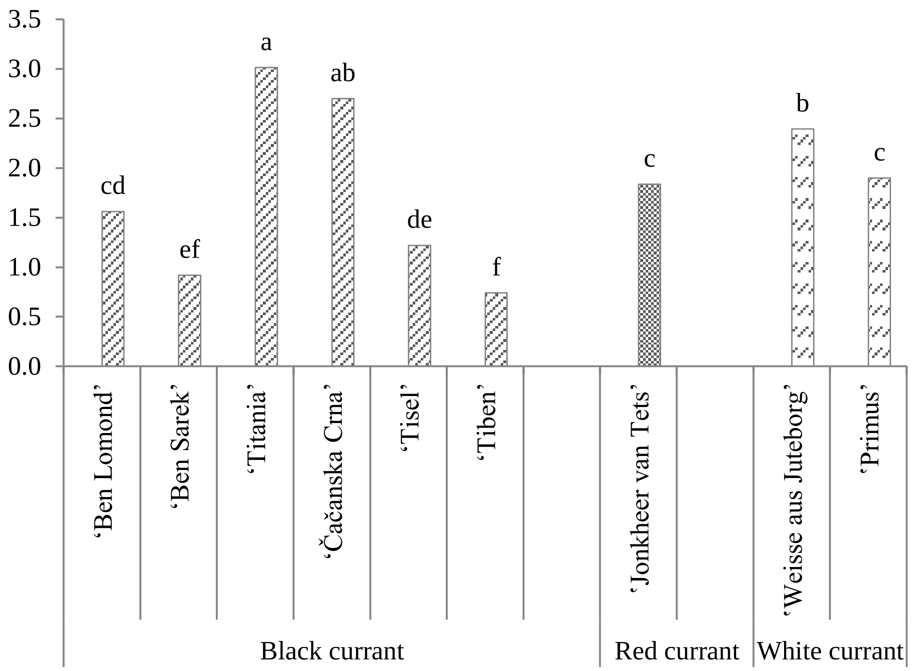

Figure 1. Sugars/Organic Acids ratio in the fruit of currant cultivars. The different small letters at the top of columns indicate significant differences in Sugars/Organic Acids Ratio at P ≤ 0.05 by Duncan's test.

Conclusion

Currants are an exceptionally rich source of sugars and acids, and an interesting nutritional alternative. Berries could be of interest to industrial applications in healthcare and can be used as a new source of natural foods.

Wide variations in major taste compounds of the target analytes examined were observed, and thus, it is clear that the differences in currant cultivars play an important role in determining fruit composition. The most suitable currant cultivars for grown in Western Serbia proved to be 'Titania' and 'Čačanska Crna'.

Acknowledgment

This study was conducted under Research Project TR-31093, financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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