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RELEASE OF PHENOLIC COMPOUNDS FROM BEAN FLOUR, BEAN-DERIVED CHIPS AND BLACK CHOKEBERRY JUICE AND CHANGES IN THEIR ANTIOXIDANT ACTIVITY DURING DIGESTION IN AN *IN VITRO* GASTROINTESTINAL MODEL

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An *in vitro* gastrointestinal model, which simulates the conditions of the human digestive tract, was used in this work to determine the potential antioxidant activity in Jaś Karłowy bean flour, extruded Jaś Karłowy bean products (chips) and black chokeberry juice.

The aim of the study was to investigate changes in the level of phenolic compounds and antioxidant activity under *in vitro* conditions which closely resemble those occurring in the human digestive tract. We examined the effect of human fecal flora on the antioxidant activity of products undergoing digestion, which are a rich source of antioxidant compounds. Results obtained in the research indicate that the *in vitro* gastrointestinal tract model applied in this work can be successfully used to study changes in the level of phenolic compounds.

Our observations show that digestion of Jaś Karłowy bean flour, an extruded product from bean and black chokeberry juice in the gastrointestinal tract model has an influence on the antioxidant activity and the level of phenolic compounds in these products of Jaś Karłowy bean flour, extruded product from bean and black chokeberry juice. The highest antioxidant activity, approx. 30 mg Trolox/g during the digestion process, was noted for both flour and extruded bean products. All products examined were found to stimulate the growth of intestinal microorganisms, however black chokeberry juice decreased only the growth of bacteria from the *Enterococcus* species.

INTRODUCTION

The increasing significance of bioactive substances, such as antioxidants, in our diet induced studies on their digestion and bioavailability [Ekmekcioglu, 2002; Garret *et al.,* 1999; Hoebler *et al.*, 2002]. Yet, examining the intestinal bioavailability of food in humans is very costly and not always easy due to ethical reasons and considerable interindividual variations. To circumvent the mentioned problems experimental models were adapted which allow examining these processes *in vitro* [Ekmekcioglu, 2002]. It was demonstrated that the data on bioavailability obtained from *in vitro* studies using dynamic models was reproducible and comparable with results from *in vivo* studies [Ekmekcioglu, 2002]. It should be underlined that bioavailability of nutrients, including antioxidants, is a newly explored field. Examining bioavailability of nutritional compounds in natural conditions is limited due to the difficulty in accessing the human gastrointestinal tract, especially the small intestine. All of the above initiated numerous studies in experimental *in vitro* models using automated models of the human gastrointestinal tract [Neumann *et al.,* 2006].

In our studies, we used an "*in vitro*" model of the gastrointestinal tract that was constructed based on literature data and earlier studies of Aura *et al.* [1999], Gil-Izguierdo *et al.* [2001] and Hoebler *et al.* [2002].

Most fruits and vegetables are a rich source of bioactive compounds with high antioxidant potential [Netzel *et al*., 2005; Krul *et al*., 2001]. Food products contain protective or anticarcinogenic substances, some of which are present also in plants, *e.g.* glucosinolate in vegetables, vitamin C in fruit and polyphenolic compounds in legume plants and black chokeberries. Flavonoids belong to one of the major groups of natural antioxidants which are widespread (particularly flavonol quercetin) in food products consumed by humans [Justesen & Arrigoni, 2001; Gil-Izquierdo *et al*., 2001]. They have been reported to exhibit a wide range of biological functions, including anticarcinogenic, anti-inflammatory and antiviral activities [Aherne & O'Brien, 1999; Cook & Samman, 1996; Hollman *et al.*, 1996]. Polyphenols form a very complex group of compounds present in most plants [Scalbert *et al*., 2002]. Many foods and beverages contain high levels of phenolic compounds. Dietary phenolics contribute to the health benefits obtained from a diet rich in fruits and vegetables [Williamson *et al.*, 2000]. Despite the growing interest in the health benefits caused by polyphenols, little is known about their influence on the growth of bacteria present in the gastrointestinal tract and about their interaction with polyphenols during digestion. It has been argued that the stomach, intestinal lumen and the colon can contain substantial levels of unabsorbed phenolics, which may play a key role in the antioxidant defense of the gastrointestinal tract (GT). This is consistent with epidemiological evidence suggesting that diets rich in fruits and vegetables are associated with decreased risk of gastric colon and rectal cancer [Jenner *et al*., 2005].

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Anthocyanins belong to the flavonoid group of polyphenolic compounds and are responsible for the red and blue coloring of such plant organs as fruits, flowers and leaves [McDougall *et al.*, 2005b]. Anthocyanins also possess known pharmacological properties and are used by humans for therapeutic purposes. Previous studies on the effect of anthocyanins on tumors showed that some of them were not effective in suppressing tumor growth [Kong *et al.*, 2003]. In recent years, black chokeberries have been recognised as a food colorant and as a source of valued phytonutrients. The anthocyanin level of the berries has been reported to be as high as 1% on a dry weight basis, whereas the total phenolic content has been reported to be more than 20 mg/g (gallic acid equivalents). The high content of phenolics seems to correlate with the high antioxidant activity reported for black chokeberries [Slimestad *et al.*, 2005].

Results obtained by Rechner *et al.* [2004] show that dietary polyphenols are metabolised in the colon, depending on substrate concentration and residence time, with resultant formation of simple phenolics, which can be considered as biomarkers of colonic metabolism if subsequently absorbed. The aim of this study was to assess the impact of digestion in the *in vitro* tract model on the antioxidant activity and changes in the level of phenolic compounds in bean flour, bean-derived chips and black chokeberry juice.

MATERIALS AND METHODS

Extruded product – chips

Extrusion parameters were as determined by Czarnecka *et al.* [1998]. Chips were obtained from bean-fermentation meal and ground maize mixed at a ratio of 1:1. The process of lactic fermentation was carried out at 37°C for 18 h by *Lactobacillus plantarum* T-106 (strain supplied by The Technical University of Łódź).

Black chokeberry juice

The black chokeberry juice was obtained from Lech company and was bought at a local market (Wrocław, Poland).

The *in vitro* gastrointestinal model (Figure 1)

The digestion was conducted in a glass bioreactor placed in a container of water held at 37°C by a through-flow water bath. Digestion products were stirred by a motorized overhead paddle stirrer.

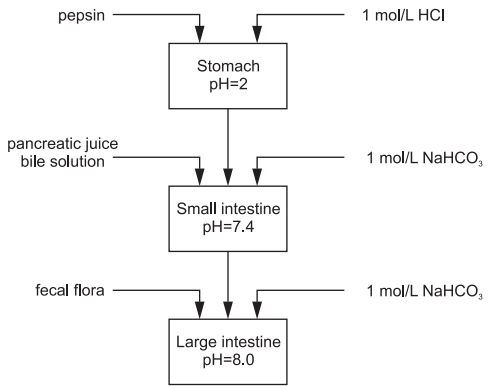

FIGURE 1. Gastrointestinal tract model.

Product samples were digested by incubating 20 g of a given product with 200 mL of a pepsin (Sigma-Aldrich) solution (0.453 g/L), pH 2.0 at 37°C for 4 h for bean products, and for 2 h for black chokeberry juice. Subsequently, the mixture was incubated with 10 mL of a bile solution (0.12 g) (Sigma-Aldrich) and pancreatic (0.02 g) juice (Sigma-Aldrich) in 0.1 mol/L $NaHCO_3$, pH 7.4 for 2 h after which incubation was continued at pH 7.4 for another 18 h with fecal flora. Addition of 1 mol/L HCl and 1 mol/L $NaHCO_3$ to the bioreactor was propelled by peristaltic pumps under the control of a pH electrode [Aura *et al.*, 1999; Gil-Izguierdo *et al.*, 2001; Gumienna *et al.*, 2006; Hoebler *et al.*, 2002].

Fecal samples were obtained from 3 healthy adult volunteers and microorganisms were isolated in accordance with Knarreborg *et al.* [2002]. The number of microorganisms in the suspensions accounted for 10^8 cfu/mL. The number of microorganisms was calculated after 2 and 21 h of digestion in the gastrointestinal tract model. The number of colony forming units (cfu)/mL was determined on modified Garche's medium for *Bifidobacterium*, on MRS for *Lactobacillus*, on medium with kanamycin, esculin and sodium azide for *Enterococcus,* and on McConkey medium for *Enterobacteriaceae* [Biedrzycka *et al*., 2005; Blake *et al.*, 2003]. Plates were incubated at 37°C for 48 h in anaerobic conditions.

Analysis of antioxidant capacity

The total antioxidant capacity in the stomach, in small intestine and large intestine was evaluated by the Trolox equivalent antioxidant activity (TEAC) assay described by Re *et al.* [1999] with some modifications. The assay is based on the ability of compounds to scavenge the free radical 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonate (ABTS). Results of the TEAC assay are expressed as the capability of antioxidants to scavenge ABTS radicals relative to that of Trolox (a water-soluble vitamin E analogue) and given as mg Trolox/g dry matter of the sample or 1 per mL of juice.

Total phenolic content

Phenolics content was measured using the modified Folin-Ciocalteau method and its values were estimated from a standard curve of gallic acid. All results have been corrected for the presence of phenols in the pancreatin/bile salts mixture. Results are expressed as equivalents of gallic acid per 1 g of dry matter or 1 mL of juice [Singelton & Rossi, 1965].

Data analysis

Each experiment was repeated three times. Analysis of data was done using Excel 2000 Microsoft Office Program.

RESULTS

Products subjected to the digestion process were characterised by different initial concentrations of phenolic compounds and antioxidant capacity. The content of phenolic compounds was 1.58 mg/g in Jaś Karłowy bean flour, 1.65 mg/g in extruded product after fermentation and 7.27 mg/mL in black chokeberry juice (Table 1). Antioxidant capacity was noted as 2.78 mg Trolox/g in flour, 3.83 mg Trolox/g in extruded product and 8.01 mg Trolox/mL in black chokeberry juice. The giv-

TABLE 1. Antioxidant capacity of flour, extruded product and chokeberry juice and polyphenols content.

Products	Antioxidant capacity (mg Trolox/g or mg Trolox/mL)	Total polyphenol content (mg gallic acid/g or mg/mL)
Instant flour	2.78±0.34	1.58±0.21
Extruded products	3.83±0.55	1.65±0.07
Black chokeberry juice	8.01±0.12	7.27±0.55

en results show that the highest concentration of phenolic compounds as well as the highest antioxidative potential was detected in black chokeberry juice. Interestingly, antioxidant capacity was higher in the extruded products after fermentation than for Jaś Karłowy bean flour.

In the case of Jaś Karłowy bean flour, the highest antioxidant capacity (16.03 mg Trolox/g) was observed after digestion of the sample in the small intestine. Further on, digestion in the large intestine with participation of specific microflora raised the antioxidant activity in flour and extruded products to 32.54 mg Trolox/g and 30.53 mg Trolox/g, respectively. In the case of bean products, considerable differences were determined after each step of digestion. However, antioxidant capacity in black chokeberry juice after digestion was significantly increased and established as 9.03 mg Trolox/mL in the presence of specific microflora (Tables 2, 3, 4). During digestion of bean products an increase was observed in the content of phenolic compounds, while the level of phenolics decreased in black chokeberry juice. Thus, it is highly conceivable that during digestion in the presence of microflora specific compounds are formed which raise the antioxidant potential, but are not polyphenols. Flavonoids reach the large intestine, where they can be transformed and/or degraded by the colonic microflora. The formed metabolites are believed to have not only a beneficial effect on the large intestine cells and/or on the residing microflora but may also be absorbed and exert a biological action away from the large intestine [Gil-Izquierdo, 2001]. Table 5 presents the number of all identified fecal bacteria which was found to be at a notably higher level after digestion in the large intestine of legume products than after 2 h of residing in the small intestinal environmental. For black chokeberry juice a significant increase was observed in the growth of *Bifidobacterium*, *Lactobacillus* and *Enterobacteriaceae* bacteria. Yet, the digestion of the juice in the small intestine considerably lowered the number of bacteria of the *Enterococcus* species and remained at the same level until the end of the digestion process.

In summary, the presented study has shown that the application of the *L. plantarum* T-106 strain in the fermentation process is advantageous as the level of phenolic compounds

TABLE 2. Antioxidant capacity of instant flour and polyphenols content detected in instant flour during digestion in the gastrointestinal tract model.

Treatment	Antioxidant capacity (mg Trolox/mL)	Total polyphenol content (mg gallic acid/g)
4 h at pH 2.0	3.02±0.21^{a}	3.21±0.43^{b}
at pH 7.4	15.04±1.06^{b}	2.81±0.18^{a}
2.5 h at pH 7.4	16.03±1.36^{c}	3.85±0.30^{c}
18 h at pH 8.0	32.54±3.14^{d}	4.02±0.28^{c}

Values are means±SD of three independent experiments. Means with different letters: $^{a, b, c, d, e}$ are significantly different (in the columns) at $p<0.05$.

TABLE 3. Antioxidant capacity of extruded products and polyphenols content detected in extruded products during digestion in the gastrointestinal tract model.

Treatment	Antioxidant capacity (mg Trolox/g)	Total polyphenol content (mg gallic acid/g)
4 h at pH 2.0	4.17±0.30^{a}	2.62±0.14^{a}
at pH 7.4	18.00±1.06^{c}	2.68±0.35^{a}
10 min after digestion with fecal flora	12.26±0.87^{b}	2.69±0.14^{a}
2.5 h at pH 7.4	18.06±1.75^{c}	3.01±0.16^{b}
at pH 8.0	21.65±2.75^{d}	4.00±0.21^{c}
18 h at pH 8.0	30.53±2.40^{e}	5.57±0.28^{d}

Values are means±SD of three independent experiments. Means with different letters: $^{a, b, c, d, e}$ are significantly different (in the columns) at $p<0.05$.

TABLE 4. Antioxidant capacity of black chokeberry juice and polyphenols content detected in black chokeberry juice during digestion in the gastrointestinal tract model.

Treatment	Antioxidant capacity (mg Trolox/mL)	Total polyphenol content (mg gallic acid/mL)
2 h at pH 2.0	5.36±0.10^{a}	6.32±0.33^{d}
at pH 7.4	7.05±0.11^{b}	4.87±0.11^{c}
2.5 h at pH 7.4	8.25±1.07^{c}	6.55±0.18^{d}
at pH 8.0	8.18±0.10^{c}	3.41±0.08^{b}
18 h at pH 8.0	9.03±1.12^{d}	2.89±0.07^{a}

Values are means±SD of three independent experiments. Means with different letters: $^{a, b, c, d, e}$ are significantly different (in the columns) at $p<0.05$.

TABLE 5. Changes in the number of intestinal microflora in the course of digestion of bean flour, extruded products and chokeberry juice.

Sample	*Bifidobacterium*	*Lactobacillus*	*Enterococcus*	*Enterobacteriaceae*
	Log $_{10}$cfu/mL after digestion±SD			
Inoculum	6.42±0.19	6.25±0.13	6.90±0.34	6.80±0.18
Instant flour				
2 h at pH 7.4	8.76±0.29	8.70±0.17	8.66±0.31	8,51±0.14
21 h	10.51±0.79	10.84±0.03	9.95±0.42	9.64±0.76
Extruded products				
2 h at pH 7.4	7.45±0.17	6.85±0.21	7.55±0.53	7.47±0.34
21 h	9.93±0.70	8.88±0.40	9.42±0.84	9.84±0.12
Chokeberry juice				
2.5 h at pH 7.4	7.45±0.23	7.13±0.11	3.29±0.12	6.49±0.16
21 h	9.41±0.51	8.87±0.25	3.16±0.31	9.25±1.30

in extruded product (chips). The highest antioxidant activity was determined in flour and extruded products after digestion in the large intestine. The total content of phenolic compounds was relatively low. Digestion of black chokeberry juice decreased the content of phenols, but increased the antioxidant activity of the juice. Bean products stimulated the growth of intestinal bacteria, while the growth of *Enterococcus* was not stimulated by chokeberry juice.

DISCUSSION

Under *in vitro* conditions that simulate the digestion process in the gastrointestinal tract the antioxidant capacity in flour, extruded products and black chokeberry juice was observed to increase. In particular, experiments showed that the antioxidant capacity and the polyphenol content of flour and extruded product digests were higher than in black chokeberry juice.

A major part of the flavonoid glycosides in food are expected to reach the colon, where they are subjected to degradation by faecal microflora [Justesen *et al.*, 2000; Rechner *et al.*, 2004; Williamson *et al*., 2000]. It has been reported that flavonoids present in foods cannot be absorbed from the intestine since they are bound to sugars and form glycosides [Crespy *et al.,* 2002]. Only free flavonoids without a sugar molecule, the so-called aglycones, are considered able of passing through the epithelial cells of the gastrointestinal tract, while no enzymes that can split the predominantly β-glycosidic bonds are secreted into the gut or present in the intestinal wall. However, absorption of quercetin glycosides from onion was in fact far better than the absorption of pure aglycones [Hollman & Katan, 1995; Scalbert *et al*., 2002]. It was suggested that absorption kinetics and bioavailability of flavonoids are probably determined by the form of glycoside. Flavonoid metabolism produces a series of phenolic compounds that have been identified as aromatic acids [Schneider & Blaut, 2000].

Previous results have shown that cell extracts from feces hydrolyze flavonoid glycosides to respective aglycones [Winter *et al.,* 1989; Aura *et al*., 1999; Justesen & Arrigoni, 2001; Day *et al*., 1998]. Hydrolysis of flavonoid glucosides occurred mainly in the jejunum [Ioku *et al*., 1998]. In contrast, *Eubacterium ramulus* not only cleaves the glycosidic bond and ferments the resulting glucose, but also attacks the flavonoid ring system [Blaut *et al*., 2003].

We propose that during digestion phenolic compounds are liberated from glycoside flavones as a result of hydrolysis of the glycoside bond, especially in bean products. Justesen *et al.* [2000] demonstrated that an active aglycone is more reactive than the glycoside form. Another factor which cannot be overlooked is the action of the intestinal microflora which, due to the metabolic process taking place, primarily, in the large intestine, may increase the antioxidative potential of the digested products [Gawęcki & Libudzisz, 2006].

Results obtained by McDougall *et al.* [2005a] suggest that polyphenols transiently bind to food matrices during digestion, which protects the more labile anthocyanins from degradation.

Anthocyanins appear to be rapidly absorbed, preferentially from the stomach and the small intestine [Talavéra *et al.*, 2003]. Due to this fact, the gastrointestinal model used in our study does not fully reflect the *in vivo* conditions. Nevertheless, as shown in the study, the model appears to be an useful tool for investigating changes of dietary compounds, especially flavonoids, and sufficient for observing tendencies in chemical transformation of nutrients, of health-promoting compounds in particular.

CONCLUSIONS

1. The *in vitro* gastrointestinal tract model applied in this work can be successfully used to study changes in the level of phenolic compounds.

2. The highest antioxidant capacity, approx. 30 mg Trolox/g during the digestion process, was noted for both flour and extruded bean products.

3. Bean products stimulated the growth of intestinal bacteria, while the growth of *Enterococcus* was not stimulated by chokeberry juice.

REFERENCES

1. Aherne S.A., O'Brien N.M., The flavonoids, myricetin, quercetin and rutin, protect against cholestan-3β, 5α, 6β-triol-induced toxicity in Chinese hamster ovary cells *in vitro*. Nutr. Res., 1999, 19, 749–760.
2. Aherne S.A., O'Brien N.M., Dietary flavonols: chemistry, food content and metabolism. Nutrition, 2002, 18, 75–81.
3. Aura A.M., Harkonen H., Fabritius M., Poutanen K., Development of *in vitro* enzymatic digestion method for removal of starch and protein and assessment of its performance using rye and wheat breads. J. Cereal Sci., 1999, 29, 139–152.
4. Biedrzycka E., Bielecka M., Amarowicz R., The effect of intestinal microflora on antioxidant activity of apple juice. J. Food Lipids, 2005, 12, 261–274.
5. Blake D.B., Hillman K., Fenlon D.R., The use of model ileum to investigate the effects of novel and existing antimicrobials on indigenous porcine gastrointestinal microflora using vancomycin as a sample. Anim. Feed Sci. Technol., 2003, 103, 123–139.
6. Blaut M., Schoefer L., Braune A., Transformation of flavonoids by intestinal microorganisms. Int. J. Vitam. Nutr. Res., 2003, 73, 79–87.
7. Cook N.C., Samman S., Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. Nutr. Biochem., 1996, 7, 66–76.
8. Crespy V., Morand C., Besson C., Manach C., Demigne C., Remesy C., Quercetin, but not its glycosides, is absorbed from rat stomach. J. Agric. Food Chem., 2002, 50, 618–621.
9. Czarnecka M., Czarnecki Z., Nowak J., Roszyk H., Effect of lactic fermentation and extrusion of bean and pea seeds on nutritional and functional properties. Nahrung, 1998, 1, 7–11.
10. Day A.J., DuPont M.S., Ridley S., Rhodes M., Rhodes M.J.C., Morgan M.R.A., Williamson G., Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver β- glucosidase activity. FEBS Lett., 1998, 436, 71–75.
11. Ekmekcioglu C., A physiological approach for preparing and conducting intestinal bioavailability studies using experimental systems. Food Chem., 2002, 76, 225–230.
12. Garret DA, Failla M.L., Sarama R.J., Development of an *in vitro* digestion method to access carotenoid bioavailability from

meals. J. Agric. Food Chem., 1999, 47, 4301–4309.
13. Gawęcki J., Libudzisz Z., Microorganisms in food and nutrition. 2006, The August Cieszkowski Agricultural University of Poznań Press, pp. 31–40 (in Polish).
14. Gil-Izquierdo A., Gil M.I., Ferreres F., Tomas-Barberan F.A., *In vitro* availability of flavonoids and other phenolics in orange juice. J. Agric. Food Chem., 2001, 49, 1035–1041.
15. Gumienna M., Goderska K., Nowak J., Czarnecki Z., Changes in the antioxidative activities of bean products and intestinal microflora in the model of the gastrointestinal tract "*in vitro*". Pol. J. Food Nutr. Sci., 2006, 56/15, SI 2, 29–32.
16. Hoebler C., Lecannu G., Belleville C., Devaux M.F., Popineau Y., Barry J.L., Development of an *in vitro* system simulating bucco-gastric digestion to assess the physical and chemical changes of food. Int. J. Food Sci. Nutr., 2002, 53, 389–402.
17. Hollman P.C.H., Hertog M.G.L., Katan M.B., Analysis and health effects of flavonoids. Food Chem., 1996, 57, 43–46.
18. Hollman P.C.H., Katan M.B., Dietary flavonoids: intake, health, effect and bioavailability. Food Chem. Toxicol., 1995, 37, 937–942.
19. Ioku K., Pongpiriyadacha Y., Kinishi Y., Takei Y., Nakatani N., Terao J., β-glucosidase activity in the rat small intestine toward quercetin monoglucosides. Biosci. Biotechol. Biochem., 1998, 62, 1428–1431.
20. Jenner A.M., Rafter J., Halliwell B., Human fecal water content of phenolics: The extent of colonic exposure to aromatic compounds. Free Rad. Biol. Med., 2005, 38, 763–772.
21. Justesen U., Arrigoni E., Electrospray ionization mass spectrometric study of degradation products of quercetin, quercetin-3-glucoside and quercetin-3-rhamnoglucoside, produced by *in vitro* fermentation with human faecal flora. Rapid Commun. Mass Spectrom., 2001, 15, 477–483.
22. Justesen U., Arrigoni E., Larsen B.R., Amado R., Degradation of flavonoid glycosides and aglycones during *in vitro* fermentation with human faecal flora. Lebensm. Wiss. Technol., 2000, 33, 424–430.
23. Knarreborg A., Simon M.A., Engberg R.M., Jensen B.B., Tannock G.W., Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl. Environ. Microbiol., 2002, 68, 5918–5924.
24. Kong J.M., Chia L.S., Goh N.K., Chia T.F., Brouillard R., Analysis and biological activities of anthocyanins. Phytochemistry, 2003, 64, 923–933.
25. Krul C., Luiten-Schuite A., Tenfelde A., Van Ommen B., Verhagen H., Havenaar R., Antimutagenic activity of green tea and black tea extracts studied in a dynamic *in vitro* gastrointestinal model. Mutat. Res., 2001, 474, 71–85.
26. McDougall G.J., Dobson P., Smith P., Blake A., Stewart D., Assessing potential bioavailability of raspberry anthocyanins using an *in vitro* digestion system. J. Agric. Food Chem., 2005a, 53, 5896–5904.
27. McDougall G.J., Fyffe S., Dobson P., Stewart D., Anthocyanins from red wine. Their stability under simulated gastrointestinal digestion. Phytochemistry, 2005b, 66, 2540–2548.
28. Netzel M., Stintzing F.C., Quaas D., Stras G., Carle R., Bitsch R., Bitsch I., Frank T., Renal extraction of antioxidative constituents from red beet in humans. Food Res. Int., 2005, 38, 1051–1058.
29. Neumann M., Goderska K., Grajek K., Grajek W., The *in vitro* models of gastrointestinal tract to study bioavailability of nutrients. Żywność. Nauka. Technologia. Jakość, 2006, 1, 30–45 (in Polish).
30. Re R., Pellegirini N., Protegente A., Pannala A., Yang M., Rice-Evans C., Antioxidant activity applying an improved ABTS radical cation decolonization assay. Free Rad. Biol. Med., 1999, 1231–1232.
31. Rechner A.R., Smith M.A., Kuhnle G., Gibson G.R., Debnam E.S., Srai S.K.S., Moore K.P., Rice-Evans C.A., Colonic metabolism of dietary polyphenols: influence of structure on microbial fermentation products. Free Rad. Biol. Med., 2004, 36, 212–225.
32. Scalbert A., Morand C., Manach C., Rémésy C., Absorption and metabolism of polyphenols in the gut and impact on health. Biomed. Pharmacother., 2002, 56, 276–282.
33. Scalbert A., Williamson G., Dietary intake and bioavailability of polyphenols. J. Nutr., 2000, 130, 2073S-2085S.
34. Schneider H., Blaut M., Anaerobic degradation of flavonoids by *Eubacterium ramulus*. Arch. Microbiol., 2000, 173, 71–75.
35. Singelton V.L., Rossi J.A., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 1965, 16, 144–158.
36. Slimestad R., Torskangerpoll K., Nateland H.S., Johannessen T., Giske N.H., Flavonoids from black chokeberries, *Aronia melanocarpa*. J. Food Compos. Anal., 2005, 18, 61–68.
37. Talavéra S., Felgines C., Texier O., Besson C., Lamaison J.L., Rémésy C., Anthocyanins are efficiently absorbed from the stomach in anesthetized rats. J. Nutr., 2003, 133, 4178–4182.
38. Williamson G., Day A.J., Plumb G.W., Couteau D., Human metabolic pathways of dietary flavonoids and cinnamates. Biochem. Soc. Trans., 2000, 28, 16–22.
39. Winter J., Moore L.H., Dowell V.R., Bokkenheuser V.D., C-ring cleavage of flavonoids by human intestinal bacteria. Appl. Environ. Microbiol., 1989, 55, 1203–1208.

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