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Italian *Lycium barbarum* L. Berry: Chemical Characterization and Nutraceutical Value

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Lycium barbarum L. has received considerable attention in recent years also in western countries because of the interesting healthy value of these berries. In this paper, goji samples cultivated in Southern Italy were analyzed for their chemical composition and nutritional profile in order to characterize fruits of Italian origin and to increase the awareness about their nutraceutical value. Lipid fraction was characterized by high percentages of unsaturated fatty acids, in particular oleic and linoleic acids, and very low values of atherogenic and thrombogenic indexes (0.1 and 0.2, respectively). In addition, goji berry was an interesting source of phytosterols (41.5 mg/100 g), essentially represented by β-sitosterol. Carotenoid analysis showed the presence of zeaxanthin, in esterified form, with high content of zeaxanthin dipalmitate (277.9 mg/100 g). Finally, in vitro antioxidant capacity and phenolic compounds were investigated. The results suggested that goji hydro-alcoholic extract possessed the ability to scavenge free radicals. Phenolic acids were clearly the most abundant compounds followed by flavonols and favanols. The results reported in this study confirm that Italian *L. barbarum* berry is a rich source of bioactive molecules with nutraceutical properties.

Keywords: *Lycium barbarum* berry, Nutritional composition, Nutraceuticals, Lipids, Carotenoids, Phenolics.

For centuries, goji berry (*Lycium barbarum* L.) has been used in traditional medicine in China [1] and, in recent years, the large customer demand for goji berry-related food products resulted in producing these berries even in western countries, among which Italy. Recently, the production and sale of raw berries and goji berry-related food products (juices, jams, bakery products, energy bars) have increased rapidly since these berries have been described as superfood [2]. Moreover, goji berry bioactives are available on the market as nutraceuticals in the form of supplements and capsules [3].

The popularity of these fruits is due to the supposed beneficial effects in the prevention of chronic diseases (cancer, atherosclerosis and diabetes), and promotion of weight loss and longevity [4]. The *L. barbarum* berry consumption and/or supplementation may be useful for the prevention of chronic eye diseases [5] and for the improvement of lipid profile and antioxidant status in patients with metabolic syndrome [6].

As regards biologically active components of *L. barbarum* fruit, polysaccharides, phenols, and carotenoids are the most studied, together with the lipid composition of seed oils or essential oils from *Lycium* species [4, 7].

In recent years, some papers have concerned the chemical composition and the biological properties of *Lycium* spp., cultivated in Italy. Some researchers studied the antioxidant compound fingerprint and the bioactivity of goji berry fruit (*Lycium* spp.), harvested in Northern Italy. They studied goji chemical-nutraceutical composition (polyphenolic compounds, monoterpenes, organic acids, and vitamin C) and bioactivity, using different liquid chromatographic methods for comprehensive authentication and quality control of these fruits [8]. The nutritional profile of fresh and dried goji berries cultivated in Southern Italy was evaluated, confirming that goji berry was a source of bioactive components, such as vitamin E, minerals and fiber [9]. It was also reported a comparative study on biological properties, in particular the antioxidant activity, of Calabrian goji *vs* Chinese goji [10]. Their results highlighted the good antioxidant, anti-inflammatory and anticholinesterase *in vitro* properties of the Italian goji extract, which was also found to be a rich source of carotenoids, compared to the Chinese goji extract. Some authors studied the antioxidant activity, cytotoxicity and cell absorption of oil obtained by supercritical CO_2 extraction from *L. europaeum* fruit (from Sardinia island) [11]. The goji oil was characterized by high levels of lipid soluble antioxidants (all-*trans*-zeaxanthin, all-*trans*-β-carotene, and α-tocopherol), phenols and flavonoids. Moreover, the oil showed radical scavenging activity and induced a significant *in vitro* inhibitory effect on the growth of colon adenocarcinoma cells [11]. Metabolite bioactives of fresh fruits, leaves, stems and flowers of Italian *L. barbarum* were studied by NMR [12]. They found kaempferol, caffeic acid, 3,4,5-trihydroxycinnamic acid and 5-hydroxyferulic acid in fresh fruits; rutin and chlorogenic acid in leaves and flowers. The phenolic profile of Italian goji berries, investigated through ultra-high-pressure liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UHPLC-QTOF-MS), showed that hydroxycinnamic acids, followed by flavonols and flavanols, were the most representative phenolics [13].

Other papers relate to the determination of zeaxanthin dipalmitate [14], sugars [15], and lipids [16] of goji berry purchased on the Italian market. While several papers on composition and biological activity of Chinese goji berries have been published [4, 17], little information is available on Italian goji berries. Therefore, the aim of the present study is to determine the chemical composition and nutritional profile of fresh goji berries cultivated in Southern Italy, with a particular focus on fatty acids, sterols, carotenoids and phenolic compounds. The characterization of bioactives in *L. barbarum* fresh fruits can be useful to increase the awareness about their nutraceutical value. Figure 1 shows the chemical composition of fresh Italian goji berries.

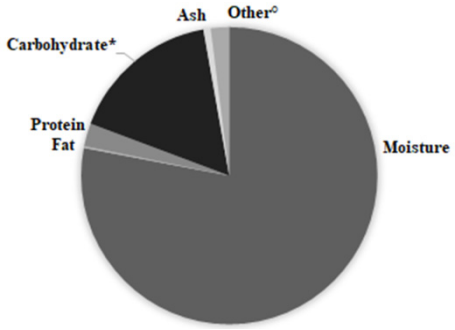

Figure 1: Plot of chemical composition of fresh goji berries (g/100g) (mean values, *n*=3). Carbohydrate*, simple sugars (glucose, fructose, sucrose); Other°, digestible and non-digestible polysaccharides.

They have, in descending order, 78.0% moisture, 16.5% simple sugar, 2.5% protein, 0.8% ash and 2% other compounds (digestible and non-digestible polysaccharides). Similar results were reported for fresh Italian berries (77.4% moisture, 1.1% fats, 2.5% proteins, 15.3% carbohydrates and 2.9% fibre) [9]. The gross composition of dried ground flour from Turkish goji was studied and the following proximate composition data were reported: 10.34% moisture, 4.11% crude oil, 8.90% crude protein, 7.30% fibre [18]. Table 1 shows the mineral composition of fresh Italian goji berries. Potassium (K) is the predominant element, followed by sodium (Na), with values of 150.8 and 61.4 mg/100g, respectively.

Table 1: Mineral composition of goji berries (mg/100g, mean values ± SD, *n*=3) and contribution to the RDA

Minerals	mg/100g	% RDA*
Ca	8.9±0.0	1
K	150.8±3.6	8
Mg	21.5±0.2	6
Na	61.4±1.8	4
Fe	0.4±0.0	3
P	4.5±0.0	1

*calculated for 100g of fresh goji berries, according to Reg. EU 1169/2011 and LARN (2014) for Na.

Other authors also reported that potassium was the main mineral (13447.4 mg/kg), but they found mineral contents rather different [18]. Moreover, the results obtained in this study shows that magnesium (Mg) content was higher than that (12.7 mg/100g) reported by other authors [9]. According to literature [18], the mineral composition of foods of vegetable origin, as goji berry fruit, varies greatly according with climatic conditions, geographical origin, harvesting time, postharvest treatments, and the use of fertilizers. Table 1 shows also the percentage contribution to the RDA (100 g of fresh goji), according to Reg. EU 1169/2011 [19] and LARN (2014) [20]. From data, fresh goji berries can be considered a good source of K, in fact 100 g of fresh goji berries contribute to about 8% of the RDA. High dietary potassium (>3.5 g/day) is associated with a decrease in blood pressure, an effect which has been consistently documented in several meta-analyses of randomized clinical trials [21]. Vitamin C content in fresh goji berries was 35 mg/100g for which 100 g of fresh berries contribute approximately to 44% of the RDA, according to the RDA reported in Reg. EU 1169/2011 [19]. Other authors reported lower [8] or similar [9] contents. Table 2 shows the FA percentage composition of goji seed oil, obtained by HRGC analysis.

Table 2: Fatty acid composition (% mol) of goji seed oil (mean values ± SD, *n*=3).

Fatty acid		%
Saturated (SFA)		
Myristic acid	C14:0	0.2 ± 0.0
Palmitic acid	C16:0	8.4 ± 0.1
Margaric acid	C17:0	0.2 ± 0.0
Stearic acid	C18:0	2.8 ± 0.2
Arachidic acid	C20:0	0.5 ± 0.0
Behenic acid	C22:0	0.5 ± 0.0
Lignoceric acid	C24:0	0.2 ± 0.0
Monounsaturated (MUFA)		
Hexadecenoic acid	C16:1n-7+n-9	0.9 ± 0.1
Heptadecenoic acid	C17:1n-8	0.1 ± 0.0
Octadecenoic acid	C18:1n-9+n-7	34.7 ± 0.3
Polyunsaturated (PUFA)		
Linoleic acid	C18:2n-6	48.8 ± 0.6
α-Linolenic acid	C18:3n-3	1.6 ± 0.0
γ-Linolenic acid	C18:3n-6	1.1 ± 0.1
Index		
AI Atherogenic Index		0.1
TI Thrombogenic Index		0.2
HI Hypocholesterolemic Index		10.0

AI=C12:0+4×C14:0+C16:0)/(MUFA+PUFAn-6+PUFAn-3)
TI=(C14:0+C16:0+C18:0)/[0.5×MUFA+0.5×PUFAn-6+3×PUFAn-3+(n-3/n-6ratio)]
HI=(C18:1n-9+C18:1n-7+C18:2n-6+C18:3n-6+C18:3n-3+C20:3n-6+C20:4n-6+C20:5n-3+C22:4n-6+C22:5n-3+C22:6n-3)/(C14:0+C16:0)

The FA percentage contents, grouped as polyunsaturated (PUFA), monounsaturated (MUFA), and saturated (SFA), together with some ratios (PUFA/SFA and PUFA/MUFA), are reported in Figure 2.

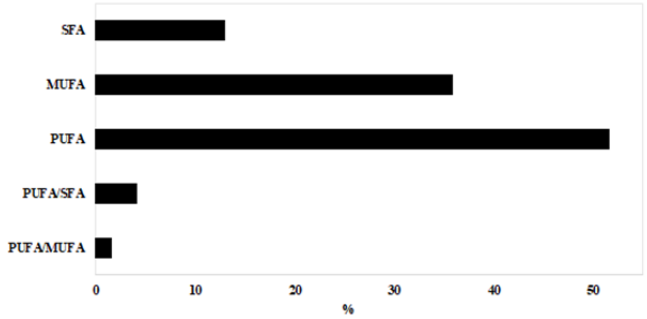

Figure 2: Plot of FA grouped as saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and some ratios (PUFA/SFA; PUFA/MUFA).

It can be noted that the unsaturated FA (UFA) were the most abundant (87.2%, of which 35.7% MUFA and 51.5% PUFA) in respect to SFA (12.8%), in fact the PUFA/SFA and PUFA/MUFA ratios were 4.0 and 1.4, respectively. The SFA were essentially represented by palmitic and stearic acid, on average 8.4% and 2.8%, respectively. The other SFA (myristic C14:0, margaric 17:0, arachidic C20:0, lignoceric C24:0 acids) were present in an amount less than 0.5%. Octadecenoic acids (C18:1 n-9+n-7) were the main MUFA (34.7%), while linoleic acid (C18:2 n-6) was the principal PUFA (48.8%). Only few and not extensive papers reported on the FA composition of seed oils obtained from goji berries. The FA composition of mature Italian *L. europaeum* berry oil was investigated and a concentration of approximately 21% of SFA (mainly palmitic 16:0 and stearic C18:0 acids), 17% of MUFA (mainly oleic C18:1 n-9 and palmitoleic C16:1 n-7 acids) and 60% of PUFA, mainly constituted by linoleic acid (C18:2 n-6) was found [11]. In order to discriminate geographic origin of *L. barbarum* berry FA and sterol composition were studied, and it was found that Italian samples had high content of linoleic acid (40.5%), followed

by octadecenoic acids (C18:1 n-9+n-7, 31.7%) [22]. Some authors studied the FA composition of goji seed oils obtained by different extraction methods and found that UFA were from 84.21% to 87.91% for CO_2 acetone and CO_2 extraction methods, while SFA were 15.79 and 12.09%, respectively [23]. Other researchers reported linoleic (60.77%), oleic (21.69%), and palmitic (8.23%) acids as major FA [18]. The FA composition of *L. intricatum* seed oil was investigated and it was found that the content of SFA, MUFA and PUFA were 5.93, 44.57, and 49.47%, respectively [24].

As known, FA composition is of crucial importance for determining the properties and health benefits of a food, particularly for cardiovascular health. The nutritional quality of the lipid fraction was determined by examining the FA profile and taking into consideration the following indexes: atherogenicity (AI) and thrombogenicity (TI) indexes [25] and hypocholesterolemic (HI) index [26]. These indexes, reported in Table 2, show interesting values of AI (0.1), TI (0.2) and HI (10.0), since the low values of these indexes indicate high quantities of anti-atherogenic FA in oil or fat. The lower AI and TI index values, the healthier the food. Further, some authors [27] underscored that oil or fat was nutritionally the most adequate based on the relation between hypocholesterolemic and hypercholesterolemic FA. Based on these results, goji berry could represent a novel dietary source of essential FA, especially linoleic acid. An alkaline hydrolysis was carried out on goji seed oil to obtain data relative to the qualitative and quantitative composition of sterol fraction. Sterol identification was carried out by HRGC-MS and the typical fragmentation is reported in Table 3, together with the sterol composition (% and mg/100g).

Table 3: Fragmentation ions of TMSE sterols of goji seed oil and sterol composition (% and mg/100g, mean values ± SD, *n*=3).

Sterols	EI-MS *m/z*	%	mg/100g
Cholesterol	458,443,368,353,329	7.9 ± 0.5	3.2 ± 1.8
Ergosterol	468,453,378,363,339	11.9 ± 0.8	4.5 ± 2.1
Stigmasterol	484,469,394,379,355	5.9 ± 0.3	2.8 ± 1.8
Δ-5,23-stigmastadienol	486,469,394,379,355	2.0 ± 0.4	1.2 ± 1.0
β-sitosterol	488,471,396,381,357	59.3 ± 1.5	23.8 ± 5.4
β-sitostanol	484,473,398,3883,359	4.1 ± 0.3	2.0 ± 1.9
Δ5-avenasterol	484,469,394,379,355	7.1 ± 0.6	3.0 ± 1.3
Δ7-stigmastenol	486,471,396,381	0.3 ± 0.0	0.1 ± 0.2
Δ7-avenasterol	484,469,394,379	1.5 ± 0.3	0.9 ± 1.3
Δ5-sterol (total)		98.2	40.5
Δ7-sterol (total)		1.8	1.0

TMSE sterols give a not abundant molecular ion, while the first significant ion observed in the high mass range was usually equivalent to $[M-15]^+$, due to the loss of the methyl terminal group. Other main fragment ions useful to identify the single sterol compounds were $[M-90]^+$, $[M-105]^+$, and $[M-129]^+$. They correspond to the loss of the trimethylsilanol, methyl group with trimethylsilanol and to the fragmentation of 1,2-cyclopenthanophenanthrene structure, respectively. It can be noted that Δ^5-sterols, mainly represented by β-sitosterol, were the major components (98.2%) in respect to Δ^7-sterols (1.8%). These data confirmed those reported in a previous study [22], where β-sitosterol, generally the most abundant sterol in vegetable edible oils, was the main sterol of Italian samples. Phytosterols have been studied for their role in lowering cholesterol levels. In addition to this property, plant sterols possess anti-atherogenicity, anti-inflammatory, anti-cancer, and anti-oxidation activities [28].

Based on these results, goji berry could represent a novel dietary source of phytosterols, interesting functional components actually added to many foods, as spread, oils, and butter [29]. Carotenoid extract of goji berries showed a complex composition. Figure 3 shows the HPLC-DAD profile obtained with C30 column and the quantitative results are reported in Table 4.

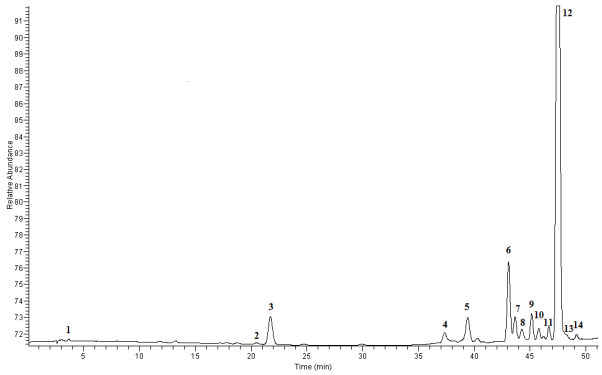

Figure 3: HPLC-DAD profile of the carotenoidic fraction of goji berries. **1**. (all-*E*)-zeaxanthin, **2**. (all-*E*)-lutein palmitate, **3**. (all-*E*)-zeaxanthin palmitate, **4**. (all-*E*)-violaxanthin caprate laurate, **5**. (all-*E*)-β-cryptoxanthin palmitate, **6**. (all-*E*)-antheraxanthin dipalmitate, **7**. (all-*E*)-luteoxanthin dipalmitate, **8**. (all-E)-zeazanthin palmitoleate palmitate, **9**. (all-*E*)-zeaxanthin myristate palmitate, **10**. (all-*E*)-lutein palmitate oleate, **11**. (all-*E*)-zeaxanthin palmitate oleate, **12**. (all-*E*)-zeaxanthin dipalmitate, **13**. (*Z*)-zeaxanthin dipalmitate, **14**. (all-*E*)-zeaxanthin palmitate stearate.

It is possible to observe a very low concentration of free xanthophylls such as zeaxanthin (<1 mg/100g), while the monoester forms are present mainly as zeaxanthin monopalmitate (14 mg/100g) and to a lesser extent as lutein monopalmitate (<5 mg/100g). After the free and monoester xanthophylls, the diester forms, represented mainly by zeaxanthin dipalmitate that accounts for 78% of carotenoid total area, were eluted.

The main advantage of the extraction procedure followed in this work [30], is represented by the possibility to study the native forms of carotenoids in fact, avoiding the saponification step, all the esterified xanthophylls can be detected by HPLC. The evaluation of carotenoid ester profile represents a very studied topic in the last years [5,31], while the determination of carotenoids after saponification gives only information about the free xanthophyll's and carotenoids, losing all the information about the individual carotenoid compound. This last approach was used by some researchers that studied the carotenoid composition in *L. barbarum* berry [32]. They reported the concentration of β-carotene, zeaxanthin, kryptoxanthin, zeaxanthin in *cis* and *trans* forms; in particular, all-*trans*-zeaxanthin accounted for 132.6 mg/100 g while a high concentration of zeaxanthin dipalmitate, equal to 277.9 mg/100g dw, was found in Italian berries (Table 4). Other authors reported that in Chinese goji berry zeaxanthin dipalmitate content ranged from 280 to 540 mg/100g of edible portion and represented more than 85% of total carotenoids [14]. In another paper, lower amount (114 mg/100g) of zeaxanthin dipalmitate was found [33].

It should be highlighted that the variability in carotenoid profile depends on several factors, among which the degree of ripeness, in fact unripe and fully ripe fruit contained very different concentration of the individual carotenoids [5]. For example, a great variation of zeaxanthin dipalmitate content, ranging from 0% in mature green fruits to 80% in fully ripe fruits, was observed.

This high amount of carotenoids in goji berry is closely related to amount of carotenoids in goji berry is closely related to various biological/pharmacological properties of this fruit. As an example, it has been reported that the zeaxanthin from goji berry showed an anti-inflammatory potential tested in Blab/c mice [34].

Other authors [35] reported zeaxanthin as a promising agent for the prevention and treatment of uveal melanoma, the most common malignant intraocular tumor in adults. Consumption of dietary

Table 4: Identification data and composition (% and mg/100g, mean values ± SD, *n*=3) of carotenoids of goji berry, on dry weight.

N°	Tr (min)	Identity	UV/VIS λmax (nm)	ESI (+)-MS *m/z*	%	mg/100g
1	3.6	(all-*E*)-zeaxanthin	426/450/476	591-569	0.2 ± 0.0	0.6±0.1
2	20.5	(all-*E*)-lutein palmitate	424/445/472	830-789-533	1.3 ± 0.2	4.8±0.9
3	21.7	(all-*E*)-zeaxanthin palmitate	426/450/476	830-789-533	4.0 ± 0.1	14.4±0.2
4	37.3	(all-*E*)-violaxanthin caprate laurate	418/440/469	962-739	1.5 ± 0.7	5.3±2.7
5	39.4	(all-*E*)-β-cryptoxanthin palmitate	426/451/477	791-535	4.2 ± 2.7	15.1±9.3
6	43.0	(all-*E*)-antheraxanthin dipalmitate	424/446/473	1084-805-549	4.1 ± 0.3	14.9±1.4
7	43.6	(all-*E*)-luteoxanthin dipalmitate	407/428/453	1078-799	0.5 ± 0.1	1.8±0.2
8	44.2	(all-*E*)-zeazanthin palmitoleate palmitate	426/450/476	1067-789-533	2.4 ± 0.1	8.5±0.1
9	45.1	(all-*E*)-zeaxanthin myristate palmitate	426/450/476	1040-789	0.6 ± 0.2	2.0±0.6
10	45.8	(all-*E*)-lutein palmitate oleate	424/445/472	1094-789	1.5 ± 0.0	5.5±0.1
11	46.7	(all-*E*)-zeaxanthin palmitate oleate	426/450/476	1094-789	0.4 ± 0.0	1.6±0.1
12	47.5	(all-*E*)-zeaxanthin dipalmitate	426/450/476	1069-789-533	77.5 ± 1.8	277.9±9.8
13	48.3	(*Z*)-zeaxanthin dipalmitate	424/448/474	1069-789-533	1.2 ± 0.3	4.2±1.0
14	49.1	(all-*E*)-zeaxanthin palmitate stearate	426/450/476	1096-817-789	0.7 ± 0.1	2.4±0.3

wolfberry could be beneficial to retinoprotection through reversal of mitochondrial function in diabetic mice. Goji berry attenuated hypoxia and mitochondrial stress as demonstrated by declined expression of hypoxia-inducible factor-1-α vascular endothelial growth factor, and heat shock protein 60 [36]. The results reported in this study confirm that Italian goji berry is a rich source of carotenoids, containing remarkable amounts of zeaxanthin dipalmitate that presents the interesting advantage of a better solubility and bioavailability in comparison to free zeaxanthin [14].

Total phenolic content (TPC), together with the antioxidant capacity of goji berry sample, was investigated by means of Folin-Ciocalteu and ORAC assays, respectively. The mean value of TPC detected using the Folin assay was around 7.6 mg GAE/g, and this latter was very closed to what obtained from semi-quantitative analysis. Overall, our results fitted with previous findings [37], analyzing the phenolic composition and antioxidant capacities in four red goji berries from China. In particular, these authors pointed out a mean value of 3.2 mg GAE/g. Subsequently, the antioxidant capacity of goji berries was evaluated by means of ORAC radical scavenging test, in order to understand the possible health-promoting effects provided by this matrix. An ORAC mean value of 6.3 μmol TE/g was obtained, in line with other works investigating the ORAC values in Calabrian and Chinese goji extracts [10].

These results suggested that the hydro-alcoholic fraction of goji berries, characterized by phenolic compounds, possessed the ability to scavenge free radicals. Therefore, our results sustain the use of goji berry extracts to promote health-benefits thanks to their wide phenolic composition and *in vitro* antioxidant potential.

The combination of a comprehensive database on polyphenols (*i.e.*, Phenol-Explorer) with recursive analysis and frequency filters applied allowed to annotate 379 compounds. Flavonoids were definitely the most common class of phenolics detected, with 201 compounds (being 79 flavonol, 71 flavones, and 51 anthocyanin equivalents), followed by 72 phenolic acids (hydroxybenzoic and hydroxycinnamic acids), 59 tyrosol equivalents, 24 lignans (with 13 dibenzylbutyrolactones and 11 furofurans, respectively), 14 alkylphenols, and 9 stilbenes. The entire list of phenolic compounds identified is provided as supplementary data, together with annotations (raw formula, identification score) and composite mass spectrometry spectra. The untargeted screening of goji berry extracts suggested a wide and diverse profile in phenolic compounds detected.

However, considering the semi-quantitative values for phenolic class equivalents (Table 5), phenolic acids were clearly the most abundant compounds with a mean value of 7536.1 mg/kg (expressed as ferulic acid equivalents) followed by flavonols and favanols (expressed as catechin equivalents), with a mean value of 1066.0 mg/kg.

Table 5: Semi-quantitative values for phenolic class equivalents (mean values ± SD, *n*=3) of goji berries, on dry weight.

Compounds	mg/kg
Luteolin Eq.	122.6 ± 12.8
Cyanidin Eq	275.3 ± 70.5
Catechin Eq.	1066.0 ± 171.6
Ferulic acid Eq.	7536.1 ± 949.9
Tyrosol Eq.	729.7 ± 119.3
5-Pentadecylresorcinol Eq.	209.0 ± 65.3
Sesamin Eq.	227.2 ± 29.0
Matairesinol Eq.	448.2 ± 38.5
Resveratrol Eq.	11.2 ± 0.8

As regard the phenolic distribution, the most abundant phenolics were glycosidic forms of quercetin and kaempferol (supplementary data), while among hydroxycinnamics an abundance of 4-hydroxybenzoic acid, hydroxycaffeic acid and coumaric acid derivatives was noted. These findings are in agreement with the results reported by other authors [38], reviewing the *L. barbarum* composition and its health benefits. In particular, these authors underlined the importance of hydroxycinnamic acids (*i.e.*, ferulic acid, caffeic acid, chlorogenic acid and coumaric acid) and flavanols (*i.e.*, catechin and epicatechin) in goji berry samples. Interestingly, the UHPLC-QTOF analysis allowed us to confirm previously findings [39] regarding the anthocyanin identification in this food matrix. In particular, an average content of 275.3 mg/kg was found, even though the goji berry color is clearly given by carotenoids when considering *L. barbarum* species. At this regard, glycosidic forms of pelargonidin and delphinidin were the most abundant anthocyanins detected (supplementary data). Moreover, as can be observed by semi-quantitative values, the distribution of lignans was different; in fact, matairesinol derivatives were more abundant than sesamin equivalents (448.2 *vs* 227.2 mg/kg, respectively). From a general point of view, these findings confirm that Italian goji berry sample is very rich in polyphenols. However, it's important to underline that the actual phenolic composition depends on several factors, including geographical origin, variety and genetic background, environmental and harvest conditions, and post-harvest factors [40]. The results reported in this study highlighted that goji berries, cultivated in Italy, are interesting fruits because important source of healthy compounds, among which unsaturated fatty acids, phytosterols, carotenoids and phenolic compounds. Knowledge gained from this research will help to determine the potential of these berries to be commercially exploited for nutraceutical applications and incorporation into food formulations to improve human health.

Experimental

Samples: Fresh mature fruits of *L. barbarum* were provided by Sud Rienergy S.r.l., a farm located in the area of Corigliano Calabro (Cosenza, Calabria, Italy). The latitude and longitude of the collection site were 39°129 13' 27,69" N and 9° 01' 29,69" E, respectively. Three samples of goji berries were randomly collected in the orchard from different plants in the 2016 harvest season

(July-August). The samples were taxonomically identified by Rizzo Nicola (Sud Rienergy farm). Representative specimens of *L. barbarum* fruits were deposited at Orto Botanico, Centro di Ateneo per i Musei Scientifici, University of Perugia (Italy). The samples were analyzed immediately after arrival to the laboratory or after being stored for few days at 4 °C. An aliquot of fruit samples was also stored at -20 °C.

Analysis of chemical and nutritional composition: Fresh fruits were analyzed for moisture, ash, and protein (N×6.25) contents, according to standard methods [41]. Sugars were identified and quantified by HPLC equipped with evaporative light scattering detector, as reported in a previous paper [15]. Lipid fraction was quantified after extraction, following a published previously method [16]. Mineral analysis was carried out by ICP-OES [9]. Vitamin C was determined by 2,6-dichloroindophenol titrimetric method [41].

Fatty acid analysis: The fatty acid methyl esters (FAME) of total lipid fraction were obtained by transesterification at room temperature in capped screw top tubes, according to the method published previously [42]. The samples were analyzed by high-resolution gas chromatography (HRGC) coupled with flame ionization detector (FID) as reported in a previous paper [16].

Sterol analysis: Initial alkaline hydrolysis was performed according to the AOCS method (Ch 6-91), after 5-α-colestane (internal standard) addition. Trimethylsilyl ether (TMSE) derivatives, prepared as described in a previous paper [43], were analyzed by HRGC-FID and identified by HRGC coupled with mass spectrometry (MS) detector [22].

Carotenoid extraction and analysis: Goji fruit samples, sun-dried for 1 week until to reach about 16% moisture, were milled by electric blender with distilled water in order to remove all polar compounds and prepare the matrix for the extraction of carotenoids.

Then the sample were washed with absolute methanol and extracted with hexane-acetone mixture (3:2, v/v) until the residue became colorless. Goji fruit carotenoids were by HPLC with diode-array and mass spectrometry detection systems (DAD-MS), using the validated method described in a previous paper [30].

Total phenolic content and in vitro antioxidant capacity determination: TPC was determined colorimetrically, according to the Folin-Ciocalteu assay. Briefly, absorbance was recorded at 765 nm after a 40 min at 20 °C incubation step. A calibration curve was prepared using gallic acid as standard compound, and the results were expressed as mg of gallic acid equivalents (GAE)/g of dried sample [13]. Regarding the determination of *in vitro* antioxidant capacity, the oxygen radical antioxidant capacity (ORAC) test was used, according to a previously reported procedure [13]. Results were expressed as μmol trolox equivalents (TE)/g of dried sample.

UHPLC-QTOF-MS phenolic profiling of goji berries: Phenolic compounds were then screened by means of a hybrid quadrupole-time-of-flight mass spectrometer coupled to UHPLC-QTOF-MS system, on the basis of previous experiments on goji berries [13]. Furthermore, in order to provide quantitative information and considering the availability of nine phenolic standards (methanolic standard solutions of pure individual phenolics, from Extrasynthese, Lyon, France), cumulative intensities for each phenolic class were calculated, and results finally converted into quantitative data, using calibration curves. In particular, ferulic acid (for hydroxycinnamic acids and other phenolic acids), matairesinol (for dibenzylbutyrolactone and dihydroxydibenzylbutane lignans), sesamin (furan and furofuran lignans), cyanidin (anthocyanins), catechin (flavanols), luteolin (flavones and other remaining flavonoids), resveratrol (stilbenes), 5-pentadecylresorcinol (alkylphenols) and tyrosol (tyrosols and other remaining phenolics) were used as representatives of their respective phenolic classes.

Statistical analysis: Analytical procedures were carried out in duplicate. Data were processed and edited with Microsoft Excel 2016 (Microsoft Office, USA). Metabolomics data on phenolic profiling were interpreted using the software Agilent Mass Profiler Professional B.12.06 (from Agilent Technologies). Phenolic compounds were filtered by abundance and by frequency (only those compounds with an area > 10000 counts and appearing in 100% of samples in at least one condition were considered), normalized at the 75th percentile, and then baselined to the median of each compound in all replicates.

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