Biochemical characterization of artisanal and industrial Moroccan table olives

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Abstract— The study aim the evaluation of industrial and artisanal table olive. The methodology of evaluation followed is done by dimensional analysis (principal component analysis: PCA) and Pearson correlation analysis. The targeted table olives were green olives, red olives and black olives. Belonging to seven regions in Morocco (Guersif, Chawn, Tawnate, Marrakesh, Lamata and El Kalaalagouira). Artisanal table olives are rich in phenolic compounds (4.22g/100g) and carotenoids (0.00181g/100g), industrial table olives are rich in flavonoids (0.0297g/100g) and proteins (9g/100g). PCA analysis shows that distributions of biochemical analysis carried out are able to group the table and artisanal olives to characterize them according to their biochemical contents. We also observed that there is some discrimination between artisanal olives and industrial olives according to the bioclimatic stage.

Key words: Antioxidant activity, Industrial olives, Artisanal olives, phenolic compounds, PCA, Biochemical analysis, flavonoids.

I. INTRODUCTION

A great number of people in developed countries reach an advanced age, but with concomitant diseases such as Alzheimer's, Parkinson's, vascular dementia and diabetes. A diet rich in table olive, high in monounsaturated fats, is associated with a reduced risk from age-related diseases. The mediterranean countries, which in their diet consume considerable amounts of table olive, are characterized by a low level of heart attacks (Guarchet and al.,2014) . The olive tree is one of the most cultivated fruit trees on the mediterranean coast: Spain, Portugal, Italy, Greece, Turkey, Tunisia and Morocco (UNCTD., 2015). Olive production reached 1.56 million, achieving the highest production rate in Morocco so far (M.W.N. 2017). The Moroccan olive picholine (MOP) is a variety that adapts well to the soil and climatic conditions of the whole country and presents the typical characteristics of the double aptitude variety, the olives produced are used to any type of confectionery: broken green olives with ripe black olive (Rokni and al., 2015).

The beneficial effects of table olives consumption have been attributed to monosaturated fatty acids, proteins, total phenolic compounds, total carotenoids, total flavonoids compounds and antioxidant activity (Bianchi 2003 ; Turkmen 2006). However the phenolic compounds have been reported to show the chemo-protective effects against certain cardiovascular illnesses and cancers, for example breast cancer and colon cancer (Covas and al., 2006; Sirianni and al., 2010; Bouallagui and al, 2011)..

Table olives fermentation differs from other nutrients (small weights, beans, cabbage, carrots) from its chemical composition, by the presence of the oleuropein molecule which is responsible for the bitter taste. It contains low proteins (1-2 g/100g), sugars (2,6-6 g/100g) and high fat levels (12-30 g/100g), mainly oleic acid, although these values can change depending on the olive cultivar (Skevin and al., 2003), the ripening stage (Sakouhi and al., 2008), the agricultural techniques (Marsilio and al., 2006) and the preparation methods (Montano and al.,2005).

For industrial preparations we find the Spanish method for green olives, the Californian style for black oxidized olives, and the Greek preparation for black olives (Hui. and al., 2006).

Studies have clearly shown that differences in the factors of geographical origins, process and genetic cause actual differences in the physical and chemical quality and sensory characteristics of table olives (including intrinsic factors, such as those related to the characteristic of the cultivated olive tree variety) and extrinsic factors such as soil, climate, cultivation and manufacturing methods used for industrial olives and storage conditions actually cause differences in composition of the produced table olives and olive oils (Issaoui and al., 2009 ;Karabekir and al, 2013 ; Ben Hassine and al. 2013 ; Noor et al, 2014).

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Many studies have targeted the characterization and composition of types of olives (green olives, red olives and black olives). Different methodology have been used to classify table olives (Ollivier and al, 2006, Runico and al, 2008). Among these methods, dimensional analysis (Principal Component Analysis), have been applied to identify variables and their correlations.

In spite of the large volumes of the Moroccan table olive production and also exportations, the nutritional and the chemical characterizations of the Moroccan fermented olives are not valued. To our knowledge, the chemical changes occurring during fermentation of industrial environment from Moroccan Picholine (Kiai and al.,2014).

In order to identify the main factors those affect the installation and the evolution of the industrial and artisanal fermentation process, which will allow its optimization and mastery. We have prepared artisanal and industrial olives from different regions (Guersif, Chawn, Tawnate, Marrakesh, Lamata and El Kalaalagouira) to compare their biochemical quality and their composition in polyphenols and to study the effect of the terroir on these parameters.

II. MATERIELS ET METHODS

1. PLANT MATERIAL

The present work was carried out on monovarietal olive fruits from the Moroccan variety Moroccan Picholine 'olea europaea'. The olive samples were collected and isolated to three pools (green table olive, red table olive and black table olive) located in the olive growing country side areas of Morocco (figure 1). The locations where the olive samples were obtained were Guersif, Chawn, Tawnate, Marrakesh, Lamta and El Kalaalagouira. The olive fruits were hand-picked and 5 kg of olives were collected from the same trees for each harvest. Only healthy fruits without any kind of infection or physical damage were selected. After harvesting, the olive fruits were immediately transported to the laboratory in cool bags.

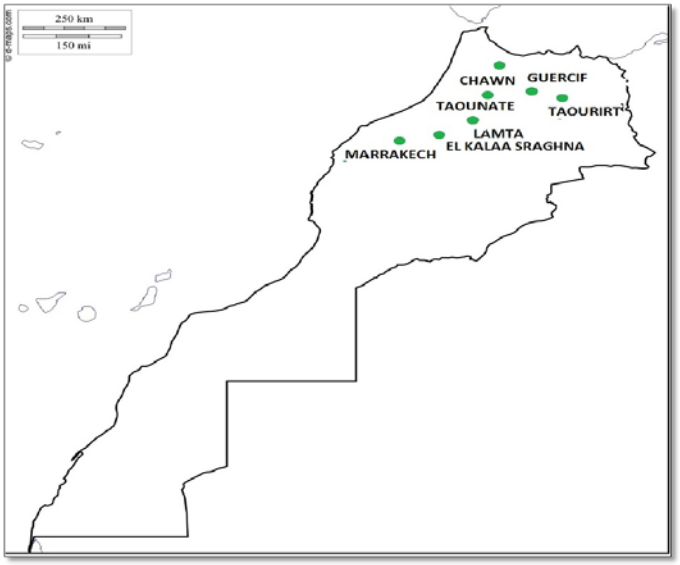

Figure 1: Geographical map of studied area.

2. INDUSTRIAL AND ARTISANAL TABLE OLIVE PROCESSING

After debittering of industrial and artisanal table olives, they are prepared as follows:

TABLE 1: CHARACTERISTICS OF INDUSTRIAL AND ARTISANAL OLIVES

Type of olive	Process
Industrial olives	Water (15 %), salt (4.5%), and gluconate for physico-chemical propriety: 5.5 ≤pH≤ 7; salt 2 to 4%; ferrous gluconate< 0.15 g /kg of total iron.
artisanal olives	Dry salt (2-4 x %), olive oil (1-3 %), garlic (2 %) and thyme (2 %).

The storage was done in one place at a temperature not exceeding 37 ° C.

3. DETERMINATION OF PHENOLIC CONTENT

Phenol content methanolic extracts was analyzed using the modified isolation method described by (Barros and al.,2007). Polyphenol was estimated with Folin–Ciocalteau reagent at 725 nm. The results were expressed as mg of Gallic Acid Equivalent (GAE) (g/100g) of olive paste.

4. DETERMINATION OF FLAVONOID CONTENT

Flavonoid content was determined using aluminum chloride (AlCl3) according to (Ordonez and al.,2006). Using quercetin as a standard. Olives extract (0.1ml) was added to 0.3 ml distilled water followed by 5% NaNO2 (0.03 ml) after 5 min at 25°C, AlCl3 (0.03 ml, 10%) was added. After further 5min, the reaction mixture was treated with 0.2 ml of 1mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The results were expressed as mg of quercetin.

5. DETERMINATION OF LIPID CONTENT

The olive pulp oil was extracted by a Soxhlet system using n-hexane with certain modifications. The fat content is calculated according to (Abaza and al.,2002).

6. DETERMINATION OF PROTEIN CONTENT

The proteins were assayed by modified the method of Lowry (Kumar and al.,2013). Copper sulphate in alkaline medium and Folin-ciocalteu reagent yellow acid consisting of a mixture of phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMo12O40) "in the presence of a protein gives a blue color.

7. DETERMINATION OF CAROTENOID

Carotenoid estimation was done according to analytical procedure by(Nayek and al., 2014). Accurately weighted 0.5g of olive paste sample was taken, and homogenized with 10 ml of acetone. The mixture was centrifuged (10000 g) for 15min at 4 °C. The supernatant were separated and 0.5ml of it is mixed

with 4.5ml of the respective solvent. Chlorophyll-a, Chlorophyll-b and carotenoids content were spectrophotometry determined by using the following equations:

$$Ch.a = 12.25 * A663.2 - 279 * A646.8$$

$$Ch.b = 21.5 * A646.8 - 5.1 * A663.2$$

$$Total\ carotenoids = 1000 * A470 - 1.82 * Ch.a - 85.02 * Ch.b\ 198$$

8. Antioxidant capacity determinations

The DPPH RSA (radical scavenging activity) of paste olive extract was determined according to the method of (Jeong and al.,2004). After a 0.1 ml aliquot of extract dissolved in methanol was mixed with 0.9 ml of 0.041 mM DPPH for 30 min, the optical density (OD) of the sample was measured at 517 nm using a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan). RSA was expressed as a percentage inhibition and it was calculated by the following equation:

DPPH RSA (%) = [1 – (sample OD/control OD)] * 100

9. Statistical analysis

Experimental data was statistically analyzed using PCA (Past Software),Correlation analysis (Pearson).

III. Results and discussion

Biochemical analysis of artisanal olives (Table 2) reveal a distribution that does not vary according to their nature (green olives, red olives and black olives). For these types of table olives, it is noted that lipids are the highest contents, followed gradually by proteins and phenolic compounds. This order of major composition of olives concording to those reported by (Hui and al.,2006; Cicerale and al., 2010) have evaluated that olive contains (1.5-5%) phenolic compounds play significant roles in human nutrition and health .Flavonoids and carotenoids of artisanal olive paste is more rich in carotenoids (moyart = 0.00149 g/100g) and less riche in flavonoids (moyart =0.0208g/100g) compared respectively with industrial olives (moyind = 0.002733g/100g); (moyind=0.04g/100g). Variance analysis of assays done on the three types of artisanal olives shows that almost of the constituents do not show statistical variability in the artisanal olive samples studied.

Table 2: Quantitative distributions of polyphenols, flavonoids, carotenoids, lipids and proteins in different types of artisanal and industrial olives.

analysis	Polphenol		Flavonoids		Lipids		Proteins		Carotenoids	
process	art	ind***	art	ind	art**	ind**	Art	ind*	art	Ind*
Green olive	3.21	0.90	0.0297	0.04	07.91	16.67	6.55	4	0.00181	0.00028
Red olive	4.14	2.43	0.0137	0.02	17.24	29.67	3.38	5	0.0011	0.00044
Black olive	4.22	2.81	0.019	0.06	26.55	46.00	3.7	9	0.00156	0.0001

values are given as mean of three repetitions standard deviation; Art: artisanal, Ind: industrial; *** Significance at P < 0.001 **Significance at P < 0.01; * Significance at P < 0.05.

The results of biochemical analysis of industrial olives presented in table 2 show that lipids are the highest contents (46g/100g) for black olive, followed gradually by proteins (9g/100g) for black olives and phenolic compounds (m= 2.62g/100g) for paste black and red olive. From this it can be deduced that red olives and black olives are richer in phenolic compounds, lipids and proteins than green olives. Which is relatively similar to the results found by (Mahhou and al.,2011) who found that there is a strong correlation between maturity index and lipid content whose lipids can reaches a content of 54%. Also the results of (Suarez and al.,2011) proves that red and black olives have the ability to preserve proteins (8-16%). In addition (Ollivier and al.,2004) has shown that the polyphenol content of olive increases during ripening. In other side these results are agree with those reported by (Kiai and al.,2014). Showing higher concentration in Flavonoids for green olive (1.2g/100g) and less riche in phenolic compounds (2.8g/100g). Variance analysis of these assays in the three types of olives shows that overall the constituents present variability in the industrial samples studied.

In Summary Olive fruit contains many substances belonging to different nature and chemical structure such as chlorophylls and carotenoids pigments, tocopherols, polyphenols, and volatile compounds. They are minority compounds but contain the "fingerprint" of the table olive and olive oil. These compounds also play an important role in the stability and organoleptic characteristics of taste, aroma and color (Hui and al.,2006).

To have a dimensional view of the analysis obtained on the three types of olives, we used the principal components analysis PCA of all merged data done on several types of olives belonging to the three types of table olives (green olives, red olives and black olives). The results obtained are summarized in (figure 2). Analysis shows that green olives are grouped and contribute negatively to component 1. The red olives are also grouped in individual pool and contributed negatively to component 2 (PC2). The black olives are grouped and contributed to component 1 (PC1). These distributions show that the biochemical analysis carried out are able to group the table olives and to characterize them according to their biochemical contents.

Biochemical analysis distribution carried out shows that artisanal olive content in carotenoids, flavonoids and proteins are strongly correlated by contributing negatively to the component 1 and positively to the PC2. These three constituents (carotenoids, the flavonoids and proteins) characterize green olive. Carotenoids in Industrial olives and polyphenols in artisanal table contribute negatively to PC2 and strongly correlate with red olives.

Flavonoids, proteins, lipids in industrial table olive, lipids in artisanal table olive and polyphenols in artisanal table olive contribute to the positive part of component 1 and come into association with black olives.

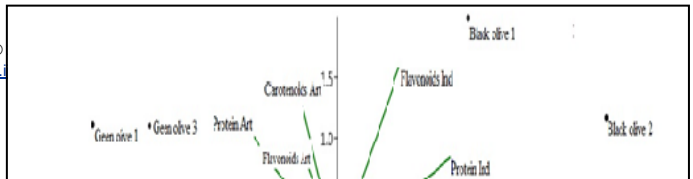

Figure 2: Dimensional analysis of industrial and artisanal olives

In summary, the analysis done on industrial and artisanal table olive shows that black olives are more adapted to industrial treatments. Green olives are more suitable for artisanal treatments. While red olives are more suited to industrial treatments for flavonoids valuation and artisanal treatment for phenolic compounds valuation. The results of principal component analysis of industrial olives for the polyphenols, lipids, flavonoids and carotenoids concurs with research of (Haddam and al.,2014). They found of PCA similar results for orthodiphenol, total polyphenol, tocopherol and fatty acid composition

The following table (table 3) shows the biochemical analyzes carried out on green table olives according to six regions targeted in this study. The principal component analysis (fig.3) of the different olives and their biochemical contents shows that the olives are grouped in two pools. The first is formed by olives from two regions Chawn and Taounate. The second pool is formed by olives from the Lamta region, K Sraghna, Marrakech and Guersif. The first pool is characterized by a subhumid and humid climate while the second pool is characterized by an arid or semi-arid climate. . And this confirmed by the study (Hui and al.,2006) that deduced the biochemical composition of olive allows the characterization of olive trees, the identification of the geographical location, the knowledge of the bioclimatic stage.

These results lead us to suggest that the principal component analysis is a very advisable and reliable approach to trace the origin of biochemical test from different provenance geographical indications systems in Morocco; some other authors have observed for Moroccan olive oils from 92 olive samples other cultivars originating from similar areas (Chefchaoune, Taounate, Fès, Ouazzane, and Meknès) a similar results (Bajoub and al.,2015; Bajoub and al.,2016).

Table 3: Effect of the region on biochemical analyzes performed on green table olives.

Regions Content (g/100g)	Guersif	Chawn	Tawnate	K Sraghna	Marrakech	Lamta
Polyphenols	4.34	3.87	1.42	3.73	4.55	4.14
Flavonoids	0.02	0.03	0.02	0.02	0.02	0.03
Carotenoids	0.013	0.009	0.011	0.020	0.015	0.017
Lipids	24.73	26.55	31.68	16.66	17.24	20.83
Proteins	7.8	6.2	5.65	6.55	2.25	4.5

Values are given as mean of three repetitions standard deviation;

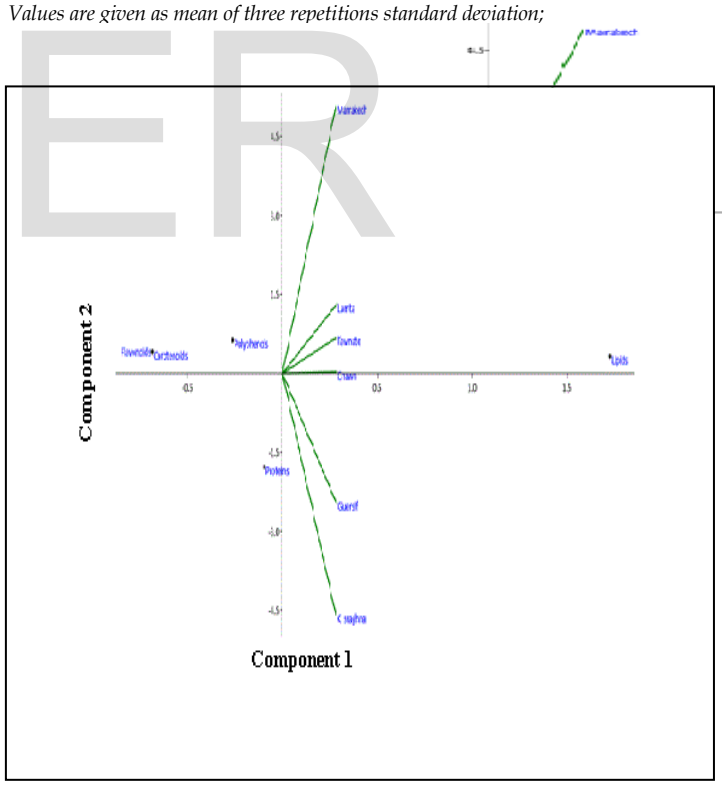

Figure 3: principal component analysis of biochemical tests performed on green table olives.

The most important phenolic compounds contained in table olive, and therefore in the Mediterranean diet, are tyrosol, hydroxytyrosol and oleuropein, which are absorbed by the human digestive system. These components have both antioxidant and chemoprotective activity and a role in endothelial

function improvement (Tuck and al.,2002). To evaluate the role of polyphenols in the antioxidant quality of black olives we found it useful to study the correlation between the antioxidant activity of methanolic extract of the black olive paste. The results obtained show the existence of a linear correlation between antioxidant activities and phenolic contents. The linear model excites in the two types of black artisanal olives (a= 5.023) and industrial table olives (a= 2.670) the only difference is the existence of a difference in the coefficient of direction of two equations. This shows that the polyphenol contents change qualitatively and quantitatively and this according to the manufacturing process (artisanal or industrial table olive). Concerned to industrial table black olives (Haddam and al.,2014) we shows a positive correlation (R^2= 0.6690) between the oxidative stability and total polyphenol content of the oils produced from the Moroccan picholine were in agreement with those found by our study.

Conclusion

The results obtained in this preliminary study showed variation of the physicochemical characteristics of industrial olives, whereas the artisanal olives represent a stability of characteristics.
Green artisanal olives contain high levels of carotenoids, flavonoids and proteins.
Black industrial olives contain high levels of lipids, flavonoids and proteins.
Red olives contain high levels of polyphenols and carotenoids.
The experiment done on green olives show variation of physicochemical properties that is according to the region. The correlation between the phenols content and antioxidant activity are best for the industrial olives (R2=0.815).

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