Variation in the content of flavonols and main organic acids in the fruit of European cranberry (*Oxycoccus palustris* Pers.) growing in peatlands of North-Western Poland

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S u m m a r y

This paper documents phytochemical variation of European cranberry (*Oxycoccus palustris* Pers. = *Vaccinium oxycoccos* L.) growing in peatlands of North-Western Poland. Thirty four fruit samples collected in 2008–2009 at 15 sites in northern Wielkopolska (Greater Poland) and Western Pomerania were used in the study. The flavonol content, expressed as quercetin equivalent, was determined spectrophotometrically. The amount of main organic acids: citric, malic, and quinic, was determined using the HPLC-DAD method. The obtained results show high phytochemical differentiation of European cranberry fruit. The flavonol content ranged from 57 to 298 mg% of dry matter. The organic acids in question accounted for, respectively: 8.57–21.32% (citric acid), 2.18–14.24% (malic acid), and 2.96–8.79% (quinic acid) of fruit dry matter. There was also a large variation in the ratio of quinic acid to malic acid (from 0.27 to 3.83). A strong negative correlation was found between the above-mentioned acids (r= -0.74, p=0.000). This indicates the possibility of occurrence of two chemotypes of European cranberry, differing in the content of quinic acid and malic acid.

Key words: *Oxycoccus palustris, medicinal plants, phytochemical variability*, *organic acids*, *flavonoids*

INTRODUCTION

Cranberry fruit is widely used in the food industry [1-4] and in phytotherapy – mainly in urinary tract infections [5-8]. It can also be used in the prevention of cardiovascular disease [9-11] and ulcer diseases of the digestive system [12-14].

The pharmacological activity of the plant material under discussion is associated with a high content of organic acids, flavonols (mainly quercetin), anthocyanins, proanthocyanidins, and other phenolic compounds [3, 15-17].

A large majority of papers describing the chemical composition of cranberry fruit relates to *Oxycoccus macrocarpos* (Aiton) Pursh (American cranberry) – a species native to North America and widely cultivated in the USA and Canada [18-29]. There are relatively few studies on European cranberry (*Oxycoccus palustris* Pers.) occurred in Poland, and most frequently, they do not take into account intraspecific variations in the level of active compounds [30-35]. Therefore, it would be interesting to carry out phytochemical analysis on possibly large plant material that would allow us to estimate differentiation of the species in question.

The aim of the present study was to determine phytochemical variation in the fruit of wildly growing European cranberry in terms of the content of flavonols and main organic acids: citric, malic, and quinic.

MATERIAL AND METHODS

Plant material

34 fruit samples of European cranberry (*Oxycoccus palustris* Pers. = *Vaccinium oxycoccos* L.) originating from 15 peat bogs located in northern Wielkopolska and Western Pomerania were used in the present study (tab. 1). Plant material (well-developed and ripe fruit) was collected during the period from September to October of 2008–2009. Cranberry fruit was lyophilized at a temperature of –50°C and under a pressure of 0.5 hPa (Heto Dry Winner model DW3, Heto Holten A/S, Allerød, Denmark).

Flavonol analysis

The total flavonol content was determined spectrophotometrically from 1.00 g of powdered cranberry fruit, according to Christ-Müller's method described by Polish Pharmacopoeia VI [36]. The absorbance was measured at $\lambda=425.0$ nm on a Cintra 20 UV-VIS spectrometer (GBC) [35]. All solvents of analytical grade were purchased from POCH S.A. The flavonol content was expressed as a quercetin equivalent, in mg/100 g [mg%] of dry matter (DM) of cranberry fruit.

Table 1.

The location of peatlands and the number of samples of cranberry fruit

No.	Peatlands	District, Province	Geographical coordinates	No. of samples	Year of collection
1	Peatland „Mszar nad Jeziorem Piaski” near Karnice [PIA]	Łobez, Zachodniopomorskie	N 53° 41’ 54” E 15° 27’ 25”	3	2008
2	Peatland „Mszar koło Starej Dobrzycy” [DOB]	Łobez, Zachodniopomorskie	N 53° 48’ 07” E 15° 31’ 48”	2	2008
3	Peatland by lake Rzecińskie [RZE]	Szamotuły, Wielkopolskie	N 52° 45' 45" E 16° 18' 45"	1	2009
4	Peatland by lake Pustelnik Mały [PuM]	Czarnków-Trzcianka, Wielkopolskie	N 52° 46’ 51” E 16° 19’ 06”	1	2009
5	Peatland by lake Pustelnik Duży [PuD]	Czarnków-Trzcianka, Wielkopolskie	N 52° 46’ 44” E 16° 19’ 04”	1	2009
6	Peatland by lake Pokraczyn [POK]	Czarnków-Trzcianka, Wielkopolskie	N 52° 47’ 03” E 16° 21’ 24”	2	2009
7	Peatland by lake Kuźnik Olszowy [KuO]	Piła, Wielkopolskie	N 53° 12’ 46” E 16° 43’ 39”	3	2008-2009
8	Peatland by lake Kuźnik Bagienny [KuB]	Piła, Wielkopolskie	N 53° 12’ 50” E 16° 43' 51"	4	2008-2009
9	Peatland by lake Kuźniczek [KUZ]	Piła, Wielkopolskie	N 53° 12’ 00” E 16° 44' 21"	1	2009
10	Peatland by lake Kuźnik Mały [KuM]	Piła, Wielkopolskie	N 53° 11’ 53” E 16° 44’ 24”	1	2009
11	Peatland by lake Okoniowe [OKO]	Piła, Wielkopolskie	N 53° 11’ 19” E 16° 48’ 04”	4	2009
12	Peatland between Skórka and Jeziorki [SKO]	Piła, Wielkopolskie	N 53° 10’ 13” E 16° 52’ 09”	3	2008
13	Peatland near Zelgniewo [ZEL]	Piła, Wielkopolskie	N 53° 11’ 06” E 16° 54’ 39”	3	2008
14	Peatland by lake Czarne near Jeziorki [JEZ]	Piła, Wielkopolskie	N 53° 09’ 45” E 16° 51' 51"	2	2008
15	Peatland by lake Czarne near Kaczory [KAC]	Piła, Wielkopolskie	N 53° 07’ 23” E 16° 54' 56"	3	2008
	Total			34	

Organic acid analysis

The organic acid content was determined using the HPLC-DAD method after water extraction [37]. The freeze-dried and powdered cranberry fruit (0.25–0.35 g) was extracted twice for 30 min with 10.0 ml of water by sonification. All aqueous extracts were combined and diluted with water to 25 ml, and then centrifuged. The HPLC analysis was performed on an Agilent 1100 HPLC system, equipped with a photodiode array detector (DAD). For all separations, a Lichrospher 100

RP18 column (250.0 x 4.0 mm, 5.0 μm) purchased at Merck was used. The mobile phase consisted of 27.2 g/l K_2HPO_4 adjusted to pH=2.40 with 25% H_3PO_4, applied in isocratic elution for 30 min. The flow rate was adjusted to 0.6 ml/min, the detection wavelength set to DAD at λ=215.0 nm and then 20.0 μl of sample was injected. All separations were performed at a temperature of 24.0°C. Peaks were assigned by spiking the samples with standard compounds and compared with the UV-spectra and retention times. All solvents used were of HPLC grade (Merck). Reference substances were obtained from Sigma-Aldrich. The content of organic acids was given in g/100 g [%] of dry matter of cranberry fruit.

Statistical analysis

In statistical analysis, the Kruskal-Wallis test was applied, using Statistica 7.1 software [38]. Pearson's coefficient of correlation was used to evaluate correlations between variables. The Shapiro-Wilk test was applied to assess the normality of variable distribution. The phytochemical similarity of cranberry fruit samples was determined based on cluster analysis of the standardized contents of flavonols and organic acids. The Euclidean distance was used as a measure of distance, while UPGMA as the clustering method.

RESULTS

The obtained results show a large range of phytochemical variability of European cranberry fruit (tab. 2). The study found significant differences in relative and absolute content of the compounds under investigation. Among the organic acids, citric acid clearly dominated. It accounted for 37% to 64% of all acids under study, whereas malic acid and quinic acid made up 8–43% and 11–32%, respectively. The ratio of quinic acid to malic acid was characterized by very high variability (from 0.27 to 3.83). The content of both above-mentioned acids in the plant material was strongly negatively correlated (fig. 1).

Phytochemical variation of cranberry fruit was not connected with the geographical distribution of the investigated samples. Significant differences were recorded in the level of the compounds under study in particular peat bogs, irrespective of the year of harvest. This is demonstrated by cluster analysis of phytochemical similarity of the samples (fig. 2). This analysis allowed the identify 5 groups which were statistically significantly different in terms of the content of the compounds under discussion (figs 2–4). For example, group I was distinguished by the lowest average content of flavonols (fig. 3) and quinic acid as well as the highest content of malic acid (fig. 4). In turn, by far in group III the highest content of citric acid was found, whereas in cranberry fruits originating from the peat bog "Mszar near Stara Dobrzyca" (group V) – the highest content of flavonols.

Table 2.

Variability of the content of flavonols and organic acids in freeze dried fruit of European cranberry (n=34)

Variables	Mean ± SD	Min.	Max.	V [%]
Flavonols [mg%]	138.10 ± 47.5	57	298	34
Citric acid [%]	14.72 ± 3.22	8.57	21.32	22
Malic acid [%]	7.45 ± 2.91	2.18	14.24	39
Quinic acid [%]	5.78 ± 1.62	2.96	8.79	28
Sum of organic acids [%]	27.94 ± 3.76	21.20	36.86	13
Quinic/malic acid	1.03 ± 0.81	0.27	3.83	78

Flavonol content – expressed as quercetin equivalent; quinic/malic acid – the ratio of quinic to malic acid; SD – standard deviation; V – variability coefficient.

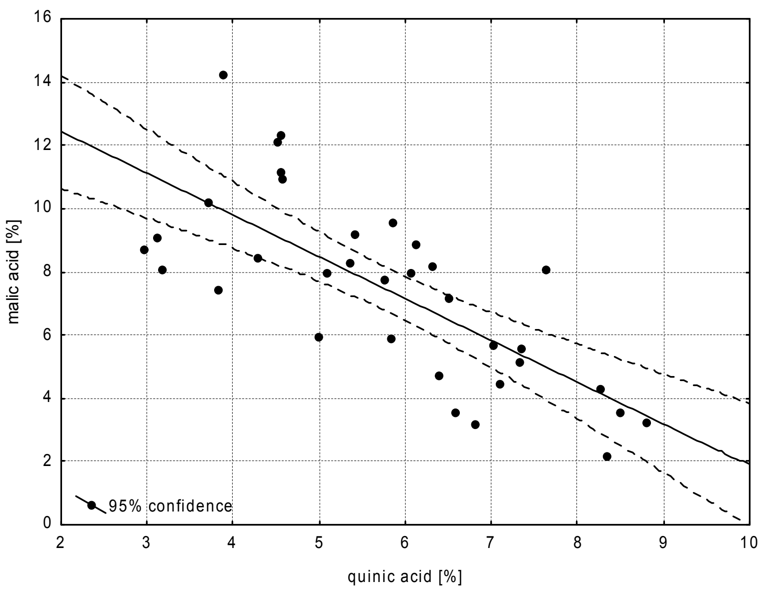

Figure 1.
Correlation between the malic and quinic acid content in freeze dried fruit of European cranberry Pearson's coefficient of correlation: –0.74; p=0.000; n=34.

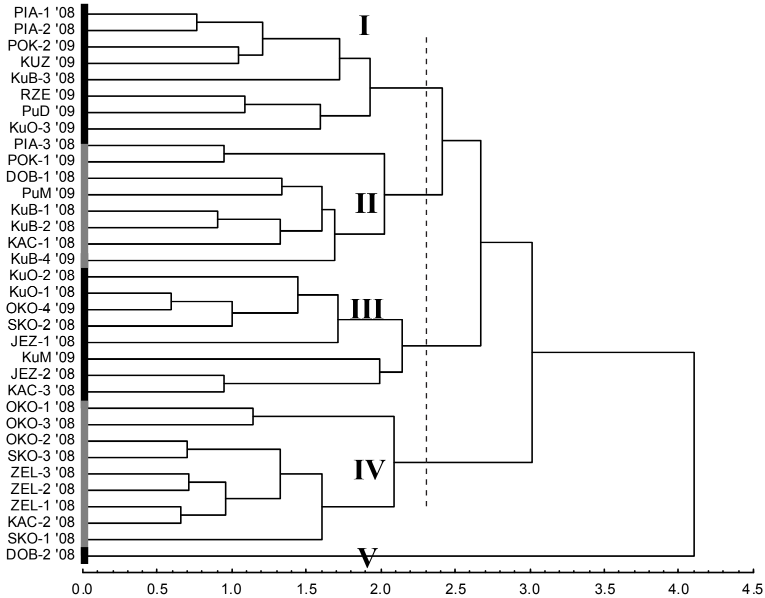

Figure 2.
The UPGMA cluster analysis based on Euclidean distance of the contents of flavonols and organic acids in fruit of European cranberry (n=136)
Abbreviations of the names of peatlands – like in table 1.

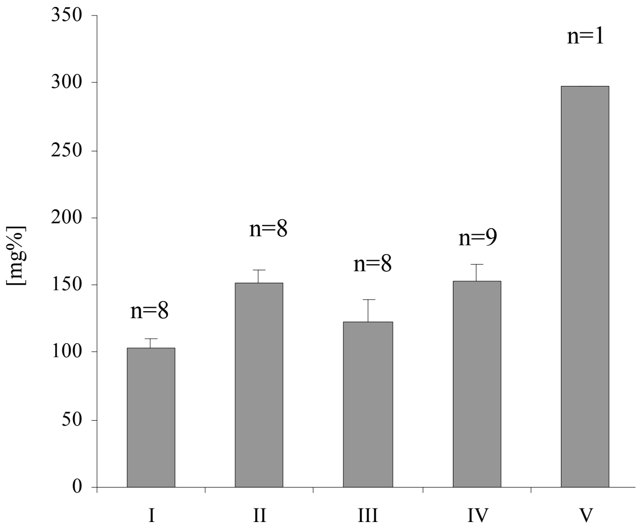

Figure 3.
Differentiation of the content of flavonols expressed as quercetin equivalent (mean ± SE) in sample groups of fruit of European cranberry
Kruskal-Wallis test: 13.10376, p=0.0108, n=34; except V group: 10.89732, p=0.0123, n=33.
Groups of samples – like in figure 2.

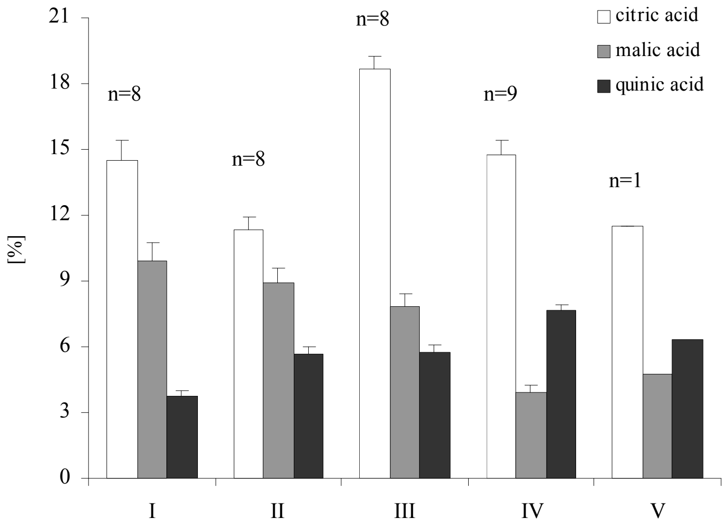

Figure 4.
Differentiation of the contents of the main organic acids (mean ± SE) in sample groups of fruit of European cranberry
Kruskal-Wallis test for citric acid: 22.87185, p=0.0001, n=34; malic acid: 22.32479, p=0.0002, n=34; quinic acid: 24.32437, p=0.0001, n=34. Groups of samples – like in figure 2.

DISCUSSION AND CONCLUSIONS

Wild berry fruits (cranberry, cowberry, bilberry, and others) are rich sources of phenolics and organic acids. They are characterized by a high content of flavonoids, including anthocyanins and flavonols [3, 22, 39]. Among the flavonols present in cranberry fruit, quercetin is predominant. Myricetin and kaempferol are found in much smaller amounts [15, 19, 31, 34].

The total flavonol content, as reported by different authors, ranges from 11.6 to 35.3 mg% of fresh matter [22, 28, 40] and, respectively, from 78 to 274 mg% of dry matter [6, 35, 41-42]. This is in agreement with the results presented in this article (tab. 2).

Comparative studies show that European cranberry contains less flavonols and a similar amount of organic acids, or even more, as compared to American cranberry [6, 33, 35]. The main organic acids in cranberry fruit include citric, malic, and quinic acids. In the studied samples of European cranberry, the citric acid content generally was distinctly higher than the amount of malic and quinic acids. In several cases, the level of malic acid was similar or even slightly higher than that for citric acid.

According to several authors, citric acid is the dominant organic acid in the fruit of European and American cranberry [6, 26]. Nevertheless, some data show that citric and quinic acids can co-dominate in cranberry fruit [33], or malic acid can predominate [43]. The monographic study of Watson [20] provides interesting results. According to this work, malic acid by far dominates in fresh fruit of American cranberry (64% of total), while citric acid (42%) and malic acid (41%) are predominants in frozen fruit. The above-cited author suggests that the metabolic processes taking place in frozen macerated berries cause these differences. Our previous study [44] shows that proportions of main organic acids in European cranberry fruit are similar in the lyophilized and thermally dried plant material. In both cases, citric acid is predominant; on average, it accounts for more than 50% of total acids under investigation.

Klein [45] reports that quinic acid content and the ratio of quinic acid to malic acid are reasonably constant and this fact is used to determine the percentage content of cranberry juice in beverages and to assess the authenticity of cranberry juices. However, the results obtained in European cranberry demonstrate large differences in quinic acid content in its fruit and high variations in the ratio of quinic acid to malic acid (tab. 2). A strong negative correlation was found between the above-mentioned acids (fig. 1).

To sum up, European cranberry fruit is characterized by high phytochemical variation in terms of both the content of flavonols and the content of main organic acids. This variability is unrelated to the geographical distribution of the studied species in the western Poland. On the other hand, the differences in ratio of quinic acid to malic acid indicate the possibility of occurrence of two chemotypes of cranberry, determined by genetically and (or) by environmental factors.

ACKNOWLEDGEMENTS

The authors would like to thank to employes of Department of Quality Control of Medicinal Products and Dietary Supplements, Institute of Natural Fibres and Medicinal Plants (Agnieszka Frąckowiak, Małgorzata Grześkowiak, Bogna Opala, Aurelia Pietrowiak) for performing of the phytochemical analysis. We would thank to dr Maciej Gąbka, Department of Hydrobiology and dr Paweł M. Owsianny, Department of Geomorphology (Adam Mickiewicz University) – for their felp in field work.

The study was supported by the Ministry of Science and Higher Education under a research project No. N405022 31/1319.

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ZRÓŻNICOWANIE ZAWARTOŚCI FLAWONOLI I GŁÓWNYCH KWASÓW ORGANICZNYCH W OWOCACH ŻURAWINY BŁOTNEJ (*OXYCOCCUS PALUSTRIS* PERS.) NA TORFOWISKACH PÓŁNOCNO-ZACHODNIEJ POLSKI

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S t r e s z c z e n i e

Niniejsza praca dokumentuje zróżnicowanie fitochemiczne żurawiny błotnej (*Oxycoccus palustris* Pers. = *Vaccinium oxycoccos* L.) na torfowiskach północno-zachodniej Polski. W badaniach wykorzystano 34 próby owoców zebranych w latach 2008–2009 na 15 stanowiskach w północnej Wielkopolsce i na Pomorzu Zachodnim. W liofilizowanym surowcu określono zawartość flawonoli w przeliczeniu na kwercetynę (metodą spektrofotometryczną) oraz ilość głównych kwasów organicznych: cytrynowego, jabłkowego i chinowego (metodą HPLC-DAD). Uzyskane wyniki wskazują na dużą zmienność fitochemiczną owoców żurawiny błotnej. Zawartość flawonoli wahała się w granicach od 57 do 298 mg% suchej masy. Analizowane kwasy organiczne stanowiły odpowiednio: 8,57–21,32% (kwas cytrynowy), 2,18–14,24% (kwas jabłkowy) oraz 2,96–8,79% (kwas chinowy) suchej masy owoców. Duże zróżnicowanie występowało także w proporcji kwasu chinowego do jabłkowego (od 0,27 do 3,83). Między wymienionymi kwasami stwierdzono silną ujemną korelację (r= -0,74; p=0,000). Wskazuje to na możliwość występowania dwóch chemotypów żurawiny błotnej, różniących się zawartością kwasu chinowego i jabłkowego.

Słowa kluczowe: *Oxycoccus palustris*, *rośliny lecznicze*, *zmienność fitochemiczna*, *kwasy organiczne*, *flawonoidy*