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Porównanie zawartości związków fenolowych oraz aktywności przeciwutleniającej ekstraktów wodnych z białych, czerwonych oraz czarnych porzeczek (*Ribes* sp.)

Streszczenie: *Celem przeprowadzonych badań było porównanie zawartości związków o charakterze przeciwutleniaczy oraz aktywności biologicznej wodnych ekstraktów z białej, czerwonej i czarnej odmiany porzeczki (Ribes sp.). Charakterystyka chemiczna ekstraktów została ustalona poprzez oznaczenie związków z grupy fenoli z użyciem techniki HPLC-DAD-MS, całkowitej aktywności przeciwutleniającej za pomocą standardowych testów spektrofotometrycznych (ABTS, DPPH, FC), a także sporządzenie profili przeciwutleniaczy z użyciem technik TLC z wizualizacją odczynnikami (ABTS, DPPH i FC) oraz deprywatyzacji postkolumnowej z użyciem odczynnika ABTS. Otrzymane wyniki wykazały, że badane odmiany porzeczek różnią sie głównie zawartością związków z grupy antocyjanów, co wiąże się z różnicami w aktywności przeciwutleniającej tych odmian. Najwyższą zawartością związków fenolowych, jak również najwyższą aktywnością przeciwutleniającą charakteryzował się ekstrakt z odmiany czarnej, następnie odmiany czerwonej i białej.*

Słowa kluczowe: *porzeczki, antocyjany, całkowity potencjał przeciwutleniający*

Comparison of the content of phenolic compounds and antioxidant activity of white, red and black currants (Ribes sp.) extracts

Abstract: *The aim of this study was to compare the content of antioxidant compounds and the antioxidant activity of extracts from white, red and black currants (Ribes sp.). The chemical properties verified included determinations of anthocyanins and other phenols by HPLC-DAD-MS, total antioxidant activity by standard spectrophotometric tests (ABTS, DPPH and FCR), and profiles of antioxidants by TLC with visualization reagents (ABTS, DPPH, FC) and post-column derivatization with ABTS reagent. The results obtained showed that the studied varieties of Ribes sp. differed mostly in the content of anthocyanins and hence in antioxidant activity. The highest content of phenolic compounds, as well as the highest antioxidant activity exhibited black currant extract, followed by red currant extract and white variety.*

Keywords: *currants, anthocyanins, total antioxidant potential*

1. Introduction

The research carried out over past two decades suggested that biological activity of phytochemicals may differ depending whether they are studied as purified compounds or in combination with food matrix as they occur in nature. To recognize how food matrix influences the biological properties of phytochemicals of interest, the approach involving comparison of plant material derived from the same species, but from varieties differing in colour, thus in the content of specific components such as e.g. anthocyanins, was developed. Also other researchers employed this strategy to study matrix impact on bioactive potential of phytochemicals. The most frequent are comparisons of the contents of nutrients (sugars, fatty acids, amino acids), non-nutrients (organic acids, phenolic acids) and antioxidant potentials of different fruit or vegetable varieties [1, 2, 3]. In some studies, additionally cytotoxic or antimicrobial activities are evaluated.

In our investigations, the comparison of total antioxidant potential (ABTS, DPPH, FCR), composition of phenolic compounds (HPLC-DAD-MS), antioxidant profiles by TLC and post-column derivatization were carried out for extracts from fruits differing mainly in the content of phenols, *i.e*. white, red and black currants (*Ribes sp.)*. Although in recent years a growing number of publications about anthocyanins content in black and red currants can be observed, to our best knowledge the comparison of these two varieties with the white variety have not been done before.

2. Experimental

2.1. Chemicals and reagents

The following chemicals were used: 1,1-diphenylo-2-picrylhydrazyl (DPPH), 2,2-azinobis-(ethyl-2,3-dihydrobenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), Folin-Ciocalteu's phenol reagent (FC), acetonitrile and formic acid, all of which were purchased from Merck (Germany). Standard compound 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Sigma–Aldrich (USA). HPLC grade methanol and pure p.a. methanol were from Chempur (Poland), ethyl acetate pure p.a. was from POCH (Poland). Water was purified with a QPLUS185 system from Millipore (USA).

2.2. Plant material

Water extracts prepared from white, red and black currants (*Ribes* sp.) were used throughout this study. All currant fruits were obtained from the local market and were cultivated in the North of Poland. Before extraction, the fruits were kept at -20°C, then freeze-dried using Christ Alpha 2-4 LSC. To obtain extracts, 0.2 g of each lyophilizate was mixed with 5 mL of deionized water and transferred to an ultrasound bath Unitra U4 (Unitra-Unima, Poland) for 10 min. Subsequently, the extracts were centrifuged (Heraeus Megafuge 16R Centrifuge) at 5000 rpm for 20 min at 4°C, to remove particulates. The collected supernatants were used in all experiments.

2.3. Profiles of antioxidants using TLC with antioxidant-directed visualization

Water extracts of lyophilizates of currants were applied onto TLC plates (silica gel HPTLC 60 F254, 20 × 10 cm; 0.25 mm; Merck, Germany) using glass capillary. The mobile phase consisted of ethyl acetate, formic acid, and water (6:1:1 v/v/v). Detection of the separated antioxidants was achieved by spraying plates with methanolic solution of either DPPH or ABTS radicals, or FC reagent diluted with water as described previously by Kusznierewicz [4].

2.4. Determination of phenolic compounds in fruit extracts by HPLC-DAD-MS

The analysis of phenolic compounds was performed according to Kusznierewicz [5] using the Agilent 1200 Series HPLC-DAD-MS system (Agilent Technologies, USA). Phenomenex Kinetex XB-C18 column, size 150 x 4.6 mm, particle size 5 µm was used for separation of phytochemicals. If the standard compounds were not available, the comparison of retention time, mass signal ([M+H]+, [M+H]- and fragment (m/z) with available literature data were the basis for the identification of individual components.

2.5. On-line profiling of antioxidants by HPLC-coupled post-column derivatization

The chromatographic profiles of antioxidants in plant extracts studied were obtained by online post-column derivatization with ABTS radical using Pinnacle PCX Derivatization Instrument (Pickering Laboratories, Inc., USA) according to the procedure previously described by Kusznierewicz [4]. Separation conditions were the same as described in section 2.4.

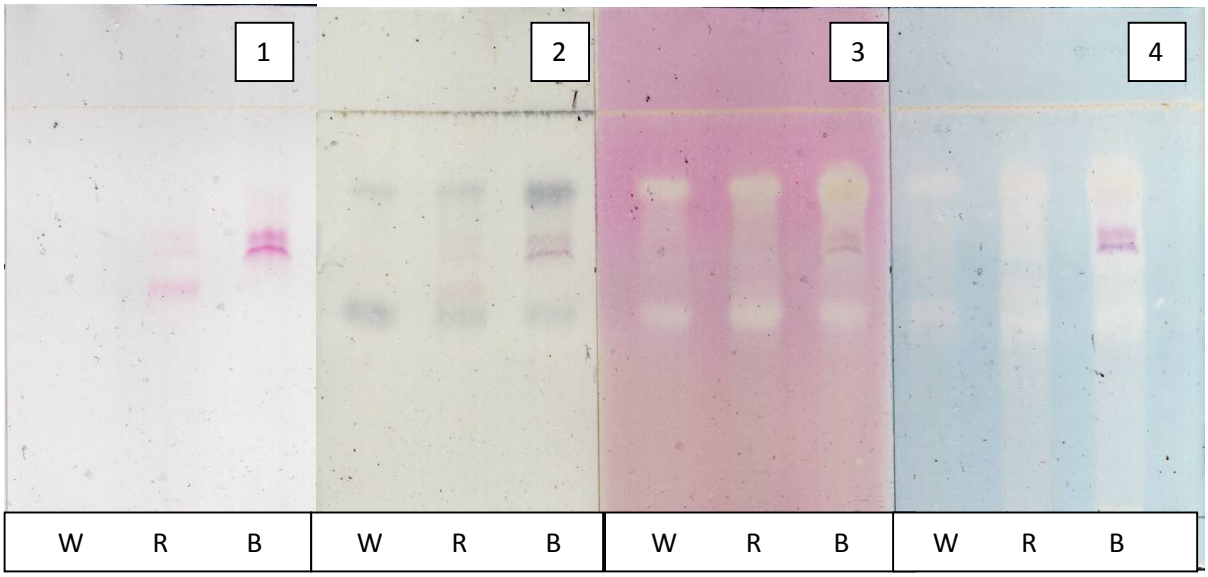
2.6. Determination of antioxidant activity by spectrophotometric methods

The popular spectrophotometric methods employing ABTS and DPPH radicals, as well as FC reagent were used for the colorimetric determination of antioxidant activity as described earlier [4]. All determinations were carried out in disposable cuvettes at room temperature with Thermo Scientific NanoDrop 2000c spectrophotometer.

3. Results and discussion

3.1. Profiles of antioxidants using TLC

TLC analysis of antioxidants present in white, red and black currant fruit extracts are shown in Fig. 1. Antioxidant profiles detected in the tested samples differed significantly. For the white variety, they were much poorer than the ones obtained for red and black cultivars. Visualization reagents showed that additionally to coloured antioxidants visible on the chromatogram without visualization reagents, in all three varieties of currants, there are also present compounds with antioxidant activity other than anthocyanins.



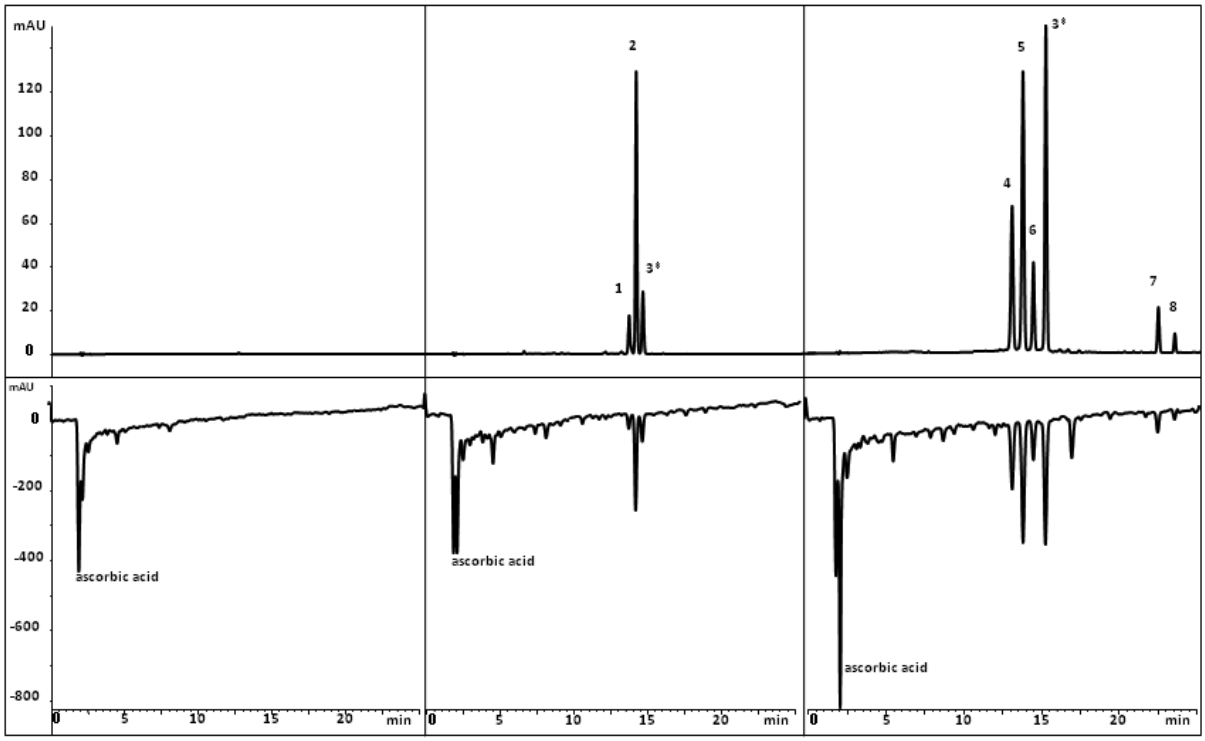
Rys. 1. Profile przeciwutleniaczy dla wodnych ekstraktów z białych (W), czerwonych (R) i czarnych (B) porzeczek uzyskane za pomocą techniki TLC bez (1) oraz z wizualizacja odczynnikami FC (2), DPPH (3) oraz ABTS (4).

Fig. 1. Antioxidant profiles of extracts from white (W), red (R) and black (B) currants obtained using TLC without (1) and with FC (2), DPPH (3) and ABTS (4) reagent visualization.

3.2. Qualitative and quantitative determination of phenolic compounds and on-line profiling of antioxidants in currant extracts by HPLC-DAD-MS coupled with post-column derivatization

Fig. 2 shows chromatograms obtained using HPLC-DAD-MS analysis of phenolic compounds for white (left panel), red (middle panel) and black (right panel) currant extracts. The main phenols detected belonged to anthocyanins. As expected, the richest source of anthocyanins from *Ribes* sp. turned out to be black currant, followed by red variety. White currants did not contain any compounds from this group of antioxidants. At the 325 nm, also some minor peaks were seen (data not shown) which could represent phenolic acids, however in post-column derivatization they displayed only minor antioxidant activity (Fig. 2, bottom panel).

The HPLC-MS data for major anthocyanins present in currant varieties studied are assembled in table 1. The identification of major peaks was confirmed by comparison with previously published data [6-10]. Quantitative analysis of data on anthocyanins content in red and black currants is also shown in table 1. Total anthocyanin content in black variety is four times higher than in red variety.



Rys. 2. Profile chromatograficzne uzyskane przed - (górne chromatogramy śledzone przy 525 nm) oraz po procesie derywatyzacji z użyciem odczynnika ABTS - (dolne chromatogramy śledzone przy 734 nm) dla wodnych ekstraktów z białych (panel A), czerwonych (panel B) i czarnych (panel C) porzeczek. Ponumerowane piki reprezentuja: 3-O-sambubiozyd cyjanidyny (1), 3-O-(2-ksylozylorutynozyd) cyjanidyny (2), 3-O-rutynozyd cyjanidyny (3), 3-O-glukozyd delfinidyny (4), 3-O-rutynozyd delfinidyny (5), 3-O-glukozyd cyjanidyny (6), 3-O-(6”-kumaryloglukozyd) delfinidyny (7) oraz 3-O-(6”-kumaryloglukozyd) cyjanidyny (8).

Fig. 2. Combined plots of profiles before - (top chromatograms traced at 525 nm) and after derivatization with ABTS reagent - (bottom chromatograms traced at 734 nm) obtained for white (panel A), red (panel B) and black currant water extracts (panel C). Numbered peaks represent: cyanidin 3-O-sambubioside (1), cyanidin 3-O-(2-xylosyl)-rutinoside) (2), cyanidin 3-O-rutinoside (3), delphinidin 3-O-glucoside (4), delphinidin 3-O-rutinoside (5), cyanidin 3-O-glucoside (6), delphinidin-3-O-(6”-coumaroylglucoside) (7) and cyanidin3-O-(6”-coumaroylglucoside) (8).

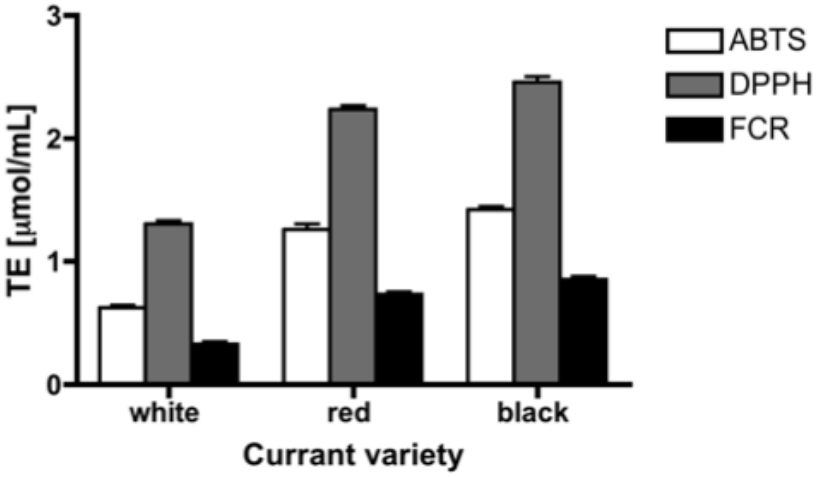
Tabela 1. Identyfikacja antocyjanów w ekstraktach z porzeczek techniką HPLC-MS
***Table 1.** HPLC-MS identification of anthocyanins present in currant extracts*

Peak number	Tentative identification	MS (m/z)	Anthocyanin content [mg/g d.w.]*	
			Red currants	Black currants
1	Cyanidin 3-*O*-(2-xylosylrutinoside)	727	0,109	-
2	Cyanidin 3-*O*-sambubioside	581	0,711	-
3	Cyanidin 3-*O*-rutinoside	595	0,195	0,746
4	Delphinidin 3-*O*-glucoside	465	-	1,338
5	Delphinidin 3-*O*-rutinoside	611	-	0,381
6	Cyanidin 3-*O*-glucoside	449	-	1,351
7	Delphinidin 3-*O*-(6”-coumaroylglucoside)	611	-	0,158
8	Cyanidin 3-*O*-(6”-coumaroylglucoside)	595	-	0,062

*Data are the mean values of three independent measurements. Standard deviations were within ± 5 % of the means.

3.3. Total antioxidant activity

In all three spectrophotometric tests, the highest antioxidant exhibited black currant extract, followed by red currant extract and the least active was the white variety (Fig. 3), which points to anthocyanins as the main reducing components. However, antioxidant activity of black currant extract turned out to be only twice higher than that of white currant extract. This may suggest that some unidentified antioxidants are also present in the white variety. This suggestion is supported by the results of on-line antioxidant profiling with ABTS (Fig. 2, bottom panels) where the increased background along the whole chromatograms reveals the presence of non-identified components with a weak yet measurable antiradical activity.



Rys. 3. Całkowity potencjał przeciwutleniający oznaczony za pomocą testów ABTS, DPPH i FC dla wodnych ekstraktów z białych, czerwonych i czarnych porzeczek. Wyniki przedstawiają wartość średnią ± *odchylenie standardowe dla trzech niezależnych doświadczeń.*

Fig 3. Total antioxidant potential determined by ABTS, DPPH or FC test for water extract from white, red and black currants. The results are means ± SD for three independent determinations.

Post-column derivatization with ABTS radical, confirmed results obtained by standard spectrophotometric tests. The highest antioxidant activity exhibited the extracts from black currants, followed by red and white varieties. Ascorbic acid was the major identifiable contributor to the total antioxidant activity of tested extracts, especially in the case of white currants.

4. Literature

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