Valorisation of local almond genotypes regarding their biochemical and mineral compositions

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Abstract. The new tendency for intensification of almond plantations has induced a clear neglect of local ecotypes and cultivars that have proved high performances and good adaptation to many biotic and abiotic stresses. Prospecting the main producing regions in Tunisia has demonstrated a high genetic diversity. In addition, all of the pomological, biochemical and mineral characterizations have confirmed the potentialities of some local genotypes in comparison to the newly introduced cultivars. The analysis of their content in some biochemical compounds such as antioxidant has furthermore shown that some landraces are highly rich in α, γ and δ-tocophérols. Our result reported that the mineral composition of this nut is dominated by phosphor, calcium, iron, sodium, zinc and copper. In comparison to some introduced cultivars such as 'Mazzetto', 'Lauranne' and 'Supernova' many local ecotypes presented high performances. In fact the ecotype 'BF2', was highly rich in potassium and phosphor while 'TL7' in calcium. Additionally, the dominance of 'khoukhi', 'Dillou' and 'Blanco' for the iron element composition is another statement in favour of existent possibilities for a better valorisation of the local germplasm and consequently for its better preservation.

Keywords. *Prunus dulcis* L. – Local landraces – Antioxydants – Fat acids.

Valorisation des génotypes locaux d'amandier par analyse de leurs compositions biochimiques et minérale

Résumé. *La tendance actuelle vers l'intensification des plantations d'amandier a induit une nette négligence des variétés et des écotypes locaux. Ceux-ci ont montré des potentialités agronomiques très intéressantes ainsi que des niveaux satisfaisant de tolérance à divers stress biotiques et abiotiques. Les travaux de caractérisation pomologique, biochimique et minérale ont prouvé les performances de ces individus vis a vis des variétés introduites comme 'Mazzetto', 'Lauranne' et 'Supernova'. En effet, l'analyse du contenu des fruits en antioxydants a montré la richesse de certains écotypes en α, γ and δ-tocophérols. D'autre part, nos résultats ont confirmé que la composition minérale des fruits d'amandier est dominé par les éléments phosphore, calcium, fer, sodium, zinc et cuivre. D'ailleurs, les fruits de l'écotype 'BF2' sont remarquablement riche en potassium et en phosphore. De plus, la richesse en élément fer des écotypes du nord comme 'khoukhi', 'Dillou' et 'Blanco' sont tous des arguments en faveur de la possibilité d'une meilleure valorisation des ressources génétique d'amandier en cosmétique et dans l'industrie alimentaire ainsi que leurs conservations.*

Mots-clés. Prunus dulcis *L. – Écotypes locaux – Antioxydants – Acides gras.*

I – Introduction

Almond (*Prunus dulcis* L.) is a major nut tree grown around the Mediterranean area. In Tunisia it occupies the second position after olive tree with more than 250,000 ha. It is spread all over the country under different bioclimatic stages and it represents a principal income for many farmers living in extreme climate condition regions. Fruits are generally kept in the tree until their hulls are almost dry for an easier removal at harvesting time. In the local market quality and consumer choice is based on the presence of the main cultivars traditionally known as good products for consumption and culinary preparations. In fact, the two cultivars 'Achaak' and 'Porto' are the more desirable and quoted in the almond market. Unknown ecotypes are generally mixed altogether and sold at very low prices. The prospecting effort started few years ago has demonstrated the presence of a large almond diversity with two distinguished genetic pools one in the north and a second in the central and southern part of the country (Gouta *et al.*, 2010). Unfortunately, the Tunisian almond germplasm is actually threatened by lost and genetic erosion.

As the kernel is the edible part of the nut and the main fraction of it are lipids, many researchers have evocated that this fraction constitutes an important caloric source. It does not contribute to cholesterol formation in humans due to the high level of unsaturated fatty acids which are negatively correlated with serum lipid profiles and cholesterol status associated with a lower risk of cardiovascular diseases (Sabate and Hook, 1996; Jenkins *et al.*, 2008). Tocopherols with the four different homologues acting as antioxidants are believed to be involved in a diversity of physiological, biological and biochemical functions (Reische *et al.*, 1998) and are considered as a valuable added compound in almond (Marwede *et al.*, 2004). Moreover, sweet almonds are considered to be as an important source of macro and micro-elements especially potassium, calcium, magnesium and manganese (Souty *et al.* 1971; Saura Calixto and Canellas, 1982; Schirra, 1997).

The aim of this study is the evaluation of the biochemical and mineral compositions of the main almond cultivars in order to find an added value for the local almond genotypes for a better valorisation and consequently a better preservation.

II – Materials and methods

Fruits were harvested at maturity stages from 10 years old almond trees grafted on 'Garnem' rootstock and preserved at the national collection of Sidi Bouzid (35.117 N, 9.567 E; 369 m above sea level.). Trees were drip irrigated and conventional technical practices were applied. For each genotype, three replicates of 50 fruits were randomly collected. After cracking, seed coats were removed and kernels were dried at room temperature for 2 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 h with petroleum ether as solvent. 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 (EEC Regulation 2568/91). These methyl esters were separated by use of a flame ionization detector (FID) gas chromatograph. The identification of the FAMEs was achieved by comparison with relative retention times in a reference sample that contained standard methyl esters. The individual tocopherol isomers were analyzed using a reversed phase by high performance liquid chromatography, model 360 (Kontron, Eching, Germany) (Kodad *et al.*, 2006).

For mineral analyses K, Na and Ca were analysed using a Spectrophotometric method while Fe and Cu were analysed by using an Atomic Absorption Spectrophotometer according to Pauwels *et al.*, 1992.

Statistical analysis: The one factor ANOVA and the principal component analysis were done with the software SPSS, 17.0. The mean separation was done with the Duncan test at a probability of 0.05.

III – Results and discussion

The concentrations of most of the mineral elements in the kernel for the fifteen almond genotypes of this study revealed significantly differences (Table 1). Potassium (K) was the most predominant mineral element with values ranging from 595.12 mg/100g for 'Supernova' to 1381.87 mg/100g for the local ecotype 'BF2'. In the second position we found the phosphorus (P) and as for potassium the ecotype 'BF2' has the highest content (768.33 mg/100g) compared to the lowest (367.71 mg/100g) presented by 'KF1'. This last value does not differ significantly with those presented by the introduced cultivars 'Supernova' (396.30 mg/100g), 'Mazzetto' (436.67 mg/100g) and 'Lauranne' (470.83 mg/100g). Values observed for the well known local cultivars 'Achaak'; 'Fekhfekh', 'Ksantini' and 'Zahaaf' for K and P were important but not the highest. In fact, they were respectively 897.56, 1151.22, 1146.34 and 1073.17 mg/100g for K and 478.33, 467.29, 646,04 and 472.29 mg/100g for P. The dominance of these two macroelements (K and P) in almond was also confirmed by previous reports (Prats-Moya *et al.*, 1997; Saura Calixto *et al.*, 1981; Schirra, 1997). Almond kernels of the genotypes studied are also an important source of calcium. Values were 367.67 mg/100g for the ecotype 'TL7', 346.67 mg/100g for 'TL6' and 316.67 mg/100g for 'BF2'. Relatively low values were observed for 'Mazzetto' (191.67 mg/100g), 'Lauranne' (200.00 mg/100g) and 'Supernova' (137.5 mg/100g).

Regarding microelements our results showed a clear superiority of cultivars from the north of Tunisia for iron (Fe). This was clear for the cultivars 'Khoukhi' (133.7 mg/100g) and 'Blanco' (103.3) and for the ecotype 'Dillou' (112.8 mg/100g). For the remaining elements copper (Cu) and zinc (Zn) the performances of the ecotype 'BF2' were also confirmed by the high values of 3.63 mg/100g and 5.52 mg/100g, respectively.

The significant differences in the contents of individual mineral elements can have different origins starting by genetic to ecological, culture (soil, water availability, rootstocks) or climate conditions (Aslantas *et al.*, 2001). Socias *et al.* (2008) showed that genetic variability coefficients for calcium, magnesium and potassium tend to be less significant among years and suggested opportunities for genetic manipulation.

The high content of macro and microelements observed for some ecotypes was clearly confirmed by the principal component analysis (Fig. 1). The two first components explained 60% of the variability observed. The first component was correlated with P, Cu, K and Zn contents, while the second one was correlated with Ca, Fe and Na contents. The presence of the ecotypes 'BF5' on the extreme left and 'TL7', 'TL6' and 'Dillou' on the upper party reflects their potentialities regarding mineral contents. These can be valorised as important dietary source for these essential elements. Also with the actual development of processing of almond these nuts can be used as natural additives and sources of iron, calcium or potassium. Moreover, almond milk is considered as a vegetable milk substitute recommended in cases of intolerance to cow's milk (Cotta Ramusino *et al.*, 1961).

In a second step we were interested in the biochemical analyses of the main Tunisian almond cultivars (Table 2). Although, the local cultivars 'Mahsouna' and 'Faggoussi' showed the highest lipid content (respectively 59.2 and 59.3%) no large range was observed for the ten genotypes concerned by this study. In fact the lowest value was noted for 'Elloumi' but this value does not differ significantly from the contents of the foreigner cultivars: 'Mazzetto' (54.3%), 'Lauranne' (55.5%) and 'Supernova' (55.5%). These values are relatively higher then those reported by Ahrens *et al.* (2005) for the Californian commercial cultivars (from 35 to 54%).

Regarding fatty acid composition we confirmed as reported in the literature (Yada *et al.*, 2011) the dominance of five major fatty acids (FA). They were in decreasing order (Table 2), oleic (18:1) with values ranging from 78.3% for 'Elloumi' to 64% for 'Porto'. The second is linoleic (18:2) with a highest value of 25.9% for 'Porto' followed by palmitic (16:0) with two superior values for 'Elloumi' and 'Ksontini'. These two local cultivars dominate for the stearic (18:0) with a value of 3.3%. The last is the palmitoleic (16:1) and is generally presented as traces (<1%). Our study revealed an exception with the cultivar 'Elloumi' that present a small content (3.9%) of gadoleic (20:1). According to many authors these differences are genetically and independent of growth conditions. Prats-Moya *et al.* (1999) showed that triacylglycerol composition could be used to distinguish among almond genotypes.

Table 1. Kernel mineral composition of the main Tunisian almond cultivar in comparison to some foreigner cultivars

Cultivar	Cu (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)	K (mg/100 g)	P (mg/ 100 g)	Ca (mg/100 g)	Na (mg/100 g)
'BF2'	3.63 a	24.97 gh	5.52 a	1381.87 a	768.33 a	316.67 cb	83.33 ab
'Achaak'	1.16 ef	84.53 de	3.90 bc	897.56 cd	478.33 cdef	225.00 efg	76.7 abc
'Fekhfekh'	1.70 cb	28.77 g	4.60 ab	1151.22 b	467.29 def	191.67 fgh	82.5 ab
'Ksantini'	1.49 cdef	72.05 e	3.14 cd	1146.34 b	646.04 ab	291.67 bcd	75.0 abc
'Zahaaf'	1.19 ef	85.50 de	4.14 cb	1073.17 bc	472.29 cdef	175.00 gh	70.0 bcd
'TL7'	1.22 def	86.13 de	2.66 d	790.24 d	404.38 ef	376.67 a	78.3 abc
'KF1'	1.60 bcde	11.05 h	2.62 d	1151.22 b	367.71 f	183.33 gh	68.3 cd
'TL6'	1.97 b	93.97 cd	3.74 bcd	926.83 cd	572.08 bcd	346.67 ab	80.3 abc
'TL8'	1.21 de	86.13 de	4.16 bc	1180.49 b	624.38 abc	160.00 h	79.3 abc
'Blanco'	0.79 f	103.30 bc	4.84 ab	1195.12 b	608.44 bc	255.00 def	51.3 e
'Dillou'	1.63 bde	112.80 b	4.24 bc	1056.91 cb	555.83 bcde	253.33 def	85.0 a
'Khoukhi'	1.96 b	133.70 a	3.81 bcd	1089.43 bc	433.54 def	282.67 cde	77.6 abc
'Mazzetto'	1.66 bcd	34.87 g	4.04 bc	1087.80 bc	436.67 def	191.67 fgh	67.5 cd
'Lauranne'	1.78 bc	52.40 f	4.36 abc	892.68 cd	470.83 cdef	200.00 fgh	58.3 de
'Supernova'	1.10 f	49.15 f	3.31 cd	595.12 e	396.30 ef	137.50 h	77.5 abc

a,b,c,d,e,f,g,h Significant difference at Duncan's multiple Range Test (5%).

Table 2. Oil content, fatty acid and α, γ and δ- tocophérol compositions of the main Tunisian almond cultivars

Cultivar	Oil content (%)	C16:0 (%)	C16:1 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C20:1 (%)	α-tocophérol (mg/kg)	γ-tocophérol (mg/kg)	δ-tocophérol (mg/kg)
'Zahaaf'	54.0 d	6.8 b	0.6 ab	1.6 e	73.9 bc	16.9 bc	–	344.4 c	5.0 e	.09 e
'Ksontini'	54.3 cd	9.0 a	0.5 cde	3.3 ab	76.2 ab	10.0 d	–	296.8 d	7.0 c	.15 cd
'Mahsouna'	59.2 a	6.6 b	0.6 b	2.7 c	75.9 bc	14.0 bc	–	257.0 e	10.2 b	.57 a
'Faggoussi'	59.3 a	6.2 b	0.5 bc	1.8 de	71.5 c	19.7 b	–	269.5 de	6.1 d	.087 e
'Elloumi'	53.3 d	10.0 a	0.4 e	3.3 a	78.3 a	3.5 e	3.9	208.4 f	3.41 f	.02 f
'Abiodh Sfax'	58.1 ab	6.2 b	0.5 cd	2.8 ab	73.3 bc	16.9 bc	–	277.6 de	5.41 de	.14 de
'Porto'.	57.0 abc	7.1 b	0.5 cd	2.2 cd	64.0 d	25.9 a	–	340.1 c	6.0 d	.19 c
'Mazzetto'	54.3 dc	6.6 b	0.5 cd	2.6 c	73.5 d	16.5 a	–	495.9 a	12.5 a	.37 b
'Lauranne'	55.5 bcd	6.5 b	0.7 a	1.7 de	74.4 abc	16.4 bc	–	289.81 d	3.1 f	.08 e
'Supernova'	55.5 bcd	6.4 b	0.5 de	2.5 bc	74.5 abc	15.9 bc	–	403.6 b	6.9 c	.017 f

a,b,c,d,e,f,g,h Significant difference at Duncan's multiple Range Test (5%).

Finally, it is actually well known that almond kernel is a good source of tocopherols with a domination of α-tocophérol followed by γ and δ isomers (Kodad *et al.* 2006).

In our study (Table 2), the concentration of α-tocophérol ranges from 495.9 mg/kg of oil for 'Mazzetto' to 208.6 mg/kg for 'Elloumi' but many other local cultivars presented high values. In fact we registered also the values of 344.4 mg/kg for 'Zahaaf' and 340.1 mg/kg for 'Porto'. For the other isomers high values of γ tocopherol were obtained for 'Mazzetto' (12.5 mg/kg) and 'Mahsouna' (10.2 mg/kg) while only traces (<1 mg/kg) were observed for the δ tocopherol.

While α-tocophérol is the form of vitamin E efficiently used by the human body and is yet often deficient in modern diet local (Pongracz *et al.*, 1995; Krings and Berger, 2001) genotypes with high content could be used in almond confectioneries, with chocolates or to prepare sweets and syrup.

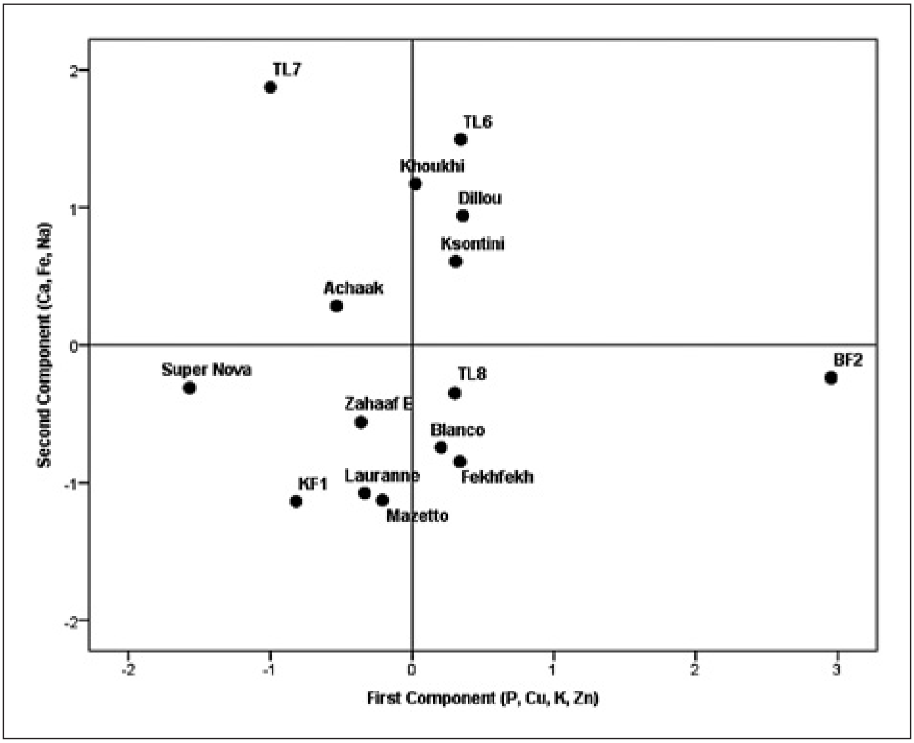

Fig. 1. PCA on the mineral composition of some local almond genotypes.

IV – Conclusions

The biochemical and mineral composition analyses carried out in this study have demonstrated that many local cultivars and unknown ecotypes showed high performances and can be easily valorised and, therefore, preserved from genetic erosion. This fruit consumption either roasted, crude, as natural food additives or as supplements for some deficiencies such as anaemia or bones calcium degradation can incite a national effort to preserve an important heritage in degradation.

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