

Food Chemistry 83 (2003) 363–369

Food Chemistry

[www.elsevier.com/locate/foodchem](http://www.elsevier.com/locate/foodchem/a4.3d)

Changes in sugars, organic acids and amino acids in medlar (Mespilus germanica L.) during fruit development and maturation

Robert H. Glew^a, Faik A. Ayaz^{b,*}, Carlos Sanz^c, D.J. VanderJagt^a, H.-S. Huang^d, L.-T. Chuang^d, M. Strnad^e

a Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine, Albuquerque, New Mexico, USA

bDepartment of Biology, Karadeniz Technical University, 61080 Trabzon, Turkey

^c Department of Physiology and Technology of Plant Products, Instituto de la Grasa, CSIC, Padre García Tejero 4, 41012-Seville, Spain

^dRoss Products Division, Abbott Laboratories, Colombus, Ohio, USA

e Laboratory of Growth Regulators, Palacky University, Slechtitelu 11, 783 71 Olomouc, Czech Republic

Received 7 January 2003; accepted 11 February 2003

Abstract

The contents of sugars, organic acids, and amino acids (after acid hydrolysis) were determined during development and maturation of medlar (Mespilus germanica L.) fruit from 39 days after full bloom (DAF) until 2 weeks after the beginning of fruit drop (161 DAF). Fructose, glucose and sucrose were identified as the principal sugars and their levels varied remarkably during development. The fructose level increased continually through development reaching its maximum by 161 DAF (1200 mg/100 g fresh weight) while the increase of sucrose reached maximum at 131 DAF and had decreased at 161 DAF. After some fluctations at 69 DAF, glucose level remained high (686 mg/100 g fresh weight) at 161 DAF, when compared with Stage IV (131 DAF). While the level of malic acid increased continually, the ascorbic acid level decreased dramatically through fruit development; both acids reached their maximum and minimum levels at 161 DAF, i.e. 428 and 8.4 mg/100 g fresh weight, respectively. The total amino acid composition also changed in decreasing trend throughout development and remained low at 161 DAF. In the ripe fruit, glutamate and aspartate were the major amino compounds identified. These changes in the identified compounds can be related to the metabolic activity during fruit development and maturation.

 \odot 2003 Elsevier Ltd. All rights reserved.

Keywords: Medlar (Mespilus germanica L.); Sugar; Organic acid; Amino acid; Fruit development; Maturation

1. Introduction

Corresponding author.

Free sugars, organic acids and amino acids (free and in proteins) are natural components of many fruits and vegetables and they play important roles in maintaining fruit quality and determining nutrititive value (Ashoor $\&$ Knox, 1982). The nature and the concentration of these constituents in fruits have been of interest because of their important influence on the organoleptic properties. Therefore, food analysts and plant physiologists have been interested in changes in the nature and amounts of the various chemical components occurring during ripening in the edible parts of fruits because of their impact on the market quality of the food product [\(Wrolstad, 1981](#page-6-0)).

Numerous studies have been reported on the metabolism and physiology of many fruits, including free sugar, organic acid, and amino acid compositions. The relationships between these compounds and fruit ripening have been extensively studied in different fruits such as peach ([Chapman & Horvat, 1990](#page-6-0)), mayhaw [\(Chap](#page-6-0)[man & Horvath, 1993;](#page-6-0) [Chapman, Horvat, & Payne,](#page-6-0) [1991\)](#page-6-0), persimmon [\(Ayaz & Kadioglu, 1998; Senter,](#page-5-0) [Chapman, Forbus, & Payne, 1991](#page-5-0)), apples ([Ackerman,](#page-5-0) [Fischer, & Amado, 1992\)](#page-5-0), strawberry (Pérez, Rios, [Sanz, & Olias, 1992](#page-6-0)), blueberry [\(Ayaz, Kadioglu, Ber](#page-5-0)[toft, Acar, & Turna, 2001](#page-5-0)).

Medlar, Mespilus germanica L. (fam: Rosaceae), is a spiny shrub that grows to a height of 2–3 m in the wild and 6 m when cultivated. The fruits of medlar are subglobose or pyriform and crowned by foliaceous sepals, ranging from 1.5 to 3 cm in diameter ([Browicz, 1972\)](#page-6-0).

E-mail address: faa@ktu.edu.tr (F. A. Ayaz).

^{0308-8146/03/\$ -} see front matter © 2003 Elsevier Ltd. All rights reserved. doi:10.1016/S0308-8146(03)00097-9

The pome-like fruit originates from the inferior ovary, generally with five stony seeds. The skin colour is brown, sometimes tinged reddish. Several common varieties of medlars are also well-known through Europe and Asia. Among these Dutsch (with big fruit), Common (with medium fruit), Royal (with small fruit), Nottingham (with small fruit, tasty) and Stoneless are probably the best known. The most common use of medlar fruits is for raw consumption after bletting. The harvest of fruit bletted on the plant in late autumn or the harvest of fruits at physiological ripening and their storage in straw until over-ripening are well known traditions, still alive today. The bletted fruits (bletting: the ripening of fruit, especially of fruit stored until the desired degree of decay and softness is attained) have a sweet and slightly acid flesh. Jams and jellies can be obtained. In cookery, a surprisingly long list of recipes can be found. The astringency of the fruits has been well known since ancient times. Medlar bletted pulp or syrup was a popular remedy against enteritis ([Bignami, 2000\)](#page-6-0) and modern medicine has recognized (in the 1920s) its healing properties, to treat constipation, kidney and bladder stones and as a diuretic [\(Baytop, 1999\)](#page-5-0).

The medlar has been of recent interest for its edible fuits. Recently, the physical (weight, colour, firmness), physicochemical (pH, soluble solids) and chemical (moisture, soluble sugars, starch) changes during maturation of Spanish medlar have been reported [\(Romero-Rodriguez, Simal-Lozano, Vazguez-Oderiz,](#page-6-0) [Lopez-Hernandez, & Gonzalez-Castro, 2000](#page-6-0)) and [de](#page-6-0) [Pascual-Teresa, Santos-Buelga, and Rivas-Gonzalo](#page-6-0) [\(2000\)](#page-6-0) have analysed flavanols in medlar fruit. More recently, changes in mineral composition, at different stages of maturity of medlar [\(Glew, Ayaz, VanderJagt,](#page-6-0) [Millson, Dris, & Niskanen, 2002](#page-6-0)) and fatty acid composition, during ripening of medlar [\(Ayaz, Glew,](#page-5-0) [Huang, Chuang, VanderJagt, & Strnad, 2002\)](#page-5-0) have been reported. Besides these studies, data on the composition and nutritional value of medlars are still scarce. As far as we know, there are no data in the literature about changes in the fatty acid composition during development and maturation of medlar fruit.

Medlar fruit is also widely consumed in Turkey. Harvest takes place through October, storing part of the crop in cold, dark, and aerated places, to induce the fruit to soften. Part of the crop is used to make pickles of this fruit to be consumed as an appetizer on winter days. The determination of sugar, organic acid and amino acid compositions of medlar may be useful for several reasons, first, in order to know how important medlar fruit is nutritionally and second, because the marked changes that occur in the sugar, organic acid and amino acid contents and profiles of fruits can have deleterious effects on their acceptability as a food source. The objective of this study was to study the changes of sugars, organic acids, and amino acids in medlar during fruit development and maturation.

2. Materials and methods

2.1. Fruit material

Medlar (Mespilus germanica L., wild type) fruit was randomly harvested from fourteen 20-year-old trees in the early morning from various single genotypes of bulk populations in the native habitat of the species located in the grounds of hillsides of Caykara (Trabzon) and neighbouring lands that are 500–600 m over sea level, northeast Anatolia (Turkey). The blossoms were considered to be in full bloom on 8 May 2000 and five maturity categories were collected by sampling the fruits at 39, 69, 100, 131 and 161 days after full bloom (DAF). These sampled fruits were subdivided into immature, mid-ripe and ripe maturity stages, on the basis of skin and state of maturity, considering the earlier DAFs. For the immature stage, the entire medlar had a light green skin. For the mid-ripe stage, the skin was ca. 60% light brown and 40% pale green. For the ripe stage, the skin colour of medlar fruit was completely brown and inside was completely white [\(Table 1](#page-2-0)). One kilogram of medlar fruit was gathered in triplicate at each collection time and stored at -35 °C. All reagents, solvents and standards were of analytical reagent grade.

2.2. Sugar and organic acid extraction

For the ethanolic extraction of sugars and organic acids, fruit flesh, without seeds, was blended in the dark with 95% ethanol for 3–5 min, depending on tissue softness, at maximum speed with a blender. The homogenate was vacuum-filtered through Whatman No. 1 filter paper and the residue washed twice with 80% ethanol. The filtrates were combined and adjusted to 5 ml/g fresh weight (FW) with ethanol. Henceforth, this is considered the ethanolic extract.

2.3. HPLC analysis of sugars and organic acids

Sugars and organic acids were analysed in a Hewlett-Packard 1090 liquid chromatograph equipped with a photodiode array detector and a Waters 410 differential refractometer (Millipore) connected in series. Data were processed by means of Hewlett-Packard 85-B computing system and a Beckman Analogue Interface Module 406 and Gold V.711 software, respectively. Isocratic separations of the compounds were made on a stainless steel Ion-300 (300 mm \times 7.8 mm, 10 µm) column, containing a cation-exchange polymer in the ionic hydrogen form, with an IonGuard GC801 guard column (Interaction, San Jose, CA), and thermostatted at 23 °C. The mobile phase utilized for the elution consisted of a filtered

Maturity	Harvest no.	Harvest date	Days after full bloom (DAF)	State of fruit maturity
Immature		15 June 2000	39	Unripe, skin light green-greenish, pulp white, fruit hard
		15 July 2000	69	Unripe, skin ca. 60% light brownish and 40% pale greenish, pulp white, fruit hard
Mid ripe		15 August 2000	100	Unripe, skin completely brownish, pulp white, fruit hard
		15 September 2000	131	Table ripe, skin completely brownish, pulp white
Ripe		15 October 2000	161	Ripe, skin completely brownish, pulp white

Table 1 Collection dates, stages as days after full bloom (DAF) and the colour of fruit with state of fruit maturity of medlar (Mespilus germanica L.)

 $(0.22 \mu m \text{ nylon})$ and degassed solution of 0.0085 N H₂SO₄ and a flow rate of 0.4 ml/min. UV detection was selected at 195 and 245 nm, the refractive index detector was used at sensitivity $16x$, and the injection volume was 20μ .

2.4. Protein and amino acid analysis

Samples were ground to a fine powder with the aid of a mortar and pestle and dried under vacuum at 25° C until a constant weight was reached. Each sample was analyzed in triplicate. Samples of 2–3 mg of each fruit specimen were placed in three 2-ml glass ampoules containing 50 ml of internal standard (norleucine) and 0.35 ml of 6 N HCl. The ampoules were frozen using liquid nitrogen, evacuated, sealed, and then placed in an oven at 110 °C for 24 h. The ampoules were allowed to cool and were then placed in a vacuum centrifuge to remove acid. Samples were redissolved in 0.4 ml of 1 mM HCl, and a 20-µl aliquot was removed from each ampoule for derivatization. A single set of samples was analysed separately for cysteine content. These samples were first oxidized with performic acid [\(Hirs, 1967\)](#page-6-0) at room temperature for 18 h. Performic acid was removed in a vacuum centrifuge and samples were hydrolysed according to the technique described earlier.

Tryptophan analysis was performed separately on a single set of samples. Dried plant specimens were placed in polypropylene tubes and hydrolysed in 4.2 M KOH containing 1% thiodiglycol (w/v) [\(Hugli & Moore,](#page-6-0) [1972\)](#page-6-0) at 110 \degree C for 18 h. The KOH was neutralized with 4.2 M perchloric acid. The supernatant was then removed and adjusted to pH 3 with dilute acetic acid, and then a 50-µl aliquot was removed for derivatization. Quantitative analysis was performed using a Pierce standard H amino acid calibration mixture suplemented with tryptophan. Norleucine was used as the internal standard in all determinations.

The Pico-tag system (Waters, Millfor, MA) was used for quantification of amino acids. Aliquots were dried and hydrolysed, mixed with $10 \mu l$ of redrying solution (ethanol:water:triethylamine, 2:2:1), and dried again. The samples were then reacted with $20 \mu l$ of phenylisothiocyanate (water:ethanol:triethylamine:phenylisothiocyanate, 7:1:1:1) at room temperature for 20 min [\(Cohen & Straydom,](#page-6-0)

[1988](#page-6-0)), and then excess reagent was removed in a vacuum centrifuge. Derivatized samples were dissolved in 0.1 ml of 0.14 M sodium acetate that had been adjusted to pH 6.4 with acetic acid. A 10 -µl aliquot was injected into the column for analysis. A Waters C_{18} column (3.9×300) mm) was used with conditions described by [Buzzigoli et](#page-6-0) [al. \(1990\)](#page-6-0) in order to obtain complete resolution of tryptophan and the ornithine produced as a result of alkaline hydrolysis of arginine. Analysis of all other amino acids was conducted using a Waters C18 column $(3.9\times150$ mm) with gradient conitions described by [Bidlingmeyer, Cohen, and Tarvin \(1984\).](#page-5-0) Egg white lysozyme was used as the control protein.

3. Results and discussion

Fructose, glucose and sucrose were the major soluble sugars. The level of fructose was 197 mg 100 g^{-1} fresh weight at 39 DAF, and then increased continually throughout ripening, reaching a maximum level of 1200 mg 100 g^{-1} fresh weight in the ripest fruit at 161 DAF. Despite decreasing to its minimum level (219 mg 100 g^{-1} fresh weight) at 161 DAF, sucrose level increased throughout immature and mid-ripe maturity stages, ranging between 159 and 918 mg 100 g^{-1} fresh weight between 39 and 131 DAFs. Although there was a sudden increase in the level of glucose at immature maturity (at 69 DAF) and a sudden decrease at mid-ripe maturity at 100 and 131 DAFs, the level of glucose reached a value of 686 mg 100 g^{-1} fresh weight in the ripe medlar harvested at 161 DAF (15 October). A continual increase in total sugar content (sum of individuals sugars) of the fruit was determined, reaching a maximum value of 2105 mg 100 g^{-1} fresh weight by the mid-October (161 DAF) ([Table 2](#page-3-0)).

There have been numerous studies attributed to increasing levels of fructose, glucose and sucrose at advanced stages of fruit maturity. In an apple variety (var. Glockenapfel) ([Ackerman et al., 1992](#page-5-0)), the content of sucrose increased through ripening, and also after several weeks of storage, reaching its maximum of 6000 mg 100 g^{-1} dry weight, while the contents of fructose and glucose (fructose being the major soluble sugar)

Table 2

Sugar and organic acid compositions (mg/100 fresh weight) of medlar (Mespilus germanica) fruit at various stages of development and maturation. Results are expressed as the means \pm S.D. of three separate extractions and determinations

Compounds	Immature		Mid ripe		Ripe	
	39 DAF	69 DAF	100 DAF	131 DAF	161 DAF	
Sucrose	$15 + 0.7$	$184 + 0.3$	$572 + 0.4$	$918 + 0.2$	$219 + 0.6$	
Fructose	$197 + 0.2$	230 ± 0.9	$437 + 1.8$	$722 + 0.3$	$1200 + 2.3$	
Glucose	$298 + 0.3$	$788 + 0.3$	619 ± 0.3	$352 + 0.1$	686 ± 0.8	
\sum Sugar ^a	$654 + 0.4$	$1202 + 0.5$	$1628 + 0.5$		$1992 + 0.2$ 2105 + 1.2	
Citric acid	$528 + 0.1$	$318 + 0.2$	$418 + 0.0$	$486 + 0.0$	404 ± 0.2	
Malic acid	$126 + 0.1$	$226 + 1.2$	$309 + 0.3$	$374 + 0.4$	$428 + 0.3$	
Ascorbic acid	41.7 ± 0.1	17.0 ± 0.0	13.2 ± 0.0	11.3 ± 0.0	8.4 ± 0.0	
\sum Acid ^b	$692 + 0.1$	$561 + 0.6$	$740 + 0.1$	$872 + 0.1$	$841 + 0.3$	

 \sum Sugar is the sum of sucrose, glucose and fructose.

 \overline{D} Acid is the sum of citric, malic and ascorbic acids.

decreased slightly toward the end of ripening (just before harvest) to \sim 3000 and 500 mg 100 g⁻¹ dry weight, respectively. The same authors explained the rapid increase in the glucose concentration by a mechanism related to starch synthesis and hydrolysis, and the sudden drops of all three sugars at the beginning of the storage period were explained by the fact that apples were harvested just before the climacteric. In two particular mayhaw (Crataegus aestivalis and C. opaca) fruits, major levels of fructose and glucose increased from the immature to ripe stage, beginning from ~ 0.8 and 1.1 to ~ 1.6 and 1.5% at the ripe stage, respectively ([Chapman & Horvath, 1993](#page-6-0)). Apart from some fluctations that occurred during early stages of fruit growth (80 and 95 DAFs), sucrose content increased continually during maturation of peach fruit soaring to 6 and 7% of fresh weight at 135 DAF, while the contents of both fructose and glucose were around 1 and 2% of fresh weight [\(Chapman & Horvath, 1990\)](#page-6-0). [Senter et al. \(1991\)](#page-6-0) reported progressive increases in both fructose and glucose levels throughout fruit development and maturation in several persimmons (except Kijiro in which the sugars remained fairly constant) and more especially in the fruit of Fuyu cultivar, in which the levels were $9-22.2$ and $12-24.4$ g/100 g dry weight (immature to ripe). In addition, sucrose content showed some fluctations (Giambo and A. Shiraza), sudden drop (Fuyu and Jiro) and increase (Giambo) and sometimes maintaining levels (22.0 g 100 g⁻¹ dry weight, in Ichi Kjiro cv.) equal to the levels of fructose and glucose at ripe maturity.

Arecent study of Spanish medlar has revealed a progressive increase in fructose and glucose contents through September and November (8 week period during maturation, 1-week intervals), ranging from 21.6 to 34.7 $g/100$ g dry weight for fructose and 12.8 to 21.2 $g/$ 100 g dry weight for glucose. Besides these two sugars,

sucrose content (except during the second week of maturity) increased until the sixth week of ripening (4.53 $g/100$ g dry weight), and then, as the medlar ripened, it levelled to 1.24 g/100 g dry weight at the eighth week of maturation ([Romero-Rodriguez et al., 2000\)](#page-6-0).

It was concluded that the concentration of sucrose, rather than glucose or fructose, most nearly parallels the rate of respiration of a fruit during its development. Sucrose can be replaced by glucose, but fructose seems to be more effective than these two sugars in promoting fruit set [\(Nitsch, 1953](#page-6-0)).

Citric, malic and ascorbic acids, the major organic acids in medlar, were determined, and their levels changed significantly during fruit development. The levels of citric and malic acids were 528 and 126 mg 100 g^{-1} fresh weight at the beginning of immature maturity stage (at 39 DAF, 15 June), and then the malic acid level increased continually, reaching a maximum value of 428 mg 100 g^{-1} fresh weight in the ripe maturity harvested at 161 DAF (15 October). The level of citric acid remained high, reaching a value of 486 mg 100 g⁻¹ fresh weight at midripe stage (131 DAF); it then decreased to a level of 404 mg 100 g^{-1} fresh weight at 161 DAF. Ascorbic acid exhibited a steady decrease throughout maturation, beginning with a value of 41.7 mg 100 g^{-1} fresh weight and reaching 8.4 mg 100 g^{-1} fresh weight at 161 DAF in the ripe fruit. The total acid content (sum of individuals acids) of the fruit throughout development and maturation showed fluctations, reaching a maximum value of 871 mg/100 g fwt by the mid-September (131 DAF) and remaining thereafter at a value of 841 mg 100 g^{-1} fresh weight in the fully ripe fruit at 161 DAF (Table 2).

It has been reported that the content of quinic acid was highest (1.2% of fresh weight) at the beginning of peach maturation and then, after a continual decrease throughout maturation, remained low (ca. 0.2% fresh weight) in the ripest peach fruit. However, citric acid content continually declined in parallel with quinic acid after 110 DAF. Malic acid content, after fluctuating around 0.2–0.3% of fresh weight, jumped to a maximum (0.5% of fresh weight) and again started to decline, keeping its content about 0.4% of fresh weight [\(Chapman & Horvath, 1990](#page-6-0)). It is known that malic acid is the predominant acid during maturation of Crataegus fruits (Chapman et al., 1991) and, comparatively, the level of this acid in two cvs of Crataegus declined more rapidly in C. apaca than in C. aestivalis, as did the content of quinic acid, while citric acid, the second most abundant acid, showed an increase at the midripe stage, followed by a decrease at the ripe stage [\(Chapman &](#page-6-0) [Horvath, 1993\)](#page-6-0).

[Ackerman et al. \(1992\)](#page-5-0) reported citric acid (\sim 8 to 5 g 100 g^{-1} fresh weight) as the most and malic acid (1–0.6) g 100 g^{-1} fresh weight) as the second most abundant acid determined in an apple variety (Glockenapfel)

throughout ripening and storage in a decreasing trend. Persimmons ([Senter et al., 1991](#page-6-0)) showed a fair increase in their malic acid contents and a decrease in citric acid contents, except Ichi Kijiro and Aizumi cultivars, in which citric acid content was roughly the same during development. Consequently, we conclude that medlar fruit exhibited a steady increase in malic acid content and decrease in ascorbic acid content during development and maturation, while the level of citric acid did not show such behaviour.

The methodology used in the present study for the separation of both sugars and organic acids in a single injection was previously evolved and used in strawberries and other fruits (Pérez et al., 1997). This analytical procedure is fairly simple and rapid and allows for the detection and quantitation of the main sugars and organic acids found in fruits, in general. The HPLC column utilized was found to be reliable and operated well under continual use.

3.1. Amino acid composition during fruit ripening

Table 3 summarizes the contents of 18 of the amino acids commonly found in proteins. Glutamine and asparagine are not reported since they are deaminated and converted into glutamine and aspartic acid, respectively, by the acid hydrolysis step that precedes column chromatography of the free amino acids. Our results have revealed that the total amount of most of the amino acids declined between 39 and 161 DAFs of development. Aspartate and glutamate were the major

amino acids in the ripe medlar harvested at 161 DAF (15 October). In general, the total amino acid content of the fruit was relatively low at all stages of development, ranging from 0.88 to 1.86% of dry weight. The protein content is relatively constant during the first three stages of development, but then declines by about one half by 161 DAF (15 October) ([Table 4](#page-5-0)).

In terms of nutritional quality, which was assessed by comparing the percentages of the essential amino acids versus those of a World Health Organization ([WHO\)](#page-6-0) standard protein, as shown in [Table 4](#page-5-0), the protein of medlar at all stages of maturation compares well with that of the WHO standard. Only two of the amino acids or amino acid pairs had scores that fell below 100%, and these differed from standard protein by only a few percentage points (phenylalanine/tyrosine) or 16% (lysine). Noteworthy is the exceptionally high content of isoleucine in all five stages of the medlar specimens. Most of the common amino acids in fruits were present [\(Borroughs, 1970](#page-6-0)), asparagine, glutamine, and alanine being the three most prominent.

Asparagine and glutamine are the major N-transport compounds found in plants and consequently in fruits [\(Atkins, Pate, & Sharley, 1975](#page-5-0)). During ripening of strawberry, the level of asparagine was determined to be about 50% of total amino acid content ([Perez et al.,](#page-6-0) [1997\)](#page-6-0). [Kuneman, Braddock, and McChesney \(1988\)](#page-6-0) reported that strawberry might be distinquishable from other soft fruits by examining asparagine values. Aspartic acid and asparagine were found to be the principal amino acids and accounted for about 70% of

Table 3

Amino acid content (mg g^{-1} dry weight) of medlar fruit at various stages of development. Results are expressed as the means \pm SD of three separate extractions and determinations

Amino acids	Immature		Mid ripe		Ripe	
	39 DAF	69 DAF	100 DAF	131 DAF	161 DAF	
Trytophan	0.31 ± 0.002	0.23 ± 0.004	0.20 ± 0.025	0.22 ± 0.002	0.16 ± 0.004	
Threonine	0.95 ± 0.06	0.62 ± 0.085	0.65 ± 0.074	0.43 ± 0.014	0.49 ± 0.49	
Isoleucine	1.01 ± 0.086	0.79 ± 0.017	1.01 ± 0.01	0.73 ± 0.001	0.67 ± 0.016	
Lysine	0.99 ± 0.08	0.68 ± 0.28	0.67 ± 0.014	0.54 ± 0.013	0.45 ± 0.005	
Methionine	0.47 ± 0.016	0.26 ± 0.001	0.33 ± 0.011	0.24 ± 0.019	0.20 ± 0.012	
Cysteine	0.09 ± 0.013	0.08 ± 0.001	0.17 ± 0.01	0.06 ± 0.004	0.05 ± 0.001	
Phenylalanine	0.84 ± 0.07	0.61 ± 0.036	0.81 ± 0.02	0.52 ± 0.016	0.47 ± 0.005	
Tyrosine	0.24 ± 0.008	0.17 ± 0.025	0.26 ± 0.003	0.18 ± 0.011	0.15 ± 0.002	
Valine	1.05 ± 0.067	0.75 ± 0.039	0.88 ± 0.016	0.61 ± 0.014	0.57 ± 0.004	
Arginine	1.38 ± 0.142	1.00 ± 0.096	1.42 ± 0.083	0.68 ± 0.004	0.42 ± 0.009	
Histidine	0.34 ± 0.022	0.23 ± 0.013	0.31 ± 0.003	0.13 ± 0.012	0.11 ± 0.011	
Alanine	0.89 ± 0.052	0.67 ± 0.041	0.66 ± 0.041	0.41 ± 0.017	0.37 ± 0.003	
Aspartate	3.33 ± 0.219	2.69 ± 0.147	2.02 ± 0.007	1.51 ± 0.024	1.13 ± 0.045	
Glutamate	2.50 ± 0.363	2.21 ± 0.16	3.93 ± 0.376	1.53 ± 0.08	1.22 ± 0.015	
Glycine	0.72 ± 0.079	0.51 ± 0.057	0.79 ± 0.025	0.42 ± 0.007	0.38 ± 0.002	
Proline	0.99 ± 0.085	0.74 ± 0.051	0.94 ± 0.002	0.65 ± 0.011	0.63 ± 0.008	
Serine	0.99 ± 0.066	0.68 ± 0.072	0.82 ± 0.038	0.58 ± 0.007	0.54 ± 0.008	
Σ protein mg g ⁻¹ dry weight)	18.6	14.6	17.2	10.2	8.01	

Abbreviations of amino acids: ile, isoleucine; leu, leucine; lys, lysine; met+cys, methionine+cysteine; phe+thr, phenylalanine+threonine; thr, threonine; trp, tryptophan; val, valine.

^a WHO (1985).

^b AA, amino acid.

^c Average of 39–161 DAF.

the amino acid content in an apple variety, followed by glutamic acid/glutamine, serine, glycine, and phenylalanine (Ackerman et al., 1992). During the first 10 weeks of the apple maturation, they obtained a dramatic drop in amino acid content of the fruit flesh and then the values stayed more or less constant. They conclude that the variation is probably related to the processes of protein synthesis and degradation during maturation as well as to the dilution effect. [Tressl and Drawert \(1973\)](#page-6-0) have reported that leucine and valine concentrations increased about three-fold following the climacteric rise in strawberry cv. Chandler, whereas leucine was detected only in trace amounts in the cultivar. An increase in phenylalanine content during ripening was seen in banana [\(Tressl & Drawert, 1973\)](#page-6-0). More significant changes during ripening of strawberry fruit were found in alanine content which sharply increased between 30 and 39 DAFs, remained almost constant until 41 DAF, and decreased in fully ripe fruits ([Perez, Rios, Sanz, &](#page-6-0) [Olias, 1992\)](#page-6-0). As a result, the protein content of medlar was lower at all stages of medlar fruit (about 2%) development. However, the quality of the medlar protein seemed to be good relative to the WHO standard, indicating that its nutritional quality was relatively high.

4. Conclusions

In conclusion, there are significant differences in the levels of sugars, organic acids and amino acids of medlar fruit, between maturity stages during fruit development. Medlar fruit contains important amounts of flavouring amino acids, sugars and organic acids that might play a significant role in its flavour. It can be recommended that a harvest date for medlar fruit, in the middle of October at ripe stage, will give appropriate levels of sugars, organic acids and amino acids for people who consume the fruit in their diets. However some climatic and agronomic changes can occur and need to be examined. This type of information could provide local populations (where medlar is a native species in their flora) with a basis for food choices.

Acknowledgement

Support by the Scientific and Research Council of Turkey (TUBITAK)-NATO is greatly appreciated.

References

- Ackerman, J., Fischer, M., & Amado, R. (1992). Changes in sugars, acids, and amino acids during ripening and storage of apples (cv. Glockenapfel). Journal of Agricultural and Food Chemistry, 40, 1131–1134.
- Ashoor, S. H., & Knox, J. M. (1982). Determination of organic acids in foods by high-performance liquid chromatography. Journal of Chromatography, 299, 288–292.
- Atkins, C. A., Pate, J. S., & Sharkey, P. J. (1975). Asparagine metabolism-key to the nitrogen nutrition of developing legume seeds. Plant Physiology, 56, 807–812.
- Ayaz, F. A., Glew, R. H., Huang, H. S., Chuang, L. T., VanderJagt, D. J., & Strnad, M. (2002). Evolution of fatty acids in medlar (Mespilus germanica L.) mesocarp at different stages of ripening. Grasas y Aceites, 53, 352–356.
- Ayaz, F. A., & Kadioglu, A. (1998). Nonvolatile acid composition during fruit development of Diospyros lotus L. Turkish Journal of Botany, 22, 69–72.
- Ayaz, F. A., Kadioglu, A., Bertoft, E., Acar, C., & Turna, I. (2001). The effect of fruit maturation on sugar and organic acid composition in two blueberries $(V.$ arctostaphylos L. and V. myrtillus L.) native to Turkey. New Zealand Journal of Crop and Horticultural Science, 29, 137–141.
- Baytop, T. (1999). Türkiye'de Bitkiler ile Tedavi, Geçmi°te ve Bugün [Therapy with medicinal plants in Turkey-past and present] (2nd ed.). Capa-Istanbul: Nobel Tip Basimevi.
- Bidlingmeyer, B. A., Cohen, S. A., & Tarvin, T. L. (1984). Rapid analysis of amino acids using precolumn derivitization. Journal of Chromatography, 336, 93–104.

Bignami, C. (2000). Il nespolo comune. L'Informace Agrario, 25, 43– 46.

- Borroughs, L. F. (1970). Amino acids. In A. C. Hulme (Ed.), Biochemistry of fruits and their products. London: Academic Press.
- Browicz, K. (1972). Mespilus L. In P. H. Davis (Ed.), Flora of Turkey and the East Aegean Islands, Vol. 4 (pp. 128–129). Edinburgh: Edinburgh University Press.
- Buzzigoli, G., Lanzone, L., Ciorciaro, D., Fascerra, S., Cerri, M., Scandroglio, A., Coldani, R., & Ferrannini, E. (1990). Characterization of a reversed-phase high performance liquid chromatographic system for determinationof blood amino acids. Journal of Chromatography, 507, 85–93.
- Chapman, G. W., & Horvat, R. J. (1990). Changes in nonvolatile acids, sugars, pectin and sugar composition of pectin during peach (cv. Monroe) maturation. Journal of Agricultural and Food Chemistry, 38, 383–387.
- Chapman, G. W., & Horvat, R. J. (1993). Chemical compositional changes in two genetically diverse cultivars of mayhaw fruit at three maturity stages. Journal of Agricultural and Food Chemistry, 41, 1550–1552.
- Chapman, G. W., Horvat, R. J., & Payne, J. A. (1991). The nonovdatile acid and sugar composition of mayhaw fruits (Crataegus aestivalis, C. opaca, C. rufula). Journal of Food Quality, 14, 435–439.
- Cohen, S. A., & Straydom, D. J. (1988). Amino acid analysis utilizing phenylisothiocyanate derivatives. Analytical Biochemistry, 174, 1– 16.
- de Pascual-Teresa, S., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2000). Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. Journal of Agricultural and Food Chemistry, 48, 5331–5337.
- Glew, R. H., Ayaz, F. A., VanderJagt, D. J., Millson, M., Dris R., & Niskanen, R. Mineral composition of medlar (Mespilus germanica) fruit at different stages of maturity. Journal of Food Quality (in press).

Hirs, C. W. H. (1967). Performic acid oxidation. Methods Enzymology, 1, 197–199.

- Hugli, T. E., & Moore, S. (1972). Determination of tryptophan content of proteins by ion exchange chromatography of alkaline hydrolysates. Journal of Biological Chemistry, 247, 2828–2834.
- Kuneman, D. W., Braddock, J. K., & McChesney, L. L. (1988). HPLC profile of amino acids in fruits juices as their (1-fluoro-2,4 dinitrophenyl)-5-L-alanine amide derivatives. Journal of Agricultural and Food Chemistry, 36, 6–9.
- Nitsch, J. P. (1953). The physiology of fruit growth. Annual Review of Plant Physiology, 4, 199–236.
- Pérez, A. G., Olías, R., Espada, J., Olías, J. M., & Sanz, C. (1997). Rapid determination of sugars, nonovolatile acids, and ascorbic acid in strawberry and other fruits. Journal of Agricultural and Food Chemistry, 45, 3545–3549.
- Pérez, A. G., Rios, J. J., Sanz, C., & Olías, J. M. (1992). Aroma components and free acids in starwberry variety Chandler during ripening. Journal of Agricultural and Food Chemistry, 40, 2232–2235.
- Romero-Rodriguez, A., Simal-Lozano, J., Vazguez-Oderiz, L., Lopez-Hernandez, J., & Gonzalez-Castro, M. J. (2000). Physical, physicochemical and chemical changes during maturation of medlars and persimmons. Deutsche Lebensmittel-Rundschau, 96, 142–145.
- Senter, S. D., Chapman, G. W., Forbus, W. R., & Payne, J. A. (1991). Sugar and nonvolatile acid composition of persimmons during maturation. Journal of Food Science, 56, 989–991.
- Tressl, R., & Drawert, F. (1973). Biogenesis of banana volatiles. Journal of Agricultural and Food Chemistry, 21, 562–565.
- WHO. (1985). WHO/FAO report: Energy and protein requirements. WHO technical report series no. 724. Geneva: World Health Organization.
- Wrolstad, R. E. (1981). Use of sugar, sorbitol, and nonvolatile acid profile in determining the authenticity of fruit juice concentrates. In Proceedings of the Symposium on Technological Problems of Fruit Juice Concenrates (pp. 27–39). Corvallis, OR: Oregon State University.