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ANTIOXIDANT ACTIVITY OF GOJI BERRIES AND BILBERRY AT PARTICULAR DIGESTION STAGES IN AN *IN VITRO* MODEL SIMULATING THE HUMAN ALIMENTARY TRACT

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Abstract: The aim of the study was to evaluate the antioxidant activity of Chinese boxthorn fruit (goji) and bilberry digestion products. It has been shown that the simulated digestion in the stomach has no significant impact on the compounds of antioxidant nature. Bilberry fruit had a significantly higher antioxidant activity of the insoluble fraction of polyphenols when compared with goji fruit. Goji berries were characterized by a high migration rate of polyphenolic compounds through the dialysis membrane. The intestinal microflora had a significant influence on the release of antioxidant components from fruit.
Key words: antioxidant activity, polyphenols content, *in vitro*, bilberry, Chinese boxthorn

INTRODUCTION

Chinese boxthorn (goji) fruit is known in traditional Chinese medicine for centuries. For the antioxidant properties are responsible polyphenolic compounds, vitamins and bioactive polysaccharide complex (LBP). Polyphenolic compounds present in these fruits belong mainly to the group of phenolic acids and flavonoids. The main flavonoids present in goji berries are quercetin rhamnoside and rutin. Among the phenolic acids in wolfberry chlorogenic acid, p-coumaric acid and caffeic acid are mentioned (**Cieślik and Gębusia, 2012**). One of the most important representatives of the berries that grow wildly is bilberry. The main group of polyphenolic compounds present in these berries are anthocyanins, which are responsible for their color and constitute 90 % of total polyphenols in fruits. Glycosides of cyanidin, malvidin, delphinidin, peonidin, and petunidin are located mainly in the skin of bilberries. The main flavonols found in these berries include quercetin glycosides and phenolic acids - chlorogenic acid and caffeic acid (**Määttä-Riihinen et al., 2004**).

Fruits are the source of many bioactive components, including antioxidant compounds. These are mainly vitamins and polyphenolic compounds, which play an important role in the prevention of degenerative diseases, such as tumors or cardiovascular diseases. In nature, most polyphenolic compounds are present in food as esters, glycosides and polymers, which have to be hydrolyzed by digestive enzymes or the intestinal microflora in order to become absorbed (**Nyska et al., 2003**). Hydrolysis of flavonoid glycosides starts in the mouth, under the influence of β-glycosidases. In the stomach, the majority of the flavonols oligomers, due to the low pH of gastric juice, is degraded into smaller units. In the small intestine free phenolic acids are absorbed, while their esters have to be degraded by esterases produced by microflora present in the colon. Polyphenols, which have not been absorbed in the small intestine, go to the large intestine. There, colonic bacteria hydrolyze glycosides to aglycones, which are then converted to various acids. Microbiota can also catalyze the reactions of hydrolysis, dehydroxylation, demethylation and decarboxylation, and the resulting metabolites may be absorbed and transported by the portal vein to the liver (**Aura, 2005**).

The aim of the experiments was to evaluate the antioxidant activity of digestion products of goji fruit and bilberry. The evaluation was made at subsequent stages of digestion process in a simulated human digestive tract.

MATERIAL AND METHODS

The fruits that were subjected to *in vitro* digestion process were bilberry (*Vaccinium myrtillus*) and Chinese boxthorn – goji berry (*Lycium chinense*). The digestion process simulation consisted of following stages: digestion in the stomach and duodenum (pepsin, pH = 2), absorption through intestinal epithelium (pancreatin, bile, pH = 7, dialysis membranes) and digestion in the intestine by the intestinal microflora (inoculum of *Bacteroides galacturonicus, Enterococcus caccae, Bifidobacterium catenulatum, Ruminococcus gauvreauii, Lactobacillus* sp., and *Escherichia coli*). The antioxidant activity and total polyphenol content were determined in extracts of fruits and dialysates obtained after each digestion step.

As a result of the digestion process the followed dialysate were obtained:

(a) supernatant – soluble fraction of polyphenols digested in the stomach and duodenum,

(b) precipitate – insoluble fraction of polyphenols digested in the stomach and duodenum,

(c) contents of dialysis bags – soluble fraction of polyphenols not subjected to absorption by the intestinal epithelium,

(d) PBS buffer – soluble fraction of polyphenols absorbed by the intestinal epithelium,

(e) fermentation residue – insoluble fraction of polyphenols digested in large intestine.

The alcohol extracts of analysed fruits (10 g fruit + 90 mL methanol) were also prepared by high speed homogenization (19000 rpm, 5 min). Obtained solutions were filtered and filled up to 100 mL with solvent. Antioxidant activity was determined according to **Tarko et al. (2009)** and expressed in mg of Trolox/100 g. Total polyphenols content was determined according to **Tarko et al. (2009)** and expressed as mg of catechin/100 g, based on the standard plot.

RESULT AND DISCUSSION

The analyzed fruits have been subjected to the process of *in vitro* digestion using dialysis membranes. Antioxidant activity and total polyphenols content were determined both in the initial material and dialysates at every stage of digestion. Table 1 shows the results of the total content of polyphenolic compounds in fruits tested during the digestion process.

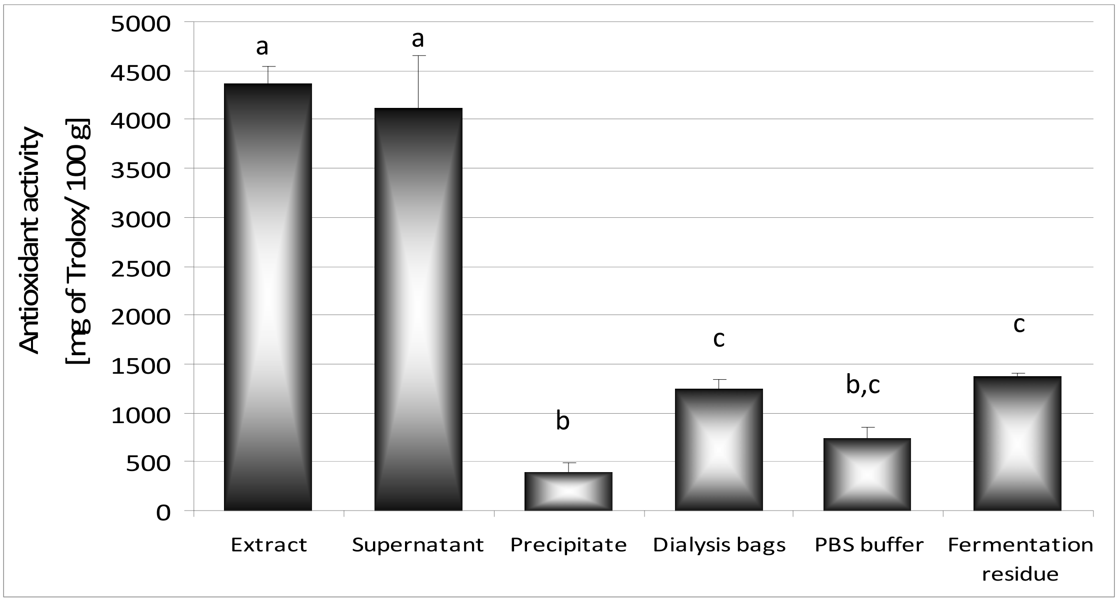
Table 1. Total polyphenols content in analyzed fruits at particular digestion stages

Specification	Total polyphenols content (mg of catechin/100 g)	
	Chinese boxthorn (goji)	Bilberry
Fruit extract	1431 ± 60^{a}	4271 ± 473^{a}
Supernatant	62 ± 10^{b}	48 ± 15^{b}
Precipitate	20 ± 13^{b}	21 ± 10^{b}
Contents of dialysis bags	43 ± 5^{b}	33 ± 8^{b}
PBS buffer	54 ± 20^{b}	27 ± 6^{b}
Fermentation residue	31 ± 9^{b}	41 ± 11^{b}

a,b - identical letters within the same fruit denote the lack of statistically significant differences ($p<0.05$)

Antioxidant activity of goji berries determined in the study was 4373 mg Trolox/100 g (Fig. 1). This value is in accordance with results of other authors (Luo et al., 2004; Huiping et al., 2010) that determined antioxidant activity of goji between 491 and 6110 mg Trolox/100 g. In the study, the total content of polyphenolic compounds in the Chinese boxthorn was 1431 mg catechin/100 g, and was higher than described in other papers. In the Medina (2011) study, the level of polyphenols ranged from 895 to 1036 mg/100 g. The discrepancies in the presented results are influenced by many factors, including temperature and method of fruit drying.

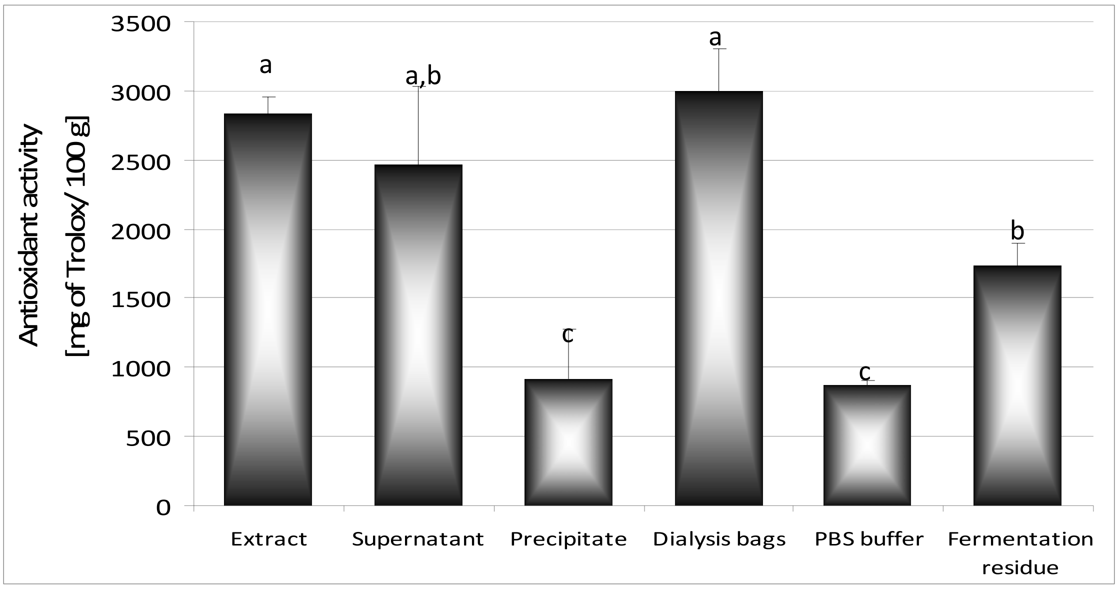
Antioxidant activity of bilberries analyzed in this study was 2838 mg Trolox/100 g (Fig. 2), while total polyphenols content was 4271 mg catechin/100 g (Tab. 1). Marinova et al. (2005) showed that polyphenols content in bilberries is 190.3 mg catechin/100 g. Antioxidant activity of bilberry fruit examined by Jabłońska-Ryś et al. (2009) reached the level of 1687 mg Trolox/100 g. The factors determining the content of polyphenolic compounds in bilberries can be environmental conditions and genetic determinants of fruit. The level of anthocyanins, the major polyphenols in bilberry, is strongly dependent on the action of visible light and ultraviolet radiation on the fruit.



a,b,c - identical letters denote the lack of statistically significant differences (p<0.05)

Fig. 1. Antioxidant activity of boxthorn fruit at particular digestion stages

Analysis of the antioxidant capacity of supernatants obtained after the first stage of digestion of goji berries (Fig. 1) and bilberry (Fig. 2) showed no significant changes in antioxidant activity after this stage, compared to adequate methanol extracts. At the same stage of digestion, however, a decrease in polyphenol content in dialysates of goji and bilberry fruits was demonstrated (23- and 89-fold, respectively).



a,b,c - identical letters denote the lack of statistically significant differences (p<0.05)

Fig. 2. Antioxidant activity of bilberry at particular digestion stages

The antioxidant activity of the precipitate obtained after bilberry digestion in the small intestine was more than 2-fold higher than the antioxidant activity of the precipitate after boxthorn digestion. At the same time, it was observed that the polyphenols concentration in the precipitates of those fruits was similar.

Based on these results, it appears that the insoluble fraction of polyphenols from bilberry contains powerful antioxidants. Chinese boxthorn fruit had a high migration rate of polyphenolic compounds through the dialysis membrane (87% of supernatant polyphenols was then found in soluble fraction of polyphenols absorbed by the intestinal epithelium). For bilberry the migration rate was lower (56%). Dialysates obtained at this stage of digestion were characterized by similar antioxidant activity, even though the concentration of polyphenols in the bilberry dialysate was twice lower. Bilberry is the fruit rich in anthocyanin pigments. The obtained results suggest that during digestion high concentrations of cyanidin and its glycosides are released. These are compounds with strong antioxidant potential, and their presence may result in the high ability to free radicals scavenging of dialysates of bilberry.

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