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Influence of region and variety on fatty acid and tocopherol concentration of almond

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Abstract. This paper describes an investigation into the fatty acid and tocopherol concentration of several varieties of almonds grown in different almond-producing regions of Australia, as well as almonds grown in Spain and California. Considerable variation was observed not only in linoleic acid (15.7% – 29.9% of total lipids) and Vitamin E (8.2 mg – 21.5 mg/100 g) content, but also lipid (46.1 – 63.5 g/100 g), oleic acid (58.5% – 71.3% of total lipids), palmitic acid (5.9% – 7.5% of total lipids) and stearic acid (1.0% – 2.4% of total lipids) content. Understanding the influence of region and variety on almond composition will enable industry to make informed decisions with regards to almond breeding programs and the suitability of almond cultivars to particular growing regions.

Keywords. Almond – Fatty acid – Linoleic acid – Lipophilic antioxidant – Vitamin E – Tocopherol.

Influence de la région et de la variété sur la concentration en acides gras et tocophérol des amandiers

Résumé. *Ce document décrit une enquête sur la concentration en acides gras et tocophérol pour plusieurs variétés d'amandiers cultivés dans différentes régions productrices d'amandes de l'Australie, ainsi que pour les amandiers cultivés en Espagne et en Californie. Une variation considérable a été observée non seulement pour la teneur en acide linoléique (15,7% – 29,9% des lipides totaux) et en vitamine E (8,2 mg – 21,5 mg/100 g), mais aussi en lipides (46,1 – 63,5 g/100 g), acide oléique (58,5% – 71,3% des lipides totaux), acide palmitique (5,9% – 7,5% des lipides totaux) et acide stéarique (1,0% – 2,4% des lipides totaux). La compréhension de l'influence de la région et de la variété sur la composition des amandes permettra à l'industrie de prendre des décisions éclairées en ce qui concerne les programmes d'amélioration de l'amandier et l'adéquation des cultivars d'amandier à des régions particulières de culture.*

Mots-clés. *Amandier – Acides gras – Acide linoléique – Antioxydant lipophile – Vitamine E – Tocophérol.*

I – Introduction

Almonds are a nutrient-rich source of lipids, protein, dietary fibre, minerals, vitamins and polyphenols (Esfahlan *et al*., 2010). The health benefits afforded by almonds have been largely attributed to lipids and vitamins, with numerous clinical and pre-clinical trials and epidemiologic studies showing that regular consumption of almonds can: significantly reduce low density lipoprotein (LDL), cholesterol, postprandial glycaemia and insulinaemia; improve body weight control; and reduce the risk of obesity-related diseases such as coronary heart disease (CHD) and type II diabetes (Jenkins *et al*., 2008, Rajaram *et al*., 2010, Wien *et al*., 2010, Damasceno *et al*., 2011). All of these health benefits have been largely attributed to high proportions of the unsaturated fatty acids (USFA) oleic acid, linoleic acid and Vitamin E.

Linoleic acid, an omega-6 fatty acid, is an essential fatty acid for humans and is involved in children's growth and the prevention of cardiovascular disease (Jenskin *et al*., 2008, Wien *et al*., 2010). However, high levels of linoleic acid have been considered as markers of almond spoilage, since linoleic acid's double bonds are susceptible to oxidation, thus, high levels of oleic acid and low lev-

els of linoleic acid have been associated with prolonged shelf-life of almonds and are often advocated (Zaplin *et al*., 2013). However, another property in lipids, Vitamin E, can improve almond shelf-life (Socias i Company *et al*., 2010). Of the various tree nuts, almonds typically contain the most Vitamin E; with two handfuls of almonds providing the average daily-recommended dose of Vitamin E, being 15 mg per day (Institute of Medicine, 2000). Previous studies have shown almond Vitamin E largely comprises α-tocopherol and γ-tocopherol, with lesser amounts of β-tocopherol, δ-tocopherol and α-tocotrienol also present; the relative proportions of which are thought to be influenced by genotype and region of origin (Yildirim *et al*., 2010; Kodad *et al*., 2011a).

Very little is known about the Vitamin E and fatty acid composition of almonds grown in different Australian regions. This study aimed to investigate the influence of environmental conditions (i.e. regionality) and genetics (i.e. variety) on almond fatty acids in particular linoleic acid and Vitamin E. Improved knowledge of the regional and/or varietal differences in almond composition may enable industry to make more informed decisions with respect to varietal selection.

II – Materials and methods

Australian almond samples were collected from Willunga, Adelaide, Riverland (South Australia) and Sunraysia (Victoria) during the 2012 season. Spanish almonds were sourced from Zaragoza and North American almonds were sourced from Merced, (California). The cultivars selected were: 'Nonpareil' (from Sunraysia, Adelaide, Willunga, Riverland, Zaragoza and Merced), 'Johnston' (from Adelaide and Willunga), 'Somerton' (Willunga), 'Carmel' (Riverland and Merced) and 'Guara' (Riverland and Zaragoza). Samples (100 kernels randomly harvested per tree per cultivar) were harvested when the mesocarps had naturally split, indicating ripening. For each cultivar, 25 trees from growers' orchard were harvested. Kernels were dried at 50°C for 48 hours and the moisture content (approximately 2%) confirmed by the gravimetric technique. Dried kernels were ground using a coffee grinder to a fine powder, sieved through a 1000 μM mesh and then stored under nitrogen prior to analysis.

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 reported by Makrides and colleagues (Makrides *et al*., 1996). Fatty acid composition was determined using an HP 6890 Gas Chromatograph (Hewlett Packard, Palo Alto, CA. USA) equipped with a flame ionization detector (FID), split/splitless injection, HP 7683 autosampler and HP Chemstation. A capillary GC column SGE BPX 70 (50 m, 0.32 mm ID, 0.25 μm) was used (SGE Analytical Science Pty. Ltd. RingWood, VIC. Australia). Helium was the carrier gas and the split-ratio was 20:1, the injector temperature was set at 250°C and the detector temperature at 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 based on the retention time of the internal standard free fatty acid C17.

Tocol extraction was based on the alkaline saponification and hexane extraction method used previously for cereals and nuts (Xu, 2002). HPLC analysis protocol referred to Lampi and colleagues (Lamp *et al*., 2008; Lampi, 2011), specifically, the isocratic mobile phase was hexane (with 2% 1,4-dioxane), flow rate 1.0 mL/min, injection volume 20 μL, column temperature 25°C. HPLC analysis was performed using an Agilent 1200 HPLC (Agilent Technologies, Deutschland, Germany) coupled with a diode array detector (DAD), fluorescence detector (FLD), autosampler, quaternary pump, Agilent Chemstation and Grace Alltime HP silica column (150 mm, 3 mm, 3 μm; Grace Discovery Sciences, Deerfield, IL. USA). α, β, γ, δ-Tocopherol and α-tocotrienol were quantified using calibration curves prepared from external standards. α-Tocopherol was measured by DAD at a signal wavelength of 292 nm, while β, γ, δ-tocopherol and α-tocotrienol were analysed by FLD at signal wavelengths of 292 nm (excitation) and 325 nm (emission).

Chemical data were analysed by ANOVA using GenStat (14th Edition, VSN International Limited, Herts, UK) and GraphPad Prism 5 (Version 5.01 GraphPad Software, Inc. La Jolla, CA, USA). Mean comparisons were performed by least significant difference (LSD) multiple-comparison test at $p < 0.05$. Pearson's co-efficient was used for correlation analysis.

III – Results and discussion

1. Influence of region and genotype on almond lipid content

The lipid content of the almonds studied ranged from 46.1 to 63.5 g/100 g (Table 1). The lowest lipid content (46.1 g/100 g) was observed in 'Carmel' almonds from California, while 'Nonpareil' almonds from Willunga (Australia) contained the highest lipid level (63.5 g/100 g). The lipid concentrations of Australian grown almond samples were 53.1 to 63.5 g/ 100 g. This was considered representative of the major commercial varieties grown in the four key regions of Australia. In this study environment appeared to have a significant effect on the lipid content of 'Nonpareil', 'Carmel' and 'Guara' almonds, with the northern hemisphere grown almonds containing lower lipid levels (between 46.1-51% of kernel weight) than almonds grown in the southern hemisphere (between 53.7-63.5% of kernel weight) (Table 1). For example, Californian 'Nonpareil' lipid content (47.1 g/100 g) was lower than the levels reported in an earlier study (Sathe *et al*., 2008), but similar with a recent study (Yada *et al*., 2013). However, 'Somerton' and 'Johnston' varieties showed no compositional differences between sites (Table 1). In contrast, genotype had a greater influence on almond lipid content. In the Riverland, lipid content varied significantly between genotypes ($P< 0.05$).

2. Influence of region and genotype on almond fatty acid composition

Unsaturated fatty acids comprised oleic acid (58.1-71.3% of total lipids), linoleic acid (15.7-29.9% of total lipids), palmitoleic acid (0.20-0.62% of total lipids) and vaccenic acid (0.77-2.17% of total lipids), which made up more than 90% of the total lipids. Saturated fatty acids, including palmitic acid (5.9-7.5% of total lipids), stearic acid (1.0-2.4% of total lipids), arachidic acid (0.07-0.10% of total lipids) and myristic acid (0.02-0.05% of total lipids, data not shown in the table) accounted for the remaining 10%. These results demonstrate the influence of environment and genotype on almond fatty acid composition (Table 1). Oleic acid and linoleic acid were the most abundant fatty acids, in agreement with previous studies on almonds grown around the world, i.e. in Turkey, Iran, Spain, Italy, China, India, California (Kodad *et al*., 2010; Moayedi *et al*., 2011; Tian *et al*., 2011). A negative correlation was observed between oleic acid and linoleic acid concentrations (Pearson r -0.993, *p* value (two tails) < 0.0001) as in other studies (Sathe *et al*., 2008; Kodad *et al*., 2010; Kodad *et al*., 2011b,). No correlation was observed between any lipid fractions and the total lipid content not even with the major fatty acid, oleic acid, which was also consistent with previous reports (Sathe *et al*., 2008; Kodad *et al*., 2011b).

Significant differences were observed in the oleic acid levels of almonds harvested from different genotypes and from different regions. The highest oleic acid level (68.1% of total lipids) was observed in 'Nonpareil' almonds from Spain, followed by 'Nonpareil' almonds (65.6% of total lipids) from California (Table 1). 'Nonpareil' almonds produced in Australia generally contained lower oleic acid levels, albeit 'Nonpareil' almonds from Adelaide plains were similar to almonds from California, with 64.2% of total lipids. Likewise, 'Guara' almonds from Spain contained oleic acid levels greater than 'Guara' almonds from Australian Riverland, but the oleic acid content of 'Carmel' almonds showed no significant difference when grown in California or Australia (Table 1). On the other hand, genotype had a significant impact on oleic acid levels for almonds grown at the same sites; i.e. in Willunga, 'Somerton' almonds had the highest oleic acid levels, followed by 'Johnston' and 'Nonpareil' almonds (Table 1). In Adelaide Plains, the local variety 'Johnston' yielded almonds that

Table 1. Lipid and fatty acid compositions of almonds from different genotypes

Sample		Concentration†							
Genotype	Region	Lipids (g/100g)	Palmitic (C16:0)	Palmitoleic (C16:1n-7)	Stearic (C18:0)	Vaccenic (C18:1n-7)	Oleic (C18:1n-9)	Linoleic (C18:2n-6)	Ratio O/L
'Nonpareil'	Willunga	63.5 ± 0.7 a	6.8 ± 0.0 c	0.41 ± 0.01 d	1.4 ± 0.1 a	1.2 ± 0.0 d	58.5 ± 0.4 d	29.9 ± 0.6 a	1.96 ± 0.04 d
	Adelaide	60.0 ± 1.1 ab	6.9 ± 0.1 bc	0.49 ± 0.01 c	1.4 ± 0.0 a	1.3 ± 0.0 c	64.2 ± 0.4 bc	23.9 ± 0.5 c	2.69 ± 0.04 c
	Riverland	56.5 ± 2.8 b	6.7 ± 0.1 c	0.45 ± 0.02 d	1.3 ± 0.0 b	1.3 ± 0.0 c	63.6 ± 0.7 c	24.8 ± 0.9 b	2.57 ± 0.08 c
	Sunraysia	57.7 ± 1.3 ab	6.9 ± 0.0 b	0.52 ± 0.01 b	1.1 ± 0.0 c	1.5 ± 0.0 b	63.8 ± 0.3 c	24.4 ± 0.4 bc	2.61 ± 0.03 c
	Spain	51.0 ± 2.0 bc	7.5 ± 0.0 a	0.62 ± 0.00 a	1.4 ± 0.0 ab	2.2 ± 0.0 a	68.1 ± 0.1 a	19.0 ± 0.0 e	3.58 ± 0.00 a
	California	47.1 ± 3.2 c	6.6 ± 0.0 c	0.48 ± 0.01 cd	1.2 ± 0.0 b	1.5 ± 0.0 b	65.6 ± 0.1 b	22.6 ± 0.0 d	2.90 ± 0.01 b
	P	*0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
'Somerton'	Willunga	60.5 ± 1.5	6.1 ± 0.0 a	0.41 ± 0.00	2.4 ± 0.0 a	1.0 ± 0.0 b	67.0 ± 0.1 a	21.4 ± 0.2 a	3.13 ± 0.02 b
	Riverland	59.4 ± 0.8	5.9 ± 0.0 b	0.40 ± 0.00	1.7 ± 0.0 b	1.2 ± 0.0 a	70.1 ± 0.2 a	18.9 ± 0.2 b	3.71 ± 0.03 a
	P	*ns*	*< 0.01*	*ns*	*< 0.001*	*< 0.001*	*ns*	*< 0.001*	*< 0.001*
'Johnston'	Willunga	60.8 ± 0.8	6.9 ± 0.0	0.44 ± 0.00 b	2.0 ± 0.0 a	1.1 ± 0.0 b	63.0 ± 0.1 b	25.0 ± 0.1 a	2.52 ± 0.01 b
	Adelaide	58.2 ± 0.7	6.7 ± 0.1	0.56 ± 0.01 a	1.8 ± 0.1 a	1.3 ± 0.0 a	67.0 ± 0.4 a	21.0 ± 0.6 b	3.19 ± 0.07 a
	P	*ns*	*ns*	*< 0.001*	*< 0.05*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
'Carmel'	Riverland	53.7 ± 1.0 a	6.8 ± 0.0 b	0.39 ± 0.01 b	1.2 ± 0.0	1.2 ± 0.0 b	58.6 ± 0.4	29.8 ± 0.5	1.96 ± 0.03
	California	46.1 ± 1.5 b	7.2 ± 0.0 a	0.44 ± 0.00 a	1.2 ± 0.0	1.4 ± 0.0 a	58.1 ± 0.1	29.7 ± 0.1	1.96 ± 0.00
	P	< 0.05	< 0.001	< 0.05	ns	< 0.001	ns	ns	ns
'Guara'	Riverland	60.4 ± 2.0 a	5.9 ± 0.0 b	0.23 ± 0.00 b	2.4 ± 0.0 a	0.8 ± 0.0 b	66.4 ± 0.0 b	22.3 ± 0.1 a	2.97 ± 0.01 b
	Spain	50.7 ± 2.1 b	6.9 ± 0.0 a	0.45 ± 0.00 a	2.3 ± 0.0 b	1.2 ± 0.0 a	71.3 ± 0.1 a	15.7 ± 0.0 b	4.53 ± 0.01 a
	P	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Values are means of three replicates ± standard error. Values followed by different letters within a column are significantly different ($P < 0.05$); ns = not significant.
† Fatty acid content as a percentage of lipids.

also contained more oleic acid than 'Nonpareil' almonds. In the Riverland, 'Somerton' almonds had the highest oleic acid levels (Table 1). The differentiation of oleic acid and linoleic acid concentrations led to variation in the O/L ratio (oleic/linoleic) for almonds of different varieties and from different regions. We found that the O/L ratio largely depended on linoleic acid rather than oleic acid levels, due to a highly negative relationship between the O/L ratio and linoleic acid (Pearson r 0.9984, 95% CI 0.9974-0.9991, *p* value (two tailed) <0.0001) which is greater than the positive relationship between the O/L ratio and oleic acid (Pearson r 0.9892, 95% CI 0.9819-0.9936, *p* value (two tailed) <0.0001). The O/L ratio typically represents almond kernel shelf-life: with higher O/L ratio indicating greater storage capacity (Piscopo *et al*., 2010; Kodad *et al*., 2011b). However, a higher O/L ratio means a lower proportion of linoleic acid, i.e. the healthiest fatty acid present in almond lipids. Therefore, consideration of high O/L ratio for long storage and high linoleic acid for more nutrition has opened space to further research.

Linoleic acid concentrations were significantly affected by both environment and genotype (Table 1). Willunga grown almonds contained more linoleic acid than almonds from other sites. For example, 'Nonpareil' almonds grown in Willunga contained significantly higher linoleic acid levels than 'Nonpareil' almonds grown in the Riverland, Sunraysia and Adelaide plains, by 17%, 19% and 20%, respectively. Similarly, 'Somerton' and 'Johnston' almonds grown in Willunga contained 12% and 16% more linoleic acid than Riverland and Adelaide plains almonds, respectively. Considering the environmental influence, solar radiation and temperature (data not shown) varied between sites during the 2012 growing season. However, differences observed in rainfall would likely be negated by irrigation. Willunga experienced less solar radiation than the other sites, which may lead to higher linoleic acid content. Willunga also experienced comparatively lower temperatures than the other sites, which could influence linoleic acid concentrations, i.e. high temperatures have been shown to negatively impact linoleic acid synthesis in sunflower seeds (Harris *et al*., 1978).

Australian grown almonds also had higher linoleic acid levels between 18.1-29.9% than Spanish almonds (Table 1). According to the literature, Spanish almonds contain linoleic acid levels ranging from 12.6-27.1% while Mediterranean almonds contained between 12.9-25.9% linoleic acid (Kodad *et al*., 2011b). Californian almonds were reported to contain 21.5-31.1% linoleic acid (Sathe *et al*., 2008). Noticeably, the regions producing almonds with lower linoleic acid are not irrigated, whereas Californian and Australian regions routinely apply irrigation to their orchards. Irrigation could therefore be a reason for linoleic acid differentiation. Nanos and colleagues found irrigation resulted in lower linoleic acid in Texas almond variety, but not in 'Ferragnès' almond variety (Nanos *et al*., 2002). Apparently, environmental factors such as irrigation, as well as genotype, can influence almond linoleic acid levels.

3. Influence of variety/region on almond tocols

Almond storage time not only depends on the O/L ratio, but also the relative concentration of Vitamin E compared to almond lipids; since higher Vitamin E content can improve lipid resistance to rancidity (Socias i Company *et al*., 2010). Therefore, breeding programs aim to improve the O/L ratio and Vitamin E content of almonds. As such, the Vitamin E content of different varieties and selections grown in particular regions was investigated. Vitamin E comprises eight tocopherol homologues: α, β, γ and δ-tocopherol and α, β, γ and δ-tocotrienol. In this study, four homologues α-tocopherol, β-tocopherol, γ-tocopherol and α-tocotrienol, were quantified in each of the different samples (Table 2). α-Tocopherol was the major component and accounted for more than 90% of the total tocols, followed by γ-tocopherol, in agreement with previous studies (Matthäus and Özcan, 2009; Kodad *et al*., 2011a). β-Tocopherol was the third most abundant tocol in Australian grown almonds, but not in Spanish or Californian almonds, which instead, contained higher concentrations of α-tocotrienol. δ-Tocopherol was only observed in trace amounts, as reported elsewhere (Kornsteiner *et al*., 2006; Matthäus and Özcan, 2009). α-Tocotrienol was detected in all samples,

but considerable variation was observed between Australian almonds and almonds grown overseas (Table 2). Previous studies by Kodad and colleagues (Kodad *et al*., 2011a) reported high concentrations of δ-tocopherol, while, Zacheo and colleagues (Zacheo *et al*., 2000) detected β-tocopherol at concentrations greater than γ-tocopherol in almonds from an Italian growing region. The variation in tocol concentrations observed in almonds from different origins indicated that both genotype and environment likely affect almond tocol composition.

Table 2. Tocol composition of almonds from different genotypes

Sample		Concentration (mg/100 g)				
Genotype	**Site**	**Total tocols**	**α-tocopherol**	**γ-tocopherol**	**β-tocopherol**	**α-tocotrienol**
'Nonpareil'	Willunga	14.4 ± 0.5 b	13.7 ± 0.5 b	0.6 ± 0.0 a	0.09 ± 0.01a	0.05 ± 0.01b
	Adelaide	14.4 ± 1.3 b	13.9 ± 1.2 b	0.3 ± 0.0 b	0.08 ± 0.00 b	0.04 ± 0.00 b
	Riverland	11.0 ± 0.4 b	10.7 ± 0.4 b	0.2 ± 0.0 cd	0.06 ± 0.01 b	0.02 ± 0.00 b
	Sunraysia	11.0 ± 0.8 b	10.7 ± 0.8 b	0.2 ± 0.0 d	0.07 ± 0.00 b	0.03 ± 0.00 b
	Spain	18.5 ± 1.7 a	17.9 ± 1.7 a	0.3 ± 0.0 c	0.09 ± 0.01 a	0.26 ± 0.02 a
	California	18.3 ± 1.6 a	17.9 ± 1.6 a	0.1 ± 0.0 e	0.01 ± 0.00 c	0.30 ± 0.03 a
	P	< 0.05	< 0.05	< 0.001	< 0.001	< 0.001
'Somerton'	Willunga	9.5 ± 0.1	9.3 ± 0.1	0.2 ± 0.0	0.04 ± 0.00 b	0.04 ± 0.00
	Riverland	12.1 ± 1.3	11.8 ± 1.3	0.2 ± 0.0	0.06 ± 0.00 a	0.03 ± 0.00
	P	*ns*	*ns*	*ns*	*< 0.05*	*ns*
'Johnston'	Willunga	11.8 ± 1.8	11.5 ± 1.7	0.3 ± 0.0 a	0.04 ± 0.00 b	0.01 ± 0.00
	Adelaide	10.1 ± 0.2	9.8 ± 0.2	0.2 ± 0.0 b	0.07 ± 0.00 a	0.01 ± 0.00
	P	*ns*	*ns*	*< 0.05*	*< 0.001*	*ns*
'Carmel'	Riverland	14.7 ± 0.8	14.4 ± 0.8	0.2 ± 0.0 a	0.07 ± 0.00 a	0.02 ± 0.00 b
	California	14.9 ± 1.7	14.6 ± 1.7	0.1 ± 0.0 b	0.02 ± 0.00 b	0.20 ± 0.02 a
	P	*ns*	*ns*	*< 0.001*	*< 0.001*	*< 0.001*
'Guara'	Riverland	18.9 ± 1.2	18.4 ± 1.1	0.4 ± 0.0 a	0.08 ± 0.00	0.09 ± 0.01 b
	Spain	21.5 ± 0.9	20.9 ± 0.9	0.3 ± 0.0 b	0.12 ± 0.01	0.24 ± 0.02 a
	P	*ns*	*ns*	*< 0.05*	*ns*	*< 0.05*

Values are means of three replicates ± standard error. Values followed by different letters within a column are significantly different ($P < 0.05$); ns = not significant.

In this study, we found environment significantly influenced α-tocopherol levels in 'Nonpareil' almonds (Table 2), but not in almonds of other varieties. Genotype affected α-tocopherol concentrations in Riverland almonds ($p < 0.001$). We observed that 'Guara' had the highest α-tocopherol concentration (18.4 mg per 100 g dry weight), followed by 'Carmel' (14.4 mg per 100 g dry weight). In contrast, γ-tocopherol concentration was more strongly affected by environment and genotype than α-tocopherol concentration; an environmental effect on γ-tocopherol levels occurred in 'Nonpareil', 'Johnston', 'Carmel' and 'Guara' almonds, but not in 'Somerton' almonds (Table 2). Variation due to genotype was observed at all three sites, i.e. Adelaide plains, Willunga and Riverland. β-Tocopherol was influenced by environment and genotype, with significant differences observed in all cases (Table 2). Noticeably, α-tocotrienol showed little difference among Australian almonds from different growing regions, but varied widely between southern and northern regions: i.e. Spanish and Californian almonds contained higher concentrations of α-tocotrienol than Australian almonds (Table 2). The levels of α-tocotrienol observed equaled or surpassed the γ-tocopherol levels of Spanish and Californian almonds. This finding has not been reported in previous research. One possible explanation is due to geographical origin, but the exact factors influencing γ-tocopherol remain unclear. Further research is required to investigate the factors affecting tocol composition of almonds from different growing regions.

Intriguingly, we found an environmental influence on almond tocol homologues that may also depend with genotype. For example, a large difference was seen in the α-tocopherol concentration of 'Nonpareil' almonds, with 23% higher levels corresponding to Willunga and Adelaide plains 'Nonpareil' than Riverland and Sunraysia 'Nonpareil'. This could be due to the lower solar radiation experienced in Willunga, compared to the Riverland and Sunraysia. However, differentiation did not occur in other genotypes. In addition, α-tocopherol was quite stable in 'Carmel' almonds grown in distinct regions; Australian Riverland 'Carmel' and Californian 'Carmel' contained 14.4 mg/100 g and 14.6 mg/100 g, respectively. Likewise, 'Guara' almonds had similar β-tocopherol levels when grown in the Australian Riverland or Spain, i.e. 0.08 mg/100 g and 0.10 mg/100 g, respectively (Table 2). These results therefore suggest that the environmental influence on almond tocol homologues also depends on the genotype involved.

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