

Polyphenol content and antioxidant activity of California almonds depend on cultivar and harvest year[☆]

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ARTICLE INFO

Article history:

Received 9 September 2009

Received in revised form 27 January 2010

Accepted 16 March 2010

Keywords:

Almonds
Flavonoid
Polyphenols
Antioxidant
Year
Folin
FRAP
LC-MS
Skin

ABSTRACT

The polyphenol content and antioxidant activity of Nonpareil, Carmel, Butte, Sonora, Fritz, Mission, and Monterey almond cultivars harvested over three seasons in California were examined. LC-MS was employed to quantify 16 flavonoids and two phenolic acids in acidified methanol extracts of almond skins. The 3-year mean polyphenol content of cultivars ranged from 4.0 to 10.7 mg/100 g almonds. Isorhamnetin-3-O-rutinoside was the most abundant flavonoid, present at 28–49% of total polyphenols among cultivars. Almonds from 2006 and 2007 had 13% fewer polyphenols than 2005, but FRAP and total phenols were comparable. Cultivar, but not season, had a differential impact on individual polyphenol synthesis. Using the results of polyphenol, total phenol, and FRAP, multivariate analysis distinguished harvest years and most cultivars with 80% confidence. Flavonoid content and antioxidant activity of almonds may be more dependent on cultivar than on seasonal differences.

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1. Introduction

For more than 100 years, tree nuts have been advocated as a nutritious component of diet (Reiling, 2008). Almonds are the most popularly consumed tree nut, and increasing experimental evidence suggests that almonds improve serum lipid profiles and cholesterol status (Chen, Milbury, Lapsley, & Blumberg, 2005; Jenkins et al., 2008; King, Blumberg, Ingwersen, Jenab, & Tucker, 2008),

changes associated with reduced risk of cardiovascular disease. Almonds are a good dietary source of vitamin E, sterols, and flavonoids, each of which has been suggested to play a role in the promotion of health. In particular, increased consumption of flavonoids has been associated with an anti-obesity effect in women and reduced risk of stroke, cardiovascular disease, and some forms of cancer (Geleijnse, Launer, Hofman, Pols, & Witteman, 1999; Hertog, Hollman, Katan, & Kromhout, 1993; Hughes et al., 2008; Keli, Hertog, Feskens, & Kromhout, 1996; Yochum, Kushi, Meyer, & Folsom, 1999). Previous efforts by our laboratory and others have focused on the identification and quantification of almond polyphenols to ascertain their contribution to flavonoid intake and health outcomes (Alasalvar & Shahidi, 2009; Garrido, Monagas, Gomez-Cordoves, & Bartolome, 2008; Harnly et al., 2006; Milbury, Chen, Dolnikowski, & Blumberg, 2006).

Almond polyphenols are concentrated in the skins, which can be removed by blanching. Almonds are often blanched for commercial reasons even though this lowers their polyphenol content. Flavonoids and other polyphenols comprise 0.2–0.8% of the dry weight of almond skins (Garrido et al., 2008). Flavonoid-containing almond skin extracts can inhibit LDL-oxidation and DNA damage in vitro (Wijeratne, Abou-Zaid, & Shahidi, 2006). Polyphenols identified from almonds also have strong 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Sang et al., 2002).

Abbreviations: B, Butte; C, Carmel; CA, catechin; EC, epicatechin; DiOHK, dihydroxykaempferol; E, eriodictyol; ECD, electrochemical detection; F, Fritz; GAE, gallic acid equivalents; FRAP, ferric reducing antioxidant power; Iso, isorhamnetin; Iso3Glu, isorhamnetin-3-O-glucoside; Iso3R, isorhamnetin-3-O-rutinoside; K, kaempferol; K3Gal, kaempferol-3-O-galactoside; K3Glu, kaempferol-3-O-glucoside; K3R, kaempferol-3-O-rutinoside; M, Mission; Mo, Monterey; N, naringenin; N, Nonpareil; N7Glu, naringenin-7-O-glucoside; ORAC, oxygen radical absorbance capacity; PA, procatechuic acid; pHBA, *p*-hydroxybenzoic acid; Q, quercetin; Q3Gal, quercetin-3-O-galactoside; R, rutin; TE, Trolox equivalents.

[☆] Supported by the Almond Board of California, US Department of Agriculture (USDA)/Agricultural Research Service under Cooperative Agreement No. 58-1950-7-707, and K12GM074869 from the National Institute of General Medical Sciences. The contents of this publication do not necessarily reflect the official views or policies of the NIGMS, NIH, or USDA nor does mention of trade names, commercial products or organisations imply endorsement by the US government.

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We previously reported that flavonoid content varied at least 2-fold among eight California almond cultivars harvested in 2004 (Milbury et al., 2006). Barreira, Ferreira, Oliveira, and Pereira (2008) found that flavonoid content varied 4-fold in 10 Portuguese almond cultivars. Besides cultivar differences, climate and geography may also affect almond flavonoid concentration. These parameters also affect the content of fibre, total phenols, minerals, and tocopherols in almonds and other tree nuts (Amaral, Valentao, Andrade, Martins, & Seabra, 2008; Laverdrine, Ravel, Villet, Ducros, & Alaray, 2000; Parcerisa et al., 1995; Sanchez-Bel, Egea, Martinez-Madrid, Flores, & Romojaro, 2008). However, data regarding the seasonal variability of almond flavonoids are lacking. This information may contribute to better estimates of flavonoid intakes, broaden the understanding of cultivar-specific flavonoid and phenolic syntheses in almonds, and inform agricultural practices to increase the flavonoid content of almonds or improve their resistance to pests.

California is the largest almond growing region in the world. In 2008, 1.38 billion pounds of almonds were harvested, accounting for 80% of global production (Almond Board of California, 2008). Nonpareil, Carmel, and Butte are the main almond cultivars and represented 60% of the almond harvested in 2007 (Bolling, Dolnikowski, Blumberg, & Chen, 2009). The objective of this study was to quantify the major flavonoids and phenolic acids, total phenols, and antioxidant activity from Nonpareil, Carmel, Butte, Monterey, Fritz, Mission, and Sonora almonds harvested over a 3-year period.

2. Materials and methods

2.1. Chemicals and reagents

Quercetin-4-O-glucoside, quercetin-3-O-galactoside, isorhamnetin, kaempferol-3-O-rutinoside, isorhamnetin-3-O-glucoside, isorhamnetin-3-O-rutinoside, naringenin-7-O-glucoside, and rutin were obtained from Extrasynthese (Genay, France). Naringenin, quercetin, and eriodictyol were acquired from Indofine (Belle Mead, NJ). Methanol was HPLC grade from Fischer (Fair Lawn, NJ). Water was ultrapure grade. All other chemicals and reagents were acquired from Sigma-Aldrich (St. Louis, MO).

2.2. Almond samples

Raw almonds harvested in 2005, 2006, and 2007 from California were generously provided by the Almond Board of California. Monterey, Fritz, Mission, and Sonora almond samples had one sample from one orchard each in the central California growing region. Nonpareil, Carmel, and Butte almonds had one sample from three different orchards. In total, 13 samples of seven cultivars were collected from the same orchards in three consecutive years across the counties of Yolo, Sacramento, Stanislaus, Fresno, and Kern. A total of 39 almond samples were included in the study. For each cultivar, the mass of skin in 100 g almonds was determined by blanching almonds in hot water for 3 min, removing skins by hand, and air-drying them at room temperature. The fractional percentage of almond skin was used to calculate the polyphenol content and antioxidant activity per 100 g almonds to facilitate comparison to the USDA flavonoid database.

2.3. Method of extraction

Skins from raw almonds were removed and extracted according to Bolling et al. (2009). Briefly, almonds were blanched three times in liquid nitrogen, hand peeled, and then the skins were pulverised under liquid nitrogen to a powder. Almond powder was steeped twice in 3.5% acetic acid, 50% methanol in water at 4 °C over

20 h. Aliquots of extract were dried under nitrogen gas and stored at –20 °C in darkness until analysis.

2.4. Analysis of polyphenols

For LC–MS analysis of polyphenols, dried almond skin extract was first suspended in 497 μ L of 50% methanol with 3 μ L of 1 mM daidzein as an internal standard, diluted 10-fold with 1% formic acid, and centrifuged at 12,000 \times g for 10 min prior to injection. The concentration of 16 flavonoids and two phenolic acids were quantified by LC–MS as described by Bolling et al. (2009). Briefly, an Agilent 1100 MSD quadrupole with electrospray ionisation (ESI) was equipped with a 250 \times 4.60 mm Synergi 4 μ MAX-RP 80A column (Phenomenex, Torrance, CA) and set to a constant temperature of 25 °C. The polyphenols were eluted by an increasing gradient of 1% formic acid and 100% methanol at a flow rate of 0.2 mL/min. MSD signals were acquired in selected ion monitoring mode in three groups as negative ions: m/z 137, 153, 289 from retention time (Rt) of 0–22.6 min; m/z 137, 287, 433, 447, 463, 477, 593, 609, and 623 from Rt 22.6–28.2 min; and m/z 253, 271, 285, 287, 301, 315 from Rt 28.2 to 50 min.

Prior to calibration curve runs, all standards were mixed in a stock solution of methanol, aliquoted, and stored at –80 °C in amber screw-capped glass vials. On each day of analysis, different concentrations of standard solution, consisting of (+)-catechin (CA), (–)-epicatechin (EC), daidzein, eriodictyol (E), *p*-hydroxybenzoic acid (pHBA), isorhamnetin (Iso), isorhamnetin-3-O-glucoside (Iso3Glu), isorhamnetin-3-O-rutinoside (Iso3R), kaempferol (K), kaempferol-3-O-glucoside (K3Glu), kaempferol-3-O-rutinoside (K3R), naringenin (N), naringenin-7-O-glucoside (N7Glu), protocatechuic acid (PA), quercetin (Q), quercetin-3-galactoside (Q3Gal), and rutin (R), were serially diluted with 1% aqueous formic acid. Dihydrokaempferol was quantified on the basis of eriodictyol equivalents and kaempferol-3-O-galactoside on the basis of kaempferol-3-O-glucoside equivalents. Quercetin-3-O-galactoside coeluted with quercetin-3-O-glucoside. Routine intra- and inter-day assay coefficients of variation (CV) were 2.4% and 6.8%, respectively.

2.5. Total phenols and ferric reducing antioxidant power (FRAP)

The total phenol content of almond skin extract was determined according to Singleton, Orthofer, and Lamuela-Raventos (1999) with results expressed as mg gallic acid equivalents (GAE) per 100 g raw almonds. The FRAP assay assesses total antioxidant capacity via a redox-linked reduction of Fe³⁺-2,4,6-tri-pyridyl-S-triazine to a blue-coloured Fe²⁺ complex at pH 3.5 (Benzie & Strain, 1996). Following reconstitution in methanol, almond skin extracts were incubated at ambient temperature with the FRAP reagent and the absorbance measured at 593 nm after 1 h (Chen & Blumberg, 2008). FRAP reducing power is expressed as μ mol TE/100 g almonds. Routine intra- and inter-day assay CV were 0.7% and 4.2%, respectively.

2.6. Statistics and data analysis

For all experiments, samples were analysed in duplicate. Statistical significance was determined by a mixed model ANOVA of cultivar and seasonal variation, followed by Tukey's Honestly Significant Difference as post hoc analysis by JMPIN v 3.2.6 software (SAS Institute Inc., Cary, NC). Pearson's correlation analysis was performed using GraphPad Prism v 5.01 (GraphPad Software Inc., La Jolla, CA). Differences were considered significant at $P \leq 0.05$. Canonical discriminant analysis was performed by SAS v 9.1.3 program (SAS Institute Inc., Cary, NC) using standard pooled variance and 80% confidence ellipses. Cultivar and season were

independently modelled using polyphenol, FRAP, and total phenol results.

3. Results

3.1. Cultivar

Over three seasons, the mean flavonoid and phenolic acid content in seven almond cultivars ranged from 3.96 to 10.7 mg/100 g almonds (Table 1). This represents a 2.7-fold difference between Fritz and Sonora cultivars, which had the lowest and highest polyphenolic content, respectively. Carmel had the second highest polyphenol content at 8.0 mg/100 g almonds, 25% less than that of Sonora. Nonpareil, which represented 40% of California almond acreage in 2007, had midrange polyphenol content of 6.2 mg/

100 g almonds. Butte, Carmel, and Nonpareil had similar levels of polyphenols ranging from 6.2 to 8.0 mg/100 g.

Polyphenolic content and total phenols were slightly correlated among cultivars ($R^2 = 0.70$, $P = 0.083$). However, FRAP values and polyphenol content were significantly correlated ($R^2 = 0.85$, $P = 0.016$). Similar to their polyphenolic content, Sonora almonds had the highest total phenol concentration of 159 mg GAE/100 g and FRAP value of 891 $\mu\text{mol TE}/100\text{ g}$, although these values were not significantly different from the Carmel, Mission, and Nonpareil cultivars.

Of the 18 polyphenols quantified by the LC–MS analysis, catechin (CA), epicatechin (EC), naringenin-7-*O*-glucoside (N7Glu), kaempferol-3-*O*-rutoside (K3R), dihydroxykaempferol (DiOH), isorhamnetin-3-*O*-rutoside (Iso3R), isorhamnetin-3-*O*-glucoside (Iso3Glu), and naringenin (N) were the major flavonoids, each representing $\geq 3\%$ of the total polyphenol content (Table 2). In Sonora almonds, CA, Iso3R, and Iso3Glu comprised 75% of the polyphenol content. Butte, Carmel, and Mission almonds were particularly high in K3R with 0.8–1.4 mg/100 g and low in Iso3G with 0.2–0.3 mg/100 g almonds. Sonora, Nonpareil, and Monterey almonds were high in Iso3G with 2.0–2.9 mg/100 g almonds and relatively lower in K3R with 0.2–0.4 mg/100 g almonds. Iso3R was the predominant polyphenol among almond cultivars, since it comprised 30% or more of the adjusted total polyphenol content (Table 2). Relative to other cultivars, Butte almonds had greater ratios of K3R and Iso3R, and Nonpareil and Sonora had greater ratios of CA and Iso3G. Further, Mission almonds had a unique flavonoid profile, in that N7Glu and N were at least 2-fold greater than other cultivars.

Canonical discriminant analysis of polyphenolic content and antioxidant activity of seven almond cultivars resulted in six variables with P -values less than 0.05 (Table 3). Reduced-space plots of

Table 1
Polyphenol content, total phenols, and FRAP values of seven almond cultivars over 3 years.

Cultivar (skin%)	Polyphenols (mg/100 g)	Total phenols (mg GAE/100 g)	FRAP ($\mu\text{mol TE}/100\text{ g}$)
Sonora (4.0)	10.7 \pm 2.90 ^a	159 \pm 21 ^a	891 \pm 139 ^a
Carmel (4.5)	7.96 \pm 1.44 ^{ab}	101 \pm 30 ^a	888 \pm 216 ^a
Mission (4.4)	6.91 \pm 0.51 ^b	102 \pm 60 ^a	609 \pm 267 ^{ab}
Butte (4.4)	6.62 \pm 0.79 ^{bc}	58 \pm 7 ^b	368 \pm 78 ^b
Nonpareil (4.3)	6.19 \pm 0.78 ^{bc}	108 \pm 25 ^a	645 \pm 87 ^{ab}
Monterey (4.4)	4.88 \pm 1.08 ^c	81 \pm 12 ^b	530 \pm 53 ^b
Fritz (4.6)	3.96 \pm 2.34 ^c	58 \pm 7 ^b	565 \pm 274 ^b

Data are expressed as mean \pm SD, $n = 9$ (Butte, Carmel, Nonpareil) and $n = 3$ (Sonora, Mission, Monterey, Fritz). Values within columns bearing different letters differ, tested by ANOVA and Tukey's HSD, $P \leq 0.05$. Abbreviations: GAE, gallic acid equivalents; TE, Trolox equivalents.

Table 2
Polyphenolic content of almond cultivars.

Cultivar	Catechin	Epicatechin	Naringenin-7- <i>O</i> -glucoside	Kaempferol-3- <i>O</i> -rutoside	Isorhamnetin-3- <i>O</i> -rutoside	Isorhamnetin-3- <i>O</i> -glucoside	Naringenin
Butte	0.536 \pm 0.114 ^c (8.2) ^a	0.316 \pm 0.131 ^{bc} (4.7)	0.136 \pm 0.064 ^b (2.0)	1.38 \pm 0.50 ^a (20)	2.98 \pm 0.65 ^a (44)	0.290 \pm 0.183 ^d (4.7)	0.380 \pm 0.074 ^b (5.6)
Carmel	0.505 \pm 0.157 ^c (7.3)	0.378 \pm 0.167 ^c (5.3)	0.210 \pm 0.151 ^b (2.8)	1.02 \pm 0.24 ^b (15)	3.33 \pm 0.50 ^a (49)	0.294 \pm 0.090 ^d (4.3)	0.405 \pm 0.105 ^b (5.8)
Fritz	0.569 \pm 0.148 ^{bc} (15)	0.351 \pm 0.209 ^c (8.3)	0.132 \pm 0.200 ^b (2.1)	0.567 \pm 0.228 ^c (14)	1.65 \pm 0.98 ^c (39)	0.111 \pm 0.088 ^d (2.4)	0.220 \pm 0.314 ^c (3.7)
Mission	0.793 \pm 0.120 ^{bc} (11)	0.408 \pm 0.213 ^a (5.6)	0.402 \pm 0.128 ^a (5.6)	0.772 \pm 0.036 ^{bc} (11)	2.73 \pm 0.14 ^{ab} (38)	0.223 \pm 0.043 ^d (3.1)	0.801 \pm 0.028 ^a (11)
Monterey	0.568 \pm 0.216 ^{bc} (11)	0.346 \pm 0.168 ^c (6.5)	0.107 \pm 0.031 ^b (2.1)	0.446 \pm 0.221 ^{cd} (9.2)	1.98 \pm 0.37 ^{bc} (39)	0.605 \pm 0.386 ^c (11)	0.299 \pm 0.050 ^{bc} (5.9)
Nonpareil	0.958 \pm 0.167 ^b (3.0)	0.504 \pm 0.123 ^{ab} (7.9)	0.204 \pm 0.093 ^b (3.1)	0.218 \pm 0.051 ^d (3.4)	2.00 \pm 0.31 ^c (31)	1.04 \pm 0.18 ^b (16)	0.395 \pm 0.087 ^b (6.2)
Sonora	2.99 \pm 1.35 ^a (26)	0.692 \pm 0.411 ^a (6.0)	0.187 \pm 0.043 ^b (1.7)	0.254 \pm 0.098 ^d (2.3)	2.90 \pm 0.33 ^a (28)	2.09 \pm 0.30 ^a (20)	0.306 \pm 0.106 ^{bc} (2.8)

^a Values in parentheses are per cent of total polyphenols measured by LC–MS. Data are expressed in mg/100 g almonds as mean \pm SD, $n = 9$ (Butte, Carmel, Nonpareil) and $n = 3$ (Sonora, Mission, Monterey, Fritz). Values bearing different letters differ among columns, tested by ANOVA and Tukey's HSD, $P \leq 0.05$.

Table 3
Canonical discriminant analysis parameters for California almond cultivars and harvest season models.

Model	Variable number	Canonical correlation	F-value	DF	P-value ^a	Eigen value	Proportion of variance
Cultivar	1	0.9772	13.5061	48	<0.0001	21.1585	0.5941
	2	0.9334	9.4895	35	<0.0001	6.7794	0.1904
	3	0.9029	7.6686	24	<0.0001	4.4097	0.1238
	4	0.8049	5.7487	15	<0.0001	1.8399	0.0517
	5	0.7270	4.8069	8	0.0001	1.1207	0.0315
	6	0.4829	3.0410	3	0.0441	0.3041	0.0085
Season	1	0.9155	3.1476	38	0.0004	5.1773	0.7189
	2	0.8182	2.1371	18	0.0546	2.0246	0.2811

^a Probability that canonical correlation and all smaller ones are zero in the population (SAS Inc.).

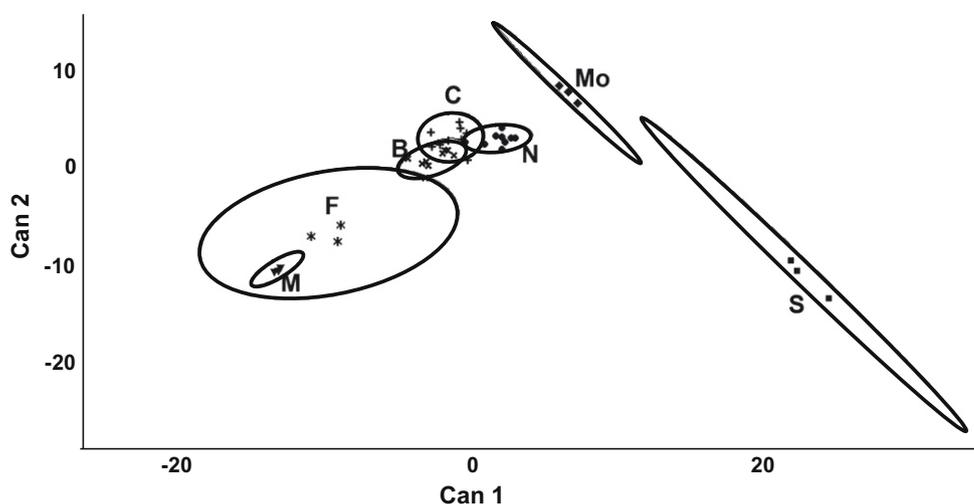


Fig. 1. Canonical discriminant analysis of almond cultivars based on polyphenol content and antioxidant activity. Labels: M, Mission; F, Fritz; B, Butte; C, Carmel; N, Nonpareil; Mo, Monterey; S, Sonora; Can1, first canonical variable; Can2, second canonical variable. Data represents the first two canonical variables of almond samples by cultivar with 80% confidence ellipses.

the first two canonical variables distinguished cultivars on the basis of 80% confidence ellipses (Fig. 1). The first and second canonical variables accounted for 59% and 18% of the variation between cultivars, respectively. The flavonoids Iso, Iso3Glu, K, Q3Gal, CA, K3Glu, K3Gal, and Q provided the best discrimination between cultivars, having absolute CV1 values greater than 0.5 for these factors (Table 4). Butte, Carmel and Nonpareil almonds were the least distinguishable cultivars, having reduced space distances ranging from 2.5 to 4.9. Fritz and Mission were also less distinct, separated by a distance of 5.1. Monterey was closest to Sonora, with a distance of 6.7. All other distances ranged from 9.2 to 36.

3.2. Season

The impact of season on polyphenol content and antioxidant activity was examined only on Butte, Carmel, and Nonpareil cultivars. Butte, Carmel, and Nonpareil almonds ($n = 9$) had significant differences in flavonoid and phenolic acid content, but not total

Table 4
Canonical discriminant analysis factors for the first and second canonical variables (Can1, Can2) for cultivar and season models.

Cultivar	Season				
	Factor	Can1	Can2	Factor	Can1
Iso	0.8629	-0.4245	EC	0.5565	-0.2427
Iso3Glu	0.8383	-0.2245	PA	0.4348	0.403
K	0.8156	-0.372	R	0.4024	-0.5197
Q3Gal	0.7536	-0.4402	pHBA	0.4007	0.2846
CA	0.7135	-0.5134	E	0.3926	-0.1597
K3Glu	0.6835	-0.1091	DiOHK	0.2199	-0.2429
K3Gal	0.5292	-0.1619	N7Glu	0.1942	-0.1788
Q	0.5279	0.233	Q3Gal	0.1862	-0.2296
Total Phenols	0.4243	-0.2972	N	0.1679	-0.1766
EC	0.3879	-0.2102	Q	0.1557	-0.424
FRAP	0.3424	-0.1496	K3Glu	0.1398	-0.1788
E	0.1191	-0.3778	CA	0.1346	-0.0433
PA	0.0166	-0.1056	K3Gal	0.129	-0.1451
Iso3R	0.014	-0.0108	K	0.1223	-0.1039
pHBA	-0.0205	0.0174	Iso3R	0.12	-0.0602
DiOHK	-0.1117	-0.0785	K3R	0.0697	0.1583
R	-0.1687	-0.234	Iso3Glu	0.0635	-0.1113
N7Glu	-0.186	-0.2591	Iso	0.0323	-0.1007
N	-0.3595	-0.2375	Total Phenols	0.0202	-0.3386
K3R	-0.3774	0.0986	FRAP	-0.0461	-0.2351

Table 5

The effect of harvest season on polyphenol content and antioxidant activity of Butte, Carmel, and Nonpareil almonds.

Season	Polyphenols (mg/100 g)	Total Phenols (mg GAE/100 g)	FRAP ($\mu\text{mol TE}/100 \text{ g}$)
2005	7.019 \pm 0.103 ^a	87 \pm 26 ^a	584 \pm 207 ^a
2006	6.284 \pm 0.124 ^b	96 \pm 39 ^a	616 \pm 251 ^a
2007	6.117 \pm 0.959 ^b	87 \pm 38 ^a	630 \pm 200 ^a

Data are expressed as mean \pm SD, $n = 9$. Values within the same column bearing different letters differ, tested by ANOVA and Tukey's HSD, $P \leq 0.05$. Abbreviations: GAE, gallic acid equivalents; TE, Trolox equivalents.

phenols or FRAP values between seasons (Table 5). Polyphenols measured by LC-MS were the highest at 7.0 mg/100 g almonds in the 2005 harvest year, 13% greater than 2007. Among years, EC was the only major flavonoid that had a significant seasonal difference. Almonds from 2005 and 2006 had 0.479 ± 0.174 and 0.434 ± 0.100 mg EC/100 g, respectively, while 2007 had 0.284 ± 0.132 mg EC/100 g, 1.7-fold less than 2005 ($P < 0.05$). The polyphenolic profile (ratio of individual flavonoid or phenolic acid to total polyphenols) was unchanged between harvest years.

Canonical discriminant analysis of seasonal differences in Butte, Carmel, and Nonpareil cultivars resulted in two canonical variables that accounted for 72% and 28% of variation, respectively (Fig. 2). EC was the strongest factor for the first canonical variable, which best distinguished almonds from 2005 and 2007, with a distance of 5.9 (Table 4). For the second canonical variable, rutin (R) and procatechuic acid (PA) best separated 2006 from the 2005 and 2007 seasons.

4. Discussion

Polyphenols and other antioxidant constituents may contribute to the health promoting effect of fruits, vegetables, whole grains, and nuts. Revealing an association between the dietary intake of polyphenols and their potential health benefits requires an accurate assessment of the content of these constituents in plant foods. The polyphenol content of many plant foods have been reported in the flavonoid, isoflavone, and proanthocyanidin databases published by the USDA. However, the scope of this information is limited, because of a number of unaccounted factors such as differences in cultivars, growing environments, seasons, process-

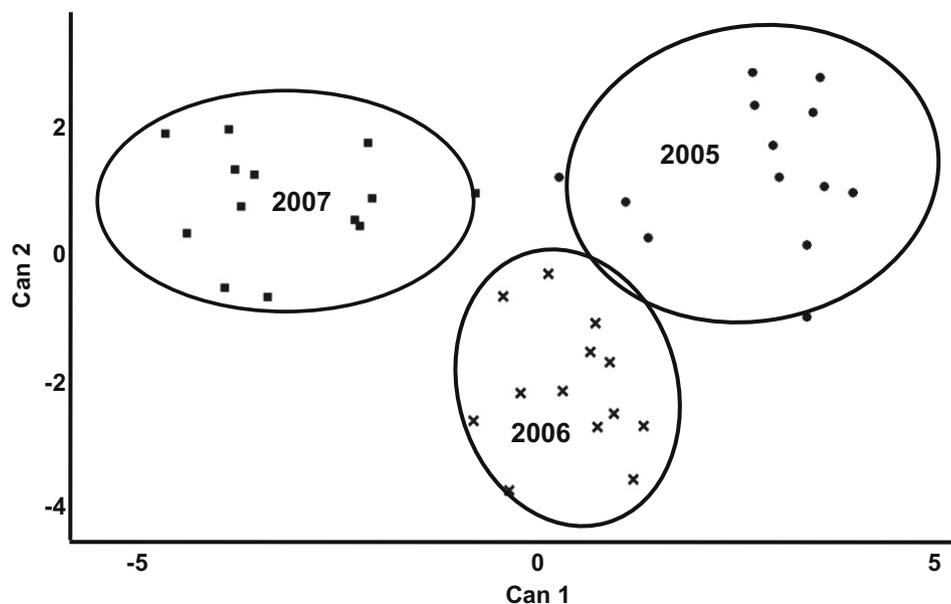


Fig. 2. Canonical discriminant analysis of almond harvest seasons based on polyphenol content and antioxidant activity. Data represents the first two canonical variables of Butte, Carmel, and Nonpareil almond samples by harvest year with 80% confidence ellipses. Labels: Can1, first canonical variable; Can2, second canonical variable.

ing, and storage. These factors are known to affect polyphenol content and profiles of plant foods. In our previous study with one almond sample each for eight cultivars, we found that almond flavonoid content might be dependent on cultivar (Milbury et al., 2006). Almond polyphenols include simple phenols, flavonoids, tannins, condensed or polymerised flavonoids or phenols, and proanthocyanidins. We have further characterised here the impact of cultivar and harvest year on almond polyphenol profile and antioxidant activity.

4.1. Cultivar

The cultivars reported here represented 82% of almond commercial acreage in 2007 (Almond Board of California, 2008). The 3-year mean phenolic acid and flavonoid content of seven California almond cultivars varied by 2.7-fold. This is comparable to our previous study where total phenols and polyphenols varied by 2.7-fold (60.2–175.1 mg GAE/100 g) and 1.9-fold (14.6–27.2 mg/100 g almonds), respectively, in a single year across eight almond cultivars; similarly, Fritz and Monterey were low polyphenol-producing cultivars (Milbury et al., 2006). Hughey et al. (2008) reported that Carmel almonds had 47% more flavonoids than Nonpareil almonds harvested in 2006. This degree of variation is similar to the 29% difference of the same cultivars over a 3-year period reported here. In contrast to our finding that Carmel almonds contain more polyphenols than Nonpareil, we previously found that Nonpareil almonds harvested in 2004 had 14% more polyphenols than Carmel (Milbury et al., 2006). Season and growing region may account for this discrepancy.

The major California almond cultivars had less variability in flavonoid content, total phenols, and antioxidant activity than almonds harvested in Portugal and Iran. A study of 10 Portuguese almond cultivars found four and 18-fold differences in flavonoids and total phenols between cultivars, respectively (Barreira et al., 2008). Analysis of 18 Iranian almond genotypes showed a 4.6-fold variation in the phenolic content of hulls and a similar variability of the antioxidant activity of extracts (Sfahlan, Mahmoodzadeh, Hasanzadeh, Heidari, & Jamei, 2009).

Relative to other reports of polyphenol content variability, the 3-year variation of California almond polyphenols appears to be

equivalent or less than tree fruits. Over a 1-year period, plum, peach, and nectarine skin total phenols varied 2.0, 2.7, and 4.8-fold, respectively, between cultivars (Gil, Tomas-Barberan, Hess-Pierce, & Kader, 2002). Apple polyphenols varied at least 5.2-fold between 67 cultivars (Wojdylo, Oszmainski, & Laskowski, 2008).

In addition to the variation in polyphenol content between cultivars, the seven California almond cultivars had unique polyphenolic profiles (Table 2). Flavonoids are products of the shikimate pathway from phenylalanine and acetate in plants. The genetic variation in the shikimate pathway of almond cultivars is likely responsible for the different flavonoid profiles between cultivars. Since polyphenols are phytoalexins, overlaying cultivar-specific polyphenol composition could yield insight into genetic traits favourable for stress adaptation and disease resistance. Therefore, more rigorous investigations into the extent of cultivar-environment interactions that affect almond polyphenol content are warranted.

Polyphenol and antioxidant content could differentiate almond cultivars and harvest years. Using multivariate analysis, studies of the chemical composition of wines (Sivertsen, Holen, Nicolaysen, & Risvik, 1999), olives (Ocakoglu, Tokatli, Ozen, & Korel, 2009), and cabbage (Sousa et al., 2008) have also distinguished cultivar and fertilisation methods. We found that canonical discriminant analysis of polyphenol content and antioxidant activity could distinguish almonds harvested in different seasons and from some cultivars with 80% confidence. Similarly, fatty acid composition (Carratala, Garcia-Lopez, Berenguer-Navarro, & Grane-Teruel, 1998), free amino acid profile (Seron et al., 1998), and triglycerides (Martin-Carratala, Llorens-Jorda, Berenguer-Navarro, & Grane-Teruel, 1999) also have distinguished almond cultivars. Thus, compiling cultivar nutrient composition data may help inform selection of cultivars, agricultural practices, and growth environments that improve almond nutrient content.

Using a canonical discriminant analysis, we found that polyphenol content and antioxidant activity could corroborate known almond cultivar inheritance. Fritz is an offspring of the Mission cultivar, Butte is a cross of Mission and Nonpareil cultivars, and Carmel, Monterey, and Sonora are offspring of Nonpareil (Bartolozzi, Warburton, Arulsekhar, & Gradziel, 1998; Hauagge, Kester, Arulsekhar, Parfitt, & Liu, 1987). In a reduced-space plot

(Fig. 1) of almond cultivars, offspring are the nearest neighbours to parents. It is notable that although Monterey and Sonora are both offspring of Nonpareil, Sonora is a high polyphenol producer, while Monterey is a low polyphenol producer. Sonora, the offspring of Nonpareil and Eureka cultivars, also shares a different origin than Padre, another high polyphenol-producing cultivar (Bartolozzi et al., 1998; Milbury et al., 2006). Further monitoring of polyphenol content in almonds of different cultivars may lead to more information about gene–environment interactions.

Region could also affect concentration of polyphenols in almond skin among different cultivars. Preliminary analysis of compositional data pooled by geographic region indicated changes to polyphenols and total phenol concentrations. Because agronomic conditions and orchard locations were not controlled by this study, a more strictly-controlled study controlling for agronomic practice is warranted to characterise factors responsible for regional differences in flavonoid content.

4.2. Season

Climate variability, agricultural practice, or other factors may lead to the seasonal variation in almond polyphenol content. We found 13% greater polyphenol content in 2005 compared to 2007 among Butte, Carmel, and Nonpareil cultivars. The degree of this variation was less than that reported by Garrido et al. (2008) of unidentified American almond cultivars which reported differences of 54% for polyphenols and 36% for ORAC values between 2004 and 2006. Interestingly, results from this study and ours demonstrate that the seasonal differences in total phenols and antioxidant activities were less than polyphenol content. This implicates antioxidant constituents in almonds besides the 18 polyphenols measured in our study in maintaining total antioxidant activity between years. However, unlike the differential effect of cultivar on polyphenol synthetic pathways, season may affect pathways similarly.

4.3. Methods of analysis

Quantitative LC–MS analysis of polyphenols limits potential interference from co-eluting compounds. Differences in the absolute amount of polyphenols recovered from almond skins between studies may arise from methods of extraction and analysis (Bolling et al., 2009). Previously, we found hot water blanching followed by acidified methanol extraction recovers nearly 50% more polyphenols from almond skins (Bolling et al., 2009). The range of 4.0–10.7 mg polyphenols/100 g almonds in the present study is less than the mean value of 15.24 mg/100 g almonds reported in the USDA flavonoid database. The flavonoid database values for almonds are based on our prior study (Milbury et al., 2006), and data from the Food Composition Nutrient Data Laboratories of the USDA (Harnly et al., 2006). These data utilise extracts from whole almonds, whereas the present study has only analysed skin, which accounted for 78–98% of the flavonoid content from whole almonds (Milbury et al., 2006). The flavonoid database also reports 2.46 mg cyanidin and 2.59 mg (–)-epigallocatechin/100 g almonds. These flavonoids were not determined in the present study and contribute to the polyphenol content of almond skins. Therefore, interpretation of polyphenol concentrations between studies should consider the effect of analytical methods.

5. Conclusion

Over three seasons, California almond cultivars had unique polyphenol profiles. The flavonoid content almond cultivars varied between seasons. This information provides evidence for variation of

almond polyphenols and antioxidant activity due to genes and environment and could inform cultivation practices to enhance the polyphenol and antioxidant quality of almonds. Knowledge of cultivar-specific polyphenol profiles may also allow optimisation of agronomic and post-harvest handling to maximise health benefits of polyphenols. However, more information is needed regarding the bioavailability of individual phenolic and flavonoid constituents from almond skins to determine if these changes are nutritionally relevant. Further work is also required to determine the effect of processing and storage on the polyphenolic content of different almond cultivars.

Acknowledgement

This work was supported by US Department of Agriculture (USDA)/Agricultural Research Service under Cooperative Agreement No. 58-1950-7-707 and a grant from the Almond Board of California. Dr. Bolling was supported by award K12GM074869 from the National Institute of Medical Sciences. The authors are grateful for the excellent technical assistance of Jennifer O'Leary, Desire Kelley, and Sisca Bolling.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.foodchem.2010.03.068.

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