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SMALL BERRIES – ATTRACTIVE SOURCE OF BIOACTIVE COMPOUNDS FOR CONSUMERS

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Small berries nowadays start be very popular not only in medicine, pharmacy but also in gastronomy, due to the high amount of biologically active compounds and pleasant sensory characteristic. These kinds of fruits is rich for natural colorants, flavonoids, phenolic acids, vitamins and mineral compounds. Aim of this study was to investigate the biological activity (antioxidant activity, natural colorants, polyphenols and flavonoids) of selected kind of small berry fruits – acai, goji, schisandra, mulberry and sea buckthorn berries. The values of antioxidant activity by DPPH method was the highest in sea buckthorn berries 8.96 ±0.09 mg TEAC/g; values by molybdenum reducing antioxidant power method ranged from 94.69 (sea buckthorn) to 337.56 (acai) mg TEAC/g. The total polyphenol content in evaluated small berries ranged from 4.29 (mulberry) to 19.66 (acai) mg GAE/g; total flavonoid content was from 0.86 (goji) to 13.01 (acai) µg QE/g. Results showed that goji, schisandra and sea buckthorn berries are also rich for carotenoids, with the highest value in goji berry – 0.38 mg/g. In acai berry was detected high level of total anthocyanins – 10.74 mg/g. The results showed that small berries can be important tools for increasing nutritional value of diet, foods and can improve sensory properties of these products.

Keywords: antioxidant activity; polyphenols; flavonoids; fruit; natural colorants

Introduction

Small berries contain significant levels of micronutrients and phytochemicals with important biological properties. Consumption of this kind of fruits has been associated with diverse health benefits, such as prevention of heart disease, hypertension, certain forms of cancer and other degenerative or age-related diseases (Viskelis et al., 2012). Berries have been traditionally consumed worldwide to prevent and treat many diseases. In the last decennium some of the berry products, traditionally used in certain parts of the world, have appeared on market shelves of the other parts under the label "superfoods". Berry fruits are known for having antioxidant potential due to the various active compounds from several different classes, such as polyphenols, isoprenoids (carotenoids, terpens, phytosterols) or organic sulphur compounds (glucosinolates, glutamylcysteine sulphoxide). The most abundant active compounds in edible berries are phenolic acids, tannins, and flavonoids, especially anthocyanins (more than 550 anthocyanins have been reported), which give the nice and very attractive color for many flowers or berries (Diaconeasa et al., 2015). Because of the positive effects of small berries polyphenols on human health, the interest in consuming these kinds of fruits

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and their products is growing. In order to fully understand the roles of fruits polyphenols, further studies are needed (Jakobek et al., 2007).

The main objective of this study was to evaluated biological activity of small berries – acai, goji, schisandra, mulberry and sea buckthorn.

Materials and methodology

Materials

Small berries – acai (*Euterpe oleracea* Mart.), goji (*Lycium chinense* Mill.), schisandra (*Schisandra chinensis* Baill.), mulberry (*Morus alba* L.) and sea buckthorn (*Hippophae rhamnoides* L.) were purchased from local market in dry form. All chemicals used in this work were analytical grade and were purchased from Sigma-Alrich (USA) and CentralChem (SK).

Methods

Prepare of extracts

0.2 g of sample was extracted with 20 mL of 80% ethanol for 24 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids). Extraction was carried out in triplicate.

For carotenoid determination (goji, schisandra, sea buckthorn) 1 g of sample and acetone were added into the mortar and the sample was completely broken into pieces. The obtained extract was extracted with petroleum ether in separatory funnel and filtered into 50 mL flasks through a common filter paper.

For anthocyanin determination (acai) 1 g of sample was extracted with acidified ethanol. The obtained extract was extracted was filtered into 50 mL flasks through a common filter paper.

Radical scavenging activity

Radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Yen and Chen, 1995). Absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Molybdenum reducing antioxidant power method

Molybdenum reducing antioxidant power method of samples was measured according Prieto et al. (1999) method with a slight modification. Absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 700 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Total polyphenol content

Total polyphenol content extracts was measured by the method of Singleton and Rossi (1965) using Folin-Ciocalteu reagent. Gallic acid was used as the standard and the results were expressed in mg/g gallic acid equivalents.

Total flavonoid content

Total flavonoids were determined using the modified method of Lamaison and Carnat (Quitter – Deleu et al., 2000). Quercetin was used as the standard and the results were expressed in µg/g quercetin equivalents.

Total carotenoid content

Total carotenoid content was determined spectrophotometrically according STN (1986) method. Absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 449 nm. Results were expressed in mg β carotene equivalent per g of sample.

Total anthocyanin content

Total anthocyanin content was determined spectrophotometrically according Lee et al. (2005) method. Absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 520 and 700 nm. Results were expressed in mg cyanidine-3-glucoside equivalent per g of sample.

Statistical analysis

The basic statistical analyzes were realized in SAS programming packages (THE SAS SYSTEM V 9.2.). Correlation coefficients were calculated by CORR analysis (SAS, 2009).

Results and discussion

Radical scavenging activity

The highest activity (Table 1) of small berries by this method was detected in sea buckthorn and decreased in following order: acai > goji > schisandra > mulberry. Guo et al. (2017) similarly determined antioxidant activity of four subspecies of sea buckthorn berries, and results ranged from 266 to 369 µmol TEAC/g, with the best results in *Hippophae rhamnoides* L. subs. *sinensis*. Sea buckthorn berries start be more using in Slovak republic, due to the health benefits. From these berries is possible prepare widely kind of products, such as juice, syrup, jam, dry berries, pulp and also oil from small semen situated in inside part of berries. Very strong activity by DPPH method was also detected in acai berry, which is very popular in Slovakia mainly in beverage industry. Garzón et al. (2017) tested fresh acai berries and found antioxidant activity by DPPH method in amount 2.69 µmol TEAC/g. Acai berries similarly like sea buckthorn berries content high level of organic acids, and from this reason are very sour, which is for many consumers unpleasant. But in market nowadays consumers can find widely kind of products regarding beverages and candied berries which are very preferable for many consumers.

Table 1 Radical scavenging activity (DPPH) and molybdenum reducing antioxidant power (MRAP) of evaluated berries

Sample	DPPH, mg TEAC/g	MRAP, mg TEAC/g
Sea buckthorn	8.96 ±0.09	94.69 ±2.95
Goji	7.22 ±0.21	337.56 ±6.71
Schisandra	5.85 ±0.01	148.87 ±18.21
Mulberry	4.48 ±0.53	286.66 ±33.09
Acai	8.03 ±0.12	137.53 ±6.67

TEAC – Trolox equivalent antioxidant capacity; ±standard deviation

Molybdenum reducing antioxidant power method

The strongest activity by this method was detected in goji berry (Table 1) and decreased in following order: mulberry > schisandza > acai > sea buckthorn. Higher activity by this method was determined in mulberry berries. Dimitrova et al. (2014) also tested antioxiodant activity by reducing power method and found in mulberry water extract activity 4.53 µmol TEAC/g. Hamza et al. (2013) tested antioxidant activity of mulberry fruit powder in male rats exposed to gamma radiation and published that administration of mulberry fruit powder to irradiated rats was found to offer protection against irradiation induced oxidative stress by elevating the activity of antioxidant enzymes, enhancing liver function in addition to improving the lipid metabolism. From their results it could be concluded that the berries might be considered a natural antioxidant substance that can protect from radiation hazards.

The results of antioxidant activity are different with compare to DPPH method. The main cause is that DPPH and molybdenum reducing antioxidant power method run in different principle. In DPPH is possible activity of sample to eliminate synthetic radical; as eliminators works mainly mineral compounds, vitamins as well as poylphenols. In molybdenum reducing antioxidant power method antioxidants (mainly polyphenols and carotenoids) from sample act as reductones and reduce Mo^{VI} to Mo^{V}. From this reason is necessary for testing antioxidant activity of sample use more than one method.

Total polyphenol content

Polyphenols are ubiquitous secondary metabolites in plants. They are known to have antioxidant activity and it is likely that the activity of these extracts is due to these compounds (Tepe et al., 2006).

Total polyphenol content in tested berries was the highest in acai and schisandra berries. The lowest content (Table 2) was found in sea buckthorn sample. Schisandra berries are also called five-flavor fruits is known mainly in Chinese medicine, when are used for treatment of several diseases. Mocan et al. (2014) determined in schisandra berries total polyphenols in amount 9.20 mg GAE/g, and also these authors found that in schisandra berries is dominant from polyphenols chlorogenic acid and rutine, whereas isoquercetin and quercetin are present in smaller amount. Wang et al. (2011) found 26.79 ±17.06 mg GAE/g of total polyphenols in dry schisandra fruits.

Total flavonoid content

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants (Suhartono et al., 2012). Total flavonoid content (Table 2) in tested berries ranged from 0.86 to 13.01 µg QE/g, with the best results in acai and sea buckthorn. Role of flavonoids in human health is supported by the ability of the flavonoids to induce human protective enzyme systems, and by a number of epidemiological studies suggesting protective effects against cardiovascular diseases, cancers, and other age-related diseases (Yao et al., 2004). Small berries fruits can effectively increase amount of flavonoids in human diet. The highest amount of total flavonoids was determined in acai berries. Kang et al. (2010) isolated seven major flavonoids from freeze-dried acai pulp by various chromatographic methods. Their structures were elucidated as orientin, homoorientin, vitexin, luteolin, chrysoeriol, quercetin, and dihydrokaempferol. Sea buckthorn berries was also rich for flavonoids. Rop et al. (2014) tested six cultivars of sea buckthorn and amount of total flavonoids ranged from 4.18 to 7.97 mg RE/g (rutine equivalent). Suomela et al. (2006) reported that the flavonoids in sea buckthorn fruit pulp are mainly flavonols, of which isorhamnetin is typically found in the largest amounts. Quercetin is also present, as well as small amounts of kaempferol.

The most important flavonol glycosides in sea buckthorn are isorhamnetin-3-O-sophoroside-7-O-rhamnoside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside, quercetin-3-O-rutinoside, and quercetin-3-O-glucoside.

Table 2 Total polyphenol and flavonoid content of evaluated berries

Sample	Total polyphenols, mg GAE/g	Total flavonoids, µg QE/g
Sea buckthorn	7.81 ±0.95	6.81 ±0.54
Goji	13.51 ±1.58	0.86 ±0.06
Schisandra	15.55 ±0.45	3.66 ±0.47
Mulberry	4.29 ±0.53	4.84 ±0.21
Acai	19.66 ±0.52	13.01 ±0.76

GAE – Gallic acid equivalent; QE – quercetin equivalent; ±standard deviation

Total carotenoid and anthocyanin content

In sea buckthorn, goji and schisandra was also determined total carotenoid content, with the best results in goji (0.38 ±0.05 mg/g), following by sea buckthorn (0.15 ±0.01mg/g) and schisandra (0.12 ±0.02 mg/g). Carotenoids are known as photoprotection agents, defending the existing fotodestruction many biologically active substances in cells and tissues. Carotenoids are naturally pigments, with different colors like red, orange or yellow. Carotenoids are used as natural colorants for food and cosmetics (Bunghez et al., 2012). Protti et al. (2017) determined in dry goji berry presence of zeaxantin (dominant), β-cryptoxanthin, β-carotene and lutein. Statistically strong correlation ($p \leq 0.05$) was observed in our study between total carotenoid content and molybdenum reducing antioxidant power method ($\rho = 0.817$).

In acai berry was detected also amount of total anthocyanins – 10.73 ±0.57 mg/g, which indicated that this kind of berry is very rich for this important natural colorant.

Mertens-Talcott et al. (2008) tested pharmacokinetics of anthocyanins after the consumption of anthocyanin rich acai juice and pulp in human healthy voluntreers and published that anhocyanins from acai are bioavailable in healthy human volunteers upon the consumption of acai juice and pulp in moderate amounts and acai. Acai pulp caused a significant increase in the antioxidant capacity of plasma, which indicates the *in vivo* antioxidant potential of acai.

Conclusions

Small berries being rich in phytochemicals and incorporation of these into food diet and food products will add health benefits. As consumption of berries is becoming popular worldwide, mainly in gastronomy due to the positive sensory profiles of these berries and this using can supply essential nutrition as well as health benefits.

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