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Evolution of fatty acids in medlar (*Mespilus germanica* L.) mesocarp at different stages of ripening

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RESUMEN

Evolución de los ácidos grasos en el mesocarpo del níspero (*Mespilus germanica*. L.) a diferentes estados de maduración.

La composición en ácidos grasos del níspero (*Mespilus germanica* L.) varió significativamente entre los estados de maduración muestreados a los 157, 172 y 187 DAFs (días después de la floración). Veinte y un ácidos grasos diferentes fueron detectados en el fruto preclimatérico y 17 cuando comenzó el climaterio. Los ácidos grasos principales encontrados en nísperos, recolectados desde Octubre (157 y 172 DAFs) hasta Noviembre (187 DAF), fueron principalmente ácido palmítico (16:0), ácido linoléico (18:2n-6), y ácido α-linolénico (18:3n-3). En tanto que el contenido en ácidos grasos saturados (ácido palmítico (16:0) y ácido esteárico (18:0)) aumentó, el contenido en ácidos grasos esenciales (ácido linoleico (18:2n-6) y ácido linolénico (18:3n-6)) disminuyó durante la maduración, en paralelo con el oscurecimiento de la pulpa. El porcentaje de ácido linoleico y de ácido α-linolénico en frutos maduros sin reblandecer fue de 60.0 y 13.5% del peso seco a 157 DAF, disminuyendo durante la maduración, y permaneciendo a 28.7 y 56% del peso seco, respectivamente, en la pulpa completamente blanda y oscura. También se observó durante la maduración del níspero una marcada disminución en el número de dobles enlaces, en el tanto por ciento de instauración y en la relación instauración / saturación. La contribución de los ácidos grasos insaturados al contenido de ácidos grasos totales disminuyó marcadamente cuando el níspero comenzó progresivamente a reblandecerse y oscurecerse.

PALABRAS-CLAVE: Acidos grasos (evolución) – Maduración – Níspero (Mespilus germanica L.) – Reblandecimiento.

SUMMARY

Evolution of fatty acids in medlar (*Mespilus germanica* L.) mesocarp at different stages of ripening.

The fatty acid composition of medlar (*Mespilus germanica* L.) varied significantly among the ripening stages sampled at 157, 172 and 187 DAFs (days after full bloom). Twenty-one different fatty acids were detected in preclimacteric fruit and 17 when the climacteric began. Principal fatty acids, determined in medlar fruit harvested from October (157 and 172 DAFs) to November (187 DAF) were mainly palmitic acid (16:0), linoleic acid (18:2n-6), and α-linolenic acid (18:3n-3). While the content of saturated fatty acids [palmitic acid (16:0) and stearic acid (18:0)] increased, the content of the essential polyunsaturated fatty acids [linoleic acid (18:2n-6) and linolenic acid (18:3n-3)] decreased through ripening, in parallel with pulp darkening. The percentage of linoleic acid and α-linolenic acid in ripe, hard fruits was 60.0 and 13.5 % of dry wt at 157 DAF which decreased throughout ripening, remaining at 28.7 and 5.6 % of dry wt, respectively, in the fully softened and darkened pulp. A marked decreases in the double bond index, percentage of unsaturation and the ratio of unsaturation/saturation were also seen throughout the medlar ripening. The contribution of unsaturated fatty acid to the total fatty acid content decreased markedly as the medlar fruit became progressively softer and darkened.

KEY-WORDS: Fatty acids - Fruit ripening - Medlar (Mespilus germanica L.).

1. INTRODUCTION

Medlar (*Mespilus germanica* L., Fam: Rosaceae) is a spiny shrub that has been cultivated in many countries of Europe and Asia for its edible fruits and ornamental qualities. The pear- and apple-shaped fruits are subglobose or pyriform (drupes) and range in diameter from 1.5-3 cm. In general, ripening occurs late in medlar development. Unripe fruits are hard and must spend several weeks to soften and sweeten. After a frost or cold exposure, fruits on the trees or after harvest become brown when ready to consume (Browicz, 1972; Dirr, 1990).

Lipid components in fruits are presumed to contribute to characteristic aroma and flavour during ripening. These are essentially considered as precursors for various odorous volatile compounds

(Gholap and Bandyopadhyay, 1980) and also contribute to nutritional value of fruit (Supran, 1978).

Reports on the chemical composition of medlar fruit during growth and development have appeared in the literature are so scarce. In a recent study, Romero-Rodriguez et al. (2000) described some of the physical, physicochemical and chemical changes (sugars, organic acids, minerals, etc) during maturation of medlar. As far as we know, there are no reports concerning changes of fatty acid composition during ripening as well as pulp darkening for medlar in the literature. We are interested in the fatty acid composition of medlar fruit for two reasons: first, because the marked changes in their content and profile during ripening can have deleterious effects on the acceptability of medlar as a food source; and second, because medlar fruit could contribute significant quantities of essential fatty acids to the diets of populations who consume them. In this paper we report changes in free fatty acids during medlar fruit ripening.

2. MATERIALS AND METHODS

2. 1. Fruit sampling

The wild medlar (*Mespilus germanica* L.) fruits were harvested from fourteen 20-year-old trees grown in Trabzon (Caykara) and surrounding lands, in Turkey, and harvested in the Fall of 2000. The blossoms were considered to be in full bloom on May 8th of 2000. Fruits were sampled at 157, 172 and 187 days after full bloom (DAF) (Table I). One kg of medlar fruit was gathered in triplicate at each harvest time. The harvested fruits were freeze-dried and stored at -80^{o}C. After lyophilization, the hard, dried fruits were ground to a fine powder using a stainless steel mill.

2. 2. Extraction

Three different powdered mesocarp samples (5 g) were extracted with chloroform:methanol 2:1, (v/v) as described by Chamberlain et al. (1993). The solid, non-lipid material was removed by filtration and the lipid material was recovered after solvent removal in a stream of nitrogen. The residue was redissolved in anhydrous chloroform/methanol 19:1 (v/v) and clarified by centrifugation at 10,000 x *g* for 10 min. Fatty acid methyl esters were obtained using 14% (w/v) boron trifluoride (BF_3) in methanol (Morrison and Smith, 1964). Fifty nanograms of heptadecanoic acid (internal standard) and a 1 ml aliquot of lipid sample were transferred to a 15 ml teflon-lined screw tube. After removal of solvent by nitrogen gassing, the sample was mixed with 0.5 ml of the BF_3 reagent placed in a water bath at 100^{o}C for 30 min. After cooling, fatty acids methyl esters were extracted into hexane. A calibration mixture of fatty acid standards was processed in parallel.

Table I

Variations in the color of the skin and pulp of medlar fruit (*Mespilus germanica* L.) at different stages of ripening

Harvest no.	Days after full bloom (DAF)	State of ripeness, fruit skin and pulp color
I.	157	Ripe, skin light brown, fruit hard, pulp white
II.	172	Ripe, skin partly dark brown, fruit table soften, pulp whitish and partly brownish
III.	187	Very ripe, skin fully dark brown, fruit soften, pulp fully dark brown

2. 3. Gas chromatography analysis of fatty acids

Aliquots (1-2 μl) of the hexane solution of the fatty acid methyl esters were analyzed by gas chromatography, in a Hewlett-Packard (5890 Series II) apparatus equipped with a fused-silica capillary column (Omegawax; 30 m x 0.32 mm I.D., Supelco, Bellefonte, PA) and a flame–ionization detector (FID). The injector temperature was set at 200^{o}C, detector at 230^{o}C, oven at 120^{o}C initially, then 120-205^{o}C at 4^{o}C/min, and finally 205^{o}C for 18 min. The carrier gas was helium and the flow rate was approximately 50 ml/sec. Electronic pressure control in the constant flow mode was used. The fatty acid data reported represent the average of three determinations conducted on three independent assays. The double bond index (DBI) was calculated as described previously (Navari-Izzo et al., 1991).

2.4. Statistical analysis

Data on fatty acids composition were evaluated by analysis of variance, using the general linear procedure, a package program of the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). The design used was a completely randomized block design. Specific differences were determined by LSD. All comparisons were made at 5% (P = 0.05) level of significance.

3. RESULTS AND DISCUSSION

Medlar fruit that was ripening between October and November 2000 were analyzed for fatty acid content. Variations in the color of the skin and mesocarp of medlar fruit at various stages of ripening are described in Table I. The data in Table II show that palmitic acid (16:0), linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) were the major

Table II

Fatty acid composition (%) of the total lipid of medlar (*Mespilus germanica* L.) fruit at different stages of ripening. Values represent the mean ± standard deviation of three separate extractions and determinations. n.d.; not detected (< 0.01%). DBI; Double bond index, LSD (*P* = 0.05); Least significant difference. CV; coefficient variation

	DAF (days after full bloom)				
Fatty acids	**157**	**172**	**187**	**LSD (*P* = 0.05)**	**CV%**
C10:0	0.3±0.01	n.d.	n.d.	n.d.	n.d.
C12:0	0.3±0.10	0.5±0.11	1.4±0.40	0.77	128
C13:0	0.3±0.11	n.d.	n.d.	n.d.	n.d.
C14:0	0.4±0.02	0.4±0.30	1.1±0.17	2.05	96
C14:1	0.3±0.10	n.d.	n.d.	n.d.	n.d.
C15:0	0.1±0.01	0.3±0.04	0.9±0.11	0.21	130
C16:0	16.0±0.15	24.3±1.47	36.9±1.13	3.42	272
C16:1	0.3±0.02	0.3±0.00	0.6±0.03	0.07	268
C18:0	2.6±0.21	5.5±1.40	7.9±1.19	3.41	211
C18:1n-9	9.5±0.24	2.0±0.16	3.5±0.03	0.54	140
C18:1n-7	0.9±0.05	0.9±0.03	1.5±0.01	0.12	362
C18:1n-5	0.1±0.01	0.1±0.00	0.1±0.02	0.05	294
C18:2n-6	60.0±0.76	44.3±0.81	28.7±1.65	3.73	401
C18:3n-3	13.5±0.04	17.3±1.97	5.6±0.38	3.68	224
C20:0	1.5±0.03	1.6±0.13	4.2±0.23	0.46	175
C20:1n-9	0.2±0.00	0.1±0.00	0.2±0.11	0.18	181
C20:1n-7	0.4±0.04	n.d.	n.d.	n.d.	n.d.
C20:2n-6	0.4±0.04	0.1±0.04	0.2±0.15	0.31	148
C22:0	1.6±0.01	1.5±0.11	4.4±0.83	1.53	162
C22:1n-9	0.1±0.00	0.2±0.04	0.7±0.18	0.33	125
C24:0	0.9±0.02	0.6±0.12	2.1±0.23	0.46	167
% Unsat.	76.2±0.32	65.3±2.61	41.1±2.01	6.09	377
% Sat.	23.8±0.34	34.7±2.60	58.9±2.02	6.07	243
U/S	3.2±0.12	1.9±0.22	0.7±0.06	0.43	171
DBI	8.3±0.21	4.9±0.71	1.8±0.11	1.38	170

predominant fatty acids detected throughout medlar pulp softening. The percentages of 16:0 and 18:0 acids in the ripe hard medlar were 16.0 and 2.6% of dry wt, and these values increased throughout ripening, reaching 36.9 and 7.9 % of dry wt, respectively, in very ripe-fully dark brown and soft medlar. In contrast, the proportion of linoleic acid and α-linolenic acid in the ripe hard fruits was 60.0 and 13.5 % of dry wt, respectively, and then their level decreased throughout ripening, remaining low (28.7 and 5.6 % of dry wt) at 187 DAF. The values decreased progressively throughout ripening reaching 28.7 and 5.6 % of dry wt, respectively, in fully dark brown and soft medlar. In addition to these prominent fatty acids, a remarkable increase was also observed in the content of some minor saturated fatty acids (18:0, 20:0, 22:0, and 24:0) during medlar ripening. Though a constant level was observed between 157 and 172 DAFs, a sudden increase in the content of some minor unsaturated fatty acids (16:1, 18:1n-7, and 22:1n-9) was seen at 187 DAF in the ripest, fully softened and darkened (brown) pulp of medlar. A progressive and significant decrease in the double bond index (8.3 to 1.8) between 157 and 187 DAFs was also observed. The percent of unsaturation (76.2 to 41.1%) and the unsaturation/saturation ratio (3.2 to 0.7) decreased significantly during ripening (Table II), while the percentage of lipid saturation increased through ripening reaching a peak of 58.9% in fully softened and darkened medlar pulp.

Our results clearly indicate that the amounts of both saturated and unsaturated fatty acids composition changed markedly throughout fruit ripening in association with pulp softening, as does the ratio of unsaturation/saturation and the double bond index. It is well known that as the peach fruit

matures, the degree of unsaturation of fatty acids increases (Izzo *et al*., 1995). In fruits, generally, oleic acid (18:1), linoleic acid (18:2) and α-linolenic acid (18:3) are the predominant fatty acids throughout development and senescence, although their specific contents vary among species and during maturation (Nordby and Nagy, 1979; Ratovohery *et al*., 1988; Nieto and Romero, 1995; Ayaz *et al*., 1997; Ayaz and Kadioglu, 1999; Ayaz and Kadioglu, 2000). The present study has revealed that the degree of unsaturation decreases during ripening as the medlar fruit over matures and the pulp softens in parallel with pulp darkening. It has been reported that an increase in DBI occurs in lipids of peach mesocarp due to increased activities of desaturase enzymes which appear to be activated when membrane bilayer fluidity decreases (Izzo *et al*., 1995).

The data reported here show that the greatest changes in fatty acid composition of medlar pulp take place during climacteric fruit ripening. During fruit ripening and senescence, cytoplasmic structures reorganize within the cells (Nagy *et al*., 1978) and cellular disorganization resulting from catabolism during senescence is accompanied by enzymatic breakdown of lipoprotein membranes (Galliard, 1975). Previously, it has been reported that the greatest changes in membrane properties in apple (Lurie and Ben-Arie, 1983) and avocado (Dallman *et al.,* 1988) fruits occur as fruits reach the climacteric. These changes were correlated with variations in lipid composition of cell membranes (Lurie and Ben-Arie, 1983).

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 (Sacher, 1973), and ethylene plays a large 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 de-greening and color formation that occurs in many fruits (Goldschmidt, 1980). A second possible mechanism for the marked decline in the fatty acid content 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 (Paliyath and Thompson, 1987). 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 sterol: fatty acid ratio, and a selective depletion of unsaturated fatty acids (Fobel *et. al*, 1987; Borochov *et al*., 1982). Our results suggest a rapid and marked decrease in total lipid fatty acids in medlar may also be due to an increase of fatty acid β-oxidation during the ripening process.

In summary, we report nutritionally important fatty acids and their accumulation profiles in ripening medlar fruit. It seems that much of the potential 18:2n-6 and 18:3n-3 fatty acid benefit will be lost if one waits to consume the fruit until 187 DAF (3rd November) and presumably later. In fact, people in northeast Anatolia (Turkey) who use very soft medlar fruit in their diet should be aware that the fruit has lost a great percentage of these essential fatty acids. Such people should be encouraged to use the harder medlar (harvested in October) in their diets. This work represents the first report of fatty acid composition of ripening medlar fruit. A comparative study including other wild genotypes and cultivars of medlar may also provide additional information about regulation of lipid biosynthesis in medlar and additional fruit species.

4. CONCLUSION

There are significant differences in the levels of fatty acids of medlar fruit among analyzed ripening stages through pulp darkening. It can be recommended that a harvest date for medlar fruit in mid-October, will yield maximum fatty acid benefit. How earlier harvest and consumption may impact agricultural procedures and productivity should be examined. The information reported here could provide local populations that consume medlar with basic information that could positively impact their nutrition and health.

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