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Antioxidant properties of polyphenolic extracts from chokeberry, blackcurrant, blackberry, and raspberry fruits

Przeciwutleniajace właściwości ekstraktów polifenolowych z owoców aronii, czarnej porzeczki, jeżyny i maliny

INTRODUCTION

Polyphenols are the biologically precious constituents of many plants. A rich source of them are fruits, of the *Rosaceae* family in particular, to which belong, among others, such fruits as: raspberry (*Rubus idaeus L.*) (RB), blackberry (*Rubus plicatus* W. et N.) (BB), chokeberry (*Aronia melanocarpa* (Michx.) Elliott) (CB), and blackcurrant (*Ribes nigrum* L.) (BC). Polyphenolic compounds possess antioxidant, antiseptic and bactericidal properties, their structure being much differentiated. The polyphenolic antioxidants of fruits belong to the group of flavonoids (flavonols, flavanols, anthocyanins, flavanons, and flavons) and a group of phenolic acids (hydroxycinnamic and hydroxybenzoic acids).

The main phenolic compounds of the *Rosaceae* family that occur in plants are, among others: *p-coumaric* acid, caffeic acid, ferulic, anthocyanins (mainly cyanidin derivatives), flavonols (quercetin, mirycitin and kaempferol glycosides), flavanols ((+)catechin and (-)epicatechin).

In some species there are large amounts of ellagic acid derivatives (hydrolysable tannins), e.g. in raspberry and blackberry [2], flavan-3-ols and their oligomers (mainly dimmers) in raspberry [6], hydroxycinnamic acid derivatives, represented mainly by chlorogenic acid and neochlorogenic acid, e.g. in chokeberry [7].

The large differentiation in quality and quantity of polyphenolic compounds of the *Rosaceae* family may explain the diverse antioxidant, antiseptic and bactericiadal properties. Extracts obtained from fruits using different solvents have different hydrophobicity [1], and also different antioxidant activity that depends on the amount and kind of phenolic compounds contained in an extract.

The aim of the work was to test the antioxidant activity of four fractions of phenolic compounds which were obtained with methanol (WA), ether (WB), acetate (WC) and water (WD) extraction, from fruits of chokeberry (CB), blackcurrant (BC), blackberry (BB) and raspberry (RB).

MATERIALS AND METHODS

The material to study was chokeberry (CB) fruit of the Galician variety, picked from the Sady Trzebnickie plantation, blackcurrant (BC) of the Tisel variety from the Lubin area, and blackberry (BB) of Orkan and raspberry (RB) of the Polka variety purchased on detail. The fruits were frozen and then freeze-dried (CHRIST ALPHA 1-4 LSC), and just before extraction they were disintegrated with an analytical mill (A11 basic of IKA-Werke, Germany). The commuted lyophilizate (50,00 ± 0,01g) was subjected to extraction twice with 80% methanol in water solution (analytical grade) with HCl (0.1%) - (1350 ml total), keeping it twice for 15 min under sonication (Senorex Digital 10P of Bandeli, Germany) at 25°C and 60% power, and in a fridge at 4°C for 24 h. After filtering, from the alcohol-water extract 20% of its volume was taken (1/5 of total) and alcohol was removed with a vacuum evaporator (40°C) and residue was dried at 40°C on water bath and in air dryer until dry extract (WA) was obtained. From the rest of the methanol-water extract (80%) the methanol was removed under reduced pressure, mixed with water at 45°C and left in a fridge for 24 h at 4°C. After removing precipitate, water solution was extracted in a separator with diethyl ether and then with ethyl acetate of 100 and 200 ml volume. The obtained extracts were condensed under reduced pressure to obtain WB and WC fractions, respectively. The aqueous solution after extraction was evaporated with a vacuum evaporator and dried to get the extract WD.

The fractions obtained were assayed for total phenolic content, anthocyanins and antioxidant activity. Total phenolic were assayed using the method with the Folin-Ciocalteau reactant according to Gao [4], the results expressed as mg gallic acid (GAE) in 1g fraction. The content of anthocyanins was determined with the method given by Giusti and Wrolstad [5], and the results expressed as mg cyanidine-3-glucoside (Cy-3-glu) in 1g fraction. Antioxidant activity was measured with the radical cation $ABTS^{\bullet+}$ [8] and expressed in micromoles of Trolox/1 g fraction. The correlation coefficient between total phenolic content and antioxidant activity was calculated, and variance analysis was done with the Duncan test at confidence level 0.05; the Statistica 9.0 program being used for the calculations.

RESULTS AND DISCUSSION

By extracting with various solvents, four fractions of different hydrophobicity were obtained. A preliminary HPLC chromatographic analysis showed that in the methanol and water extracts there were anthocyanins (520 nm), and in the ether and acetate fractions there were phenolic acids (320 nm) and flavonols (360 nm) at different proportions.

Table 1 shows the contents of total phenolic, anthocyanins, and the antioxidant activity of the extracts obtained from the fruits. The richest in polyphenols proved to be extracts from blackcurrant, then chokeberry, raspberry and blackberry. The blackcurrant fractions contained from 31 mg/g (WD) to 331 mg/g (WB); those from chokeberry - from 20 mg/g (WD) to 197 mg/g (WC); raspberry - from 22 mg/g (WD) to 210 mg/g (WB); blackberry - from 46 mg/g (WA) to 113 mg/g (WC).

Tabela 1. Total phenolics and anthocyanins contents, as well as antioxidant activity ($ABTS^{\bullet+}$) of chokeberry, blackcurrant, blackberry, and raspberry extracts.

Variety of fruits	Extract	Total phenolics (mg GAE/g)	$ABTS^{\bullet+}$ (μM Trolox/g)	Anthocyanins (mg Cy-3-glu/g)
		Average ± SD	Average ± SD	Average ± SD
Chokeberry	W A	22.64 ±4.854 ^{f}	116.8 ± 28.31 ^{h}	3.33 ± 1.246^{d}
	W B	195.62 ±19.788^{b}	1115.2 ± 64.82^{b}	-
	W C	197.34 ±6.907^{b}	1049.2 ± 11.62^{c}	1.66 ± 0.545$^{e.f}$
	W D	19.77 ±0.607 ^{f}	143.4 ± 8.79 ^{h}	3.65 ± 0.487$^{c.d}$
Blackcurrant	W A	34.18 ± 1.947 ^{f}	172.5 ± 3.21 ^{h}	4.48 ± 0.311^{c}
	W B	331.00 ±24.042^{a}	1598.0 ± 32.53^{a}	-
	W C	86.91 ±3.689 $^{c.d}$	443.8 ± 32.48 ^{f}	-
	W D	30.91 ±0.888 ^{f}	144.4 ± 5.17 ^{h}	1.26 ± 0.048^{f}
Blackberry	W A	45.79 ±1.845$^{e.f}$	311.3 ± 19.89 ^{g}	9.46 ± 0.042^{b}
	W B	79.20 ±4.612 ^{d}	437.5 ± 9.90 ^{f}	-
	W C	113.23 ±7.242^{c}	780.3 ± 52.61^{d}	0.85 ± 0.102^{f}
	W D	64.78 ±0.342$^{d.e.}$	446.2 ± 2.36 ^{f}	14.35 ± 0.213^{a}
Raspberry	W A	28.55 ±0.820 ^{f}	162.8 ± 3.11 ^{h}	2.32 ± 0.028^{e}
	W B	210.04 ±37.166^{b}	592.9 ± 37.05^{e}	-
	W C	89.30 ±14.177 $^{c.d}$	453.1 ± 10.75^{f}	-
	W D	22.46 ±0.068 ^{f}	118.9 ± 2.97 ^{h}	1.01 ± 0.167^{f}

Different letters a, b, c…in the same column indicate statistically significant differences ($p < 0.05$).

From the results obtained, it is clear that the content of polyphenolic compounds was highest in the ether (WB) and acetate (WC) fractions, whereas the total polyphenol content was the least in the water (WD) and methanol (WA) fraction.

Anthocyanins were assayed in some fractions only, mainly methanol and aqueous, and in the case of chokeberry and blackberry, which are fruits especially rich in those compounds, in the acetate fraction also. The content of colorants in the fractions was 3.33 – 24.6 mg/g fraction, the most in blackberry and chokeberry extracts, and least in blackcurrant and raspberry extracts.

The greatest antioxidant activity was characteristic for ether and acetate fractions that contained most phenolic compounds. It was confirmed by the high correlation coefficient between total polyphenols content and $ABTS^{\bullet+}$ test ($r = 0.94$). The fruits used for making the extracts differed with respect to the traits studied. The overall activity of the extract towards $ABTS^{\bullet+}$ cation-radicals decreased according to the sequence: chokeberry > blackcurrant > blackberry > raspberry. Of all the fractions tested, the highest activity of 1598 μM Trolox/g fraction was found in the ether blackcurrant extract, next in the ether (WB) and acetate (WC) extracts from chokeberry, 1115 and 1049 μM Trolox/g, respectively. It should be noted that the activity of fractions containing anthocyanins was not high, which may be due to the low participation of those compounds in the antioxidant potential of the fruits tested. A smaller participation of anthocyanins than polyphenols in the antioxidant potential was reported also by Vangdal et al. [11].

The polyphenolic composition of the extracts could be differentiated, both with respect to the number of OH groups and amount and kind of substituents, e.g. methyl or glycoside in a molecule. As reported by Rice-Evans et al. [9], antioxidant activity increases with increasing number of –OH groups, while with increasing number of sugar substituents and O-Me groups it decreases. Some authors [3, 10] report that activity of phenolic compounds does not depend on the type of family they belong to, but more on their specific structure, kind and number of substituents, position of –OH groups (*orto-, meta-, para-*), lipo- or hydrophilic character and substances that are present in the heterogenic medium of a polyphenolic fraction.

CONCLUSIONS

Among the fractions studied, obtained from chokeberry, blackcurrant, blackberry and raspberry, the richest in phenolic compounds were the ether (WB) and acetate (WC) fractions, whereas smallest amounts of polyphenols were found in the methanol (WA) and water (WD) fractions.

The overall antioxidant activity of the extracts towards the $ABTS^{\bullet+}$ cation radical decreased in order: chokeberry > blackcurrant > blackberry > raspberry.

Research is in progress to identify the constituents of WB and WC extracts and explain their high antioxidant activity.

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SUMMARY

Berry fruits are valuable source of polyphenolic compounds, which show high biological activity. They are characterized by great diversity in structure and antioxidant properties. The main phenolic compounds found in investigated fruits from *Rosaceae* family are: p-coumaric, caffeic and ferulic acids; anthocyanins, mainly cyaniding-3-glucoside derivatives; flavonols, commonly quercetin, myrycetin and kaempferol, and flavanols like (+) catechin and (-) epicatechin. In some species, in raspberry and blackberry for example, ellegic acid derivatives (hydrolysable tannins) are found in large quantities. In other ones, e.g. in chokeberry and blackberry, hydroxycinnamic acid derivatives such as chlorogenic and neochlorogenic acids dominate. Large variations in quality and quantity of phenolic compounds in fruits may explain their different antioxidant properties. The aim of this study was investigation of antioxidant activity of phenolic compounds in extracts obtained from chokeberry (CB), blackcurrant (BC), blackberry (BB), and raspberry (RB). The determination of total phenolic and anthocyanins content as well as $ABTS^{\bullet+}$ test, were done in four fractions obtained with methanol (WA), ether (WB), acetate (WC) and water (WD). The richest in phenolic compounds were blackcurrant extracts (31-331 mg/g), then extracts from raspberry (22-210 mg/g), chokeberry (20-197 mg/g), and blackberry (46-113 mg/g). The highest content was determined in WB fraction (BC, CB, RB) and WC fraction (CB, BB). Total antioxidant activity against ABTS radical cation decreased in order: CB >BC >BB > RB. The most active were ether (WB) and acetate (WC) fractions, which contain the majority of phenolic compounds. The correlation coefficient between total phenolic content and ABTS test was high ($r = 0.94$).

Keywords: polyphenolic fractions, total phenolics, anthocyanins, $ABTS^{\bullet+}$, *Rosaceae*

STRESZCZENIE

Owoce jagodowe są cennym źródłem związków z grupy polifenoli, wykazujących wysoką aktywność biologiczną. Charakteryzują się one dużą różnorodnością w budowie i właściwościach przeciwutleniających. Główne związki fenolowe występujące w badanych owocach z rodziny *Rosaceae* to: kwas p-kumarowy, kawowy i ferulowy; antocyjany głównie pochodne cyjanidyny; flawonole, zwykle kwercetyna, mirycetyna i kempferol, a także flawanole jak (+)katechina i (-) epikatechina. W niektórych gatunkach owoców występują w dużych ilościach pochodne kwasu elagowego (taniny hydrolizujące), np. w malinach i jeżynach, flawan-3-ole oraz ich oligomery, przykładowo w malinach; pochodne kwasu hydroksycynamonowego jak kwas chlorogenowy i neochlorogenowy, np. w aronii i jeżynach. Duże zróżnicowanie w jakości i ilości związków

polifenolowych w owocach może wyjaśniać ich odmienne właściwości przeciwutleniające. Celem pracy było zbadanie aktywności przeciwutleniającej związków fenolowych w ekstraktach z aronii (CB) czarnej porzeczki (BC), jeżyny (BB) i maliny (RB). Oznaczenia zawartości polifenoli ogółem, antocyjanów i aktywności wobec kationorodników $ABTS^{\bullet+}$ wykonano w czterech frakcjach: metanolowej (WA), eterowej (WB), octanowej (WC) i wodnej (WD). Najbogatsze w polifenole były ekstrakty z czarnej porzeczki (31-331 mg/g), następnie z maliny (22-210 mg/g), aronii (20-197 mg/g) i jeżyny (46-113 mg/g), przy czym najwięcej tych związków zawierały frakcje WB (CzP, Ar, M) i WC (Ar, J). Sumaryczna aktywność ekstraktów wobec $ABTS^{\bullet+}$ zmniejszała się w kolejności: Ar > CzP > J > M. Najbardziej aktywne były frakcje eterowe (WB) i octanowe (WC), zawierające najwięcej związków fenolowych, co potwierdza wysoki współczynnik korelacji pomiędzy zawartością polifenoli ogółem i testem ABTS ($r = 0{,}94$).

Słowa kluczowe: frakcje polifenolowe, polifenole ogółem, antocyjany, $ABTS^{\bullet+}$, *Rosaceae*

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