Kernel Fatty Acid and Triacylglycerol Composition for Three Almond Cultivars During Maturation

Ammar Cherif[a], Khaled Sebei[a], Sadok Boukhchina[a], Habib Kallel[a], Khaled Belkacemi[b,*], and Joseph Arul[c]

[a]Laboratoire de Biochimie des Lipides, Faculté des Sciences de Tunis, Campus Universitaire, 2094 Elmanar II, Tunisie, and Departments of [b]Soil Science and Agri-Food Engineering and [c]Food Science and Nutrition, Université Laval, Sainte-Foy, Québec, Canada, G1K 7P4

ABSTRACT: The kernel oil content, kernel FA and TAG composition, kernel moisture content, and kernel weight as well as fruit weight of three almond cultivars (Achaak, Mazetto, and Perlees) were monitored during the maturation of kernels. Lipid fractions of all almond samples were extracted using a mixture of chloroform and methanol. FAME and TAG contained in these fractions were analyzed by GC and HPLC, respectively. The ratio of kernel to fruit weight appears to be a good indicator of almond kernel development. The total lipid content of developing almond kernels exhibited a sigmoidal pattern with time, similar to seeds and kernels of other higher plants; the cultivar Achaak showed a higher rate of lipid accumulation. The proportion of oleic acid (0) dominated at the later stage of maturation for all three almond cultivars. Although there was no significant difference in the FA composition for the three cultivars studied, marked differences were observed in their TAG profiles. Ten TAG species identified were LLL, LLO, LnOO, LOO, LOP, PLP, OOO, POO, POP, and SOO, where L represents linoleic acid; Ln, linolenic acid; P, palmitic acid; and S, stearic acid. The difference in the TAG profile can be useful for distinguishing various cultivars. The oil of Mazetto cultivar kernels exhibited a TAG composition comparable to that of olive oil.

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KEY WORDS: Almond oil, fatty acids, maturation, *Prunus amygdalus*, triacylglycerol.

Nuts of the almond tree (*Prunus amygdalus* Batsch), which belongs to the *Rosaceae* family, are among the most popular tree nuts worldwide (1). Almonds constitute an important part of the human diet. They are typically used as snack foods as well as ingredients in a variety of processed foods, especially in bakery and confectionery products. It is a widely grown nut crop in the Mediterranean region and the United States of America. The fruit of this crop is highly valued for its dietetic, cosmetic, and pharmaceutical properties. The origin and specificity of agricultural crops are often used to define their quality and commercial value. This generality applies to almonds, for which many cultivars are known.

The lipid content in almonds ranges from 50 to 60% (w/w) (2). The FA composition has been extensively reported for almond cultivars from different geographical origins including Italian (3), American (4), Spanish (5), and Tunisian (6). The major FA in almonds are oleic (18:1), representing 60–70% of the total FA; linoleic (18:2), 14–26%; and α-linolenic (18:3), below 1% (7). However, the TAG composition of almond nuts has scarcely been reported (8). Recently, the TAG composition of almond kernel oil from 19 cultivars was reported, and the cultivars were classified by means of principal component analysis (9). The TAG compositions of almond oil from different cultivars were found to be similar. However, the evolution of FA and TAG composition during maturation of the almond kernels is not known. Thus, the objective of this work was to identify and compare the FA and TAG composition of almond oils from three cultivars—Tunisian (Achaak), American (Perlees), and Italian (Mazetto)—during different stages of almond kernel maturation.

*To whom correspondence should be addressed.
E-mail: khaled.belkacemi@sga.ulaval.ca

EXPERIMENTAL PROCEDURES

Almond kernels and dry matter content. Almond kernels were collected from Tunisian (Achaak), American (Perlees), and Italian (Mazetto), cultivars grown in the experimental plots of Groupement Obligatoire des Viticulteurs et Producteurs de Fruits (GOVPF) (Tunis, Tunisia). Nuts were harvested every week, starting at 40 d after flowering, for 25 wk. The nuts and kernels were milled separately with a universal cutting mill (Laval Lab Inc., Quebec City, Canada) and weighed, and their dry matter content was then determined after vacuum oven drying at 60°C for 72 h.

Lipid extraction, FA and TAG analyses. Lipids were extracted from almond powder with 1:1 (vol/vol) chloroform/methanol by the Soxhlet method (10). The lipid content was determined by the method of Folch *et al.* (11). The FA analysis was carried out by converting FA into their methyl esters. FAME were recovered using the procedure of Metcalfe *et al.* (12). They were separated on a Supelco SP2380 capillary column (30 m length; 0.25 mm i.d.; 0.20 mm film thickness) of fused silica (Bellefonte, PA) and quantified by using a gas chromatograph (Model 5890A; Hewlett-Packard, Palo Alto, CA) equipped with an FID. The carrier gas was nitrogen. The injector and detector temperatures were maintained at 230 and 250°C, respectively, whereas the column temperature was maintained at 220°C. FA were identified by comparison with known standards. TAG were analyzed by using a high-performance liquid chromatograph (HPLC) equipped with an RP18 stainless-steel column (250 mm length × 4.6

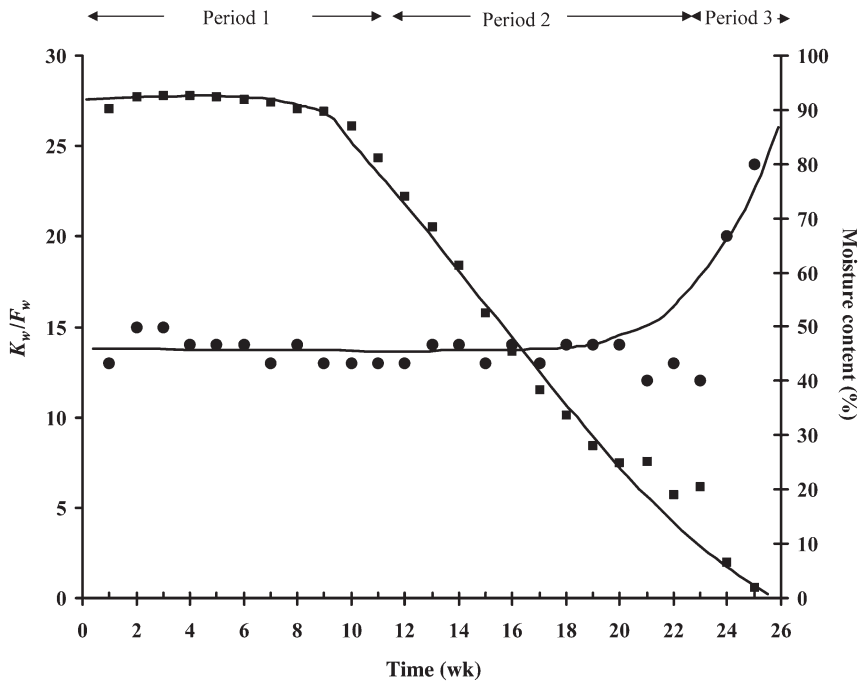

FIG. 1. Evolution of kernel weight/fruit weight ratio (K_w/F_w; ●) and kernel moisture content (■) during maturation of almond cultivar Achaak. Time indicates weeks after the 40th day after flowering.

mm i.d.) from Knauer (Berlin, Germany), a guard column, and a refractive index detector (differential refractometer, model 410; Waters SA, Saint-Quentin, France). The mobile phase was 1:1 acetonitrile/acetone. The flow rate was isocratically controlled at 1.5 mL/min. The column temperature was maintained at 30°C. Identification of TAG was identical to that reported by Gigliotti *et al*. (13).

RESULTS AND DISCUSSION

During early development, the almond kernel is translucent, but it solidifies with the accumulation of dry matter. Figure 1 shows the evolution of almond kernel moisture content as well as the ratio of kernel weight (K_w) to fruit weight (F_w). Three characteristic periods are distinguishable. In the first 9 wk of maturation, almond moisture and K_w/F_w remain constant and equal to 90% and 13, respectively. During this period, structures and cellular organs develop. The second period (9–23 wk) is characterized by a rapid decrease in the moisture content of fruits but little change in K_w/F_w. Synthesis of organic matter such as lipids, protein bodies, and carbohydrates occurs during this period, leading to an increase in the kernel weight, K_w, which compensates for the moisture loss of the fruits, thereby stabilizing the ratio K_w/F_w at 13. As the kernel matures, lipid accumulation ceases and protein bodies continue to accrue, although the moisture content of the almonds decreases further and reaches approximately 2% at the end of maturation. This characterizes the third period, where the ratio K_w/F_w increases to 24 at the end point of maturation. This increase is attributed to the stabilization of the kernel weight, K_w, and the decrease of the fruit weight, F_w. Moreover, syntheses of storage substances such as lipids reach their limiting values, but the mature fruit continues to dry (Fig.1).

The profiles of the total lipid content of almond kernels over maturation time for the three cultivars show a sigmoidal pattern (Fig. 2). During the lag phase, from 4 to 6 wk of maturation, depending on the cultivar, the kernels contain a small amount of lipids. These lipids are essentially membrane lipids, as seen by the FA composition showing a high proportion of linoleic (18:2) and linolenic (18:3) FA (Fig. 3). The

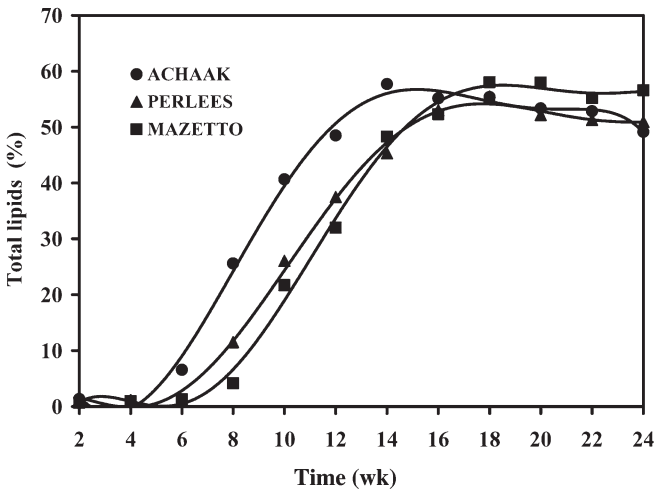

FIG. 2. Time course of the total lipid profile for the three cultivars during maturation. Time indicates weeks after the 40th day after flowering.

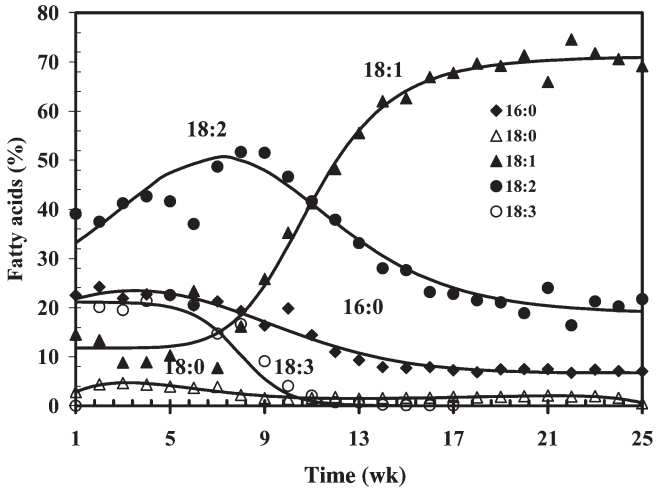

FIG. 3. FA composition of Achaak cultivar kernels. Time indicates weeks after the 40th day of flowering.

second phase is characterized by a dramatic rise in oil content and the development of oil bodies, accompanied by an increase in oleic acid.

The dramatic increase in oil content may be due not only to a high rate of oil synthesis, but also to an increase in kernel dry weight because of a loss of moisture (Fig. 1). The third phase, after 14–18 wk of maturity, is accompanied by saturation levels of lipids and fully developed oil bodies. This pattern is quite similar to the seeds and kernels of other higher plants (14). Figure 2 shows the parallel lines of Achaak and Mazetto during weeks 8–12. At least during this phase, the rates were the same. Among the three cultivars, the lipid synthesis rate for Achaak was higher than that of Perlees and Mazetto. However, the lipid content at saturation was nearly the same (50–55%) for all three cultivars, and the lipid content was well within the range reported for almond kernels (17).

The evolution of the profile of major FA in the cultivar Achaak during kernel maturation is presented in Figure 3. At about 7 wk, marked changes occurred in the FA profile. The proportion of 18:2, which had gradually increased to 54%, started to decline steadily until 17 wk, reaching a final value of 22%. The initial increase in 18:2 is likely due to continued synthesis of this acid for incorporation into half-membranes of the oil bodies. The decline in the proportion of this acid marks the development of spherosomes or vesicles into oil bodies. At about 7 wk of maturation, the proportions of 18:3, 16:0, and 18:0 started to decline, and that of 18:1 started to increase. Furthermore, the concentration of 18:3 dropped from its initial value of 22.5% to *ca*. 8% after 15 wk of maturation. To a lesser extent, a decrease in the 18:0 level was also observed. On the other hand, 18:3 declined from 20% and was undetected after 11 wk of maturation. The proportion of oleic acid, which remained at about 10% until 7 wk of maturation, increased to a constant value of about 70% after 17 wk of maturation. The total oil content steadily increased during this period. Since only the proportions of FA rather than the absolute quantities of FA per kernel were monitored during maturation, it is not possible to draw inferences about interconversions of FA on the synthetic pathway.

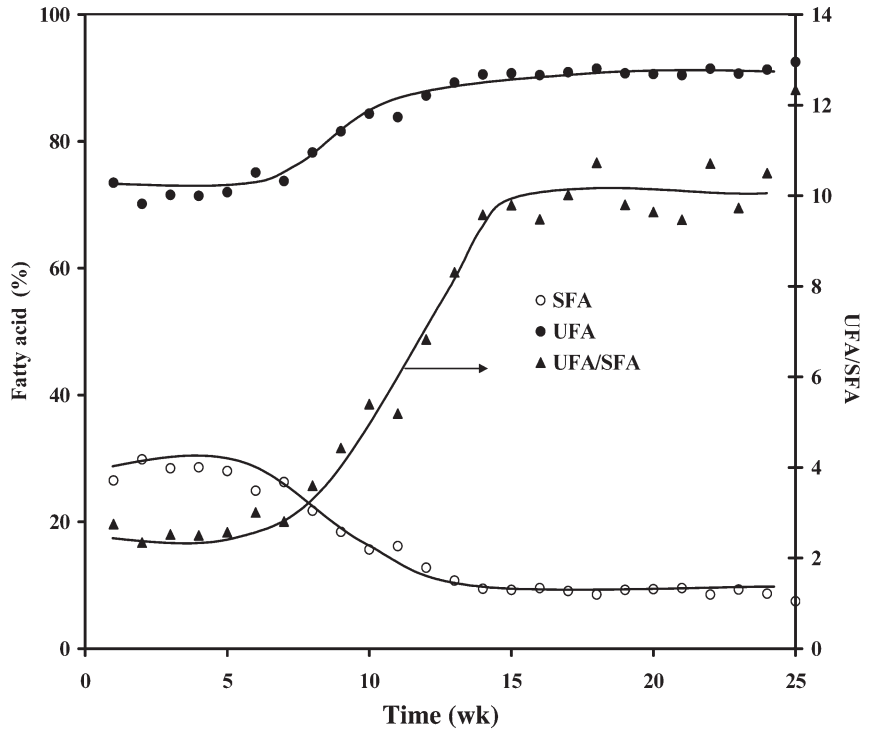

FIG. 4. Evolution of saturated FA (SFA) and unsaturated FA (UFA) during maturation of Achaak cultivar kernels. Time indicates weeks after the 40th day after flowering.

Figure 4 shows the evolution of total unsaturated FA (UFA) and total saturated FA (SFA) levels during maturation of almond kernels for the cultivar Achaak. UFA accounted for 73.5% of total FA in the early stages of maturation, and their content reached a level of 93% at the later stages of maturation. This evolution is characterized by a transition period occurring at 12 to 13 wk of maturation when the UFA level reached about 87%. The evolution of the UFA/SFA ratio followed a sigmoidal pattern. It increased from 3 to a final value of 10.5 to 12 at the later stages of maturation, with a value of 7.0 at the transition. This pattern is nearly identical among the cultivars and indicates that almond kernel oil is essentially unsaturated oil.

Ten species of TAG were detected during the maturation of almond kernels. Six of them were major ones (LLL, LLO, LOO, OOO, LOP, and POO) and four were minor (POP, SOO, PLP, and LOO, where P = palmitic, O = oleic, S = stearic, and L = linoleic). The changes in the proportion of the major species during maturation of the kernels of Achaak cultivar is shown in Figure 5. The major species represent 84% of the total TAG during the early stages of maturation: LLO (22%), LOO (20%), POO (15%), LOP (14%), OOO (13%), and LLL (5%). The proportion of each of the four minor TAG was <5% and remained unchanged during maturation. Among the major species, OOO exhibited the greatest increase during kernel development, from 12 to 36%. The species LLO, LLL, and LOP decreased during maturation. The most notable decline was in LLO, from 22% to a steady value of 11%, whereas the decline in LLL and LOP was small, e.g., <4%. The proportions of POO and LOO remained fairly stable during maturation. These two species appear to have been synthesized during the early stages of kernel development. The decline in LLL, LLO, and LOP and the strong increase in OOO reflect the increase in oleic acid and the decline in linoleic and palmitic acids during maturation. Thus, it appears that OOO, LOO, and POO are the major species synthesized during the later stages of maturation. The increase in oleic acid in later stages of almond maturation (Fig. 3) reflects the increases in OOO, LOO, and POO.

Figure 6 is a comparison of TAG for the three cultivars carried out at the last stage of maturation, e.g., 25 wk after the 40th day after flowering. For all three cultivars, the major TAG were OOO, LLO, LOO, LOP, and POO. The Mazetto cultivar had the highest levels of OOO, LLO, and LOO but the lowest levels of LLO and LOP. On the other hand, the Perlees cultivar was richer in LLO and LOP but was poorer in OOO as well as POO. The Achaak cultivar had an intermediate TAG composition. The total content of the major TAG species among cultivars was nearly the same, where OOO and LOO together accounted for 60–70% of total TAG. The predominant TAG was OOO for the three cultivars, also reported by Martin-Carratala *et al.* (9) for 19 almond kernel cultivars. In the present work, 10 TAG species including two minor ones, i.e., POP and LnOO (where Ln = linolenic acid), yet unreported in almond oil, were identified. Moreover, the difference in TAG composition suggests that OOO can be used to differentiate among almond cultivars, for example, in detecting the adulteration of almond oil.

The profile of almond oil TAG is qualitatively similar among the cultivars, and their overall unsaturation level is also nearly the same, even though there are differences in the TAG composition among them, as seen by the same average number of double bonds, i.e., 3, for all three cultivars. Furthermore, the

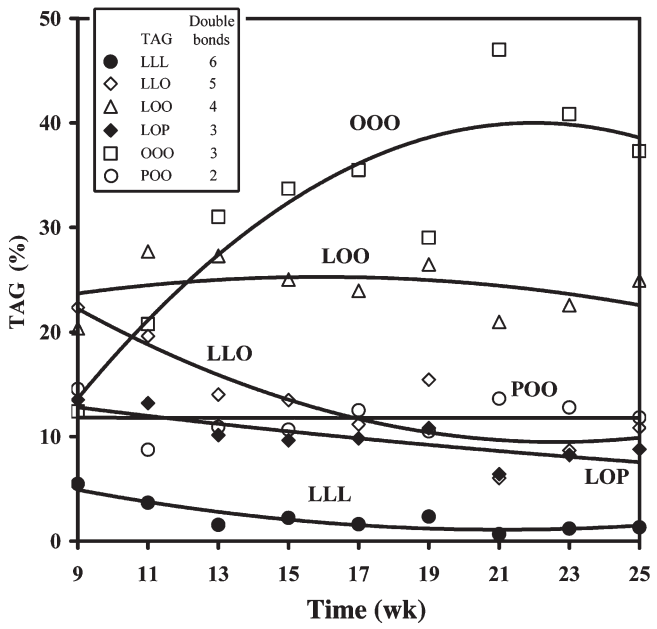

FIG. 5. Evolution of TAG during maturation of Achaak cultivar kernels. O, oleic acid; L, linoleic acid; P, palmitic acid. Time indicates weeks after the 40th day after flowering.

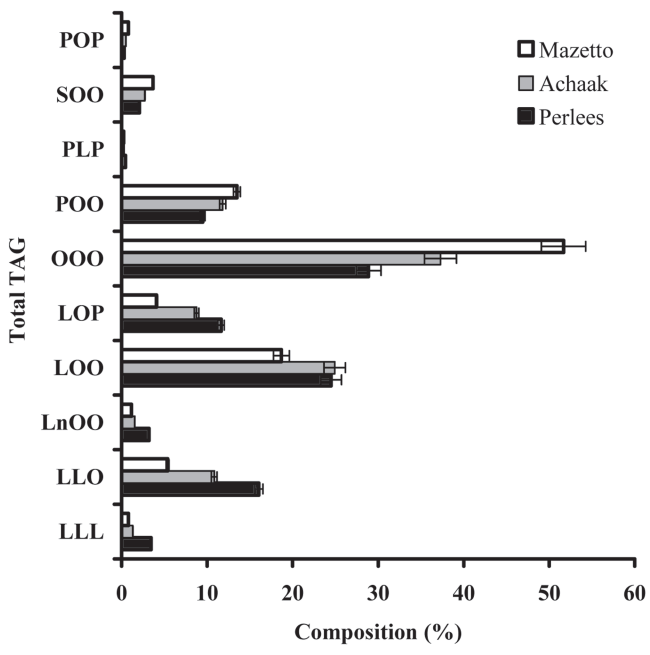

FIG. 6. Comparison of the TAG composition of mature kernels of cultivars Mazetto, Achaak, and Perlees. Values are presented as mean ± SD (n = 3). Ln, linolenic acid; for other abbreviations see Figure 5.

TAG composition of almond kernel of some cultivars may be comparable to olive oil. The levels of OOO, POO, POL, and SOO in olive oil, 43.5, 18.4, 4.1, and 3.7%, respectively (13), compares well with those of the Mazetto cultivar, i.e., 50.1, 16.6, 5.0, and 4.0%, respectively.

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