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Microbial quality and yeast population dynamics in cracked green table olives' fermentations

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ABSTRACT

Cracked green table olives, from the Manzanilla variety, are a fermented food produced and consumed in Portugal. The objective of the present work was to study the microbiological characteristics and yeast population evolution during the fermentation of cracked green olives. The predominant microorganisms were yeasts while lactic acid bacteria were not detected and a clear decrease of the mould population was observed. At the end of the fermentations, no viable counts of *Enterobacteriaceae* were found. Yeast isolates were identified by the 5.8S rRNA-ITS region restriction analysis and by sequencing the D1/D2 region of the 26S rRNA gene. During the initial phases of the fermentations a great diversity of yeasts was observed. However, as the processes evolved the biodiversity decreased with the fermentative yeasts *Citeromyces matritensis, Zygotorulaspora mrakii* and *Saccharomyces cerevisiae* becoming the dominant species. The presence of these fermentative yeasts at the end of the production processes may explain the instability of the final product and is associated to a risk of spoilage. The results obtained represent a first attempt towards the comprehension of the microbiota of this type of "Natural olives" that constitute an important component of the Mediterranean diet.

Key words: Green table olives, fermentation, yeasts, RFLP, LSUrDNA, spoilage

1. Introduction

Olives are the fruits of *Olea europeae* L., which offer two relevant components of Mediterranean diet: olive oil and table olives. Table olives are classified according to the degree of ripeness of the fresh drupes used in the production: green olives, turning color olives and black olives (IOOC, 2004). The most significant industrial production methods of table olives are the Spanish-type olives and the Californian style olives involving both a de-bittering process with sodium hydroxide solutions (Gómez, García & Navarro, 2006). However, around the Mediterranean fringe there are other methods to produce table olives where the fruits are brined after harvesting, without going through a NaOH treatment to debitter and left to ferment until they lose their bitterness, at least partially (Hurtado, Reguant, Esteve-Zarzoso, Bordons & Rozès, 2008; Bautista-Gallego, Arroyo-López, Durán-Quintana & Garrido-Fernández, 2010). In some cases, the drupes undergo pre-treatments in water to fasten the debitterisation process (Valenčič, Mavsar, Bučar-Miklavčič, Butinar, Čadež, Golob, Raspor, & Možina, 2010). The "natural green olives" are different from the lye treated ones, mainly due to their taste, color and residual bitterness (Garrido-Fernández, Fernández Díez, & Adams, 1997).

The most important microbial groups involved in olive fermentations are mainly lactic acid bacteria and yeasts. When the growth of lactic acid bacteria overcomes the growth of yeasts, lactic acid fermentation is favoured and a final food product with lower pH value is obtained, as in Spanish style olives. However, if yeasts become the dominant microorganisms, the olives produced will have higher pH values as in Greek style olives (Gómez et al., 2006). The main roles of yeasts in the processing of fermented olives, are associated with the production of alcohols, ethyl acetate, acetaldehyde and organic acids, compounds that are relevant for the development of

taste and aroma and for the preservation characteristics of this fermented food (Hernández, Martín, Aranda, Pérez-Nevado, & Córdoba, 2007; Arroyo-López, Querol, Bautista-Gallego, & Garrido-Fernández, 2008). Nevertheless, in certain processing conditions and after packing, yeasts may have a negative role as they are responsible for the production of CO_2, softening of the fruits due to pectinolitic activity, clouding of brines, biofilm production and, probably, production of off flavors (Arroyo-López et al., 2008).

In Portugal there are few reports related to microbial characteristics of black/green olives. They refer manly to olives produced and commercialized in the North and Center of the country where varieties such as Galega, Gordal, Cordovil, Negrinha do Freixo (*Olea europeae* L.) are the most frequent (Oliveira, Brito, Catulo, Leitão, Gomes, Silva, Vilas-Boas, Peito, Fernandes, Gordo, & Peres, 2004, Pereira, Pereira, Bento & Estevinho, 2008). In the Southern part of Portugal cracked green table olives, from the Manzanila variety, not debittered with lye solutions is one of the most popular methods of producing table olives. The process remains empirical as only the salt content and the overall sensorial characteristics are verified. The main problem of these olives is related to the instability of the packed product after fermentation.

In this context, the objective of the present work was to study the microbiological characteristics, yeast population diversity and succession during the production of Portuguese cracked green olives.

2. Material and methods

2.1. Fermentation conditions

Olives from the Manzanilla variety were harvested, by hand, in the Southern part of Portugal during October of 2008, when the surface colour was green and were transported to the factory, located in the Algarve. On arrival, fruits were washed and selected to remove damaged fruits. After selection they were cracked by passing the olives between two stainless steel plates. Then, fruits were calibrated, transferred to screw-capped fermentation vessels and covered with a freshly prepared brine (6-12 g NaCl/100 ml). Fermentations were carried out at room temperature, due to microorganisms present on the fruits and environment, for periods of 52 days.

During the fermentation period, brine samples were collected from two independent vessels (A and B) under sterile conditions, using 0.5 m length disinfected plastic tubes. The collected samples were aseptically transferred, to sterile 50 ml tubes and transported, under refrigeration, to the laboratory where the microbiological study took place.

2.2. Microbiological analysis

From each sample obtained, 1 mL of fermenting brine was treated, following the decimal dilution protocol, in sterile ¼ Ringer solution. Aliquots were plated, in duplicate, by surface spreading or incorporation, on media for the detection and enumeration of microorganisms: a) Tryptic Soy Agar (TSA, Sharlau, Spain) (pH 5) for total viable count, incubated at 25 ºC for 5 days; b) de Man, Rogosa and Sharpe agar (MRS, Sharlau, Spain) (pH 5) with cycloheximide (0,05 %) overlaid with the same medium without cycloheximide, for lactic acid bacteria, incubated at 25ºC for 5 days; c) Malt Extract Agar (MEA, Sharlau, Spain) (pH 5) for yeasts, incubated at 25ºC for 5 days (this medium was used to count the yeasts and to obtain isolates for further

identification); d) Rose Bengal Chloramphenicol Agar (Biokar Diagnostics) for moulds, incubated at 25ºC for 5 days; e) Chromocult Agar (Difco, England) for *Enterobacteriaceae* and *Escherichia coli* incubated at 35ºC for 2 days.

Yeast colonies were selected from those grown on MEA, according to their macro morphology and isolated in proportion to their frequencies. Isolates were purified by successive streaking in YEPD (yeast extract, peptone, dextrose, agar) (Sharlau) or Potato Dextrose Agar (Sharlau). A total of 108 colonies from the fermentations was obtained and identified. The isolated strains were preserved at -80ºC with glycerol (20% v/v) as the cryprotectant agent.

2.3. Yeast Identification

Yeasts isolates identification was performed by the PCR-RFLP method described by Esteve-Zarzoso, Belloch, Uruburu, & Querol, (1999) in combination with sequence analysis of the D1/D2 domain of ribossomal DNA (rDNA). Genomic DNA from YEPD liquid cultures were extracted according to Querol, Barrio, & Ramon, (1992). PCR reaction mixtures (75 µl) containing 0.5 µM primer ITS1 (5'TCCGTAGGTGAACCTGCGG3') and 0.5 µM primer ITS4 (5'TCCTCCCGCTTATTGATATGC3') (White, Bruns, Lee, & Taylor, 1990), 10 µM deoxynucleotides (Promega), 1.5 mM MgCl2 and 1 unit DNA polymerase (Promega) were prepared. Amplifications were performed in a thermocycler (Thermo Electron, USA) under the following conditions: initial denaturation at 95ºC for 5 min; 30 cycles of denaturating at 94ºC for 1 min; annealing at 55.5ºC for 2 minutes; extension at 72 ºC for 2 minutes and a final extension step at 72ºC for 10 min. PCR products (10 µl) were

142 digested without further purification with the restriction endonucleases *Cfo*I, *Hae*III and
143 *Hin*fI (Roche). The PCR products and their restriction fragments were separated on 1%
144 and 3% agarose gels, respectively, with 1xTAE buffer. After electrophoresis, gels were
145 stained with etidium bromide, visualized under UV light in a G-Box Syngene-Genesis
146 10 UV Scanner (UK). Fragment sizes were estimated by comparison against a DNA
147 ladder (100 bp BioRad). Yeasts were identified to the species level by comparison of
148 the amplified products and their restriction-fragments profiles with those described by
149 Guillamón, Sabaté, Barrio, Cano, & Querol, 1998; Esteve-Zarzoso et al., 1999; Esteve-
150 Zarzoso, Zorman, Belloch & Querol, 2003; de Llanos-Frutos, Fernandez-Espinar, &
151 Querol, 2004 and Coton, Coton, Levert, Casaregola, & Sohier, 2006. A representative
152 number of isolates corresponding of each PCR-RFLP profile was selected and sequence
153 analysis of the domains D1 and D2 of the 26S rDNA region was accomplished. PCR
154 amplification of the referred region in the 26S rDNA gene was performed with the
155 primers NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'-
156 GGTCCGTGTTTCAAGACGG-3') (Kurtzman & Robnett, 1998). The amplification
157 reaction and PCR conditions were identical to those described above for the analysis of
158 the ITS 5.8 rRNA region except for the primers used (NL1 and NL4). Amplified
159 products were sequenced elsewhere (LGC Genomics, Germany) and sequences were
160 compared to those available in GenBank database at the National Center for
161 Biotechnology Information (NCBI) using BLAST
162 (http://www.nvbi.nlm.nih.gov/BLAST/).

163

164 **3. Results and discussion**

165

166 *3.1. Microbiological analysis*

The evolution of total viable counts, yeasts, moulds and *Enterobacteriaceae* in cracked green olives' brines of the two independent fermentation vessels A and B is represented in Fig. 1 (Fig. 1A, Fig. 1B, Fig 1C and Fig 1D). During the first 8 days, the total counts and the yeast population showed an adaptation phase which was followed by exponential growth reaching the stationary phase, after, approximately 17 days. At the start of the processes, counts of the yeast population increased from 4.9-5.0 log 10 CFU/ mL of brine to maximum values of 6.0-6.5 log 10 CFU/ mL of brine, in both vessels (Fig. 1A and 1B). Yeasts were present all along the fermentation period studied in both fermenters (Fig. 1B) and seem to be the group of organisms responsible for the main changes occurring in the table olive fermentation studied in the present work. The yeast counts obtained were higher than the values obtained by Hernández et al. (2007) in Manzanilla variety olives but in the range of those described in the review of Arroyo–López et al. (2008) who reported yeasts to be present throughout fermentation processes reaching populations that ranged from 4.0 to 6.0 log 10 CFU/mL of brine.

The mould population was present since the beginning of fermentations (4.4 and 3.9 log CFU/ ml of brine respectively in both Vessels A and B) and a decline of its number was observed until day 45 of the fermentation (Fig. 1C). The presence of *Penicillium* and *Aspergillus* fungi are reported to cause softening of the olives and may be responsible by the production of malodorous compounds (Hutkins, 2006). Additionally, the presence of moulds in olive´s brines can compromise the safety of the product, especially in the case of fungi producers of mycotoxins (Zinedine & Mañes, 2009).

The results obtained showed that the lactic acid bacteria group was not detected in the brines. Aponte, Ventorino, Blaiotta, Volpe, Farina, Avellone, Lanza, & Moschetti (2010) also did not find lactic acid bacteria in some varieties of Sicilian olives, including Manzanilla, when culture-dependent and independent methods were used. In addition, this group of bacteria was not detected as well in fermentations of Slovenian olives (Valenčič et al., 2010). The most relevant factors related to the absence of lactic acid bacteria in cracked green table olives may be the initial NaCl concentration (6-12%) and the presence of natural inhibitors compounds, such as phenolic compounds, that quickly diffuse from the broken drupes into the brines. Ruiz-Barba, Rios-Sanchez, Fedriani-Iriso, Olias, Rios, & Jimenez-Diaz (1990) studied the antimicrobial properties of olive phenols against *Lactobacillus plantarum* in non-alkali treated olives, indicating that phenolic compounds have a pronounced bactericidal effect on this microorganism, through the alteration of the cellular ultrastructure on two targets: cell wall and cytoplasmic membrane. For many years, oleuropein and its hydrolysis products (aglycon, elenoic acid and hydroxytyrosol) have been pointed out as responsible for the growth inhibition of lactic acid bacteria (Ruiz-Barba, Brenes, Jiménez, García, & Garrido, 1993). Recently, Medina, Romero, Castro & Brenes (2008) reported that the main lactic acid bacteria inhibitors in brines are the dialdehydic form of decarboxymethyl elenolic acid free (EDA) or linked to hydroxytyrosol (HyEDA). As referred by Medina, García, Romero, de Castro, & Brenes (2009) HyEDA may be formed during olive brining as a consequence of the enzymatic hydrolysis of oleuropein by β-glucosidase which is present in brines.

In the case of the *Enterobacteriaceae,* this population was detected in the beginning of both fermentations at levels ranging from 2.6 to 3.5 log 10 CFU/ mL of brine during the first 30 days. Thereafter, a decline of those bacteria was observed and no viable

counts (<10 CFU/ml) were found at the end of both fermentations studied (Fig. 1D). Typical *Escherichia coli* colonies were not detected throughout the entire study. A waning in the *Enterobacteriaceae* population followed by its disappearance has been also described in various olives' fermentation processes (Tassou, Panagou, & Katsaboxakis, 2002; Bautista-Gallego, et al., 2010). High numbers of *Enterobacteriaceae* represent a risk of deterioration due to the production of off flavors and gas pocket spoilage in the olives' surface (Garrido-Fernández et al., 1997).

3.2. Yeast identification

The study of the microbial populations evolution presented previously showed that the yeasts was a quite relevant microbial group in the processing of cracked green table olives which led to the identification of that microbiota. A total of 108 yeast isolates was obtained from the two independent olive fermentations studied. Their identification was performed by the ITS1-5.8 rRNA-ITS2 region PCR-RFLP, using the restriction enzymes *Cfo*I, *Hae*III and *Hinf*I, in combination with the sequence analysis of the D/D2 26S rRNA region. The ITS region of the yeast isolates originated PCR products with size ranging from 450 bp to 850 bp. The yeast isolates gave origin to nine ITS-RFLP different restriction fragment length polymorphism profiles which were compared to patterns published previously (Guillamón et al., 1998; Esteve-Zarzoso et al., 1999; Esteve-Zarzoso et al., 2003; de Llanos-Frutos et al., 2004; Coton et al., 2006). This comparison allowed the assignment of the following species: *Aureobasidium pullulans* (yeast like species), *Candida boidinii, Candida diddensiae, Candida oleophila, Citeromyces matritensis, Rhodotorula mucilaginosa, Saccharomyces cerevisiae and*

Zygotorulaspora mrakii (previously *Zygosaccharomyces mrakii* and reassigned to a new genus by Kurtzman (2003) (Table 1). From the nine RFLP profiles obtained only one (Profile V) did not match any of the already published pattern. In addition to PCR-RFLP identification method, 22 representative strains of the ITS-RFLP profiles had their 26S rRNA gene' D1/D2 region sequenced and the obtained sequences were compared to the NCBI GeneBank data base using BLAST. Table 2 resumes the results of the percentages of sequence similarity in one selected strain (as an example) of each RFLP group sequenced, after BLAST analysis, as well as the GeneBank *accession numbers* of the species used in the comparisons. All the isolates corresponding to the different RFLP profiles, showed a sequence similarity of the D1/D2 region higher than 98%. These results allowed the confirmation of the identifications performed through restriction analysis in eight of the nine profiles obtained (I, II, III, IV, VI, VII, VIII and IX) and the identification of the isolates characterized by RFLP-Profile V as *Candida quercitrusa*.

As fermentations evolved, a succession of microbial species took place (Table 3). The earlier fermentation samples, corresponding to the first four days, contained a mixture of species including *A. pullulans* (yeast like fungus), *C. boidinii*, *C. diddensiae*, *C. oleophila*, *C. quercitrusa* and *R. mucilaginosa*. Although these species were isolated in low percentages (<6.4%) (Table 1) they might have had an influence in the fermentations conditions in the processing of olives. As fermentations progressed, the majority of those species were not detected with the exception of *C. diddensiae* which was isolated during 2 weeks (Table 3). The presence of *C. diddensiae* was also referred by Arroyo-López, Durán-Quintana, Ruiz-Barba, Querol, & Garrido-Fernández (2006) in green table olives (Aloreña cultivar) and by Hurtado et al. (2008) in *Arbequina* table olives. The decreasing of oxygen concentration in the fermentation vessels, the levels of

phenolic compounds or the accumulation of fermentation products in the brines may explain why those earlier species were not detected after the first days. For example, *A. pullulans* is an oxidative ubiquitous yeast like species found on fruits surfaces and phyllosphere (Zalar, Gostinčar, Hoog, Uršič, Sudhadham, & Gunde-Cimerman, 2008). It was recently referred in olives by Nisiotou, Chorianopoulos, Nychas, & Panagou (2010) and Valenčič et al. (2010) but has been commonly isolated from the surface of grapes and during the initial phases of wine production, being inhibited after the first fermentation days (Sabate, Cano, Esteve-Zarzoso, & Guillamón, 2002). The basidiomicetous yeasts of the genus *Rhodotorula* have been identified in the early stages of olive fermentation since the beginning of this kind of studies (Vaughn, Jakubczyk, MacMillan, Higgins, Davé, & Crampton, 1969; Arroyo-López et al., 2006, Nisiotou et al., 2010). An important aspect of this pink species' role in olive's production environment is their involvement in the softening of olives due to the production of polygalacturonases (Vaughn, et al., 1969). The yeast *C. boidinii* was isolated in a relative frequency of 2.8% in the early phase of the fermentation, although it has been detected in high percentages either in black olives of the Hojiblanca (Arroyo-López et al., 2006) and Conservolea varieties (Nisiotou et al., 2010) as well as in green *Arbequina* olives (Hurtado et al., 2008). The species *C. oleophila* was also found in brines from Slonenian Istria (Valenčič et al., 2010). On the other hand, *C. quercitrusa*, although in low percentage, was described for the first time in an olive environment. This specie has mainly been associated to wine ecosystems (Chavan, Mane, Kulkarni, Shaikh, Ghormade, Nerkar, Shouche, & Deshphande, 2009). The greatest number of yeast isolates from cracked green table olives' brines corresponded to *Cit. matritensis* (9,2%), *S. cerevisiae* (11,9%) and *Z. mrakii* (59,6%) (Table 2). These species were isolated some days after fermentation had initiated and lasted until the end

of the process with the exception of *Cit. matritensis* which is a fermentative yeast able to grow in the presence of high concentrations of NaCl. *Cit matritensis* was reported in French black olives by Coton et al. (2006). *Z. mrakii* is a fermentative yeast (Kurtzman, 2003) that has never been described in table olives while *S. cerevisiae* is also fermentative and its presence in olives' brines has been recorded in black or green olives (Marquina, Peres, Caldas, Marques, Peinado & Spencer-Martins, 1992; Arroyo López et al., 2006; Rodríguez–Gómez et al., 2010). Both of these species have been associated to vigorous production of gas which may explain the instability of the packed olives characterized by brine spills resulting in a very short shelf life. The genus *Zygosaccharomyces* to which *Zygotorulaspora mrakii* previously belonged to is known for including the major spoiler microorganisms in fruit juices, sauces, soft drinks, alcoholic beverages among others. In fact, the presence of species of that genus is normally an indicator of future spoilage despite their capacity of eventually confer to fermented food useful organoleptic qualities (Esteve-Zarzoso et al., 2003; Loureiro & Malfeito-Ferreira, 2003).

In the wine industry, *S. cerevisiae* is responsible for the production of wine but is considered a spoilage yeast in bottled wines due to its capacity to cause refermentation when sugars are available and ethanol is present (Loureiro & Malfeito-Ferreira, 2003). In the case of olive production, *S. cerevisiae* has also been attributed positive roles and negatives activities as discussed by Arroyo-López et al. (2008). In fermented food, the line between beneficial fermenting activity and spoilage activity of yeasts is difficult to draw depending on the phase of the processing/storage the yeast activity takes place. In view of these facts, the production process of cracked green olives should include an operation/strategy to reduce the yeast number and limit their growth before storage and packaging in order to improve its stability and shelf life.

On the other hand, it is interesting to note that species commonly related to olive production previously described in Portugal, Morocco and Spain, belonging to the genera *Pichia, Debaryomyces* or *Kluyveromyces* were not isolated during the present study (Marquina et al., 1992; Hernández et al., 2007; Rodríguez–Gómez et al., 2010). Yeasts biodiversity reported in distinct olive types may explain the different characteristics in terms of chemical, microbiological and sensorial quality of table olives. It would be interesting to study the relation between the microbiota present during the processing and the development of the final product´s organoleptic profile.

4. Conclusions

Cracked green table olives from the Manzanilla variety are characterized by a fresh natural green colour, a rich aroma and typical bitter taste. These olives are included in the category of "natural olives" which undergo a spontaneous fermentation carried out by fruit and environmental microbiota, with yeasts found to be the dominant group. The identification methods used (PCR-RFLP and 26S rDNA partial sequencing analysis) appeared to be adequate to characterize the yeast biota, revealing that the most representative species present at the end of the fermentation processes were *Z. mrakii* and *S. cerevisiae* which active fermenting metabolism compromises the stability of the final product during the storage. The olives' fermentations studied guaranteed the absence of *E. coli* and other *Enterobacteriaceae* in the table olives as well as a reduction in the mould viable counts. However, an optimization of the production process to improve the stability and consequently, the shelf life of cracked green table olives in necessary. The knowledge gathered during the course of the described work could

enable the selection of native strains to be used as starter cultures to improve the quality of the product and prevent spoilage during the storage period. Using controlled and defined inocula to initiate the fermentation may be a way to obtain more reliable and better quality food products. The production of good quality food products creates economic gains and avoids financial losses.

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Figure 1

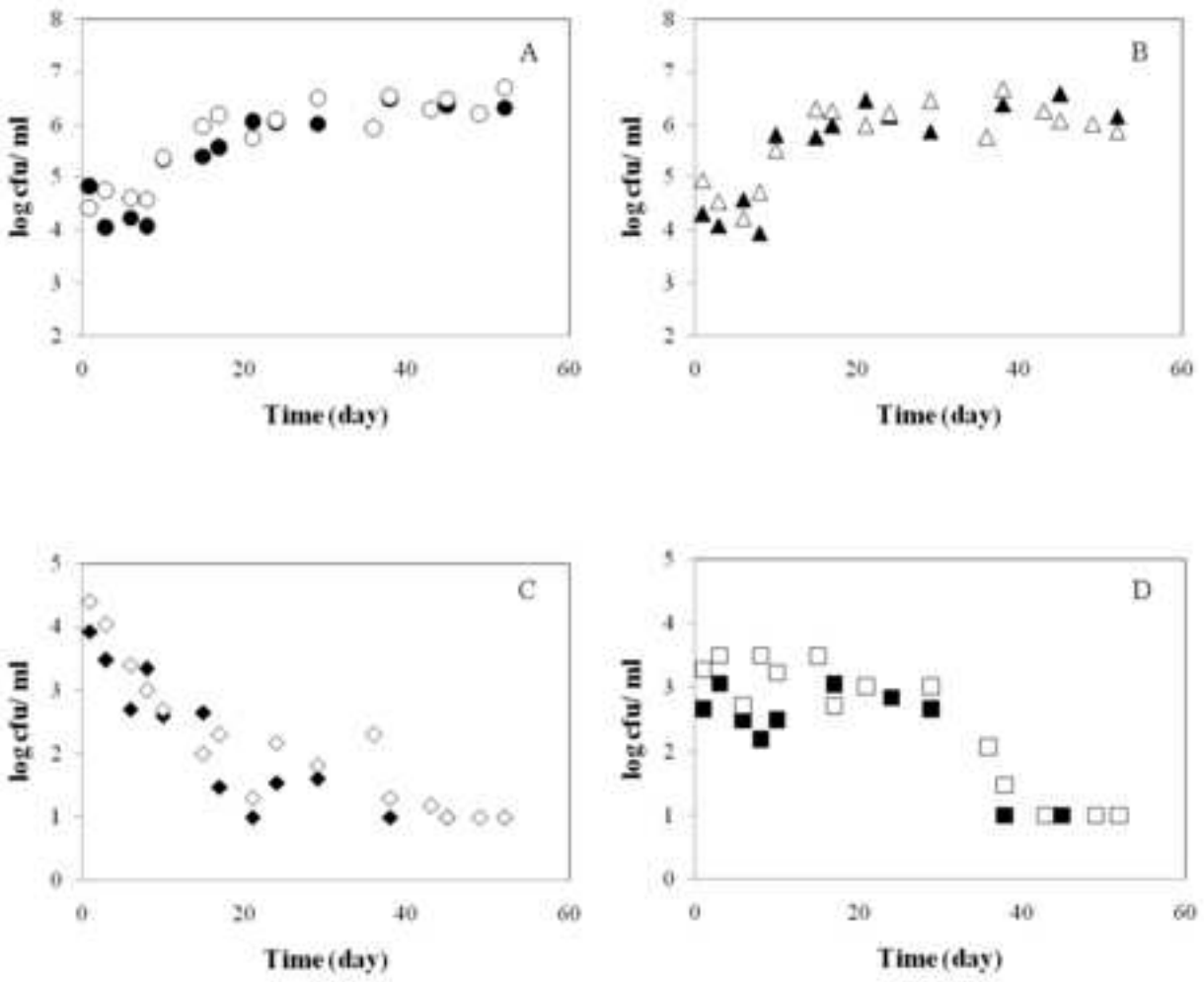

Figure Legends

Fig. 1 Evolution of microbiota during the fermentation of cracked green table olives. Total microbiota (Fig 1A), yeasts (Fig 1B), moulds (Fig 1C) and *Enterobacteriaceae* (Fig 1D) in fermentation Vessel A (open symbols) and Vessel B (closed symbols).

Table 1

Table 1 Identification of yeast isolates obtained from the fermentations of cracked green table olives through PCR-RFLP method (RFLP profiles, PCR products, restriction fragments and identified species (* Non identified by RFLP).

RFLP Profile Nº	PCR Product (bp)	Restriction Fragments (bp)			Identified species	Isolation (%)
		CfoI	*HaeIII*	*HinfI*		
I	590	190+180+100	450+150	280+180+130	*Aureobasidium pullulans*	4.6
II	750	350+310+90	700	390+190+160	*Candida boidinii*	2.8
III	630	290+170+130	420+130+90	310+310	*Candida diddensiae*	6.4
IV	630	290+290	420+140+80	310+310	*Candida oleophila*	0.9
V	600	280+205	400+120+90	300+190+110	*	1.8
VI	700	320+200+100+80	420+200+100	400+320	*Cyteromyces matritensis*	9.2
VII	640	320+240+80	430+210	340+220+80	*Rhodotorula mucilaginosa*	1.8
VIII	850	380+320+150	330+230+170+120	380+360+110	*Saccharomyces cerevisiae*	11.9
IX	660	300+300	400+100+80	320+190+120	*Zygotorulaspora mrakii*	59.6

Table 2

Table 2 Results of the comparison of the isolate sequences with those present in GeneBank from NCBI database (ATCC- American Type Culture Collection, USA; CBS-Centraalbureau voor Schimmelcultures, Nederland; NRRL- Agricultural Research Culture Collection, USA; [a] Relation of similarity of number of nucleotides in Domain D1/D2 between isolates and GeneBank accession strains).

Profile	Fragment sequenced (bp)	GeneBank accession number – Species and strain designation	Similarity [a] (%)
I	530	FJ150916 A*ureobasidium pullulans* var. *pullulans* Strain CBS 146.30	530/530 100%
II	494	FJ914947 *Candida boidinii* Srain ATCC 90438	493/494 99.7%
III	443	U45750 *Candida diddensiae* Strain NRRL Y-7589	443/443 100%
IV	507	AF178050 *Candida oleophila* Strain CBS 6106	507/507 100%
V	533	DQ655691 *Candida quercitrusa* Strain NRRL Y-27941	533/533 100%
VI	502	EF550346 *Citeromyces matritensis* Strain NRRL Y-2407	497/502 99%
VII	493	AF335986 *Rhodotorula mucilaginosa* Strain ATCC 32763	492/493 99.7%
VIII	535	AY048154 *Saccharomyces cerevisiae* Strain NRRL Y-12632	535/535 100%
IX	534	U72159 *Zygosaccharomyces mrakii* Strain NRRL Y-12654	533/534 99.8%

Table 3 Succession of yeasts during the fermentation of cracked green table olives.