PAPER

EVALUATION OF THE CHEMICAL AND NUTRITIONAL PROPERTIES OF TUNISIAN ALMOND CULTIVARS

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ABSTRACT

The aim of this research was to evaluate for the first time protein, oil content, fatty acid profile and sugar composition for the main commercial almond cultivars in Tunisia in comparison to foreigners. Thus, fruits from twelve locals and five introduced cultivars from France, Italy and Spain were analyzed over two years. In fact, total oil content varied from 52.28% ('Blanco') to 60.95% ('Lsen Asfour') in the first year and from 47.75% ('Zahaaf') to 56.15% ('Mahsouna') in the second. However, the highest oleic acid content was noted in 'Francoli' (76.2%) for both years. It was followed by 'Sahnoun' (75.11%) firstly and 'Abiodh' (73.02%) secondly. Likewise, the highest linoleic acid content was observed in 'Porto' for both studied years (22.87% and 23.67%). The highest palmitic acid content was detected in 'Porto' (7.02%) and in 'Tuono' for the consecutive years. Sugars profile was quite distinctive among cultivars. The cultivar 'Porto' presented the highest total sugars (5.8 g/100g DW) and sucrose contents (4.96 g/100g DW). Nevertheless, protein content doesn't show extreme values. For both years, the local cultivar 'Zahaaf' presented the highest protein content (27 g/100g DW) while introduced French cultivar 'Fournat de Breznaud' presented the lowest protein content (17 g/100g DW). All the analyzed components were different significantly according to cultivar and year effects. Results evidenced that the local Tunisian cultivars are highly rich in oil and fatty acids particularly oleic and linoleic acids, confirm the almond kernel as a high nutritional dietetic source and underline the high adaptability of some introduction.

Keywords: fatty acid composition, local cultivars, oil quality, *Prunus dulcis* L., sugar content

1. INTRODUCTION

The cultivated almond [*Prunus dulcis* (Miller) Webb] is a tree species whose domestication and spread has closely paralleled the rise of Eurasian civilizations. This tree-crop species is mainly planted for its edible seeds (kernels). Today, almonds are cultivated in more than 50 countries (http://faostat.fao.org), with approximately 95% produced in California, Australia and the Mediterranean Basin. In Tunisia, almond cultivation is present around the country mainly under rainfed conditions (GOUTA *et al.,* 2019). Moreover, the almond kernel represents the main nutrient source for many rural populations in the central and southern parts of the country. The high nutritive value of the almond kernel comes mainly from its high lipid content. In fact, it contains 52% of lipids, 20% of proteins and 20% of carbohydrates including 5% of water and 3% of soluble sugars (KADER, 1996). Almond quality was formerly related to the kernel flavor in addition to its physical parameters such as kernel size, percentage of double kernel and kernel rate without any attention to its nutraceutical composition (ROMOJARO *et al.,* 1988; NANOS *et al.,* 2002). At this moment, however, the nutritive value of almond kernel related to lipid, sugar, protein and mineral richness are being evaluated as main component of the almond kernel quality.
Different studies have reported that almonds consumption can significantly lower total and low-density-lipoprotein (LDL) cholesterol in plasma, reduce risk for heart disease and prevent several forms of cancer and inflammation (JENKINS *et al.,* 2008). The beneficial health effect of almond was attributed to its high content of mono and poly-unsaturated fatty acids (ROS and MATAIX, 2006). Moreover, the high (oleic acid/linoleic acid) ratio is used in determining the kernel quality due to its preventive effect on lipid oxidation and oil stability (KODAD *et al.,* 2010). In addition, negative cholesterol effects can be treated by an equilibrate lipid diet based on nut consumption including almonds (MUSA-VELASCO *et al.,* 2016). Furthermore, almond oil contains antioxidants and fat-soluble bioactive compounds that make it oil with interesting nutritional and cosmetic properties (RONCERO *et al.,* 2016). In this context several studies have been published about total oil and fatty acid profile of some almond cultivars (ÖZCAN et al., 2010; YILDIRIM *et al.,* 2016; ČOLIĆ *et al.,* 2017; SOCIAS I COMPANY *et al.,* 2018). In addition, sugars composition in the almond kernel has been reported in many studies (KAZANTZIS *et al.,* 2003; BALTA *et al.,* 2009). However, as far as we know, very few researches were carried out to characterize the nutraceutical values together with these chemical compositions (SOCIAS I COMPANY *et al.,* 2010; KODAD, 2017). This information is null regarding the rich almond germplasm from Tunisia considered as an almond diversification center.
The objective of this study was to determine for the first time the chemical and nutritional composition (including total oil, protein contents, fatty acid and sugar composition) of most important Tunisian almond cultivars. Moreover, the interaction of genotype x environment would be deeply discussed. Findings of the present work will be important for selecting cultivars with more stable macronutrients composition from year to year and consequently less subject to climate changes.

2. MATERIALS AND METHODS

2.1. Plant material

Plant material assayed included twelve Tunisian almond cultivars ('Dillou', 'Khoukhi', 'Blanco', 'Abiodh', 'Lsen Asfour', 'Achaak', 'Zahaaf', 'Fekhfekh', 'Ksontini', 'Sahnoun',

'Porto', and 'Mahsouna') and five almond cultivars originating from Italy ('Mazetto' and 'Supernova'), Spain ('Francoli'), France ('Lauranne' and 'Fournat de Breznaud') assayed as reference. The local cultivars used in this work (Fig. 1) are early flowering, auto-incompatible and their pomological and agronomical characteristics were previously well described (GOUTA *et al.*, 2011; GOUTA *et al.*, 2019). All studied almonds were collected from the national collection in Sidi Bouzid in central-western part of Tunisia during two consecutive years 2009 and 2010.

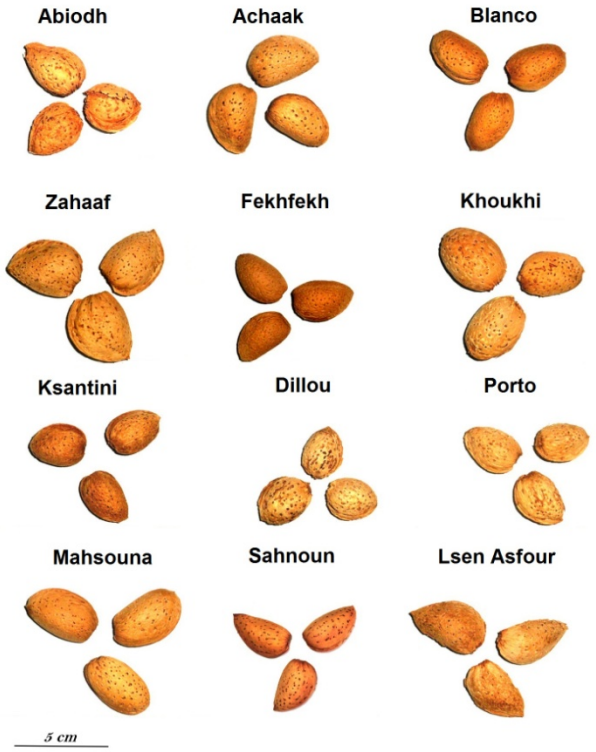

Figure 1. Pomological characteristics among the native Tunisian almond cultivars.

2.2. Oil and fatty acid determination

Kernels were preliminary blanched for 3 min in boiling water eliminating seed coat. The kernels were dried at 25°C until constant weight and then ground. Oil was extracted using about 5g of ground almond in a Soxtec Avanti 2055 fat extractor (Foss Tecator, Höganäs, Sweden) for 2 h using 70 ml of petroleum ether as solvent and keeping temperature at 135°C. To remove any residual ether, the extract was subject successively to vacuum evaporation for 15 min in a vacuum desiccator. Ten microliters of Butylated hydroxyl toluene methanol solution (BHT) as an antioxidant agent was added to each oil sample which was kept in an amber vial at -20°C until analysis. The percentage of the different fatty acids in oil samples was determined by capillarity gas chromatography of the fatty acid methyl esters (FAMEs). Methyl esters of the corresponding fatty acids were obtained by trans-esterification with KOH of each almond oil sample according to the official method UNE-EN (ISAO 5509, 2000). They were separated using a flame ionizing detector

(FID) gas chromatograph HP-6890 equipped with HP-Innowax column (30 m × 0.25 mm i.d.) and 0.25 μm film thinness (Agilent Technologies, Waldron, Germany). The FAMES identification was realized by comparison with relative chromatographic retention times of standard methyl esters mixture (Sigma-Aldrich, Madrid, Spain).

2.3. Sugar determination

Free sugar profiles were determined by a high performance liquid chromatography (HPLC, Agilent 1100, Germany) during the two consecutive years 2009 and 2010. In first step kernels samples were dried in an oven at 25°C until weight stabilized, ground in a mortar and then defatted using a soxhlet and ether petroleum as a solvent. Once defatted, a sample of 0.7 to 1.3 g of the remaining powder was moved to a falcon tube and mixed with of 9 ml MilliQ water. For protein denaturation, 0.5 ml of Carrez I (potassium ferrocyanide 15% w/v) and 0.5 ml of Carrez II (zinc acetate 30% w/v) solutions were added and kept under agitation in an agitator (Reax, Madrid, Spain) for 10 min. The resulting suspension was centrifuged at 8000 rpm for 20 min. The supernatant was recuperated and passed throw a nylon filter 0.45 μm before injection in a HPLC apparatus. A volume of 20 μl of the filtrate was injected in an interchange cationic column (Pb) CHO-682 (Transgenomic, Madrid, Spain). Sugar detection was performed according to the detection time of reference samples (Sigma, Madrid, Spain) of raffinose, sucrose, glucose and fructose.

2.4. Total protein determination

Protein fraction was obtained by the following formula:

$$\text{Protein percentage} = \text{total nitrogen percentage} \times Kc$$

with Kc presenting a conversion factor equal to 6.25 for almond. The total nitrogen content was obtained by the Dumas method (DUMAS, 1826). Almond kernels for each genotype were defatted as already mentioned (using soxhlet and ether petroleum solvent) and then analyzed by a LECO FP-528 Protein/Nitrogen Analyzer (LECO cooperation, Saint Joseph, MI, USA). A sample of 0.2 g of the resulting powder was incinerated at 850°C and the gases generated were passed through hot copper to remove oxygen. Nitrogen molecules with helium were measured in a cell differential thermo-conductivity. Then, data were read and interpreted with CPU-CAR-02 software. Results were expressed as percentage of nitrogen by kernel powder weight.

2.5. Statistical analysis

Three replicates of 20 kernels from each genotype were evaluated. The significance of cultivar, year and cultivar × year interaction effects for all studied components were tested on the 17 cultivars by ANOVA using SPSS 20.0. Differences between means were evaluated by using Duncan multiple range test. Correlations between traits were calculated from raw data of the two years using Pearson correlation coefficient. Trait mean values were used to perform a Principal Component Analysis (PCA).

3. RESULTS

3.1. Effect of the year and its interaction with the cultivar

The analysis of variance showed significant effect of cultivar and year for the fatty acids and sugars compositions and oil and protein contents in the seventeen almond cultivars assayed during two consecutive years. In addition, the interaction cultivar × year exhibited considerable variation for all analyzed parameters (Table 1). Besides the significant effect of the cultivar, a clear and significant environmental effect was noted in the oil content for all studied cultivars due to the specific climatic conditions of years tested. Some almond cultivars have shown high year to year stability in their fatty acids content compared to other cultivars. These results indicate that the year effect on the fatty acids composition in almond mainly depends on genotype. Stable values for some fatty acids were observed in cultivars such as 'Dillou', 'Sahnoun', 'Mazetto' and 'Mahsouna' for arachidic acid; 'Dillou', 'Khoukhi', 'Lsen Asfour', 'Sahnoun', 'Super Nova', 'Lauranne' and 'Mahsouna' for linolenic acid; 'Porto', 'Abiodh' and 'Mahsouna' for palmitic acid; 'Lsen Asfour' and 'Mahsouna' for palmitoleic acid; 'Lauranne' and 'Mahsouna' for stearic acid (Table 2).
The year effect was significant for different sugar amounts except raffinose percentage (Table 1). Moreover, studied cultivars show stable and similar year to year sugar percentage excepting the glucose percentage, confirming that the year to year stability depends on the specific characteristics of the genotype.

3.2. Oil content

The mean value of oil content over the 2 years varied from 47.75% for 'Zahaaf' to 60.95% for 'Lsen Asfour' (Table 2). In 2009, the mean value of total lipid was 56.23%, ranged for the local cultivars from 52.28% for Blanco to 60.95% for 'Lsen Asfour' and for the foreign cultivars from 53.36% for 'Francoli' to 55.93 % for 'Breznaud'. In 2010, the mean value of total oil was 51.39%, ranged for the local cultivars from 47.75% for 'Zahaaf' to 56.15% for 'Mahsouna' and for the foreign cultivars total lipid content ranged from 48.37% for 'Francoli' to 54.45% for 'Lauranne'. The values of total lipid content were found to be low for the European cultivars compared to the values registered in the Tunisian local cultivars. In fact, it was found in the range of 47-56% for 'Francoli' (Spain), 'Super Nova' and 'Mazetto' (Italy) and 'Laurane' and 'Fournat de Breznaud' (France).

3.3. Protein content

The mean value of the protein content was for almost studied cultivars higher in 2010 than in 2009, contrarily to the oil content which was higher in 2009 (Table 2). For the year 2009, the lowest contents were showed by the local cultivars 'Lsen Asfour' (14.49%) and 'Mahsouna' (17.34%) and the French cultivar 'Fournat de Breznaud' (17.84%) while the highest values ranged between 23 and 21.2% for 'Francoli', 'Zahaaf', 'Ksontini', 'Super nova' and 'Mazetto', respectively. In 2010 these same cultivars showed the highest protein content with a greater range of variation (27.15-23.35%). Likewise 'Mahsouna', 'Fournat de Breznaud' and 'Lsen Asfour' showed the lowest protein content (17.14-18.11%) the second year of study. However, the cultivars 'Dillou', 'Mahsouna' and 'Fournat de Breznaud' showed stable mean value of the protein content over the two year.

Table 1. Analysis of variance of fatty acid (Palmitic, Palmitoleic, Stearic, Oleic and Linoleic) content, total lipid content, sugar composition (Raffinose, Sucrose, Glucose, Fructose), total sugar content and protein content in the 17 assayed almond cultivars.

Source of variation	Df[1]	Mean squares											
		Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Total lipid	Raffinose	Sucrose	Glucose	Fructose	Total Sugar	Protein
Genotype (G)	16	**0.636**	**0.027**	**1.722**	**32.18**	**28.40**	**25.25**	**0.541**	**2.908**	**0.028**	**0.021**	**4.33**	**37.55**
Year (Y)	1	**1.547**	**0.048**	**1.498**	**189.36**	**172.58**	**596.627**	0.065	**15.514**	**0.036**	**0.003**	**19.651**	**50.28**
G × Y	16	**0.141**	**0.006**	**0.238**	**8.055**	**5.56**	**11.365**	**0.054**	**1.144**	**0.005**	**0.003**	**1.511**	**8.723**
Error	68	0.000	0.000	0.013	0.587	0.788	0.328	0.012	0.042	0.000	0.000	0.065	0.159

Mean squares in bold case present a level of significance of $P<0.001$.
[1]Df: Degree of freedom.

Table 2. Fatty acid (palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, α-Linolenic), protein content and total lipid for each almond cultivar assayed during two consecutive years (2009 and 2010).

	Miristic		Palmitic		Palmitoleic		Margaric		Margaroleic		Stearic		Oleic	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
	Tunisian almond cultivars													
Dillou	0,04^{a}	0,05^{b}	6,19^{a}	6,46^{b}	0,52^{a}	0,56^{b}	0,04^{a}	0,05^{b}	0,09^{a}	0,10^{b}	1,36^{a}	1,53^{b}	72,10^{a}	69,49^{b}
Khoukhi	0,05^{a}	0,06^{b}	6,32^{a}	7,23^{b}	0,51^{a}	0,48^{b}	0,04^{a}	0,06^{b}	0,09^{a}	0,10^{b}	1,31^{a}	1,70^{b}	73,59^{a}	66,70^{b}
Blanco	0,04^{a}	0,04^{b}	6,14^{a}	6,29^{b}	0,41^{a}	0,43^{b}	0,04^{a}	0,05^{b}	0,09^{a}	0,09^{b}	1,40^{a}	1,35^{b}	70,65^{a}	70,42^{b}
Abiodh	0,03^{a}	0,06^{b}	6,88^{a}	6,85^{a}	0,59^{a}	0,54^{b}	0,05^{a}	0,02^{b}	0,09^{a}	0,05^{b}	1,75^{a}	1,62^{b}	73,56^{a}	73,02^{b}
Lsen Asfour	0,03^{a}	0,04^{b}	6,56^{a}	6,85^{b}	0,54^{a}	0,52^{a}	0,04^{a}	0,05^{b}	0,07^{a}	0,09^{b}	2,84^{a}	1,82^{b}	71,22^{a}	67,62^{b}
Achaak	0,02^{a}	0,05^{b}	6,62^{a}	7,25^{b}	0,53^{a}	0,40^{b}	0,05^{a}	0,03^{b}	0,08^{a}	0,06^{b}	2,69^{a}	2,40^{b}	73,06^{a}	67,95^{b}
Zahaaf	0,04^{a}	0,11^{b}	6,61^{a}	6,44^{b}	0,55^{a}	0,43^{b}	0,04^{a}	0,02^{b}	0,09^{a}	0,05^{b}	1,46^{a}	1,72^{b}	75,06^{a}	71,91^{b}
Fekhfekh	0,01^{a}	0,05^{b}	5,86^{a}	6,12^{b}	0,35^{a}	0,31^{b}	0,05^{a}	0,04^{b}	0,06^{a}	0,06^{a}	2,82^{a}	2,53^{b}	72,95^{a}	69,65^{b}
Ksontini	0,03^{a}	0,05^{b}	7,01^{a}	6,91^{b}	0,34^{a}	0,39^{b}	0,06^{a}	0,02^{b}	0,08^{a}	0,07^{b}	3,75^{a}	2,66^{b}	67,50^{a}	67,90^{b}
Sahnoun	0,03^{a}	0,03^{a}	6,28^{a}	6,42^{b}	0,53^{a}	0,46^{b}	0,05^{a}	0,05^{b}	0,09^{a}	0,10^{b}	2,00^{a}	1,92^{b}	75,11^{a}	71,05^{b}
Porto	0,03^{a}	0,03^{b}	7,02^{a}	7,04^{a}	0,52^{a}	0,43^{b}	0,05^{a}	0,06^{b}	0,08^{a}	0,10^{b}	2,38^{a}	2,13^{b}	66,70^{a}	66,28^{b}
Mahsouna	0,02^{a}	0,02^{a}	6,77^{a}	6,85^{a}	0,50^{a}	0,43^{a}	0,04^{a}	0,05^{b}	0,07^{a}	0,09^{b}	2,58^{a}	2,43^{a}	73,14^{a}	70,38^{a}

International reference almond cultivars														
Mazetto	0,02[a]	0,03[b]	6,77[a]	7,53[b]	0,47[a]	0,30[b]	0,05[a]	0,07[b]	0,08[a]	0,09[b]	2,63[a]	2,52[b]	72,56[a]	65,29[b]
Francoli	0,02[a]	0,04[b]	6,25[a]	6,40[b]	0,50[a]	0,49[b]	0,05[a]	0,06[b]	0,08[a]	0,10[b]	3,05[a]	2,50[b]	76,21[a]	76,15[b]
SuperNova	0,02[a]	0,02[b]	6,61[a]	7,24[b]	0,46[a]	0,48[b]	0,05[a]	0,06[b]	0,08[a]	0,10[b]	2,72[a]	2,17[b]	73,15[a]	69,89[b]
Lauranne	0,02[a]	0,02[b]	6,63[a]	6,79[b]	0,62[a]	0,55[b]	0,05[a]	0,05[b]	0,10[a]	0,10[a]	1,79[a]	1,79[a]	73,77[a]	70,67[b]
F. Breznaud	0,03[a]	0,03[b]	6,76[a]	6,84[b]	0,52[a]	0,51[b]	0,05[a]	0,05[b]	0,08[a]	0,09[b]	2,32[a]	1,94[b]	69,94[a]	69,56[b]
Min	0,01	0,02	5,86	6,12	0,34	0,30	0,04	0,02	0,06	0,05	1,31	1,35	66,70	65,29
Max	0,05	0,11	7,02	7,53	0,62	0,56	0,06	0,07	0,10	0,10	3,75	2,66	76,21	76,15
Mean	0,03	0,04	6,55	6,79	0,50	0,45	0,05	0,05	0,08	0,08	2,29	2,04	72,37	69,64
SD	0,01	0,02	0,33	0,39	0,07	0,08	0,01	0,01	0,01	0,02	0,70	0,40	2,53	2,65
CV	31,64	48,45	4,97	5,76	14,65	16,82	11,36	31,47	12,09	20,70	30,58	19,81	3,50	3,80

Table 2. Continues.

	Linoleic		**Arachidic**		**α-Linolenic**		**Gadoleic**		**Protein**		**Total Lipid**		**O/L ratio**		**USFA**	
	2009	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
Tunisian almond cultivars																
Dillou	19,40[a]	21,37[b]	0,05[a]	0,03[a]	0,02[a]	0,02[a]	0,06[a]	0,07[b]	19,75[a]	19,49[a]	55,22[a]	52,81[b]	3,72[a]	3,25[b]	91,50[a]	90,86[b]
Khoukhi	17,86[a]	23,27[a]	0,05[a]	0,06[b]	0,01[a]	0,01[b]	0,06[a]	0,06[a]	20,48[a]	21,65[b]	55,80[a]	50,60[b]	4,12[a]	2,87[b]	91,45[a]	89,96[b]
Blanco	21,01[a]	20,84[b]	0,06[a]	0,06[b]	0,02[a]	0,02[b]	0,06[a]	0,07[b]	19,67[a]	18,39[b]	52,28[a]	53,83[b]	3,36[a]	3,38[b]	91,65[a]	91,26[b]
Abiodh	16,85[a]	17,54[b]	0,07[a]	0,07[b]	0,01[a]	0,07[b]	0,06[a]	0,03[b]	19,94[a]	18,48[b]	58,66[a]	48,61[b]	4,36[a]	4,16[b]	90,41[a]	90,55[b]
Lsen Asfour	18,40[a]	22,27[b]	0,09[a]	0,06[b]	0,02[a]	0,02[a]	0,07[a]	0,07[a]	14,49[a]	18,12[b]	60,95[a]	52,51[b]	3,90[a]	3,04[b]	89,62[a]	89,89[a]
Achaak	16,60[a]	21,51[b]	0,09[a]	0,09[b]	0,02[a]	0,06[b]	0,07[a]	0,03[b]	17,91[a]	18,37[b]	58,26[a]	55,53[b]	4,40[a]	3,16[b]	89,66[a]	89,46[b]
Zahaaf	15,91[a]	19,03[b]	0,06[a]	0,07[b]	0,02[a]	0,08[b]	0,06[a]	0,00[b]	22,89[a]	24,83[b]	54,01[a]	47,75[b]	4,712[a]	3,78[b]	90,97[a]	90,94[a]
Fekhfekh	17,60[a]	20,53[b]	0,10[a]	0,09[b]	0,02[a]	0,07[b]	0,07[a]	0,00[b]	17,74[a]	22,84[b]	57,76[a]	52,57[b]	4,14[a]	3,39[b]	90,55[a]	90,17[b]
Ksontini	20,89[a]	21,68[b]	0,12[a]	0,10[b]	0,02[a]	0,06[a]	0,08[a]	0,04[b]	21,95[a]	24,28[b]	54,33[a]	50,77[b]	3,23[a]	3,13[b]	88,38[a]	89,57[b]
Sahnoun	15,67[a]	19,34[b]	0,08[a]	0,08[a]	0,02[a]	0,02[a]	0,06[a]	0,07[a]	18,84[a]	20,17[b]	56,69[a]	49,96[b]	4,80[a]	3,67[b]	90,78[a]	90,39[b]
Porto	22,87[a]	23,67[a]	0,08[a]	0,08[b]	0,02[a]	0,00[b]	0,07[a]	0,07[a]	19,92[a]	22,71[b]	56,99[a]	49,44[b]	2,92[a]	2,80[b]	89,57[a]	89,95[b]
Mahsouna	16,54[a]	18,93[a]	0,10[a]	0,10[a]	0,02[a]	0,02[a]	0,08[a]	0,07[a]	17,35[a]	17,15[a]	60,37[a]	56,15[b]	4,70[a]	3,72[a]	89,67[a]	89,31[a]

International reference almond cultivars																
Mazetto	17,06[a]	23,67[b]	0,12[a]	0,12[a]	0,02[a]	0,03[b]	0,07[a]	0,06[a]	21,27[a]	27,15[b]	54,30[a]	48,82[b]	4,25[a]	2,76[b]	89,62[a]	88,96[b]
Francoli	13,45[a]	13,92[b]	0,11[a]	0,10[b]	0,02[a]	0,03[b]	0,08[a]	0,07[b]	23,02[a]	23,35[b]	53,36[a]	48,37[b]	5,66[a]	5,47[b]	89,66[a]	90,07[b]
SuperNova	16,58[a]	19,69[b]	0,11[a]	0,11[b]	0,03[a]	0,03[a]	0,08[a]	0,08[a]	21,63[a]	26,33[b]	55,48[a]	50,60[b]	4,41[a]	3,55[b]	89,73[a]	89,58[b]
Lauranne	16,79[a]	19,75[b]	0,08[a]	0,08[b]	0,03[a]	0,01[a]	0,06[a]	0,08[b]	20,55[a]	18,02[b]	55,52[a]	54,45[b]	4,39[a]	3,58[b]	90,55[a]	90,42[b]
F. Breznaud	20,08[a]	20,77[b]	0,08[a]	0,07[b]	0,02[a]	0,00[b]	0,06[a]	0,07[a]	17,84[a]	17,79[a]	55,93[a]	50,91[b]	3,43[a]	3,35[b]	90,02[a]	90,33[b]
Min	13,45	13,92	0,05	0,03	0,01	0,00	0,06	0,00	14,49	17,15	52,28	47,75	2,92	2,76	88,38	88,96
Max	22,87	23,67	0,12	0,12	0,03	0,08	0,08	0,08	23,02	27,15	60,95	56,15	5,67	5,47	91,65	91,26
Mean	17,86	20,46	0,08	0,08	0,02	0,03	0,07	0,06	19,72	21,12	56,23	51,39	4,15	3,47	90,22	90,10
SD	2,34	2,42	0,02	0,02	0,00	0,02	0,01	0,03	2,20	3,25	2,39	2,55	0,67	0,64	0,87	0,61
CV	13,11	11,81	28,85	27,81	22,02	79,53	12,56	46,97	11,18	15,39	4,24	4,97	16,24	18,33	0,96	0,68

Mean values of each parameter in each genotype in different years followed by a different lower-case letter are significantly different at P=0.01 by the Duncan test.

3.4. Fatty acid composition

The fatty acid profile of almond oil consisting of mystiric (C14:0), palmitic (C16:0), palmitoleic (C16:1), margaric (C17:0), margaroleic (C17:1 n-8), stearic (C18:0), oleic (C18:1 n-9), linoleic (C18:2 n-6), α-linolenic (C18:3 n-3), arachidic (C20:0), and gadoleic (C20:1 n-11) (Table 2). Fatty acid composition of studied almond kernel oil has shown three predominant fatty acids regardless of cultivar or year. The oleic acid is the main monounsaturated fatty acid, followed by linoleic acid the main polyunsaturated fatty acid and the palmitic acid the main saturated fatty acid. The ranges of variation of these three fatty acids were 65-76%, 13-23% and 5.8-7.5%, respectively. The contents of stearic and palmitoleic acids were <4%, and ranged between 1.3-3.7% and 0.3-0.6%, respectively.
In both years, the oleic, linoleic and palmitic acids varied among cultivars. In 2009, the highest values of oleic acid content were determined in 'Francoli' (76.21%), followed by cultivars 'Sahnoun' (75.11%) and 'Zahaaf' (75.06%). However, the lowest values were found in 'Porto' (66.70%) and 'Breznaud' (69.94%). For linoleic acid, 'Porto' represented the highest value (22.87%) and 'Francoli', 'Sahnoun' and 'Zahaaf' showed the lowest value (13.45-15.91%). For Palmitic acid, 'Porto' and 'Ksontini' demonstrated the highest palmitic content (7%) while 'Fekhfekh' showed the lowest value (5.86%). In 2010, the cultivar 'Francoli' showed the highest value of oleic acid content (76.15%), followed by 'Abiodh', 'Zahaaf' and 'Sahnoun' while 'Mazetto' recorded the lowest value (65.29%). For linoleic acid, the highest value was obtained for 'Porto' (23.67%) whereas the lowest value was obtained for 'Francoli' (13.92%). 'Mazetto' represented the highest palmitic acid content (7.53%) and 'Fekhfekh' represented the lowest palmitic content (6.12%). Thus the varieties 'Sahnoun', 'Zahaaf' and 'Francoli', are superior in marketing quality with high oleic acid content and low linleic and palmitic contents.
The oleic/linoleic (O/L) ratio showed a large variability among cultivars because of the high variability in oleic and linoleic acids contents. This ratio was generally higher in 2009 than in 2010 (Table 2). The cultivars 'Francoli' showed the higher (oleic/linoleic) ratio (5.6-5.4) during the two consecutive years followed by the cultivars 'Sahnoun', 'Zahaaf' and 'Mahsouna' (Table 2). Owing to their highest (O/L) ratio, these cultivars represented the greatest stability of almond kernels and oil. However, 'Porto' cultivar showed the lowest (oleic/linoleic) ratio (2.8-2.9) followed by the varieties 'Ksontini' and 'Mazetto' in 2009 and 2010, respectively. For the local cultivars it was noted that oleic and linoleic acids together accounted from 88.38 to 91.65% of the total extracted almond oil.

3.5. Sugar composition

Total sugar content varied from 2.3 to 6.5 g 100 g^{-1} of dry weight (DW), with an average content of 3.98 g 100 g^{-1}DW (Table 3). Sucrose, raffinose, glucose and fructose contents were analyzed separately. Sucrose was the sugar present at the highest concentration in all studied cultivars (1.9 to 5.8 g 100 g^{-1}DW) followed by raffinose (0.04 to 1.36 g 100 g^{-1}DW), glucose (0.019 to 0.43 g 100 g^{-1}DW) and fructose (0.007 to 0.322 g 100 g^{-1}DW).
The year effect was significant on the total sugar content (Table 1). The mean value of total sugar was higher in 2009 than in 2010 for all studied cultivars excepting 'Blanco' (Table 3).

Table 3. Sugar composition (Raffinose, sucrose, glucose, fructose), total sugar content and percentage of each type of sugars for each almond cultivar in two consecutive years (2009 and 2010).

	Raffinose		Sucrose		Glucose		Fructose		Total sugar		%raffinose		%sucrose		%glucose		%fructose	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Tunisian almond cultivars																		
Dillou	1,049^{a}	0,958^{b}	3,592^{a}	3,558^{a}	0,061^{a}	0,062^{a}	0,010^{a}	0,010^{a}	4,712^{a}	4,589^{b}	0,223^{a}	0,209^{a}	0,762^{a}	0,775^{a}	0,013^{a}	0,014^{a}	0,002^{a}	0,002^{b}
Khoukhi	0,720^{a}	0,724^{a}	3,447^{a}	3,456^{a}	0,120^{a}	0,114^{a}	0,010^{a}	0,010^{b}	4,297^{a}	4,304^{a}	0,168^{a}	0,168^{a}	0,802^{a}	0,803^{a}	0,028^{a}	0,026^{a}	0,002^{a}	0,002^{a}
Blanco	1,046^{a}	1,365^{a}	3,131^{a}	4,139^{b}	0,067^{a}	0,053^{a}	0,010^{a}	0,010^{b}	4,254^{a}	5,567^{a}	0,246^{a}	0,245^{a}	0,736^{a}	0,743^{a}	0,016^{a}	0,010^{b}	0,002^{a}	0,002^{b}
Abiodh	0,612^{a}	0,301^{b}	5,807^{a}	2,741^{b}	0,076^{a}	0,069^{a}	0,010^{a}	0,040^{a}	6,505^{a}	3,151^{b}	0,094^{a}	0,095^{a}	0,893^{a}	0,870^{a}	0,012^{a}	0,022^{b}	0,002^{a}	0,013^{a}
Lsen Asfour	0,151^{a}	0,369^{b}	3,737^{a}	3,265^{b}	0,050^{a}	0,041^{a}	0,010^{a}	0,010^{b}	3,949^{a}	3,684^{b}	0,038^{a}	0,100^{b}	0,946^{a}	0,886^{b}	0,013^{a}	0,011^{a}	0,003^{a}	0,003^{b}
Achaak	0,516^{a}	0,390^{b}	3,256^{a}	2,722^{b}	0,058^{a}	0,031^{b}	0,007^{a}	0,010^{b}	3,837^{a}	3,154^{b}	0,134^{a}	0,124^{b}	0,849^{a}	0,863^{b}	0,015^{a}	0,010^{b}	0,002^{a}	0,003^{b}
Zahaaf	0,318^{a}	0,145^{b}	2,846^{a}	2,202^{a}	0,034^{a}	0,028^{a}	0,010^{a}	0,010^{b}	3,207^{a}	2,385^{a}	0,099^{a}	0,061^{b}	0,887^{a}	0,923^{b}	0,010^{a}	0,012^{a}	0,003^{a}	0,004^{a}
Fekhfekh	0,369^{a}	0,182^{b}	3,977^{a}	2,760^{b}	0,092^{a}	0,026^{b}	0,010^{a}	0,010^{b}	4,449^{a}	2,978^{b}	0,083^{a}	0,061^{b}	0,894^{a}	0,927^{b}	0,021^{a}	0,009^{b}	0,002^{a}	0,003^{b}
Ksontini	0,134^{a}	0,472^{b}	3,569^{a}	2,576^{b}	0,042^{a}	0,046^{a}	0,010^{a}	0,010^{b}	3,755^{a}	3,104^{b}	0,036^{a}	0,152^{b}	0,950^{a}	0,830^{b}	0,011^{a}	0,015^{b}	0,003^{a}	0,003^{b}
Sahnoun	0,279^{a}	0,227^{a}	4,657^{a}	2,790^{b}	0,063^{a}	0,023^{b}	0,010^{a}	0,040^{b}	5,009^{a}	3,080^{b}	0,056^{a}	0,074^{a}	0,930^{a}	0,906^{b}	0,013^{a}	0,007^{b}	0,002^{a}	0,013^{b}
Porto	0,714^{a}	0,789^{a}	5,074^{a}	4,845^{a}	0,060^{a}	0,063^{a}	0,010^{a}	0,050^{a}	5,858^{a}	5,747^{b}	0,122^{a}	0,137^{b}	0,866^{a}	0,843^{b}	0,010^{a}	0,011^{a}	0,002^{a}	0,009^{b}
Mahsouna	0,450^{a}	0,318^{a}	2,411^{a}	1,976^{b}	0,065^{a}	0,033^{b}	0,010^{a}	0,010^{b}	2,936^{a}	2,338^{b}	0,153^{a}	0,136^{a}	0,821^{a}	0,845^{a}	0,022^{a}	0,014^{b}	0,003^{a}	0,004^{b}
International reference almond cultivars																		
Mazetto	0,413^{a}	0,225^{b}	3,286^{a}	2,428^{b}	0,027^{a}	0,019^{a}	0,026^{a}	0,024^{b}	3,752^{a}	2,695^{b}	0,110^{a}	0,083^{b}	0,876^{a}	0,901^{b}	0,007^{a}	0,007^{a}	0,007^{a}	0,009^{b}
Francoli	0,416^{a}	0,214^{b}	3,990^{a}	2,536^{b}	0,141^{a}	0,032^{b}	0,116^{a}	0,036^{b}	4,663^{a}	2,818^{b}	0,089^{a}	0,076^{b}	0,856^{a}	0,900^{b}	0,030^{a}	0,011^{b}	0,025^{a}	0,013^{b}
Super Nova	0,326^{a}	0,152^{b}	3,238^{a}	2,611^{b}	0,056^{a}	0,052^{a}	0,038^{a}	0,038^{a}	3,658^{a}	2,854^{b}	0,089^{a}	0,053^{b}	0,885^{a}	0,915^{b}	0,015^{a}	0,018^{a}	0,010^{a}	0,013^{b}
Lauranne	0,234^{a}	0,042^{b}	3,903^{a}	3,128^{b}	0,434^{a}	0,212^{b}	0,322^{a}	0,174^{b}	4,893^{a}	3,556^{b}	0,048^{a}	0,012^{b}	0,798^{a}	0,880^{b}	0,089^{a}	0,060^{b}	0,066^{a}	0,049^{b}
F. Breznaud	0,230^{a}	0,244^{a}	5,083^{a}	4,025^{b}	0,161^{a}	0,077^{b}	0,104^{a}	0,067^{a}	5,578^{a}	4,413^{b}	0,041^{a}	0,055^{b}	0,911^{a}	0,912^{a}	0,029^{a}	0,017^{b}	0,019^{a}	0,015^{a}
Min	0,134	0,042	2,411	1,976	0,027	0,019	0,007	0,010	2,936	2,338	0,036	0,012	0,736	0,743	0,007	0,007	0,002	0,002
Max	1,049	1,365	5,807	4,845	0,434	0,212	0,322	0,174	6,505	5,747	0,246	0,245	0,950	0,927	0,089	0,060	0,066	0,049
Mean	0,469	0,419	3,824	3,045	0,095	0,058	0,042	0,033	4,430	3,554	0,108	0,108	0,863	0,866	0,021	0,016	0,009	0,009

Mean values of each parameter in each genotype in different years followed by a different lower-case letter are significantly different at P=0.01 by the Duncan test.

Taking into account both years of study, the Tunisian variety 'Porto' and French variety 'Fournat de Breznaud' represented the higher sugar (5.8 and 4.9 g 100 g^{-1}DW) and sucrose content (4.9 and 4.5 g 100 g^{-1}DW), while the varieties 'Zahaaf' and 'Mahsouna' showed the lowest contents. 'Blanco', 'Dillou', 'Porto' and 'Khoukhi' have the highest raffinose levels, in decreasing order, for both years. Concerning the fructose and glucose percentages, the French varieties 'Lauranne' and 'Fournat de Breznaud' demonstrated the highest mean values for the two years of study (Table 3). However, the Italian variety 'Mazetto' represented the lowest glucose content (0.02 g 100g^{-1}DW).
The two local varieties 'Achaak' and 'Porto' are the two most appreciated almond kernel by consumers. 'Porto' seems to be sweeter than 'Achaak' and showed two times more total sugar and four times more fructose percentage.

3.6. Correlation among nutraceutical properties

The correlation among oil content and fatty acids of the studied almond kernel cultivars is reported in Table 4. High significant negative correlation was found between linoleic and oleic acid contents (r= -0.969). Significant positive correlations were also found between stearic and arachidic acid contents (r= 0.848) and in margaric versus margaroleic and gadoleic (r= 0.686 and r= 0.705, respectively). A significant negative correlation was found between gadoleic versus myristic and linolenic (r= -0.704 and r= -0.810, respectively). For the sugar composition, total sugar content was positively and highly correlated with sucrose (r= 0.974) and raffinose (r= 0.539). Also, a significant and high correlation was found between glucose and fructose contents (r= 0.933).
Moreover, significant and negative correlations were observed between total sugar content and arachidic acid (r= -0.537) and linolenic acid (r= -0.547). Similarly, a significant and negative correlation was also found between raffinose and stearic acid (r= -0.527) and arachidic acid (r= -0.589). Significant positive correlation was found between glucose and palmitoleic acid (r= 0.511). These relationships between different biochemical traits of almond suggest that the selection for one of these fatty acids or sugars could negatively or positively modify the amount of the other. Finally, a significant negative correlation was found between the oil and protein contents (r= -0.647).

3.7. Chemical diversity analysis

A principal component analysis (PCA) was performed on biochemical data (fatty acid, total oil and protein contents and sugar composition) for screening and describing the similarities among the 17 studied almond cultivars (Fig. 2). The PCA yielded six significant components with eigenvalues ≥ 1 and accounting for 91% of the total variance in the dataset (Table 5). The first two PCs (PC1 and PC2) accounted for 48.24% of the total of variance. PC-1 and PC-2 represented 27.41% and 20.83% of the variance, respectively. Eigen analysis of the correlation matrix revealed that PC-1 was mainly contributed by total sugar, sucrose and raffinose contents. PC-2 was correlated to arachidic, gadoleic, margaric and stearic acids. The third and fourth PC accounted for 16.83% and 11.35%, respectively. PC-3 was represented by oleic, linoleic, fructose and glucose contents while PC-4 was highly correlated to oil and protein contents.

Table 4. Correlations between fatty acid and oil content composition, protein content, total lipid, sugar composition and total sugar content.

	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Arachidic	α-Linolenic	Protein	Total Lipid	Raffinose	Sucrose	Glucose	Fructose	Total sugar
Palmitic	1													
Palmitoleic	-0,047	1												
Stearic	0,165	**-0,400**	1											
Oleic	**-0,607**	**0,458**	-0,047	1										
Linoleic	**0,474**	**-0,405**	-0,175	**-0,968**	1									
Arachidic	**0,237**	**-0,436**	**0,848**	0,026	**-0,232**	1								
α-Linolenic	0,006	**-0,322**	0,055	-0,010	0,001	0,143	1							
Protein	**0,252**	**-0,373**	0,094	-0,119	0,080	**0,285**	**0,297**	1						
Total Lipid	-0,192	**0,294**	**0,223**	**0,260**	**-0,294**	0,055	**-0,322**	**-0,647**	1					
Raffinose	**-0,217**	0,060	**-0,458**	-0,117	**0,250**	**-0,521**	**-0,264**	-0,128	0,065	1				
Sucrose	-0,080	**0,346**	-0,066	0,005	0,032	**-0,238**	**-0,485**	**-0,271**	**0,362**	**0,305**	1			
Glucose	-0,067	**0,455**	-0,164	**0,205**	-0,170	-0,131	-0,166	-0,136	0,175	-0,103	**0,287**	1		
Fructose	0,010	**0,374**	-0,045	0,178	-0,181	0,069	-0,109	-0,002	0,014	**-0,269**	0,166	**0,896**	1	
Total sugar	-0,136	**0,363**	**-0,206**	-0,005	0,078	**-0,361**	**-0,503**	**-0,277**	**0,336**	**0,530**	**0,961**	**0,333**	0,181	1

Correlations shown in bold case are significant at $P<0.05$.

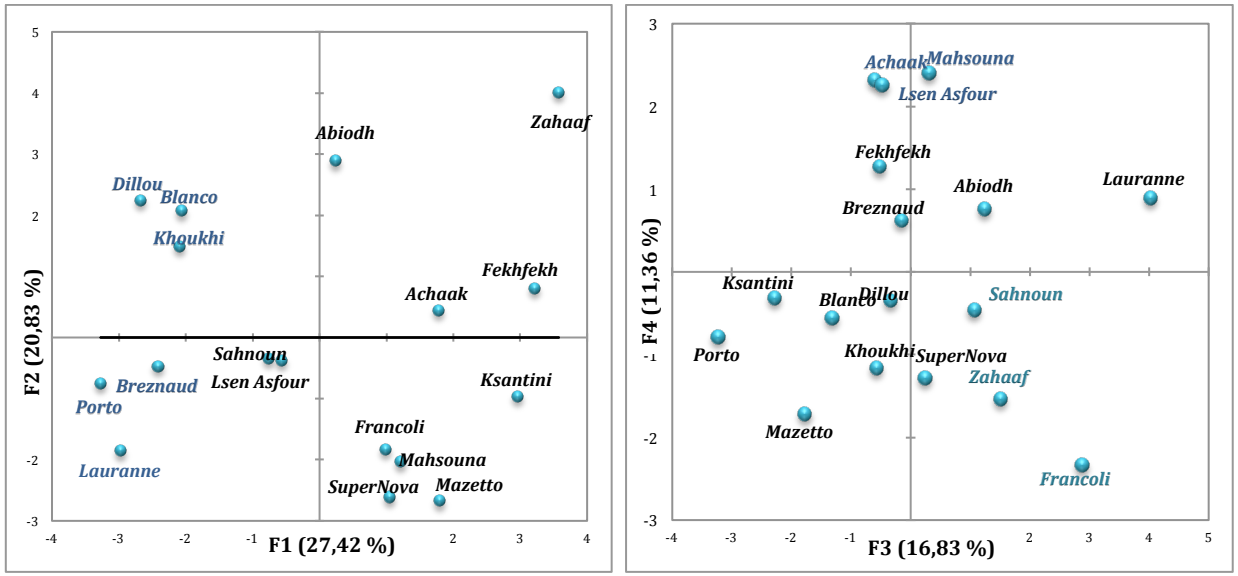

Figure 2. Score plot showed the Principal component analysis (PCA) based on nutraceutical data (fatty acid, total oil and protein contents and sugar composition) describing the similarities among the 17 studied almond cultivars.

Table 5. Eigenvectors of the four principal components axes from PCA analysis of the 17 almond cultivars for fatty acid and oil content composition, protein content, total lipid, sugar composition) and total sugar content. Eigenvalues and their contribution to total variation are listed at the bottom of columns.

Variable	F1	F2	F3	F4
Palmitic	-0,010	-0,400	-0,310	0,042
Palmitoleic	-0,588	0,161	0,581	0,114
Stearic	0,570	-0,663	-0,230	0,121
Oleic	0,228	0,160	0,860	-0,158
Linoleic	-0,375	0,062	-0,823	0,115
Arachidic	0,581	-0,777	-0,056	-0,050
α-Linolenic	0,856	0,312	0,154	0,063
Protein	0,419	-0,133	-0,056	-0,842
Total Lipid	-0,084	-0,133	-0,027	0,935
Raffinose	-0,485	0,440	-0,435	-0,156
Sucrose	-0,682	0,152	-0,203	-0,028
Glucose	-0,504	-0,183	0,586	0,114
Fructose	-0,391	-0,367	0,643	0,021
Total sugar	-0,798	0,240	-0,228	-0,067
Eigenvalue	4,935	3,749	3,030	2,045
Variance (%)	27,415	20,827	16,831	11,359
Cumulative (%)	27,415	48,243	65,073	76,433

Based on the PCA results (Fig. 2), same studied almond cultivars could be described by similarities in chemical characteristics considering oil and sugar composition while others had different chemical profile. PC-1 allowed the separation of 'Porto', 'Fournat de Breznaud', 'Blanco', 'Dillou', 'Khoukhi' and 'Lauranne' which are rich in total sugar, sucrose and raffinose. The cultivars 'Sahnoun', 'Zahaaf', 'Francoli' and 'Lauranne', separated along the positive direction of PC-3, were characterized by high oleic, fructose and glucose contents and low linoleic content. 'Mahsouna', 'Achaak', 'Lsen Asfour', 'Fekhfekh' and 'Lauranne' were situated in the positive side of PC-4 owing their high oil content opposing to 'Mazetto', 'Supernova', 'Zahaaf', 'Francoli' and 'Ksontini' on the negative direction with the highest protein content. This data suggests that almond kernels of 'Lauranne' cultivar offer unique nutritional potential, with high oil content, oleic acid and oleic to linoleic acids ratio and with superior total sugar content, especially fructose content. Moreover, the cultivars 'Lsen Asfour', 'Achaak' and 'Mahsouna' were associated together and represented some similarities in their composition.

4. DISCUSSION

The results showed that the main cultivated almonds in Tunisia are a potentially rich source of protein, unsaturated fatty acids and sugars. However, their contents on nutritional compound was affected by both genotype and harvest year. The year-to-year variation in fruit quality parameters may be explained by the differences in annual temperatures and precipitation over the two years of study (data not shown). The hard climatic conditions prevailing (dry and hot season) during 2010 were believed to be a contributing factor to the reported variation in sugar and oil content.

Significant genotypic and environmental effects were noted in the oil content for studied cultivars in the present study. The discrepancies in the possible year effect on oil content could be the result of the specific climatic conditions of the years tested (SOCIAS I COMPANY *et al.*, 2008). The variation between years indicated that climatic conditions had an effect on almond fruit development and thus severe deficiencies influenced lipid content (ZHU *et al.*, 2015). Therefore, the oil content trait appears to be under polygenic control (FONT I FORCADA *et al.*, 2011), with a clear environmental effect (ABDALLAH *et al.*, 1998; SATHE *et al.*, 2008; KODAD *et al.*, 2010). Moreover, the effect of harvest year on almond kernel oil content has been widely reported in the literature to be significant (BARBERA *et al.*, 1994; ABDALLAH *et al.*, 1998; SATHE *et al.*, 2008). YILDIRIM *et al*. (2016) reported that the total oil content changed significantly by year in fifteen commercial almond cultivars with the exception of cultivar 'Sonora'. However, no significant year effect was found by KODAD *et al.* (2011) in extensive two-year studies, although the interaction of genotype × year was significant. The magnitude of the effect of the external factors such as the climatic condition of the year probably depends on the genetic background of each cultivar, explaining the significant effect of the interaction genotype × year (KODAD *et al.*, 2011).

The variability range in total oil content in the present study was similar to the range of variability reported in previous studies. SATHE *et al.* (2008) have reported that oil content for eight almond Californian cultivars varied from 49.10% to 66.38%. ASKIN *et al.* (2007) reported that kernel oil content of 26 almond genotypes from eastern Anatolia (Turkey) varied from 25.19% to 60.77%. ČOLIĆ *et al.* (2017) reported that the range in total oil content for twenty almond spontaneous selections varied between 36.3 and 62.8%. Oil content of local almond genotype from Argentine varied from 48% to 57.5% (MAESTRI *et*

al., 2015). KODAK *et al.* (2008) found that total lipid contents ranged from 54 to 64.5% for European cultivars. They reported also that total lipid contents ranged from 35 to 53% for Australian cultivars and from 35 to 61% for Californian cultivars. Similarly, YADA *et al.* (2011) reported the variation range of kernel lipid contents of the most important commercial and local almond cultivars growing in USA-California (35-66%), Greece (56-61%), Italy (42-57%), Portugal (48-59%), Spain (40-67%), Turkey (25-61%), Afghanistan (43-63%), Egypt (55-59%), India (44-56%) and Iran (55-62%).

The heritability described for oil content is high (0.57) indicating an additive gene action, being a trait less influenced by environmental effects (FONT I FORCADA *et al.,* 2011). Consequently, selection for this trait will be more effective because it is less influenced by the environment (KODAD *et al.,* 2013). The local Tunisian cultivars with high and stable oil content could be incorporated into the almond breeding program in order to increase the oil content. In addition, the lipid portion, followed by the protein fraction, is the main component of the almond kernel, and is a major determinant of kernel flavor particularly following roasting (SOCIAS *et al.,* 2008). However, kernels with a relatively low percentage of oil such as 'Blanco'; 'Francoli', 'Ksantini' and ' Zahaaf' are required to produce almond milk, a dietetic product; because it's caloric level must be similar to that of cow's milk. Low lipid contents ('Lsen Asfour', 'Fourna de Breznaud') are also suitable for production of almond flour because of their correlation with high protein content (LONGHI, 1952).

For protein content, stability from year to year was observed for the cultivars 'Dillou' 'Mahsouna' and 'Fournat de Breznaud'. DROGOUDI *et al.* (2012), studying protein and mineral nutrient contents in kernels of 72 sweet almond cultivars and accessions grown in France, Greece and Italy, reported that the higher temperatures may have favored growth and nutrient utilization, resulting in greater nutrient contents in warmer year. Protein content in the seventeen studied almond cultivars ranged from 14 to 27%, which presented an interested range of variability compared with previous studies. In fact, protein contents ranged from 18.5 to 24.0 g $100g^{-1}$ of almond among all samples for the top ten almond-producing varieties in California and presently account for about 80% of the total commercial almond acreage (YADA *et al.,* 2013). KODAD *et al.* (2013) reported that the protein content ranging between 14.1 and 35.1% for 41 native almond genotypes grown in different geographical regions in Morocco. ÖZCAN *et al.* (2011) noted that crude protein content of five Turkish almonds varied from 12.7% to 16.3%. ASKIN *et al.* (2007) reported a wider range of protein content variability (16-31%) in 26 native genotypes from Turkey. All these results indicate the high range of variability of protein content depending on the genotype and the environmental conditions of the growing region (KODAD *et al.,* 2006). FONT I FORCADA *et al.* (2011) reported that the heritability estimate of protein content in almond is very low (h^2= 12.1%), confirming the strong effect of environmental conditions on its expression.

Almond oil has been reported to be very rich in monounsaturated fatty acids (MUFAs), especially in oleic and linoleic acids, whereas saturated fatty acids, especially palmitic, palmitoleic and stearic, are very low (YADA *et al.,* 2011). In commercial almond cultivars grown in various regions of the world, oleic and linoleic acids together accounts for about 90% of the total lipids, whereas, other fatty acids, including saturated fatty acids accounts for less than 10% (YADA *et al.,* 2011). This was consistent for the cultivars originate from the north of Tunisia that are 'Dillou', 'Khoukhi', 'Blanco' and 'Abiodh'. But overall the fatty acid composition, in the present paper, was in agreement with previous studies on almond grown around the world (SATHE *et al.,* 2008; MAESTRI *et al.,* 2015; ZHU *et al.,* 2015; ČOLIĆ *et al.,* 2017).

The variety 'Mahsouna' appears to present the most stable oil composition. Moreover, it presented stable value for oleic and linoleic acid contents. However, the oil composition of the varieties 'Blanco', 'Achaak', and 'Francoli' was more affected by the climatic conditions of the year studied. This confirmed that the year-on-year stability of each fatty acid depended on the specific characteristics of the genotype (ABDALLAH *et al.*, 1998; SATHE *et al.*, 2008; KODAD *et al.*, 2008, 2010; YADA *et al.*, 2011). KODAD *et al.* (2010) reported stable values for some fatty acids in some genotypes such as 'Marcona', 'Del Cid', and 'Castilla' for palmitic acid; 'Marcona' and 'Khoukhi' for palmitoleic acid; Desmayo Largueta and 'Del Cid' for stearic acid; 'Brézenaud' and 'Vivot' for oleic acid; and 'Desmayo Largueta', 'Khoukhi', 'Marcona', 'Retsou', and 'Vivot' for linoleic acid.

ABDALLAH *et al.* (1998) reported that the year effect was significant for all fatty acids except palmitoleic acid in twenty one Californian cultivars growing at four different sites. KODAD *et al.* (2010), after studying seventeen almond cultivars, reported that the year effect was significant for all fatty acids, except palmitic acid. Similarly, KODAD *et al.* (2011) noted that the year effect was not significant for palmitic and stearic acids. Furthermore, KODAD *et al.* (2010) reported that the genotype× year interaction was significant for all fatty acids except oleic acid, showing that the magnitude of the values changed each year. YILDIRIM *et al.* (2016) reported also that the effect of the cultivar, year and the interaction cultivar×year were significant for all fatty acids except heptadecanoic acid in fifteen commercial Turkish almond cultivars. Finally, SATHE *et al.* (2008) reported that the year effect was significant for all fatty acids in Californian cultivars growing at different sites, but stated that the year-to-year variability in fatty acid composition depended on the specific climatic conditions in that year.

Comparing linoleic acid levels in Spanish, Mediterranean, Californian and Australian almonds, ZHU *et al.* (2015) noticed that the regions producing almonds with lower linoleic acid were not irrigated, whereas Californian and Australian regions routinely apply irrigation to their orchards. NANOS *et al.* (2002), based on oil composition data, noted that irrigation resulted in almonds with superior oil quality as the oil had higher oleic acid content and oleic/linoleic acid ratio than almonds from non-irrigated trees. Consequently, Irrigation can affect almond kernel oil composition. For the others fatty acids, NANOS *et al.* (2002) reported that irrigation decreased the amounts of palmitic and palmitoleic acids, but did not affect the amount of stearic acid in 'Ferragnès' and 'Texas'.

The high content of unsaturated fatty acids, mainly of oleic acid, increases the phytonutrient value of the almond because this type of fatty acids does not contribute to the formation of cholesterol (KODAD *et al.*, 2011). Moreover, High levels of oleic acid and low levels of linoleic acid have been associated with prolonged shelf-life of almonds and are often advocated (ZHU *et al.*, 2015). Thus the varieties 'Sahnoun', 'Zahaaf' and 'Francoli', are superior in marketing quality with high oleic acid content and low linoleic and palmitic contents.

Furthermore, the higher oleic/linoleic (O/L) ratio was reported on 'Francoli' followed by 'Sahnoun', 'Zahaaf' and 'Mahsouna' cultivars. This ratio is considered a significant quality criterion of the oil kernel due to its preventive effect on lipid oxidation especially where almonds will be stored for long periods (KODAD *et al.*, 2010). In fact, a high O/L ratio is considered as an important factor providing stability in oils as well as a higher nutritional value and healthiest almond lipids (KODAD *et al.*, 2013; YILDIRIM *et al.*, 2016). For this, all oils of 'Abiodh', 'Francoli', 'Mahsouna', 'Sahnoun' and 'Zahaaf' can be considered of highly perfromant (Oleic/linoleic ratio > 4.2).

The two cultivars 'Achaak' and 'Francoli' were proved to be highly affected by the climatic conditions for sugars composition while 'Dillou' and 'Khoukhi' presented the most stable

sugar composition regarding harvest year. Sugar composition of almond kernel has vital value for good flavor and taste (NANOS *et al.,* 2002). It depends on the cultivar as well as the maturity stage but some sugar composition changes during maturation are cultivar-specific (NANOS *et al.,* 2002; KAZANTZIS *et al.,* 2003). In fact, KAZANTZIS *et al.* (2003) indicated that early harvested 'Ferragnes' almonds had higher raffinose content than late harvested almonds (due to sucrose accumulation with maturation and the preferential production of sucrose from raffinose and the other sugars) while the opposite held true for 'Texas' almonds. However, the effect of the year was reported to be non-significant on the expression of the sucrose content (YADA *et al.,* 2013). SÁNCHEZ-BEL *et al.* (2008) reported that sucrose and glucose contents in kernels of 'Guara' grown under drip-irrigated orchards were higher than those from non-irrigated orchards. The effect of year was reported to be significant on the total sugar content of the kernel of 'Ferragnes' and 'Mazeratto' varieties (BARBERA *et al.,* 1994).

Soluble sugars, while present in relatively low amounts, are sufficient to make kernels sweet-tasting (SCHIRRA, 1997). Free sugars are important nutritional components that affect the kernel flavor of almond (BALTA *et al.,* 2009). 'Porto' and 'Fournat de Breznaud' represented the higher sugar and sucrose content. Data regarding sucrose contents of this study were similar to those by KAZANKAYA *et al.* (2008) and BALTA *et al.* (2009). The prevalence of sucrose as the main sugar in almond is in agreement with previous works (FOURIE and BASSON, 1990; KADER *et al.,* 1996; NANOS *et al.,* 2002; KAZANKAYA *et al.,* 2008; BARREIRA *et al.,* 2010). They found, also, that sucrose was the main sugar constituent in almond followed by raffinose, glucose and fructose. FOURIE and BASSON (1990) obtained individual sugar contents of five almond cultivars ranging between 3.10 to 4.68 g 100 g^{-1}DW, 0.02 to 0.07 g 100 g^{-1}DW and 0.05 to 0.13 g 100 g^{-1}DW for sucrose, glucose and fructose, respectively. YADA *et al.* (2011) reported that the range variation of almond sugar contents (percentage of total weights) of commercially and locally almond cultivars growing in California is from 2.1 to 7%, in Greece from 2.6 to 4%, in India from 3.6 to 12%, in Italy from 2.1 to 5.5%, in Portugal from 2.5 to 7.1%, in Spain from 1.8 to 7.6%, and in Turkey from 2.5 to 13%.

Regarding relationships among the different nutraceutical almond parameters some interesting correlations were demonstrated in this work. Oleic and linoleic acids presented a conversely relationship. In the literature it has been reported that the proportion of oleic acid among total fatty acids is highly and negatively correlated with the linoleic acid levels with similar correlation coefficient (r= -0.9) (ABDALLAH *et al.,* 1998; ASKIN *et al.,* 2007; SATHE *et al.,* 2008; KODAD *et al.,* 2011; ZHU *et al.,* 2015). This high correlation between the two predominant fatty acids of almond kernels would allow accurate future predictions of total fatty acid composition by analyzing only linoleic acid level (ABDALLAH *et al.,* 1998). This higher correlation, could be considered as an index in any almond breeding program to improve almond quality (WANG *et al.,* 2019). In addition, the proportion of oleic acid was negatively correlated with palmitic level (r= -0.607). Similar results were reported in other almond cultivars (ASKIN *et al.,* 2007; SATHE *et al.,* 2008; KODAD *et al.,* 2011). Moreover, the correlation between stearic and arachidic was also approved by other authors (SATHE *et al.,* 2008).

Correlation coefficients, greater than 0.71 or smaller than -0.71, have been suggested to be biologically meaningful showing that this correlation is not influenced by climatic and environmental conditions and is genotype-dependent (KODAD *et al.,* 2011). No correlations were observed between the oil content and the percentages of the different fatty acids, even with the major fatty acid, which was also consistent with previous studies (SATHE *et al.,* 2008; KODAD *et al.,* 2011).

The negative correlation between protein and oil contents observed was previously reported by KODAD *et al.* (2013). On the other hand, BALTA *et al.* (2009) reported a positive correlation between maltose, glucose and fructose in sweet almond while this relationship was negative in bitter almond. Accordingly, these correlation findings indicate that inter-relationship among sugar contents vary according to kernel taste.

5. CONCLUSIONS

This work represents one of the most complete chemical and nutritional studies in almond characterizing the main almond cultivars grown in Tunisia. Results evidenced that oil, sugar and protein contents in almond depend of a polygenic background with a clear environment effect. Local Tunisian cultivars are highly rich in oil and fatty acids particularly oleic and linoleic acids with percentages between 88.4 and 91.6% of the extracted oil. In addition, the local cultivar 'Mahsouna' identified after a prospecting effort in the region of Sfax (South Tunisia) presented the most stable characteristics over the years regarding oil composition and protein content. The cultivar 'Porto' from the north of the country was performing in terms of sucrose and total sugar contents. This information would be essential to increase our knowledge on the local Tunisian almond diversity and their biochemical performance regarding traits to select adequate parents for future breeding programs. These results also support the importance of the characterization and preservation of genetic diversity being the granary for selecting in the coming future cultivars with high quality in a context of global warming offering valuable information for breeders about limits and capacities of the Tunisian almond cultivars to be used for breeding programs.

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