Protein Content and Oil Composition of Almond from Moroccan Seedlings: Genetic Diversity, Oil Quality and Geographical Origin

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Abstract The protein and oil content and the fatty acid profile of the kernels of selected almond genotypes from four different Moroccan regions were determined in order to evaluate the kernel quality of the plant material of these different regions. The ranges of oil content (48.7-64.5% of kernel DW), oleic (61.8-80.2% of total oil), linoleic (11.4-27.0%), palmitic (5.6-7.7%), stearic (1.3-3.1%), and palmitoelic (0.4-0.9%) acid percentages agreed with previous results of other almond genotypes, but the protein content (14.1-35.1% of kernel DW) showed that some genotypes had higher values than any previously recorded in almond. Some genotypes from mountainous regions showed kernels with very high oil content as well as high and consistent oleic and linoleic ratio, establishing a possible differentiation according to the geographical origin. These differences may allow establishing a geographical denomination for almond products. In terms of genetic diversity, oleic and linoleic acids were confirmed to be the most variable components of almond oil chemical composition among genotypes. Additionally, the genotypes with extreme favorable values, such as high protein content, could be incorporated into an almond breeding program aiming at an increase in kernel quality.

Keywords *Prunus amygdalus* · Protein content · Oil content · Fatty acids · Quality · Genetic resources · Breeding

Abbreviations

DW	dry weight
MUFA	mono-unsaturated fatty acids
O/L	oleic acid percentage/linoleic acid percentage ratio
FAME	fatty acid methyl ester
PUFA	poly-unsaturated fatty acids

Introduction

Almond (*Prunus amygdalus* Batsch) is a major tree nut grown in areas of Mediterranean climate. In Morocco, almond is grown in several regions from north to south, under different environmental conditions, mostly on non-irrigated areas of poor soils and receiving little attention from farmers. The climate is primarily Mediterranean, becoming more extreme towards the inland regions and Saharian in the south. The resultant variability in the environment and climate has turned into an extensive diversity of almond genotypes in each productive region, due to the fact that about 50% of the almond trees grown in Morocco are seedlings, located primarily in the north and the south [1, 2]. As a consequence, the genetic variability of the local Moroccan almond populations is assumed to be very large, including the possibility of peach introgression through natural peach × almond hybrids [3]. Several studies on the genetic structure of these populations have shown the presence of a great variability among genotypes of the same population [4], but also between populations [1]. Selection of local almond genotypes for late-bloom, and frost and disease resistance have been carried out since 1975 [5, 6]. These studies have allowed selecting genotypes with kernels of high physical quality [4, 7] or of high yielding potential due to high spur density [4]. However, the chemical quality of the kernel of these local populations has not been approached. The knowledge of the chemical components of the commercial and local almond genotypes has allowed their better characterization in Spain [8], the United States [9], Australia [10] and Turkey [11]. These results have opened the possibilities of incorporating the best genotypes in breeding programs for improving kernel quality. Almond breeding programs throughout the world have released many cultivars during the last decade [12], with varying nutrient composition, including differences in the fatty acid profile and tocopherol concentration of the kernel oil [13]. However, the use of a reduced number of cultivars as

parents has resulted in the appearance of different symptoms of inbreeding in the progeny, stressing the importance of including other unrelated promising genotypes in future breeding crosses [14].

The authenticity and traceability of the almond kernel and its by-products are of great interest for the protection of the consumer. Determination of the compositional variability of the oil from different countries, locations or cultivars could be imperative for proper classification of the product and the protection of its authenticity in the market. The modern almond industry requires commercial cultivars characterized by kernels with high quality attributes, because the best end-use for each cultivar is a function of its chemical composition [13] and of the consumers' trend for foods without synthetic additives [15]. Kernels with a high percentage of oil could be used to produce nougat or to extract oil for utilization in the cosmetic and pharmaceutical industries [13]. In addition, high oil content is desirable because higher oil contents result in less water absorption by the almond paste [16]. On the contrary, low oil contents are preferable for the production of almond flour or almond milk. In the case of individual fatty acids, low content of linoleic acid is correlated with high oil stability [17], whereas high content of oleic acid is considered a positive trait from the nutritional point of view [13].

The high nutritive value of almond kernels arises mainly from their high lipid content, which constitutes an important caloric source, but does not contribute to cholesterol formation in humans, due to their high level of unsaturated fatty acids, mainly mono-unsaturated fatty acids (MUFA), because MUFAs are inversely correlated to serum cholesterol levels [18]. In commercial almond cultivars grown in various regions, the oil from almond kernels is reported to be very rich in MUFAs, especially oleic acid and linoleic acids, whereas the levels of saturated fatty acid, especially palmitic, palmitoleic and stearic acids, are very low [19]. Kernel tendency to rancidification during storage and transport is a quality loss and is related

to oxidation of the kernel fatty acids [20]. Thus, oil stability and fatty acid composition, essentially the ratio of oleic to linoleic (O/L) acids, are considered important criteria for evaluating kernel quality [13]. It has also been reported that almond oil content and composition depend primarily on the genotype effect, but also on the environmental conditions [9, 21, 22]. In addition, almond protein contains a good balance of essential amino acids with exception of methionine, and is easily hydrolyzed by common digestive protease, producing high-quality protein hydrolysates in relation to essential amino acid balance [23].

The main objective of the present study was the evaluation of the quality and genetic diversity of local almond seedlings in Morocco based on the protein and oil content, and on the fatty acid profile, thus allowing the selection of promising genotypes for commercial utilization and to be incorporated into almond breeding programs for kernel quality.

Materials and Methods

Plant Material

This study was carried out with almond genotypes from four different regions with wealthy genetic resources: Aknoul situated in the Rif Mountains (North of Morocco), Azilal in the high Atlas Mountains (South Central Morocco), and two valleys in Central Morocco, Saïs (village of Sfasif) and Tadla (region of Bni Mellal). A total of 41 local genotypes from different zones of each region were selected because of the general status of the plant (vigor, ramification, foliar density and appearance), late blooming, and kernel physical quality appreciated by the local population. These genotypes were marked and fruits were collected in summer (7-10 August) during two consecutive years (2009-2010).

Determination of Oil and Fatty Acids

Two replicates of 20 fruits of each genotype were randomly collected. After cracking, seed coats were removed by pouring in warm water. Kernels were dried at room temperature for two days and ground in an electrical grinder. Oil was extracted from 4-5 g of ground almond kernels in a commercial fat-extractor (Selecta, Barcelona, Spain) for 2 hours using petroleum ether as a solvent and keeping the heating source at 135 ºC [24]. The fat content was determined as the difference in weight of the dried kernel sample before and after extraction. The oil sample was utilized to prepare the methyl esters of the corresponding fatty acids (FAME) according to the EU official method [25]. These methyl esters were separated using a flame ionization detector gas chromatograph equipped with a HP-88 capillary column (100m × 0.25mm i.d. 0.2 mm film.) (Agilent Technologies, Santa Clara, CA, USA). The initial column temperature was 100ºC. The oven temperature was then increased from 100 to 175ºC at 13ºC/min ramp rate, from 175 to 200ºC at 4ºC/min ramp rate. The temperature was maintained at 200ºC for 20min. The injection volume was 1.0 μL. The identification of the FAMEs was achieved by comparing with relative retention times in a reference sample that contained standard methyl esters (Sigma-Aldrich, Madrid, Spain).

Determination of Total Protein Content

The protein fraction was determined through the total N content obtained by the Dumas method and applying the conversion factor of 6.25. A sample of 0.2 g of almond flour was weighed and introduced into the analyzer LECO FP-528 Protein/Nitrogen Analyzer (LECO Corporation, Saint Joseph, MI, USA). The results were read and interpreted with the software

CPU-CAR-02. The sample was burned at 850°C, and during the incineration the gases CO_2, H_2O, N_2 and NO_X were generated. The gas was passed through hot copper to remove oxygen and then conducted through two filters. Finally, the molecular nitrogen with helium was measured in a cell differential thermoconductivity. The results were expressed as percentage of nitrogen by weight.

Statistical Analysis

All statistical analyses were performed with the SAS program [26]. Analysis of variance was performed with a three random factors design. Season and population were orthogonal factors whereas the factor tree was hierarchical to the factor population because the trees were not repeated between sites. To draw a general conclusion among the four almond locations, the population was considered as a random effect [27]. The Principal Component Analysis (PCA) was applied to describe the pattern of almond diversity. In PCA, intercorrelation among variables (component) was removed [28], thus reducing the number of variables by linear combination of correlated characters into principal orthogonal axes (PC1, PC2, PCn) which are not correlated [29]. The maximal amount of variance in the data set and its direction are often explained by the first PC. Each PC is defined by a vector known as the eigenvector of the variance-covariance matrix. PCA is used to establish correlations between variables and to visualize the relationships of individuals in two or three dimensional graphs.

Results and Discussion

Variability Analysis

The statistical analysis showed that the effects of year, population, genotype/population, the interaction year × population as well as the interaction year × genotype/population were significant for oil and protein content (Table 1). Large differences in oil content and major fatty acids were found among the genotypes studied (Table 2). The year effect has been reported to be significant for oil content when the evaluation of this trait was conducted under several growing conditions [9, 22], but not when the evaluation was conducted under the same growing conditions [21, 30]. The mean value of oil content was significantly lower in 2009 (56.1%) than in 2010 (57.1%), although the difference was small. The mean value of oil content over the two years for the individual genotypes varied from 48.7 to 64.5%, being over 60% in SA-5, AK-3, AZ-2, AZ-3 and AZ-10 (Table 2). The range of variability for oil content in these Moroccan genotypes was lower than that reported in other regions: 40-68% for European cultivars [23, 31, 32], 35-61% for Australian cultivars [10], 36-53% for Californian cultivars [9], 43–63% for Afghan selections [33], and 25.1-60.8% for Turkish genotypes [11].

Conversely, the mean value of the protein content was slightly higher in 2009 (25.3%) than in 2010 (24.8%), confirming the negative correlation between oil and protein contents (Table 3). The protein content showed a higher variability between genotypes, with the two-year mean ranging between 14.1 and 35.1% (Table 2). Contrary to the oil content, the range of variability for protein content was larger than that previously reported: 22.5-25.8% by Barbera et al. [34], 11.8-31.8% by Kodad et al. [35], 16.1-31.5% by Askin et al. [11] and 8.4-24.7% by Font i Forcada [36]. The highest protein content so far reported was 32% for various Indian almond selections [37]. However, three Moroccan genotypes (AG4, BM5, and BM13) showed higher values than these Indian genotypes (Table 2). Abdallah et al. [9] reported the highest protein content for genotypes partly deriving from peach and this possibility cannot be discarded for the Moroccon seedlings with high protein content due to

the presence of natural peach × almond hybrids in Morocco [3] and the possibility of peach gene introgression into these populations, specially the Sfasif population. The same peach parentage could also be suggested for some Indian genotypes [14].

When the contents of oil and protein were examined for each population, the Azilal pool had the highest mean value for oil content as well as the lowest for protein content (Table 3). The lowest value for oil content was recorded for the Bni Mellal pool, and the highest for protein for the Sfasif population (Table 3). Oil and protein contents appear to be under polygenic control [36], with a clear environmental effect [21, 22]. Thus, the magnitude of the effect of the external factors, such as the climatic condition of the year, would probably depend on the genetic background of each genotype. This would explain the significant effect of the interaction year × genotype/population (Table 1) and that the rank of the different population means would depend on the year conditions. No causal relationship has been so far established between oil and protein content and the changes in the production factors (soil type, irrigation method, and temperature range) determining the different growing conditions [9, 21, 38]. Thus, the consistent high values of oil content for the Azilal genetic pool must be mostly attributed to the ability of this genetic pool in producing higher oil amounts.

As expected, the concentrations for the different fatty acids were low for the saturated fatty acids (palmitic and stearic), intermediate for the polyunsaturated fatty acids (PUFA) (linoleic), and high for MUFAs, especially oleic acid (Table 2). Several reports have shown that almond cultivars have a concentration of MUFAs generally higher than 90% of the total lipid content, whereas for the saturated fatty acid is lower than 10% [9, 22, 24, 38, 39]. The range of concentration over the total lipid content was of 5.6-7.7% for palmitic acid, 0.4-0.9% for palmitoleic acid, 1.3-3.1% for stearic acid, 61.8-80.2% for oleic acid, and 11.4-26.9 for linoleic acid (Table 2). A similar fatty acid profile has also been reported for local selections from Afghanistan [33], India [37], Iran [40] and Portugal [41]. The genotype AG-6 showed a

very high oleic acid percentage (80%), similar to those reported for some local Turkish genotypes [11].

The statistical analysis showed that the effect of the year, population and genotype/population were significant for palmitic, palmitoleic, oleic and linoleic acids (Table 1). In spite of the significance of the year effect on individual fatty acids, the magnitude of the variation between years is negligible as compared with the variability between genotypes (Table 3), as already reported [9, 21, 22]. The lack of significance of the interactions year × population and year × genotype/population for palmitoleic, stearic, and linoleic fatty acids (Table 1), indicates that the mean value of each population and genotype was only slightly changed over the two years (Table 3). The highest mean values of palmitic and palmitoleic acids were recorded in the Azilal pool, significantly higher than in the other pools (Table 3). A consistent year to year high mean value for oleic acid was recorded in the Aknoul pool, and consistent low in the Bni Mellal and Sfasif pools (Table 3). Conversely, for linoleic fatty acid the lowest mean value was recorded in the Aknoul pool, and the highest in the Sfasif pool (Table 3).

The most important MUFA in almond was oleic acid [32]. It has been reported that MUFAs were as effective as PUFAs in the reduction of low-density-lipoprotein cholesterol in humans [42], mainly for oleic acid [18]. Kernel tendency to rancidification during storage and transport is a quality loss and is related to oxidation of the kernel fatty acids [43]. Thus, fatty acid composition, essentially the ratio of oleic to linoleic (O/L) acids, is considered an important criterion to evaluate kernel quality, as a high O/L ratio is essential for maintaining oil stability [20]. Additionally, the saturated fatty acids (palmitic and stearic) give more stability to the fat [44]. In the Moroccan genotypes the O/L ratio ranged between 2.34 (SA-2) to 7.29 (AG-5) (Table 2). When comparing the populations, the highest mean O/L ratio was recorded at Aknoul and the lowest at Sfasif (Table 3). Consequently, the oil from the

genotypes selected at Aknoul is very interesting from the point of view of its stability and of its nutritional and healthy properties, more than the oil from genotypes of the other regions, because of the high oleic acid percentage, the high O/L ratio and the intermediate percentages of palmitic and stearic acids (Table 3). However, some genotypes from Azilal (AZ-5 and AZ-9) also have an oil of high quality, with low linoleic and high oleic acids and high O/L ratio (Table 2).

Diversity Analysis

Multivariate statistical techniques have already been used to classify almond cultivars based on the similarity in their fatty acid profile (percentages of the major and minor fatty acids), or in the triacylglycerol composition of the kernel oil [29, 45-47]. Thus the PCA and cluster analysis was applied to the data in order to describe the similarities among genotypes and to identify the promising ones to be possibly included in almond breeding programs. To select the best model with the minimum number of dimensions explaining the data structure, the exclusion rule was employed based on the amount of residual variability to be tolerated, retaining a sufficient number of PCs capable of explaining a percentage of variance > 80%. Using this rule, the first three PCs are enough because they described 84.8% of the sample variability. The contribution of each principal component to the total variance is shown in Table 4. Oleic and linoleic fatty acids and the ratio O/L were primarily responsible for separation on PC1 (Table 4). The separation along PC2 was due to variation in oil and protein contents and the O/L ratio (Table 4). The separation along PC3 was due to palmitic, palmitoleic and stearic acids (Table 4). Similar results were reported in previous PCA applications to almond research [30, 45], and confirming that oleic and linoleic acids are the most variable components of almond kernel oil among genotypes.

When the means were plotted on the three principal axes (Fig 1), the genotypes BM-3 and BM-15 from Beni Mellal; AZ-4, AZ-5, AZ-9, and AZ-10 from Azilal; and SA-6, AG-1, AG-3, AG-5, and AG-6 from Aknoul, had high positive values on PC1, characterized by high values for oleic acid and the O/L ratio, and low values for linoleic acid (Table 4). The genotypes BM-12 from Beni Mellal, AK-1 AG-4, AG-2, AK-3 from Aknoul and AZ-3 from Azilal, had intermediate values of oleic acid and the O/L ratio. The rest of the genotypes had negative values on PC1, showing low oleic acid and the O/L ratio, and high linoleic acid, with the most representative genotypes being SA-2 and SA-3 from Aknoul; and BM-7, BM-8, BM-4, BM-5, BM-2, BM-1, BM-6, BM-14, BM-10 from Bni Mellal (Table 2). These results point out that most genotypes from Azilal (high Atlas Mountains) and Aknoul (Rif Mountains) have an oil of higher quality than the genotypes from Bni Mellal (Tadla plain) and Sfasif (Saiss plain), with significant differences among these population, as shown by the mean values of oleic acid and the O/L ratio, which in both years were higher at Azilal and Aknoul than at Bni Mellal and Sfassif (Table 3). Although irrigation was not applied in all prospected sites, the rainfall is generally higher and the temperature is cooler in the mountains than in the plains. These facts could affect the oleic and linoleic acids accumulation in the almond kernel. Nanos et al. [48] and Barbera et al. [34] reported that the oleic acid content of ‘Ferragnès’ increased in irrigated plants as compared to stressed plants. Similarly Sánchez-Bel et al. [49] found slightly higher oleic acid and lower linoleic and palmitic acid percentages in ‘Guara’ from drip-irrigated plots as compared with non-irrigated plots in the same Spanish region. In other species, such as olive, Montefredine and Laporta [50] suggested that low temperatures resulted in higher contents of oleic acid in disfavor of linoleic. Concerning the palmitic, palmitoleic and stearics acids, the genotypes AZ-1, AZ-5, AZ-7 and AZ-10 from Azilal; BM-5 and BM-9 from Bni Mellal, and AK-1 from Aknoul showed very high palmitic and

palmitoleic and very low stearic acid, in contrast to AZ-2, AK-3 and AG-3, with a very high stearic acid percentage (Table 2).

The genotypes AZ-1, AZ-7, AZ-5, AZ-3, AZ-10 and AZ-2 from Azilal, and SA-1, SA-2 and SA-3 from Aknoul showed a very high oil content and a very low protein content and, as a consequence, a very high oil/protein ratio (Table 2). Opposite results were obtained in six genotypes from Bni Mellal (BM-1, BM-4, BM-5, BM-6, BM-10 and BM-13), four from Aknoul (AG-3, AG-4, AG-5, and AK-2), and SF-1 from Sfassif (Table 2, Fig 1). The remaining genotypes showed intermediate values (Table 2). The oil/protein ratio is important for the confectionary industry, especially for marzipan production, since it influences water absorption by the almond paste: the higher the lipid content, the lower the water absorption [16]. Thus, the genotypes with high oil/protein ratio could be destined to the confectionary industry and to the extraction of almond oil, especially in the mountain locations of Azilal and Aknoul, considered the most important areas of almond seedling production in Morocco. However, the clustering of genotypes using the variability observed did not allow their classification according to their geographical origin, confirming that the oil content and the fatty acid profile depend primarily on the genotype and not on the geographical origin.

Conclusion

The present results reveal that the kernel oil of the Moroccan population of almond seedlings show a similar oil content and fatty acid profile than other almond genotypes [19]. However, the Moroccan almond seedlings are characterized by high protein content. As a consequence, these genotypes could be used as a source of protein for industrial processes and food additives. Some genotypes had higher oil and protein contents and oleic acid percentage than

others so far reported and could be used as parents to improve these traits in an almond breeding program. The differences between the almond seedling population for almost all the chemical components studied, especially for oil content, and for oleic and linoleic acids, may offer the opportunity to establish their commercial differentiation. These differences could be the basis of legislation for protecting a denomination of origin or a geographical origin, thus providing the possibility of labeling food products by growing area, and offering extra economical benefits for the farmers. Thus these results represent a step towards the objective characterization and classification of economical areas for almond production, particularly for the production of oil of higher quality. With this reference it could be possible to establish the criterion of geographical origin and to increase the competitiveness of the almond production and the almond by-products on the local markets.

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References

1. Lansari A, Azoulay H, Kester DE (1998) The morphological structure of almond seedling populations in Morocco. Acta Hort 470:95-100
2. Anonymous (2011) Situation de l'Agriculture Marocaine. N° 9. Ministère de l'Agriculture et de la Pêche Maritime. Rabat, Maroc

3. Barbeau G, El Bouami A (1980) Prospections 'Amandier' dans le sud Marocain. Fruits 35:39-50

4. Lansari A, Iezzoni AF, Kester DE (1994) Morphological variation within collections of Moroccan almond clones and Mediterranean and North American cultivars. Euphytica 78:27-41

5. Barbeau G, El Bouami A (1979) Prospection de tardivité de floraison chez l'amandier dans le sud Marocain. Fruits 34:131-137

6. Laghezali M (1985) L'amandier au Maroc. Options Méditerr CIHEAM/IAMZ 85/I:91-96

7. Oukabli A, Mamouni A, Laghezali M, Chahbar A, Mekkaoui A, Lahlou M, Bari A (2007) Caractérisation de la diversité génétique des populations locales d'amandier cultivés [*Prunus dulcis* (Miller) Webb] au Maroc. Proc IV Journées Nationales de Biodiversités

8. Romojaro F, Riquelme F, Jimémez JJ, Llorente S (1988) Fat content and oil characteristics of some almond varieties. Fruit Sci Rep 15: 53-57

9. Abdallah A, Ahumada MH, Gradziel TM (1998) Oil content and fatty acid composition of almond kernels from different genotypes and California production regions. J Am Soc Hort Sci 123:1029-1033

10. Vezvaei A, Jackson JF (1996) Almond nut analysis. In: Linskens HF, Jackson JF (eds.). Modern methods of plant analysis. Vol. 18. Fruit analysis. Springer-Verlag, Berlin, Germany, p 135-148

11. Askin MA, Balta MF, Tekintas FE, Kazankaya A, Balta F (2007) Fatty acid composition affected by kernel weight in almond [(*Prunus dulcis* (Mill.) D.A. Webb.]. J Food Comp Anal 20:7-12

12. Socias i Company R, Gómez Aparisi J, Alonso JM, Rubio-Cabetas MJ, Kodad O (2009) Retos y perspectivas de los nuevos cultivares y patrones de almendro. Inf Técn Econ Agrar 105:99-116
13. Socias i Company R, Kodad O, Alonso JM, Gradziel TM (2008) Almond quality: a breeding perspective. Hort Rev 34:197-238
14. Socias i Company R (2002) Latest advances in almond self-compatibility. Acta Hort 591:205-212
15. Krings U, Berger RG (2001) Antioxidant activity of some roasted foods. Food Chem 72:223-229
16. Alessandroni A (1980) Le mandorle. Panif Pasticc 8:67-71
17. Zacheo G, Capello MS, Gallo A, Santino A, Capello AR (2000) Changes associated with postharvest ageing in almond seeds. Lebens.-Wissens. Technol. 33:415-423
18. Sabate J, Hook DG (1996) Almonds, walnuts, and serum lipids. In: Spiller, GA (ed) Lipids in human nutrition. CRC Press, Boca Raton, FL, p 137-444
19. Yada S, Lapsley K, Huang G (2011) A review of composition studies of cultivated almonds: Macronutrients and micronutrients. J Food Comp Anal 24:469–480
20. Kester DE, Cunningham S, Kader AA (1993) Almonds. In: Encyclopedia of Food Science, Food Technology and Nutrition. Academic Press, London, pp 121-126
21. Kodad O, Estopañán G, Juan T, Molino M, Mamouni A, Messaoui Z, Lahlou M, Socias i Company R (2010) Plasticity and stability in the major fatty acid content of almond kernels grown under two Mediterranean climates. J Hort Sci Biotechnol 85:381-386
22. Sathe SK, Seram NP, Kshirsagar HH, Heber D, Lapsley KA (2008) Fatty acid composition of California grown almonds. J Food Sci 73:C607-C614
23. Sathe SK (1993) Solubilization, electrophoretic characterization and in vitro digestibility of almond (*Prunus amygdalus*) proteins. J Food Biochem 16:249–264

24. Kodad O, Socias i Company R (2008) Variability of oil content and of major fatty acid composition in almond (*Prunus amygdalus* Batsch) and its relation with kernel quality. J Agric Food Chem 56:4096–4101

25. EEC Commission regulation N° 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Official Journal of the European Communities, 5 Sep 1991, L 248:1-83

26. SAS INSTITUTE. (2000) *SAS/STAT User's Guide*. SAS Institute, Cary, NC, USA

27. Steel RGD, Torrie JH (1960) Principales and Procedures of Statistics. McGraw-Hill Book Company Inc., New York-Toronto-London, 481 p

28. Broschat TK (1979) Principal component analysis in horticultural research. HortScience 14:114-117

29. Phillipeau G (1986) Comment interpréter les résultats d'une analyse en composantes principales. Institut Technique des Céreales et des Fourrages, p 1-28

30. Kodad O, Alonso JM, Espiau M, Estopañán G, Juan T, Socias i Company R (2011) Chemometric characterization of almond germplasm: compositional aspects involved in quality and breeding. J Am Soc Hort Sci 136:273–281

31. Romojaro F, García JE, López Andreu FJ (1977) Estudio sobre la composición química de variedades de almendra del sureste español. An Edafol Agrobiol 36:121-131

32. Saura-Calixto F, Cañellas MJ, Soler L (1988) La Almendra: Composición, Variedades, Desarrollo y Maduración. INIA, Madrid, 173 pp

33. Kaska N, Kafkas S, Padulosi S, Wassimi N, Ak BE (2006) Characterization of nut species of Afghanistan: I–Almond. Acta Hort 726:147–155

34. Barbera G, Di Marco L, La Mantia T, Schirra M (1994) Effect of rootstock on productive and qualitative response of two almond varieties. Acta Hort 373:129-134

35. Kodad O, Socias i Company R. Gracia Gómez MS, Martínez Lázaro JM, Bonilla A (2004) La composición de la almendra como criterio para su utilización industrial y como base para la selección en un programa de mejora genética. Actas III Congreso Español de Ingeniería de Alimentos, Pamplona, Spain, ISBN 86-688-7989-4, p 1094-1102

36. Font i Forcada C, Kodad O, Juan T, Estopañán G, Socias i Company R (2011) Genetic variability and pollen effect on the transmission of the chemical components of the almond kernel. Span J Agr Res 9:781-789

37. Kumar K, Ahuja KL, Uppal DK (1994) Kernel quality of almonds (*Prunus amygdalus* Batsch) in terms of oil content, fatty acid composition and phospholipid content. J Food Sci Tech 31:335–337

38. Schirra M, Agabbio M (1989) Influence of irrigation on keeping quality of almond kernels. J Food Sci 54:1642-1465

39. Ruggeri S, Cappelloni M, Gambelli L, Nicoli S, Carnovale E (1998) Chemical composition and nutritive value of nuts grown in Italy. Ital J Food Sci 3:243–251

40. Mehran M, Filsoof M (1974) Characteristics of Iranian almond nuts and oils. J Am Oil Chem Soc 51:433–434

41. Neto-Martins A, Gomes C, Ferreira L (2000) Almond production and characteristics in Algarve, Portugal. Nucis 9:6–9

42. Mensik RP, Katan MB (1989) Effect of a diet enriched with monounsaturated or polyunsaturated fatty acid on the levels of low-density and high-density lipoprotein cholesterol in healthy women and men. N Engl J Med 321:436–441

43. Senesi E, Rizzolo A, Colombo C, Testoni A (1996) Influence of pre-processing storage conditions on peeled almond quality. Ital J Food Sci 2:115–125

44. Borges OP, Carvalho JS, Correia PR, Silva AP (2007) Lipid and fatty acid profiles of *Castanea sativa* Mill. chestnut of 17 native Portuguese cultivars. J Food Comp Anal 20:80–89

45. García-López C, Grané-Teruel N, Berenguer-Navarro V, García-García, JE, Martín-Carratalá, ML (1996) Major fatty acid composition of 19 almond cultivars of different origins. A chemometric approach. J Agric Food Chem 46:963-967

46. Martín-Carratalá ML, García-López C, Berenguer-Navarro V, Grané-Teruel N (1998) New contribution to the chemometric characterization of almond cultivars on the basis of their fatty acid profiles. J Agric Food Chem 46:963–967

47. Martín-Carratalá ML, Llorens-Jordá C, Berenguer-Navarro V, Grané-Teruel N (1999) Comparative study on the triglyceride composition of almond kernel oil. A new basis for cultivar chemometric characterization. J Agric Food Chem 47:3688–3692

48. Nanos GD, Kazantzis I, Kefalas P, Petrakis C, Stavroulakis GG (2002) Irrigation and harvest time affect almond kernel quality and composition. Scientia Hort 96:249-254

49. Sánchez-Bel P, Egea I, Martínez-Madrid MC, Flores B, Romojaro F (2008) Influence of irrigation and organic/inorganic fertilization on chemical quality of almond (*Prunus amygdalus* cv. Guara). J Agric Food Chem 56:10056–10062

50. Montefredine A, Laporta L (1963) Ricerche gas-chromatografiche sulla composizione degli olii d'oliva italiana. Riv Ital Sost Grasse 40:379-381

Table 1 Global analysis of variance for oil content (%), protein content (%) and fatty acid composition of Moroccan almond seedling

Variable	df	Mean square	*F*-Value	*P*
Oil content				
Year	1	24,31	27,90	<.0001
Population	3	99,21	113,87	<.0001
Year × population	3	3,07	3,52	0.0185
Genotype/population	37	40,93	46,98	<.0001
Year × Genotype/population	37	3,42	3,92	<.0001
Error	82	0,87		
Protein content				
Year	1	10,82	11,72	0.0010
Population	3	240,57	260,66	<.0001
Year × population	3	2,90	3,14	0.0297
Genotype/population	37	69,77	75,60	<.0001
Year × Genotype/population	37	1,92	2,08	0.0030
Error	82	0,92		
Palmitic acid.				
Year	1	4,30	24,63	<.0001
Population	3	4,19	24,00	<.0001
Year × population	3	0,65	3,75	0.0141
Genotype/population	37	0,78	4,44	<.0001
Year × Genotype/population	37	0,11	0,62	0.9478
Error	82	0,17		
Palmitoleic acid				

Year	1	0,07	12,78	0.0006
Population	3	0,24	46,34	<.0001
Year × population	3	0,01	1,28	0.2866
Genotype/population	37	0,04	7,80	<.0001
Year × Genotype/population	37	0,01	0,57	0.9699
Error	82	0,01		
Stearic acid				
Year	1	0,40	15,11	0.0002
Population	3	0,02	0,58	0.6325
Year × population	3	0,00	0,09	0.9630
Genotype/population	37	0,62	23,66	<.0001
Year × Genotype/population	37	0,03	1,18	0.2623
Error	82	0,03		
Oleic acid				
Year	1	15,62	15,46	0.0002
Population	3	72,29	71,52	<.0001
Year × population	3	9,84	9,73	<.0001
Genotype/population	37	57,84	57,22	<.0001
Year × Genotype/population	37	2,78	2,75	<.0001
Error	82	1,01		
Linoleic acid				
Year	1	5,85	9,16	0.0033
Population	3	83,07	130,10	<.0001
Year × population	3	0,46	0,72	0.5402
Genotype/population	37	43,91	68,77	<.0001

Year × Genotype/population	37	0,73	1,15	0.2974
Error	82	0,64		

Table 2 Oil, protein content and fatty acids of the kernels of 41 Moroccan almond seedlings from different geographical origin (mean of the two years)

Region of origin	Genotypes	Code number [a]	Oil content (Ol) (% of kernel DW)	Protein content (P) (% of kernel DW)	R1 (Ol/P ratio)	Palmitic (% of total oil content)	Palmitoleic (% of total oil content)	Stearic (% of total oil content)	Oleic (O) (% of total oil content)	Linoleic (L) (% of total oil content)	R2 (O/L ratio)
Aknoul	AG-1	1	55.8±0.99	28.2±0.96	2.0±0.07	6.2±0.25	0.8±0.16	2.0±0.17	74.4±1.42	16.5±1.14	4.6±0.39
	AG-2	2	56.5±1.26	25.7±2.32	2.2±0.18	6.6±1.06	0.7±0.15	1.8±0.15	70.4±1.72	19.5±1.20	3.6±0.28
	AG-3	3	56.0±1.08	24.9±0.47	2.3±0.07	6.7±0.18	0.7±0.18	2.3±0.18	74.3±1.42	15.5±0.63	4.8±0.27
	AG-4	4	54.8±0.02	33.5±0.60	1.6±0.03	6.3±0.69	0.6±0.16	2.2±0.16	71.5±0.99	18.7±0.85	3.8±0.23
	AG-5	5	50.4±0.56	28.6±0.59	1.8±0.05	6.3±0.09	0.6±0.07	1.5±0.11	75.3±1.01	14.9±1.14	5.1±0.35
	AG-6	6	58.5±1.06	21.7±1.96	2.7±0.22	5.6±0.10	0.5±0.08	1.7±0.30	80.2±2.56	11.4±2.25	7.3±1.66
	AK-1	7	56.2±1.45	28.3±0.54	2.0±0.02	7.3±0.13	0.6±0.07	1.7±0.12	69.4±1.07	19.6±0.84	3.6±0.10
	AK-2	8	54.1±1.49	28.5±0.48	1.9±0.08	5.8±0.26	0.4±0.06	2.2±0.12	71.2±0.46	20.0±0.43	3.6±0.13
	AK-3	9	60.3±0.21	22.4±1.71	2.7±0.20	5.6±0.14	0.4±0.06	2.3±0.11	73.1±0.61	18.0±0.21	4.1±0.02

	SA-1	10	56.5±5.84	19.4±0.54	2.9±0.30	6.9±0.37	0.5±0.03	1.9±0.01	65.2±2.06	23.2±0.48	2.8±0.14
	SA-2	11	59.1±1.85	18.6±1.07	3.2±0.20	6.5±0.60	0.4±0.01	1.9±0.02	61.8±1.39	26.9±0.86	2.4±0.05
	SA-3	12	57.3±0.47	25.5±0.41	2.3±0.04	6.4±0.23	0.4±0.02	2.0±0.06	65.4±2.16	23.8±0.83	2.8±0.17
	SA-5	13	60.0±2.94	21.3±0.43	2.8±0.18	7.1±0.42	0.5±0.03	2.2±0.54	65.0±2.94	23.5±0.52	2.8±0.17
	SA-6	14	58.1±0.72	24.6±1.83	2.4±0.21	6.7±0.47	0.7±0.05	1.5±0.15	72.5±1.59	17.2±2.31	4.3±0.57
	AZ-1	15	57.7±1.54	23.6±0.67	2.5±0.04	7.7±0.48	0.7±0.09	2.0±0.25	65.2±0.99	23.0±1.24	2.8±0.16
	AZ-10	16	62.7±0.83	18.1±0.80	3.5±0.20	7.5±0.45	0.9±0.04	1.7±0.08	71.5±0.93	17.8±0.57	4.0±0.11
	AZ-2	17	64.6±0.81	14.1±0.09	4.6±0.05	6.7±0.47	0.5±0.02	3.1±0.33	67.7±0.47	21.3±0.59	3.2±0.10
	AZ-3	18	63.0±0.36	18.1±0.15	3.5±0.03	6.9±0.52	0.6±0.06	1.8±0.06	71.0±0.74	19.2±0.40	3.7±0.05
Azilal	AZ-4	19	55.7±0.84	23.1±0.10	2.4±0.04	6.8±0.47	0.6±0.01	1.6±0.05	71.7±0.48	17.9±0.03	4.0±0.03
	AZ-5	20	59.1±0.83	22.3±0.29	2.7±0.02	7.3±0.59	0.8±0.04	1.3±0.06	75.5±0.98	14.6±0.41	5.2±0.09
	AZ-7	21	54.6±1.12	22.5±0.36	2.4±0.01	7.0±1.08	0.8±0.04	2.1±0.07	66.0±0.88	23.2±0.63	2.9±0.11
	AZ-8	22	57.0±0.54	22.1±0.13	2.6±0.04	7.0±0.90	0.5±0.03	1.9±0.06	66.1±0.90	23.4±0.65	2.8±0.12
	AZ-9	23	54.2±0.83	29.1±0.71	1.9±0.07	6.9±0.44	0.6±0.01	2.0±0.06	72.9±0.77	16.6±0.43	4.4±0.08

Bni Mellal	BM-1	24	52.9±1.04	28.7±0.54	1.9±0.06	6.4±0.11	0.5±0.01	1.9±0.06	66.9±0.66	23.2±0.30	2.9±0.06
	BM-10	25	49.1±0.71	31.0±0.78	1.6±0.06	6.2±0.10	0.4±0.01	2.0±0.07	68.9±0.22	21.6±0.45	3.2±0.07
	BM-11	26	57.4±2.21	25.8±4.39	2.3±0.47	6.1±0.53	0.5±0.04	1.6±0.06	69.5±0.76	21.3±0.89	3.3±0.18
	BM-12	27	55.7±0.46	24.4±1.15	2.3±0.12	6.0±0.56	0.4±0.01	2.3±0.41	72.1±0.83	18.1±0.17	4.0±0.07
	BM-13	28	59.4±0.59	35.1±1.87	1.7±0.11	6.3±0.45	0.5±0.02	2.3±0.35	67.6±2.65	21.4±0.46	3.2±0.12
	BM-14	29	57.5±1.30	25.3±2.18	2.3±0.26	6.9±0.19	0.5±0.01	1.7±0.23	64.9±3.95	21.8±0.57	3.0±0.13
	BM-15	30	59.3±0.61	23.4±0.92	2.5±0.10	6.5±0.41	0.5±0.04	1.6±0.09	71.9±1.38	17.5±1.50	4.2±0.38
	BM-2	31	57.2±0.66	26.9±0.76	2.1±0.04	6.4±0.05	0.5±0.12	2.9±0.02	67.4±0.76	22.2±0.19	3.0±0.03
	BM-3	32	57.6±1.46	27.3±0.17	2.1±0.05	6.2±0.12	0.6±0.11	1.8±0.14	74.3±0.31	16.5±0.34	4.5±0.09
	BM-4	33	52.8±0.70	26.4±0.54	2.0±0.02	6.5±0.33	0.5±0.04	2.1±0.09	66.1±0.19	23.7±0.59	2.8±0.07
	BM-5	34	48.7±1.07	34.3±0.82	1.4±0.06	6.4±0.26	0.4±0.00	1.5±0.07	66.8±0.56	23.7±0.51	2.8±0.05
	BM-6	35	54.2±1.23	28.0±0.48	1.9±0.07	5.8±0.66	0.5±0.01	2.2±0.08	67.2±0.21	22.7±0.53	3.0±0.07
	BM-7	36	57.0±0.89	195±0.39	2.9±0.04	6.2±0.38	0.5±0.11	2.0±0.01	64.6±1.56	25.7±1.16	2.5±0.16
	BM-8	37	51.2±0.46	21.4±0.35	2.4±0.05	6.2±0.12	0.5±0.10	1.8±0.02	65.5±0.43	25.2±0.15	2.6±0.02
	BM-9	38	56.7±0.97	23.3±0.22	2.4±0.04	7.6±0.40	0.7±0.10	1.8±0.04	68.4±0.38	20.8±0.51	3.3±0.07

	SF-1	39	54.8±0.86	30.7±0.47	1.8±0.03	6.1±0.15	0.5±0.04	2.7±0.30	69.0±0.90	21.1±1.02	3.3±0.16
Sfasif	SF-2	40	57.5±0.31	26.0±0.82	2.2±0.06	6.5±0.34	0.5±0.01	1.6±0.04	68.0±0.25	22.4±0.43	3.0±0.07
	SF-3	41	55.7±1.01	26.3±0.54	2.1±0.06	6.3±0.33	0.6±0.04	1.5±0.04	67.0±0.82	23.6±0.47	2.8±0.03

a Code number for genotype identification in Fig. 1.

Table 3 Mean values of the each chemical component of each population for two crop years

Variable	Population	Year [a]		mean [b]
		2009	2010	
Oil content (Ol) (% of kernel DW)	Aknoul	55.9	57.5	56.7 b
	Bni Mellal	54.7	55.6	55.1 d
	Azilal	58.6	58.9	58.7 a
	Sfassif	55.7	56.3	56.0 c
	Mean	56.1	57.1*	56.6
Protein content (P) (% of kernel DW)	Aknoul	25.2	25.0	25.1 c
	Bni Mellal	27.3	26.2	26.7 b
	Azilal	21.4	21.4	21.4 d
	Sfassif	27.8	27.5	27.7 a
	Mean	25.3	24.8*	25.1
R1 (Ol/P ratio)	Aknoul	2.3	2.4	2.3 b
	Bni Mellal	2.1	2.2	2.1 c
	Azilal	2.9	2.9	2.9 a
	Sfassif	2.0	2.1	2.0 d
	Mean	2.3	2.4*	2.4
Palmitic (% of total oil content)	Aknoul	6.3	6.6	6.4 b
	Bni Mellal	6.2	6.5	6.4 b
	Azilal	6.7	7.4	7.15 a
	Sfassif	6.3	6.4	6.3 b
	Mean	6.3	6.7ns	6.5
Palmitoleic (% of total oil content)	Aknoul	0.5	0.6	0.6 b
	Bni Mellal	0.5	0.5	0.5 c

	Azilal	0.7	0.7	0.7 a
	Sfassif	0.5	0.5	0.5c
	Mean	0.5	0.6	0.6
	Aknoul	1.9	2.0	2.0 a
	Bni Mellal	1.9	2.0	2.0 a
Stearic (% of total oil content)	Azilal	1.9	2.0	1.9 a
	Sfassif	1.8	2.0	1.9 a
	Mean	1.9	2.0*	2.0
	Aknoul	71.4	70.0	70.7 a
	Bni Mellal	68.6	67.7	68.1 c
Oleic (O) (% of total oil content)	Azilal	69.4	70.0	69.7 b
	Sfassif	67.6	68.4	68.0 c
	Mean	69.7	69.0*	69.4
	Aknoul	18.8	19.5	19.2 d
	Bni Mellal	21.5	21.8	21.7 b
Linoleic (L) (% of total oil content)	Azilal	19.4	19.9	19.7 c
	Sfassif	22.3	22.5	22.4 a
	Mean	20.2	20.7*	20.4
	Aknoul	4.1	3.8	4.0 a
	Bni Mellal	3.2	3.2	3.2 c
R2 (O/l ratio)	Azilal	3.7	3.6	3.7 b
	Sfassif	3.1	3.0	3.0 d
	Mean	3.6	3.5*	3.6

a Mean difference between year significant at $P > 0.5$ (*) or non-significant (ns).

b Population means for each compound or ratio followed by different letters are significantly different at $P > 0.5$.

Table 4 Eigenvectors of the 3 principal components axes from PCA analysis of the Moroccan almond seedlings[z]

Variable	PC1	PC2	PC3
Oil content	0.11	**0.48**	-0.24
Protein content	-0.01	**-0.52**	0.16
R1 (Oil/Protein)	0.01	**0.55**	-0.24
Palmitic acid	0.02	0.35	**0.57**
Palmitoleic acid	0.27	0.24	**0.51**
Stearic acid	-0.19	0.05	**-0.48**
Oleic acid	**0.54**	-0.11	-0.15
Linoleic acid	**-0.55**	0.05	0.09
R2 (Oleic/Linoleic)	**0.54**	-0.06	-0.15
Eigenvalue	3.16	2.88	1.58
Proportion of total (%)	35.16	32.03	17.63

[z] Eigenvalues and their contribution to total variation are listed at the bottom of columns

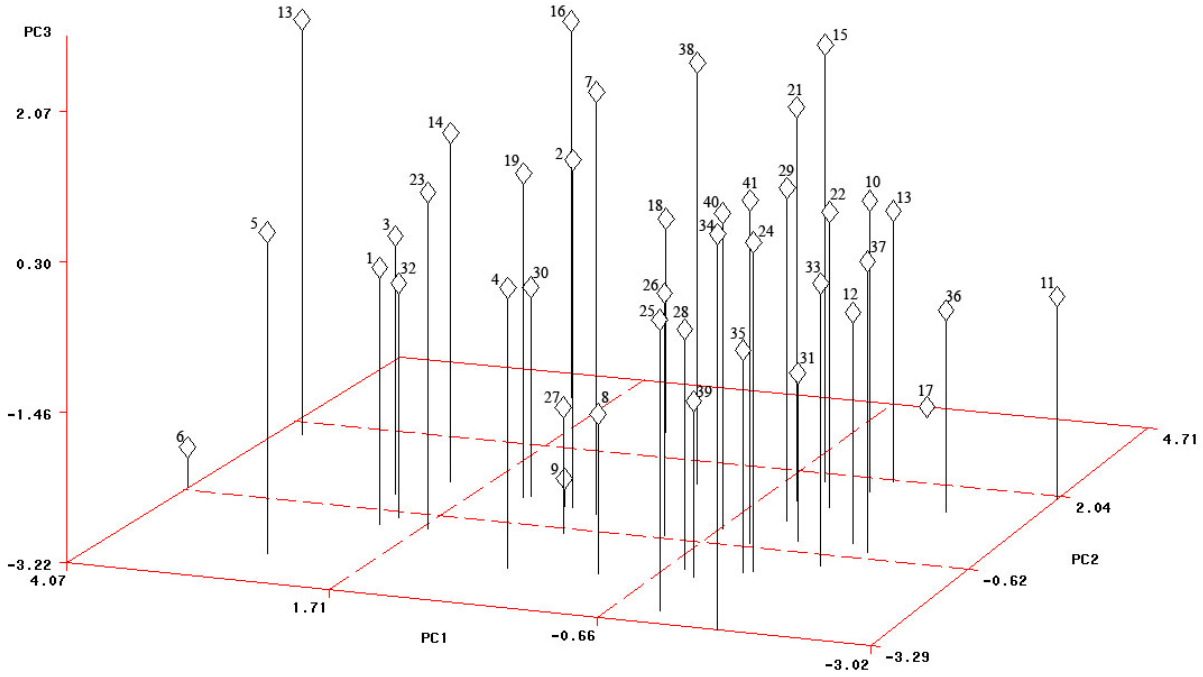

Fig. 1 Position of the principal component (PC) scores of the almond kernel composition for 41 Moroccan almond seedlings. Numbers refer to the seedling code number (Table 2)