Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino

Largo Braccini 2, 10095 - Grugliasco (TO), ITALY

GOJI BERRY FRUIT (*LYCIUM* SPP.): ANTIOXIDANT COMPOUND FINGERPRINT AND BIOACTIVITY EVALUATION

Running head: GOJI FRUIT ANALYTICAL FINGERPRINT

Donno D.*, Beccaro G.L., Mellano M.G., Cerutti A.K., Bounous G.

*Corresponding author: e-mail: dario.donno@unito.it

Number of Figures: 3

Number of Tables: 6

ABSTRACT

The antioxidants in goji berry (*Lycium* spp.) fruits might confer many health protective benefits by alleviating oxidative stress. The aim of this study was to describe quality traits and the level of potentially bioactive compounds (HPLC fingerprint) and their influence on fruit phytocomplex and antioxidant activity in goji in comparison to the most common fruits. Goji berry was identified as a rich source of antioxidant compounds, with health-promoting properties comparable to other common fruit species.

The obtained fingerprint may be useful to better understand the nutraceutical traits of this species recently considered as functional food thanks to its antioxidant properties.

Keywords: quality, bioactive compounds, antioxidant activity, recent-discovered fruit species

1. INTRODUCTION

Goji berry is a *Solanaceous* deciduous shrubbery that grows in China, Tibet, and other parts of Asia, and its fruits are 1–2 cm-long, bright orange-red ellipsoid berries. There are two closely related species, *Licium barbarum* L. and *L. chinense* Miller, which both have a long tradition as food and medicinal plants in China and other Asian countries. These species possess a highly similar anatomy and tissue structure. Differentiation based on morphological and histological analyses is very delicate. Confident distinction requires molecular techniques, such as random amplified polymorphic DNA (RAPD) analyses (Potterat, 2010), since confusion on the different genotypes is found at the nursery and commercial levels.

Goji berry has different vernacular names; the most common name, “wolfberry,” comes from the character “gou” as it is related to the one that means wolf. The name goji is an extrapolation of a number of native words, and it was originally coined in 1973 by researchers at the Tanaduk Botanical Research Institute (TBRI) (Amagase & Farnsworth, 2011). The original area of *Lycium* spp. is not definitively established but is likely found in the Mediterranean Basin. Meanwhile, the plant is widely distributed in warm regions of the world, in particular, in the Mediterranean area and Southwest and Central Asia. It is also cultivated in North America and Australia as a hedge plant (Potterat, 2010).

L. barbarum grows up to 3 m high, while *L. chinense* is somewhat smaller, and its gray-green leaves are alternate, lanceolate, and gradually narrow to the petiole. The species presents one to three axillary flowers. The calyx and pistils are fused: the calyx is bilabial with a double-toothed lower lip. The corolla is funnel-shaped, light purple or violet with a 5-lobed margin. There are four stamens, which are

hairy at the base. The ovary is two-chambered with one style (Amagase, Sun, & Borek, 2009; Anonymous, 2010). The fruit is ovoidal with acute apex, 6–20 mm in length, 3–8 mm in diameter, and pericarp red to dark red (Potterat, 2010; Zhao, Zhang, He, Wang, Zhang, & Zhang, 2009b). The fruits are collected in late summer to autumn, dried in the shade till the skin shrinks, and then exposed to the sun until the outer skin becomes dry and hard but the pulp is still soft (Amagase & Farnsworth, 2011; Amagase, et al., 2009). There are many pests that can occur on goji and many natural enemies for the control of *Aphis* and *Paratrioza* (Ale-Agha, Brassmann, & Jensen, 2009; Chen, Cheng, Zhang, Zhang, & Ding, 2003; Zhao, Li, Li, & Zhang, 2009a).

Studies indicate effects of goji fruit on aging, neuroprotection, general well-being, fatigue/endurance, metabolism/energy expenditure, glucose control in diabetics and glaucoma, antioxidant properties, immunomodulation, anti-tumor activity, and cytoprotection. Widely used in the traditional Chinese medicine, *L. barbarum* and *L. chinense* can also be sold as a dietary supplement or classified as a nutraceutical food for its long and safe traditional use (Amagase, et al., 2009; Zhao, et al., 2009a; Zhao, et al., 2009b). In general, studies have confirmed the health benefits coming from high fruit and vegetable consumption (Deng, Lin, Xu, Gao, Xie, & Li, 2013). Low intake of fruits and vegetables is estimated to cause about 19% of gastrointestinal cancer, 31% of ischaemic heart disease, and 11% of strokes (Costa, Garcia-Diaz, Jimenez, & Silva, 2013; Isabelle, Lee, Lim, Koh, Huang, & Ong, 2010; Medina, 2011); indeed, oxidative stress is implicated in a number of diseases, including cardiovascular dysfunction, various typologies of cancer, rheumatism, diabetes, rheumatoid arthritis, pulmonary emphysema, dermatitis, cataract, neurodegenerative diseases, endothelian cell dysfunction, and several autoimmune diseases linked to the degenerative process of ageing (Contessa, Mellano, Beccaro, Giusiano, & Botta, 2013; Dell'Agli, Di Lorenzo, Badea, Sangiovanni, Dima, Bosisio, & Restani, 2013; Donno, Beccaro, Mellano, Canterino, Cerutti, & Bounous, 2013a). Some authors report that antioxidant molecules in goji fruit might confer many health protective benefits by alleviating oxidative stress, i.e.,

preventing free radicals from damaging proteins, DNA, and lipids (Lako, Trenerry, Wahlqvist, Wattanapenpaiboon, Sotheeswaran, & Premier, 2007); through additive and synergistic effects, the complex mixture of phytochemicals in fruits or herbal products may provide better protection than a single phytochemical (Donno, Beccaro, Mellano, Bonvegna, & Bounous, 2014a; Durgo, Belscak-Cvitanovic, Stancic, Franekic, & Komes, 2012).

Moreover, the different *Lycium* spp. fruits are most often incorporated into complex herb formulae in traditional medicines, in a 6–18 g/100 g ratio as dried material. In case of decoction, scientific references indicate 5–15 g/100 ml of goji, equivalent to 25–120 g of fresh berries. There were few reports on the use of goji fresh fruit, as a single component or a major component in a recipe (Amagase & Farnsworth, 2011; Amagase, et al., 2009; Anonymous, 2010). Researchers set a recommended amount/volume of 30 ml of decoction four times daily (total 120 ml/day), which is equivalent to approximately 150 g of fresh berries (servings can be combined, if desired) (Ionica, Nour, & Trandafir, 2012; Potterat, 2010). As other commonly eaten fruits, goji has been traditionally used as a food and herbal medicine for over 2500 years without any specific toxicity. There were only two recent case reports of a possible interaction of goji fruit tea with warfarin (Coumadin) (Amagase & Farnsworth, 2011).

For all these reasons, the goji fruit market today is significantly expanding; China, the main supplier of goji products in the world, had total exports generating US$ 120 million in 2010. This production derived from 82,000 ha cultivated nationwide, yielding 95,000 tons of wolfberries (Amagase & Farnsworth, 2011). Most commercially produced wolfberries come from *L. barbarum* and *L. chinense* plantations in the Ningxia Hui Region in North-central China and the Xinjiang Uyghur Region in Western China. In addition, goji is also grown in Mongolia valleys (Potterat, 2010). The fruits are sold, dried, or squeezed to obtain juice (Amagase, et al., 2009; Anonymous, 2010).

Many goji products are sold on the health food market, in particular, through the internet market, praised for well-being and longevity, and they are usually very expensive. Goji is also found in conventional food products, such as yoghurt. However, a clear identification of the different wolfberry species and cultivars is difficult, so that adulteration in commercial products cannot be excluded (Potterat, 2010). Ten *Lycium* spp. genotypes are found to be substitutes or adulterants in the commercial market in Hong Kong and China; it is difficult to identify the *Lycium* species by traditional morphological and histological analysis (Amagase & Farnsworth, 2011; Amagase, et al., 2009). Now that the goji has gained worldwide recognition with strong market demand, the much more cheaply cultivated *L. chinense* is being passed off as *L. barbarum* fruit. For this reason, the analytical fingerprint could be considered an easy and reliable technique to characterize and differentiate *Lycium* species and to control the product quality and standardization (Le, Chiu, & Ng, 2007; Zhou, Jiang, Ding, Cheng, Xu, But, & Shaw, 2008).

By definition, a chromatographic fingerprint is a chromatographic pattern of the extract of the most common pharmacologically active compounds. It is suggested that using the obtained chromatographic fingerprints, the authentication and identification of fruits or fruit-derived products, can be accurately conducted even if the amount and/or concentration of the main chemical constituents are not exactly the same for different samples or the chromatographic fingerprints could successfully demonstrate both the “sameness” and “differences” between different samples (Donno, Beccaro, Mellano, Cerutti, & Bounous, 2014b; Zhou, et al., 2008). The chromatographic techniques could be used to obtain a relatively complete picture of the fruit extracts, which is usually called analytical fingerprint, in order to represent the so-called phytocomplex; in order to understand bioactivities and possible side effects of active compounds of fresh fruit or fruit extracts and to enhance product quality control, it needs to determine most of the fruit phytochemical constituents (Canterino, Donno, Mellano, Beccaro, & Bounous, 2012; Donno, Beccaro, Mellano, Torello Marinoni, Cerutti, Canterino, & Bounous, 2012; Donno, Cavanna, Beccaro, Mellano, Torello Marinoni, Cerutti, & Bounous, 2013d).

As previously mentioned, the interest in the composition of goji fruit has intensified because of an increased awareness of their possible health benefits, as they are rich sources of micronutrients and phytochemicals, such as organic acids, sugars, and phenolic compounds (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012a; Mikulic-Petkovsek, Slatnar, Stampar, & Veberic, 2012b). Some of these phytochemicals, which act as antioxidants, have recently been identified, and recent data show that they help to optimize human health by neutralizing free radicals in the body (Amagase, et al., 2009; Amaral, Mira, Nogueira, da Silva, & Florencio, 2009; Borges, Degeneve, Mullen, & Crozier, 2009). For example, in the pharmaceutical industry, organic acids are used as antioxidants, preservatives, acidulants, and drug absorption modifiers. Organic acids can also maintain the quality and nutritive value of fruit; goji is considered a good source of bioactive components and the manipulation of their contents, specifically those linked to fruit quality parameters, is a legitimate objective of crop improvement (Mikulic-Petkovsek, et al., 2012a).

The aim of this study was to describe goji fruit quality traits and report on the level of potentially bioactive compounds and their influence on total fruit phytocomplex and antioxidant activity. This study emphasizes that quality parameters are not enough for a full evaluation of the these fruits but it is also necessary to consider nutraceutical features, defining an effective chemical fingerprint, used as a quality control tool; as little information is currently available on the chemical fingerprint of goji fruit, the results of the present study may encourage a deeper evaluation of the effective nutraceutical value for different genotypes. Regarding the growing interest in introducing goji cultivation in different pedoclimates, the sustainability of its production compared to common local fruit species should also be evaluated (Cerutti, Beccaro, Bruun, Bosco, Donno, Notarnicola, & Bounous, 2013; Cerutti, Bruun, Donno, Beccaro, & Bounous, 2013).

2. MATERIALS AND METHODS

2.1 Plant material

Samples of goji fruit were picked up in a farm located in Alzate di Momo (Northern Italy) in October 2013; the fruits (0.5 kg for each plant) were manually picked from three plants for each replication.

This study focused on quality traits and health-promoting effects based on the nutraceutical fingerprint and antioxidant activity; because of the confusion and difficulty of distinguishing between *L. barbarum* and *L. chinense*, even by botanists and nurserymen, there are many problems in understanding the genetic origin of the analyzed commercial genotype: morphological, quality, and nutraceutical information indicate that probably *L. chinense* or, more likely, a hybrid cultivar between the two species was analyzed. This genotype is one of the most cultivated in small family-managed farms and nurseries with commercial purposes. The same analyses were performed on some common temperate and subtropical fruit species grown in the same pedoclimatic conditions in order to understand if this species presents a real added nutritional value compared with others. All harvested fruits were collected randomly in the orchard from different plants and analyzed fresh or after being stored for few days at 4°C and 95% relative humidity (RH).

2.2 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).

Ethanol was purchased from Fluka Biochemika (Buchs, Switzerland). Analytic HPLC grade solvents, methanol, and formic acid were purchased from Sigma Aldrich and Fluka Biochemika, respectively; potassium dihydrogen phosphate, ammonium 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).

Cetyltrimethylammonium bromide (cetrimide) was purchased from Extrasynthése (Genay, France), while 1,2-phenylenediamine dihydrochloride (OPDA) was purchased from Sigma Aldrich.

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

2.3 Qualitative analysis

2.3.1 Physical parameters

Average fruit weight (g) was evaluated by Mettler PM460 DeltaRange Electronic Balance (Mettler, Greifensee, Switzerland), while a digital caliper (Traceable Digital Caliper-6'', VWR International, Milano, Italy) was used for measuring fruit size (mm). For each analysis, three replications, each obtained from 15 fruits, were considered.

2.3.2 Chemical parameters

TSS (°Brix) were recorded with a digital refractometer DBR35 (Tsingtao Unicom-Optics Instruments, Laixi, China); TA ($meq \cdot L^{-1}$) and pH (pH-units) were determined by titrating 10 mL of pulp juice (rising to 100 ml final volume with Milli-Q water) with a solution of NaOH (0.2 $mol \cdot L^{-1}$), using an automatic titrator (Crison Titromatic 2S, Crison, Alella, Spain).

2.4 Spectrophotometric analysis

2.4.1 Total polyphenolic compounds (TPC)

For the extraction of polyphenolic compounds, samples were placed in 50 mL test tubes, and 25 mL of extraction solution (a solution of methanol and water acidified with HCL 37%) were subsequently added to the weighed samples; after 60 minutes 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 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 at 765 nm (Slinkard & Singleton, 1977).

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

2.4.2 Antioxidant bioactivity

Antioxidant activity in the goji fruit pulp was evaluated by ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1999). 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+}). Absorbance at 595 nm with a UV/Vis spectrophotometer (1600-PC, VWR International) was recorded.

The standard curve was obtained using $FeSO_4 \cdot 7H_2O$ (concentration range: 100–1000 $\mu mol \cdot L^{-1}$), and results were expressed as millimoles of Fe^{2+} equivalents per kilogram (solid food) of FW.

2.5 Chromatographic analysis

2.5.1 Sample preparation protocols

2.5.1.1 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.

2.5.1.2 Monoterpenes and organic acids

For the extraction of organic acids and monoterpenes, three replications, each obtained from 30 fruits, were considered. Five grams of fruit pulp 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 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.

2.5.1.3 Vitamin C

Ten grams of fruit pulp 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 at room temperature in an ALC Centrifuge PK 120. 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 (Sanchez, Gil-Izquierdo, & Gil, 2003).

Acidified samples were centrifuged for 5 min at 200 Hz 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 C_{18} 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 (Gonzalez-Molina, Moreno, & Garcia-Viguera, 2008).

2.5.2 Standard preparation

Chemical structures of all the biomarkers are shown in Fig. 1.

Stock solutions of monoterpenes, ascorbic and dehydroascorbic acids, cinnamic acids, and flavonols with a concentration of 1.0 $mg \cdot mL^{-1}$ were prepared in methanol: four calibration standards were prepared by dilution with methanol; stock solutions of benzoic acids and catechins with a concentration of 1.0 $mg \cdot mL^{-1}$ were prepared in 95% methanol and 5% water. In this case, four calibration standards were prepared by dilution with 50% methanol–water.

Stock solutions of organic acids with a concentration of 1.0 $mg \cdot mL^{-1}$ were prepared in ultrapure water; from these solutions, four calibration standards were prepared by dilution with water.

2.5.3 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 GI315D UV-Vis diode array detector (Agilent Technologies, Santa Clara, CA, USA), was used for the analysis.

Five different chromatographic methods were used to analyse the samples, two for polyphenols and one for terpenic compounds, organic acids, and vitamins, respectively.

In all of the used methods, bioactive compound separation was achieved on a ZORBAX Eclipse XDB – C18 column (4.6 × 150 mm, 5 µm, Agilent Technologies).

Different mobile phases were used: methanol and a solution of 40 mM potassium dihydrogen phosphate in water with a flow rate of 1.0 $mL \cdot min^{-1}$ (method A, 60 minute gradient analysis of cinnamic acids and flavonols), a solution of methanol/water/formic acid (5:95:0.1 v/v/v) and a mix of methanol/formic acid (100:0.1 v/v) with a flow rate of 1.0 $mL \cdot min^{-1}$ (method B, 35 minute gradient analysis of benzoic acids and catechins), water and methanol with a flow rate of 1.0 $mL \cdot min^{-1}$ (method C, 75 minute gradient analysis of monoterpenes), 0.5% $(NH_4)H_2PO_4$ aqueous solution (pH 2.8, adjusted with

phosphoric acid) with a flow rate of 0.5 mL·min^{-1} (method D, 20 minute isocratic analysis of organic acids), and methanol – water (5:95, v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate with a flow rate of 0.9 mL·min^{-1} (method E, 15 minute isocratic analysis of ascorbic and dehydroascorbic acids) (Donno, et al., 2014a).

UV spectra were recorded at 330 nm (A); 250, 280, and 320 nm (B); 220 and 235 nm (C); 214 nm (D); 261; and 348 nm (E) .

2.5.4 Identification and quantification of bioactive compounds

All 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. The external standard method was used for quantitative determinations. Calibration curves in the 125–1000 mg·L^{-1} range with good linearity for a four point plot were used to determine the bioactive compound concentration in goji fruit samples; the linearity for each compound was established by plotting the peak area (*y*) versus the concentration (*x*) of each biomarker. The limit of detection (LOD) and the limit of quantification (LOQ) of the five chromatographic methods were defined as the lowest amount of analyte that gives a reproducible peak with a signal-to-noise ratio (S/N) of 3 and 10, respectively. The main analytical method validation data are summarized in Table 1.

All samples were analysed in triplicate, and standard deviations are given in order to assess the repeatability of the used methods. Accuracy was checked by spiking samples with a solution containing each bioactive compound in a concentration of 10 mg·mL^{-1}.

Examples of goji chromatographic profiles are reported in Fig. 2. Total bioactive compound content (TBCC) was determined as the sum of the most important classes of bioactive compounds present in the samples. Five polyphenolic classes were considered: benzoic acids (ellagic and gallic acids),

catechins (catechin and epicatechin), cinnamic acids (caffeic, chlorogenic, coumaric, and ferulic acids), flavonols (hyperoside, isoquercitrin, quercetin, quercitrin, and rutin), and tannins (castalagin, vescalagin); one terpenic class was considered: monoterpenes (limonene, phellandrene, sabinene, γ-terpinene, and terpinolene). Organic acids (citric, malic, oxalic, quinic, succinic, and tartaric acids) and vitamin C (ascorbic and dehydroascorbic acids) were also considered to obtain a specific analytical fingerprint. All results were expressed as mg per 100 g of FW.

2.6 Statistical Analysis

Results were subjected to analysis of variance (ANOVA) test for mean comparison (SPSS 18.0 Software) and HSD Tukey multiple range test ($P < 0.05$). Principal component analysis (PCA) was performed on the chemical and nutraceutical data.

3. RESULTS

3.1 Chemical – nutraceutical analysis and antioxidant bioactivity

All quality data are reported in Table 2. Results showed that the fruit is ovoidal with an acute apex (11.50 mm in length and 8.15 mm in width), a mean weight value of 0.67 g, a bright red pericarp, and white endocarp.

Regarding chemical qualitative parameters, TSS showed a mean value of 11.63°Brix, while TA ranged from 264.80 meq·L^{-1} to 272.50 meq·L^{-1} with a pH mean value of 3.41 pH-units.

The content of total polyphenolic compounds in the extracts is reported in Table 3a. Results showed that the lowest TPC value was 255.87 $mg_{GAE}/100g_{FW}$ (sample G1) and the highest value was 281.91 $mg_{GAE}/100g_{FW}$ (sample G3). G2 and G3 samples showed higher antioxidant activity than the G1

sample; the lowest FRAP value was observed in G1 (18.00 mmol $Fe^{2+}\cdot kg^{-1}$) and the highest in G3 (20.89 mmol $Fe^{2+}\cdot kg^{-1}$) (Table 3a).

These analyses were also performed on some common temperate and subtropical fruit species in order to compare goji chemical and nutraceutical properties to other common species (Table 3b). Guava showed the highest TSS value (15.16 °Brix), followed by blackcurrant and kiwifruit (14.00 °Brix), apple (13.43 °Brix), orange (12.53 °Brix), and goji (11.63 °Brix). Guava (538.30 meq$\cdot L^{-1}$) had the highest TA value, followed by raspberry (413.57 meq$\cdot L^{-1}$) and orange (383.10 meq$\cdot L^{-1}$), while the TA of apple (49.01 meq$\cdot L^{-1}$) was the lowest. The pH values ranged from 2.92 pH-units in guava to 3.81 pH-units in apple.

The content of total polyphenolic compounds was statistically different among the different species. Kiwifruit and apple contained small quantities of polyphenolic compounds (70.23–83.40 $mg_{GAE}/100g_{FW}$), while a significantly higher polyphenolic content was observed in strawberry (323.39 $mg_{GAE}/100g_{FW}$) and blackcurrant (434.43 $mg_{GAE}/100g_{FW}$). Goji (268.5 $mg_{GAE}/100g_{FW}$) was in a medium position between orange (158.70 $mg_{GAE}/100g_{FW}$) and guava (310.10 $mg_{GAE}/100g_{FW}$).

Regarding the values of the total antioxidant capacity, expressed as FRAP assay, the results showed large statistical variations among the different species. Berries and, in particular, blackcurrant (76.86 mmol $Fe^{2+}\cdot kg^{-1}$) and blueberry (49.36 mmol $Fe^{2+}\cdot kg^{-1}$), showed the highest antioxidant capacity. Goji presented a higher FRAP value (19.36 mmol $Fe^{2+}\cdot kg^{-1}$) than kiwifruit, raspberry, and orange.

Significant differences in vitamin C content were recorded in the different species. Blackcurrant showed the highest vitamin C content (162.73 mg/100 g_{FW}), followed by kiwifruit (74.56 mg/100 g_{FW}), orange (71.12 mg/100 g_{FW}), strawberry (57.95 mg/100 g_{FW}), and goji (48.94 mg/100 g_{FW}). The lowest vitamin C value was recorded in apple (3.91 mg/100 g_{FW}).

Principal component analysis was performed on all samples and it reduced the initial variables (TSS, TA, pH, TPC, antioxidant activity, and vitamin C content) into three principal components (88.14% of

total variance) and divided samples in two groups (berries and no-berry fruit), confirming the statistically significant differences of the ANOVA test on quality and nutraceutical data (Fig. 3); goji fruit could be considered chemically similar to other berry fruits. The PCA graph showed a correlation between the nutraceutical variables (TPC, antioxidant activity, and vitamin C content) and PC1 (42.04% of total variance), while TSS and TA presented a correlation with PC2 (29.08% of total variance). The pH was in an intermediate position between PC1 and PC2.

3.2 Total bioactive compound content (TBCC) and single compound profile

All data (with mean values) are reported in Table 4 (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 analysed samples showed a lower TBCC value of 5357.22 mg/100 g_{FW} (sample G1) and a higher value of 6048.24 mg/100 g_{FW} (sample G3); the TBCC mean value was 5806.80 mg/100 g_{FW}.

Goji samples showed the following bioactive compound composition: four cinnamic acids (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid), one flavonol (hyperoside), one benzoic acid (gallic acid), two catechins (catechin, epicatechin), three monoterpenes (phellandrene, sabinene, γ-terpinene), five organic acids (citric acid, malic acid, oxalic acid, quinic acid, tartaric acid), and one vitamin (vitamin C expressed as the sum of ascorbic acid and dehydroascorbic acid); isoquercitrin, quercetin, quercitrin, rutin, ellagic acid, castalagin, vescalagin, limonene, terpinolene, and succinic acid were not detected. Single bioactive compound content ranged from 13.08 mg/100 g_{FW} (oxalic acid, G3 sample) to 2544.31 mg/100 g_{FW} (quinic acid, G3 sample).

Correlation among antioxidant activity and TPC, TBCC, and single bioactive classes are reported in Table 5; monoterpenes and vitamins showed a negative correlation with antioxidant capacity (strong and weak, respectively), while polyphenols and organic acids presented a strong positive correlation (0.8290

and 0.8606, respectively). TPC (R=0.9996) and TBCC (R=0.8363) showed a strong positive correlation with antioxidant activity; TBCC correlation value was lower than TPC because of the negative influence of monoterpenes and vitamins.

3.3 Fingerprinting

The chemical fingerprint of goji fruit was reported: in total, 17 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.

The chemical fingerprint showed the prevalence of organic acids and polyphenolic compounds (as the sum of cinnamic acids, flavonols, benzoic acids, catechins, and tannins) in chemical composition of all the analyzed samples (mean values were considered); the most important class was organic acids (76.82%), followed by polyphenols (16.20%), monoterpenes (6.13%), and vitamins (0.84%) (Table 6).

Therefore, organic acids and polyphenolic compounds were two major groups of bioactive compounds in the evaluated goji fruit; in the polyphenol group, the most important classes were cinnamic acids (7.94%) and catechins (5.99%), followed by flavonols and benzoic acids (all percentages refer to the total content of bioactive compounds). Tannins were not detected.

4. DISCUSSION

Goji has achieved widespread popularity in the past decade due to its acceptance by the public as a “super fruit” or “super food” with highly advantageous nutritive properties (Jamin, 2009; Karp, 2012). As a result of the continued use of this fruit in soft drinks and other foods, sales have extended out from

traditional Chinese communities and from specialized health food stores into the mainstream market in many countries (Amagase & Farnsworth, 2011; Amagase, et al., 2009). Under the Dietary Supplement Health and Education Act of 1994, goji can be sold in the USA and UE as an ingredient in dietary supplement or foods; however, these products cannot be promoted as drugs, and therapeutic claims are prohibited. Import of the berries does not automatically mean that they were consumed as such, however, since it is known that dietary supplements containing goji berries were being marketed in the EU before 1997 and in the US before 1994 (Potterat, 2010).

During the past several years, the quest for alternative crops with high nutritional value has increased interest in goji (Liu, 2003; Mikulic-Petkovsek, et al., 2012b); however, TPC, antioxidant activity, and most of the potential health-promoting agents of wolfberry remain unscrutinized. Despite many reports of commonly available fruits, such as blueberry, kiwifruit, orange and apple, on their TPC and antioxidant activity (Canterino, et al., 2012; Donno, et al., 2013a; Donno, et al., 2012; Pellegrini, Serafini, Colombi, Del Rio, Salvatore, Bianchi, & Brighenti, 2003), little information is available for currently underused fruits. Goji fruits may contain a significant amount of phytochemicals or even unique compounds that are health-promoting (Ionica, et al., 2012; Le, et al., 2007); this study showed that the analyzed quality and nutraceutical parameters of the fruits of this species are comparable to those of other common fruit species, such as as *Ribes nigrum*, *Actinidia deliciosa*, and *Citrus sinensis*.

In general, major fruit species showed different chemical characteristics compared to berry fruits (Contessa, et al., 2013). In order to simplify the multivariate model based on the analysis of six parameters (in particular, TPC, TBCC, and antioxidant activity) and classify the species according to their quality and nutraceutical characteristics, a PCA was carried out. As in other studies (Amagase, et al., 2009; Mikulic-Petkovsek, et al., 2012a; Mikulic-Petkovsek, et al., 2012b), results showed that goji is very similar to other berry fruits (same PCA group). In this case, antioxidant activity and bioactive compound contribution to total fruit phytocomplex were also used to highlight goji nutraceutical properties;

antioxidant activity was considered an important method to evaluate the nutraceutical properties of fruit, as shown in other previous studies (Amaral, et al., 2009; Chen, Xin, Yuan, Su, & Liu, 2013; Isabelle, et al., 2010). In particular, in this study, the correlation between TPC/TBCC and antioxidant activity was useful to show that the detected single compounds were strongly related to some nutraceutical properties (antioxidant capacity). Moreover, an ANOVA test and PCA confirmed the TPC and antioxidant activity results of other authors (Anonymous, 2010; Contessa, et al., 2013), significantly contributing to improve the knowledge of this species.

Specific bioactive compounds can be used collectively as representative standards of a plant sample in quantification (Tsao & Yang, 2003), as done in this study. HPLC data can be used as TBCC for the quantification of health-promoting agents because HPLC methods give more information on individual compounds or groups of compounds than the TPC by the Folin-Ciocalteu method (Gudej & Tomczyk, 2004). In this study, an innovative approach has been applied to evaluate the goji fruit chemical composition and medicinal properties; an analytical fingerprint was used to show the single bioactive class contribution to the total fruit phytocomplex. Indeed, synergistic or additive biological effects of different bioactive compounds could contribute to disease prevention more than a single compound or a group of compounds (Benvenuti, Pellati, Melegari, & Bertelli, 2004; Bolwell, 1990). In previous studies (Donno, Beccaro, Mellano, Cerutti, & Bounous, 2013b; Donno, Beccaro, Mellano, Cerutti, Marconi, & Bounous, 2013c), this approach was only considered in relation to the medical property evaluation of herbal preparations, while in this research, it was applied to the study of fresh fruit quality. The main aim was to obtain a fingerprint of wolfberry fresh fruits by reverse phase mode HPLC/DAD analyses. By different elution methods, all of the metabolites in the fruit extracts of the considered species were simultaneously determined; the obtained fingerprints were useful for authentication, and quality control purposes. To identify the bioactive compounds, the UV–vis absorption spectra and the chromatographic retention times were used and combined for tentative identification of the selected biomarkers. The

methods showed a good resolution for most peaks and could be routinely used to evaluate overall fruit quality; it could be also applied for other species and genera, as shown in other studies (Canterino, et al., 2012; Donno, et al., 2012).

The HPLC analysis of bioactive compounds is nowadays a widespread and well-developed characterization tool, and some analytical reports were found in the literature (Anttonen & Karjalainen, 2006; Aprea, Carlin, Giongo, Grisenti, & Gasperi, 2009; Donno, et al., 2013c). Based on the obtained results, many studies pointed out that the identified polyphenolic compounds significantly contribute to the goji phytocomplex and antioxidant activity (Amagase, et al., 2009; Potterat, 2010); the present study confirmed these results, adding organic acids, vitamins, and terpenic compounds also significantly contributed to the wolfberry fruit phytocomplex, as antioxidant and anti-inflammatory health-promoting agents. No studies emphasized the complete identification of single bioactive compounds in goji fresh fruit by HPLC analysis.

In this study, effective HPLC–DAD methods were used for fingerprint analysis and nutraceutical identification of goji fruit. Comparing with other analytical studies (Mikulic-Petkovsek, et al., 2012a; Mikulic-Petkovsek, et al., 2012b), the chromatographic conditions were optimized to obtain an analytical fingerprint containing complete information of chemical composition with a good resolution and a reasonable analysis time. Different linear gradients in different slopes were used for optimizing the molecule separation; indeed, some compounds were similar in structure with each other in the same chemical class. Adding formic and phosphoric acid was necessary for enhancing the resolution and eliminating peak tailing because most of the compounds were also weakly acidic, according to other studies (Donno, et al., 2013b; Donno, et al., 2013c; Zhao, Dong, & Lin, 2009c). The wavelength selection was an important step for developing a reliable fingerprint; only selected wavelengths were suitable to achieve more specific peaks as well as a smooth baseline after a full-scan on the chromatogram from 190

to 400 nm, according to other similar research (Donno, et al., 2013d; Serafini, Stanzione, Foddai, Anton, & Delmulle, 2012).

This study is only preliminary about goji fruit chemical composition; this information is actually completely missing for this species. Genotype is an important variable to define the nutraceutical and quality traits (Beccaro, Torello-Marinoni, Binelli, Donno, Boccacci, Botta, Cerutti, & Conedera, 2012) but, in this case, this research only focused on the antioxidant activity and chemical profile of a commercial cultivar. This first overview could be extended in further studies with a careful comparison of different wolfberry genotypes and *Lycium* species; the diversity in total bioactive compound content and antioxidant activity between cultivars in other species (Canterino, Donno, & Mellano, 2010; Canterino, et al., 2012; Mellano, Beccaro, Donno, Marinoni, Boccacci, Canterino, Cerutti, & Bounous, 2012) emphasizes the need for additional screening to identify goji species and cultivars with high antioxidant capacity and health-promoting potential.

5. CONCLUSIONS

A number of things lead to the confusion between the different species and genotypes of cultivated *Lycium*. In this study, wolfberry was identified as a rich source of antioxidant compounds; the observed analytical fingerprint demonstrated that the species represents a rich source of organic acids and polyphenolic compounds, especially cinnamic acids and catechins; this research suggested that identified nutraceuticals might contribute to the total phytocomplex of these fruits. This study developed an important tool to assess goji chemical composition and bioactivity, using different chromatographic methods for comprehensive authentication and quality control of its fruits: well-designed clinical trials with phytochemically well-characterized extracts are required before the potential of goji as a medicinal plant or food can be definitively assessed. Goji berry fruit is devoid of toxicity but caution is advised with regard to possible drug interactions as well as with products of unknown or dubious origin; for this

reason, the development of rigorous quality control protocols for goji products is urgently needed: this research showed that analytical fingerprinting could be an important tool to assess quality, chemical composition, and bioactivity of wolfberry fruits, helping to find new sources of natural health-promoting compounds.

6. REFERENCES

Ale-Agha, N., Brassmann, M., & Jensen, M. (2009). Biodiversity and new records of microfungi in the Ruhrarea (north Rhine Westfalia), Germany. *Commun Agric Appl Biol Sci, 74*, 785-796.

Amagase, H., & Farnsworth, N. R. (2011). A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of Lycium barbarum fruit (Goji). *Food Research International, 44*, 1702-1717.

Amagase, H., Sun, B., & Borek, C. (2009). Lycium barbarum (goji) juice improves in vivo antioxidant biomarkers in serum of healthy adults. *Nutr Res, 29*, 19-25.

Amaral, S., Mira, L., Nogueira, J. M. F., da Silva, A. P., & Florencio, M. H. (2009). Plant extracts with anti-inflammatory properties-A new approach for characterization of their bioactive compounds and establishment of structure-antioxidant activity relationships. *Bioorganic & Medicinal Chemistry, 17*, 1876-1883.

Anonymous. (2010). Are some of the more exotic berry types, such as goji and acai berries, better for health than the more common berries such as strawberries, blueberries and raspberries? *Mayo Clinic health letter (English ed.), 28*, 8.

Anttonen, M. J., & Karjalainen, R. O. (2006). High-Performance Liquid Chromatography Analysis of Black Currant (Ribes nigrum L.) Fruit Phenolics Grown either Conventionally or Organically. *Journal of Agricultural and Food Chemistry, 54*, 7530-7538.

Aprea, E., Carlin, S., Giongo, L., Grisenti, M., & Gasperi, F. (2009). Characterization of 14 Raspberry Cultivars by Solid-Phase Microextraction and Relationship with Gray Mold Susceptibility. *Journal of Agricultural and Food Chemistry, 58*, 1100-1105.

Beccaro, G. L., Torello-Marinoni, D., Binelli, G., Donno, D., Boccacci, P., Botta, R., Cerutti, A. K., & Conedera, M. (2012). Insights in the chestnut genetic diversity in Canton Ticino (Southern Switzerland). *Silvae Genetica, 61*, 292 - 300.

Benvenuti, S., Pellati, F., Melegari, M., & Bertelli, D. (2004). Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of Rubus, Ribes, and Aronia. *Journal of Food Science, 69*, C164-C169.

Benzie, I. F., & Strain, J. J. (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.

Bolwell, G. P. (1990). Plant Polyphenols: Vegetable tannins revisited (1989). By E. Haslam. Chemistry and Pharmacology of Natural Products (J. D. Phillipson, D. C. Ayres and H. Baxter, Eds). Cambridge University Press: Cambridge, Pp. 230, £35/$70. *BioEssays, 12*, 453-453.

Borges, G., Degeneve, A., Mullen, W., & Crozier, A. (2009). Identification of Flavonoid and Phenolic Antioxidants in Black Currants, Blueberries, Raspberries, Red Currants, and Cranberries†. *Journal of Agricultural and Food Chemistry, 58*, 3901-3909.

Canterino, S., Donno, D., & Mellano, M. (2010). Sensorial Analysis and Fruit Quality in Citrus sinensis (L.) Osbeck from Northern Italy (Piedmont). *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 928*, 389-394.

Canterino, S., Donno, D., Mellano, M. G., Beccaro, G. L., & Bounous, G. (2012). Nutritional and sensory survey of Citrus sinensis (L.) cultivars grown at the most Northern limit of the Mediterranean latitude. *Journal of Food Quality, 35*, 108-118.

Cerutti, A. K., Beccaro, G. L., Bruun, S., Bosco, S., Donno, D., Notarnicola, B., & Bounous, G. (2013). LCA application in the fruit sector: state of the art and recommendations for environmental declarations of fruit products. *Journal of Cleaner Production*.

Cerutti, A. K., Bruun, S., Donno, D., Beccaro, G. L., & Bounous, G. (2013). Environmental sustainability of traditional foods: the case of ancient apple cultivars in Northern Italy assessed by multifunctional LCA. *Journal of Cleaner Production, 52*, 245-252.

Chen, J., Cheng, H., Zhang, J., Zhang, G., & Ding, W. (2003). Investigation on occurrence of lycium barbarum pests and its natural enemies at Ningxia. *Zhong Yao Cai, 26*, 391-394.

Chen, L., Xin, X., Yuan, Q., Su, D., & Liu, W. (2013). Phytochemical properties and antioxidant capacities of various colored berries. *J Sci Food Agric*.

Contessa, C., Mellano, M. G., Beccaro, G. L., Giusiano, A., & Botta, R. (2013). Total antioxidant capacity and total phenolic and anthocyanin contents in fruit species grown in Northwest Italy. *Scientia Horticulturae, 160*, 351-357.

Costa, A. G. V., Garcia-Diaz, D. F., Jimenez, P., & Silva, P. I. (2013). Bioactive compounds and health benefits of exotic tropical red–black berries. *Journal of Functional Foods, 5*, 539-549.

Dell'Agli, M., Di Lorenzo, C., Badea, M., Sangiovanni, E., Dima, L., Bosisio, E., & Restani, P. (2013). Plant food supplements with anti-inflammatory properties: a systematic review (I). *Crit Rev Food Sci Nutr, 53*, 403-413.

Deng, G.-F., Lin, X., Xu, X.-R., Gao, L.-L., Xie, J.-F., & Li, H.-B. (2013). Antioxidant capacities and total phenolic contents of 56 vegetables. *Journal of Functional Foods, 5*, 260-266.

Donno, D., Beccaro, G. L., Mellano, M. G., Bonvegna, L., & Bounous, G. (2014a). Castanea spp. buds as a phytochemical source for herbal preparations: botanical fingerprint for nutraceutical identification and functional food standardisation. *Journal of the Science of Food and Agriculture*, n/a-n/a.

Donno, D., Beccaro, G. L., Mellano, M. G., Canterino, S., Cerutti, A. K., & Bounous, G. (2013a). Improving the nutritional value of kiwifruit with the application of agroindustry waste extracts. *Journal Of Applied Botany And Food Quality, 86*, 11-15.

Donno, D., Beccaro, G. L., Mellano, M. G., Cerutti, A. K., & Bounous, G. (2013b). Medicinal plants, chemical composition and quality: may blackcurrant buds and blackberry sprouts be a new polyphenol source for herbal preparations? *Journal Of Applied Botany And Food Quality, 86*, 79-89.

Donno, D., Beccaro, G. L., Mellano, M. G., Cerutti, A. K., & Bounous, G. (2014b). Chemical fingerprint as nutraceutical quality differentiation tool in *Asimina triloba* L. fruit pulp at different ripening stages: an old species for new health needs. *Journal Of Food And Nutrition Research, -*, First Online.

Donno, D., Beccaro, G. L., Mellano, M. G., Cerutti, A. K., Marconi, V., & Bounous, G. (2013c). Botanicals in Ribes nigrum bud-preparations: An analytical fingerprinting to evaluate the bioactive contribution to total phytocomplex. *Pharm Biol, 51*, 1282-1292.

Donno, D., Beccaro, G. L., Mellano, M. G., Torello Marinoni, D., Cerutti, A. K., Canterino, S., & Bounous, G. (2012). Application of sensory, nutraceutical and genetic techniques to create a quality profile of ancient apple cultivars. *Journal of Food Quality, 35*, 169-181.

Donno, D., Cavanna, M., Beccaro, G. L., Mellano, M. G., Torello Marinoni, D., Cerutti, A. K., & Bounous, G. (2013d). Currants and strawberries as bioactive compound sources: determination of antioxidant profiles with HPLC-DAD/MS. *Journal Of Applied Botany And Food Quality, 86*, 1-10.

Durgo, K., Belscak-Cvitanovic, A., Stancic, A., Franekic, J., & Komes, D. (2012). The bioactive potential of red raspberry (Rubus idaeus L.) leaves in exhibiting cytotoxic and cytoprotective activity on human laryngeal carcinoma and colon adenocarcinoma. *J Med Food, 15*, 258-268.

Gonzalez-Molina, E., Moreno, D. A., & Garcia-Viguera, C. (2008). Genotype and harvest time influence the phytochemical quality of Fino lemon juice (Citrus limon (L.) Burm. F.) for industrial use. *Journal of Agricultural and Food Chemistry, 56*, 1669-1675.

Gudej, J., & Tomczyk, M. (2004). Determination of Flavonoids, Tannins and Ellagic acid in leaves from *Rubus* L. species. *Archives of Pharmacal Research, 27*, 1114-1119.

Ionica, M. E., Nour, V., & Trandafir, I. (2012). Polyphenols content and antioxidant capacity of goji fruits (Lycium chinense) as affected by the extraction solvents. *South-Western Journal of Horticulture Biology and Environment, 3*, 121-129.

Isabelle, M., Lee, B. L., Lim, M. T., Koh, W. P., Huang, D. J., & Ong, C. N. (2010). Antioxidant activity and profiles of common fruits in Singapore. *Food Chemistry, 123*, 77-84.

Jamin, E. (2009). Superfruits: are they authentic? *Fruit Processing, 19*, 170-175.

Karp, D. (2012). Frontiers of fruit in California: new and rediscovered fruit types with potential for commercial cultivation. *Journal of the American Pomological Society, 66*, 125-132.

Lako, J., Trenerry, V. C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., & Premier, R. (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry, 101*, 1727-1741.

Le, K., Chiu, F., & Ng, K. (2007). Identification and quantification of antioxidants in Fructus lycii. *Food Chemistry, 105*, 353-363.

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr, 78*, 517S-520S.

Medina, M. B. (2011). Determination of the total phenolics in juices and superfruits by a novel chemical method. *Journal of Functional Foods, 3*, 79-87.

Mellano, M. G., Beccaro, G. L., Donno, D., Marinoni, D. T., Boccacci, P., Canterino, S., Cerutti, A. K., & Bounous, G. (2012). Castanea spp. biodiversity conservation: collection and characterization of the genetic diversity of an endangered species. *Genetic Resources and Crop Evolution, 59*, 1727-1741.

Mikulic-Petkovsek, M., Schmitzer, V., Slatnar, A., Stampar, F., & Veberic, R. (2012a). Composition of Sugars, Organic Acids, and Total Phenolics in 25 Wild or Cultivated Berry Species. *Journal of Food Science, 77*, C1064-C1070.

Mikulic-Petkovsek, M., Slatnar, A., Stampar, F., & Veberic, R. (2012b). HPLC–MSn identification and quantification of flavonol glycosides in 28 wild and cultivated berry species. *Food Chemistry, 135*, 2138-2146.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., & Brighenti, F. (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J Nutr, 133*, 2812-2819.

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*, 7-19.

Sanchez, A. C. G., Gil-Izquierdo, A., & Gil, M. I. (2003). Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *Journal of the Science of Food and Agriculture, 83*, 995-1003.

Serafini, M., Stanzione, A., Foddai, S., Anton, R., & Delmulle, L. (2012). The European role on traditional herbal medicinal products and traditional plant food supplements. *J Clin Gastroenterol, 46 Suppl*, S93-94.

Slinkard, K., & Singleton, V. L. (1977). Total Phenol Analysis: Automation and Comparison with Manual Methods. *American Journal of Enology and Viticulture, 28*, 49-55.

Tsao, R., & Yang, R. (2003). Optimization of a new mobile phase to know the complex and real polyphenolic composition: towards a total phenolic index using high-performance liquid chromatography. *Journal of Chromatography A, 1018*, 29-40.

Zhao, R., Li, Q. W., Li, J., & Zhang, T. (2009a). Protective effect of Lycium barbarum polysaccharide 4 on kidneys in streptozotocin-induced diabetic rats. *Can J Physiol Pharmacol, 87*, 711-719.

Zhao, Z. H., Zhang, R., He, D. H., Wang, F., Zhang, T. T., & Zhang, Z. S. (2009b). Risk assessment and control strategies of pests in Lycium barbarum fields under different managements. *Ying Yong Sheng Tai Xue Bao, 20*, 843-850.

Zhao, Z. Y., Dong, L. L., & Lin, F. (2009c). Fingerprint Analysis of Euonymus alatus (Thuhb) siebold by LC-DAD and LC-ESI-MS. *Chromatographia, 69*, 429-436.

Zhou, Y., Jiang, S. Y., Ding, L. S., Cheng, S. W., Xu, H. X., But, P. P. H., & Shaw, P. C. (2008). Chemical Fingerprinting of Medicinal Plants "Gui-jiu" by LC-ESI Multiple-Stage MS. *Chromatographia, 68*, 781-789.

Tables

Table 1. Calibration curve equations, R^2, LOD, and LOQ of the used chromatographic methods for each calibration standard (Donno, et al., 2014a).

Class	Standard	Identification code	Method	Calibration curve equations (peak area = y; concentration = x)	R^2	LOD (mg/L)	LOQ (mg/L)
Cinnamic acids	caffeic acid	1	A	y = 10.155x + 13.008	0.985	1.232	4.107
	chlorogenic acid	2	A	y = 7.165x + 95.749	0.995	0.627	2.091
	coumaric acid	3	A	y = 10.904x + 187.144	0.999	1.037	3.456
	ferulic acid	4	A	y = 6.181x - 273.562	1.000	1.012	3.373
Flavonols	hyperoside	5	A	y = 14.315x - 262.753	1.000	0.549	1.829
	isoquercitrin	6	A	y = 11.437x + 100.974	0.998	0.475	1.585
	quercetin	7	A	y = 5.505x - 418.512	0.996	1.897	6.323
	quercitrin	8	A	y = 5.162x - 168.272	0.996	1.072	3.575
	rutin	9	A	y = 8.213x + 105.923	0.999	0.672	2.241
Benzoic acids	ellagic acid	10	B	y = 5.766x + 281.063	0.988	1.881	6.271
	gallic acid	11	B	y = 10.703x + 59.149	0.998	0.283	0.944
Catechins	catechin	12	B	y = 6.567x - 178.554	0.999	1.207	4.024
	epicatechin	13	B	y = 6.104x - 172.263	0.997	0.362	1.206
Tannins	castalagin	14	B	y = 3.261x - 65.994	0.995	1.755	5.850
	vescalagin	15	B	y = 19.124x - 42.783	0.996	1.749	5.829
Monoterpenes	limonene	16	C	y = 1.347x + 30.797	0.997	2.108	7.026
	phellandrene	17	C	y = 4.488x - 39.986	1.000	1.312	4.374
	sabinene	18	C	y = 29.237x - 296.283	1.000	0.026	0.087
	γ-terpinene	19	C	y = 2.461x + 205.211	0.993	2.758	9.194
	terpinolene	20	C	y = 0.056x - 1.809	0.995	7.479	24.930
Organic acids	citric acid	21	D	y = 1.695x + 16.075	1.000	1.065	3.549
	malic acid	22	D	y = 1.962x - 16.921	0.998	0.688	2.295
	oxalic acid	23	D	y = 20.034x + 287.523	0.999	0.098	0.328
	quinic acid	24	D	y = 1.193x - 3.232	1.000	2.054	6.845
	succinic acid	25	D	y = 0.845x + 47.492	0.997	1.492	4.972
	tartaric acid	26	D	y = 4.609x - 73.283	1.000	0.401	1.335
Vitamins	ascorbic acid	27	E	y = 40.541x - 798.702	0.998	0.236	0.786
	dehydroascorbic acid	28	E	y = 5.844x + 197.332	0.999	0.836	2.786

Table 2. Physical and chemical quality parameters in goji samples.

Sample	*Physical qualitative parameters*								
	weight (g)			*width (mm)*			*length (mm)*		
		mean	*SD*		*mean*	*SD*		*mean*	*SD*
G_1	0.73	0.67	0.07	10.30	8.15	1.86	12.57	11.50	1.33
G_2	0.59			6.98			10.01		
G_3	0.68			7.17			11.92		

Sample	*Chemical qualitative parameters*								
	total soluble solids (°Brix)			*titratable acidity (meq/L)*			*pH (upH)*		
		mean	*SD*		*mean*	*SD*		*mean*	*SD*
G_1	11.60	11.63	0.06	265.70	267.67	4.21	3.47	3.41	0.09
G_2	11.70			264.80			3.45		
G_3	11.60			272.50			3.31		

Table 3a. TPC and antioxidant activity data in analysed goji extracts.

Sample	TPC ($mg_{GAE}/100\ g_{FW}$)	Mean value ($mg_{GAE}/100_{FW}$)	SD	Antioxidant activity ($mmol\ Fe^{2+}/kg$)	Mean value ($mmol\ Fe^{2+}/kg$)	SD
G_1	255.87	268.35	13.05	18.00	19.36	1.46
G_2	267.28			19.19		
G_3	281.91			20.89		

Table 3b. Goji nutraceutical and quality traits compared to main common fruit. Mean values of each sample is given (N = 3). Different letters for each sample indicate the significant differences at $P < 0.05$.

Sample	TSS (°Brix)	Tukey test	SD	TA (meq/L)	Tukey test	SD	pH (upH)	Tukey test	SD
Apple	13.43	de	0.53	49.01	a	3.28	3.81	d	0.04
Blackcurrant	14.00	ef	0.23	183.08	b	3.80	2.93	a	0.04
Blueberry	9.20	a	0.87	166.70	b	12.45	3.21	bc	0.12
Guava	15.16	f	0.80	538.30	e	90.37	2.92	a	0.12
Kiwifruit	14.00	ef	0.40	352.90	cd	15.48	2.97	a	0.09
Goji	11.63	bc	0.06	267.67	bc	4.21	3.41	c	0.09
Orange	12.53	cd	0.21	383.10	d	20.54	3.36	c	0.05
Raspberry	10.70	b	0.36	413.57	d	51.62	3.03	ab	0.04
Strawberry	8.05	a	0.24	184.65	b	5.98	3.37	c	0.03
Sample	**TPC** ($mg_{GAE}/100_{FW}$)	**Tukey test**	**SD**	**Antioxidant activity** ($mmol\ Fe^{2+}/kg$)	**Tukey test**	**SD**	**Vitamin C** ($mg/100_{FW}$)	**Tukey test**	**SD**
Apple	83.40	a	13.24	5.62	a	1.12	3.91	a	0.48
Blackcurrant	434.43	d	99.66	76.86	f	8.55	162.73	f	7.17
Blueberry	299.60	c	44.12	49.36	e	5.05	12.60	ab	2.79
Guava	310.10	c	6.73	25.07	cd	1.45	19.36	ab	0.82
Kiwifruit	70.23	a	17.74	13.39	ab	1.01	74.56	e	9.84
Goji	268.35	bc	13.05	19.36	bc	1.46	48.94	cd	17.01
Orange	158.70	ab	1.91	12.43	ab	0.18	71.12	e	1.96
Raspberry	322.36	cd	7.15	13.02	ab	0.54	31.93	bc	4.36
Strawberry	323.39	cd	57.80	35.43	d	4.69	57.95	de	2.60

Table 4. Single compound profile of analysed samples**.**

mg/100 g_{FW}	*Cinnamic acids*			
sample	***caffeic acid***	***chlorogenic acid***	***coumaric acid***	***ferulic acid***
G_1	110.45	114.87	111.38	125.18
G_2	110.44	112.78	110.91	126.04
G_3	111.65	111.89	111.66	126.17
Mean value	110.84	113.18	111.32	125.80
SD	0.70	1.53	0.38	0.54

mg/100 g_{FW}	*Flavonols*				
sample	***hyperoside***	***isoquercitrin***	***quercetin***	***quercitrin***	***rutin***
G_1	115.57	n.d.	n.d.	n.d.	n.d.
G_2	116.90	n.d.	n.d.	n.d.	n.d.
G_3	116.34	n.d.	n.d.	n.d.	n.d.
Mean value	116.27	/	/	/	/
SD	0.67	/	/	/	/

mg/100 g_{FW}	*Benzoic acids*		*Catechins*		*Tannins*	
sample	***ellagic acid***	***gallic acid***	***catechin***	***epicatechin***	***castalagin***	***vescalagin***
G_1	n.d.	15.39	118.26	227.74	n.d.	n.d.
G_2	n.d.	15.24	118.68	230.54	n.d.	n.d.
G_3	n.d.	15.31	119.35	229.26	n.d.	n.d.
Mean value	/	15.31	118.76	229.18	/	/
SD	/	0.08	0.55	1.40	/	/

mg/100 g_{FW}	*Monoterpenes*				
sample	***limonene***	***phellandrene***	***sabinene***	***γ-terpinene***	***terpinolene***
G_1	n.d.	221.85	57.70	87.13	n.d.
G_2	n.d.	213.53	56.42	83.17	n.d.
G_3	n.d.	212.49	56.10	80.15	n.d.
Mean value	/	215.96	56.74	83.48	/
SD	/	5.13	0.84	3.50	/

mg/100 g_{FW}	*Organic acids*					
sample	***citric acid***	***malic acid***	***oxalic acid***	***quinic acid***	***succinic acid***	***tartaric acid***
G_1	262.44	451.43	13.44	1919.56	n.d.	1362.51
G_2	291.15	719.74	13.72	1571.32	n.d.	2056.09
G_3	208.67	633.11	13.08	2544.31	n.d.	1322.46
Mean value	254.09	601.43	13.41	2011.73	/	1580.35
SD	41.87	136.93	0.32	493.00	/	412.49

mg/100 g_{FW}	**Vitamins**	***TBCC***
sample	***vitamin C***	
G_1	42.32	5357.22
G_2	68.26	6014.94
G_3	36.24	6048.24
Mean value	48.94	5806.80
SD	17.01	389.70

Table 5. Correlation among antioxidant activity and TPC\TBCC\all single bioactive compounds.

Pearson correlation coefficient (R)						
	TPC	***TBCC***	***Polyphenols***	***Monoterpenes***	***Organic acids***	***Vitamins***
Antioxidant activity	0.9996	0.8363	0.8290	-0.9263	0.8606	-0.2756
correlation	positive strong	positive strong	positive strong	negative strong	positive strong	negative weak

Tab. 6. Contribution of antioxidant classes to the fruit phytocomplex in analysed extracts.

mg/100 g_{FW}	*Cinnamic acids*	*Flavonols*	*Benzoic acids*	*Catechins*	*Tannins*	*Monoterpenes*	*Organic acids*	*Vitamins*
G_1	461.88	115.57	15.39	345.99	0.00	366.67	4009.39	42.32
G_2	460.17	116.90	15.24	349.22	0.00	353.13	4652.03	68.26
G_3	461.37	116.34	15.31	348.60	0.00	348.74	4721.64	36.24
Mean value	461.14	116.27	15.31	347.94	0.00	356.18	4461.02	48.94
Phytocomplex	7.94%	2.00%	0.26%	5.99%	0.00%	6.13%	76.82%	0.84%

Figures

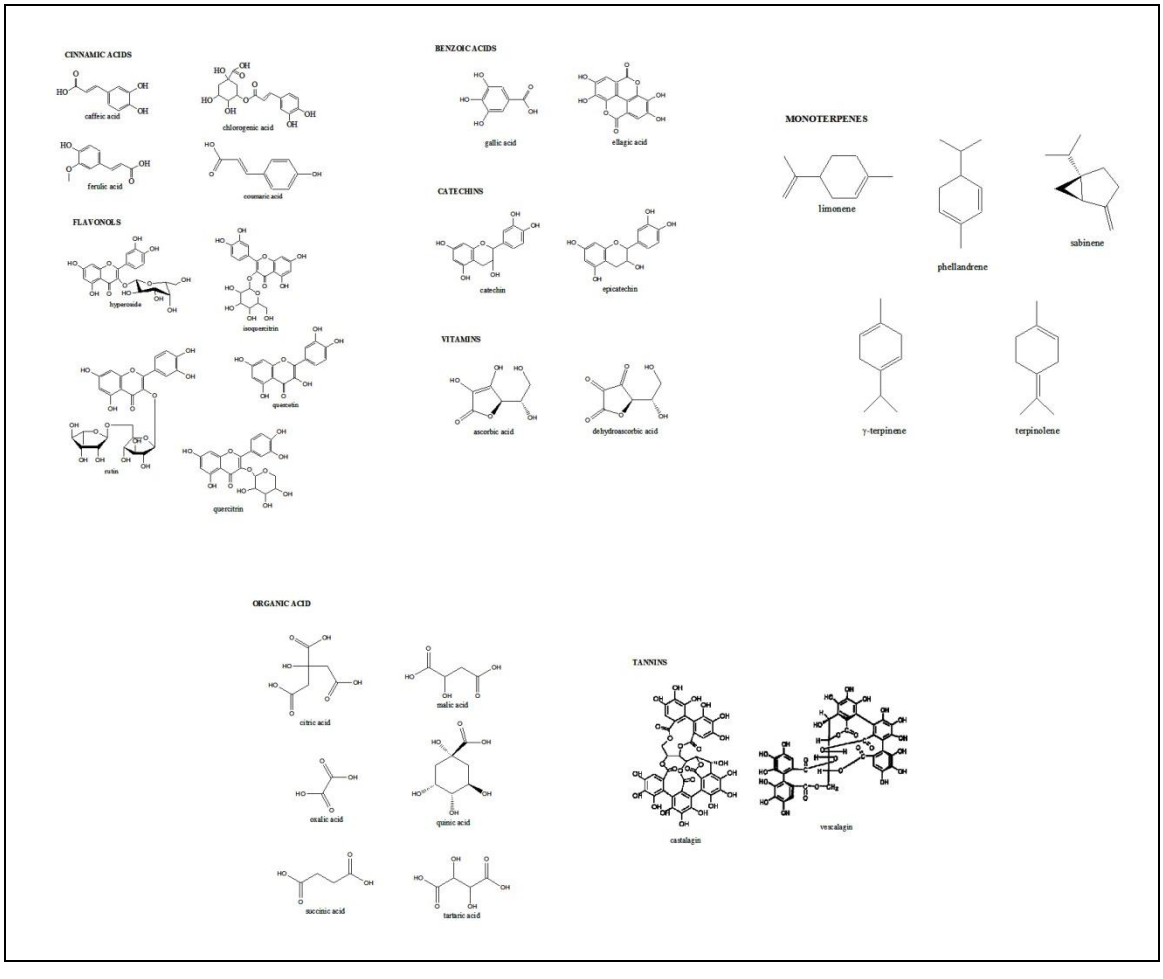
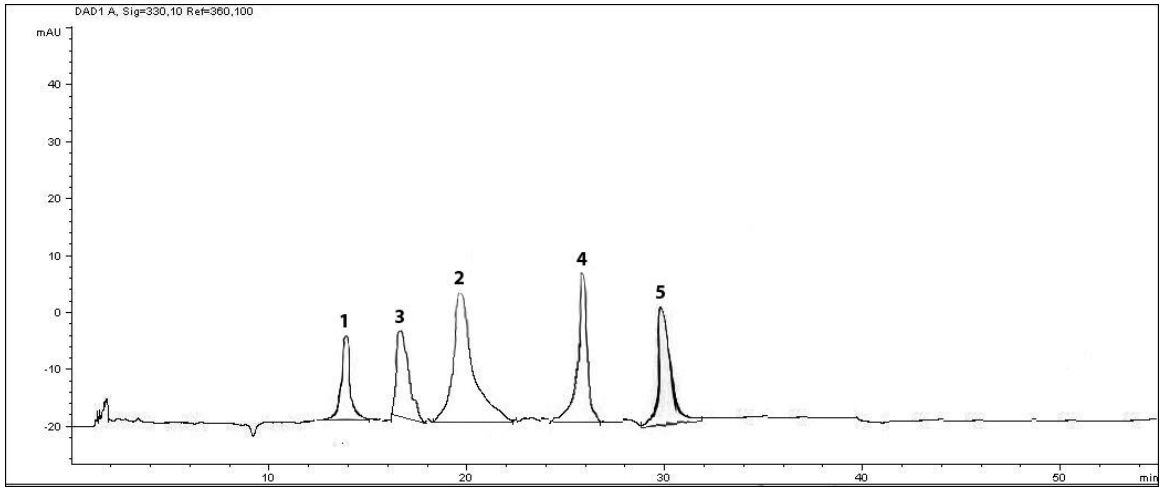
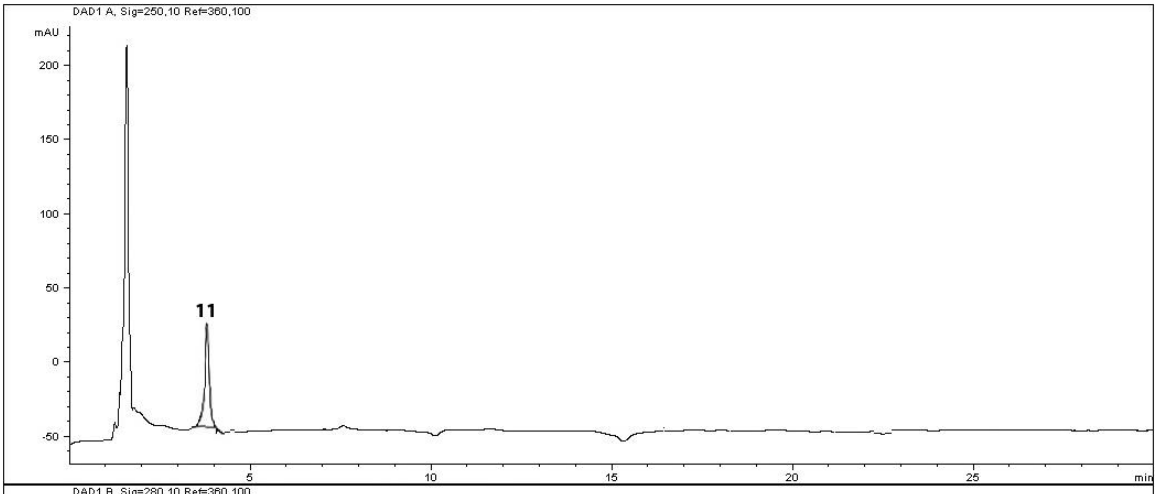
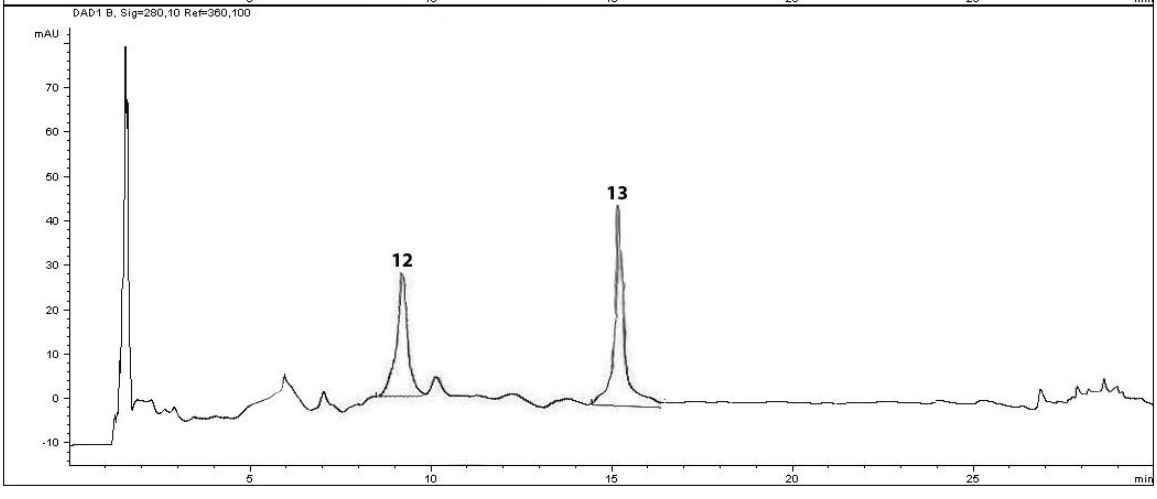
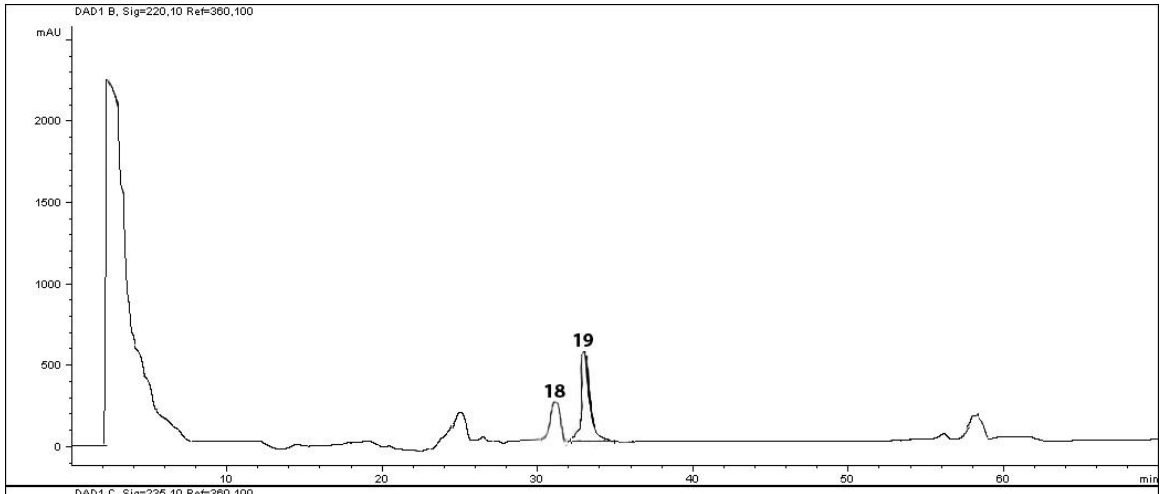


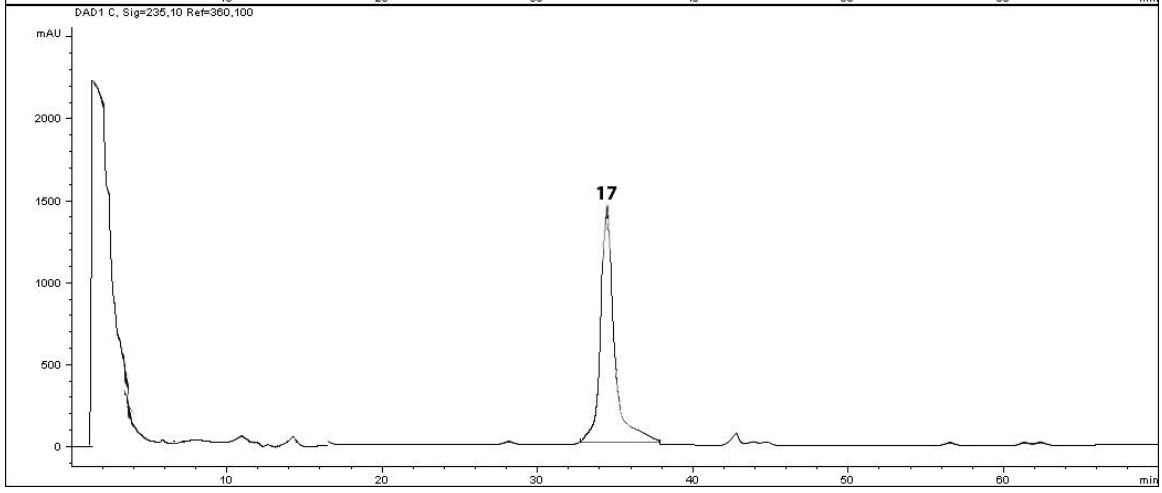
Fig. 1. Chemical structures of the detected bioactive compounds.

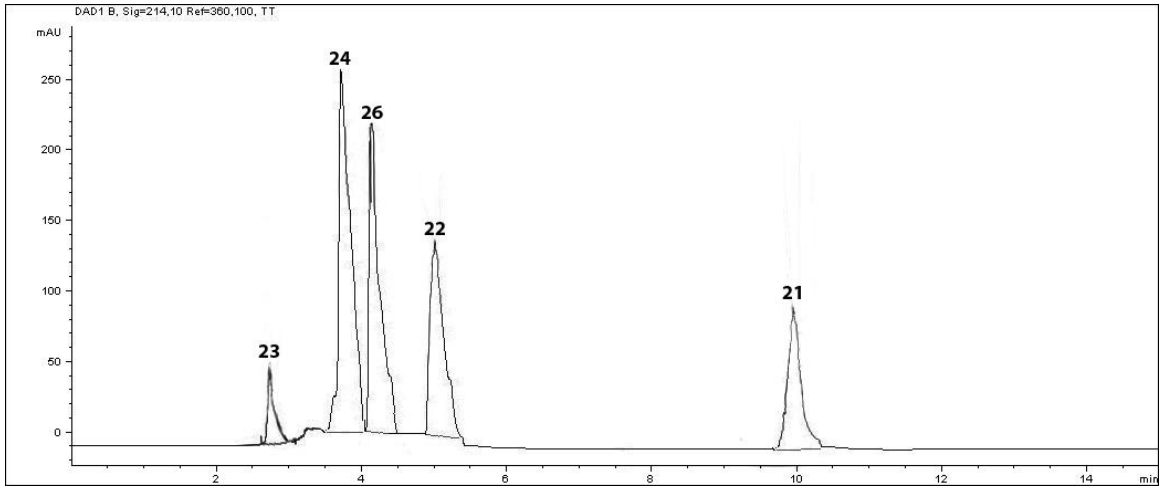

DAD1 A, Sig=330,10 Ref=360,100
mAU
40
30
20
10
0
-10
-20
1
3
2
4
5
10
20
30
40
50
min


DAD1 A, Sig=250,10 Ref=360,100
mAU
200
150
100
50
0
-50
11
5
10
15
20
25
min


DAD1 B, Sig=280,10 Ref=360,100
mAU
70
60
50
40
30
20
10
0
-10
12
13
5
10
15
20
25
min


DAD1 B, Sig=220,10 Ref=360,100
mAU
2000
1500
1000
500
0
19
18
10
20
30
40
50
60
min


DAD1 C, Sig=235,10 Ref=360,100
mAU
2000
1500
1000
500
0
17
10
20
30
40
50
60
min


DAD1 B, Sig=214,10 Ref=360,100, TT
mAU
250
200
150
100
50
0
24
26
22
21
23
2
4
6
8
10
12
14
min

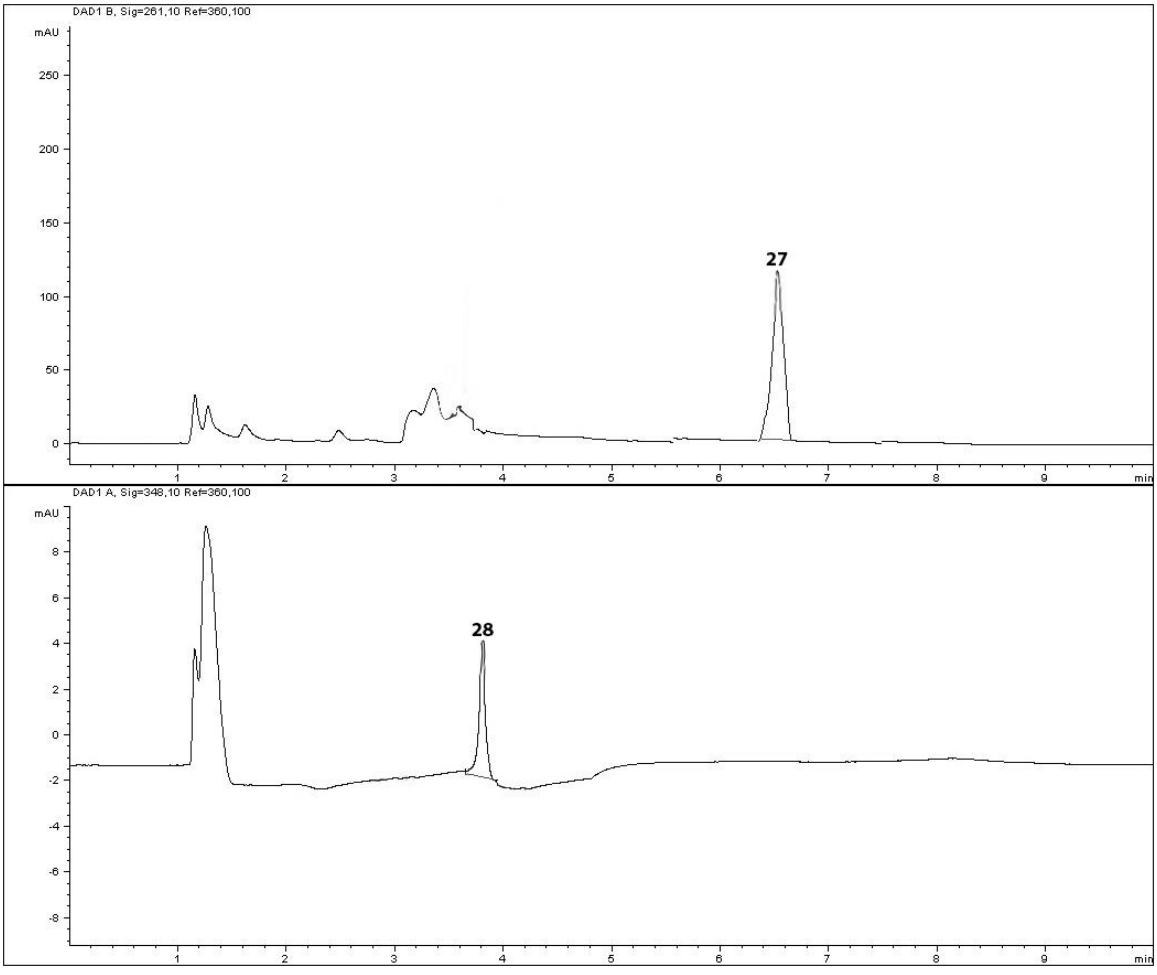


Fig. 2. HPLC/DAD bioactive compound profile. Standard identification code was reported in Table 1.

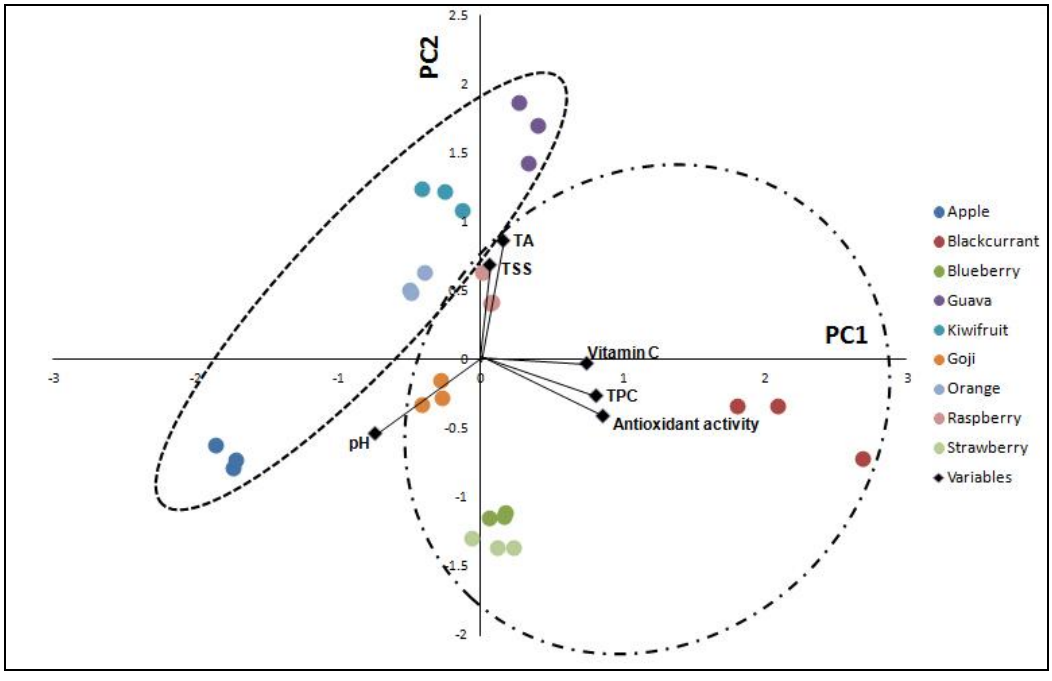


Fig. 3. PCA individual/variable graphs of fruit extract samples.