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Variability of oil content and its physico-chemical traits from five almond (*Prunisdulcis*) cultivars grown in northern Morocco.

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Abstract

In Morocco, almond (*Prunusdulcis* [Mill.] D.A. Webb) is the most important nut tree and the second after olive in terms of commercial production. In this work, we investigated almond oil content and its physico-chemical traits from five cultivars widely grown in northern Morocco. Oil quality determinations consisted of polyphenols content (PP), acid value (AV), peroxide value (PV), and UV absorption coefficients (K_{232} and K_{270}). For all the studied traits, cultivar was the main source of variability, although site and cultivar×site effects were also significant. Mean values averaged between sites of oil content ranged from 49.50% dry weight of total kernel in FournatdeBreznaud to 56.75% in Marcona. Low oil yielding cultivars with early maturity were the highest in terms of PP, resulting in a negative correlation between PP and oil content($r = -0.917^{***}$). K_{232} and K_{270} were significantly correlated to PV. Principal component analysis (PCA) allowed a better discrimination between the five cultivars and sites. In fact, 70% of total variability was genotypic-dependent while 15% was attributed to environmental effect. Oil samples analyzed were generally of excellent quality with low values of AV, PV, K_{232}, and K_{270} and higher values of PP.

1. Introduction

Almond is the most important nut worldwide in terms of commercial production. USA and countries of the Mediterranean basin are the main producers. According to FAOSTAT'syearbook[1],the world production reached 2,917,894 tonnes of unshelled almond, of which Morocco (4th worldwide ranking) produced 96,523 tonnes.

Sweet almond is commercially grown worldwide.It is cultivated for its kernel, which is the edible part of the nut and it is known to be of high nutritional value.It can be consumed raw, roasted, unblanched or blanched, and incorporated into other products after transformation (mainly sliced and slivered almonds). The important nutritive value of almond kernel arises from its high lipid content, which forms an important source of caloric energy of 6 Kcal/g [2]. In the literature, the majority of almond composition researches have an emphasis on lipid fraction and its composition, particularly fatty acids. In almonds, lipids are present as intracellular oil droplets which are linked to many cellular functions, including lipid storage [3]. These droplets accounted for about 1-3 µm of diameter in the cotyledon tissues of kernels [3, 4].A wide range of variability in lipid content has been reported for almond from different geographical origins [5-9].Sweet almonds are rich sources of lipids, protein, dietary fiber, minerals, polyphenols, and vitamins [8, 10,11].

The Committee on Fats and Oils of the Codex Alimentarius does not describe physico-chemical characteristics of almond oil since it is produced at a small scale in few countries like France, Spain and USA. Besides, almond oil has long been used in complementary medicine circles for its numerous health benefits [12]. It has been demonstrated that almond oil has several properties including anti-inflammatory, immunity-boosting and anti-hepatotoxicity effects [13-16]. It is also used as component of dry skin creams, anti-wrinkle, and anti-aging products in the cosmetic industry as well as for pharmaceutical purposes. The increase of almond production

globally with demand of new specialty oils encourages screening of both almond cultivars and genotypes for higher oil content but also evaluate its quality under different environments.
Northern Morocco hosts a wide genetic diversity both of commercial cultivars and seedlings (local genotypes). This region presents approximately 37% of cultivated area and ensures 18% of national production of almonds. As far as we know, no previous studies were carried out to characterize oil content and its physico-chemical traits in commercial cultivars grown in northern Morocco. Therefore, the objectives of the present work were: (i) to determine oil content in the five widely grown almond cultivars of northern Morocco, and (ii) to investigate some physico-chemical traits of extracted oils from these cultivars.

2. Experimental details

2.1. Plant material and sampling

Plant material consisted of five almond commercial cultivars namely: Marcona, Fournat de Breznaud, Ferragnès, Ferraduel, and Tuono, known to be the most important in northern Morocco. Prior to harvesting, three individual orchard trees (considered as replicates) of these cultivars were marked in three different sites of northern Morocco (Figure 1): Aknoul (60 km from Taza, 34°39′0″ N, 3°52′0″ W), BniHdifa (50 km from Al Hoceima, 35°1′22″ N, 4°8′27″ W), and Tahar Souk (50 km from Taounate, 35°1′22″ N, 4°8′27″ W).Northern Morocco is characterized by a Mediterranean climate humid in winter and semi-arid in summer. BniHdifa, under coastal influence, has an annual rainfall of 441 mm with average temperature of 15 °C. Aknoul and Tahar Souk, both semi-continental to Mediterranean influence, receive about 461 mm and 472 mm of annual precipitations with average temperature of 14.5 °C and 14.2 °C, respectively.
At physiological maturity stage, which fits 89 on the BBCHphenological scale, we harvested about 1.5 Kg of fruits around the canopy fromeach of all marked trees across the three sites during the 2015growing season. Samples were brought to laboratory in black bags until analysis.

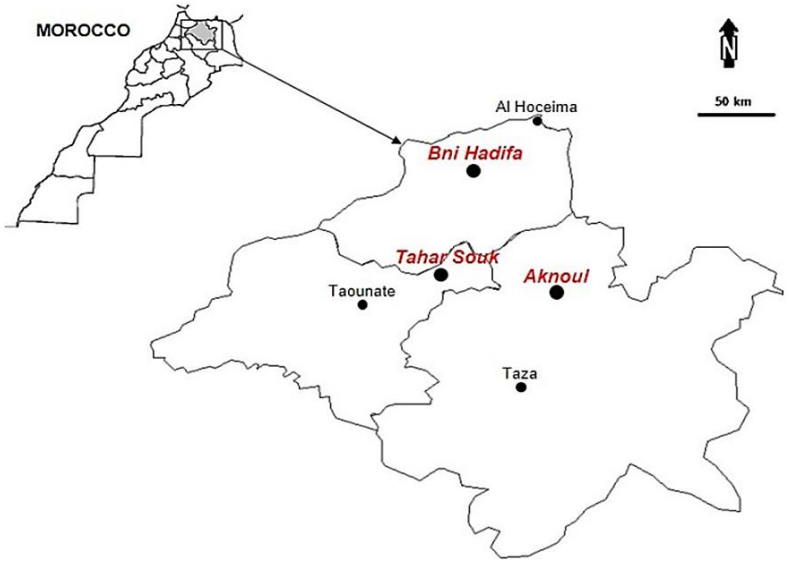

Figure 1. Geographical localisation of sampling sites.

2.2. Oil content determination

Firstly, fruits were shelled and cracked using a hammer to release kernels. Dry weight was determined on sub-samples of about 200g of kernels. Oil extraction was achieved with a Komet screw press (Model DD85G, Germany), with a screw speed of 20 rpm. The screw press was first run for 15 min without kernel material but with heating means. During extraction period, temperature of pressing was maintained at 40°C according to Martínez*et al.* [17]. After each extraction, all press devices were cleaned and dried. Oil content (OC) was expressed as percentage of dry weight using the following formulae:

$$\mathbf{OC}=\frac{(\boldsymbol{M1}-\boldsymbol{M2})}{\boldsymbol{M}}\times 10$$

Where *M* is the weight of the dry kernels, *M1* the weight of the flask containing the oil, and *M2* the weight of the empty flask. Oil samples were transferred to dark glass vial and stored at -20°C until analysis.

2.3. Physico-chemical traits measurements

Acid value known also as free acidity (AV), peroxide value (PV), specific extinction coefficients K_{232} and K_{270} were determined according to standard methods of AOCS[18] with slight modifications.Concerning the AV determination, 500 mg oil was dissolved in ethanol and then neutralized by KOH 0.01N.PV was measured by dissolving about 1 g oil in 30 ml of acetic acid and chloroform (3:2, v/v) mixture. 500 µl of saturated KI were added to the solution and left to react for one minute before adding 30 ml of distilled water. The titration of the iodine excess was performed using $Na_2S_2O_3$ 0.01N and the PV was expressed as meq peroxide per kg of oil.The extinction coefficients $K_{232}$and $K_{270}$of almond oil were calculated respectively from the absorption at 232 and 270 nm. A 1 g of oil was dissolved in cyclohexane and the UV absorbance was measured in an UV spectrophotometer (RAYLEIGH, UV-1800, Beijing Rayleigh Analytical Instrument Corporation (BRAIC), Beijing, China).Total polyphenols (PP) content in oil samples was determined in methanolic extract by Folin–Ciocalteau method, the absorbance was measured at 760 nm as wavelength. A calibration curve was done using gallic acid (GA) and the results were expressed as mg GA/g oil.

2.4. Statistical analysis

All determinations were performed in triplicate. Combined analyses of variance (ANOVA) were carried out using the general linear model procedure over the three sites. Least significance differences (LSD) were computed at 5% as probability level. The relationship between the studied traits was established and the most important among them were graphically represented. A multivariate statistical analysis using principal component analysis (PCA) was performed on means of the whole studied traits to discriminate between cultivars and sites. All statistical analyses were carried out by means of STATGRAPHICS package version XVI (Statpoint Technologies, Inc., Virginia, USA).

3. Results

3.1. Analyses of variance

Table 1 summarizes mean squares from the combined analyses of variance for the studied traits. Cultivar effect was predominant for the majority of traits and explained more than 50% for AV, K_{232}, and K_{272}, and more than 90% for OC, PP, and PV. Site and site×cultivar effects were of minor magnitude.

Table 1. Mean squares of the combined analyses of variance for almond oil content (OC), polyphenols (PP), acid value (AV), peroxide value (PV), and UV specific coefficients of absorption(K_{232} and K_{270}).

Source of variance	df	OC	PP($\times10^{-3}$)	AV	PV($\times10^{-3}$)	K_{232}	K_{270}
Site	2	7.16***	8.00***	0.065*	2.31***	0.767***	0.018***
Cultivar	4	80.46***	63.34***	0.083**	55.96***	1.084***	0.030***
Replicate (Site)	6	0.03*	0.06	0.003	0.01	0.051***	0.001
Site×Cultivar	8	0.50***	0.42***	0.111***	2.48***	0.112***	0.010***
Residual	24	0.10	0.05	0.016	0.01	0.006	0.001
Total	44						

*, **, and *** indicate significance at 0.05, 0.01, and 0.001 levels of probability, respectively.

3.2. Mean Comparison among sites

Averaged values recorded, for the three sites, are presented in Table 2. LSD's test revealed that there were significant differences ($P<0.05$) between sites for almost all traits. Aknoul exhibited the highest values of OC (53.50 %DW), AV (0.84 % oleic acid), PV (0.36 meqO_2/kg of oil), and K_{270} (0.147). However, higher scores of PP (0.65 mg GA/g oil) and K_{232} (1.69) were recorded in BniHadifa and Tahar Souk, respectively. The lowest values of OC (52.13%DW), PP (0.61 mg GA/g oil), AV (0.71% oleic acid), and PV (0.33 meqO_2/kg of oil) were observed in Tahar Souk. Regarding specific coefficients of extinction, lower records for K_{232} (1.24) and K_{270} (0.086) were displayed by Aknoul and BniHadifa, respectively.

Table 2. Mean values of sites for almond oil content (OC), polyphenols (PP), acid value (AV), peroxide value (PV), UV specific coefficients of absorption(K_{232} and K_{270}). Means for each character followed by the same letter are not significantly different at $P < 0.05$.

Sites	OC (%DW)	PP (mg GA/g oil)	AV (% Oleicacid)	PV (meqO2/kg of oil)	K_{232}	K_{270}
Aknoul	53.50 a	0.64 b	0.84 a	0.36 a	1.24 c	0.147 a
BniHadifa	52.94 b	0.65 a	0.75 ab	0.34 b	1.46 b	0.086 b
Tahar Souk	52.13 c	0.61 c	0.71 b	0.33 c	1.69 a	0.145 a

3.3. Mean comparison among cultivars

Mean values of the investigated traits for cultivars averaged between sites are summarized in Table 3. Wide variations were shown between all cultivars for the majority of studied characters. Marcona had the highest OC (56.75%), while Fournat de Breznaud presented the lowest one (49.50%). PP content was found to vary significantly among cultivars and ranged from 0.56 mg GA/g oil (Marcona) to 0.76 (Fournat de Breznaud). The values obtained for AV and PV were higher in Fournat de Breznaud. K_{232} varied from 1.18 (Marcona) to 1.90 (Ferragnès). Regarding K_{270}, significant differences were found among cultivars with highest value for Ferraduel (0.19) and the lowest on for Tuono (0.06).

Table 3. Mean values of cultivars for almond oil content (OC), polyphenols (PP), acid value (AV), peroxide value (PV), UV specific coefficients of absorption(K_{232} and K_{270}). Means for each character followed by the same letter are not significantly different at $P < 0.05$.

Cultivars	OC (%DW)	PP (mg GA/g oil)	AV (% Oleicacid)	PV ($meqO_2$/kg of oil)	K_{232}	K_{270}
Ferraduel	53.78 c	0.58 d	0.80 ab	0.39 b	1.59 b	0.19 a
Ferragnès	54.04 b	0.59 c	0.63 c	0.39 b	1.90 a	0.14 c
Fournat de Breznaud	49.50 e	0.76 a	0.90 a	0.41 a	1.61 b	0.16 b
Marcona	56.75 a	0.56 e	0.77 b	0.23 d	1.18 c	0.07 d
Tuono	50.20 d	0.67 b	0.74 bc	0.30 c	1.04 d	0.06 e

3.4. Relationships among traits

Table 4 shows the matrix correlations between studied traits. Important associations were highlighted between some characters. In this regard, a high negative correlation was found between OC and PP (r=–0.917***). PV was positively associated with K_{232} (r= 0.586*), but also with K_{270} (r = 0.547*). Specific coefficients of extinction K_{232} and K_{270} were also positively correlated to each other (r=0.546*). PP content was also positively correlated to AV(r=0.329). No significant correlations were found between the remaining traits.

Table 4. Coefficients of Correlation among the studied traits: oil content (OC), polyphenols (PP), acid value (AV), peroxide value (PV), UV specific coefficients of absorption (K_{232} and K_{270}).

	OC	PP	AV	PV	K_{232}	K_{270}
OC		–0.917***	–0.164	–0.389	–0.045	–0.067
PP			0.329	0.404	–0.070	0.010
AV				0.089	–0.181	0.056
PV					0.586*	0.547*
K_{232}						0.546*
K_{270}						

*, **, and *** indicate significance at 0.05, 0.01, and 0.001 levels of probability, respectively.

It is worthy to point out that cultivars of earliest maturity (Fournat de Breznaud and Tuono) are characterized by lower OC, whereas later maturity cultivars (Marcona, Ferragnés, and Ferraduel) are found to be of high OC resulting in a positive correlation between OC and ripening time as shown in Figure 2 .

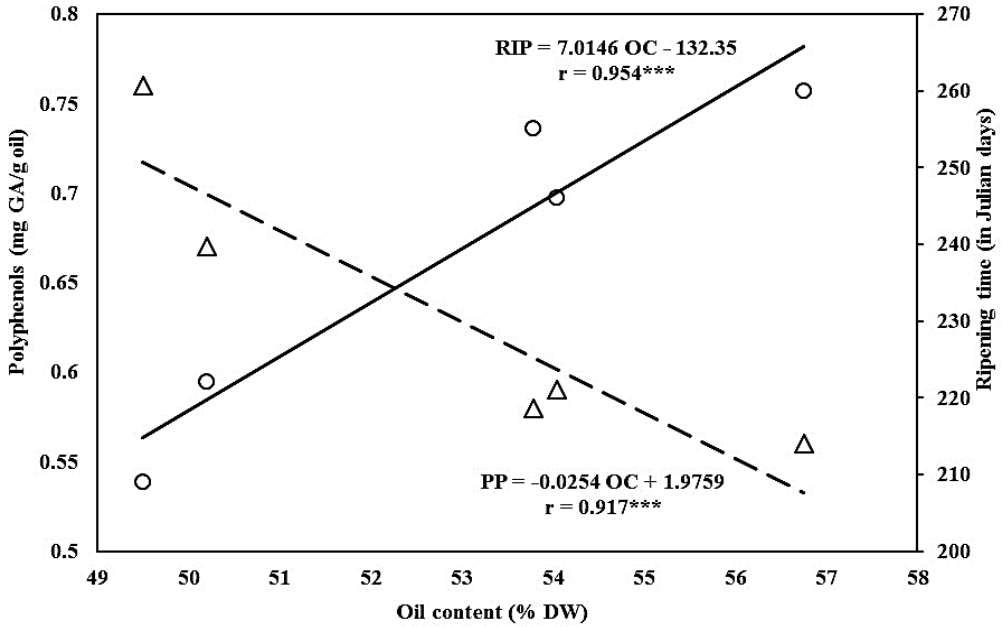

Figure 2 : Regression of polyphenols (PP) and ripenning time (RIP) on oil content (OC) . Points plotted are cultivars mean values averaged over sites for polyphenols (△) and oil content (○). DW = dry weight; r = correlation coefficient. *** indicate significance at 0.001 levels of probability.

3.5. Principal component analysis

The results showed that the three first PCAs explained over 85% of the total observed variance. PC1, PC2 and PC3 accounted for 40%, 30% and 15% of total variability, respectively. Points plotted on the surface delimited by PC1 and PC2 (Figure 3) are related to cultivars. PC1 allowed the separation of Marcona and its pollinator Fournat de Breznaud. In addition, Marcona interacted with OC on the positive direction of PC1, however Fournat de Breznaud was associated with PP and AV on its negative side. Nevertheless, along PC2 separated the three remaining cultivars. Ferragnès and Ferraduel were situated together on the positive direction of PC2 and interacted with K_{232} and K_{270}, while Tuono was on the negative side with the lowest values for K_{232}, K_{270}, and PV.

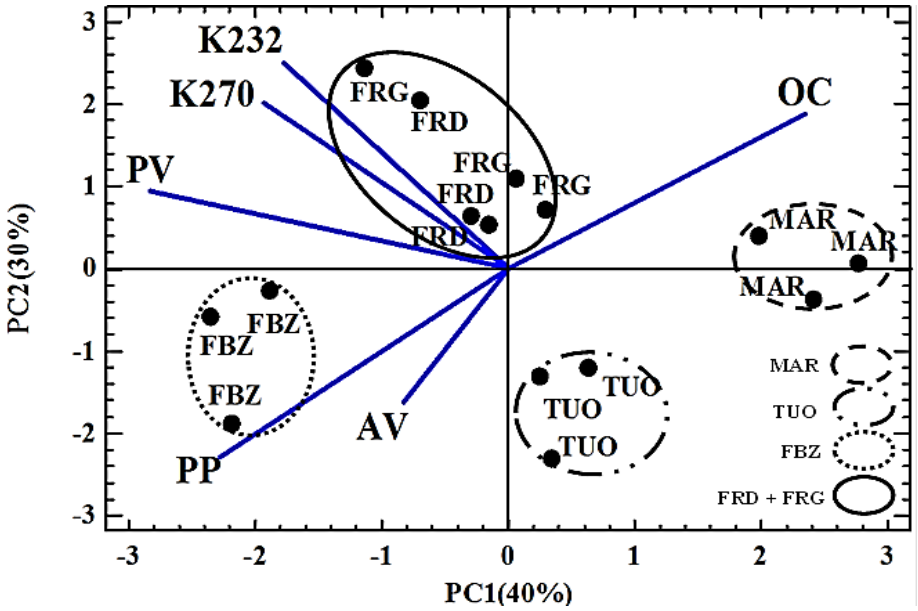

Figure 3 : Principal component analysis (PCA) projections on PC1 and PC2. The eigenvalues are symbolized as blue segments representing traits that most affect each principal cmoponent. OC=oil content, PP=polyphenols content, AV= acid value, PV=peroxide value, K_{232}=UV specific coefficient of absorption at 232nm, and K_{270}= UV specific coefficient of absorption at 270nm. The 15 points are cultivar mean values of each studied trait. FRD=Ferragnès, FRG=Ferragnès, FBZ= Fournat de Breznaud, MAR=Marcona, and TUO=Tuono.

Representative eigenvectors of PV, K_{232}, and K_{270}onFigure 3were plotted inthesame direction resulting in positive correlation between them. The same picture was reflected for PP and AV indicating a positive association between these two traits.Such associations confirmed the results of correlations analyses shown in Table 4. Moreover, eigenvectors of OC and PP on Figure 3 were opposed, which indicated a high and significant negative correlation between these two traits (r = –0.917***), already displayed in Table 4 and clearly manifested in Figure 2,which indicated that high oil yielding cultivars such as Marcona produced less PP and vice versa.

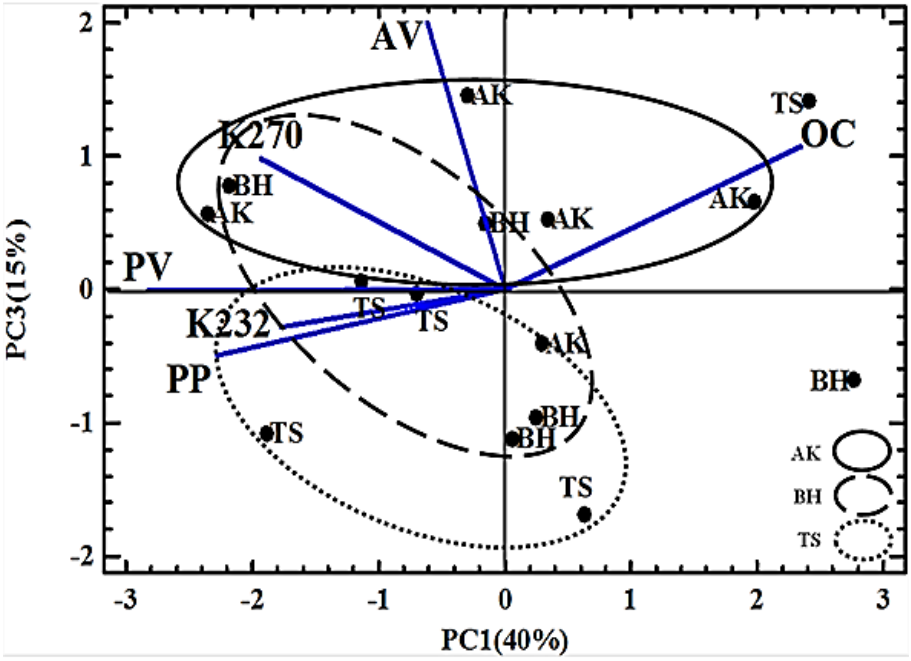

Figure 4:PCA projections on PC1 and PC3. The eigenvalues are symbolized as blue segments representing traits that most affect each principal component. OC=oil content, PP=polyphenols content, AV= acid value, PV=peroxide value, K_{232}=UV specific coefficient of absorption at 232nm, and K_{270}= UV specific coefficient of absorption at 270nm. The 15 points are site mean values of each studied trait. AK=Aknoul, BH=BniHadifa, and TS=Tahar Souk.

In the Figure 4are projected points related to sites on the plane delimited by PC1 and PC3. This latter allowed a clear separation between sites. In fact, Aknoul with higher values of OC, AV, and K_{270} was positioned towards the positive side of PC3, whileTahar Souk in the opposite direction interacted with K_{232} and PP.Points related to BniHadifa overlapped between Aknoul and Tahar Souk.

Discussion

In this research work, we focused on evaluation of oil content and its physico-chemical traits in five almond cultivars grown at three different sites in northern Morocco. Statistical analyses performed showed considerable variations in the studied traits for all cultivars and sites. OC varied significantly among sites in agreement with other research works [8, 19], these differences could be explained by pedoclimatic conditions varying from an environment to another. However, cultivar effect was the main variability source indicating that OC is genetically dependent. Previous works have reported significant effects of genotype on total oil content from seven Californian (USA) cultivars [6].In the present study, values of OC ranged between 49.5 and 56.75% and were slightly lower than those recorded in Spanish cultivars [5, 7]. Similar values were also reported in commercial cultivars grown in Argentina [8] and Australia [20]. Values of OC found in our study were lower than those of Moroccan genotypes [21], but higher than native and wild almonds from Iran [9, 22].

High oil yielding cultivars in our work, were the late ripening ones, indicating a positive association between oil content and ripening date. This correlation was in line with that found by Nanos *et al*., [23]forFerragnés and Texas.Such relationship could be assigned to cultivar differences regarding fruit development period (FDP). Forexample,Marcona, for which FDPwerelonger, accumulated the highest OC among cultivars.

Regarding the physico-chemical traits, polyphenols (PP) are bioactive compounds, which act as free radical scavengers. Owing to this propriety, PP play a crucial role for preventing the auto-oxidation of unsaturated fatty acids, and thereby increasing an oil's shelf life, but also they have preventive effects against reactive oxygen

species (ROS) involved in many pathologies[24] . In almond, the majority of polyphenolic compounds are concentrated in kernel skins. According to Bolling*et al*., [25], cultivar effect was the main source of variability of PP in agreement with our work. Total PP values found here are in line with previous findings[26]. The negative correlation between PP and OC means that an increase in oil content will be accompanied by a decrease of polyphenols content. Dag *et al*., [27] reported a rapid decline in oil polyphenols with late harvest and advanced maturation in two olive cultivars Barnea and Souri.Such associations were also demonstrated for other species [28, 29].

Quality parameters of almond oil investigated in our experiment were mainly under genetic determination in accordance with previously reported works [20, 30]. AV is defined as % of free oleic acid in an oil sample; in our results Ferragnès had the lowest AV, however the highest one was displayed by Fournat de breznaud. Low acidity value indicates the oil freshness, good quality, and no hydrolysis of glycerolipids. PVassesses the presence of peroxides and hydroperoxides and therefore gives information on oil oxidation. Freshly extracted oil is known to be of low PV, values found in our work ranged from 0.30 meqO2/kg of oil in Tuono to 0.41 in Fournat de Breznaud. These values of PV were below the normal range recommended by Codex Alimentarius with a maximum of 15 meqO2/kg of oil. Comparable values were reported by several authors for almond [20], Amashindwi[31], and Chia [32]. Moreover, oxidation products like hydroperoxides and derivatives measured by PV are considered as conjugated diene (CD) and conjugated triene (CT). CD and CT absorb light at 232 and 270nm respectively; this may explain the positive correlation between (PV, K_{232}) on one hand and between (PV, K_{270}) on the other hand.

Specific coefficients of absorption K_{232} and K_{270} are also useful tools in oil quality evaluation.UV absorbance at 232 and 270 nm is due to the presence of CD and CT, respectively, being products of oxidation occurring in oils. Hence, they indicate the degree of oil oxidation. The major fraction of almond oil is unsaturated fatty acids[9], their oxidation leads to the formation of hydro peroxides (the primary oxidation products), which absorb at 232nm. These primary oxidation products are unstable; thereafter they are quickly converted to secondary oxidation products mainly diketones and unsaturated ketones that absorb at 270 nm[33]. So the strong and positive correlation between K_{232} and K_{270} found its explanation in the fast transformation of primary oxidation products into secondary ones. In our work, K_{232} and K_{270} ranged from1.04 to1.90 and from 0.06 to 0.19 respectively, these finding are comparable to those reported by Maestri*et al*., [20].

Conclusions

A wide variability was observed between cultivars and sites for oil content and its physico-chemical traits, for which the genotype was the main variability source. High oil content in cultivars investigated here, make them good source of vegetable oil for cosmetic and pharmaceutical purposes. In addition, oils recovered from these cultivars were of good quality, which is demonstrated by low values of AV, PV, K_{232}, K_{270}, and higher PP content. These promising results should be completed by and analysis of fatty acids profile, tocopherols, and phytosterols.

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