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Changes in Fatty Acid and Tocopherol Content during Almond (*Prunus dulcis*, cv. Nonpareil) Kernel Development

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**abstract**

Lipids are the major nutritional component of almonds and almond lipids comprise a range of fatty acids from C14 up to C20, including saturated, monounsaturated and polyunsaturated fatty acids, and oil soluble compounds such as plant sterols and tocopherols. This study investigated the change in fatty acid and tocopherol levels during almond kernel maturation, in the variety Nonpareil, grown in the Adelaide Plains of South Australia. The investigation was carried out between November 2012 and February 2013. The accumulation of lipids was determined over six timepoints, commencing at 74 days post-anthesis, and then at 20 day intervals. Almond lipid accumulation occurred rapidly.
between 95 and 115 days post-anthesis, i.e. at a rate of up to 1.83 g/day per 100 g fresh weight but then slowed. Tocopherols accumulated steadily and were positively correlated with lipid development; with α-tocopherol forming at the highest rate, being 0.58 mg/day in 100 g lipid, between the first two timepoints. The key timing for accumulation of the major fatty acid, oleic acid, was between 95 and 115 days post-anthesis, after which accumulation remained constant, at 0.57% of total lipids per day. In contrast, linoleic acid accumulated during the first two timepoints then declined to 23% of final lipid content.

This study aimed to determine the timing of almond lipophilic antioxidant production, to inform almond orchard management practices, such as irrigation and fertilisation, which may impact kernel composition, and therefore, quality.

**Keywords:** Almond, Fatty acids, Fruit development, Lipids, *Prunus*, Tocopherols

**INTRODUCTION**

Lipids represent the major nutritional component of almond kernels and account for more than 50% of total kernel dry weight (Kodad et al. 2011a, Zhu et al. 2015). Isotope labelling experiments have previously been employed to study changes in the composition of lipids and fatty acids in almonds during development (Cherif et al. 2004, Munshi and Sukhija 1984, Soler et al. 1988). These studies, using [1-14C] acetate incorporation, or organic solvents to extract fatty acid and triacylglycerol, monitored almond fatty acid biosynthesis.
However, these studies were based on cultivars and development stages for almonds grown under northern hemisphere climatic conditions. So far, studies concerning the accumulation of fatty acids during almond kernel maturation have not been undertaken in the southern hemisphere where almond fruits are exposed to more solar radiation during maturation (Zhu 2014 PhD thesis), in particular, solar UV radiation in the southern hemisphere is stronger than the northern hemisphere (Gies et al. 2004). Australia has a long history of almond production, and Australian production has increased dramatically over the last decade from 16,000 t in 2006 to over 81,000 t in 2016 (ABA 2016). Australian almond producing regions experience unique environmental conditions, for example, limited rainfall (and frequent droughts), intense ultraviolet radiation (UVR), and predominantly red loamy and sandy soils, i.e. conditions which influence almond kernel development (Mousavi and Alimohamadi 2006). Kodad et al (2010) also pointed out the climatic conditions prevalent during the growing season, along with genotype and environment together influence almond oil content and fatty acid composition. It is therefore worth studying the changes in fatty acid profiles of Australian grown almonds during kernel development.

To date, the accumulation of tocopherols during almond lipid maturation has not been reported in the literature. Among the various tree nuts, almonds have the highest vitamin E (tocopherol) content (Kodad et al. 2011b, Zhu et al. 2015). Tocopherol concentration is therefore a key nutritional measure of almond kernel quality. Almond lipids predominantly
comprise the monounsaturated fatty acid, oleic acid, and the polyunsaturated fatty acid, linoleic acid (Kodad et al. 2011a, Zhu et al. 2015) together with tocopherols collectively, these constituents have been shown to play an important lipophilic antioxidant role in human metabolism (Damasceno et al. 2011, Hollis and Mattes 2007, Rajaram et al. 2010, Wien et al. 2010). The concentrations of tocopherols in fully ripened almond kernels has been well documented (Kodad et al. 2011b, Kornsteiner et al. 2006, Lopez-Ortiz et al. 2008, Madawala 2012, Matthäus and Ozcan 2009), but the accumulation of tocopherols during kernel development has not been extensively studied. In this study, changes in four tocopherol homologues over six stages of almond kernel development were followed, to determine the key timing of tocopherol formation.

Given the nutritional importance of lipids, unsaturated fatty acids and tocopherols, insight into their accumulation during almond kernel maturation might be used to inform the timing of almond orchard management practices, such as irrigation and fertilisation, in order to enhance kernel quality. Nanos and colleagues (Nanos et al. 2002) found irrigation enhanced oleic acid content in almond lipids compared with no irrigation. However, our previous study (Zhu et al. 2015) observed that moderate deficient irrigation increased oleic acid in comparison to the control. Therefore, the present study aimed to investigate the key time points for almond lipids during drupe maturation, providing useful data for future studies. This study was performed on Nonpareil almonds, a cultivar grown extensively throughout Australia, as the basis for decision-making in the orchard.
Material and Methods

Plant materials. Almonds were harvested from 26-year-old trees (*Prunus dulcis*, cv. Nonpareil) grown in an orchard in the North Adelaide Plains (34°92’S, 138°60’E, elevation 48 m above sea level), during the 2012-2013 growing season; with the orchard managed according to typical commercial practices. The soil comprised red-brown earth, with a high clay content. Climate data (Table 1) was sourced from the Australian Bureau of Meteorology (www.bom.gov.au). Almonds were sampled at six different timepoints starting at 74 days post-anthesis (t=1) and then at approximately 20 day intervals thereafter (i.e. t=2, 3, 4 and 5), until commercial maturity (t=6). Two almonds were randomly selected from each of 40 trees at each timepoint. Kernels were opened and photographed with a Canon EOS500 digital camera. Kernels collected at t=1, 2, 3, 4 and 5 were ground to a slurry and analysed in fresh form only; while fully ripened kernels (sampled at t=6, i.e. 167 days post-anthesis, at commercial maturity when the mesocarp of almond drupes were dry and split, which denotes the almond fruit is fully mature), were analysed in both fresh and dried forms. Kernels were dried by heating at 50°C for 48 hours, to achieve a final moisture content of approximately 2%, measured according to the gravimetric technique (Zhu et al. 2015). Dried kernels were ground to a fine powder with a coffee grinder, then sieved through a 1000 µm mesh, prior to compositional analysis.

Chemical reagents. Analytical grade hexane, ethanol, methanol, chloroform, *n*-heptane,
sodium chloride, butylated hydroxyanisole (BHA), sulphuric acid, ascorbic acid and potassium hydroxide were purchased from Merck (French Forest, Australia), Scharlau (Gillman, Australia) and Sigma Aldrich (Castle Hill, Australia). A C17 free fatty acid (>99% purity) was sourced from Nucheck Prep Inc. (Elysian, MN, USA) and used as an internal standard for determining the fatty acid profile of almond lipids. For identification and quantification of tocopherols, external standard curves were developed using an α, β, γ, δ-tocopherol standards set and an α-tocotrienol standard, sourced from Calbiochem (San Diego, CA, USA) and Cayman Chemicals (Ann Arbor, MI, USA), respectively.

**Fatty acid determination.** Lipid extraction and fatty acid determinations were performed (in triplicate) using chloroform-methanol extraction and methanol-sulphuric acid FAME formation (fatty acid methylation), based on methodology previously described by Makrides et al. (1996) with some modification (Zhu et al. 2015). Briefly, almond powder (0.05 g) was mixed with 0.9% aqueous sodium chloride (2 mL), methanol (3 mL, containing 0.005% BHA), C17 free fatty acid (400 µL, 0.16% in methanol) as an internal standard and chloroform (6 mL), and allowed to stand for 1 hour. After extraction, samples were centrifuged (3000 x g for 10 min) and the organic phase separated and concentrated using a nitrogen evaporator (N-EVAP 112, Organomation Associates Inc., Berlin, MA. USA) at 45°C. After evaporation, the vial containing the extract was weighed, and the difference between the vial with extract and the initial empty vial is the amount of the sample lipid. After drying, methylation was achieved by adding chloroform:methanol (9:1
v/v, 1 mL, containing 0.005% BHA) and methanol (5 mL, containing 1% sulphuric acid), and heating to 70°C for 3 hours. After samples had cooled, n-heptane (2 mL) and water (0.75 mL) were added and samples were mixed thoroughly. The organic layer was transferred to a GC vial for analysis. Fatty acid composition was determined using an HP 6890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with a flame ionisation detector (FID) and HP 7683 autosampler. Separation was performed on an SGE BPX 70 capillary column (50 m, 0.32 mm ID, 0.25 µm; SGE Analytical Science Pty. Ltd., Ringwood, Vic., Australia). Helium was used as the carrier gas and the split-ratio was 20:1. The injector temperature was 250°C and the detector temperature was 300°C. The initial oven temperature was 140°C, increasing to 220°C at 5°C/min, and then held at this temperature for 3 min. FAMEs were identified and quantified based on the retention time and peak area of the C17 free fatty acid internal standard.

**Tocol determination.** Tocol extraction was based on the alkaline saponification and hexane extraction method used previously for analysis of cereals and nuts (Xu 2002) and described previously (Lampi 2011, Lampi et al. 2008). Briefly, almond powder (0.25 g) was mixed with ascorbic acid (0.025 g), ethanol (2.5 mL) and 80% aqueous potassium hydroxide solution (0.25 mL). After being vortexed for 30 s, the samples were incubated in a water bath at 70°C for 30 min, with (vortex) mixing at 10 min intervals. Samples were then placed in ice water for 5 min, before water (1.5 mL) and hexane (2.5 mL) were added, the resulting mixture vortexed for 30 s. Samples were then centrifuged (1000 x g for 10 min). The
hexane layer was transferred to vials and the residue extracted again, before the combined hexane extracts were concentrated using a nitrogen evaporator (N-EVAP 112) at 45°C. The resulting residue was re-dissolved in hexane (1 mL) prior to HPLC analysis, using previously published protocols (Lampi 2011, Lampi et al. 2008); i.e. the isocratic mobile phase was hexane (with 2% 1,4-dioxane), with a flow rate of 1.0 mL/min, an injection volume of 20 µL and column temperature of 25°C. HPLC analysis was performed using an Agilent 1200 HPLC (Agilent Technologies, Waldbronn, Germany) coupled with diode array and fluorescence detectors (DAD and FLD, respectively). Separation was achieved using a Grace Alltime HP silica column (150 mm, 3 mm ID, 3 µm; Grace Discovery Sciences, Deerfield, IL, USA). α, β, γ, δ-Tocopherol and α-tocotrienol standards were used to prepare external calibration curves. α-Tocopherol was detected by DAD at a wavelength of 292 nm, while β, γ, δ-tocopherol and α-tocotrienol were detected by FLD at wavelengths of 292 nm (excitation) and 325 nm (emission).

**Data analysis.** Chemical data were analysed by one-way ANOVA using GenStat (15th Edition, VSN International Limited, Herts, UK) and GraphPad Prism 5 (Version 5.01 GraphPad Software Inc., La Jolla, CA, USA) for graph presentation. Mean comparisons were performed by Tukey’s multiple-comparison test at $P<0.05$. Pearson’s co-efficient was used for correlation analysis.'
The study of fruit morphology is important for orchard management, because via plant morphology, both the vegetative and reproductive structures of the plant are observed. Plant morphology also examines the process in which structures originate and mature as a plant grows. This information is the key to predict crop yield. Compositional changes that occur during fruit morphological development could have a significant role in determining orchard practice, in a manner similar to that employed by the wine industry to determine the timing of vineyard management practices. In this study, we measured changes in almond morphological and compositional characteristics during development, to determine to what extent this information could be used by industry to inform orchard management decisions.

**Fruit appearance during almond kernel development**

Changes in the appearance of almonds during their development and ripening are shown in Figure 1. At the first and second timepoints (i.e. at t=1 and t=2, being 74 and 95 days post-anthesis respectively), almond kernels contained clear endosperm, while the outer hull (mesocarp) was bright green in color. As kernels developed, the endosperm decreased in size as the embryo developed which was cream in color, but by the third and fourth timepoints (i.e. t=3 and t=4, being 136 and 156 days post-anthesis respectively), the kernel skin remained pale and fruit color was unchanged. By the fifth timepoint (i.e. t=5, being 156 days post-anthesis), the kernel had become firm and the skin had browned. The fruit mesocarp had become dry and exhibited a leathery texture. At commercial maturity, (i.e.
t=6, being 167 days post-anthesis or commercial maturity) kernels were fully ripe and comparatively dry; i.e. moisture levels had decreased to approximately 5% (data not shown). These anatomical observations were similar to those described by Munshi and Sukhja (1984), and Hawker and Buttrose (1980), despite different varieties being studied; i.e. Nonpareil in this study, the regional selection H5 in the Munshi and Sukhja study, and the local varieties Chellaston and Johnston were comprehensively illustrated of almond kernel anatomical features during maturation in the Hawker and Buttrose study. In addition to those early studies, Martínez-Gómez et al. (2008) also finely observed the dissected parts of five almond cultivars during drupe development, and had similar but with subtle differences and specific descriptions for individual cultivars.

**Lipid accumulation during almond kernel development**

Lipid accumulation is shown in Figure 2. Based on the rate differences, accumulation patterns could be seen. From 74 to 95 days post-anthesis the rate was 0.38 g/100 g/day; from 95 to 115 days post-anthesis the rate was 1.83 g/100 g/day; from 115 to 156 days post-anthesis the rate was 0.05 g/100 g/day; from 156 to 167 days post-anthesis the rate was 0.62 g/100 g/day. The ANOVA analysis showed there was no significant difference between t=3, t=4, and t=5, and there was a significant difference between t=5 and t=6, likewise, a significant difference between t=2 and t=3 (Table 2). During t=1 to t=2, lipid accumulation was slow; from t=2 to t=3 had the highest development of almond lipid synthesis, within 20 days, lipid synthesis increased to 46.46 g/100g fresh kernels at stage 3, which is regarded
as the critical time for lipid accumulation. Thereafter, the rate of almond lipid synthesis declined to 0.05 g/100 g/day, where t=3 to t=5 lasted 41 days. During this time, other compounds are being actively metabolized, for example, significant quantities of protein form, and sugar and moisture content decrease (Cherif et al. 2004). The results show that lipids accumulate in the first period of development. This could have implications for early harvesting of almonds to produce high quality almond oil. This has been done in other crops such as grapes resulting in high quality grape seed oil (Rubio et al. 2009).

Approaching the ripening period (t=6), lipid accumulation increased again to 0.62 g/100 g/day and reached the final amount of 53.70 g/100 g dry kernels. This could in part be attributed to moisture loss which resulted in the kernel dry mass being concentrated. Harvest occurs in the summer season; in the current study, the weather prior to harvest comprised low rainfall and high daily maximum temperatures (Table 1). The long-term average maximum temperature for the Adelaide Plains is 28.1°C, compared to 28.5–28.7°C for January and February in the 2013 growing season. Conditions were also much drier in 2013, with just 9.0 and 12.4 mm of rainfall in January and February respectively, compared with long-term averages of 21.2 and 20.7 mm for these months respectively. Warmer and drier climatic conditions can give increased seed dry mass (Monga et al. 1983, Munshi and Sukhija 1984, Onemli 2012). Warmer and drier climatic conditions also affect almond lipid fraction composition. It was observed that almonds grown in the Riverland region (a hot and dry almond growing region in Australia) had a higher portion of linoleic acid than almonds grown in Willunga (a relatively mild and humid region) (Zhu et al. 2015). Further
studies should be designed to test the effect of water and fertiliser on almond kernel development. For example, applying varying fertiliser and water amounts at time point 2 which is the start time for almond lipid accumulation at the highest rate, and measure the difference between treatments and control.

Fatty acid composition during almond kernel development

Changes in the fatty acid composition of almonds included comparisons of myristic, palmitic, palmitoleic, vaccenic, stearic, oleic, linoleic, linolenic and arachidic acids during kernel development (Table 2). Accumulation of some fatty acids, oleic and linoleic acids in particular, changed considerably between the initial (t=1) and final (t=6) timepoints. ANOVA showed significant differences between t=2 and t=3 in all fatty acids, with the exception of myristic and arachidic acids (Table 2). This suggests that between 95 and 115 days post-anthesis is a key time for fatty acid formation and it may be an optimal time to manipulate fatty acid composition by possibly increasing fertilisation or increasing light penetration into the canopy, to achieve, for example a higher oleic acid to linoleic acid ratio, in order to extend kernel shelf-life, (Kodad et al. 2011a).

Indeed, oleic and linoleic acids showed opposing accumulation patterns after t=2; levels of both fatty acids increased to 39% of total lipids between t=1 and t=2, (being 10.8 and 24.4% of total lipids, respectively). Thereafter, oleic acid continued to accumulate until the maximum value of 63% of total lipids was achieved, which explains the high correlation
(R=0.8651) with lipid development that was observed. In contrast, linoleic acid concentrations reached a peak at t=2 then decreased until t=5 after which it remained fairly constant to commercial maturity (t=6). In a study of fatty acid synthesis in sunflower seeds, Onemli (2012) reported a different situation: i.e. at the second stage, the cross-point was the maximum value for oleic acid, rather than linoleic acid, thereafter, oleic acid concentration decreased. Yet, there is a similarity: no linear response of linoleic acid to sunflower oil accumulation was found but a negative correlation between oleic acid and sunflower oil content was observed. Moreover, in an early study of almond lipid development (Soler et al. 1988), oleic acid and linoleic acid exhibited the same trend as the present study during lipid accumulation. Noticeably, the concentration of linoleic acid was high at 59.2% of total lipids and then declined to 29% at maturity (Soler et al. 1988). Concentrations of linoleic acid did not reach such high levels during this study. These differences could reflect the differences in sampling times. There are no other studies reporting such high concentrations of linoleic acid in almond lipids or during lipid maturation. Future studies could consider sampling kernels over the ripening period to explore oleic and linoleic acid synthesis in almond lipids.

The pattern of linoleic acid accumulation during almond lipid maturation was quite similar to those observed for accumulation of some saturated fatty acids, such as myristic, and palmitic, i.e. maximum concentrations were achieved in the early stages of kernel development and then decreased to relatively constant levels prior to commercial maturity. This agreed with findings reported by Munshi and Sukhija (1984), who performed $^{14}$C
labelling experiments to study almond lipid biosynthesis. Linolenic acid, a poly-
unsaturated fatty acid, followed a similar type of accumulation pattern.

Correlation coefficients between fatty acids and total lipids are shown in Table 4. A strong
positive correlation was found between vaccenic and palmitic acids (R=0.9592), and
between oleic and palmitic acids (R=0.8828). This might reflect similarities between
metabolic pathways for C18:1 and C16:0 production, but this has not been reported in the
literature.

**Tocopherols accumulation during almond kernel development**

Figure 2 shows the accumulation of almond lipids and tocopherols between t=1 and t=6,
and Table 3 demonstrates the key timing of tocopherol formation. α-Tocopherol
concentration showed a very strong positive correlation with almond lipid accumulation
content (R=0.864, p<0.0001). β-Tocopherol and α-tocotrienol also showed a strong
positive correlation with lipid accumulation (R=0.824, 0.761 respectively, p<0.0001),
while γ-tocopherol showed a moderate correlation with almond lipid accumulation (R=
0.502 p=0.02). This result reflects the natural pathway of tocol accumulation as α-
tocopherol, β-tocopherol and α-tocotrienol are end products whereas γ-tocopherol is an
intermediate product towards α-tocopherol.

In the present study, the rates of α-tocopherol synthesis varied considerably. From t=1 to
t=2 (74 to 95 days post-anthesis) it was 0.58 mg/day in 100 g lipids; from t=2 to t=4 (from
95 to 136 days post-anthesis) it was 0.09 mg/day in 100 g lipids; and from \( t=4 \) to the final stage (from 136 to 167 days post-anthesis) the rate was 0.28 mg/day in 100 g lipids. The highest accumulation rate took place in the first period.

Through the whole kernel development, the transformation between the homologues was not observed. For example, from the early stage to the final stage, \( \alpha \)-tocopherol was always the predominant constituent, no other homologues like \( \gamma \)-tocopherol and \( \alpha \)-tocotrienol were higher than \( \alpha \)-tocopherol. \( \alpha \)-Tocopherol is synthesized, via \( \gamma \)-tocopherol methyltransferase, and the levels of \( \gamma \)-tocopherol were at least ten times less than the final product which shows an efficient turnover of substrate. Future research could involve harvesting at earlier stages of almond kernel development and refine the time line of sampling.

We recognize that there is a limitation in the present study, i.e. single year, single variety and single locality. Year variation needs to be carried out in future studies, to determine if this has any bearing on kernel development. Any variation seen between years will most likely be due to the climate. Regarding variety and locality, these two factors could be in another study, taking into account agronomic and genotypic differences. Some cultivars have shorter ripening times and therefore kernel development should be faster in those.

**CONCLUSION**

This study determined the changes in fatty acid and tocopherol composition during kernel development for almonds grown in the Adelaide Plains in Australia. Results suggest the
key timing of almond lipid accumulation was between 95 and 115 days post-anthesis, while tocopherols predominantly accumulated between 74 and 95 days after anthesis. Especially, the time between 95 days and 115 days post-anthesis is a crucial period to apply orchard management techniques such as increased water and fertilisation, to enhance the lipids and tocopherol in almond, as well as to influence oleic acid and linoleic acid maturation to control the O/L ratio for long shelf life of kernels.

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REFERENCES


prediabetes. J Am Coll Nutr 29:189-197


Table 1. Climatic conditions in the Adelaide Plains during the 2012/13 growing season.

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>$T_{\text{max}}^*$ (°C)</th>
<th>$T_{\text{min}}^*$ (°C)</th>
<th>Rainfall* (mm)</th>
<th>Solar Radiation* (MJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>September</td>
<td>19.1 (18.3)</td>
<td>9.0 (8.9)</td>
<td>21.6 (54.4)</td>
<td>16.7 (15.5)</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>21.9 (21.0)</td>
<td>9.6 (10.6)</td>
<td>15.6 (44.9)</td>
<td>23.4 (20.6)</td>
</tr>
<tr>
<td></td>
<td>November</td>
<td>26.6 (24.0)</td>
<td>14.5 (12.8)</td>
<td>16.4 (30.5)</td>
<td>28.9 (24.7)</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>27.0 (25.7)</td>
<td>15.5 (14.5)</td>
<td>13.6 (27.4)</td>
<td>30.3 (26.7)</td>
</tr>
<tr>
<td>2013</td>
<td>January</td>
<td>28.5 (28.1)</td>
<td>15.7 (16.0)</td>
<td>9.0 (21.2)</td>
<td>27.6 (27.7)</td>
</tr>
<tr>
<td></td>
<td>February</td>
<td>28.7 (28.1)</td>
<td>17.3 (16.2)</td>
<td>12.4 (20.7)</td>
<td>23.7 (24.4)</td>
</tr>
</tbody>
</table>

Data from the Bureau of Meteorology website (www.bom.org.au)

* Seasonal data (and long term average data)
Table 2. Fatty acid composition of almonds at different stages of kernel development.

<table>
<thead>
<tr>
<th>Sampling time: (days post-anthesis)</th>
<th>74 days</th>
<th>95 days</th>
<th>115 days</th>
<th>136 days</th>
<th>156 days</th>
<th>167 days</th>
<th>167 days*</th>
</tr>
</thead>
<tbody>
<tr>
<td>t=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid (g/100 g)</td>
<td>3.9 ± 0.1 c</td>
<td>7.9 ± 0.05 c</td>
<td>44.5 ± 2.32 b</td>
<td>45.7 ± 0.69 b</td>
<td>46.5 ± 0.30 b</td>
<td>53.3 ± 2.17 a</td>
<td>53.3 ± 0.70 a</td>
</tr>
<tr>
<td>myristic(C14:0)</td>
<td>nd</td>
<td>0.06 ± 0.00 a</td>
<td>0.07 ± 0.00 a</td>
<td>0.05 ± 0.00 b</td>
<td>0.05 ± 0.00 b</td>
<td>0.04 ± 0.00 b</td>
<td>0.05 ± 0.00 b</td>
</tr>
<tr>
<td>palmitic(C16:0)</td>
<td>7.7 ± 0.1 c</td>
<td>9.0 ± 0.05 a</td>
<td>8.1 ± 0.09 b</td>
<td>7.3 ± 0.02 d</td>
<td>7.2 ± 0.03 d</td>
<td>7.3 ± 0.05 d</td>
<td>7.3 ± 0.04 d</td>
</tr>
<tr>
<td>palmitoleic(C16:1)</td>
<td>nd</td>
<td>0.40 ± 0.00 d</td>
<td>0.53 ± 0.01 a</td>
<td>0.48 ± 0.00 c</td>
<td>0.51 ± 0.01 ab</td>
<td>0.50 ± 0.00 bc</td>
<td>0.48 ± 0.01 c</td>
</tr>
<tr>
<td>stearic(C18:0)</td>
<td>nd</td>
<td>0.98 ± 0.02 d</td>
<td>1.4 ± 0.02 c</td>
<td>1.80 ± 0.02 a</td>
<td>1.7 ± 0.01 a</td>
<td>1.6 ± 0.03 b</td>
<td>1.6 ± 0.01 b</td>
</tr>
<tr>
<td>vaccenic(C18:1n=7)</td>
<td>0.75 ± 0.07 c</td>
<td>1.4 ± 0.00 b</td>
<td>1.5 ± 0.01 a</td>
<td>1.4 ± 0.01 ab</td>
<td>1.4 ± 0.00 ab</td>
<td>1.4 ± 0.01 ab</td>
<td>1.4 ± 0.01 ab</td>
</tr>
<tr>
<td>oleic(C18:1n=9)</td>
<td>10.8 ± 0.7 e</td>
<td>39.1 ± 0.17 d</td>
<td>52.4 ± 0.30 c</td>
<td>60.1 ± 0.24 b</td>
<td>63.3 ± 0.09 a</td>
<td>63.7 ± 0.18 a</td>
<td>62.6 ± 0.16 a</td>
</tr>
<tr>
<td>Fatty Acid</td>
<td>74 days</td>
<td>95 days</td>
<td>115 days</td>
<td>136 days</td>
<td>156 days</td>
<td>167 days</td>
<td>167 days*</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>linoleic(C18:2)</td>
<td>24.4 ± 1.6 cd</td>
<td>38.5 ± 0.23 a</td>
<td>33.7 ± 0.15 b</td>
<td>26.8 ± 0.28 c</td>
<td>23.9 ± 0.06 cd</td>
<td>23.4 ± 0.16 d</td>
<td>24.7 ± 0.15 cd</td>
</tr>
<tr>
<td>linolenic(C18:3)</td>
<td>nd</td>
<td>0.32 ± 0.02 a</td>
<td>0.11 ± 0.00 b</td>
<td>0.07 ± 0.00 c</td>
<td>0.05 ± 0.00 c</td>
<td>0.07 ± 0.00 bc</td>
<td>0.08 ± 0.01 bc</td>
</tr>
<tr>
<td>arachidic(C20:0)</td>
<td>nd</td>
<td>0.11 ± 0.00 a</td>
<td>0.10 ± 0.01 a</td>
<td>0.09 ± 0.01 a</td>
<td>0.09 ± 0.01 a</td>
<td>0.11 ± 0.01 a</td>
<td>0.09 ± 0.00 a</td>
</tr>
</tbody>
</table>

Values are means of three replicates ± standard error. Fatty acid content expressed as a percentage of total lipids.

Means within a row followed by different letters indicate significantly different ($P = 0.05$, one-way ANOVA).

nd = not detected.

* results from analysis of dried kernels.

Table 3. Tocol composition (mg/100 g) of almonds at different stages of kernel development.
<table>
<thead>
<tr>
<th></th>
<th>1.2 ± 0.02 c</th>
<th>12.1 ± 0.33 b</th>
<th>14.3 ± 0.82 b</th>
<th>15.7 ± 0.72 b</th>
<th>21.1 ± 0.98 a</th>
<th>20.5 ± 1.84 a</th>
<th>21.3 ± 1.14 a</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>0.01 ± 0.00 c</td>
<td>0.68 ± 0.05 a</td>
<td>0.50 ± 0.02 b</td>
<td>0.42 ± 0.01 b</td>
<td>0.51 ± 0.03 b</td>
<td>0.71 ± 0.03 a</td>
<td>0.52 ± 0.01 b</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>nd</td>
<td>0.08 ± 0.01 b</td>
<td>0.10 ± 0.00 a</td>
<td>0.10 ± 0.00 a</td>
<td>0.10 ± 0.00 a</td>
<td>0.10 ± 0.00 a</td>
<td>0.10 ± 0.00 a</td>
</tr>
<tr>
<td>β-tocopherol</td>
<td>0.09 ± 0.02 de</td>
<td>0.04 ± 0.01 e</td>
<td>0.20 ± 0.00 c</td>
<td>0.42 ± 0.03 a</td>
<td>0.18 ± 0.02 cd</td>
<td>0.27 ± 0.03 bc</td>
<td>0.35 ± 0.02 ab</td>
</tr>
</tbody>
</table>

Values are means of three replicates ± standard error.

Means within a row followed by different letters indicate significantly different ($P < 0.001$, one-way ANOVA).

nd = not detected.

* results from analysis of dried kernels.
Table 4. Correlation coefficients for individual fatty acids against total lipid content.

<table>
<thead>
<tr>
<th>lipids</th>
<th>myristic (C14:0)</th>
<th>palmitic (C16:0)</th>
<th>palmitoleic (C16:1n-7)</th>
<th>vaccenic (C18:1n-7)</th>
<th>oleic (C18:1n-9)</th>
<th>linoleic (C18:2n-6)</th>
<th>arachidic (C20:0)</th>
<th>linolenic (C18:3n-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lipids</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myristic</td>
<td>0.20*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>palmitic</td>
<td>0.36**</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>palmitoleic</td>
<td>0.67***</td>
<td>0.73***</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vaccenic</td>
<td>0.55***</td>
<td>0.80***</td>
<td>0.00</td>
<td>0.96***</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oleic</td>
<td>0.87***</td>
<td>0.41**</td>
<td>0.17</td>
<td>0.88***</td>
<td>0.78***</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>linoleic</td>
<td>0.15</td>
<td>0.37**</td>
<td>0.87***</td>
<td>0.02</td>
<td>0.08</td>
<td>0.03</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>arachidic</td>
<td>0.32**</td>
<td>0.78***</td>
<td>0.04</td>
<td>0.80***</td>
<td>0.85***</td>
<td>0.60***</td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>linolenic</td>
<td>0.09</td>
<td>0.38**</td>
<td>0.73***</td>
<td>0.07</td>
<td>0.13</td>
<td>0.00</td>
<td>0.73***</td>
<td>0.33**</td>
</tr>
</tbody>
</table>

Pearson r values which indicate significant correlations (CI 95%, *P ≤ 0.05, **P ≤0.005, *** P ≤0.001).
Figure 1. Kernel appearance at different developmental stages.
Lipid content

α-Tocopherol

Time (days post-anthesis)

Lipid content (g/100 g)

α-tocopherol (mg/100 g)
Figure 2. Lipid and tocopherol isomer accumulation during almond kernel development. Bars show ± S.E.

* results from analysis of dried kernels