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Functional lipid characteristics, oxidative stability, and antioxidant activity of macadamia nut (*Macadamia integrifolia*) cultivars

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ABSTRACT

Phytochemical compounds (tocopherols, tocotrienols, and squalene) were measured in seven macadamia cultivars harvested from four locations on Hawaii island to establish whether these compounds enhance the oxidative stability of roasted kernels. Cultivars that had the greatest oxidative stability also had high total lipid-soluble antioxidant capacity. Tocopherols [delta (δ), gamma (γ), alpha (α)] were not detected in most macadamia nut samples, but macadamia kernels contained significant amounts of tocotrienols (31–92 μg/g oil) and squalene (72–171 μg/g oil) for all cultivars tested. This is the first report of variation for three tocotrienol homologs (δ-, γ-, α-T3) and total antioxidant capacity in macadamia nut cultivars. No statistical correlations were found between oxidative stability and tocopherol, tocotrienol, and squalene concentrations. However, two cultivars (HAES 294 and HAES 835) were identified with superior oxidative stability, suggesting that the kernel quality of these cultivars is more stable during storage.

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1. Introduction

Phytochemicals are biologically active compounds present in natural foods including fruits, vegetables, grains, nuts, and seeds that have the potential to prevent or delay the onset of chronic diseases, such as various cancers and cardiovascular disease (CVD). The presence of phytochemicals in macadamia nuts (*Macadamia integrifolia*) may contribute positively to human health, and this information could be useful for macadamia nut growers, processors, and consumers. In addition, these compounds may protect the kernels from oxidation reactions during storage and marketing, thereby extending shelf-life.

Macadamia nuts are rich in monounsaturated fatty acids (MUFA), predominantly oleic (~60%) and palmitoleic (~20%) acids (Cavaletto, 1983). Macadamia nuts contain higher levels of MUFA than any other food source known. Diets containing high MUFA-rich foods reduce plasma LDL cholesterol levels and decrease the risk of CVD (Garg, Blake, & Wills, 2003; Kris-Etherton et al., 1999a). This cholesterol-lowering and coronary-protective effect may be due to the high percentage of unsaturated fatty acids, as well as other bioactive constituents such as tocopherols, phytosterols, and squalene (Kris-Etherton et al., 1999b).

Tocopherols, tocotrienols, and squalene are present in macadamia kernels and have antioxidant properties, but their contributions to oxidative stability of the kernels have not been defined (Franke, Murphy, Lacey, & Custer, 2007; Kaijser, Dutta, & Savage, 2000; Kornsteiner, Wagner, & Elmadfa, 2006; Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004; Tsumura, 1988; Wang, 1972). Tocols are powerful antioxidants against lipid oxidation and may delay rancidity during storage. Tocopherols have been shown to contribute to the kernel stability of hazelnuts and walnuts (Savage, Dutta, & McNeil, 1999; Savage, McNeil, & Dutta, 1997). Macadamia kernels contained low concentrations of α-tocopherol and δ-tocopherol compared to almonds and pecans (Fourie & Basson, 1989), but Kaijser et al. (2000) found high concentrations of α-tocotrienol in *M. tetraphylla*, a species grown in Australia. Both α-tocopherol and α-tocotrienol have been identified in *M. integrifolia* kernels, but there have been no studies examining their relationship to oxidative stability (Franke et al., 2007; Wang, 1972).

Tocotrienols (T3) are found in seeds of certain plant species. Palm oil and rice bran oil are the richest sources of T3, but wheat germ oil, coconut oil, barley and soybeans also contain these compounds (Gruszka & Kruk, 2007; Packer, Weber, & Rimbach, 2001). Tocotrienols contain multiple double bonds which quench free radical reactions more readily than tocopherols (Packer et al., 2001), possibly increasing the oxidative stability of macadamia kernels. T3 have cholesterol-lowering, anticarcinogenic and neuroprotective properties, in addition to enhanced antioxidant properties (Packer et al., 2001; Sen, Khanna, & Roy, 2007). Macadamia nuts also contain significant concentrations of phytosterols, especially β-sitosterol (Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004; Phillips, Ruggio, & Ashraf-Khorassani, 2005). Squalene is

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converted into phytosterols in plant cells. Squalene quenches singlet oxygen and prevents lipid peroxidation (Kohno et al., 1995; Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004). Squalene is abundant in olive oil, pumpkin seeds, and quinoa (Ryan, Galvin, O'Connor, Maguire & O'Brien, 2007). In one report for macadamia nuts, squalene concentration was 185 μg/g oil (Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004).

In macadamia breeding programs, emphasis has been placed on developing high-yielding cultivars adapted to specific locations. The chemical composition and nutritional aspects of kernels have been mostly disregarded. Cultivar comparisons for phytochemical content are limited, and there have been no reports comparing antioxidant capacity, tocotrienol contents or squalene concentrations in Hawaii's macadamia nut cultivars. The objectives of this research were to identify and quantify specific phytochemicals (tocols and squalene) in macadamia nuts and establish whether these compounds enhance the oxidative stability of dried and roasted kernels.

2. Materials and methods

2.1. Standards and reagents

HPLC-grade acetonitrile and ethanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). HPLC-grade methanol and analytical grade petroleum ether, hexane, and isopropanol were from VWR International, Inc. (West Chester, PA, USA). Reagents and Trolox standard for total antioxidant activity measurements were purchased from Analytic Jena USA (The Woodlands, TX, USA). Standards of α-tocopherol, γ-tocopherol, δ-tocopherol, and squalene were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Macadamia samples

Macadamia nuts of seven cultivars or advanced selections released by the Hawaii Agricultural Experiment Station (HAES) were collected from four production areas on the island of Hawaii, USA. Nuts were harvested from trees of HAES 246 (cv. Keauhou), HAES 294 (cv. Purvis), HAES 344 (cv. Kau), HAES 508 (cv. Kakea), HAES 788 (cv. Pahala), HAES 835, and HAES 856. Freshly harvested, green-in-husk mature nuts were dehusked and dried to a kernel moisture of 1.5% using an incremental drying process in a forced air oven (Shel Lab; Sheldon Manufacturing, Inc., Cornelius, OR, USA) (Wall & Gentry, 2007). Dried nuts were mechanically cracked and shelled, and subsamples of the dried kernels were vacuum-packed and frozen (−20 °C) for later analyses. The remainder were roasted in a convection oven (Lindbergh/Blue, Asheville, NC, USA) at 125 °C for 20–25 min. Subsamples of roasted kernels were frozen for later analyses.

2.3. Total oil content

The total oil content for each kernel sample was measured using an Ankom Xt10 extraction system (Ankom Technology, Macedon, NY) (AOCS, 2004). Dried, ground kernel samples (1 g) were placed into sample bags and further dried for 3 h at 100 °C in an oven. Samples were weighed and placed into the extractor with petroleum ether and extracted for 40 min. After extraction, samples were dried for 30 min at 100 °C, reweighed, and total oil content was calculated.

2.4. Oxidative stability and total antioxidant activity

Macadamia oil for oxidative stability and antioxidant activity measurements was obtained through cold press extraction (with a Carver hydraulic press, Model C, Sterling Hydraulic Equipment, Inc., Menomonee Falls, WI) of subsamples from dried and roasted kernels for each cultivar. The cold pressed oils were centrifuged for 15 min at 15,000 rpm to obtain clarified oil for assays, flushed with N_2, and frozen for later use in analyses. Oxidative stability of the oils was evaluated using a Metrohm 743 Rancimat instrument (Brinkman, Herisau, Switzerland), which measures the rate of oxidation under accelerated conditions (Frank, Geil, & Freaso, 1982). Clarified, cold pressed oil (2.5 g) was added to the reaction vessel with 60 mL distilled water, and heated to 130 °C with an air flow rate of 20 L/h. The oxidative stability was expressed as the induction time for oxidation of the oils.

Total antioxidant activity was determined for the oils using an automated photo-chemiluminescent (PCL) system (Photochem©, Analytik Jena Model AG; Analytic Jena USA, The Woodlands, TX, USA), which measures the capacity to quench free radicals (Popov & Lewin, 1996). Oil samples (20 μL) were added to reagent kits supplied by the manufacturer and the automated PCL system measured the total antioxidant capacity of the lipids (ACL mode). Trolox was used as a standard, and results were expressed in Trolox equivalents (nmol TE/g oil).

2.5. Oil extraction

Oils were extracted for tocol and squalene analyses using modified methods of Maguire et al. (2004) and Savage et al. (1999). Finely ground kernels (20 g) were prepared, and a subsample (3 g) was extracted with 9 mL of hexane/isopropanol (3:2 v/v) under vigorous stirring for 1 h, then filtered under vacuum. The filter residue was re-extracted twice with hexane/isopropanol (3:2), then dried with 6.7% sodium sulfate. The filtrate was centrifuged at 2000 rpm for 10 min. The extracts were dried under N_2 to yield pure oil, and samples were frozen at −80 °C for later analyses.

2.6. HPLC analysis

The tocol and squalene contents of the extracted oil were quantified using high pressure liquid chromatography (HPLC). For HPLC analysis, the extracted oil (0.08 g) was resuspended in 0.4 mL HPLC-grade ethanol and filtered through a 0.2 μm nylon membrane filter. Phytochemicals were analysed by injecting 20 μL of sample into an Agilent 1100 series liquid chromatograph (Agilent Technologies, Wilmington, Del., USA) with HPLC-grade acetonitrile:methanol:water (72:8:1) as the mobile phase (Gruszka & Kruk, 2007) and a Prodigy ODS-2 column (2 × 150 mm, 5 μm; Phenomenex, Torrance, CA, USA) as the stationary phase followed by a diode array detector (DAD) and a fluorescence detector (FLD) in sequence. The flow rate was 1 mL/min and the column temperature was 25 °C. Sample peaks for α-tocopherol, γ-tocopherol, δ-tocopherol, and squalene were identified according to HPLC retention times and absorbance spectra in comparison with authentic standards. Palm oil was used to assist in identification of tocotrienol peaks. Squalene was detected and quantified using the DAD set at 205 nm wavelength, whereas the tocols were detected and quantified with the FLD set at 290 nm excitation and 320 nm emission. Tocol homologs were also detected using the DAD at a wavelength of 292 nm. Quantitation was based on authentic standards of α-tocopherol, γ-tocopherol, δ-tocopherol, and squalene. Concentrations of standards were determined using spectrophotometry and molar extinction coefficients. The extinction coefficients ($E^{1\%}_{1\ cm}$) used for α-tocopherol, γ-tocopherol, and δ-tocopherol in ethanol were 3270 (292 nm), 3810 (298 nm), and 3520 (298 nm), respectively (Podda, Weber, Traber, & Packer, 1996). Tocotrienols were identified according to HPLC retention times and quantified based on detailed calibration curves and response factors for corresponding tocopherol standards. The fluorescence intensity for

corresponding tocopherol and tocotrienol peaks is equivalent at the same concentration, because fluorescence is emitted only by the chromanol ring of the tocols and is not influenced by the isoprenoid side chain (Gruszka & Kruk, 2007). Each sample was analysed in duplicate. For recovery tests, samples were spiked with standard solutions before extraction.

2.7. Statistical analysis

Data were analysed using the general linear models (GLM) procedure of SAS (SAS Institute, 2008) (SAS, Cary, NC, USA) according to a randomised complete block design where locations served as blocks for each cultivar. Nuts were harvested twice per year from the Kau and Keaau locations and once from the Captain Cook and Waiakea sites, for a total of 6 blocks. Data were presented as means ± standard errors (SE), and where appropriate, mean separations were performed by Fisher's protected LSD test at $P = 0.05$. Correlations between phytochemical contents and total antioxidant activity and oxidative stability were determined for roasted macadamia kernels.

3. Results and discussion

3.1. Oil content

Macadamia kernels contained 68–72% oil in 2006 and 64–69% oil in 2007 (Table 1). These values are lower than the oil content of premium macadamia nuts (>72%), and may indicate sub-optimal cultural practices in the orchards or variability in harvest maturity. Kaijser et al. (2000) reported 69–78% oil contents in four macadamia cultivars, whereas Maguire et al. (2004) measured 59% oil in macadamia kernels. Macadamias are considered a high-oil nut, with similar amounts as pine nuts (68–75%), pecans (70–72%), and walnuts (63–70%) (Amaral, Casal, Pereira, Seabra, & Oliveira, 2003; Miraliakbari & Shahidi, 2008a, 2008b). The seven Hawaii-grown cultivars did not differ in oil content (Table 1), but nuts harvested from the Waiakea orchard (elevation 180 m) in 2007 had slightly more oil than nuts harvested from the Captain Cook site (elevation 600 m) (Table 2). Small differences among orchard locations could be expected, because oil accumulation during nut maturation can be influenced by a number of environmental and horticultural factors, such as temperature, water stress and nitrogen fertility (Stephenson & Gallagher, 1986; Stephenson, Gallagher, & Doogan, 2003; Stephenson, Gallagher, Doogan, & Mayer, 2000).

3.2. Oxidative stability

Lipid oxidation imparts off-flavors and aromas to nuts and compromises nutritional quality. Therefore, identification of cultivars with improved oxidative stability is key to breeding programs and the nut processing industry. The oxidative stability of macadamia oils was expressed as the induction time (hours) that preceded a rapid increase in auto-oxidation, and was measured using the Rancimat system (Frank et al., 1982). Oil from HAES 294 kernels had high oxidative stability (>9 h) for both years, whereas HAES 856 oil had the shortest induction time (6.8–7.3 h) (Table 1). In 2006, HAES 344 ranked second for oxidative stability, followed by HAES 835. In 2007, oil from HAES 835 kernels had the greatest oxidative stability at 10.1 h. The oxidative stability of macadamia oil (7–10 h) is low when compared to hazelnut, pistachio, or almond oils, but greater than walnut oil (Amaral et al., 2003; Arranz, Cert, Perez-Jimenez, Cert, & Saura-Calixto, 2008; Savage et al., 1999). The results are slightly higher than those of Tsumura (1988), who measured 6–8 h induction times for several Hawaii-grown macadamia cultivars. Other tree nut oils have been ranked for oxidative stability with pecan oil > pistachio oil > hazelnut oil > almond oil > Brazil nut oil > pine nut oil > walnut oil (Miraliakbari & Shahidi, 2008b).

In 2006, the cultivars with the greatest oxidative stability (HAES 294, HAES 344, HAES 835) also had the highest antioxidant activity (Table 1). In 2007, HAES 835 had the greatest oxidative stability and high antioxidant activity. HAES 508 also had high antioxidant activity. A positive correlation ($r = 0.25$, $P = 0.03$) was detected between oxidative stability and antioxidant activity when values for all cultivars were considered for both years.

Nuts harvested from Keaau (elevation 100 m) and Kau (elevation 240 m) orchards had the highest oxidative stability in both years, and nuts harvested from Captain Cook had the shortest induction times (Table 2). The Keaau and Kau sites consisted of well-managed commercial orchards, whereas the Captain Cook site was a high elevation (600 m) experimental orchard. As with oil content, kernel oxidative stability could be influenced by environmental, cultural and edaphic conditions, however the study was not designed to control or document these factors.

Table 1
Total oil content, oxidative stability, antioxidant activity, squalene and tocotrienol concentrations of macadamia kernels harvested in 2006 and 2007 from seven cultivars.

Cultivar	Oil content (%)	Oxidative stability[A] (hours)	Antioxidant activity[B] (nmolTE/g oil)	Squalene (μg/g oil)	Tocotrienols (μg/g oil)
2006					
HAES 856	71.26 ± 1.37 a[C]	7.30 ± 0.36 b[C]	42.53 ± 3.26 c[C]	110.92 ± 21.92 a	65.94 ± 22.18 ab
HAES 835	71.89 ± 0.46 a	8.53 ± 0.51 ab	64.63 ± 4.65 a	92.01 ± 12.10 a	52.65 ± 5.18 ab
HAES 788	69.74 ± 0.99 a	8.23 ± 0.96 ab	48.56 ± 7.29 bc	93.89 ± 28.12 a	61.33 ± 6.85 ab
HAES 508	69.36 ± 0.96 a	8.12 ± 0.32 ab	48.94 ± 5.63 bc	89.31 ± 22.68 a	59.53 ± 6.76 ab
HAES 344	70.48 ± 0.69 a	9.18 ± 0.60 ab	60.61 ± 3.72 ab	73.70 ± 8.57 a	49.84 ± 4.43 ab
HAES 294	69.45 ± 0.88 a	9.22 ± 0.69 a	65.78 ± 4.86 a	101.67 ± 14.04 a	91.59 ± 40.60 a
HAES 246	68.42 ± 0.97 a	8.27 ± 0.31 ab	44.43 ± 3.59 c	103.99 ± 25.84 a	46.56 ± 8.72 b
2007					
HAES 856	65.79 ± 1.45 a[C]	6.82 ± 0.69 c[C]	40.86 ± 2.21 ab[C]	129.05 ± 28.00 ab[C]	30.69 ± 10.23 b[C]
HAES 835	63.93 ± 1.19 a	10.08 ± 0.73 a	46.81 ± 2.37 a	72.44 ± 10 b	37.59 ± 6.80 ab
HAES 788	65.64 ± 2.47 a	7.27 ± 0.51 c	37.48 ± 2.22 b	97.16 ± 23.40 ab	43.44 ± 3.56 ab
HAES 508	65.22 ± 0.74 a	8.03 ± 0.32 bc	47.04 ± 3.01 a	171.26 ± 31.56 a	30.15 ± 8.72 b
HAES 344	69.18 ± 0.89 a	6.95 ± 0.53 c	43.68 ± 3.36 ab	78.08 ± 16.76 b	50.16 ± 3.14 a
HAES 294	65.95 ± 1.02 a	9.32 ± 0.79 ab	40.03 ± 1.62 ab	110.40 ± 33.83 ab	41.49 ± 8.26 ab
HAES 246	65.99 ± 0.98 a	7.59 ± 0.48 c	40.94 ± 4.01 ab	113.50 ± 27.47 ab	42.62 ± 0.92 ab

[A] Induction time (hours) for auto-oxidation of macadamia nut oils, measured using the Rancimat system.
[B] Total lipid-soluble antioxidant capacity of the macadamia nut oils, measured as Trolox equivalents (TE) using a photo-chemiluminescence (PCL) system.
[C] Means are averaged for dried and roasted kernels harvested from 4 locations. Values are means (±standard errors) of 8–12 observations. Means within columns followed by the same letter are not significantly different ($P > 0.05$).

Table 2
Total oil content, oxidative stability, antioxidant activity, squalene and tocotrienol concentrations of macadamia kernels harvested in 2006 and 2007 from four locations in Hawaii.

Location	Oil content (%)	Oxidative stability[A] (hours)	Antioxidant activity[B] (nmolTE/g oil)	Squalene (μg/g oil)	Tocotrienols (μg/g oil)
2006					
Kau	70.09 ± 0.56 a[C]	8.96 ± 0.39 ab[C]	48.17 ± 3.10 a[C]	97.35 ± 11.22 a[C]	65.53 ± 15.21 a[C]
Keaau	68.66 ± 0.72 a	9.35 ± 0.34 a	59.23 ± 3.08 a	107.63 ± 19.83 a	52.48 ± 4.98 a
Caption Cook	70.83 ± 0.73 a	6.82 ± 0.15 c	54.90 ± 4.96 a	85.41 ± 10.06 a	62.32 ± 11.86 a
Waiakea	70.52 ± 1.02 a	7.94 ± 0.23 b	58.82 ± 5.84 a	79.87 ± 21.31 a	56.05 ± 3.13 a
2007					
Kau	65.88 ± 0.83 ab[C]	8.49 ± 0.32 a[C]	41.94 ± 1.84 a[C]	94.98 ± 16.30 a[C]	42.68 ± 4.61 a[C]
Keaau	66.63 ± 0.72 ab	8.68 ± 0.60 a	45.72 ± 2.94 a	134.42 ± 24.44 a	43.70 ± 3.17 a
Caption Cook	64.30 ± 0.85 b	6.59 ± 0.37 b	40.94 ± 1.61 a	92.29 ± 17.35 a	30.94 ± 5.46 a
Waiakea	68.59 ± 1.48 a	7.27 ± 0.86 ab	40.81 ± 3.10 a	124.42 ± 23.80 a	39.63 ± 3.70 a

[A] Induction time (hours) for auto0oxidation of macadamia nut oils, measured using the Rancimat system.
[B] Total lipid-soluble antioxidant capacity of the macadamia nut oils, measured as Trolox equivalents (TE) using a photo-chemiluminescence (PCL) system.
[C] Means are averaged for dried and roasted kernels of 7 cultivars. Values are means (±standard errors) of 10–28 observations. Means within columns followed by the same letter are not significantly different ($P > 0.05$).

3.3. Antioxidant capacity

The PCL inhibition assay measured the superoxide scavenging capacity of the macadamia nut oils. Cultivar differences were detected ($P < 0.05$) for both years. Total lipid-soluble antioxidant capacity ranged from 42.53 to 65.78 nmolTE/g oil in 2006, and from 37.48 to 46.81 nmolTE/g oil in 2007 (Table 1), indicating a seasonal effect on antioxidant activity. There were no differences among orchard locations for antioxidant capacity (Table 2).

The total antioxidant activity for macadamia kernels has not been reported previously. For other nut oils measured with the PCL inhibition assay, antioxidant capacity was calculated as α-tocopherol equivalents and was highest for pecan and walnut oils, followed by pistachio, hazelnut, almond, and Brazil nut oils (Miraliakbari & Shahidi, 2008a, 2008b). However, when free radical scavenging activity was measured with the DPPH (2,2-diphenyl-1-picrylhydrazl) assay, the ranking of antioxidant capacity was pistachio > hazelnut > walnut > almond > peanut (Arranz et al., 2008).

Tocopherols are considered the most important natural antioxidants in nut oils (Arranz et al., 2008; Fourie & Basson, 1989). Tocotrienols, phenolics, phytosterols, squalene, and phospholipids could enhance antioxidant capacity as well (Miraliakbari & Shahidi, 2008a; Villarreal-Lozoya, Lombardini, & Cisneros-Zevallos, 2007). However, previous reports have shown that macadamia nuts contain only small amounts of phenolics, similar to almonds without skins (Kornsteiner et al., 2006; Quinn & Tang, 1996). Therefore phenolics are not likely to be important sources of macadamia antioxidant capacity or oxidative stability. Differences among macadamia cultivars for antioxidant capacity were most apparent in 2006, with HAES 294 and HAES 835 having the highest activity (65.78 and 64.63 nmol TE/g oil, respectively). This superior activity may be explained by favorable combinations of minor antioxidant components, other than tocopherols.

3.4. Tocopherols

Tocopherols [delta (δ), gamma (γ), alpha (α)] were not detected in most macadamia nut samples, except for low amounts of γ-tocopherol (γ-toc) and α-tocopherol (α-toc) in HAES 856 in 2006 and HAES 294 in 2007 (Table 3). Delta-tocopherol was not detected in any macadamia nuts analysed. The reverse-phase HPLC system that was used for tocol analysis did not enable separation of beta (β)- and γ-tocopherols. However β-toc is a minor component of most nut oils, and the reverse-phase system provided good selectivity for T3 isomers, a short analysis time, and reproducible chromatographic peaks (Abidi, 2000; Gruszka & Kruk, 2007).

Our results are similar to previous reports for macadamia nuts in which tocopherols were either not detected (Kornsteiner et al., 2006), or present in low amounts ranging from 0.6 to 2.8 μg/g oil for α-toc (Franke et al., 2007; Kaijser et al., 2000; Tsumura, 1988). However, δ-toc was detected and ranged from 3.5 to 4.8 μg/g oil in macadamia nuts analysed by Kaijser et al. (2000). Contrary results were reported by Maguire et al. (2004), in which macadamia nuts contained 122 μg α-toc/g oil – a low level when compared to almonds (452 μg) and hazelnuts (371 μg).

Vitamin E consists of homologs of tocopherols (toc) and tocotrienols (T3), although the US recommended dietary allowance (RDA) for vitamin E is based only on α-toc, the most biologically active form (Institute of Medicine, 2000). Macadamia nuts contained trace amounts of α-toc, and therefore would not contribute to meeting the RDA for vitamin E. Also, the tocopherol content was too low to influence kernel stability. Nevertheless, macadamia kernels did have significant amounts of T3 for all cultivars tested (Tables 1 and 3). While α-toc is the most active E vitamer, the unique biological and nutritional properties attributed to T3 homologs remain poorly studied (Sen et al., 2007).

3.5. Tocotrienols

In 2006, T3 concentrations ranged from 10.48 to 17.66 μg/g oil for δ-T3, 10.51–34.28 μg/g oil for γ-T3, and 17.44–46.83 μg/g oil for α-T3 (Table 3). Total T3 content was highest for HAES 294 (91.59 μg/g oil) and lowest for HAES 246 (46.56 μg/g oil) (Table 1). Levels were lower in 2007, suggesting considerable environmental effects on T3 accumulation during kernel development. T3 ranged from 3.00 to 8.45 μg/g oil for δ-T3, 8.75–17.30 μg/g oil for γ-T3, and 15.91–24.40 μg/g oil for α-T3 (Table 3). In 2007, total T3 content was highest for HAES 344 (50.15 μg/g oil) and lowest for HAES 508 (30.15 μg/g oil) (Table 1).

This is the first report of detection of three tocotrienol homologs (δ-, γ-, α-T3) in different macadamia cultivars. Previous studies did not compare cultivars or measured only α-T3 concentrations. Dry-roasted macadamia nuts of an unidentified cultivar had <0.5 μg δ-T3, 1.7 μg γ-T3, and 18 μg α-T3 per gram of kernel in a study by Franke et al. (2007). The results for the seven Hawaiian macadamia cultivars (*M. integrifolia*) are similar to the range of α-T3 (12.5–48.4 μg/g oil) measured in four cultivars of *M. tetraphylla* grown in New Zealand (Kaijser et al., 2000). Others did not detect any α-T3 (Kornsteiner et al., 2006), or very low amounts (<2 μg/g oil) in macadamia kernels (Tsumura, 1988).

The total T3 content of macadamia kernel oil (30–92 μg/g oil) can be compared to the total T3 contents of 21 species of plant seed oils reported by Gruszka and Kruk (2007). Nearly all the oils tested

Table 3
Tocopherol and tocotrienol contents of roasted macadamia kernels harvested in 2006 and 2007 from seven cultivars.

Cultivar	γ-tocopherol (μg/g oil)	α-tocopherol (μg/g oil)	δ-tocotrienol (μg/g oil)	γ-tocotrienol (μg /g oil)	α-tocotrienol (μg/g oil)	Total (μg/g oil)
2006						
HAES 856	0.59 ± 0.59[A]	2.25 ± 2.25[A]	16.52 ± 3.23 a[A]	17.35 ± 4.06 ab[A]	32.07 ± 15.80 ab[A]	68.78 ± 22.18 ab[A]
HAES 835	ND[B]	ND	15.50 ± 3.18 a	12.81 ± 1.22 ab	24.34 ± 1.14 ab	52.65 ± 5.18 ab
HAES 788	ND	ND	16.46 ± 2.83 a	17.21 ± 3.99 ab	27.66 ± 2.67 ab	61.33 ± 6.85 ab
HAES 508	ND	ND	15.25 ± 2.51 a	15.03 ± 2.25 ab	29.25 ± 2.23 ab	59.53 ± 6.76 ab
HAES 344	ND	ND	13.00 ± 1.29 a	10.51 ± 2.15 b	26.34 ± 1.56 ab	49.84 ± 4.43 ab
HAES 294	ND	ND	10.48 ± 3.34 a	34.28 ± 21.27 a	46.83 ± 21.85 a	91.59 ± 40.60 a
HAES 246	ND	ND	17.66 ± 2.40 a	11.47 ± 2.05 ab	17.44 ± 5.84 b	46.56 ± 8.72 b
2007						
HAES 856	ND	ND	6.03 ± 1.48 a[A]	8.75 ± 4.81 b[A]	15.91 ± 5.51 a[A]	30.69 ± 10.23 b[A]
HAES 835	ND	ND	6.97 ± 1.64 a	14.17 ± 2.97 ab	16.45 ± 5.65 a	37.59 ± 6.80 ab
HAES 788	ND	ND	6.00 ± 1.11 a	15.18 ± 2.81 ab	22.26 ± 1.85 a	43.44 ± 3.56 ab
HAES 508	ND	ND	3.00 ± 1.96 a	9.04 ±.36 b	18.11 ±.86 a	30.15 ± 8.72 b
HAES 344	ND	ND	8.45 ± 1.53 a	17.30 ± 2.32 a	24.40 ± 0.95 a	50.15 ± 3.14 a
HAES 294	2.03 ± 2.03	3.12 ± 3.12	12.52 ± 2.39 ab	22.23 ± 5.47 a	46.64 ± 8.26 ab	
HAES 246	ND	ND	7.11 ± 1.20 a	16.61 ± 1.60 ab	18.92 ± 0.93 a	42.64 ± 0.92 ab

[A] Values are means (±standard errors) of 6 observations. Means within columns followed by the same letter are not significantly different ($P > 0.05$).
[B] ND = not detected. Delta-tocopherol was not detected in any samples.

contained low but detectable amounts of T3, but the highest concentrations were found in rice bran (177.5 μg/g oil), grape seed (27.9 μg/g oil), corn (24.3 μg/g oil), and milk thistle oils (13.4 μg/g oil) (Gruszka & Kruk, 2007). Palm oil contains the highest amount of T3s (up to 800 μg/g) among all oil seed crops (Sen et al., 2007).

Alpha-tocotrienol has been reported to have three times the *in vitro* free radical scavenging activity of α-toc (Packer et al., 2001), and this could enhance kernel oxidative stability. HAES 294 had the longest Rancimat induction time, the highest lipid-soluble antioxidant activity, and the greatest T3 contents in 2006 (Tables 1 and 3). However, this relationship between oxidative stability, antioxidant activity and T3 levels was not observed for the other cultivars, or in the second year (Tables 1 and 3). Kaijser et al. (2000) showed similar results for *M. tetraphylla* varieties. The cultivar with the highest oxidative stability also contained the highest α-T3 content, suggesting that T3 concentration contributes to kernel stability, but the trend was not consistent for the other cultivars.

Although T3s are more potent antioxidants than tocopherols, their bioavailability is lower after oral ingestion (Packer et al., 2001). Nevertheless, T3s are bioavailable to all vital organs, protect against stroke-associated brain damage, and exhibit cholesterol-lowering and anticarcinogenic properties (Packer et al., 2001; Sen et al., 2007). Also, T3s penetrate quickly through the skin and minimise UV-induced oxidative stress. Topical application of T3 is an efficient way to enrich skin with vitamin E, thus macadamia oil could be well-suited for skin care products.

3.6. Squalene

Squalene, like T3, is an effective and stable antioxidant. Squalene is a major component of human skin surface lipids, and protects skin from UV-induced lipid peroxidation (Kohno et al., 1995). Macadamia nuts appear to be a significant source of dietary squalene. Squalene concentrations ranged from 73.7 to 110.92 μg/g oil in 2006 and 72.44 to 171.26 μg/g oil in 2007 (Table 1). The squalene levels for the different cultivars were fairly consistent across harvest seasons, with the exception of HAES 508. In 2007, HAES 508 had the greatest squalene concentration and total antioxidant activity among cultivars (Table 1).

Information on the squalene levels of nut oils is lacking in the literature, but in one report for an unidentified macadamia cultivar, the squalene content was 185 μg/g oil (Maguire et al., 2004). Hazelnuts, peanuts, almonds, and walnuts contained 186 μg, 98 μg, 95 μg, and 9 μg squalene per gram oil, respectively (Maguire et al., 2004). Squalene is abundant in olive oil (2000–7000 μg/g oil), amaranth (1300–4200 μg/g), pumpkin seeds (890 μg/g), and quinoa (584 μg/g) (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007). Animal studies suggest that squalene inhibits tumor formation, and that a lower risk of various cancers associated with high olive oil consumption may be due to the presence of squalene (Sotiroudis & Kyrtopoulos, 2008).

3.7. Orchard location effects on phytochemicals

Macadamia nuts harvested from the different orchard locations had similar levels of squalene and T3 (Table 2). In general, phytochemical contents appeared to be influenced by cultivar variation, more so than by orchard location. There are no other reports comparing orchard locations for macadamia nut phytochemical composition. More research is needed to clarify the effects of environment, crop load, and horticultural practices on the phytochemical content of macadamia kernels.

3.8. Correlations between oxidative stability, antioxidant capacity and phytochemicals

No statistical correlations were detected between squalene or T3 and the oxidative stability or antioxidant activity of macadamia oils (Table 4). In a similar report, no correlations were observed between rancidity (measured as peroxide value) and the tocol content or unsaturated fatty acid content for macadamia nuts, hazelnuts, peanuts, and walnuts (Maguire et al., 2004). Nevertheless, antioxidant compounds such as T3 may contribute to oxidative stability for certain macadamia cultivars. HAES 294 had the

Table 4
Correlations between oxidative stability, total antioxidant activity, and phytochemical concentrations of macadamia kernels harvested in 2006 and 2007.

	Tocopherols	Tocotrienols	Squalene
Oxidative stability[A]	0.124 ($P = 0.32$)	0.113 ($P = 0.36$)	−0.07 ($P = 0.57$)
Antioxidant activity[B]	−0.034 ($P = 0.78$)	0.092 ($P = 0.46$)	−0.145 ($P = 0.24$)

[A] Induction time (hours) for auto-oxidation of macadamia nut oils, measured using the Rancimat system.
[B] Total lipid-soluble antioxidant capacity of the macadamia nut oils, measured as Trolox equivalents (TE) using a photo-chemiluminescence (PCL) system.

longest oxidation induction time, the greatest antioxidant activity, and the highest T3 concentrations in 2006 (Table 1). Tsumura (1988) also reported that HAES 294 had the greatest oxidative stability among eight cultivars tested over 2 years. In 2007, HAES 508 ranked highest for antioxidant capacity and squalene concentrations, whereas HAES 835 had high oxidative stability and antioxidant activity but average T3 concentrations (Table 1).

There appears to be a complex relationship between oxidative stability, fatty acid composition, and phytochemical composition that needs further elucidation. HAES 294 seems to be an ideal cultivars to include in future studies. In walnuts, reduced oxidative stability (measured with a Rancimat) was correlated with higher levels of the polyunsaturated fatty acids (PUFA) in extracted oils (Amaral et al., 2003; Savage et al., 1999). Similarly, in a study of New Zealand-grown macadamia nuts, the cultivar with the lowest oxidative stability also had the highest content of the PUFA, linoleic acid (Kaijser et al., 2000). Monounsaturated fatty acids (MUFA) are more stable than PUFA to oxidation, therefore cultivars with a higher MUFA to PUFA ratio may be more resistant to oxidation. Macadamia nuts contain high amounts of palmitoleic (18–23%) and oleic (56–65%) MUFA (Cavaletto, 1983; Kris-Etherton et al., 1999b; Maguire et al., 2004). Macadamia kernel stability may depend on the interaction between fatty acid composition and the amount and type of minor phytochemical compounds in the oil.

4. Conclusions

Macadamia kernels contained significant amounts of T3 and squalene, and these phytochemicals may confer antioxidant, anticancer, and cholesterol-lowering properties to consumers. This is the first study in which three homologs of T3 were detected and compared among cultivars, and in which squalene was extensively analysed for macadamia kernels. Macadamia nuts appear to be a good source of dietary squalene and T3. Also, macadamia oil may be a useful ingredient in skin care products, because T3 and squalene are effective at preventing sunlight-induced oxidative stress to the skin. No clear relationship existed between oxidative stability and phytochemical contents. However, oils extracted from macadamia cultivars HAES 294 and HAES 835 had consistent oxidative stability for both years, and HAES 294 had high T3 concentrations. These Hawaii-grown cultivars may maintain superior quality during long-term storage or marketing.

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