Transformations of Phenolic Compounds in an *in vitro* Model Simulating the Human Alimentary Tract

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Summary

The aim of this work is to establish the antioxidant properties of polyphenolic compounds of selected fruits before and after their transformations during digestion. The experiment was conducted in *in vitro* conditions on a set of dialysis membranes which simulated the human digestive tract. Apples of the Šampion, Malinowka and Golden Delicious cultivars, black chokeberry, banana, Wegierka zwykla blue plum, melon and Lukasowka pear were chosen for examination. It was found that compounds obtained after simulated digestion of chokeberries, pears and bananas showed lower antioxidant potential than fresh fruits, while the opposite results were obtained for apples and plums. All dialysates obtained after digestion were characterized by lower content of total polyphenols in comparison with raw material (fresh fruits). It was found that the polyphenols were hydrolyzed, especially glycosides of quercetin and cyanidin. Phenolic acids and cyanidin were characterized by low availability for absorption, whereas catechin and quercetin had a very high level of accessibility in the model small intestine.

Key words: phenolic compounds, fruits, *in vitro* digestion, dialysate membranes, antioxidant activity

Introduction

Vitamins C, E and A, carotenoids and a very numerous group of phenolic compounds belong to substances which can neutralize free radicals. The basic sources of polyphenols in the diet are fruits (apples, berries, citruses) and vegetables (onion, broccoli, cucurbits, soybean), but they can also be identified in herbs, coffee and tea.

Intake of a large amount of polyphenol-rich products is not directly linked with the concentration of these compounds in blood and tissues. This may be due to either low uptake rate from the alimentary tract or their rapid metabolic transformation and elimination (*1*). On top of that, the products of polyphenol metabolism often differ from the native forms as far as antioxidant activity is concerned, once they reach the blood and tissues (*2,3*). Therefore, it is important to recognize the mechanisms of digestion of polyphenols and their assimilation.

The effectiveness of absorption of polyphenols from the alimentary tract is influenced by many factors, *e.g.* molecular mass, environmental pH, hydrophobicity, degree of polymerization and kind of sugar present in the molecule. Aglycons, because of their hydrophobic character, penetrate biological membranes by passive diffusion, whereas most flavonoids exist as glycosides and have to be hydrolyzed by intestinal enzymes or intestinal microflora before diffusion (*4*). However, it was found that daidzein and genistein are absorbed in the stomach but only as aglycons (*5*), while glycosides of quercetin can be assimilated in the small intestine (*6*).

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Flavonoid glycosides do not undergo acid hydrolysis in the stomach and pass unchanged to the intestine. There, enzymatic hydrolysis and cleavage of the sugar moiety take place (*7*). Absorbed products of intestinal metabolism are transported with the blood to the liver and are enzymatically transformed in deglycosidation, glucuronisation and sulphonation reactions (*4*). Some polyphenols and their metabolites are transported from the liver with the blood to tissues and kidneys, where their subsequent transformation occurs, or they are excreted in the bile (*8*). Polyphenol metabolites which are not digested and absorbed in the small intestine are actively metabolized by large intestine microflora.

In many experiments *in vitro* and *in vivo* the health-promoting properties of polyphenolic compounds have been demonstrated (*9,10*). However, despite their presence in the diet, many of them cannot develop biological activity because of the metabolic changes they are subject to in the human body (*8*). It is necessary to revise ideas in this area and recognise which of these components are best absorbed and can be transformed into active metabolites.

The main aim of this work is to assess antioxidant activity of polyphenolic compounds which passed from some fruits into dialysates during their simulated digestion process with dialysis membranes. In our experiments a simple model of membranes with mapping of proper pH, temperature and digestion time was used. The used model did not take into account the interactions connected with the activity of alimentary tract microflora. Since lipids in fruits are trace elements, there was no addition of bile salts, which could complicate the model and would not add anything important to the experiment. The experiment was planned to show how much of any polyphenolic component despite the composition of the intestinal microflora passes into the dialysate and how high its antioxidant activity is.

Materials and Methods

The experimental material consisted of the Šampion, Malinowka and Golden Delicious cultivars of apples, black chokeberry, banana, Wegierka zwykla blue plum, Lukasowka pear and melon, bought in the local supermarket.

Chemicals

Chemicals used in this research were the following: diammonium salt of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid (ABTS diammonium salt), (±)-6--hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), a phosphate buffer saline (PBS) containing: 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride at pH=7.4; and the enzymes: pepsin (activity 3300 U/mg), pancreatin (activity equivalent to $8 \times$ USP (U.S. Pharmacopeia)), β-glucosidase, β-xylosidase, β-galactosidase and β-hesperidase. All the chemicals listed, as well as HPLC standards and dialysis sacks (D9777--100FT), were purchased from the Sigma-Aldrich Company (Munich, Germany). The chemicals: hydrochloric acid (HCl), sodium hydrogencarbonate $(NaHCO_3)$, sodium carbonate (Na_2CO_3), potassium persulphate $(K_2S_2O_8)$, methanol (analytically pure), and other basic chemicals were obtained from the POCh Company (Gliwice, Poland).

In vitro simulation of digestion process

The method described by Żyła *et al.* (*11*) was utilized to perform *in vitro* digestion, with modifications to obtain conditions of the human alimentary tract. The samples were subjected to two subsequent incubations in order to simulate the stomach (pH=2.0) and small intestine (pH=7.0) conditions. For this purpose, the analyzed fruit (0.5 g) was acidified to pH=2.0 (by the addition of 1 M HCl in the amount determined on the basis of earlier titrations), then 0.75 mL of pepsin were added and made up to 3 mL with redistilled water. The obtained mixture was agitated and incubated in a water bath for 2 h (at 37 °C). The samples were neutralized (1.25 M $NaHCO_3$ was added in the amount estimated on the basis of earlier titrations), 0.375 mL of pancreatin was added and made up to 1.15 mL with redistilled water. The resulting mixture was transferred to the dialysis sacks, placed in an Erlenmeyer flask containing 50 mL of phosphate buffer (PBS) and incubated in a rotary shaker (4 h, 80 cpm, 37 °C). The PBS buffer together with the compounds that passed through the membrane (dialysate) were treated as an equivalent of the raw material absorbed in the intestine after digestion. The results obtained were calculated and expressed per 100 g of fresh mass of the raw material.

Antioxidant activity assay

Antioxidant activity of the raw material and dialysate was determined according to Re *et al.* (*12*) with slight modifications. ABTS radical was generated in the chemical reaction between the 7 mM diammonium salt of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid and the 2.45 mM potassium persulphate. In order to terminate the reaction and to stabilize the ABTS cation radical, the solution was kept overnight in the dark at ambient temperature. Prior to analysis, the radical solution was diluted with phosphate buffer saline (pH=7.4) in such a way that allowed obtaining the final absorbance of A=(0.70±0.02) ($ABTS_{0.7}$) measured at 734 nm (Beckman DU 650 spectrophotometer, Beckman Coulter, Fullerton, CA, USA).

Fruits were freeze-dried and then extracted with methanol (0.5 g in 25 mL), while the dialysates were analyzed with no additional preparation. Aliquots (100 µL) of diluted samples or Trolox solution (γ=1–10 mg/100 mL) were added to 1 mL of $ABTS_{0.7}$ and the absorbance was measured 6 min after mixing. Antioxidant capacity was calculated using a standard curve obtained by measuring the absorbance of synthetic vitamin E solutions (Trolox) and expressed in mg of Trolox per 100 g of fresh mass.

Determination of total polyphenolic content using Folin-Ciocalteu method

Total polyphenols were measured using Swain and Hillis method (*13*). A volume of 45 mL of redistilled water, 0.25 mL of Folin-Ciocalteu reagent (water dissolved at 1:1 by volume) and 0.5 mL of 7 % Na_2CO_3 were added to 5 mL of fruit extract (0.5 g in 25 mL) or dialysate (not diluted). The mixture was left for 30 min in the dark.

Then the absorbance was measured on a spectrophotometer (Beckman DU 650, $\lambda=760$ nm). The obtained results of total polyphenolic content were expressed as g of catechin per 100 g of fresh mass based on the standard plot.

Analysis of the content of polyphenolic compounds using an HPLC method

The polyphenols in the fruits and dialysate were determined by HPLC method proposed by Oszmiański *et al.* (*14*). Prior to digestion, freeze-dried material (2 g) was extracted 3 times with 80 % methanol (ultrasound bath, 15 min) in order to obtain 50 mL of the extract. The extracts and dialysates were filtered with the use of Schott funnel G4 and centrifuged twice (14 000 rpm, 10 min).

Thus prepared extract was analyzed using high performance liquid chromatography (HPLC), on a Merck--Hitachi L-7455 (Hitachi Ltd., Tokyo, Japan) apparatus with a diode array detector (DAD). Phenolic compounds were separated in a column type Synergi Fusion RP-80A 150×4.6 mm (4 μm), Phenomenex (Torrance, CA, USA). The mobile phase consisted of 2.5 % acetic acid (solution A) and acetonitrile (solution B), applied in the following gradient: linearly from 0 % B to 25 % B during a period of 36 min; then, the column was treated with a pure solution A. The flow of the liquid phase was 1 mL per min, and the detection was conducted by measuring absorbance at 280 nm for flavanols, at 320 nm for phenolic acids, 360 nm for flavonols and 520 nm for anthocyanins. In order to identify the compounds, retention times of the compounds under analysis and standard compounds were compared. In addition, enzymatic hydrolysis of flavonol glycosides and cyanidin glycosides in a

citrate buffer solution (citric acid and sodium citrate), its pH value being 5, was performed for identification. The disappearance of single peaks in the chromatogram and formation of the corresponding aglycones was observed using HPLC after 1-hour incubation at 38 °C with a specific enzyme: β-glucosidase, β-xylosidase, β-galactosidase and β-hesperidase. The calibration curves were made from (–)epicatechin, (+)catechin, chlorogenic acid, phloridzin, isoquercitrin, and cyanidin-3-glucoside as standards. Procyanidin B2, C1, and B1 used as standards were obtained by the method of Oszmiański and Bourzeix (*15*). For those analytes where no standard was available, standards of the same family were used; thus quercetin-3--galactoside, quercetin-3-glucoside, quercetin-3-arabinoside, quercetin-3-xyloside and quercetin-3-rhamnoside were quantified as quercetin-3-glucoside (isoquercitrin); phloretin-2-glucoside as phloridzin; *p*-coumaric derivative as *p*-coumaric acid; and caffeic acid derivatives as chlorogenic acid. Results were expressed as mg per 100 g of fresh mass.

Statistical analysis

There were a minimum of three repetitions of the whole analysis. The results are shown as the arithmetic mean (±standard deviation). A single-factor analysis of variance test (ANOVA) with a *post hoc* Tukey's test was applied to assess the differences between the means. A Kolmogorov-Smirnov test was applied to examine the normality of distribution.

Results

Antioxidant activity of the examined fresh fruits was very different. As it can be seen in Fig. 1, the highest value of the examined parameter was shown in black
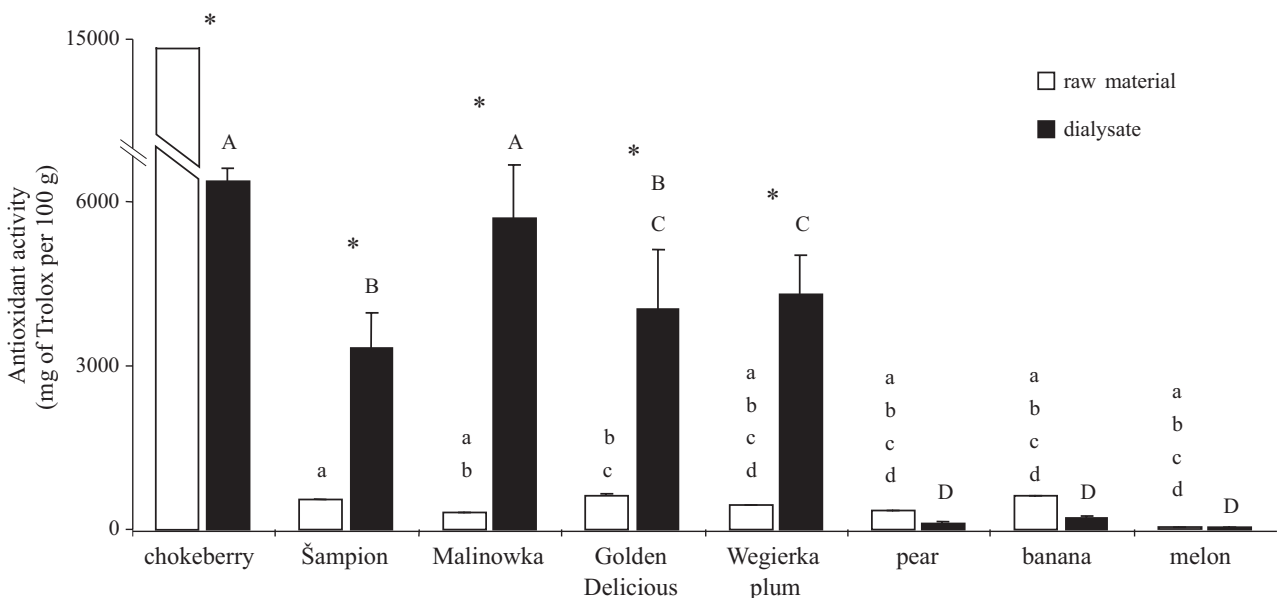
*statistically significant differences at p<0.05 as compared to raw material before digestion
a, b, c, d=the same letters represent statistically not significant differences (p<0.05) (raw material)
A, B, C, D=the same letters represent statistically not significant differences (p<0.05) (dialysate)

Fig. 1. Antioxidant activity of raw material and dialysates obtained in simulated digestion determined by assay with ABTS radical and expressed as mg of Trolox per 100 g of fresh mass (*N*=5)

chokeberry (13 587 mg of Trolox per 100 g). The rest of the fruits were characterized by significantly lower antioxidant activity. Among the examined apples, Šampion and Golden Delicious were comparable in terms of free radical scavenging capacity (552 and 618 mg of Trolox per 100 g, respectively), which was twice higher than for Malinowka cultivar.

Total content of polyphenols (Fig. 2) was closely correlated with the antioxidative activity of fruits (R^2=0.997). It was found that the mass fraction of phenolic compounds in black chokeberry (16 031 mg of catechin per 100 g) was significantly higher than in the rest of the raw fruits. Among the analyzed apple varieties, a high mass fraction of polyphenols was found in Malinowka and Golden Delicious (1639 and 1161 mg per 100 g, respectively). Plums, pears and bananas were characterized by significantly lower mass fractions of the evaluated compounds and simultaneously by lower antioxidant potential.

Dialysates obtained from all apple cultivars and plums were characterized by higher antioxidant activity (Fig. 1), while in other fruits a 2- to 3-fold reduction of the analyzed parameters was found. The digestion did not change the antioxidant activity of melon. The greatest capacity for free radical scavenging was shown by dialysates of black chokeberry (6385 mg of Trolox per 100 g) and Malinowka apple (5714 mg of Trolox per 100 g). About 30 % lower activity was shown by dialysates obtained from plums and Golden Delicious apples. All analyzed samples showed reduction of the polyphenolic mass fraction in dialysates (Fig. 2), and those changes were statistically significant for black chokeberry and Malinowka and Golden Delicious apples.

For the estimation of polyphenolic profiles of fruits and dialysates, black chokeberry, plums and Malinowka apples were chosen, as they were characterized by high antioxidant activity and total polyphenolic content (Table 1).

Among the obtained flavonoids in black chokeberry (83 %) there were mainly cyanidin glycosides, with a particularly high mass fraction of cyanidin-3-galactoside and cyanidin-3-arabinoside, which made up 50.8 and 22.2 % respectively, of the identified polyphenols. The phenolic acid mass fraction of all the analyzed compounds was 17 %; the simulated process of digestion and assimilation reduced 2–3 times the mass fraction of the examined phenolic compounds of black chokeberry. Up to 30 % decrease of neochlorogenic and chlorogenic acids, quercetin-3-vicianoside and eriodictyol was found after the digestion. It was also noticed that dialysates contained derivates of caffeic and *p*-coumaric acids not present in fresh fruits.

For Malinowka variety, 63 % of all the examined compounds were flavonoids, mainly epicatechin and procyanidins (Table 1), whereas the highest mass fraction of all estimated compounds was ascribed to chlorogenic acid (86.3 mg/100 g). In the dialysate obtained after simulated digestion of apple, a decrease of most of the examined phenolic compounds was detected. It is worth noting that in the dialysate there were no quercetin glycosides (with the exception of trace amounts of quercetin-3-galactoside), chlorogenic acid and procyanidin C1, which were present in the fruit before digestion. The amount of (−)epicatechin was lowered by almost 11 times.

Plum fruits were characterized by a high mass fraction of phenolic acids (neochlorogenic and chlorogenic)
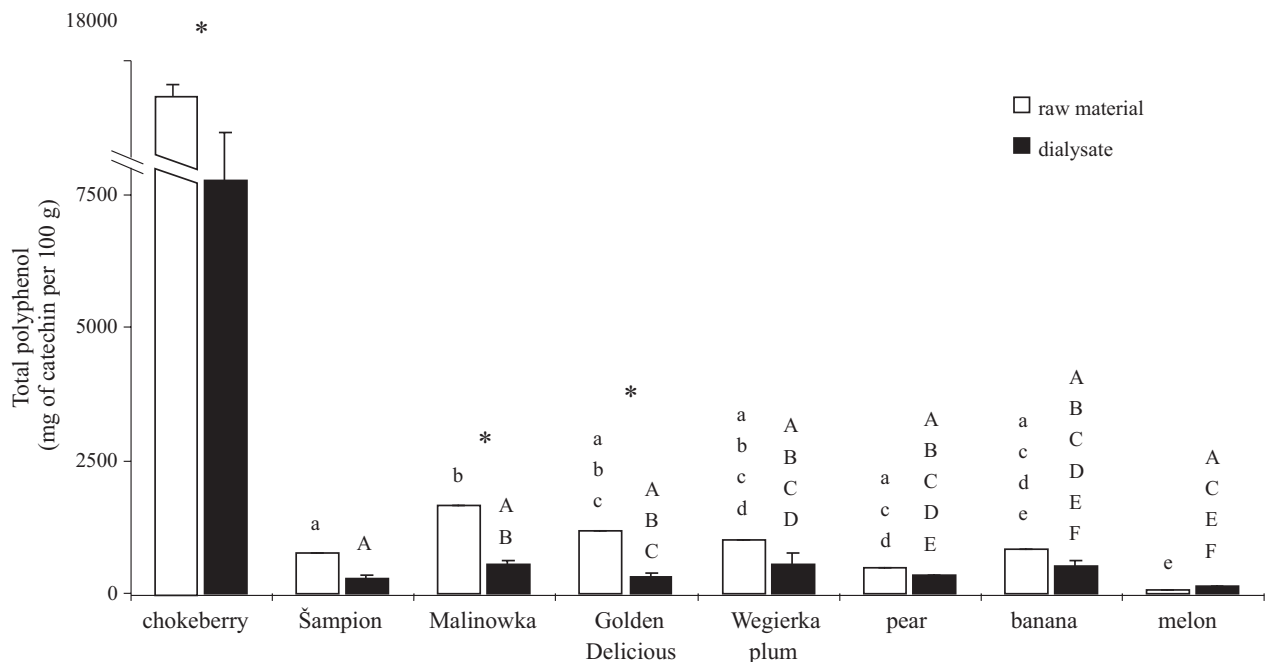
*statistically significant differences at p<0.05 as compared to raw material before digestion
a, b, c, d, e=the same letters represent statistically not significant differences (p<0.05) (raw material)
A, B, C, D, E, F=the same letters represent statistically not significant differences (p<0.05) (dialysate)

Fig. 2. Content of polyphenolic compounds in raw material and dialysates obtained in simulated digestion determined by Folin-
-Ciocalteu method and expressed as mg of catechin per 100 g of fresh mass (*N*=5)

Table 1. Profile of polyphenols in raw material and dialysates after digestion determined by HPLC method (arithmetic mean±S.D., *N*=3)

Specification	w/(mg per 100 g of fresh mass)					
	Chokeberry		Wegierka plum		Malinowka apple	
	Fresh fruit	Dialysate	Fresh fruit	Dialysate	Fresh fruit	Dialysate
Quercetin-3-galactoside	28.3±0.4	(16.2±0.2)*	0	0	4.9±0.1	(1.90±0.02)*
Quercetin-3-glucoside	20.8±0.3	(11.2±0.1)*	1.90±0.02	0*	3.2±0.1	0*
Quercetin-3-arabinoside	NA	NA	NA	NA	4.7±0.1	0*
Quercetin-3-vicianoside	8.5±0.1	(6.2±0.1)*	NA	NA	NA	NA
Quercetin-3-rhamnoside	NA	NA	NA	NA	2.6±0.1	0*
Quercetin-3-xyloside	NA	NA	NA	NA	6.60±0.03	0*
Quercetin-3-robinoside	11.1±0.1	(5.3±0.1)*	NA	NA	NA	NA
Cyanidin-3-galactoside	1243±16	(356±4)*	NA	NA	15.2±0.2	0*
Cyanidin-3-glucoside	46.2±0.6	(11.6±0.1)*	10.0±0.1	0*	NA	NA
Cyanidin-3-arabinoside	544±7	(144±2)*	NA	NA	NA	NA
Cyanidin-3-rutinoside	NA	NA	20.1±0.2	(10.7±0.1)*	NA	NA
Cyanidin-3-xyloside	73±1	(17.5±0.1)*	22.0±0.2	0*	NA	NA
Neochlorogenic acid	189±2	(154±2)*	181±2	(23.7±0.3)*	NA	NA
Chlorogenic acid	218±3	(153±2)*	43.3±0.5	(15.8±0.2)*	86.3±1	0*
Protocatechuic acid	0	(46.1±0.5)*	0	(46.1±0.5)*	NA	NA
Caffeic acid derivative	0	(104±1)*	0	(16.1±0.2)*	0	(8.1±0.1)*
p-coumaric acid derivative	NA	NA	NA	NA	0	(3.60±0.04)*
5-(*p*-coumaroyl)quinic acid	NA	NA	NA	NA	21.4±0.2	(3.10±0.1)*
Peonidin-3-glucoside	NA	NA	3.00±0.04	0*	NA	NA
Phloretin xyloglucoside	NA	NA	NA	NA	16.3±0.2	(4.6±0.1)*
Rutin	12.6±0.2	(6.4±0.1)*	21.3±0.2	(3.10±0.04)*	NA	NA
Eriodictyol	51.4±0.7	(46.2±0.5)*	NA	NA	NA	NA
(+)Catechins	NA	NA	10.0±0.1	(3.70±0.04)*	7.10±0.08	(6.3±0.1)*
(–)Epicatechin	NA	NA	NA	NA	32.2±0.4	(3.00±0.03)*
Phloridzin	NA	NA	NA	NA	18.3±0.2	(5.4±0.1)*
Procyanidin B1	NA	NA	NA	NA	13.2±0.2	(9.3±0.1)*
Procyanidin B2	NA	NA	NA	NA	41.3±0.5	(8.2±0.1)*
Procyanidin C1	NA	NA	NA	NA	18.8±0.2	0*

NA=not assayed
*statistically significant (p<0.05) as compared to fresh fruit

and the flavonoids group, with mainly cyanidin glycosides and rutin dominating (Table 1). In dialysates obtained after digestion *in vitro*, the mass fractions of the above compounds were significantly lowered, and the mass fractions of cyanidin-3-glucoside and cyanidin-3--xyloside were lower than the detection threshold. Also, quercetin-3-glucoside and peonidin-3-glucoside were not detected although they were present in the raw material. Similarly as in the case of black chokeberry, the presence of protocatechuic acid was noted, whereas it was not present in fresh plums.

Discussion

Fruits are a source of precious bioactive compounds for the organism, *i.e.* vitamins, fibre, sugars, minerals and antioxidants (*16,17*). Consumption of large amounts of those substances is not connected directly with the increase of their concentrations in the blood stream and tissues. Mechanisms of digestion and assimilation play a

very important role in these circumstances. For example, although orange juice is a very rich source of flavanones, only a limited quantity is soluble. In addition, under the alkaline conditions in the small intestine and digestion by pancreatin and bile, some of the flavanones are transformed into insoluble chalcones (*18*). The precipitated antioxidant compounds are not available for absorption in the small intestine.

For very high antioxidant activity of black chokeberry vitamins (mainly C, E and A), carotenoids and phenolic compounds are most often responsible. Total phenolic content of black chokeberry fruits estimated in this work was 16 000 mg of catechin per 100 g and was closely correlated with antioxidant activity (R^2=0.93). Almost twice lower levels of polyphenols in chokeberry were noted by Bienvenuti *et al.* (*19*). Many factors may be responsible for the divergence among results. Cheynier (*20*) showed that variety, level of maturity, conditions of ripeness and storage influence the quantitative and qualitative composition of phytocompounds, and

the differences among the compound levels can reach several tens of percent. For scavenging of free radicals not only the amount of polyphenolic compounds is responsible, but also their kind (21,22). Our results show that cyanidin glycosides, mainly cyanidin-3-galactoside and cyanidin-3-arabinoside are the dominating phytocompounds in black chokeberry (Table 1). Cyanidin, quercetin and their glycosides are characterized by high antioxidant potential (23) and are responsible for high antioxidant activity of black chokeberry fruit.

Antioxidant activity of apples mainly depended on quantitative and qualitative composition of polyphenolic compounds. In fruit, mainly phenolic acids (*i.e.* chlorogenic), procyanidins and dihydrochalcons dominated (20). The profile depended on vegetation conditions, ripeness, storage conditions and cultivar (24–27). In this work, it was found (for Malinowka variety) that dominating phytocompounds were phenolic acids (mainly chlorogenic and *p*-coumaroyl quinic), epicatechin and procyanidins (Table 1). Antioxidant potential of apples was influenced mainly by procyanidins and epicatechin, although the fraction of phenolic acids in all examined compounds was 30 %. Phenolic acid activity is rather low in comparison with *e.g.* quercetin (22,23). Heo *et al.* (23) arranged antioxidants in order of their free radical scavenging ability: epicatechin>cyanidin>quercetin>catechin>cyanidin-3-glucoside>peonidin>quercetin-3-galactoside>cyanidin-3-rutinoside>quercetin-3-glucoside>peonidin-3-glucoside>quercetin-3-rutinoside>chlorogenic acid.

The fruits of plums and pears were characterized by similar antioxidant activity, whereas the concentration of polyphenols in plums was twice as high (Figs. 1 and 2). Plum fruits were characterized by the presence of phenolic acids, especially neochlorogenic and chlorogenic acids, which according to Rice-Evans *et al.* (22) have low antioxidant capacity.

Schieber *et al.* (28) showed that the dominant compound in three pear varieties is chlorogenic acid. Also, the presence of quercetin-3-glucoside, epicatechin and arbutin was measured. Similar antioxidant potential of pears and plums with different concentrations of polyphenols supports the conclusion that plums contain more phenolic acids and fewer components of high antioxidant activity.

The analysis of antioxidant activity of dialysates obtained after digestion of black chokeberry, pears and bananas showed a 2- to 3-fold decrease of free radical scavenging capacity in comparison with raw material (Fig. 1). Also, lowering of polyphenolic content was measured in dialysates of those fruits. In experiments similar to ours, Gil-Izquierdo *et al.* (29) showed that the content of total flavanones decreased after simulated digestion of different orange juices. The levels of compounds able to permeate through the dialysis membrane and of those remaining in the retentate were lower than in the initial juice. The transformation into non-red forms and/or degradation of anthocyanins (97 %) as well as vitamin C degradation (>95 %) were also observed in a pomegranate juice during gastrointestinal digestion (30).

The antioxidant activity of dialysates was influenced by, first of all, membrane permeable phenolic compounds. The effectiveness of polyphenol absorption in the digestive tract depends, among other things, on molecule dimensions, level of polymerization, presence and kind of sugar in the molecule and on the hydrophobicity of the compound (8). Polyphenol glycosides and procyanidins are absorbed with low yield (2). Effectiveness of procyanidin absorption is low and closely connected to molecule dimensions (8). Besides, because of different levels of binding with the food matrix, polyphenolic compounds are released from it in different ways. Some food constituents can affect the dialysis rates, and results from *in vitro* digestion should be treated with caution. Particularly when the release of phenolic compounds from non-liquid foods is evaluated, the food matrix effect should be taken into account. The research of Mazzaracchio *et al.* (31) showed that polyphenols were easily absorbed in the case of such food compounds as cellulose, pectins and lignins. Gil-Izquierdo *et al.* (32) presented a new method that allowed evaluation of the *in vitro* availability of phenolic compounds in non-liquid foods, such as cloudy orange juice, strawberry fruit and strawberry jam. They demonstrated that crucial points were the close contact between food and dialysis membrane, as well as monitoring and faster equilibration of pH values.

The estimation of antioxidant activity of dialysates of Šampion, Malinowka and Golden Delicious apples, and plum showed greater free radical scavenging capacity in comparison with fruits before digestion, but the total polyphenolic content in dialysates was lower. During the digestion process, polyphenolic compounds present in fruits probably underwent changes into forms showing high antioxidant potential. In dialysates of Malinowka apples, cyanidin and quercetin glycosides were not present, whereas they were present in fresh fruit. According to Scalbert and Williamson (4), Olthof *et al.* (2) and Spencer (33), those compounds are hydrolyzed by digesting enzymes (pepsin and pancreatin) to quercetin and cyanidin with very high antioxidant potential.

The changes in antioxidant activity of dialysates in comparison with fresh fruits depended on species and their chemical composition. High concentration of phenolic acids in raw material causes a drop of antioxidant potential because of digestion and because of low level of intestinal absorption of those compounds. Conversely, quercetin glycosides undergo acidic hydrolysis releasing aglycones with high potential for the increase of antioxidant activity. Also, large amounts of procyanidins in raw material influenced the high antioxidant potential in dialysate. Anthocyanins present in strawberries under alkaline conditions in the intestine are degraded (transformed) and free ellagic acid is released from ellagitannins, leading to a tenfold increase of this compound during digestion (32).

During analysis of the phenolic compound profile of black chokeberry, plum and Malinowka apple, it was observed that the mass fraction of chlorogenic acid in dialysates had dropped significantly. It can be concluded that the effectiveness of intestinal absorption of that phenolic acid is low. Research conducted by Gonthier *et al.* (34) and Olthof *et al.* (2) showed that chlorogenic acid was not digested and absorbed before the human large intestine. Under the influence of microbial enzymes,

chlorogenic acid decomposed to cinnamic, phenylacetic and benzoic acids, but the main metabolite was hippuric acid (*2*). In the *in vitro* model used in this work the intestinal microflora activity was not taken into account, which may explain the low concentration of chlorogenic acid and its metabolites in the dialysate.

The difference in catechin content in fresh apples and their dialysates was not significant (Table 1), which may indicate its good digestion and absorption rate in the small intestine. Large amount of catechin in the dialysate may originate from decomposition of procyanidins (especially B2) during digestion, although Rios *et al.* (*35*) showed that procyanidin decomposition in the acidic environment of the stomach was low. Absorption of catechin was connected with the structure of that compound. Aglycones were better absorbed in the small intestine than glycoside derivatives (*4,8*), although Murota and Terao (*5*) showed that the quercetin-3-glucoside was also absorbed. Walle (*36*) proved an active influence of membrane transporters SGLT1, MCT, MRP1 and MRP2 on transportation of polyphenol glycosides through the intestinal epithelium. However, permeation of aglycones occurred faster than of glycosides and was more effective because it was based on a diffusion rule and did not need the presence of membrane transporters and energy.

As a result of digestion, the content of quercetin and cyanidin glycosides was significantly lowered, which proves that they were hydrolyzed to aglycones (Table 1), but part of those compounds was absorbed in the small intestine in unchanged form. The kind of sugar in the molecule is crucial both for enzymatic hydrolysis (*33*) and for affinity to the proper membrane transporter – polyphenol compounds with glucose in their molecule had the greatest affinity for membrane transporters (*36*).

The metabolism and absorption of polyphenols are still not known and understood entirely. The techniques used for research of these processes are being improved and the obtained results are extending the knowledge about polyphenols, but the research should still be continued. The greater the knowledge about absorption of polyphenols into the human body, the more easily the biological activity of these compounds will be used.

Conclusions

The antioxidant activity of the compounds in dialysates of apples and plums increased as a result of the action of enzymes together with pH and temperature in the *in vitro* digestion model. On the contrary, in black chokeberry, pears and banana dialysates, the decrease of antioxidant activity was observed. Firstly, this was connected with the increased amount of polyphenolic molecules as the result of disruption of polymers to monomer and aglycones. Secondly, an improvement of their absorption was caused by their transformations into simple compounds (monomers, aglycones) and facilitated penetration through the dialysate membrane. During the simulated digestion process, the hydrolysis of polyphenols, especially quercetin and cyanidin glycosides occurred. It was shown that phenolic acids and procyanidins were characterized by low levels of intestinal absorption, whereas catechin and eriodictyol were charac-

terized by very high (over 80 %) absorption in the small intestine.

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