The Black Olive Fruits of Jijelian Sigoise Variety (Eastern Algeria): Quality Evaluation for Possible Use as Table Olives and Pesticides Research

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Abstract
Black olive fruits of Jijelian *Sigoise variety* (Eastern Algeria) were investigated mainly for physico-chemical composition, microbiological parameters and pesticide research. Carpological results reveal that black olive fruits of Jijelian *Sigoise variety* are suitable for processing as table olives (weight fruits: 3.40 ± 0.28 to 5.95±0.42g). The moisture contents of olive fruit varied between 9.95% and 17.94%. Ash was in the range of 4.60 ± 0.08 % in the samples S03; 7.10 ± 0.03% in the sample S04 and 7.99 ± 0.14% in the sample S02. The olive fruits of sample S01 were characterized by the lowest organic matter (74.25 ± 0.57%) and the highest ash matter (12.00 ± 1.08%). Titrable acidity value for all samples ranges between 7.21 ± 0.30 and 12.46 ± 0.22 (mg/g of flesh). The total amount of phenols among olive fruits was different. The total phenol content as gallic acid equivalent in all samples ranged from 578.40 ± 1.76 to 1059.04 ± 2.35 (µg/ml of flesh). Pesticide research has revealed the presence of an adjuvant to some pesticides (abamectin, delthamethrine) which is butylated hydroxytoluene (BHT) with a concentration of 0.43%.The black olive fruits used in this study were considered valuable for table olives production and one out of three samples contained pesticides

Keywords: Black olives, Sigoise variety, Table olives, Physico-chemical properties, Pesticides

Introduction

The production of table olives in the world is mainly concentrated in the Mediterranean region. In European Union, Spain, Italy, Greece, Portugal and France are the best producer countries. Table olives are a traditional Mediterranean product and, like olive oil and red wine, are one of the most important components of the Mediterranean diet. The Trade Standard Applying to table olives (COI/OT/NC N°1 2004) defines table olives as "the product prepared from the sound fruits of varieties of the cultivated olive tree (*Oleae europaea* L.) that is chosen for their production of olives whose volume, shape, flesh-to-stone ratio, fine flesh, taste, firmness and ease of detachment from the stone make them particularly suitable for processing". Different kinds should be classified according to the fruit ripeness stage, trade preparation, styles and sizing.

In East Algeria (Jijel) olive tree is one of the major agricultural trees with an area of 14,500 hectares distributed essentially in mountainous areas**.** The majority of the olives grown are dedicated to the production of olive oil. In this area, *Sigoise variety* of *Olea europea* was implanted at the end 1990, to develop the production of table olives, whose production is exclusive to Western Algeria (Anonymous, 2009). Sigoise variety is characterized by ratio flesh / stone (average 6.44); high sugar content (greater than 4%) (Balastouras, 1976), salt water tolerant, resistant to cold and drought; precocious flowering; easy harvesting, performed almost entirely by hand; good pollinator of *Chemlal variety* (Mendil and Sebai, 2006)**.**
In East Algeria, olive fruits are harvested when fully matured and completely dark by hand or by knocking the fruits from the tree with a slender pole or by shaking the branches and collecting the fruits from the ground. Contamination of the fruits by hazardous microorganisms may occur while falling unavoidably on the ground or by workers while harvesting handling and working the fruits. The natural fermentation is particularly influenced by the characteristics of the natural olive cultivar, so investigation on olive productions is necessary. In the present study black olive fruits of *Sigoise variety* were evaluated for their physico-chemical composition, microbiological characteristics and pesticide research with the main objective of evaluating the availability of the olives for table olives processing.

Materials and Methods

Samples collection

Four samples of black olives, Sigoise variety were collected from four regions such as Texanna (S01); Kaous (S02); Sidi Abdelaziz (S03) and Settara (S04) in Jijel, East of Algeria. Fruits were harvested between the end of September and the second week of November, at a maturity stage suitable for processing. For each sampling, five Kg of black olives was collected and immediately transported to the laboratory.

Microbiological analysis

In order to evaluate the microbiological quality of black olives fruits (*Sigoise* variety)**,** 10g of each sample was diluted in 90 ml of Ringer solution, homogenized and the appropriate dilutions were plated in triplicate onto appropriate media. The analyses were done in triplicate and the plates were subjected to microbiological numbering by CFU counting.

The media and the conditions used for microbial numeration were the following : Plate Count Agar (PCA), incubated at 37 °C for 48 h for mesophilic bacteria ; Man Rosa-Sharpe Agar (MRS) incubated at 32°C for 48h to 72h in anaerobiosis for lactic acid bacteria; Violet Red Bile Glucose Agar (VRBG) incubated at 37°C for 24-48h for Enterobacteria; King A Medium, incubated at 30°C for 48 h for *Pseudomonas* ; Violet Red Bile Lactose Agar (VRBL) incubated at 37 °C for 24-48 h for total coliforms, and 44 °C for 48 h for fecal coliforms; Oxytetracycline Glucose Agar (OGA) pH 3.5 incubated at 25°C for 5 days for yeast and moulds, Baird –Parker Agar base, incubated at 37°C for 24-48 h for staphylococci and micrococacea (Campaniello et al., 2005; Kacem and Karam, 2006 and Idoui et al., 2009).

Physico-chemical determination

Carpological parameters: length (mm), diameter (mm), fruit weight (g), stone weight (g), flesh weight (g) and ratio flesh /pit were carried out on 50 fruits randomly sampled from the entire amount (Poiana and Romeo, 2006).The analyses were done in triplicate.pH measurements were determined by the use of pH-meter (HANA pH-521). Free acidity was determined according to AOAC (1990). Ten ml of extract olives was dissolved in a mixture of 10 ml of isobutanol- ethanol solvent and 10 ml of alcoholic potassium hydroxide. Titration was achieved by HCl solution (0.5 N) and using a few drops of phenolphthalein as indicator solution. The analyses were done in triplicate.

Determination of moisture content was made according to the following procedure: an initial mass of olives was drying at 105°C ± 1 °C. Sample weight was determined after each time interval (5 min) until stabilization. Method described by Fernandez-Diez and Adams (1997) was used for the determination of dry matter content. It was assessed by oven drying at 105°± 1°C. Olive samples were transferred to a muffle furnace at a temperature of 900°C to complete the combustion of carbon. Once the residue of combustion was colored white, the residue of combustion was weighted. Organic matter content was calculated by the difference between dry matter and ash. The analyses were done in triplicate.

Total polyphenols were extracted after vortexing 5 g of olive flesh with 10 ml of hexane and 10 ml of methanol-water mixture (6v / 4v). Total volume was separated by centrifugation. The lower phase was collected. Methanol-water mixture was added to the upper phase, and the process of centrifugation was repeted. Solvent was added to the lower volume obtained. 0.5 ml of Folin-Ciocalteu's reagent was added to 0.2 ml of the total polyphenols extract. After 3 min, 4 ml of sodium carbonate (1M) was added to the solution and supplemented with distilled water. Preparation were kept in the dark for 90 min. Concentration of total polyphenols was measured spectrophotometrically at 725 nm, and expressed as mg/kg of gallic acid by means of a calibration plot using pure gallic acid as standard (Nassif, 2004). The estimation of total phenolics compounds in the extract was carried out in triplicate.

Pesticides research

A sample preparation procedure for the analysis of pesticides in fresh olives was described by Jon (2008). Olive sample of 25 g were extracted in 60 ml of acetonitrile for 1 min, 50 g anhydrous sodium sulfate was added, and the extraction continued for another 2 min using an homogenizer at 9500 rpm. After centrifugation at 4000 rpm for 3 min, the extraction vessel was transferred to a freezer for a minimum of 4 h or over night. Upon removal from the freezer, part of the organic phase was transferred to a small beaker leaving the solids behind (including

the frozen oil). A 12 ml (9.43 g) portion of the extract, measured by mass, was transferred to a 100 ml round-bottom flask and evaporated to dryness. The residue was reconstituted in 2 ml acetonitrile. Purification step was performed on activated silica gel (45 °C for 4 h). The elution of the sample was made by 10 ml of acetonitrile. The eluate was concentrated at second time in a rotary evaporator (40 °C), then again with 2 ml of acetonitrile. 1µl of the final extract was injected into the GC-MS.

Statistical analysis

Statistical comparison of data was performed by ANOVA to reveal significant differences for each parameter among samples. A probability value of $P < 0.05$ was adopted as the criteria for significant differences.

Results and Discussion

Microbiological Analysis

The microbial count of Sigoise olive fruits was reported in Table 1. The count of mesophilic bacteria is between 18 and 32 $\times 10^7$cfu/g. The enterobacteria counts ranged from a count of 3 and 7$\times$ 10^5cfu /g. *Staphylococci*, *Micrococcaceae* and fecal coliforms were not detected in all samples. Also, counts of lactic acid bacteria and total coliforms were between 13 and 20$\times$ 10^7cfu/ g and 9 and 33 $\times$ 10^3cfu / g respectively. The presence of total coliform bacteria is probably due to olives fecal contamination by contact with the ground during harvesting and storage in unhealthy places.

Table1. Counts of microbial population in sigoise olive samples

Flora	Sample			
	S 01	**S 02**	**S 03**	**S 04**
Mesophilic bacteria (10^7cfu/ g)	18 ±0,01^a	20±0,05^a	26±0,10^a	32±0,04^a
Total coliforms (10^3cfu/ g)	9±0,02^b	12±0,04^b	12±0,01^b	33±0,06^b
Thermotolerant coliforms (10^2cfu/g)	00	00	00	00
Enterobacteria (10^5cfu/ g)	4±0,0^a	6±0,09^a	3±0.4^b	7±0,03^b
Yeast (10^6cfu/ g)	41±0,06^a	47±0,08^c	70±0,05^c	67±0,14^d
Mould (10^5cfu/ g)	26±0,22^a	24±0,12^b	10±0,06^c	34±0,0^d
Lactic acid bacteria (10^7cfu/ g)	13±0,01^a	20±0,09^c	13±0,18^a	14±0,02^a
Staphylococci (10 cfu/g)	absence	absence	absence	absence
***Micrococaceae* (10cfu/g)**	absence	absence	absence	absence

Results are expressed as means ± standard deviation of three measurements. Means followed by a different letter are significantly different.

It should be emphasized that yeasts and moulds were present in high numbers relatively to the other groups of microorganisms, ranging between 41×10^6 and 70×10^6 cfu / g and 10 and 34 $\times$ 10^5 cfu / g respectevly. According to Guiraud and Rosec (2004) the pH of yeast overgrowth is pH 3 - 7.5, which explains their presence in all samples. According to Garcia and Yousfi (2005) damaged olives fruit in cases are a nest of infection from microorganisms. This gradual deterioration is accelerated by the presence of mechanically damaged fruits, which are particularly susceptible to infection by fungi. Stoning olives promote access of *Penicillium* and mould to nutrients. Lipid residue suggests contamination by *Penicillium* with high lipolytic activity. Such activity has been studied in some species of *Penicillium* and *Aspergillus* isolated from olives (Fares et al., 1985).

Carpological parameters

The carpological parameters of Sigoise black olives were reported in Table 2. Other carpological parameters, such as the length, stone, flesh and flesh to pit ratio, were quite significant for all samples. Carpological data reveal that cultivars are suitable for processing as table olives. Only sample S04 was classified, according to the IOOC (2000) as medium weight fruits (from 2 to 4g). Samples S01, S02, S03 have a high weight fruits (from 4 to 6 g). Balastouras (1976) showed that the average weight of olives "varieties Sigoise" varies from 4.5 to 5.5g.Values of the length and diameter of olives "Sigoise" present an apparent variability.

Table 2. Carporogical characteristics of sigoise olive samples

Sample	Length (mm)	Diameter (mm)	Fruit (g)	Stone (g)	Flesh (g)	Flesh / pit
S01	18.42±1.33[a]	14.50±0.68[a]	5.80±0.57[b]	0.41±0.23[a]	5.39±0.31[a]	13.15±0.76[a]
S02	20.52±1.22[b]	14.82±0.65[a]	5.95±0.42[b]	0.63±0.13[b]	5.32±0.46[b]	8.44±1.08[b]
S03	26.09±1.26[c]	18.41±1.23[a]	5.61±0.65[b]	0.56±0.14[c]	5.05±0.55[c]	9.01±0.63[c]
S04	18.41±1.18[d]	14.50±0.51[a]	3.40±0.28[b]	0.31±0.16[d]	3.39±0.20[d]	10.62±0.40[d]

Results are expressed as means ± standard deviation of three measurements.
Means followed by a different letter are significantly different.

Poiana and Romero (2006) described some Italian olive varieties "Nocellara enta, Nocellara missinese, Moresca and Tonda Oliaglora iblea" destined to the production of table olive, the length and diameter ranged from (21.51-27.89) mm and (16.53-22.35) mm, respectively. Compared to our results, sample S03, which was characterized by a length of 26.09 mm and diameter 18.41mm is between the values found for "Oglialora" (21.51mm length, 16.53 mm diameter) and those of 'Tonda iblea "(26.89mm length, 20.37mm diameter). Based on the classification proposed by Brighigna (1998) all samples of Sigoise variety studied had a flesh/pit higher than 5 and so considered very good for table olives. It has been established that fruit quality varies from the seasons and this depend largely on several factors, and, according to Ouaouicha and Chimi (2007) the high temperatures of the spring causes the early loss of fruits and slowing the process of growth due to the excessive effect of evapotranspiration.

Physicochemical characteristics

Dry, ash, organic matter content and moisture of Sigoise olive fruits were reported in Table 3. The results showed that moisture varied between 9.95% and 17.94%; these variations were probable due to the difference in growing conditions, method of harvesting and irrigation (Tanilgan et al., 2007).As shown in the same table, Ash was in the range of 4.60 ± 0.08 % in the samples S03; 7.10 ± 0.03% in the sample S04; 7.99 ± 0.14% in the sample S02 and 12 ± 1.08 % in the sample S01. From the above data, significant differences between samples were observed. Our results are not in agreement with those reported by Ryan and Robards (1998) the sample S01 is characterized by the lowest organic matter (74.25± 0.57%) and the highest ash matter (12.00 ± 1.08%). However, Lopez et al. (2008) reported that variations of this parameter are due to the distribution of minerals in the soil, the stage of maturation, fertilization methods and chemical composition of fertilizers. In addition, dry mater content was not explained by the ripening stage of the fruit olives.

Table3. Dry, ash, organic matter content and moisture of sigoise olive samples

Samples	Ash (%)	Dry matter (%)	Organic matter (%)	Moisture (%)
S01	12.00±1.08[a]	86.25±0.51[a]	74.25± 0.57[a]	15.75±0.91[a]
S02	7.99± 0.14[b]	84.25±0.09[b]	76.26± 0.05[b]	13.04±0.76[b]
S03	4.60 ±0.08[c]	90.05±0.07[c]	85.45± 0.01[c]	9.95±0.77[c]
S04	7.10±0.03[d]	82.02 ±0.05[d]	74.92± 0.02[d]	17.94±0.78[d]

Results are expressed as means ± standard deviation of three measurements.
Means followed by a different letter are significantly different.

Titrable acidity value for all samples ranges between 7.21 ± 0.30 and 12.46 ± 0.22 (mg/g of flesh). According to Boskou (2009) the factors that lead to high acidity in the olives are: the invasion of the fruit by coachman, delays between harvesting and extraction, especially if the fruit was damaged or was bruised during harvest and fungal diseases in the fruit (*Gloesporium*, *Macrophoma*, etc). pH values of samples ranged from pH5.24 ± 0.27 and pH 5.96 ± 0.75. Similar results are found by Ghattas (2004).

Table4. pH, titrable acidity and total polyphenols of sigoise olive samples

Samples	**pH**	**Titrable acidity** (mg/g of flesh)	**Total polyphenol** (µg/ml of flesh)
S01	5.73± 0.50[a]	8.80± 0.55[c]	710.68±1.55[a]
S02	5.24± 0.27[a]	12.46 ± 0.22[c]	578.40±1.76[b]
S03	5.26± 0.11[a]	9.75± 0.07[c]	581.27± 3.99[c]
S04	5.96± 0.75[a]	7.21± 0.30[c]	1059.04±2.35[d]

Results are expressed as means ± standard deviation of three measurements.
Means followed by a different letter are significantly different.

As shown in Table 4, important differences were found in polyphenols content among black olives samples. Total polyphenols content of olives samples presented was variable, ranging from 578.40 ± 1.76 to 1059.04 ± 2.35 (µg/ml of flesh). From the above data, significant differences between samples were observed. These variations are probably due to maturity degree. According to Stupans et al. (2002) the polyphenol content decreases if the optimum stage of maturity is exceeded. Boskou et al. (2006); Tasioula and Okogero (2001); Visioli et al. (2000); Tanilgan et al. (2007) reported that infestation and geographical climate can influence the levels of these compounds. In addition, the water status of the tree affects the synthesis of phenolic compounds in olives.

Pesticides research

We detected and identified a peak corresponding to butylated hydroxytoluene (BHT) (peak 55, rt = 10 603 min) with a concentration of 0.43% (Figure 1); the NIST library integrated in our unit has confirmed the mass Spectrum identity of this molecule which seem to be an adjuvant to some pesticides (abamectin, delthamethrin) (EC-ISO 11014-1 2006). Cultures has not undergone any pesticide treatment, we believe that detection of butylated hydroxytoluene allows us to suspect the presence of pesticides which were degraded and eventually transferred by wind and / or groundwater from nearby greenhouses.

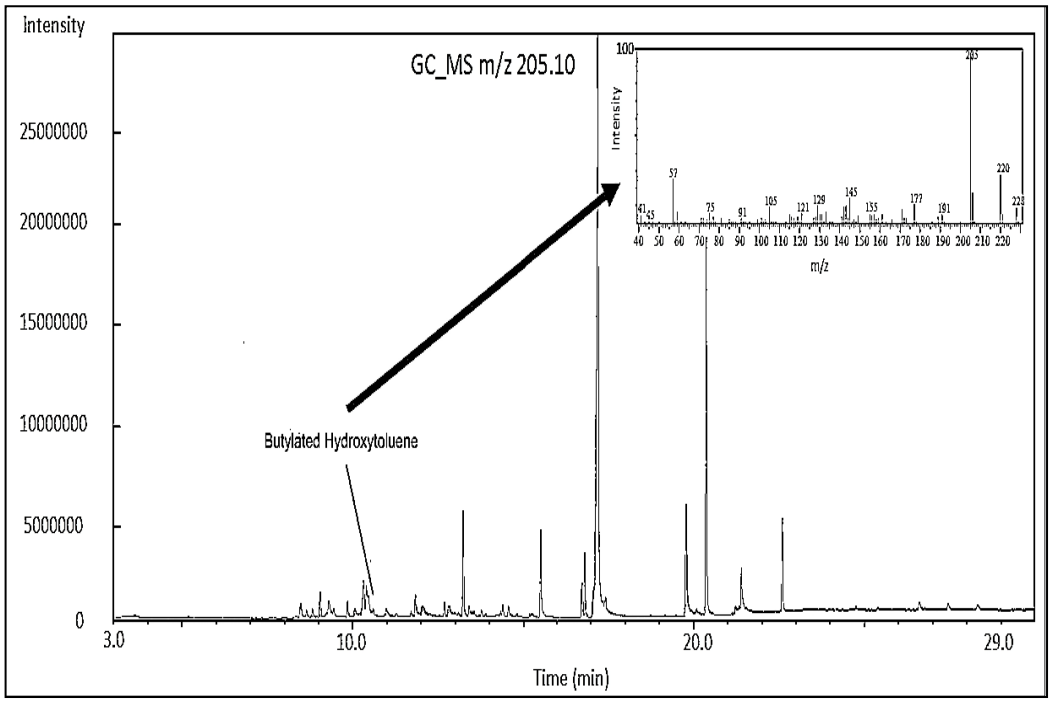

Figure1: Pesticides research in olive (case of sample"$S0_3$)

Also, the analysis of our olive sample shows peaks of varying concentration that revealed the presence of various constituents such as the matrix components which are mainly represented by fatty acids; oleic acid as a major peak (peak 104, rt = 17 195 min) with high concentration 26.58%, linoleic acid (peak 105, rt = 17,344 min) with a concentration of 0.13%, palmitoleic acid (pic89, rt = 14,941 min) with a concentration of 0.10% .Palmitic, stearic and eicosanoic acid (peaks 16, 22, 95) with the same concentration 0.02%. Carbohydrates (sugars): The 1Hydroxy, 3Hydroxy pyrano [3,4-c] pyran-5-carboxaldehyde (peak 87, rt = 14.56 min).Amino acids: The carbobenzenoxy-dl-histidine (peak 51, rt = 10.06 min). Alcohols: Benzyl alcohol (peak 7, rt = 4149 min).Esters: The 4 - pentanoic acid, methyl 3-methyl ester (peak 4, rt = 3511 min).Acids: The nonanoic acid (peak 33, rt = 7572 min). And contaminants whose 1, 2-Benzene decarboxylic (peak 125, rt = 20 336 min) with a concentration of 17.28%.

Conclusion

To our knowledge, no information existed on the quality of black olives fruits (Sigoise variety) cultivated especially in the East of Algeria. Our results showed that the characteristics of the olive fruits revealed their good quality for production of table olives and solely the sample coded SO_3 contained pesticides.

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