

Influence of applied drying methods on phytochemical composition in fresh and dried goji fruits by HPLC fingerprint

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Abstract *Lycium* spp. fruit (goji or wolfberry) has become more popular due to its public acceptance as a functional food or “super food/fruit”. The aim of this study was to describe the influence of two different drying systems (air-thermal and freeze drying) on fresh and dried goji fruit nutraceutical traits and report on the level of potentially bioactive compounds and their effects on total fruit phytochemical and antioxidant activity. Spectrophotometric and chromatographic methods coupled to multivariate analysis were performed on all the goji samples. In this research, goji fresh and dried fruits were identified as a source of compounds with potential health-promoting properties. The results of this study showed that freeze drying allowed to preserve most of the phytochemical characteristics of the fresh fruits, as phenolic acids (ferulic and ellagic acids) and vitamin C content. Moreover, polyphenols could be selected as biomarkers in order to distinguish different *Lycium* species or the same *Lycium* genotype from different geographical areas. This research emphasizes that an adequate evaluation of nutraceutical traits is necessary in order to select the higher-quality raw materials (cultivated varieties): as a cost-effective and efficient way to trace food, this study showed that HPLC fingerprint can be used to identify and track different raw materials (fresh fruits) and derived food products (dried fruits).

Keywords *Lycium* spp. · Drying system · Chemical composition · Food security · HPLC analysis

Introduction

The international food trade is continuously growing, led by the sophisticated preservation technology and rapid transport methods. Additionally, consumers’ tastes and food habits are changing, becoming more varied, and stimulating the demand for traditional and new foods from other regions, attractive not only for their culinary characteristics but also for their novel properties [1]. However, the sustainability of these new productions from other areas compared with common local fruit species should also be evaluated [2]. In particular, the so-called functional foods are one of the most interesting areas of research and innovations in the food industry [3]: the growing demand for functional food may be associated with the rising costs of health care, the steady increase in life expectancy and the interest of the elderly in the improvement of life quality [4].

This growing consumer’s awareness coupled with progress in nutrition science provides companies with opportunities to develop a range of new functional products [5, 6]. However, consumer choices of functional foods involve increasing perceived risks and complexity [7]. Moreover, food trade globalization requires the development of an integrated approach for tracing the origin, quality, and authenticity of commercialized products to ensure food safety [8]. Food traceability is essential to boost the reliability of labeling, certify the origin and quality of market produce, and prevent labeling fraud [9]. To address these problems, different marker methods are used, as molecular techniques [10], phytochemical fingerprinting [11, 12], sensory analysis [13]. In this context, *Lycium* spp. fruit,

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commonly known as goji (or wolfberry), has become more popular for the last few years due to its public acceptance as a functional food or “super food/fruit” with highly advantageous nutritive and antioxidant properties [14].

Lycium spp. fruit is a famous traditional medicine in Asian countries [15]. There are two closely related species, *Lycium barbarum* L. and *L. chinense* Miller, used as a functional food for more than 2500 years [16]. Through specialized health food stores, *L. barbarum* has penetrated the world health product market with a total export of US\$ 120 million in China. The closely related species *L. chinense* is also sold as goji in grocery stores; however, the price of *L. chinense* is much lower than that of *L. barbarum*, which has steeply decreased the cost of using goji products. Another member of *Lycium* spp., *L. ruthenicum*, is extremely scarce and thus has become costly because of demand [9]. The identification of *L. barbarum* and its closely related species is important not only for food origin monitoring but also for ensuring clinical drug safety [17]: as the morphology and tissue structure of the *L. barbarum* fruit resemble those fruit of other *Lycium* species, species closely related to the official goji dominate the market [18].

Modern studies indicate that extracts from *Lycium* spp. possess a range of biologic activities, including antioxidant properties [14]. The extracts also exhibit antiaging effects, neuroprotection, promotion of endurance, increased metabolism, improved control of glucose and other diabetic symptoms, antiglaucoma effects, immunomodulation, anti-tumor activity, and cytoprotection [19].

An increased awareness of their possible health benefits intensified the interest in the goji chemical composition: *Lycium* fruits are a rich source of nutrients and phytochemicals as organic acids, sugars and polysaccharides, carotenoids (zeaxanthin), vitamins (vitamin C, vitamin B complex, vitamin E), flavonoids and phenolic acids, minerals and trace elements (zinc, iron, calcium, selenium, germanium, and phosphorus), 18 amino acids (including eight essential amino acids), alkaloids, and fat [14].

Goji products are sold on the health food market as fresh fruits, but especially as dried products [20]. Drying is probably the oldest known method of food preservation: the sun drying of fruits and the smoking of fish and meat are both well-known processes that originated in antiquity. A dried food product offers the advantage of decreased weight, which has the potential for savings in the cost of transporting the product. However, there is often a decrease in the quality of the dried product because most of the conventional techniques use high temperatures during the drying process [21]. As many of the new techniques use lower temperatures and/or decreased drying times, they should be considered for use by the food industry. The terms “dried” or “dehydrated” are not synonymous: the US Department of Agriculture lists dehydrated foods as those with no more

than 2.5 % water, while dried foods apply to any food product with more than 2.5 % water [22].

The drying system technology should be selected considering the final destination of the produce, its specifications and characteristics. Solar (open-air) drying is the traditional drying method for goji fruits and probably the oldest industrial process still being used. However, this process has several drawbacks, as the requirement for large areas of space and for high labor inputs, difficulty in controlling the drying rate, insect infestation and microbial contamination [21]. Convection drying is a simple technique, which is in some ways similar to solar drying and has many of the same drawbacks, as quality losses regarding color, flavor (taste and aroma), and texture, limited rehydration, case hardening (the formation of a hard outer shell), and shrinkage [23]. The drying process takes place in an enclosed, heated chamber. The drying medium—in most cases, hot air—is allowed to pass over the product, which has been placed in open trays [24]. In contrast, another technique, freeze drying, utilizes the principle that, under high vacuum, frozen water can be removed from a food and collected without going through a liquid phase. In this way, freeze drying produces the highest-quality dried food product: the freeze-dried fruits become very light and crispy and retain much of their original flavor (taste and aroma) and phytochemicals. This is largely because the structure of the fruits is not severely damaged as in other drying procedures. However, this method is a slow and expensive process [21, 23].

Dried fruits possess much lower moisture content as a large proportion of their original water has been removed, either naturally through sun drying or through the use of specialized dryers or dehydrators [20]. Epidemiologic studies have found an association between dried fruit consumption and diet quality [25]. Indeed, dried fruits are rich sources of essential nutrients and health-promoting bioactive compounds [26]: they are low in their fat and sodium content, devoid of trans-fats and cholesterol and good sources of dietary fiber and minerals (copper, iron, magnesium, manganese, phosphorus, and potassium) [27]. Dried fruits are also excellent sources of phytochemicals with high values of antioxidant activity, as phenolic compounds and vitamins. In particular, dried fruits contain both water-soluble (betaine, choline, folate, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, and vitamin C) and fat-soluble vitamins (A, E, and K) [19]. In general, they are not good sources of amino acids due to their low protein content, even if dried fruits contain all indispensable amino acids.

Numerous health benefits of dried fruits have been reported [28]. Indeed, dried fruit consumption is associated with a lower body weight, improved adiposity measures, and higher overall diet quality [20] thanks to their essential

Table 1 Origin and identification code for each goji fruit sample

Sample	Sampling site	Identification code
Fresh fruit genotype 1	Boves	GBovF
Dried fruit genotype 1	Boves	GBovD
Fresh fruit genotype 2	Bagnasco	GBagnF
Dried fruit genotype 2	Bagnasco	GBagnD

Freeze-drying system was considered

nutrients and phytochemicals as well as their antioxidant activities [26].

Dried fruits are widely used as ingredients in packaged snacks, confectionary products, baked goods, cereals, energy and nutritional bars, ready-to-eat salads, and sweet industries, among many other specialty foods [29].

This study focused on nutraceutical traits and health-promoting effects based on the chemical fingerprint and antioxidant activity of fresh and dried fruits despite the confusion and difficulty of distinguishing between *L. barbarum* and *L. chinense* cultivated varieties, morphological, quality, and nutraceutical information indicates that probably hybrid cultivars between the two species were analyzed. These genotypes are actually the most cultivated in small family-managed farms and nurseries with commercial purposes (juice products). The aim of this study was to describe the influence of two different drying systems on fresh and dried goji fruit nutraceutical traits and report on the level of potentially bioactive compounds and their effects on total fruit phytocomplex and antioxidant activity. This research emphasizes that it is necessary define an effective phytochemical fingerprint, used as a quality and traceability control tool; as few information is currently available on the bioactive compound fingerprint of goji fresh and dried fruit, the results of the present study may encourage a deeper evaluation of the effective nutraceutical value for different *Lycium* spp. genotypes and different commercial products.

Materials and methods

Plant material

Samples of goji fruit (hybrid cultivars between *L. barbarum* and *L. chinense* were analyzed) were harvested in two farm located in Boves and Bagnasco (Cuneo province, Northern Italy) in October 2014; the fruits (0.5 kg for each plant) were manually picked from three plants for each replication. Each sample was identified with a code (Table 1).

In this research, first of all, two drying methods were compared for each genotype and then, due to its

better performance, the innovative freeze-drying system was selected in order to compare fresh and dried fruits: the same analyses were performed on fresh and dried fruits in order to understand the effects of drying processes on the real added nutritional value of this fruit species.

A part of all the harvested fruits were immediately analyzed fresh or after being stored for few days at 4 °C and 95 % relative humidity (RH). The second part has been subjected to a drying process with two different methods (air-thermal and freeze drying). Air-thermal drying (convective system) is a classic process used to dry foods: analyzed fruits were exposed to a continuously flowing hot stream of air (temperature: 60 °C; system power: 1.39 kW/h) in a stove (WIPA, Stadtlohn, Germany) at very low relative humidity (less than 0.5 %) for 3 days in order to reach a changeless weight (loss of water = −84 %). Freeze drying, one of the best methods of water removal from biological materials, was also used: in this case, water was removed from analyzed fruits by dehydration, through sublimation of ice in the materials by an air flow (temperature: 35–40 °C; humidity: 2–3 %) blown toward the fruits to be dried. The equipment for freeze drying (Model NWT-25, North West Technology, Cuneo, Italy) was based on the “vaporization chain system” (system power: 0.72 kW/h; drying cycle capacity: 40 kg; drying cycle time: 6–48 h).

Solvents and chemicals

Sodium carbonate, Folin–Ciocalteu phenols reagent, sodium acetate, citric acid, hydrochloric acid, iron(III) chloride hexahydrate, 2,4,6-tripyridyl-*S*-triazine (TPTZ), and 1,2-phenylenediamine dihydrochloride (OPDA) were purchased from Sigma Aldrich (St. Louis, MO, USA), while acetic acid was purchased from Fluka Biochemika, Buchs, Switzerland. Ethylenediaminetetraacetic acid (EDTA) disodium salt was purchased from AMRESCO (Solon, OH, USA), while sodium fluoride was purchased from Riedel–de Haen (Seelze, Germany). Cetyltrimethylammonium bromide (cetrimide) was purchased from Extrasynthèse (Genay, France).

Ethanol was purchased from Fluka Biochemika (Buchs, Switzerland). Analytic HPLC grade solvents, methanol and acetonitrile, and formic acid were purchased from Sigma Aldrich and Fluka Biochemika, respectively; potassium dihydrogen phosphate, and phosphoric acid were also purchased from Sigma Aldrich. Milli-Q ultrapure water was produced by using Sartorius Stedium Biotech mod. Arium (Sartorius, Goettingen, Germany).

All polyphenolic and monoterpenic standards were purchased from Sigma Aldrich. Organic acids were purchased from Fluka Biochemika, while ascorbic acid and dehydroascorbic acid were purchased from Extrasynthèse.

Spectrophotometric analysis

Total polyphenolic compounds (TPC)

For the extraction of polyphenolic compounds, samples (10 g) were placed in 50 mL test tubes, and 25 mL of extraction solution (methanol and water acidified with 37 % conc. HCL, 95:4.5:0.5 v/v/v) were subsequently added to the weighed samples (whole fruit); after 60 min in the dark, the extracts were homogenized with an Ultra-Turrax (T25, IKA WERKE, Staufen, Germany) for about 1 min and then centrifuged for 15 min at 50 Hz (3000 rpm or $1008\times g$) in an ALC Centrifuge PK 120 (ALC International, Cologno Monzese, Italy). The method used for the determination of total polyphenol content (TPC) was based on Folin–Ciocalteu phenol reagent and spectrophotometric determination [30]. A total of 0.5 g of fruit extract, weighted before dilution, and 30 mL of bi-distilled water was added to 2.5 mL of Folin–Ciocalteu reagent and 10 mL of Na_2CO_3 15 %. Then, water was added up to 50 mL (total volume); after 2 h in the dark, absorbance was 765 nm.

The standard calibration curve was plotted using gallic acid at concentrations of 0.02–0.1 mg mL⁻¹. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight (FW).

Antioxidant activity

Antioxidant activity in the goji fruit pulp was evaluated by ferric reducing antioxidant power (FRAP) assay [31]. The extracts used for analysis were those used previously for quantification of total polyphenols.

The method was based on the reduction of the ferric (Fe^{3+}) TPTZ (2,4,6-tripyridyl-*S*-triazine) complex to its ferrous form (Fe^{2+}). The FRAP reagent was prepared daily by mixing 2.5 mL of TPTZ solution and 2.5 mL of $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ solution with 25 mL acetate buffer (0.3 M) and then warmed at 37 °C before using; 30 mL of sample (15 mL of extract sample and 15 mL of extraction buffer, dilution 1:2) was added to 90 mL of bi-distilled water and 900 mL of FRAP reagent in a 2-mL micro tube and then incubated at 37 °C for 30 min in a Shaking Water Bath (G.F.L. Shaking Water Bath mod.1083, Burgwedel, Germany). Absorbance at 595 nm with a UV/Vis spectrophotometer (1600-PC, VWR International) was recorded.

The standard curve was obtained using $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ (concentration range: 100–1000 $\mu\text{mol L}^{-1}$), and results were expressed as millimoles of Fe^{2+} equivalents per kg (solid food) of FW.

Chromatographic analysis

Sample preparation protocols

Polyphenolic compounds Methanolic extracts used for the previous analysis were filtered with circular pre-injection filters (0.45 μm , polytetrafluoroethylene membrane, PTFE) and then stored for a few days at normal atmosphere (N.A.), 4 °C and 95 % RH.

Organic acids For the extraction of organic acids, three replications, each obtained from 30 fruits (whole fruit), were considered. Five grams of fruit were put into a test tube and 25 mL of 95 % ethanol solution were then added. After 10 min in the dark, the extracts were homogenized with an Ultra-Turrax (T25, IKA WERKE, Staufen, Germany) for about 1 min and then centrifuged for 10 min at 66 Hz (4000 rpm or $1792\times g$) in an ALC Centrifuge PK 120 (ALC International, Cologno Monzese, Italy).

Samples were then stored in at N.A., at 4 °C and 95 % R.H until analysis.

Vitamin C Ten grams of fruit (whole fruit) were put into a test tube with 10 mL of extraction solution (0.1 mol L⁻¹ citric acid, 2 mmol L⁻¹ ethylenediaminetetraacetic acid (EDTA) disodium salt, and 4 mmol L⁻¹ sodium fluoride in methanol—water 5:95 v/v) were then added.

The extracts were homogenized with an Ultra-Turrax (IKA WERKE T25) for about 1 min and then centrifuged for 10 min at 66 Hz (4000 rpm or $1792\times g$) at room temperature in an ALC Centrifuge PK120. The supernatants were recovered and transferred to a second test tube through filter cloth and then acidified with 4 mol L⁻¹ HCl to decrease the pH solution to a value of 2.2–2.4 [32].

Acidified samples were centrifuged for 5 min at 200 Hz (12,000 rpm or $16,128\times g$) at 4 °C with an ALC Multi Speed refrigerated centrifuge PK 121R (ALC International), and the supernatants were then filtered through a 0.45 μm filter (Titan 2 HPLC filter 17 mm PTFE Membrane); polyphenolic compounds were absorbed on a C₁₈ cartridge for solid-phase extraction (Sep-Pak® C-18, Waters, Milford, MA, USA). Then, 250 μL of OPDA solution (18.8 mmol L⁻¹) was added to 750 μL of extracted samples for DHAA derivatization into the fluorophore 3-(1,2-dihydroxyethyl)furo(3,4-b)quinoxalina-1-one (DFQ). After 37 min in the dark, the samples were analyzed with the HPLC–DAD system [33].

Table 2 Main validation characteristics of the used chromatographic methods for each calibration standard

Class	Standard	Identification code	Retention time (t_R) (min)	Method	Calibration curve equation	R^2	Calibration curve range (mg L^{-1})	LOD (mg L^{-1})	LOQ (mg L^{-1})
Cinnamic acids	Caffeic acid	1	4.54	A	$y = 59.046x + 200.6$	0.996	111–500	0.305	1.016
	Chlorogenic acid	2	3.89	A	$y = 13.583x + 760.05$	0.984	111–500	0.940	3.134
	Coumaric acid	3	6.74	A	$y = 8.9342x + 217.4$	0.997	111–500	2.907	9.690
	Ferulic acid	4	7.99	A	$y = 3.3963x - 4.9524$	1.000	111–500	1.245	4.150
Flavonols	Hyperoside	5	10.89	A	$y = 7.1322x - 4.583$	0.999	111–500	3.372	11.241
	Isoquercitrin	6	11.24	A	$y = 8.3078x + 26.621$	0.999	111–500	0.252	0.840
	Quercetin	7	17.67	A	$y = 3.4095x - 98.307$	0.998	111–500	4.055	13.518
	Quercitrin	8	13.28	A	$y = 2.7413x + 5.6367$	0.998	111–500	5.456	18.187
	Rutin	9	12.95	A	$y = 6.5808x + 30.831$	0.999	111–500	2.937	9.790
Benzoic acids	Ellagic acid	10	18.65	B	$y = 29.954x + 184.52$	0.998	62.5–250	0.611	2.035
	Gallic acid	11	4.26	B	$y = 44.996x + 261.86$	0.999	62.5–250	0.435	1.451
Catechins	Catechin	12	10.31	B	$y = 8.9197x + 66.952$	1.000	62.5–250	2.343	7.809
	Epicatechin	13	14.30	B	$y = 12.88x - 43.816$	0.999	62.5–250	0.763	2.543
Organic acids	Citric acid	16	5.30	C	$y = 1.0603x - 22.092$	1.000	167–1000	18.805	62.682
	Malic acid	17	4.03	C	$y = 1.415x - 80.254$	0.996	167–1000	15.721	52.404
	Oxalic acid	18	7.85	C	$y = 6.4502x + 6.1503$	0.998	167–1000	0.550	1.835
	Quinic acid	19	3.21	C	$y = 0.8087x - 38.021$	0.998	167–1000	26.106	87.021
	Succinic acid	20	3.46	C	$y = 0.9236x - 8.0823$	0.995	167–1000	7.135	23.783
Vitamins	Tartaric acid	21	5.69	C	$y = 1.8427x + 15.796$	1.000	167–1000	8.520	28.401
	Ascorbic acid	22	4.14	D	$y = 42.71x + 27.969$	0.999	100–1000	0.836	2.786
	Dehydroascorbic acid	23	3.41	D	$y = 4.1628x + 140.01$	0.999	30–300	1.095	3.649

Apparatus and chromatographic conditions

An Agilent 1200 high-performance liquid chromatograph, equipped with a G1311A quaternary pump, a manual injection valve, and a 20 μL sample loop, coupled to an Agilent G1315D UV–Vis diode array detector (Agilent Technologies, Santa Clara, CA, USA), was used for the analysis.

The external standard method was used for quantitative determinations. The external standard calibration curves were generated using different data points. Twenty μL aliquots of each standard solution were used for HPLC analysis, and manual injections were performed in triplicate for each concentration level. The calibration curves were obtained by plotting the peak area (y) of the compound at each level versus the concentration of the sample (x). For reference compounds, the limit of detection (LOD) and the limit of quantification (LOQ) were experimentally determined by HPLC analysis of serial dilutions of a standard solution to reach a signal-to-noise (S/N) ratio of 3 and 10, respectively. The chromatographic methods, already validated for herbal preparation analysis [34], in this case, are used and adapted for fruit phytochemical analysis for the first time. The validation was performed in agreement

with international guidelines for analytical techniques for the quality control of biopharmaceuticals (ICH guidelines) [35]. All samples were analyzed in triplicate, and standard deviations are given in order to assess the repeatability of the used methods. Accuracy was checked using the recovery test by spiking samples with a solution containing each bioactive compound (10 mg mL^{-1}) to reach 100 % of the test concentration. The main analytical method validation data are summarized in Table 2.

Four different chromatographic methods were used to analyze the samples, two for polyphenols and one for organic acids, and vitamins, respectively: all the single compounds were identified in samples by comparison and combination of their retention times and UV spectra with those of authentic standards in the same chromatographic conditions. In all of the used methods, bioactive compound separation was achieved on a KINETEX—C18 column ($4.6 \times 150 \text{ mm}$, $5 \mu\text{m}$, Phenomenex, Torrance, CA, USA). Different mobile phases were used for a specific bioactive compound identification and UV spectra were recorded at 330 nm (A); 280 nm (B); 214 nm (C); 261, and 348 nm (D). The chromatographic conditions of each method are reported in Table 3.

Table 3 Chromatographic conditions of each used method

Method	Compounds of interest	Stationary phase	Mobile phase	Flow (mL min ⁻¹)	Time of analysis (min)	Gradient ^b	Wavelength (nm)
A	Cinnamic acids, flavonols	KINETEX—C18 column (4.6 × 150 mm, 5 μm)	A: 10 mM KH ₂ PO ₄ /H ₃ PO ₄ , pH = 2.8 B: CH ₃ CN	1.5	20 + 2 (CT) ^a	Yes	330
B	Benzoic acids, catechins	KINETEX—C18 column (4.6 × 150 mm, 5 μm)	A: H ₂ O/CH ₃ OH/HCOOH (5:95:0.1 v/v/v), pH = 2.5 B: CH ₃ OH/HCOOH (100:0.1 v/v)	0.6	23 + 2 (CT) ^a	Yes	280
C	Organic acids	KINETEX—C18 column (4.6 × 150 mm, 5 μm)	A: 10 mM KH ₂ PO ₄ /H ₃ PO ₄ , pH = 2.8 B: CH ₃ CN	0.6	13 + 2 (CT) ^a	No	214
D	Vitamins	KINETEX—C18 column (4.6 × 150 mm, 5 μm)	A: 5 mM C ₁₆ H ₃₃ N(CH ₃) ₃ Br/50 mM KH ₂ PO ₄ , pH = 2.5 B: CH ₃ OH	0.9	10 + 5 (CT) ^a	No	261, 348

Method A gradient: 5 %B to 21 %B in 17 min + 21 %B in 3 min

Method B gradient: 3 %B to 85 %B in 22 min + 85 %B in 1 min

Method C ratio of phase A and B: 95:5

Method D ratio of phase A and B: 95:5

^a CT Conditioning time

^b Elution conditions

According to “multi-marker approach” [36], total bioactive compound content (TBCC), intended as synergistic or additive effects of the selected phytochemicals present in the samples (phytochemical complex), was determined as the sum of the most important classes of bioactive compounds present in the samples. Bioactive markers were selected comparing health-promoting properties and the most important compounds in literature with an important role in the positive effects on human organism. Four polyphenolic classes were considered: benzoic acids (ellagic and gallic acids), catechins (catechin and epicatechin), cinnamic acids (caffeic, chlorogenic, coumaric, and ferulic acids), and flavonols (hyperoside, isoquercitrin, quercetin, quercitrin, and rutin). The phenolic classes were arranged according to previous studies [34, 37]. Organic acids (citric, malic, oxalic, quinic, succinic, and tartaric acids) and vitamin C (ascorbic and dehydroascorbic acids) were also considered to obtain a complete analytical fingerprint. All results were expressed as mg per 100 g of fresh weight (FW).

Statistical analysis

Results were subjected to analysis of variance (ANOVA) test for mean comparison (SPSS 22.0 Software) and HSD Tukey multiple range test ($P < 0.05$).

In fresh and dried fruits, synergistic or additive therapeutic effects of several phytochemicals (phytochemical complex), rather than a single compound, could contribute to disease

prevention: for this reason, single compounds belonging to the same chemical class were combined in bioactive classes for multivariate data handling. Bioactive classes were determined as sum of the single content of nutraceuticals belonging to the same chemical class. In order to amplify the differences in the goji fruit extracts and easily differentiate them with a better graphic visualization, principal component analysis (PCA) was performed on the nutraceutical data: the data matrix was defined as 12 objects (3 repetitions for 4 samples) and 5 variables (bioactive classes coupled according to the chromatographic methods). Scaling was carried out as data pre-treatment on the original data: Z score scaling was carried out on the data matrix.

Results

Nutraceutical analysis and antioxidant activity

Air-thermal dried goji fruit showed lower values of TPC than freeze-drying ones: 420.00 ± 16.13 mg_{GAE}/100 g_{FW} (air-thermal) versus 502.13 ± 71.41 mg_{GAE}/100 g_{FW} (freeze-drying) for Boves samples and 470.56 ± 4.19 mg_{GAE}/100 g_{FW} (air-thermal) vs 486.49 ± 3.52 mg_{GAE}/100 g_{FW} (freeze-drying) for Bagnasco samples. Antioxidant activity values showed the same trend: 19.58 ± 0.67 mmol Fe²⁺ kg⁻¹ (air-thermal) versus 22.79 ± 0.36 mmol Fe²⁺ kg⁻¹

Table 4 TPC and antioxidant activity data in analyzed goji extracts

Sample	Total polyphenol content (TPC)			Antioxidant activity (AA)		
	Mean value	SD	Tukey test	Mean value	SD	Tukey test
	(mg _{GAE} /100 g _{FW})			(mmol Fe ²⁺ /kg)		
GBovF ^a	199.465	19.444	a	21.826	0.992	ab
GBovD ^b	502.126	71.410	b	22.794	0.362	bc
GBagnF ^c	240.268	8.582	a	21.242	0.439	a
GBagnD ^d	486.488	3.523	b	23.562	0.094	c

Mean values of each sample is given ($N = 3$). Different letters for each sample indicate the significant differences at $P < 0.05$

^a GBovF = fresh goji fruits from Boves

^b GBovD = dried goji fruits from Boves (freeze-drying)

^c GBagnF = fresh goji fruits from Bagnasco

^d GBagnD = dried goji fruits from Bagnasco (freeze-drying)

(freeze-drying) for Boves samples and 20.89 ± 0.25 mmol Fe²⁺ kg⁻¹ (air-thermal) versus 23.56 ± 0.09 mmol Fe²⁺ kg⁻¹ (freeze-drying) for Bagnasco samples. For this reason, freeze-drying method was selected to compare fresh and dried fruits.

The content of total polyphenolic compounds in the extracts is reported in Table 4. Results showed that the lowest TPC value was 199.47 ± 19.44 mg_{GAE}/100 g_{FW} (sample GBovF) and the highest value was 502.13 ± 71.41 mg_{GAE}/100 g_{FW} (sample GBovD). GBagnD and GBovD samples showed higher antioxidant activity than GBagnF and GBovF samples: in particular, the lowest FRAP value was observed in GBagnF (21.24 ± 0.44 mmol Fe²⁺ kg⁻¹) and the highest in GBagnD (23.56 ± 0.09 mmol Fe²⁺ kg⁻¹) (Table 4).

Multivariate analysis

The initial variables were bioactive classes (cinnamic acids + flavonols, benzoic acids + catechins, organic acids, vitamins, TBCC) and, on the basis of eigenvalues >1, PCA reduced them into two principal components (85.47 % of total variance) and confirmed the initial four groups as showed in the PCA plot (Fig. 1), and the statistically significant differences showed in TBCC and fingerprint profile (ANOVA test). PCA loadings showed a correlation between TBCC, organic acids, and vitamins and PC1 (91.7, 92.7, and 79.4 % of total variance, respectively) and a correlation between cinnamic acids + flavonols, and benzoic acids + catechins and PC2 (93.1, and 92.4 % of total variance, respectively). Organic acids, vitamins and cinnamic acids have been identified as bioactive classes with the most discriminating power: these three nutraceutical classes included the compounds with higher statistical differences in their bioactive content.

PCA plot showed two directions of sample differentiation: “drying process” direction related to PC1 (organic acids and vitamins) and “genotype/origin” direction related to PC2 (polyphenolic compounds).

Total bioactive compound content (TBCC) and fingerprinting

All data (with mean values) are reported in Table 5 (TBCC and single compounds).

The content of total bioactive compounds in the evaluated samples was calculated as the sum of the most important biologically active molecules detected in the extracts. The analyzed samples showed a lower TBCC value of 453.34 ± 25.28 mg/100 g_{FW} (sample GBovD) and a higher value of 905.09 ± 293.80 mg/100 g_{FW} (sample GBagnD).

Goji samples showed the following bioactive compound composition (fingerprint): four cinnamic acids (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid), one flavonol (hyperoside) except GBovD and GBagnD samples, one benzoic acid (ellagic acid), two catechins (catechin, epicatechin), six organic acids (citric acid, malic acid, oxalic acid, quinic acid, succinic acid, tartaric acid), and vitamin C (expressed as the sum of ascorbic acid and dehydroascorbic acid); isoquercitrin, quercetin, quercitrin, rutin, and gallic acid were not detected.

The chemical fingerprint of goji fruit analyzed samples was defined: in total, 15 bioactive compounds were identified by HPLC/DAD. By single bioactive compound profile, health-promoting agents were grouped into different classes to evaluate the single contribution of each class to total fruit phytocomplex composition.

In all the goji fruit samples, phytochemical composition was influenced by the used drying system: in total fruit phytocomplex, the content of phenolic acids, catechins and vitamins in samples dried by air-thermal system showed

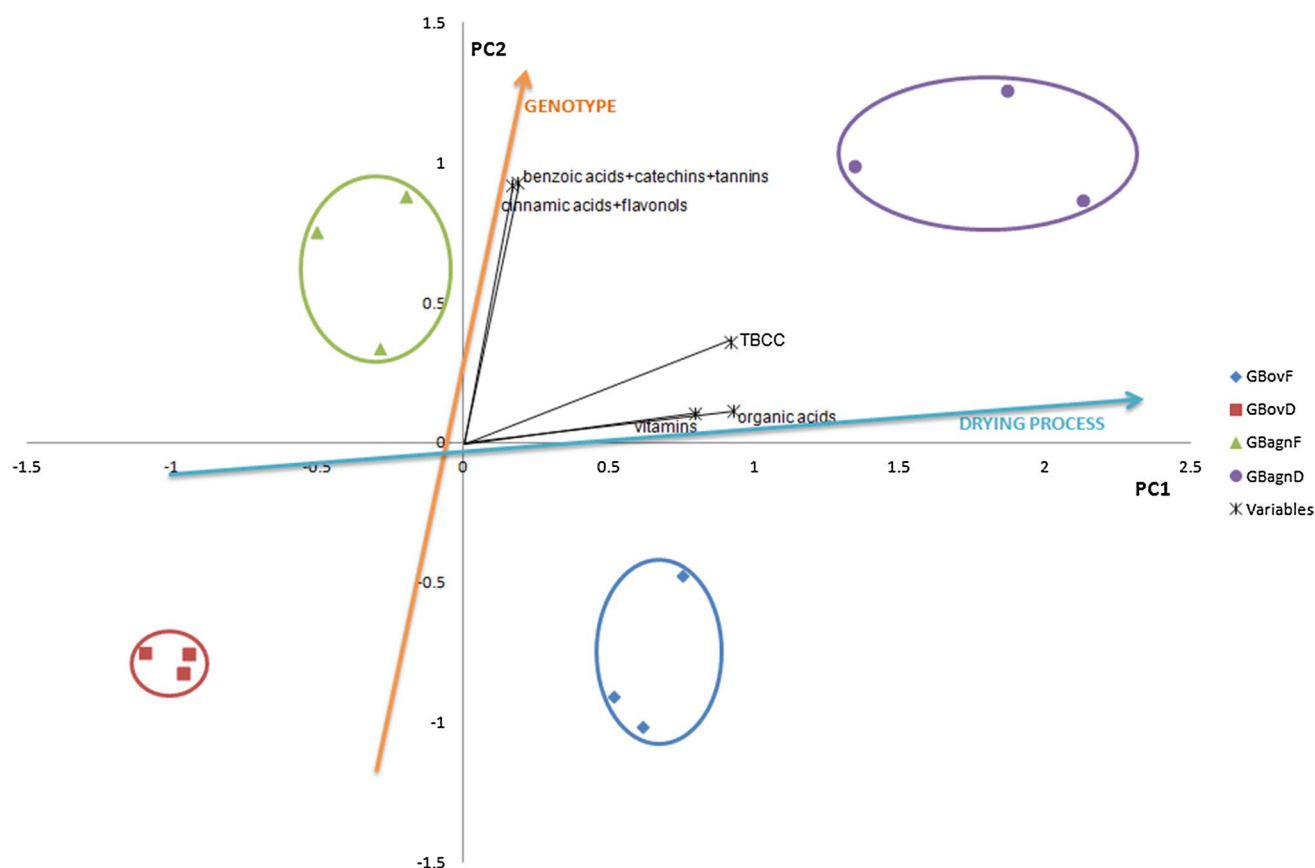


Fig. 1 PCA plot of fruit extract samples

lower values than freeze-drying system. For this reason, freeze-drying system was selected in order to compare fresh and dried fruits.

The chemical fingerprint showed the prevalence of organic acids (62.74 %) and vitamins (25.52 %) in chemical composition of all the analyzed samples (mean values were considered; Fig. 2), followed by catechins (6.97 %) and cinnamic acids (4.26 %; Table 6).

Moreover, polyphenols could be divided in several subclasses with different contribution to total polyphenolic composition; in the polyphenol group, the most important classes were catechins (59.60 %) and cinnamic acids (36.92 %), followed by benzoic acids and flavonols (mean values were considered in the evaluation of polyphenol percentage composition; Fig. 3).

Discussion

In this study, polyphenolic composition was evaluated by spectrophotometric and chromatographic analysis, but vitamins and organic acids were also considered in order to assess a more complete bioactive compound

profile. Moreover, evaluation of antioxidant activity was considered an important method to evaluate the nutraceutical properties of fruit, as shown in other previous studies [38]. Goji TPC values (Table 4) were similar to blueberry fruits [39] and comparable with those of other common fruit species, such as *Ribes nigrum* [40], *Actinidia deliciosa* [41], and *Citrus sinensis* [13]. Drying process may hydrolyze the phenolic compounds, and sometimes hydrolyzed compounds may give a higher response in FC method. Antioxidant activity showed values similar to other berry species [42] in a range between raspberry and strawberry fruits [43]. Similar results were reported in other researches on phytochemical composition and bioactivity of goji fruits [16, 19, 20]. TPC values were much higher for dried fruits than the corresponding values for their fresh counterparts because antioxidants became concentrated after the drying process. While there was a loss or modification of some specific phytochemicals during drying, antioxidant activity remains relatively unchanged during the process, implying that some bioactive compounds were unidentified, as shown in other researches [20, 27]: Table 5 highlighted that dried fruits, in general, presented a smaller amount of vitamin C than fresh fruits. In phytocomplex composition of Boves

Table 5 Single compound profile of analyzed samples

Sample	Cinnamic acids					
	Caffeic acid (mg/100 g _{FW})		Chlorogenic acid (mg/100 g _{FW})		Coumaric acid (mg/100 g _{FW})	
	Mean value	SD	Tukey test	Mean value	SD	Tukey test
GBovF ^a	0.832	0.076	a	0.329	0.012	a
GBovD ^b	1.369	0.202	a	0.944	0.014	ab
GBagnF ^c	0.996	0.545	a	4.687	1.556	b
GBagnD ^d	1.617	0.183	a	8.947	2.562	c
Sample	Flavonols					
	Hyperoside (mg/100 g _{FW})		Isoquercitrin (mg/100 g _{FW})		Quercitrin (mg/100 g _{FW})	
	Mean value	SD	Tukey test	Mean value	SD	Tukey test
GBovF	0.993	0.165	a	n.d.	–	n.d.
GBovD	n.d.	n.d.	–	n.d.	–	n.d.
GBagnF	3.848	0.342	b	n.d.	–	n.d.
GBagnD	n.d.	n.d.	–	n.d.	–	n.d.
Sample	Catechins					
	Ellagic acid (mg/100 g _{FW})		Gallic acid (mg/100 g _{FW})		Catechin (mg/100 g _{FW})	
	Mean value	SD	Tukey test	Mean value	SD	Tukey test
GBovF	0.012	0.002	a	n.d.	–	n.d.
GBovD	0.157	0.008	a	n.d.	–	n.d.
GBagnF	0.816	0.191	a	n.d.	–	n.d.
GBagnD	10.156	4.061	b	n.d.	–	n.d.
Sample	Organic acids					
	Citric acid (mg/100 g _{FW})		Malic acid (mg/100 g _{FW})		Quinic acid (mg/100 g _{FW})	
	Mean value	SD	Tukey test	Mean value	SD	Tukey test
GBovF	180.608	41.383	a	75.769	22.342	a
GBovD	65.528	6.776	a	37.136	7.334	a
Sample	Tartaric acid (mg/100 g _{FW})					
	Succinic acid (mg/100 g _{FW})		Tartaric acid (mg/100 g _{FW})		Tartaric acid (mg/100 g _{FW})	
	Mean value	SD	Tukey test	Mean value	SD	Tukey test
GBovF	156.283	35.891	b	156.283	35.891	b
GBovD	56.294	25.029	a	56.294	25.029	a

Table 5 continued

Sample	Organic acids									
	Citric acid		Malic acid		Oxalic acid		Quinic acid		Succinic acid	
	(mg/100 g _{FW})		(mg/100 g _{FW})		(mg/100 g _{FW})		(mg/100 g _{FW})		(mg/100 g _{FW})	
	Mean value	SD	Tukey test	Mean value	SD	Tukey test	Mean value	SD	Mean value	Tukey test
GBagnF	81.765	31.197	a	68.856	25.345	a	13.302	5.967	39.678	2.065
GBagnD	187.805	105.405	a	44.101	30.190	a	10.113	0.975	60.733	21.281
Sample	TBCC (mg/100 g _{FW})									
	Vitamins									
	Vitamin C (mg/100 g _{FW})									
	Mean value	SD	Tukey test	Mean value	SD	Tukey test	Mean value	SD	Mean value	Tukey test
GBovF	203.271	16.439	b	736.014	76.686	ab	54.511	20.196	106.421	39.823
GBovD	117.389	7.575	a	453.341	25.279	a	54.511	20.196	106.421	39.823
GBagnF	185.976	7.223	b	634.797	48.874	ab	54.511	20.196	106.421	39.823
GBagnD	174.547	35.178	b	905.085	293.796	b	54.511	20.196	106.421	39.823

^a GBovF = fresh goji fruits from Boves^b GBovD = dried goji fruits from Boves (freeze-drying)^c GBagnF = fresh goji fruits from Bagnasco^d GBagnD = dried goji fruits from Bagnasco (freeze-drying)

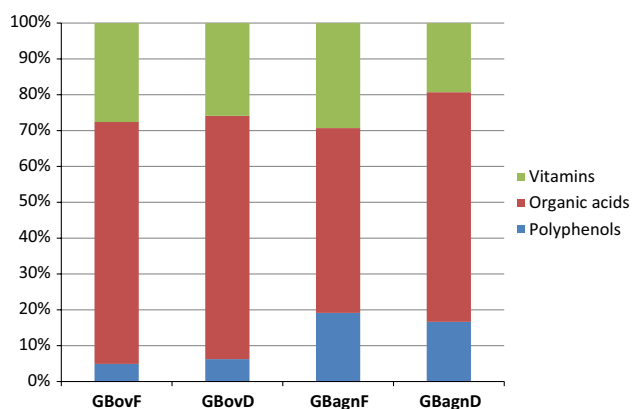


Fig. 2 Antioxidant phytochemical representation for each analyzed extract. The first two vertical bars on the left are referred to samples from Boves (fresh and dried, respectively), while the other ones are referred to samples from Bagnasco (fresh and dried, respectively). Freeze-drying system was considered

samples, vitamin C decreased from 27.62 % (fresh) to 25.89 % (dried), while in Bagnasco samples from 29.30 % (fresh) to 19.29 % (dried). There is often a decrease in the dried product quality, as a reduction of nutraceutical properties or undesirable changes in the sensory traits, because most conventional techniques use high temperatures during the drying process: the results of this study confirmed that freeze drying allowed to preserve most of the phytochemical characteristics of the fresh fruits, as TPC and relative antioxidant activity, phenolic acids (ferulic and ellagic acids) and vitamin C content (Tables 7, 8).

Thanks to these nutraceutical properties, the *Lycium* spp. fruit is now increasingly being presented as a functional food throughout Europe and in Asian countries [44], even if, considering the results of this study, nutraceutical value of hybrid cultivars between the two main *Lycium* species is no greater than that of other commonly grown berry fruits. The introduction of new foods into the diet becomes responsible for new allergic sensitizations [45]. For this reason, traceability of goji-derived products (genotype and origin) and differentiation among different *Lycium* species

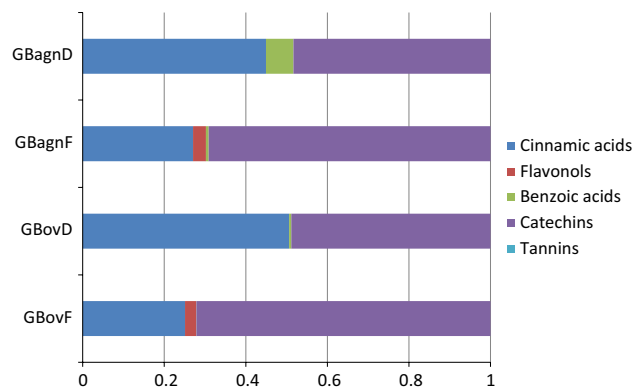


Fig. 3 Phenolic compound class composition of goji fruit samples. The first two vertical bars at the bottom are referred to samples from Boves (fresh and dried, respectively), while the other ones are referred to samples from Bagnasco (fresh and dried, respectively). Freeze-drying system was considered

and their hybrids can play an important role in the wolfberry market and in meeting the demands of both producers and consumers.

The traditional approaches for the characterization of goji fruits are mainly attributed to physicochemical and sensory analysis, even if they are time consuming, require a very experienced analyst, and are strongly dependent on the expert's ability and judgment [15]. In other studies, different genetic, analytical and quantitative methods, including DNA barcode [9], Random Amplified Polymorphic DNA [46], LC-MS and GC-MS [47], NIR spectroscopy [48] have also been used to classify *Lycium* fruits according to their origin and genotype. In this research, high-performance liquid chromatography (HPLC) fingerprint have been used for species and origin differentiation and quality control: the selected phytochemicals were identified as biomarkers with high concentration as shown in other similar studies [16, 17] and significant statistical differences were found in phytochemical profile of different considered genotypes. Analytical fingerprint, used to detect the complex constituents in goji extracts, was suitable for quality control

Table 6 Contribution of antioxidant classes to the fruit phytocomplex in analyzed extracts

	Cinnamic acids (%)	Flavonols (%)	Benzoic acids (%)	Catechins (%)	Organic acids (%)	Vitamins (%)
GBovF ^a	1.23	0.13	0.00	3.55	67.46	27.62
GBovD ^b	3.16	0.00	0.03	3.05	67.86	25.89
GBagnF ^c	5.18	0.61	0.13	13.23	51.56	29.30
GBagnD ^d	7.48	0.00	1.12	8.05	64.07	19.29

^a GBovF = fresh goji fruits from Boves

^b GBovD = dried goji fruits from Boves (freeze-drying)

^c GBagnF = fresh goji fruits from Bagnasco

^d GBagnD = dried goji fruits from Bagnasco (freeze-drying)

Table 7 TPC and antioxidant activity data in analyzed extracts derived from dried goji fruits

Sample	Drying method	Total polyphenol content (TPC)			Antioxidant activity (AA)		
		Mean value (mg _{GAE} /100 g _{FW})	SD (mg _{GAE} /100 g _{FW})	Tukey test (mg _{GAE} /100 g _{FW})	Mean value (mmol Fe ²⁺ /kg)	SD (mmol Fe ²⁺ /kg)	Tukey test (mmol Fe ²⁺ /kg)
GBov	Air-thermal	420.002	16.125	a	19.578	0.669	a
GBov	Freeze drying	502.126	71.410	c	22.794	0.362	bc
GBagn	Air-thermal	470.556	4.186	ab	20.887	0.245	ab
GBagn	Freeze drying	486.488	3.523	b	23.562	0.094	b

Mean values of each sample is given ($N = 3$). Different letters for each sample indicate the significant differences at $P < 0.05$

Table 8 Contribution of antioxidant classes to the fruit phytochemical complex in the extracts derived from dried goji fruits

	Drying method	Cinnamic acids (%)	Flavonols (%)	Benzoic acids (%)	Catechins (%)	Organic acids (%)	Vitamins (%)
GBov	Air-thermal	3.06	0.00	0.01	2.93	71.15	22.86
GBov	Freeze drying	3.16	0.00	0.03	3.05	67.86	25.89
GBagn	Air-thermal	6.71	0.00	0.73	7.35	67.58	17.63
GBagn	Freeze drying	7.48	0.00	1.12	8.05	64.07	19.29

of different genotypes as shown in other research [49]: in this study, the chromatographic conditions were optimized to obtain a chemical pattern containing complete information of phytochemical composition with a good resolution and a reasonable analysis time for each used methods. In addition, a diode array detector with selected wavelengths was used, but the chemical information of each extract could also be identified using other detectors as mass spectrometry (MS). These results have provided important and critical evidence for future development of goji fruits and derived products under direction of their optimized quality control methods.

PCA has been implemented to examine the qualitative and quantitative differences among the four samples of the goji berries used in this study: PCA plot showed that phytochemical complex of each sample depended on two different factors: “drying process” correlated with PC1 (organic acids and vitamins) and “genotype/origin” correlated to PC2 (polyphenolic compounds) as shown in Fig. 1. Organic acids and, especially, vitamin C are heat-sensitive molecules, and they degrade during the drying process, while polyphenols are components with a higher molecular weight and resistant to thermal degradation: the quality of dried foods is dependent in part on changes occurring during processing and storage [23] and vitamin C content could be one of the main markers to select the best drying process as discussed in this study. Moreover, polyphenolic compound composition in fruits and other plant parts strongly depends on plant genotype and pedoclimatic conditions [33]: for this reason, they could be selected as biomarkers in order to distinguish different *Lycium* species or the same *Lycium* genotype from different geographical areas.

Finally, in this work, it was verified that HPLC fingerprint technique based on chemometrics has high potential to control the quality of goji fresh and dried fruits for sale in an efficient and accurate way and it could be used as traceability control and differentiation tool: the identification based on HPLC–DAD technique is considered a “preliminary identification” since the conclusive phytochemical identification could be better achieved after NMR and MS analysis.

Conclusions

An intensive research for extraction of phytochemicals from vegetables and fruits and for identification and quantification of new lead compounds for the development of novel pharmacological therapeutics is extremely important. This study developed an important tool to assess goji product chemical composition and bioactivity, using different chromatographic methods for comprehensive authentication and quality control of fresh and dried fruits, showing that the selection of a suitable drying systems could be important in order to increase the antioxidant and health properties of wolfberry derived product.

The results presented in this study enhance the knowledge of the phytochemical composition of goji berries. Additionally, this work could help aggregate information in the field of biological activities and nutraceutical properties of *Lycium* spp. fruits. In particular, this research highlighted that goji dried fruits, with their unique combination of phytochemicals and antioxidant compounds, are a convenient step toward healthier eating and a means to bridge

the gap between recommended intake of fruits and actual consumption. The results of this study showed that freeze drying allowed to preserve most of the phytochemical characteristics of the fresh fruits: thanks to this technique, goji dried fruits could be included together with fresh fruit recommendations around the world since they help meet dietary guidelines for daily fruit serving and address barriers to fruit intake.

Because free radicals are implicated in many diseases and age-related conditions, the antioxidant dependent actions of *Lycium* spp. may have a wide range of beneficial effects. On the other hand, goji berries show a high allergenic potential and the prevalence of sensitization in the Western country population should be carefully considered. For these reasons, an adequate evaluation of nutraceutical properties and a complete assessment of wolfberry fruit phytochemical composition is necessary in order to establish genuine and safe raw materials: as a cost-effective and efficient way to trace food, this study showed that phytochemical fingerprint can be used to identify and track different raw materials (fresh fruits) and derived food commodities (dried fruits).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements The authors declare that the research did not involve Human Participants and/or Animals.

References

1. Roberfroid MB (2002) Global view on functional foods: European perspectives. *Br J Nutr* 88(SUPPL. 2):S133–S138
2. Cerutti AK, Bruun S, Donno D, Beccaro GL, Bounous G (2013) Environmental sustainability of traditional foods: the case of ancient apple cultivars in Northern Italy assessed by multifunctional LCA. *J Clean Prod* 52:245–252. doi:10.1016/j.jclepro.2013.03.029
3. Donno D, Cerutti AK, Prgomet I, Mellano MG, Beccaro GL (2015) Foodomics for mulberry fruit (*Morus* spp.): analytical fingerprint as antioxidants' and health properties' determination tool. *Food Res Int* 69:179–188. doi:10.1016/j.foodres.2014.12.020
4. Roberfroid MB (2000) Concepts and strategy of functional food science: the European perspective. *Am J Clin Nutr* 71(6):1660s–1664s
5. Carnes J, de Larramendi CH, Ferrer A, Huertas AJ, Lopez-Matas MA, Pagan JA, Navarro LA, Garcia-Abujeta JL, Vicario S, Pena M (2013) Recently introduced foods as new allergenic sources: sensitisation to Goji berries (*Lycium barbarum*). *Food Chem* 137(1–4):130–135. doi:10.1016/j.foodchem.2012.10.005
6. Donno D, Beccaro GL, Mellano MG, Bonvegna L, Bounous G (2014) *Castanea* spp. buds as a phytochemical source for herbal preparations: botanical fingerprint for nutraceutical identification and functional food standardisation. *J Sci Food Agric* 94(14):2863–2873. doi:10.1002/jsfa.6627
7. Dolgoplova I, Teuber R, Bruschi V (2015) Consumers' perceptions of functional foods: trust and food-neophobia in a cross-cultural context. *Int J Consum Stud*. doi:10.1111/ijcs.12184
8. Dalvit C, De Marchi M, Targhetta C, Gervaso M, Cassandro M (2008) Genetic traceability of meat using microsatellite markers. *Food Res Int* 41(3):301–307. doi:10.1016/j.foodres.2007.12.010
9. Xin TY, Yao H, Gao HH, Zhou XZ, Ma XC, Xu CQ, Chen J, Han JP, Pang XH, Xu R, Song JY, Chen SL (2013) Super food *Lycium barbarum* (Solanaceae) traceability via an internal transcribed spacer 2 barcode. *Food Res Int* 54(2):1699–1704. doi:10.1016/j.foodres.2013.10.007
10. Donno D, Beccaro GL, Mellano MG, Torello-Marini D, Cerutti AK, Canterino S, Bounous G (2012) Application of sensory, nutraceutical and genetic techniques to create a quality profile of ancient apple cultivars. *J Food Qual* 35(3):169–181. doi:10.1111/j.1745-4557.2012.00442.x
11. Donno D, Beccaro GL, Mellano MG, Cerutti AK, Bounous G (2014) Chemical fingerprint as nutraceutical quality differentiation tool in *Asimina triloba* L. fruit pulp at different ripening stages: an old species for new health needs. *J Food Nutr Res* 53(1):81–95
12. Donno D, Beccaro GL, Mellano MG, Cerutti AK, Marconi V, Bounous G (2013) Botanicals in *Ribes nigrum* bud-preparations: an analytical fingerprinting to evaluate the bioactive contribution to total phytocomplex. *Pharm Biol* 51(10):1282–1292. doi:10.3109/13880209.2013.786101
13. Canterino S, Donno D, Mellano MG, Beccaro GL, Bounous G (2012) Nutritional and sensory survey of *Citrus sinensis* (L.) cultivars grown at the most Northern limit of the Mediterranean latitude. *J Food Qual* 35(2):108–118
14. Amagase H, Sun B, Borek C (2009) *Lycium barbarum* (goji) juice improves in vivo antioxidant biomarkers in serum of healthy adults. *Nutr Res* 29(1):19–25. doi:10.1016/j.nutres.2008.11.005
15. Yao X, Peng Y, Xu LJ, Li L, Wu QL, Xiao PG (2011) Phytochemical and biological studies of lycium medicinal plants. *Chem Biodivers* 8(6):976–1010
16. Donno D, Beccaro GL, Mellano MG, Cerutti AK, Bounous G (2014) Goji berry fruit *Lycium* spp.: antioxidant compound fingerprint and bioactivity evaluation. *J Funct Foods*. doi:10.1016/j.jff.2014.05.020
17. Potterat O (2010) Goji (*Lycium barbarum* and *L. chinense*): phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. *Planta Med* 76(1):7–19. doi:10.1055/s-0029-1186218
18. Cheng K-T, Chang H-C, Huang H, Lin C-T (2000) RAPD analysis of *Lycium barbarum* medicine in Taiwan market. *Bot Bull Acad Sin* 41:11–14
19. Zhong Y, Shahidi F, Nacz M (2013) Phytochemicals and health benefits of goji berries. In: *Dried fruits: phytochemicals and health effects*. Blackwell Publishing Ltd., pp 133–144
20. Shahidi F (2012) *Dried fruits: phytochemicals and health effects*, vol 8. Wiley
21. Cohen JS, Yang TCS (1995) Progress in food dehydration. *Trends Food Sci Technol* 6(1):20–25. doi:10.1016/S0924-2244(00)88913-X
22. Vega-Mercado H, Marcela Góngora-Nieto M, Barbosa-Cánovas GV (2001) Advances in dehydration of foods. *J Food Eng* 49(4):271–289. doi:10.1016/S0260-8774(00)00224-7
23. Nijhuis HH, Topping HM, Muresan S, Yuksel D, Leguijt C, Kloek W (1998) Approaches to improving the quality of dried fruit and vegetables. *Trends Food Sci Technol* 9(1):13–20. doi:10.1016/S0924-2244(97)00007-1

24. Koyuncu T, Pinar Y, Lule F (2007) Convective drying characteristics of azarole red (*Crataegus monogyna* Jacq.) and yellow (*Crataegus aronia* Bosc.) fruits. *J Food Eng* 78(4):1471–1475. doi:[10.1016/j.jfoodeng.2005.09.036](https://doi.org/10.1016/j.jfoodeng.2005.09.036)
25. Williamson G, Carughi A (2010) Polyphenol content and health benefits of raisins. *Nutr Res* 30(8):511–519
26. Vinson JA, Zubik L, Bose P, Samman N, Proch J (2005) Dried fruits: excellent in vitro and in vivo antioxidants. *J Am Coll Nutr* 24(1):44–50
27. Alasalvar C, Shahidi F (2013) Composition, phytochemicals, and beneficial health effects of dried fruits: an overview. In: *Dried fruits: phytochemicals and health effects*. Blackwell Publishing Ltd., pp 1–19
28. Vayalil PK (2012) Date fruits (*Phoenix dactylifera* Linn): an emerging medicinal food. *Crit Rev Food Sci Nutr* 52(3):249–271
29. Ratti C (2001) Hot air and freeze-drying of high-value foods: a review. *J Food Eng* 49(4):311–319. doi:[10.1016/S0260-8774\(00\)00228-4](https://doi.org/10.1016/S0260-8774(00)00228-4)
30. Slinkard K, Singleton VL (1977) Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic* 28(1):49–55
31. Benzie IF, Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol* 299:15–27
32. Sanchez ACG, Gil-Izquierdo A, Gil MI (2003) Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *J Sci Food Agric* 83(10):995–1003. doi:[10.1002/jsfa.1436](https://doi.org/10.1002/jsfa.1436)
33. Gonzalez-Molina E, Moreno DA, Garcia-Viguera C (2008) Genotype and harvest time influence the phytochemical quality of Fino lemon juice (*Citrus limon* (L.) Burm. F.) for industrial use. *J Agric Food Chem* 56(5):1669–1675. doi:[10.1021/jf073282w](https://doi.org/10.1021/jf073282w)
34. Donno D, Boggia R, Zunin P, Cerutti AK, Guido M, Mellano MG, Prgomet Z, Beccaro GL (2015) Phytochemical fingerprint and chemometrics for natural food preparation pattern recognition: an innovative technique in food supplement quality control. *J Food Sci Technol*. doi:[10.1007/s13197-015-2115-6](https://doi.org/10.1007/s13197-015-2115-6)
35. Kamboj A (2012) Analytical evaluation of herbal drugs. INTECH Open Access Publisher, Rijeka, Croatia
36. Mok DKW, Chau FT (2006) Chemical information of Chinese medicines: a challenge to chemist. *Chemom Intell Lab Syst* 82(1–2):210–217. doi:[10.1016/j.chemolab.2005.05.006](https://doi.org/10.1016/j.chemolab.2005.05.006)
37. Sakakibara H, Honda Y, Nakagawa S, Ashida H, Kanazawa K (2003) Simultaneous determination of all polyphenols in vegetables, fruits, and teas. *J Agric Food Chem* 51(3):571–581. doi:[10.1021/jf020926l](https://doi.org/10.1021/jf020926l)
38. Isabelle M, Lee BL, Lim MT, Koh WP, Huang DJ, Ong CN (2010) Antioxidant activity and profiles of common fruits in Singapore. *Food Chem* 123(1):77–84. doi:[10.1016/j.foodchem.2010.04.002](https://doi.org/10.1016/j.foodchem.2010.04.002)
39. Contessa C, Mellano MG, Beccaro GL, Giusiano A, Botta R (2013) Total antioxidant capacity and total phenolic and anthocyanin contents in fruit species grown in Northwest Italy. *Sci Hortic* 160:351–357. doi:[10.1016/j.scienta.2013.06.019](https://doi.org/10.1016/j.scienta.2013.06.019)
40. Donno D, Cavanna M, Beccaro GL, Mellano MG, Torello-Marinoni D, Cerutti AK, Bounous G (2013) Currants and strawberries as bioactive compound sources: determination of antioxidant profiles with HPLC-DAD/MS. *J Appl Bot Food Qual* 86:1–10. doi:[10.5073/JABFQ.2013.086.001](https://doi.org/10.5073/JABFQ.2013.086.001)
41. Donno D, Beccaro GL, Mellano MG, Canterino S, Cerutti AK, Bounous G (2013) Improving the nutritional value of kiwifruit with the application of agroindustry waste extracts. *J Appl Bot Food Qual* 86:11–15. doi:[10.5073/JABFQ.2013.086.002](https://doi.org/10.5073/JABFQ.2013.086.002)
42. Nile SH, Park SW (2014) Edible berries: bioactive components and their effect on human health. *Nutrition* 30(2):134–144. doi:[10.1016/j.nut.2013.04.007](https://doi.org/10.1016/j.nut.2013.04.007)
43. Crespo P, Bordonaba JG, Terry LA, Carlen C (2010) Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. *Food Chem* 122(1):16–24. doi:[10.1016/j.foodchem.2010.02.010](https://doi.org/10.1016/j.foodchem.2010.02.010)
44. Abdennacer B, Karim M, Mr Yassine, Nesrine R, Mouna D, Mohamed B (2015) Determination of phytochemicals and antioxidant activity of methanol extracts obtained from the fruit and leaves of Tunisian *Lycium intricatum* Boiss. *Food Chem* 174:577–584. doi:[10.1016/j.foodchem.2014.11.114](https://doi.org/10.1016/j.foodchem.2014.11.114)
45. Larramendi C, Garcia-Abujeta J, Vicario S, García-Endrino A, López-Matas M, García-Sedeño M, Carnés J (2012) 4 Goji berries (*Lycium barbarum*): risk of allergic reactions in individuals with food allergy. *J Investig Allergol Clin Immunol* 22(5):345
46. Zhang KYB, Leung HW, Yeung HW, Wong RNS (2001) Differentiation of *Lycium barbarum* from its related *Lycium* species using random amplified polymorphic DNA. *Planta Med* 67(04):379–381. doi:[10.1055/s-2001-14310](https://doi.org/10.1055/s-2001-14310)
47. Bondia-Pons I, Savolainen O, Törrönen R, Martinez JA, Poutanen K, Hanhineva K (2014) Metabolic profiling of Goji berry extracts for discrimination of geographical origin by non-targeted liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *Food Res Int* 63(Part B (0)):132–138. doi:[10.1016/j.foodres.2014.01.067](https://doi.org/10.1016/j.foodres.2014.01.067)
48. Reich G (2005) Near-infrared spectroscopy and imaging: basic principles and pharmaceutical applications. *Adv Drug Deliv Rev* 57(8):1109–1143. doi:[10.1016/j.addr.2005.01.020](https://doi.org/10.1016/j.addr.2005.01.020)
49. Xu C, Yang B, Zhu W, Li X, Tian J, Zhang L (2015) Characterisation of polyphenol constituents of *Linderae* aggregate leaves using HPLC fingerprint analysis and their antioxidant activities. *Food Chem* 186:83–89. doi:[10.1016/j.foodchem.2014.11.042](https://doi.org/10.1016/j.foodchem.2014.11.042)