Preliminary Evaluation of Nutritional and Medicinal Components of *Crataegus azarolus* Fruits

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Abstract

Azarole (*Crataegus azarolus* L.) is a fruit and ornamental tree cultivated for centuries in the Mediterranean area, which is presently becoming rare in Italy. Programs directed to the collection and characterisation of its genetic resources have been started, in order to contribute to the exploitation of this species.

The main pomological traits and the content of sugars, acids and total polyphenols were determined in fruits at the ripening stage of five azarole accessions collected in two regions of Italy. The accessions were clearly differentiated by both biometric and chemical fruit characteristics. GHI4 and GHI7 were distinguished by bigger fruits and more tasty flesh than the other accessions. Sugar profiles of these two accessions were clearly differentiated by the high levels of sucrose, whereas this sugar was almost absent in the other accessions. Similar contents of total polyphenols, flavonoids and procyanidins were measured in all the accessions. The presence of good levels of these components with medicinal properties makes azarole fruits susceptible to exploitation in the field of food with nutraceutical interest.

INTRODUCTION

Azarole (*Crataegus azarolus* L.) is a multipurpose fruit tree cultivated or naturalised in the Mediterranean region and in Italy, with good potential as an ornamental, fruit and medicinal plant (Ker'y et al., 1989; Bignami and Kurzmann, 1998; Bignami and Kurzmann, 2000). Similarly to *Crataegus monogyna*, flowers and leaves are considered able to exert antioxidant activities and a positive action on the heart, due to the content of flavonoids and procyanidins (Twaij et al., 1987; Ker'y et al., 1989), but their medicinal properties are still not fully understood and not yet exploited. Furthermore, little research has been devoted to the composition and the presence and function of bioactive compounds in azarole fruits (Twaij et al., 1987; Ker'y et al., 1989; Schussler et al., 1991).

In Italy azarole has become very rare. Presently a few specimens are still cultivated in domestic orchards and gardens and their fruits are sometimes sold in the market. A program of conservation and characterisation of these threatened genetic resources started in 1996 within a EU project (Resgen 29), dealing with the exploitation of minor fruit tree species. Accessions of *Crataegus azarolus* have been individualised and collected in different regions of Italy and preliminarily described (Bignami and Kurzmann, 2000). The presence of flavonoids in leaf and flowers has been revealed by chemical analysis directed to the chemotaxonomy of these Italian ecotypes (Bignami and Kurzmann, 1998), but the composition of their fruits is still unknown.

The use of food as a medicine has gained increasing attention in the last years. The evaluation of the nutritional and medicinal value of the fruits could then support the exploitation of azarole. The pomological and analytical characterisation of the fruits of some accessions collected in Lazio and Emilia-Romagna was carried out in order to evaluate the potential for fresh consumption and for medicinal purposes.

MATERIAL AND METHODS

Samples of fruits were harvested at ripening time in 1998-1999. The pomological traits were described or measured on 20-30 fruits per accession. Titratable acidity and the

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refractometric index were measured on fresh samples of fruits by means of a titrator and digital refractometer (Atago PR-101). Qualitative and quantitative determination of sugars, sugar alcohols and organic acids was carried out by gas-liquid chromatography (GC), according to Bartolozzi et al. (1997). 0.5 g of lyophilised flesh and skin were extracted in 100 ml imydazol 0.05 M, pH 7.2-ethanol (50:50 v:v), adding β–phenyl-glucopyranoside (2.5 mg) as an internal standard. 2 ml of the extracts were dehydrated and treated with 600 ml of pyridine, 200 ml of trimethyldisilazane and 100 ml of trimethylchlorosilane, and then heated at 50° for 1 hour. The trimethylsilyl derivatives were injected into a GC Chrompack CP 9000, equipped with a splitter injector and a flame ionisation detector and a capillary column CP-Sil-5CB- 10 m, 0,25 mm ID, 0.12 mm df (Chrompack, Middelburg, Netherlands). The temperature of injector and detector were 300° and 320° respectively. The temperature program was: 120° for 1 min, and then from 120 to 180° at 10°/min; 180 to 210° at 15°/min; 210 to 300° at 20°/min. Flow rates of He, H_2, air and N_2 were 1, 30, 250 and 30 ml/min respectively, with a split ratio of 80:1. Retention times of the standards of the main sugars and organic acids present in the fruits were used for the qualitative determination. Procyanidins were analysed by the official colorimetric method, according to the European Pharmacopeia (1998), with cyanidin chloride as a standard; total flavonoid content was measured by HPLC, with naringenin as an internal standard, and calculated as % hyperoside. The estimation of total polyphenols was performed by the Folin-Ciocalteau method, and expressed as gallic acid (Scalbert et al., 1989).

RESULTS

Fruit Traits

The accessions were clearly differentiated by several pomological traits, such as fruit size and shape, skin colour and flesh taste (Tab. 1). GHI4 and GHI7 showed the biggest fruits, with an average weight of 7-8 g, and POM1 the smallest. The shape was roundish-flat, with the lowest height/width ratio in GHI5 and POM1. The ratio between seed and fruit weight was quite high in all the accessions (16-23%). On the basis of fruit size and taste, the most interesting accessions for fresh consumption and processing are GHI4 and GHI7, as already stated by Bignami and Kurzmann (1998).

Fruit Composition

1. Sugars and Acids The refractometric index ranged from 14 to 18%, with the higher values in GHI4, GHI7 and POM2 (Tab. 2). The titratable acidity was quite high, with respect to other edible *Maloideae*, like apple, and variable among the accessions, ranging from 0.5% to 2% (Tab. 2). The total content of sugar is high with respect to other *Crataegus* species of food interest (Chapman and Horvat, 1991; Chapman and Horvat, 1993).

The accessions were well differentiated both by sugar and acid profile (Fig 1). The identification of sugars, acids and alcohols showed that the main sugars in azarole are represented by fructose, glucose and sucrose and the acids are malic, citric, quinic and succinic acid (Fig. 1 and Tab. 3-4). Sorbitol was the main polyalcohol, accounting for 1.3-2.3 % of fresh weight. The qualitative and quantitative determinations showed differences among accessions. GHI4 and GHI7 revealed in fact a richest composition of acids and sugars. A high quantity of sucrose (4-6% of fresh weight) was found in two accessions, GHI4 and GHI7, whereas only trace amounts were observed in GHI5, POM1 and POM2. Glucose and fructose accounted for 5-6 % of fresh weight in the accessions with low sucrose content, and was 3-4% in the accessions with high sucrose content. Malic acid was the major nonvolatile acid, accounting for about 80% of total acids in GHI5, POM1 and POM2, and for 50-60% in GHI4 and GHI7 (Tab. 4). Low content of succinic acid has been observed in GHI4 and traces in the other accessions. Quinic acid was present in relevant concentration in GHI4 and GHI7 and citric acid in GHI4. The highest content of sorbitol was found in GHI5 and POM1.

2. Polyphenols The content of total polyphenols was 1.8- 2.4 % of fresh weight, that is about 0.4-0.6 % on a fresh weight basis, depending on the accession. Like other *Crataegus* (Chelaninskaya and Dovnar, 1977), the species *azarolus* is therefore quite rich in polyphenols (Tab.5). The values were in the range observed in other small fruits, such as raspberry, blackberry and red-currant (Melegari and Benvenuti, 1998). The levels of total flavonoids and procyanidins were similar to that of the azarole ecotypes analysed in Iraq (Ker'y et al., 1987). The content of procyanidins did not greatly differ from the values indicated in the European Pharmacopoeia for hawthorn (*Crataegus monogyna* L.).

CONCLUSIONS

The pomological and analytical measurements revealed the existence of interesting aspects worthy of exploitation for different kinds of fruit use.

Flavour and size of the fruits, together with the high level of sugars and acids, point out GHI4 and GHI 7 as the best accessions with good potential for fresh consumption and for processing into different products, such as jams, juices and candies.

As a complex, the presence of components with medicinal properties makes azarole fruits of all the accessions susceptible to exploitation in the field of herb preparations and food with medicinal and nutraceutical interest. Compared to other *Crataegus* spp., *C. azarolus* could offer some advantages for qualitative and agronomic aspects, such as the good or excellent fruit and juice taste; the easier fruit harvest, the dependability of the fruit size; and the high yield per plant which can be obtained in favourable conditions.

From the chemotaxonomic point of view, the sugar and acid profile offered an effective tool for accession discrimination, with sucrose as the main marker among the sugars. On the basis of both pomological traits and chemical composition, the two accessions GHI5 and POM1 were quite similar and seem to have a common genetic origin.

Studies are advisable on a wider range of ecotypes, on the environmental effect (year and location) and on the modification of the main components during fruit ripening and processing (juice, jams) are advisable before starting selection activity and an effective program of exploitation more clearly directed to the different uses.

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Tables

Table 1. Fruit traits of azarole accessions (±standard deviation).

Accession	**Weight g**	**Eight cm**	**Width cm**	**Shape h/w**	**Seed/fruit%**	**Skin colour**
GHI4	7.9±1.7	2.0±0.1	2.3±0.2	0.87 ±0.06	20±4	pale yellow
GHI5	5.5±0.9	2.8±0.1	2.4±0.1	0.78±0.03	16±2	yellow
GHI7	6.9±1.0	2.1±0.2	2.4±0.1	0.89±0.07	25±4	red-orange
POM1	5.6±0.9	1.8±0.1	2.3±0.1	0.79±0.03	17±2	yellow
POM2	2.9±0.4	1.5±0.8	1.8±0.1	0.86±0.09	23±2	red

Table 2. Refractometric index, pH, titratable acidity and total sugars and acids in azarole fruits (±standard deviation).

Accession	**RI °Brix**	**pH**	**Titratable acidity (%)**	**Total sugars (% f.w.)**	**Total acids (% f.w.)**
GHI4	18.8±1.6	3.2±0.1	1.56±0.54	14.08±0.73	2.72±0.04
GHI5	15.2±1.2	3.3±0.2	0.56±0.58	15.29±0.02	2.31±0.05
GHI7	17.6±1.4	3.3±0.2	1.98±0.70	13.79±0.54	2.29±0.09
POM1	13.6±2.1	3.5±0.2	1.23±0.60	13.06±0.38	1.52±0.01
POM2	18.9±0.8	3.5±0.1	0.84±0.57	15.87±0.15	2.45±0.01

Table 3. Mean content of the main organic acids, sugars and sugar alcohols in the fruits of five azarole accessions (% of fresh weight; ±standard deviation).

Accession	**malic acid**	**citric acid**	**quinic acid**	**fructose**	**glucose**	**sucrose**	**sorbitol**
GHI4	1.36±0.04	0.64±0.01	0.65±0.02	4.31±0.24	3.94±0.05	4.05±0.35	1.74±0.07
GHI5	2.27±0.03	0.33±0.01	0.18±0.01	6.76±0.10	6.12±0.02	0.07±0.04	2.30±0.01
GHI7	1.35±0.03	0.32±0.01	0.61±0.03	3.19±0.49	3.04±0.09	6.24±0.05	1.31±0.01
POM1	1.19±0.01	0.19±0.01	0.13±0.005	5.97±0.03	5.30±0.15	0.03±0.008	2.03±0.04
POM2	2.00±0.01	0.36±0.02	0.08±0.001	7.26±0.13	6.73±0.04	0.07±0.00	1.76±0.03

Table 4. Incidence (%) of the main compounds on total acids and sugars in the azarole accessions.

accession	malic acid	citric acid	succinic acid	quinic acid	fructose	glucose	sucrose	sorbitol
GHI4	50.1	23.7	2.2	23.9	30.6	28.0	28.7	12.4
GHI5	81.5	11.8	0.4	6.4	44.2	40.0	0.5	15.0
GHI7	58.7	14.1	0.5	26.7	23.1	22.01	45.3	9.5
POM1	78.3	12.6	0.4	8.7	44.7	39.6	0.2	15.2
POM2	81.4	14.8	0.5	3.3	45.7	42.4	0.4	11.1

Table 5. Biologically active components in azarole fruits (±standard deviation).

Accession	*Procyanidins (cyanidin chloride % d.w.)	*Total flavonoids (hyperosyde % d.w.)	Total polyphenols (% d.w.)
GHI4	1.79	0.066	2.3 ± 0.2
GHI5	-	-	2.4 ± 0.1
GHI7	1.33	0.065	2.4 ± 0.1
POM1	1.27	0.069	2.3 ± 0.1
POM2	1.03	0.081	1.8 ± 0.1

<u>Figures</u>

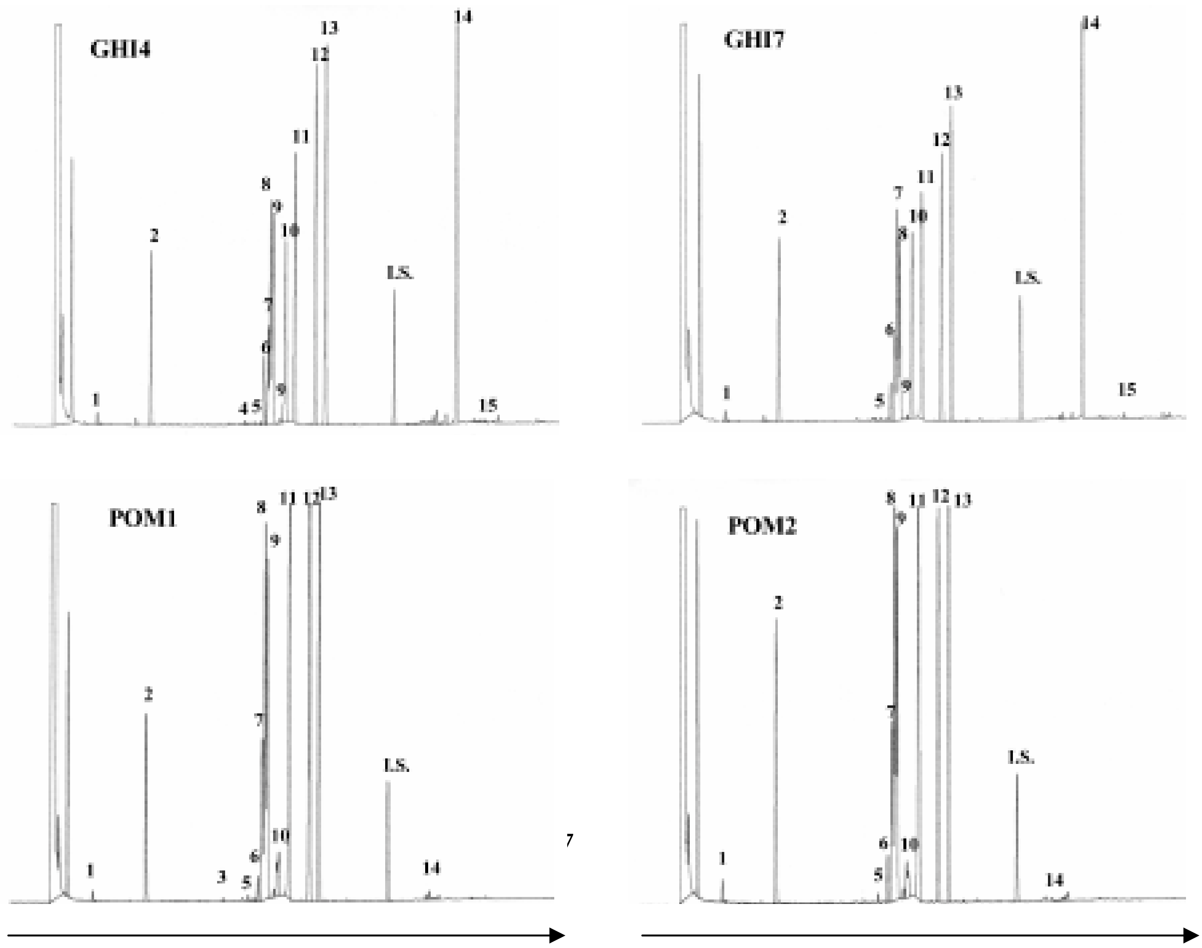

Fig. 1. GLC identification of acid and sugars in fruits of four azarole accessions. 1=succinic acid; 2=malic acid; 3=xylose; 4=xylitol; 5=xylose2; 6=citric acid; 7=fructose$_1$; 8=fructose$_2$; 9=fructose$_3$; 10=quinic acid; 11=glucose$_1$; 12=sorbitol; 13=glucose$_2$; I.S.= Internal standard: β−phenyl-glucopyranoside; 14=sucrose; 15=trihalose.