Phytochemical Compounds and Antioxidant Activity in Different Cultivars of Cranberry (*Vaccinium Macrocarpon* L)

Jan Oszmiański, Joanna Kolniak-Ostek, Sabina Lachowicz, Józef Gorzelany, and Natalia Matłok

Abstract: Cranberries can be a component of a healthy diet, because they are a great source of health-promoting compounds and nutrients. The aims of this study were to evaluated phytochemicals and antioxidant activity in 6 cultivars of cranberry fruit grown in Poland. The content of polyphenols, carotenoids, chlorophylls, and triterpenoids were determined with the use of UPLC-PDA-MS/MS, although antioxidant activity was examined with DPPH, ABTS, and FRAP assays. The cvs. "Franklin," "Howes," and "Stevens" were characterized by the highest concentration of total polyphenols (4219, 3995, and 3584 mg/100 g dm), triterpenoids (3582, 3671, and 3451 mg/kg dm), carotenoids (9.75, 8.52, and 7.94 mg/kg dm), and antioxidant activity (ABTS: 226, 264, 246; FRAP: 102, 139, 124; DPPH: 235, 320, 284 μmolTE/g dm), making these 3 cultivars especially recommendable for consumption. Furthermore, a positive correlation between content of phytochemicals and antioxidant activity was found.

Keywords: antioxidant properties, carotenoids, chlorophylls, triterpenoids, polyphenols

Practical Application: The manuscript "Phytochemical compounds and antioxidant activity in different cultivars of cranberry (*Vaccinium macrocarpon* L)" represents cultivars commonly grown in Poland that maybe beneficial offer the food industry, to develop attractive foods with a high content of biologically active substances.

Introduction

Cranberry (*Vaccinium macrocarpon* L.) is an evergreen, perennial shrub of the heath family *Ericaceae* and of genus *Vaccinium*. It is native to wetlands in eastern parts of North America, hence it is also commonly known as American cranberry. Today the plant is not only grown in North America but also in some regions of South America and in Eastern Europe, including Poland (Reid and others 2001; McKenna and others 2002). There are approximately 200 varieties of the plant and they mainly differ in terms of shape, size, color of fruit, appearance of leaves, and fertility. Other variety specific properties include tolerance to pests and diseases, sensitivity to frost, and usefulness for processing and storability. The most widely grown cranberry crops are cvs "Ben Lear," "Stevens," and "Pilgrim" (Hołderna-Kędzia 2006).

Cranberry, fresh, and dried, are a great source of vitamins (provitamine A, C, B1, B2, and B3), organic acids, mineral salts (potassium, sodium, calcium, phosphorus, magnesium, and iron rod), sugars (glucose constituting 3.1% and fructose 1%), dietary fiber, and pectins (McKenna and others 2002; Hołderna-Kędzia 2006). Cranberries are particularly rich in bioactive compounds for a healthy diet, especially phenolic compounds, triterpenoids, and carotenoids. Besides, these compounds are responsible for qualitative properties of raw and processed products. They mainly impact tartness, taste, flavor, color, and texture. Moreover, seems cranberries promising in prevent ion of urinary tract infections (Howell and others 2010; Kim and others 2012), reducing the risk of cardiovascular diseases (Ruel and others 2005), atopic dermatitis, Alzheimer's disease, macular degeneration, neurodegenerative diseases and diabetes (Krishnaiah and others 2011; Hannoufa and Hosain 2012), and show anti-mutagenic, antiinflammatory, antibacterial, properties (Vattem and others 2006; Mueller and others 2013).

Furthermore, recent research has shown that the cranberry cultivar may substantially influence total phytochemical compounds, such as color, pH, dry matter, organic acids, sugars, fibers, polyphenols, carotenoids, triterpenoids, and antioxidant activity (Ercisli and others 2012; Hannoufa and Hossain 2012). This information will help select cultivars of cranberries with high nutritional value.

Therefore, the aim of this study was to examine and compare phytochemicals composition and antioxidant activity (ABTS, FRAP) in 6 different cultivars of cranberry grown in Poland. An additional aim of this study was to selected cultivars with high nutritional value. Characterization of triterpenoids, carotenoids, and chlorophylls with the use of LC–MS–QTof and UPLC–PDA–FL was done for the 1st time in the scientific literature of cranberry fruit.

Materials and Methods

Reagents and standards

Acetonitrile, formic acid, methanol, all-*trans*-β-carotene, α-carotene, all-*trans*-lutein, neoxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, chlorophyll *a*, betulinic, oleanolic, and ursolic acid, ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), methanol acetic

JFDS-2017-0688 Submitted 4/27/2017, Accepted 8/23/2017. *Authors Oszmiański, Kolniak-Ostek, and Lachowicz are with Dept. of Fruit, Vegetable and Plant Nutraceutical Technology, Wroclaw Univ. of Environmental and Life Science, 37 Chełmońskiego Street, 51–630 Wroclaw, Poland. Authors Gorzelany and Matłok are with Dept. of Agro and Food Production Engineering, Rzeszow Univ. Biology and Agriculture, 4 Zelwerowicza Street, 35–601 Rzeszów, Poland. Direct inquiries to author Lachowicz (E-mail: sabina.lachowicz@upwr.edu.pl).*

doi: 10.1111/1750-3841.13924

Journal of Food Science A Publication of the Institute of Food Technologists

Table 1–Mean temperatures and total precipitation, per quarter and annually during 2016 at the plantation of Radomyśl nad Sanem.

Period	Mean temperature (°C)	Total precipitation (mm)
January to March	2.7	80.0
April to June	12.7	120.0
July to September	19.0	150.0
Annually	11.4[a]	350.0

[a]For the period up until SEPTEMBER.

acid, and phloroglucinol were purchased from Sigma-Aldrich (Steinheim, Germany). (−)-Epicatechin, (+)-catechin, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, di-caffeic quinic acid, procyanidin B2, p-coumaric acid, myricetin, isoquercitrin, caffeic acid, cyanidin-3-*O*-galactoside, and cyanidin-3-*O*-glucoside were purchased from Extrasynthese (Lyon, France). Acetonitrile for ultra-phase liquid chromatography (UPLC; Gradient grade) and ascorbic acid were from Merck (Darmstadt, Germany).

Plant materials

Six cranberry (*Vaccinium macrocarpon* L.) cvs. "Ben Lear," "Franklin," "Howes," "Pilgrim," "Red Star," and "Stevens," were used in this study. Cranberry fruits were obtained from a horticultural farm in Nowiny, commune of Radomyśl nad Sanem, Podkarpackie Region, Poland (N: 50O41′59″–50O42′5″ E: 21O55′21″–21O55′8″). Soilless culture of cranberry was conducted on a substrate of pure quartz sand, where it was possible to regulate water and fertilizer ratios. The examined cultivars were grown in 1-ha beds with dykes for flooding with water during harvest and in winter. In 2016, from 24 March to 28 August in the course of 7 procedures the following fertilizers were applied: Wigor S, triple superphosphate, potassium sulfate, Polimag S, ammonium sulfate, magnesium sulfate (7–hydrate). In July 2016, Dursban was used as a treatment for May bug larvae, in an amount of 2.5 kg/ha. In each cultivar, samples of 2 kg were collected manually, using an identical method. Harvesting time: 8 to 20 September. The total amount of fertilizers administered to the plants in 2016 was: sodium 75.5 kg, phosphorus 132 kg, potassium 185 kg, magnesium 52.5 kg, sulfur 512.85 kg per 1 ha of land. The mean temperatures and total precipitation, per quarter and annually during 2016 at the palntation of Radomyśl and Sanem showed Table 1.

The raw material (∼2.0 kg each) was collected at the optimum ripening stage recommended for consumption. Next, the samples were frozen and dried using an Alpha 1–4 LSC freeze dryer (Christ, Osterode, Germany). The homogeneous dry material was obtained by crushing the dried tissues using a closed laboratory mill (IKA A.11, Germany). The powders were kept in a refrigerator (−80 °C) until extract preparation. The basic parameters of the chemical composition—dry matter (PN-90/A-75101/03), total soluble solids (PN-90/A-75101.07), vitamin C (PN-A-04019:1998), pectin, and acidity (PN-90/A-75101/04)—were determined in fresh fruit according to Polish standards. Results were reported as the arithmetic mean of 3 independent repetitions ($n = 3$), taking into account the standard deviation (SD).

Total soluble solids, pH, and total titratable acidity

Total soluble solids (TSS) were measured with a digital Atago refractometer (N-20 model; Atago, Bellevue, Wa., U.S.A.) at 20 °C with values being expressed as °Brix. The titratable acidity (TA) and pH were determined by acid–base potentiometer (877 Titrino plus; Metrohm ion analyses CH9101, Herisau, Switzerland), using 0.1 N NaOH up to pH 8.1; values were expressed as g citric acid/L. Analyses were made in 3 replications ($n = 3$).

Extraction procedure

The samples of cranberry (2 g) were extracted with 50 mL of methanol acidified with 2.0% formic acid. The extraction was performed twice on the same cultivars of cranberry by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland) and with occasional shaking. Next, the slurry was centrifuged at 19000 *g* for 10 min, and the supernatant was filtered through a Hydropilic PTFE 0.20 μm membrane (Millex Simplicity Filter, Merck) and used for analysis. The content of polyphenols in individual extracts was determined by means of the ultra-performance liquid chromatography-photodiode array detector-mass spectrometry (UPLC−PDA−MS) method. All extractions were carried out in triplicate ($n = 3$).

Identification and quantification of polyphenols by the UPLC−PDA−MS method

Identification and quantification of polyphenols of cranberry extracts was carried out using LC/MS Q-TOF and quantitative UPLC–PDA–FL analysis of polyphenols (anthocyanin, flavan-3-ol, flavonol, and phenolic acid) was performed as described previously by Oszmiański and others (2015). All measurements were repeated 3 times ($n = 3$). The results were expressed as mg per 100 g dry matter (dm).

Analysis of proanthocyanidins by phloroglucinolysis method

An analysis of polymeric procyanidins by phloroglucinolysis method was performed according to the protocol described previously by Oszmiański and others (2015). All measurements were repeated 3 times ($n = 3$). The results were expressed as mg per 100 g dm.

Identification and quantification of triterpenoids

Sample extraction was performed as described previously by Farneti and others (2015). The column used was a UPLC BEH C18 column (1.7 μm, 2.1 × 150 mm, Waters Corp., Milford, Mass., U.S.A.). The elution solvents were 100% methanol (A) and 100% acetonitrile (B; 15:85, v/v). Ursolic, oleanolic, and betulinic acids were eluted isocratically at a flow rate of 0.1 mL/min for 10 min at 20 °C. All incubations were done in triplicate ($n = 3$). The results were expressed as mg per kg of dm.

Identification and quantification of carotenoids and chlorophylls

Sample extraction was performed as described previously by Lin and Chen 2003. Compounds were separated with the ACQUITY UPLC BEH RP C18 column (1.7 μm, 2.1 × 100 mm, Waters Corp., Milford, Mass., U.S.A.) at 32 °C. The elution solvents were ACN:MeOH (7:3, v/v) (A) and 0.1% formic acid (B). Samples (10 μL) were eluted according to the linear gradient described by Delphino-Rius and others (2014). The runs were monitored at 450 and 650 nm. The PDA spectra were measured over the wavelength range of 200 to 700 nm in steps of 2 nm. The retention times and spectra were compared to those of the authentic standards. All incubations were done in triplicate. The results were expressed as mg per kg of dm.

Determination of antioxidant activity

A solvent for the extraction was prepared as described previously by Lachowicz and others (2017). The DPPH, ABTS, and FRAP assays were prepared as previously described by Yen and Chen (1995), Re and others (1999), and Benzie and Strain (1996), respectively. The antioxidant activity was expressed as mMol of Trolox per 100 g DW. Measurements by means of DPPH, ABTS, and FRAP methods involved use of a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan).

Analysis of sugar by the HPLC-ELSD method

An analysis of sugar by the HPLC–ELSD method was performed according to the protocol described previously by Oszmiański and others (2015). All measurements were repeated 3 times. The results were expressed as mg per 100 g dm.

Statistical analysis

Statistical analysis, 1-way ANOVA, and principal component analysis (PCA) were conducted using Statistica version 12.5 (StatSoft, Kraków, Poland). Significant differences ($P \leq 0.05$) between mean values were evaluated by one-way ANOVA and Duncan's multiple range test.

Results and Discussions

Basic chemical composition

The total dry matter, total acidity, pectins, ascorbic acid, pH, total sugar in 6 cultivars of cranberry are presented inTable 2. Contents of dry matter in the 6 cultivars of *Vaccinium macrocarpon* ranged from 10.06% in cv. "Ben Lear" to 13.34% in cv. "Howes." The examined samples of cranberry were characterized by low acidity from 2.35 to 1.95 g/100 g in fresh matter (fm). The highest and the lowest acidity was observed in cvs. "Red Star" and "Pilgrim," respectively. The findings show that cranberries are a good source of vitamin C. The highest concentrations of vitamin C, which are important health-promoting compounds, was identified in cv. "Pilgrim" (20.74 mg/100 g fm), and the lowest content was found in "Red Star" (10.07 mg/100 g fm). The content of Vitamin C in fresh fruit, identified by McKay and Blumberg (2007) amounted to 12.6 mg fm. According to Mazur and others (2009), the concentration of Vitamin C in cvs. "Ben Lear," "Stevens," and "Pilgrim" of cranberry was 1.2, 1.4-time higher, and 1.7-time lower than the same cultivars grown in horticultural farm in Poland. The examined cranberries were found with the contents of pectins at the level ranging from 0.75% in cv. "Red Star" to 1.11% in cv. "Franklin." Pectins are a soluble fraction of dietary fiber, beneficially impacting metabolic, and physiological processes of the organism (Nawirska and Kwaśniewska 2004). It has been established (Jackson and others 2007) that pectins show anti-neoplastic effects, and play important biological functions in the development and growth of plants and in protecting them against pathogens. The findings acquired by Popov and others (2006) during experiments involving mice with induced colitis showed that soluble fraction of dietary fiber extracted from cranberry produced antiinflammatory effects. The contents of sugar in different cultivars of cranberries ranged from 3.83 in cv. "Howes" to 4.82 mg/100 g fm in cv. "Red Star." The glucose was the predominant sugar in cranberry and ranged from 3.36 in cv. "Howes" to 4.72 mg/100 g fm in cv. "Pilgrim." According Teleszko (2011) the total sugars in cv. "Ben Lear" of *Vaccinium macrocarpon,* was 1.4-time higher than "Ben Lear" grown in horticultural farm in Poland.

Polyphenol contents in cranberry fruit

Polyphenols are the most important bioactive compounds in cranberries. It was shown that the contents and profiles of these compounds were significantly different by cultivar-specific traits (Table 3).

The higher contents of total phenolic compounds (TPC) were observed in cv. "Franklin" and "Howes" (4219 and 3995 mg/100 g dm, respectively) and the lower value was in cv. "Red Star" (2628 mg/100 g dm). The TPC in pomace cv. "Pilgrim" of cranberry was 3.0-time lower than fruit cv. "Pilgrim" grown in horticultural farm in Poland (Oszmiański and others 2015).

The higher concentration of total anthocyanins (TA), was identified in cv. "Franklin," and the lowest in cv. "Red Star" (1716 and 695 mg/100 g dm, respectively). According Viskelis and others (2009) the TA in cv. "Stevens," "Pilgrim," "Ben Lear" of cranberry grown in America were 10.0, 9.0, and 5.0-times lower than cvs. "Stevens," "Pilgrim,"" "Ben Lear" grown in horticultural farm in Poland. Similar results obtained by Wang and Stretch (2001). The contents of TA in cvs. "Pilgrim," "Ben Lear," "Franklin," "Howes," and "Stevens" of cranberry from America were 4.0, 2.4, 2.8, 3.4, and 3.5-times lower than the same cultivars grown in horticultural farm in Poland.

The content of phenolic acids (PA) ranged from 649 to 327 mg/100 g dm for the cv. "Howes," "Pilgrim," respectively (Table 3). According to White and others (2010), the mean content of PA in pomace of cranberry was 358 mg/100 g dm and this quantity was similar to the value determined in this study in the case of "Red Star." The study by Oszmiański and others (2015) showed the content of PA in pomace cv. "Pilgrim" of cranberry was 13% higher than cv. "Pilgrim" grown in horticultural farm in Poland. These differences may be linked with varied weather conditions, sunlight, harvesting time, and place. The obtained result was similar to the value identified in the case of cv. "Franklin." The next group of compounds were flavonols (F); their contents were the highest in cv. "Franklin" (1088 mg/100 g dm) and the lowest in cv. "Pilgrim" (643 mg/100 g dm; Table 3). White and others (2010) reported the contents of *F* in cranberry pomace to be at the level of 358 mg/100 g dm. This quantity is 1.8-time lower than in the findings related to cv. "Pilgrim."

The last group of compounds identified in cranberries were flavan-3-ols (F3o). The contents of F3o ranged from 1283 in cv. "Howes," to 860 mg/100 g dm in cv. "Red Star" (Table 3). A study carried out by White and others (2010) showed the contents of procyanidins in cranberry pomace at the level of 167 mg/100 g dm. Prior and others (2010), demonstrated that the contents of procyanidins in commercially available cranberry powders ranged from 0.63 to 177 mg /100 g dm.

Carotenoid and chlorophyll contents in cranberry fruit

Examination of the relevant cultivars of large cranberry was carried out using high performance liquid chromatography LC-PDA-ESI-MS/MS (Table 4). Among the carotenoids group, all-trans -lutein and 1, 3-*cis*-lutein with [M + H]+ at $m/z = 569$ at wavelengths characteristic for this compound $\lambda = 431$ and 662, was found and identified compared to the standard. Also all-trans-β-carotene, 9-*cis*-β-carotene with [M + H] + at $m/z = 537$ and $\lambda = 462$ and 648 was determined in all cultivars of cranberry. Besides carotenoids, also 4 compounds belonging to the group of chlorophylls were detected. The main of one was chlorophyll *b* (with [M + H] + at $m/z = 901$). In addition, metabolites of the previously mentioned compounds were identified as pheophytin a ([M + H] + at $m/z = 872$), and pheophytin *b* ([M +

H] + at $m/z = 886$). Furthermore, carotenoids and chlorophylls were detected as well, the content of which in apple fruit and Amazonian fruit was confirmed by other authors (de Rosso and Mercadante 2007; Delgado-Pelayo and others 2014). The presence of carotenoids and chlorophyll pigments has not been investigated in the earlier conducted studies on the chemical composition of cranberry fruit. Therefore, in our study, the individual carotenoids and chlorophylls present in all cultivars of cranberry fruit have been identified for the 1st time ever.

The main carotenoids was all-trans-β-carotene (ranged from 86% to 89%) followed by 9-*cis*-β-carotene, all-trans-lutein and 13-*cis*-lutein. The lower contents of the compounds were observed in cv. "Red Star" (5.80 mg/kg dm), and the higher contents was in cv. "Howes" (9.75 mg/kg dm; Table 4). Pappas and Schaich (2009) reported that cranberries contained lutein (0.28 to 0.2 mg/kg) and β-carotene (0.2 to 2.6 mg/kg). The contents identified by Pop and others (2014), depending on the variety of sea buckthorn berry, ranged from 96.7 mg/100 g dm in cv. "Serbanesti" to 53.1 mg/100 g dm in cv. "Serpenta." A study by Pertuzatti and others (2015) showed that passion fruit grown in organic and conventional cultivation systems had different contents of carotenoids, at the level of 13.99 mg/100 g dm, and 25.10 mg/100 g dm, and were 2.3 and 2.5-times higher than cvs. "Red Star" and "Howes" of cranberry. A study carried out by Carvalho and others (2013), assessing 6 raspberry cultivars showed that the contents of lutein ranged from 1.14 in "Tulameen" to 2.08 mg/100 g in cvs. "Alpen Gold" and

Table 2–Major chemical composition of 6 cultivars of cranberry fruit.

	Cultivars					
Chemical compounds	**Ben Lear**	**Red Star**	**Howes**	**Franklin**	**Pilgrim**	**Stevens**
Dry substance (%)	10.06 ± 0.05e	12.34 ± 0.07d	13.34 ± 0.08a	12.62 ± 0.07c	12.79 ± 0.07b	12.52 ± 0.07c
Total acidity (g/100 g fm)[a]	2.29 ± 0.1b	2.35 ± 0.04a	2.20 ± 0.03c	2.13 ± 0.03d	1.95 ± 0.02e	2.25 ± 0.04c
pH	3.02 ± 0.03a	2.81 ± 0.02b	2.767 ± 0.01c	2.74 ± 0.01d	2.78 ± 0.01c	2.61 ± 0.01e
Extract (°Brix)	9.90 ± 0.07c	9.30 ± 0.09e	10.20 ± 0.02a	9.60 ± 0.05d	10.00 ± 0.02b	9.50 ± 0.07d
Pectins (%)	1.09 ± 0.01b	0.75 ± 0.01e	1.04 ± 0.02c	1.11 ± 0.02a	1.09 ± 0.03b	0.91 ± 0.01d
Vitamin C (mg/100 g fm)	14.19 ± 0.12e	10.07 ± 0.20f	18.99 ± 0.09c	19.31 ± 0.09b	20.74 ± 0.24a	18.01 ± 0.11d
Fructose (g/100 g fm)	0.65 ± 0.04a	0.37 ± 0.03e	0.46 ± 0.03c	0.38 ± 0.03e	0.51 ± 0.04b	0.42 ± 0.02d
Glucose (g/100 g fm)	3.41 ± 0.06e	4.46 ± 0.07b	3.36 ± 0.04f	3.83 ± 0.08c	4.72 ± 0.03a	3.65 ± 0.04d
Total sugar	4.06 ± 0.08d	4.82 ± 0.05a	3.83 ± 0.06e	4.21 ± 0.03c	4.79 ± 0.06b	4.08 ± 0.03d

[a]fm, fresh matter.
a–c, Means ± SD followed by different letters within the same line represent significant differences ($P < 0.05$). Data are the averages of triplicates.

Table 3–Polyphenol contents [mg/100 g dm[a]] and antioxidant activity [μmol TE/g dm[a]] in the different cultivar cranberry fruit.

	Ben Lear	**Red Star**	**Howes**	**Franklin**	**Pilgrim**	**Stevens**
Anthocyanins						
Delfinidyn derivatives	31.27 ± 0.25d	42.00 ± 0.33b	43.87 ± 0.35a	40.77 ± 0.32c	40.16 ± 0.32c	41.60 ± 0.29bc
Cyanidin derivatives	442 ± 3e	759 ± 5b	967 ± 5a	668 ± 5c	604 ± 4d	757 ± 5b
Peonidin derivatives	192 ± 1d	311 ± 2cd	666 ± 5a	496 ± 3b	392 ± 3c	308 ± 2cd
Malwidin derivatives	29.85 ± 0.25c	39.58 ± 0.31b	38.72 ± 0.39b	35.65 ± 0.28bc	58.85 ± 0.47a	39.56 ± 0.31b
Total anthocyanins	1147 ± 4c	695 ± 3e	1152 ± 3c	1716 ± 3a	1241 ± 3b	1095 ± 4d
Phenolic acid						
p-Coumaroyl derivatives	227 ± 1d	451 ± 3a	256 ± 2c	210 ± 1e	350 ± 2b	265 ± 2c
Chlorogenic acid	72.00 ± 0.57d	129.62 ± 1.03a	75.38 ± 0.63cd	72.88 ± 0.58d	85.82 ± 0.67b	79.42 ± 0.62c
Caffeoyl and derivatives	42.83 ± 0.34c	68.28 ± 0.54a	45.55 ± 0.36c	43.76 ± 0.35c	54.28 ± 0.41b	39.93 ± 0.31d
Total phenolic acid	385 ± 2c	342 ± 1e	649 ± 3a	377 ± 2d	327 ± 2f	491 ± 2b
Flavonols						
Sinapoyl derivatives	4.66 ± 0.03c	5.82 ± 0.04a	5.06 ± 0.04ab	4.36 ± 0.03c	5.73 ± 0.05b	5.01 ± 0.04ab
Myricetinderivatives	556 ± 4c	642 ± 5b	926 ± 5a	496 ± 3d	618 ± 4bc	635 ± 3bc
Quercetin derivatives	137 ± 1d	221 ± 1ab	117 ± 1e	107 ± 1ef	151 ± 1c	225 ± 1a
Metoxyquercetin derivatives	33.31 ± 0.27d	42.02 ± 0.33a	40.10 ± 0.32b	35.21 ± 0.28c	42.00 ± 0.33a	43.04 ± 0.34a
Total flavonols	908 ± 3 b	731 ± 2d	911 ± 2b	1088 ± 2a	643 ± 2e	817 ± 3c
Flavan-3-ols and procyanidins						
(+)-Catechin	3.65 ± 0.02c	3.49 ± 0.02cd	2.79 ± 0.02d	5.65 ± 0.04b	7.53 ± 0.06a	5.89 ± 0.04b
B-typePA-dimer	12.62 ± 0.10e	22.27 ± 0.17c	16.75 ± 0.13d	12.62 ± 0.10e	32.67 ± 0.26b	36.75 ± 0.29a
(−)-Epicatechin	38.90 ± 0.31c	37.44 ± 0.29cd	27.46 ± 0.21d	49.07 ± 0.39b	56.84 ± 0.45a	49.73 ± 0.39b
A-typePA-dimer	17.80 ± 0.14c	16.94 ± 0.13cd	28.62 ± 0.22b	17.80 ± 0.14c	16.94 ± 0.13cd	32.07 ± 0.25a
A-typePA-trimer	76.94 ± 0.61b	27.82 ± 0.22f	38.78 ± 0.31d	76.04 ± 0.60a	43.82 ± 0.35c	31.06 ± 0.21e
A-typePA-tetramer	59.09 ± 0.47c	65.61 ± 0.52a	48.51 ± 0.38d	59.09 ± 0.47c	63.61 ± 0.50b	41.51 ± 0.33e
Polymeric procyanidins	651 ± 5f	1109 ± 8a	875 ± 5d	751 ± 6e	959 ± 7b	901 ± 5c
Total flavan-3-ols and procyanidins	1098 ± 2c	860 ± 1f	1283 ± 2a	1038 ± 3d	971 ± 2e	1181 ± 2b
TPC[b]	3538 ± 28c	2629 ± 21e	3995 ± 31b	4219 ± 33a	3181 ± 25d	3584 ± 28c
DPPH[c]	234 ± 0.0c	239 ± 0c	235 ± 0c	320 ± 0a	214 ± 0d	284 ± 0b
ABTS[c]	218 ± 0d	245 ± 0b	226 ± 0c	264 ± 0a	189 ± 0e	246 ± 0b
FRAP[c]	102 ± 0c	104 ± 0c	102 ± 0c	139 ± 0a	93.25 ± 0.00 d	124 ± 0b

Means ± SD followed by different letters (a–e) within the same line represent significant differences ($P < 0.05$). Data are the averages of triplicates.
[a]DM, dry matter; TE, Trolox equivalents.
[b]TPC, total phenolic compounds.
[c]DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid; FRAP, ferric reducing antioxidant power.

Table 4–Carotenoid, chlorophylls and triterpenoids composition of different cultivar of cranberry fruit (mg/kg dm).[a]

	tR (min)	λ (nm)	[M+H]+ (*m/z*)		Ben Lear	Red Star	Howes	Franklin	Pilgrim	Stevens
			MS	MS/MS						
All *trans* lutein	5.21	267, 445, 474	568	551/553	0.76 ± 0.02d	0.76 ± 0.01d	0.89 ± 0.02a	0.81 ± 0.02c	0.84 ± 0.06b	0.66 ± 0.03e
13 *cis*-lutein	5.85	335, 420,446, 474	568	551/553	0.03 ± 0.00b	0.03 ± 0.00b	0.04 ± 0.00a	0.04 ± 0.00a	0.03 ± 0.00b	0.03 ± 0.00b
all-*trans* β-carotene	8.21	452, 479	537	445, 203, 177, 149, 137,	6.58 ± 0.07c	4.42 ± 0.06f	7.80 ± 0.08a	6.64 ± 0.05b	6.32 ± 0.07d	6.27 ± 0.08e
9-*cis* β-carotene	8.23	345, 447, 475	537	444,430, 269	1.04 ± 0.04a	0.59 ± 0.01e	1.02 ± 0.04b	1.02 ± 0.04b	0.87 ± 0.02d	0.99 ± 0.03c
Total					8.41 ± 0.05	5.80 ± 0.06	9.75 ± 0.08	8.52 ± 0.07	8.06 ± 0.08	7.94 ± 0.06
Chlorophyll *b*	7.30	462, 648	907	687,629,597, 571,569,533	0.25 ± 0.00d	0.51 ± 0.01c	0.53 ± 0.01b	0.71 ± 0.02a	0.16 ± 0.00f	0.18 ± 0.00e
Pheophytin *b*	8.42	430, 653	886	607,	2.19 ± 0.04b	1.71 ± 0.03d	1.69 ± 0.03d	1.53 ± 0.03e	2.62 ± 0.05a	1.75 ± 0.02c
Pheophytin *a*	8.80	408, 666	872	593, 533	3.30 ± 0.05c	2.99 ± 0.04e	3.80 ± 0.06a	3.29 ± 0.03c	3.54 ± 0.04b	3.21 ± 0.03d
Total					5.74 ± 0.04c	5.21 ± 0.03e	6.02 ± 0.05b	5.52 ± 0.04d	6.33 ± 0.04a	5.14 ± 0.03f
Betulinic acid	6.89		455		638 ± 2d	635 ± 2d	753 ± 2c	820 ± 2a	824 ± 2a	808 ± 1b
Oleanolic acid	7.11		455		894 ± 2d	1056 ± 3b	1070 ± 3b	1137 ± 3a	1025 ± 2b	980 ± 2c
Ursolic acid	7.59		455		1530 ± 3c	1647 ± 2b	1759 ± 2a	1714 ± 2a	1044 ± 2d	1663 ± 2b
TTC[b]					3061 ± 459d	3338 ± 121c	3582 ± 514b	3671 ± 452a	2892 ± 508e	3451 ± 451b

[a]Values are means ± standard deviation. $n = 3$. Mean values within a row with different letters are significantly different at $P < 0.05$.
[b]TTC, total triterpenoids compounds.

were 5.0 and 4.6-lower than cvs. "Red Star" and "Howes" of cranberry. In general, the average content of the carotenoids in cranberry cultivars were 49% higher than the raspberry fruit (Carvalho and others 2013) and 81% and 80% lower than saskatoon berry and buckthorn berry, respectively (Pop and others 2014; Lachowicz and others 2017).

The lowest contents of chlorophyll were observed in "Red Star" (5.21 mg/kg dm), and the highest in "Pilgrim" cultivar (6.33 mg/kg dm). The main chlorophylls were pheophytin a (ranged from 27% to 41%) followed by pheophytin b and chlorophyll b. The content of chlorophyll green apples (162 to 1359 mg/kg dm) differed significantly ($P < 0.05$) from the content of cranberry fruit (Delgado-Pelayo and others 2014). The cranberry cultivars were characterized by low contents of chlorophyll, as opposed to the high contents of anthocyanins, as reflected by the predominance of red in comparison to green color.

Triterpenoids contents in cranberry fruit

The relevant cultivars of cranberry fruit were examined for the presence of triterpenoids with the use of high performance liquid chromatography LC-PDA-ESI-MS/MS (Table 4). The detected compounds were identified as oleanolic, betulinic, and ursolic acids based on their molecular ion [M−H]− at *m/z* 455. The triterpenoid compounds identified by MS profile with fragmentation pathways, the retention times and UV spectra of authentic standards Betulinic, oleanolic, and ursolic acid have not been determined in cranberry fruit.

Betulinic acid, oleanolic acid, and ursolic acid were identified in the fruit. The higher total contents of triterpenoids (TTC), were found in cv. "Franklin" (3671 mg/kg dm) and the lower was in cv. "Pilgrim" (2892 mg/kg dm). In all investigated cultivars, ursolic acid was the major triterpenoid (ranged from 50% to 37%), then oleanolic acid (ranged from 28% to 35%), and betulinic acid (ranged from 19% to 28%). The contents of specific acids varied between the cultivars. In all investigated cultivars of cranberry fruit, only cv. "Pilgrim" showed a balanced percent ratio of oleanolic and ursolic acid (of 35% to 37%). Kondo (2006) reported the contents of ursolic acid in cranberry to range from 60 to 110 mg/100 g fm. According to Szakiel and others (2012), the ursolic acid as the predominant triterpenoids were present in the cranberry (20% of all wax extract), apple (98%), and sweet cherry (60%). McKenna and others (2002) a study of cranberry pomace and wax coat showed the presence of polymeric terpenes belonging to the group of phytosterols (β-sitosterol and stigmasterol).

Antioxidant activity of cranberry fruit

Analysis of antioxidant activity (AA), identified in the cranberry cultivars with the use of ABTS, DPPH, and FRAP assays, showed significant differences (Table 3). The results were presented in μmol TE/g dm. The highest AA were identified by ABTS, DPPH and FRAP assays in cv. "Franklin," that is, 264, 320, and 139 μmol TE/g dm, respectively. The lowest ABTS, DPPH, and FRAP potentials were observed in cv. "Pilgrim," that is, 189, 214, and 93.25 μmol TE/g dm, respectively.

A study published by Oszmiański and others (2015) presented that the AA (ABTS, DPPH, and FRAP assays) in cv. "Pilgrim" of cranberry fruit was at the level of 145 and 63.68 μmol TE/g dm, and was 1.1-time higher and 1.5 and 1.6-times lower than cv. "Pilgrim" grown in horticultural farm in Poland. Paredes-López and others (2010) reported the value of AA in DPPH in examined blackberries and raspberries ranging from 25.3 to 35.5 μmol TE/g and was 8.0 and 6.0-time lower than cv. "Pilgrim" of cranberry

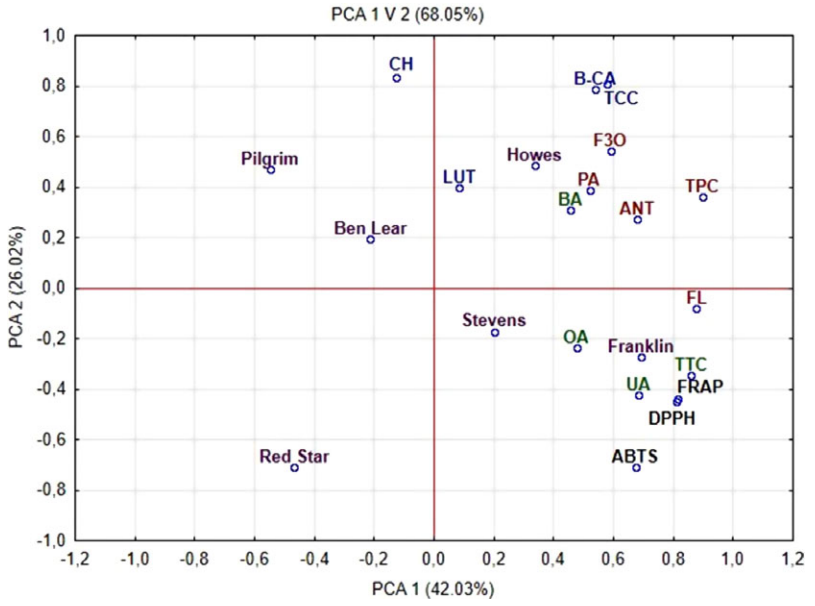

Figure 1–PCA mam showing the relationship among phytochemical compounds and antioxidant activity of 6 cultivars of cranberry. Abbreviations: UA, ursolic acid; OA, oleanolic acid; BA, betulinic acid; TTC, total triterpenoids compounds; FL, flavonols; ANT, anthocyanins; PA, phenolic acid; F3o, falavan-3-ols; TPC, total phenolic compounds; B-CA, β-caroten; LUT, lutein; TCC, total carotenoids compounds; CH, chlorophylls.

Table 5–Correlation between bioactive compounds and antioxidant activity

Compounds	DPPH	ABTS	FRAP
Betulinic acid	0.430	0.429	0.436
Oleanolic acid	0.447	0.457	0.454
Ursolic acid	0.559	0.833	0.553
Total triterpenoids	0.737	0.852	0.736
Chlorophylls	0.570	0.806	0.558
Lutein	−0.302	−0.331	−0.290
β-Caroten	0.112	0.168	0.119
Total carotenoids	0.089	0.184	0.097
Anthocyanins	0.602	0.675	0.614
Phenolic acids	0.028	0.116	0.025
Flavonols	0.724	0.646	0.728
Flavan-3-ols	0.132	0.022	0.131
Total polyphenols compounds	0.584	0.508	0.591

fruit. According to Namiesnik and others (2013) the AA of ABTS and FRAP assays in gooseberries, cranberries fruit were 12.0, 10.0, and 2.4, 3.1-times lower and in blueberry fruit was 1.1-time higher than average AA in cranberry fruit. The study by Mazur and others (2009) investigated cranberry cvs. "Ben Lear," "Pilgrim," and "Stevens" grown in Warsaw (Poland), and determined AA, with ABTS assay, were 6.0, 8.9, and 3.1 times lower than "Ben Lear," "Pilgrim," and "Stevens" grown in horticultural farm, near Rzeszów (Poland).

The AA (ABTS, DPPH, FRAP) correlated with TPC (for example, $R^2 = 0.508$, 0.584, and 0.591, $P < 0.05$), but a significant positive correlation was observed between AA and: A ($R^2 = 0.675$, 0.602, and 0.614, $P < 0.05$), F ($R^2 = 0.646$, 0.724, and 0.728, $P < 0.05$; Table 5). Tsao and others (2003) also demonstrated significant positive correlations between AA (FRAP) and total phenolic, especially anthocyanins and flavonols in cranberry fruit. Besides, the AA of all cultivars of cranberry fruit was positive correlated with the content of triterpenoids ($R^2 = 0.852$, 0.737, and 0.736, $P < 0.05$). The strongest correlation was found for ursolic acid and the ABTS assays ($R^2 = 0.833$, $P < 0.05$) and positive correlation with the DPPH and FRAP assays ($R^2 = 0.559$ and 0.553, $P < 0.05$). Positive correlation was found between chlorophylls and AA ($R^2 = 0.806$, 0.570, and 0.558, $P < 0.05$). The AA (ABTS, DPPH, FRAP) poorly correlated with total carotenoids, (for example, $R^2 = 0.184$, 0.089, and 0.097, $P < 0.05$) but a negative correlation was observed between AA and lutein ($R^2 = -0.331$, −0.302, and −0.290, $P < 0.05$).

Principal component analysis

The average results of 6 cultivars of cranberry fruit of their bioactive compounds and antioxidant activity, were emphasized during PCA analysis. Two main PCAs for the analyzed 6 cultivars of cranberry fruit accounted for 68.05% of the total variability, PC1 for 42.03% and PC2 for 26.03% are presented in Figure 1. The results obtained in the analysis of PCA, using the relationships among studied groups, indicated the presence of 4 clusters:

- Cluster 1: Cultivars "Stevens" and "Franklin" with a higher concentration of oleanolic acid (OA), ursolic acid (UA), total triterpenoids compounds (TTC), flavonols (FL). In addition, a positive correlation with antioxidant activity (ABTS, FRAP, and DPPH) was detected.
- Cluster 2: Cultivar "Howes" with high total content of carotenoids (TCC), lutein (LUT), β-carotene (*B*-CA), betulinic acid (BA), anthocyanins (ANT), phenolic acid (PA), flavan-3-ols (F3o), and total phenolic compounds (TPC). In addition, a positive correlation with antioxidant activity (ABTS, FRAP, and DPPH) was detected.
- Cluster 3: Cultivars "Pilgrim" and "Ben Lear" with high content of chlorophylls (CH) and negative correlation with antioxidant activity.
- Cluster 4: Cultivar "Red Star" with a positive correlation with antioxidant activity.

Conclusion

Basic chemical composition, phytochemical compounds (polyphenols, carotenoids, chlorophylls, and triterpenoids) and antioxidant activity in 6 different cultivars of cranberry fruit grown in horticultural farm in Poland were examined. Cranberry fruit has been shown to contain high quantities of health-promoting

compounds and their concentration depends on a cultivar. The cvs. "Franklin," "Howes," and "Stevens" were characterized by the highest concentration of vitamin C, pectins, phenolic compounds, triterpenoids, carotenoids, and high antioxidant activity, making these 3 cultivars especially recommendable for consumption. Furthermore, a positive correlation between content of phytochemicals and antioxidant activity was found. These cultivars of cranberries can be used to develop attractive foods with a high content of biologically active substances.

Authors' Contributions

Important contributions to design and in preparation of the manuscript: S.L. Contributions to sample and analysis experiments: S.L., J.O. i J.K-O. Analysis of the experimental data: S.L. i J.O. Critical revision for important intellectual content: S.L., J.O. i J.K-O. Delivery of the material from a horticultural farm: J.G. i N.M. All authors helped preparing the paper and approved the final version.

Conflicts of Interest

The authors declare no conflict of interest.

References

Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem 239:70–6.

Carvalho E, Fraser PD, Martens S. 2013. Carotenoids and tocopherols in yellow and red raspberries. Food Chem 139(1):744–52.

Delgado-Pelayo R, Gallardo-Guerrero L, Hornero-Méndez D. 2014. Chlorophyll and carotenoid pigments in the peel and flesh of commercial apple fruit varieties. Food Res Intern 65:272–81.

Delphino-Rius A, Eras J, Marsol-Vall A, Vilaró F, Balcells M, Canela-Garay R. 2014. Ultra-performance liquid chromatography analysis to study the changes in the carotenoid profile of commercial monovarietal fruit juices. J Chromatogr A 1331:90–9.

De Rosso VV, Mercadante AZ. 2007. Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from apple fruit Amazonian fruits. J Agric Food Chem 55(13):5062–72.

Ercisli S, Tosun M, Karlidag H, Dzubur A, Hadziabulic S, Aliman Y. 2012. Color and antioxidant characteristics of some fresh fig (*Ficus carica* L.) genotypes from northeastern Turkey. Plant Foods Hum Nutr 67(3):271–6.

Farneti B, Masuero D, Costa F, Magnago P, Malnoy M, Costa G, Vrhovsek U, Mattivi F. 2015. Is there room for improving the nutraceutical composition of apple? J Agric Food Chem 63(10):2750–9.

Hannoufa A, Hossain Z. 2012. Regulation of carotenoid accumulation in plants. Biocatal Agric Biotechnol 1:198–202.

Hołderna-Kędzia E. 2006. Botanical characteristics, chemical composition and biological properties of American cranberry (*Vaccinium macrocarpon* Aiton). Phytotherapy Progr 1:41–46.

Howell AB, Botto H, Combescure C, Blanc-Potard AB, Gausa L, Matsumoto T, Tenke P, Sotto A. Lavigne JP. 2010. Dosage effect on uropathogenic *Escherichia coli* anti-adhesion activity in urine following consumption of cranberry powder standardized for proanthocyanidin content: A multicentric randomized double blind study. BMC Infec Dis 10(1):1–11.

Jackson CL, Dreaden TM, Theobald LK, Tran NM, Beal TL, Eid M, Gao MY, Shirley RB, Stoffel MT, Kumar MV, Mohnen D. 2007. Pectin induces apoptosis in human prostate cancer cells: correlation of apoptotic function with pectin structure. Glycobiol 17(8):805–19.

Kim SH, Ahn YO, Ahn MJ, Lee HS, Kwak SS. 2012. Down regulation of β-carotene hydroxylase increases β-carotene and total carotenoids enhancing salt stress tolerance in transgenic cultured cells of sweet potato. Phytochem 74:69–78.

Kondo M. 2006. Phytochemical studies of extracts from cranberry (*Vaccinium macrocarpon*) with anticancer, antifungal, and cardioprotective properties (dissertation). University of Massachusetts Dartmouth: North Dartmouth, MA. p 71–97.

Krishnaiah D, Sarbatly R, Nithyanandam R. 2011. A review of the antioxidant potential of medicinal plant species. Food Bioprod Proc 89:217–33.

Lachowicz S, Kolniak-Ostek J, Oszmiański J, Wiśniewski R. 2017. Comparison of phenolic content and antioxidant capacity of bear garlic (*Allium ursinum* L.) in different maturity stages. J Food Process Preserv 41(1).

Lachowicz S, Oszmiański J, Pluta S. 2017. The composition of bioactive compounds and antioxidant activity of Saskatoon berry (*Amelanchier alnifolia* Nutt.) genotypes grown in central Poland. Food Chem 235:234–43.

Lin CH, Chen BH. 2003. Determination of carotenoids in tomato juice by liquid chromatography. J Chromatogr A 1012(1):103–9.

Mazur B, Borowska EJ, Polak M. 2009. Contents of Vitamin C and antioxidative capacity of fruit and pomace obtained from swamp and large cranberry. Food Sci Technol Qual 2(63): 130–7.

McKay DL, Blumberg JB. 2007. Cranberries (*Vaccinium macrocarpon*) and cardiovascular disease risk factors. Nutr Review 65(11):490–502.

McKenna DJ, Jones K, Hughes K, Humphrey S. 2002. Botanical medicines. The desk reference for major herbal supplements. 2nd Ed. The Haworth Herbal Press, Inc., New York-London-Oxford.

Mueller D, Triebel S, Rudakovski O, Richling E. 2013. Influence of triterpenoids present in apple peel on inflammatory gene expression associated with inflammatory bowel disease (IBD). Food Chem 139:339–46.

Oszmiański J, Kolniak-Ostek J, Lachowicz S, Gorzelany J, Matłok N. 2015. Effect of dried powder preparation process on polyphenolic content and antioxidant capacity of cranberry (*Vaccinium macrocarpon*). Ind Crop Prod 77:658–65.

Namiesnik J, Vearasilp K, Kupska M, Ham K-S, Kang S-G, Park Y-K, Barasch D, Nemirovski A, Gorinstein S. 2013. Antioxidant activities and bioactive components in some berries. Eur Food Res Technol 237:819–29.

Nawirska A, Kwaśniewska M. 2004. Fiber fractions in fruit pomace. Acta Scientarium Polonorum. Tech Aliment 3(1):13–20 (in Polish).

Pappas E, Schaich KM. 2009. Phytochemicals of cranberries and cranberry products: characterization, potential health effects, and processing stability. Critical Rev Food Sci Nutr 49(9):741–81.

Paredes-López O, Cervantes-Ceja ML, Vigna-Pérez M, Hernández-Pérez T. 2010. Berries: improving human health and healthy aging, and promoting quality life—a review. Plant Food Hum Nutr 65:299–308.

Pertuzatti PB, Sganzerla M, Jacques AC, Barcia MT, Zambiazi RC. 2015. Carotenoids, tocopherols, and ascorbic acid content in yellow passion fruit (*Passiflora edulis*) grown under different cultivation systems. LWT-Food Sci Technol 64(1):259–63.

Pop RM, Weesepoel Y, Socaciu C, Pintea A, Vincken J-P, Gruppen H. 2014. Carotenoid composition of berries and leaves from 6 Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. Food Chem 147:1–9.

Popov SV, Markov PA, Nikitina IR, Petrishev S, Smirnov V, Ovodov YS. 2006. Preventive effect of a pectic polysaccharide of the common cranberry *Vaccinium oxycoccos* L. on acetic acid- induced colitis in mice. World J Gastroentero 12(41):6646–51.

Prior RL, Fan E, Ji H, Howell A, Nio C, Payne MJ, Reed J. 2010. Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. J Agr Food Chem 90:1473–8.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med 26(9/10):1231–7.

Reid G, Hsiehl J, Potter P, Mighton J, Lam D, Warren D, Stephenson J. 2001. Cranberry juice consumption may reduce biofilms on uroepithelial cells: pilot study in spinal cord injured patients. Spinal Cord 39:26–30.

Ruel G, Pomerleau S, Couture P, Lamarche B, Couillard C. 2005. Changes in plasma antioxidant capacity and oxidized low-density lipoprotein levels in men after short-term cranberry juice consumption. Metabolomism 54(7):856–61.

Szakiel A, Pączkowski C, Pensec F, 2012. Bertsch C, Fruit cuticular waxes as a source of biologically active triterpenoids. Phytochem Rev 11(2–3):263–84.

Teleszko M. 2011. Large cranberry – possible uses in production of bio-foods. Food Sci Technol Qual 6(79):132–41 (in Polish).

Tsao R, Yang R, Young JC, Zhu H. 2003. Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). J Agric Food Chem 51(21):6347–53.

Vattem DA, Jang HD, Levin R, Shetty K. 2006. Synergism of cranberry phenolics with ellagic acid and rosmarinic acid for antimutagenic and DNA protection functions. J Food Biochem 30(1):98–116.

Viskelis P, Rubinskiene M, Jasutiene I, Sarkinas A, Daubaras R, Cesoniene L. 2009. Anthocyanins, antioxidative, and antimicrobial properties of American cranberry (*Vaccinium macrocarpon* Ait.) and their press cakes. J Food Sci 74(2):157–61.

Wang SY, Stretch AW. 2001. Antioxidant capacity in cranberry is influenced by cultivar and storage temperature. J Agric Food Chem 49:969–74.

White LB, Howard RL, Prior RL. 2010. Proximate and polyphenolic characterization of cranberry pomace. J Agric Food Chem 58:4030–6.

Yen GC, Chen HY. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 43:27–32.