Physico-Chemical Properties, Fatty Acid Composition and Total Phenol Contents of Olive Oil Extracts by Traditional Method in East Algeria

Tayeb Idoui

University of Jijel, Laboratory of Biotechnology, Environment and Health, (18000) Algeria

tay_idoui@yahoo.fr

Abstract: The purpose of this study was to evaluate the microbiological characteristics of olives then evaluated the physical and chemical characteristics of their oils. Ten olives samples were used in this study, and then aliquots were used for microbiological analysis. The olives oils obtained by traditional method from the same samples were examined for physical and chemical properties (acidity (%), peroxide values, saponification number, pH, moisture and impurities), fatty acids composition and total phenols content.
Results indicate that olives samples reveal a diversity of microflora (mesophilic bacteria, enterobacteria, lactic acid bacteria, yeast and moulds); the physical and chemical characteristics of their olive oils were altered and were not favourable for Algerian Official Journal limits. Oleic acid was found in highest percentage, followed by palmitic, linoleic, stearic and linolinic. Total phenols contents expressed as gallic acid of olive oils values ranged from 60.1 and 97.1mg /kg.

Keywords: Olive, Olive Oils, Composition, Fatty acids, Total Phenols.

Introduction

The Mediterranean coastal areas have a mild, warm climate that fully meets the climatic requirements of *Olea europaea* trees, and they are thus considered an ideal habitat for their growth and development (Boggia et al., 2005). The olive tree grows in a subtropical climate as a traditional main crop, familiar in Mediterranean countries. It probably originates from Mesopotamia and has been cultivated from many centuries in southern European countries bordering the Mediterranean and in North Africa (Murkovic et al., 2004 and Tanilgan et al., 2007).
The olive tree is one of the major agricultural trees in Jijel (Algeria), with an area of 14000 hectares distributed essentially in mountainous areas (Anonymous, 2006). The major part of production is generated in the production of olive oil.
Olive oil has a unique position among edible oils due to its delicate flavour, stability and health benefits (Vekiari et al., 2007). The Mediterranean people considered olive oil not only an excellent food but also a healing agent. During the past four decades a renewed interest in the nutritional and health aspects of olive oil has been generated. Olive oil is a key component of the traditional Mediterranean diet, which is believed to be associated with a relatively long life in good health (Visioli and Galli, 1998 and Tanilgan et al., 2007).
An abundance of oleic acid, a monounsaturated fatty acid, is the feature that sets olive oil apart from other vegetable oils. The Mediterranean diet includes the consumption of large amounts of olive oil, which contains high amounts of phenolic substances (Garcia et al., 2003).
The quality of the olive oil may depend on the composition of the fresh fruits, environmental conditions during the transformation process and the production technology. Spontaneous olive fermentation has shown some problems concerning the acidity of the final product (olive oil) (Poiana and Romeo, 2006). Fermentation is a spontaneous phenomenon in a traditional way, including several stages, depending on spontaneous colonisation by microbial strains, associated with the raw materials and local environmental conditions, which still takes place in olive then the quality of oil olive would be affected.
The annual olive oil production in Jijel (Eastern Algeria) was estimated to 34. 10^3 hectolitres, (Anonymous, 2006), but the quality of this product is affected because before processing extraction, olives are stored at environmental temperature for every week for what the spontaneous fermentation take place. Much work has been done on the effect of storage conditions and packaging materials on olive oil quality but a few works has been done on the effect of olive storage conditions on olive oil quality (Vekiari et al., 2007).
The objective of this study was to evaluate the microbiological quality of olives and then evaluated the physical characteristics and chemical composition of their oils extracted by traditional methods in East Algeria.

Materials and Methods

Olives and olive oil samples

Ten (10) olives fruits samples were collected from different location (Texenna, Tassoust, Kaous, El Emir, Taher and Beni-Ahmed) in East and South-East Jijel (Algeria). Samples were collected during the period when olives are usually harvested for oil production. For each sample aliquots of olives were used for microbiological analysis. From each sample, olive oil is extracted by traditional method and final products were submitted to physical and chemical characteristics determination, fatty acid composition and total phenols contents.

Microbiological analysis

Twenty g (20g) of olives of each sample was diluted in physiologic water (180 ml 0.9% NaCl), homogenized and the dilutions were plated in duplicate onto appropriate media.

The media and the conditions using for microbial numeration were the following: Plate count agar (PCA) incubated at 37°C for 48h for mesophilic bacteria; violet red bile glucose agar (VRBG), incubated at 37°C for 24h for enterobacteria; Baird-Parker agar base, incubated at 37°C for 48h for staphylococci; MRS agar, incubated at 32°C for 48h to 72h in anaerobiosis for lactic acid bacteria; Oxytetracyclin glucose agar (OGA), incubated at 25°C for 3-7 days for yeasts and moulds (Campaniello et al., 2005; Idoui et al., 2009).

Physical and chemical analysis

The pH measurements of olive oils were obtained with a pH meter (HANNA), calibrated with two standard solutions buffered at pH= 4.00 and pH= 7.00. The impurities and moisture were determined according to the method described by Tafalo et al. (2012)

Chemical analysis (Free oil acidity, peroxide value) was performed according to AOAC (1990). Saponification number was determined using the method described by Tafalo et al. (2012).

For the free oil acidity, a known weight of olive oil was dissolved in a mixture of diethyl ether / ethanol (1:1 v/v). The mixture was titrated with potassium hydroxide in methanol (0.05M) in the presence of phenolphthalein as indicator. For peroxide value, about 5g of olive oil was dissolved in a mixture of acetic acid/ chloroform (3:2 v/v), and saturated solution of KI (1ml) was then added. The liberation iodine was titrated with sodium thiosulphate solution (0.05M) in the presence of starch as indicator. For saponification number, a known weight of olive oil (1g) was dissolved in alcoholic potassium hydroxide (25 ml) then evaporated for 30mn. The sample was titrated with chlorydric acid (0.5N) in the presence of phenolphthalein as indicator.

Analysis of fatty acids composition

The analyses of fatty acids were performed according to the official method of the European Community Regulation (1991). The olive oil samples were esterified in a methanol solution of 2N KOH for 30minutes at 50°C. The gaschromatographic analyses of fatty acid methyl esters were performed on a Perkin Elmer gas chromatograph, equipped with a flame ionisation detector (Shimadzu QP2010): The column was a fused silica capillary SE30 length 25meters, diameter 0.25 µm. Helium was the carrier gas. The column temperature program was: initially isotherm at 140°C for 10min, an initial programmed rate of 1°C/min up to 160°C, then a second rate of 2°C/min up to 220°C and a final isotherm for 15min. Samples were injected into the split mode. The apparatus itself carried out recording and integration.

The gas-chromatographic peaks were identified as corresponding fatty acid methyl esters by check of the elution order on the column and compared the retention times with those of pure standards.

Determination of total phenols content

The total phenol content was determined according to the methods described by Tsimidou (1999). 100g of oil was extracted three times with 500ml of methanol (methanol / Water: 40v/60v). The total phenols in the oil extracts were measured by the Folin-Ciocalteu assay. The measurement was carried out at 765nm via UV-spectrophotometer. Results were expressed as mg of gallic acid equivalent in one kg oil.

Statistical Analysis

Significant differences between the results were calculated by analysis of variance (ANOVA). Differences at $p < 0.05$ were considered to be significant. Where there were differences, a Duncan test was applied to indicate the samples between which there were differences.

Results and Discussion

Microbiological analysis of olives

The results of microbiological characteristics of the olives samples are summarized in table 1. Results indicate that olives samples reveal a diversity of microflora. This diversity could be linked to environmental conditions, specially the spontaneous fermentation. Count of mesophilic bacteria is between 2.40 to 9.76 log cfu/g. Samples 01 and 03 contained the highest count of mesophilic bacteria. This result is in agreement with those reported on Sicily olives samples by Poiana and Romeo (2006). The enterobacteria counts ranged from a count of 1.01 log cfu/g to 5 log cfu /g. Staphylococci were not detected in 6 out of 10 samples. Also, counts of lactic acid bacteria

and yeast and moulds were between 1.20 to 8.00 log cfu/g and 1.25 to 8.00 log cfu/g respectively. These results show the changes in the lactic acid bacteria and yeast and mould populations, which grew in all olives samples. The counts of lactic acid bacteria are higher than yeast and moulds. Poiana and Romeo (2006) reported that in general, yeasts coexisted with lactic acid bacteria throughout the whole fermentation period. Their counts were lower than those of the lactic acid bacteria through the most active fermentation period and their presence was stable. These results are in agreement with those reported on Algerian table olives samples by Kacem and Karam (2006). In the same way, Sousa et al. (2006) reported that the fermentation of table olives involves a complex microflora of lactic acid bacteria, yeasts, Gram-positive and Gram-negative bacteria.

Table 1. Counts of microflora olives samples**.**

Sample	Mesophilic bacteria (log cfu/ g)	Enterobacteria (log cfu/ g)	Staphylococci (log cfu/ g)	Lactic acid bacteria (log cfu/ g)	Yeasts and moulds (log cfu/ g)
01	6.40	4.00	0.00	3.24	ND
02	3.24	4.01	ND	3.82	6.80
03	9.76	2.96	0.00	4.40	ND
04	4.00	1.01	0.00	8.00	4.80
05	3.40	1.20	1.40	5.00	3.70
06	2.80	4.00	0.00	3.24	2.20
07	2.40	5.00	ND	5.20	5.00
08	4.00	1.01	1.20	1.20	1.25
09	ND	1.02	0.00	6.00	1.30
10	ND	1.40	0.00	5.20	8.00

ND: Non determined

In the study of Campaniello et al. (2005), microbiological analyses of lactic acid bacteria and yeasts in olives showed that their cell load was higher on the raw material and during the first fermentation phase and increased during the storage of products. The same authors found that staphylococci were undetectable in samples and Enterobacteriaceae remained constant in all samples of analyzed olives, during the 80 days of fermentation.
As reported in literature, the predominant microorganisms in Spanish style treated table olives are lactic acid bacteria; yeasts, instead, are the organisms responsible for fermentation of olives in natural processing (Garrido-Fernandez et al., 1997). In Algerian table olives, lactic acid bacteria and yeasts are also the predominant microorganisms (Kacem and Karam, 2006; Idoui et al., 2009).
Many studies have been conducted regarding the microbial characterization of table olives and results showed the beneficial effects of microflora to maintained the quality of the final product. In contrary, spontaneous olive fermentation used for oil production has shown some problems concerning the acidity of the final product (Poiana and Romeo, 2006). Then it is recommended that olive oil should be extracted just after olives gathering.

Chemical composition and physical properties of olive oils

The characteristics of chemical and physiological properties of olive oils extracted from all samples are shown in table 2. As is shown, important differences were found in chemical values (free fatty acid, peroxide value) among olive oils samples. These properties especially depend on the initial quality of the olives samples.
Acidity (% oleic acid) was in the range of 1.57% and 9.11%. It was higher in all samples excepted in samples 05 and 08. According to Algerian Official Journal and C.O.I (2003), olive oils should have acidity (%) ≤ 3.3% and the acidity contents of 8 out of 10 samples were higher than this limit. These results are not in agreement with those reported on Turkish olive oils samples (Acidity: 0.5%- 1.7%) by Tanilgan et al. (2007). Moussa et al. (1995) established free fatty acid in olive oil as 0.55%- 0.62%.
The results obtained also indicated that peroxide values were higher and ranged between 19 meq O_2/kg – 27.05 meq O_2/kg. It is clear that peroxide values of 80% olive oils samples exceed the value of 20 meq O_2/kg of olive oil, which is the maximum established by the Council for International Olive Oil and Algerian Official Journal. Our results are not in agreement with those found in a study conducted by Vekiari et al. (2007) on the effects of processing methods and commercial storage conditions on the extra virgin olive oil quality indexes, peroxide values in all cases did not exceed 20 meq O_2/kg of olive oil. Similar results are found by Kiritsakis and Dugan (1984), the peroxide values of olive oils obtained from olive fruits collected with different methods in Greece were found between 6.0- 47.7 meq O_2/kg.
Saponification number of all olive oils samples ranged from 102.85 mg KOH/ g to 162.82 mg KOH/ g. All samples were above the limits established by C.O.I (2003) and Algerian Official Journal (184- 196 mg KOH/ g).

In table 2, pH, moisture and impurities values of all olive oils samples are ranged from pH4.65- pH5.73, 0.95% - 3.33% and 0.06% -10.00% respectively. According to C.O.I and Algerian Official Journal, the moisture and impurities of all olives oils samples were higher than the limits. These results are not in agreement with those obtained by Vekiari et al. (2007), impurities values were between 0.21% and 0.43%.
Our observations and results confirmed the negative effect of microbial quality of olives on the final products. Authors reported that during storage, the growth of cellulolytic yeasts and contaminant microorganisms can cause the softening of olives (Lanciotti et al., 1999), and then olive oil quality is affected. In the same way, the significant factor affecting the oxidative reactions was present, water activity and high fatty acids (table2). The increase in oxidation was confirmed by the increase of peroxide values.

Table 2. Chemical and physical properties of olive oils (Mean ± SD).

Sample	Free fatty acid (% oleic cid)	Peroxide value (meq O_2/ kg)	Saponification number (mg KOH/ g)	pH	Moisture (%)	Impurities (%)
01	7.00 ± 0.5	23.00 ± 1.23	146.97 ± 2.75	ND	2.00 ± 0.10	3.75 ± 0.17
02	9.11 ± 1.5	27.05 ± 1.19	147.96 ± 3.35	4.72 ± 0.63	3.00 ± 0.50	4.60 ± 0.13
03	4.51 ± 1.2	26.90 ± 1.50	162.82 ± 2.10	5.63 ± 0.60	2.00 ± 0.45	2.00 ± 0.14
04	6.25 ± 0.9	27.00 ± 1.20	137.92 ± 1.40	4.96 ± 0.53	0.95 ± 0.52	0.06 ± 0.21
05	1.57 ± 1.3	19.00 ± 1.95	128.05 ± 2.50	5.73 ± 0.50	3.33 ± 0.60	3.00 ± 0.19
06	5.12 ± 0.7	23.00 ± 1.25	108.69 ± 1.20	5.63 ± 0.49	2.00 ± 0.35	4.60 ± 0.23
07	6.21 ± 0.5	ND	102.85 ± 1.35	4.74 ± 0.58	3.13 ± 0.44	3.30 ± 0.20
08	3.12 ± 1.4	18.4 ± 0.80	134.77 ± 2.50	ND	2.00 ± 0.34	10.00 ± 0.18
09	3.67 ± 0.3	23.50 ± 0.55	132.73 ± 2.20	4.65 ± 0.55	1.00 ± 0.10	3.75 ± 0.15
10	6.13 ± 0.7	25.00 ± 1.15	157.42 ± 2.30	4.94 ± 0.45	ND	1.19 ± 0.12

ND: Non determined

Fatty acid composition of olive oils

The fatty acid compositions of the ten olive oils samples were determined by gas chromatography and the results are shown in table 3. When examining the fatty acid composition, differences among the samples were observed. It is clear that oleic acid was present in the highest concentration; the values were ranged between 64.62% and 80.60%. It was followed by palmitic acid (10%- 17.30%), linoleic acid (5.45% - 14.26%), stearic acid (3.06% - 7.00%) and linolenic acid (0.82% -2.24%). Sample coded 02 contained the highest concentration of oleic acid (80.60%) but sample coded 05 has a lowest percentage of the same fatty acid (64.62%). These results showed that the total unsaturated fatty acid contents such as oleic, linoleic and linolenic acids were in high levels. Unsaturated fatty acid values were between 76.68% and 86.94%. Differences in these values can be due to species, genetics, variety, growing conditions, locality, climatic conditions and postharvest treatment (Kiritsakis and Markakis, 1984; Aparicio et al., 1994). To our knowledge there is still no information about the fatty acid composition of our local olive oils (Olive oils produced in Jijel's area) and there is not any studies carried out to determine this chemical parameter. The results found in this study were in agreement with those reported by several authors. Tanilgan et al. (2007) determined that the contents of the main fatty acid of olive oils from five Turkish olive varieties ranged between 65.7-81.1%oleic, 3.5-15.5% linoleic, 0.1-3.0% linolenic, 8.1-15.2% palmitic and 2.0-5.6% stearic acids. Ollivier et al. (2005) reviewed 8.49-13.72% palmitic, 2.11-2.6%stearic, 66.36-79.39%oleic, 5.82-11.85%linoleic and 0.61-0.65% linolenic acids. In a study conducted by Aparicio and Luna (2002), the main fatty acids of monovarietal virgin olive oils was ranged between 9.17-11.6% palmitic, 2.2-2.4% stearic, 78.1-80.3% oleic, 4.8-5.7%linoleic and 0.4-0.8% linolenic acid respectively.

Table 3. Fatty acid composition (%) and total phenol contents (mg/kg) of olive oils (Mean ± SD).

Sample	Fatty acid composition (%)					Gallic acid equivalent
	Palmitic	Stearic	Oleic	Linoleic	Linolenic	(Mg /kg)
01	13.50	5.02	71.98	6.25	1.25	65.3 ± 1.2 $^{(bc)}$
02	10.00	3.06	80.60	5.45	0.89	60.1 ± 1.8 $^{(bc)}$
03	17.30	6.02	68.42	7.36	0.90	63.2 ± 1.6 $^{(bc)}$
04	15.70	4.00	73.52	5.83	0.95	61.0 ± 1.3 $^{(bc)}$
05	11.80	7.08	64.62	14.26	2.24	85.2 ± 1.7$^{(b)}$
06	12.74	5.00	73.12	8.14	1.00	64.2 ± 1.9 $^{(bc)}$
07	15.50	4.01	70.45	9.12	0.92	75.0 ± 1.8 $^{(c)}$
08	15.50	5.00	69.21	9.40	0.89	97.1 ± 1.9 $^{(a)}$

09	14.01	5.00	72.24	7.65	1.10	$69.4 \pm 1.5^{(c)}$
10	12.08	3.30	74.85	8.95	0.82	$63.0 \pm 1.6^{(bc)}$

Total phenols contents of olive oils

The results of total phenol contents of olive oils samples are shown in table 3. Total phenol contents expressed as gallic acid of olive oils values ranged from 60.1 and 97.1mg /kg. Total phenol content as gallic acid equivalent in sample coded 08 was the highest (97.1 mg/kg) but sample coded 02 has a lowest percentage of these components (60.1%). These results showed a difference in total phenol contents of olive oils samples. These differences may be due to maturation state and nature of cultivar. The phenol contents of olive oils were found higher than reported by Tanilgan et al. (2007). these authors determined that the contents of the total phenol of olive oils from five Turkish olive varieties ranged between 22.5-97.1mg/kg. The results of study conducted by Garcia et al. (2003) showed that the total phenol content of commercial olive oil is about 400mg/kg as caffeic acid equivalent**.**

Conclusions

To our knowledge, no information existed on physical characteristics and chemical composition of olive oil produced in East Algeria and especially in regions of Jijel. The physical and chemical characteristics of samples collected from different regions in Jijel showed considerable differences. The fatty acid compositions are useful for distinguishing the monovarietial olive oils belonging to particular cultivars. Our results showed that samples of olive oils are extracted from different cultivars.

The concept of total antioxidant capacity of processed foods is gaining momentum and emerging as an important parameter to assess the quality of the product. Our results showed that the analysed samples contained height concentration of phenols. Further studies about the total antioxidant capacity of these samples in vitro and in-vivo were needed.

References

Algerian Official Journal. (1990). Specifications of olive oils, NA1166.

Anonymous, (2006). Statistique 2006-2007. Agriculture Chamber, Jijel, Algeria.

AOAC, (1990). Official methods of analysis, 15th Edition. Association of Analytical Chemists, Washington, DC.

Aparicio, R. Ferreiro, L. Alonso, V. (1994). Effect of climate on the chemical composition of virgin oil. Analytica Chimica Acta, 292, 235-241.

Aparicio, R. Luna, G. (2002). Characterization of monovarietal virgin olive oils. European Journal Lipid Science and Technol, 104, 614-627.

Boggia, R. Evangelisti, F. Rossi, N. Salvadeo, P. Zunin, P. (2005). Chemical composition of olive oils of the cultivar Colombaia. Grasas y Aceites, 56, 276-283.

Campaniello, D. Bevilacqua, A. Damato, D. Corbo, M.R. Altieri, C. Sinigaglia, M. (2005). Micobial characterization of table olives processed according to Spanish and natural styles. Food Technol Biotechnol, 43**,** 289-294.

C.O.I . (2003). Normes Commerciales applicaples aux huiles d'olive et aux huiles de grignons d'olive. C.O.I/ T.15/ N.C.N 3/ Rev. 1.

European Community Regulation. (1991). Official Journal of European Community, L248, Sptember 5th.

Garcia, A. Brenes, M. Garcia, P. Romero, C. Garrido, A. 2003. Phenolic content of commercial olive oils. European Food Rescus Technol, 216, 520-525.

Garrido-Fernandez, A. Fernandez-diez, M.J. Adams, M.R. (1997). Table olives, Production and processing; Chapman and Hall, London; 495 pp.

Idoui, T. Boudjerda, D. Leghouchi, E. Karam, N.E. (2009). Naturally fermented Jijelian black olives: microbiological characteristics and isolation of lactic acid bacteria. Grasas y Aceites, 60, 516-520.

Kacem, M. Karam, N.E. (2006). Microbiological study of naturally fermented Algerian green olives: isolation and identification of lactic acid bacteria and yeasts along with the effects of brine solutions obtained at the end of olive fermentation on Lactobacillus plantarum growth. Grasas y Aceites, 57, 292-300.

Kiritsakis, A. Dugan, L.R. (1984). Effect of selected storage conditions and packaging materials on olive oil quality. Journal American Oil Chemistry Society, 61, 1868-1870.

Lanciotti, R. Corbo, M.R. Sinigaglia, M. Guerzoni, M.E. (1999). Microbial characterization of table olives from the Apulian market. Advanced Food Science, 21, 159-165.
Moussa, Y.M. Metzidakis, I. Gerasopoulos, A. Kiritsakis, A. (1995). The effect of field storage of Koroneiki and Mastoides olives on oil quality characteristics. Rivista Italiana Delle Sost Grasse LXXII, **5**, 253- 259.

Murkovic, M. Lechner, S. Pietzka, A. Bratocos, M. Katzogiannos, E. (2004). Analysis of minor components in olive oil. Journal of Biochemical Biophysic and Methods, 61, 155-160.

Ollivier, D. Artaud, J. Pinatel, C. Durbec, J.P. Guerere, M. (2005). Differentiation of French virgin olive oil RDOs by sensory characteristics, fatty acid and triacylglycerol compositions and chemometrics. Food Chimistry, 97, 382-393.

Poiana, M. Romeo, F.V. (2006). Changes in chemical and microbiological parameters of some varieties of Sicily olives during natural fermentation. Grasas y Aceites, 57, 402-408.

Sousa, A. Ferreira, I.C.F.R. Calhelha, R. Andrade, P.B. Valentão, P. Seabra R. Estevinho L. Bento, L. Pereira, J.A. (2006). Phenolics and antimicrobial activity of traditional stoned tableolives 'alcaparra' Bioorganic and Medicinal Chemistry, 14 (24), 8533-8538

Tanilgan, K. Ozcan, M. Unver, A. (2007). Physical and chemical characteristics of five Turkish olive (*Olea europea L.*) varieties and their oils. Grasas y Aceites, 58, 142-147.

Tofalo, R. Schirone, M. Perpetuini, G. Angelozzi, G. Suzzi, G. Corsetti, A. (2012). Microbiological and chemical profiles of naturally fermented table olives and brines from different Italian cultivars. Antonie van Leeuwenhoek, 102, 121-131.

Tsimidou, Z.M. (1999). Analysis of virgin olive oil polyphenols. Sem Food Analyse, 4, 13-29.

Vekiari, S.A. Papadopoulou, P. Kiritsakis, A. (2007). Effects of processing methods and commercial storage conditions on the extra virgin olive oil quality indexes. Grasas y Aceites, 58, 237-242.

Visioli, F. Galli, C. (1998). The effect of minor constituents of olive oil on cardiovascular disease: New findings. Nutrition Reviews, 56, 142-147.