Research Article

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Evaluation of the chemical and nutritional characteristics of almonds (*Prunus dulcis* (Mill). D.A. Webb) as influenced by harvest time and cultivar

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Abstract

BACKGROUND: Several workers have studied the effect of harvest time on chemical and nutritional composition of almonds, but the results are partly conflicting, probably due to differences in the cultivars considered and to different agronomic and climatic conditions in the growing areas. In this paper, the influence of harvest time and cultivar on the chemical and nutritional composition of almonds (*Prunus dulcis* (Mill). D.A. Webb) were evaluated. Ten cultivars were considered, grown in the same orchard and subjected to the same agronomical regime. Almonds were collected at two different harvest times: (i) when the fruits were unripe, but already edible, and showed green and moist hull; and (ii) when the fruits were ripe, with dry brown hull. The analyses of proximate composition, fatty acid profile, total phenolic compounds, and antioxidant activity were carried out.

RESULTS: Lipid content increased ($P < 0.001$) during ripening, while both protein and carbohydrate content decreased ($P < 0.01$). Fatty acid composition showed a not univocal behavior during ripening and was highly influenced by cultivar. Total phenolic compounds and antioxidant activity varied among cultivars but increased during ripening with the exception of cv Marcona. The 'Genco' and 'Francolì' cultivars were found to be phenolic rich.

CONCLUSION: Harvest time and cultivar significantly influenced the chemical and nutritional composition of almonds. Genotype strongly influenced fatty acid composition and total phenolic compounds. The changes of bioactive compounds and antioxidant activity suggest that the synthesis of antioxidants also occurs in the last stage of ripening. Unripe almonds, a valuable niche product, showed interesting nutritional value.

Keywords: almonds; fatty acid composition; total phenolic compounds; antioxidant activity; harvest time

INTRODUCTION

Almond (*Prunus dulcis* (Mill.) D.A. Webb) is one of the oldest cultivated nut trees in the world and a major nut tree crop in hot-arid countries of the Mediterranean basin,[1,2] including southern Italy and, particularly, the Apulia region.[3]

Almond trees produce nutrient-dense nuts appreciated for their favorable lipid profile, and for high contents of vitamin E and polyphenols. Almonds are mostly consumed without removing the skin, but may also be blanched and peeled, then milled and processed to nondairy beverages, or confectionery delicacies.[4]

The importance of almonds from an agronomical and nutritional point of view explains the presence of numerous studies related in particular to the characterization of the chemical composition of fruit, with particular emphasis on the lipid fraction.[5] The latter is characterized by the predominance of unsaturated fatty acids, such as oleic and linoleic acid, and low amounts of saturated fatty acids, as well as by the presence of antioxidant compounds.[6] Previous studies showed the influence of several factors, such as genotype and harvest year, on the antioxidant compounds of almonds. In particular, Bolling *et al.*[7] reported that the synthesis of the individual polyphenols was related only to the cultivar. Flavonoids, antioxidant activity, and total polyphenols instead showed a significant interaction between genotype and environmental conditions.

Furthermore, almond genotype is the main cause of variability of fatty acid composition, oleic/linoleic acid ratio, and content of minor compounds (squalene and α-tocopherol).[8,9] At the same time, several studies[10,11] reported that both lipid content and fatty acid composition were affected by growing region, pointing out the significant interaction between genotype and environment. Finally, Yada *et al.*[12] showed that moisture, total lipid, monounsaturated fatty acids, dietary fiber, and ash content of almonds were significantly affected by the harvest year, although this aspect was not univocally demonstrated in literature.

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Moreover, many other studies were carried out in order to investigate the use of almonds to produce processed food, such as almond milk,[13] almond fermented milk,[14] and almond paste for desserts,[15] all pointing out the health benefits of almonds and related products.[16–19] At the same time, to take advantage of the high phenolic content of almond skins,[4] several studies evaluated the potential use of this by-product of almond blanching as a new ingredient, with the aim of improving the health value of foods.[20,21]

The composition of almonds is also strongly influenced by harvest time; that is, it varies at increasing ripening degree, as reported by several studies. Nanos *et al.*[1] carried out a study on two cultivars ('Texas' and 'Ferragnès') studying the effect of both harvest time and irrigation strategies on lipid content, lipid quality and sugar content. Cherif *et al.*[22] studied three cultivars ('Achaak', 'Perlees', and 'Mazzetto') and reported an increase of lipid content during ripening, with significant changes in fatty acid composition. Hawker and Buttrose[23] considered two cultivars ('Chellaston' and 'Johnston Prolific') and studied the anatomy and the chemical composition of different parts of the fruit, reporting the evolution of lipid, protein, and sugar contents during ripening. Soler *et al.*[24] reported the changes of carbohydrates, proteins, and free amino acids during almond fruit developing, considering a single cultivar. Egea *et al.*[25] investigated the changes of both carbohydrate and protein content during fruit development in a single almond cultivar ('Marta'), under deficit irrigation conditions.

However, these studies focused on very few cultivars, and their results were partly conflicting. This could be due to a difference in the cultivars considered as well as to different agronomic and climatic conditions in the growing areas.

In this framework, the aim of this study was to determine the influence of harvest time and cultivar on the chemical composition of almond fruit by considering ten different cultivars, all grown in the same orchard. Two different harvest times were considered. The first corresponded to an early stage of almond maturity, when the fruit was still unripe but could be already consumed as fresh product, whereas the second harvest time corresponded to ripe fruits with dry hull.

EXPERIMENTAL

Plant material and sampling

The research was carried out on adult almond trees (30 years old) belonging to the germplasm collection of the Department of Soil, Plant and Food Science (DISSPA) of the University of Bari, grown in Valenzano, near Bari (Apulia region, southeastern Italy). Ten commercially important cultivars were studied, of which eight were selected among the most widespread in the top producing countries (Australia, California, Italy, and Spain),[26–29] and two were new cultivars obtained in recent breeding programs.[27,28] In detail, the cultivars examined were the Australian 'Johnston Prolific' (JP_{cv}), the Californian 'Texas' (TE_{cv}) and 'Thompson' (TH_{cv}), the Italian 'Filippo Ceo' (FC_{cv}), 'Genco' (GE_{cv}) and 'Tuono' (TU_{cv}), the Spanish 'Desmajo Largueta' (DL_{cv}), and 'Marcona' (MA_{cv}), as well as 'Francolì' (FR_{cv}) and 'Ferragnès' (FE_{cv}), the latter two being the new ones, recently grown in Italian and Spanish new plantations.[27,28] Among these cultivars, the Italian 'Tuono', is also grown in Greece, Libya, Tunisia and in Spain (where it is known as 'Guara').[30] All almond cultivars were grafted, on sweet almond cv Don Carlo, by T-budding in the fall onto almond seedling rootstocks, a common grafting technique used for almond orchards located in the Mediterranean region and already used in other studies.[31,32] Almond cultivars were grown under rainfed conditions and with a tree spacing of 6.0 m × 6.0 m. All trees had the same age, and standard cultural practices were performed. Fruits were randomly collected from four different trees for each cultivar at two different harvest times, as follows: (i) T_1 (14 July 2016) corresponding to stage 'J'[33] – that is, an early stage of almond maturity, when the fruit was still unripe, with green and moist hull, but with developed cotyledons, easily separable from almond skin; (ii) T_2 (between the second half of August and the beginning of September 2016), corresponding to stage 'L'[33] – that is, ripe fruits with dry brown hull. Whole fruits collected at T_1 and T_2 were quickly stored at −18 °C until analysis. Then, the hull and shell were removed to obtain the kernel. Finally, almonds were finely milled by an electric grinder (B7301, Imetec, Azzano S. Paolo, Bergamo, Italy) and analyzed with three replicates. Samples at T_1 were lyophilized (De Mori, Milan, Italy) prior to milling.

Morphological features of almonds

Weight was determined by an analytical scale on 100 fruits. Width, length, and thickness were measured by a caliper on 100 fruits. All parameters were measured on fruits collected at T_1 and T_2.

Chemical composition

Protein (total nitrogen × 5.18), ash, and moisture content were determined according to the AACC methods 46-11A, 0801, and 44-15A respectively.[34] Fat content was determined by means of a Soxhlet apparatus using diethyl ether (Sigma Aldrich, Milan, Italy) as extracting solvent.[35] Total carbohydrates were calculated by difference. Energy value was expressed as kilocalories per kilogram and was calculated using Atwater's coefficients.[36]

Fatty acid composition

The fatty acid composition was determined by gas chromatographic analysis of fatty acid methyl esters according to AOCS method Ch 1–91.[37]

The gas chromatography system used consisted of a 7890A gas chromatograph (Agilent Technologies, Palo Alto, USA) equipped with a flame ionization detector and an SP2340 fused-silica capillary column 60 m × 0.25 mm × 0.2 μm film thickness (Supelco Park, Bellefonte, PA, USA). The gas chromatography conditions used were the same as those reported in our previous study.[38] In particular, the temperature of the split injector was 230 °C, with a splitting ratio of 1 : 50; the detector temperature was 290 °C. The oven temperature was programmed from 60 to 180 °C, with increments of 5 °C min^{-1}, then to 240 °C with increments of 3 °C min^{-1}, and a final isothermal of 20 min. Helium was utilized as carrier gas at a constant flow rate of 3 mL min^{-1}. The identification of each fatty acid was carried out by comparing the retention time with that of the corresponding methyl ester standard (Sigma Aldrich, Milan, Italy). The results were expressed as grams per kilogram.

Determination of antioxidant activity and total phenolic compounds

Radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and content of total phenolic compounds were measured on the methanol extract prepared as follows: 0.5 g of sample powder was mixed with 10 mL of aqueous methanol (70% v/v) and stirred for 2 h. After centrifugation at 10 000 rpm for 10 min, the supernatant was utilized for the determination of the antioxidant activity, as reported in Cosmai *et al.*[39] with some modifications. In particular, 100 μL of extract were diluted ten times

and added at 900 μL of 60 μmol L^{-1} DPPH methanol solution (Sigma Aldrich, Milan, Italy). Samples were stored in dark condition for 1 h, then the absorbance at 517 nm was read for each sample with a Cary 60 UV–Vis spectrometer (Agilent Technologies, Santa Clara, CA, USA). A calibration curve was prepared with (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, Sigma Aldrich, Milan, Italy) in order to express antioxidant activity in equivalents of this compound.

Total phenolic compounds were determined using the Folin–Ciocalteu method, previously reported in Singleton and Rossi[40] with some variations. Basically, 100 μL of extract was added to 100 μL of Folin and Ciocalteu's phenol reagent (Sigma Aldrich, Milan, Italy) and to 800 μL of sodium carbonate (5% w/v). The mixture was kept stirring in dark condition for 1 h, then was centrifuged at 10 000 rpm for 3 min. Finally, the absorbance at 765 nm was read. Total phenolic compounds were expressed as milligrams per kilogram of gallic acid, previously used to obtain the calibration curve.

Statistical analysis

To analyze the differences among samples, one-way analysis of variance (ANOVA), followed by Fisher's test (least significant difference) for multiple comparisons at $P < 0.05$, was carried out on the experimental data by means of the XLStat software (Addinsoft SARL, New York, NY, USA). Principal component analysis was applied to define the influence of cultivar and harvest time.

RESULTS AND DICUSSION

Morphological and basic nutritional data

Table 1 reports the mean values of weight and size of kernels collected at two different harvest times: T_1, corresponding to unripe fruits with green and moist hull, traditionally consumed fresh, and T_2, corresponding to drier fruits collected at full ripening time. Almond size's indices at T_1 were characterized by a great variability; in particular, kernel weight ranged from 1.63 to 3.14 g. Kernel weight remarkably decreased at T_2, due to moisture loss, with a lower variability than at T_1 (range 1.21 – 1.78 g). JP_{cv} showed the highest kernel weight both at T_1 and T_2 and was affected by the highest weight loss (exceeding 43%) during ripening. The same cultivar showed the longest kernels. The cultivars under investigation were characterized by different kernel shape, varying from round (MA_{cv}, with 0.80 and 0.76 width/length ratio at T_1 and at T_2 respectively) to elongated (JP_{cv}, with 0.43 and 0.47 width/length ratio at T_1 and at T_2 respectively).

Table 2 reports the chemical composition of almond cultivars at the two different harvest times considered. All almond cultivars showed a remarkable variability for the chemical parameters evaluated. T_1 almonds were characterized by a high moisture content (450.8 g kg^{-1} as mean value, reaching 562.0 g kg^{-1} in TE_{cv}), which dramatically decreased to 58.3 g kg^{-1} in T_2 almonds. The lipid content at T_1 was between 200.6 g kg^{-1} (in TE_{cv}) and 301.1 g kg^{-1} (in DL_{cv}) on fresh matter, and considerably increased at T_2, reaching 561.7 g kg^{-1} (in FC_{cv}). The latter cultivar also showed the lowest protein content at both T_1 and T_2 (101.1 g kg^{-1} and 141.2 g kg^{-1} respectively). In this regard, other authors[25] reported that the highest lipid content was coupled with the lowest protein content.

MA_{cv} showed the highest protein content at T_2 (220.8 g kg^{-1}), whereas JP_{cv} showed the highest protein content at T_1 (151.7 g kg^{-1}). Carbohydrates mean content accounted for 184.3 g kg^{-1} and 226.3 g kg^{-1} at T_1 and T_2 respectively. Ash content was below 20.0 g kg^{-1} at T_1, rising to a range of 24.9 – 34.9 g kg^{-1} at T_2.

Owing to the strong difference in moisture content (much lower at T_2 than at T_1), the total energy value (expressed on fresh matter) of almonds collected at T_2 was dramatically higher (6082.5 kcal kg^{-1}) than at T_1 (3374.0 kcal kg^{-1}). These observations could induce modern consumers, searching for less energetic food, to prefer fresh unripe almonds over ripe fruits.

The differences between T_1 and T_2 are highlighted in Fig. 1, which reports the mean value and the results of statistical analysis (one-way ANOVA) of proteins, lipids, carbohydrates, and ash content expressed on dry matter. Harvest time significantly influenced the lipid content, making it strongly increase during ripening ($P < 0.001$). This trend was due to the incomplete biosynthesis of triacylglycerols at T_1. Cherif *et al.*[22] Egea *et al.*,[25] and Piscopo *et al.*[2] previously reported similar results, whereas Nanos *et al.*[1] reported no significant differences in lipid content during ripening, considering two cultivars (TE_{cv} and FE_{cv}) under two different irrigation strategies.

Protein content exhibited a significant decrease ($P < 0.01$) in almonds harvested at T_2, ranging from 246.0 to 210.2 g kg^{-1}. A previous study carried out over 3 years in cv Marta reported a sharp decrease of protein content immediately after fruit dehydration, but just in 1 year of production.[25] Thus, protein content could be influenced by several factors in addition to cultivar, such as climatic conditions during kernel filling stage. Changes in protein content during ripening were previously studied by Soler *et al.*[24] who reported a protein increase as ripening proceeded. This trend was not found in our samples.

Paralleling the same trend of protein content, a drop of total carbohydrates on dry matter was observed, from an average of 276.7 g kg^{-1} (T_1) to 240.4 g kg^{-1} (T_2). Carbohydrates could be used as substrate for the biosynthesis of other chemical compounds during ripening. Finally, ash content remained constant during almond ripening ($P = 0.252$).

The changes that occurred in chemical composition could also be explained by considering the differences in kernel weight at the two harvest times (Table 1). Considering the chemical composition on a dry basis (data not shown), a significant and negative correlation was found between kernel weight and lipid content ($R = -0.756$), whereas a positive correlation occurred with carbohydrate content ($R = 0.634$). Thickness, length and width were not significantly correlated with chemical composition. Greater variations in kernel weight during ripening (such as in FC_{cv} and JP_{cv}) corresponded to stronger changes in the chemical composition.

Considering the data obtained in our investigation, unripe almonds (T_1) revealed interesting nutritional characteristics compared with fully ripe almonds (T_2). As a matter of fact, almonds have much lower lipid content and higher protein content at T_1 than at T_2. Moreover, owing to their high moisture content, unripe almonds have a lower energy value than fully ripe fruits. However, such a high moisture level determines a very low shelf-life, limiting the consumption of unripe almonds, as fresh product, to a short period of the year.[41] Frozen storage could be effective to extend the shelf-life of this nutritionally valuable product.

Fatty acid composition

Table 3 reports the fatty acid composition and the results of statistical analysis of almond kernels collected at T_1 and T_2, expressed as grams per kilogram. All the cultivars showed a predominance of

Table 1. Mean value of kernel weight, length, width and thickness of almonds examined at two different harvest times

	T_1					T_2				
Cultivar	Weight (g)	Thickness (mm)	Length (mm)	Width (mm)	Width/length ratio	Weight (g)	Thickness (mm)	Length (mm)	Width (mm)	Width/length ratio
DL_{cv}	2.20	8.52	27.93	14.73	0.52	1.43	7.35	28.45	15.02	0.53
GE_{cv}	1.94	10.41	22.33	14.94	0.67	1.56	8.46	23.88	15.52	0.65
JP_{cv}	3.14	9.52	35.77	15.57	0.43	1.78	7.19	32.14	15.15	0.47
MA_{cv}	1.78	9.62	20.30	16.26	0.80	1.45	8.54	21.90	16.69	0.76
TH_{cv}	1.84	8.76	25.13	13.31	0.52	1.31	7.77	24.22	13.89	0.57
TU_{cv}	1.86	8.70	25.38	15.02	0.59	1.35	7.08	25.07	15.93	0.63
FC_{cv}	2.16	11.09	24.73	15.88	0.64	1.21	7.73	24.88	14.98	0.60
FE_{cv}	2.12	8.89	31.20	14.76	0.47	1.62	8.28	28.74	14.04	0.49
FR_{cv}	2.01	9.26	26.51	14.10	0.53	1.39	6.90	25.94	15.02	0.58
TE_{cv}	1.63	9.61	22.37	13.51	0.60	1.46	9.42	23.48	13.80	0.59

T_1, unripe, but already edible, drupes with green and moist hull; T_2, ripe almonds. DL_{cv}, cv Desmajo Largueta; FC_{cv}, cv Filippo Ceo; FE_{cv}, cv Ferragnès; GE_{cv}, cv Genco; MA_{cv}, cv Marcona; TH_{cv}, cv Thompson; TU_{cv}, cv Tuono; TE_{cv}, cv Texas; JP_{cv}, cv Johnston Prolific; FR_{cv}, cv Francolì.

Table 2. Chemical composition (g kg^{-1} on fresh weight) and energy value (kcal kg^{-1} on fresh weight) of almonds examined at two different harvest times ($n = 3$)

	T_1						T_2					
Cultivar	Moisture content	Lipid content	Protein content	Carbohydrate content	Ash content	Energy value	Moisture content	Lipid content	Protein content	Carbohydrate content	Ash content	Energy value
DL_{cv}	394.0	301.1	140.1	145.5	19.3	3852.3	56.8	505.7	196.6	208.9	32.0	6173.3
GE_{cv}	482.2	233.2	124.4	144.5	15.7	3174.4	65.3	423.9	214.6	266.3	29.9	5738.7
JP_{cv}	438.6	210.9	151.7	184.3	14.5	3242.1	54.3	469.7	201.6	248.1	26.3	6026.1
MA_{cv}	421.0	264.1	147.9	150.7	16.3	3571.3	60.9	527.1	220.8	157.1	34.1	6255.5
TH_{cv}	430.7	268.4	149.2	134.8	16.9	3551.6	52.7	480.3	212.3	223.6	31.1	6066.3
TU_{cv}	422.4	269.3	148.9	140.9	18.5	3582.9	53.0	473.0	204.5	239.3	30.2	6032.2
FC_{cv}	476.0	227.0	101.1	181.5	14.4	3173.4	53.8	561.7	141.2	218.4	24.9	6493.7
FE_{cv}	439.0	247.1	138.7	157.4	17.8	3408.3	65.0	501.3	181.4	223.7	28.6	6132.1
FR_{cv}	442.3	266.3	123.9	149.4	18.1	3489.9	56.7	442.9	204.9	260.6	34.9	5848.1
TE_{cv}	562.0	200.6	121.9	100.1	15.4	2693.4	64.5	487.5	201.4	217.4	29.2	6062.7
Mean	450.8	248.8	134.8	148.9	16.7	3374.0	58.3	487.3	197.9	226.3	30.1	6082.5
SD	46.8	30.8	16.5	23.7	1.7	438.0	5.2	39.7	22.7	31.0	3.1	572.1

T_1, unripe, but already edible, drupes with green and moist hull; T_2, ripe almonds. DL_{cv}, cv Desmajo Largueta; FC_{cv}, cv Filippo Ceo; FE_{cv}, cv Ferragnès; GE_{cv}, cv Genco; MA_{cv}, cv Marcona; TH_{cv}, cv Thompson; TU_{cv}, cv Tuono; TE_{cv}, cv Texas; JP_{cv}, cv Johnston Prolific; FR_{cv}, cv Francolì. SD, standard deviation.

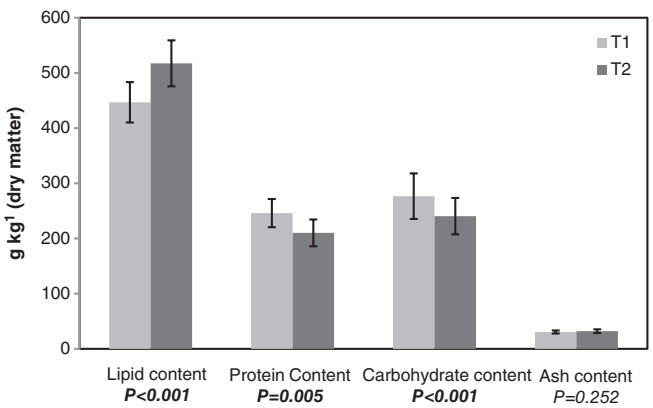
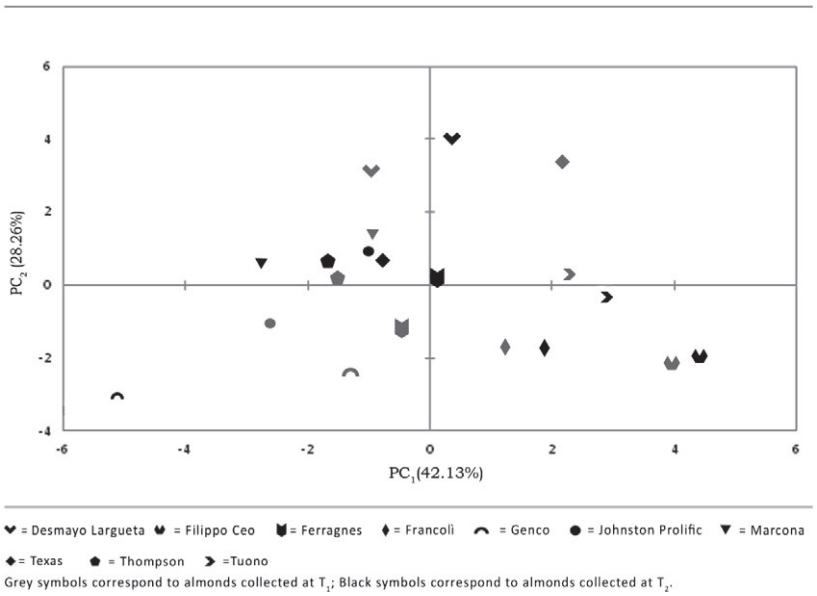


Figure 1. Mean value, with standard deviation, and results of statistical analysis of lipid, protein, carbohydrate, and ash contents of almonds collected at two different harvest times: T_1, unripe, but already edible, drupes with green and moist hull; T_2, ripe almonds.

Table 3. Mean value ($n = 3$), standard deviation (SD) and results of statistical analysis of the fatty acid composition (g kg^{-1}) of the lipid fraction of almonds collected at two different harvest times

		DL_{cv}	FC_{cv}	FE_{cv}	FR_{cv}	GE_{cv}	JP_{cv}	MA_{cv}	TE_{cv}	TH_{cv}	TU_{cv}
$C_{14:0}$	T_1	0.28 ± 0.02^a	0.23 ± 0.02	0.26 ± 0.01	0.16 ± 0.03	0.24 ± 0.01^b	0.44 ± 0.13	0.21 ± 0.14	0.14 ± 0.03^b	0.26 ± 0.07	0.20 ± 0.03
	T_2	0.20 ± 0.00^b	0.28 ± 0.03	0.28 ± 0.01	0.11 ± 0.02	0.49 ± 0.09^a	0.32 ± 0.11	0.29 ± 0.11	0.32 ± 0.07^a	0.17 ± 0.06	0.16 ± 0.04
$C_{14:1}$	T_1	0.28 ± 0.01	0.17 ± 0.00	0.21 ± 0.02	0.20 ± 0.01	0.20 ± 0.03	0.26 ± 0.03	0.22 ± 0.15	0.32 ± 0.03	0.30 ± 0.01	0.24 ± 0.02
	T_2	0.30 ± 0.01	0.17 ± 0.01	0.26 ± 0.03	0.22 ± 0.01	0.24 ± 0.01	0.32 ± 0.02	0.30 ± 0.12	0.27 ± 0.03	0.30 ± 0.01	0.22 ± 0.01
$C_{15:0}$	T_1	0.11 ± 0.01^b	0.09 ± 0.00	0.09 ± 0.03	0.09 ± 0.02	0.09 ± 0.00	0.12 ± 0.00	0.07 ± 0.01	0.16 ± 0.03	0.11 ± 0.01	0.11 ± 0.02
	T_2	0.24 ± 0.07^a	0.10 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.07 ± 0.11	0.13 ± 0.01	0.08 ± 0.11	0.13 ± 0.02	0.12 ± 0.00	0.12 ± 0.01
$C_{16:0}$	T_1	76.72 ± 0.29	56.59 ± 0.15	64.82 ± 1.23	63.81 ± 1.25	55.61 ± 1.00	63.18 ± 1.08^b	66.49 ± 0.22	71.49 ± 0.39^a	63.56 ± 0.27	71.28 ± 0.40^a
	T_2	77.03 ± 0.19	59.09 ± 0.29	68.61 ± 1.31	61.56 ± 1.14	60.10 ± 1.76	68.02 ± 0.91^a	70.17 ± 0.30	64.39 ± 0.22^b	67.50 ± 0.34	69.22 ± 0.33^b
$C_{16:1}$	T_1	4.90 ± 0.11	3.14 ± 0.23	4.23 ± 0.12^b	3.71 ± 0.22	4.18 ± 0.32^b	4.97 ± 0.61	5.12 ± 0.06^b	4.07 ± 0.01	3.99 ± 0.31	4.10 ± 0.21
	T_2	5.35 ± 0.23	3.79 ± 0.21	5.60 ± 0.22^a	4.32 ± 0.34	6.17 ± 0.16^a	6.15 ± 0.81	7.26 ± 0.07^a	4.10 ± 0.05	4.90 ± 0.42	4.60 ± 0.25
$C_{17:0}$	T_1	0.60 ± 0.02	0.70 ± 0.03	0.55 ± 0.01	0.56 ± 0.02	0.52 ± 0.04	0.56 ± 0.04	0.54 ± 0.06	0.64 ± 0.06	0.56 ± 0.01	0.61 ± 0.03
	T_2	0.59 ± 0.01	0.62 ± 0.02	0.54 ± 0.01	0.55 ± 0.10	0.48 ± 0.01	0.53 ± 0.06	0.45 ± 0.04	0.57 ± 0.04	0.54 ± 0.00	0.60 ± 0.03
$C_{17:1}$	T_1	1.02 ± 0.03	0.86 ± 0.03	0.94 ± 0.02	0.93 ± 0.11	1.00 ± 0.02	1.03 ± 0.05	0.98 ± 0.02	0.89 ± 0.05	1.04 ± 0.02	0.89 ± 0.03
	T_2	0.98 ± 0.03	0.78 ± 0.04	0.90 ± 0.00	0.89 ± 0.13	1.05 ± 0.08	0.92 ± 0.04	0.94 ± 0.03	0.91 ± 0.05	1.05 ± 0.02	0.89 ± 0.03
$C_{18:0}$	T_1	20.62 ± 0.12	39.73 ± 0.22	20.20 ± 0.23	23.40 ± 0.91	18.46 ± 1.02	17.38 ± 1.02^b	20.00 ± 1.13	24.72 ± 0.13^a	17.35 ± 0.15	24.21 ± 0.10^b
	T_2	21.29 ± 0.21	39.25 ± 0.26	21.43 ± 0.37	26.82 ± 0.94	14.35 ± 1.06	21.74 ± 1.10^a	18.11 ± 0.80	21.94 ± 0.10^b	16.86 ± 0.17	30.50 ± 0.21^a
$C_{18:1}$	T_1	628.20 ± 1.32	685.36 ± 0.81^a	729.68 ± 1.17^a	720.68 ± 0.3	752.80 ± 0.94^b	760.11 ± 1.41^a	694.8 ± 1.39	599.14 ± 1.37^b	721.99 ± 0.16	671.56 ± 0.19
	T_2	631.46 ± 2.12	667.26 ± 0.73^b	685.13 ± 0.91^b	727.98 ± 0.49	782.75 ± 0.83^a	682.80 ± 1.50^b	689.01 ± 1.08	678.30 ± 1.13^a	716.68 ± 0.26	671.15 ± 0.98
$C_{18:2}$	T_1	265.98 ± 1.10	210.89 ± 0.09^b	177.44 ± 0.82^b	184.58 ± 1.11	165.49 ± 4.02^a	150.69 ± 0.08^b	210.18 ± 0.18	296.81 ± 1.11^a	189.38 ± 0.46	224.62 ± 0.74
	T_2	261.08 ± 1.12	226.26 ± 0.03^a	215.48 ± 1.01^a	175.40 ± 1.32	133.04 ± 1.23^b	217.94 ± 0.13^a	212.18 ± 0.18	227.78 ± 1.18^b	190.78 ± 0.36	220.18 ± 1.69
$C_{20:0}$	T_1	0.65 ± 0.02	1.35 ± 0.01	0.75 ± 0.01	0.94 ± 0.04	0.63 ± 0.21	0.55 ± 0.13	0.63 ± 0.03	0.79 ± 0.15	0.69 ± 0.23	1.25 ± 0.05
	T_2	0.69 ± 0.01	1.38 ± 0.01	0.74 ± 0.01	1.09 ± 0.05	0.50 ± 0.12	0.31 ± 0.44	0.50 ± 0.07	0.57 ± 0.10	0.32 ± 0.45	1.37 ± 0.03
$C_{18:3}$	T_1	0.53 ± 0.01	0.72 ± 0.01	0.68 ± 0.02	0.76 ± 0.07	0.66 ± 0.04	0.56 ± 0.14	0.64 ± 0.03	0.69 ± 0.02	0.62 ± 0.03	0.71 ± 0.01
	T_2	0.61 ± 0.03	0.79 ± 0.02	0.72 ± 0.02	0.77 ± 0.05	0.69 ± 0.05	0.70 ± 0.14	0.54 ± 0.07	0.61 ± 0.02	0.64 ± 0.02	0.71 ± 0.03
$C_{22:0}$	T_1	0.12 ± 0.02	0.17 ± 0.03	0.15 ± 0.01	0.17 ± 0.00	0.11 ± 0.08	0.13 ± 0.00	0.11 ± 0.01	0.15 ± 0.00	0.14 ± 0.01	0.23 ± 0.00
	T_2	0.17 ± 0.01	0.23 ± 0.04	0.17 ± 0.02	0.19 ± 0.01	0.06 ± 0.08	0.12 ± 0.03	0.18 ± 0.03	0.11 ± 0.03	0.16 ± 0.04	0.28 ± 0.03

Different letters in column, for the same fatty acid, indicate significant differences at $P < 0.05$.
T_1, unripe, but already edible, drupes with green and moist hull; T_2, ripe almonds. DL_{cv}, cv Desmajo Largueta; FC_{cv}, cv Filippo Ceo; FE_{cv}, cv Ferragnès; GE_{cv}, cv Genco; MA_{cv}, cv Marcona; TH_{cv}, cv Thompson; TU_{cv}, cv Tuono; TE_{cv}, cv Texas; JP_{cv}, cv Johnston Prolific; FR_{cv}, cv Francolì.



	$C_{14:0}$	$C_{14:1}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{18:3}$	$C_{22:0}$
PC1	0.344	0.220	0.091	0.003	**0.440**	**0.572**	**0.646**	**0.818**	0.279	0.227	**0.825**	**0.382**	**0.631**
PC2	0.103	**0.593**	**0.450**	**0.783**	0.050	0.051	0.026	0.042	**0.620**	**0.663**	0.080	0.209	0.003

Figure 2. Loading plot of the principal component analysis carried out on the fatty acid composition of almonds collected at two different harvest times: T_1, unripe, but already edible, drupes with green and moist hull; T_2, ripe almonds. The cultivar has been considered as supplementary variable. The table at the bottom reports the contribution of the single fatty acid to the PC_1 and PC_2.

oleic acid, ranging from 599.14 g kg^{-1} (TE_{cv} at T_1) to 782.75 g kg^{-1} (GE_{cv} at T_2). Linoleic acid was the second most abundant fatty acid, with the lowest content in JP_{cv} at T_1 (150.69 g kg^{-1}) and the highest content in DL_{cv} at T_2 (261.08 g kg^{-1}). At the same time, DL_{cv} showed the highest content of palmitic acid at both T_1 and T_2, with values of 76.72 g kg^{-1} and 77.03 g kg^{-1} respectively. The lowest contents of palmitic acid were found in GE_{cv} and FC_{cv} at both harvest times. Therefore, almonds showed a well-balanced and healthy fatty acid composition, even at the earliest stages of ripeness.

The results of the statistical analysis (one-way ANOVA) revealed that fatty acid composition was influenced by harvest time, pointing out significant differences among seven out of ten cultivars under investigation. The evolution of fatty acid composition during ripening did not exhibit univocal behavior.

In particular, GE_{cv} and TE_{cv} showed a significant increase of oleic acid content during ripening and, oppositely, FC_{cv}, FE_{cv}, and JP_{cv} revealed a significant decrease of the same fatty acid. In a previous study, Nanos *et al.*[1] examined TE_{cv} and FE_{cv} and reported a higher oleic content in early-harvested almonds than in late-harvested ones. On the other hand, both Cherif *et al.*[22] and Piscopo *et al.*[2] reported an increase of oleic acid during ripening.

In FC_{cv}, FE_{cv}, and JP_{cv}, the decrease of oleic acid corresponded to a significant increase of linoleic acid during ripening, whereas GE_{cv} and TE_{cv} showed a decrease. Palmitoleic acid significantly decreased in FE_{cv}, GE_{cv}, and MA_{cv}.

The saturated lipid fraction was represented mostly by palmitic acid, which also exhibited divergent trends among cultivars. Its content significantly decreased in TE_{cv} and TU_{cv}. Stearic acid increased in JP_{cv} and TU_{cv}, but significantly decreased in TE_{cv}.

To better point out the influence of cultivar in our samples, fatty acid compositional data were submitted to principal component analysis, setting the cultivar as supplementary variable (Fig. 2). As expected, the distribution of samples was strongly influenced by the variable 'cultivar', regardless of harvest time. However, we found an irregular behavior in sample distribution among the two principal components. PC_1 explained over the 42% of total variability and was influenced by minor and saturated fatty acids. In this case, some cultivars, such as GE_{cv}, JP_{cv}, TE_{cv}, and DL_{cv}, exhibited a great variability on this axis. PC_2, instead, was influenced by major fatty acids (oleic and linoleic acids), and both JP_{cv} and TE_{cv} showed a large variability related to harvest time.

On the whole, our results agreed with existing literature[1,2,5,8,12,42] and highlighted that the evolution of fatty acid composition during ripening was related to varietal factors.

The positive effects of almond consumption on health are widely reported in the literature.[43] These properties are mainly related to almond fatty acid composition, which contributes to enrich the diet in monounsaturated fatty acids. The latter have a more favorable effect on health than polyunsaturated fatty acids,[44,45] besides the obvious positive effect over saturated fatty acids.[46,47] A moderate and regular consumption of almonds and nuts (~30 g daily) is associated with health-promoting effects, and the use of almonds as a nutraceutical tool is conceivable in metabolic diseases because they reduce low-density lipoprotein and total cholesterol and improve glycemic control.[18,48–50] Thus, the consumption of almonds is gaining interest both locally, in the producing areas, and worldwide.

Antioxidant activity and total phenolic compounds

The total phenolic compounds and the antioxidant activity of almond cultivars at both T_1 and T_2 are reported in Table 4. The total phenolic compounds showed a great variability among cultivars, ranging from 943.84 (JP_{cv}) to 2751.22 mg kg^{-1} gallic acid (FR_{cv}) on

Table 4. Mean value ($n = 3$), plus/minus standard deviation, and results of statistical analysis of the antioxidant activity (μmol Trolox equivalents g^{-1} on dry matter) and of the total phenolic compounds (mg kg^{-1} gallic acid on dry matter) of almonds collected at two different harvest times

	Antioxidant activity			Total phenolic compounds		
	T_1	T_2	*P-value*	T_1	T_2	*P-value*
DL_{cv}	25.00 ± 0.22	28.80 ± 3.87	*0.164*	1669.74 ± 33.59	1853.56 ± 322.04	*0.23*
GE_{cv}	27.25 ± 2.12	60.30 ± 3.31	***<0.001***	2171.66 ± 191.45	11 030.53 ± 54.91	***<0.001***
JP_{cv}	14.13 ± 1.33	16.27 ± 1.68	*0.158*	943.84 ± 19.24	782.05 ± 51.08	***0.002***
MA_{cv}	15.04 ± 0.40	12.69 ± 1.35	***0.044***	1015.24 ± 8.84	391.98 ± 15.71	***<0.001***
TH_{cv}	13.26 ± 1.19	21.97 ± 0.55	***<0.001***	806.49 ± 12.99	1538.54 ± 46.36	***<0.001***
TU_{cv}	18.25 ± 0.84	40.98 ± 1.56	***<0.001***	1285.69 ± 11.84	4887.99 ± 146.36	***<0.001***
FC_{cv}	20.59 ± 2.76	28.19 ± 2.58	***0.025***	1258.26 ± 17.12	2659.81 ± 417.69	***0.001***
FE_{cv}	29.08 ± 0.20	44.36 ± 2.20	***<0.001***	2505.83 ± 203.26	4170.8 ± 876.93	***0.011***
FR_{cv}	29.96 ± 2.89	60.99 ± 3.61	***<0.001***	2751.22 ± 264.81	7272.32 ± 602.92	***<0.001***
TE_{cv}	14.71 ± 1.77	19.57 ± 4.84	*0.178*	1500.65 ± 16.93	1483.25 ± 19.89	*0.726*

T_1, unripe, but already edible, drupes with green and moist hull; T_2, ripe almonds. DL_{cv}, cv Desmajo Largueta; FC_{cv}, cv Filippo Ceo; FE_{cv}, cv Ferragnès; GE_{cv}, cv Genco; MA_{cv}, cv Marcona; TH_{cv}, cv Thompson; TU_{cv}, cv Tuono; TE_{cv}, cv Texas; JP_{cv}, cv Johnston Prolific; FR_{cv}, cv Francolì. Bold values indicate significant differences at $P < 0.05$.

dry matter. The phenolic compounds were more abundant at T_2 than at T_1, with the highest value in GE_{cv} (11 030.53 mg kg^{-1}). MA_{cv} at T_2 was characterized by the lowest phenolic content, with a mean value of 391.98 mg kg^{-1} gallic acid. This unusual result was also found by Čolić *et al.*,[51] who reported a total phenolic compound content of 204 mg kg^{-1} gallic acid. Overall, our results agree with the existing literature that reports a wide variability in the content of total phenolic compounds among almond cultivars.[7,52] It should be underlined that almond phenolic compounds have positive health effects, such as the reduction of oxidative stress and inflammation.[53,54] In particular, the most abundant class of polyphenols in almonds is represented by proanthocyanidins,[4] recognized as strong contributors of the stability of intestinal microbiota, improving the immune response.[55,56] Therefore, the variations observed among cultivars could have consequences on the health benefits associated with almond consumption.[43,47,57] Moreover, some phenolic-rich cultivars, in particular GE_{cv} and FR_{cv}, could be used in the formulation of food products in order to extend their shelf-life, by reducing the lipid oxidation and preventing the formation of off-flavors.[58] These cultivars will be the object of further studies of shelf-life assessment.

Considering the effect of harvest time, eight out of ten cultivars showed significant differences between T_1 and T_2. In particular, six cultivars showed a significant increase during ripening, probably as a consequence of the incomplete biosynthesis of phenolic compounds in unripe green almonds. Two cultivars (MA_{cv} and JP_{cv}), on the contrary, showed a significant decrease of the total phenolic compounds during ripening, while for DL_{cv} and TE_{cv} the variation observed during ripening was not significant.

The antioxidant activity was significantly correlated with the total phenolic content ($R = 0.9306$ and 0.9408 at T_1 and T_2 respectively). Also, the antioxidant activity varied greatly among cultivars, in particular at T_2, when the lowest value accounted for 12.69 μmol Trolox equivalents per gram for MA_{cv} and the highest value, observed in FR_{cv} and GE_{cv}, was five times higher.

With the exception of MA_{cv}, JP_{cv}, and TE_{cv}, the antioxidant activity at T_2 was significantly higher than at T_1. Even for this parameter, MA_{cv} showed a peculiar behavior, with a significant decrease during ripening. The differences in the content of total phenolic compounds and antioxidant activity observed among cultivars could be due to several factors, such as genetic influence and harvest year, as reported by numerous workers.[7,20,59] Furthermore, total phenolic compounds and antioxidant activity were influenced by harvest time.

CONCLUSIONS

In this study, the influence of harvest time and cultivar on the chemical composition of almonds was evaluated considering ten cultivars grown in the same orchard. Harvest time significantly influenced the chemical composition of almonds, showing an increase of the lipid content, and, at the same time, a decrease in carbohydrates and protein content. Ash content remained constant during ripening.

The fatty acid composition was also affected by harvest time, showing no univocal behavior among the cultivars and then pointing out a strong varietal influence.

A great variability of antioxidant activity and content of total phenolic compounds was found among the ten cultivars considered, pointing out the strong influence of the genotype. These parameters tended to increase with harvest time, suggesting that the synthesis of antioxidant compounds also occurred in the last stage of ripening.

Data variability, also considering the influence of kernel weight, suggests that each cultivar has a particular attitude to different purposes. Lower lipid content and higher levels of phenolic compounds could positively influence the shelf-life, by limiting the oxidative process during almond storage.

Moreover, in this study we also carried out a nutritional characterization of unripe almonds. Owing to lower lipid content and higher moisture content, they show a markedly lower energy value than fully ripe fruits.

Unripe almonds are a valuable niche product. Owing to their very low shelf-life, unripe almonds are traditionally consumed fresh in the producing area during a very short period of the year when they are naturally available. However, unripe almonds could be stored frozen and then marketed all the year round and far beyond the area of production, fulfilling the expectation of consumers aware of the relation between healthy diet and well-being.

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