Diversity and bioavailability of fruit polyphenols

TOMASZ TARKO – ALEKSANDRA DUDA-CHODAK – PAWEŁ SROKA – PAWEŁ SATORA – DOROTA SEMIK-SZCZURAK – ŁUKASZ WAJDA

Summary
The aim of the study was to evaluate changes of phenolic compounds of eight fruit types during digestion in the simulated human digestive tract. Significant differences in antioxidant activity and the content of polyphenols were found among species. All fractions obtained after in vitro digestion demonstrated lower content of polyphenols than in fruits. Antioxidant activity of supernatants after digestion was usually higher than antioxidant activity of the plant material before digestion. Moreover, migration rate of phenolic compounds through dialysis membrane depended on the fruit species. Chokeberry and elderberry are good sources of antioxidants. Polyphenols absorbed in the digestion tract after their consumption demonstrated high antioxidant potential. Biotransformation of phenolic compounds by the intestinal microbiota mostly depends on the content and profile of those compounds. Solid residues of bilberry fruit remaining after microbial fermentation showed the highest polyphenol content and antioxidant activity.

Keywords
antioxidant activity; polyphenolic compounds; in vitro model; digestion; bioavailability; fruits

Humans are permanently exposed, during their life, to the adverse effects of by-products of oxygen metabolism, including free radicals. These compounds cause so called “lifestyle diseases”, including diseases of cardiovascular and nervous systems, diabetes, obesity, allergy and cancer [1]. Polyphenols scavenge free radicals and act as their natural neutralizers. They also influence the activity of antioxidative enzymes, particularly superoxide dismutase, catalase and glutathione peroxidase, which catalyse inactivation of reactive oxygen species [2].

Metabolism and absorption of phenolic compounds in the human digestive tract has a huge impact on their health-promoting properties to human body. Polyphenols usually have to be released from the food matrix in the small and large intestine before their absorption. The efficiency of polyphenol absorption from the gastrointestinal tract is influenced by many factors, including pH, molecular weight and hydrophilicity of phenolic compounds, polymerization degree and the type of sugar molecule bound to their aglycones. Certain aglycones penetrate biological membranes by passive diffusion, however, most flavonoids are present as glycosides which must be hydrolysed before absorption [3].

Effectiveness of polyphenol digestion and absorption in the human digestive tract has significant impact on health-promoting properties of those compounds. Fruits are the major source of antioxidants and their deficiency in the diet may lead to the development of many diseases. Recommended daily intake of vegetables, fruits and products made of them (400–500 g) helps protecting people against lifestyle diseases.

The main goal of the study was to determine the antioxidant activity (*AOX*), total polyphenol content (*TPC*) and polyphenol profile in selected fruits and evaluate the bioavailability of phenolic compounds at every stage of in vitro digestion.

Tomasz Tarko, Aleksandra Duda-Chodak, Paweł Sroka, Paweł Satora, Dorota Semik-Szczurak, Department of Fermentation Technology and Technical Microbiology, Faculty of Food Technology, University of Agriculture in Krakow, ul. Balicka 122, 30-149 Krakow, Poland.
Łukasz Wajda, Malopolska Centre of Biotechnology, Jagiellonian University, ul. Gronostajowa 7A, 30-387 Krakow, Poland.

Correspondence author:
Tomasz Tarko, tel.: +48126624792, fax: +4812 6624798, e-mail: t.tarko@ur.krakow.pl

MATERIALS AND METHODS

Fruits

Fruits used in the experiment were purchased from the pomological orchard of the University of Agriculture in Krakow, located in Garlica Murowana near Krakow, or from the organic farms in Malopolska and Podkarpacie in Poland. Dried goji berries (Chinese wolfberry) were purchased in stores distributing organic food in Poland. Experimental materials used in the study were fruits of: black chokeberry (*Aronia melanocarpa* (Michx.) Elliott), bilberry (*Vaccinium myrtillus* L.), elderberry (*Sambucus nigra* L.), 'Golden Delicious' apples (*Malus domestica* Borkh. 'Golden Delicious'), goji berries (*Lycium chinense* Mill.), Japanese quince (*Chaenomeles japonica* (Thunb.), black currant (*Ribes nigrum* L.), and cranberry microcarpa (*Oxycoccus microcarpus* Turcz. ex Rupr.). Fruits were washed in tap water, dried with a paper towel and frozen at –80 °C to inhibit transformation of compounds with antioxidant properties.

In vitro digestion model

Fruits (0.5 g) were accurately weighed into screw-capped vials and acidified to pH 2 with HCl (0.5 mol·l^{-1}; POCh, Gliwice, Poland). Then, 0.75 ml of pepsin solution (62.6 mg of pepsin, EC 3.4.23.1, with activity 3 440 U·mg^{-1}; Sigma-Aldrich, St. Louis, Missouri, USA), dissolved in 20 ml of 0.1 mol·l^{-1} HCl (POCh) and re-distilled water (obtained by ultrafiltration; Simplicity, Milipore, Billerica, Massachusetts, USA) were added to a total volume of 3 ml. One unit (1 U) is the enzyme activity that will produce an increase of absorbance at 280 nm (ΔA_{280}) of 0.001 per min at pH 2.0 at 37 °C, measured as trichloroacetic acid-soluble (TCA-soluble) products and using hemoglobin as substrate. After thorough mixing, test tubes were incubated in a water bath at 37 °C for 2 h.

Then, $NaHCO_3$ (POCh) in an amount providing pH 7, and 0.375 ml of pancreatin and bile solution (66.7 mg of pancreatin, EC 232-468-9, with activity 8 × USP (Sigma-Aldrich) and 833.3 mg of bile (EC 232-369-0, Sigma-Aldrich) dissolved in 10 ml of 0.1 mol·l^{-1} $NaHCO_3$) were added. One unit of enzyme activity, according to United States Pharmacopeia (USP), is defined as the amount of the substance that decomposes the given substrate at a specified rate under standard assay conditions. The volume was adjusted to 5 ml with re-distilled water. Vials were incubated in a water bath (37 °C, 4 h) and centrifuged (1 380 ×*g*, 10 min).

Supernatants were decanted and solid residue was rinsed twice with 3 ml of re-distilled water. Each time the sample was mixed, centrifuged and the supernatants were collected. Solid residues obtained after centrifugation (sediments) were used for the simulation of digestion in the small intestine. Combined supernatants were transferred into the dialysis membrane (Sigma-Aldrich) and dialysed in a shaking water bath (85·min^{-1}, 37 °C, 3 h) against phosphate buffered saline (PBS, Sigma-Aldrich).

The contents of dialysis bags (retentates) were combined with sediments (obtained after centrifugation at previous digestion stage) and alkalized to pH 8. The mixture was transferred to a glass screw-capped tube and inoculated with a mixture of intestinal bacteria (*Bacteroides galacturonicus, Enterococcus caccae, Bifidobacterium catenulatum, Ruminococcus gauvreauii, Lactobacillus* sp., *Escherichia coli*; Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). The final number of bacterial cells in the sample subjected to the fermentation was 10^9 CFU·ml^{-1}. The test tube was saturated with inert gas (CO_2) and incubated at 37 °C for 16 h (fermentation under anaerobic conditions), then the samples were centrifuged and supernatants collected.

As a result of the in vitro digestion, the following fractions were obtained:

- Supernatant, a soluble fraction of phenolic compounds subjected to the digestion in stomach and duodenum, obtained by centrifugation of the sample after enzymatic digestion step;
- Sediment, representing an insoluble fraction of phenolic compounds subjected to the digestion in stomach and duodenum, the residue from the centrifugation of the sample obtained after enzymatic digestion;
- Retentate, a remnant obtained after dialysis representing soluble fraction of phenolic compounds that did not migrate through the dialysis membrane;
- Permeate, PBS buffer containing the ingredients that have passed through the dialysis membranes, representing the soluble fraction of phenolic compounds absorbed by the epithelium of the small intestine;
- Post-fermentation residue, a fraction of phenolic compounds that remained after bacterial digestion of sediments and the retentate in the colon.

The experiments were carried out in at least three replicates. Subsequently, the antioxidant activity, total polyphenol content and the polyphenol profile of all obtained fractions were analysed.

Determination of total polyphenol content and antioxidant activity

The samples were centrifuged (1120 ×*g*, MPW-350R, Merazet, Poznan, Poland) before analysis.

Total polyphenol content (*TPC*) was assessed by Folin-Ciocalteu method according to the European Pharmacopoeia [4] but results were expressed as grams of (+)-catechin per kilogram of fresh weight (FW).

Antioxidant activity (*AOX*) was determined using the active cation-radical of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS, Sigma Aldrich) [5]. *AOX* was calculated on the basis of calibration curve, prepared each time from synthetic vitamin E ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Trolox, Sigma Aldrich) and expressed as grams of Trolox per kilogram of FW.

Determination of polyphenol profile by HPLC method

For quantitative analyses, standard curves were prepared for the following standards: ferulic acid, caffeic acid, chlorogenic acid, gallic acid, hippuric acid, *p*-coumaric acid, protocatechuic acid, ellagic acid, (+)-catechin, quercetin, resveratrol, kaempferol (Sigma Aldrich), phloridzin, (–)-epicatechin, procyanidins B1 and B2, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-sambubioside, cyanidin-3-*O*-arabinoside cyanidine-3-*O*-glucoside, delphinidin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, peonidin-3-glucoside (Extrasynthese, Genay, France).

Prior to analysis, the samples were filtered through a nylon syringe filter (pore size 0.45 μm; Chemland, Stargard Szczecinski, Poland). The analysis of polyphenol profile was carried out using high-performance liquid chromatography (HPLC) in a Flexar instrument (Perkin-Elmer, Waltam, Massachusetts, USA) equipped with UV-Vis detector. A Synergi Fusion RP-80A column (200 mm × 4.6 mm, particle size 4 μm; Phenomenex, Torrance, California, USA), thermostated at 30 °C was used for all analyses. A 2.5% aqueous solution of acetic acid (solution A, POCh) and acetonitrile (solution B, POCh) were used as a mobile phase.

Gradient programme for the analysis of gallic acid, (+)-catechin, (–)-epicatechin, (–)-epigallocatechin, epigallocatechin gallate, phloridzin, hesperetin, hesperidin, procyanidins B1 and B2 (detection at 280 nm) was as follows: linearly from 5% B to 20% B for 30 min, then linearly from 20% B to 100% B for 3 min, isocratically for 7 min 100% B and linearly 100% B to 5% B within 4 min (flow rate 0.5 ml·min^{-1}). After each separation, the column was washed with 5% B solution.

The gradient program for the determination of caffeic acid, *p*-coumaric acid, chlorogenic acid, ferulic acid, vitexin, isovitexin, apigenin and resveratrol (detection at 325 nm) was carried out isocratically 20% B (15 min), linearly 20% B to 100% B within 30 min, isocratically 100% B for 4 min, linearly 100% B to 20% B within 3 min time (the flow at 0.5 ml·min^{-1}) and then washed with 20% B solution.

In cases of quercetin, quercetin-3-*O*-glucoside, rutin, kaempferol (flow rate 1 ml·min^{-1}, detection at 360 nm) and hippuric acid, protocatechuic acid, ellagic acid, daidzin, daidzein and genistein (flow rate 1 ml·min^{-1}, 250 nm) the following programme was applied: linearly from 5% B to 20% B within 20 min, then linearly 20% B to 100% B within 10 min, isocratically for 3 min 100% B, linearly 100% B to 5% B within 4 min and washing the column with 5% B solution.

Anthocyanins (myrtillin, ideain, kuromanin, keracyanin, cyanidin-3-*O*-arabinoside, callistephin, peonidin-3-*O*-glucoside, cyanidin-3-*O*-sambubioside, cyanin) were analysed (flow rate 0.5 ml·min^{-1}, detection at 520 nm) using the following program: linearly from 5% B to 20% B within 30 min, then linearly to 100% B within 3 min, isocratically for 4 min 100% B and linearly 100% B to 5% B within 4 min.

Limit of detection (*LOD*) and limit of quantification (*LOQ*) for flavonoids and phenolic acids were 0.02 mg·kg^{-1} and 0.1 mg·kg^{-1} respectively, and for anthocyanins 0.05 mg·kg^{-1} and 0.2 mg·kg^{-1} respectively. Polyphenols not detected in any experimental variants were not included in tables.

Statistical analysis

All experiments and analyses were carried out in at least three repetitions and results were shown as arithmetic mean ± standard deviation (*SD*). Statistical analysis was performed using InStat v. 3.01 (GraphPad Software, La Jolla, California, USA). A single-factor analysis of variance (ANOVA) with post hoc Tukey's test was applied to determine the significance of differences between means. The Kolmogorov-Smirnov test was carried out to assess normality of distribution.

RESULTS

The fruits of black chokeberry demonstrated the highest *AOX* (54.27 g·kg^{-1} FW) and the highest *TPC* (115.15 g·kg^{-1} FW). Golden Delicious apples

Tab. 1. Total polyphenol content and antioxidant activity of fruits at different digestion stages.

Sample	Fruits	Supernatant	Sediment	Retentate	Permeate	Post-fermentation residue
	Total polyphenol content [g·kg^{-1}]					
Chokeberry	115.15 ± 19.20^{a}	6.18 ± 0.78^{b}	1.12 ± 0.28^{c}	1.00 ± 0.10^{c}	1.74 ± 0.54^{d}	0.52 ± 0.14^{e}
Elderberry	80.71 ± 0.92^{a}	4.44 ± 0.78^{b}	0.20 ± 0.08^{c}	0.82 ± 0.14^{d}	1.48 ± 0.14^{e}	0.80 ± 0.08^{d}
Bilberry	42.71 ± 4.73^{a}	2.88 ± 0.89^{b}	0.42 ± 0.08^{c}	0.66 ± 0.16^{c}	0.54 ± 0.12^{c}	0.82 ± 0.20^{d}
Apple	8.49 ± 0.82^{a}	0.48 ± 0.06^{b}	0.12 ± 0.02^{c}	0.28 ± 0.03^{d}	0.14 ± 0.02^{c}	0.20 ± 0.04^{e}
Goji berries	14.31 ± 0.60^{a}	3.72 ± 0.60^{b}	0.40 ± 0.07^{c}	0.86 ± 0.10^{d}	1.08 ± 0.30^{d}	0.62 ± 0.18^{c}
Japanese quince	41.05 ± 0.66^{a}	2.76 ± 0.48^{b}	1.08 ± 0.33^{c}	0.80 ± 0.12^{c}	0.84 ± 0.02^{c}	0.64 ± 0.04^{d}
Blackcurrant	23.25 ± 0.34^{a}	1.38 ± 0.46^{b}	0.24 ± 0.04^{c}	0.34 ± 0.10^{cd}	0.32 ± 0.04^{c}	0.46 ± 0.16^{d}
Cranberries	27.09 ± 1.67^{a}	1.74 ± 0.06^{b}	0.24 ± 0.02^{c}	0.42 ± 0.08^{d}	0.38 ± 0.06^{d}	0.26 ± 0.06^{c}
	Antioxidant activity [g·kg^{-1}]					
Chokeberry	54.27 ± 1.45^{a}	73.76 ± 4.10^{b}	32.04 ± 4.58^{c}	24.36 ± 3.22^{d}	36.09 ± 3.69^{c}	13.04 ± 5.22^{e}
Elderberry	44.02 ± 0.72^{a}	75.19 ± 11.96^{b}	7.55 ± 1.14^{c}	25.23 ± 6.35^{d}	26.28 ± 4.79^{d}	15.02 ± 1.74^{e}
Bilberry	28.38 ± 1.20^{a}	24.61 ± 5.66^{a}	9.15 ± 1.57^{c}	14.97 ± 2.11^{b}	5.72 ± 0.35^{d}	17.29 ± 1.69^{b}
Apple	5.60 ± 0.29^{a}	6.82 ± 1.05^{b}	2.20 ± 0.33^{c}	2.42 ± 0.20^{c}	3.14 ± 0.72^{d}	4.83 ± 0.64^{e}
Goji berries	43.73 ± 1.75^{a}	41.20 ± 5.27^{a}	3.96 ± 0.97^{b}	12.51 ± 0.91^{c}	7.48 ± 0.99^{d}	13.66 ± 0.42^{c}
Japanese quince	22.16 ± 0.39^{a}	44.61 ± 0.83^{c}	13.09 ± 2.07^{b}	23.80 ± 1.55^{a}	15.79 ± 3.47^{b}	12.12 ± 1.39^{b}
Blackcurrant	10.92 ± 0.75^{a}	35.10 ± 4.65^{b}	10.19 ± 2.56^{a}	8.47 ± 1.57^{c}	7.49 ± 0.94^{c}	8.25 ± 0.97^{c}
Cranberries	18.23 ± 0.98^{a}	26.09 ± 3.89^{b}	6.63 ± 0.81^{c}	9.92 ± 0.88^{d}	6.81 ± 0.50^{c}	6.85 ± 1.88^{c}

Means marked with the same superscript letter in a row are not significantly different at $p < 0.05$, $n = 3$.
Total polyphenol content is expressed as grams of (+)-catechin per kilogram of fresh weight. Antioxidant activity is expressed as grams of Trolox per kilogram of fresh weight.

showed the lowest *AOX* (5.6 g·kg^{-1} FW), and the lowest *TPC* (8.49 g·kg^{-1} FW) among all tested fruits (Tab. 1).

According to the results obtained from simulated digestion of apple fruits, the supernatant fraction demonstrated lower *TPC* and slightly higher *AOX* than fruits (Tab. 1). Procyanidins and (+)-catechin dominated in polyphenol profile of apple fruits but they were not detected in the supernatant (Tab. 2). A small amount of phenolic compounds with low *AOX* went to sediments (insoluble fraction subjected to digestion in stomach and duodenum). Moreover, polyphenols present in analysed apples demonstrated moderate migration rate through the dialysis membrane (29 % in relation to the supernatant). Most of polyphenols (58 %), mainly phenolic acids and phloridzin (Tab. 2), remained in the retentate. After subject-

Tab. 2. Profile of phenolic compounds at individual stages of digestion of apple.

Compound [mg·kg^{-1}]	Raw material	Supernatant	Sediment	Retentate	Permeate	Post-fermentation residue
(–)-Epicatechin	8.1 ± 7.0	nd	nd	nd	nd	nd
Phloridzin	5.1 ± 0.8^{ab}	7.4 ± 1.6^{a}	5.5 ± 1.5^{ab}	4.6 ± 0.9^{b}	3.3 ± 0.0^{c}	1.4 ± 0.5^{d}
(+)-Catechin	33.5 ± 10.2	nd	nd	nd	nd	nd
Kaempferol	1.2 ± 0.3^{a}	0.9 ± 0.2^{a}	nd	0.5 ± 0.0^{b}	nd	nd
Chlorogenic acid	4.3 ± 0.8^{a}	2.8 ± 0.7^{b}	nd	1.4 ± 0.2^{c}	nd	3.6 ± 1.2^{b}
Ferulic acid	2.0 ± 0.2^{a}	0.7 ± 0.0^{b}	nd	0.2 ± 0.0^{c}	nd	0.6 ± 0.0^{b}
Hippuric acid	0.8 ± 0.1^{a}	0.7 ± 0.1^{a}	nd	0.6 ± 0.2^{a}	nd	6.0 ± 1.6^{b}
Caffeic acid	nd	nd	2.5 ± 0.0^{a}	0.7 ± 0.0^{b}	nd	0.2 ± 0.0^{b}
Quercetin	nd	0.7 ± 0.0	nd	nd	nd	nd
Quercetin-3-*O*-glucoside	21.5 ± 7.1^{a}	1.6 ± 0.5^{b}	nd	nd	nd	nd
Procyanidin B1	19.6 ± 8.1	nd	nd	nd	nd	nd
Procyanidin B2	93.9 ± 21.2	nd	nd	nd	nd	nd

Values are expressed per kilogram of fresh weight. Means marked with the same superscript letter in a row are not significantly different at $p < 0.05$, $n = 3$.
nd – not detected, content of the compound was below method sensitivity.

ing the retentate and sediment to bacteria, post-fermentation residue demonstrated higher *AOX* (Tab. 1).

Bilberry fruits contained relatively high levels of anthocyanins (Tab. 3) demonstrating a high content of (–)-epicatechin (633 mg·kg^{-1} FW). *AOX* of bilberry fruits was 28.38 g·kg^{-1} FW. Quercetin derivatives, (+)-catechin and phenolic acids (chlorogenic, *p*-coumaric and caffeic) dominated in goji berries (Tab. 4) and *TPC* was 14.31 g·kg^{-1} FW. *AOX* of goji berries was 43.73 g·kg^{-1} FW (Tab. 1). There were no statistically significant differences of *AOX* between results obtained for fruits and supernatants collected after the first digestion step of bilberry and goji berries (Tab. 1). However, *TPC* of supernatants was lower in case of both tested fruits. In the case of goji, *TPC* in the supernatant comprised 26% of their quantity detected in fruits and, in the case of bilberry, it was only 7% (Tab. 1). Moreover, both bilberry fruits and the supernatant obtained after the first digestion step were rich in anthocyanins, mostly cyani-

Tab. 3. Profile of phenolic compounds at individual stages of digestion of bilberry.

Compound [mg·kg^{-1}]	Raw material	Supernatant	Sediment	Retentate	Permeate	Post-fermentation residue
Cyanidin-3-*O*-arabinoside	69.8 ± 11.1^{a}	10.1 ± 3.2^{b}	nd	nd	nd	nd
Cyanidin-3-*O*-galactoside	51.3 ± 11.3^{a}	17.0 ± 2.8^{b}	nd	nd	nd	nd
Cyanidin-3-*O*-glucoside	68.9 ± 12.2^{a}	10.7 ± 2.8^{b}	nd	nd	nd	nd
(–)-Epicatechin	633.0 ± 72.2^{a}	350.3 ± 72.5^{b}	nd	217.6 ± 33.4^{c}	3.7 ± 0.0^{d}	122.1 ± 33.8^{e}
(+)-Catechin	12.8 ± 3.4^{a}	14.0 ± 0.5^{a}	nd	nd	nd	3.0 ± 0.9^{b}
Chlorogenic acid	41.2 ± 10.0^{a}	25.5 ± 8.5^{b}	0.6 ± 0.0^{c}	5.5 ± 0.7^{d}	nd	7.6 ± 1.6^{d}
Ferulic acid	8.7 ± 1.2^{a}	7.8 ± 2.1^{a}	nd	3.0 ± 0.7^{b}	nd	0.2 ± 0.0^{c}
Gallic acid	6.6 ± 1.1^{a}	106.7 ± 23.7^{b}	1.5 ± 0.0^{c}	6.7 ± 0.5^{a}	nd	13.6 ± 1.8^{d}
Hippuric acid	1.5 ± 0.5^{a}	0.8 ± 0.2^{b}	nd	nd	nd	2.5 ± 0.2^{c}
Caffeic acid	29.9 ± 7.3^{a}	23.9 ± 6.2^{a}	0.6 ± 0.0^{b}	6.0 ± 1.6^{c}	2.5 ± 0.0^{d}	6.9 ± 2.1^{c}
Quercetin	0.2 ± 0.0^{a}	18.9 ± 2.1^{b}	nd	nd	nd	3.0 ± 0.7^{c}
Quercetin-3-*O*-glucoside	73.7 ± 13.7^{a}	25.8 ± 3.0^{b}	nd	0.7 ± 0.5^{c}	nd	1.4 ± 0.7^{c}
Quercetin-3-*O*-rutinoside	52.4 ± 10.4^{a}	34.7 ± 11.7^{b}	0.9 ± 0.3^{c}	1.8 ± 0.0^{d}	nd	1.6 ± 0.2^{d}
Peonidin-3-*O*-glucoside	10.1 ± 3.3^{a}	2.1 ± 0.5^{b}	nd	nd	nd	nd
Procyanidin B2	76.9 ± 12.1^{a}	1.2 ± 0.2^{b}	nd	nd	nd	nd
Resveratrol	0.4 ± 0.1^{a}	0.2 ± 0.0^{a}	0.6 ± 0.0^{b}	0.5 ± 0.0^{b}	nd	0.5 ± 0.0^{b}

Values are expressed per kilogram of fresh weight. Means marked with the same superscript letter in a row are not significantly different at $p < 0.05$, $n = 3$.
nd – not detected, content of the compound was below method sensitivity.

Tab. 4. Profile of phenolic compounds at individual stages of digestion of goji berries.

Compound [mg·kg^{-1}]	Raw material	Supernatant	Sediment	Retentate	Permeate	Post-fermentation residue
Cyanidin-3-*O*-glucoside	1.2 ± 0.3	nd	nd	nd	nd	nd
Phloridzin	17.4 ± 7.1^{a}	9.4 ± 2.3^{b}	0.6 ± 0.0^{c}	7.4 ± 1.8^{ab}	nd	3.9 ± 0.5^{d}
(+)-Catechin	31.2 ± 8.7^{a}	22.5 ± 11.3^{a}	nd	12.7 ± 2.8^{b}	nd	12.9 ± 3.2^{b}
Chlorogenic acid	35.6 ± 12.1^{a}	13.3 ± 2.5^{b}	nd	6.2 ± 0.7^{c}	2.5 ± 0.2^{d}	10.4 ± 2.8^{bc}
Ellagic acid	3.6 ± 0.9^{a}	3.7 ± 0.9^{a}	nd	0.6 ± 0.2^{b}	nd	1.4 ± 0.2^{c}
Ferulic acid	2.6 ± 0.7^{a}	1.2 ± 0.5^{b}	nd	0.5 ± 0.0^{c}	nd	0.2 ± 0.0^{c}
Hippuric acid	0.8 ± 0.2^{a}	0.4 ± 0.0^{b}	nd	0.3 ± 0.0^{b}	nd	3.4 ± 1.2^{c}
Caffeic acid	17.8 ± 4.1^{a}	9.4 ± 1.8^{b}	nd	3.0 ± 0.9^{c}	nd	3.0 ± 0.5^{c}
p-Coumaric acid	22.5 ± 8.7^{a}	17.0 ± 3.2^{a}	nd	4.1 ± 0.5^{b}	nd	3.2 ± 0.9^{b}
Quercetin	1.2 ± 0.3^{a}	1.2 ± 0.5^{a}	nd	0.5 ± 0.0^{b}	nd	2.8 ± 0.7^{c}
Quercetin-3-*O*-glucoside	11.9 ± 3.7^{a}	4.4 ± 1.8^{b}	nd	2.5 ± 0.5^{bc}	nd	2.3 ± 0.7^{c}
Quercetin-3-*O*-rutinoside	57.4 ± 14.2^{a}	32.9 ± 8.5^{b}	nd	13.6 ± 3.0^{c}	nd	18.4 ± 4.8^{c}
Peonidin-3-*O*-glucoside	1.4 ± 0.5^{a}	1.8 ± 0.2^{b}	nd	nd	nd	nd

Values are expressed per kilogram of fresh weight. Means marked with the same superscript letter in a row are not significantly different at $p < 0.05$, $n = 3$.
nd – not detected, content of the compound was below method sensitivity.

Tab. 5. Profile of phenolic compounds at individual stages of digestion of Japanese quince.

Compound [mg·kg^{-1}]	Raw material	Supernatant	Sediment	Retentate	Permeate	Post-fermentation residue
(–)-Epicatechin	52.4 ± 10.3^{a}	50.4 ± 14.0^{a}	5.8 ± 0.9^{b}	nd	nd	nd
Phloridzin	11.1 ± 3.7^{a}	12.7 ± 2.5^{a}	10.3 ± 2.6^{a}	3.5 ± 0.7^{b}	3.6 ± 0.6^{b}	2.5 ± 0.7^{b}
(+)-Catechin	3.4 ± 0.9^{a}	38.0 ± 7.8^{b}	nd	nd	nd	nd
Chlorogenic acid	1.0 ± 0.2^{a}	0.6 ± 0.0^{b}	2.3 ± 0.3^{c}	0.3 ± 0.0^{b}	0.4 ± 0.0^{b}	0.6 ± 0.2^{b}
Gallic acid	33.3 ± 9.2^{a}	17.3 ± 4.8^{b}	2.6 ± 0.3^{c}	1.8 ± 0.5^{c}	4.9 ± 0.9^{d}	2.3 ± 0.7^{c}
Hippuric acid	0.9 ± 0.4^{a}	0.3 ± 0.1^{b}	nd	0.3 ± 0.1^{b}	nd	6.9 ± 2.1^{c}
Caffeic acid	7.9 ± 1.1^{a}	2.5 ± 0.7^{b}	1.2 ± 0.3^{c}	0.3 ± 0.0^{d}	nd	0.4 ± 0.0^{d}
p-Coumaric acid	10.2 ± 3.7^{a}	2.1 ± 0.5^{b}	nd	0.5 ± 0.0^{c}	nd	nd
Quercetin	6.8 ± 1.3^{a}	1.8 ± 0.5^{b}	1.2 ± 0.3^{b}	1.6 ± 0.2^{b}	nd	11.3 ± 2.8^{c}
Quercetin-3-*O*-glucoside	44.4 ± 2.9^{a}	37.5 ± 8.7^{a}	nd	10.3 ± 1.6^{b}	nd	14.7 ± 3.2^{b}
Quercetin-3-*O*-rutinoside	13.4 ± 3.3^{a}	11.7 ± 2.5^{a}	nd	5.1 ± 1.6^{b}	nd	4.7 ± 0.7^{b}
Procyanidin B1	19.0 ± 3.4^{a}	14.3 ± 5.8^{a}	nd	nd	nd	17.5 ± 2.5^{a}
Procyanidin B2	70.7 ± 20.3^{a}	67.8 ± 27.8^{a}	nd	nd	nd	nd

Values are expressed per kilogram of fresh weight. Means marked with the same superscript letter in a row are not significantly different at $p < 0.05$, $n = 3$.
nd – not detected, content of the compound was below method sensitivity.

din glycosides, (–)-epicatechin and phenolic acids (Tab. 3). After simulated digestion in stomach and duodenum, the content of anthocyanins decreased significantly (by more than 80 %) with 98 % and 55 % losses of procyanidin B2 and (–)-epicatechin, respectively. On the other hand, the content of gallic acid increased after those processes. Degradation of quercetin glycosides occurred at this stage of simulated digestion (Tab. 3). Higher amounts of aglycones (quercetin) were detected in the supernatant than in the fruits.

TPC in Japanese quince determined in the present study was 41.05 g·kg^{-1} FW (Tab. 1), and the analysis of the polyphenol profile showed that derivatives of quercetin and catechins, phenolic acids and procyanidins were major compounds (Tab. 5). *AOX* of Japanese quince fruit was 22.16 g·kg^{-1} FW (Tab. 1). *TPC* in supernatants obtained after digestion of Japanese quince was lower than in fruits (Tab. 1) but *AOX* was significantly higher.

AOX of cranberry fruits was 18.23 g·kg^{-1} FW (Tab. 1), which is relatively low comparing to other tested fruits. Cranberry fruits were rich in quercetin glycosides, phenolic acids and procyanidins. Cyanidin-3-*O*-galactoside and delphinidin-3-*O*-glucoside dominated among the identified anthocyanins (Tab. 6). Supernatants obtained after digestion of cranberries had a lower *TPC* and slightly higher *AOX* comparing to fruits (Tab. 1).

Chokeberry was the richest source of phenolic compounds, in particular anthocyanins (cyanidin glucoside), procyanidins B1 and B2, and phenolic acids, mainly caffeic acid (Tab. 7) among all tested fruits. Chokeberry also demonstrated the highest *AOX* among tested fruits (Tab. 1). After simulated digestion of chokeberry, *TPC* in the supernatant was 18-fold lower than in fresh fruits, while *AOX* increased during that process by approximately 35 % (Tab. 1). The permeate obtained after dialysing chokeberry suspension demonstrated the highest *TPC* (1.74 g·kg^{-1} FW) and *AOX* (36.09 g·kg^{-1} FW) among all tested fruits. Intestinal bacteria contributed significantly to degradation of phenolic compounds of chokeberry fruits. Anthocyanins, which persisted at early digestion stages of chokeberry, were decomposed by bacteria (Tab. 7). This resulted in a significant decrease of *AOX* of the post-fermentation residue in comparison to other fractions collected during simulated digestion (Tab. 1).

Elderberry fruits demonstrated a high antioxidant activity (44.02 g·kg^{-1} FW) and a high level of polyphenols (80.71 g·kg^{-1} FW) (Tab. 1). Anthocyanins were the main group of elderberry polyphenols, representing 87 % of all identified substances with cyanidin-3-*O*-sambubioside (7 782 mg·kg^{-1} FW) being the dominant compound (Tab. 8). The contents of quercetin glycosides, procyanidin B1 and phenolic acids were also relatively high. Supernatants obtained after simulated digestion of elderberry fruits demonstrated a significantly lower (18-fold) level of *TPC* than fruits and by more than 70 % higher *AOX* (Tab. 1). Supernatant obtained from the first stage of elderberry digestion demonstrated the highest *AOX* (75.19 g·kg^{-1} FW) among all the tested fruits. The presence of anthocyanins (cyanidin-3-*O*-sambubioside and quercetin-3-*O*-rutinoside) was re-

sponsible for *AOX* of the elderberry supernatant (Tab. 8). Moreover, the sediment obtained after simulated digestion of elderberry demonstrated the largest decrease of *TPC* and *AOX* in relation to corresponding supernatants (Tab. 1). After dialysing elderberry supernatant, a 3-fold decrease in *TPC* was noted and a 30% decrease of *AOX* of the permeate occurred in comparison to the supernatant (Tab. 1). Intestinal bacteria were only slightly involved in the degradation of phenolic compounds from elderberry fruits (Tab. 1), mainly affecting glycosides of cyanidin and quercetin (Tab. 8). A significant increase in the content of quercetin, originating from the decomposition of its glycosides, took place in the post-fermentation residues.

Black currant fruits demonstrated one of the lowest *AOX* (10.92 g·kg^{-1} FW) and relatively low

Tab. 6. Profile of phenolic compounds at individual stages of digestion of cranberry.

Compound [mg·kg^{-1}]	Raw material	Supernatant	Sediment	Retentate	Permeate	Post-fermentation residue
Cyanidin-3-*O*-rutinoside	4.7 ± 1.2	nd	nd	nd	nd	nd
Cyanidin-3-*O*-galactoside	29.9 ± 11.2^{a}	20.9 ± 7.1^{a}	nd	nd	nd	nd
Delphinidin-3-*O*-glucoside	14.2 ± 3.7	nd	nd	nd	nd	nd
Phloridzin	11.4 ± 5.7^{a}	2.3 ± 0.7^{b}	nd	1.4 ± 0.7^{b}	nd	0.2 ± 0.0^{c}
Chlorogenic acid	1.2 ± 0.1^{a}	nd	nd	nd	nd	2.2 ± 0.7^{a}
Ferulic acid	4.5 ± 0.3^{a}	2.3 ± 0.7^{b}	0.3 ± 0.0^{c}	nd	0.2 ± 0.0^{c}	0.7 ± 0.0^{d}
Gallic acid	12.7 ± 2.3^{a}	19.3 ± 2.8^{a}	0.6 ± 0.0^{b}	5.5 ± 1.4^{c}	1.2 ± 0.0^{d}	1.7 ± 0.2^{d}
Hippuric acid	2.8 ± 0.4^{a}	2.6 ± 0.2^{a}	nd	0.5 ± 0.2^{b}	0.6 ± 0.3^{b}	3.9 ± 0.9^{c}
Caffeic acid	9.7 ± 3.3^{a}	16.1 ± 0.7^{b}	nd	2.2 ± 0.7^{c}	3.4 ± 0.3^{c}	1.9 ± 0.0^{d}
p-Coumaric acid	14.6 ± 4.1^{a}	3.5 ± 1.2^{b}	nd	3.2 ± 0.2^{b}	nd	0.5 ± 0.0^{c}
Quercetin	0.3 ± 0.0^{a}	0.4 ± 0.0^{a}	nd	nd	nd	0.7 ± 0.0^{b}
Quercetin-3-*O*-glucoside	154.0 ± 17.1^{a}	76.0 ± 16.9^{b}	nd	5.2 ± 1.2^{c}	2.5 ± 0.2^{d}	0.7 ± 0.1^{e}
Quercetin-3-*O*-rutinoside	9.4 ± 1.3	nd	nd	nd	nd	nd
Pelargonidin-3-*O*-glucoside	9.3 ± 2.4	nd	nd	nd	nd	nd
Procyanidin B1	23.9 ± 9.9^{a}	23.1 ± 9.7^{a}	nd	23.5 ± 4.6^{a}	nd	nd
Procyanidin B2	44.5 ± 10.4^{a}	8.1 ± 3.0^{b}	0.6 ± 0.2^{c}	2.1 ± 0.0^{d}	nd	1.2 ± 0.5^{d}

Values are expressed per kilogram of fresh weight. Means marked with the same superscript letter in a row are not significantly different at $p < 0.05$, $n = 3$.
nd – not detected, content of the compound was below method sensitivity.

Tab. 7. Profile of phenolic compounds at individual stages of digestion of chokeberry.

Compound [mg·kg^{-1}]	Raw material	Supernatant	Sediment	Retentate	Permeate	Post-fermentation residue
Cyanidin-3-*O*-galactoside	53.0 ± 7.0	nd	nd	nd	nd	nd
Cyanidin-3-*O*-glucoside	355.2 ± 22.5^{a}	58.7 ± 10.1^{b}	26.1 ± 0.6^{c}	38.2 ± 8.5^{d}	nd	nd
Delphinidin-3-*O*-glucoside	25.5 ± 2.1^{a}	4.8 ± 0.9^{b}	nd	nd	nd	nd
Chlorogenic acid	4.5 ± 0.3^{a}	2.3 ± 0.0^{b}	0.6 ± 0.0^{c}	2.3 ± 0.0^{b}	0.2 ± 0.0^{d}	2.1 ± 0.0^{b}
Ferulic acid	1.1 ± 0.2^{a}	1.2 ± 0.0^{a}	nd	2.1 ± 0.2^{b}	nd	0.9 ± 0.0^{c}
Gallic acid	47.6 ± 4.1^{a}	40.3 ± 8.3^{a}	3.8 ± 0.0^{b}	19.1 ± 2.1^{c}	1.5 ± 0.0^{d}	9.7 ± 1.6^{e}
Hippuric acid	2.6 ± 0.2^{a}	1.5 ± 0.2^{b}	1.9 ± 0.0^{b}	1.5 ± 0.6^{b}	1.5 ± 0.0^{b}	12.5 ± 2.5^{c}
Caffeic acid	172.9 ± 11.5^{a}	3.3 ± 0.2^{b}	0.3 ± 0.0^{c}	2.6 ± 0.5^{b}	nd	5.5 ± 0.5^{d}
p-Coumaric acid	7.2 ± 1.0^{a}	3.3 ± 0.2^{b}	nd	3.2 ± 0.0^{b}	nd	3.2 ± 0.2^{b}
Quercetin	0.3 ± 0.0^{a}	0.5 ± 0.0^{a}	nd	nd	nd	7.1 ± 0.7^{b}
Quercetin-3-*O*-glucoside	43.2 ± 7.5^{a}	27.1 ± 3.2^{b}	nd	15.2 ± 0.7^{c}	nd	0.6 ± 0.0^{d}
Quercetin-3-*O*-rutinoside	24.0 ± 1.2^{a}	13.6 ± 0.7^{b}	nd	9.9 ± 0.5^{c}	nd	8.1 ± 0.7^{c}
Peonidin-3-glucoside	2.6 ± 0.0	nd	nd	nd	nd	nd
Procyanidin B1	71.1 ± 1.1	nd	nd	nd	nd	nd
Procyanidin B2	97.2 ± 9.3	nd	nd	nd	nd	nd

Values are expressed per kilogram of fresh weight. Means marked with the same superscript letter in a row are not significantly different at $p < 0.05$, $n = 3$.
nd – not detected, content of the compound was below method sensitivity.

Tab. 8. Profile of phenolic compounds at individual stages of digestion of elderberry.

Compound [mg·kg^{-1}]	Raw material	Supernatant	Sediment	Retentate	Permeate	Post-fermentation residue
Cyanidin-3-*O*-glucoside	22.3 ± 5.2^{a}	5.1 ± 0.2^{b}	nd	6.0 ± 0.5^{b}	nd	nd
Cyanidin-3-*O*-sambubioside	7782.1 ± 982.1^{a}	1676.2 ± 268.9^{b}	555.7 ± 91.4^{c}	764.4 ± 123.1^{c}	121.1 ± 10.8^{d}	99.8 ± 11.2^{d}
Chlorogenic acid	0.4 ± 0.0^{a}	4.1 ± 0.5^{b}	nd	4.4 ± 0.5^{b}	1.2 ± 0.0^{c}	1.2 ± 0.0^{c}
Gallic acid	75.3 ± 10.2^{a}	86.0 ± 7.6^{a}	12.2 ± 2.3^{b}	13.6 ± 1.8^{b}	4.9 ± 0.0^{c}	3.5 ± 0.5^{c}
Hippuric acid	5.6 ± 1.2^{a}	2.5 ± 0.7^{b}	2.3 ± 0.6^{b}	2.7 ± 0.5^{b}	1.5 ± 0.0^{c}	9.8 ± 1.8^{d}
Caffeic acid	41.9 ± 7.1^{a}	27.8 ± 2.5^{b}	0.5 ± 0.0^{c}	1.2 ± 1.2^{c}	nd	19.6 ± 2.1^{d}
p-Coumaric acid	55.5 ± 4.9^{a}	12.3 ± 0.7^{b}	2.9 ± 0.0^{c}	nd	nd	nd
Protocatechuic acid	3.2 ± 0.2^{a}	43.5 ± 6.2^{b}	1.5 ± 0.0^{c}	31.7 ± 3.9^{d}	23.4 ± 4.9^{e}	12.7 ± 1.6^{e}
Quercetin	0.8 ± 0.0^{a}	0.2 ± 0.0^{a}	nd	nd	3.0 ± 0.0^{b}	10.8 ± 2.8^{c}
Quercetin-3-*O*-glucoside	71.3 ± 10.8^{a}	8.9 ± 1.2^{b}	2.9 ± 0.0^{c}	6.4 ± 0.7^{d}	nd	2.8 ± 0.2^{c}
Quercetin-3-*O*-rutinoside	467.2 ± 31.1^{a}	304.8 ± 30.6^{b}	16.5 ± 0.6^{c}	167.0 ± 22.8^{d}	nd	12.4 ± 0.7^{c}
Procyanidin B1	162.4 ± 8.5^{a}	59.6 ± 2.3^{b}	nd	30.1 ± 2.5^{c}	nd	36.3 ± 2.8^{c}
Procyanidin B2	20.4 ± 1.3^{a}	15.9 ± 3.2^{b}	nd	nd	nd	nd

Values are expressed per kilogram of fresh weight. Means marked with the same superscript letter in a row are not significantly different at $p < 0.05$, $n = 3$.
nd – not detected, content of the compound was below method sensitivity.

Tab. 9. Profile of phenolic compounds at individual stages of digestion of blackcurrant.

Compound [mg·kg^{-1}]	Raw material	Supernatant	Sediment	Retentate	Permeate	Post-fermentation residue
Cyanidin-3-*O*-arabinoside	28.5 ± 4.9^{a}	nd	nd	nd	nd	nd
Cyanidin-3-*O*-galactoside	43.2 ± 11.2^{a}	nd	nd	nd	nd	nd
Cyanidin-3-*O*-glucoside	85.7 ± 22.1^{a}	nd	nd	nd	nd	nd
Phloridzin	11.3 ± 4.1^{a}	2.8 ± 1.2^{b}	nd	1.4 ± 0.5^{c}	nd	nd
Chlorogenic acid	5.1 ± 0.7^{a}	2.8 ± 0.5^{b}	nd	0.5 ± 0.2^{c}	nd	0.9 ± 0.2^{c}
Ferulic acid	5.1 ± 1.3^{a}	2.1 ± 0.9^{b}	nd	0.5 ± 0.0^{c}	nd	0.2 ± 0.0^{c}
Gallic acid	24.1 ± 7.2^{a}	18.9 ± 2.1^{a}	0.6 ± 0.0^{b}	0.5 ± 0.0^{b}	1.2 ± 0.0^{b}	0.5 ± 0.0^{b}
Hippuric acid	1.6 ± 0.4^{a}	0.8 ± 0.3^{b}	nd	nd	0.5 ± 0.1^{b}	6.9 ± 3.0^{c}
Caffeic acid	31.4 ± 9.1^{a}	17.5 ± 3.9^{b}	0.6 ± 0.1^{d}	2.5 ± 0.5^{c}	2.5 ± 0.9^{c}	5.5 ± 2.1^{c}
Protocatechuic acid	31.5 ± 10.1^{a}	41.4 ± 16.3^{a}	nd	10.6 ± 2.8^{b}	2.5 ± 0.1^{c}	12.0 ± 0.1^{b}
Quercetin	1.1 ± 0.2^{a}	0.7 ± 0.0^{a}	nd	0.2 ± 0.0^{b}	nd	0.5 ± 0.0^{ab}
Quercetin-3-*O*-glucoside	12.5 ± 4.1^{a}	4.4 ± 0.9^{b}	0.6 ± 0.0^{c}	3.2 ± 0.7^{b}	nd	0.9 ± 0.1^{c}
Quercetin-3-*O*-rutinoside	11.2 ± 2.9^{a}	11.5 ± 2.8^{a}	nd	5.5 ± 1.4^{b}	nd	2.3 ± 0.7^{c}
Pelargonidin-3-*O*-glucoside	99.1 ± 24.9^{a}	46.2 ± 13.8^{b}	nd	nd	nd	nd

Values are expressed per kilogram of fresh weight. Means marked with the same superscript letter in a row are not significantly different at $p < 0.05$, $n = 3$.
nd – not detected, content of the compound was below method sensitivity.

TPC (23.25 g·kg^{-1} FW) (Tab. 1). Anthocyanins were the largest group of polyphenols in the black currant and significant quantities of phenolic acids were detected as well (Tab. 9). After simulated digestion in stomach and duodenum, *AOX* increased more than 3-fold while *TPC* of the supernatant was 17-fold lower (Tab. 1). Phenolic compounds of black currant migrated through the dialysis membrane with a low efficiency (only 23 % of the initial quantity got to the permeate), which was accompanied by a 20% decrease in the antioxidant capacity of the permeate. Intestinal bacteria did not play any important role in decomposition of phenolic compounds of black currant.

DISCUSSION

The lowest values of *TPC* and *AOX* were determined for apples but these fruits should be considered as one of the major sources of polyphenols in the diet of most European countries due

to their frequent consumption [5]. Procyanidins and (+)-catechin were dominant polyphenols in apples, which confirms the findings of Alonso-Salces et al. [6]. Those authors reported that, in addition to (+)-catechin and procyanidins, dihydrochalcones, flavonols, glycosides of cyanidin and hydroxycinnamic acid derivatives could also be detected in apples. It was shown that the content of quercetin glycosides was 6.7–24.0 mg·kg^{-1} FW, while the content of (–)-epicatechin in Golden Delicious cultivar was 50.8–74.2 mg·kg^{-1} FW [5, 7]. *AOX* of the supernatant determined after the first digestion step of apples increased but *TPC* of this fraction decreased. The appearance of the metabolites of polyphenol digestion with high AOX could explain this phenomenon. Polymeric compounds were probably decomposed to monomers, which have a greater antioxidant potential. Bouayed et al. [8] reported that polyphenols present in apples were decomposed mainly in stomach (65%) during digestion and only 10% of phenolic compounds was degraded during further digestion steps in the small intestine. This indicates the sensitivity of those compounds to the alkaline environment as well as to bile salts and pancreatin. Our study showed that a small amount of phenolic compounds with low *AOX* went to the sediments. This was related to the high proportion of pulp compared to peel and seeds in apple fruits. *AOX* of the post-fermentation residue was higher in comparison to the fraction subjected to that digestion step. In this fraction, phenolic acids demonstrating average *AOX* were the most abundant but the products of degradation of procyanidins and phlorizin, which have a high antioxidant activity, were probably present as well.

Anthocyanins responsible for the colour of bilberry are the main group of phenolic compounds and they constituted 90% of polyphenols contained in those fruits. Glycosides of cyanidin, malvidin, delphinidin, peonidin and petunidin are located mostly in the peel of bilberry, while they occur in the form of granules of various sizes in vacuoles [9]. Gao and Mazza [10] reported that main polyphenols in bilberry are galactosides, glycosides and arabinosides of petunidin, delphinidin and malvidin. Main flavonols present in those berries are quercetin galactoside, glucoside and rhamnoside. Other phenolic compounds with a high content in bilberries were chlorogenic, caffeic and ferulic acids, and (–)-epicatechin [9, 11]. Our study showed that bilberry fruits contained relatively high levels of anthocyanins. However, it should be noted that only derivatives of cyanidin were considered in the current study, while berries contain more delphinidin and malvidin derivatives [10]. This indicates that the actual level of anthocyanins in those fruits was probably higher. Jabłońska-Ryś et al. [12] demonstrated that *AOX* of bilberries was 16.87 g·kg^{-1} FW, while, in our research, *AOX* of bilberry fruits was 28.38 g·kg^{-1} FW.

Ripe, dried goji berries contain large quantities of vitamin C (25 g·kg^{-1} of fruit) and carotenoids (0.5% dry weight, DW). An important component of this fruit is a bioactive polysaccharide complex LBP (*Lycium barbarum* polysaccharides), which account for 5–8% of the dry matter of the berries [13]. Medina [14] stated that the amount of polyphenols in goji berries was 8.95–10.36 g·kg^{-1} FW (expressed as gallic acid equivalents). Klenow et al. [15] showed that the main flavonoids present in goji berries are rhamnoside and rutinoside of quercetin, while chlorogenic, caffeic and *p*-coumaric acids are the dominant phenolic acids. In our study, we confirmed that quercetin derivatives, (+)-catechin and phenolic acids dominated in goji berries and *TPC* was 14.31 g·kg^{-1} FW. *AOX* of goji berries was previously found to range from 4.91 g·kg^{-1} FW [5] to 61.10 g·kg^{-1} FW [16]. This lack of data consistency may be caused by many factors, including the differences in methods and temperatures applied during fruit drying.

The content of identified phenolic compounds in goji berries was much lower than in bilberries. Due to the activity of acids and enzymes in stomach and duodenum, glycosides were the most degradable compounds. Quercetin glycosides were also metabolized by the intestinal microbiota and the content of quercetin aglycone in the post-fermentation residue was 2-fold higher than in fresh goji berries. Phenolic compounds of goji fruits efficiently migrated trough the dialysis membrane (29% of the quantity detected in the supernatant), while the migration rate was lower for bilberry (19%). Dialysates obtained at that stage of simulated digestion demonstrated similar *AOX*, even though *TPC* in the bilberry dialysate was two times lower than in goji berries. During the digestion of bilberry that is rich in anthocyanins, high amounts of cyanidin and its glycosides were released. The presence of these compounds that are strong antioxidants may be responsible for high antioxidant activity [17]. It was also demonstrated, in the present study, that polyphenols extracted from goji berries were more efficiently metabolized by the intestinal bacteria than compounds from bilberry. High content of (–)-epicatechin detected in the retentate might be toxic for the colon bacteria and inhibit the processes of polyphenols biotransformation and metabolism. Sun et al. [18] showed very strong antibacterial properties of catechins

against a wide spectrum of bacteria. As a result of the degradation of phenolic compounds, phenolic acids were mainly detected in the digestion residues of bilberries and goji berries.

Du et al. [19] indicated catechins and leucoanthocyanins as the main phenolic compounds in ripe Japanese quince fruits. Polyphenol profile obtained in our study indicated that the derivatives of quercetin and catechins, phenolic acids and procyanidins were major compounds. *AOX* of Japanese quince (22.16 $g \cdot kg^{-1}$ FW) was moderate in comparison to other tested plant materials. In addition to phenolic compounds, high content of vitamin C might also contribute to the antioxidant activity of the Japanese quince [19]. *TPC* in the supernatants obtained after digestion of Japanese quince was lower than in fruits but *AOX* was significantly higher. The fruits of Japanese quince are rich in procyanidins – polyphenols with high antiradical activity, which are digested in stomach and duodenum to a small extent. Probably degradation of procyanidins resulted in the increased levels of (+)-catechin in the supernatant comparing to fruits.

Feghali et al. [20] stated that *TPC* in cranberries was approx. 20 $g \cdot kg^{-1}$ DW. Glycosides of cyanidin, pelargonidin, petunidin and malvidin are dominant anthocyanins of that fruit. Ehala et al. [11] indicated cranberries as a source of phenolic acids and quercetin. Current study showed that cranberry fruits were rich in quercetin glycosides, phenolic acids and procyanidins. As in the case of Japanese quince, supernatants obtained after digestion of cranberries demonstrated lower *TPC* in comparison to fruits but they held only slightly higher *AOX* than fruits. The analysis of sediments obtained after simulated digestion of Japanese quince and cranberries showed substantially higher *TPC* in the case of Japanese quince, which resulted from the high content of peel in relation to the pulp in Japanese quince fruit. Simulation of passive transport through the dialysis membrane also showed significantly greater migration rate of polyphenols from quince fruits than from cranberries.

In the studies involving Caco-2 cell line, it was shown that only dimers and trimers formed during the degradation of proanthocyanidins penetrated the epithelium of the small intestine. Polymeric forms of those compounds also demonstrated low absorption by the colon epithelium [20]. Comparing the last stage of simulated digestion to the previously analysed fractions, it can be concluded that intestinal bacteria metabolized polyphenols present in the fruits of cranberry and Japanese quince to the same extent, but metabolites derived from cranberries had higher *AOX*.

Oszmiański and Wojdyło [21] showed that proanthocyanins and cyanidin glycosides were dominant polyphenols of chokeberry fruits consituting 66 % and 25 % of total phenolic compounds, respectively. Moreover, phenolic acids (chlorogenic and neochlorogenic acids) and flavonols, mainly represented by quercetin glycosides, were also detected in the fruits of black chokeberry. Our study confirmed that chokeberry fruits contain high content of phenolic compounds, mainly anthocyanins, procyanidins B1 and B2, and phenolic acids. *AOX* and *TPC* of the supernatant and the permeate showed that chokeberry is not only the richest source of antioxidants but also that those antioxidants are bioaccessible. Bermúdez-Soto et al. [22] studied the stability of phenolic compounds of black chokeberry during in vitro digestion. They found that main polyphenols of chokeberry were stable in stomach but they were degraded in contact with pancreatin. The losses of anthocyanins subjected to pancreatin for 2 h reached 43 % and were the largest among phenolic compounds of chokeberry. At the same time, the content of neochlorogenic and chlorogenic acids decreased by 28 % and 24 %, respectively. It was also found that alkaline environment had greater impact on transformation of chokeberry polyphenols than the activity of digestive enzymes. Our study showed that also intestinal bacteria contributed significantly to degradation of phenolic compounds of chokeberry fruits.

Wu et al. [17] showed that *TPC* in elderberry fruits was about 64 $g \cdot kg^{-1}$ FW, while the *AOX* was approximately 62 $g \cdot kg^{-1}$ FW. In our study, *AOX* and *TPC* were 44.02 $g \cdot kg^{-1}$ FW and 80.71 $g \cdot kg^{-1}$ FW, respectively. Galić et al. [23] showed that elderberry fruits were rich in biologically active flavonoids, anthocyanins and vitamins. The total content of those compounds in fruits ranged from 2 $g \cdot kg^{-1}$ to 10 $g \cdot kg^{-1}$ FW and the following anthocyanins dominated in polyphenol profile: cyanidin-3-*O*-sambubioside, cyanidin-3-*O*-glucoside and cyanidin-3,5-*O*-diglucoside. Wu et al. [17] also showed that anthocyanins present in elderberry were absorbed during digestion moderately, in comparison to other flavonoids. In the current study, anthocyanins (cyanidin-3-*O*-sambubioside and quercetin-3-*O*-rutinoside) were responsible for *AOX* of the supernatant obtained during simulated digestion of chokeberry fruits. The sediment obtained after simulated digestion of elderberry demonstrated the largest decrease in *TPC* and *AOX* in relation to the adequate supernatants. This proved that the share of the insoluble fraction of phenolic compounds in those fruits was low.

Black currant fruits demonstrated low *AOX* (10.92 g·kg^{-1} FW) and *TPC* (23.25 g·kg^{-1} FW). Gramza-Michałowska and Człapka-Matyasik [24] obtained similar results (18.61 g·kg^{-1} FW) and classified black currant to the group of fruits with low *AOX*. In the same study, *TPC* was 10.6 g·kg^{-1} (expressed as gallic acid). Black currant fruits are a rich source of vitamin C, which demonstrates *AOX* lower than most of phenolic compounds. Numerous papers [17, 25] classified those fruits as food rich in anthocyanin pigments, mainly glycosides of delphinidin and cyanidin, and – in smaller quantities – glycosides of petunidin and pelargonidin. Except for anthocyanin derivatives of phenolic acids, proanthocyanidins and flavonol glycosides were also present. Rechner et al. [26] found that anthocyanin glycosides were the main metabolites of black currant present in the human body.

It was demonstrated that mostly phenolic acids with a low *AOX* [1] pass to the permeate. The permeate obtained after apple or quince digestion additionally contained phloridzin, while quercetin glucosides were present in the cranberry permeate. Moreover, elderberry permeate contained cyanidin-3-*O*-sambubioside and quercetin. It was demonstrated that, in comparison to glycosides, aglycones had a high *AOX* [26], so the presence of quercetin in the elderberry permeate could contribute to its high *AOX*. Flavonoids and their derivatives which passed to permeates are mostly active in epithelium cells, however, their presence was also demonstrated in brain and prostate of rats [3]. On the other hand, phenolic acids were associated with anticarcinogenic effect and some of them demonstrated higher activities than the parent anthocyanins [27]. Next to the passive diffusion, active transport is also involved in polyphenol absorption in the human digestive tract.

It can be concluded that *TPC* was lower at all digestion stages than in plant materials. However, in most cases, *AOX* of supernatants obtained after the first digestion step was significantly higher than in fruits, which suggest that phenolic compounds were transformed to derivatives with higher *AOX* after subjecting them to digestive enzymes. Fruits with higher peel content in relation to the flesh demonstrated higher *TPC* in the insoluble fraction. Tested fruits showed various migration rates of phenolic compounds through the dialysis membrane. Black chokeberry and elderberry fruits are good sources of polyphenols in the human diet. Insoluble phenolic compounds in the studied fruits, including condensed tannins, are partially or completely decomposed by bacterial fermentation during the digestion in the large intestine. It was shown that bilberry fruits had the highest *TPC* and *AOX* in the post-fermentation residue. Many phenolic compounds of bilberry fruits, despite their high *AOX*, were not metabolized at the stage of gastric and duodenum digestion, and did not pass through the membrane to permeate. However, those compounds were decomposed by the colon microflora. Concluding, the structure and character of phenolic compounds, raw material properties and interactions with other food ingredients have the greatest impact on the processes taking place during digestion of polyphenols.

Acknowledgements

This project was financially supported by the research grant (decision number DEC-2011/01/B/NZ9/00218) from the National Science Centre, Krakow, Poland.

REFERENCES

1. Rice-Evans, C. A. – Miller, N. J. – Paganga, G.: Antioxidant properties of phenolic compounds. Trends in Plant Science, *2*, 1997, pp. 152–159. DOI: 10.1016/S1360-1385(97)01018-2.
2. Molina, M. F. – Sanchez-Reus, I. – Iglesias, I. – Benedi, J.: Quercetin, a flavonoid antioxidant, prevents and protects against ethanol-induced oxidative stress in mouse liver. Biological and Pharmaceutical Bulletin, *26*, 2003, pp. 1398–1402. DOI: 10.1248/bpb.26.1398.
3. Manach, C. – Scalbert, A. – Morand, C. – Remsy, C. – Jimenez, L.: Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition, *79*, 2004, pp. 727–747. ISSN: 0002-9165. <http://ajcn.nutrition.org/content/79/5/727.full.pdf+html>
4. ISO 14502-1:2005. Determination of substances characteristic of green and black tea – Part 1: Content of total polyphenols in tea – Colorimetric method using Folin-Ciocalteu reagent. Geneva : International Organization for Standardization, 2005.
5. Lee, K. W. – Kim, Y. J. – Kim, D.-O. – Lee, H. J. – Lee, C. Y.: Major phenolics in apple and their contribution to the total antioxidant capacity. Journal of Agricultural and Food Chemistry, *51*, 2003, pp. 6516–6520. DOI: 10.1021/jf034475w.
6. Alonso-Salces, R. M. – Korta, E. – Barranco A. – Berrueta, L. A. – Gallo, B. – Vicente, F.: Pressurized liquid extraction for the determination of polyphenols in apple. Journal of Chromatography A, *933*, 2001, pp. 37–43. DOI: 10.1016/S0021-9673(01)01212-2.
7. Duda-Chodak, A. – Tarko, T. – Satora, P. – Sroka, P. – Tuszyński, T.: The profile of polyphenols and antioxidant properties of selected apples cultivars grown in Poland. Journal of Fruit and Ornamental Plant Research, *18*, 2010, pp. 39–50. ISSN: 1231-0948. <http://www.inhort.pl/files/journal_pdf/journal_2010_2/full4%202010(2).pdf>
8. Bouayed, J. – Hoffmann, L. – Bohn, T.: Total pheno-

lics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. Food Chemistry, *128*, 2011, pp. 14–21. DOI: 10.1016/j.foodchem.2011.02.052.
9. Määttä-Riihinen, K. R. – Kamal-Eldin, A. – Mattila, P. H. – González-Paramás, A. M. – Törrönen, A. R.: Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 4477–4486. DOI: 10.1021/jf049595y.
10. Gao, L. – Mazza, G.: Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. Journal of Food Science, *59*, 1994, pp. 1057–1059. DOI: 10.1111/j.1365-2621.1994.tb08189.x.
11. Ehala, S. – Vaher, M. – Kaljurand, M.: Characterization of phenolic profiles of northern european berries by capillary electrophoresis and determination of their antioxidant activity. Journal of Agricultural and Food Chemistry, 53, 2005, pp. 6484–6490. DOI: 10.1021/jf050397w.
12. Jabłońska-Ryś, E. – Zalewska-Korona, M. – Kalbarczyk, J.: Antioxidant capacity, ascorbic acid and phenolics content in wild edible fruit. Journal of Fruit and Ornamental Plant Research, *17*, 2009, pp. 115–120. ISSN: 1231-0948. <http://www.insad.pl/files/journal_pdf/journal2009_2/full11%202009(2).pdf>
13. Cieślik, E. – Gębusia, A.: Charakterystyka właściwości prozdrowotnych owoców roślin egzotycznych. (Characteristics of health properties of exotic fruit). Postępy Fitoterapii, *2*, 2012, pp. 93–100. ISSN: 1509-8699. In Polish.
14. Medina, M.: Determination of the total phenolics in juices and superfruits by a novel chemical method. Journal of Functional Food, *3*, 2011, pp. 79–87. DOI: 10.1016/j.jff.2011.02.007.
15. Klenow, S. – Latté, K. P. – Wegewitz, U. – Dusemund, B. – Pöting, A. – Appel, K. E. – Großklaus, R. – Schumann, R. – Lampen, A.: Risikobewertung von Pflanzen und pflanzlichen Zubereitungen. Berlin : Bundesinstitut für Risikobewertung, 2012. ISBN: 3938163763. <http://www.bfr.bund.de/cm/350/risikobewertung-von-pflanzen-und-pflanzlichen-zubereitungen.pdf>
16. Fan, H. – Mazza, G. – Liao, X.: Purification, composition and antioxidant activity of polysaccharides from wolfberry, cherry, kiwi and cranberry fruits. Croatian Journal of Food Science and Technology, *2*, 2010, pp. 9–17. ISSN: 1848-9923. <http://hrcak.srce.hr/file/89739>
17. Wu, X. – Gu, L. – Prior, R. L. – McKay, S.: Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 7846–7856. DOI: 10.1021/jf0486850.
18. Sun, L. – Zhang, C. – Li, P.: Characterization, antimicrobial activity, and mechanism of a high-performance (–)-epigallocatechin-3-gallate (EGCG)-CuII/polyvinyl alcohol (PVA) nanofibrous membrane. Journal of Agricultural and Food Chemistry, *59*, 2011, pp. 5087–5092. DOI: 10.1021/jf200580t.
19. Du, H. – Wu, J. – Li, H. – Zhong, P.-X. – Xu, Y.-J. – Li, C.-H. – Ji, K.-X. – Wang, L.-C.: Polyphenols and triterpenes from *Chaenomeles* fruits: Chemical analysis and antioxidant activities assessment. Food Chemistry, *141*, 2013, pp 4260–4268. DOI: 10.1016/j.foodchem.2013.06.109.
20. Feghali, K. – Feldman, M. – La, V. D. – Santos, J. – Grenier, D.: Cranberry proanthocyanidins: natural weapons against periodontal diseases. Journal of Agricultural and Food Chemistry, *60*, 2012, pp. 5728–5735. DOI: 10.1021/jf203304v.
21. Oszmiański, J. – Wojdyło, A.: *Aronia melanocarpa* phenolics and their antioxidant activity. European Food Research and Technology, *221*, 2005, pp. 809–813. DOI: 10.1007/s00217-005-0002-5.
22. Bermúdez-Soto, M. J. – Tomas-Barberan, F. A. – Garcia-Conesa, M. T.: Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to *in vitro* gastric and pancreatic digestion. Food Chemistry, *102*, 2007, pp. 865–874. DOI: 10.1016/j.foodchem.2006.06.025.
23. Galić, A. – Dragović-Uzelac, V. – Levaj, B. – Bursać-Kovacević, D. – Pliestić, S. – Arnautović, S.: The polyphenols stability, enzyme activity and physicochemical parameters during producing wild elderberry concentrated juice. Agriculturae Conspectus Scientificus, *74*, 2009, pp. 181–186. ISSN: 1331-7776. <https://acs.agr.hr/acs/index.php/acs/article/download/447/431>
24. Gramza-Michałowska, A. – Człapka-Matyasik, M.: Evaluation of the antiradical potential of fruit and vegetable snack. Acta Scientiarum Polonorum, Technologia Alimentaria, *10,* 2011, pp. 61–72. ISSN: 1644-0730. <http://www.food.actapol.net/pub/5_1_2011.pdf>
25. Mattila, P. H. – Hellström, J. – McDougall, G. – Dobson, G. – Pihlava, J.-M. – Tiirikka, T. – Steward, D. – Karjalainen, R.: Polyphenol and vitamin C contents in European commercial blackcurrant juice products. Food Chemistry, *127*, 2011, pp. 1216–1233. DOI: 10.1016/j.foodchem.2011.01.129.
26. Rechner, A. R. – Kuhnle, G. – Hu, H. – Roedig-Penman, A. – van den Braak, M. H. – Moore, K. P. – Rice-Evans, C. A.: The metabolism of dietary polyphenols and the relevance to circulating levels of conjugated metabolites. Free Radical Research, *36*, 2002, pp. 1229–1241. DOI: 10.1080/246-1071576021000016472.
27. Duda-Chodak, A. – Tarko, T. – Satora, P. – Sroka, P.: Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. European Journal of Nutrition, *54*, 2015. pp. 325–341. DOI: 10.1007/s00394-015-0852-y.

Received 19 January 2017; 1st revised 9 March 2017; accepted 10 April 2017; published online 18 May 2017.