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Effects of olive storage and packaging on microbial and fatty acids profiles of olive oil produced in traditional mills in Morocco

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Abstract
The aim of this work was to evaluate the effects of storage time of olive fruits and packaging type on the microbial and chemical quality of virgin olive oil produced in traditional mills in Morocco. 72 samples of olive oil of Moroccan Picholine variety were recovered in PET and dark glass bottles and analyzed after 3 and 6 months of storage. Results indicated that mesophilic and psychrotrophic bacteria, yeasts and moulds showed similar profiles in both containers, while, indices of quality and saturated fatty acids (SFA) increased, and unsaturated fatty acids (UFA) decreased. Significant changes were shown in palmitic, stearic, and oleic acids. Furthermore, accelerated degradations of oils were observed in plastic container. Statistical analysis showed a strong negative correlation between UFA and SFA, free fatty acids contents (FFAs), K232 and K270. Discriminate analysis showed that storing olive fruits plays important role in the classification of olive oil. In fact, long storage linked to the presence of microorganisms was responsible for significant changes produced in fatty acids profile and olive oil quality.

1. Introduction
Olive oil is the primary fat source in traditional Mediterranean diets [1]. It is particularly appreciated for its healthy benefits mainly attributed to its antioxidants components and to high content of unsaturated fatty [2, 3]. The monounsaturated fatty acid (MUFA), principally oleic acid, and the major essential fatty acids (linoleic and linolenic acids) are involved in reducing the risk of coronary heart diseases [4]. In addition, olive oil is appreciated for its high stability compared to other vegetable oils [5]. This resistance to oxidative degradation is mainly attributed to the fatty acid composition, particularly the unsaturated fatty acid fraction. However,olive oil is susceptible to lipid oxidation from theproduction until the consumption [6], generating formation of hydroperoxides, evaluated by peroxide index (PV) that indicates the early stages of oxidation and formation of the conjugated dienes and trienes, compounds formed from the hydroperoxides of unsaturated fatty acids and their fragmentation products [7]. These compounds contents, indicating the olive oil quality, are influenced by several factors lonked to the quality of olive fruits and procedures of harvesting, handling, transportation and storage prior to olive milling [8], the extraction system [9, 10] and to the storage time and conditions. During storage, olive oil is subject to hydrolysis, oxidation, auto-oxidation [11, 12] and polymerization, leading to the deterioration of its components, quality and nutritional values [13], and altering its oxidative stability and reducing its health benefits. Since olive oil is produced during a limited period, it must be stored throughout the rest of the year until the next olive-campaign [14].
In a previous investigation, we reported the presence of microorganisms in olives destined to milling [15]. These microorganisms, known for their lipolytic activity, are highly involved in olive oil biodegradation releasing free fatty acids [16, 17, 18], exposed to oxidation.
In Morocco, olive oil production is increasing continuously, due tothe “Green Morocco Plan” set by the Moroccan government, which encloses among others, olive area extension and olive oil processing [19]. Over

the last six years, Morocco has produced an annual average of about 123,000 tones of olive oil [20]. The mayor amount of these oils (71%) is extracted in industrial mills, although, the other type of mills; traditional mills or "maâsras" represent the big party(98 %) of olive oil production sector [19]. In traditional mills the olive oil is obtained without any quality control system, and is destined exclusively to local consumption. General steps of olive oil production in traditional mills in Morocco have been described by El haouhay et al. [15].
The aims of this work were to determine the effect of olive storage on fatty acids profile of olive oil extracted from Moroccan Picholine variety fruits, in rural region of Eastern of Morocco, and to assess the influence of microorganisms, type of container and oil storage on the fatty acid behaviors.

2. Materials and methods

2.1. Sampling

Olive oil of the Moroccan Picholine variety was sampled, during 2011, from traditional mills in the rural area of Eastern Morocco (Taourirt-Oujda). Some olive fruits, separated in 3 groups A, B and C, were stored respectively for 7, 15 and 30 days before milling. Then, olive oil was extracted according to the method practiced in this region. The olive fruitsstorage was performed in plastic bags in environmental conditions (around 11°C and 71% of relative humidity). At the end of storage, the olives of each group were milled in traditional mill. From olive groups (A, B and C), 72 olive oil sampleswere packed in polyethylene terephtalate (PET) (n=36, 12/ group) and amber glass bottles (n=36, 12/group), and then stored in darkness at ambient temperature (17–23°C) for 3 and 6 months of storage, before their analysis.

2.2. Microbiological analysis: mesophilic and psychrotrophic bacteria, moulds and yeasts

Samples of olive oil were analyzed according to the methods described by Majadahonda [21]. Results were expressed as colony forming units (CFU) of micro-organisms per mL of olive oil (CFU/mL). The CFU numbers were also transformed into corresponding logarithmic numbers.

2.3. Determination of conventional quality indices

The free acidity and the peroxide values were expressed as percentage of oleic acid (%) and milliequivalents of oxygen per kg of oil (meq O_2/kg), respectively. The K232 and K270, UV spectrophotometric indices, were measured according to the official methods described in Regulation EC 2568/91 of the European Commission[22]. All parameters were determined in triplicate.

2.4. Fatty acids

The fatty acid composition of oils was analyzed according to the Commission Regulation (EC) No 796/2002 [23] and amending Regulation EC 2568/91 of the European Commission[22], with some modifications. Methylation by heating was used, with sodium methylate in methanol (0.2 N) followed by esterification in sulphuric acid in methanol (4%). Collection of fatty acid methyl esters (FAMEs) was realized after addition of 5 ml of hexane followed by solution of saturated NaCl. The hexane layer (1 mL) was kept into a vial and stored at -20ºC until analysis. Individual fatty acid methyl esters (FAMEs) from the oil samples were separated and quantified by a gas chromatography apparatus (Perkin-Elmer) equipped with a flame ionization detector (FID) and a capillary column SP-2560 (100 m x 0,25 µm film x 0,20 d.i) from SUPELCO (USA). Hydrogen was used as carrier gas with a flow rate of 25.0 mL/min. Oven set with a temperature programme; 60 ° C (the initial temperature) during 3 min, increased to 170 ºC at a rate of 5 ºC/min, during 9 min, then to 230 ºC at a rate of 10º/ min, during 5 min. The injector and detector temperature were set at 230ºC and 250ºC respectively, and the injection volume was 1 µL. Fatty acids peak identification was accomplished by comparing the peak retention times with standard compounds from Sigma (St. Louis, MO, USA) injected under the same gas chromatographic conditions. Results were expressed as relative percent of total area [24]. Three replicates were prepared and analyzed per sample.

2.5. Statistical analysis

All the assays were made in triplicate. Statistical analysis was performed using SPSS 20.0 (IBM® SPSS® Statistics 20.0) and Statgraphics1 Plus 4.1 software. Differences between means of $p < 0.05$ were considered significant. One-way analysis of variance (ANOVA) was used to determine the influence of storage time, when the variables fulfilled the parametric conditions. When the variables were non-parametric, the Kruskal–Wallis test was used. Fisher's least significant difference procedure was used to discriminate between the means of the variables when necessary. The Kolmogorov–Smirnov test and the Bartlett test, as well as the Wilcoxon test, were used to test the normal distribution of variables and the homogeneity of variances.

3. Results and discussion

3.1. Microbiological analysis: mesophilic and psychrotrophic bacteria, moulds and yeasts

The results of microbial contents (mesophilic and psychrotrophic bacteria, moulds and yeasts) obtained in olive oil were summarized in table (1).

Allgroups of oil samples A (olive oil extracted from olives stored for 7 days), B (olive oil extracted from olives stored for 15 days) and C (olive oil extracted from olives stored for 30 days) were obtained from olive fruits, in which, we reported the presence of the same groups of microorganisms [15].The micro-organisms found in oils samples were derived from the olives' phyllosphera which, during the crushing of the olives, migrate into the oil together with the solid parts of the fruit and micro-drops of vegetation water [17].The presence of vegetation water with oil, caused by lack of oil filtration may lead to formation of oil-in-water emulsion, which had an important role in both the growth of microorganisms and their enzyme function [25].

Theevolution of mesophilic and psychrotrophic bacteria in the three oil groups A, B and C showed a slight difference between containers PET and glass bottles. After 6 months of storage, number ofbacteria decreased significantly ($p < 0.05$) in all oil samples. Furthermore, the dynamic mould population in theoil samples was similar in the two types of containers, and after 6 months of storage moulds were completely eliminated.

Table 1: Quantity of micro-organisms (log10 CFU/mL) of Moroccan Picholine olive oil produced in traditional mill and stored in PET and glass bottles.

Microorganisms /group of oils		Mesophilics [a] (log10 CFU/mL)	Psychrotrophics [a] (log10 CFU/mL)	Moulds [a] (log10 CFU/mL)	Yeasts [a] (log10 CFU/mL)
PET: (n=36)					
3 months	A	3.41 ± 0.52 a	4.11 ±0.89 a	1.65 ± 0.95 a	3.00 ± 1.46 a
	B	3.35 ± 0.50 a	3.75 ±1.13 a	1.25 ± 1.30 a	2.95 ± 0.98 a
	C	3.68 ± 0.89 a	3.14 ±1.58 a	1.52 ± 1.06 a	3.27 ± 1.29 a
6 months	A	2.28 ± 0.48 b	2.51 ± 0.72 b	-	2.97 ± 1.44 a
	B	2.36 ± 0.50 b	2.39 ± 0.90 b	-	3.60 ± 0.86 a
	C	2.61 ± 0.75 b	2.02 ± 1.32 b	-	2.07 ± 1.28 b
Glass: (n=36)					
3 months	A	3.21 ± 0.34 a	3.73 ±1.03 a	1.45 ± 0.87 a	2.73 ± 1.53 a
	B	3.19 ± 0.26 a	3.83 ± 1.03 a	1.00 ± 0.97 a	3.11 ± 1.12 a
	C	3.38 ± 1.08 a	2.88 ± 1.62 a	1.37 ± 1.09 a	3.32 ± 1.19 a
6 months	A	2.14 ± 0.47 b	2.76 ± 1.08 b	-	2.77 ± 1.42 a
	B	2.27 ± 0.63 b	2.07 ± 1.09 b	-	3.28 ± 0.54 a
	C	1.97 ± 1.01 b	1.81 ± 1.13 b	-	2.18 ± 1.09 b

Note: A, B and C: oil obtained from olives stored for 7, 15 and 30 days, respectively. [a] Means ± SD. Means followed by the same small letter are not significantly different ($p > 0.05$), consideringthe same type of microorganism of the same type of bottle. Means followed by the same symbol:* are significantly different ($p < 0.05$), considering the type of bottle.

Additionally,yeasts did not change significantly during storage and showed similar counts both in PET and glass bottles. These results showed that content of the type of microorganisms (mesophilic and psychrotrophic bacteria, moulds and yeasts) found in these oils was not influenced by the material of these containers. However, the presence of microorganisms in olive oil may have a negative influence in its compounds and quality. The reduction of microorganisms in oil during storage may be due to the separation of the two phases of the emulsion, water and oil, giving the water phase in the bottom of the containers, fact, the presence of water in oil showed an important role in both the growth of micro-organisms and their enzyme function [18]. In these conditions, only anaerobic microorganisms, including some bacteria and yeasts continue to survive. However, the moulds, which are aerobic, are eliminated. This phenomenon may lead to a natural improvement in the hygienic quality, and to a decrease in the nutritional properties of olive oil by degrading its components [18].

3.2. Conventional quality parameter analysis

Of the 72 olive oil samples analyzed (Table 2); only 9 samples (3 in PET and 6 in glass bottles) were classified as extra virgin. From 3 months to 6 months of storage, the percentage of acidity increased in all samples, with significant differences between the three groups A (oil obtained from olives stored during 7 days), B (oil

obtained from olives stored during 15 days) and C (oils obtained from olives stores during 30 days). The acidity washigher in oils packaged in PET than in glass.

Table 2: Quality parameters of Moroccan Picholine olive oil produced in traditional mill and stored in PET and glass bottles.

Quality parameters /group of oils		Acidity (%oleic acid) [a]	Peroxide index (meq O2/kg)[a]	K232 [a]	K270 [a]	Category (number of samples)		
						EVOO	VOO	L
PET: (n=36)								
3 months	A	0.90 ± 0.26 a*	3.49 ± 0.73 a	1.87 ± 0.03a*	0.18 ± 0.03 a*	3	7	2
	B	1.80 ± 0.65 b	5.37 ± 1.30 b*	2.69 ± 0.38 b*	0.24 ± 0.02 b*	0	6	6
	C	4.26 ± 0.84 c*	6.72 ± 1.58 c*	4.23 ± 0.80 c*	0.35 ± 0.06 c*	0	0	12
6 months	A	1.60 ± 0.92 d*	7.04 ± 1.56 c	2.66 ± 0.23 d*	0.23 ± 0.03 d*	0	5	7
	B	3.73 ± 1.02 e*	9.76 ± 2.45 d	3.76 ± 0.47 e*	0.31 ± 0.05 e*	0	2	10
	C	5.61 ± 1.35 f*	13.70 ± 3.13 e*	5.53 ± 1.01 f*	0.47 ± 0.07 f*	0	0	12
Glass: (n=36)								
3 months	A	0.62 ± 0.20 a*	3.17 ± 1.32 a	1.50 ± 0.22 a*	0.13 ± 0.05 a*	5	6	1
	B	1.44 ± 0.41 b	3.87 ± 1.18 b*	2.18 ± 0.14 b*	0.19 ± 0.03 b*	0	9	3
	C	2.75 ± 0.89 c*	4.34 ± 1.19 c*	2.88 ± 0.54 c*	0.20 ± 0.05 b*	0	0	12
6 months	A	0.95 ± 0.37d*	5.96 ± 2.68 d	1.90 ± 0.27 d*	0.17 ± 0.04 c*	1	5	6
	B	2.09 ± 0.76 e*	7.14 ± 2.14 e	2.69 ± 0.24 e*	0.24 ± 0.03 d*	0	4	8
	C	3.47 ± 0.75 f*	9.27 ± 2.36 f*	3.58 ± 0.53 f*	0.31 ± 0.08 e*	0	0	12

Note:A, B and C: oil obtained from olives stored for 7, 15 and 30 days, respectively. [a] Means ± SD. Means followed by the same small letter are not significantly different ($p > 0.05$), comparing, between the same type of parameter of the same type of container. Means followed by the same symbol:* are significantly different ($p < 0.05$), considering the same type of parameter of the same type of bottle. Means followed by the same symbol:* are significantly different ($p < 0.05$), considering the type of bottle. EVOO: Extra Virgin Olive Oil, VOO: Virgin Olive Oil, L: Lampante Virgin Olive Oil.

Free fatty acids (FFAs), are formed by hydrolysis of triglycerides in presence of lipase. degrading triglycerides to FFAs and glycerol [16, 17, 18]. In fact, high contents of FFAs were shown in oils stored in PET, indicating that plastic container may be favorable for the hydrolyze of triglycerides.

The values of peroxide value (PV) and extinction coefficients K232 and K270 increased during storage in all oil samples of the three groups A, B and C. Significant differences were shownbetween oils of the A, and C. Furthermore, these values were lower in container of glass than in PET bottles, indicating a higher transmission of light, oxygen by the PET material. Moreover, the FFAs were also indicated as accelerators of oxidation [26, 27].At the end of storage (6 months) more than 80% of oilstored in PET material were lampante, while, in glass the value was about 72%, according to the Regulation EC 2568/91 of the European Commission[22]. Thesevalues demonstrated that quality parameter may be correlated with possible changes in fatty acids profile of olive oil.

3.3. Fatty acids composition

Twelve fatty acidswere identified and classified into two groups: unsaturated fatty acids UFA (Table 3) and saturated fatty acids (SFA) (Table 4).

Within the unsaturated group (Table 3), the oleic acid (C18:1) (monounsaturated fatty acid: MUFA) was the most abundant, ranging between 75.28 % ± 2.04 (in oils from group A) and 68.15 % ± 1.87 (in oils from group C). From 3 to 6 months of storage there was a significant decrease of oleic acid. Comparing between containers, oil stored in PET showed reduced values, indicating that plastic bottles probably accelerate degradation of oleic acid. The other determined MUFA (Palmitoleic acid (C16:1), eicosanoic acid (C20:1) and heptadecenoic acid (C17:1)) showed a slow decrease during storage, without important differences between containers. Two main polyunsaturated fatty acids (PUFA) composed this group: linoleic acid (C18:2), ranging between 11.19 % ± 1.44 in oil of group A and 10.63 % ± 1.34 in oil of group C; and linolenic acid (C18:3) ranging between 0.76 % ± 0.28 in oil from A and 0.52 % ± 0.29 in oil of group C. These results are in agreement with the previous

work reported by Sun-Waterhouse et al. (2011), where unsaturated fatty acids, especially oleic acid, decreased during olive oil storage.

Within the saturated fatty acids group (SFA) (Table 4), palmitic acid (C16:0) was the most prevalent compound, with mean values varying significantly between 10.00 % ± 1.43 in oils of A and 14.98 % ± 1.11 in oils of C. Stearic acid (C18:0) was, in order of relative abundance, the second most abundant component from the SFA, with mean values varying between 1.26 % ± 0.26 in oils of A and 3.42% ± 0.81 in oil of group C.

Other saturated fatty acids were also found at very low concentrations: margaric acid (C17:0), arachidic acid (C20:0), behenic acid (C22:0), and lignoceric acid (C24:0), with mean values ranging from 0.14 % ± 0.05 to 0.23 % ± 0.03, 0.19 % ± 0.09 to 0.34 % ± 0.07, 0.06 % ± 0.04 to 0.12 % ± 0.05, and 0.04 % ± 0.04 to 0.11% ± 0.05, respectively.

Table 3: Unsaturated fatty acid profile (%) of Moroccan Picholine olive oil produced in traditional mill and stored in PET and glass bottles.

Fatty acids (%) [a] / Group of oils		C16:1 (%)	C17:1 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)	C20:1 (%)
PET: (n=36)							
	A	0.65 ± 0.15 a	0.08 ± 0.07 a	73.14 ± 1.32 a	11.19 ± 1.44 a	0.70 ± 0.30 a	0.32 ± 0.08 a
3 months	B	0.57 ±0.11 a	0.07 ± 0.07 ac	71.87 ± 2.11 b*	11.07 ± 1.88 a	0.64 ± 0.32 a	0.31 ± 0.08 ab
	C	0.54 ± 0.12 a	0.03 ± 0.03 bc	70.17 ± 1.98 c *	10.96 ± 1.30 a	0.53 ± 0.29 a	0.27 ± 0.08 bc
	A	0.63 ± 0.17 a	0.08 ± 0.07 a	71.72 ±1 .32 d*	11.06 ± 1.04 a	0.68 ± 0.30 a	0.31 ± 0.07 a
6 months	B	0.55 ± 0.12 a	0.05 ± 0.06 ad	69.87 ± 2.17 e*	10,84 ± 1.86 a	0.62 ± 0.31 a	0.30 ± 0.08 a
	C	0.51 ± 0.12 a	0.02 ± 0.03 bd	68.15 ± 1.87 f*	10.77 ± 1.14 a	0.52 ± 0.29 a	0.26 ± 0.08 ac
Glass: (n=36)							
	A	0.66 ± 0.17 a	0.12 ± 0.07 a	75.28 ± 2.04 a	11.12 ± 1.73 a	0.76 ± 0.28 a	0.31 ± 0.08 a
3 months	B	0.66 ± 0.16 a	0.10 ± 0.19 ab	73.20 ± 2.28 b*	11.03 ± 1.40 a	0.73 ± 0.26 a	0.29 ± 0.09 ab
	C	0.55 ± 0.12 a	0.05 ± 0.05 b	71.01 ± 1.50 c*	10.78 ± 1.40 a	0.64 ± 0.27 a	0.22 ± 0.15 b
	A	0.65 ± 0.17 a	0.12 ± 0.07 a	73.75 ± 2.04 d*	11.02 ± 1.67 a	0.73 ± 0.26 a	0.30 ± 0.08 a
6 months	B	0.64 ± 0.19 a	0.09 ± 0.18 ab	71.89 ± 2.36 e*	10.97 ± 1.54 a	0.72 ± 0.26 a	0.28 ± 0.09 a
	C	0.54 ± 0.14 a	0.05 ± 0.04 b	68.18 ± 1.40 f*	10.63 ± 1.34 a	0.63 ± 0.27 a	0.21 ± 0.15 ab

Note:A, B and C: oil obtained from olives stored for 7, 15 and 30 days, respectively. [a] Means ± SD. Means followed by the same small letter are not significantly different ($p > 0.05$), considering the same type of fatty acid of the same type of bottle. Means followed by the same symbol:* are significantly different ($p < 0.05$) considering the type of bottle.

Table 4: Saturated fatty acid profile (%)of Moroccan Picholine olive oil produced in traditional mill and stored in PET and glass bottles.

Fatty acids (%) [a] / Group of oils		C16:0 (%)	C17:0 (%)	C18:0 (%)	C20:0 (%)	C22:0 (%)	C24:0 (%)
PET: (n=36)							
	A	11.63 ± 1.34 a	0.18 ± 0.06 a	1.61 ± 0.47 a	0.30 ± 0.05 a	0.08 ± 0.05 a	0.07 ± 0.06 a
3 months	B	12.81 ± 1.24 b	0.15 ± 0.05 a c	2.11 ± 0.66 ad	0.25 ± 0.08 a	0.09 ± 0.04 a	0.07 ± 0.05 a
	C	13.52 ± 0.93 b	0.20 ± 0.03 b c	2.91 ± 0.89 bd	0.26 ± 0.08 a	0.10 ± 0.06 a	0.09 ± 0.05 a
	A	12.66 ± 1.4 1 c*	0.21 ± 0.06 a	2.21 ± 0.43 c	0.34 ± 0.07 a	0.10 ± 0.05 a	0.10 ± 0.06 a
6 months	B	13.82 ± 1.10 d	0.17 ± 0.06 a c	2.93 ± 0.75 ce	0.29 ± 0.09 a	0.11 ± 0.04 a	0.10 ± 0.05 a
	C	14.97 ± 1.11 e	0.23 ± 0.03 b c	3.42 ± 0.81 bec	0.31 ± 0.08a	0.12 ± 0.05 a	0.11 ± 0.05 a
Glass: (n=36)							
	A	10.00 ± 1.43 a	0.14 ± 0.05 a	1.26 ± 0.26 a	0.19 ± 0.09 a	0.06 ± 0.04 a	0.04 ± 0.04 a
3 months	B	11.59 ± 1.46 a b	0.17 ± 0.04 a	1.84 ± 0.77 bc	0.24 ± 0.07 a	0.08 ± 0.06 a	0.07 ± 0.04 a
	C	13.47 ± 0.99 b	0.17 ± 0.05 a	2.33 ± 0.52 b	0.20 ± 0.13 a	0.09 ± 0.05 a	0.07 ± 0.04 ab
	A	11.24 ± 1.58 a*	0.16 ± 0.05 a	1.87 ± 0.40 c	0.24 ± 0.11 a	0.10 ± 0.07 a	0.06 ± 0.04 a
6 months	B	12.31 ± 1.33 a b	0.18 ± 0.04 a	2.27 ± 0.75 ca	0.28 ± 0.08 a	0.10 ± 0.04 a	0.10 ± 0.03 ab
	C	14.98 ± 1.11 c	0.19 ± 0.06 a	2.89 ± 0.84 a	0.25 ± 0.14 a	0.11 ± 0.05 a	0.10 ± 0.03 b

Note:A: oil obtained from olives stored for 7 days, B: oil obtained from olives stored for 15 days and C: oil obtained from olives stored for 30 days. [a] Means ± SD. Means followed by the same small letter are not significantly different ($p > 0.05$), considering the same type of fatty acid of the same type of bottle. Means followed by the same symbol:* are significantly different ($p < 0.05$) considering the type of bottle.

Similar results were reported in a previous study [28], where saturated fatty acids, particularly palmitic and stearic acids, increased during olive oil storage. Considering the limits established by EC 2568/91 Regulation of the of the European Commission [22], the fatty acid levels found in the analyzed samples covered the normal ranges considered for virgin olive oil.
Furthermore, ratios between oleic and linoleic acids (C18:1/C18:2), unsaturated and saturated fatty acids (UFA/SFA) and MUFA/PUFA, indicated as important parameters in the evaluation of the quality and stability of olive oils[7], were also determined in this work (Table 5).

Table 5: Profile of fatty acids; MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids, UFA/SFA: unsaturated saturated fatty acid ratio, MUFA/PUFA: monounsaturated polyunsaturated fatty acid ratio and O/L: oleic linoleic ratio of Moroccan Picholine olive oils produced in traditional mill and stored in PET and glass bottles.

Fatty acids [a] / group of oils		MUFA (%)	PUFA (%)	SFA (%)	UFA/ SFA	MUFA/ PUFA	O/L
PET: (n=36)							
	A	74.19 ± 1.82 a*	11.89 ± 1.84 a	13.87 ± 1.53 a*	6.20 ± 0.80 a*	6.23 ± 1.07 a	6.53 ± 0.98 a
3 months	B	72.82 ± 1.40 b*	11.71 ± 2.23 a	15.48 ± 1.20 b*	5.46 ± 0.32 b*	6.21 ± 1.39 a	6.49 ± 1.38 a
	C	71.01 ± 1.66 c*	11.49 ± 1.58 a	17.08 ± 0.94 c	4.83 ± 0.31 c*	6.18 ± 0.83 a	6.40 ± 0.89 a
	A	72.74 ± 1.48 d*	11.74 ± 3.27 a	15.62 ± 1.50 d*	5.40 ± 0.70 a*	6.19 ± 1.38 a	6.48 ± 1.34 a
6 months	B	70.77 ± 1.50 e*	11.46 ± 2.08 a	17.42 ± 0.98 e*	4.72 ± 0.25 b*	6.17 ± 1.32 a	6.44 ± 1.30 a
	C	68.9 ± 1.53 f*	11.29 ± 1.39 a	19.16 ± 0.79 f*	4.18 ± 0.20 c*	6.10 ± 0.76 a	6.32 ± 0.81 a
Glass: (n=36)							
	A	76.37 ± 1.80 a *	11.88 ± 1.47 a	11.69 ± 1.11 a*	7.54 ± 0.71 a *	6.42 ± 0.95 a	6.76 ± 0.93 a
3 months	B	74.25 ± 2.97 b *	11.76 ± 1.56 a	13.99 ± 1.30 b*	6.14 ± 0.74 b *	6.31 ± 0.90 a	6.63 ± 1.07 a
	C	71.83 ± 3.79 c *	11.42 ± 1.29 a	16.33 ± 2.02 c	5.09 ± 0.80 c *	6.28 ± 0.97 a	6.58 ± 1.00 a
	A	74.22 ± 1.95 d *	11.75 ± 1.77 a	13.67 ± 0.85 d*	6.28 ± 0.41 d *	6.31 ± 0.91 a	6.63 ± 0.70 a
6 months	B	72.9 ± 1.90 e *	11.69 ± 1.47 a	15.24 ± 1.19 e*	5.55 ± 0.49 e *	6.23 ± 0.88 a	6.55 ± 1.04 a
	C	68.98 ± 2.88 f*	11.26 ± 1.04 a	18.52 ± 2.51 f*	4.33 ± 0.68 f*	6.12 ± 0.66 a	6.41 ± 0.73 a

Note: A, B and C: oil obtained from olives stored for 7, 15 and 30 days, respectively. a Means ± SD. Means followed by the same small letter are not significantly different ($p > 0.05$),considering the same type of fatty acid of the same type of bottle. Means followed by the same symbol:* are significantly different ($p < 0.05$) considering the type of bottle.

As can be observed on table 5, MUFA was the most abundant group of fatty acids in all analyzed samples, being the highest average content found in oil of group A (oil of olives stored for 7 days) with 76.37 % ± 1.80, followed by oil of group B (oil of olives stored for 15 days) and the lowest (68.9 % ± 1.53) in oil of group C (oil of olives stored for 30 days). Olive oil samples of group A showed the highest values of C18:1/C18:2, UFA/SFA and MUFA/PUFA ratios (6.76 ± 0.93 %, 7.54 ± 0.71 % and 6.42 ± 0.95 %, respectively). Hence, olive oil of group A seems to be more stable to oxidative deterioration.
In fact, microorganisms found in olive oil (Table 1) may be responsible for the modification of fatty acids profile. However, changes in fatty acids profile were indicated as a result of enzymes produced by microorganisms [29]. Some bacteria are involved in strong degradation of long fatty acids, especially oleic acid[30]. The lipolytic activity of yeasts leads to the increase of palmitic acid and reduction of oleic acid in olive oil [18, 31]. Furthermore, the exposure of olive fruits to microorganisms during long time of storage [15], lead to significant physical and chemical changes in their olive oil (group C).
Comparing between containers, PET bottles presented the highest behavior observed in fatty acid profile. In fact, plastic container accelerated the degradation of olive oil. Although, the contents of microorganisms showed in these oils (Table 1) were similar in glass as in PET bottles, we could emphasize that plastic material may favor the lipolytic activity of microorganisms.

3.4. Multivariate analysis

This analysis was realized in order to disclose relationships between studied variables (Free fatty acids (FFAs), peroxide value (PV), K232, K270, oleic acid (C18:1), palmitic acid (C16:0), stearic acid (C18:0), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA) and ratios between oleic and linoleic acids (Oleic/Linoleic: C18:1/C18:2), unsaturated and saturated fatty acids (UFA/SFA) and monounsaturated and polyunsaturated fatty acids (MUFA/PUFA)) and to find overall differences. For the Principal Component Analysis (PCA), data on microorganisms, palmitoleic acid, margaric

acid, margaroleic acid, linoleic acid, linolenic acid, gadoleic acid, arachic acid, behenic acid, and lignoceric acid were not taken into account, because of the absence of variability between the samples. In this case, three factors were extracted, with eigenvalues greater than 1, and together they account for 74.7187% of the variability in the original data.

The correlations showed that Factor 1 (41.203% variance) was, among others, linked to: SFA (correlation coefficient, 0.893), MUFA (-0.763), UFA/SFA (- 0.916), Palmitic acid (+ 0.796), Stearic acid (+ 0.549), Oleic acid (- 0.626), FFAs (+ 0.770), K270 (+ 0.751) and K232 (+ 0.775). Factor 2 (23.455% variance) was associated with MUFA/PUFA (0.988), Oleic/Linoleic (0.987) and PUFA (-0.934), while Factor 3 (10.061% variance) was correlated with PV (0.429).

Furthermore, the projection of the original variables onto the plane of the first two principal components demonstrated the relationships among the various quantitative variables (Fig. 1). Hence, strong relationship between MUFA and C18:1 or between C18:0, C16:0 and SFA was shown. Strong positive association of MUFA / PUFA and Oleic / Linoleic has also been observed. Both variables were negatively correlated with PUFA. Figure (1) showed that MUFA and oleic acid are negatively correlated with SFA, stearic, palmitic acid, and also negatively linked to FFAs, K232 and K 270. Figure (1) also indicated that PV was not related to PUFA because they formed an angle close to 90º between them.

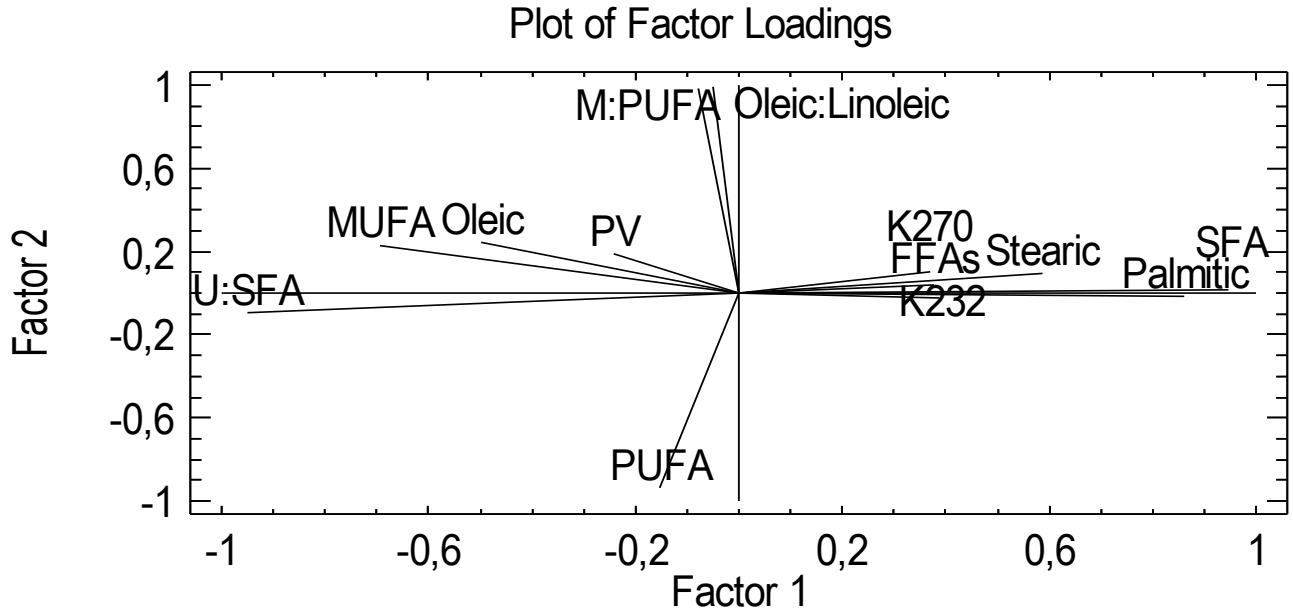

Figure 1: Plot of weights for the two factors for studied variables in Picholine olive oils produced in traditional mill.

Additionally, multivariable analysis may also provide an overview of the overall effects of olive storage and the processing steps on the quality parameters and fatty acid composition.
This is achieved by the plot of two discriminant functions (Fig. 2), demonstrating a significant separation between group A (oil of olives stored for 7 days) and group C (oil of olives stored for 30 days).

Based on the PCAs, the variables that most contributed to the differentiation among oil groups A, B and C, were those strongly correlated (positively or negatively) with Factor 1 (Fig. 1). From the comparison between the spatial distribution of variables (Fig.1) and oil groups (Fig. 2) on their respective projection planes, it is deduced that group A was richer in C18:1 and MUFA while group C had higher proportions of SFA, C16:0, C18:0, FFAs, K270 and K232. However, oil of group C, lampante oil (as was demonstrated above) showed characteristics and composition of fatty acids that differentiated them from other categories of olive oil [32].
The existence of negative and strong correlation between MUFA or C18:1, and FFAs demonstrated that the main olive oil hydrolysis product was oleic acid [33]. FFAs were also involved in initiation of oxidation chain reactions [34] to increase the oxidation and auto-oxidation of olive oil [25], which may explain the positive correlation between FFAs and K232, and K270. Furthermore, the ability of monounsaturated fatty acids to

promote lipid oxidation [26] may explain the negative and strong correlation between MUFA or C18:1 and the tree quality parameters (FFAs, K232 and K270).

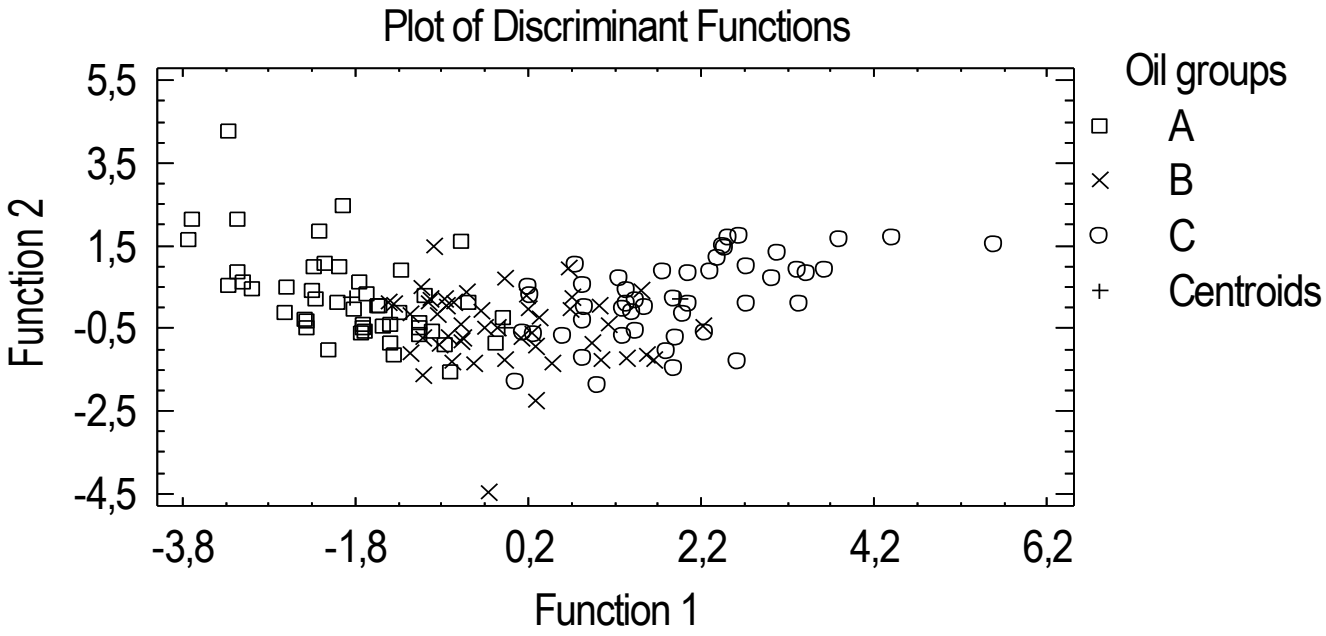

Figure 2: Plot of discriminant functions separating the three groups of traditional Moroccan Picholine olive oils A, B and C, obtained from olive fruits stored for 7, 15 and 30 days, respectively.

In contrast, other studies revealed that the susceptibility of fatty acids to oxidation was correlated with the degree of unsaturation, indicating that PUFA were more prone to oxidative rancidity than MUFA [35]. Although, the main parameter influencing lipid oxidation was the fatty acid composition of the triglycerides [36], the oxidative degradation of olive oil is related not only to the fatty acid composition of its lipid matrix, but also to the presence of other factors such as water. Hence, in oil-in-water emulsions the formation of lipid hydroperoxide concentration (PV) was strongly increased in the presence of oleic acid than in presence of linoleic or linolenic acid (PUFA) [26].
Furthermore, microorganisms found in these studied oils, and in their corresponding olive fruits [15], may have power effects to select the concentration and type of fatty acid as carbon source for their metabolism.
The existence of negative and strong correlation between fatty acids indicates that saturated fatty acids may be the results of conversion of unsaturated fatty acids. Hence, palmitic acid has been indicated as the primary detected product from oleic acid degradation [37, 38, 39].

Conclusions

During olive oil storage, mesophilic and psychrotrophic bacteria, moulds and yeasts had similar profile in both containers, while indices of quality and saturated fatty acids (SFA) increased, and unsaturated fatty acids (UFA) decreased. Significant changes were shown in palmitic, stearic, and oleic acids.

Furthermore, accelerated degradation of olive oils was observed in plastic containers. Factorial analysis showed a strong negative correlation between UFA and SFA, free fatty acids contents (FFAs), K232 and K270. Discriminate analysis showed that storage of olive fruits play important role on the classification of olive oil.

Thus, the prolonged time of olive fruits storage, linked to the presence of microorganisms, may influence the classification and composition of olive oil, degrading principally triglycerides and fatty acid and consequently modifying their quality, and altering their oxidative stability and health benefits.

In fact, to protect the olive oil quality, in terms of its fatty acid composition,factors accelerating their hydrolysis and oxidation should be controlled during all stages of processing, mainly the extended olive storage in uncontrolled conditions.

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