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Phenolic and carotenoid profile of new goji cultivars and their anti-hyperglycemic, anti-aging and antioxidant properties

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ABSTRACT

The aim of the study was to analyze potential health-promoting components of 21 new cultivars of goji (*Lycium barbarum* L.). Polyphenols and carotenoids were identified by LC-MS-Q/TOF and quantified by UPLC-PDA. Moreover, vitamin C, sugar and organic acid profile, antioxidant ($ABTS^{+}\cdot$, FRAP) and in vitro antidiabetic potential (inhibition of α-amylase and α-glucosidase) and anti-aging (acetylcholinesterase (AChE) and butyrylcholinesterase BuChE) activity were evaluated. Some new cultivars contained high amounts of polyphenols (g8, g13 and g43) and carotenoids (g11, g26 and g12), and showed antioxidant (g8, g17, g22, g23), antidiabetic (g26), and anti-aging activity (g4, g6, g37). They were also content of vitamin C (2.39 to 6.24 mg/100 g), organic acids (1.95–4.30 g/100 g), and sugars (1.59–5.11 g/100 g). In conclusion, selected new cultivars might be used for the production of functional foods, as well as for medical or/and cosmetic purposes.

1. Introduction

Lycium barbarum L., known as wolfberry or goji berry, is one of boxthorn shrub species in the nightshades family. Goji berries are orange-red, approximately 2 cm long ellipsoids with sweet-and-tangy flavor. They are an excellent source of carotenoids (0.03–0.5% d.w.), mainly of zeaxanthin that represents 31–56% of total carotenoids. The berries contain also lower amounts of neoxanthin, cryptoxanthin, and ß-carotene (Peng et al., 2005; Wang, Chang, Inbaraj, & Chen, 2010), which are natural pigments responsible for yellow, orange, and red color (Amagase, Sun, & Borek, 2009). Additionally, goji berries are rich in ascorbic acid (approx. 42 mg/100 g) (Llorent-Martínez, Fernández-De Córdova, Ortega-Barrales, & Ruiz-Medina, 2013), thiamin, riboflavin and vitamins E, B1, B2, and B6.

Some other compounds identified in the fruits include polyphenols – with quercetin and kaempferol derivatives of rutinoside as dominant fractions – chlorogenic and caffeic acids, and small amounts of caffeoylquinic and *p*-coumaric acids (Inbaraj, Lu, Kao, & Chen, 2010; Rocchetti et al., 2018; Wang et al., 2010).

Further, goji berries contain carbohydrates (arabinose, rhamnose, xylose, galactose, mannose and glucose) (Montesano et al., 2016), organic acids (malic acid, citric acid, shikimic acid and fumaric acid) (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012), and many minerals (potassium, sodium, phosphorus, magnesium, iron, calcium, zinc, and selenium) (Cieślik & Gębusia, 2012; Llorent-Martínez et al., 2013). Interestingly, iron content of 5.5 mg/100 g is high with respect to Dietary Reference Intake (DRI). Goji berries comprise also fatty acids (hexadecanoic acid, linoleic acid and myristic acid) (Blasi, Montesano, Simonetti, & Cossignani, 2017) and amino acids (proline, betaine and taurine) (Potterat, 2010).

In the last decades, numerous publications have reported goji berries as a rich source of phytocompounds with biological activity, and they are now receiving increasing attention. Due to numerous pro-health features, goji berries have been used in traditional Chinese medicine for over 2300 years (Cieślik & Gębusia, 2012). Nowadays, epidemiological studies suggest that goji berries exhibit antioxidant, anticarcinogenic and immunity enhancing properties, and exert anti-tumor, antidiabetic, cytoprotective, neuroprotective, and immunomodulatory effects (Chang & So, 2008; He, Yang, Jiao, Tian, & Zhao, 2012; Kulczyński & Gramza-Michałowska, 2016). These variable nutritional and health promoting properties are provided by a structurally varied range of components, including phenolics, carotenoids and polysaccharides (Kulczyński & Gramza-Michałowska, 2016; Le, Chiu, & Ng, 2007; Luo, Cai, Yan, Sun, & Corke, 2004). Additionally, the

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fruits are used in the treatment of heart, kidney, eye, lung, liver, and skin diseases (Zengping, Zulfiqar, & Ikhlas, 2008). The goji-related benefits have been attributed to carotenoid and polyphenolic compounds.

Current production of goji berries is 95,000 tonnes per year, and the crop acreage is 82,000 ha (Kulczyński & Gramza-Michałowska, 2016). Goji berries are mostly grown for dry fruits but also for juices, liquors, wines, spices for soups, meat and vegetarian dishes and tea amendments (Amagase & Farnsworth, 2011; Benzie & Wachtel-Galor, 2011). Apart from being natural, nontoxic colorants in drinks and cosmetics, goji carotenoids show biological activity, e.g. they act as antioxidants or precursors of vitamin A.

Goji bush comes from south-eastern Asia (Mongolia, Tibet, India and China), and its acreage is growing steadily in Japan, Korea and other countries of south-western Asia. Due to its short vegetation period, goji berry is successfully cultivated in Europe, including Poland. In Poland, this species was previously grown as a wild form on the loess hillsides of the Wisła and the Bug rivers, where it reinforced the riversides (Bogacz, 2009). Currently, five varieties of goji berries are available in Poland. The first in cultivation was 'Korean Big' stained for centuries in Chinese medicine, also of Chinese origin grows a variety 'Amber Swett Goji' with fruits of yellow, another is 'New Big' bred in Germany distinguished by large fruit with a length of 2 cm and width 1 cm. In 2013, in Poland was selected in the first variation of the sweet fruit without seeds ('NO 1'). High interest in goji berries encouraged breeders to develop new varieties adapted to moderate climatic conditions and doing well on commercial plantations in this part of the world. Environmental factors have significant effects on authentically and the content of main active components of *L. barbarum* fruits. The principle factors of content or accumulation in fruits of polysaccharides, carotenoids and polyphenols are soil available phosphorus, temperature-sunshine and accessibility to organic matter, respectively (Dong, Wang, Zhu, & Wang, 2012). Liu et al. (2017) postulated that environmental temperature play one of the most an important factor influencing on the phenolic compositions and contents in the leaves and stems. The higher and lower levels of phenolic components in the samples were observed as they were harvested in higher (April) and lower (February) temperature months, respectively. From an agronomical point of view, each region produces specific cultivars that may differ in chemical composition and biological properties. The differences between goji berries growing in China and Italy was previously evaluated by Rocchetti et al. (2018) or in different localization in China (Dong et al., 2012).

To the best of our knowledge, there are insufficient literature data for a comprehensive comparison of the new cultivars in terms of their antioxidant and enzymatic activity. Hence, the aim of this study was to evaluate the antidiabetic (α-amylase and α-glucosidase inhibitory activities), anti-aging (AChE and BuChE inhibitory activities) and antioxidant (ABTS and FRAP) properties of different new cultivars of Europe-grown goji berries and to prepare their phytochemical profile (carotenoids and polyphenols). The findings should be useful in the utilization of Lycium germplasm for new cultivars.

2. Material and methods

2.1. Reagents and standards

All standards of polyphenolic compounds and carotenoids were purchased from Extrasynthese (Lyon, France). Acetonitrile for ultra-performance liquid chromatography (UPLC; Gradient grade) and ascorbic acid were from Merck (Darmstadt, Germany). The rest reagents were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Plant material

Twenty-one new cvs. of goji berries (*Lycium barbarum* L.; Fig. 1) were investigated, namely, g4 (4v342), g6 (6g123), g8 (8g734), g10 (10a31), g11 (11z34), g12 (12g72), g13 (13b17), g17 (17k21), g20 (20g23), g21 (21a18), g22 (22g22), g23 (23j45), g25 (25v34), g26 (26a37), g27 (27g85), g35 (35g13), g36 (36a3), g37 (37z6), g41 (41b42), g43 (43s43), g44 (44g23). Fruit samples were collected from bushes grown in a field trial established in 2016 at the Experimental Orchard at Wrocław (51 07° 02.0″ N, 17°04′ 25.0″ E). All fruits pooled and then a subsample of 1 kg were taken from each lot. From the 1 kg subsamples, 0.5 kg fruits were collected randomly for the final lab analysis. The fresh fruits were directly frozen at −50 °C, and then freeze-dried (24 h; Christ Alpha 1–4 LSC; Germany). The homogeneous dry material was obtained by crushing the dried tissues using a closed laboratory mill (IKA A.11, Germany).

2.3. Identification and quantification of polyphenols

The powder samples of fruits (~1 g) were extracted with by 9 mL of mixture containing HPLC-grade methanol:H_2O (30:70%, v/v), ascorbic acid (2%) and acetic acid (1%) of reagent. The extraction was performed twice by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland) and with occasional shaking. Next, the slurry was centrifuged at 19,000*g* for 10 min, and the supernatant was filtered through a Hydrophilic PTFE 0.20 μm membrane (Millex Samplicity Filter, Merck, Darmstadt, Germany) and used for analysis. All extractions were carried out in triplicate. Qualitative (LC-MS-Q/TOF) and quantitative (UPLC-PDA) analysis of polyphenols (flavonols at 360 nm, and phenolic acids at 320 nm) were performed as described previously by Wojdyło, Carbonell-Barrachina, Legua, and Hernández (2016). Separations of individual polyphenols were carried out using a ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm, Waters Corporation, Milford, USA) at 30 °C. The samples (5 μl) were injected, and the elution was completed in 15 min with a sequence of linear gradients and isocratic flow rates of 0.42 mL/min. The mobile phase consisted of solvent A (2.0% formic acid, v/v) and solvent B (100% acetonitrile). The program began with gradient elution with 99–65% solvent A (0–12 min), and then lowering solvent A to 0% for condition column (12.5–13.5 min), the gradient returned to the initial composition (99% A) until 15 min for held constant to re-equilibrate the column. All measurements were repeated three times. The results were expressed as mg per *kg* of dry matter (dm).

2.4. Identification and quantification of carotenoids

The powder samples of fruits (0.20 g) containing 10% $MgCO_3$ and 1% butylhydroxytoluene (BHT) to prevent oxidation were continuously shaken with 5 mL of a ternary mixture of methanol/acetone/hexane (1:1:2, by vol.) at 300 rpm (DOS-10L Digital Orbital Shaker, Elmi Ltd., Riga, Latvia) for 30 min in the dark. Recovered supernatants were obtained after the 4–5 times re-extracted of solid residue. All combined fraction collected after centrifugation (4 °C, 7 min at 19,000*g*; MPW-350, Warsaw, Poland) were evaporated to dryness. The pellet was diluted using 2 mL of 100% methanol, filtered through a hydrophilic polytetrafluoroethylene (PTFE) 0.20-μm membrane (Millex Samplicity® Filter, Merck, Darmstadt, Germany) and used for analysis.

Carotenoids were carried out on LC-MS-Q/TOF and UPLC-PDA (ide)ntification and quantification, respectively) on a ACQUITY UPLC BEH RP C18 column being protected by guard column of the same materials (1.7 mm, 2.1 mm x 100 mm, Waters Corp., Milford, MA, USA) was operated at 30 °C. The elution solvents were linear gradient of acetonitrile:methanol (70:30%, v/v) (A) and 0.1% formic acid (B) as flow rates of 0.42 mL/min. The runs were monitored at 450 nm. The PDA spectra were measured over the wavelength range of 200–700 nm in steps of 2 nm. The retention times and spectra were compared to those of the authentic standards. All incubations were done in triplicate. The results were expressed as mg per *kg* of dm.