Oil Content and Fatty Acid Composition of Almond Kernels from Different Genotypes and California Production Regions

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ABSTRACT. Seed of California almond [*Prunus dulcis* (Mill.) D.A. Webb, syn. *P. amygdalus* Batsch, and *P. communis* (L.) Arcangeli, non-Huds.] genotypes contained very low saturated fatty acids, high monounsaturated fatty acids, and low polyunsaturated fatty acids. Kernel oil consisted primarily of five fatty acids: palmitic, palmitoleic, stearic, oleic, and linoleic. Linolenic acid was only present in amounts of <0.02% and only in a few samples. Small but significant differences among genotypes and sampling sites were found in the proportions of palmitic, palmitoleic, and stearic fatty acids. The major differences in fatty acid composition among genotypes was found in the proportions of oleic, a monounsaturated fatty acid, and linoleic, a polyunsaturated fatty acid. The proportion of oleic acid was highest, ranging from ≈62% to 76%, and was highly and negatively correlated with linoleic acid levels. Usable genetic variation and a significant genotype × environment interaction were identified for oil content and composition. The introgression of new germplasm from peach and related species does not appear to reduce oil quantity or quality, and may offer opportunities for further genetic improvement of kernel oil composition.

During the last 20 years, almond [*Prunus dulcis* (Mill.) D.A. Webb, syn. *P. amygdalus* Batsch, and *P. communis* (L.) Arcangeli, non-Huds.] has become a major temperate tree nut crop due to the very large increase in production in California, where ≈70% of the world's supply is produced (Kester et al., 1993). Nut meat production was 555 million pounds in 1995, with 30% going to domestic consumption and ≈70% exported as whole or partial nuts, paste (marzipan), and oil. Loss of nut quality during storage or transport is attributed primarily to the oxidation of kernel fatty acids, which constitute as much as 60% of total kernel mass (Guadagni et al., 1978; Senesi et al., 1996).

The fatty acid composition of almond oil also has dietary importance. Several studies have shown that almond oil is very rich in monounsaturated fatty acid (MUFA), particularly oleic acid (18:1) (Kafkas et al., 1995; Senesi et al., 1991; 1996; Vezvaei and Jackson, 1996). Recent studies suggest that there is an inverse relationship between dietary MUFAs and serum cholesterol levels. A MUFA-rich diet from almonds lowered low-density lipoprotein cholesterol (LDLC) and total cholesterol (TC) without altering beneficial high-density lipoprotein cholesterol (HDL) levels (Sabate and Hook, 1996; Sabate et al., 1996).

While the important California almonds cultivars have been evaluated for several fruit quality attributes (Kester and Gradziel, 1996; Kester and Micke, 1984; Kester et al., 1980), they have not been analyzed for their oil content and composition. Almond oil content and composition has been reported to be primarily genotype dependent (Romojaro et al., 1988), although environmental conditions also appear to be important determinants (Barbera et al., 1994; Kafkas et al., 1995). Several important California almond selections have recently resulted from the introgression of genes for self-compatibility and disease resistance from peach and re-

lated germplasm that has had no previous selection for kernel oil quality. Lower nut oil quality has been reported in wild and less-intensively selected walnut germplasm (Greve et al., 1992) and in the generative organs of hybrids between almond and peach (Rikhter, 1990). The effect on oil quality of mature kernels from almond interspecific crosses with its close relatives has not yet been studied.

The aim of this study was to evaluate variation in the oil content and fatty acid composition among currently important California almond cultivars and breeding selections resulting from peach gene introgression and to determine environmental effects of different production regions on kernel oil content and composition.

Materials and Methods

Oil content and fatty acid composition were determined for 21 sweet almond genotypes harvested in 1995 and 1996 (Table 1). No previous deliberate selection for either higher oil or higher MUFA had occurred within this germplasm. Cultivars were grown in a Central Valley regional trial site at Manteca, Calif. To assess site effects, 'Nonpareil', the major cultivar grown in California (47% of total acreage), and three of its important pollinizers, 'Carmel', 'Sonora', and 'Mission' (26% of acreage when combined), were harvested from almond variety regional trial sites at Chico, Winters, Manteca, and Bakersfield, Calif., during the 1996 season. Harvest sites represent the northern and southern Sacramento Valley and northern and southern San Joaquin Valley production areas respectively (Fig. 1) and vary in climate, soil type, and cultural methods (Table 2). USDA-NRCS classifications for soil series are Vina loam-Mollisols, Xerolls, Haploxerolls, Pachic; Yolo loam-Entisols, Orthents, Xerorthents, Typic; Delhi loamy sand-Entisols, Psamments, Xeropsamments, Typic; and Milham sandy loam-Aridisols, Argids, Haplargids, Typic. Weather information for harvest sites were obtained from the University of California Statewide Integrated Pest Management Program at Davis.

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Table 1. Kernel oil [% dry mass (DM)] and fatty acid composition (% of total oil) for almond genotypes sampled in 1995 and 1996. Data sorted on the proportion of oleic acid (18:1) for 1996 samples.

Genotype	Oil (% DM)		Palmetic C 16:0		Palmetoleic C 16:1		Stearic C 18:0		Oleic C 18:1		Linoleic C 18:2	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
LeGrand	51	48	6.1	5.8	0.4	0.4	2.0	1.5	71.9	76.0	19.5	16.3
Rosetta	42	42	6.6	5.4	0.4	0.4	1.3	1.6	67.0	73.4	24.7	19.2
PA-F7,5-9	47	43	5.7	5.9	0.5	0.6	1.6	1.5	76.8	73.0	15.4	19.1
Price	38	38	6.3	5.0	0.4	0.4	1.5	1.5	65.6	72.8	26.3	20.3
PA-F7,1-1	46	44	6.2	5.8	0.5	0.5	1.6	1.4	74.1	72.8	17.6	19.5
Mission	52	47	5.6	5.3	0.3	0.3	2.0	1.8	70.4	71.9	21.7	20.6
Thompson	44	41	6.0	5.6	0.3	0.4	2.0	1.6	72.6	71.5	19.1	20.8
PA-F7,5-11	47	45	5.6	6.3	0.4	0.4	1.6	1.3	75.7	71.3	16.6	20.6
Aldrich	52	48	6.2	5.5	0.3	0.3	1.4	1.4	68.9	71.0	23.1	21.8
Padre	52	51	6.1	5.3	0.3	0.3	2.3	1.6	64.8	70.9	26.5	21.8
Ne Plus Ultra	50	46	6.3	6.1	0.3	0.5	1.9	1.3	65.5	70.7	26.0	21.4
Fritz	49	38	6.5	5.8	0.5	0.4	1.3	1.3	68.0	70.6	23.7	21.8
Wood Colony	44	46	6.2	5.6	0.4	0.4	1.3	1.1	72.0	70.5	20.1	22.4
Sauret #2	43	39	6.5	5.8	0.5	0.5	1.5	1.2	69.8	70.2	21.5	22.3
Sonora	41	44	7.3	5.8	0.5	0.4	1.6	1.6	61.7	69.3	28.9	22.9
Monterey	48	36	6.7	6.3	0.4	0.5	2.2	1.4	68.2	67.5	22.5	24.2
Nonpareil	48	39	6.8	6.4	0.5	0.5	1.6	1.2	68.1	66.8	23.0	25.0
Carmel	49	41	6.4	6.0	0.4	0.5	1.3	1.4	62.4	66.6	29.5	25.5
Butte	53	48	6.6	6.2	0.4	0.5	1.8	1.6	63.9	64.7	27.0	26.9
Peerless	44	39	6.5	5.8	0.4	0.5	1.4	1.3	65.8	64.6	25.8	27.7
PA-F7,5-7	42	46	6.6	6.7	0.4	0.4	2.3	1.9	64.0	61.8	26.7	29.2
LSD (5%)	5	3	0.7	0.6	0.2	0.1	0.3	0.3	3.0	3.2	2.7	2.8

Mature nuts were harvested using standard commercial practices. Nuts were considered mature when the fruit mesocarp (hull) was fully dried and split along the fruit suture and when peduncle abscission was nearly complete, allowing ready harvest of nuts by limb shaking. Production from at least 20 trees per cultivar was used to determine cultivar yield. Almond samples were collected and dried at 60 °C for 3 d to a moisture content of 3% to 4%. Three replications of five fruit each were randomly collected for each genotype. Kernels were removed and the distal half of the kernel (away from the embryo) was peeled with a scalpel. Kernel sections were cut transversely in the peeled areas and a combined sample of 100 mg (from five kernels) of very thin slices was used for oil analysis.

Total fat content and fatty acid methyl esters (FAMES) were determined according to the procedure of Garces and Mancha (1993). This method allows complete oil extraction and fatty acid transmethylation in the same tube. A 100-mg sample of kernel tissue and 0.5 mL (10 g·L⁻¹ in methanol) of margaric acid (17:0) were boiled (at 80 °C for 2 h under N₂ gas) with a reagent mixture containing 37 methanol : 36 heptane : 20 benzene : 5 2,2-dimethoxypropane : 2 H₂SO₄ (by volume). After cooling at room temperature, two phases were formed. The upper phase, containing FAMES, was collected. FAMES were separated using an flame ionization detector gas chromatograph equipped with a SP-2330 column (30 m, 0.25 mm I.D., 0.2 µm film thickness) (Supelco, Bellefonte, Pa.). The FA^s were identified based on R_f of known standards (Sigma, St. Louis, Mo.). The presence of 17:0 as an internal standard allowed the calculation of the total lipids based on the area of the standard. The extraction solutions were prepared as follows: Solution A contained 185 mL methanol, 100 mL benzene, 25 mL 2,2-dimethoxypropane, and 10 mL H₂SO₄; solution B contained heptane. For each sample, 0.5 mL of 17:0, 3 mL from A, and 2 mL from B were added.

Data were analyzed using SAS's ANOVA procedure for balanced data (SAS, Cary, N.C.). Fisher's LSD was used for comparison of genotype effect on oil composition.

Results and Discussion

VARIATION AMONG GENOTYPES. Oil content varied significantly among genotypes for both seasons, ranging from 36% to 53% of the kernel dry mass (Tables 1 and 3) and was similar to the range

Fig. 1. Sampling sites within the Central Valley almond production area of California.

Table 2. Comparison of the environmental differences in the regional sampling sites, and in the 1996 yields of 'Nonpareil' relative to the Bakersfield sampling site.

Harvest site	Avg. August temp (°C)		Soil type ^a	Irrigation method	Relative yield of Nonpareil
	Max	Min			
Chico	35.9	15.8	Vina loam	Solid-set sprinkler	0.86
Winters	36.4	16.2	Yolo loam	Microsprinkler	0.37
Manteca	35.8	15.7	Delhi loamy sand	Flood	0.17
Bakersfield	36.9	20.0	Milham sandy loam	Microsprinkler	1.00

^aUSDA-NRCS soil taxonomic classification provided in text.

Table 3. Analysis of variance of kernel oil [% dry mass (DM)] and fatty acid composition (% of total oil) for almond genotypes sampled in 1995 and 1996.

Source	df	Mean square ^a					
		Oil (% DM)	Palmetic C 16:0	Palmetoleic C 16:1	Stearic C 18:0	Oleic C 18:1	Linoleic C 18:2
Genotype (G)	20	70.0 ^{**}	0.69 ^{**}	0.024 ^{**}	0.32 ^{**}	70.1 ^{**}	60.3 ^{**}
Year (Y)	1	494.1 ^{**}	8.19 ^{**}	0.010	1.77 ^{**}	67.2 ^{**}	17.3 [*]
G × Y	20	30.1 ^{**}	0.36 ^{**}	0.007 [*]	0.11 ^{**}	20.0 ^{**}	16.9 ^{**}
Error	84	6.7	0.15	0.004	0.05	3.3	2.6

^{*},^{**}Significant at $P = 0.05$ or 0.01 , respectively.

of 35% to 61% recently reported by Vezvaei and Jackson (1996) for Australian cultivars. The oil content of 'Price' was consistently low, while that for 'Padre' was consistently high, with the remaining genotypes being intermediate. Oil content from 1995 harvested kernels was generally slightly higher than those from 1996; both year and genotype × year differences were significant (Table 3). Significant differences among genotypes are indicated by LSD values (Table 1). The oil content of selections possessing peach germplasm (having PA prefix) was generally, though not always, higher and more stable between seasons. This was contrary to expectations, since peach germplasm has had no previous direct selection for kernel oil quality, as the mesocarp or flesh is the commercial product (Rikhter, 1990). Most selections resulted from using peach as a pollen parent followed by one to two backcrosses, or, in the case of 'PA-F7,5-7', one backcross from *Prunus mira* Koehne (a wild peach species) to almond. Peach and almond are fully interfertile (Kester et al., 1991), thus peach genes should constitute ≈12% to 25% of the selection's genome. The peach genes are probably active in kernel oil production since fatty acid metabolism is controlled by a large number of diverse genes (Kester and Gradziel, 1996). The absence of any observable detrimental effect of introgressed genes on kernel oil quality reinforces the value of this exotic germplasm to almond cultivar improvement. No previous direct selection for either higher oil or higher MUFA had taken place for any of the almond genotypes tested, although any selection for long storage of almond would likely affect oil quality since oil composition is a major determinant of stored product quality (Kester et al., 1993).

Almond samples analyzed contained very low (<10%) saturated fatty acids, high MUFA, and low PUFA (polyunsaturated fatty acid) compared to other nut crops (Kester et al., 1993). Almond oil consisted primarily of five fatty acids: palmetic (16:0), palmetoleic (16:1), stearic (18:0), oleic (18:1), and linoleic (18:2) (Table 1). Linolenic acid (18:3) was not at detectable levels for most samples tested, although it was found in proportions of <0.02% of total kernel oil in a few genotypes (data not shown). Small differences were found in the proportions of 16:0, 16:1, and 18:0 fatty acids. Palmetic (16:0), which ranged from 5.0% to 7.3% of total kernel oil, was the major saturated fatty acid in all genotypes tested. Palmetoleic acid (16:1) ranged from 0.3% to

0.7% of total kernel oil. Stearic acid (18:0) was also a minor saturated fatty acid, ranging from 1.1% to 2.3%. The proportion of the saturated palmetic and stearic fatty acids was generally slightly higher in 1995 than 1996 (Table 1).

The major differences in fatty acid composition among genotypes was found in the proportions of the MUFA [oleic (18:1)] and PUFA [linoleic (18:2)]. The proportion of oleic acid in the two seasons was highest, ranging from ≈62% to 77%. Most genotypes contained a similar proportion of oleic acid in 1996 as in 1995. A few cultivars, including 'Rosetta', 'Price', 'Ne Plus Ultra', 'Padre', and 'Sonora', had lower proportions of oleic acid in 1995.

Most selections resulting from the introgression of peach germplasm contained high proportions of oleic (18:1) for both seasons tested. The exception was the selection 'PA-F7,5-7', from a cross

Fig. 2. Relationship between oleic and linoleic acids as percent kernel oil, all samples, both years.

Fig. 3. Ratio of kernel 18:1 (oleic acid) to 18:2 (linoleic acid) for almond genotypes sampled in 1995 and 1996.

to *P. mira*, followed by a single backcross to almond, which showed low oleic and high linoleic acid levels similar to ratios reported by Rikhter (1990) in the generative organs of similar hybrids. The proportion of linoleic acid (18:2) was highly and negatively correlated with oleic levels for both years for all samples (Fig. 2), similar to previous observations by Garcia et al. (1992, 1994) for pistachio (*Pistacia vera* L.) (1992) and hazelnut (*Corylus avellana* L.) (1994). This high correlation between the two predominant fatty acids of almond kernels should allow accurate future predictions of total fatty acid composition by analyzing only linoleic acid levels. The general applicability of this finding to almond and its close relatives is supported by the maintenance of this relationship even in interspecific almond x peach hybrids and their backcross progeny. The pool of oleic acid

(18:1) thus appears to be controlled by its conversion to linoleic acid (18:2). Oleic desaturase has been proposed by Garcia et al. (1992) to control the variation in fatty acid composition in pistachio. A higher ratio of oleic to linoleic acid (18:1/18:2) is associated with lower rancidity and greater oil stability (Kester et al., 1993). High ratios were observed in 'Le Grand' and often, though not always, in breeding selections having peach germplasm (Fig. 3). 'Le Grand' is unique among the cultivars tested in that it possesses partial self-compatibility, probably derived from peach (Kester et al., 1991). As with kernel oil content, results suggest that introgression of genes from related species such as peach (where no selection has been applied for kernel storage or oil quality) do not necessarily result in loss in oil quality in backcross progeny but may offer opportunities for improved quality.

The ratio of oleic to linoleic acid was generally lower in 1996 than 1995 samples, although genotype rankings remained similar. Tree yields in 1995 and 1996 were lower than normal due to unfavorable weather at bloom (Micke, 1996). No strong relationship was observed between oil content or composition and cultivar yield or kernel size (data not shown).

VARIATION AMONG PRODUCTION REGIONS. Oil content (percent fat) and composition varied significantly among genotypes and production regions tested, being consistently lower in the Manteca plot (Table 4, Fig. 4). Yields also varied greatly with region. The southernmost region, Bakersfield, produced the highest yields, followed by the northernmost region, Chico. The central regions of Winters and Manteca had 'Nonpareil' yields only 37% and 17% as large as similar test plantings in Bakersfield (Table 2). Very similar yield ratios were reported for 'Carmel', 'Sonora', and 'Mission', with regional differences being attributed to differences in weather conditions at bloom (Micke, 1996). Contrary to expectations, higher yields were associated with higher oil content (percent kernel dry mass) and higher oleic/linoleic acid ratios (Table 2, Fig. 4). However, due to the range of important production factors varying at these sites (Table 2), causal relationships cannot be determined from this data. Differences in oil content were not due to differences in kernel moisture content since moisture content for all genotypes at all locations ranged from only 2% to 3%.

Small but significant changes in the proportions of 18:0, 18:1, and 18:2 were observed among production regions (Table 4). Variation in oleic acid (18:1) generally mirrored differences in total oil content but to a lesser degree (Fig. 4). Variations in linoleic acid (18:2) closely followed the inverse relationship previously reported with oleic acid levels. 'Nonpareil' displayed the greatest variation over regions, with 'Carmel' and 'Mission' being the most uniform. Kafkas et al. (1995) had previously reported the greater environmental stability of 'Mission' (Syn. "Texas Prolific") oil content compared to 'Nonpareil'. Schirra and Agabbio (1989) also noted a greater environmental stability of 'Mission' when grown in different European environments.

The performance of these cultivars at Manteca, in terms of

Table 4. Analysis of variance of kernel oil [% dry mass (DM)] and fatty acid composition (% of total oil) for almond genotypes sampled in four different Central Valley locations.

Source	df	Mean square ^a					
		Oil (% DM)	Palmitic C 16:0	Palmitoleic C 16:1	Stearic C 18:0	Oleic C 18:1	Linoleic C 18:2
Genotype (G)	3	510.0**	304.7**	7.89**	1.95**	796.3*	341.2*
Location (L)	3	942.2**	154.8	3.52	0.32*	660.6*	322.3*
G x L	9	219.7*	126.0*	2.92	0.23*	408.1	200.7*
Error	32	78.4	57.3	1.64	0.08	187.7	83.7

*Significant at $P = 0.05$ or 0.01 , respectively.

Fig. 4. Variation in oil content and composition for major California almond cultivars when grown in different production regions.

ranking and actual oil composition, is in good agreement with the separate evaluation of genotype performance at this regional variety trial site reported in Table 1. The considerable variation in cultivar performance among regional sampling sites supports the significant genotype by environment interactions shown for oil content and oleic acid (18:2) composition and is similar to that reported by Greve et al. (1992) for walnut.

Opportunities thus exist for optimizing oil content and quality through the proper selection of genotype and production environment. The introgression of new germplasm from peach and related species does not appear to result in reduced oil quantity or quality and may offer opportunities for further genetic improvement of oil quality. The importance of production environment on final genetic potential, however, supports the need for improved understanding of the environmental factors affecting kernel oil quality, including the possible relationship between oil content and yield.

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