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Microbiological characterization of table olives commercialized in Portugal in respect to safety aspects

Ana Paula Pereira, José Alberto Pereira, Albino Bento, M. Letícia Estevinho *

CIMO/Escola Superior Agrária, Instituto Politécnico de Bragança, Campus Santa Apolónia, Apartado 1 172, 5301-855 Bragança, Portugal

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ABSTRACT

Table olives are a traditional component of the Mediterranean diet and are largely consumed in the world. There are different trade treatments that originate different types of olives. The aim of the present work was to proceed to the microbiological characterization of table olives commercialized in the Portuguese market, with respect to their microbiological safety. The microbiological characterization was made in the olive pulp and packing brine of thirty-five table olives samples of different types and trade treatments, namely natural olives *Cv.* Galega (NOG), natural turning colour olives (NTCO), green olives (GO), black ripe olives (BO) and natural olives purchased in the traditional market and obtained from traditional producers (NOt). Simultaneously it was verified specific legislation of the table olives in what it concerns to labeling rules, pH values and the identification of isolated yeasts. In general, table olives consumed showed acceptable security with exception of four NOt samples that presented *Staphylococcus aureus*. In a considerable number of samples high number of microorganisms indicators of contaminations were observed that reveals the need of optimization the hygienic procedures during production process to improve the quality and safety of table olives. During the work seven yeasts were isolated from olive pulp and brine.

1. Introduction

The production of table olives in the world is mainly concentrated in the Mediterranean region. And in European Union, Spain, Italy, Greece, Portugal and France are 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 no. 1, 2004) define table olives as the product "prepared from the sound fruits of varieties of the cultivated olive tree (*Oleae europaea* L.) that are 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.

There are three main trade preparations of table olives, namely green or Spanish-style olives, black ripe or Californian-style olives and naturally black or turning colour olives (Romero et al., 2004; Fernández et al., 1997). The Spanish processing method includes treatment with sodium hydroxide solution, for the total removal of the bitter compound oleuropein, washing, brining and fermentation, sorting and size grading and packaging (Romero et al., 2004). The Californian method of treatment includes lye treatment, washing, iron-salt treatment and air-oxidation, washing, sizing, canning and sterilisation (Marsilio et al., 2001). The Greek-style method of treatment is milder and includes washing, natural fermentation in brine, air-oxidation for colour improvement, sizing and packing (Boskou et al., 2006).

No official microbiological criteria for table olives are available. However, the Standard 66-1981 (Rev. 1-1987) of the Codex Alimentarius (Anonymous, 1987) prescribed the minimum requirements related to hygiene for table olives. And the final product shall be free from microorganisms and parasites in amounts which may represent a hazard to health and shall not contain any substance originating from microorganisms in amounts which may represent a hazard to health. According to COI (2004) fermented olives held in bulk in a covering liquid may contain lactic bacteria and/or yeasts used for fermentation. The number of such microorganisms in a selective culture medium may, for each one, be up to 10^9 colony forming units/mL of brine or per gramme of flesh depending on the level of fermentation. On the other hand, olives preserved by heat sterilisation (such as olives darkened by oxidation) shall have received a processing treatment sufficient both in time and temperature to destroy spores of *Clostridium botulinum* (COI, 2004).

The most important safety issue in fermented olives appears to be the risk of *C. botulinum* growth and toxin formation. In spite of black olives were incriminated in a small outbreak of botulism of

* Corresponding author. Tel.: +351 273 303342; fax: +351 273 325405.
E-mail address: leticia@ipb.pt (M.L. Estevinho).

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the B type, the occurrence of *C. botulinum* appears, however, to be rare (Nout and Rombouts, 2000).

The aim of this work was to proceed to the microbiological characterization of table olives commercialized in the Portuguese market. The microbiological characterization occurred in olive pulp and packing brine of thirty-five table olives samples of different types and trade treatments, namely natural olives *Cv.* Galega (NOG), natural turning colour olives (NTCO), green or Spanish-style olives (GO), black ripe or Californian-style olives (BO) and natural olives purchased in the traditional market and obtained from traditional producers (NOt). Simultaneously it was verified specific legislation of the table olives in what it concerns to labeling rules. The isolated yeasts were also identified.

2. Materials and methods

2.1. Samples

Thirty-five table olives samples were studied (Table 1). Seven samples were classified as natural olives in brine (black and turning colour olives) *Cv.* Galega (NOG) and were purchased in a supermarket. Seven samples were from natural turning colour olives in brine (NTCO) and were also obtained in a supermarket. Seven samples were classified as green olives (GO), produced by the Spanish method and were purchased in a supermarket. Seven samples were classified as ripe black olives (BO), produced by the Californian method, of which four samples were purchased in a supermarket; two were purchased in the traditional market and one was obtained directly from an industrial producer. The last seven samples, obtained from traditional producers and purchased in traditional market, were produced by the traditional method and classified as turning colour olives in brine (NOt).

2.1.1. Labeling

The labeling of the table olives purchased in the supermarket were analysed in agreement with the requirements of the current Portuguese legislation, Dec.-Lei no. 170/92 of 8th August, and the specific mentions referred in the Portuguese Standard NP – 3034 (1987).

2.1.2. pH

The pH was determined, both in the pulp and packing brine of table olives, directly with a Basic 20 pH Meter.

2.2. Microbiological determinations

The microbiological analysis was made on the olive pulp and on the packing brine of the container. In order to evaluate the microbiological quality of the table olives it were researched pathogenic microorganisms – *Salmonella*, and *Staphylococcus aureus*, – microorganisms indicators of microbial contamination – total coliforms, *Escherichia coli*, faecal *Streptococcus*, sulphite reducing *Clostridium* spores and moulds – and microorganisms involved in the fermentation of the product – mesophilic microorganisms and yeasts.

2.2.1. Samples preparation

The samples preparation was accomplished according to the Portuguese Standard ISO 6887 – 1 (1999), NP – 1829 (1982), and NP – 3005 (1985). For the olive pulp analyses, two grams of each sample was weighted aseptically and homogenized in a stomacher Seward 400 for two minutes with 20 mL of sterile Ringer solution (10^{-1} dilution). Further 1 mL of 10^{-1} dilution was added to 9 mL of Ringer solution, and then homogenized (10^{-2} dilution). A series of decimal dilutions until 10^{-5} dilution, were made with the same solvents. In order to analyse the olives packing brine, 20 mL of each sample was taken aseptically. Then 1 mL of the sample was homogenized with 9 mL of sterile water (10^{-1} dilution). Several decimal dilutions were made with the same solvents until 10^{-4} dilution was achieved. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems).

2.2.2. Enumeration of the total mesophilic microorganisms

Enumeration was made on Plate Count Agar (Himedia), incubated at 37 °C for 48 h. Results were expressed as colony forming units of mesophilic microorganisms per gram of pulp olive (CFU/g) or per mL of packing brine (CFU/mL).

2.2.3. Yeast and moulds

Enumeration was made on Potato Dextrose Agar Medium (Himedia) pH 3.5, acidified with tartaric acid, incubated at 25 °C for 5 days. Results were expressed as colony forming units of yeast and moulds per gram of pulp olive (CFU/g) or per mL of packing brine (CFU/mL).

2.2.4. Salmonella

Detection according to ISO 6579 (2002). Results were expressed as presence or absence of *Salmonella*.

2.2.5. Staphylococcus aureus

Enumeration was made on CHAPMAN Agar (Culimed Panreac Quimica), incubated at 35 °C for 48 h. Results were expressed as colony forming units of *S. aureus* per gram of pulp olive (CFU/g) or per mL of packing brine (CFU/mL).

2.2.6. Total coliforms

Enumeration was made according to the conventional method described in Bacteriological Analytical Manual *Online* of U.S. Food and Drug Administration (http://www.cfsan.fda.gov/~ebam/bam-toc.html), using the most probable number (MPN) technique. The MPN in each sample was determined using the FDA online MPN table (http://www.cfsan.fda.gov/~ebam/bam-a2.html). Results were expressed as most probable numbers of coliforms per gram of pulp olive (MPN/g) or per mL of packing brine (MPN/mL).

2.2.7. Escherichia coli

The positive results for total coliforms were researched for *E. coli*. Enumeration was made on T.B.X. Agar (Oxoid), incubated at 44 °C for 24 h. Results were expressed as colony forming units of *E. coli* per gram of pulp olive (CFU/g) or per mL of packing brine (CFU/mL).

2.2.8. Fecal streptococci

Detection on BAGG Broth (HiMedia), incubated at 44 °C for 48 h. Results were expressed as presence or absence of faecal streptococci.

2.2.9. Sulphite reducing Clostridium spores

Detection according to Portuguese Standard NP 2262 (1986). Results were expressed as presence or absence of sulphite reducing *Clostridium* spores.

2.3. Isolation and identification of yeasts

The yeasts isolated from samples of pulp and packing brine were identified with the API 20C Aux system. The system consists of a disposable plastic strips containing 20 cupules. The first copula is a negative control, while the second contains glucose and serves as a positive control. The remaining 18 cupules each contain a specific substrate that may be assimilated by the test organism. After the distribution of about 5 mL of distilled water in the incubation box it was placed the strip. Isolates, which were cultured in YPD agar plates and incubated 48 h at 25 °C, were picked up with a sterile loop from the YPD plates and were added to a NaCl 0.85% medium to constitute a suspension. The turbidity of the suspension was equal to 2 MacFarland standard. Hundred microliters of this suspension were transferred to an ampule of API C Medium and homogenized. The cupules were filled with the suspension obtained in the ampule of API C Medium according with the manufacturer's directions. The strips were incubated at 25 °C and read at 24, 48, and 72 h. A profile number was generated for each isolate depending upon the reactions it produced. Identifications were made by referring to the API 20C AUX – Analytical Profile Index (Anonymous, 1997).

3. Results and discussion

3.1. Labeling and information to consumers

The analysis of the samples label is summarized in Table 1. Table olives shall be labeled according to Portuguese legislation (D.L. no. 170/92 of August 8th) and NP-3034 (1987) for generic and specific inscriptions, respectively. The generic inscriptions, obligatory mentioned in the label of the container, are the name of the product, the list of ingredients, the net weight, the date of minimum durability, the lot identification and the name, firm or social denomination and the address of the manufacturer, packer, distributor or vendor of the product, established in the European Union. All analyzed samples presented the name of the product, "olives" or "table olives", the list of ingredients listed in descending order

Table 1
Registration in the label of table olive samples

Sample	Product name	Size grading	Trade category	List of ingredients	Net drained weight	Net weight	Lot	Notes
Natural olives Cv.Galega (NOG)								
1	Black olives Galega. Natural fermentation	–	–	Whole black olives Galega, water, salt, acidifying agent (E 270)	520 g	900 g	Yes	–
2	Whole natural black olives Galega in brine	300/400	–	Olives, water, salt and acidifying agents (E 270, E 327 and E 330)	220 g	380 g	Yes	Pasteurized product
3	Natural black olives Galega	–	–	Whole olives, water, salt, acidifying agent (E 270)	220 g	360 g	Yes	–
4	Natural black olives Galega	411/460	2nd	Olives, water, salt, acidifying agent (E 270), preservative agent (E 202)	300 g	580 g	Yes	–
5	Black olives Galega from Serpa	–	–	Olives, water, salt, acidifying agent (E 270)	180 g	310 g	No	–
6	Whole natural turning colour olives Galega	360/460	1st	Olives, water, salt and acidifying agents (E 270, E 327 and E 330)	420 g	720 g	Yes	–
7	Turning colour olives Galega	340/400	–	Turning colour olives Galega, water, salt and acidifying agent (E 270)	250 g	500 g	Yes	–
Natural turning colour olives (NTCO)								
1	Split turning colour olives	231/260	1st	Olives, water, salt, acidifying agent (E 270), antioxidant (E 300/E 330), preservative agent (E202), aromatic herbs	200 g	360 g	Yes	Designation of origin (DO) olives
2	Turning colour olives	240/260	–	Turning colour olives, water, salt and acidifying agent (E 270)	250 g	500 g	Yes	–
3	Whole natural turning colour olives in brine	230/260	–	Olives, water, salt, acidifying agents (E 270, E 327 and E 330) and preservative agent (E 202)	300 g	550 g	Yes	–
4	Natural turning colour olives	–	–	Whole olives, water, salt, acidifying agent (E 270), antioxidant (E 300)	220 g	360 g	Yes	–
5	Turning colour olives of Cordovil from Serpa	–	–	Olives, water, salt, acidifying agent (E 270), antioxidant (E 300)	180 g	310 g	No	–
6	Split turning colour table olives	–	–	Olives, water, salt, aromatic herbs, acidifying agent (E 270), antioxidant (E 330/E 300), preservative agent (E 202)	800 g	1325 g	Yes	
7	Whole natural turning colour olives in brine	230/260	–	Olives, water, salt and acidifying agents (E 270, E 327 and E330)	220 g	380 g	Yes	Pasteurized product
Green olives (GO)								
1*	Olives Gordal	120/140	–	Green olives gordal, water, salt, antioxidant (E 300), preservative agent (E 202), acidifying agents (E 270 and E 330)	198 g	360 g	Yes	–
2	Green olives with aromatic herbs	200/220	–	Green olives with aromatic herbs, water, salt and acidifying agent (E 270)	250 g	500 g	Yes	–
3	Green table olives	–	–	Olives, water, salt, acidifying agent (E 270), antioxidant (E 330/E 300), preservative agent (E 202)	800 g	1400 g	Yes	–
4	Green olives	201/220	1st	Olives, water, salt, acidifying agents (E 330 and E 270), antioxidant (E 300)	300 g	580 g	Yes	–
5	Green olives from Elvas	201/220	–	Olives, water, salt, acidifying agents (E 270 and E 330), antioxidant (E 300)	200 g	317 g	Yes	–
6	Whole green olives in brine	180/200	–	Olives, water, salt, acidifying agents (E 270, E 327 and E 330) and antioxidant (E 300)	220 g	380 g	Yes	Pasteurized product
7	Whole green olives in brine	231/260	1st	Green olives, water, salt, acidifying agents (E 270, E 327 and E 330), antioxidant (E 300)	220 g	380 g	Yes	Pasteurized product
8	Whole green olives in brine	200/230	–	Green olives, water, salt, acidifying agents (E 270, E 330 and E327), antioxidant (E 300)	220 g	380 g	Yes	–
Black ripe olives (BO)								
1	Black ripe olives	280/300	–	Black ripe olives, water, salt, acidifying agent (E 270) and stabilizer (E 579)	250 g	500 g	Yes	–
2	Whole black ripe olives	260/290	–	Olives, water, salt, acidifying agent (E 270) and colour stabilizer (E 579)	220 g	380 g	Yes	Sterilised product
3	Whole black ripe olives	231/260	1st	Olives, water, salt, acidifying agent (E 270), colour stabilizer (E 579)	220 g	380 g	Yes	Sterilised product
4	Whole black ripe olives	260/290	–	Olives, water, salt, acidifying agent (E 270) and colour stabilizer (E 579)	220 g	380 g	Yes	–
5	Black ripe olives *Cv.* Negrinha de Freixo	–	Not packaged	–	–	–	TM	
6	Black ripe olives	–	Not packaged	–	–	–	TM	
7	Black ripe olives	–	Not packaged	–	–	–	TM	
Natural turning colour olives from traditional market (NOt)								
1	Natural turning colour olives *Cv.* Negrinha de Freixo	–	Not packaged	–	–	–	TM	
2	Natural turning colour olives	–	Not packaged	–	–	–	TM	
3	Natural turning colour olives	–	Not packaged	–	–	–	TM	

(*continued on next page*)

Table 1 (*continued*)

Sample	Product name	Size grading	Trade category	List of ingredients	Net drained weight	Net weight	Lot	Notes
4	Natural turning colour olives	–	Not packaged	–	–	–	TM	
5	Natural turning colour olives	–	Not packaged	–	–	–	TM	
6	Natural turning colour olives	–	Not packaged	–	–	–	TM	
7	Split natural turning colour olives	–	Not packaged	–	–	–	TM	

of ingoing weight and the date of minimum durability declared by month and year, and in some cases by day. The net weight and drained weight were declared in all samples but in six of them (samples 3 and 7 of NOG, 2 and 4 of NTCO, 2 of GO and 1 of BO), the units were not showed in the metric system. Lot identification was not referred in two samples (samples 5 of NOG and 5 of NTCO). The remaining inscriptions were fulfilled in all samples (Table 1).

According to the NP-3034 (1987) the specific inscriptions that shall be in the label of the container are the name of product "Olives" or "Table olives" followed by type of olives ("Green", "Turning colour" or "Black"), style ("Whole olives", "Split olives", "Stoned olives", "Stuffed olives" and "Sliced olives"), size grading, trade category ("Extra", "First" or "Second") and net drained weight. One sample (sample 1 of GO) did not refer the name of the product. Twelve samples had a reference to the style "whole" or "split" olives. The size grading was declared in nineteen samples and the trade category was referred only in six samples (samples 4 and 6 of NOG, 1 of NTCO, 4 and 7 of GO and 3 of BO) (Table 1).

Only one sample (sample 1 of GO) presented additional nutrition labeling per 100 g of olives and per dosis (9 olives ≈ 19 g). According the producer 100 g of olives have energetic value of 578.5 kJ/140 kcal, 0.0 g of protein; 5.3 g of carbohydrates and 13.2 g of lipids. Per dosis was mentioned: energetic value of 109.5 kJ/26.5 kcal, 0.0 g of protein; 1 g of carbohydrates and 2.5 g of lipids. Sodium level was also mentioned, less than 1000 mg/100 g of olives and 190 mg/dosis. However, no information was given about saturated fatty acids and crude fiber content (D.L. no. 167/2004 of July 7th).

Concerning to additives several are mentioned in the labeling: acidifying agents (lactic acid – E 270 and calcium lactate – E327), antioxidants (ascorbic acid – E 300 and citric acid – E 330), preservative agent (potassium sorbate – E 202), colour stabilizer (E 579). All these chemical products could have toxicological implications; however additives have also an important role in food preservation and in the increasing and maintenance of their biological properties. In the samples of this work, all NOG, NTCO, GO and BO mentioned the presence of lactic acid (E 270) as acidifying agent. With the same functions calcium lactate (E 327) were added at two NOG, two NTCO and three GO samples. Two samples of NOG, three of NTCO and all of GO refers the presence of citric acid as antioxidant, and four NTCO and all the GO refers also the use of ascorbic acid (E 300). Only BO refers the use of ferrous gluconate (E 579) which is related with the production process and the necessity to stabilize the colour (black).

3.2. Hygienic status of samples and influence of table olive type

The pH values measured for the table olive pulp and packing brine are shown in Table 2. The maximum pH limits of brines must comply with NP-3034 (1987), and are different according to type and treatment. The pH levels stipulated should be between 4 and 4.5 for GO whereas for pasteurized BO the pH could be up to 5.5. The maximum pH for pasteurized green and turning colour olives and for sterilised olives is 4.3 and 8.0, respectively.

Generally, it was verified that the pH value was similar both in the olive pulp and in the packing brine, with the exception of sample one of NOG, in which the pH value of the pulp was lower (4.3) than the one of the brine (5.9). The raising of pH of this sample may be related with microbiological changes that might be happening in the packing brine. Since the standard does not refer the maximum pH limit for NTCO, it can be applied the maximum pH limit of 4.0 for GO, treated or untreated, in hermetically-sealed containers (Fernández et al., 1997). Thus, almost all samples presented a pH lower to the established, with the exception of the packed samples 3 and 4 of GO and NOt.

Concerning the microbiological analysis of table olives samples, shown in Tables 3 and 4, great variability was observed between pulp and packing brine and between trade treatments.

Total mesophilic microorganisms, in which are included the lactic acid bacteria and yeasts were the dominant microflora, once they play an important role in the fermentation of table olives. These microorganisms were detected essentially in NOt and were

Table 2
pH value of the pulp and packing brine of the table olives samples

Samples	pH	
	Pulp	Brine
Natural olives Cv. Galega		
1	4.3	5.9
2	3.8	3.7
3	3.4	3.5
4	3.9	3.9
5	4.0	4.1
6	3.7	3.6
7	3.4	3.4
Natural turning colour olives		
1	3.7	3.6
2	4.0	4.0
3	3.7	3.7
4	3.6	3.5
5	3.4	3.6
6	3.7	3.7
7	3.4	3.4
Green olives		
1	3.6	3.7
2	3.7	3.8
3	4.5	4.5
4	4.2	4.3
5	3.5	3.6
6	3.4	3.5
7	4.1	4.1
8	3.7	3.8
Black ripe olives		
1	4.5	4.6
2	6.2	6.1
3	7.0	6.7
4	7.1	6.7
5	5.8	6.3
6	5.4	5.5
7	5.4	5.5
Natural turning colour olives from traditional market/producer		
1	5.5	5.5
2	4.4	4.3
3	4.5	4.4
4	4.2	4.1
5	4.4	4.4
6	5.3	5.3
7	4.4	4.4

Table 3
Microbiological characterization of the pulp of table olives samples

Samples	Total mesophilic microorganisms (CFU/g)	Yeasts (CFU/g)	Moulds (CFU/g)	Total coliforms (MPN/g)	*E. coli* (CFU/g)	*Salmonella*	*S. aureus* (CFU/g)	Fecal Streptococci	SRC spores
Natural olives Cv. Galega									
1	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
2	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
3	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
4	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
5	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
6	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
7	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
Natural turning colour olives									
1	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
2	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
3	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
4	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
5	0.0	2.0×10^3	0.0	0.0	0.0	–	0.0	–	+
6	0.0			0.0	0.0	–	0.0	–	–
7	0.0	0.0	0.0	0.0	0.0	–	0.0	–	–
Green olives									
1	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
2	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
3	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
4	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
5	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
6	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
7	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
8	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
Black ripe olives									
1	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
2	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
3	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
4	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
5	1.4×10^5	1.1×10^6	0.0	0.0	0.0	–	0.0	–	+
6	0.0	4.9×10^4	0.0	0.0	0.0	–	0.0	–	+
7	0.0	8.3×10^5	0.0	0.0	0.0	–	0.0	–	+
Natural turning colour olives from traditional market/producer									
1	1.0×10^3	7.0×10^3	0.0	0.0	0.0	–	0.0	–	+
2	0.0	2.0×10^5	0.0	0.0	0.0	–	0.0	–	+
3	Uncountable	1.1×10^5	0.0	0.0	0.0	–	0.0	–	+
4	Uncountable	7.6×10^4	0.0	0.0	0.0	–	0.0	–	+
5	1.5×10^5	8.2×10^4	0.0	0.0	0.0	–	0.0	–	+
6	1.0×10^3	1.2×10^5	1.3×10^4	0.0	0.0	–	0.0	–	+
7	3.2×10^4	1.9×10^6	2.0×10^3	0.0	0.0	–	0.0	–	+

present in higher levels in packing brine than in the pulp, probably due to the values of water activity (a_w) and to the phenolic compounds present in the olive's pulp (Pereira et al., 2006; Sousa et al., 2008). Natural olives are fermented predominantly by yeasts (Fernández et al., 1997; Nout and Rombouts, 2000), which could explain their detection in higher levels than total mesophilic microorganisms. In fact, unlike lactic acid bacteria, yeasts are more tolerant to high NaCl concentrations and to phenolic compounds, such as oleuropein (Asehraou et al., 2000; Borcakli et al., 1993). The exception was NOG that presented more samples with total mesophilic than yeasts. Similar results were mentioned by Oliveira et al. (2004) for Galega olives at the end of fermentation with the number of yeasts of was about 10^5 CFU/mL. Lactic acid bacteria play a dominant role in the fermentation of green olives in brine (Asehraou and Faid, 1993; Nout and Rombouts, 2000). The pH values determined in the samples 3 and 4 of GO were higher than the stipulated and were near the pH optimum value for the growing of yeasts, detected in these two samples. The fermentative yeasts contribute to the organoleptic characteristics of table olives but oxidative yeasts that occur in superficial films should be kept low by anaerobiosis, as they oxidize lactic acid, raise the pH, and thereby may favour malodorous spoilage by *Clostridium* spp. (Nout and Rombouts, 2000).

Moulds counts are used to detect fungal contamination (Anonymous, 2004), and were present in two pulp and six brine samples mainly from NOt. Probably this situation was related with air surface of brine in tanks or barrels. Excessive mould growth origin a mouldy taste in olives and can cause spoilage by consuming the acids produced during fermentation (Anonymous, 2004). Eltem (1996) isolated *Aspergillus flavus* strains from natural black olives in brine and Roussos et al. (2006) confirmed the accumulation of micotoxins in the brines of fermentation that could compromises the safety of the product.

Concerning the microorganisms indicators of contamination, it can be inferred that most of the NOt were produced under deficient hygiene conditions. Total coliforms were only detected in packing brine of table olive samples that came from traditional market. These microorganisms are indicators of recent faecal contamination, so their presence in the samples reveals lack of good practices of hygiene during or after the manufacturing, but the levels determined do not represent a hazard to health. However, coliform bacteria are responsible for gassy spoilage. *E. coli* is used to monitor the potential for food poisoning and should be tested in pasteurized packed olives for health and safety purposes (Anonymous, 2004).

Faecal streptococci or enterococci were absent in the pulp but were detected in six samples of brine, namely in one sample of

Table 4
Microbiological characterization of the packing brine of table olives samples

Samples	Total mesophilic microorganisms (CFU/mL)	Yeasts (CFU/mL)	Moulds (CFU/mL)	Total coliforms (MPN/mL)	*E. coli* (CFU/mL)	*Salmonella* (CFU/mL)	*S. aureus* (CFU/mL)	Fecal Streptococci	SRC spores
Natural olives Cv. Galega									
1	6.0×10^2	0.0	0.0	0.0	0.0	–	0.0	–	+
2	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
3	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
4	0.0	1.4×10^5	0.0	0.0	0.0	–	0.0	–	+
5	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
6	8.0×10^3	0.0	0.0	0.0	0.0	–	0.0	–	+
7	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
Natural turning colour olives									
1	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
2	0.0	1.3×10^5	0.0	0.0	0.0	–	0.0	–	+
3	0.0	3.0×10^3	1.0×10^2	0.0	0.0	–	0.0	–	+
4	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
5	0.0	5.5×10^2	0.0	0.0	0.0	–	0.0	–	+
6	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
7	1.0×10^2	0.0	0.0	0.0	0.0	–	0.0	–	+
Green olives									
1	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
2	1.0×10^2	0.0	0.0	0.0	0.0	–	0.0	–	+
3	6.5×10^2	1.1×10^4	0.0	0.0	0.0	–	0.0	+	+
4	0.0	1.0×10^5	0.0	0.0	0.0	–	0.0	–	+
5	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
6	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
7	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
8	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
Black ripe olives									
1	1.0×10^2	7.8×10^4	0.0	0.0	0.0	–	0.0	–	–
2	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
3	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
4	0.0	0.0	0.0	0.0	0.0	–	0.0	–	+
5	0.0	Uncountable	0.0	3.6	0.0	–	0.0	–	+
6	1.6×10^5	Uncountable	3.8×10^3	93.0	0.0	–	10.0	+	+
7	1.5×10^6	2.5×10^6	0.0	93.0	2.3×10^4	–	40.0	+	+
Natural turning colour olives from traditional market/producer									
1	3.7×10^3	Uncountable	0.0	0.0	0.0	–	0.0	–	+
2	1.4×10^4	Uncountable	0.0	0.0	0.0	–	0.0	–	+
3	Uncountable	3.2×10^6	0.0	0.0	0.0	–	0.0	–	+
4	Uncountable	Uncountable	8.1×10^3	11.0	0.0	–	10.0	+	+
5	4.6×10^4	6.7×10^4	7.3×10^3	9.2	0.0	–	0.0	–	+
6	7.4×10^5	3.8×10^5	6.3×10^4	9.2	0.0	–	0.0	+	+
7	3.2×10^5	5.3×10^5	8.6×10^3	23.0	0.0	–	10.0	+	+

GO packed olives and five samples of NOt. The presence of enterococci in fermented vegetables it is often not clear, whether hey originate from the plant material or as environmental contaminants (Franz and Holzapfel, 2004). However, enterococci have already been isolated from Spanish-style green olive fermentations by Floriano et al. (1998), in which *E. faecalis* is a frequent contaminant.

The sulphite reducing *Clostridium* (SRC) spores are indicators of remote faecal contamination. They were detected in almost all samples with the exception of two, being even present in pasteurized and sterilised samples. Their presence in pasteurized olives is due to the occurrence of anaerobic fermentations of the product or to the resistance of spores to pasteurization. However, the spores should be destroyed by sterilisation as its presence in a sterilised product indicates either inadequate heat treatment or post-sterilisation contamination. Clostridial bacteria are relatively common in the environment. This kind of bacteria are able to produce spoors that can survive under harsh conditions, such as high temperature and humidity, and have latent potential for spoilage namely malodorous deterioration (putrid, butyric and zapatera spoilage) (Anonymous, 2004).

The levels of the pathogens *Salmonella*, and *S. aureus* were accessed since they are a potential food hazard. None of the analysed samples presented *Salmonella*, but *S. aureus* was detected in small numbers in the packing brine of four NOt samples. These samples should be considered as unacceptable/potentially hazardous and so improper for consumption, because they can cause food-borne illness. The occurrences of *S. aureus* in dry-salted olives were reported by Asehraou et al. (1992) in samples from Moroccan.

3.3. Isolation and identification of yeasts

Seven yeasts species were identified in the analysed samples (Table 5). Five of the identified species belong to *Candida* genus. Marked differences were observed between pulp and packing brine of table olives. In olive pulp, yeasts were identified in 11 samples, one (*C. boidini*) in NTCO (sample 5); three in BO (samples 5–7) with the same species and all NOt showed the presence of yeasts. On the other side, in olive package brine yeasts were identified in 13 samples, namely one in NOG (sample 4 with *C. glabrata*); two in NTCO (samples 3 and 5 with *C. krusei*); two in GO (samples 3 and 4), three in BO (samples 1, 6 and 7); and five in NOt (samples 3–7).

C. boidinii was the most frequent specie in the pulp, and it was detected mainly in NOt. The presence of *C. boidinii* in table olives has already been mentioned by Arroyo-López et al. (2006) that found this specie was the most frequent in aerobically processed

Table 5
Yeasts isolated from pulp and packing brine of table olives samples

Yeasts	Pulp		Brine	
	No. of samples	%	No. of samples	%
Candida boidinii	9	62.50	1	4.20
Candida famata	1	6.25	0	0.00
Candida glabrata	3	18.75	3	12.50
Candida krusei	0	0.00	12	70.80
Candida utilis	1	6.25	0	0.00
Geotrichum penicillatum	1	6.25	2	8.30
Kloeckera spp.	0	0.00	1	4.20

The number of samples with yeast occurs and occurrence (%).

black table olives and it was also isolated from anaerobically processed black table olives. The dominant yeast in the packing brine was *C. krusei* and it was isolated essentially from brine of olives that come from traditional market and producers (NOt). Its absence in the pulp of olives could be related to its sensitivity to phenolic compounds and to low value of a_w. In fact previous works (Pereira et al., 2006; Sousa et al., 2006, 2008) proved that olive pulp is rich in phenols that showed antimicrobial activity.

C. glabrata and *C. krusei* have emerged as important pathogens, whose clinical isolates have been increasing, being *C. glabrata* associated, specially, to urinary tract, mucosal areas and lungs (Hazen, 1995). On the other hand, *C. krusei* is an opportunistic yeast of clinical significance that has already been isolated from vaginal tract (Parazzini et al., 2000), skin, fingernails and oral mucosas (Crocco et al., 2004), blood (Pfaller et al., 2003) and mucosal surfaces (Samaranayake, 1997). The presence of *C. krusei* in brine may be associated to contamination due to lack of hygiene during the processing or manipulation of table olives and it is a matter of concern because it is a multidrug-resistant opportunistic fungal pathogen (Pfaller et al. 2008). This situation is in accordance with the results obtained in microbiological characterization (see Table 3 and 4). On the other side, Fernández et al. (1997) referred that *C. krusei*, isolated from naturally black olive, was able to produce gas-pocket spoilage that alter the quality and safety of table olives.

In general, the results obtained have shown that the optimization of hygienic procedures in the production process is necessary to improve the quality and safety of table olives, especially of the ones from the traditional market/producers. To reduce the risk of food-borne illness and spoilage, good practices in agriculture (GAP), hygiene (GHP) and manufacturing (GMP) most be improved.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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