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REGULAR ARTICLE

Physicochemical, nutritional and microbiological characteristics of traditional table olives from Southern Portugal

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ABSTRACT

Table olives (*Olea europaea*) are an important fermented product of the Mediterranean diet food pattern and Portugal is one of the biggest producers in Europe. Three different cultivars produced traditionally in Southern Portugal were studied, Maçanilha Algarvia, Cobrançosa and Galega, which were all processed through natural fermentations, although prepared differently. Maçanilha green were cracked, Cobrançosa turning color split, and Galega black prepared as a whole. At the end of the fermentation, the microbiological, physicochemical and nutritional characteristics were studied following standard methodologies. All the fruits showed a good pulp/stone ratio, confirming their aptitude to produce table olives, though their color and texture were statistically different. The three table olives showed suitable acidity and pH values, in addition to a high water content, followed by fat, low-sugar content and fairly low dietary fiber, resulting in a valuable energy food product. Nutritionally, the olives were not significantly different, however Galega had the highest total phenolic content. Statistical differences were observed among their fatty acid composition, with Galega and Maçanilha having the highest oleic and linoleic contents, respectively. The mesophilic microorganisms, the fungi and the LAB were 5.3, 4.7, 4.0 Log CFU/g, 5.9, 5.0, 4.4 Log CFU/g and 5.6, 3.7, 3.7 Log CFU/g for Maçanilha, Cobrançosa and Galega, respectively. No *E. coli*, staphylococci, *Salmonella* sp. and *Listeria monocytogenes* were detected in the samples. The table olives studied revealed an excellent microbial quality and are a good source of phenolics and total unsaturated fatty acids, namely linoleic and oleic acids.

Keywords: *Olea europaea*; Natural fermentation; Nutritional characterization; Maçanilha Algarvia, Cobrançosa and Galega Cultivars

INTRODUCTION

According to the International Olive Oil Council (IOOC, 2016), the most consumed fermented food in Europe are table olives. These processed fruits of *Olea europaea* are of high nutritional value and their consumption is recommended every day in a serving portion, such as a handful (Bach-Faig et al., 2011; Uylaşer and Yildiz, 2014).

In the 2015/2016 crop year, the International Olive Oil Council published that world table olives production was about 2,700,000 ton, in which 770,000 ton were European. Spain was the largest producer with 490,800 ton, followed by Greece (204,000 ton), Italy (50,500 ton) and Portugal (20,700 ton) and finally by France with 1,100 ton (IOOC, 2016). Table olives production is a valuable contribution to the economy of Mediterranean countries.

The olive fermentation processing aims to eliminate its natural bitterness associated with phenolic compounds, enhance the preservation characteristics and improve the organoleptic, nutritional and functional features of the final product. According to the processing operations, the most common commercialized preparations are classified as Treated olives, Natural olives and Olives darkened by oxidation. Treated olives, such as Spanish-style olives, are green, changing color or black fruits that undergo alkaline treatment, followed by complete or partial fermentation (IOOC, 2004). The objective of the lye treatment is to debitter the olives through the chemical hydrolysis of oleuropein and increase the permeability of the olive's skin, resulting in an increase of the efflux of nutrients into the brines. Natural olives, for example, Greek olives, are green, changing color or black fruits, placed directly in brines where they undergo a complete or partial fermentation and

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oleuropein removal is slow and partial. Olives darkened by oxidation, represented mainly by Californian olives, are lye-treated, where the oxidation occurs due to the introduction of air and to the addition of ferrous gluconate or ferrous lactate, and, at the end of the process, thermally treated (Alves and Quintas, 2016; Garrído-Fernández et al., 1997; IOOC, 2004).

The most common olive varieties in Portugal, according Lopes et al., (2004), are Carrasquenha, Cobrançosa, Cordovil de Castelo Branco, Cordovil de Serpa, Galega Vulgar, Maçanilha Algarvia, Redondil and Negrinha (also known as Negrinha do Freixo). In the Southern part of the country, the Algarve, the cultivars mainly used to produce table olives are Maçanilha Algarvia, Galega and more recently, Cobrançosa. The most common way of processing is directly brine olives to obtain Natural olives according to the Trade Standard classification (IOOC, 2004).

This work aimed to characterize table olives produced in southern Portugal using traditional procedures, namely the cultivars Maçanilha Algarvia, Galega and Cobrançosa, regarding their physicochemical properties, nutritional composition and microbiological quality.

MATERIALS AND METHODS

Preparation and Fermentation operations

The olives of the Maçanilha Algarvia, Cobrançosa and Galega cultivars harvested in the crop year of 2014 were supplied by producers from the Algarve and Alentejo regions (Portugal), respectively. In a local medium size factory (Hélder Madeira - Indústria e Comércio de Azeitonas, Unipessoal Lda. Tavira, Portugal) olives were submitted to washing and screening to remove the damaged fruits. Then, Maçanilha Algarvia were cracked, Cobrançosa were split and Galega were prepared as whole olives. Then, the fruits underwent a 'spontaneous' fermentation in a brine prepared with 8% of sea salt until the desirable organoleptic characteristics developed. At the end of fermentation, which usually takes five, six and seven months for cracked Maçanilha Algarvia, split Cobrançosa and Galega cultivars, that is, at the end of February, March and April, respectively, table olive samples were analyzed for physical properties (olive weight, length and diameter, surface color and hardness), pH, total acidity, reducing sugars concentration, total phenolic content, antioxidant activity, fatty acid profile, nutritional value and salt content. Additionally, the microbiological characteristics were also studied, namely the hygienic and safety parameters.

All solvents and reagents used in the methodologies were of chromatographic or analytical grade.

Physical properties

Olive morphology: A sample of 20 olives was used for morphology study. Fruits were weighed individually using a digital balance Mettler PM 300 (± 0.01 g) (Greifensee, Switzerland) and the length and mid-section diameter were measured with a caliper micrometer Powerfix, Z22855 (± 0.01 mm) (Neckarsulm, Germany). After individually measuring the whole olives, the weight, length and mid-section diameter of the stone were also measured. The weight of each fraction was used to calculate the pulp/stone ratio. The edible portion, expressed in percentage, was obtained by the ratio of pulp and whole olive fruit weights. The study of the olives surface color was done with a Dr. Lange Spectro-colour (Berlin, Germany) tri-stimulus colorimeter by the CIE *L a b* system, where the *L* parameter indicates lightness (0 for black to 100 for white), *a* represents green to red (negative to positive values) and *b* represents blue to yellow (negative to positive values) on the hue-circle. The *Hue angle* ($\tan^{-1} b/a$) and *chroma* ($a^2 + b^2)^{0.5}$ parameters were also calculated. One point in each of the 20 fruits were analyzed and the mean average of these values were calculated.

A compression test was performed on ten randomly chosen olives to evaluate the olive's hardness, ie, the maximum force (N) required to compress the sample, using a texture profile analyzer Brookfield, LFRA 1500 (Middleboro, USA) equipped with a flat surface compression probe (TA39) of 2 mm diameter and 20 mm length. The test speed and the total deformation were 0.5 mm/s and 2 mm, respectively.

Chemical analyses

Twenty olives previously depitted were chopped and homogenized in an Ultra-Turrax homogenizer, T25, IKA-Laborthechnik, (Staufen, Germany). The obtained paste was immediately analyzed in triplicates.

The pH values of the brines were measured in a digital Crison instrument, GLP 21pH meter (Barcelona, Spain), at 21 °C.

The total acidity was calculated by the sum of the free and combined acidities and expressed as g lactic acid/100 ml brine (%, w/v) according to Saúde et al. (2017). A titration method described by Fernández-Díez et al. (1985) was used to obtain the free and combined acidities.

The levels of reducing sugars in the olive paste pulp were determined according to an adaptation of the Miller's method (Miller, 1959) described in Maldonado et al. (2008) and expressed as g/kg.

The phenolic compounds were extracted using 10 g of olive paste pulp and 25 ml of pure methanol. The total phenolic

content (TPC) expressed as mg gallic acid equivalent per 100 g fresh olive was determined with the Folin-Ciocalteu test, as described by Singleton et al. (1999) with some modifications (Saúde et al., 2017).

Antioxidant activity: The capacity to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) was monitored according to the Boskou et al. (2006) method with some modifications. A stock solution was prepared by dissolving 5.9 mg $DPPH^{\bullet}$ with 250 ml methanol (6×10^{-5} M) and then stored at – 20 °C until needed. 0.1 ml of olive extract solution was mixed with 3.9 ml of methanolic solution containing $DPPH^{\bullet}$ radicals. The mixture was shaken vigorously and left to stand for 60 min in the dark at room temperature. The absorbance was taken at 517 nm in the spectrophotometer. For the $ABTS^{\bullet+}$ (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) assay, the procedure followed the method described by Thaipong et al. (2006) with some modifications. A working solution of $ABTS^{\bullet+}$ was prepared by mixing two stock solutions of 7.4 mM $ABTS^{\bullet+}$ and 2.6 mM potassium persulfate solution in equal quantities and allowing them to react for 12 h in the dark at room temperature. Then this $ABTS^{\bullet+}$ solution was diluted by mixing 1 ml with 60 ml methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer. 0.05 ml of olive extract solution was added to 0.1 ml of methanol and were allowed to react with 2.85 ml of methanolic solution of $ABTS^{\bullet+}$ for 2 h in a dark condition. Then the absorbance was taken at 734 nm. $DPPH^{\bullet}$ or $ABTS^{\bullet+}$ scavenging effect were calculated as the percentage of DPPH or ABTS discoloration using eq. 1:

$$\%\ \text{scavenging effect} = \frac{A_{DPPH/ABTS} - A_S}{A_{DPPH/ABTS}} \times 100 \qquad \text{(eq. 1)}$$

where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and $A_{DPPH/ABTS}$ is the absorbance of the $DPPH^{\bullet}$ or $ABTS^{\bullet+}$ solution. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of the scavenging effect percentage against the extract concentration in the solution. Standard curves for both assays were obtained by measuring the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activities of 25 - 1000 μM of Trolox solutions (TE). The $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activities were expressed as μmol TE/100 g fresh olive.

Nutritional composition

The contents of macronutrients, moisture, protein, fat, ash, fiber and carbohydrates were determined according to Saúde et al. (2017) using the standard methods of AOAC.

Moisture content was calculated as the difference between 100% and the dry matter percentage. The dry matter was obtained oven-drying 15 g of samples, at 105 ± 2 °C, until a constant weight was attained, according to AOAC 934.01 method (AOAC, 1990a).

The protein content of the wet pulp, expressed as a percentage, was obtained multiplying the total nitrogen content by the 6.25 conversion factor. Five grams of samples were used to determine the total nitrogen content by the Kjeldahl method according to AOAC 920.152 (AOAC, 1990b).

The fat content of the wet pulp, expressed as a percentage, was obtained by extraction in a Soxhlet apparatus, with a minimum time of 13 h, using 5 g of dry matter and *n*-hexane as solvent, following the AOAC 948.22 methodology (AOAC, 2000).

The percentage of ash in the wet pulp was obtained by incineration of 5 g of drupe pulp paste in a muffled furnace at 550 ± 15 °C until consistent weight was reached, in accordance to AOAC 940.26 method (AOAC, 1990c).

Three grams of fat free samples were submitted to successive digestion with acid (H_2SO_4, 1.25%) and alkali (NaOH, 1.25%), drying and incineration to obtain the dietary fiber content, according to AOAC 978.10 method (AOAC, 2005). The results were expressed as a percentage of the wet pulp.

The content of carbohydrates was calculated as the difference between 100% and the sum of percentages of the other macronutrients (moisture, protein, fat, fiber and ash).

The energy value, in kilocalories per 100 g of fresh drupe, was calculated using the conversion factors established in the European regulation (EC, 2008), which are 4 for protein and carbohydrates, 9 for fat and 2 for dietary fiber contents.

The mineral amounts of Na, K and Ca were measured by flame photometry (Jenway, PFP 7, Essex, England) following the methodology reported in Saúde et al. (2017). Each measurement was done in triplicates and expressed in percentage as g cation/100 g wet pulp.

The total mineral content obtained was used to calculate the total salt content of olive pulp (%, equivalent to g NaCl/100 g wet pulp).

Fatty acid profile

Fatty acid methyl esters (FAME) were prepared by the method described by Nunes et al. (2011), using 5 g of olive paste pulp. FAME were dissolved in 100 μl of *n*-hexane solution and 1 μl of this solution was GC analyzed. The relative content of FAME was calculated as a molar percentage on the basis of the molecular weight of each one.

Microbiological characteristics

Twelve samples of each cultivar were examined for aerobic mesophilic and psychrotrophic microorganisms, yeasts and molds, *Enterobacteriaceae*, *Escherichia coli*, lactic acid bacteria (LAB), sulphite reducing clostridium spores and coagulase positive staphylococci. The safety parameters *Salmonella* spp. and *L. monocytogenes* were also investigated. The microbiological study was done using the methods identified in Table 1. *Pseudomonas* sp. were enumerated using Pseudomonas agar base with CFC (Cetrimide, Fucidin, Cephalosporin) Supplement (Scharlau, Spain), incubated at 30 °C for 48 h.

Statistical analyses

The results obtained were expressed as the means of several measurements (depending on the method of analysis) and the standard deviation. Analysis of variance (ANOVA) was done and a Scheffe's multiple-range test was used to compare the average values. Differences were statistically significant at $p < 0.05$. SPSS statistical software version 22.0 (IBM SPSS Statistics 22.0, Faro, Portugal) was used for statistical analyses.

RESULTS AND DISCUSSION

Olive cultivars grown in Southern Portugal, Maçanilha Algarvia and Galega from Algarve and Cobrançosa from Alentejo regions demonstrated biometric variability due to the influence of variety and a result of the biodiversity of this environmental zone. Giuffrè (2017) also mentioned this aspect in a biometric evaluation of olive cultivars from the region of Calabria in South Italy. Table 2 shows the results regarding carpological and color analysis of the epicarp and the consistency of the different cultivars of table olives. According to carpological data, significant differences among cultivars were observed ($p < 0.05$). Galega had the smallest and the lightest fruits and stones, however its drupes had the highest edible portion. The pulp/stone ratio (P/S) is an important quality parameter for table olives and should be higher than 4. A good ratio was obtained for Galega cultivar, confirming its aptitude for the processing of table olives. Although the Maçanilha Algarvia and Cobrançosa cultivars had a P/S ratio inferior to 4, they are processed and consumed as table olives due, mainly, to their sensorial properties, in the case of Maçanilha Algarvia, and the market demand as well as sensorial characteristics in the case of Cobrançosa.

The parameters examined for color showed statistically significant differences among the olive varieties ($p < 0.05$). The Galega was the least bright, followed by Maçanilha and Cobrançosa cultivars, as can be seen by the *L* parameter values, a measurement of olive superficial luminance

Table 1: Methodologies used to determine microbiological quality of table olives

Determination	Methodology	Description
Aerobic Mesophilic Count	ISO 4833-1:2013	Microbiology of food and animal feeding stuffs - Horizontal methods for enumeration of microorganisms. Colony-count at 30°C.
Psychrotrophic microorganisms	ISO 17410: 2001	Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of psychrotrophic microorganisms.
Yeasts and molds	ISO 21527-1:2008	Microbiology - General guidance for enumeration of yeasts and molds. Colony-count at 25°C.
Enterobacteriaceae	ISO 21528-2:2004	Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method.
Escherichia coli β-glucuronidase positive	ISO 16649-2:2001	Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase positive *Escherichia coli* -Part 2: Colony-count at 44°C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide.
Lactic acid bacteria (LAB)	ISO 15214 (British Standard), 1998	Microbiology of food and animal feeding stuffs - Horizontal methods for enumeration of mesophilic lactic acid bacteria. Colony-count at 30°C.
Sulphite reducing *clostridium* spores	NP 2262:1986	General rules to detect spores of sulphite reducing *Clostridium* spores (Regras gerais para a pesquisa de esporos de Clostridios sulfito redutores).
Coagulase positive *staphylococci*	ISO 6888-1:1999; Amd 1:2003	Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium Amendment 1: Inclusion of precision data.
Salmonella spp.	ISO 6579:2002	Microbiology of food and animal feeding stuffs - Horizontal methods for the detection of *Salmonella* spp.
Listeria monocytogenes	ISO 112901:1996 FDAM1:2004	Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method.

($L_{Galega} < L_{Maçanilha} < L_{Cobrançosa}$). The higher the value of this parameter, the better the color. The *a* and *b* parameters displayed a distinct toning of slightly yellow-red for Maçanilha compared with the other varieties (lower +*a* and higher +*b* values). The *hue angle* and *chroma* values indicated a greater intensity of color for Maçanilha and Cobrançosa olives.

The results of texture analysis showed significant differences among the olive varieties ($p < 0.05$). Maçanilha had the highest hardness pulp, followed by Cobrançosa and Galega cultivars. These results are explained by the degree of ripeness of the fruits at the time of processing. According to the traditional processing methods, while the Maçanilha Algarvia fruits were processed in a green state of maturation, the Cobrançosa drupes were processed in a medium degree of ripeness and the Galega olives were only processed when they were mature, showing a softer texture.

Regarding the physicochemical analysis of the brines, namely total acidity and pH (Table 3), the results obtained, in the three varieties studied, are in accordance with the values mentioned in the Trade Standard Applying to Table Olives (IOOC, 2004), which are acidity ≥ 0.3 g lactic acid/100 ml of brine and pH ≤ 4.3. Maçanilha cultivar had the highest acidity and the lowest pH, which were statistically different from the corresponding values of the other olive varieties ($p < 0.05$). Nevertheless, all cultivars showed suitable acidity and pH values, allowing the preservation of table olives, avoiding pathogens and spoilers' growth during storage.

The results of the olive pulp analysis revealed that reducing sugars were still detected at low values (Table 3) in all the studied cultivars, comparing with their content before fermentation, that it was about ten times higher (data not shown). The values obtained were significantly different ($p < 0.05$): Galega olives had the highest reducing sugar concentration, followed by Cobrançosa and Maçanilha cultivars. The different fruits from the different cultivars and preparation methods, before fermentation, may explain the differences observed, as also reported by other authors,

Table 2: Physical properties of table olives

Biometric parameters	Table olive cultivars		
	Maçanilha algarvia	Cobrançosa	Galega
Fruit weight (g)	4.1 ± 0.8^b	4.4 ± 0.7^a	2.3 ± 0.3^a
Stone weight (g)	1.1 ± 0.2^c	1.0 ± 0.2^b	0.41 ± 0.05^a
Pulp/stone ratio	2.7 ± 0.5^a	3.2 ± 0.6^a	4.5 ± 0.7^b
Edible portion (%)	72 ± 4^a	76 ± 3^b	82 ± 3^c
Fruit size (cm)			
Length	1.8 ± 0.2^a	2.4 ± 0.2^b	1.9 ± 0.1^a
Diameter	1.6 ± 0.1^b	1.7 ± 0.1^c	1.38 ± 0.08^a
Stone size (cm)			
Length	1.4 ± 0.1^b	1.8 ± 0.2^c	0.96 ± 0.09^a
Diameter	1.0 ± 0.2^c	0.80 ± 0.07^b	0.24 ± 0.03^a
Color and texture parameters			
L	43 ± 3^a	45 ± 2^c	30 ± 5^a
A	1.51 ± 0.07^a	3.5± 0.9^c	2.6 ± 0.9^b
B	16 ± 2^b	15 ± 2^b	10 ± 2^a
Hue angle	1.48 ± 0.01^b	1.3 ± 0.1^b	1.1 ± 0.3^a
Chroma	18 ± 2^b	16 ± 1^b	10 ± 2^a
Hardness (N)	550 ± 70^c	400 ± 50^b	280 ± 50$^\alpha$

Different letters in the same row indicate significant differences according to a Scheffe's multiple-range test (p<0.05).

Table 3: Physicochemical characteristics of table olives

	Table olive cultivars		
	Maçanilha algarvia	Cobrançosa	Galega
pH	4.2 ± 0.2^a	4.3 ± 0.1^{a,b}	4.4 ± 0.1^b
Total acidity (% w/v, lactic acid)	0.67 ± 0.07^b	0.51 ± 0.04^a	0.50 ± 0.03^a
Reducing sugars (g/kg)	1.9 ± 0.7^a	3.5 ± 0.9^b	4.1 ±0.5^c
Total salt content (%, equivalent to g NaCl/100 g)	3.4 ± 0.6^a	4.7 ± 0.5^b	5.3 ± 0.6^c
TPC (mg/100 g)	120 ± 10^a	160 ± 40^b	230 ± 10^c
EC_{50} (mg/ml)	2.0 ± 0.2^c	0.87 ± 0.09^a	1.07 ± 0.05^b
$ABTS^{\bullet+}$ (mmol TE/100 g)	680 ± 30^a	840 ± 40^c	800 ± 40^b
$DPPH^{\bullet}$ (mmol TE/100 g)	230 ± 20$^\alpha$	480 ± 50^b	440 ± 40^b

Different letters in the same row indicate significant differences according to a Scheffe's multiple-range test (p<0.05).

namely when olives are debittered and rinsed before brining with successive treatments using high lye concentrations of NaOH (Maldonado et al., 2008). In fact, as Maçanilha olives were cracked, Cobrançosa were split and Galega were brined as a whole, the diffusion of reducing sugars from the olive tissues to the brines during fermentation is easier for Cobrançosa and Maçanilha olives, explaining the lower reducing sugar concentrations obtained.

The total salt values acquired in all the studied olive cultivars (Table 3) were lower than the indicated minimal limit value of 6% for natural fermented olives (IOOC, 2004). These results are justified by the fact that, for marketing, table olives were subjected to a new washing phase and packed in an immersion brine with a lower NaCl concentration (~ 2%) than the brines used for olive fermentation (~ 8% NaCl) before seasoning. This is why a lower salt concentration is obtained after an equilibrium between the olive pulp tissues and the new brine has been established. In addition, the lower values of the total salt content in table olives meet the concerns for reducing salt intake in food. The decrease of salt may have severe repercussions in the risk of the safety and spoilage of table olives. However, according to the microbial characteristics studied in the present study (Table 6), *Salmonella* spp. and *L. monocytogenes*, considered as food safety parameters were not detected as in the study of Mateus et al. (2016).

Olive polyphenols have major functions in human nutrition as preventive agents against numerous illnesses (Boskou *et al.*, 2006). Statistically significant differences of olive polyphenols (TPC, Table 3) were obtained among studied varieties ($p < 0.05$), with the Galega showing the highest content, followed by olives of the Cobrançosa and Maçanilha Algarvia cultivars. As reported by several authors in studies with other cultivars, the differences observed are due to the influence of cultivar (Malheiro et al., 2011; Pereira et al., 2006; Romero et al., 2004a; Vinha et al., 2005), to the degree of ripeness of the fruit when it is processed (Gutiérrez et al., 1999; Othman et al., 2009; Ryan et al., 1999; Sousa et al., 2014) and to the elaboration methods (Marsilio et al., 2001; Pereira et al., 2006; Romero et al., 2004a). In fact, Maçanilha olives lost significant quantities of phenolic compounds during fermentation because they were previously cracked, exposing a higher area of olive flesh which increases the compounds' diffusion. On the other hand, Galega cultivar natural black olives contained the most phenolics, indicating that during processing loss was minimized, as their drupes were fermented as a whole. As reported by several authors (Garrído-Fernández et al., 1997; Tuna and Akpinar-Bayizit, 2009), lye treatments and subsequent washing phases before fermentation of olives prepared Spanish-style, involves an intricate mechanism in the removal of some water-soluble substances from the olive drupe, as well as oleuropein, the most abundant biologically active coumarin-like phenolic compound, and their final content in table olives largely depends on the processing method, corroborating the results of the present study. According to several authors, there is a linear correlation between the total polyphenol content of table olives and their antioxidant activity (Romero *et al.*, 2004b; Sousa et al., 2008, 2014). The results obtained with the three studied varieties showed the same trend, with the $DPPH^{\bullet}$ or $ABTS^{\bullet+}$ scavenging effect of Maçanilha cultivar presenting the lowest values. Cobrançosa table olives showed the highest antioxidant potential, with the corresponding highest values of $DPPH^{\bullet}$ or $ABTS^{\bullet+}$ scavenging effect and the lowest concentration providing 50% inhibition measured by EC_{50} parameter. As a result, table olives represent an important supply of antioxidant phenolic compounds. The consumption of a portion of table olives (50 g) provides about 58 – 116 mg of total phenolic compounds, which corresponds to 342 – 419 μmol Trolox equivalent. These values were approximately 10% of the estimated total phenolic intake in Spain, which is 1171 mg galic acid per person and per day, and also, 10% of the correspondent total dietary antioxidant capacity of this diet of 3549 mmol Trolox equivalent by ABTS test (Saura-Calixto and Goňi, 2006).

Table 4 shows the mean nutrient composition of the studied table olives and the daily nutrient reference intakes proposed by World Health Organization (WHO, 2015), for comparison between the amount existing in these table olives and the human requirements.

The macronutrients found at lower concentrations were protein, dietary fiber, ash and carbohydrates, followed by fat. Water was the major component. Galega cultivar had significantly higher contents of ash and carbohydrates than the other table olives ($p < 0.05$), as well as dietary fiber than Maçanilha Algarvia table olives ($p < 0.05$), which can be attributed to the different maturation stages of the three olive cultivars when they were processed, as it was also observed by other authors (Sousa et al., 2011). In addition, the drupes of Maçanilha and Cobrançosa were fermented with the pulp, cracked or split, in opposition to the Galega ones that were processed as a whole, and once in contact with the brine, a reduction in the substrate amounts of the Maçanilha and Cobrançosa could easily occur.

A portion of 50 g of table olives can provide about 5% of the daily energy requirements and 15 to 30% of the total lipid needed, considering the 33 – 67 g/day recommended amount.

Regarding cations contents, Galega had the higher significant amounts of sodium comparing to the other

Table 4: Nutritional composition of maçanilha algarvia, cobrançosa and galega table olives (per 100 g of fresh weight of edible olive) and daily nutrient reference intakes proposed by world health organization (WHO, 2015)

	Table olive cultivars			WHO nutrient reference intakes (/Day)
	Maçanilha algarvia	Cobrançosa	Galega	
Energy (kcal)	210 ± 20^{a}	220 ± 40^{a}	220±20^{a}	2000
Water (g)	69 ± 2^{a}	67 ± 4^{a}	66 ± 2^{a}	
Protein (g)	1.2 ± 0.2^{a}	1.2 ± 0.2^{a}	1.2 ± 0.1^{a}	50-75
Fat (g)	21 ± 2^{a}	23 ± 4^{a}	22 ± 3^{a}	33-67
Carbohydrate (g)	2.7 ± 0.9^{a}	3.2 ± 0.9^{a}	4.3 ± 0.9^{b}	275-375
Dietary fiber (g)	2.3 ± 0.5^{b}	1.2 ± 0.4^{a}	1.8 ± 0.8^{b}	> 25
Ash (g)	3.4 ± 0.3^{a}	4.4 ± 0.4^{b}	4.6 ± 0.9^{b}	
Na (mg)	1140 ± 190^{a}	1640 ± 200^{b}	1860 ± 230^{c}	< 2000
K (mg)	130 ± 30^{b}	100 ± 10^{a}	145 ± 5^{b}	> 3510
Ca (mg)	100 ± 40$^{\alpha}$	110± 10$^{\alpha,b}$	127 ± 8^{b}	< 3000

Different letters in the same row indicate significant differences according to a Scheffe's multiple-range test (p<0.05).

cultivars, the higher significant content of potassium than Cobrançosa and the higher significant content of calcium than Maçanilha Algarvia table olives ($p < 0.05$), probably due to the ripeness degree of the cultivar and the elaboration method used before fermentation. In addition, despite the variability of the different types of table olives, on average, a portion provides a very high amount of sodium, nearly a third to half of the daily limit (< 2000 mg/day, according to WHO recommendations (WHO, 2015), specifically 46.5% for Galega, 41.1% for split Cobrançosa and 28.6% for cracked Maçanilha cultivars. This high Na concentration results from the processing and preservation of olive products which rely on salt. Therefore, the differences observed are due to the elaboration style used. Table olives contribute with about 2% of the potassium recommendation needs (> 3510 mg/day) and also 2% of the calcium tolerable upper intake level (< 3000 mg/day (WHO, 2015)).

Table olives also have important compounds with relevant active properties, such as fatty acids, mainly monounsaturated fatty acids (Bianchi, 2003; Al-Bachir, 2017). When considering the fatty acid profile (Table 5), all table olives studied showed high amounts of total unsaturated fatty acids of 77.1, 78.1 and 78.8 mol% for Maçanilha, Cobrançosa and Galega cultivars, respectively. Five major fatty acids were identified, palmitic, palmitoleic, stearic, oleic and linoleic. Oleic acid is the predominant one, ranging from 61 mol% (Maçanilha) to 78 mol% (Galega). Palmitic was the second most abundant fatty acid (22 mol%), followed by linoleic, stearic and palmitoleic acids. Maçanilha cultivar had the highest linoleic content (16 mol%), followed by Cobrançosa (9 mol%) and Galega (0.7 mol%), and conversely the lowest oleic content. Significant differences were observed among these results ($p < 0.05$), making Maçanilha the healthiest cultivar in terms of its fatty acid profile. A portion (50 g) of Maçanilha, Cobrançosa or Galega table olives provides an average of about 2.2, 2.4 or 2.2 g of total saturated fatty acids, approximately 10% of the upper intake level recommended of 22 g/day (WHO, 2015), 6.7, 8.1, or 8.9 g of monounsaturated fatty acids and 1.7, 1.1 or 0.1 g of polyunsaturated fatty acids, which correspond to 13.1, 8.1 or 0.6% of polyunsaturated fatty acids needs (13 – 22 g/day (WHO, 2015)).

The results of the microbial quality of the three types of table olives studied are summarized in Table 6. The safety microbial parameters, *Salmonella* sp. and *Listeria monocytogenes* were not detected in the olives, which meets the microbiological criteria of food safety according to the European regulation (EC, 2005, 2007). The countings of *Enterobacteriaceae*, *Escherichia coli*, sulphite reducing *Clostridium* spores, coagulase positive staphylococci and *Pseudomonas* sp., in all the studied samples, were inferior to 10 CFU per gram of olives. The aerobic mesophilic, the psychrotrophic microorganisms, the yeasts and molds and LAB were also enumerated and the values found were within the limits expected for fermented foods and described in the Trade Standard Applying to Table Olives (IOOC, 2004). The microbial counts of mesophilic microorganisms and yeasts obtained were in the range of those described by Alves et al. (2015) and in the review of Arroyo-López et al. (2012) for yeasts. The presence of mesophilic and psychrotrophic groups also warnings for the potential of the table olives to undergo alteration processes mediated by microorganisms, during the shelf-life period, especially when high levels of reducing sugars are available in the brines or in the olives (Alves et al., 2015). The occurrence of psychrotrophic microorganisms alerts to the fact that even at refrigeration temperatures, the product may be altered, though more slowly. The absence of *Enterobacteriaceae* including *E. coli* in the table olives is a desirable result, which could be related to the increasing acidity in brine (Romeo et al., 2012). This microbial group should be monitored as it is responsible for an increased risk of spoilage of table olives (off flavors and gas pocket in the surface of olives) (Garrído-Fernández et al., 1997). Galega olives presented lower levels of the various microbial groups in comparison to the other

Table 5: Fatty acid profile of table olives

	Table olive cultivars		
	Maçanilha algarvia	**Cobrançosa**	**Galega**
Free fatty acids (mol%)			
Palmitic acid (C16:0)	22.0 ± 3.0^a	22.0 ± 1.0^a	21.3 ± 0.2^a
Palmitoleic acid (C16:1)	0.049 ± 0.004^a	0.09 ± 0.02^b	0.06 ± 0.01^a
Stearic acid (C18:0)	0.030 ± 0.007^a	0.25 ± 0.06^b	0.016 ± 0.004^a
Oleic acid (C18:1)	61.0 ± 8.0^a	69.0 ± 2.0^b	78.0 ± 0.7^c
Linoleic acid (C18:2)	16.0 ± 3.0^c	9.0 ± 1.0^b	0.70 ± 0.04$^\alpha$

Different letters in the same row indicate significant differences according to a Scheffe's multiple-range test (p<0.05).

Table 6: Microbial characteristics and safety parameters of table olives of the maçanilha algarvia, cobrançosa and galega cultivars

	Table olive cultivars (Log CFU/g)		
	Maçanilha algarvia	**Cobrançosa**	**Galega**
Aerobic Mesophilic Count	5.7 ± 0.2^b	4.7 ± 0.7^a	4.0 ± 0.4^a
Psychrotrophic microorganisms	5.3 ± 0.7^b	5.2 ± 0.8^b	3.8 ± 0.7^a
Yeasts and molds	5.9 ± 0.4^c	5.0 ± 0.7^b	4.4 ± 0.4^a
Enterobacteriaceae	< 1	< 1	< 1
Escherichia coli β-glucuronidase positive	< 1	< 1	< 1
Lactic acid bacteria (LAB)	5.6 ± 0.2^b	3.7 ± 0.9^a	3.7 ± 0.4^a
Sulphite reducing *clostridium* spores	< 1	< 1	< 1
Coagulase positive staphylococci	< 1	< 1	< 1
Pseudomonas sp.	< 1	< 1	< 1
Salmonella spp.	ND	ND	ND
Listeria monocytogenes	ND	ND	ND

Different letters in the same row indicate significant differences according to a Scheffe's multiple-range test (p<0.05). ND - Not detected

cultivars' table olives ($p < 0.05$), which may be explained due to the fact that they were processed as a whole while the others were cracked (Maçanilha Algarvia) or split (Cobrançosa). In both the latter types of processing, there is an enhancing of the diffusion of various compounds, especially reducing sugars from the cells, making then more easily usable by microorganisms. This facilitates their growth and explains the lower level of microbiota in the Galega olives when compared with the other two types.

CONCLUSION

Cracked (Maçanilha Algarvia cultivar), split (Cobrançosa cultivar) and whole table olives (Galega cultivar) are processed according to traditional methods categorized as "Natural Olives" as they are not debittered with lye solutions, and thus characterized by singular organoleptic properties with the residual phenols being responsible for their typical bitter taste. Galega had the smallest fruits and stones and the highest edible portion while Maçanilha showed the highest hardness of the pulp. The table olives studied possessed the expected acidity values and good microbial quality according to the European microbiological criteria. Nutritionally, the three table olives have low-sugar content, fairly low but important dietary fiber as well as energy value and are a good source of healthy compounds, especially phenolics, to which antioxidant properties are associated, with Cobrançosa showing the highest antioxidant potential. The three table olives showed high amounts of total unsaturated fatty acids, however Maçanilha cultivar had the highest linoleic content and Galega the highest oleic acid content.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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