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Effect of potassium sorbate and sodium benzoate on microbial population and fermentation of black olives

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Abstract: Black olive fermentation characteristics and diffusion of preservatives into olives were evaluated in brines containing 500 ppm potassium sorbate, 1000 ppm sodium benzoate, 500 ppm sodium benzoate + 250 ppm potassium sorbate and no preservative (control). Changes in brine pH, acidity and microbial population (lactic acid bacteria, yeasts, moulds) were followed during fermentation and storage. Results indicated that K-sorbate when used at 500 ppm concentration in black olive fermentation had a slight stimulatory effect on the growth of lactic acid bacteria. The yeast counts of brines containing 500 ppm K-sorbate and 1000 ppm Na-benzoate were lower than for the brine containing 250 ppm K-sorbate + 500 ppm Na-benzoate and for the control with no preservative, while mould growth was completely inhibited in all treatments during fermentation. Mould counts stayed below the detection limit (<10 cfu g^{-1}) during 214 days of vacuum-packaged storage. Yeast counts showed a progressive decline within 28 days of storage and then stayed relatively constant in all treatments thereafter. The level of yeast population was higher in the control sample than in the sample containing both preservatives during storage. The diffusion of Na-benzoate and K-sorbate into the olives after equilibrium was 64% and 80% during fermentation respectively.

Keywords: potassium sorbate; sodium benzoate; black olives; lactic acid fermentation

INTRODUCTION

Fermentation is one of the most important methods of food preservation as it imparts certain desired organoleptic qualities to products, while providing a means for extending the processing season for fruits and vegetables and requiring comparatively little mechanical energy input.[1] The most important pickled products are cucumber, cabbage (sauerkraut) and olive, which is a fruit of considerable economic importance especially for the Mediterranean countries.

The production of pickled olives consists of selecting suitable varieties, harvesting, grading, treating with lye, washing with water, brining and packaging. The normal fermentation flora of olives comprise lactic acid bacteria and oxidative yeasts. The lactic acid bacteria which take part during fermentation of olives and many other vegetables are *Enterococcus faecalis, Leuconostoc mesenteroides. Lactobacillus brevis. Pediococcus damnosus* and *Lactobacillus plantarum*.[2] Despite the use of considerable care in each of the processing steps, pickled olives may suffer from a number of spoilage problems caused by bacteria, yeasts and moulds. These microbial spoilage problems include gassy spoilage by mainly coliform bacteria, butyric acid fermentation by *Clostridium* spp, scum formation by yeasts and tissue softening by pectinolytic microorganisms.[2]

Benzoic acid is especially suitable for inhibiting yeasts and moulds, but it is less effective against bacteria. Sorbic acid is a potent inhibitor of the growth of a wide variety of yeasts, moulds and bacteria, with less effect on lactic acid bacteria. Because of their high solubility in water, sodium salts of benzoic acid and potassium salts of sorbic acid are used in the pickle industry. The effectiveness of sorbates and benzoates increases with increased acidity, but sorbic acid is more effective than benzoic acid in the weak acid pH range. Sorbic acid has a lower order of toxicity than benzoic acid. This is probably because of its similar metabolism to fatty acids in the human body and animals.[3–5] The maximum approved amounts of potassium sorbate and sodium benzoate in table olives are 1000 and 500 mg kg^{-1} respectively.[6]

Many studies have been reported on the antimicrobial activities and uses of benzoates and sorbates.

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Antimicrobial activity and food applications of sorbic acid and sorbates were reviewed by Sofos and Busta[7] and Lück.[4] Manganelli and Casolari[8] and Warth[9] studied the effects of sorbic acid and benzoic acid on the growth and survival of some yeast strains. Eklund[10] determined the minimum inhibitory concentrations of sorbic acid for some bacteria. Liewen and Marth[11] studied environmental factors affecting the antimicrobial effectiveness of sorbate in foods, and Sofos *et al*[12] reviewed the mode of action of sorbic acid on bacterial cells and spores. Banks *et al*[13] investigated the growth of food poisoning and spoilage organisms in the presence of sodium benzoate and potassium sorbate. Göksungur *et al*[5] studied the effect of sodium benzoate and potassium sorbate on the fermentation of cucumber pickles.

There has been no detailed research focused on the use of these preservatives in pickled table olives. Therefore the objective of this study was to investigate the effect of Na-benzoate and K-sorbate on the fermentation parameters and microbial population during the fermentation of black olives. The diffusion of these preservatives into the olives was also estimated.

MATERIALS AND METHODS

Material

Black olives (var Memecik) were obtained from a local brinery a few hours after delivery to the plant. They were commercially washed, sorted for size (320–360 olives kg^{-1}) and microbial or mechanical damage and placed into experimental brine solution within 12 h.

Methods

Brine preparation, fermentation and storage

Four different brine solutions containing 500 ppm potassium sorbate (Merck 5119), 1000 ppm sodium benzoate (Merck 6290), 500 ppm sodium benzoate + 250 ppm potassium sorbate and no preservative (control) were prepared with three replicates. Olives and brine were added to 2 kg standard glass jars in a ratio of 60:40 (w/w) for fermentation in each condition. During fermentation the NaCl concentration was determined and maintained at the initial concentration of 8% (w/v) by regularly adding salt to the brine at the rate absorbed by the olives. Fermentation was performed in glass jars incubated at 20 °C in the dark for 140 days. After fermentation the olives were vacuum packed in 250 g, 160 μm thick commercial packages produced from Coex material (polyamide + polyethylene + ionomer), using a Multivac A 300/16 tabletop model vacuum-packaging machine under a partial vacuum of 10^5 Pa, and stored in the dark at 20 °C for another 7 months. Samples were taken for analysis at defined intervals during fermentation and storage.

Chemical analysis

The pH of brines was measured using an NEL Model 821 pH meter with an accuracy of 0.01 units. The acidity of brines was determined by titrating with standardised NaOH solution using phenolphthalein as indicator.[14] The acid content of the brine was calculated as % lactic acid. The salt content of the brine was determined by titrating with standardised $AgNO_3$ (Merck 1512) solution using K_2CrO_4 (Merck 4952) as indicator.[14] Sodium benzoate and potassium sorbate in the brine and in the flesh of olives were determined by a spectrophotometric method.[14]

Microbiological analysis

During fermentation, 1 ml of brine sample was mixed with 9 ml of sterile peptone water (1 g l^{-1}), and a series of decimal dilutions were prepared. During the storage period, 15 olives from each of the three vacuum-packed samples were picked and aseptically destoned. Then the triplicate flesh samples were mixed and 10 g of subsample was homogenised for 1 min in a stomacher (Seward Medical, Model 400) with 90 ml of sterile peptone water (1 g l^{-1}), and a series of decimal dilutions were prepared. Enumeration of lactic acid bacteria was performed on Man Rogosa Sharp Agar (Oxoid, CM 361) by double-layer plating in duplicate series of appropriate dilutions and incubating at 30 °C for 3 days. For the enumeration of yeasts and moulds, Potato Dextrose Agar (Oxoid, CM 139) was employed and duplicate plates of appropriate dilutions were incubated at 25 °C for 5 days. All analyses were carried out on triplicate samples, and the results are presented as the average of triplicate measurements.

Statistical analysis

A two-way analysis of variance was carried out on the data to determine the significance of individual differences. Here $p < 0.05$ was used as the criterion for statistical significance, and analyses were conducted using the Tarist commercial statistical package.

RESULTS AND DISCUSSION

Effect of potassium sorbate and sodium benzoate on fermentation

The development of brine acidity and decrease in pH during fermentation are shown in Fig 1. As seen in Fig 1a, the acid production rate was high during the first 90 days of fermentation, and no increase in acidity was observed after 120 days. The mean acidity and pH values corresponding to the treatments are presented in Table 1. As seen in Table 1, the acidity of the brine containing 500 ppm potassium sorbate was significantly higher than for the other samples ($p < 0.05$), while no significant differences were observed in the acidity of brines containing 1000 ppm sodium benzoate and 500 ppm sodium benzoate + 250 ppm potassium sorbate and of the control. The titratable acidity of the brine containing 500 ppm K-sorbate increased from 0% to 1.12%. The acidity of the other samples

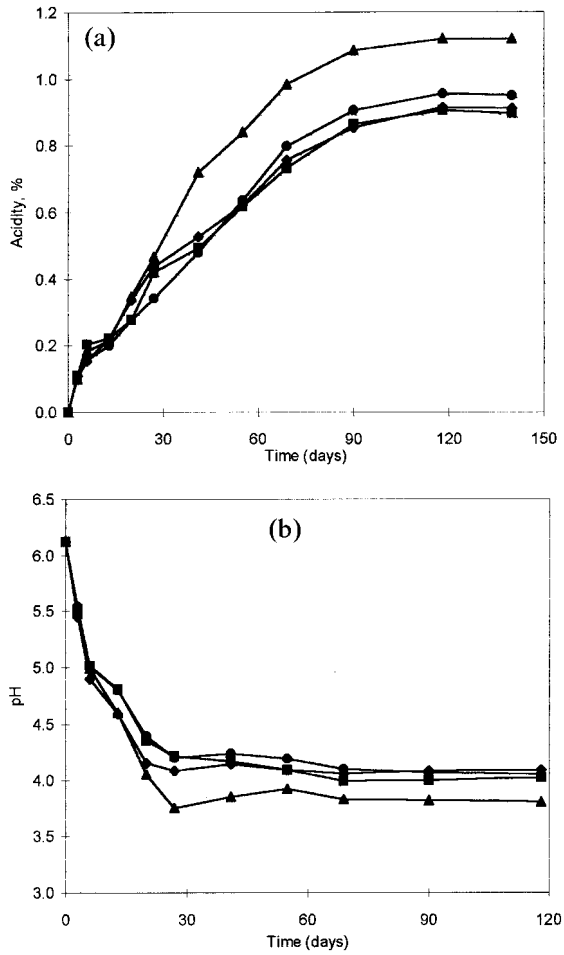

Figure 1. (a) Development of titratable acidity and (b) decrease in pH during fermentation in brines containing (◆) no preservative (control), (■) 1000 ppm Na-benzoate, (▲) 500 ppm K-sorbate, (●) 500 ppm Na-benzoate + 250 ppm K-sorbate.

reached a level of 0.90–0.95% at the end of fermentation.

The pH of all brines decreased from an initial value of 6.2–6.3 to 3.7–4.2 within the first 4 weeks of fermentation owing to the production of various acids by lactic acid bacteria as the end-products of their metabolism. As seen in Table 1, the brine with 500 ppm K-sorbate had a significantly lower pH value than the other samples ($p < 0.05$).

Table 1. Mean acidity and pH values corresponding to various treatments after 120 days of fermentation: A, no preservative (control); B, 1000 ppm Na-benzoate; C, 500 ppm K-sorbate; D, 500 ppm Na-benzoate + 250 ppm K-sorbate

Treatment	*Mean acidity*[a]	*Mean pH*[b]
A	0.493	4.565
B	0.485	4.629
C	0.606*	4.442*
D	0.485	4.665

[a] Sem (standard error of the mean) = 0.0055.
[b] Sem = 0.0129.
* $p < 0.05$.

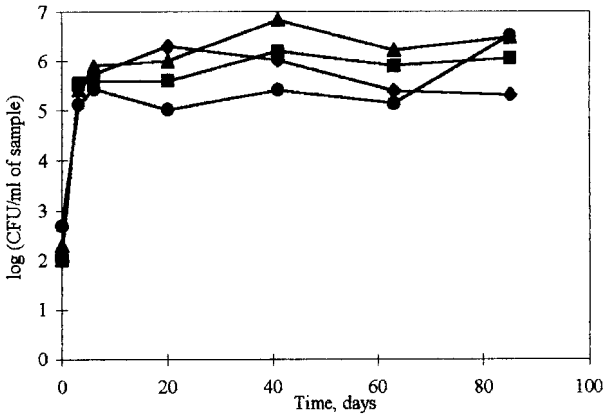

Figure 2. Effect of preservatives on lactic acid bacterial growth during fermentation in brines containing (◆) no preservative (control), (■) 1000 ppm Na-benzoate, (▲) 500 ppm K-sorbate, (●) 500 ppm Na-benzoate + 250 ppm K-sorbate.

These results clearly indicate that Na-benzoate and K-sorbate which are used as preservatives in the fermentation of olives did not have any adverse effect on fermentation. In fact, the development of acidity and decrease in pH in the brine containing only K-sorbate were higher than for the control sample, the sample with only Na-benzoate and the sample containing both preservatives. Hence it was concluded that K-sorbate enhanced the acid production during fermentation of olives.

Effect of potassium sorbate and sodium benzoate on microbial population

The microbiological status (lactic acid bacteria, yeast and mould counts) of brines and olive samples was determined during fermentation and storage periods. Lactic acid bacteria (LAB) grew rapidly and produced a vigorous fermentation in all treatments, including the control sample with no sorbate or benzoate (Fig 2). The lactic acid bacteria increased from an initial population of 10^2–10^3 cfu ml^{-1} to a level of approximately 10^5–10^6 cfu ml^{-1} within 1 week of fermentation, and no significant increase in the LAB count was observed thereafter. K-sorbate and Na-benzoate did not seem to have any inhibitory effect on the growth of LAB. The brine sample containing 500 ppm K-sorbate exhibited higher LAB counts than the other samples during fermentation. This accords with the development of acidity and decrease in pH during fermentation as shown in Fig 1. These results show that K-sorbate when used at 500 ppm concentration in black olive fermentation seemed to have a slight stimulatory effect on the growth of LAB.

There are conflicting reports in the literature on the effect of sorbate on LAB. These differences, of course, emerge from the concentrations and test media used and the micro-organisms tested. Some reports suggest that LAB generally tolerate sorbate and that sorbate exerts a selective inhibition against all catalase-positive micro-organisms and can be used as a selective agent for catalase-negative LAB.[11] Hamdan *et al*,[15] however,

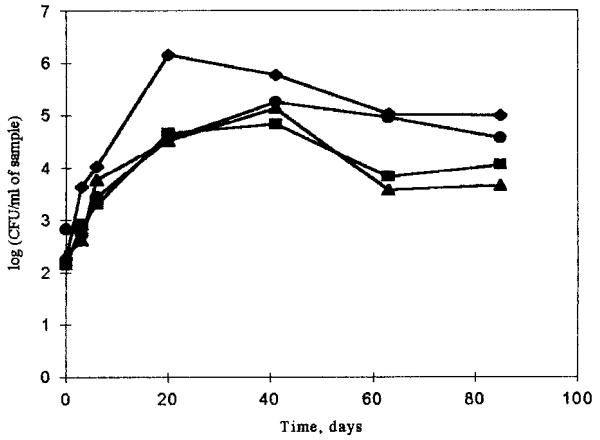

Figure 3. Effect of preservatives on yeast growth during fermentation in brines containing (◆) no preservative (control), (■) 1000 ppm Na-benzoate, (▲) 500 ppm K-sorbate, (●) 500 ppm Na-benzoate + 250 ppm K-sorbate.

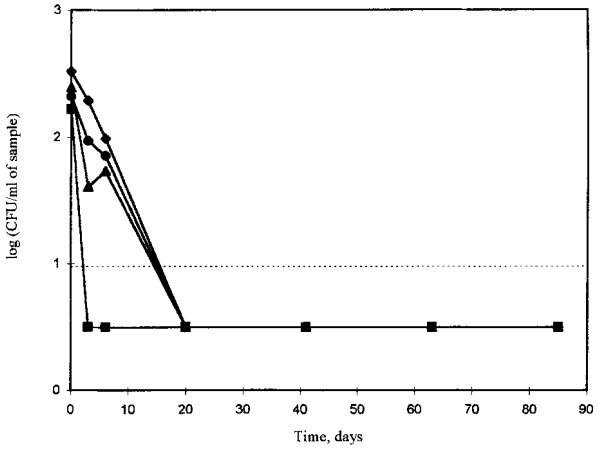

Figure 4. Effect of preservatives on mould growth during fermentation in brines containing (◆) no preservative (control), (■) 1000 ppm Na-benzoate, (▲) 500 ppm K-sorbate, (●) 500 ppm Na-benzoate + 250 ppm K-sorbate (points below the dotted line are $<10\,\text{cfu ml}^{-1}$).

reported that catalase-negative yoghurt starter bacteria were inhibited by 1000 ppm sorbic acid. According to Lück,[4] sorbate concentrations of 1000–2000 ppm slightly inhibit the desired lactic acid fermentation in fermented vegetables. Our results clearly indicate that no consistent and significant inhibitory action of benzoate and sorbate on LAB is observed when brines containing 500 ppm K-sorbate, 1000 ppm Na-benzoate and 500 ppm Na-benzoate + 250 ppm K-sorbate are compared with the control sample.

Results of yeast enumeration indicated that yeasts grew rapidly, and from an initial population of approximately $10^2\,\text{cfu ml}^{-1}$ reached a level of $10^6\,\text{cfu ml}^{-1}$ in the brine containing no preservative (Fig 3). The yeast growth rate was high during the first 20 days of fermentation, and a continuous decrease in yeast count was observed in the control brine sample after 20 days, as seen in Fig 3. The yeast counts of brines containing 1000 ppm Na-benzoate and 500 ppm K-sorbate were lower than for the other samples. These results are in support of the findings of Sofos and Busta,[7] Lück[4] and Wind and Restaino.[16] The mixture of 500 ppm Na-benzoate and 250 ppm K-sorbate moderately suppressed yeast growth in brine. It is well known that the antimicrobial effect of sorbate and benzoate is enhanced by the presence of organic acids such as lactic, citric and acetic acid.[17] The results obtained in this study show that the inhibitory action of sorbate and benzoate on yeast growth increased as the acidity increased during fermentation (Fig 1). Accordingly, a decreasing trend in the viable yeast count was observed in control and sorbate- and/or benzoate-containing brines after 20 days of fermentation respectively (Fig 3). These results are in good agreement with the findings of Costilow *et al* (cited by Naewbanij *et al*[18]), who demonstrated that the addition of sorbic acid (100–1000 ppm) to the brine inhibited yeast activity during cucumber fermentation. Similar findings were also reported by others.[16,18–21] Guillou *et al*,[22] (1992), however, determined that 2000 ppm K-sorbate in brines containing no NaCl and $CaCl_2$ was not sufficient to inhibit yeast growth in natural cucumber fermentation, and they concluded that there was a synergistic action between NaCl, $CaCl_2$ and K-sorbate which allowed good-quality pickles to be produced when moderate amounts of all three were present in the brine.

The results of mould enumeration of brine samples are presented in Fig 4. As seen in Fig 4, mould growth was completely inhibited in all treatments. Mould counts decreased from an initial population of (1.6–3.3) $\times 10^2\,\text{cfu ml}^{-1}$ to below the detection limit ($<10\,\text{cfu ml}^{-1}$) within 20 days of fermentation in all treatments, including the control sample with no preservative added. Guillou *et al*[22] reported that mould growth was rapid when sorbate and NaCl were absent, delayed when sorbate was present at 200 ppm and very slow at 400 ppm concentration in the absence of NaCl in brine during natural cucumber fermentation. Similar but lower inhibitory activities were also found in brines at NaCl concentrations up to 10% when sorbate was absent, indicating that NaCl alone inhibited the mould growth in brine during fermentation. In the present study the inhibitory effect on mould growth in the absence of sorbate may not only be due to the high salt concentration (8%) but may also arise from the experimental conditions which partially exclude oxygen.

The effect of preservatives on the yeast and mould counts of olives during storage is shown in Fig 5. Mould growth was completely inhibited in vacuum-packaged olives in all treatments, including the control sample, and the counts stayed below the detection limit ($<10\,\text{cfu g}^{-1}$) during 214 days of storage. The yeast counts showed a progressive decline from approximately $10^5\,\text{cfu g}^{-1}$ to a level of $10^3\,\text{cfu g}^{-1}$ within 28 days of storage and then stayed relatively constant at a level of 10^3–$10^4\,\text{cfu g}^{-1}$ in the control sample during the rest of the storage period. Yeast counts were higher in the control sample than in samples containing 1000 ppm Na-benzoate, 500 ppm

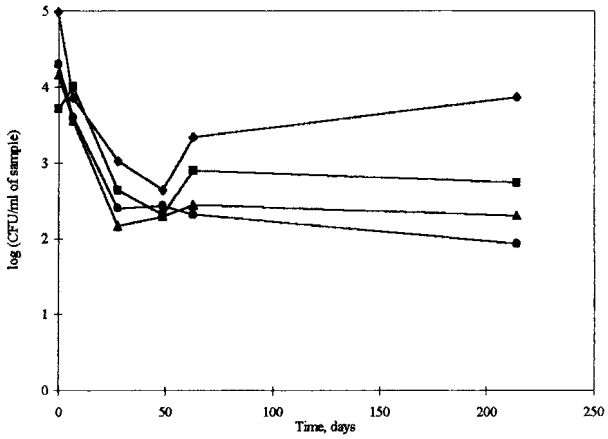

Figure 5. Effect of preservatives on yeast growth during storage in brines containing (◆) no preservative (control), (■) 1000 ppm Na-benzoate, (▲) 500 ppm K-sorbate, (●) 500 ppm Na-benzoate + 250 ppm K-sorbate.

Na-benzoate + 250 ppm K-sorbate and 500 ppm K-sorbate, and the lowest counts were obtained in samples containing 500 ppm K-sorbate and 500 ppm Na-benzoate + 250 ppm K-sorbate, indicating the inhibitory action of sorbate and benzoate on yeast growth during vacuum-packaged storage.

Diffusion of potassium sorbate and sodium benzoate into olives

K-sorbate and Na-benzoate concentrations of brines

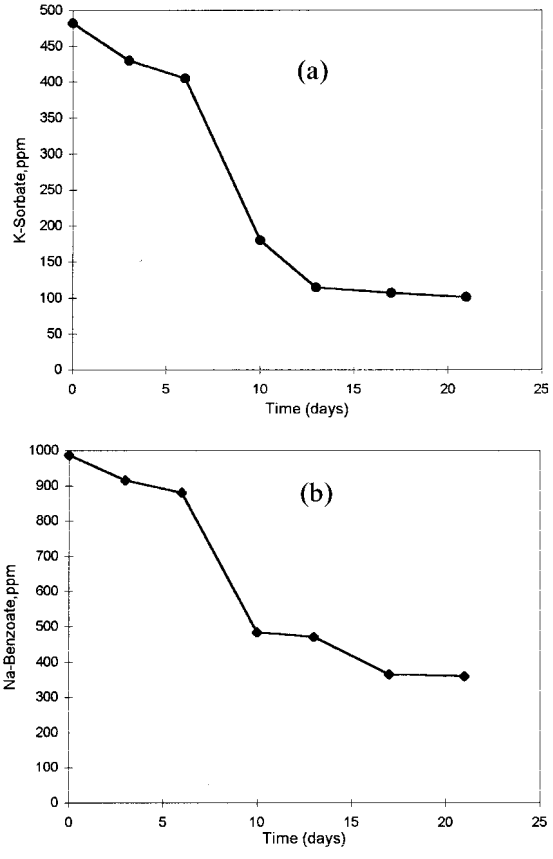

Figure 6. (a) K-sorbate and (b) Na-benzoate concentrations in brine during fermentation.

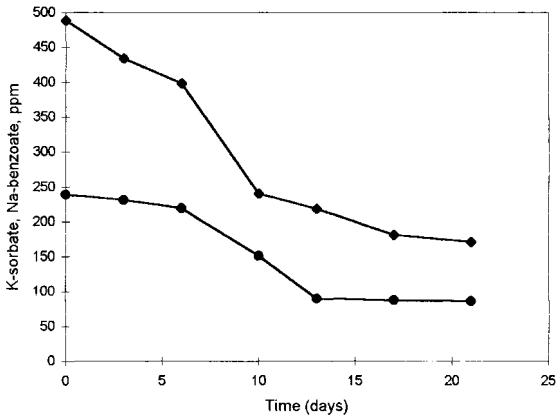

Figure 7. K-sorbate (●) and Na-benzoate (◆) concentrations in brine during fermentation when used together.

were determined during fermentation. Analysis of sorbate and benzoate in the brine and flesh of olives at different times during lactic acid fermentation showed that the concentrations of preservatives in the brine and flesh summed up approximately to the initial concentrations of preservatives added to the brine at the beginning, indicating that sorbate and benzoate in brine were not metabolised by the microbial population present (data not shown). When K-sorbate was used alone at a concentration of 500 ppm, the diffusion was almost over in 13 days, as seen in Fig 6a. There was 101 ppm K-sorbate in the brine and 80% of the preservative had diffused into the olives when equilibrium was reached. When Na-benzoate was used alone at a concentration of 1000 ppm, 64% of the preservative had diffused into the olives at the end of 17 days. The diffusion was higher during the first 10 days, as seen in Fig 6b, and at the end of 17 days there was 359 ppm Na-benzoate in the brine.

When K-sorbate (250 ppm) and Na-benzoate (500 ppm) were used together, the diffusion of preservatives was almost identical, with 65% and 66% of their initial concentrations respectively (Fig 7). The diffusion was rather slow in the first 6 days of fermentation, especially for sorbate, after which it accelerated up to 13 days for sorbate and 10 days for benzoate. The remaining concentrations of sorbate and benzoate in the brines were 86 and 171 ppm respectively.

CONCLUSION

In many countries, sodium benzoate and potassium sorbate can be used in various food products, including pickles and soft drinks, up to 0.1%. As a result of the findings of this study, use of these preservatives in brined olives has the advantage of inhibiting yeast growth during fermentation and storage periods without adverse effects on fermentation. Besides, potassium sorbate enhances lactic acid fermentation as determined by both lactic acid bacteria counts and increase in acidity in the brines. Normally, higher salt concentrations (10% or 12%)

have been used in brined olive fermentation by the industry to inhibit microbial spoilage. By the use of K-sorbate or Na-benzoate, rather lower salt concentrations, eg 8%, can be used without significant microbial spoilage. Further studies will focus on the equilibrium and dynamic behaviour of the system by determining partition coefficients of sorbate and benzoate between tissue and brine and by generating adsorption isotherms and kinetics.

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