

Content in organic acids of *Mespilus* spp. and *Crataegus* spp. genotypes

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Abstract

Medlar and hawthorn genotypes were analysed and also their individual organic acids, in order to better understand their use as functional foods, but also as ingredients in pharmaceutical, nutritional products and in medicine. HPLC analysis was carried out using a Surveyor Thermo Electron system. Ascorbic, oxalic, tartaric, malic, citric, succinic and fumaric acids were detected. On average, malic acid was predominant with a range of 415.08 mg/100 g FW in *Mespilus germanica* and 1,128.68 mg/100 g FW in *Crataegus pentagyna*. The order of organic acid depending on their content/100 g FW was: for *Mespilus germanica* - malic > tartaric > oxalic > citric > fumaric > ascorbic > succinic; for *Crataegus monogyna* - malic > oxalic > citric > succinic > tartaric > ascorbic > fumaric; for *Crataegus pentagyna* - malic > citric > oxalic > succinic > tartaric > ascorbic > fumaric. The data obtained in this study do confirm that medlar and hawthorn fruits are a rich source of organic acid, and their organic acid content within the 100 g was greater than human daily consumption for this required level.

Keywords: fruits; genotype; hawthorn; HPLC; medlar; organic acids

Introduction

Despite the increased interest for fruit species with lesser known fruits, as a source of new valuable compounds and with pharmaceutical properties, they are insufficiently used for this purpose. A wide range of active biological products with applications in phyto-chemistry have been found in fruits of plants of *Rosaceae* family (Halvorsen *et al.*, 2002; Kraft *et al.*, 2008). *Mespilus* and *Crataegus* genus belongs to *Rosaceae* family, the species belonging to them are found both in spontaneous flora and in culture flora, being cultivated and searched for edible fruits (Ayaz *et al.*, 2002). Also other parts of plant contain many chemical compounds useful in the food and pharmaceutical industry (Liu *et al.*, 2009). Hawthorn species (*Crataegus* spp.) have attracted attention in the food and medical field due to their features and for health benefits (Jurikova *et al.*, 2012). Either fresh or dried fruits of hawthorn are used for preparation of teas, or as a source of extracts, or for production of food supplements (Bekbolatova *et al.*, 2018). Medlar is also used in folk medicine, especially by people in south-eastern Europe, Turkey and Iran, primarily for the treatment of constipation, as a diuretic, and

regular consumption of peeled medlar fruits helps in treating kidney stones by dissolving them gradually. Medlar paste or syrup is a popular remedy against enteritis (Glew *et al.*, 2003a). Among the constituents of biological samples, organic acids are of increasing interest because of their role in plant physiology as cofactors, buffering agents, and intermediates of the most important metabolic pathways of carbohydrates, lipids, and proteins (Koyuncu, 2004). The organic acid content of fruits differs depending on species. Some organic acids have antioxidant role (tartaric, malic and citric acids), acidulant (tartaric, malic, citric and ascorbic acids) or preservative (sorbic and benzoic acids). Fruit acids that allow nutrient digestion and stimulate blood circulation are considered among the quality parameters of hawthorn fruits (Gundogdu *et al.*, 2014). Numerous studies have quantified and detailed the organic acid content of hawthorn fruits and there have been differences between them due to the species, location (Liu *et al.*, 2009). Medlar is a typical fruit that has gained great value in human consumption and commercial importance in recent years, attracting researchers to study its chemical or nutritional compositions (Ayaz *et al.*, 2002; Dincer *et al.* 2002). Sugar content such as fructose and glucose (Glew *et al.*, 2003b,c), linoleic acid and palmitic acid (Ayaz *et al.*, 2008), organic acids such as malic acid and citric acid (Glew *et al.* 2003a,c), potassium (Glew *et al.*, 2003b), amino acids (Glew *et al.*, 2003a) and volatile components (Pourmortazavi *et al.*, 2005) were determined in high amounts in medlar fruits. Bioactive components of medlar fruits, their characterization and use in functional foods, as well as human health assessment are among the main objectives of contemporary research (Gülçin *et al.*, 2011). The purpose of this study was to identify and quantify the main organic acids in fruits that have reached full maturity of genomes of medlar (*Mespilus germanica* L.) and hawthorn (*Crataegus monogyna* Jacq., *Crataegus pentagyna* Waldst. Et Kit.), using HPLC method.

Materials and Methods

Biological material

Four medlar genotypes (*Mespilus germanica* L.) and thirtieth hawthorn genotypes (twentieth of *Crataegus monogyna* (L.) Jacq. and eight genotypes of *Crataegus pentagyna* (L.)Waldst. et Kit.) were studied. Genotypes were identified in spontaneous flora in ecosystems in the south-west of Oltenia region (Romania), showing interest in terms of fruit shape, size and colour. From the ripened fruits the seeds were extracted, and the pulp was stored in the freezer, for preservation in order to carry out the analyses of identification and quantification of the content in organic acids and dry matter.

Methods

To determine the organic acids, an amount of each genotype (~ 2 g) was weighed using the Radwag AS 220 RD analytical balance. Each sample was homogenized with the Ultra-Turrax apparatus, stored in tubes and diluted with 30 mL of 2% HCl solution (Sigma-Aldrich). The resulting mixtures were shaken with the Vortex shaker, then introduced into the Fungilab ultrasound bath, at a temperature under observation so as not to exceed 25 °C, for 60 minutes. The samples were centrifuged (Eppendorf Centrifuge 5430 R), 5 minutes, at 4000 rpm, then filtered and introduced into HPLC for analysis based on organic acid standards purchased from Sigma-Aldrich (ascorbic, oxalic, tartaric, malic, citric, succinic, and fumaric acid). HPLC analysis was performed with a SurveyorThermo Electron system containing a Surveyor Plus LCPMPP pump, a Surveyor Plus ASP autosampler and a 5 cm diode flow cells detector. Integration, storage and processing of data were performed by Chrom Quest 4.2 software. Concentration of organic acids was determined using an Acclaim OA column (5 µm, 4 x 250 mm, 100 mM Na₂SO₄ mobile phase, pH 2.65, adjusted with methanesulfonic acid), at a temperature of 30 °C, a flow rate of 0.6 mL / min and a 5 µL injection. Detection of organic acids was carried out by UV absorption at 214 nm, while the detection of ascorbic acid was at 243 nm. All results were expressed in mg / 100 g FW.

Statistical analysis

The data obtained were statistically processed using the statistical program (StatPoint Technologies, Warrenton, VA, SUA).

Results

The results obtained from the analysis made on organic acids contained in medlar and hawthorn fruits are found in Tables 1, 2 and 3. The order of organic acid depending on their content/100 g was: for *Mespilus germanica* - malic > tartaric > oxalic > citric > fumaric > ascorbic > succinic; for *Crataegus monogyna* - malic > oxalic > citric > succinic > tartaric > ascorbic > fumaric; for *Crataegus pentagyna* - malic > citric > oxalic > succinic > tartaric > ascorbic > fumaric. The most predominant acid according to the amount obtained from the analyses was the malic acid in all studied genotypes from all three species. Ascorbic acid, even in small amounts was indentified in all genotypes from all the three studied species (for medlar 0.49 mg/100 g to 0.88 mg/100 g; *C. monogyna* from 1.04 mg/100 g to 29.62 mg/100 g and *C. pentagyna* from 2.49 mg/100 g to 21.26 mg/100 g). Citric acid was also identified in all genotype's species, with the highest value of 1,554.92 mg/100 g in hawthorn. Succinic acid was found only in one medlar genotype (32.10 mg/100 g), and as for hawthorn genotypes the values were from 69.51 mg/100 g to 328.73 mg/100 g for red hawthorn and from 89.49 mg/100 g to 574.79 mg/100 g for black hawthorn, the rest being bellow the limit of detection. Fumaric acid was present in all studied samples, ranging from 0.15 mg/100 g to 1.28 mg/100 g in medlar, and 0.37 mg/100 g to 4.96 mg/100 g in hawthorn species. Oxalic acid ranged from 29.73 mg/100 g to 72.92 mg/100 g in medlar fruits, and relatively close values in both red and black hawthorn from 120.51 mg/100 g to 511.43 mg/100 g. The highest values regarding tartaric acid was found in *C. pentagyna* (from 62.22 mg/100 g to 241.48 mg/100 g), followed by medlar (70.84 mg/100 g to 169.47 mg/100 g) and *C. monogyna* (37.68 mg/100 g to 177.93 mg/100 g).

Table 1. Organic acids content in *Mespilus germanica* L. (mg/100 g sample)

Organic acids	Ascorbic acid	Oxalic acid	Tartaric acid	Malic acid	Citric acid	Succinic acid	Fumaric acid
Mean ± SD	0.7±	54.73±	111.57±	415.08±	16.41±	n.d*	0.79±
Variations limit	0.49-0.88	29.73-72.92	70.84-169.47	293.92-583.66	14.53-20.42	-	0.15-1.28
CV%	0.23	0.34	0.37	0.33	0.16	-	0.62

Note: SD = standard deviation; CV% = coefficient of variation; *n.d. = not detected

Table 2. Organic acids content in *Crataegus monogyna* (L.) (mg/100 g sample)

Organic acids	Ascorbic acid	Oxalic acid	Tartaric acid	Malic acid	Citric acid	Succinic acid	Fumaric acid
Mean ± SD	14.65 ± 8.98	234.24 ± 93.19	101.88 ± 36.31	1,070.45 ± 297.82	206.25 ± 107.81	185.00 ± 74.17	0.73 ± 0.83
Variations limit	1.04 – 29.62	120.51 – 493.03	37.68 – 177.93	631.17 – 1,827.65	84.63 – 398.84	69.51 – 328.73	0.37 – 1.47
CV%	3.98	41.32	16.10	132.04	47.80	42.82	0.18

Note: SD = standard deviation; CV% = coefficient of variation

Table 3. Organic acids content in *Crataegus pentagyna* (L.) Kit. (mg/100 g sample)

Organic acids	Ascorbic acid	Oxalic acid	Tartaric acid	Malic acid	Citric acid	Succinic acid	Fumaric acid
Mean \pm SD	11.75 \pm 6.23	312.49 \pm 125.24	120.01 \pm 57.20	1,128.68 \pm 471.87	604.54 \pm 584.24	301.61 \pm 187.42	2.49 \pm 1.68
Variations limit	2.49 – 21.26	134.84 – 511.43	62.22 – 241.48	553.79 – 1,823.47	68.31 – 1,554.92	89.49 – 574.79	0.78 – 4.96
CV%	5.21	104.71	47.82	394.49	488.44	173.34	1.77

Note: SD = standard deviation; CV% = coefficient of variation

Discussion

Ascorbic acid is an organic acid with antioxidant properties. It is involved in many functions of the body, including collagen formation, iron uptake, immunity system, wound healing and maintenance of cartilage, bones and teeth (Lee and Kader, 2000). Ascorbic acid was identified in all medlar genotypes under study, an average value of 0.7 mg/100 g was obtained in the 4 genotypes, the limits of variation between 0.49 mg/100 g (N1) and 0.88 mg/100 g (E1) (Table 1). Similar studies by Ayaz *et al.* (2007) on medlar fruits showed that both ascorbic acid and citric acid were present in undetectable quantities. Although present in relatively small amounts, ascorbic acid was identified in all 30 genotypes analyzed, with a minimum of 1.04 mg/100 g (G1) and a maximum value of 29.62 mg/100 g (G12), for *C. monogyna* species (Table 2), values that are close to those found by Ruiz-Rodríguez *et al.* (2014) who recorded 30.35 mg/100 g ascorbic acid sample. The ascorbic acid recorded in genotypes of *C. pentagyna*, had a minimum value of 2.49 mg/100 g (G14) and a maximum of 21.26 mg/100 g (G22) (Table 3). With respect to changes in the content of sugars and organic acids in medlar fruits during fruit development and ripening, Glew *et al.* (2003a) noted that while the level of malic acid increased continuously, the level of ascorbic acid decreased through fruit development, both acids reaching the maximum and minimum limits at 161 days after complete flowering, respectively 428 and 8.4 mg/100 g FW. Kayashima and Katayama (2002) considered that oxalic acid is available as a natural antioxidant and may play an important role in natural and artificial preservation of oxidized materials. As regards oxalic acid, the highest content was T1 genotype (72.92 mg/100 g) and N1 (64.84 mg/100 g), the lowest content being in C1 genotype (29.73 mg/100 g). Oxalic acid had relatively close values between the two *Crataegus* species: with a minimum value of 120.51 mg/100 g (G12) and a maximum value of 493.03 mg/100 g (G26) in *C. monogyna* genotypes (Table 2); a minimum of 134.84 mg/100 g (G13) and a maximum value of 511.43 mg/100 g (G22) in *C. pentagyna* (Table 3). Gundogdu *et al.* (2014) conducted a study on *C. atrosanguinea* Pojark fruits grown in Erzincan province of Turkey and reported highest oxalic acid content (12.419 g/100 g). Regarding tartaric acid, it was detected in medlar in concentrations between 70.84 mg/100 g (E1) and 169.47 mg/100 g (N1) (Table 1). Tartaric acid present in hawthorn fruit had higher values in *C. pentagyna* (between 62.22 mg/100 g in G27 and 241.48 mg/100 g in G22) than in *C. monogyna* (37.68 mg/100 g in G21 and 177.93 mg/100 g in G8) (Table 2 and Table 3). Malic acid plays an important role for human body as it is often found in fruits. For malic acid the content ranged from 583.66 mg/100 g (N1) to 293.92 mg/100 g (E1) (Table 1). Similar studies by Romero Rodriguez *et al.* (1992) showed that malic acid had a concentration of 562.6 mg/100 g FW in medlar fruits, being lower than the one recorded in N1 genotype, and Glew *et al.* (2003a) identified a concentration of 428 mg/100 g FW. The predominant acid in all genotypes analyzed from both *Crataegus* species was malic acid with the highest value recorded at G16 (1,827.65 mg/100 g) and a minimum at G15 (631.17 mg/100 g) for *C. monogyna* (Table 2). In the fruits of *C. pentagyna* the minimum value was 553.79 mg/100 g (G27) and a maximum value of 1,823.47 mg/100 g (G22) (Table 3). Liu *et al.* (2010) conducted a study on *C. pinnatifida* var. major fruits grown in China and reported malic acid content as 0.72 g/100 g. Citric acid was found in all

the fruits of the moss, with the highest content in T1 genotype (20.42 mg/100 g), followed by N1 genotype (15.73 mg/100 g sample), and the lowest concentration was 14.53 mg/100 g to C1 genotype (Table 1). Significant differences between the two *Crataegus* species are given by citric acid, *C. pentagyna* recorded both the minimum and maximum values of all the studied genotypes (68.31 mg/100 g, G27 respectively 1,554.92 mg/100 g, G23), and *C. monogyna* had a minimum of 84.63 mg/100 g at G24 and a maximum of 398.84 mg/100 g at G5 (Table 2 and Table 3). The results are like those obtained by Gundogdu *et al.* (2014). Citric acid is the most used acid, while malic acid and tartaric acid are important natural compounds of fruits used alongside fumaric acid in fruit flavored drinks (Shui and Leong, 2002). The succinic acid was found in a single medlar genotype (T1) at a concentration of 32.10 mg/100 g, and for the other genotypes it was present in undetectable quantities. Fumaric acid was found in the medlar in relatively small quantities, in all 4 genotypes studied, the limits varying between 1.07 mg/100 g sample (T1) and 0.15 mg/100 g (N1) (Table 1). Below detection limit, in some genotypes of *C. monogyna*, the content of succinic and fumaric acid was also present, the data being similar to those made by Pereira *et al.* (2013). A maximum of 328.73 mg/100 g succinic acid at G28 (the minimum value was 69.51 mg/100 g at G15) and a maximum of 1.47 mg/100 g fumaric acid at G5 (the minimum was recorded at G10, 0.37 mg/100 g) was found in *C. monogyna* genotypes (Table 2). The succinic acid also had values below the detection limit for some genotypes of *C. pentagyna*, the minimum value obtained was 89.49 mg/100 g (G14) and maximum of 574.79 mg/100 g (G23) (Table 3). Fumaric acid recorded a minimum of 0.78 mg/100 g at G30 and a maximum of 4.96 mg/100 g at G19.

Conclusions

The data obtained in this study do confirm that medlar and hawthorn fruits are a rich source of organic acid, and their organic acid content within the 100 g was greater than human daily consumption for this required level. The results obtained regarding the organic acid content of medlar and hawthorn fruits are necessary, in order to better understand their use as functional foods, but also as ingredients in pharmaceutical, nutritional products and in medicine.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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