Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Determination of fruit characteristics, fatty acid profile and total antioxidant capacity of *Mespilus germanica* L. fruit

Hale Seçilmiş Canbay[1*], Ersin Atay[2], Serdal Oğüt[3]

[1]*Research and Practice Center, Mehmet Akif Ersoy University, 15100, Burdur, Turkey*

[2]*TAGEM, Furuit Research Institue, 32500, Isparta Turkey*

[3]*Department of Nutrition and Dietetics, Aydin Health School, Adnan Menderes University, Aydin, Turkey*

ARTICLE INFO

ABSTRACT

Objective: To determine fruit characteristics, fatty acid profile and total antioxidant capacitiy of first cultured *Mespilus germanica* L.
Methods: A total of 15 fruits were taken randomly from four directions of adult trees. Then the physical and chemical properties of first cultured medlar fruit (Istanbul/Turkey) were measured by using refractometer, colorimeter, spectrophotometer and gas chromatograph mass spectrometer, respectivly.
Results: In the fruit studied, the results showed that palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid and behenic acid were the most abundant fatty acids (FAs), and the main FA was palmitic acid [(35.35 ± 1.20)%]. The percentage of linoleic acid and stearic acid in this fruit oil were (29.10 ± 1.70)% and (8.53 ± 0.25)%, respectively. As a result of the analysis, the total antioxidant capacity of medlar fruit was (1.1 ± 0.2) mmol trolox equivalents/L.
Conclusions: The present study has demonstrated the concentrations of FAs and the antioxidantive capacity of first cultured Istanbul medlar fruits by using many tested methods. It is proved that in our daily life, medlar fruit plays a significant role with its nutrition and health effect.

1. Introduction

Medlar is the fruit of *Mespilus germanica* L. (*M. germanica*) in the family of Rosaceae. It consists of two species which are widespread in the Asia, southeastern part of Europe and Northern America[1]. It grows poorly in frost-free areas, poor soils and on rocks. In Turkey, this kind of fruit grows wildly in different regions, especially in the Northern and Western Anatolia and Marmara regions[2].

Medlar fruit has been gaining commercial importance as a foodstuff for human consumption, and nutrient content during fruit maturation and ripening stage[3-6]. Medlar grows to spiny shrub or small tree (2–3 m), with a height of 4–7 m in cultivated form and has long leaves. The brown, sometimes reddish tinged fruit of medlar with yellowish or brown stony seeds (1–1.25 cm in lenght) are subglobose or pyriform. It is crowned by foliaceous sepals ranging from 1.5 to 3 cm in diameter and weighing from about 10 g to more than 80 g[1,2]. The fruit is harvested from the medlar trees in October, stored as a part of the crop in cold, dark and aerated places, to bring on the fruit to soften[5-7]. Medlar fruits are very rich sources of bioactive compounds such as phenolics, and also be of different fatty acids (FAs). Fruits and vegetables are elementary food sources providing essential nutrients for sustaining life. They also contain a variety of phytochemicals which provide important health benefits[8].

Phenolics and lipids are natural components of many fruits and vegatables. They play an important role in maintaining fruit quality (characteristic aroma and flavors) and determining nutritive value. Regular consumption of fruits and vegetables is associated with reduced risks of chronic diseases, such as cancers and cardiovascular disease. Lipids are partly accountable for the physical and chemical properties of foods. Many lipid characteristics in foods are explained in terms of their fatty acid composition[4]. Many kinds of FAs play a key role in trace quantities in the regulation of a variety of physiological, pharmacological and biological effects on human health. In fact, they were benificial to the reduction of coronary artery disease, so they are commercialized as nutraceuticals under the form of capsules or used as ingredient in infant food products. The FAs composition of food is very important because lipids are one of the three major constituents of food. Their roles in biological tissues are: (a) source of energy; (b) components of biological membranes; (c) precursor for many different molecules and (d) transport vehicle for vitamin A, D, E and K[9]. The main sources of lipids in our diet are vegetable and different foodstuffs

　*Corresponding author: Hale Seçilmi Canbay, Research and Practice Center, Mehmet Akif Ersoy University, 15100, Burdur, Turkey.
　Tel: +90 248 211 3244
　E-mail: halecanbay@gmail.com

of animal source oils[9]. Fruit usually contains minor amounts of lipids. Lipid components in fruits are presumed to contribute to characteristic aroma and flavor during ripening. These are essentially considered as precursors for various odorous volatile compounds and also contribute to nutritional value of fruit. Medlar fruits can contain the FAs[4]. Prior to the analysis, lipids were obtained by different solvent extraction. The obtained lipids are then converted into fatty acid methyl esters (FAMEs) for gas chromatography/flame ionization detector or gas chromatography-mass spectrometer (GC-MS) analysis. Many different methylation and silylation methods are described in papers[4,7]. A free radical is any atom or molecule that has a single unpaired electron in an outer shell. Free radical-induced oxidative stress has been associated with several toxic cellular processes including oxidation damage to protein and DNA, membrane lipid oxidation, enzyme inactivation, and gene mutation that may lead to carcinogenesis[10]. Antioxidants are reducing agents, and limit oxidative damage to biological structures by passivating free radicals. They are compounds to be added to lipids and lipid-containing foods to increase their shelf-life by retarding the process of lipid peroxidation. Also, they have been widely used as food additives to avoid food degradation, and played an important role in preventing many lifestyle-related diseases and aging, being closely related to the formation of reactive oxygen species (ROS) and to lipid peroxidation[11]. Production of ROS during normal cell metabolism is a normal and necessary process that provides important physiological functions. An imbalance between ROS production and antioxidant defences results in oxidative stress which has been recognized as playing a prominent role in the causation of several age-related and chronic diseases, neurodegenerative and cardiovascular diseases. Intake of sufficient amounts of antioxidants is necessary to prevent and/or inhibit these harmful reactions. It has been reported that most of the antioxidant capacity of fruits and vegetables may come from total phenolics, anthocyanins, and flavonoids[12]. Several methods have been developed to determine the antioxidant potential of fruits and vegetables products. The trolox equivalent antioxidant capacity by using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as an oxidant, the ferric reducing antioxidant power, the 2,2'-diphenyl-1-picrylhydrazyl free radical scavenging potential, the oxygen radical absorption capacity, the total radical absorption potentials, and the photochemiluminescence assays are some of the most commonly used assays. Antioxidants can reduce radicals primarly by two mechanisms: single electron transfer and hydrogen atom transfer[13]. Ayaz *et al*. have analyzed the changes in FA composition[4]. Altuntaş *et al*. have described some of the physical (dimension, geometric mean diameter, sphericity, bulk density) and chemical (moisture, crude protein, energy) properties of medlar fruit[1]. Also, they have reported the mineral content (Al, B, Ca, Cr, Fe, In, K, P, Pb, S, Se, Ti, V, Zn) of medlar fruit and Glew *et al*. have described the changes of sugars, organic acids, and amino acids in medlar during fruit development and maturation[5-7]. The polyphenol contents and antioxidant properties of medlar have been investigated by Gülcin *et al*.[11]. The aim of our study was to determine the fruit characteristics, FA composition and the total antioxidant capacity of first cultured Istanbul medlar fruits. Experiments were carried out on "Istanbul"/quince A rootstock/scion combination. "Istanbul" was the only local medlar variety registered to Variety Registration and Seed Certification Center of Turkey, which associated with the International Union for the Protection of New Varieties of Plants.

2. Materials and methods

2.1. Chemicals and reagents

Methanol (HPLC grade), chloroform (HPLC grade) and *n*-hexane (HPLC grade) were obtained from Merck (Dermstadt, Germany).

NaCl and HCl were also purchased from Merck (Dermstadt, Germany).

2.2. Sample description

This study was carried out over 2 consecutive years (2008–2009). Fruits were taken from adult trees (9 years old in 2008) of the Istanbul medlar variety grafted onto quince A rootstock. The orchard planted in Fruit Research Institue in Isparta (Eğirdir) in Turkey is located at 37°49' N and 30°52' W. Its elevation is 940 m high. In the region, summers are hot and rarely rainy while winters are cold and rainy. Springs and autumns on the other hand show mild and rainy climate characteristics. The soil of the research area had a loamy character.

2.3. Physical and chemical analysis of fruits

A total of 15 fruits were taken randomly from four directions of trees. The diameter and length of fruit were measured as millimeter with the help of a digital compass. In order to determine the average fruit weight, samples were weighted after harvesting one by one. The rates of soluble solids content (SSC) were measured by refractometer (Hanna, HI 96801).

The skin colors of the fruits were measured by using Minolta CR-400 (Konica Minolta) color device. Color measurements were done through two different areas of the equatorial part of the fruits in a way that forms an angle of 180° between them. The mean of the two measurements was taken as the mean value of a fruit. Color measurements were made only in 2009.

2.4. Extraction

For the extraction of lipid in *M. germanica*, the liquid extraction method was used. The lipid extraction of *M. germanica* were performed as described by Chamberlain *et al*. with minor modifications[14]. Samples of finely ground powder of medlar fruit (10 g) in triplicate were weighed and extracted with 30 mL chloroform: methanol (2:1, v/v), and a saline solution (NaCl, 0.9%) was added at a rate of 20% of the extraction volume by using an Heidolph Diax 900 homogeniser (Burladingen, Germany). After extraction, the extracts were evaporated by rotary vacuum evaporation (Laborta 4001, Germany) at 40 °C and stored at -20 °C until the time of analysis.

2.5. Preparation of FAMEs

A preparation step was necessary prior to introduction of the oil into the GC-MS for the individual determination of FA composition. FAMEs were obtained by trans-esterification with 1.5 mol/L HCl in methanol and 100 μL oil were mixed and shaken vigorously for 15 min in Bandelin ultrasonic shaker (Berlin, Germany)[15]. Oil was methylated for 2 h at 80 °C, and then 1 mL hexane was added to collect the FAME in hexane as above and FAME was analysed by GC-MS.

2.6. Analysis of FAME by GC-MS

GC analysis were performed on GC-17A (Shimadzu, Kyoto, Japon) equipped with mass spectrometry detector (QP5050, Shimadzu, Kyoto, Japon) and split/splitless injector. The injector and detector temperatures were fixed at 250 °C. The split ratio was 1:20 and the flow-rate of carrier gas was 2 mL/min. A CP-Wax (50 m × 0.32 mm ID, 0.32 μm film thickness) fused capillary column (Varian, Lake Forest, USA) and a GC-MS Real Time Analysis software system (Shimadzu, Kyoto, Japon) were used for analysing

FAMEs. The temperature for the column was hold at 60 °C for 1 min, increased by 13 °C/min to 175 °C, increased at 4 °C/min to 215 °C, and then hold at 215 °C for 35 min. The total run took 86 min.

2.7. Analysis of total antioxidant capacity by UV-visible spectrophotometer

Total antioxidant levels were measured by the Erel methods (Rel Assay Diagnostic, Turkey). These methods were automatic and colorimetric. The total antioxidant status measurement was based on the bleaching of the characteristic colour of a more stable ABTS radical cation by antioxidants[16]. Analysis of samples therefore involved spectrophotometric measurement of this reaction in the automatic analyzer (Perkin Elmer, UV-visible spectrophotometer model Lambda 20). Total antioxidant capacity measurements were carried out by spectrophotometer at 660 nm.

3. Results

3.1. Physical and chemical analysis

Colour is one of the most important quality components of fresh fruits. Fruit ripening is a complex, genetically programmed process that culminates in dramatic changes in texture, colour, flavour and aroma. Color spaces and numerical values are used to create, represent and envision colors in two and three dimensional field. Usually, the color of foods has been measured in $L^*a^*b^*$. The $L^*a^*b^*$ color space or CIELAB is an international standard for color measurements, adopted by the Commission Internationale d'Eclairage in 1976. L^* is the luminance or lightness component, which ranges from 0 to 100, and parameters a^* (from green to red) and b^* (from blue to yellow) are the two chromatic components, which range from -120 to 120[17]. The L*C*h color space uses the same diagram as the L^*a^*b color space, but uses cylindrical coordinates in place of rectangular coordinates. In this color space, C^* is chroma. The value of C^* is 0 at the center and increases according to the distance from the center. Hue angle is defined as starting at the $+a^*$ axis and is expressed in degrees[18]. The dimensional properties of medlar are given in Table 1.

Table 1

Dimensional properties of *M. germanica*.

Year	Diameter (mm)	Length (mm)	Weight (g)	SSC (%)	Color values				Hue angle (°)
					L^*	a^*	b^*	C^*	
2008	32.3	32.2	21.6	19.2	51.0	8.3	27.9	29.0	72.3
2009	31.2	31.9	18.6	19.3	51.5	8.2	27.8	29.1	72.4

3.2. FAs of medlar fruit

The exracted FAs, methyl esters and the derivatives of FAs compositions were determined by GC-MS. The identification of componenets are given in Table 2.

In the medlar fruit, 19 different FAs were studied during the two-years harvest where some of the FAs such as capric acid (10:0) and tridecanoic acid (13:0) were not detected. Fruit species have characteristic FA compositions and profiles during development and ripening. In general, the palmitic acid (16:0), stearic acid (18:0), linoleic acid (18:2n-6) linolenic acid (18:3n-3), oleic acid (18:1n-9), arachidic acid (20:0) and behenic acid (22:0) are the most predominant and abundant FAs. The results had shown that the main FA was palmitic acid with percentages of (35.35 ± 1.20)%. The percentage of linoleic acid and stearic acid in this fruit oil were (29.10 ± 1.70)% and (8.53 ± 0.25)%, respectively. The quantities of unsaturated FAs in medlar fruits were high and percentages of

unsaturated FAs in fruit oil were (40.13 ± 2.03)%.

Table 2

FA compositions of *M. germanica* fruit. %.

Component name	Percentage
C10:0 (Capric acid methyl ester)	n.d.
C12:0 (Lauric acid methyl ester)	0.80 ± 0.11
C13:0 (Tridecanoic acid methyl ester)	n.d.
C14:0 (Myristic acid methyl ester)	1.50 ± 0.02
C14:1 (Myristoleic acid methyl ester)	0.30 ± 0.09
C15:0 (Pentadecanoic acid methyl ester)	0.10 ± 0.01
C16:0 (Palmitic acid methyl ester)	35.35 ± 1.20
C16:1 (Palmitoleic acid methyl ester)	0.30 ± 0.03
C18:0 (Stearic acid methyl ester)	8.53 ± 0.25
C18:1n-9 (Oleic acid methyl ester)	4.35 ± 0.37
C18:1n-7 (Vaccenic acid methyl ester)	0.85 ± 0.11
C18:2n-6 (Linoleic acid methyl ester)	29.10 ± 1.70
C18:3n-3 (α-Linolenic acid Methyl ester)	4.93 ± 0.79
C20:0 (Arachidic acid methyl ester)	3.20 ± 0.85
C20:1n-9 (cis-11-Eicosenoic acid methyl ester)	0.12 ± 0.08
C20:2n-6 (cis-11,14-Eicosadienoic acid methyl ester)	0.11 ± 0.01
C22:0 (Behenic acid methyl ester)	4.00 ± 0.75
C22:1n-9 (Erucic acid methyl ester)	0.50 ± 0.03
C24:0 (Lignoceric acid methyl ester)	2.50 ± 0.25
% Unsatisfactory	40.13 ± 2.03
% Satisfactory	55.70 ± 2.05

n.d.: Not detected.

3.3. Total antioxidant capacity

Analysis of total antioxidant capacity of medlar fruit was carried out by using spectrophotometer. Antioxidant activity was measured by using the ABTS test in the medlar fruit after the calculation on trolox equivalents. As a result of the analysis, the total antioxidant capacity of medlar fruit was (1.1 ± 0.2) mmol Trolox equivalents/L. Antioxidants were known to participate in reducing reactions. The present study has demonstrated concentrations of FAs and the antioxidantive capacity of first cultured Istanbul medlar fruits. Medlar fruit played a significant role in its nutrition and health effect.

4. Discussion

While the fruit diameter and weight values of medlar fruits were found to be high, the length value was found similiar and in accordance with literature values[1]. SSC of medlar genotypes was previously reported between 12.5% and 26.0%[3,4,17,19,20]. Colors of medlar fruits (as hue) were reported between 61.92% and 80.54%[3].

Ayaz *et al*. reported palmitic acid and linoleic acid as the major FAs[4]. The percentages of palmitic acid and linoleic acid in very ripe soften fully dark brown medlar were 36.9% and 28.7%, respectively. Similarly, in the present work, we also found palmitic acid and linoleic acid the most predominant FAs. In medlar fruits, generally 18:2n-6, 18:1 and 18:3n-3 are predominant FAs throughout development and senescence[4-7], although their specific contents vary among species. The data reported here showed that the greatest changes in FA composition of medlar fruit oil took place during climactic fruit ripening. During fruit ripening and senescence, cytoplasmic structures reorganize within the cells, and cellular disorganization resulting from catabolism during senescence is accompanied by enzymatic breakdown of lipoprotein membranes[21,22]. These changes correlated with variations in lipid composition of cell membranes[22]. Decreases in the chemical constituents in fruits during ripening can be explained by two possible mechanisms. First, as a result of senescence,

rapid metabolic changes occur during fruit ripening. Ripening is considered to be an early stage in the senescence of climacteric fruits[23], and ethylene plays an important role in this process. Sometimes ripening proceeds in parallel with fruit softening, in which case ethylene appears to be involved in tissue softening during ripening and in degreening and color formation that occur in many fruits[24]. A second possible mechanism for the marked decline in the FA composition of fruit ripening could involve degradative lipolytic enzymes (*e.g.*, phospholipase D, phosphatidic acid phosphatase, lipolytic acyl hydrolase, and lipoxygenase). Such lipid-metabolizing enzymes are associated with microsomal membranes from senescing tissues. These enzymes are capable of degrading endogenous lipids in senescing membranes and causing many chemical changes in the lipid bilayer, including loss of lipid phosphate and acids, an increase in the ratio of sterol to FA, and a selective depletion of unsaturated FAs[25]. Several authors have reported a high correlation dependence between low-molecular phenolics and antioxidant activity in fruits[26,27]. The ABTS test used for the detection of antioxidant activity is based on the monitoring of the course of inactivation of the cation ABTS^{+}, which is produced by reacting ABTS stock solution with potassium persulfate. Ercisli *et al.* reported that antioxidant capacity (in α-carotene linoleic acid assay) were between 64.6% and 92.9%[3]. Rop *et al.* investigated that antioxidant capacity values were 100 to 180 ascorbic acid equivalent/g fresh matter[28].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

References

[1] Altuntaş E, Gül EN, Bayram M. The physical, chemical and mechanical properties of medlar (*Mespilus germanica* L.) during physiological maturity and ripening period. *J Agric Fac Gaziosmanpasa Univ* 2013; **30**: 33-40.

[2] Browicz K. *Mespilus* L. In: Davis PH, editor. *Flora of Turkey and the East Aegean Islands, Vol 4*. Edinburgh: Edinburgh University Press; 1972, p. 128-9.

[3] Ercisli S, Sengul M, Yildiz H, Sener D, Duralija B, Voca S, et al. Phytochemical and antioxidant characteristics of medlar fruits (*Mespilus germanica* L.). *J Appl Bot Food Qual* 2012; **85**: 86-90.

[4] Ayaz FA, Huang HS, Chuang LT, VanderJagt DJ, Glew RH. Fatty acid composition of medlar (*Mespilus germanica* L.) fruit at different stages of development. *Ital J Food Sci* 2002; **14**: 439-46.

[5] Glew RH, Ayaz FA, Sanz C, VanderJagt DJ, Huang HS, Chuang LT, et al. Changes in sugars, organic acids and amino acids in medlar (*Mespilus germanica* L.) during fruit development and maturation. *Food Chem* 2003; **83**: 363-9.

[6] Glew RH, Ayaz FA, VanderJagt DJ, Millson M, Dris R, Niskanen R. Mineral composition of medlar (*Mespilus germanica*) fruit at different stages of maturity. *J Food Qual* 2003; **26**: 441-7.

[7] Glew RH, Ayaz FA, Sanz C, VanderJagt DJ, Huang HS, Chuang LT, et al. Effect of postharvest period on sugars, organic acids and fatty acids composition in commercially sold medlar (*Mespilus germanica* 'Dutch') fruit. *Eur Food Res Technol* 2003; **216**: 390-4.

[8] Oktay M, Gülçin I, Küfrevioğlu Öİ. Determination of *in vitro* antioxidant

[9] Petrović M, Kezić N, Bolanča V. Optimization of the GC method for routine analysis of the fatty acid profile in several food samples. *Food Chem* 2010; **122**: 285-91.

[10] Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. *Toxicol Pathol* 2010; **38**: 96-109.

[11] Gülçin I, Mshvildadze V, Gepdiremen A, Elias R. Antioxidant activity of saponins isolated from ivy: alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F. *Planta Med* 2004; **70**: 561-3.

[12] Gülçin I, Bursal E, Sehitoğlu MH, Bilsel M, Gören AC. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. *Food Chem Toxicol* 2010; **48**: 2227-38.

[13] Ozgen M, Reese RN, Tulio AZ Jr, Scheerens JC, Miller AR. Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (abts) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. *J Agric Food Chem* 2006; **54**: 1151-7.

[14] Chamberlain J, Nelson G, Milton K. Fatty acid profiles of major food sources of howler monkeys (*Alouatta palliata*) in the neotropics. *Experientia* 1993; **49**: 820-4.

[15] Nejad MS, Niroomand A. Study on lipid changes of leaves and fruits olive adapted to high temperature condition Inkhuzestan. *Pak J Biol Sci* 2007; **10**: 4535-8.

[16] Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem* 2004; **37**: 112-9.

[17] León K, Mery D, Pedreschi F, León J. Color measurement in *L* *a* *b** units from RGB digital images. *Food Res Int* 2006; **39**: 1084-91.

[18] Konica Minolta. Precise Color Communication. Tokyo: Konica Minolta; 2015. [Online] Available from: http://www.konicaminolta.com/instruments/knowledge/color/part1/08.html [Accessed on 6th May, 2015]

[19] Cavusoglu A, Sulusoglu M. *In vitro* pollen viability and pollen germination in medlar (*Mespilus germanica* L.). *Int Res J Biol Sci* 2013; **2**: 49-53.

[20] Uzun I, Bayır A. Horticultural biodiversity in Turkey. *Bull UASVM Hortic* 2009; **66**: 536-43.

[21] Ayaz FA, Kadioğlu A. Fatty acid compositional changes in developing persimmon (*Diospyros lotus* L.) fruit. *N Z J Crop Hortic Sci* 1999; **27**: 257-61.

[22] Resende ECO, Martins PF, de Azevedo RA, Jacomino AP, Bron IU. Oxidative processes during 'Golden' papaya fruit ripening. *Braz J Plant Physiol* 2012; **24**: 85-94.

[23] Halevy AH, Mayak S. Senescence and postharvest physiology of cut flowers. Part 2. In: Janick J, editor. *Horticultural reviews*. Westport: AVI Publishing; 1981, p. 59-143.

[24] Pesaresi P, Mizzotti C, Colombo M, Masiero S. Genetic regulation and structural changes during tomato fruit development and ripening. *Front Plant Sci* 2014; **5**: 124.

[25] Sun J, Li C, Prasad KN, You X, Li L, Liao F, et al. Membrane deterioration, enzymatic browning and oxidative stress in fresh fruits of three litchi cultivars during six-day storage. *Sci Hortic* 2012; **148**: 97-103.

[26] Gazdik Z, Krska B, Adam V, Saloun J, Pokorna T, Reznicek V, et al. Electrochemical determination of the antioxidant potential of some less common fruit species. *Sensors* 2008; **8**: 7564-70.

[27] Moyer RA, Hummer KE, Finn CE, Frei B, Wrolstad RE. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: vaccinium, rubus, and ribes. *J Agric Food Chem* 2002; **50**: 519-25.

[28] Rop O, Sochor J, Jurikova T, Zitka O, Skutkova H, Mlcek J, et al. Effect of five different stages of ripening on chemical compounds in medlar (*Mespilus germanica* L.). *Molecules* 2010; **16**: 74-91.