PAPER

NUTRITIONAL EVALUATION OF FRESH AND DRIED GOJI BERRIES CULTIVATED IN ITALY

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ABSTRACT

The nutritional profile of fresh and dried goji berries cultivated in Italy was investigated. The obtained data confirm goji berries as a source of nutritional and healthy components, such as vitamin E, minerals and fibre**.** Taking into account the Recommended Daily Allowance (RDA) for minerals and vitamins established by the Commission of the European Communities, Goji berries provide significant amounts of dietary fibre and zeaxanthin and can be declared on the label as a potential source of vitamins E and C. Moreover, dried goji berries can be declared as a source of K, P, Cu, Fe Mn, Zn.

Keywords: goji berries, *Lycium barbarum*, superfruit, wolfberries

1. INTRODUCTION

Fruits of *Lycium barbarum* L., belonging to Solanaceae family, commonly known as goji berries or wolfberries, have been used in Chinese traditional medicine for centuries. *Lycium barbarum* grows in China, Tibet and other parts of Asia and its fruits are 1-2 cm-long, bright orange-red ellipsoid berries. The native area of Lycium is not definitively established but it is likely found in the Mediterranean Basin (POTTERAT, 2010). Traditionally, goji berries are collected in summer and autumn. The fruits can be eaten fresh or dried, and they are also found in conventional food products, such as yoghurt, fruit juices, bakery foods, chocolate and others (MIKULIC-PETKOVSEK *et al*., 2012a, 2012b). The drying process is intended to remove water from foodstuff in order to prevent microbial spoilage and chemical alterations, thus prolonging shelf life, while realizing space and weight saving (CINQUANTA *et al*., 2010; CUCCURULLO *et al*., 2012; FRATIANNI *et al*, 2013). Traditionally, the berries are dried in the shade until the skin shrinks and then exposed to the sun until the outer skin becomes dry and hard but the pulp is still soft (AMAGASE and FARNSWORTH, 2011). The sun drying method is cheap, but there is a risk of damage due to dust and insect infestation. An alternative is hot air drying. Today the goji fruit market is significantly expanding because of an increased awareness of the possible health benefits, as fruits contain different nutrients, such as polysaccharide complexes, organic acids, phenolic compounds and antioxidants with high biological activity. Dietary fibre provides several health benefits, including the reduction of the risk of coronary heart disease, of diabetes, hypertension, obesity, stroke and some gastrointestinal disorders (EFSA, 2010). Recent studies indicate that polysaccharides from *Lycium barbarum* possess a range of biological activities, including antioxidant properties (AMAGASE and NANCE, 2008; CHANG and SO, 2008). Goji fruits constitute a variety of antioxidants such as ascorbic acid, different carotenoids (KULCZYŃSKI and GRAMZA-MICHAŁOWSKA, 2016) and high levels of phenolic compounds (ZHANG *et al*., 2016). Carotenoids are a significant group of biologically-active constituents with health promoting properties (AMAGASE *et al*., 2009; DONNO *et al*., 2014) responsible for the colour of a wide variety of foods (FRATIANNI *et al*., 2005).

The reddish-orange colour of *L. barbarum* fruits derives from a group of carotenoids, which make up only 0.03–0.5 % of the dried fruit. Zeaxanthin is the major carotenoid found in goji. This is a yellow pigment, an isomer of lutein and a derivative of β-carotene. When ingested, zeaxanthin accumulates in fatty tissues, but especially in the macula, a region of the retina, helping in protecting the macula from degeneration, which can be induced by excessive sun exposure (UV light) and by other oxidative processes. In goji, zeaxanthin is present as an ester of dipalmitate. Studies focusing on carotenoid goji berries are few and mainly aimed at the identification and quantification of ester-form carotenoids. INBARAJ *et al*. (2008) and ZHAO *et al*. (2013), in particular, identified free-forms and ester-forms of carotenoids. Beta-carotene, neoxanthin, and cryptoxanthin are also present at low concentrations (PENG *et al*., 2005; WANG *et al*., 2010). Regarding other antioxidants, studies made on *Lycium chinense Miller* reported high amounts of α-tocopherol**,** together with other vitamin E compounds (ISABELLE *et al*., 2010). Vitamin E is a generic term indicating structurally related compounds, namely tocols, comprising two groups of vitamers, i.e. tocopherols and tocotrienols, which occur in eight forms: α-tocopherol (α-T), β-tocopherol (β-T), γ-tocopherol (γ-T), and δ-tocopherol (δ-T) and α-tocotrienol (α-T3), β-tocotrienol (β-T3), γ-tocotrienol (γ-T3), and δ-tocotrienol (δ-T3). The potential health benefits of tocols have been the subject of several reviews (TIWARI and CUMMINS, 2009). Vegetable oils are the main tocol source; however, substantial amounts of these compounds are also reported in most cereal grains (FRATIANNI *et al*., 2013; MIGNOGNA

et al., 2015; PANFILI *et al*., 2003). To our knowledge, no literature data are available on the composition and content of tocols in *L. barbarum* fruits. Goji is also an extremely rich source of many essential minerals, which are essential for many actions in the body, like muscle contraction, normal heart rhythm, nerve impulse conduction, oxygen transport, oxidative phosphorylation, enzyme activation, immune functions, antioxidant activity, bone health, and acid-base balance of the blood (WILLIAMS, 2005; SALDAML and SAĞLAM, 2007). An adequate daily amount of minerals is necessary for an optimal functioning of the body. For the above reported reasons, goji berries are often proposed as functional foods and have been included in the novel category of "superfruits" or "superfoods".
Superfruits, a subcategory of superfoods, is a relatively recent word and is considered a new marketing approach to promoting common or rare fruits which can be consumed as foodstuffs or used as ingredients by manufacturers of functional foods, beverages and nutraceuticals. Superfruits have a high nutritional value due to their richness in nutrients, antioxidants, proven or potential health benefits and taste appeal (FELZENSZWALB, 2013). In the functional foods market, the products targeting health and mental well-being have prompted the food industry to increase the research and the development of these new foods, outlining a rapid expansion market in several countries (VICENTINI *et al*., 2016). In the last years, goji berries have been cultivated in Italy and are available both as fresh and dried fruits. While several papers on the medical effect of goji berries have been published, little information is available on the nutritional composition of dried and, above all, fresh goji berries. The aim of the present study is therefore to determine the compositional and nutritional value of fresh and dried goji berries cultivated in Italy, with a particular focus on minerals and some antioxidant compounds, such as carotenoids, tocols and vitamin C, to increase the awareness about their nutritional profile.

2. MATERIAL AND METHODS

2.1. Sample collection and preparation

Fresh goji berries (*L. barbarum L.*) were provided by *Favella Spa farm* (Sibari, Southern Italy). The farm has 21000 plants in 5 Ha (2.5 m x 1 m), with a drip irrigation system. Goji berries were cultivated in two consecutively growing seasons (2014 and 2015) and were collected in July. All harvested fruits were randomly collected in the orchard from different plants and analysed fresh and air-dried. Fresh goji berries (about 1 cm size) were subjected to freeze-drying before analyses, as reported by FRATIANNI *et al*., 2013 (fresh fruits). One-half of collected goji berries were air-dried in a convective dryer (B80FCV/E6L3, Termaks, Norway), at 60 °C, with an air velocity of 2.1 m/s, until a constant weight was reached (dried fruits). The drying time was about 21 h.
Results are reported as the average of the two growing seasons (2014-2015).

2.3 Proximate composition analysis

Fresh and dried fruits were analysed for moisture, ash, fat, and protein (N×6.25) contents, according to standard methods of AOAC (2000). Dietary fibre content was determined according to AOAC method 991.43 (1995) and AACC method 32-07 (1995). Total dietary fibre content was the sum of insoluble and soluble dietary fibre content. Vitamin C was determined by using an enzymatic kit (Megazyme International, Ireland), following the manufacturer instructions.

2.4. Mineral analysis

Ultrapure nitric acid for trace analysis, sulfuric acid (96 %) and standard mono elements in nitric acid 2 % were purchased from Sigma-Aldrich (20151 Milan, Italy). The determination of metals (K, Ca, Co, Cu, Fe, Mg, Mn, Mo, Na, P, Se, Zn) in goji samples was carried out by using the technique of nitric mineralization and the analysis by spectrophotometry plasma emission (*Varian ICP 710, OES* Inductively Coupled Plasma-Optical Emission Spectrometers, Palo Alto, CA 94304-1038). Samples were ground and 0.5 g was digested with 10 ml of nitric acid with a mineralizer (SCP Science DIGIprep, Quebec H9X 4B6, Canada), with the following instrumental conditions: start at 40 °C for 15 minutes; heating at 60 °C for 15 minutes; stay at 60 °C for 15 minutes; heating to 90 °C for 20 minutes. The digested samples were cooled and brought to a volume of 50 ml with bidistilled water and analysed with the optical ICP. The precision was calculated as a mean deviation of three measurements.

2.5. Carotenoid extraction and determination

Carotenoid extraction was carried out using the direct solvent extraction method reported in FRATIANNI *et al*. (2013) with slight modifications due to the complex structure of goji berries. About 0.1 g of milled freeze-dried samples (fresh fruit) and air-dried samples (dried fruit) was weighed and placed in a screw-capped tube. Then, 5 ml of ethanolic pyrogallol (60 g/L) was added as an antioxidant. The sample was stirred for 10 minutes. After that, 2 ml of absolute ethanol was added and the sample was stirred again for a few minutes. The suspension was then extracted with 15 mL of n-hexane/ethyl acetate (9:1 v/v) and stirred; after that 15 mL of sodium chloride (10 g/L) was added. Further extractions with n-hexane/ethyl acetate (9:1 v/v) were made until the organic layer was colourless. Finally, the organic layer was collected and evaporated to dryness, and the dry residue was dissolved in methanol: MTBE 50:50 (v/v). This sample was used to determine the free carotenoids not esterified with the lipid components and carotenoids esterified with fatty acids (unsaponified). A volume of 2 ml of this extract was evaporated to dryness and subjected to alkaline hydrolysis under a nitrogen flux for 1 minute in a screw-capped tube with 1 ml of ethanolic pyrogallol (60 g/L), 10 ml of solvent HEAT (hexane: ethanol: acetone: toluene 10: 6: 7: 7 v/v/v/v), 2 ml of methanolic KOH (40 %) and glass balls. The tubes were placed in a 56 °C water bath and mixed every 5 to 10 min. After alkaline digestion at 56 °C for 20 minutes, the tubes were cooled in an ice bath, and 15 mL of sodium chloride (10 g/L) were added. The suspension was then extracted with 15 mL of n-hexane/ethyl acetate (9:1 v/v) until the organic layer was colourless. The organic layer was collected and evaporated to dryness, and the dry residue was dissolved in methanol: MTBE 50:50 (v/v). This sample was used to determine carotenoids esterified with lipid components (saponified). An aliquot of the carotenoid extract was separated, as in MOULY *et al*. (1999), by a reverse-phase HPLC system. An HPLC Dionex (Sunnyvale, CA) analytical system, consisting of U3000 pumps, and an injector loop (Rheodyne, Cotati) were used. Separation was performed as in FRATIANNI *et al*. (2013) by using a YMC (Hampsted, NC, USA) stainless steel column (250×4.6 mm i.d.) packed with 5 μm silica spheres that were chemically bonded with a C30 material at a flow rate of 1 mL/min. The mobile phase was methanol: MTBE (v/v). The eluted compounds were monitored by a photo-diode array detector (Dionex, Sunnyvale) set at 430 nm.

2.6. Carotenoid identification and quantification

Carotenoids were identified on the basis of their diode array spectral characteristics, retention times, and relative elution order, compared with known commercially available standards. All-trans-β-carotene and lutein were from Sigma Chemicals (St. Luis, MO, USA); zeaxanthin and β-cryptoxanthin were obtained from Extrasynthese (Z.I. Lyon-Nord, Genay, France). Zeaxanthin dipalmitate was identified by means of its spectral characteristics found in literature (INBARAJ *et al*., 2008). Compounds were identified by comparison of their retention times with those of known available standard solutions and quantified on the basis of the calibration curves of standard solutions. Zeaxanthin dipalmitate was quantified as zeaxanthin.

2.7. Tocol analysis

Tocols were determined after the saponification method of the extract described for carotenoids. An aliquot of the carotenoid extract was collected and evaporated to dryness, and the dry residue was dissolved in 2 ml of isopropyl alcohol (1 %) in n-hexane and was analysed by HPLC under normal phase conditions, using a 250 x 4.6 mm i.d., 5 mm particle size, and Kromasil Phenomenex Si column (Torrance, CA, USA) (PANFILI *et al*., 2003). Fluorometric detection of all compounds was performed at an excitation wavelength of 290 nm and an emission wavelength of 330 nm by means of an RF 2000 spectrofluorimeter (Dionex, Sunnyvale, USA). The mobile phase was n-hexane/ethyl acetate/acetic acid (97.3:1.8:0.9 v/v/v) at a flow rate of 1.6 mL/min (FRATIANNI *et al*., 2002; PANFILI *et al*., 2003). Compounds were identified by comparison of their retention times with those of known available standard solutions and quantified on the basis of the calibration curves of standard solutions. The concentration range was 5-25 μg/ml for every tocol standard. Vitamin E activity was expressed as Tocopherol Equivalent (T.E.) (mg/100 g product), calculated as reported by SHEPPARD *et al*. (1993).

3. RESULTS AND DISCUSSION

3.1. Nutritional composition

The nutritional composition of fresh and dried goji berries is shown in Table 1. Fresh goji berries have 77.4 % moisture, 1.1 % fats, 2.5 % proteins, 15.3 % carbohydrates and 2.9 % fibre. In dried goji berries, 4.4 % fats, 10.2% proteins, 61.3 % carbohydrates and 11.4 % fibre were found. Similar results on dried goji were reported by ENDES *et al*. (2015). Our data suggest that dried fruits contain notable levels of dietary fibre, either as water-soluble form (2.6 %) or as insoluble form (8.8 %). The ratio between insoluble and soluble fibre is about 3:1. Dietary fibre intake recommendation for adults is 25 g/day (LARN, 2014). With the consumption of a portion of 30 g of dried fruits, dietary fibre intake for the adults is about 14 % of its daily recommended intake. Taking into account the European law (Regulation CE 1924/2006), dried goji can be declared in label with the claim "high fibre content", since it contains at least 6 g of fibre per 100 g. Finally, fresh and dried goji berries provide about 87 and 348 kcal/100 g, respectively.

Table 1. Nutritional composition of fresh and dried goji berries (g/100 g) (mean±standard deviation).

	Moisture	Fats	Proteins	Carbohydrates*	Fibre			Ash
					Soluble	Insoluble	Total	
Fresh	77.4±0.4	1.1±0.02	2.5±0.12	15.3	0.7±0.17	2.2±0.02	2.9	0.84±0.11
Dried	9.3±0.02	4.4±0.45	10.2±0.22	61.3	2.6±0.06	8.8±0.01	11.4	3.4±0.16

* Calculated by difference.

3.2. Mineral composition

The content of both macro and microelements in goji berries is reported in Table 2. Potassium (K) is the predominant element (276.2 mg/100 g and 881.9 mg/100 g for fresh and dried fruits, respectively), followed by sodium (Na). Potassium and sodium play an important role in regulating blood pressure and the body's acid-base balance (CLAUSEN *et al*., 2013). Goji could also be a good source of phosphorus (P) and calcium (Ca), with an appreciable concentration of magnesium (Mg), which is needed to prevent heart disease and growth retardation (CHATURVEDI *et al*., 2004). A discrete amount of copper (Cu), iron (Fe) and manganese (Mn) were also found. BELLAIO *et al*. (2016), ENDES *et al*. (2015) and LLORENT-MARTÍNEZ *et al*. (2013) reported slightly different results. As in any other plant food, the mineral content of berries reflects the soil in which they are grown. It is important to highlight that essential and nonessential element concentration is dependent on the soil characteristics, the physiology of the plant, the water source composition, and fertilizers, insecticides, pesticides, and fungicides used in the plantations. Plants can absorb, carry, and accumulate chemical elements. Each species has its own requirements and differing levels of tolerance when absorbing and accumulating an element. The movement of the inorganic constituents is selectively controlled by the plant, with some being easily absorbed and others impeded to a different degree (NAOZUKA *et al*., 2011).

Table 2. Average values of mineral elements in fresh and dried goji berries (mg/100 g) (mean±standard deviation).

	Fresh	Dried
Ca	26.6±4.90	101.3±22.60
K	276.2±41.00	881.9±239.70
Mg	12.7±2.80	45.9±9.20
Na	57.3±8.70	209.8±72.30
P	48.4±9.26	174.3±32.10
Co	0.001	0.001
Cu	0.3±0.04	0.8±0.25
Fe	0.9±0.22	3.4±1.57
Mn	0.2±0.03	0.5±0.18
Zn	0.5±0.12	1.5±0.62
Se (μg/100g)	0.1±0.01	0.17±0.03
Mo (μg/100g)	0.00	0.00

Table 3 reports the percentage contribution to the RDA of 100 g of fresh and dried goji berries, according to Reg. EU 1169/2011. For dried goji berries, the percentage of RDA per portion (30 g) is also reported. From data, fresh goji berries can be declared on the label as a source of Cu; in fact, 100 g of fresh goji berries contributed to about 25% of the RDA. The contribution of other minerals from fresh goji is low. Dried goji berries can be declared as a source of K, P, Cu, Fe Mn, Zn. A consumption of 30 g of dried goji per day contributes to the RDA approximately of 25 % for Cu, 13 % for K and less than 10 % for other elements.

Table 3. Percentage contribution to the RDA of minerals in fresh and dried goji berries.

	Reg. RDA	Fresh	Dried	Dried
	mg/day	% RDA	% RDA	% RDA x 30g
Ca	800	3	13	4
K	2000	14	44	13
Mg	375	3	12	4
P	700	7	25	7
Cu	1	25	84	25
Fe	14	6	24	7
Mn	2	8	26	8
Zn	10	5	15	5
Se (μg)	55	0	0	0

3.3. Carotenoid, tocol and ascorbic acid amounts

Table 4 shows HPLC carotenoid analysis of fresh and dried fruits. Drying of fruits did not cause significant changes in carotenoid amounts (data not shown). Unsaponified carotenoids, determined after solvent extraction, and saponified carotenoids, determined after saponification of the extract, are reported.

Table 4. Average carotenoid amounts in fresh and dried goji berries (mg/100 g) (mean±standard deviation).

	Fresh		Dried	
	Unsaponified	Saponified	Unsaponified	Saponified
Lutein	0.0	1.1±0.02	0.0	5.7±0.44
Zeaxanthin	0.0	53.8± 0.82	0.0	186.0±3.80
β-cryptoxanthin	0.0	2.3± 0.25	0.0	6.1±0.14
Zeaxanthin dipalmitate	47.8±2.32	0.0	158.8±1.53	0.0
Esters	8.5±1.24	0.0	24.5±2.30	0.0
β-carotene	0.2±0.01	0.1±0.01	0.9±0.09	1.0±0.20
Total carotenoids	56.4±1.23	57.3±0.80	184.2±1.52	198.8±3.80

Unsaponified carotenoids are characterized by a significant peak, identified as zeaxanthin dipalmitate, the dominant ester of goji berries (WELLER and BREITHAUPT, 2003; INBARAJ *et al*., 2008). Beta-carotene is also present. Before zeaxanthin dipalmitate peak, other unidentified peaks, probably carotenoid esters (INBARAJ *et al*., 2008), were also

detected. The amount of zeaxanthin dipalmitate in dried fruits is about 159 mg/100 g and of β-carotene is about 1 mg/100 g. INBARAJ *et al*. (2008) found values of zeaxanthin dipalmitate and of β-carotene of 114.3 mg/100 g and 2.4 mg/100 g, respectively. This difference is probably due to the fact that carotenoid levels can be influenced by different harvest stage fruits, geographical origin, and seasonality (WEN-PING at al., 2008). Saponification of the extract is necessary to convert esters to free-compounds and it is often used to remove chlorophylls, lipids and other analytical interferences (FRATIANNI *et al*., 2015). The saponified extract of dried fruits shows high zeaxanthin contents (about 190 mg/100 g) and small lutein and β-cryptoxanthin amounts (about 6 mg/100). Small amounts of lutein were also found, after saponification, in a work of ZAO *et al*., 2013. As a dietary supplement for eye health (CHENG *et al*., 2005), a dose of 15 g per day was deemed beneficial in supplying adequate zeaxanthin (estimated at 3 mg/day). Thirty g of our goji samples provide a zeaxanthin amount of 14 mg/day (fresh fruit) and 48 mg/day (dried fruit). Table 5 shows the tocol amounts in fresh and dried goji berries. As for carotenoids, the drying treatment did not cause significant changes in tocol contents (data not shown).

Table 5. Average tocopherol amounts in fresh and dried goji berries (mg/100 g) (mean±standard deviation).

	Fresh	Dried
α-tocopherol	1.4±0.10	5.5±0.48
β -tocopherol	1.0±0.01	4.2±0.04
Total tocopherols	2.4±0.04	9.7±0.20
Tocopherol Equivalent (TE) [§]	2	8

§ Calculated as in SHEPPARD *et al*., 1993.

Goji berries were found as a source of α- and β-tocopherol (about 1.4 and 1.0 mg/100 g, respectively, in fresh fruits, and 5.5 and 4.2 mg/100 g, respectively, in dried fruits). A paper by ISABELLE *et al*. (2015) reports, in *Lycium chinenses,* belonging to the same *Lycium* species, the presence of α-tocopherol (3.9 mg/100 g), together with γ-tocopherol (0.46 mg/100 g), δ-tocopherol (0.12 mg/100 g), and traces of α-γ-δ tocotrienol (< 0.1 mg/100 g). Table 5 also reports values of vitamin E activity provided by 100 g of product, expressed as Tocopherol Equivalent (TE) (mg/100 g product) (SHEPPARD *et al*., 1993). Taking into account the Recommended Daily Allowance (RDA) for vitamin E, which is of 12 mg/day (Regulation EU 1169/2011), 100 g of fresh goji berries contribute approximately 16 % of the RDA, while 100 g of dried fruits contribute approximately 66 % of the RDA, so that to be declared in label as a source of vitamin E. A portion of dried goji berries (30 g) contributes approximately 20 % of the RDA. The concentration of vitamin C was about 40 mg/100 g in fresh fruits and 38 mg/100 g in dried fruits. DONNO *et al.* (2015) report an amount of about 42 mg/100 g in dried goji berries. Taking into account the Recommended Daily Allowance (RDA) for vitamin C of 80 mg/day (Regulation EU 1169/2011), 100 g of fresh or dried goji berries contribute approximately 50 % of the RDA, so that they can be declared on the label as a source of vitamin C. A portion of dried goji berries (30 g) contributes about 16 % of the RDA.

4. CONCLUSIONS

Goji berries cultivated in Italy were confirmed as an important source of healthy compounds, providing a significant contribution to the diet, in terms of some inorganic nutrients, and of dietary fibre, zeaxanthin, vitamins E and C. In particular, taking into account the Recommended Daily Allowance (RDA) for minerals and vitamins established by the Commission of the European Communities, dried goji berries can be declared as a source of K, P, Cu, Fe Mn, Zn. Moreover, both fresh and dried berries can be declared on the label as a potential source of vitamins E and C.
The presented results enhance the knowledge of the composition and the nutritional characteristics of fresh and dried goji berries cultivated in Italy and will help in verifying the information reported in the label.

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Paper Received October 12, 2017 Accepted February 26, 2017