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RESEARCH ARTICLE

CONTRIBUTION TO THE CHARACTERIZATION OF STEROL COMPOSITION OF THE OLIVE OILS EXTRACTED FROM VARIETIES AND LOCAL TYPES COMPARED WITH THOSE ISSUED FROM SOME INTRODUCED FOREIGN VARIETIES IN COLLECTION AT OUAZZANE GROWING AREAS (NORTHERN MOROCCO)

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ABSTRACT

The aim of the present study is the characterization and evaluation of purity parameters of oils extracted from studied varieties and local types, in comparison with those obtained from transplanted foreign varieties with to regard to elaborate sterols profiles. The β-sitosterol rates are very high (82%-87%) in Bka; BM3; BMK; BMR; BM4; M1; S1; S2; G9; G10; BLg; BRK; Dahbia; Picual; Gordal; Manzanille and Picholine of Languedoc varieties and high in BMM; M6, and Ascolana Tenera (77%-81%). For Δ5-avenasterol, BMM recorded the highest rate (14%). The Manzanille variety gave the lowest one (3.82%). The proportions of campesterol and stigmasterol are not significantly different and are relatively lower than the standard limit (4%). For cholesterol, higher rates were found in M1; Picual; BM4; BMR; Dahbia varieties, and the lowest rates were recorded in BM2; BM3; BMM; BKa; BB; S1; S2 and Gordal varieties. While the Picholine of Languedoc; BRK; G9; BLg; M6; Manzanille; A BRK scolana Tenera and BMK varieties have given intermediate levels. For the erythrodiol and uvauol, the highest proportion was found in the Manzanille and the lowest rate was found in G9. The PCA has allowed constituting three groups. The group1: Ascolana Tenera; Bka; BM2; BM3; BB; BMM; joined by Δ^5-avenasterol, Δ^7-stigmasterol and Δ^7-avenasterol. The group2: BM4; M1; BMC; Manzanille, grouped by cholesterol, campesterol, erythrodiol and uvauol. The groupe3: S1; S2; Picual; Gordal; BLg; G10, G9; Picholine of Langudoc; BRK; Dahbia; gathered by the stigmasterol and β-Sitoserol.

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INTRODUCTION

Morocco has important olive genetic resources poorly known, not exploited or not deeded rationally and little valued. This genetic patrimony (germplasm) of unprecedented wealth and of great genetic and biochemical diversity is in regression and runs the risk of loss and disappearing if nothing is done to characterize and identify it, in order to be more informed about it, for its preservation, conservation, protection, and to safeguard and improve it. This is to be the question to valorize and optimize the uses values, the permanent and equitable traditional economic values of spontaneous, wild and related forms and domesticated or cultivated biodiversity and to reinforce and restore its ability of adaptation to their environment of origin and evolution. The approach followed in this study is to characterize and evaluate the physical and chemical performances of the oil obtained from quasi all studied varieties and local types, as much so, very recently, it have been elaborated methods detecting the richness of the olive oil in minor components of the non-glyceride fraction (unsaponifiable) which it's averred to be of special interest for checking authenticity of all cultivars (sterols, phenolic compounds, tocopherols) (COI,1996, 2003, 2009, 2011 et

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2013;Virginie, 2001; Ben Temime *et al*., 2008; Douzane *et al*., 2010; Ouazzani *et al*., 2013 et 2014; El Antari, 2013 et 2014).

Non-glyceride fraction (unsaponifiable)

Generally, the minor components of the unsaponifiable olive oil unrefined, represent a small proportion, ranging from 1% to 2% in mass. This non-glyceride fraction constituted by classes of compounds comporting several constituents, which may be represented in the trace amount. These compounds are made up in general of minor constituents such as hydrocarbons, pigments (chlorophylls, carotenoids, carotenes), tocopherols, phenols, triterpene dialcohols, methyl sterols and phytosterols. They confer to the extracted olive oil its physical and chemical characteristics and are responsible of its quality and organoleptic properties (bitterness, fruity taste green and black, aroma, astringency, pungent) (Bruni *et al*., 1994b), dietary virtuousness (nutritional), therapeutic, cosmetic and specific benefits, since as brought by food and consumed in small quantities it can reduce significantly the bad cholesterol and therefore participate to prevent the risk of cardiovascular disease, diabetes, cancer, thrombosis, atherosclerosis, arteriosclerosis, neurodegeneressence (COI, 2011 et 2013; Montero, 2015; Maria *et al*., 2015, Harrabi *et al.,* 2016).

Some like the unsaponifiable fraction of soybean, sunflower, rapeseed, hazelnut and milk thistle seeds are found in many pharmaceutical, nutritional and cosmetics applications and gated into the industrial production (food supplement or products preparation, human nutrition, soaps, detergents...). The sterols are tricyclic compounds comporting usually from 27 to 29 carbon atoms and constitutes an important part of the lipid fraction of unsaponifiables (10% to 15%) implied efficaciously in retarding oil deterioration (Casas *et al*., 2004). The phytosterols patrimony of olive oil contains a high proportion of ß-sitosterol, substance which block the intestinal cholesterol absorption and possess interesting biological characteristics (hypocholestorelemy, anti-inflammatory, immune regulation, anti hypertensive, antioxidant, anti carcinogenic) (Yoshida *et al*., 2003; Carr *et al*., 2010; Maria *et al*., 2015; Harrabi *et al.,*2016). The variety, fruit ripeness, soil and climatic conditions (topography, soil moisture, rain fall, temperature), agronomic practices related to irrigation treatment and the methods of extraction and conservation of olive oil, significantly influence the sterolic composition of the olive oil (Di-Giovacchino *et al*., 2002; Ben Temime *et al*., 2008; Carrelli *et al*., 2008; Guillaume *et al*., 2010).

The table variety Manzanille devoted to canning, with large-medium size of fruit less rich in oil and with high moisture, presented the lower unsaponifiable and Δ^7-stigmastenol rates (0.4%) compared to other studied varieties (0.5%) (Nabali, K18) presenting fairly small size of fruit (thinner fruits) and lower water content destined for oil production (Abu-Alruz *et al*., 2011). Indeed according to this author, the Δ^7-stigmasterol content of olive oil is affected mainly by soil type, geographical location, maturity of the olives and degree of fly infestation. As for the extraction temperature, variety, storage conditions of olives before extraction of the oil, the pressing with or without olive seed and the topography of the site exerted only a moderate effect (Abu-Alruz *et al*., 2011). The total sterols content decreases slowly during the olives maturity (Salvador *et al*., 2001; Lazzez *et al*., 2008) and on the contrary, increases gradualy with increasing olives storage duration before trituration (Gutierrez *et al*., 2000). The Δ^7-stigmastenol content is slightly higher in oil obtained from entire olives or those picked up in plastic bags than those pitted or stored in fruit storage boxes.

The olive kernel (stone, pit) contains more and higher level of phytosterols and Δ^7-stigmastenol than the fruit flesh (pulp) (ElAntari *et al.,* 2003b; Abu-Alruz *et al*., 2011). The oil from the olive pomace is rich in sterols than that obtained from complete or entire olives (Rodriguez *et al*., 2008). In olive oil extracted from the paste malaxation (kneading) at high temperature, the stigmasterol concentration is higher than that of Δ^5-avenasterol (Ranalli *et al*., 2001). The Δ^7-stigmastenol content is high in olive oil obtained from orchards planted in red soil (clay sand) compared with marly soil (calcareous and argilous). This white soil reflects direct sunlight and heat and thus reduces evaporation of the water (Abu-Alruz *et al*., 2011). After six months of olive oil storage, Δ^7-stigmastenol increased in different materials, classed or ranked in descending order: ceramic, plastic and glass in the diffused light, tin plated metal, plastic or glass in the dark. The olive oil stored in tins bags(stainless steel) and glass in the darkness, showed the same Δ^7-stigmastenol content throughout the total experimental period (6.5 months). The increment of Δ^7-stigmastenol under different storage conditions (ceramic, plastic and glass in the diffused light), was due to oxidation of the total sterols (the high degree of oxidation, reduces the total sterols and increases Δ^7-stigmastenol levels)(Bendini *et al*., 2009), favored by the permeability of the oxygen (ceramic, plastic), the diffused light (plastic, glass) and catalyzed by the prooxydants metals (tins) (Vekiari *et al*., 2007; Guil-Guerrero *et* Urda-Romacho, 2009; Abu-Alruz *et al*., 2011).

In fact the study of the unsaponifiable fraction through the separation and analysis of certain minor constituents such as, sterols, tocopherols and phenols, appeared to be of a great interest. from one part, the sterols are a biochemical criterion (chemometric marker) for control of the intrinsic quality, purity and verification of varietal authenticity of extracted virgin olive oils (Stiti *et al*., 2002; Matos *et al*., 2007; Ben Temime *et al*., 2008; Al-Ismail *et al*., 2010; Homapour *et al*., 2014) and are unable to detect adulteration by the presence of the adulterant oils of lower quality from different origins (pomace oil, lampante or deodorized olive oil, seed oils of soybean, sunflower, rapeseed, palm, hazelnut, flax, milk thistle seeds) (Al-Ismail *et al*., 2010; Homapour *et al*., 2014, Harrabi *et al.,* 2016). So, the high campesterol content, Δ^7-stigmasterol and brassicasterol detection are indicators or tracers to ascertain the fraudulent mixture of extra virgin olive oil with other vegetable or seed oils (soybean, colza, sunflower, mustard, hazelnut, flax, milk thistle seeds) (Soulhi, 2000 et 2002; Ben Azzouz et Maata, 2008 et 2009; Maata, 2014, Harrabi *et al.,*2016).

The most important sterols are ß-sitosterol, campesterol and stigmasterol, which are consumed in small quantities in a traditional Mediterranean diet (300 mg/day). The composition and sterols spectrum, can be used to identify and classify extra

virgin olive oils according to the varietal origin (Stiti *et al*., 2002; Sanchez *et al*., 2004; Sara *et al*., 2006; Matos *et al*., 2007) and is affected by the geographical origin of olive oil (Youssef *et al.*, 2011). On the other hand, phenols and tocopherols, although they have a minor proportion of olive oil, they are interesting for several reasons: they possess or exhibit a vitamin E and K activity and operate the roles or act as effective antioxidants, influencing the oxidative stability and helping to prolong the olive oil storage period or its shelf life. In the literature no work has been established on the characteristics of quality and purity (sterol composition) of olive oil produced by these emphasized cultivars grown in the Ouazzane region. Then it seems to be of particular interest to undertake or attempt this work, which vise as objective or contemplate to characterize the olive oils extracted from studied varieties and local types and determine their phytosterols composition and other constituents of the unsaponifiable (phenols, tocopherols) according to the geographical growing location (rainfall, temperature, humidity), soils types of the cultivation region and planted cultivars (Manai Djebali *et al*., 2012).

Study of the non-saponifiable fraction

MATERIALS AND METHODS

Plant material

The studied plant material is composed of 5 traditional Moroccan varieties (Bouchouk Laghlide, Bouchouk Rkike, Bakhboukh Beldi, Bouchouika, Dahbia), 6 Moroccan Picholine types (M1, M6, G9, G10, S1, S2), 6 oleasters types (BM2, BM3, BMK, BMM, BMR, BM4) and 5 foreign varieties (Picholine of Languedoc, Manzanille, Gordal, Picual, Ascolana Tenera) in collection (in situ,ex situ) gathered from high performing farmers in terms of quality of extracted olive oil.

Analysis methods

Collection of olives and oil samples

The maâsras selected in various implantation sites of studied varieties and local types, have each one supplied genuine fresh samples of olive oil of highest quality. The olives used for the extraction of oil were taken at human height over the all canopy of old trees of the Moroccan Picholine variety, indigenous varieties and oleasters types grown in private groves at the black maturity stage (maturity index:4). Other samples were hand-picked from varieties of foreign origin planted in the collections. The olive harvest was realized by hand and the fruits were immediately transported to traditional mill (maâsra) for their trituration within the 3 days after harvest. The olives grinding or crushing is executed by rock roller (grinding-stone) getten or drawn by animal traction. The malaxation of paste is performed at the same time as the pulverizing of fruit. Then the olive paste is as soon as putted in copper maked of beton or concreted filled with water, after, the olive oil is separated from water after decantation of the liquid phase (vegetation water) by difference of density and retrieved by hand and then filtered to remove solid impurities. The oil extracted is packaged in opaque or brown glass bottles putted in black plastic sacks (occupied volume 100%), regularly stored at low appropriate temperature (+4°C) in the darkness (refrigeration) until analysis of the parameters of quality and purity performed in agreed laboratory of chemical analysis and researches of Casablanca in Morocco (COI, 2009 et 2013).

Sterols determination (Norm EEC / 2568/91)

Olive oil is characterized by its particular composition in minor components which are extractable by lipid solvents (hexane, diethyl ether, petroleum ether), after treatment of fatty matter with an ethanol potassium hydroxide solution (saponification). Several parameters influence the unsaponifiable proportions of a fatty matter such as genetic origin (genotype of cultivar), the geographical area and pretreatments, as well as the nature of the used extraction solvent. To isolate the unsaponifiable fraction, in a conical flask of 250 ml, the olive oil samples (2.5 g) are saponified in the presence of an ethanolic potassium hydroxide solution 2 N (25 ml) by heating at reflux for 30 min. Then 25 ml of distilled water is added to the still hot saponified samples and the all is versed into an ampoule to decanting.

The extraction of the unsaponifiable is performed by a solvent (hexane or petroleum ether: 70 ml). The washing is performed 5 times with 40 ml of distilled water until total elimination of the saponifiable portion at neutral pH (coloration test of the aqueous solution with distilled water + 3 gouts of phenolphthalein). Then the organic phase is retrieved and the extraction solvent (hexane or petroleum ether) is evaporated in a rotary evaporator, and dried in drying stove for about 5 to 10 minutes (to remove traces of water and hexane or petroleum ether). For dilution of the unsaponifiable, 0.5 to 1 ml of a solvent is added (hexane, isooctane, petroleum ether, then the sterols are separated by thin layer chromatography (TLC). The silica plates (silica gel 60, 0.2 mm, with fluorescent indicator UV254), are soaked in a migration solvent (hexane: 65%+diethyl ether: 35%) for one hour. The revelation of the unsaponifiable on silica plates (TLC) is carried out by pulverization with the 2, 7 dichlorofluoroseine to 2% (Figure 1).

≈≈≈≈≈ Saturated hydrocarbons
≈≈≈≈≈ Unsaturated hydrocarbons
≈≈≈≈≈ Tocopherols
≈≈≈≈≈ Aliphatic alcohols
≈≈≈≈≈ Sterols
≈≈≈≈≈≈ Erythrodiol+ Uvaol
——————— Deposit

Figure 1: Revelation silica plate (TLC)

Then, the bands of sterols, sterol methyl, erythrodiol and uvauol are delimited and retrieved together in a flask of 100 ml, to which is added 5 to 10 ml of cold chloroform to free sterols of the silica plates. The silica plates are liberated by filtration through a glass tube and the chloroform is evaporated by a dry nitrogen stream for 30 minutes in the laboratory ambient temperature.

Silylation

The dry sample contained in the glass tube was then diluted with 500µl of the solution composed by a mixture of three products in liquid form (hexamethyl disilazane 3%, trimethyl

chlorosilane 1% and pyridine 9%). The hexamethyl disilazane is the active part of the reactive. The trichlorosilane, catalyze the reaction of silylation. The pyridine acts as a solvent. The pyridine is evaporated by a dry stream of nitrogen at laboratory ambient temperature for 30 minutes until dryness. Then are added 300 to 500µl of hexane or isooctane and injected to the gas chromatography analysis. The quantification of sterols is carried out by gas chromatography (Type Haweltt Packard 6890, Series GC System, Inlet Front S/Sl, temperature 280 °C, Pressure: 23.8 to 26.2, Carrier gas: Nitrogen (N2), the isothermal oven temperature: 280°C, Injector temperature: 300 °C, Detector temperature: 300 °C).

RESULTS AND DISCUSSION

The sterol profiles and levels of erythrodiol + uvaol of virgin olive oil have been proposed to characterize and identify the olive tree varieties (Stefanoudaki *et al*., 1999; Sancchez *et al*., 2003; Aguilera *et al*., 2005; Fuentes *et al.*, 2015). In all varieties, ß-sitosterol is present in high proportions and dominates the sterol fraction, followed by Δ^5-avenasterol, campesterol and stigmasterol, which represent 96% of the sterol content of the studied varietal virgin oils (Sanchez et *al*., 2004). The other sterol compounds present in the low content included cholesterol, Δ^7-stigmasterol and Δ^7-avenasterol (Philips *et al*., 2002; Cunha *et al*., 2006). The ß-sitosterol content is higher in Bouchouika, oleaster types (BM3, BMK, BMR, BM4), Moroccan Picholine types (M1, S1, S2, G9, G10), Bouchouk Laghlide, Bouchouk Rkike, Dahbia, Picual, Gordal, Manzanille and Picholine of Languedoc varieties (82% to 87%) and neatly higher in analyzed varieties, BMM, Moroccan Picholine type M6 and Ascolana Tenera (77% to 81%) (Table 1 and Figure 2). The Moroccan Picholine, Haouzia, Menara varieties have given at Sais region a high percentage of ß-sitosterol (78.82%-87.6%) and the Arbequine variety presented a lower rate (78.82%-81.45%) (Essiari *et al*., 2014). The Mari Iranian variety presented the highest content of ß-sitosterol (92.08%) and Rowghani variety has the lowest content (87.11%) (Homapour *et al*., 2014).

In Greece varieties (Cobrançosa, Koroneiki), the content of ß-sitosterol decreases with irrigation (Stefanoudaki *et al*., 2009; Fernandez-Silva *et al.*, 2013). For Spanish varieties, Morisca and Carrasqueña, showing a significant differences in ß-sitosterol rate at green maturity stage, there is a negative correlation between the ß-sitosterol and Δ^5-avenasterol (Sanchez *et al*., 2004; Fuentes *et al*., 2015). The Δ^5-avenasterol rate is remained steadily stable from the green stage to turning purple (skin purple) in Monica variety, while it diminishes in the Carrasqueña variety during ripening (Fuentes *et al*., 2015). In later stages of maturity, the activity of the sterol biosynthesis enzyme (Δ^7-sterol Δ^5-desaturase, Δ^7- sterol reductase...) is stopped and the conversion of the sterols in other forms of sterols is stimulated or spurred (hydrogenation, dehydrogenation) (Sakouihi *et al*., 2009; Lukic *et al*., 2013; Harrabi *et al.,* 2016). In Tunisia, the Jarboui variety presented the highest percentage of ß-sitosterol (85.2%) and lowest rate of Δ^5-avenasterol; while an opposite tendency was recorded in the Chetoui variety (Haddada *et al*., 2007; Ben Temime *et al*., 2008). For Δ^5-avenasterol, the greatest rate was found in the oleaster type BMM (14%), while the least one is found in the Manzanille variety (3.82%). The Δ^5-avenasterol content of the Iranian variety Zard, Moroccan Picholine, Haouzia, Menara, Arbequine varieties is higher (5.62% -7.76 %) than those recorded in other varieties (Mari, Gilvan, Picholine) (1.43%-1.78%) (Homapour *et al*., 2014; Essiari *et al.*, 2014). It is reported that the campesterol, stigmasterol and clerosterol, exerted anti oxidative activity or effects on oxidation of a methyl linoleate oil solution (Yoshida et Niki*,* 2003) and the Δ^5-avenasterol at high temperatures improves the oxidative stability of olive oil under frying condition (Wang *et al*., 2002). The campesterol and stigmasterol, present in low proportions, in examined varieties and local types, did not present significant differences, and didn't exceed the imposed maximum limit (4%) (Codex 2003; COI, 2013).

At common wild plant milk thistle seeds oil, the campesterol and stigmasterol levels increased gradually during seed maturation. While the Δ^7-avenasterol and Δ^7-stigmasterol contents decreased and the Δ^5-avenasterol were relatively constant as the seed developed. The rate of cholesterol was very low in immature seeds and to be greatest at the later stage of seed maturation (Harrabi *et al.,* 2016). The campesterol has a higher power of differentiation between varieties that than stigmasterol, being that it is insensitive to variation in environmental factors (water stress, geographical site, conservation) (Sanchez *et al*., 2004).The elevated acidity and lower organoleptic and sensory qualities of olive oil are due to the highest rates of stigmasterol (BenTemime *et al*., 2008). The low proportions of this sterol that did not vary with the ripeness stage of fruit (green, black) indicate that a virgin olive oil is obtained from healthy fruit (Fuentes *et al*., 2015). The Rowghani Iranian variety, presents a campesterol rates shifting from 2.65% (Gilvan region) to 5.49% (Fadak region) and the stigmasterol reached in Fadak 0.48%. For the Zard variety, this rate appeared to be significantively affected by the geographical location (Salvador *et al*., 2001; Ben Temime *et al*., 2008; Homapour *et al.*, 2014). The stigmasterol is present in Moroccan Picholine, Haouzia, Menara and Arbequine varieties at rates ranging of 0.6% to 1% (Essiari *et al*., 2014). At the Cobrancosa variety, the irrigation increases stigmasterol rate (80% to 140%) compared to the control (non-irrigated) (Fernandez-Silva *et al*., 2013). Concerning cholesterol, the greater rates are recorded in (M1, Picual, BM4, BMR, Dahbia) varieties (0.284% -0.431%). The lower rates were recorded in BM2, BM3, BMM, BKa, BB, S1, S2, Gordal and BRK varieties (0.11% - 0.16%). The rates of Picholine of Languedoc, BRK, G9, G10, BLg, M6, Manzanille, Ascolana Tenera, BMK varieties are intermediate (0.19%-0.272%). For Δ^7-stigmasterol and Δ^7-avenasterol, despite the intervarietal variations, recorded proportions are situated within the upper fixed limits (0.5%; 3%-14%) (COI, 2013). In medicinal crop of milk thistle seeds oil, at early stages of seed maturation, the Δ^7-stigmastenol was the most abundant sterol followed by β-sitosterol. Whereas at full maturity, the β-sitosterol was the most predominant sterol and the level of Δ7-stigmasterol is reduced from 54.84% to 27.81% of the total sterol content (Harrabi *et al.,* 2016). Finally, for erythrodiol and uvaol, located in the mesocarp of the olive, the higher proportion was quantified in the Manzanille variety (4.6%) and the lowest rate was found in Moroccan Picholine type G9 (0.611%).

Table 1. Sterols composition of mono varietal olive oils (unsaponifiable) and limit proportions (Codex, 2003; COI, 2013)

Varieties and local types	S1	S2	S3	S4	S5	S6	S7	S8
Limit norms	< 0.5%	< 4%	<Camp	≥ 93%	3%-14%	< 0.5%	< 1%	< 4.5%
B Lg	0.22726	3.2027	2.62688	84.97324	5.80971	0.08972	0.28811	1.04731
BRK	0.20938	2.58007	1.85048	84.64737	6.88007	0.15458	0.47666	1.02824
B.Beldi	0.122588	3.09736	1.36167	81.55416	9.27854	0.76759	0.63633	0.82856
BKa	0.14207	2.9528	1.56251	82.18298	8.14198	0.61809	0.56039	1.40463
BM 2	0.11424	2.62775	1.94511	81.50361	9.42614	0.73908	0.33414	0.53876
BM 3	0.11217	2.67058	2.00732	82.49427	8.83835	0.51284	0.25586	0.45363
BM K	0.27249	3.05214	2.24755	83.32895	4.89589	0.25733	0.52603	2.13347
BMR	0.40927	2.64425	1.3479	83.24882	9.40334	0.43146	0.37726	0.81432
BMM	0.115	2.72702	1.67845	77.47981	14.04118	0.10608	0.91884	1.03489
BM 4	0.34101	3.38295	3.64356	84.88466	4.99345	0.19697	0.26968	1.14465
PM M1	0.28418	2.87784	2.69316	83.53183	6.638	0.08384	0.29576	1.3757
PM M6	0.23231	3.12345	1.00627	80.97651	8.14257	0.42507	0.66045	1.95371
PM S1	0.12688	3.17347	1.90183	83.60457	7.32769	0.16103	0.37329	0.93155
PM S2	0.16929	3.07643	1.90478	84.07566	7.85404	0.1413	0.35699	0.89395
PM G9	0.2221	3.02147	2.15086	85.37905	5.93122	0.18221	0.41434	0.93164
PM G10	0.22004	2.99338	2.13086	84.58536	5.87609	0.19547	0.41049	0.9296
Dahbia	0.43115	3.98391	0.63966	86.81464	5.70286	0.15779	0.35542	0.89136
P. Languedoc	0.19074	2.98835	2.71651	87.46198	4.81673	0.15007	0.29349	1.76014
Picual	0.32346	2.73517	1.13976	83.946	7.03727	0.26095	0.61027	1.07777
A.Tenera	0.25464	2.79769	2.15378	81.31301	8.20722	0.1954	0.52881	1.71834
Gordal	0.09538	2.99892	1.81711	84.5263	5.72614	0.6201	0.27874	1.17094
Manzanille	0.23297	3.15633	1.00841	84.62695	3.82897	0.10944	0.28718	4.60853

+**S1**-Cholestérol +**S5**- ^{5}-avenastérol
+**S2**-Campéstérol +**S6**- ^{7}-stigmastérol
+**S3**-Stigmstérol +S7- ^{7}-avénastérol
+**S4**-ß-sitostérol +**S8**-Erythrodiol+Uvaol

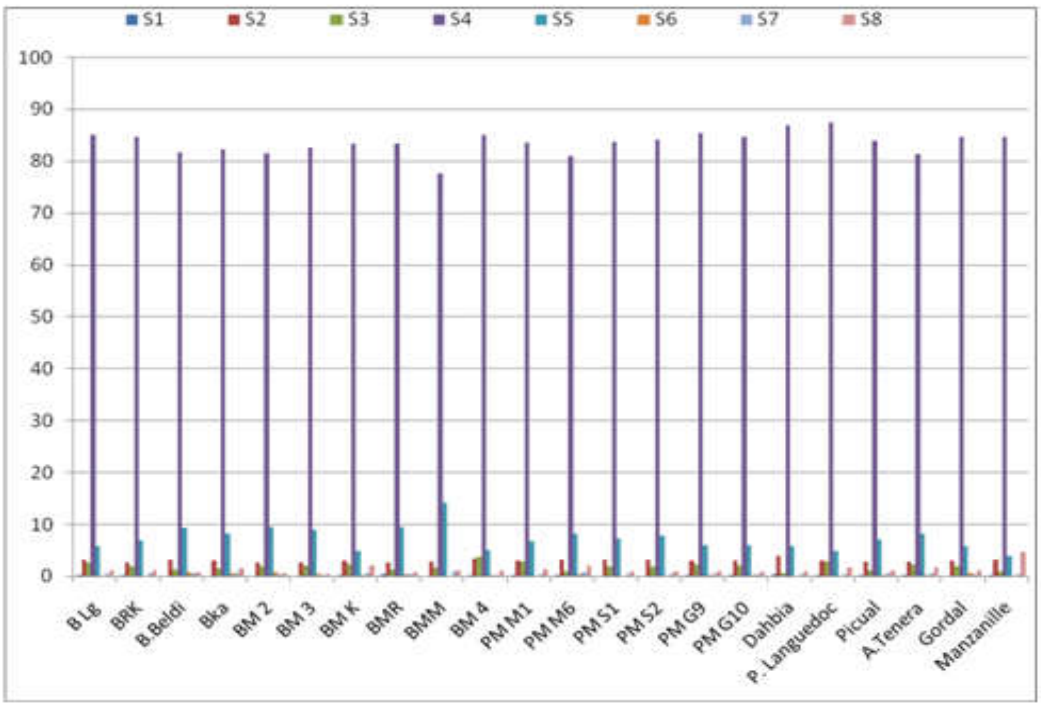

Figure 2. Sterol composition of mono varietal olive oils (unsaponifiable)

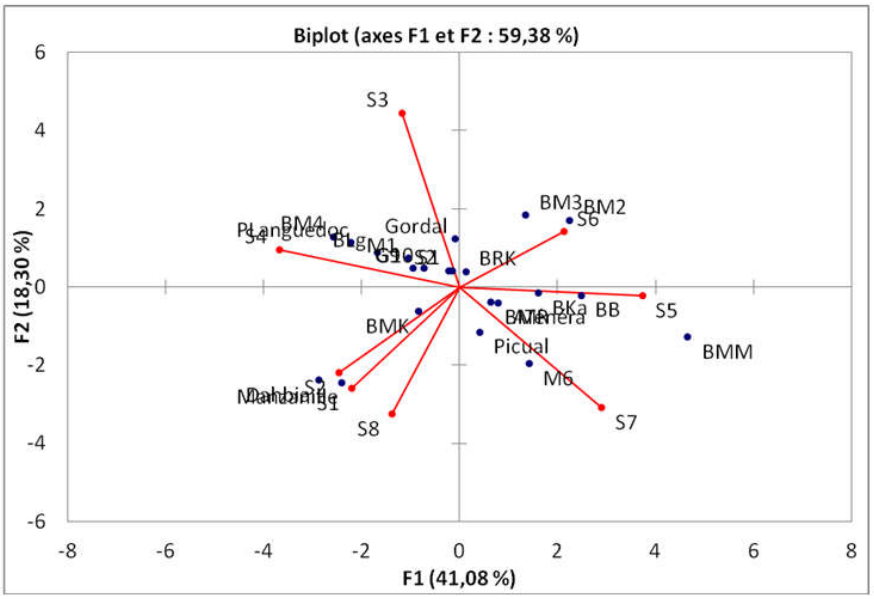

Figure 3. PCA applied to sterols of olive oil issued from studied varieties and local types

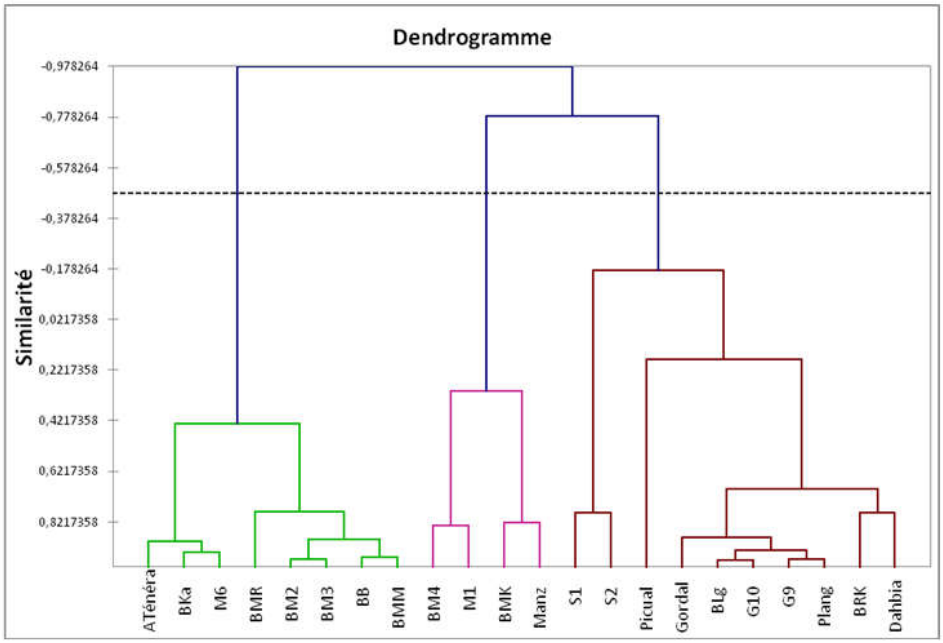

Figure 4. Clustering Hierarchical Analysis of olive oil sterols issued from studied varieties and local types

The principal component analysis (PCA) is used to form groupings between varieties taking into regard the sterol proportions individually or simultaneously of the analyzed monovarietal olive oil (Figures 3 and 4). -The group 1 (Ascolana Tenera, BKa, BMR, BM2, BM3, BB, BMM) can be hampered by the sterols:

Δ^5-avenasterol (S5), Δ^7-stigmasterol (S6), and Δ^7-avenasterol (S7). -The group 2 (BM4, M1, BMK, Manzanille), gathered by the cholesterol (S1), campesterol (S2) and the erythrodiol + uvaol (S8).-The group 3 (S1, S2, Picual, Gordal, BLg, G10, G9, Picholine of Languedoc, BRK, Dahbia), grouped by stigmasterol (S3) and β-sitosterol (S4).

The contents obtained for the main sterols are conform to the normative characteristics and international trade standard limits applicable to virgin olive oils (COI, 2009, 2011, 2013). This is in accordance with European legislation (EC No. 2568) and with the results reported for other Tunisian, Algerian, Spanish, Italian, Portuguese and Iranian oils varieties (Sanchez, 1999; Abaza, 2002; Matos *et al*., 2007; Ben Temime *et al*., 2008; Monia *et al*., 2010; Ilysoglu, 2010; Youssef *et al*., 2011; Pardo *et al*., 2011; Homapour *et al*., 2014; Essiari *et al*., 2014; Fuentes *et al*., 2015). For Tunisian (Oueslati, Chetoui) and Iranian cultivars (Zard, Rowghani, Mari), the sterol profile of olive oils extracted is largely represented by ß-sitosterol, Δ^5-avenasterol, campesterol and stigmasterol. Other types of

sterols, such as cholesterol, $\Delta^{5,24}$ methylene cholesterol, clerosterol, brassicasterol, campestanol, sitostanol, Δ^{7}-stigmastenol, $\Delta^{5,24}$ stigmastadienol and Δ^{7}-avenasterol, were found in all oil samples in small proportions. Triterpenic alcohols (erythrodiol + uvaol) were detected in the analyzed olive oils (Ben Temime *et al*., 2008; Youssef *et al*., 2011; Homapour *et al*., 2014).

In seven predominant Spanish varieties (Comezudo Corniche, Cacerena, Carrasqueira, Morisca, Verdal Bajadoz, Picual), the sterol profile and the erythrodiol + uvauol content were determined at three stages of maturity (green, semi-black, black) and have allowed to classify correctly these varieties in separate groups (Sanchez *et al*., 2004). In Italy, the oil cultivar Morailo has a higher sterols content than the cultivar Leccino (1390 and 1150 mg/Kg of oil respectively). Frantoio cultivar has an intermediate concentration of sterols. The sterol content decreases in fruit harvested at late maturity (Caselli *et al*., 1993) and with increase in altitude in the Spanish varieties (Picual, Hojiblanca) grown in Andalusia (Ferreiro et Aparicio, 1992). The olive oil sterol composition, influenced by genotype, was used to differentiate between the Portuguese (Cobrancosa, Madural, Verdeal, Transmontana) (Matos *et al*., 2007) and Iranian varieties (Zard, Rawghani, Mari) (Homapour *et al*., 2014). In Tunisia, the sterol compounds analysis, triterpenic alcohols, antioxidant content and consequently the oxidative stability reveals significant differences between the studied varieties (Grati Kamoun, 2010). For the majority of sterols and related compounds analyzed, the differences between varieties of the same region and those belonging to other regions are significant, and result from the existence of different environmental conditions (soil type, rainfall, temperatures variations). Variations in the composition of olive oil, taken from the same cultivar were observed depending on the growing area and geographical origins (climate, soil types) (Abaza 2002, Cunha *et al*., 2006; Matos *et al*., 2007; Ben Temime *et al*., 2008; Youssef, 2011). Furthermore, there is an inter-varietal variability in the various sterolic compounds and total sterols content in Tunisian varieties, Chemlali ontha and Jemri benguerdane (1149 to 2693 mg/Kg). The latter variety, presented by a few specimens in the extreme south of the country, has the highest content of β-sitosterol, the main olive oil sterol compound. Total sterols richness of cultivars (Chemlali Sfax, Chemlali North and Chemlali Djerba) was demonstrated (>2000 mg/Kg). It is the variety Chemlali North which has the highest content of total sterols (2515 mg/Kg) (Grati Kamoun, 2010).

At the medicinal crop milk thistle seeds oil the sterols content was affected by the ripening degree of the seeds, the genotype of cultivar (Egyptian, Tunisian, Iranian, Jordanian, European) and environmental conditions (Harrabi *et al.,* 2016). Others estimate that the influence of the variety is predominant and remains the main genetic factor of variation that causes significant differences between the oils on the sterol fraction composition, minor components of olive oil (Sanchez, 1999; Matos *et al*., 2007; BenTemime *et al*., 2008; Ilysoglu, 2010). The olive oil of low land contains low Δ^{7}-stigmastenol content, compared to mountainous areas, this can be due to low moisture availability in their soil (Abu-Alruz *et al*., 2011).

Conclusion

The results of this study helped to highlight the sterol fraction of the unsaponifiable oils extracted from local types and varieties of olive tree planted in the area of Ouazzane. Four sterolic compounds are most widely represented in the analyzed oils, β-sitosterol, Δ^{5}-avenasterol, campesterol and stigmasterol. Quantitative differences (ß-sitosterol, stigmasterol, Δ^{5}-avenasterol) dependent on varieties are influenced by climate changes, frequent and very characteristic of the Mediterranean climate (low rainfall in autumn and winter, hot and dry summer, overwhelming summer drought) and the geographic origin of the oil (Boggia *et al*., 2005; Ben Temime *et al*., 2008; Grati Kamoun, 2010; Youssef *et al*, 2011). In Palestine, the total sterols content of olive oil decreases, this decrease is due to a stable and high percentage of Δ^{7}-stigmastenol (Abu-Alruz *et al*., 2011).

The contents of these minor components are depending on the variety (Olivier, 2006; Caselli, 1993; BenTemime *et al*., 2008; Ilyasoglu, 2010; Grati Kamoun, 2010; Youssef *et al*, 2011; Homapour *et al*., 2014). Similarly, geographical origin (climate, topography, soil types) can influence these sterolic proportions (Boggia *et al*., 2005; Oliver, 2006; Cunha *et al*., 2006; BenTemime *et al*., 2008; Youssef *et al*., 2011; Rondanini, 2011). In the end, this type of study should be widened to other genetic resources present, as it has established the conformity of olive oils from studied varieties and local types with international specifications (COI, 2009, 2011, 2013), and European regulations (CEE, 2009) and contribute to characterize the olive oil produced and to make known their biological value in the national and international market and their valuation as specific regional products. Moreover, it was recently demonstrated that the quantities of total phytosterols, made by a diet rich in extra virgin olive oil, have extra valuable therapeutic properties by reducing the rate of plasma cholesterol in hyperlipidemic and anti carcinogenic by preventing different types of cancers (Ben Temime *et al*., 2008; Grati Kamoun, 2010; Moreno *et al*., 2015; Montero, 2015).

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