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Variability in almond oil chemical traits from traditional cultivars and native genetic resources from Argentina



Damián Maestri ^{a,*}, Marcela Martínez ^a, Romina Bodoira ^a, Yanina Rossi ^b, Alejandro Oviedo ^c, Pierluigi Pierantozzi ^{a,c}, Mariela Torres ^d

- ^a Instituto Multidisciplinario de Biología Vegetal (IMBIV, CONICET), Cátedra de Química Orgánica (FCEFyN Universidad Nacional de Córdoba), Av. Vélez Sarsfield 1611, X5016GCA Córdoba. Argentina
- ^b Instituto de Ciencia y Tecnología de los Alimentos Córdoba (ICYTAC Universidad Nacional de Córdoba CONICET), X5016GCA Córdoba, Argentina
- ^c Estación Experimental Agropecuaria San Juan (EEA INTA San Juan), Ing. Marcos Zalazar (Calle 11) y Vidart, Villa Aberastain, Pocito, 5427 San Juan, Argentina
- ^d Estación Experimental Agropecuaria San Juan (EEA INTA San Juan), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ing. Marcos Zalazar (Calle 11) y Vidart, Villa Aberastain, Pocito, 5427 San Juan, Argentina

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ABSTRACT

Almond (*Prunus dulcis* (Miller) D.A. Webb) genetic resources (Marcona, Guara, Non Pareil, IXL, AI, Martinelli C, Emilito INTA, Cáceres Clara Chica, Javier INTA) were studied during two consecutive crop years in order to evaluate variations in kernel oil yield and composition, and oil oxidative parameters. Total oil, oleic acid, α -tocopherol and squalene contents were found to range between 48.0% and 57.5%, 65% and 77.5%, 370 and 675 μ g/g oil, and 37.9 and 114.2 μ g/g oil, respectively. The genotype was the main variability source for all these chemical traits. The α -tocopherol content seems to be the most important contributor to both the radical scavenging capacity and the oxidative stability of almond oils analysed. Results obtained from the local genotypes namely Martinelli C, Emilito INTA and Javier INTA may be of interest for almond breeding focused to improve kernel oil yield and composition.

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1. Introduction

Almond is a major tree nut crop cultivated mainly in the Mediterranean region and the USA, which is the world's major producer. The kernel is the edible part of the nut and is considered an important snack and confectionary food, with a high nutritional value arising primarily from its high lipid content. Almond kernel mainly contains lipids (45–60%, dry basis); proteins and carbohydrates are present in similar amounts (20% each, in average).

Almond oil (AO) contains a substantial quantity of triacylglycerols (TAG), which are stored as intracellular oil droplets (approximately 1–3 μ m in diameter) in the cotyledon tissues of the kernel (Ren, Waldron, Pacy, Brain, & Ellis, 2001). AO is composed predominantly of mono and di-unsaturated fatty acids (FA). The FA composition of AO has been extensively reported for several

Abbreviations: AO, almond oil; AV, acid value; CD, conjugated diene; CT, conjugated triene; CY, crop year; DW, dry basis; FA, fatty acid; HPLC, high-performance liquid chromatography; OC, oil content; O/L, oleic/linoleic acid ratio; OSI, oxidative stability index; PV, peroxide value; RSC, radical scavenging capacity; SQ, squalene; α -Toc, α -tocopherol; I_2 V, iodine value.

cultivars from different geographical origins including the USA, Spain, Italy, Tunisia and Turkey (Askin, Balta, Tekintas, Kazankaya, & Balta, 2007; Kodad & Socias i Company, 2008; Martín-Carratalá, Llorens-Jordá, Berenguer-Navarro, & Grané-Teruel, 1999; Piscopo, Romeo, Petrovica, & Poiana, 2010; Prats-Mova. Grané-Teruel. Berenguer-Navarro. & Martín-Carratalá. 1999; Yada, Lapsley, & Huang, 2011). The major FA in AO is oleic acid, representing 50-80% of the total FA content. Linoleic, palmitic and stearic acids are present at levels of 10-26%, 5-9%, and 1.5-4%, respectively. Linolenic acid may be found at very low concentrations (<0.1%). Minor components characterised in AO include: tocopherols (about 450 μ g/g oil), sterols (2200 μ g/g) and squalene (95 μg/g) (Kornsteiner, Wagner, & Elmadfa, 2006; López-Ortiz et al., 2008; Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004). The major tocopherol is α -Toc (240–440 $\mu g/g$); the sterols are mainly β-sitosterol (95% of the total sterols), and campesterol and stigmasterol in similar amounts.

Freshly extracted AO has low peroxide value (lesser than $0.5 \text{ meq } O_2/\text{kg oil}$). This means that AO is readily preserved from oxidation. Besides tocopherols, which exert a protective role against lipid oxidation, recent studies have shown that almonds also contain a diverse array of phenolic and polyphenolic compounds. These chemical components are mainly present in the skin

^{*} Corresponding author. Tel.: +54 0351 4334141; fax: +54 0351 5334439. E-mail address: dmaestri@efn.uncor.edu (D. Maestri).

(seed coat) and they are effective inhibitors of oil oxidative degradation (Bolling, Dolnikowski, Blumberg, & Chen, 2010).

In Argentina, almond cultivation was introduced by European immigrants. Improvement of almond cultivation through breeding was gaining importance and allowed to get some local genotypes such as Emilito INTA, Javier INTA, Cáceres Clara Chica and Martinelli C, which are well adapted to the warm and dry areas of the Argentinean Middle West (provinces of Mendoza and San Juan). Besides giving the morphological and agronomical description of these promising almond genetic resources (Carra de Tolosa & Herrera, 2006), studies should also define their nutritional value. A lot of studies have been carried out on the characterisation of different almond cultivars and criteria for almond kernel quality evaluation, and some works have focused on fatty acid profile, triacylglycerol and tocopherol composition as discriminant parameters in order to differentiate almond genotypes (Askin et al., 2007: García-López, Grané-Teruel, Berenguer-Navarro, García-García, & Martín-Carratalá, 1996; Grane-Teruel, Prats-Moya, Berenguer-Navarro, & Martín-Carratala, 2001; Kodad et al., 2006; López-Ortiz et al., 2008; Martín-Carratalá et al., 1999).

Almond oil content and composition depend primarily on the genotype but may be affected by factors such as the crop year, and the specific environmental conditions of the growing region (Yada et al., 2011). Irrigation has shown to increase almond yields (Sánchez-Bel, Egea, Martínez-Madrid, Flores, & Romojaro, 2008) but appears to have little or no effect on both oil content and composition (Egea et al., 2009). Interestingly, Askin et al. (2007) have reported that kernel weight affects significantly the FA composition of AO showing a positive correlation with oleic acid (OA) content. The different uses of almonds may require kernels with specific physical and compositional characteristics. For oil production, besides high oil content, the FA composition and parameters related to oil oxidative stability should be important criteria for selection.

Almond oil can be extracted easily by screw pressing (Martínez, Penci, Marin, Ribotta, & Maestri, 2013). Employing a pilot plant screw-press, the highest oil recovery (440 g/kg kernel) was achieved at $8\ g/100\ g$ kernel moisture and $40\ ^{\circ}\text{C}$ pressing temperature.

Increasing worldwide almond production and increasing demand of new specialty oils, encourage screening of new almond genotypes with higher kernel oil content and improved fatty acid (higher OA concentration) profile. This study evaluates some oil chemical parameters of almond genetic resources from Argentina, and compares them with those from some selected commercial cultivars growing under the same agro-ecological conditions.

2. Materials and methods

2.1. Plant material

Almond fruits (*Prunus dulcis* (Mill.) D.A. Webb, Syn. *Prunus amygdalus* Batsch) were collected during two consecutive crop years (CY) from nine genotypes (Marcona, Guara, Non Pareil, IXL, AI, Martinelli C, Emilito INTA, Cáceres Clara Chica, Javier INTA) growing in an experimental orchard (10-year-old, tree spacing 5 m \times 5 m, 400 trees/ha, grafted on "Nemared" peach rootstock) at the Experimental Station (INTA) located at Pocito (San Juan Province, Argentina). Pocito (lat. 31°37′ S, long. 68°32′ W) is located in the Monte phytogeographical province, in arid northwestern Argentina, at 620 m above sea level. The climate in this area represents a typical arid climate with great annual temperature variations (absolute maximum values exceeding 45 °C, and absolute minimum values ranging between 5 °C and 10 °C below zero). The average annual rainfall is below 100 mm concentrated mainly in summer. The region has high heliophany, low cloudiness and

intensive solar radiation. The frost-free period comprises about 220–300 days extending from October to May. Table 1 summarises climatic conditions during both 2010 and 2011 CY evaluated. Almonds plants were grown under natural rainfall, plus supplemental irrigation of 1357 mm/year (net irrigation requirement).

From each almond genotype, three fruit samples (1 kg each) were taken from the entire canopy of five selected trees. Fruits were hand-harvested at full maturity and then dried in a vacuum oven (30 $^{\circ}$ C) until the kernel moisture content reached a value of about 5%.

2.2. Dry matter and oil contents

Fruits were cracked and shelled manually, and kernels were ground using a universal cutting mill (model Super Junior, Moulinex, France). Dry matter content was determined after oven drying at 80 °C for 72 h. Lipid extraction for total oil content was performed using Soxhlet devices with n-hexane as solvent. The solvent was removed using a rotary vacuum evaporator at 40 °C. The oil content was gravimetrically determined and expressed as weight percent on dry basis (g/100 g kernel, DB) (AOCS, 1998).

2.3. Oil analyses

For analytical determinations, oils were extracted by screw-pressing at room temperature. Briefly, kernels containing about 5% moisture (w/w) were ground and particles between 2.4 and 4.8 mm were selected using an automated screen. Oil expression was carried out with a Komet screw press (Model CA 59 G, IBG Monforts, Mönchengladbach, Germany), with a 5-mm restriction die and a screw speed of 20 rpm. The screw press was first run for 15 min without seed material but with heating *via* an electrical resistance-heating ring attached around the press barrel, to raise and maintain the screw-press barrel temperature to the desired temperature (25 °C) (Martínez et al., 2013).

Acid (AV), peroxide (PV), conjugated diene (CD) and conjugated triene (CT) values of the oil samples were analysed according to standard methods of AOCS (1998). The oxidative stability indices (OSI) were determined by Rancimat (Metrohm, Herisau, Switzerland) analysis and corresponded to the break points in the plotted curves. Air flow rate was set at 20 L/h and temperature of the heating block was maintained at 110 °C.

To evaluate the radical scavenging capacity (RSC), four concentrations (75, 100, 125 and 150 mg of oil in 1 mL toluene) of each AO sample were prepared. Each oil/toluene solution was vortexed (20 s, ambient temperature) with 3.9 mL toluene solution of the free stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (DPPH') at a concentration of 10⁻⁴ mol/L. Against a blank of pure toluene,

Table 1Average monthly temperatures (°C) and rainfall (mm) measured at the Experimental Station (INTA) located at Pocito (San Juan Province, Argentina) during 2010 and 2011 crop years.

Month	2010 crop year		2011 crop year		
	Temperature	Rainfall	Temperature	Rainfall	
January	27.5	4.6	25.9	54.0	
February	26.2	1.8	24.2	28.4	
March	24.5	0.0	22.0	9.4	
April	16.7	0.0	18.2	3.8	
May	12.1	11.4	12.6	0.0	
June	9.6	0.0	8.8	0.2	
July	6.8	0.0	7.8	2.8	
August	10.1	0.6	10.3	0.0	
September	14.4	5.6	15.9	0.0	
October	18.6	6.2	18.5	0.2	
November	22.2	10.0	23.6	26.0	
December 25.0		15.8	25.9	0.8	

the absorption at 515 nm was measured in 1 cm quartz cells using an UV–visible spectrophotometer (Perkin-Elmer Lambda 25, Shelton, CT, USA). RSC toward DPPH was estimated by mean of the following equation:

$$DPPH' = 1 - \left[\frac{absorbance\ of\ control - absorbance\ of\ test\ sample}{absorbance\ of\ control} \times 100 \right]$$

where $DPPH_r$ expresses the amount of the radical that remains in the medium after antioxidants present in the oil sample are depleted (Espín, Soler-Rivas, & Wichers, 2000). The RSC was expressed as IC_{50} which reflects the depletion of the free radical to 50%. A lower IC_{50} value indicates higher antiradical activity.

For FA composition determinations, each oil sample (0.5 g) was subjected to alkaline saponification by reflux (45 min) using 30 mL 1 N KOH in methanol. Unsaponificable matter was extracted with n-hexane (3 \times 30 mL). The fatty acids were converted to methyl esters (FAME) by reflux (45 min) using 50 mL 1 N H_2SO_4 in methanol and analysed by gas chromatography (GC) (Perkin-Elmer, Shelton, CT, USA) using a fused silica capillary column (30 m \times 0.25 mm i.d. \times 0.25 μm film thickness) CP Wax 52 CB (Varian, Walnut Creek, CA, USA); carrier gas N_2 at 1 mL/min; split ratio 100:1; column temperature programmed from 180 °C (5 min) to 220 °C at 2 °C/min; injector and detector temperatures at 250 °C, FID. The identification of FAME was carried out by comparison of their retention times with those of reference compounds (Sigma–Aldrich, St. Louis, MO, USA).

lodine values (I_2V) were calculated from fatty acid percentages (Torres & Maestri, 2006) by using the formula:

$$\begin{split} I_2V = (\% \ Palmitoleic \ acid \times 1.001) + (\% \ oleic \ acid \times 0.899) \\ + (\% \ linoleic \ acid \times 1.814) \end{split}$$

Tocopherols were analysed by HPLC (Perkin-Elmer, Shelton, CT, USA). Samples of 1 g oil were placed into 10 mL volumetric flasks. A quantity of *n*-hexane was added, swirling to dissolve the sample and making up to volume with the same solvent. An aliquot of 20 uL of this solution was injected on to a Supelcosil LC-NH₂-NP column (25 cm \times 4.6 mm. Supelco. Bellefonte. PA. USA). The mobile phase was *n*-hexane/ethyl acetate (70/30 v/v) with a flow rate of 1 mL/min. UV detection at 295 nm was performed. Individual tocopherols were identified by comparison of their retention times with those of authentic standards (α - and γ -tocopherols, ICN Biomedicals, Costa Mesa, CA). The limit of detection (LOD) i.e. the lowest concentration that could be detected - was 6.5 ppm, whereas the limit of quantification (LOQ) was equal to 9.5 ppm. α -Toc was quantified by the external standard method. The linearity of the response was verified by fitting to line results of twenty level calibration curve covering the concentration range from 2 to 1000 ppm, with a linearity regression coefficient $R^2 = 1$.

Squalene determinations were done from 400-mg oil aliquots. The oil sample was mixed with 1 mL *n*-hexane, 1 mL squalene solution (1 mg/mL n-hexane) and 2 mL KOH solution (2 N in methanol). After 1 min of vigorous shaking, the mixture was left to react for 10 min (the time required for hydrolysis of glycerides). After decanting, the upper phase (n-hexane) was extracted and washed twice (5 mL every time) with ethanol/water (50/50 v/v). The n-hexane phase was recovered and used to gas chromatography (GC) and GC-mass spectrometry (GC-MS) analyses. GC (Perkin-Elmer, Shelton, CT, USA) used a VF - 5 ms (Varian, Walnut Creek, CA, USA) capillary column (30 m \times 0.25 mm i.d.) coated with a 0.25 µm layer of 5% phenyl, 95% polydimethylsiloxane. The column temperature was set at 270 °C, injector and detector temperatures at 290 °C, carrier gas N₂ at 1 mL min⁻¹. GC-MS (Hewlett-Packard, Palo Alto, CA, USA) used helium (flow rate 1 mL min⁻¹) as carrier gas. The column, injector and detector temperatures were as for GC analysis. Squalene was identified by comparison of its mass spectra data with those of the Wiley mass spectra search library. Squalene concentration was calculated on the basis of the standard internal (squalene) concentration.

2.4. Statistical analyses

Analytical determinations reported in this study were the average of triplicate measurements from three independent samples for each almond genotype. Statistical differences were estimated from ANOVA test at the 5% level (P < 0.05) of significance for all parameters evaluated. Whenever ANOVA indicated a significant difference, a pair-wise comparison of means by least significant difference (LSD) was carried out. Except for kernel weight, which showed no significant differences between the two crop years (CY) evaluated, the chemical data set from each CY was analysed separately. In order to assess the comparative responses of almond genotypes (AG) in both 2010 and 2011 CY and the possible interactions AG x CY, chemical data were also analysed by two-way ANOVA. Correlation analyses were performed employing Pearson's test. A multivariate statistical analysis of the whole chemical data set was performed using principal component analysis (PCA). All statistical analyses were done using the InfoStat program (InfoStat version 2008, National University of Córdoba, Córdoba, Argentina).

3. Results and discussion

3.1. Kernel weight, oil content and oil analytical parameters

One important aspect in almond breeding programs is focused to increase kernel size and weight, and efforts have been conducted to obtain almond genotypes with kernel weight greater than 1 g. Because of the high stability of this physical trait across the two CY evaluated, data from these ones were averaged (Table 2). Kernel weight differed significantly among almond genotypes. Considering mean values from both 2010 and 2011 CY, it varied in the range 0.86-1.56 g. The highest kernel weight was found in the local genotype Cáceres CC and resulted remarkably higher than those registered in some Spanish, Italian, French and North American commercial cultivars (Sathe, Seeram, Kshirsagar, Heber, & Lapsley, 2008; Spiegel-Roy & Kochba, 1981), as well as from various Turkish almond genotypes (0.50-1.34 g) (Askin et al., 2007). Almond genotypes analysed in this study did not vary significantly in dry matter content; this parameter ranged from 94.8 to 96 g/100 g kernels (Table 2).

Total oil content accounted for 48.0–57.5% of the dry matter content (Table 3). Oil content varied from 48.0 to 53.5% in the first CY evaluated, and from 50.7 to 57.5% in the next year. Guara and

Table 2Origin, kernel weight (g) and dry matter content (g/100 g kernels) of some selected almond genotypes.

Genotype	Origin	Kernel weight ^a	Dry matter ^a
Marcona	Spain	1.01 ^{ab} ± 0.01	94.8 ^a ± 1.10
Guara	Spain	$0.90^{a} \pm 0.07$	$95.0^{a} \pm 1.28$
Non Pareil	USA	$0.86a \pm 0.08$	95.1 ^a ± 1.47
IXL	USA	$0.98^{ab} \pm 0.06$	$94.8^{a} \pm 0.99$
AI	France	1.34 ^{cd} ± 0.11	$95.3^{a} \pm 1.06$
Martinelli C	Argentina	$1.08^{ab} \pm 0.16$	$96.0^{a} \pm 0.26$
Emilito INTA	Argentina	1.17 ^{bc} ± 0.25	$95.3^{a} \pm 0.89$
Cáceres CC	Argentina	$1.56^{d} \pm 0.10$	$95.8^{a} \pm 0.45$
Javier INTA	Argentina	1.08 ^{ab} ± 0.12	95.6a ± 0.63

^a Data are the average of two crop years. For each crop year three independent kernel samples (50 kernels each) were used. Values in each column with the same superscript letters are not significantly different (P > 0.05) among almond genotypes.

Table 3Oil content and oil analytical parameters from kernels of some selected almond genotypes.

Parameter	Almond genotype								
	Marcona	Guara	Non Pareil	IXL	AI	Martinelli C	Emilito INTA	Cáceres CC	Javier INTA
CY 2010									
OC	$51.4^{bc} \pm 0.60$	$49.9^{ab} \pm 0.75$	53.0 ^{cd} ± 0.92	$50.0^{b} \pm 0.86$	$49.8^{ab} \pm 0.10$	$54.8^{e} \pm 0.29$	$54.7^{e} \pm 0.23$	$48.0^{a} \pm 0.21$	53.5 ^d ± 0.11
AV	$0.77^{g} \pm 0.02$	$0.51^{c} \pm 0.01$	$0.16^{b} \pm 0.01$	$0.06^{a} \pm 0.01$	$0.69^{e} \pm 0.01$	$0.59^{d} \pm 0.01$	$0.07^{a} \pm 0.01$	$0.73^{\rm f} \pm 0.03$	$0.08^{a} \pm 0.01$
PV	$0.10^{c} \pm 0.01$	$0.10^{c} \pm 0.01$	$0.03^{a} \pm 0.001$	$0.05^{a} \pm 0.001$	$0.10^{c} \pm 0.001$	$0.04^{a} \pm 0.001$	$0.06^{b} \pm 0.001$	$0.09^{c} \pm 0.001$	$0.28^{d} \pm 0.01$
CD	1.31 ^{cd} ± 0.04	$1.22^{a} \pm 0.02$	$1.35^{d} \pm 0.02$	1.31 ^{cd} ± 0.03	$1.32^{cd} \pm 0.04$	$1.25^{ab} \pm 0.01$	$1.29^{bc} \pm 0.02$	$1.43^{e} \pm 0.01$	$1.25^{ab} \pm 0.02$
CT	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$
RSC (IC_{50})	582.6g ± 3.59	437.5 ^b ± 3.54	553.6e ± 3.75	514.1° ± 1.91	412.9a ± 3.13	$588.3^{g} \pm 2.33$	567.8 ^f ± 3.19	$540.9^{d} \pm 0.85$	705.0 ^h ± 7.01
OSI	$11.9^{b} \pm 0.07$	$12.6^{\circ} \pm 0.35$	$12.6^{\circ} \pm 0.01$	13.7 ^e ± 0.14	$13.5^{e} \pm 0.35$	$11.6^{b} \pm 0.14$	$13.1^{d} \pm 0.14$	11.1 ^a ± 0.07	12.9 ^{cd} ± 0.14
CY 2011									
OC	$52.3^{abc} \pm 0.42$	$50.7^{a} \pm 1.05$	53.6 ^{bcd} ± 0.78	54.2 ^{cd} ± 0.72	52.2 ^{abc} ± 1.36	57.1 ^{ef} ± 1.52	$57.5^{\rm f} \pm 0.60$	51.2 ^{ab} ± 1.22	54.9 ^{de} ± 0.95
AV	$0.73^{d} \pm 0.04$	$0.07^{a} \pm 0.01$	$0.10^{a} \pm 0.01$	$0.10^{a} \pm 0.03$	$0.62^{c} \pm 0.03$	$0.10^{a} \pm 0.01$	$0.25^{b} \pm 0.01$	$0.08^{a} \pm 0.01$	$0.12^{a} \pm 0.01$
PV	$0.04^{b} \pm 0.01$	$0.16^{d} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.09^{c} \pm 0.01$	$0.15^{d} \pm 0.01$	$0.08^{c} \pm 0.01$	$0.24^{e} \pm 0.01$	$0.27^{f} \pm 0.01$
CD	1.35 ^{cd} ± 0.01	$1.21^{a} \pm 0.02$	$1.37^{de} \pm 0.02$	$1.32^{c} \pm 0.01$	$1.39^{e} \pm 0.01$	$1.43^{\rm f} \pm 0.03$	$1.38^{de} \pm 0.02$	$1.53^{g} \pm 0.01$	$1.26^{b} \pm 0.01$
CT	$0.04^{b} \pm 0.01$	$0.04^{b} \pm 0.01$	$0.04^{b} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.04^{b} \pm 0.01$	$0.05^{c} \pm 0.01$	$0.04^{b} \pm 0.01$	$0.06^{d} \pm 0.01$	$0.04^{b} \pm 0.01$
RSC (IC_{50})	575.8° ± 1.20	$427.5^{a} \pm 3.04$	552.6 ^d ± 3.62	$505.0^{b} \pm 7.07$	$427.6^{a} \pm 3.75$	576.7 ^e ± 2.47	$552.6^{d} \pm 3.62$	$527.7^{c} \pm 3.61$	700.1 ^f ± 2.10
OSI	12.1 ^b ± 0.42	12.5 ^{bc} ± 0.01	12.7 ^{cd} ± 0.21	13.7 ^e ± 0.28	$13.0^{d} \pm 0.01$	$11.4^{a} \pm 0.21$	12.7 ^{cd} ± 0.07	$11.0^a \pm 0.14$	12.8 ^{cd} ± 0.01

Abbreviations and units: CY, crop year; OC, oil content (g/100 g kernel, dry basis); AV, acid value (% oleic acid); PV, peroxide value (meq O_2/kg); CD, conjugate dienes (K_{232}); CT, conjugate trienes (K_{270}); RSC, radical scavenging capacity expressed as IC₅₀ (mg oil/mg DPPH'); OSI, oxidative stability index (hours). Mean values (\pm standard deviation) were the averages of three independent measurements. Values in each row with the same superscript letters are not significantly different (P > 0.05) among almond genotypes.

Cáceres CC genotypes had the lowest oil content (about 50% considering both 2010 and 2011 CY); Martinelli C and Emilito INTA had the highest one (56% in average). Analysis from two-way ANOVA (Table 4) showed that the genetic factor was the main variability source for kernel oil content. The CY effect and the interaction genotype × CY were also significant.

Considerable variability in lipid content has been reported for commercial almonds (Yada et al., 2011). García-López et al. (1996) have found lipid contents ranging from 53 to 62% in almond kernels from a wide range of geographical origins. Piscopo et al. (2010) reported lower values from same Italian, Spanish and French cultivars. In both studies, the highest oil yields were obtained from almond cultivars proceeding from Spain and Italy. The USDA National Nutrient Data Base for Standard Reference gives an average value of 49.4 g total lipids per 100 g natural almonds (USDA, 2010). Evaluation of local almonds selections throughout the world has showed that oil content in almond kernels also varies widely. Kodad & Socias i Company (2008) reported values between 50.7 and 67.5% in 47 advanced almond selections developed in Spain. Askin et al. (2007) found a wider range (25.2–60.1%) in almond genetic resources from Turkey. An average oil content of 51.4% was observed by Moayedi, Rezaei, Moini, and Keshavarz (2011) from an Iranian almond genotype. Keeping in

Table 4Variability expressed as percentage of the total sum of squares for some selected chemical parameters from almond kernels.

Parameter	Genotype (G)	Crop year (CY)	G x CY
OC	75.5 ^{**}	18.3**	6.17*
C16:0	57.4**	26.3**	16.2**
C16:1	91.2**	2.92	5.88*
C18:0	62.2**	9.37*	28.4*
C18:1	64.8**	25.6**	9.59**
C18:2	49.6**	37.2**	13.1**
O/L	54.3**	30.0**	15.7**
α-Toc	96.1**	2.62**	1.31*
SQ	91.0**	1.90*	7.08**
RSC	99.6**	0.16	0.28
OSI	97.7**	0.67	1.59

Abbreviations: OC, oil content; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; O/L, oleic/linoleic acid ratio; α -Toc, α -tocopherol content; SQ, squalene content; RSC, radical scavenging capacity; OSI, oxidative stability index.

mind the scientific literature available on macronutrient contents of almonds from a wide range of commercial cultivars, the mean value for oil content could be around 50%. So, the local genotypes named Martinelli C and Emilito INTA represent a promising base to obtain new almond cultivars with high oil content.

The press-extracted oils from all almond genotypes had very low acid and peroxide values (AV and PV, respectively) (Table 3). Considering both 2010 and 2011 CY, the highest AV and PV were observed in Marcona (0.73–0.76% oleic acid) and Javier INTA (0.27–0.28 meq O_2/kg) cultivars, respectively. Minor differences in the UV absorption coefficients (CD and CT values, 1.21–1.53, and 0.03–0.06, respectively) were observed in fresh oils from all tested almond genotypes. The values obtained for AV, PV, CD and CT were much lower than the maximum values established by the Codex Alimentarius (2001) for non-refined vegetable oils.

3.2. Fatty acid, tocopherol and squalene contents, and oxidative stability of oils

The FA composition from almond kernels is given in Table 5. In agreement with previous studies (Askin et al., 2007; García-López et al., 1996; Kodad & Socias i Company, 2008; Piscopo et al., 2010; Sathe et al., 2008) three main FA (palmitic, oleic and linoleic acids) and two minor ones (palmitoleic and stearic acids) were found in all almond genotypes. In addition, α-linolenic, arachidic and gadoleic acids were detected in trace (<0.1%) amounts. Significant variations were found among genotypes in all FA examined. Saturated FA (palmitic and stearic) were found at levels lesser than 10%; oils from Javier INTA genotype had the lowest values. Oleic acid was the major FA and accounted for 65-77.5% of the total FA content. These percentages fall within the range of 62-80% reported by Yada et al. (2011) for a wide range of commercial almonds and breeding selections. The local genotypes Martinelli C and Javier INTA had the highest oleic acid contents (72.7-77.5% and 74.7–75.2%, respectively). These percentages are among the highest ones as compared with those reported from some commercial European almond cultivars such as Ramillete, Supernova and Ferragnes (Piscopo et al., 2010). Linoleic acid concentration ranged between 12.9 and 26.1%. Oleic and linoleic acids together accounted more than 91% of the total FA content. Considering the whole data set from genotypes and CY, a highly significant negative correlation was found between both oleic and linoleic FA at r = -0.97 (P < 0.0001). Statistical correlations of oleic and linoleic

^{*} Significant at $P \leq 0.05$.

^{**} Significant at $P \le 0.01$.

Table 5Fatty acid, tocopherol and squalene contents from kernel oils of some selected almond genotypes.

Parameter	Almond genotype								
	Marcona	Guara	Non Pareil	IXL	AI	Martinelli C	Emilito INTA	Cáceres CC	Javier INTA
CY 2010									
C16:0	$6.55^{ab} \pm 0.08$	$7.30^{\circ} \pm 0.41$	$6.87^{bc} \pm 0.16$	$6.83^{bc} \pm 0.16$	$6.76^{bc} \pm 0.29$	$7.26^{\circ} \pm 0.52$	6.93 ^{bc} ± 0.11	$7.37^{c} \pm 0.38$	$6.01^{a} \pm 0.07$
C16:1	$0.53^{b} \pm 0.01$	$0.55^{b} \pm 0.04$	$0.70^{\circ} \pm 0.06$	$0.72^{c} \pm 0.07$	$0.66^{\circ} \pm 0.01$	$0.53^{b} \pm 0.01$	$0.54^{b} \pm 0.01$	$0.43^{a} \pm 0.02$	$0.55^{b} \pm 0.05$
C18:0	$1.66^{ab} \pm 0.03$	$2.21^{b} \pm 0.35$	$1.32^{a} \pm 0.14$	$1.20^{a} \pm 0.13$	$1.44^{a} \pm 0.29$	1.77 ^{ab} ± 0.34	$1.40^{a} \pm 0.04$	1.95 ^{ab} ± 0.81	$1.23^{a} \pm 0.04$
C18:1	$71.5^{bc} \pm 0.16$	$70.6^{ab} \pm 0.93$	$71.9^{bc} \pm 0.80$	$73.0^{\circ} \pm 1.61$	$73.2^{\circ} \pm 0.98$	77.5 ^e ± 1.07	$73.2^{\circ} \pm 0.04$	$69.4^{a} \pm 0.11$	$75.2^{d} \pm 0.13$
C18:2	19.7 ^{cd} ± 0.09	19.3 ^{bcd} ± 1.64	19.2 ^{bcd} ± 1.03	18.2 ^{bc} ± 1.83	17.9 ^{bc} ± 1.48	$12.9^{a} \pm 0.23$	17.9 ^{bc} ± 0.18	20.8 ^d ± 1.30	$17.0^{b} \pm 0.14$
O/L	$3.62^{ab} \pm 0.03$	$3.68^{ab} \pm 0.36$	$3.75^{ab} \pm 0.24$	$4.03^{bc} \pm 0.49$	$4.10^{bc} \pm 0.40$	$6.01^{d} \pm 0.19$	$4.08^{bc} \pm 0.04$	$3.33^{a} \pm 0.21$	$4.42^{\circ} \pm 0.04$
I_2V	$100.6^{b} \pm 0.04$	99.0 ^b ± 2.18	100.1 ^b ± 1.22	99.4 ^b ± 1.95	98.9 ^b ± 1.82	$93.6^{a} \pm 0.56$	$98.9^{b} \pm 0.32$	100.6 ^b ± 2.25	$99.0^{b} \pm 0.10$
α-Toc	595 ^{de} ± 4.8	608 ^{de} ± 2.1	$629^{e} \pm 0.71$	$630^{e} \pm 0.71$	$559^{d} \pm 6.71$	$370^a \pm 0.71$	493° ± 4.24	$396^{ab} \pm 5.4$	445 ^{bc} ± 0.71
SQ	$52.9^{a} \pm 7.4$	$74.8^{b} \pm 6.90$	$94.9^{\circ} \pm 4.84$	45.1 ^a ± 1.66	$108.0^{\circ} \pm 10.6$	$50.6^{a} \pm 5.84$	$44.0^{a} \pm 5.01$	$52.4^{a} \pm 5.91$	$51.6^{a} \pm 1.80$
CY 2011									
C16:0	$6.22^{bc} \pm 0.10$	$6.12^{ab} \pm 0.01$	$6.13^{ab} \pm 0.01$	$6.28^{\circ} \pm 0.02$	$6.66^{e} \pm 0.05$	$6.62^{e} \pm 0.08$	$6.50^{d} \pm 0.01$	$7.23^{\rm f} \pm 0.01$	$6.05^{a} \pm 0.02$
C16:1	$0.49^{a} \pm 0.02$	$0.65^{bc} \pm 0.05$	$0.70^{cd} \pm 0.03$	$0.81^{d} \pm 0.11$	$0.64^{bc} \pm 0.01$	$0.50^{a} \pm 0.01$	$0.58^{ab} \pm 0.06$	$0.50^{a} \pm 0.02$	$0.51^{a} \pm 0.02$
C18:0	$1.75^{d} \pm 0.05$	1.91 ^d ± 0.06	$1.49^{c} \pm 0.16$	1.17 ^{ab} ± 0.11	$1.31^{abc} \pm 0.23$	$1.17^{ab} \pm 0.08$	$1.28^{abc} \pm 0.01$	$1.06^{a} \pm 0.03$	$1.34^{bc} \pm 0.10$
C18:1	$70.1^{d} \pm 0.19$	$65.2^a \pm 0.02$	$69.9^{d} \pm 0.18$	$68.8^{b} \pm 0.01$	$70.5^{e} \pm 0.21$	$72.7^{f} \pm 0.06$	$69.5^{\circ} \pm 0.01$	$68.8^{b} \pm 0.19$	$74.7^{g} \pm 0.20$
C18:2	$21.4^{d} \pm 0.01$	$26.1^{g} \pm 0.01$	$21.7^{d} \pm 0.01$	$22.9^{f} \pm 0.21$	$20.8^{\circ} \pm 0.07$	$19.0^{b} \pm 0.07$	$22.1^{e} \pm 0.07$	$22.4^{e} \pm 0.16$	$17.4^{a} \pm 0.04$
O/L	$3.27^{e} \pm 0.01$	$2.49^{a} \pm 0.001$	$3.22^{e} \pm 0.01$	$3.00^{b} \pm 0.03$	$3.38^{f} \pm 0.001$	$3.83^{g} \pm 0.07$	$3.14^{d} \pm 0.01$	$3.07^{c} \pm 0.03$	$4.29^{h} \pm 0.01$
I_2V	$102.4^{d} \pm 0.11$	$106.7^{g} \pm 0.08$	$103.0^{e} \pm 0.13$	104.3 ^f ± 0.25	$101.9^{\circ} \pm 0.32$	$100.3^{b} \pm 0.25$	$103.2^{e} \pm 0.07$	$103.0^{e} \pm 0.13$	$99.2^{a} \pm 0.13$
α-Toc	$606^{\circ} \pm 4.95$	$602^{d} \pm 6.36$	$662^{d} \pm 4.95$	$663^{d} \pm 7.07$	$574^{\circ} \pm 13.4$	$396^{a} \pm 9.90$	571° ± 36.0	437 ^b ± 27.5	$443^{b} \pm 2.12$
SQ	$50.9^{b} \pm 2.63$	93.6° ± 4.57	$79.4^{d} \pm 9.24$	$37.9^a \pm 3.71$	114.2 ^f ± 9.85	$67.9^{cd} \pm 4.84$	63.1° ± 1.67	$56.0^{bc} \pm 2.89$	$65.8^{\circ} \pm 5.25$

Abbreviations and units: CY, crop year; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; O/L, oleic/linoleic acid ratio; l_2V , iodine value; α -Toc, α -tocopherol content ($\mu g/g$ oil); SQ, squalene content ($\mu g/g$ oil). Fatty acids are expressed as % of total fatty acids. Mean values (±standard deviation) were the averages of three independent measurements. Values in each row with the same superscript letters are not significantly different (P > 0.05) among almond genotypes.

acids with kernel weight were not found to be significant ($P \le 0.05$) as it was reported by Askin et al. (2007) in some Turkish almond genetic resources.

The oleic/linoleic acid ratio (O/L) is considered an important criterion to evaluate AO quality; a large variability has been reported among almond cultivars for this chemical parameter (Kodad & Socias i Company, 2008; Sathe et al., 2008). The O/L from almond genotypes studied here varied from 3.33 to 4.42 in the first CY, and from 2.49 to 4.29 in the second CY (Table 5). Kodad & Socias i Company (2008) have found high stability of O/L in kernels of individual almond genotypes harvested during two consecutive CY from the same trial site. Over a total of 56 almond genotypes, only 10 showed significant variations (P < 0.01) in O/L between CY. In spite of the stability of environmental conditions in which the almond genotypes were cultivated, significant differences between CY were found in O/L, and consistently lower values were obtained in the second CY in all almond genotypes. Nevertheless, differences between genotypes were stronger than differences between CY, as indicated by the two-way ANOVA which showed the genetic factor as the main variability source (Table 4). On the other hand, no significant correlations were found among O/L and each of two environmental factors (mean temperatures and rainfall) evaluated. In each CY, fruits were collected at full maturity because of which this factor should not affect O/L. Perhaps, other agronomic factors, such as the crop load, could be the cause of the differences in O/L as it has been suggested for other oil-bearing fruits (Barone, Gullo, Zappia, & Inglese, 1994; Pierantozzi, Torres, Lavee, & Maestri, 2014). Although fruit production (estimated as kg fruits/ha) was higher in the second CY (data not shown), we have not available data on crop load (fruit number per tree) and its possible effect on FA composition.

As regards minor components present in almond oils, most of scientific studies have primarily focused on tocopherol content and its effect on oil preservation (Kodad, Socias i Company, Prats, & López-Ortiz, 2006; López-Ortiz, Prats-Moya, & Berenguer-Navarro, 2006; López-Ortiz et al., 2008; Zacheo, Cappello, Gallo, Santino, & Cappello, 2000). Almonds are considered one of the richest food sources of α -Toc (Chen, Lapsley, & Blumberg, 2006) – which is a well-known natural antioxidant – and its concentration was proposed as a selection criterion in almond breeding (Kodad

et al., 2006). Almond genotypes studied in the present work showed α -Toc contents ranging from 370 to 675 μ g/g oil (Table 5). Considering both 2010 and 2011 CY evaluated the highest α -Toc concentration was found in oils from Non Pareil and IXL cultivars (646 μ g/g in average). This represents an exceptionally high α -Toc level as compared with those from commercial almond cultivars (USDA, 2010; Yada et al., 2011, and references therein). The values recorded for other genotypes, such as Marcona and Guara (595-606 and 608-662 μg/g oil, respectively), also result higher than the values obtained from these cultivars grown in Spain (García-López et al., 1996). Kodad et al. (2006) have found higher tocopherol concentrations in kernels harvested in years with higher temperatures suggesting that this environmental factor could affect tocopherol synthesis during kernel development. In arid northwestern Argentina, the almond kernel development mostly coincides with spring and summer months which showed warmest mean temperatures as compared with those observed in the Mediterranean region. So, the variability in tocopherol content when comparing data from almond genotypes cultivated under different environments, such as those mentioned previously, may be justified on the basis of different climatic conditions, as a direct correlation between temperature and higher α -Toc levels, as it has been reported elsewhere (Kodad et al., 2006; López-Ortiz et al., 2008; Maranz & Wiesman, 2004). The genotype was the main variability source for α -Toc content (Table 4). For the majority of the almond genotypes studied a significant CY effect was also observed, with higher α -Toc concentrations in the second CY analysed.

From a technological standpoint, the Rancimat method is a useful analytical test in order to measure the oxidative stability of oils. OSI values ranged between 11.1-13.5 h (first CY), and 11.0-13.7 h (second CY) (Table 3). Minor but significant differences were found among genotypes; the highest OSI values were registered in IXL and AI cultivars. In general, OSI records concurred with those reported by Moayedi et al. (2011) (11.7–14 h) for almond oils oxidised under the same conditions used here. OSI values correlated positively with α -Toc content (r = 0.59, P = 0.0002).

The oxidative stability of vegetable oils is mainly related to their FA composition and also to the presence of minor endogenous antioxidant substances. The antioxidant activity of natural substances

present in whole oils may be evaluated by mean of their free radical scavenging capacity (RSC) using the free stable DPPH radical. Under the assay conditions employed in this study, IC₅₀ values of AO obtained from both 2010 and 2011 CY varied in the range 412-705 mg oil/mg DPPH (Table 3). Oils from Guara and AI genotypes showed the lowest IC50 records indicating that they had higher RSC. On the contrary, the highest IC50 values were found in oils from Javier INTA genotype. Considering the RSC data set from all almond genotypes studied here the IC₅₀ values obtained were rather lower than that reported by Arranz, Cert, Pérez-Jiménez, Cert, and Saura-Calixto (2008) for solvent-extracted almond oils. α -Toc content correlated negatively with IC₅₀ values (r = -0.49, P = 0.0025) thus suggesting an effective contribution to the RSC of almond oils evaluated. Nevertheless, almond oils with the highest α-Toc content (Non Pareil, IXL) had weaker RSC (higher IC₅₀) than other ones (Guara, AI) with relatively lower α -Toc amounts. This means that, besides tocopherols, other minor components may contribute to the total antioxidant capacity of almond oils. Polyphenols, especially ortho-diphenolic compounds, have showed to be strong active free radical scavengers (Frankel, 2005; Gordon, Paiva-Martins, & Almeida, 2001). Almond kernels are a good source of phenolic substances (Bolling et al., 2010). They are found at higher concentration in the seed coat, the skin that covers the pulp. Almond phenolics are mainly polyphenolics of the flavonoid type (Bolling et al., 2010; Wijeratne, Abou-Zaid, & Shahidi, 2006). Because of their low oil solubility, only minor amounts of polyphenolics could be present in the oils. Phenolic compounds were not found in almond oils analysed here. Attempts were made to detect them by using the Folin-Ciocalteu reagent (Torres et al., 2009) but no values were registered in any almond oil tested.

Besides tocopherols, squalene - a lipophilic triterpenoid hydrocarbon present in many fats and oils - has been also found to contribute to the oxidative stability of vegetable oils. There is a paucity of information regarding the concentration of squalene in nut oils. A work by Maguire, O'Sullivan, Galvin, O'Connor, and O'Brien (2004) informs squalene contents ranging from 9.4 to $186.4 \mu g/g$ oil from edible nuts including almonds (95 ug/g oil). Squalene was detected in all almond genotypes analysed here with levels ranging from 44.0 to 108.0 µg/g oil (first CY), and from 37.9 to 114.2 µg/g oil (second CY) (Table 5). Statistically significant differences in squalene concentrations were found among almond genotypes; genotype-related differences were stronger than those due to both the CY and the interaction genotype \times CY (Table 4). A number of reports indicate that squalene prevents lipid peroxidation acting mainly as peroxyl radical scavenger (Assunta Dessi et al., 2002; Kohno et al., 1995). A significant negative correlation was found between squalene content and IC_{50} values (r = -0.54, P = 0.0007). This suggests a role of squalene in radical-mediated oxidation reactions. However, squalene did not correlated significantly ($P \le 0.05$) with OSI values.

3.3. Principal component analysis

A multivariate analysis was carried out to select chemical and physical parameters as a mean of genotype differentiation. All parameters that presented significant differences among almond genotypes were included in a principal component analysis (PCA, Fig. 1). The resulting score plot allows to see the pattern of covariation among the almond genotypes and the variables analysed. The parameters that made the major contribution to the discrimination power were kernel weight, oil, oleic acid, α -tocopherol and squalene contents. The first principal component (PC1) explained almost 44% of the data variability and allowed the separation of Martinelli C, Emilito INTA and Javier INTA genotypes, which were associated mainly with oil and oleic acid contents, whereas Guara,

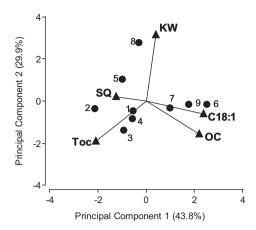


Fig. 1. Score plot of principal components 1 and 2 for chemical (OC, oil content; C18:1, oleic acid content; Toc, α-tocopherol content; SQ, squalene content), and physical (KW, kernel weight) data (♠) from almond genotypes (♠): 1, Marcona; 2, Guara; 3, Non Pareil; 4, IXL; 5, Al; 6, Martinelli C; 7, Emilito INTA; 8, Cáceres Clara Chica; 9, Javier INTA.

Non Pareil and IXL genotypes appeared related to α -Toc content. PC2 (27% of the data variability) stressed the separation of Cáceres CC genotype, which showed the highest KW, and Al genotype related to higher SQ content. These results reinforce the relevance of oil, oleic acid and α -Toc contents as discriminant parameters in order to differentiate almond genotypes.

4. Conclusions

A wide range of variability was found for kernel weight, oil content, fatty acid composition, α -Toc and squalene concentrations in almond genetic resources analysed during two crop years. The genotype was the main variability source for all parameters evaluated. Four genotypes native from Argentina were characterised for the first time. A ranking of cultivars as a function of kernel oil content showed the local genotypes Martinelli C and Emilito INTA at the top among almond genetic resources from different geographic origins. Finding connected with fatty acid composition also showed oils from Martinelli C and Javier INTA with the highest oleic acid contents. Almond oils contained significant amounts of α -Toc, but relatively low levels of squalene. The α -Toc content seems to be the most important contributor to both the radical scavenging activity and the oil oxidative stability.

This study adds new compositional data which may be of interest for almond breeding focused to improve kernel oil content and composition. Results also contribute to establish quality criteria in order to select almond genotypes for oil production. Furthermore, they stressed the importance of the local genotypes namely Martinelli C, Emilito INTA and Javier INTA as genetic sources to improve oil yield and fatty acid composition.

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