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Survival of food-borne pathogenic bacteria in table olive brines

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Abstract

Fermented or acidified vegetable foods are considered microbiologically safe although the survival of certain pathogens has occasionally been reported in these products. The aim of this research was to investigate the fate of *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes* and *Staphylococcus aureus* when they were added to different industrial olive brines, as well as to correlate their survival with the presence of phenolic and oleosidic substances. Brines of different cultivars prepared following the Spanish-style method or preserved in acidified brine were inoculated with a cocktail of four strains of each species. The evolution of their populations was analyzed by cultural methods when the brines were kept at 4 ºC or room temperature and in aerobiosis or anaerobiosis. All the pathogens investigated died off but their death rate was variable depending on the composition of the brines in phenolic compounds, temperature and oxygen availability. The time needed to reduce the inoculated pathogen populations by 5 log oscillated between less than 5 minutes and up to 17 days in the least deleterious conditions.

Keywords: viability, inhibition, *Salmonella enterica*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*

1. Introduction

Fermented or acidified vegetable products are considered microbiologically safe. However, acid resistant strains of *Salmonella* sv. Muenchen and *Escherichia coli* O157:H7 have shown that acidic foods may be a source of foodborne diseases (Centers for Disease Control and Prevention, 1996; 1999). Table olives are a traditional product in the Mediterranean basin and, nowadays, spread to almost all continents. There are many different ways of preparing table olives, depending on the different regions and countries. Only three types are economically important from a commercial perspective: Spanish-style green olives, natural olives in brine, and ripe olives darkened by oxidation. Heat treatment is only mandatory for ripe olives, which need to be sterilized because their pH values are around 5.5-6.5, far above the safety limit for preventing the growth of *Clostridium botulinum*. In contrast, Spanish-style and natural olives rely on their fermentative processes to guarantee the microbial safety of the final products, which do not need to be heat treated.

The elaboration processes of the diverse table olive types are well established (Sánchez-Gómez, García-García, & Rejano-Navarro, 2006). Basically, Spanish-style green olives are first treated with sodium hydroxide (1.8-2.5 % w/v), then washed with water and finally covered with brine (ca. 10 % w/v) where lactic acid fermentation takes place. Natural olives not treated with any alkaline treatment, are directly covered with brine and acidification with acetic acid is strongly recommended to prevent spoilage.

The predominant microorganisms in olives covered directly with brine without any alkaline treatment are yeasts. Lactic acid bacteria are the prevailing microorganisms in Spanish-style green olive brines. Salt concentration, acidity and inhibitory compounds, among other factors, limit the growth of lactic acid bacteria in non-alkali

treated olives (Medina, Romero, de Castro, Brenes, & García, 2008; Medina, Gori, Servili, de Castro, Romero, & Brenes, 2010). It has been recently demonstrated that some phenolic and oleosidic substances present in olive brines possess significant bactericidal activity against food and plant pathogens (Medina, Brenes, García, Romero, & de Castro, 2009; Brenes, García, de los Santos, Medina, Romero, de Castro, & Romero, 2011).

As mentioned above, table olives and other fermented or acidified vegetable foods, such as pickles or sauerkraut have a long history of microbial safety. In the case of olives, investigations carried out with cracked Manzanilla olives (without alkaline treatment) revealed that neither lactic acid bacteria nor *Enterobacteriaceae* were detected (Alves, Gonçalves, & Quintas). Recently, Grounda, Nychas, & Panagou (2013) investigated the survival of food-borne pathogens inoculated on natural black table olives during aerobic storage without brine. They demonstrated that natural black olives are not a favorable environment to support the growth of the investigated pathogens. However, the presence of pathogenic bacteria in brines of other types of table olives has been reported. For instance, *Listeria monocytogenes* (Caggia, Randazzo, di Salvo, Romeo, & Giudici, 2004), *Staphylococcus aureus* (Asehraou, Faid, & Jana, 1992; Pereira, Pereira, Bento, & Estevinho, 2008; Romeo Piscopo, Mincione, & Poiana, 2012), and coliforms (Asehraou et al., 1992; Pereira et al., 2008; Franzetti, Scarpellini, & Vecchio, 2011; Romeo et al., 2012) have been found in the table olives of several countries. Moreover, the survival of *E. coli* O157:H7 inoculated in fermenting olives has been observed (Spyropoulou, Chorianopoulos, Skandamis, & Nychas, 2001). Street markets were the origin of many of the samples from which these bacteria were found, whereas undesirable microorganisms were not found in most industrially packed samples. Nevertheless, taking into account that an important percentage of table olives

are sold in bulk either in retail markets or exported in big containers, there is a reasonable concern in relation to the fate of a possible contamination of olive brines with pathogenic bacteria. The same worry is present in other fermented or acidified vegetable products. For instance, survival of *E. coli* O157:H7 and *Listeria monocytogenes* during kimchi fermentation has been addressed (Cho, Lee, & Choi, 2011). Moreover, survival of *L. monocytogenes* and *Salmonella* Typhimurium in spontaneous cauliflower fermentation has been demonstrated (Paramithiotis, Doulgeraki, Tsilikidis, Nychas, & Drosinos, 2012), and these authors have raised the need for safety reassessment of fermented vegetables. Extensive research has been devoted to pickled cucumbers in order to find out the product characteristics to fulfill the US regulations, which mandate a 5-log reduction in acid-resistant bacterial pathogens (Breidt, Hayes, & McFeeters., 2007; Breidt & Caldwell, 2011; Lu, Breidt, Pérez-Díaz, & Osborne, 2011). These researchers revealed that *E. coli* O157:H7 could survive in cucumber fermentation brines, and require between 3 to 24 days to achieve the 5-log reduction. Among other factors, brine pH and acidity, temperature, and redox potential were established as the main characteristics affecting the death rate in cucumber brines.

The objective of this study were i) to determine the survival of selected pathogens in brines obtained from various types of table olives production, and ii) to correlate the survival of these microorganisms with the presence of phenolic and oleosidic substances with known antibacterial activity in the brines.

2. Material and methods

2.1 Bacterial strains and preparation of inocula

120

121 Four strains of each pathogenic species were obtained from the Spanish Type
122 Culture Collection (CECT). *Listeria monocytogenes* CECT 4031^{T} , CECT 4032, CECT
123 5366 and CECT 7467 were cultured at 37 ºC in Brain Heart Infusion (Oxoid Ltd.,
124 Basingstoke, Hampshire, England) with or without 1.5 % agar. *Staphylococcus aureus*
125 CECT 86^{T} , CECT 239, CECT 240 and CECT 976; *Salmonella enterica* sv.
126 Typhimurium CECT 722^{T} , CECT 443 and CECT 4156, and *S. enterica* sv. Enteritidis
127 CECT 4300; *Escherichia coli* CECT 434 and the strains with serotype O157:H7 CECT
128 4267, CECT 4782 and CECT 5947 were all cultured at 37 ºC in nutrient broth prepared
129 with (g/L) "Lab-Lemco" powder (Oxoid) 5, Neutralised bacteriological peptone (Oxoid)
130 10, NaCl 5 and agar 15 for solid medium (pH 7.2). For comparative purposes, four
131 strains of lactic acid bacteria (LAB) from the Instituto de la Grasa IG-CSIC culture
132 collection were used: *Pediococcus ethanolidurans* L5, *Lactobacillus pentosus* L6,
133 *Lactobacillus rapi* L8 and *Lactobacillus parafarraginis* L20 (Montaño, Sánchez,
134 Casado, Beato, & de Castro, 2013). These LAB strains have been isolated from packed
135 Spanish-style green olives and were cultured anaerobically (AnaeroGen, Oxoid) at 32
136 ºC in MRS broth (Biokar Diagnostics, Allonne, Beauvais, France) with 1.5 % agar
137 when required. All the species were maintained at -80 ºC in the adequate culture broths
138 with 15-20 % glycerol. Before the experiments, each strain was cultured twice in their
139 respective broths and centrifuged, washed and resuspended in sterilized saline (NaCl 0.9
140 %). Cocktails of each pathogenic species were obtained mixing equal quantities of the
141 corresponding strains, and a fifth cocktail with the four LAB species was also prepared.
142 The volumes were calculated to obtain ca. 8 log cfu/mL as initial inoculum when 0.1
143 mL of the suspension was added to 1.4 mL of the different test solutions and brines.
144

2.2 Olive brines

Industrial brines were obtained from a renowned table olive producer in Dos Hermanas (Seville). They were withdrawn from the respective fermentation vessels (fermenters with 10,000 kg fruits or containers with 500 kg), transported to the laboratory and kept at 4 ºC. The brines were obtained in April, what implies that they were at their preservation stage and that their main fermentation steps had already taken place. Two different brines of diverse trade preparations and varieties were chosen: HB (1&2), Hojiblanca olives preserved in acidified brine; MO (1&2), Manzanilla organic olives in vinegar acidified brine; KB (1&2), Kalamata olives in brine; MS (1&2), Spanish-style Manzanilla olives (treated with NaOH); GS (1&2), Spanish-style Gordal olives (treated with NaOH); HSP, Spanish-style Hojiblanca olives (treated with NaOH) and stuffed with pimento; MSP, Spanish-style Manzanilla olives (treated with NaOH) and stuffed with pimento.

Apart from the industrial brines, control test solutions (control brines) were prepared with NaCl (6 %) and 0.8 % free acidity using acetic or lactic acid in water. The pH values were adjusted to 3.8 with NaOH.

2.3 Survival assays

Control brines and industrial brines were filter-sterilized (0.2 μm pore size) and1.4 mL were dispensed into eppendorf tubes. The tubes with brine were placed opened in hermetic boxes (GENbox, bioMérieux), and sterilized distilled water was added in the smallest compartment of the GENbox to prevent dryness. The survival studies were performed under different temperature and atmosphere conditions. Before

inoculation, brines were previously acclimatized to temperature by placing the corresponding boxes with the brines in a refrigerator at 4±1 ºC or at room temperature (23±2 ºC). In the case of the different atmospheres, a sachet for generation of anaerobic conditions (Anaerogen, Oxoid) was introduced in the corresponding GENbox containing the opened tubes with brine, to remove the dissolved oxygen. Anaerogen sachets were not used when the brines were kept in aerobiosis. Once acclimatized, the boxes were opened and exactly 100 µL of each bacterial cocktail were added and gently mixed with each brine. A new Anaerogen sachet was introduced in the case of anaerobic conditions, and then the jars were closed and placed again at the corresponding temperatures. Samples from each tube were removed at different times, diluted in 0.1 % peptone and plated to count culturable survivors (Spiral Plater Wasp 2, Don Whitley Sci. Ltd., Shirpley, UK).

2.4 Chemical analysis

The main chemical characteristic of the olive brines were determined. The concentration of sodium chloride was analyzed by titration with a 0.1 M silver nitrate solution, with potassium chromate as indicator. Free acidity, pH and combined acidity were determined using a Metrohm 670 Titroprocessor (Herisau, Switzerland) (de Castro, García, Romero, Brenes, & Garrido, 2007).

Phenolic and oleosidic compounds in brines were measured by HPLC. A mixture of 250 µL of brine, 250 µL of internal standard (2 mM syringic acid) and 500 µl of deionized water was filtered through a 0.2 µm pore size nylon filter and an aliquot (20 µl) was injected into the liquid chromatograph. The equipment, analytical column,

mobile phases, and chromatographic conditions have been described elsewhere (Medina et al., 2008).

2.5 Statistical analysis

All the experiments were run in duplicate, and new experiments were carried out in some cases to confirm the hypotheses. Basic statistics were calculated using a Microsoft Excel spreadsheet (Microsoft). The estimated 5-log reduction times and standard errors of estimate were determined from the killing curves using the Statistica software, version 7.0 (Statsoft Inc., Tulsa, OK).

3. Results and discussion

3.1 Brine characteristics

The main chemical traits of the different industrial olive brines are displayed in Table 1. A broad range of values were measured, especially in NaCl and acidity percentages. The pH values were within 3.5 and 4.2 indicating good preservation conditions. As it is known, the pH value is correlated with the free acidity and the combined acidity. This combined acidity, which is a measurement of the buffer capacity of the brines, is composed of the sodium salts of the predominant acids (lactic, acetic, and others). The buffer system is normally stronger in Spanish-style table olives, as a consequence of the alkaline treatment with NaOH. The brines used in this study are adequate examples of the different types that exist in the Spanish table olive industry.

With regard to the concentration of phenolic compounds, Table 2 presents the content in the different brines of the compounds whose antibacterial activity has been demonstrated, as well as other compounds which indicate the differences between alkali-treated and natural olives. The high concentration in the dialdehydic forms of decarboxymethyl elenolic acid both free (EDA) and linked to hydroxytyrosol (HyEDA) is remarkable in brines HB 1&2 and MO2 of olives non-treated with alkali. In contrast, these compounds were not detected in any of the brines from olives treated with NaOH, as has already been reported (Medina et al., 2008). EDA and HyEDA are the substances in olive brines that have displayed the greatest antimicrobial activity against both Gram-negative and Gram-positive bacteria, including lactic acid bacteria (Medina et al., 2009; Brenes et al., 2011). The differences between fermenters processed in the same way and in the same factory must also be noted. For instance, brines MO1 and MO2 differ in their content in hydroxytyrosol, hydroxytyrosol 1-glucoside, tyrosol, EDA and HyEDA. Similar observations can be made when comparing samples of Spanish-style Manzanilla olives (MS1 vs. MS2) in relation to hydroxytyrosol and its glucosides, as well as samples of Spanish-style Gordal olives (GS1 vs. GS2) in relation to tyrosol concentration. All this diversity reflects the variations that are normally found in any table olive industrial processing plant.

3.2 Survival rate of the different bacteria in relation to the diverse brines and incubation environments

Bacterial survival under unfavourable conditions is higher at refrigeration temperatures (Clavero & Beuchat, 1996; Breidt et al., 2007). Table 3 presents the results of 5-log reduction times for the different brines and bacteria at 4 ºC under aerobic

243 conditions. The periods of time ranged from less than 5 minutes for all pathogens in
244 Hojiblanca olives preserved in acidified brine (HB 1&2) to around 7 days for *S. enterica*
245 in Spanish-style brines of Manzanilla olives (MS2). Clear differences were noticed in
246 relation to the distinct brines and bacterial cocktails. Hojiblanca olives without alkaline
247 treatment and preserved in acidified brine, and one of the samples of Manzanilla organic
248 olives (MO2), displayed the most lethal effect, not only against the pathogenic species,
249 but also against the LAB cocktail. When the main chemical characteristics of these
250 brines are compared with the rest (Table 1), their values cannot explain the observed
251 results. For instance, brine MO2 had less acidity and higher pH than brine MO1. On the
252 contrary, the presence of significant concentrations of EDA and HyEDA in brines
253 HB1&2 and MO2 (Table 2) do justify their lethal effect on the tested bacteria. Although
254 the combined effect of all phenolic substances that may be present in olive brines is
255 ultimately responsible for their antibacterial effect, it has been proposed that the
256 existence of dialdehydic groups in the molecules of EDA and HyEDA play a major role
257 in this effect, which is comparable with that obtained using the well-known biocide
258 glutaraldehyde (Medina et al., 2009). However, when EDA and HyEDA were not found
259 in olive brines, loss in survival was still observed, with clear differences between brines
260 for the same bacterial cocktail, and among cocktails for the same brine. The LAB
261 cocktail was the most resistant to all industrial brines, showing the longest time
262 necessary to reach the 5-log reduction of the inoculated population. This result is not
263 surprising, taken into account that vegetables in brine are usual habitats for this group of
264 microorganisms. However, it is noteworthy that the *E.coli* cocktail, with 3 strains of the
265 O157:H7 serotype, survived for a similar time as the LAB cocktail in control brines
266 prepared with only salt and acetic acid or lactic acids, which emphasize the hazard that
267 this microorganism may signify in acid foods. With respect to the other brines, *E. coli*

survived for 125.7 hours in the brine MS2, ranging from 9.3 to 46.2 hours in the rest of the industrial brines. It is remarkable that 326.9 or 259.4 hours were necessary to reduce the fixed population of *E. coli* in the acetic acid and lactic acid control brines respectively, which once again unveils that olive brines manifest bactericidal effects that are not solely due to their pH and salt and acid contents, particularly if bactericidal phenolics are present. When investigating the survival of *E. coli* O157:H7 in commercial cucumber brines, Breidt & Caldwell (2011) found a correlation between the pH of the brine and the 5-log reduction time, which varied between 22 days (pH 4.53) and 3.4 days (pH 3.16). There was one brine with pH 3.78 and only 2 days of 5-log reduction time, but contained 7.82 % NaCl. These reduction times observed in cucumber brines are longer than those we obtained for olive brines. Cucumber and olive fermentations are similar processes, but there is no evidence for natural antimicrobial compounds in brined cucumbers (Breidt et al., 2007). The same authors have preferably used *E. coli* O157:H7 as the target strain for survival studies in pickles because this microorganism has been shown to be more acid resistant than other food borne pathogens. In our case, it was not fully clear what species, in general, were the most resistant to the olive brines characteristics. As mentioned above, LAB showed the longest times to achieve 5-log reductions in all olive brines by a large margin. On the other hand, *L. monocytogenes* was the least resistant to the olive brine characteristics, requiring 66.2 hours to achieve the 5-log reduction with brine MS2, which was the brine with the lowest effect, not only against *L. monocytogenes*, but also against the other pathogens *S. aureus*, *E. coli*, and *S. enterica*. Furthermore, variations among strains of the same species in survival cannot be excluded (Grounda et al., 2013).

In short, the time necessary to reduce the population of pathogenic bacteria in some olive brines by 5 log, when kept aerobically at refrigeration temperatures, may be

as fast as 5 min in brines with typical chemical characteristics and enhanced concentration of antibacterial compounds due to the absence of alkaline treatment, or may be as long as 2.8 days (*L. monocytogenes*), 5.2 days (*E. coli*), 6.2 days (*S. aureus*), or 6.7 days (*S. enterica*) in brines with normal chemical characteristics but low concentrations of antibacterial compounds as a consequence of the alkaline treatment with NaOH.

For comparative purposes, Figure 1 shows the death rates or survival along time of the different cocktails in the industrial brine MS2, in which survival lasted more for all pathogens. Except for *E. coli* (R^2 0.80), the R^2 values obtained for the regression lines were higher than 0.90, which implies good correlation. As previously mentioned, *L. monocytogenes* died faster and LAB were the most resistant. In this brine MS2, *S. aureus* and *S. enterica* showed similar behavior and higher resistance than *E. coli*. However, it should be emphasized that the slopes were comparable for these three species. Interestingly, this order of resistance to Spanish-style olive brines agreed with the results obtained with other varieties (Argyri, Lyra, Panagou, & Tassou, 2013). These authors also revealed that survival of the pathogens lasted longer in the brines than in the fruits.

The pathogen survival times obtained at 4 ºC prompted us to investigate their fate at room temperature, which is a more realistic situation, taking into account that, to the best of our knowledge, table olives are almost never commercialized under refrigeration. For this experiment, only the brine of each type that had displayed the lowest lethal effect was chosen to determine the time necessary for the 5-log reduction of the tested pathogens. As expected, the periods of time needed to reduce the pathogen populations were much shorter at room temperature (Table 4) than at 4 ºC (Table 3). Once again, LAB were the microorganisms that survived the longest in all brines. At

318 room temperature, *S. aureus* was the second most resistant cocktail for all brines; *E. coli*
319 was the third, and *L. monocytogenes* and *S. enterica* were the least resistant to the
320 deleterious effect of the olive brines. It should be noted that the initial inocula were
321 approximately 8 log cfu/mL, and the pathogenic populations decreased below 3 log
322 cfu/mL after less than 26 hours, when the brines were tested at room temperature.
323 Although these results support to a large degree that table olive brines are not a
324 suitable environment for the foodborne pathogenic bacteria assayed in this work, new
325 experiments were carried out under anaerobic conditions. The results shown in Figure 2
326 compare the 5-log reduction times, at both temperatures and both atmospheres, for the
327 four pathogenic species when inoculated in brine MS2, which presented the lowest
328 effect against the tested bacteria. Interestingly, anaerobiosis caused a marked increase in
329 survival of *S. aureus* and *E. coli* at refrigeration temperature, with the differences being
330 less significant for the other two species at room temperature. Finally, the survival of all
331 pathogens was also investigated in the most harmful brine (HB1) at 4 ºC and
332 anaerobiosis. In these conditions, none of the bacteria were detected after 10 min (data
333 not shown).
334
335 **4. Conclusion**
336
337 Table olive industrial brines of different cultivars and elaboration processes did
338 not constitute a propitious environment for any of the pathogenic bacteria tested.
339 Although the presence of foodborne pathogenic bacteria in olive products is not likely,
340 accidental contamination cannot be ruled out. In that case, it is important to take into
341 account that survival would last longer when olives are maintained at low temperatures
342 and in anaerobiosis. In the case of olive brines which were rich in phenolic and

343 oleosidic compounds with antibacterial effect, and presented normal values of pH,
344 acidity and salt, a 5-log reduction of their initial population could be reached between 5
345 and 10 min, regardless of whether the brines were at room or refrigeration temperatures,
346 aerobiosis or anaerobiosis.
347
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351
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424 style green table olives (conservolea variety) supplemented with different carbon
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426 Figure captions

427

428 **Fig. 1.** Survival of four-strain cocktails of *L. monocytogenes* (♦), *E. coli* (○), *S. aureus*
429 (▲), *S. enterica* (■) and LAB (□) in the brine of Spanish-style Manzanilla olives (MS2)
430 at 4 ºC under aerobic conditions. The regression lines and their R^2 coefficients are also
431 shown.

432

433 **Fig. 2.** Five-log reduction times for the Spanish-style brine of the Manzanilla variety
434 (MS2) inoculated with different cocktails of foodborne pathogens under aerobic and
435 anaerobic conditions, and kept at refrigeration (4 ºC) or room (23 ºC) temperatures.
436 Error bars mean the standard error of the estimated 5-log reduction time.

Highlights

- Survival of bacterial pathogens in industrial table olive brines was assessed.
- Death rate varied according to table olive production method.
- Low temperature and anaerobiosis lengthened survival of all pathogens.
- Survival was related to the concentration of phenolic compounds in the brines.

Table 1

Chemical characteristics of the table olive brines. HB, MO, and KB brines are not alkali-treated.

Brine[a]	NaCl (%)	pH	Free acidity (%)[b]	Combined acidity (eq/L)
HB1	2.6	4.0	1.2	0.06
HB2	2.8	3.9	1.2	0.06
MO1	5.7	3.6	1.1	0.05
MO2	6.4	4.0	0.6	0.05
KB1	7.8	3.5	0.9	0.04
KB2	7.8	3.6	0.8	0.04
MS1	6.3	3.9	0.9	0.11
MS2	6.4	4.2	0.7	0.12
GS1	5.8	3.8	1.5	0.14
GS2	5.4	3.7	1.4	0.13
HSP	6.2	3.9	0.8	0.11
MSP	8.6	3.9	0.6	0.08

[a] HB, Hojiblanca olives in acidified brine; MO, Manzanilla organic olives in vinegar acidified brine; KB, Kalamata olives in brine; MS, Spanish-style Manzanilla olives; GS, Spanish-style Gordal olives; HSP, Spanish-style Hojiblanca olives stuffed with pimento; MSP, Spanish-style Manzanilla olives stuffed with pimento.

[b]Expressed as lactic acid.

Table 2

Concentration (mM) of phenolic compounds in the table olive brines. HB, MO, and KB brines are not alkali-treated.

Brine	Hydroxytyrosol	Hy 1-glucoside	Hy 4-glucoside	Salidroside	Tyrosol	EDA	HyEDA
HB1	6.3 (0.2)[a]	1.6 (0.1)	3.2 (0.1)	0.4 (0.1)	0.4 (0.1)	1.1 (0.1)	0.4 (0.1)
HB2	6.5 (0.1)	1.5 (0.1)	2.9 (0.1)	0.6 (0.0)	0.6 (0.1)	1.2 (0.1)	0.8 (0.1)
MO1	8.7 (0.1)	0.3 (0.1)	1.5 (0.1)	1.1 (0.1)	nd	nd	nd
MO2	11.0 (0.2)	1.1 (0.1)	1.7 (0.1)	1.2 (0.0)	0.2 (0.1)	1.2 (0.1)	0.5 (0.1)
KB1	9.6 (1.4)	nd[b]	nd	2.2 (0.1)	nd	nd	nd
KB2	6.1 (0.3)	nd	nd	1.6 (0.1)	nd	nd	nd
MS1	9.7 (0.4)	nd	nd	nd	1.0 (0.5)	nd	nd
MS2	11.1 (0.1)	0.5 (0.1)	0.3 (0.1)	nd	1.1 (0.1)	nd	nd
GS1	9.0 (0.1)	nd	nd	nd	0.6 (0.1)	nd	nd
GS2	9.4 (0.1)	nd	nd	nd	1.2 (0.1)	nd	nd
HSP	11.1 (0.2)	nd	nd	nd	1.0 (0.1)	nd	nd
MSP	8.3 (0.1)	nd	nd	nd	1.0 (0.1)	nd	nd

[a] Standard deviation of duplicates. [b] Not detected. Abbreviations: Hy: Hydroxytyrosol. EDA: Dialdehydic form of decarboxymethyl elenolic acid. HyEDA: EDA linked to hydroxytyrosol. For the full meaning of the brine abbreviations see Table 1.

Table 3

Five-log reduction times for the different bacterial cocktails inoculated in the distintc table olive brines and kept at 4ºC under aerobic conditions.

Brine[a]	*L. monocytogenes*		*S. enterica*		*S. aureus*		*E. coli*		LAB	
	Hours	SE[b]	Hours	SE	Hours	SE	Hours	SE	Hours	SE
HB1	<0.1	-	<0.1	-	<0.1	-	<0.1	-	0.2	0.1
HB2	<0.1	-	<0.1	-	<0.1	-	<0.1	-	<0.1	-
MO1	5.8	0.3	14.2	0.4	27.4	0.2	9.3	0.7	78.0	0.3
MO2	<0.1	-	<0.1	-	<0.1	-	<0.1	-	<0.1	-
KB1	28.1	0.2	16.1	0.2	34.2	0.1	32.4	0.2	295.4	0.4
KB2	7.8	0.4	27.7	0.3	25.6	0.2	18.9	0.5	299.4	0.4
MS1	20.5	0.1	32.5	0.1	52.8	0.4	21.0	0.1	283.3	0.3
MS2	66.2	0.5	160.4	0.3	148.3	0.4	125.7	0.5	298.1	0.2
GS1	3.5	0.1	12.4	0.2	48.6	0.2	38.6	0.6	209.4	0.3
GS2	7.5	1.1	8.4	0.4	48.9	0.3	46.2	0.4	215.1	0.3
HSP	20.9	0.2	77.8	0.3	67.8	0.5	39.4	0.4	301.6	0.3
MSP	23.5	0.2	68.7	0.3	57.2	0.2	43.0	0.4	296.2	0.3
Acetic control	183.8	1.0	141.2	0.4	198.4	0.6	326.9	0.5	312.6	0.5
Lactic control	213.5	0.8	283.5	0.5	308.0	0.3	259.4	0.5	297.6	0.3

[a] For the full meaning of the brine abbreviations see Table 1. [b]SE represents the standard error of the estimated 5 log reduction time.

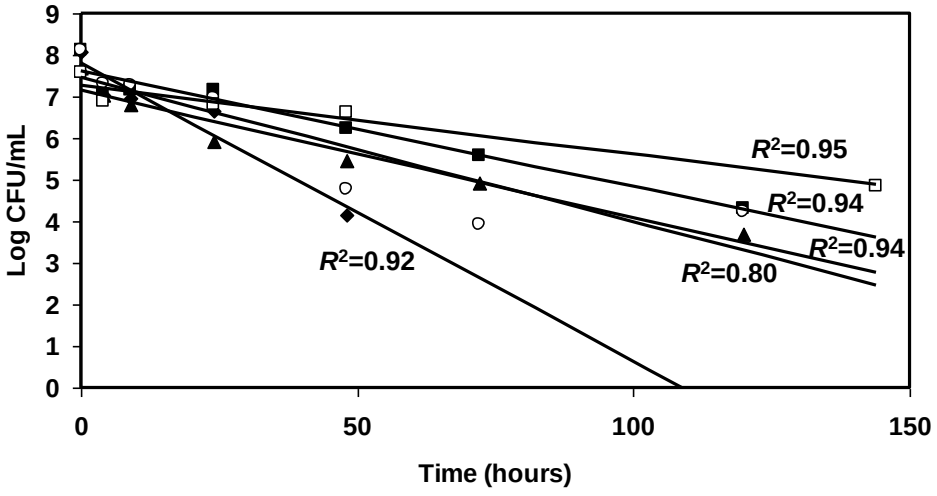
Table 4

Five-log reduction times for the different bacterial cocktails inoculated in the less harmful brine of each table olive type and kept at room temperature (23 ± 2ºC) under aerobic conditions.

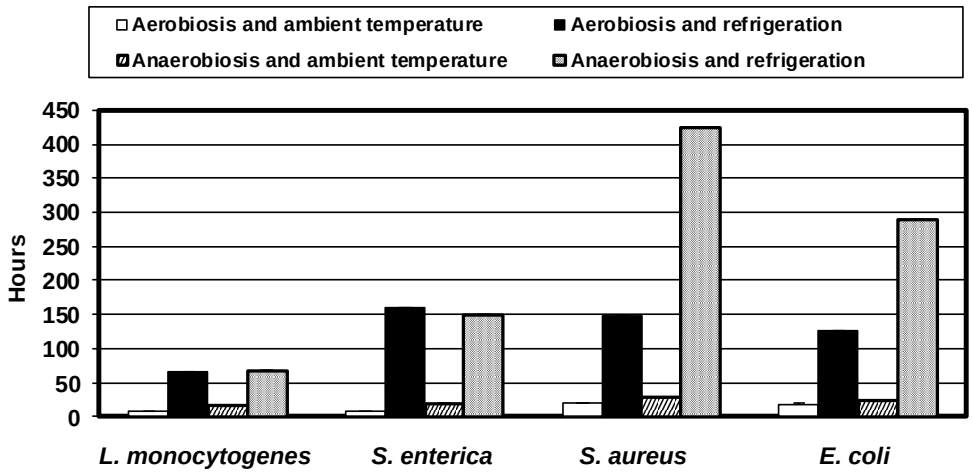
	L. monocytogenes		*S. enterica*		*S. aureus*		*E. coli*		LAB	
Brine[a]	Hours	SE[b]	Hours	SE	Hours	SE	Hours	SE	Hours	SE
HB1	<0.1	-	<0.1	-	<0.1	-	<0.1	-	<0.1	-
MO1	1.1	0.1	1.1	0.3	1.6	0.3	1.1	0.5	3.9	0.6
KB1	6.3	1.0	3.9	0.5	25.7	0.7	9.8	0.7	76.0	0.5
MS2	7.2	0.9	7.6	0.6	20.0	0.8	18.7	0.5	203.0	0.8
GS2	1.0	0.5	1.1	0.4	21.7	0.7	2.2	0.7	42.4	0.7

[a] For the full meaning of the brine abbreviations see Table 1. [b] SE represents the standard error of the estimated 5 log reduction time.

Figure


Log CFU/mL
Time (hours)
0
1
2
3
4
5
6
7
8
9
0
50
100
150
R²=0.95
R²=0.94
R²=0.94
R²=0.80
R²=0.92

Figure


Aerobiosis and ambient temperature
Aerobiosis and refrigeration
Anaerobiosis and ambient temperature
Anaerobiosis and refrigeration
Hours
450
400
350
300
250
200
150
100
50
0
L. monocytogenes
S. enterica
S. aureus
E. coli