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Physicochemical characterization and fatty acid composition of olive seed oil from the Moroccan Picholine variety

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Abstract: The preparation of table olives represents an important economic and social activity in the Mediterranean region, notably in Morocco. This sector generates large quantities of solid pollutants such as olive stones. 'Moroccan Picholine' is the most widely cultivated olive variety in Morocco due to its high adaptability as it known for its olive and oil productivity. The objective of this work is to extract the oil from the seeds of olive stones, determine its physicochemical parameters and study its fatty acid composition from the Moroccan Picholine. The seeds were obtained by crushing the stones and isolating the cellulose part, and oil was extracted from the olive seeds using the standard Soxhlet method. A series of indices have been determined in the course of the work, such as free acidity, the peroxide index, the specific extinction coefficients K232 and K270, the total phenol content and the unsaponifiable matter content. The results showed that the oils extracted from olive seeds (a by-product of table olive processing units) have an oil yield ranging from 26.5 to 43.6 %, an acidity between 5.16 and 7.87 % and normal peroxide values from 10.15 to 12.31 Meq O_2/kg. In addition, olive seed oils contain high levels of phenols varying from 6.27 to 11.82 g EAG/kg. On the other hand, gas chromatographic analysis shows that this oil is of the oleic, linoleic type. The main fatty acids are, in order of importance, oleic acid (67.1-68.5%), linoleic acid (18.9-20.6%), palmitic acid (7.8-8.2%). All these results show that olive seed oil is a new source of vegetable oil for different sectors.

Keyword: Moroccan Picholine, olive seed oil, phenolic compounds, olive stones, fatty acid composition, olive by-products.

Introduction

The olive (*Olea europaea* L.) is the primary fruit crop in the Mediterranean basin, with olive oil being a fundamental component of the regional diet (Serra-Majem et al., 2003). Mediterranean countries are the leading producers, accounting for 97% of global olive oil production, estimated at 3 159 500 tons in the 2016 crop season. Morocco ranks as the sixth-largest producer of olive oil globally, following Spain, Italy, Greece, Turkey, and Tunisia (IOC, 2016). The 'Moroccan Picholine' is the most widely cultivated variety, representing more than 96% of the nation's olive trees.

In Morocco, olive cultivation serves as a vital socio-economic pillar, contributing up to 5% of the agricultural gross domestic product and 15% of the country's agricultural foodstuff exports. Olive cultivation in Morocco is predominantly dominated by the Picholine Moroccan cultivar (up to 96%), owing to its high adaptability to various bioclimatic stages (plains, mountainous regions, arid, and Saharan areas), its favorable organoleptic characteristics (medium green fruitiness, balanced bitterness, and spiciness), its rich chemical and aromatic profiles, and its dual-purpose use for both olive oil production and canning (MAPMDREF, 2019). The chemical characteristics and quality of virgin olive oil are influenced by various factors, such as genotype, tree age, fruit ripeness, production area, pedoclimatic conditions, agronomic and irrigation practices, and the extraction process (El Yamani et al., 2020).

The olive can be divided into three anatomical parts, the skin, the pulp and the stone (endocarp of the wood containing the seed) that represents 18 to 22% of the weight of the olive (Rodríguez, 2008). The seed represents 2 to 4% of the weight of the stone and contains a significant amount of oil (22 to 27% of the mass of the seed), while the endocarp contains a maximum of 1%. The size, weight and conformation of the stone depend on the variety (Bianchi, 2003).

The olive stone has been proposed as a source of protein. However, it has mainly been used as a source of energy (Rodríguez, 2008). The preparation of activated carbon is another widely applied application for eliminating odors and contaminants (Najar-Souissi, 2005 and Ghazy, 2006). Studies carried out to characterize the sugar, cellulose, hemicellulose and lignin composition of olive stones have opened up new avenues of research and exploitation (Demirbas, 2002, Requejo, 2012). More recent studies show that the stone extracts prolong the storage times of olive oil due to their excellent antioxidant activity and can be used as a natural source of antioxidants for foods that contain an oily fraction (Hazzab et al., 2021).

When olive oil is extracted, the oil from the seeds passes into the olive oil (most of the olive oil is in the pulp). The fatty acid composition of olive oil obtained by gas chromatographic analysis shows that this acid composition is variable. Saturated fatty acids are mainly represented by palmitic acid (C16:0) (10.43%), stearic acid (C18:0) (2.16%) and arachidic acid (C20:0) (0.28%). Unsaturated fatty acids that represent up to 86.63 % include monounsaturated fatty acids such as palmitoleic acid (C16:1) (0.79%) and oleic acid (C18:1) (71.74%). Di-unsaturated fatty acid consists of linoleic acid (C18:2) (11.8%), while tri-unsaturated fatty acid includes linolenic acid (C18:3) 0.89% (Ait Yacine, 2001, Meftah, 2014). It should also be noted that the fatty acid composition shows

a predominance of monounsaturated fatty acids (Meftah, 2014).
All these parameters call for a study to develop a method for olive stone valorization, a by-product of table olive preparation units. The present work aims to extract the oil from the seeds of olive stones, determine its physicochemical parameters and study its fatty acid composition. A series of indices will be determined in the course of the work, such as free acidity, the peroxide index, the specific extinction coefficients K232, K270, the total phenol content and the unsaponifiable matter content.

Results and discussion

Oil yield

The oil content of olive seeds is not a criterion for determining the quality of olives, but it is a criterion to be considered when selecting a valorization method for the stones. During the preparation of the seeds, from one kilogram of olive stones we obtained between 50 and 60 g of seed, which represents a percentage of 5 to 6% of the stones' mass. The color of the obtained oil is noticeably greenish. GOS treated with NaOH (E2) and 20.6% for BOS treated with NaOH (E'2). The oil yields from olive seeds of the two types, green (E1) and black (E'1) are significantly different but are not influenced by treatment with NaOH or NaCl (Table 1). In general, the highest oil content is observed in oil extracted from fresh black olive seeds, with an average of 42.53%. (in relation to the mass of the seed) followed by BOS NaCl (black olive seed treated with NaCl (E'3)) 42.00 % and then BOS NaOH (black olive seed treated with NaOH (E'2)) 41.23 %. However, GOS NaOH (green olive seed treated with NaOH (E2)) presented the lowest oil content 27.30 %. These results showed that the differences observed in the olive seeds oils yield are linked to the stage of maturity of the olives.
To classify olive stone oil within the range of seed oils, we compared it with other oils, for example, the oil yield of jujube, pomegranate and prickly pear (29.25%, 23.39% and 8.74% respectively) (El Hachimi, 2015). These results show that olive seeds contain a high amount of oil. Given the large quantities of stones generated by the industry, olive stones, a by-product of the olive oil production process, exemplify agricultural food waste that can be used as a new source of vegetable oil. Olive seeds contain considerable amounts of oil, reaching about 10% w/w, depending on the cultivar and extraction method (Moghaddam et al., 2012). Additionally, the seed represents 2 to 4% of the weight of the stone and contains a significant amount of oil (22 to 27% of the mass of the stone), while the endocarp contains a maximum of 1% (Bianchi, 2003).

Free acidity

The results of the free acidity analysis, expressed as a percentage of oleic acid, revealed no significant difference between the oils from (E1) and (E'1) olive seeds. The free acidity of oil extracted from fresh (E1) was 1.84%, while the free acidity of oil extracted from (E'1) was 2.45%. However, treating both olive types with either NaCl or NaOH was associated with a noticeable increase in the acidity of their seed oils (Table 1). The results showed that NaCl increased the acidity of olive seed oil more than NaOH. Specifically, the NaOH treatment increased the acidity of green olive seed oil to 5.50% and for the black olive seed oil to 6.60%. In contrast, the NaCl treatment resulted in an increase in the acidity of green olive seed oil to 7.11% and black olive seed oil to 6.85%. This high acidity induced by NaCl and NaOH treatments could be explained by the phenomenon of hydrolysis of the fatty acids brought into contact with the brine water (El Antari, 2000). In comparison with the International Olive Oil Council's commercial standard for olive oil, we note that all the samples analyzed belong to lampante virgin olive oil class with a free acidity greater than 3% (IOC, 2013). In addition, according to N.M. 08.5.090 (Moroccan Normed) for current virgin argan oil, the acidity is around 2.5%, so we can conclude that olive seed oil is not useful for direct food consumption, but for refining or cosmetic use.

Peroxide value (PI)

The results obtained for measuring the peroxide value of olive seed oils, expressed in milliequivalents of active oxygen per kilogram of oil (meq active O_2/kg olive oil), are shown in Table 2. The two types of olive seeds oils contain approximatively the same peroxide amounts (6.26 in the (E1) type and 6.76 in the (E'1) type); however the peroxide contents are significantly increased when the both types were treated by NaOH or by NaCl. The peroxide contents in the seed oils of the treated olives are, in ascending order, 10.38 in (E2), 10.69 in GOS NaCl (green olive seed treated with NaCl (E3)), 11.19 in (E'2), and 11.96 in (E'3).
Oils extracted from green olive seeds are more resistant to oxidation than oils from black olive seeds. The oxidation of oils from residual olive seeds may be linked to the conditions under which table olives are prepared and the extraction method, which requires an increase in temperature and subsequently causes accelerated oxidation. The oxidation phenomenon may also be catalyzed by the enzyme peroxidase, which is abundant in the seeds. This hypothesis has been confirmed by other authors, who have shown that olive oil from pitted olives is more stable than that from whole olives (Servili, 1999; Del Caro, 2006).
In general, the oils studied had peroxide values below the limit set by the International Olive Oil Council's commercial standard for olive oils (20 meq active O_2/kg olive oil) (IOC, 2013). Comparatively, olive seed oil shows lower peroxide values than olive oils from the Tadla-Azilal region, which range from 12.07 to 18.66 meq active O_2/kg olive oil (Meftah, 2014 and Hirri, 2015). Additionally, our results are below the peroxide value limit for argan oil defined by the Moroccan standard (15 meq O_2/kg argan oil) (SNIMA, 2003), and also below the value specified by the Codex Alimentarius for vegetable oils (2015) (10-15 meq O_2/kg). The peroxide index indicates that the studied oil is not in an oxidation state (Benguendouz, 2017).

Ultraviolet absorbance

The specific extinction values in ultraviolet at 232 nm and 270 nm are shown in Table 2. The treatment of the two types of olives with NaOH or NaCl had a significant increase on the specific extinction values in ultraviolet at 232 nm and 270 nm. The oil samples have a value of K270 ranging from 0.083 (no treated olive) to 0.175 (treated olive), and in K232 the values are ranging from 1.067 (no treated olive) to 2.346 (treated olive). These results are still below the limit set by the International Olive Oil Council's for ordinary virgin olive oil ($K270 < 0.3$ and $K232 < 2.6$ (IOC, 2013). The differences observed in the peroxide values and the specific extinction coefficients at 232 nm and 270 nm reflect the difference in the resistance of the oils to oxidation. This resistance is influenced by the nature and content of the phenolic compounds contained in the different oils (prepared from the stones of green or black olives) (Romero, 2002). On the other hand, the source of olive stones has undergone strictly different treatments, alkaline with (NaOH) or with salt (NaCl), which leads to two different phenomena in the Saumur middle. The first is the neutralization of the phenol functions and the second is the hydrolysis of the phenolic compounds. As a result, we obtain two very distinct plant materials in terms of phenolic composition by comparison with the pre-established limits for other oils. The results obtained during this study do not exceed the limit set by the

Table 1. Different treatments to assess the quality of olive seed oils.

Green olive seeds	Treatments	Black olive seeds	Treatment
E1	No treatment	E'1	No treatment
E2	NaOH (GOS NaOH)	E'2	NaOH (BOS NaOH)
E3	NaCl (GOS NaCl)	E'3	NaCl (BOS NaCl)

Table 2. Results of physicochemical analyses of olive seeds oils, acidity, peroxide value (PI), and extinction coefficients (K232 and K270)

	Oil yield (%) mass of the seed	Acidity (%)	Peroxide (meq O_2)	K232	K270
E1	$31.06^{b} \pm 1.52$	$1.84^{b} \pm 0.17$	$6.26^{c} \pm 0.43$	$1.067^{c} \pm 0.03$	$0.083^{c} \pm 0.004$
E2 (GOS NaOH)	$27.30^{b} \pm 0.46$	$5.50^{a} \pm 0.30$	$10.38^{b} \pm 0.13$	$1.738^{b} \pm 0.06$	$0.119^{bc} \pm 0.002$
E3 (GOS NaCl)	$30.76^{b} \pm 0.83$	$7.11^{a} \pm 0.49$	$10.69^{ab} \pm 0.09$	$1.568^{b} \pm 0.11$	$0.120^{bc} \pm 0.002$
E'1	$42.53^{a} \pm 1.03$	$2.45^{b} \pm 0.40$	$6.76^{c} \pm 0.28$	$1.121^{c} \pm 0.01$	$0.095^{c} \pm 0.004$
E'2 (BOS NaOH)	$41.23^{a} \pm 0.41$	$6.60^{a} \pm 0.37$	$11.19^{ab} \pm 0.22$	$2.191^{a} \pm 0.01$	$0.142^{ab} \pm 0.018$
E'3 (BOS NaCl)	$42.00^{a} \pm 0.81$	$6.85^{a} \pm 0.51$	$11.96^{a} \pm 0.32$	$2.346^{a} \pm 0.10$	$0.175^{a} \pm 0.01$

Table 3. Total phenol content (TP) and percentage of unsaponifiable matter in olive seed oils extracted by pressing.

	TP (g EAG/kg)	Unsaponifiable %
E1	$12.35^{a} \pm 0.35$	$3.76^{a} \pm 0.19$
E2 (GOS NaOH)	$7.19^{d} \pm 0.46$	$3.27^{a} \pm 0.037$
E3 (GOS NaCl)	$10.53^{ab} \pm 0.77$	$2.92^{a} \pm 0.36$
E'1	$10.86^{ab} \pm 0.23$	$3.08^{a} \pm 0.21$
E'2 (BOS NaOH)	$7.67^{cd} \pm 0.42$	$2.96^{a} \pm 0.25$

International Olive Oil Council for current virgin olive oils (IOC, 2013), and are also below the limit for the specific extinction of argan oil at 270 nm set by the Moroccan standard (K270<0.35) (SNIMA, 2003; Belcadi-Haloui et al., 2018). All these data allowed us to conclude that this oil does not present any oxidation state.

Unsaponifiable matter content

The unsaponifiable fraction is the most important part of the oil. It contains a wide range of relevant compounds such as tocopherols, sterols and phenols (Gharby, 2013). The results showed no significant variability in the unsaponifiable percentage between the two types of olive seed oil, and that the unsaponifiable levels are not influenced by NaCl and NaOH treatments in either type, with values ranging from 2.6% to 3.76%. The results of this study (Table 3) indicate that the highest unsaponifiable matter content is found in the (E2) (3.27%), followed by the oil extracted from (E1) (3.76%). These findings suggest that the level of unsaponifiable matter in oils extracted from seeds is directly related to the maturity of the olives and the industrial method used to prepare them. For comparison, our results exceed the values reported by other authors for olive oil, which is 2.41% (Tanouti, 2011). Our values are also higher than the unsaponifiable content reported in argan oils (ranging from 0.36% to 1.1% and from 1.46% to 1.71%) (Hilali, 2005). The results of this study confirm that residual olive seed oil is a valuable source of unsaponifiable compounds.

Colorimetric determination of total phenols

The total phenolic compounds in the various olive seed oils extracted by crushing and pressing the seeds were determined using the Folin-Ciocalteu method. The results are expressed as milligrams of Gallic acid equivalent per kilogram of oil (mg GAE/kg oil) and are shown in Table 3.

In general, all the analyzed olive seed oils showed high phenolic contents, ranging from 7.19 to 12.35 g GAE/kg. The highest phenolic content was observed in the oil from (E1) (12.35 g GAE/kg), followed by (E'1) (10.86 g GAE/kg). The lowest values were observed in the (E2) and (E'2) (7.67 g GAE/kg and 7.19 g GAE/kg respectively). The results of total phenolic showed a significant difference. The treatment of olives with NaOH or NaCl influenced the total phenolic content, leading to a significant decrease in total phenolic content observed after the treatments in both (E1) and (E'1). These results clearly indicate that the phenol content in the oil depends largely on the method used to process the olives (source of the stones). This is because olive processing modifies the profile of phenolic compounds quite markedly in relation to the raw material (Marsilio, 2000; Campestre, 2002). For example, the action of sodium hydroxide on oleuropein gives 11-methylester oleoside and hydroxytyrosol (Amiot, 1986; Capozzi, 2000). In addition, during the fermentation of the olives, the basic pH of the initial medium decreases considerably to reach acidic values and then causes the hydrolysis of the unstable acetal group of the 11-methylester oleoside, which reduces the oleoside content. Similarly, all the other glycosides are hydrolysed under the same acidic conditions. Alkaline treatment transforms the free carboxylic acids and any other acidic substances into the corresponding salts (all these salts are dissolved in the brine). After treatment with NaOH, the olives are maintained in a salt solution (Saumur), at which point a complex diffusion of the olive constituents into the aqueous medium is produced, causing a reduction in the phenolic reserve (Capozzi, 2000; Marsilio, 2000). Returning to the results, the levels of phenols in the studied olive seed oils are higher than the values of olive oil provided by other authors (5.75 g/kg to 9.34 g/kg) (Meftah, 2014 and Hirri, 2015). On the other hand, seed oils have higher phenol levels than argan oil: 3.26 mg/kg and 5.01/kg (Hilali, 2005; Kouidri, 2008 and Benguendouz, 2017). The richness of olive seed oil in phenolic compounds is a crucial feature, as these are the compounds responsible for the antioxidant activity and benefits of vegetable oils. Phenol content is also a criterion to consider when choosing a vegetable oil for appropriate use. All these data confirm that olive seed are an important source of vegetable oil.

Fatty acid composition

The fatty acid composition of olive seed oils was identified using GPC. The results presented in Table 4 show that the main fatty acid present in the oil samples analyzed are: oleic acid, linoleic acid, palmitic acid and stearic acid. Indeed, the percentages of oleic acid (C18:1) vary between 65.4% for (E2) and 68.5% for (E'2). These values are of the same order as those found in olive oil (Ait Yacine, 2001; Meftah, 2014). Meanwhile, the percentages of linoleic acid (C18:2) vary between 18.9% for (E2) and 20.6% for (E'2). The percentages of linoleic acid are higher than those observed in olive oil,

Table 4. Fatty acid composition of oils extracted from olive stones and analyzed by gas chromatography, expressed as a percentage.

Fatty acid	Fresh olive stones	BOS NaCl	GOS NaCl	GOS NaOH	BOS NaOH
Saturated	11.4	12.3	12.2	12.4	12.2
Monounsaturated	69.2	67.9	68.3	68.0	66.2
Polyunsaturated	19.4	19.8	19.5	19.6	21.6
Unsaturated	88.6	87.7	87.8	87.6	87.8
Myristic C14:0	<0.05	<0.05	<0.05	<0.05	<0.05
Pentadecanoic C15:0	<0.05	<0.05	<0.05	<0.05	<0.05
Palmitic C16:0	7.9	08.2	8.1	8.2	8.2
Palimtoleic C16:1	0.1	0.1	0.1	0.1	0.2
Heptadecanoic C17:0	0.1	0.1	0.1	0.1	0.1
Heptadecanoic C17:1	<0.05	<0.05	<0.05	<0.05	<0.05
Stearic C18:0	2.9	3.4	3.4	3.5	3.3
Oleic C18:1	68.5	67.1	67.4	67.2	65.4
Linoleic C18:2	18.9	19.3	19.0	18.9	20.6
Linolenic C18:3	0.1	<0.05	<0.05	0.1	0.2
Arachidic C20:0	0.5	0.6	0.6	0.6	0.6
Gondoic C20:1 0.7	0.5	0.7	0.7	0.7	0.6
Behenic C22:0 <0.05	<0.05Method	<0.05	<0.05	<0.05	<0.05

Table 5. Comparison of olive kernel oil with other seed oils, jujube oil, pomegranate seed oil, prickly pear oil, argan oil.

	Seed oils	Jujube oil (Chouaibi M, 2012)	Pomegranate seed oil (El Hachimi, 2015)	Prickly pear oil (Ramadan, 2003)	Argan oil (Hilali, 2005)
Yield %	26.5-43.6	24.52-31.22	25.68-29.09	7.82-10.45	29.4-32.4
SFA %	12. 4	18.18	6.32	16.64	19.2
UFA%	87.6	81.83	93.67	83.48	80
MUFA %	66.2	65.57	5.98	20.70	47.2
PUFA %	21.6	16.26	87.70	62.78	34
Oleic acid %	65.4	62.49	5.39	19.48	46.5
Linoleic acid %	20.6	16.31	6.31	62.72	33.3
Palmitic acid %	8.2	10.27	3.58	11.42	13.4
Stearic acid %	3.3	6.48	2.14	4.16	4.3
Punicic acid %	ND	ND	75.39	ND	ND

ranging from 11.8 to 17.14% (Ait Yacine, 2001; Boukachabine, 2011). These two fatty acids are therefore predominant in residual olive seed oil, followed by palmitic acid (C16:0) with values ranging from 7.9 to 8.2%. These levels are lower than those found in olive oil 10.32 (Ait Yacine, 2001). For stearic acid (C18:0), we found percentages ranging from 2.9 to 3.3%, which are higher than the levels reported in olive oil (2.16 to 2.62%) (Meftah, 2014). The percentages of minor fatty acids in olive seed oils do not exceed 0.7%. From an analytical point of view, the olive processing method had no influence on the fatty acid content of the oils, whatever their origin. However, this content is influenced by the stage of maturity of the olives. Olive seed oils are richer in linoleic acid (Omega 6) than olive oils. This can be explained by the presence of an enzyme, saturase oleate, which transforms oleic acid (C18:1) into linoleic acid (C18:2) during the maturation of the fruit (Gutierre, 1999). According to the results presented in Table 4, the four main fatty acids are oleic acid (65.4%), linoleic acid (20.6%), palmitic acid (8.2%) and stearic acid (3.3%). Saturated fatty acids (SFA), mainly palmitic acid and stearic acid, vary between 11.4 and 12.4%, with a clear predominance of unsaturated fatty acid (UFA) (87.6%), with the dominance of monounsaturated fatty acid (MUFA) (66.2%) over polyunsaturated fatty acid (PUFA) (21.6%). These values are of the same order as those reported for olive oil 87.11% PUFA (Boulfane, 2015).

To position olive seed oil among other vegetable oils, we compared it with oils from jujube, pomegranate seeds, and prickly pear.

Jujube oil is characterized by a predominance of (UFA) at 81.83% compared to (SFA) at 18.18%. The percentage of (MUFA) is 65.57% and that of (PUFA) is 16.26%. Specifically, the four main fatty acids are oleic acid (62.49%), linoleic acid (16.31%), palmitic acid (10.27%) and stearic acid (6.48%) (Guil-Guerrero 2004; Chouaibi M, 2012 and El Hachimi, 2015).

The fatty acid composition of pomegranate seed oil is highly unsaturated (93.67%), with (PUFA) dominating (87.70%) over (MUFA) is (5.98%). The main acids are linoleic acid (C18:2) at 6.31%, oleic acid (C18:1) at 5.39%, and punicic acid (C18:3) at 75.39% (Parashar, 2010; El Hachimi, 2015).

Prickly pear oil presents a predominance of (UFA) at 83.48%. Its (PUFA) content is 62.78%, with linoleic acid dominating between 62.72% and 57.83%. Prickly pear oil generally contains 20.70% (MUFA), dominated by oleic acid (19.48%). However, the average (SFA) content in prickly pear oil is 16.64%. The dominant SFA are palmitic acid (11.42% to 13.98%), stearic acid (3.17% to 4.16%), and arachidic acid (0.29% to 0.47%) (Ramadan, 2003; El Hachimi, 2015).

Comparative analysis (Table 5) shows that residual olive seed oil has an oleic acid content of 65.4%. This value is higher than that observed in other oils: 62.49% for jujube; 19.48% for prickly pear and 5.39% for pomegranate. The linoleic acid content of olive seed oil is 20.6%, 62.72% for prickly pear, 16.31% for jujube and 6.31% for pomegranate. Furthermore, the percentage of IFA in the oil of olive seed, jujube, pomegranate, and prickly pear are 87.6%, 81.83%, 93.67% and 83.48% respectively. Also, the rate of MUFA is 66.2% in olive seed oil, 65.57% in jujube oil, 5.98% in pomegranate oil, and 20.70% in prickly pear oil. For PUFAs, olive seed oil could reach a percentage of 21.6%, which is higher than that of jujube oil (16.26%). However, pomegranate and prickly pear oils registered the highest values (87.70% and 62.78% respectively). In terms of SFA, olive seed oil is 12.4%, compared with 18.18% (jujube), 6.32% (pomegranate) and 16.64% (prickly pear). The results of this study show that olive seed oil has a fatty acid composition dominated by

MUFA. These oils are more stable during heating compared to those rich in polyunsaturated fatty acids (Leercf, 2011). This characteristic is very important when choosing the ingredients for a preparation that requires heating, such as cosmetic preparations. In this context, jujube, pomegranate seed and prickly pear oils have been recognized for their cosmetic uses. All these results allow us to classify this oil as an oleic-linoleic type that can be used in cosmetics, either directly or in preparations.

Materials and methods

Plant material

The study was conducted on the Picholine olive variety, with fruits harvested at two different stages in the Beni Mellal region, Morocco. The green olives, representing the pre-maturation stage, were harvested in October 2018, while the mature black olives were collected in December 2018.
Sample preparation and oil extraction

Samples preparation

In this investigation, all the studied samples were subjected to two different treatments to evaluate their impact on the quality of olive seed oils. Both green and black olive samples were divided into three groups. The first group consisted of untreated samples, the second group was treated with NaOH (2-2.5% concentration for green olives and 8% concentration for black olives for two months), and the third group was treated with NaCl (10% concentration for green olives and 12% concentration for black olives for two months) (Table 1). E1 is the oil extracted from fresh green olive seeds, E'1 is the oil extracted from fresh black olive seeds (E1 and E'1 come directly from olive trees and have not undergone any treatment).
Then, the olive stones were separated from the pulp after two months of fermentation using an industrial pitting machine with a 6 mm sieve separator. All samples were washed to remove any adherent flesh and dried for 48 hours at room temperature. The seeds were obtained by crushing the stones and isolating the cellulose part.

Extraction of oil from olive seeds

The standard Soxhlet method (NF EN ISO 659) was used as a reference for extraction and determination of the oil content. This method consists of extracting the oil using an organic solvent (Hexane) on a solid matrix (trituration) for 8 hours. The extraction was carried out using 100 g of seed paste.
The oil extracted from the olive seeds by crushing and pressing the seed using the argan oil extraction machine is intended for the determination of total phenols and the unsaponifiable fraction (Gharby et al., 2022).

Analytical methods

Oil yield

The oil yield is determined after extraction. It expresses the percentage of oil obtained in relation to the quantity of seeds used for extraction. The yield is calculated using the following formula:
Yield (%) = (H/A) × 100
H: Weight (g) of oil obtained after extraction; A: Weight (g) of powder used.

Unsaponifiable fraction

The unsaponifiable matter of an oil corresponds to all the constituents which, after basic hydrolysis (saponification), are sparingly soluble in water and soluble in organic solvents (Wolff, 1968). Unsaponifiable matter was measured in accordance with AFNOR standard NF60-206. The principle of this method is based on the saponification of a test sample of 5 g of oil in 50 ml of a hot ethanoic solution of potassium hydroxide (2N), under reflux for 20 min. After the addition of 50 ml of distilled water, the unsaponifiables were extracted with diethyl ether followed by washing with distilled water until a neutral washing reaction was obtained. The organic phase was then filtered over anhydrous sodium sulphate and evaporated in a rotary evaporator under vacuum. The residue thus obtained was dried at 103°C and then left to cool in a desiccator. This residue constitutes the unsaponifiable fraction.

Determination of total polyphenols

Total polyphenols are measured by monitoring their capacity to reduce phosphotungstic and phosphomolybdic acids, contained in the Folin reagent, to tungsten and molybdenum oxides. The latter show a bluish color measured at 760 nm.
The Folin-Ciocalteu method (Vernon, 1999) was used to determine the total polyphenol content. 1 ml of oil was mixed with 9 ml of a water/methanol mixture (25/75). The mixture was vortexed for 1 min to extract the polyphenols in the methanoic medium, then 1 ml of dichloromethane was added to allow the oily part to pass into the lower phase and facilitate the collection of 9 ml of methanoic supernatant. 1 ml of Folin reagent diluted 10-fold in water was added and the mixture was left for 2 min at room temperature (25°C) before adding 1 ml of sodium carbonate (75 g/L). The mixture was heated for 15 min at 50°C and then analyzed at 760 nm. Gallic acid with concentrations of 0 up to 100 mg/ml of the methanol/water was used to construct the calibration curve of this assay, according to the following equation:
$Y = 1.665x$ ($R^2 = 0.9913$).

Analysis of the olive seeds oil

The acidity is expressed as the percentage of oleic acid from olive oil. This is a simple and effective way for qualitative assessment and classification by commercial category of olive oil. Free acidity was given as percentage of oleic acid and determined by titration with 0.1 N KOH of an oil solution in a previously neutralized solvent (ethanol/ethyl ether 1:1) and using phenolphthalein as an indicator.
The second criterion for quality assessment is the peroxide value. This index is used to assess the oxidation state of the oil during storage which should not exceed 20 Meq (O_2)/kg for all categories of olive oil according to ICO (2013). Peroxide value was expressed as milliequivalents of active oxygen per kilogram of oil (Meq O_2/kg) and determined by a mixture of oil, chloroform, and acetic acid left to react with potassium iodide in darkness. Free iodine was then titrated with a 0.01 N sodium thiosulfate solution (ICO, 2013). The specific extinction coefficients K232 and K270 were measured from the absorption in cyclohexane solution at 232 and 270 nm respectively (ICO, 2013). UV absorbance was measured with a VWR V-3000PC spectrophotometer at 232 nm and 270 nm. K232 is useful to evaluate the formation of primary oxidation products, linolenic hydroperoxides and oxidized fatty acids that are dienes resulting from the decomposition. The secondary oxidation products hydroperoxides especially diketones and unsaturated ketones absorb light near to 270nm (K270) (Gharby, 2013; Harhar, 2011).
Determination of fatty acid composition by gas chromatography
Fatty acid methyl esters are obtained by the action of methanol in an alkaline medium on glycerides and free fatty acids using the standard method recommended by the International Olive Oil Council (IOC, 2013). To 0.1 g of seed olive oil was added 2 ml of heptane and 0.2 ml of 2N methanolic KOH. After stirring for 30 seconds, the obtained heptane was collected and injected into a gas chromatograph. The fatty acid esters obtained were analyzed using a HP 6890 phase chromatograph equipped with a gas

detector and flame ionization (T = 260°C). The column used was a Carbowax capillary column measuring 30 m x 0.32 mm x 0.25 mm. The carrier gas was nitrogen at a flow rate of 2.5 ml/min. The oven temperature program was 140°C to 200°C, 210°C to 245°C, and the temperature gradient was 10°C for 10 min. Peaks were identified in the presence of controls and the different fatty acid percentages were calculated using an automatic integrator. All analyses were performed in triplicates, and the results are expressed as mean percentages.

Statistical analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA), using SPSS (Statistical Program for Social sciences) version 23.0 for Windows. Each analysis was conducted in triplicate (n = 3) and the results were presented as means with standard deviation (SD). Significance of the F-test was estimated at $p < 0.05$. A Tukey multiple range test was used to determine specific differences between means.

Conclusion

Considering these results, we conclude that oils extracted from olive seeds (a by-product of table olive processing units) have high acidity but normal peroxide values. Additionally, olive seed oils contain higher levels of phenols compared to those found in olive and argan oils. Gas chromatographic analysis shows that this oil is of the oleic-linoleic type. The main fatty acids, in order of importance, are oleic acid, linoleic acid, palmitic acid, and stearic acid. The percentage of oleic acid is less than or equal to that found in olive oil, but the percentage of linoleic acid is higher than in olive oil. This is the main difference between olive oil and olive seed oil. Finally, residual olive seed oil has interesting physicochemical properties, a relevant fatty acid composition, and a high phenol content. Additionally, a comparison with jujube, pomegranate seed, prickly pear, and argan oils, commonly used in the cosmetics sector, shows that olive seed oil is a promising new source of vegetable oil for the cosmetics industry.

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