

## Research Note

# Evaluating the Effects of Zinc Chloride as a Preservative in Cracked Table Olive Packing

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### ABSTRACT

This survey studies the influence of different zinc chloride concentrations (0.050, 0.075, and 0.100%, wt/vol) on the shelf life of “Aceituna Aloreña de Málaga” table olives. The *Enterobacteriaceae* population significantly ( $P \leq 0.05$ ) decreased in treatments containing 0.050 and 0.100%  $ZnCl_2$ , and those with 0.075%  $ZnCl_2$  had also lower average counts than those observed under the usual packaging conditions (0.12% potassium sorbate). Lactic acid bacteria increased for treatments with 0.050 and 0.075%  $ZnCl_2$ , but in the presence of 0.100% they practically disappeared at the end of the shelf life period (~3 months). With respect to yeasts, populations of these microorganisms significantly decreased with the first two concentrations (0.050 and 0.075%) but showed a slight increase in the presence of 0.100% of  $ZnCl_2$ , although remaining markedly below populations observed with potassium sorbate packing. The use of this chloride salt also led to products with higher concentrations of sugars in brine because of its selective microbial inhibition. Finally, olives treated with 0.075%  $ZnCl_2$  showed an improved sensory profile.

Table olives have a great tradition in the countries around the Mediterranean basin. Throughout the last century, some types (such as green Spanish-style, ripe California-style, or naturally black Greek-style) reached large-scale production thanks to the introduction of new technologies and machinery. Table olives are the main fermented vegetable of the food industry, with approximately 2,200,000 tons/year (10). However, numerous local traditions have survived in specific regions. This is the case of “Aceituna Aloreña de Málaga,” which is a typical seasoned presentation from Valle del Guadalhorce (Málaga, Spain). It enjoys the only Protected Denomination of Origin (PDO) for table olives in Spain. The olives are characterized by being cracked and seasoned with garlic, pepper, fennel, and thyme. However, their shelf life is short. Diverse studies have been carried out to detect the causes of the instability of this product (3) and to extend its shelf life (1). Currently, potassium sorbate is the preservative of choice for the stabilization of Aceituna Aloreña de Málaga and other olive packing, although its effect is limited due to decomposition and absorption into the olive fat. Therefore, the screening of new preservatives against yeasts, the main cause of instability in this product (1, 4), is necessary.

The use of zinc salts has been patented for its proven antifungal activity (5). Zinc can be accumulated, mainly under aerobic conditions, in *Saccharomyces cerevisiae* and markedly influence its physiological status (17). Its presence in continuous alcoholic fermentation reduced the size of

flocks, increased tolerance to alcohol and temperature, decreased the production of glycerol, and accumulated in the yeast dry matter (21). The addition of zinc (~4 mg/liter) was convenient for producing maximum alcohol yields with *S. cerevisiae* 251 TP (19).

This mineral nutrient is also used in food technology because it forms green complexes with chlorophyll derivatives, particularly at moderately high temperatures. It has recently been applied to preserving the green color of pears, but the process required the application of heat to stabilize the color of the final product (13). Moreover, zinc salts are currently included in the strategy designed by UNICEF to combat diarrhea in children in developing countries. The doses recommend ranges from 10 to 20 mg of Zn per day (20). The use of zinc acetate, chloride, citrate, gluconate, lactate, oxide, carbonate, and sulfate is authorized in the European Union to fortify foods according to Directive 2002/46/CE of the European Union. In the United States, the same compounds are authorized for the same purpose and are generally recognized as safe (14).

The aim of this work was to assess the use of  $ZnCl_2$  as a preservative agent in Aceituna Aloreña de Málaga packing, as a substitute for potassium sorbate (habitually used by industry). However, results obtained in this matrix could be extended to other local or specific vegetable presentations that cannot be heat treated for stabilization.

### MATERIALS AND METHODS

**Samples and experimental design.** Aloreña fresh olives were used in the present study. Fruits were washed, cracked, and brined (12% NaCl) for 3 days before packing. The olives were

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placed in plastic containers (400 g of olives plus 310 ml of brine) with a 4% mixture of diced garlic, pepper strips, and small pieces of fennel and thyme. Then, four different brines were added to the containers: (i) the same brine habitually used by the industry (control), which contains 0.12% potassium sorbate, 5.0% NaCl, 0.21% citric acid, 0.06% ascorbic acid, and 0.15% lactic acid; (ii) brine with 0.050% (wt/vol) zinc chloride; (iii) brine with 0.075% zinc chloride; and (iv) brine with 0.100% zinc chloride. The last three brines did not incorporate potassium sorbate in their composition. After being completely filled, a total of 24 containers per treatment were closed and maintained at room temperature ( $20 \pm 3^\circ\text{C}$ ) for a period of time similar to that of real shelf life ( $\sim 3$  months). Periodically, two replicate containers were removed and analyzed.

**Microbial counts.** Brine samples and their decimal dilutions were plated with a Spiral System model dwScientific (Don Whitley Scientific Limited, Shipley, England) on the appropriate media. Subsequently, plates were counted, using a CounterMat v.3.10 (IUL, Barcelona, Spain) image analysis system, and results were expressed as log CFU per milliliter. *Enterobacteriaceae* were counted on crystal-violet neutral-red bile glucose agar (Merck, Darmstadt, Germany), lactic acid bacteria (LAB) were counted on de Man Rogosa Sharpe (MRS) agar (Oxoid Ltd., Basingstoke, Hampshire, England) with 0.02% (wt/vol) sodium azide (Sigma, St. Louis, MO), yeasts were counted on yeast-malt-peptone-glucose medium agar (Difco, BD, Sparks, MD) supplemented with oxytetracycline and gentamicin sulfate as selective agents for yeasts, and total viable counts were determined on plate count agar (tryptone-glucose-yeast) (Oxoid). Plates were incubated at  $30^\circ\text{C}$  for 48 to 72 h. Changes in the microbial populations versus time were assessed by estimating the area under the corresponding growth or decline curves. Areas were calculated by integration using OriginPro 7.5 software (OriginLab Corporation, Northampton, MA).

**Microbial identification.** Samples from packaging (100 ml) were collected under sterile conditions at the end of the shelf life period ( $\sim 3$  months) and plated onto the yeast and LAB selective media described above. A total of 80 isolates, 40 LAB and 40 yeasts (10 for each treatment) were randomly selected and purified by subsequent restreaking on yeast-malt-peptone-glucose medium or MRS agar, respectively. The different LAB isolates were identified to species level by multiplex PCR analysis of *recA* with species-specific primers for *Lactobacillus pentosus*, *Lactobacillus plantarum*, and *Lactobacillus paraplantarum*, following the protocol described by Torriani et al. (18). Yeasts were identified by restriction fragment length polymorphism analysis of the 5.8S internal transcribed spacer rDNA region according to the procedure described by Esteve-Zarzoso et al. (7).

**Physicochemical analyses.** The analyses of brines for pH, NaCl concentration, titratable acidity, and combined acidity were carried out using the standard methods previously described for table olives (8). Individual sugar and organic acid contents in brines were determined by using a high-performance liquid chromatography system composed of a Water 2690 Alliance according to the method developed by Montañó et al. (12).

**Sensorial analyses.** The methodology described by Hibbert (9) has been followed in this work. Data to estimate the sensory profile were obtained by quantitative descriptive analysis. Tests were conducted by a panel of 18 members of experienced judges from the staff of Instituto de la Grasa (CSIC, Seville, Spain). The

suitable descriptors to be measured were suggested during previous training sessions (6), and they were color, odor, acidity, saltiness, bitterness, firmness, fibrousness, and crispness. The panelists were asked to score the olives with a 10-cm unstructured scale. The anchor points were 0 and 10, corresponding to the least and most relevant perceptions, respectively (11, 15). The average scores of treatments for each attribute were obtained to estimate their sensory profiles. Treatments were also compared to each other, asking for differences between them but specifying if the response, the same or different, was sure or unsure; thus, each response had four possible answers: same sure, same unsure, different unsure, and different sure. This type of comparison was similar to those proposed for the determination of the *R*-index but was limited to two samples at each time. Panelists were also asked for preference. Centered (attributes) scores (9) were finally subjected to hierarchical clustering analysis based on Euclidean distances. The results permitted the detection of dissimilarities and/or similarities among runs, based on the selected attribute scores.

**Statistical data analyses.** Statistica software version 7.0 (Statsoft Inc., Tulsa, OK) was used for general linear model (GLM) and clustering data processing. GLM to data was applied according to the repeated-measurement option because results at each sampling time cannot be considered independent from previous or subsequent results.

## RESULTS AND DISCUSSION

**Microbial growth.** Total viable counts (Fig. 1a) in fresh seasoned Aceituna Aloreña de Málaga rapidly increased after packing, reaching a maximum at around  $6.5 \log \text{CFU/ml}$ . This population level was maintained with slight oscillations throughout the shelf life. Total viable counts of the control treatment (with potassium sorbate) grew rapidly and at the third sampling time were above values from data corresponding to treatments with  $\text{ZnCl}_2$ , but later on, counts were at intermediate positions due to the effect of the zinc salt on specific groups of microorganisms (Fig. 1a).

The *Enterobacteriaceae* population was present only at the beginning of packing and decreased rapidly and at a faster rate in all treatments containing  $\text{ZnCl}_2$  (Fig. 1b). Later, the populations were similar in all treatments, and a small recovery, which was more marked in the control, was noticed. In any case, the *Enterobacteriaceae* population at packing was low,  $\sim 3.0$  to  $3.5 \log \text{CFU/ml}$ , and soon markedly decreased. After 48 days, this group was not detected.

LAB were present in the product from the very beginning of packing and increased rapidly during the first days to reach a moderate level of  $\sim 4.0$  to  $6.0 \log \text{CFU/ml}$  (Fig. 1c). In the course of the shelf life, there was a tendency for the average population of LAB to increase. Apparently, after 1,000 h, LAB populations in treatments with  $\text{ZnCl}_2$  became greater than those in the control.

Yeasts were particularly affected by the presence of  $\text{ZnCl}_2$  in the packing brine (Fig. 1d). Average counts and maximum population in the control were always above those in treatments containing  $\text{ZnCl}_2$ . Yeast counts at the end of the process were particularly low in the treatment containing 0.075%  $\text{ZnCl}_2$ . Thus, the effect of this concentration of  $\text{ZnCl}_2$  was particularly efficient against

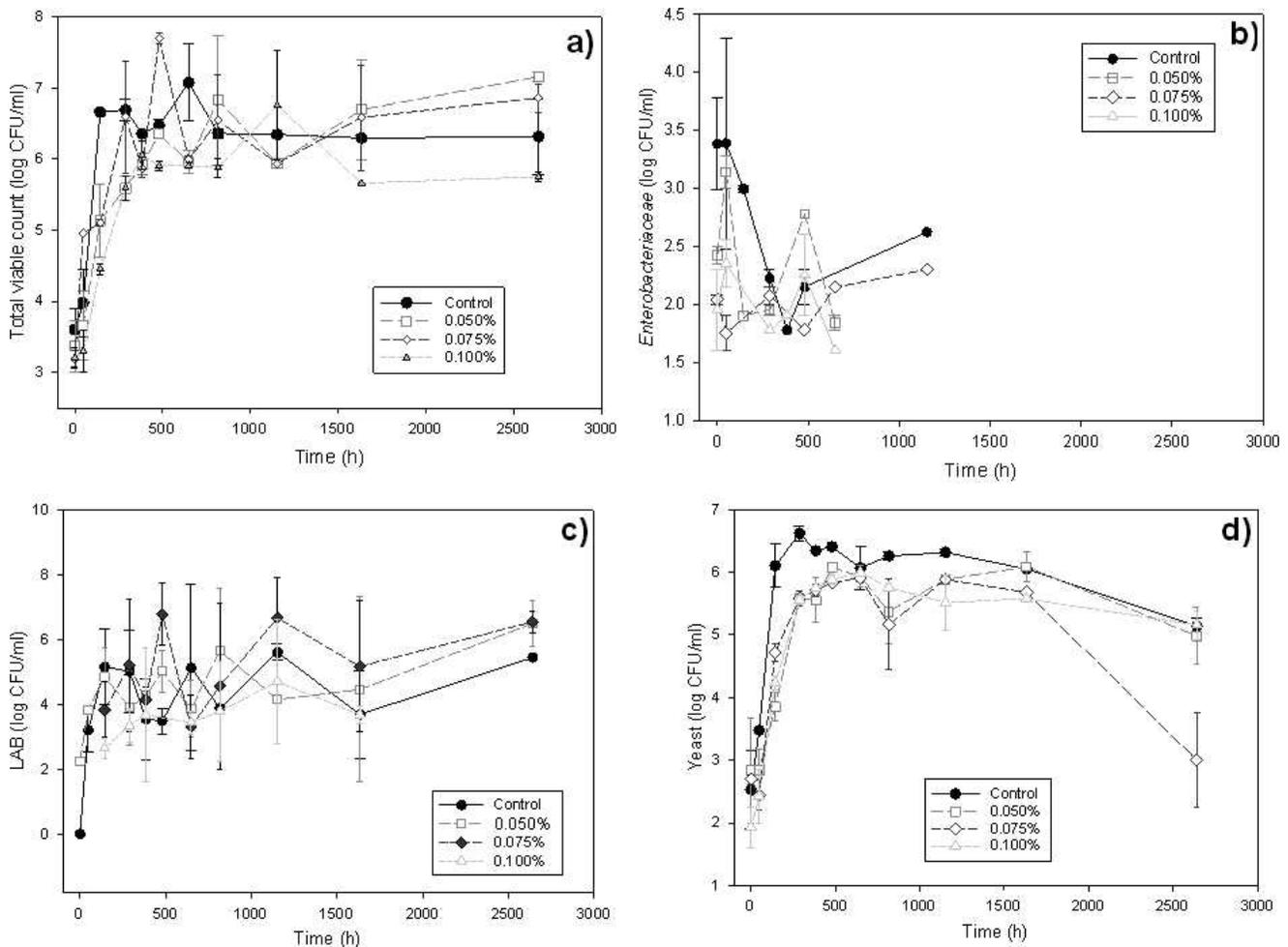


FIGURE 1. Microbial evolution in fresh-packed seasoned cracked Aceituna Aloreña de Málaga olives. Changes in total viable counts (a), Enterobacteriaceae (b), lactic acid bacteria (c), and yeasts (d) versus time according to treatments. Values are unweighted means obtained by GLM considering data as repeated measurements.

yeasts, and in this case, ZnCl<sub>2</sub> had a better inhibitory action than potassium sorbate. Bautista-Gallego et al. (5) have previously noted the inhibitory effects of ZnCl<sub>2</sub> against diverse yeast species isolated from table olives.

Growth curves for the diverse groups of microorganisms were not able to be fit by any primary model (data not shown). Thus, areas under the curves of counts versus time were selected as an approximation to estimate the overall effect of ZnCl<sub>2</sub> on microbial growth (Fig. 2). Total viable count areas increased progressively from the control to 0.075% ZnCl<sub>2</sub> but then sharply decreased when the proportion of ZnCl<sub>2</sub> was 0.100% (Fig. 2a). An explanation of such changes can be found, at least in part, by the behavior of LAB, as will be discussed below.

The presence of ZnCl<sub>2</sub> in the brine decreased the overall *Enterobacteriaceae* population (all the average areas of treatments with ZnCl<sub>2</sub> were below the control) but without significant differences among treatments with different ZnCl<sub>2</sub> concentrations (Fig. 2b). From Figure 2b, a favorable action of ZnCl<sub>2</sub> addition toward decreasing the overall *Enterobacteriaceae* population can be deduced.

The effect of ZnCl<sub>2</sub> on LAB overall population was favorable with respect to the control when the proportions

added were 0.050 and 0.075% ZnCl<sub>2</sub> (Fig. 2c); however, there was a sharp decrease with a ZnCl<sub>2</sub> concentration of 0.100%. The changes in LAB mimic those in total viable counts and show that the increase in total viable counts was due mainly to LAB growth in such treatments. At the end of fermentation, the LAB population in the different treatments was identified. There was no presence of LAB in one of the control replicates or in either of the treatments using 0.100% ZnCl<sub>2</sub> (in agreement with the changes in LAB evolution shown in Fig. 1c). In the other treatments, the species identified were *L. plantarum* and *L. pentosus*. Apparently, there was no relationship among the species identified and the concentration of ZnCl<sub>2</sub> used.

Finally, the effect of addition of ZnCl<sub>2</sub> on yeasts was opposed to that on total viable counts and LAB (Fig. 2d). There was a marked and significant decrease when 0.050 or 0.075% ZnCl<sub>2</sub> was added, but in the case of 0.100% ZnCl<sub>2</sub>, there was an increase with respect to lower proportions, although populations still maintained areas markedly below those observed with packaging using potassium sorbate. Results illustrated in this graph (Fig. 2d) agree with those observed in the growth curve of yeasts (Fig. 1d), in which the more marked decrease at the end of the storage was

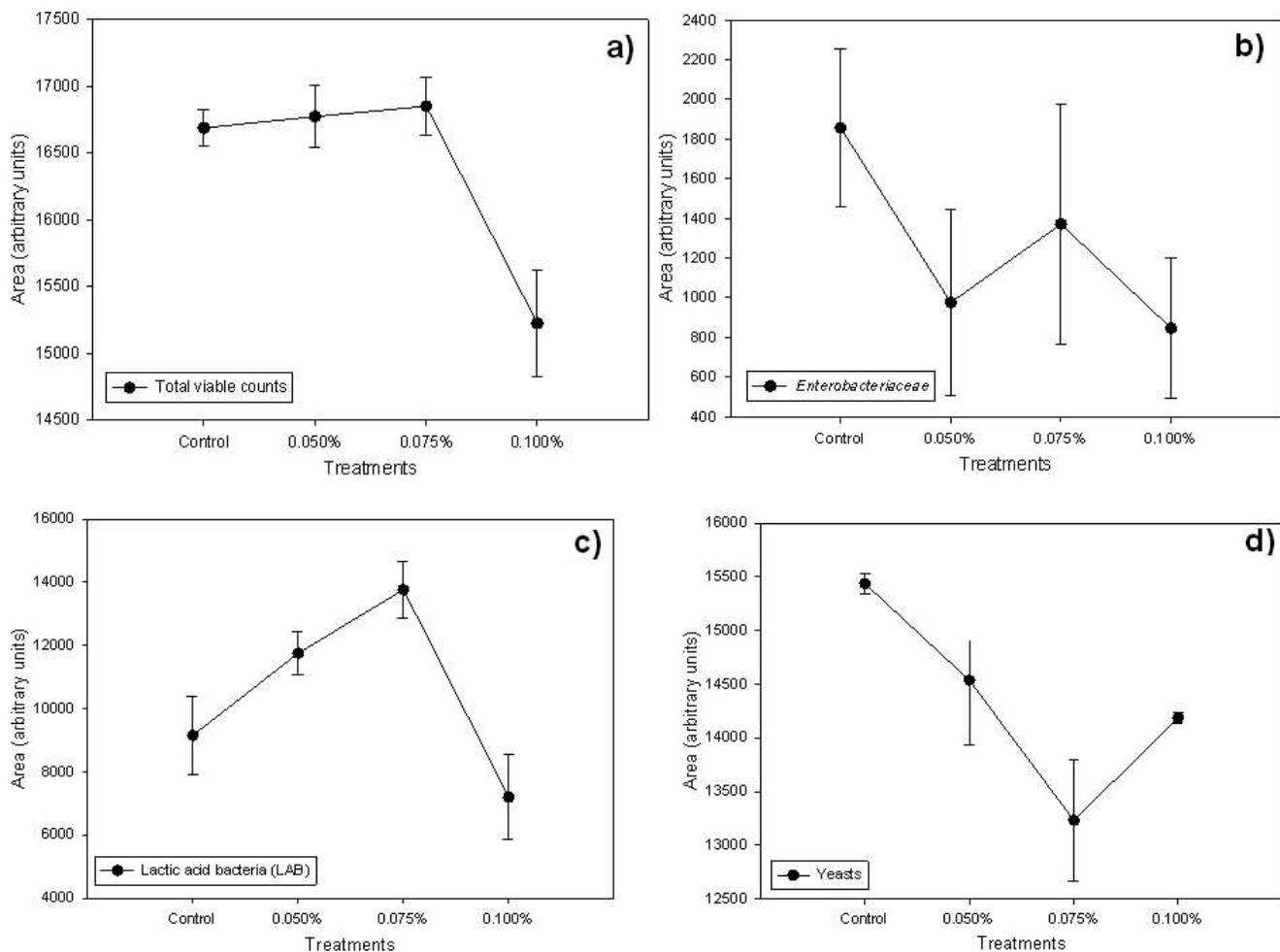


FIGURE 2. Fresh-packed seasoned cracked Aceituna Aloreña de Málaga olives. Areas under curves of total viable counts (a), Enterobacteriaceae (b), lactic acid bacteria (c), and yeasts (d) versus time, according to treatments.

found for 0.075% ZnCl<sub>2</sub>. Yeast identification at the end of the process showed that only one species (100% frequency) was found in all treatments: *S. cerevisiae*. Its presence in treatments containing ZnCl<sub>2</sub> and potassium sorbate means that this species was the most tolerant to both preservatives, among the diverse yeasts able to grow in Aceituna Aloreña de Málaga (1).

As a result, it can be deduced that neither the presence of potassium sorbate nor the use of ZnCl<sub>2</sub> in the proportions stated was able to completely stabilize the fresh-packed Aceituna Aloreña de Malaga because of the observation of LAB growth in one of the controls and in 0.050 and 0.075% ZnCl<sub>2</sub> treatments and yeast growth in all treatments. However, ZnCl<sub>2</sub> had a comparatively greater preservative action than potassium sorbate on yeasts at the levels used in the current manufacturing practices. Nevertheless, the addition of ZnCl<sub>2</sub> instead of potassium sorbate could be advisable because of its apparently higher inhibitory effect on *Enterobacteriaceae* and particularly on yeasts, which are the microorganisms of major concern in this commercial presentation (3).

**Changes in the physicochemical characteristics.** All sugars showed an increase after packing because of their diffusion from fresh fruits, followed by a rapid decrease during the first 500 h in brine and a final marked decline

(Fig. 3). The average contents versus time indicated that the most abundant substrate was glucose, followed by fructose, mannitol, and sucrose (Fig. 3). The only sugar completely exhausted at the end of the shelf life was sucrose, while by that time, there were residues of glucose, fructose, and mannitol. Changes in sugars were related to microbial activity (Fig. 1). Glucose, fructose, and mannitol curves of the control were below those of the treatments containing ZnCl<sub>2</sub>, with only slight differences among the last ones. Only the treatment containing 0.100% showed a progressive moderate increase in mannitol concentration, which persisted with time (Fig. 3d). A similar trend in sugars may be expected in olives packed at industrial scale because the containers used are the same.

There was no marked effect of ZnCl<sub>2</sub> on the initial pH of the brine, which ranged from 3.81 to 4.00. Changes in pH with time were reduced (only 0.2 units) due to the relatively high combined acidity in all treatments (see below). In fact, the pH values at the end of fermentation were very similar and ranged from 4.00 (treatment with 0.050% ZnCl<sub>2</sub>) to 4.06 (control). Thus, it is apparent that the addition of ZnCl<sub>2</sub> to the packing brines does not produce significant modifications to the pH of the products.

In processing Aceituna Aloreña de Málaga olives, there is no lye treatment, and therefore, the combined acidity is

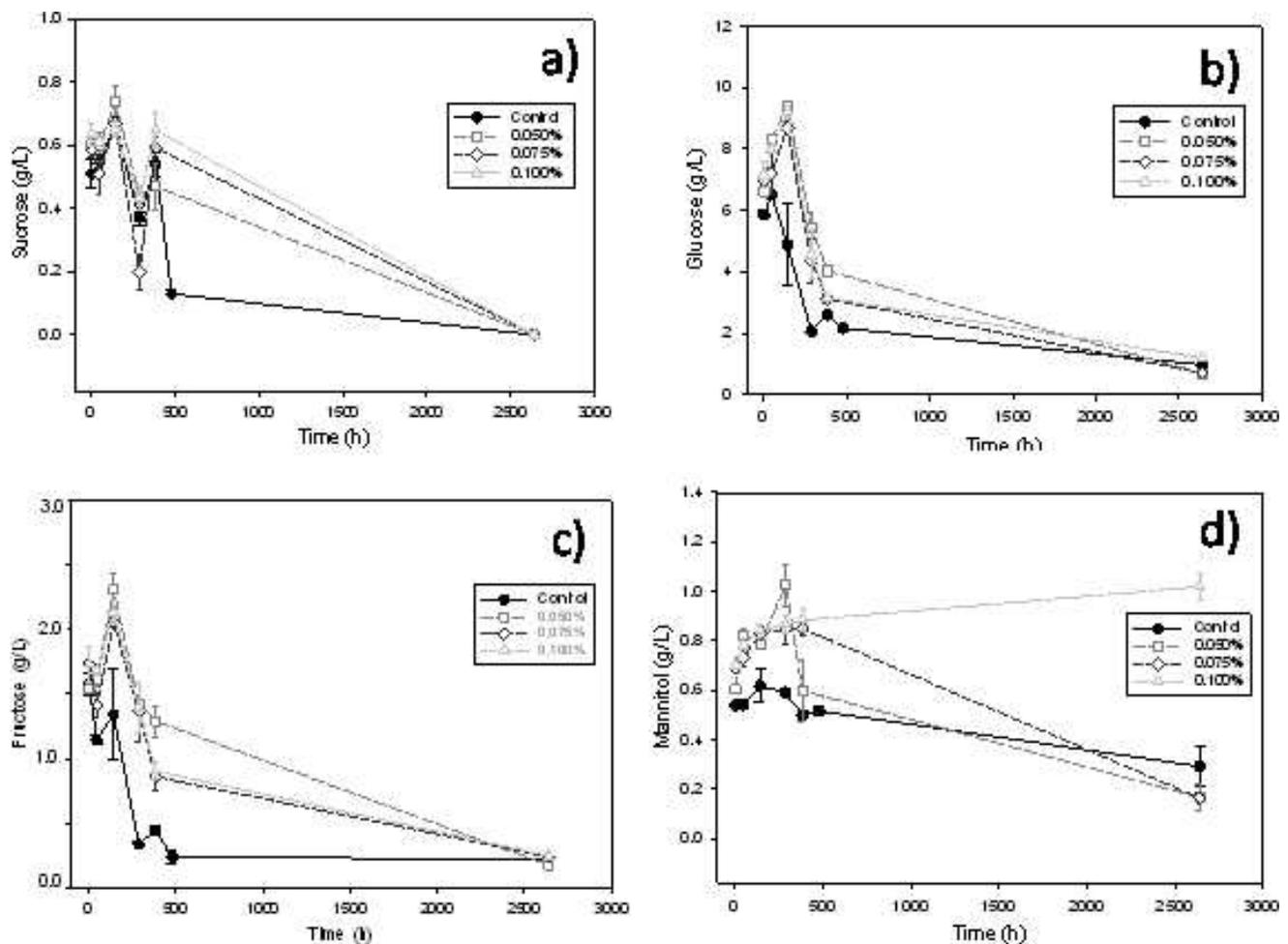


FIGURE 3. Changes in sucrose (a), glucose (b), fructose (c), and mannitol (d) according to treatments in fresh-packed seasoned cracked *Aceituna Aloreña de Málaga* olives. Values are unweighted means obtained by GLM, considering data as repeated measurements.

due only to the presence of organic salts from the fruits themselves. Initially, combined acidity ranged from 45 meq/liter (control) to 52 meq/liter (0.075 and 0.100% ZnCl<sub>2</sub>, respectively), which is a narrow band. At the end of fermentation, the values for this parameter ranged from 60 meq/liter (control and 0.100%) to 66 meq/liter (0.075% ZnCl<sub>2</sub>), while combined acidity in 0.075% ZnCl<sub>2</sub> (0.65 meq/liter) was also fairly close. Hence, the use of ZnCl<sub>2</sub> as a preservative in *Aceituna Aloreña de Málaga* olive packing does not substantially alter the combined acidity values.

Sodium chloride is completely dissociated in table olives (8). The product was prepared from fresh fruits with only 3 days in brine and with only a reduced proportion of salt absorbed into the flesh. Differences between the control and the rest of the treatments with ZnCl<sub>2</sub> were due to the diverse batch origins of both brines. After brining, there was a decrease in NaCl in the brine (~1%) because of the salt penetration into the flesh and the osmotic dehydrating effect of the brine (8). Concentrations in brine equilibrated in a few hours (the contents reached their minimum). Further, the concentrations found showed diverse oscillations, regardless of treatment, followed by a final increase. The trend was similar for all treatments. It is apparent that the higher NaCl concentration in the control (around 5%) could

have had a higher negative effect on microbial growth than in the treatments with ZnCl<sub>2</sub> (around 4.2%), although the effect was just the opposite (Fig. 2). This behavior reinforces the favorable inhibitory effect of ZnCl<sub>2</sub> with respect to treatments with potassium sorbate.

Titrateable acidity was fairly similar in all treatments during the first 500 h (Fig. 4). From that moment, changes could be observed, and particularly after 750 h, the titrateable acidity in treatments with 0.050% and mainly in those with 0.075% ZnCl<sub>2</sub> was above that in the control and in the 0.100% ZnCl<sub>2</sub> treatment. These increases in titrateable acidity were due to the acidity produced by the LAB growth in treatments having lower ZnCl<sub>2</sub> levels. These changes were outstanding in 0.075% treatment because the titrateable acidity at the end of shelf life was about 10.00 g/liter, similar to the level reached at the end of fermentation in a green (lye-treated) table olive fermentation (8). This increase must not be considered an inconvenience because the production of this additional acidity represents an extra contribution to the safety of the product.

The role played by the LAB growth during shelf life could be favorable for improving storage because such activity can promote the production of lactic acid without the formation of gas. In this case, it is probable that the growth of yeasts could be lower (just by competence) and

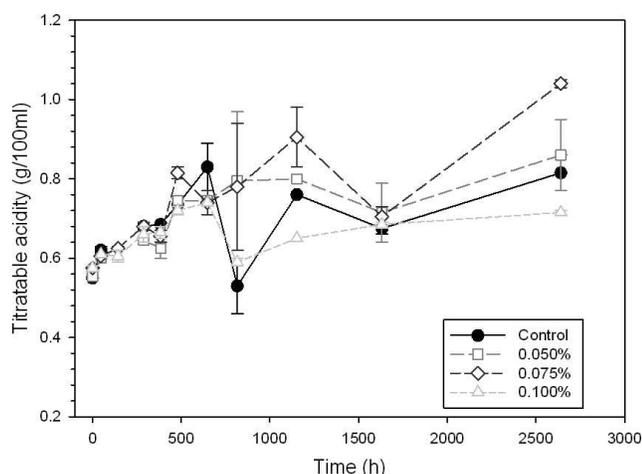


FIGURE 4. Changes in titratable acidity in brines of fresh-packed seasoned cracked *Aceituna Aloreña de Málaga* over shelf life. Values are unweighted means obtained by GLM considering data as repeated measurements.

blown-up containers or brine leakage will occur less frequently or later, with the subsequent prolongation of the product shelf life.

As shown in Table 1, acetic acid was fairly low at the beginning of shelf life and ranged from 0.166 g/liter (0.100% ZnCl<sub>2</sub>) to 0.228 g/liter (0.050% ZnCl<sub>2</sub>). At the end of storage, however, its concentration increased markedly in relative terms but not so much in absolute values as it moved from 0.425 g/liter (control) to 1.460 g/liter (0.075% ZnCl<sub>2</sub>). Lactic acid was intentionally added to the brine used to prepare the fresh seasoned cracked *Aceituna Aloreña de Málaga* olives. Hence, its concentration in the initial brine was fairly uniform and ranged from 1.100 g/liter (0.075% ZnCl<sub>2</sub>) to 1.410 g/liter (control). The concentration of this acid increased during the process, particularly in treatments in which there were marked LAB activities, reaching 4.00 and 8.97 g/liter in 0.050 and 0.075% ZnCl<sub>2</sub> treatments, respectively. In contrast, there was no acidity increment in the treatment with 0.100% ZnCl<sub>2</sub>. The rise in lactic acid (which had a limited effect on pH) cannot be considered in a negative light because its production strengthened the acidity content and decreased the probability of safety risks (8). Citric acid was also added to the initial fresh brine. The

concentration at this moment was fairly similar (Table 1) and ranged from 3.329 g/liter in control to 3.560 g/liter in 0.050% ZnCl<sub>2</sub>. However, at the end of the shelf life, its content in the brine was significantly higher than after packing. Therefore, there was a new citric acid production or solubilization from olive flesh, although this hypothesis is less reliable because the increase was higher in the control and in the treatments with 0.050 and 0.075% ZnCl<sub>2</sub> but lower in 0.100% ZnCl<sub>2</sub>. Ascorbic acid content was also determined because it was added to the fresh packing brine as antioxidant but was found only in the initial samples (24 h) and at low concentrations. This means that the effect of this acid was limited to a very short period of the shelf life. These results question the usefulness of ascorbic acid as antioxidant in this commercial presentation (and possibly in any other table olive product). This is in agreement with results obtained in a previous study in which the effectiveness of ascorbic acid was compared with that of sodium metabisulfite in cracked olives (2) and in which the ascorbic acid showed higher antioxidant activity during the first period of packing but metabisulfite was more efficient in the long term. Antioxidants are also used to mitigate the brown spots (“molestado”) during green olive postharvesting with similar behaviors: metabisulfite prevented the formation of molestado more efficiently than ascorbic acid and extended its effect even after fermentation (16).

**Sensory profile.** The sensory profile was obtained from the average scores of panelists for each descriptor. There were no differences among treatments with respect to the following descriptors: firmness, fibrousness, crispness, and color. With respect to bitterness, the highest score was given to the control and the marks decreased as the proportions of ZnCl<sub>2</sub> increased; the lowest mark was assigned to the olives preserved with 0.100% ZnCl<sub>2</sub>, while scores for 0.050 and 0.075% ZnCl<sub>2</sub> were fairly close (data not shown). Lower differences were observed for saltiness, which showed approximately the same trend as bitterness but with scores within a narrower range. Acidic taste scores were higher in the 0.075% treatment but similar in the other treatments. Great differences were also observed for odor, with the highest score for 0.075% and the lowest for 0.100%; control and 0.050% reached similar scores. Thus, in general, products containing 0.075% ZnCl<sub>2</sub> in the packing brine

TABLE 1. Changes in the organic acid concentrations over the shelf life of *Aceituna Aloreña de Málaga* olives according to treatments<sup>a</sup>

Amt of ZnCl <sub>2</sub> in treatment (%)	Acetic acid		Lactic acid		Citric acid		Ascorbic acid <sup>b</sup>
	Initial	Final	Initial	Final	Initial	Final	Initial
0 (control)	0.170 (0.021)	0.425 (0.283)	1.410 (0.035)	2.218 (0.647)	3.329 (0.249)	5.240 (0.113)	0.477 (0.539)
0.050	0.228 (0.004)	1.457 (0.844)	1.150 (0.057)	4.000 (1.202)	3.560 (0.014)	5.303 (0.088)	0.007 (0.005)
0.075	0.218 (0.004)	1.460 (1.700)	1.100 (0.014)	8.970 (3.394)	3.368 (0.265)	5.133 (0.004)	0.007 (0.007)
0.100	0.166 (0.018)	0.563 (0.105)	1.310 (0.106)	1.258 (0.103)	3.383 (0.180)	4.623 (0.110)	0.050 (0.018)

<sup>a</sup> Values are averages in grams per liter, followed by standard deviations in parentheses.

<sup>b</sup> Ascorbic acid was sporadically detected at the beginning of the shelf life and never at the end of it.

TABLE 2. Descriptive statistics obtained after grouping the treatments into two clusters<sup>a</sup>

Descriptor	Cluster 1 (0, 0.050, and 0.100% ZnCl <sub>2</sub> )		Cluster 2 (0.075% ZnCl <sub>2</sub> )	
	Avg	SD	Avg	SD
Color	4.03	0.13	4.26	0.25
Odor	3.37	0.57	4.63	0.38
Acid	3.83	0.07	4.33	0.52
Saltiness	4.01	0.32	4.22	0.34
Bitterness	4.45	0.67	4.37	0.38
Firmness	5.89	0.12	5.94	0.29
Fibrousness	5.87	0.13	5.98	0.39
Crispness	6.12	0.11	6.12	0.36

<sup>a</sup> Values are sensory scores assigned by panelists. Treatments were grouped by the *k*-means option.

were evaluated as superior with respect to acidic taste, odor, and bitterness (lower score in this case).

The sensory profile scores of treatments were subjected to cluster analysis, using the *k*-means procedure. The analysis was forced to produce only two clusters in order to obtain a further discrimination among treatments. The results observed with raw and centered data led to similar results. The first cluster included the control and 0.050 and 0.100% treatments, while cluster 2 included only 0.075% treatment (Table 2). Therefore, the treatment containing 0.075% ZnCl<sub>2</sub> could be clearly distinguished from the control and treatments containing 0.050% ZnCl<sub>2</sub>. As a result, olives containing 0.075% ZnCl<sub>2</sub> can be considered a different product with higher scores than the other treatments for color, odor, acidic taste, and saltiness (Table 2).

A methodology similar to that applied for the *R*-index estimation, but limiting the comparison to only two treatments each time, showed that the control and 0.050% ZnCl<sub>2</sub> were preferred similarly (50% each) while 0.075% ZnCl<sub>2</sub> was preferred over the control (77.8% of responses versus 22.2%, respectively) but higher proportions of ZnCl<sub>2</sub> reversed the tendency because the control was preferred to 0.100% ZnCl<sub>2</sub> (61.1 versus 38.9% of responses). Preferences between treatments with ZnCl<sub>2</sub> were always in favor of 0.075% ZnCl<sub>2</sub> (61 versus 39% of responses with respect to 0.050% ZnCl<sub>2</sub> and 78 versus 22% of responses with respect to 0.100% ZnCl<sub>2</sub>). The results of these tests were in agreement with the results obtained in the sensory profile and in clustering. Apparently, samples with 0.075% ZnCl<sub>2</sub> added were preferred to any other treatment by the majority of panelists. However, results referring to preference should be treated with care because a greater number of true consumers would be necessary to establish a definitive evaluation.

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