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CASSIS FRUITS - NATURAL SOURCE OF FOOD AND ANTIOXIDANTS THROUGHOUT THE MATURATION PERIOD

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Abstract

The paper presents the biological variation in biochemical compounds of fruits belonging to Ribes nigrum L. species during the maturation process. The fruits from two varieties were collected in the following phenological stages: early first fruits (SIII), advanced first fruit (SIV), harvesting maturity (SV), and consumption maturity (SVI). The extracts were subjected to analysis. Total phenolic content (TPC) expressed as gallic acid equivalent (GAE), total flavonoid content (TFC) expressed as rutin equivalent (RE), and free radical scavenging activity (FRSA) expressed as mg/mL ascorbic acid equivalent (AAE), and gas-chromatographic profile were determined. The phenolic content differed considerably during the maturation process. Thus, the maximum value of TPC was achieved by 'Kzvana' fruits in the SV stage with 7.36 mM GAE/ml extract. The flavonoid content was highlighted in 'Roxia' fruits in the SVI stage with 1.24 mM RE/mL extract. With regard to FRSA, 'Kzvana' fruits have better activity. Also, the aromatic profile was characterized using gas chromatographic analysis.

***Key words**: blackcurrant, maturation phenophases, phenolics, gas-chromatography.*

INTRODUCTION

Currently, there is an increasing trend of consumer acceptance for *Ribes nigrum* L. fruits, largely due to the variety of potential health benefits of active compounds such as natural antioxidants (Tabart et al., 2006; Raudsepp et al., 2010; Tabart et al., 2012). These compounds are mainly represented by flavonoids, phenolic acids, and tannins, and because of their high content the blackcurrants have come to be regarded as superfood (Ruiz del Castillio et al., 2004). There is a need also to analyse the function and structure of such compounds especially in fruits, so pleasant to us all (Milivojevic et al., 2009).
Currants are one of those fruits that have a boost intake of both pleasant-like sugars and aromatic compounds and nutraceuticals like myricetin, quercetin and isorhamnetin with neuroprotective activity and polysaccharides with immunostimulatory, antitumor, antimicrobial and antiinflammatory effects. Also, currants around the world are important for the food industry mainly because of their colour and organoleptic properties, which makes them suitable material for diverse food applications (Xu et al., 2018; Eksi Karaagac et al., 2020; Rodrigues et al., 2020).
Black currants are perennial bush plants, economically important, growing in temperate zones of Europe, Russia, northern Asia, and New Zealand (Woznicki et al., 2015).
Black currant berries, are favoured for their organoleptic properties such as pleasant color and intense taste, which are due to phenolic compounds like anthocyanins, and the presence of sugars, acids, and volatile compounds (Varming C. et al., 2004; Tarko et al., 2020).
Many morphological and biochemical changes are occurring during growth and development. This depends on the physiological state of the plant, agriculture technologies (Vagiri et al., 2013), and other factors such as genotype, climate, degree of ripening, harvesting time, and storage conditions (Rubinskiene et al.,

2006). For example, the genetic background determines the sugar accumulation of black currants and the response to weather conditions (Zheng et al., 2009). Also, accumulating evidence suggests that genotype may have a profound influence on the content of bioactive compounds in berries (Pantelidis et al., 2007). Other compounds that are accumulating are the phenolic compounds, some of the secondary metabolites occurring in plants. Their biosynthesis depends on numerous enzymes, and their metabolism is combined with morphological and biochemical regulatory patterns of plants (Thitilertdechaa and Rakariyatham, 2011). Although many studies have investigated the fruit quality and antioxidant properties of berries (Gipson et al., 2013; Ferreira Zielinski et al., 2015), little is known about variation in fruit quality, levels of phenolic compounds, and antioxidant activities of different currants varieties in relation to fruit maturation stages for varieties grown in Romania (Diaconeasa et al., 2015).

The aim of this paper was to investigate the natural variation in biochemical compounds of two varieties of berries belonging to *Ribes nigrum* L. species during maturity process. These two varieties of blackcurrant are part of a group of 12 varieties studied. All were analyzed according to the same criteria, in order to highlight the best of them, for their cultivation on large areas. The correlations between the compounds and maturation stages were also investigated in order to see the connection between the state of maturation and biosynthesized compounds.

MATERIALS AND METHODS

Biological material

The biological material consists of fruits in different maturation stages from ‘Roxia’ and ‘Kzvana’ varieties, harvested from the experimental field of the research bio-base of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Figure 1. *Whole fruit fistfruits*

Figure 2. *Advanced firstfruits*

Figure 3. *Harvesting maturity*

Figure 4. *Consumption maturity*

Fruit extracts preparation

The fruit extracts were performed using the ratio of 1:6 g L^{-1}. The fresh fruits were smashed, over which an ethanol solution was added (Saúl Olivares-Galván et al., 2020). The resulting mixture was subjected to ultrasound extraction on ice bath for the epicarp patency, for 30 minutes. Afterwards, the extracts were filtered, followed by centrifugation. The supernatant was used for the further analyses.

Determination of total phenolic content (TPC)

Total phenolic content in fruit extracts was performed spectrophotometrically using Folin-Ciocâlteu method from the literature (Sariburn et al., 2010). The total phenolic content was expressed as gallic acid equivalent (GAE) per ml of extract.

Determination of total flavonoids content (TFC)

Total phenol content in fruit extracts was determined spectrophotometrically using an adapted method (Sushant Arya et al., 2019). The total flavonoid content was expressed as rutin equivalent (RE) per ml of extract.

DPPH•free radical scavenging activity (FRSA)

Free radical scavenging activity of black currant extracts was determined using the stable radical 2,2 diphenyl-1-picrylhydrazyl (DPPH•, Sigma), according to the method described in the literature (Suleria et al., 2020). Free radical scavenging activity was expressed as mg/mL ascorbic acid equivalent (AAE).

Gas-chromatographic profile

Qualitative determination of volatile biologically active compounds was performed using a Thermo Trace 1310 gas chromatograph coupled with an ISQ system mass spectrometer, according to an adapted method (Vulic et al., 2012). The system is equipped with a chromatographic column (Thermo Scientific Trace GOLD GC Column), having a length of 30 m, an inner diameter of 0.25 mm, and a stationary phase 5% phenyl methylpolysiloxane, with a thickness of 0.25 μm. The mobile phase was helium, with a flow rate of 1 ml/min. The initial temperature was 40℃, with a ramp of 10℃/minute, reaching up to 250℃. The volume of sample introduced was 1 ml, its vaporization taking place in about 30 minutes.

Statistical analysis
A general linear model, Duncan test was used for the comparison of means for the content of bio compounds between groups, using Statistical Package for Social Science (SPSS version 21.0). The statistical significance was considered for the probability value of difference $p < 0.05$. The obtained results were expressed as mean values ± standard deviation (SD). Microcal Origin version 6.0 software was used for the charts design and Pearson correlation coefficient.

RESULTS AND DISCUSSIONS

Total phenolic content
The results of the quantitative determination of total phenolic content are shown in Figure 5. The graphs were made in the form of bars to highlight the difference between the varieties on the same maturation stage. Concerning the concentration of total phenolic accumulated during fruit maturation stage III, the upper limit is found in 'Kzvana' genotype with a concentration of 5.87 mM GAE / ml extract.

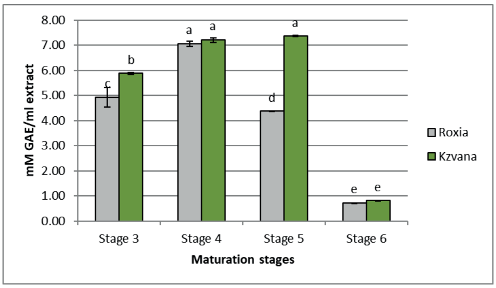

Figure 5. Total phenolic contents values for 'Roxia' and 'Kzvana' fruit genotypes

In stage IV of fruit maturation also it can be observed an accumulation of polyphenol content for both Roxia and Kzvana genotypes with the concentrations of 7.2 mM, respectively 7.06 GAE/ml extract. In stage V of fruit maturation, the phenolic content increases, reaching a highest point of 7.36 mM GAE / ml extract in the 'Kzvana' genotype case. In the final stage of maturation, both genotypes have recorded a sharp decline of phenolic content. A possible explanation can be drawn from their role in plant growth and development. Thus, with defense function, phenols are found in larger quantities in the early stages of fruit maturation when the attacks are susceptible to pests and diseases, decreasing when they reach harvest and consumption maturity.
According to the obtained results, apparently as the fruit matures, the phenolic content decreases. On one hand this may be due to the fact that during fruit maturation the plant does not need a high level of phenols, instead the amount of flavonoids may increase, with a higher ratio, both groups of active principles having the same genetic precursors.

Total flavonoid content
As regards the quantitative determination of total flavonoid content, the results are shown in Figure 6. From the quantitative analysis of fruits extracts of *Ribes nigrum* L. one can notice that the value of flavonoid content for the genotypes studied is directly proportional to the progress of fruit maturation process, except for the Roxia genotype in stage IV of maturation. For maturation stage III it can be observed that between the genotypes studied in terms of flavonoid content there is no statistically significant difference. With regard to the maturation stage IV, the highest concentration of flavonoid was recorded in the 'Roxia' genotype case with a value of 1.34 mM RE / mL extract.

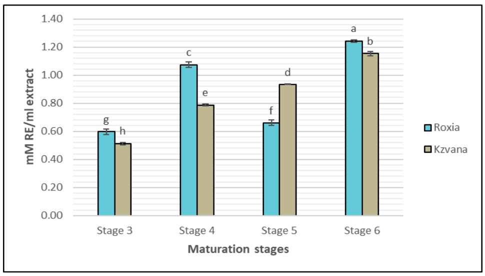

Figure 6. Total flavonoid content values for 'Roxia' and 'Kzvana' fruits

Concerning the quantity of flavonoid in the case of fruits in the maturation stage V, it can be observed that compared with stage IV, the values are decreased, the maximum concentration being reached by the 'Kzvana' genotype. The influence of maturation stage (IV) on the accumulation of flavonoid content can be concretized in a maximum value of these compounds available for both genotypes, here emphasizing the variety 'Roxia'.

Evaluation of free radical scavenging activity

The free radical scavenging activity is one of the modalities of expressing the therapeutic, biological and nutritional value of fruits and vegetables. Therefore, in Figure 7 is presented free radical scavenging activity of black currant fruit of 'Roxia' and 'Kzvana' genotypes.

In the maturation stage III, there are no significant differences of free radical scavenging activity values between the fruits of 'Roxia' and 'Kzvana' genotypes. During the fourth maturation stage analyzed, free radical scavenging activity of 'Roxia' and 'Kzvana' fruits registered increases throughout maturation, subsequently reaching a maximum activity in the maturation stage VI.

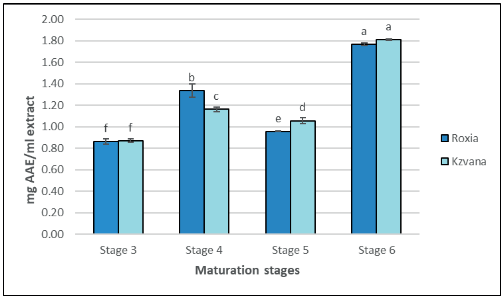

Figure 7. Free radical scavenging activity of *Ribes nigrum* L. genotypes

Thus, the values of free radical scavenging activity expressed as mg AAE / ml extract is between 0.86 - 1.77 for 'Roxia' genotype, and 0.87 - 1.81 for 'Kzvana' genotype.

Correlations between free radical scavenging activity and TPC

Correlations between free radical scavenging activity and phenolic content were tested in different stages of *Ribes nigrum* L. fruits maturation. Also, by testing the correlation between the value of free radical scavenging activity and the amount of phenolic content, it was observed that there is no direct interdependence, the correlation of those two characteristics showed that the free radical scavenging activity was not influenced by the total phenolic content, the correlation being distinct insignificant with the correlation coefficient $R^2 = 0.277$ for the probability p=0.08 for 'Roxia' (Figure 8), and $R^2 = 0.299$, p=0.078 for 'Kzvana' (Figure 9).

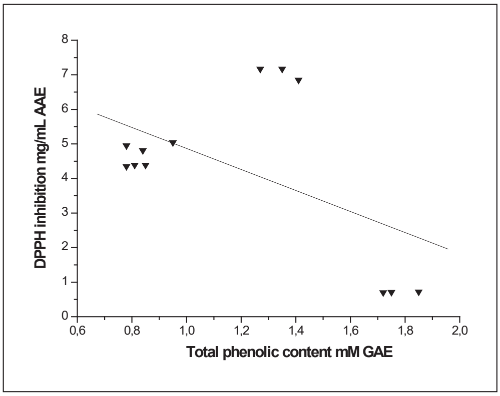

Figure 8. Correlations between the free radical scavenging activity and the total phenolic content for 'Roxia' fruits

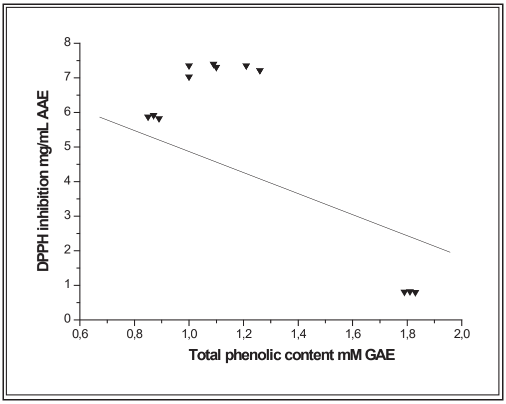

Figure 9. Correlations between the free radical scavenging activity and the total phenolic content for 'Kzvana' fruits

Correlations between free radical scavenging activity and TFC

As it is well known, the phenolic compounds have a very high antioxidant activity. Since the extracts obtained from the fruits of genotypes of the *Ribes nigrum* L. species are very complex in terms of the biochemical composition, it is very difficult to distinguish the compounds with the most antioxidant activity. Black currants are rich in active compounds that possess antiradical properties (Burdulis et al., 2009). These properties are also influenced by genotype (Nour et al., 2013). Today, in the literature, there are researches that show the link between free radical scavenging activity and flavonoids (Olajire et al., 2011; Muniyandi et al., 2019). This was also demonstrated within this study. Thus, regarding the correlation between free radical scavenging activity and flavonoid content for the genotypes studied, it can be observed that there is a linear correlation strongly positive, Pearson correlation coefficient (R^2) exceeding the value of 0.900 for the probability $p < 0.05$, for all the maturation stages of the fruit. The correlation between free radical scavenging activity and flavonoid content for the variety 'Roxia' is presented in Figure 10. As it can be seen, it was evidenced a strong positive correlation, with the value of $R^2 = 0.960$ for the probability $p < 0.001$.

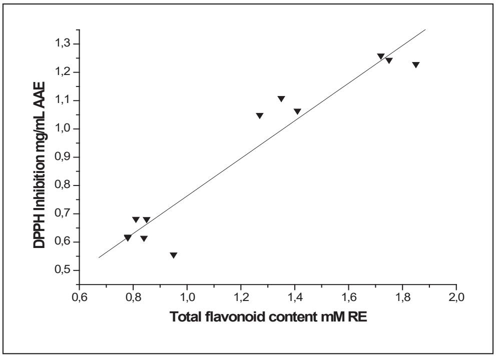

Figure 10. Correlation between the free radical scavenging activity and the total flavonoid content for 'Roxia' fruits

According to the graphical representation (Figure 11) for 'Kzvana' genotype, free radical scavenging activity has a positive linear correlation with the flavonoid content, this time more moderate, with a correlation coefficient $R^2 = 0.861$ for the probability of $p < 0.05$.

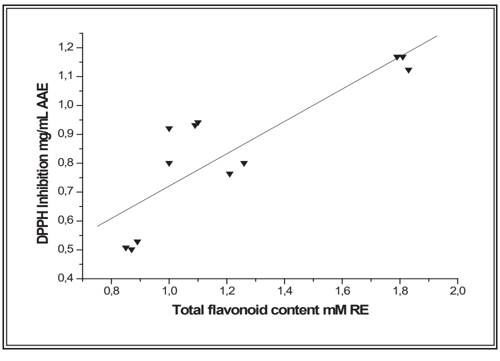

Figure 11. Correlation between the free radical scavenging activity and the total flavonoid content for 'Kzvana' fruits

However, the content of phenolics may correspond or not to the free radical scavenging activity of a fruit. This is due to the presence of other biologically active compounds with low molecular weight, such as glutathione (thiol), that participate in the redox reactions (Sochor et al., 2010).

The profile of the aroma compounds of the black and red currant (Table 1) is similar to that of most berries. According to the literature, berries may contain the following classes of compounds: aliphatic alcohols, aliphatic esters, monoterpenes, oxygenated monoterpenes, sesquiterpenes, phenols (Turemis et al., 2003). In the case of currants, over 150 volatile compounds have been identified, the most important classes being monoterpenes, sesquiterpenes, esters and alcohols (Varming et al., 2004; Liu et al., 2018)).

The most important aroma compounds for blackcurrant include esters such as 2-methylbutyl acetate, methyl butanoate, ethyl butanoate and ethyl hexanoate with sweet fruit flavor, nonanal, β-damascenone (result of carotenoid degradation), some monoterpenes (α-pinene, 1,8-cineole, linalool, terpinen-4-ol and α-terpineol), aliphatic ketones (1-octen-3-one), as well as sulfur compounds 4-methoxy-2-methyl-butanethiol, which give the specificity of smell for black currant (Vulic et al., 2012; Varming et al., 2004).

Regarding the volatile compounds identified for the varieties of *Ribes nigrum* L., alkanes and their isomers (n-Decane, n-Nonane and 2,2-Dimethoxybutane, 1,3,3-trimethoxybutane) were identified, highlighting the variety Roxia; other classes of compounds were: monoterpenes (D-Limonen), Kzvana variety with the highest content, oxygenated monoterpenes (Dihydro-Citronelol), and monoglycerides of fatty acids with antimicrobial role (Altieri et al., 2007), such as 2-mono-stearin in the case of the 'Kzvana' variety.

Also, following the evaluation of fruit extracts, components of essential oils with antimicrobial effect were determined, such as estragol (Bagamboula et al., 2004), in this case highlighting the 'Roxia' variety. The content in sugars is highlighted by the presence of sucrose in a high percentage in the case of 'Kzvana' variety.

Table 1. Profile of volatile compounds
for black and red currant

Identified compounds	*Kzvana*	*Roxia*
	Aria %	
2,2-Dimethoxybutane	9.73	19.8
2-methyl, methyl ester butanoic acid	0.82	1.48
2,4-dimethyl-1-heptene	3.05	-
1,3,3-trimethoxybutane	-	-
3,3-dimethoxy-2-butanone	0.86	1.29
2-ethyl-1,3-Dioxolan-4-methanol	2.94	6.34
n-Nonane	0.71	1.36
Itaconic anhydride	1.8	0.54
3,6-Dimethyloctane	0.98	0.54
3-Methylnonane	-	-
Phenol	0.58	0.34
n-Dean	1.46	2.06
3,3-Dimethyloctane	0.59	1.31
4-methyldecane	0.75	1.55
d-Limonene	0.59	0.28
Dihydro-Citronelol	0.9	1.47
5-ethyl-1-nonene	1	1.49
3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1.3	-
Estragole	1.24	1.73
3,5-dimethyl-Benzaldehyde	0.74	1.21
5-Hydroxymethylfurfural	1.81	-
4,6-Dimethyldodecan	0.82	1.01
4-Methyldecane	0.71	0.52
Sucrose	17.66	1.19
Fructose		30.74
d-Mannose	13.04	-
L-Sorbose	23.97	-
D-Galactose	-	-
2-methylhexadecan-1-ol	-	-
3-Icosene	1.06	-
(3E) –Isotridecanol	1.77	2.14
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	1.26	-
2-mono-stearin	3.7	-
2-mono-palmitin	-	-

Aldohexoses (mannose, fructose), phenols, monoterpenes (D-Limonen) were also found in the identification of poultry compounds of blackcurrant varieties.

There were identified monosaccharides, in nature found as hexoses, like D-mannose (in 'Kzvana'), which it seems that they may facilitate the improvement of the human diet and health (Sharma et al., 2018; Shintani, 2019; Wu et al., 2020; Lin et al., 2021).

The retention times for each identified compound are shown in Figures 12 and 13.

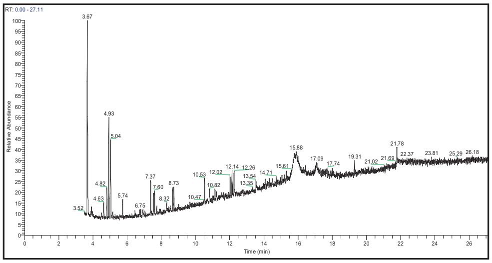

Figure 12. Gas chromatogram for the 'Roxia' variety

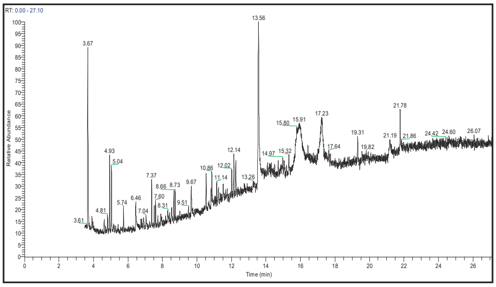

Figure 13. Gas chromatogram for the 'Kzvana' variety

CONCLUSIONS

With regard to the obtained results from the characterization of black currant extracts we may say that total phenolic and flavonoid content in black currant fruits is influenced both by the genotype and maturation stage. The influence of maturation stage on total phenolic content, the maximum values were recorded in maturation stage IV for 'Roxia' genotype, and stage V for 'Kzvana' genotype. The maximum accumulations of total flavonoid content, were recorded in the last stage analyzed for both genotypes, but emphasizing 'Roxia' genotype. Concerning free radical scavenging activity, 'Kzvana' genotype was highlighted in the last stage of maturation process.

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