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Microbial profile and bacterial characterisation of naturally debittered Hurma olives compared to non-debittered Erkence variety during ripening period

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Summary Naturally debittered Hurma olive is grown in a specific area in Karaburun peninsula in Turkey. It is characterised by its sweet taste and it differs from other varieties by losing its bitterness caused by phenolic compounds during its maturation period on the tree. Therefore, Hurma olive does not require any further debittering process to be served as table olive. This study was particularly interested in the comparison of the microbial profile of Hurma olive during its 8 weeks of maturation period in two subsequent harvest years and Erkence (not naturally debittered) olive. In addition, main bacterial profile of both Hurma and Erkence olives were isolated and identified. Aerobic mesophilic microorganism (AMM), lactic acid bacteria (LAB), Enterobacteriaceae, Pseudomonadaceae, Staphylococci, Micrococcaceae, yeasts and moulds (Y&Ms) were detected (counted and isolated) in the olive drupes during the maturation period. Isolated bacteria were identified as different spp. of Bacillaceae, Enterobacteriaceae, Micrococcoceae and Pseudomonadaceae.

Keywords Foods of plant origin, microbiology, microorganisms, olives, PCR.

Introduction

Olive is one of the traditional agricultural products of the Mediterranean countries and table olives are mostly consumed in this region. Turkey is an important table olive producing country and very rich in terms of olive varieties. Among these varieties, Hurma olive, which has characteristic sensorial properties, is a noteworthy product of Karaburun Peninsula. Hurma olive refers to the self-debittered Erkence olive variety. When Erkence olive variety gets ripened on the tree as dark brown coloured, shrivelled and especially, when it loses its bitterness itself during its maturation period, it is called anymore as Hurma olive. The exact reason of this self-debitterness is unknown. But, Hurma olive is mostly consumed by people who live in the area of Karaburun Peninsula and Izmir. They can consume Hurma olive just after collecting from the tree. So, Hurma olive does not require further debittering process. However, this phenomenon may not occur in the entire tree and in sequent harvest years. It is believed that the reason of this phenomenon is mostly dependent on climate (weather conditions, rainfall, temperature etc.) and location. Therefore, Hurma olive can be a healthier alternative for people who suffer from certain kind of diseases such as high blood pressure than the processed olives in brine with high salt content.

According to a small number of studies in literature, similar types of olive varieties 'Throuba Thassos' (known as Thrubolea) and Dhokar were also grown in Greece and Tunisia respectively (Panagou *et al.*, 2002; Jemai *et al.*, 2009). As it was cited by Aktas *et al.* (2014), according to a study performed by Kalogereas (1932) in Greece with a similar type of olive with the Hurma olive, the debittering process was attributed to the result of an action of *Phoma olea*, which is a fungus and is able to hydrolyse oleuropein. Debittering process on the tree is confined only to certain geographical locations by the local people, thus, the effect of climate and/or soil was also mentioned as the parameter (Aktas *et al.*, 2014).

Studies of raw olive fruits' microbiological characterisation are scarce in the literature (Campaniello *et al.*, 2005; Mantzouridou & Tsimidou, 2011). It is

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known that the most of the table olive fermentation processes start spontaneously and are influenced by the olive cultivar itself, its indigenous microbiota consisting mainly lactic acid bacteria (LAB) members, namely *Lactobacillus* spp., and yeasts as stated by the researchers (Nychas *et al*., 2002; Arroyo-López *et al*., 2008; Hurtado *et al*., 2012). Since culture-dependent techniques do not offer a complete profile of the microbial diversity that is present in a specific ecosystem (Hugenholtz *et al*., 1998; Cocolin *et al*., 2013), the use of molecular techniques for the evaluation of the microbial diversity during olives fermentation and the related critical aspects have been prominent.

The objective of this study was to investigate the changes of naturally debittered Hurma olive microflora during the maturation period (from the start of debittering to full ripening). To our knowledge, the present study is the first research on investigating microbial population dynamics and employing of molecular technique for the evaluation of the bacterial diversity during the maturation of the naturally black Erkence olive variety (both naturally debittered Hurma olive and non-debittered Erkence olive) and monitoring for two sequential harvest years.

Materials and methods

Materials

Both Hurma (naturally debittered Erkence) and Erkence (not naturally debittered) olives were collected manually as materials during the maturation period in the crop years of 2011 and 2012. They were collected from an orchard (latitude: 38°54′07″N, longitude: 26°57′24″E) in the village of Eglenhoca, Karaburun (İzmir) in Turkey. The olive samples were the same as used by Aktas (2013) and Aktas *et al*. (2014). The healthy olive drupes without any kind of infection or physical damage were handpicked on eight different harvest dates, corresponding to between October and December of two harvest seasons. At regular intervals during maturation periods, olives were harvested from all sides of the same three trees in the same locations for each type. Hurma and Erkence olives were differentiated in terms of their appearance. Hurma olives get shrivelled on the tree while Erkence olives have a smooth skin. The samples were brought on ice by using cool bags to the laboratory for experiments.

Microbiological analysis

Microbiological analyses were performed immediately after the samples of olive had been brought to laboratories weekly. Ten grams of each olive sample was weighed out aseptically after destoning. Afterwards, 90 mL of ¼ Ringer's (Merck, Darmstadt, Germany) solution was added into each stomacher bag in which samples were placed and homogenised for 1 min. The homogenised olive samples were serially diluted by using ¼ Ringer's solution and dilutions were plated in triplicate for microbial enumeration. The media and incubation conditions were as follows: Plate Count Agar (PCA; Difco BD, Detroit, MI, USA) for total aerobic mesophilic (AMM) counts, incubated at 30 °C for 48 h; Pseudomonas Agar supplemented with a commercial antibiotic that is known as SR 0102 E (PA; Oxoid, Milan, Italy) medium for counts of *Pseudomonas*, incubated at 25 °C for 48 h; Baird Parker Agar supplemented with egg yolk tellurite emulsion (BPA; Difco BD) for staphylococci count, incubated at 37 °C for 48 h, Violet Red Bile Glucose Agar (VRBGA; Difco BD) for enumeration of *Enterobacteriaceae*, incubated at 37 °C for 24 h; Potato Dextrose Agar (PDA; Difco BD) with the addition of tartaric acid (Merck) (pH = 3.5) used to count yeast and molds, incubated at 30 °C for 72 h. Lactobacilli MRS agar (MRS; Difco BD) poured onto petri plates including 1 mL of each samples, incubated for 30 °C for 48 h in anaerobiosis in Anaerobic jar (Merck).

Cultural bacterial isolation

In this study, during maturation period of Hurma olive 49 bacteria were isolated from all the bacterial counting media (PCA and VRBGA) and identified by biochemical tests. However, for the molecular identification and DNA sequencing, the representative isolates were selected. Purification involved two consecutive steps: culturing in nutrient broth and streaking on nutrient agar until the pure cultures were obtained. Purity of colonies was also checked under light microscopy.

Isolation of genomic DNA

Genomic bacterial DNA of samples was isolated as described by Cardinal *et al*. (1997) with slight modification. Briefly, bacterial isolate cells grown in nutrient broth for overnight were harvested by centrifugation for 2 min at 13 000 ***g*** and 4 °C. Then, pellets were washed with 500 μL of 1× TE buffer (pH8) and centrifuged for 2 min at 13 000 ***g*** and 4 °C. Before incubation for 1 h at 37 °C in water bath, the pellets were suspended into 200 μL of 1× TE buffer (pH 8) containing 25% of sucrose and 30 mg mL^{-1} lysozyme. Afterwards, 370 μL of 1× TE buffer (pH 8) containing Proteinase K (1 mg mL^{-1}) and 30 μL of 10% SDS were added respectively and samples were incubated again for 1 h at 37 °C in water bath. For the purpose of lysing of cells, 100 μL of 5 M NaCl

and 80 μL of CTAB/NaCl solution (10% of cetytrimethylammonium bromide, 0.7 M NaCl) were added respectively. After, the lysed samples were incubated for 10 min at 65 °C in water bath, they were extracted with 750 μL of chloroform/isoamylalcohol (24:1) and centrifuged for 2 min at 13 000 ***g*** and 4 °C. By transferring of upper aqueous phase into a new Eppendorf tube, the second extraction with chloroform/isoamyl alcohol was done and centrifuged for 2 min at 13 000 ***g***. This extraction step was repeated one more time and the DNA of sample was precipitated by the addition of 500 μL of 2-propanol. When the DNA wool was observed, the samples were transferred into a new Eppendorf tube containing 500 μL of 70% ethanol for washing. In the case of not observing the DNA wool, it was followed by centrifugation for 10 min at 4025 ***g*** and addition of 500 μL of 70% ethanol with centrifugation at 4025 ***g*** for 10 min. Then, the ethanol was removed and the pellets were dried at 37 °C for 10 min in an oven. Before the final step of incubation for 1 h at 37 °C in water bath, the dried pellets were dissolved in 100 μL of 1× TE buffer (pH 8) containing 100 μg mL^{-1} RNase. Finally, the alternating heat shock was applied twice to dissolve DNA (80 °C for 10 min, and −20 °C for 20 min) and 5 μL of the genomic DNA solution was used as a template. The isolation of genomic DNA was controlled by a spectrophotometer (Nano Drop 8000, Thermo Scientific, Wilmington, DE, USA).

Amplification of 16S rRNA region

Amplification PCR were performed in 25 μL of PCR mixture including 5 μL of genomic DNA as a template, 10 pmol of forward primer, 10 pmol of reverse primers, 2.5 μL of 2 μM dNTPs, 2.5 μL of Mg free Taq polymerase buffer (Fermentas, Thermo Fisher Scientific, Waltham, MA, USA), 1.5 μL of 25 mM $MgCl_2$ (Fermentas) and 1 U Taq polymerase (Fermentas). Amplification of genomic DNA was performed in a BIO-RAD C1000 thermal cycler (Hercules, CA, USA). The conditions for PCR were the incubation at 94 °C for 5 min, 40 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, elongation at 72 °C for 1 min. Final extension at 72 °C for 10 min was the last step for PCR.

PCR mixture was prepared by using the primer pair of 341 F – 518 R (forward primer, 341 F: 5′-CCTACGGGAGGCAGCAG-3′, reverse primer 518 R: 5′-ATTACCGCGGCTGCTGG-3′) (Muyzer *et al.*, 1993). Amplification of genomic DNA was visualised by agarose gel electrophoresis under UV light. The length of base pairs of the amplified PCR products was observed between 100 bp and 200 bp. *Escherichia coli* was used as a reference strain.

DNA sequencing

Since the sequencing is performed in only one direction, 341 F primer was preferred coincidently. The representative strains were chosen randomly, according to their frequency of occurrence. Also, the rarely isolated strains were all subjected to DNA sequencing analysis.

Cycle sequence was performed to label nucleotides by fluorescence in a thermocycler. PCR products were sequenced in forward direction using 341 F primer and BigDye Terminator v3.1 Cycle sequencing Kit (Appllied Biosystems, Paisley, UK), according to manufacturer's recommendations. Each sample including the mixture of 2 μL (3–10 ng μL^{-1}) of DNA, 1 μL (3.2 pmol μL^{-1}) of forward primer of 341 F, 1 μL of sequencing buffer, 2 μL of (3.2 pmole μL^{-1}) BigDye and 4 μL of deoionised water was prepared for thermocycler (C1000 Thermal Cycler, BIO-RAD) performing PCR for cycle sequencing. The sequencing in thermal cycler was initiated by incubation at 96 °C for 1 min and followed by 30 cycles of denaturation at 96 °C for 10 s, annealing at 50 °C for 5 s and elongation at 60 °C for 4 min. The sequence results were evaluated by BLAST sequence analysis tool (Madden, 2013).

Statistical analysis

Each experiment was performed in duplicate and repeated three times. The statistical comparisons of the mean values for each experiment were performed by one-way analysis of variances (ANOVA), followed by the Tukey test for comparisons between each week enumeration results and two harvest seasons using MINITAB software (Minitab, State College, PA, USA, 16.0.). Differences at $P < 0.05$ were considered as significant.

Results and discussion

Throughout the maturation stages of naturally black olive (*Olea europaea* L.) fruits of Erkence variety, the microbial composition of olive drupes was examined in 2011 and 2012 crop years.

Lactobacilli (<10 cfu g^{-1}), pseudomonads (<100 cfu g^{-1}), staphylococci (<100 cfu g^{-1}) and micrococci (<100 cfu g^{-1}) were not detected in both Hurma and Erkence olive fruits during maturation period for both seasons. This finding is consistent partially with the previous findings of the study by Borçaklı *et al.* (1993) who investigated unprocessed black olive varieties of Gemlik and Edincik in which counts of lactic acid bacteria were determined below the detection limit (<10 cfu g^{-1}). In addition, our enumeration results of staphylococci are in agreement

with the study of Mantzouridou & Tsimidou (2011) who detected no staphylococci in raw Thassos variety olives having 0.89 a_w and 10% of moisture content and total AMM count was lower compared to our results. Similar microbiological analysis' result which is no detection of LAB, enterobacteria, pseudomonads, *Staphylococcus aureus* was reported by Panagou *et al.* (2002) for dry-salted olives of Thassos variety. Differently from our findings, in the study of Panagou *et al.* (2002) raw olive fruits' microflora is comprised of mainly yeasts.

Olive is a fruit, so its microbiota mostly comes from different sources like soil, water, air, animals, insects, birds etc. However, its microbiota is affected from olives' own properties such as phenolic contents, water activity, nutrients in olive and also temperature of the environment in where olive is grown, are important as well. In the study of Aktas (2013), a_w values of Hurma and Erkence olives that were also the same samples which were used in this study were between 0.94–0.98 and 0.92–0.97 respectively in 2011; while these values were in the ranges of 0.92–0.94 and 0.94–0.96 respectively in 2012. It was also found that the a_w values of the olive samples changed significantly ($P < 0.001$) with respect to harvest year, variety and sampling time (Aktas, 2013).

Microbial profile of naturally debittered Hurma olive

Microbial population changes for total AMM count of Hurma olive for two seasons are shown in Fig. 1A. On the onset of maturation on tree for both seasons, initial count results of 2011 and 2012 (3.8 and 2.2 log cfu g^{-1}, respectively) were close to each other. Ongoing maturation trend followed an up and down line and finally peaked at 6.9 log cfu g^{-1} in the season of 2011. The increase in the microbial load of Hurma olive between the first and the last week of the maturation period was an expected result, since oleuropein content decreased as the olives got ripened (Aktas, 2013; Dağdelen *et al.*, 2013; Aktas *et al.*, 2014). Besides, the conducted study of Jemai *et al.* (2009) showed that level of oleuropein in self-debittered Dhokar olive variety which is similar to naturally debittered Hurma olive, decreased and finally reached to a negligible level (0.06 g kg^{-1}) in fully ripened olive fruit. But, there was a significant ($P > 0.05$) difference between total AMM counts of two seasons; the count of total AMM in 2012 was lower than those in 2011. This finding may be associated with the lack of *Enterobacteriaceae* in 2012, because in 2011, the presence of *Enterobacteriaceae* in high number may contributed to higher total AMM count which ranged between 2.7 log cfu g^{-1} and 5 log cfu g^{-1}. On the other hand, total AMM counts of 2012 reached to 4.2 log cfu g^{-1} that was 2.5 log units lower than those in 2011. As indicated previously, a_w of Hurma olive in 2012 (ranged between 0.92 and 0.94, determined by Aktas (2013)) and total phenolic content of Hurma olive in 2012 (Aktas *et al.*, 2014) and rainfall amount (data are not given) in the maturation period of 2012 were at a lower level than those in 2011. The difference between the counts of two years could have been related to climatic variables and alternate bearing in olive production (Fornaciari *et al.*, 2000).

Y&M growth were monitored by using PDA. The final count of Y&M for 2011 was almost as high as total AMM counts of the same year. Nonetheless, in 2012 Y&M were enumerated in the range between 3.15 and 4.45 log cfu g^{-1}. The enumeration on PDA during maturation followed a stable trend in 2011 while the trend for 2012 was not in a stable line with significant ($P > 0.05$) differences of count results as indicated with superscript in Fig. 1B.

As seen in Fig. 1C, Enterobacteriaceae reached to the highest population (4.95 log cfu g^{-1}) at the end of the sampling period in 2011, but they were at undetectable level until the fifth week in the 2012 season. Later then, the population of them ranged between 2.3 and 3.67 log cfu g^{-1} (Fig. 1C). The reason of the undetected Enterobacteriaceae may be associated with the inhibition of pathogenic bacteria such as *Staphylococcus aureus, Salmonella* Enteritidis, germination of spores of *Bacillus cereus, Escherichia coli* by high amount of phenolic compounds as stated in the study of Omar (2010).

Microbial profile of non-debittered Erkence olive

Erkence olives as stated previously in this study are smooth-surfaced olive fruits. They were analysed microbiologically to observe the difference of microbiological growth profile from Hurma olive and change of microbial load during maturation was given in (Fig. 1D–F). In 2011, all the olives on the trees were turned to Hurma olives two weeks earlier than in 2012. All types of microbial counts of Erkence olives were lower than the Hurma olive samples, since the higher content of phenolic compounds present in Erkence olive tissue (Aktas, 2013; Aktas *et al.*, 2014). Similarly in the literature, regular (non-debittered) Chemlali olive variety was compared to naturally debittered Dhokar olive variety in terms of phenolic compounds during ripening. In green maturation stage, almost two-fold higher oleuropein was observed in Chemlali olive variety than those in Dhokar variety olive fruits and the level of oleuropein did not declined as much as in Dhokar variety olive (Jemai *et al.*, 2009). On the other hand, the studies that discuss the antimicrobial action of phenolic compounds of raw olive on its microbial load are scarce. However, the major phenolic compounds of olive, oleuropein and

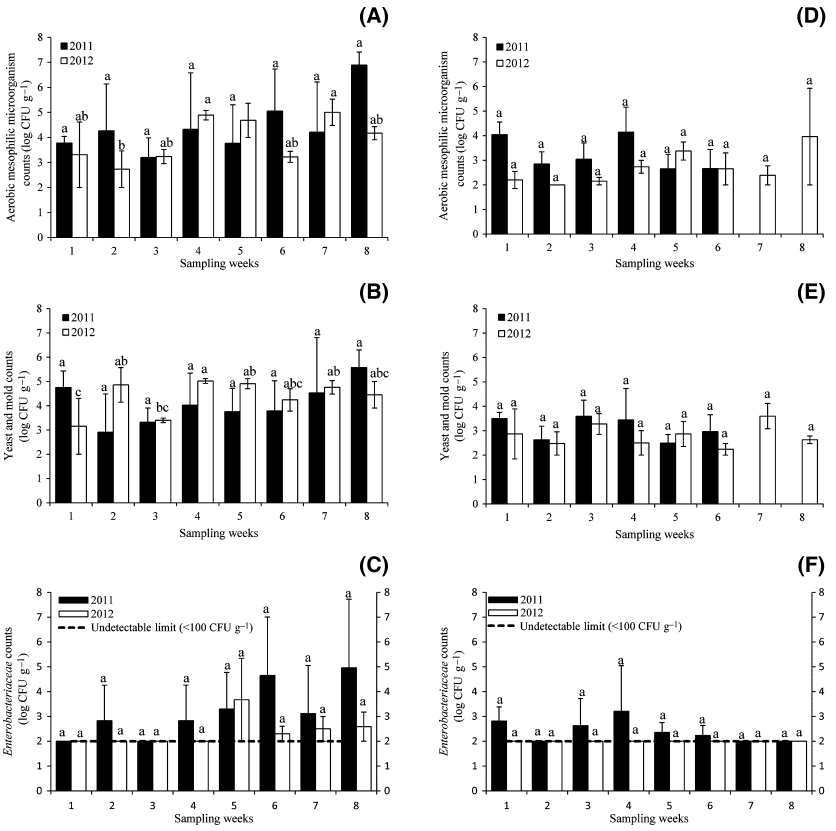

Figure 1 Microbial load (log cfu g^{-1}) of the AMM (A), Y&M (B), Enterobacteriaceae (C) found in Hurma olive and microbial load of the AMM (D), Y&M (E), Enterobacteriaceae (F) in Erkence olive during maturation. (Means for each harvest year without common letters (a–c) are significantly different ($P < 0.05$)).

hydroxytyrosol, have been studied to investigate their antimicrobial activity against various microorganisms for several times (Aziz *et al*., 1997; Ahmed *et al*., 2014). Moreover, the addition of *Olea europeae* as a food additive into the food or the usage of *O. europeae* as an antimicrobial agent for pest management programs has been proposed by Bisignano *et al*. (1999). The phenolic contents of olive may affect the types and interactions of the microbiota on olive. As stated by Canal (2016), phenolic compounds of raw olive such as hydroxytyrosol and oleuropein have also antimicrobial action on yeasts.

In 2011, total AMM counts were generally almost one logarithmic unit higher than the corresponding results of 2012 for the first half of the maturation period (Fig. 1D). In the last week of maturation in 2011 and 2012, seasons' total AMM count results were found as 2.7 and 3.96 log cfu g^{-1} respectively. But the seasonal difference must be taken into consideration since sampling of Erkence olives was finished two weeks earlier in 2011 (Fig. 1D). No significant differences ($P > 0.05$) were observed in sequential counts of total AMM during maturation in both seasons as shown statistically in Fig. 1D and *P*-values determined as 0.064 and 0.076 in 2011 and 2012, respectively.

Y&Ms were enumerated at a lower level in two seasons than those in Hurma olives. As seen in the Fig. 1E, there were no significant ($P > 0.05$) differences between sampling weeks in terms of growth and count results for both seasons.

Enterobacteriaceae count of the first year was remained in a narrow range during the maturation period, averaging about 2.4 log cfu g^{-1} of Erkence olive. On the contrary, in the second year no Enterobacteriaceae were detected above the detection limit (<100 cfu g^{-1}) (Fig. 1F).

Bacterial isolates

The classical gene used in bacterial taxonomy is the 16S rRNA gene that was also used in this study. Strains showing more than 97% of 16S rRNA sequence identity are considered to belong to the same species.

As seen in Table 1, the bacterial isolates from Hurma olives were identified resulting in five families Bacillaceae (*B. subtilis, B. simplex, Bacillus* sp.),

Table 1 Identified bacteria originated from Hurma olive by DNA sequencing method

Code	Similar genus/species from BLAST	Max ID (%)[A]
2011		
1-26	*Bacillus simplex*	100
2-19	*Klebsiella oxytoca*	98
4-14	*Pantoea* sp.	100
5-13	*Pantoea agglomerans*	100
7-13	*Bacillus* sp.	99
8-17	*Pseudomonas* sp.	97
8-20	*Pseudomonas* sp.	100
2012		
3-23	*Bacillus subtilis*	99
5-1	*Pantoea* sp.	100
6-15	*Klebsiella pneumoniea*	100
7-2	*Pantoea agglomerans*	100
8-24	*Pantoea agglomerans*	100
8-25	*Pantoea agglomerans*	100

[A]MAX ID (%) were given according to BLAST search.

Table 2 Identified bacteria originated from non-debittered Erkence olive by DNA sequencing method

Code	Similar genus/species from BLAST	Max ID (%)[A]
2011		
2-35	*Pantoea agglomerans*	99
2V-2	*Klebsiella oxytoca*	98
3-2	*Micrococcus luteus*	100
2012		
1-5	*Bacillus licheniformis*	100
1-7	*Micrococcus* sp.	100
1-21	*Pantoea* sp.	100

[A]MAX ID (%) were given according to BLAST search.

Enterobacteriaceae *(Klebsiella oxytoca, Klebsiella pneumoniea, Pantoea agglomerans, Pantoea* sp.), *Micrococcoceae (Micrococcus luteus, Micrococcus* spp.) and Pseudomonadaceae (*Pseudomonas* spp.) represented by different species. Similarly, Erkence olive's bacterial isolates belonged to the families of Enterobacteriaceae (*Pantoea agglomerans, Pantoea* sp., *Klebsiella oxytoca*), Bacillaceae (*Bacillus licheniformis*) and Micrococcoceae (*Micrococcus luteus, Micrococcus* sp.) according to DNA sequencing results (Table 2).

Although pseudomonads were not detected in related agar media during maturation period, they were identified at the end of the DNA sequencing analysis of bacterial isolates from AMM.

In both seasons, *Micrococcus luteus* was identified in the microflora of Hurma olive by DNA sequencing. These yellow pigmented Gram-positive tetrad formed cocci are predominantly found in mammalian skin and in soil, but commonly isolated from food product and air (Buchanan & Gibbons, 1974). Micrococci are widely distributed in nature in food preparation environments and also found naturally in many foods. But it is known that these cocci have either strains or species of mesothrophs and psychrotrophs (Jay, 1992). *Pantoea* spp. was one of the identified bacteria by DNA sequencing in both seasons. *Pantoea* genus which is mostly found in the bacterial isolates of Hurma olive (*Pantoea agglomerans*), is mostly found on plants and seeds and in soil (Jay, 1992). A number of coliform species in the genera *Erwinia, Pantoea, Serratia, Klebsiella* and *Enterobacter* grow predominantly on plants and vegetables (also including olives) and they are usually not found in faecal matter. The growth of these particular coliforms on olive fruit will ensure their carry-over into ready-to-eat 'Hurma' olive, thereby negating any potential utility of a coliform specification for this olive type. Also, in this study, *E. coli* which is prominent indicator of food safety was not found on Hurma olive. Moreover, saprophytic bacterium *Bacillus licheniformis* which is widely distributed in environment (Rey *et al*., 2004) was also detected in non-debittered Erkence olive microflora. On the other hand, *Phoma, Cladosporium, Alternaria, Fusarium, Aspergillus* and *Penicillium* were isolated from the Hurma olive (A.H. Baysal, D. Özcan & G.S. Sozbilen, unpublished data); that (one or several of these) also, have role, in the softening of the pickles resulting from their pectolytic activities (Jay, 1992).

Conclusions

Hurma olives can be served to the market as a healthy food alternative after washing to decrease and control microbial load and identified pathogens with salt-free or non-brined olives. Moreover, the improvement of packaging technique is essential to control hazards. Microbial characterisation of a food reveals the level of hygiene and it is a key factor in assessing the quality and the safety of food. Even though, the isolation of *Klebsiella* strains from various foods have previously been reported (Park & Sanders, 1990; Kim *et al*.,2015), to our knowledge, our study is the first report of the isolation *K. oxytoca* and *K. pneumoniae* which members of the Enterobacteriaceae, isolated from Hurma olive samples. When the results were obtained in this study, are taken into consideration, Hurma olives seem safe for human consumption as any other fruits and vegetables. However, in order to ensure safety of Hurma olive as the ready-to-eat food, determining microbial profile or detecting the bacterial foodborne pathogens of Hurma olive sold in selected retail outlets or in the market should be taken into consideration.

In conclusion, it would be the first in microbiological characterisation of Hurma olive during maturation period for two harvest years. Therefore, after maturation of Hurma olive, good handling and preventing

contamination strategies to inhibit growth of microorganisms in Hurma olive are more likely to succeed. These include cold storage such as refrigeration or freezing and packaging to reduce oxygen and increase carbondioxide.

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