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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-^{14}C] acetate incorporation, or organic solvents to extract fatty acid and triacylglycerol, monitored almond fatty acid biosynthesis.

41 However, these studies were based on cultivars and development stages for almonds grown
42 under northern hemisphere climatic conditions. So far, studies concerning the accumulation
43 of fatty acids during almond kernel maturation have not been undertaken in the southern
44 hemisphere where almond fruits are exposed to more solar radiation during maturation
45 (Zhu 2014 PhD thesis), in particular, solar UV radiation in the southern hemisphere is
46 stronger than the northern hemisphere (Gies et al. 2004). Australia has a long history of
47 almond production, and Australian production has increased dramatically over the last
48 decade from 16,000 t in 2006 to over 81,000 t in 2016 (ABA 2016). Australian almond
49 producing regions experience unique environmental conditions, for example, limited
50 rainfall (and frequent droughts), intense ultraviolet radiation (UVR), and predominantly
51 red loamy and sandy soils, i.e. conditions which influence almond kernel development
52 (Mousavi and Alimohamadi 2006). Kodad et al (2010) also pointed out the climatic
53 conditions prevalent during the growing season, along with genotype and environment
54 together influence almond oil content and fatty acid composition. It is therefore worth
55 studying the changes in fatty acid profiles of Australian grown almonds during kernel
56 development.
57

58 To date, the accumulation of tocopherols during almond lipid maturation has not been
59 reported in the literature. Among the various tree nuts, almonds have the highest vitamin E
60 (tocopherol) content (Kodad et al. 2011b, Zhu et al. 2015). Tocopherol concentration is
61 therefore a key nutritional measure of almond kernel quality. Almond lipids predominantly

62 comprise the monounsaturated fatty acid, oleic acid, and the polyunsaturated fatty acid,
63 linoleic acid (Kodad et al. 2011a, Zhu et al. 2015) together with tocopherols collectively,
64 these constituents have been shown to play an important lipophilic antioxidant role in
65 human metabolism (Damasceno et al. 2011, Hollis and Mattes 2007, Rajaram et al. 2010,
66 Wien et al. 2010). The concentrations of tocopherols in fully ripened almond kernels has
67 been well documented (Kodad et al. 2011b, Kornsteiner et al. 2006, Lopez-Ortiz et al. 2008,
68 Madawala 2012, Matthäus and Ozcan 2009), but the accumulation of tocopherols during
69 kernel development has not been extensively studied. In this study, changes in four
70 tocopherol homologues over six stages of almond kernel development were followed, to
71 determine the key timing of tocopherol formation.

72

73 Given the nutritional importance of lipids, unsaturated fatty acids and tocopherols, insight
74 into their accumulation during almond kernel maturation might be used to inform the
75 timing of almond orchard management practices, such as irrigation and fertilisation, in
76 order to enhance kernel quality. Nanos and colleagues (Nanos et al. 2002) found irrigation
77 enhanced oleic acid content in almond lipids compared with no irrigation. However, our
78 previous study (Zhu et al. 2015) observed that moderate deficient irrigation increased oleic
79 acid in comparison to the control. Therefore, the present study aimed to investigate the key
80 time points for almond lipids during drupe maturation, providing useful data for future
81 studies. This study was performed on Nonpareil almonds, a cultivar grown extensively
82 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,

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

112

113 ***Fatty acid determination.*** Lipid extraction and fatty acid determinations were performed
114 (in triplicate) using chloroform-methanol extraction and methanol-sulphuric acid FAME
115 formation (fatty acid methylation), based on methodology previously described by
116 Makrides et al. (1996) with some modification (Zhu et al. 2015). Briefly, almond powder
117 (0.05 g) was mixed with 0.9% aqueous sodium chloride (2 mL), methanol (3 mL,
118 containing 0.005% BHA), C17 free fatty acid (400 µL, 0.16% in methanol) as an internal
119 standard and chloroform (6 mL), and allowed to stand for 1 hour. After extraction, samples
120 were centrifuged (3000 x g for 10 min) and the organic phase separated and concentrated
121 using a nitrogen evaporator (N-EVAP 112, Organomation Associates Inc., Berlin, MA.
122 USA) at 45°C. After evaporation, the vial containing the extract was weighed, and the
123 difference between the vial with extract and the initial empty vial is the amount of the
124 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

146 hexane layer was transferred to vials and the residue extracted again, before the combined
147 hexane extracts were concentrated using a nitrogen evaporator (N-EVAP 112) at 45°C).
148 The resulting residue was re-dissolved in hexane (1 mL) prior to HPLC analysis, using
149 previously published protocols (Lampi 2011, Lampi et al. 2008); i.e. the isocratic mobile
150 phase was hexane (with 2% 1,4-dioxane), with a flow rate of 1.0 mL/min, an injection
151 volume of 20 µL and column temperature of 25°C. HPLC analysis was performed using
152 an Agilent 1200 HPLC (Agilent Technologies, Waldbronn, Germany) coupled with diode
153 array and fluorescence detectors (DAD and FLD, respectively). Separation was achieved
154 using a Grace Alltime HP silica column (150 mm, 3 mm ID, 3 µm; Grace Discovery
155 Sciences, Deerfield, IL, USA). α, β, γ, δ-Tocopherol and α-tocotrienol standards were used
156 to prepare external calibration curves. α-Tocopherol was detected by DAD at a wavelength
157 of 292 nm, while β, γ, δ-tocopherol and α-tocotrienol were detected by FLD at wavelengths
158 of 292 nm (excitation) and 325 nm (emission).

159

160 ***Data analysis.*** Chemical data were analysed by one-way ANOVA using GenStat (15th
161 Edition, VSN International Limited, Herts, UK) and GraphPad Prism 5 (Version 5.01
162 GraphPad Software Inc., La Jolla, CA, USA) for graph presentation. Mean comparisons
163 were performed by Tukey's multiple-comparison test at $P<0.05$. Pearson's co-efficient was
164 used for correlation analysis.

165

166 **Results and Discussion**

167 The study of fruit morphology is important for orchard management, because via plant
168 morphology, both the vegetative and reproductive structures of the plant are observed.
169 Plant morphology also examines the process in which structures originate and mature as a
170 plant grows. This information is the key to predict crop yield. Compositional changes that
171 occur during fruit morphological development could have a significant role in determining
172 orchard practice, in a manner similar to that employed by the wine industry to determine
173 the timing of vineyard management practices. In this study, we measured changes in
174 almond morphological and compositional characteristics during development, to determine
175 to what extent this information could be used by industry to inform orchard management
176 decisions.
177

178 ***Fruit appearance during almond kernel development***

179 Changes in the appearance of almonds during their development and ripening are shown
180 in Figure 1. At the first and second timepoints (i.e. at t=1 and t=2, being 74 and 95 days
181 post-anthesis respectively), almond kernels contained clear endosperm, while the outer hull
182 (mesocarp) was bright green in color. As kernels developed, the endosperm decreased in
183 size as the embryo developed which was cream in color, but by the third and fourth
184 timepoints (i.e. t=3 and t=4, being 136 and 156 days post-anthesis respectively), the kernel
185 skin remained pale and fruit color was unchanged. By the fifth timepoint (i.e. t=5, being
186 156 days post-anthesis), the kernel had become firm and the skin had browned. The fruit
187 mesocarp had become dry and exhibited a leathery texture. At commercial maturity, (i.e.

188 t=6, being 167 days post-anthesis or commercial maturity) kernels were fully ripe and
189 comparatively dry; i.e. moisture levels had decreased to approximately 5% (data not
190 shown). These anatomical observations were similar to those described by Munshi and
191 Sukhja (1984), and Hawker and Buttrose (1980), despite different varieties being studied;
192 i.e. Nonpareil in this study, the regional selection H5 in the Munshi and Sukhja study, and
193 the local varieties Chellaston and Johnston were comprehensively illustrated of almond
194 kernel anatomical features during maturation in the Hawker and Buttrose study. In addition
195 to those early studies, Martínez-Gómez et al. (2008) also finely observed the dissected parts
196 of five almond cultivars during drupe development, and had similar but with subtle
197 differences and specific descriptions for individual cultivars.
198

199 ***Lipid accumulation during almond kernel development***

200 Lipid accumulation is shown in Figure 2. Based on the rate differences, accumulation
201 patterns could be seen. From 74 to 95 days post-anthesis the rate was 0.38 g/100 g/day;
202 from 95 to 115 days post-anthesis the rate was 1.83 g/100 g/day; from 115 to 156 days post-
203 anthesis the rate was 0.05 g/100 g/day; from 156 to 167 days post-anthesis the rate was
204 0.62 g/100 g/day. The ANOVA analysis showed there was no significant difference between
205 t=3, t=4, and t=5, and there was a significant difference between t=5 and t=6, likewise, a
206 significant difference between t=2 and t=3 (Table 2). During t=1 to t=2, lipid accumulation
207 was slow; from t=2 to t=3 had the highest development of almond lipid synthesis, within
208 20 days, lipid synthesis increased to 46.46 g/100g fresh kernels at stage 3, which is regarded

209 as the critical time for lipid accumulation. Thereafter, the rate of almond lipid synthesis
210 declined to 0.05 g/100 g/day, where t=3 to t=5 lasted 41 days. During this time, other
211 compounds are being actively metabolized, for example, significant quantities of protein
212 form, and sugar and moisture content decrease (Cherif et al. 2004). The results show that
213 lipids accumulate in the first period of development. This could have implications for early
214 harvesting of almonds to produce high quality almond oil. This has been done in other
215 crops such as grapes resulting in high quality grape seed oil (Rubio et al. 2009).

216 Approaching the ripening period (t=6), lipid accumulation increased again to 0.62 g/100
217 g/day and reached the final amount of 53.70 g/100 g dry kernels. This could in part be
218 attributed to moisture loss which resulted in the kernel dry mass being concentrated.
219 Harvest occurs in the summer season; in the current study, the weather prior to harvest
220 comprised low rainfall and high daily maximum temperatures (Table 1). The long-term
221 average maximum temperature for the Adelaide Plains is 28.1°C, compared to 28.5–28.7°C
222 for January and February in the 2013 growing season. Conditions were also much drier in
223 2013, with just 9.0 and 12.4 mm of rainfall in January and February respectively, compared
224 with long-term averages of 21.2 and 20.7 mm for these months respectively. Warmer and
225 drier climatic conditions can give increased seed dry mass (Monga et al. 1983, Munshi and
226 Sukhija 1984, Onemli 2012). Warmer and drier climatic conditions also affect almond lipid
227 fraction composition. It was observed that almonds grown in the Riverland region (a hot
228 and dry almond growing region in Australia) had a higher portion of linoleic acid than
229 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

272 labelling experiments to study almond lipid biosynthesis. Linolenic acid, a poly-
273 unsaturated fatty acid, followed a similar type of accumulation pattern.

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

279

280 ***Tocopherols accumulation during almond kernel development***

281 Figure 2 shows the accumulation of almond lipids and tocopherols between t=1 and t=6,
282 and Table 3 demonstrates the key timing of tocopherol formation. α-Tocopherol
283 concentration showed a very strong positive correlation with almond lipid accumulation
284 content (R=0.864, p<0.0001). β-Tocopherol and α-tocotrienol also showed a strong
285 positive correlation with lipid accumulation (R=0.824, 0.761 respectively, p<0.0001),
286 while γ-tocopherol showed a moderate correlation with almond lipid accumulation (R=
287 0.502 p=0.02). This result reflects the natural pathway of tocol accumulation as α-
288 tocopherol, β-tocopherol and α-tocotrienol are end products whereas γ-tocopherol is an
289 intermediate product towards α-tocopherol.

290 In the present study, the rates of α-tocopherol synthesis varied considerably. From t=1 to
291 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, α-tocopherol was always the predominant constituent, no other homologues like γ-tocopherol and α-tocotrienol were higher than α-tocopherol. α-Tocopherol is synthesized, via γ-tocopherol methyltransferase, and the levels of γ-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

312 key timing of almond lipid accumulation was between 95 and 115 days post-anthesis, while
313 tocopherols predominantly accumulated between 74 and 95 days after anthesis. Especially,
314 the time between 95 days and 115 days post-anthesis is a crucial period to apply orchard
315 management techniques such as increased water and fertilisation, to enhance the lipids and
316 tocopherol in almond, as well as to influence oleic acid and linoleic acid maturation to
317 control the O/L ratio for long shelf life of kernels.

318

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

Year	Month	T_{max}* (°C)	T_{min}* (°C)	Rainfall* (mm)	Solar Radiation* (MJ/m^2)
2012	September	19.1 (18.3)	9.0 (8.9)	21.6 (54.4)	16.7 (15.5)
	October	21.9 (21.0)	9.6 (10.6)	15.6 (44.9)	23.4 (20.6)
	November	26.6 (24.0)	14.5 (12.8)	16.4 (30.5)	28.9 (24.7)
	December	27.0 (25.7)	15.5 (14.5)	13.6 (27.4)	30.3 (26.7)
2013	January	28.5 (28.1)	15.7 (16.0)	9.0 (21.2)	27.6 (27.7)
	February	28.7 (28.1)	17.3 (16.2)	12.4 (20.7)	23.7 (24.4)

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

* Seasonal data (and long term average data)

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Table 2. Fatty acid composition of almonds at different stages of kernel development.

Sampling time: (days post-anthesis)	74 days	95 days	115 days	136 days	156 days	167 days	167 days*
	t=1	t=2	t=3	t=4	t=5	t=6	t=6*
Lipid (g/100 g)	3.9 ± 0.1 c	7.9 ± 0.05 c	44.5 ± 2.32 b	45.7 ± 0.69 b	46.5 ± 0.30 b	53.3 ± 2.17 a	53.3 ± 0.70 a
myristic(C14:0)	nd	0.06 ± 0.00 a	0.07 ± 0.00 a	0.05 ± 0.00 b	0.05 ± 0.00 b	0.04 ± 0.00 b	0.05 ± 0.00 b
palmitic(C16:0)	7.7 ± 0.1 c	9.0 ± 0.05 a	8.1 ± 0.09 b	7.3 ± 0.02 d	7.2 ± 0.03 d	7.3 ± 0.05 d	7.3 ± 0.04 d
palmitoleic(C16:1)	nd	0.40 ± 0.00 d	0.53 ± 0.01 a	0.48 ± 0.00 c	0.51 ± 0.01 ab	0.50 ± 0.00 bc	0.48 ± 0.01 c
stearic(C18:0)	nd	0.98 ± 0.02 d	1.4 ± 0.02 c	1.80 ± 0.02 a	1.7 ± 0.01 a	1.6 ± 0.03 b	1.6 ± 0.01 b
vaccenic(C18:1n=7)	0.75 ± 0.07 c	1.4 ± 0.00 b	1.5 ± 0.01 a	1.4 ± 0.01 ab	1.4 ± 0.00 ab	1.4 ± 0.01 ab	1.4 ± 0.01 ab
oleic(C18:1n=9)	10.8 ± 0.7 e	39.1 ± 0.17 d	52.4 ± 0.30 c	60.1 ± 0.24 b	63.3 ± 0.09 a	63.7 ± 0.18 a	62.6 ± 0.16 a

linoleic(C18:2)	24.4 ± 1.6 cd	38.5 ± 0.23 a	33.7 ± 0.15 b	26.8 ± 0.28 c	23.9 ± 0.06 cd	23.4 ± 0.16 d	24.7 ± 0.15 cd
linolenic(C18:3)	nd	0.32 ± 0.02 a	0.11 ± 0.00 b	0.07 ± 0.00 c	0.05 ± 0.00 c	0.07 ± 0.00 bc	0.08 ± 0.01 bc
arachidic(C20:0)	nd	0.11 ± 0.00 a	0.10 ± 0.01 a	0.09 ± 0.01 a	0.09 ± 0.01 a	0.11 ± 0.01 a	0.09 ± 0.00 a

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.

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Table 3. Tocol composition (mg/100 g) of almonds at different stages of kernel development.

Sampling time:	74 days	95 days	115 days	136 days	156 days	167 days	167 days*
(days post-anthesis)							
	t=1	t=2	t=3	t=4	t=5	t=6	t=6*

α-tocopherol	1.2 ± 0.02 c	12.1 ± 0.33 b	14.3 ± 0.82 b	15.7 ± 0.72 b	21.1 ± 0.98 a	20.5 ± 1.84 a	21.3 ± 1.14 a
γ-tocopherol	0.01 ± 0.00 c	0.68 ± 0.05 a	0.50 ± 0.02 b	0.42 ± 0.01 b	0.51 ± 0.03 b	0.71 ± 0.03 a	0.52 ± 0.01 b
β-tocopherol	nd	0.08 ± 0.01 b	0.10 ± 0.00 a	0.10 ± 0.00 a	0.10 ± 0.00 a	0.10 ± 0.00 a	0.10 ± 0.00 a
α-tocotrienol	0.09 ± 0.02 de	0.04 ± 0.01 e	0.20 ± 0.00 c	0.42 ± 0.03 a	0.18 ± 0.02 cd	0.27 ± 0.03 bc	0.35 ± 0.02 ab

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.

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Table 4. Correlation coefficients for individual fatty acids against total lipid content.

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

Pearson r values which indicate significant correlations (CI 95%, *$P \leq 0.05$, **$P \leq 0.005$, *** $P \leq 0.001$).

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t=1

t=2

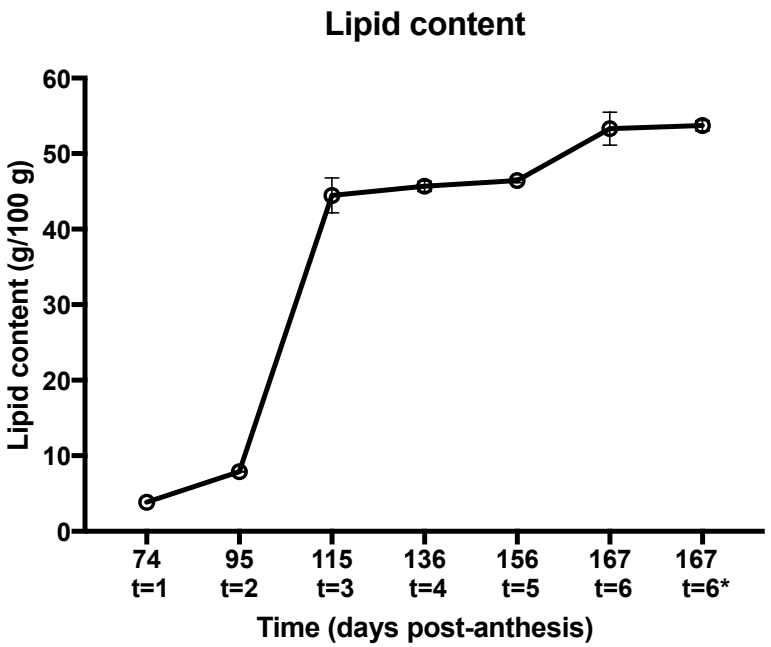
t=3

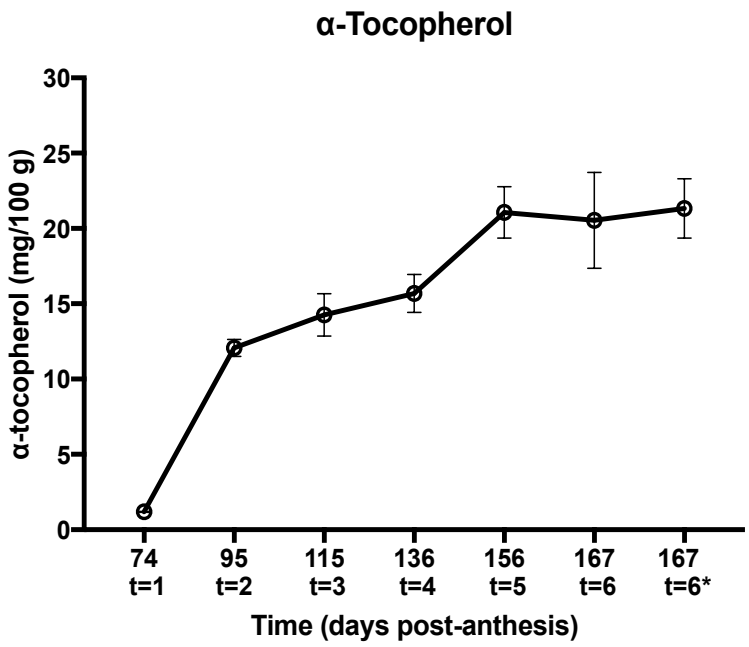
t=4

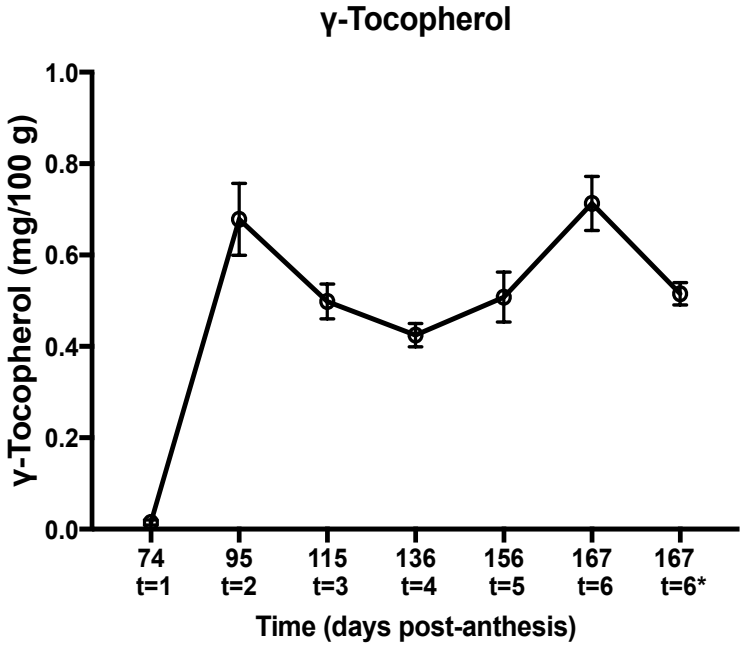
t=5

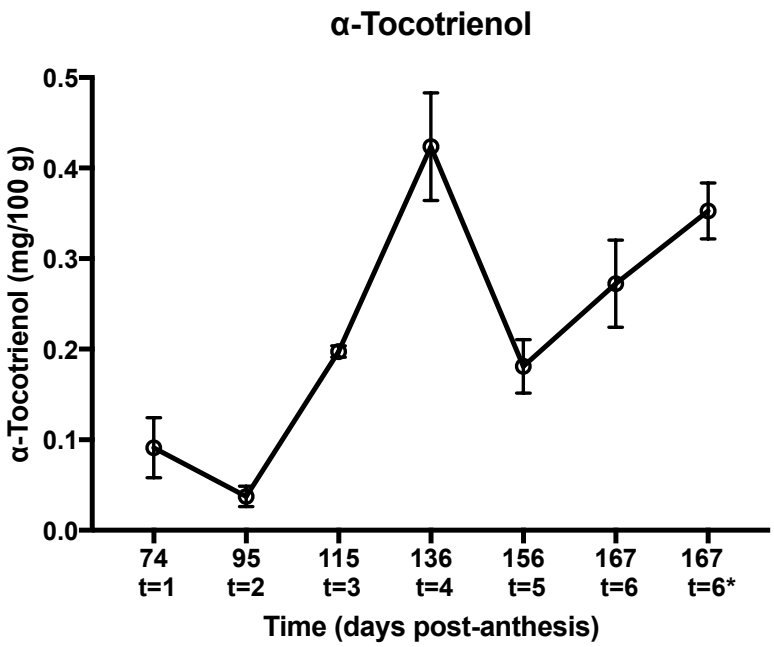
t=6 (commercial maturity)

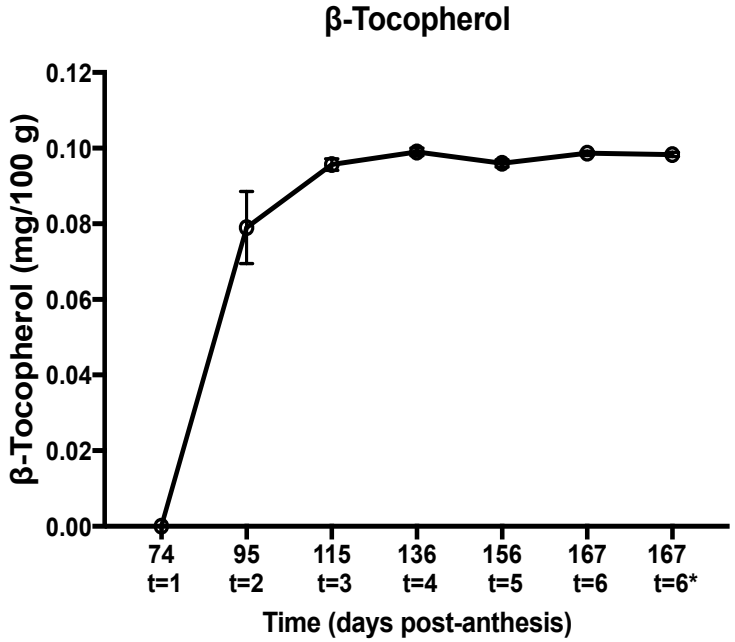
Figure 1. Kernel appearance at different developmental stages.


Lipid content
Lipid content (g/100 g)
60
50
40
30
20
10
0
74 t=1
95 t=2
115 t=3
136 t=4
156 t=5
167 t=6
167 t=6*
Time (days post-anthesis)


α-Tocopherol
α-tocopherol (mg/100 g)
30
25
20
15
10
5
0
74 t=1
95 t=2
115 t=3
136 t=4
156 t=5
167 t=6
167 t=6*
Time (days post-anthesis)


γ-Tocopherol
γ-Tocopherol (mg/100 g)
1.0
0.8
0.6
0.4
0.2
0.0
74 t=1
95 t=2
115 t=3
136 t=4
156 t=5
167 t=6
167 t=6*
Time (days post-anthesis)


α-Tocotrienol
α-Tocotrienol (mg/100 g)
0.5
0.4
0.3
0.2
0.1
0.0
74 t=1
95 t=2
115 t=3
136 t=4
156 t=5
167 t=6
167 t=6*
Time (days post-anthesis)



* results from analysis of dried kernels

Figure 2. Lipid and tocopherol isomer accumulation during almond kernel development. Bars show ± S.E.