Phytochemical characterization and antioxidant activity evaluation of Mediterranean medlar fruit (*Crataegus azarolus* L.): Preliminary study of underutilized genetic resources as a potential source of health-promoting compound for food supplements

DARIO DONNO – MARIA GABRIELLA MELLANO – ŽELJKO PRGOMET – ALESSANDRO KIM CERUTTI – GABRIELE LORIS BECCARO

Summary

Azarole fruit (Mediterranean medlar, *Crataegus azarolus* L.) has attracted increasing attention in the field of functional foods because of its widely reported health benefits. The aim of this research was to describe overall quality traits of azarole fruit, report its antioxidant activity, and identify and quantify the levels of potentially bioactive compounds by chromatographic fingerprints. Different methods were used to determine concentrations of phytochemical compounds (phenolic and organic acids, flavonols, tannins, catechins, monoterpenes, vitamin C) in the fresh fruits. For the analysis, high performance liquid chromatography with diode-array detection was used. The same analytical methods were also applied to some common temperate fruit species grown in the same pedoclimatic conditions in order to understand if *C. azarolus* presents a real added phytochemical value compared with others. The chemical fingerprints showed the prevalence of organic acids (55.0%), followed by polyphenols (25.1%), monoterpenes (15.4%) and vitamins (4.5%) in the phytochemical composition of all the analysed samples (mean values were considered). This study developed an effective tool to assess azarole fruit quality, chemical composition and antioxidant activity. The results may support the exploitation of this fruit as a potential natural source of bioactive compounds in several applications.

Keywords

underutilized fruits; red azarole; bioactive compounds; chromatographic fingerprinting; phytochemical

Throughout history, man has used different natural materials to prevent several diseases. In particular, fruits and vegetables are good sources of natural antioxidants, containing many different components. These provide protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer and cardiovascular diseases. Polyphenolic derivatives, some of the most important phytochemicals found in plants, are a large family of secondary metabolites with various roles in plant defense and with demonstrated antioxidant activity as well as beneficial health effects [1].

Recently, there has been a growing interest in underutilized fruits, also known as minor, secondary or alternative fruits. These include *Asimina triloba* L. [2], *Morus nigra* L. [3] and *Lycium* spp. [4]. The growing worldwide interest in introducing the cultivation of these species to promote the differentiation of the cultivated agrobiodiversity could also be encouraged by the management with more environmentally friendly agrotechniques (if compared with the most commonly grown fruit species), and by the greater sustainability of their production [5]. However, neglected and underutilized natural food resources are suffering from less attention and research, and their nutritional, economic and socio-cultural potentials are not fully exploited. Investigation of such properties has been of interest mainly for finding new sources for natural antioxidants, functional foods and nutraceuticals. Currently, large research data on com-

Dario Donno, Maria Gabriella Mellano, Alessandro Kim Cerutti, Gabriele Loris Beccaro, Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy.
Zeljko Prgomet, Polytechnic of Rijeka, Trpimirova 2, 51000 Rijeka, Croatia.

Correspondence author:
Dario Donno, e-mail: dario.donno@unito.it

mon fruits, as berries, kiwifruit, orange and apple, on their total polyphenolic content (*TPC*) and antioxidant activity are available [3], but little information is available on underutilized fruits.

Azarole fruits (*Crataegus azarolus* L.) have attracted increasing attention in the field of food, nutraceuticals and medicine because of their widely reported health benefits, as the risk reduction of cardiovascular diseases. The systematics of the genus *Crataegus* have been considered problematic because of hybridization, introgression, polyploidy and apomixis in this genus [6]. Considerable revisionary work has led to substantial reductions in the numbers of species that can plausibly be recognized in *Crataegus* genus. Worldwide, it appears that 150–200 species of *Crataegus* would be a reasonable estimate, which is a much better manageable number to deal with in studies of chemical variation within the genus [7].

C. azarolus tree is a multipurpose (ornamental tree, rootstock for *Pyrus communis* L., medicinal plant) small deciduous tree growing up to 3–5 m high. It has been cultivated for centuries in the Mediterranean area. Flowering occurs in April and May. The plant can grow in light, medium and heavy soils, requiring moist or wet soil and can tolerating drought. Fruits and flowers are also used for medicinal purposes, in particular, fruits are not only consumed fresh and dried but also used to produce jam, marmalade and syrup [8]. Fruit weight ranges from 2 g to 8 g and fruits are very variable in size, can be up to 25 mm in diameter, and colour, from yellow through bright red to black. There are 1–3 large seeds in the centre of the fruit [9]. The fruits contain, on average, 15 °Brix total sugars, 45 meq·l^{-1} total acidity, 300 mg·kg^{-1} vitamin C, 110 mg·kg^{-1} Ca, 100 mg·kg^{-1} P, 10 mg·kg^{-1} Fe, 1600 mg·kg^{-1} K, 70 mg·kg^{-1} Mg, 2 mg·kg^{-1} Cu, 2.5 mg·kg^{-1} Mn, 20 mg·kg^{-1} Na, 10 g·kg^{-1} proteins and 130 g·kg^{-1} carbohydrates, as reported by KOYUNCU et al. [8]. It is not easy to find enough scientific information on the azarole fruit production and consumption. Moreover, only few studies evaluated the nutritional, nutraceutical and medicinal value of this species [10]. Several bioactive phytochemicals were isolated from fruits of European and Asian *Crataegus* species. The high contents of flavonoids, proanthocyanidins, catechins, phenolic acids, essential oils and terpenoids explain their use in natural therapy for the treatment of neurodegenerative diseases, in some types of cancer, in the affectation of the immunological system and at cardiovascular disorders [11]. Indeed, *Crataegus* spp. extracts exert a wide range of pharmaceutical properties, especially on the cardiovascular system, including cardiotonic, antiarrhythmic, hypotensive, hypolipidemic and antioxidant activities. In particular, the use of fruits for the treatment of heart ailments dates back to the late 1800s, and numerous laboratory tests and clinical trials demonstrated their efficacy in the treatment or prevention of cardiovascular diseases [12].

The wide diversity and genotypic variability among *Crataegus* species suggests that accurate characterization of the fruits and determination of their antioxidant properties, studied as a functional food with potentially high nutraceutical value, could provide new production alternatives for the fruit growers. However, fruit composition regarding bioactive compounds versus efficacy is a key problem, according to present quality requirements [13].

Important progress was registered in the last decade regarding the extraction, identification and quantification of bioactive compounds needed for an adequate quality control of plant materials used as fresh food, food-derived products, food supplements or raw materials for herbal medicines [4]. Regarding analytical technologies used in industrial quality control, the most common method for analytical controls is spectrophotometric quantification of total bioactive compounds in fruits or fruit-derived products [14]. Spectrophotometric determination is a commonly adapted method, which works very well where an estimation is needed rather than an accurate quantification of bioactive compounds. Therefore, the method is a good tool for rapid screening of total nutraceutical content in plant materials as fruits [15]. Besides this, the spectroscopic method does not provide any specificity regarding a bioactive compound fingerprint in fresh fruits or food supplements [16]. For this reason, recently, a fingerprint approach has become used for identification and direct analysis of plant materials. Different kinds of features can be referred to the overall fingerprint: genetic [17], quality [18], sensory [19] or morphological [20] features could be used to create a complete fingerprint. The most advanced technique for phytochemical fingerprinting, used today, is high performance liquid chromatography (HPLC) coupled to UV–visible diode array detection (DAD) or mass spectrometry (MS) [21]. Organic solvents (methanol, ethanol) and aqueous buffers are used in the main extraction methods to extract bioactive compounds. Many sources indicate good correlations between the content of bioactive compounds and antioxidant capacity evaluated by different techniques [22].

The aim of this research was to describe azarole quality traits, identify and quantify the

main bioactive compounds, and evaluate the composition of the fruit phytocomplex as well as the antioxidant activity. This study focused on one of the most cultivated azarole genotypes with commercial utilization. The research emphasized that it is necessary to consider nutraceutical features for a complete evaluation of these fruits, using fingerprinting as a quality control tool. As little information is currently available on the chemical fingerprint of azarole fruits, the results of the present study may encourage a deeper evaluation of the nutraceutical value for the many hundreds of different fruit-bearing *Crataegus* spp. cultivars.

MATERIALS AND METHODS

Plant material

Three different red azarole fruit (variety Azzeruolo rosso d'Italia) samples of the same genotype were manually picked up from a germplasm repository of the University of Turin (Northern Italy) located in Chieri (N 45° 1', E 7° 49', 305 m above sea level) in November 2014. The climate of the area is rainy moderate temperate, with rains in spring and autumn, a mean annual temperature of 12.2 °C and a rainfall of approximately 810.1 mm annually. The soil is loam-clay. The analysed genotype is actually one of the most cultivated in small family-managed farms and nurseries with commercial utilization. An amount of 0.5 kg of physiologically mature fruits were randomly selected from three plants for each replication (n = 3). Fruits were analysed fresh, after being stored for few days at 4 °C and 95% relative humidity (*RH*).

The same analyses were also performed on some common temperate fruit species in order to understand if this species presents a real added phytochemical value compared with others. The following fruit species were involved: *Malus domestica* Borkh. (apple), *Rubus ulmifolius* Schott. (blackberry), *Ribes nigrum* L. (blackcurrant), *Vaccinium corymbosum* L. (blueberry), *Lycium barbarum* L. (goji), *Morus nigra* L. (black mulberry), *Rubus idaeus* L. (raspberry) and *Fragaria vesca* L. (strawberry). All harvested fruits were analysed after being stored in the same conditions as azarole samples.

Determination of morphological and chemical properties

To determine the morphological traits of the fresh fruits, approximately 10% of the samples were randomly taken out and their width/length and weight were measured using a digital caliper (sensitivity 0.01 mm; Traceable Digital Caliper-6"; VWR International, Milano, Italy) and a balance (sensitivity 0.01 g; Mettler, Greifensee, Switzerland), respectively.

Fruit tissues were homogenized with a blender, samples of homogenates were centrifuged and the total soluble solid (*TSS*) value was determined by a digital refractometer (Tsingtao Unicom-Optics Instruments, Laixi, China). Results were expressed as degrees Brix. Titratable acidity (*TA*, expressed in milliequivalents per litre) and pH were determined by titrating 10 ml of fruit juice aliquot (adjusted to 100 ml final volume with Milli-Q water; Sartorius, Goettingen, Germany) using 0.2 $mol \cdot l^{-1}$ NaOH and an automatic titrator (Crison, Alella, Spain).

Spectrophotometric analysis

The method used for the determination of total polyphenol content (*TPC*) was based on Folin-Ciocalteu phenol reagent and spectrophotometric determination at 765 nm [23]. Results were expressed as grams of gallic acid equivalents (GAE) per kilogram of fresh weight (FW).

The total anthocyanin content (*TAC*) in the fruit extracts was directly determined using the pH-differential method [16]. Results were expressed as milligrams of cyanidin-3-O-glucoside (C3G) per kilogram of FW.

In this research, antioxidant activity (*AA*) in the azarole fruit pulp was evaluated by ferric reducing antioxidant power (FRAP) assay [22]. Results were expressed as millimoles of Fe^{2+} equivalents per kilogram (solid food) of FW.

Chromatographic analysis

Sample preparation protocols

Polyphenolic compounds were extracted with a mixture of methanol : water : 37% HCl (95 : 4.5 : 0.5, v/v/v). Methanolic extracts used for the previous analysis were filtered through a membrane microfilter (polytetrafluoroethylene membrane, PTFE; pore size 0.45 μm) and then stored for a few days at normal atmosphere (NA), at 4 °C and 95% *RH*. Monoterpenes and organic acids were extracted with 95% ethanol. Samples were then stored until analysis in NA, at 4 °C and 95% *RH*.

Ascorbic acid and dehydroascorbic acid were extracted by an extraction solution (0.1 $mol \cdot l^{-1}$ citric acid, 2 $mmol \cdot l^{-1}$ ethylenediaminetetraacetic acid (EDTA) disodium salt, and 4 $mmol \cdot l^{-1}$ sodium fluoride in methanol-water, 5 : 95, v/v). *o*-Phenylenediamine (OPDA) solution (18.8 $mmol \cdot l^{-1}$) was added to 750 μl of extracted samples for dehydroascorbic acid (DHAA) derivatization to

a fluorophore, 3-(1,2-dihydroxyethyl)furo(3,4-b) quinoxalina-1-one (DFQ).

Standard preparation and calibration

Stock solutions of standards with a concentration of 1.0 mg·ml^{-1} were prepared in several solvents (methanol, water and ethanol). The external standard method was used for quantitative determinations. Manual injections were performed in triplicate for each concentration level.

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, California, USA), was used for the analysis. Five different chromatographic methods were used to analyse the samples: in all of the used methods, bioactive compound separation was achieved on a Kinetex C18 column (4.6 mm × 150 mm, 5 μm; Phenomenex, Torrance, California, USA). Different mobile phases were used for a specific bioactive compound identification and UV absorbance was recorded at 330 nm (A); 280 nm (B); 210 nm, 220 nm, 235 nm and 250 nm (C); 214 nm (D); 261 nm and 348 nm (E). The chromatographic conditions of each method are reported in Tab. 1. The main analytical method validation data are summarized in Tab. 2. All the samples were analysed in triplicate, and standard deviations are given in order to assess the repeatability of the used methods.

Identification and quantification of bioactive compounds in the extracts

Total bioactive compound content (*TBCC*) was evaluated as the sum of the most important classes of bioactive compounds (biomarkers) 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.

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). Monoterpenes (limonene, phellandrene, sabinene, γ-terpinene, terpinolene), 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 milligrams per kilogram of FW.

Statistical analysis

Results were subjected to analysis of variance (ANOVA) test for mean comparison (SPSS 22.0 Software, IBM SPSS, Chicago, Illinois, USA) and Tukey's multiple range test (using $p < 0.05$). Multivariate analysis (MVA) was carried out on all the samples. The data matrix was defined as 30 objects (3 repetitions for 9 samples) and 7 variables (*TSS*, *TA*, pH, *TPC*, *TAC*, vitamin C and *AA*). In order to amplify the differences in the fruit extracts and

Tab. 1. Chromatographic conditions of the used methods [36].

Method	Compounds of interest	Mobile phase	Flow [ml·min^{-1}]	Elution conditions	Wavelength [nm]
A	Cinnamic acids, flavonols	A: 10 mmol·l^{-1} KH_2PO_4/H_3PO_4, pH 2.8 B: CH_3CN	1.5	Gradient analysis: 5% B to 21% B in 17 min + 21%B in 3 min (2 min conditioning time)	330
B	Benzoic acids, catechins, tannins	A: H_2O : CH_3OH : HCOOH (5 : 95 : 0.1 v/v/v), pH 2.5 B: CH_3OH : HCOOH (100 : 0.1 v/v)	0.6	Gradient analysis: 3% B to 85% B in 22 min + 85% B in 1 min (2 min conditioning time)	280
C	Monoterpenes	A: H_2O B: CH_3CN	1.0	Gradient analysis: 30% B to 56% B in 15 min + 56% B in 2 min (3 min conditioning)	210; 220; 235; 250
D	Organic acids	A: 10 mmol·l^{-1} KH_2PO_4/H_3PO_4, pH 2.8 B: CH_3CN	0.6	Isocratic analysis: ratio of phase A and B 95 : 5 in 13 min (2 min conditioning time)	214
E	Vitamins	A: 5 mmol·l^{-1} $C_{16}H_{33}N(CH_3)_3Br$/ 50 mmol·l^{-1} KH_2PO_4, pH 2.5 B: CH_3OH	0.9	Isocratic analysis: ratio of phase A and B 95 : 5 in 10 min (5 min conditioning time)	261; 348

In all methods, the stationary phase Kinetex C18 column, 4.6 mm × 150 mm, 5 μm (Phenomenex, Torrance, California, USA) was used.

Tab. 2. Main chromatographic parameters of the used methods for each calibration standard [36].

Method	Class	Standard	ID	Retention time (t_R) [min]	Wavelength [nm]	Calibration curve equation	R^2	Calibration curve range [mg·l^{-1}]	*LOD* [mg·l^{-1}]	*LOQ* [mg·l^{-1}]
A	Cinnamic acids	Caffeic acid	1	4.54	330	$y = 59.046x + 200.6$	0.996	111–500	0.305	1.016
		Chlorogenic acid	2	3.89	330	$y = 13.583x + 760.05$	0.984	111–500	0.940	3.134
		Coumaric acid	3	6.74	330	$y = 8.9342x + 217.4$	0.997	111–500	2.907	9.690
		Ferulic acid	4	7.99	330	$y = 3.3963x - 4.9524$	1.000	111–500	1.245	4.150
	Flavonols	Hyperoside	5	10.89	330	$y = 7.1322x - 4.583$	0.999	111–500	3.372	11.241
		Isoquercitrin	6	11.24	330	$y = 8.3078x + 26.621$	0.999	111–500	0.252	0.840
		Quercetin	7	17.67	330	$y = 3.4095x - 98.307$	0.998	111–500	4.055	13.518
		Quercitrin	8	13.28	330	$y = 2.7413x + 5.6367$	0.998	111–500	5.456	18.187
		Rutin	9	12.95	330	$y = 6.5808x + 30.831$	0.999	111–500	2.937	9.790
B	Benzoic acids	Ellagic acid	10	18.65	280	$y = 29.954x + 184.52$	0.998	62.5–250	0.611	2.035
		Gallic acid	11	4.26	280	$y = 44.996x + 261.86$	0.999	62.5–250	0.435	1.451
	Catechins	Catechin	12	10.31	280	$y = 8.9197x + 66.952$	1.000	62.5–250	2.343	7.809
		Epicatechin	13	14.30	280	$y = 12.88x - 43.816$	0.999	62.5–250	0.763	2.543
	Tannins	Castalagin	14	16.35	280	$y = 4.236x - 8.535$	1.000	62.5–250	1.009	3.363
		Vescalagin	15	17.25	280	$y = 4.939x - 1.232$	1.000	62.5–250	0.603	2.010
C	Monoterpenes	Limonene	16	3.35	250	$y = 0.1894x - 5.420$	0.999	125–1 000	8.654	28.847
		Phellandrene	17	3.57	210	$y = 8.783x - 145.3$	0.998	125–1 000	0.562	1.874
		Sabinene	18	3.45	220	$y = 18.14x - 1004$	0.998	125–1 000	0.094	0.314
		γ-Terpinene	19	3.28	235	$y = 0.4886x - 23.02$	0.999	125–1 000	17.577	58.590
		Terpinolene	20	4.83	220	$y = 26.52x + 876.8$	0.999	125–1 000	0.241	0.804
D	Organic acids	Citric acid	21	5.30	214	$y = 1.0603x - 22.092$	1.000	167–1 000	18.805	62.682
		Malic acid	22	4.03	214	$y = 1.415x - 80.254$	0.996	167–1 000	15.721	52.404
		Oxalic acid	23	7.85	214	$y = 6.4502x + 6.1503$	0.998	167–1 000	0.550	1.835
		Quinic acid	24	3.21	214	$y = 0.8087x - 38.021$	0.998	167–1 000	26.106	87.021
		Succinic acid	25	3.46	214	$y = 0.9236x - 8.0823$	0.995	167–1 000	7.135	23.783
		Tartaric acid	26	5.69	214	$y = 1.8427x + 15.796$	1.000	167–1000	8.520	28.401
E	Vitamins	Ascorbic acid	27	4.14	261	$y = 42.71x + 27.969$	0.999	100–1 000	0.836	2.786
		Dehydroascorbic acid	28	3.41	348	$y = 4.1628x + 140.01$	0.999	30–300	1.095	3.649

ID – identification code, *LOD* – limit of detection, *LOQ* – limit of quantification.

easily differentiate them with a better graphic visualization, principal component analysis (PCA) was performed on all the fruit samples. Scaling was carried out as data pre-treatment on the original data. Z score scaling was carried out on the data matrix before MVA in order to scale to unit variance.

RESULTS

Morphological traits, chemical-nutraceutical analysis and antioxidant activity

Azarole fruits were broadly ovoid (16.03 mm ± 0.18 mm in length and 13.43 mm ± 0.14 mm in width), with a weight of 2.10 g ± 0.16 g, and a red colour with reflections tending to orange. Quality analysis reported a *TSS* value of (16.36 ± 1.43) °Brix, while *TA* showed a value of (68.42 ± 5.56) meq·l^{-1} with a pH value of 3.54 ± 0.24 (all the values are reported as mean value ±standard deviation). Tab. 3 presents data on quality traits of the analysed fruits of the selected common temperate fruit species compared to red azarole fruits. Black mulberry showed the highest *TSS* value (18.97 °Brix), followed by azarole (16.36 °Brix) and blackcurrant (14.43 °Brix), while raspberry (413.71 meq·l^{-1}) had the highest *TA* value, followed by goji (267.79 meq·l^{-1}) and strawberry (185.23 meq·l^{-1}). Azarole extracts showed a lower *TA* value than the most of other fruits.

Three different azarole fruit samples of the same genotype were represented by A1, A2 and A3. *TPC* values (expressed as grams of GAE) ranged from (5.335 ± 0.026) g·kg^{-1} FW (sample A2) to (6.251 ± 0.048) g·kg^{-1} FW (sample A1). Moreover, the highest *AA* value was determined

Tab. 3. Quality traits of azarole (*Crataegus azarolus* L) fruits and of common fruits.

Common name	Species	Variety	*TSS* [°Brix]	*TA* [meq·l^{-1}]	pH
Azarole	*Crataegus azarolus* L.	Azzeruolo rosso d'Italia	16.36 ± 1.43^{f}	68.42 ± 5.56^{a}	3.54 ± 0.24^{de}
Apple	*Malus domestica* Borkh.	Golden Delicious	14.13 ± 0.54^{de}	49.56 ± 4.15^{a}	4.46 ± 0.54^{e}
Blackberry	*Rubus ulmifolius* Schott.	Kiowa	12.73 ± 1.63^{cde}	149.78 ± 2.44^{b}	3.24 ± 0.14^{bc}
Blackcurrant	*Ribes nigrum* L.	Ben Lomond	14.43 ± 0.96^{e}	183.52 ± 3.89^{b}	3.13 ± 0.99^{a}
Blueberry	*Vaccinium corymbosum* L.	Duke	10.03 ± 1.21^{ab}	167.64 ± 12.53^{b}	3.31 ± 0.24^{abc}
Goji	*Lycium barbarum*L.	Sweet	12.40 ± 0.65^{cd}	267.79 ± 4.82^{c}	3.80 ± 0.65^{cd}
Black mulberry	*Morus nigra* L.	Kokuso	18.97 ± 1.75^{g}	29.57 ± 2.04^{a}	5.78 ± 0.70^{f}
Raspberry	*Rubus idaeus* L.	Heritage	10.76 ± 0.60^{bc}	413.71 ± 51.98^{d}	3.24 ± 0.97^{ab}
Strawberry	*Fragaria vesca* L.	Arosa	8.46 ± 0.92^{a}	185.23 ± 6.81^{b}	3.84 ± 0.12^{cd}

Mean and standard deviation values of each sample is given (n = 3). Different letters in superscript for each sample indicate the significant differences at $p < 0.05$.
TSS – total soluble solids, *TA* – titratable acidity.

Tab. 4. Nutraceutical traits of azarole (*Crataegus azarolus* L) fruits and of common fruits.

Common name	*TPC* [g·kg^{-1}]	*AA* [mmol·kg^{-1}]	*TAC* [mg·kg^{-1}]	Vitamin C [mg·kg^{-1}]
Azarole	5.930 ± 0.516^{d}	24.108 ± 0.583^{c}	118.709 ± 19.913^{a}	656.860 ± 6.433^{de}
Apple	0.840 ± 0.133^{a}	6.232 ± 1.125^{a}	2.365 ± 0.123^{a}	44.412 ± 12.211^{a}
Blackberry	2.625 ± 0.074^{b}	66.954 ± 1.955^{f}	1 034.247 ± 39.194^{d}	471.518 ± 50.966^{cd}
Blackcurrant	4.349 ± 0.999^{c}	77.557 ± 9.303^{f}	2 249.430 ± 40.052^{de}	1 633.764 ± 85.626^{f}
Blueberry	3.000 ± 0.443^{b}	49.960 ± 5.878^{e}	2 317.093 ± 97.511^{e}	128.523 ± 34.937^{ab}
Goji	2.692 ± 0.137^{b}	19.380 ± 1.729^{bc}	1 165.777 ± 64.912^{d}	893.414 ± 258.695^{e}
Black mulberry	2.373 ± 0.063^{b}	22.332 ± 0.632^{bc}	808.788 ± 102.893^{c}	31.828 ± 14.326^{a}
Raspberry	3.225 ± 0.076^{bc}	13.631 ± 1.143^{ab}	350.783 ± 50.580^{b}	320.422 ± 56.300^{bc}
Strawberry	3.238 ± 0.580^{bc}	35.962 ± 5.093^{d}	366.985 ± 34.320^{b}	587.755 ± 38.346^{d}

Species and variety of fruits are given in Tab. 3. Mean and standard deviation values of each sample is given (n = 3). Different letters in superscript for each sample indicate the significant differences at $p < 0.05$.
TPC – total polyphenol content (expressed as grams of gallic acid equivalents per kilogram of fresh weight, FW), *AA* – antioxidant activity (expressed as millimoles of Fe^{2+} equivalents per kilogram FW), *TAC* – total anthocyanin content (expressed as milligrams of cyanidin-3-O-glucoside per kilogram FW).

for A1 (24.78 mmol·kg^{-1} ± 1.98 mmol·kg^{-1} FW). Sample A1 also showed the highest *TAC* value (140.16 mg·kg^{-1} ± 20.01 mg·kg^{-1} FW).

In Tab. 4, nutraceutical traits of all the fruits of the selected common temperate fruit species compared to red azarole fruits are reported. The contents of total polyphenolic compounds were statistically different among the different species. Apple contained small quantities of polyphenolic compounds (0.840 g·kg^{-1}), while a significantly higher *TPC* was observed in azarole (5.930 g·kg^{-1}) and blackcurrant (4.349 g·kg^{-1}). The results showed statistical differences among the several species in the values of the antioxidant capacity determined by FRAP assay. Berries, in particular blackcurrant (77.557 mmol·kg^{-1}), blackberry (66.954 mmol·kg^{-1}) and blueberry (49.960 mmol·kg^{-1}), showed the highest antioxidant capacity, while azarole presented a higher *AA* value (24.108 mmol·kg^{-1}) than black mulberry, goji, raspberry and apple. The content of total anthocyanins was statistically different among the considered species, azarole fruits presenting one of the lowest *TAC* values (118.709 mg·kg^{-1}), which was just higher than that for apple. Significant differences in vitamin C content were also recorded: blackcurrant showed the highest vitamin C content (1633.764 mg·kg^{-1}), followed by goji (893.414 mg·kg^{-1}), azarole (656.859 mg·kg^{-1}), strawberry (587.754 mg·kg^{-1}) and blackberry (471.518 mg·kg^{-1}). The lowest vitamin C values were recorded for apple (44.412 mg·kg^{-1}) and black mulberry (31.828 mg·kg^{-1}).

In order to better visualize the possible differences in the fruit extracts and easily characterize the samples, PCA was performed. It reduced the initial variables (*TSS*, *TA*, pH, *TPC*, *AA*, *TAC* and vitamin C content) into three principal components (79.4% of total variance), placing the nine species in the PCA score plot (Fig. 1) in relation to their quality and nutraceutical traits. Principal component 1 (PC1) and Principal component 2 (PC2) well represent alone the system information (63.4% of total variance). PCA produced three groups, highlighted in Fig. 1 with circles, without statistical meaning. The groups were named A (mulberry), B (berries) and C (pommel fruit), confirming the statistically significant differences of the ANOVA test on quality and nutraceutical data. The ellipses around each object group only indicate the position of a category in the plot without statistical meaning. The PCA plot showed a correlation between the nutraceutical variables (*TPC*, *AA*, *TAC* and vitamin C content) and PC1 (37.3% of total variance), while *TSS*, *TA* and pH presented a correlation with PC2 (26.1% of total variance). In this case, nutraceutical properties were identified as variables with the most discriminating power between different species, including the traits with high statistical differences in their content.

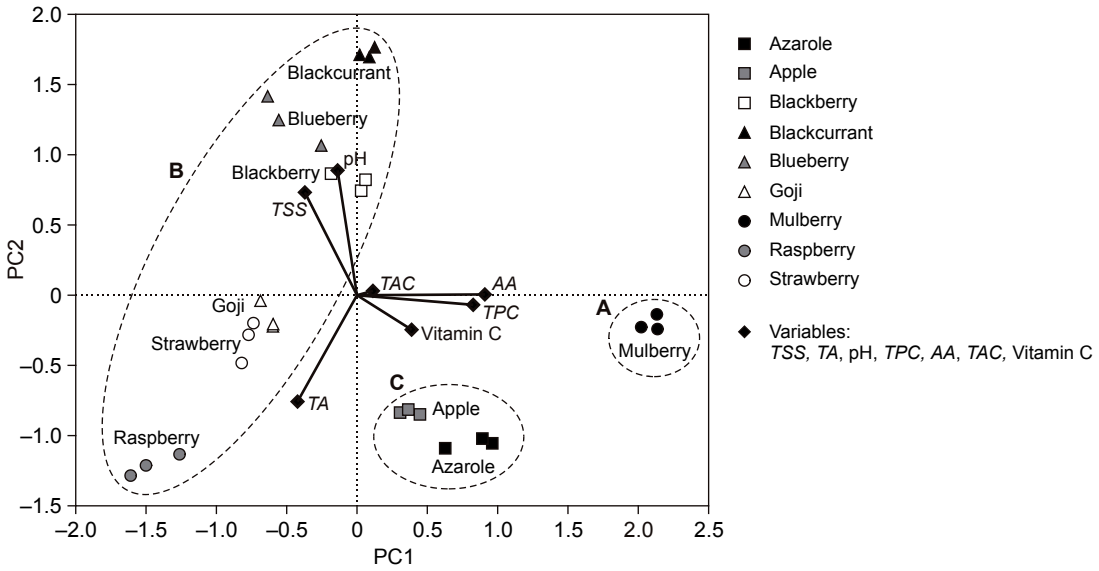


Fig. 1. Principal component analysis plot of fruit extract samples.

A – mulberry, B – berries, C – pommel fruit.
TSS – total soluble solids, *TA* – titratable acidity, *TPC* – total polyphenol content, *AA* – antioxidant activity, *TAC* – total anthocyanin content.

Tab. 5. Single compound profile of azarole (*Crataegus azarolus* L.) fruit samples.

Bioactive class	Biomarker	Selected genotype			Species (mean value among genotypes)
		A1	A2	A3	
Cinnamic acids	Caffeic acid	14.859 ± 1.086	20.916 ± 9.757	3.634 ± 0.460	13.136 ± 8.769
	Chlorogenic acid	82.921 ± 26.558	99.584 ± 29.688	40.480 ± 28.513	74.328 ± 30.475
	Coumaric acid	19.399 ± 3.168	9.546 ± 0.710	6.438 ± 0.353	11.794 ± 6.767
	Ferulic acid	32.219 ± 14.329	21.541 ± 14.797	75.224 ± 16.637	42.995 ± 28.417
Flavonols	Hyperoside	nd	nd	nd	nd
	Isoquercitrin	nd	nd	nd	nd
	Quercetin	97.640 ± 7.729	98.227 ± 2.992	95.439 ± 5.020	97.102 ± 1.470
	Quercitrin	124.594 ± 11.491	123.681 ± 7.604	117.204 ± 5.460	121.826 ± 4.029
	Rutin	nd	nd	nd	nd
Benzoic acids	Ellagic acid	41.504 ± 2.206	31.289 ± 9.389	33.947 ± 3.354	35.580 ± 5.300
	Gallic acid	146.103 ± 14.427	110.061 ± 7.645	111.593 ± 8.925	122.586 ± 20.381
Catechins	Catechin	1 020.919 ± 125.760	806.539 ± 141.822	1 033.8678 ± 138.884	953.775 ± 127.675
	Epicatechin	1 015.398 ± 242.341	771.283 ± 255.876	435.701 ± 249.417	740.794 ± 291.049
Tannins	Castalagin	507.392 ± 22.238	467.069 ± 30.534	568.083 ± 19.210	514.181 ± 50.848
	Vescalagin	917.843 ± 114.537	704.370 ± 168.792	867.301 ± 121.662	829.838 ± 111.559
Mono-terpenes	Limonene	2 126.293 ± 402.301	1 222.489 ± 395.167	1 409.546 ± 508.295	1 586.109 ± 477.071
	Phellandrene	98.601 ± 11.294	89.310 ± 9.638	74.476 ± 8.715	87.462 ± 12.168
	Sabinene	13.939 ± 6.763	7.097 ± 0.775	2.5678 ± 0.334	7.868 ± 5.725
	γ-Terpinene	711.422 ± 141.608	384.437 ± 142.875	624.622 ± 160.954	573.493 ± 169.382
	Terpinolene	3.088 ± 0.596	6.265 ± 0.209	4.282 ± 0.101	4.545 ± 1.605
Organic acids	Citric acid	3 478.978 ± 223.648	3 431.901 ± 184.986	3 108.508 ± 237.197	3 339.796 ± 201.680
	Malic acid	1 931.242 ± 208.891	1 536.628 ± 180.602	1 769.704 ± 127.016	1 745.858 ± 198.385
	Oxalic acid	197.128 ± 40.667	161.586 ± 52.062	163.243 ± 31.640	173.986 ± 20.059
	Quinic acid	nd	nd	nd	nd
	Succinic acid	nd	nd	nd	nd
	Tartaric acid	2 864.435 ± 85.066	2 814.054 ± 55.968	2 700.135 ± 83.115	2 792.875 ± 84.173
Vitamins	Ascorbic acid	94.689 ± 13.184	91.912 ± 15.558	92.548 ± 9.879	93.050 ± 1.455
	Dehydro-ascorbic acid	569.421 ± 21.990	562.719 ± 29.063	559.289 ± 35.880	563.810 ± 5.153
Total bioactive compounds content		16 110.026 ± 1 450.936	13 572.504 ± 1 606.674	13 897.831 ± 1 555.487	14 526.787 ± 1 380.741

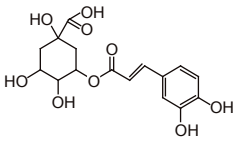
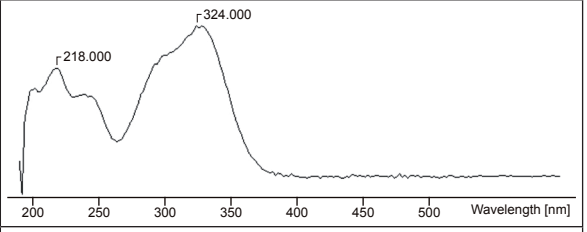
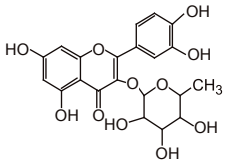
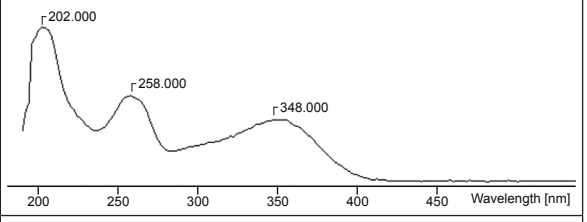
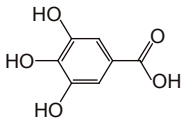
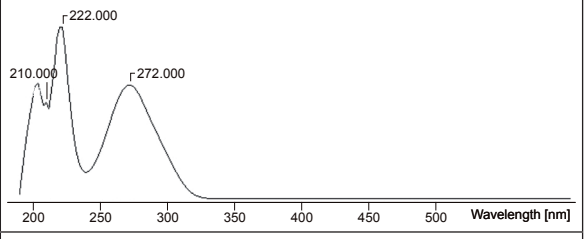
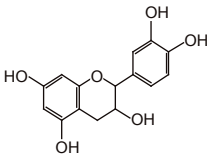
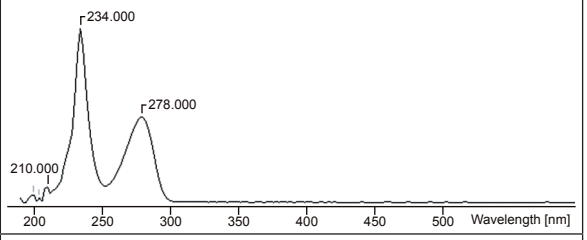
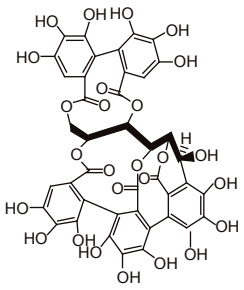
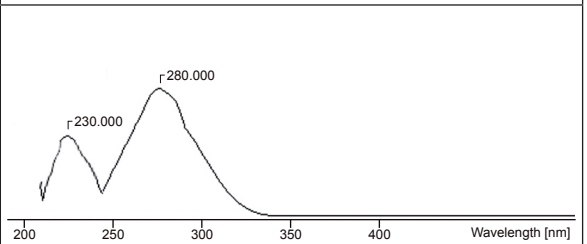
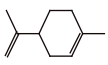
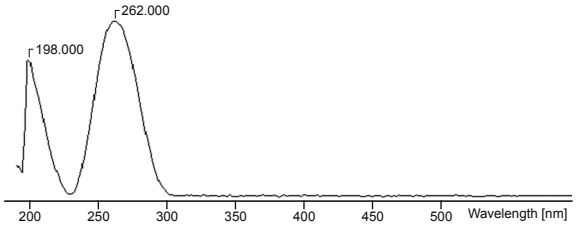
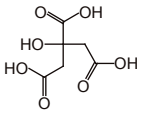
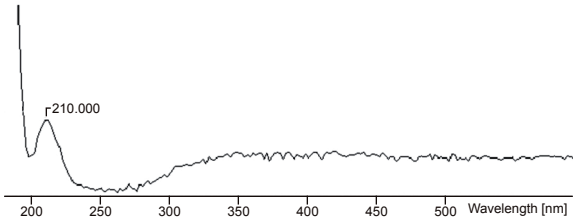
Mean and standard deviation values of each sample is given (n = 3). Results are expressed as milligrams per kilograms of fresh weight. A1, A2, and A3 represent mean values of different biological samples (three replications for each biological sample). nd – not detected.

Total bioactive compound content and the profile of single compounds

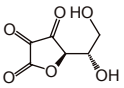
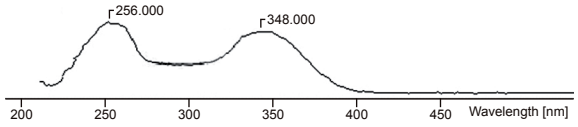
Mean values of total bioactive compound content (*TBCC*) and single compounds are presented in Tab. 5. *TBCC* was calculated as the sum of the selected biomarkers detected in the extracts. The analysed samples showed a lower *TBCC* value of (13 572.504 ± 1 606.674) mg·kg^{-1} (sample A2) and a higher value of (16 110.026 ± 1 450.936) mg·kg^{-1} (sample A1).

C. azarolus samples showed the following bioactive compound composition: four cinnamic acids (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid), two flavonols (quercetin, quercitrin), two benzoic acids (ellagic and gallic acids), two catechins (catechin, epicatechin), two tannins (castalagin, vescalagin), five monoterpenes (limonene, phellandrene, sabinene, γ-terpinene, terpinolene), four organic acids (citric acid, malic acid, oxalic acid, tartaric acid) and vitamin C (ex-

Tab. 6. Chemical and spectroscopic characteristics of the main biomarkers.

Compound	Structure	UV spectrum
Chlorogenic acid Exact mass: 354.10 *m/z*: 354.10 (100.0%), 355.10 (17.9%), 356.10 (3.3%) Elemental analysis: C, 54.24; H, 5.12; O, 40.64 pK_a (predicted): 3.91 ± 0.50 log*P*: 0.370 ± 0.461		
Quercitrin Exact mass: 448.10 *m/z*: 448.10 (100.0%), 449.10 (23.1%), 450.11 (2.6%), 450.10 (2.3%) Elemental analysis: C, 56.25; H, 4.50; O, 39.25 pK_a (predicted): 6.17 ± 0.40 log*P*: 0.579 ± 1.358		
Gallic acid Exact mass: 170.02 *m/z*: 170.02 (100.0%), 171.02 (7.8%), 172.03 (1.3%) Elemental analysis: C, 49,42; H, 3,55; O, 47,02 pK_a (predicted): 4.33 ± 0.10 log*P*: 0.531 ± 0.325		
Catechin Exact mass: 290.08 *m/z*: 290.08 (100.0%), 291.08 (16.9%), 292.09 (1.4%), 292.08 (1.2%) Elemental analysis: C, 62,07; H, 4,86; O, 33,07 pK_a (predicted): 9.54 ± 0.10 log*P*: 0.610 ± 0.454		
Vescalagin Exact mass: 934.07 *m/z*: 934.07 (100.0%), 935.07 (45.6%), 936.08 (16.0%), 937.08 (4.1%), 935.08 (1.4%) Elemental analysis: C, 52,69; H, 2,80; O, 44,51 pK_a (predicted): 4.07 ± 0.40 log*P*: 2.628 ± 0.967		
Limonene Exact mass: 136.13 *m/z*: 136.13 (100.0%), 137.13 (11.4%) Elemental analysis: C, 88,16; H, 11,84 pK_a (predicted): – log*P*: 4.552 ± 0.241		
Citric acid Exact mass: 192.03 *m/z*: 192.03 (100.0%), 193.03 (7.1%), 194.03 (1.6%) Elemental analysis: C, 37,51; H, 4,20; O, 58,29 pK_a (predicted): 2.93 ± 0.28 log*P*: –1.198 ± 0.396		

Tab. 6. *continued*

Compound	Structure	UV spectrum
Dehydroascorbic acid Exact mass: 174.02 *m/z*: 174.02 (100.0%), 175.02 (7.0%), 176.02 (1.4%) Elemental analysis: C, 41,39; H, 3,47; O, 55,14 pK_a (predicted): 12.12 ± 0.20 log *P*: –1.702 ± 0.478		

pK_a – logarithmic acid dissociation constant, log P – logarithmic partition coefficient.

pressed as the sum of ascorbic acid and dehydroascorbic acid). Hyperoside, isoquercitrin, rutin, quinic acid and succinic acid were not detected. Single bioactive compound contents ranged from (2.568 ± 0.334) mg·kg^{-1} (sabinene, sample A3) to (3 478.978 ± 223.648) mg·kg^{-1} (citric acid, sample A1). Chemical and spectroscopic characteristics of the main selected biomarkers are presented in Tab. 6.

Fingerprinting

The phytochemical fingerprint of azarole fruit was obtained as, in total, 23 bioactive compounds were identified by HPLC-DAD. 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, polyphenols (as the sum of anthocyanins, cinnamic acids, flavonols, benzoic acids, catechins and tannins), and monoterpenes in chemical composition of all the analysed samples (mean values were considered). The most important class was organic acids (55.0%), followed by polyphenols (25.1%), monoterpenes (15.4%) and vitamins (4.5%). In the polyphenolic group, the most important classes were catechins (11.6%) and tannins (9.2%), followed by flavonols, benzoic acids, cinnamic acids and anthocyanins (all percentages refer to the total content of bioactive compounds) (Tab. 7).

DISCUSSION

Many studies showed the bioactive properties of natural ingredients mainly linked to the antioxidant activity of phytochemicals, even if it has increasingly been realized that phytochemicals are much more than just antioxidants and that the modes of action are much more complex than initially assumed [10]. In particular, the phenolic compounds are the nutraceuticals that contribute the most to antioxidant activity of the fresh foods [1]. *C. azarolus* fruits may contain a significant amount of health-promoting agents [6, 8].

Tab. 7. Contribution of bioactive classes to the phytocomplex in extracts of azarole (*Crataegus azarolus* L) fruits.

Bioactive class	Selected genotype			Species (mean value among genotypes)	Phytocomplex [%]
	A1	A2	A3		
Cinnamic acids	149.397 ± 9.803	151.587 ± 9.665	125.776 ± 12.015	142.253 ± 14.312	1.0
Flavonols	222.234 ± 2.123	221.908 ± 6.863	212.643 ± 5.414	218.928 ± 5.446	1.5
Benzoic acids	187.607 ± 24.325	141.350 ± 26.115	145.540 ± 25.890	158.166 ± 25.583	1.1
Catechins	2 036.317 ± 307.179	1 577.822 ± 284.626	1 469.568 ± 306.225	1 694.569 ± 300.871	11.6
Tannins	1 425.235 ± 112.254	1 171.439 ± 145.306	1 435.384 ± 134.094	1 344.019 ± 149.545	9.2
Anthocyanins	141.552 ± 9.003	109.567 ± 16.426	105.008 ± 9.081	118.709 ± 19.913	0.8
Monoterpenes	2 953.342 ± 707.626	1 709.598 ± 645.562	2 115.493 ± 590.599	2 259.477 ± 634.250	15.4
Organic acids	8 471.784 ± 353.583	7 944.168 ± 408.282	7 741.590 ± 385.722	8 052.514 ± 376.962	55.0
Vitamins	664.110 ± 7.719	654.631 ± 5.103	651.837 ± 7.367	656.860 ± 6.433	4.5

Mean and standard deviation values of each sample is given (n = 3). Results are expressed as milligrams per kilogram of fresh weight. A1, A2 and A3 represent mean values of different biological samples (three replicates for each biological sample). Phytocomplex percentage express the contribution of single bioactive class to total bioactive compound content.

This study showed that the analysed parameters of the azarole fruits are comparable to those of other common fruit species that present a high nutraceutical value, such as *Vaccinium corymbosum*, *Fragaria vesca*, *Rubus idaeus* and *Morus nigra*. Moreover, in addition to the high antioxidant capacity, azarole showed high vitamin C and phenolics contents, which may increase its consumption as the functional food. As consumer interest in health and awareness of the potential benefits of particular foods is on the increase in the world, and this interest is reflected in the growing number of products appearing on the market, it is important to find new natural nutraceutical sources for functional foods, re-discovering alternative fruit species with a high health impact. The chemical composition of azarole is comparable with phytochemical fingerprint of fruits of other *Crataegus* species, as *C. monogyna* Jacq. This is one of the species that is highly recommended in folk medicine and fruits are widely consumed because they are considered to be 'healthy' and nutritious thanks to their high content of tocopherols, ascorbic acid, β-carotene, proanthocyanidins, phenolics and flavonoids (e.g. chlorogenic acid, hyperoside, rutin, quercetin, epicatechin, catechin). Moreover, in view of their sugar content (e.g. fructose, glucose, sucrose and trehalose), wild fruits of *C. monogyna* represent an important contribution to local daily diets, especially during autumn and early winter [24].

As in other studies [6], our results showed that azarole is very similar to apple fruits (same PCA group), while different from berry fruits and black mulberry (PCA group B and C, respectively). According to other researches [25], vitamin C content was similar to other fruit species as goji and strawberries, while *TPC* values were comparable to berry fruits, such as raspberries and blackberries.

Antioxidant activity (determined by FRAP assay) and bioactive compound contribution to total fruit phytocomplex were also used to characterize the red azarole nutraceutical properties as a functional food. Antioxidant activity was considered an important parameter to evaluate the nutraceutical properties of fruits, as shown in previous studies on other fruit species [3]. In the present study, the high content of polyphenolic compounds was not matched by an equally high value of *AA*, probably because the flavonoids were present as glycosides. In any case, it may not be the only reason for this result, since other compounds found in fruits also have antioxidant activity. The antioxidant activity is mainly associated with the content of flavonoids. However, when these compounds are present as aglycones, they have a higher activity than their respective glycosides [26]. For example, the structures as quercetin, which have hydroxyl groups in positions 3' and 4' of B ring, and OH in C-3 position, permit stable structures that efficiently capture free radicals, a requirement for maximum antioxidant capacity [27]. This information may explain the moderate antioxidant activity found in the azarole extracts of the present study. In this case, the antioxidant activity of fruits was not only associated with the presence of the phenolic acids and flavonoids (in particular, quercetin), but also was attributed to other compounds, such as vitamin C. Today, great emphasis is placed on the range and quantity of antioxidant vitamins and the percentage of daily requirements they fulfill. Thanks to the high vitamin C content and the ease of consumption compared to other fruits with similar nutraceutical properties, consumption of this fruits as functional foods could be targeted at particular population sectors, such as children, sportsmen, the elderly and pregnant women.

It appears to be universally assumed that the polyphenolic and terpenic compounds are responsible for the most of red azarole pharmacological activity, while few studies attempted to actually characterize the single active components [11]. Specific bioactive compounds can be collectively used as representative standards of a fruit extract in quantification [28], as done in this study. Chromatographic data can be used as *TBCC* for quantification of health-promoting agents because HPLC techniques give more information on individual compounds or groups of compounds than *TPC* by the Folin–Ciocalteu method or *TAC* by the pH-differential method [29]. The Folin–Ciocalteu phenol reagent is used to obtain a crude estimate of the amount of phenolic compounds present in an extract, but it is not a good method to quantify phenolic compounds because there are several other molecules able to react with phosphotungstic and phosphomolybdic acids present in Folin-Ciocalteu reagent, thereby altering the results. The assay was shown not specific to just polyphenols but to any other substance that could be oxidized by the reagent and the poor specificity of the assay was reported [30]. In addition, phenolic compounds, depending on the number of phenolic groups they have, respond differently to the Folin–Ciocalteu reagent [31]. Actually, this method is being progressively considered as a reducing power assay.

In this study, an innovative approach was applied to evaluate the *C. azarolus* fruit phytochemical composition and nutraceutical proper-

ties, namely, a specific fingerprint, coupled to the multivariate data analysis (PCA), which was used to show the single bioactive class contribution to the total fruit phytocomplex. Indeed, synergistic or additive biological effects of different phytochemicals could contribute to disease prevention more than a single compound or a group of compounds [32]. By HPLC-DAD, several metabolites in the azarole fruits were simultaneously determined and the obtained fingerprint was useful for antioxidant activity evaluation as well as phytochemical composition characterization. The chromatographic methods could be routinely used to evaluate overall fruit quality, also for other species and genera, as shown previously [4].

Many studies pointed out that the identified polyphenolic compounds significantly contribute to the *C. azarolus* phytocomplex and antioxidant activity [9]. The present study confirmed these results, indentifying organic acids, vitamins and terpenic compounds as also significantly contributing to the red azarole fruit nutraceutical composition, as antioxidant agents. Moreover, organic acids are also used by the pharmaceutical industry as preservatives, acidulants and drug absorption modifiers, and they are important in order to maintain the quality and nutritive value of fruits. Due to the correlation between organic acids and fruit quality parameters, the manipulation of their contents is a legitimate objective of crop improvement as reported by [33].

The bioactive compound composition of fruits is also determined by genetic and environmental factors [34], but it may be modified by oxidative reactions during processing and storage [26]. This research is only a preliminary study on red azarole phytochemical composition. In this case, the research only focused on the antioxidant activity and chemical profile of a single commercial genotype in a specific sampling site without considerations on post-harvest influence on quality and nutraceutical traits. The diversity in *TBCC* and antioxidant activity among cultivars of other species [35] emphasizes the need for additional screening to identify *Crataegus* species and azarole cultivars with high antioxidant capacity and health-promoting potential. Finally, further researches focused on *C. azarolus* chemistry are also necessary to distinguish the bioactive compounds from those that are pharmacologically inactive [11].

CONCLUSIONS

This study contributed to the knowledge of the phytochemical composition and antioxidant properties of the red azarole, supporting the exploitation of this fruit species. In particular, *C. azarolus* fruits could be useful for the food industry, in particular as a potential natural source of biomolecules with strong antioxidant properties due to the high contents of organic and phenolic acids, and due to a synergetic effect of vitamin C and flavonoids. These results may also provide a basis for planning breeding strategies as well as selecting cultivars with potentially high phytonutrient profiles and antioxidant capacities. However, further studies are required before these fruit extracts can be utilized in the production of health-promoting foods and as an antioxidant carrier in pharmaceutical industries, too. A more holistic approach to the study of *C. azarolus* chemistry is also necessary to further research on the species, as the current research tends to focus on the quantification of individual compounds but it fails to look at the phytocomplex as a whole. Finally, this study developed an effective tool to assess red azarole quality, chemical composition and antioxidant activity. This research showed that analytical fingerprinting could be an important tool to evaluate fruit nutraceutical properties, helping to find new sources of natural health-promoting compounds. It can also highlight the contribution of each bioactive class to the total phytocomplex, considering synergistic and additive effects of all the phytochemicals selected as biomarkers.

REFERENCES

1. Scalbert, A. – Johnson, I. T. – Saltmarsh, M.: Polyphenols: antioxidants and beyond. American Journal of Clinical Nutrition, *81*, 2005, pp. 215S–217S. ISSN: 0002-9165 (print), 1938-3207 (online). <http://ajcn.nutrition.org/content/81/1/215S.full.pdf+html>
2. Donno, D. – Beccaro, G. L. – Mellano, M. G. – Cerutti, A. K. – Bounous, G.: 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, *53*, 2014, pp. 81–95. ISSN: 1336-8672 (print), 1338-4260 (online). <http://www.vup.sk/en/download.php?bulID=1559>
3. Donno, D. – Cerutti, A. K. – Prgomet, I. – Mellano, M. G. – Beccaro, G. L.: Foodomics for mulberry fruit (*Morus* spp.): Analytical fingerprint as antioxidants' and health properties' determination tool. Food Research International, *69*, 2015, pp. 179–188. DOI: 10.1016/j.foodres.2014.12.020.
4. Donno, D. – Beccaro, G. L. – Mellano, M. G. – Cerutti, A. K. – Bounous, G.: Goji berry fruit (*Lycium* spp.): antioxidant compound fingerprint and bioactivity evaluation. Journal of Functional Foods, *18, Part B*, 2015, pp. 1070–1085, DOI: 10.1016/j.

jff.2014.05.020.
5. Cerutti, A. K. – Beccaro, G. L. – Bruun, S. – Bosco, S. – Donno, D. – Notarnicola, B. – Bounous, G.: Life cycle assessment application in the fruit sector: state of the art and recommendations for environmental declarations of fruit products. Journal of Cleaner Production, *73*, 2014, pp. 125–135. DOI: 10.1016/j.jclepro.2013.09.017.
6. Bahri-Sahloul, R. – Ammar, S. – Grec, S. – Harzallah-Skhiri, F.: Chemical characterisation of *Crataegus azarolus* L. fruit from 14 genotypes found in Tunisia. Journal of Horticultural Science and Biotechnology, *84*, 2009, pp. 23–28. DOI: 10.1080/14620316.2009.11512474.
7. Phipps, J. B. – O'Kennon, R. – Lance, R. W.: Hawthorns and medlars. Cambridge : Timber Press, 2003. ISBN: 0881925918.
8. Koyuncu, T. – Pinar, Y. – Lule, F.: Convective drying characteristics of azarole red (*Crataegus monogyna* Jacq.) and yellow (*Crataegus aronia* Bosc.) fruits. Journal of Food Engineering, *78*, 2007, pp. 1471–1475. DOI: 10.1016/j.jfoodeng.2005.09.036.
9. Hashempour, A. – Ghazvini, R. F. – Bakhshi, D. – Ghasemnezhad, M. – Sharafti, M. – Ahmadian, H.: Ascorbic acid, anthocyanins, and phenolics contents and antioxidant activity of ber, azarole, raspberry, and cornelian cherry fruit genotypes growing in Iran. Horticulture Environment and Biotechnology, *51*, 2010, pp. 83–88. ISSN: 0253-6498.
10. Bignami, C. – Paolocci, M. – Scossa, A. – Bertazza, G.: Preliminary evaluation of nutritional and medicinal components of *Crataegus azarolus* fruits. In: ISHS Acta Horticulturae 597. Leuven : International Society for Horticultural Science, 2001, pp. 95–100. ISBN: 978-90-66058-86-6. DOI: 10.17660/ActaHortic.2003.597.11.
11. Edwards, J. E. – Brown, P. N. – Talent, N. – Dickinson, T. A. – Shipley, P. R.: A review of the chemistry of the genus *Crataegus*. Phytochemistry, *79*, 2012, pp. 5–26. DOI: 10.1016/j.phytochem.2012.04.006.
12. Guo, R. – Pittler, M. H. – Ernst, E.: Hawthorn extract for treating chronic heart failure. In: Cochrane Library [online]. London: John Wiley & Sons, 23 January 2008. DOI: 10.1002/14651858.CD005312.pub2.
13. Ramawat, K. – Dass, S. – Mathur, M.: The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In: Ramawat, K.G. (Ed.): Herbal drugs: ethnomedicine to modern medicine, Berlin: Springer, 2009, pp. 7–32. ISBN: 3540791159.
14. Boggia, R. – Casolino, M. C. – Hysenaj, V. – Oliveri, P. – Zunin, P.: A screening method based on UV–Visible spectroscopy and multivariate analysis to assess addition of filler juices and water to pomegranate juices. Food Chemistry, *140*, 2013, pp. 735–741. DOI: 10.1016/j.foodchem.2012.11.020.
15. Forina, M. – Boggia, R. – Casale, M.: The information content of visible spectra of extra virgin olive oil in the characterization of its origin. Annali di chimica, *97*, 2007, pp. 615–633. DOI: 10.1002/adic.200790047.
16. Giusti, M. M. – Wrolstad, R. E.: Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Current Protocols in Food Analytical Chemistry. Chichester : John Wiley & Sons, 2001. ISBN: 9780471142911. DOI: 10.1002/0471142913.faf0102s00.
17. Beccaro, G. L. – Torello-Marinoni, D. – Binelli, G. – Donno, D. – Boccacci, P. – Botta, R. – Cerutti, A. K. – Conedera, M.: Insights in the chestnut genetic diversity in Canton Ticino (Southern Switzerland). Silvae Genetica, *61*, 2012, pp. 292–300. ISSN: 0037-5349.
18. Donno, D. – Galizia, D. – Cerutti, A. K.: Fruit nutraceutical value in ancient apple cultivars grown in Piedmont (Northern Italy). In: ISHS Acta Horticulturae 940. Leuven: International Society for Horticultural Science, 2010, pp. 131–138. ISBN: 9066056851. DOI: 10.17660/ActaHortic.2012.940.16.
19. Canterino, S. – Donno, D. – Mellano, M. G.: Sensorial analysis and fruit quality in *Citrus sinensis* (L.) Osbeck from Northern Italy (Piedmont). In: ISHS Acta Horticulturae 928. Leuven : International Society for Horticultural Science, 2010, pp. 389–394.ISBN: 9066057440. DOI: 10.17660/ActaHortic.2012.928.52.
20. Mellano, M. G. – Beccaro, G.L. – Donno, D. – Torello Marinoni, D. – Boccacci, P. – Canterino, S. – Cerutti, A. K. – Bounous, G.: *Castanea* spp. biodiversity conservation: collection and characterization of the genetic diversity of an endangered species. Genetic Resources and Crop Evolution, *59*, 2012, pp. 1727–1741. DOI: 10.1007/s10722-012-9794-x.
21. Kong, W.-J. – Zhao, Y.-L. – Xiao, X.-H. – Wang, J.-B. – Li, H.-B. – Li, Z.-L. – Jin, C. – Liu, Y.: Spectrum-effect relationships between ultra performance liquid chromatography fingerprints and anti-bacterial activities of *Rhizoma coptidis*. Analytica Chimica Acta, *634*, 2009, pp. 279–285. DOI: 10.1016/j.aca.2009.01.005.
22. Benzie, I. F. F. – Strain, J. J.: 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 in Enzymology, *299*, 1999, pp. 15–27. DOI: 10.1016/S0076-6879(99)99005-5.
23. Slinkard, K. – Singleton, V. L.: Total phenol analysis: Automation and comparison with manual methods. American Journal of Enology and Viticulture, *28*, 1977, pp. 49–55. ISSN: 0002-9254. <http://www.ajevonline.org/content/28/1/49.full-text.pdf>
24. Barros, L. – Carvalho, A. M. – Ferreira, I. C. F. R.: Comparing the composition and bioactivity of *Crataegus monogyna* flowers and fruits used in folk medicine. Phytochemical Analysis, *22*, 2011, pp. 181–188. DOI: 10.1002/pca.1267.
25. Pantelidis, G. – Vasilakakis, M. – Manganaris, G. – Diamantidis, G.: Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. Food Chemistry, *102*, 2007, pp. 777–783. DOI: 10.1016/j.foodchem.2006.06.021.
26. Robards, K. – Prenzler, P. D. – Tucker, G. – Swatsitang, P. – Glover, W.: Phenolic compounds

and their role in oxidative processes in fruits. Food Chemistry, *66*, 1999, pp. 401–436, DOI: 10.1016/S0308-8146(99)00093-X.
27. Cotelle, N. – Bernier, J.-L. – Catteau, J.-P. – Pommery, J. – Wallet, J.-C. – Gaydou, E. M.: Antioxidant properties of hydroxy-flavones. Free Radical Biology and Medicine, *20*, 1996, pp. 35–43. DOI: 10.1016/0891-5849(95)02014-4.
28. Tsao, R. – Yang, R.: 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*, 2003, pp. 29–40. DOI: 10.1016/j.chroma.2003.08.034.
29. Tranchida, P. Q. – Donato, P. – Cacciola, F. – Beccaria, M. – Dugo, P. – Mondello, L.: Potential of comprehensive chromatography in food analysis. Trends in Analytical Chemistry, *52*, 2013, pp. 186–205. DOI: 10.1016/j.trac.2013.07.008.
30. Escarpa, A. – González, M.: Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. Analytica Chimica Acta, *427*, 2001, pp. 119–127. DOI: 10.1016/S0003-2670(00)01188-0.
31. Singleton, V. L. – Orthofer, R. – Lamuela-Raventos, R. M.: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, *299*, 1999, pp. 152–178. DOI: 10.1016/S0076-6879(99)99017-1.
32. Liu, R. H.: Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. American Journal of Clinical Nutrition, *78*, 2003, pp. 517S–520S. ISSN: 0002-9165. <http://ajcn.nutrition.org/content/78/3/517S.full.pdf+html>
33. Mikulic-Petkovsek, M. – Schmitzer, V. – Slatnar, A. – Stampar, F. – Veberic, R.: Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. Journal of Food Science, *77*, 2012, pp. C1064–C1070. DOI: 10.1111/j.1750-3841.2012.02896.x.
34. Donno, D. – Beccaro, G. L. – Mellano, G. M. – Cerutti, A. K. – Canterino, S. – Bounous, G.: Effect of agronomic and environmental conditions on chemical composition of tree-species buds used for herbal preparations. Vegetos - An International Journal of Plant Research, *25*, 2012, pp. 21–29. ISSN: 0970-4078 (print), 2229-4473 (online).
35. Donno, D. – Cavanna, M. – Beccaro, G. L. – Mellano, M. G. – Torello-Marinoni, D. – Cerutti, A. K. – Bounous, G.: Currants and strawberries as bioactive compound sources: determination of antioxidant profiles with HPLC-DAD/MS. Journal of Applied Botany and Food Quality, *86*, 2013, pp. 1–10. DOI: 10.5073/JABFQ.2013.086.001.
36. Donno, D. – Boggia, R. – Zunin, P. – Cerutti, A. K. – Guido, M. – Mellano, M. G. – Prgomet, Z. – Beccaro, G. L.: Phytochemical fingerprint and chemometrics for natural food preparation pattern recognition: an innovative technique in food supplement quality control. Journal of Food Science and Technology, *53*, 2016, pp. 1071–1083. DOI: 10.1007/s13197-015-2115-6.

Received 12 July 2016; 1st revised 31 October 2016; accepted 28 November 2016; published online 27 January 2017.