Lactobacillus plantarum survival in aerobic, directly brined olives

M.C. DURAN, P. GARCIA, M. BRENES, and A. GARRIDO

ABSTRACT

Storage at 20°C improved *Lactobacillus plantarum* survival in directly brined olives. The best inoculation dates were on the first and fifth days after brining for Cacereña and Hojiblanca cultivars respectively. *Lb. plantarum* was present throughout the storage period only in Hojiblanca olives covered with initial free-salt solution and inoculated on the fifth day. In the presence of that bacterium, titratable acidity (0.5–1%) and pH (<4.0) reached proper values for olive preservation. Yeast populations, consisting of *Pichia membranaefaciens* and *Pichia fermentans*, in Hojiblanca, and *Pichia minuta, Pichia fermentans, Pichia membranaefaciens*, and *Rodotorula spp*, in Cacereña, were found in all treatments.

Key Words: lactobacillus, olives, acidity, inoculation date, aerobic brined

INTRODUCTION

PROCESSING OF RIPE OLIVES usually involves prior storage in brine. Vaughn (1985) described in detail the characteristics of fermentations of Californian cultivars (cvs). The initial population of extraneous organisms, consisting mainly of coliform bacteria and bacilli disappeared in about 10–14 days. However, they were replaced by lactic acid bacteria (LAB) which, due to comparatively high concentrations of salt, were restricted to species of *Lactobacillus plantarum*. Conditions have been changed in current industrial process to salt free storage, which was investigated by Vaughn et al. (1969). This involves addition of 0.5–1.0% (W/V) lactic or acetic acid or their mixture to the brine from the beginning of the process. Inhibition of organisms other than *Lb. plantarum* was improved by anaerobic conditions and, when necessary, preservatives.

When processing of ripe olives was introduced into Spain, a brining procedure similar to that used in California was applied (Fernández Díez et al., 1985). It has since been modified by introducing aerobic conditions (Garrido et al., 1990). In some Spanish directly brined cvs., LAB (*Pediococcus spp* and *Lb. plantarum*) are usually present during storage (García García et al., 1992), but, in others, normal microflora consists of only yeasts (Fernández González et al., 1992). The great volume of gases produced by this procedure is removed by purging with air, thus diminishing shrivelling, gas-pocket spoilage, and softening. Control of pH is impossible unless lactic or acetic acids are added. Growth of LAB in such storage should be promoted since this may produce the lactic acid required to maintain pH at low values needed for preservation (Vaughn, 1969). It could also avoid gas-pockets, because these bacteria are not generally involved in such spoilage (Durán Quintana et al., 1979) and facilitate use of lower levels of salt (which is strongly encouraged by environmental control agencies).

Colonization of solutions containing directly brined olives by LAB is difficult because of the inhibitory effect of oleuropein and other polyphenols from the olives (Walter et al., 1973; Ruiz Barba et al., 1990, 1992). However, growth of *Lb. plantarum* has been induced in the storage brine of ripe olives (Durán Quintana et al., 1993) and its survival during the first days of brining was studied (Durán et al., 1993). A more detailed study of ripe olive brining is needed to elucidate the fermentation pattern in different storage conditions and to enhance growth of LAB, especially *Lb. plantarum*. This could lead to development of a standard procedure that guarantees safe preservation.

Our objective was to study the effects of initial salt concentration and pH of brining solutions, date of inoculation, temperature, and cultivar on survival of *Lb. plantarum*, titratable acidity (pH), and carbohydrate utilization during storage of ripe olives in brine.

Authors are with the Departamento de Biotecnología de Alimentos, Instituto de la Grasa y sus Derivados (CSIC). Apartado 1078. Sevilla, Spain.

MATERIALS & METHODS

Olives (*Olea europaea* L) and fermentors

Fruits were of the Hojiblanca and Cacereña cvs, obtained from local processors. Their degree of ripeness was such that, for both types, 70% had a superficial green-yellow color whereas the other 30% had some pink color to the skin. Fermentors were made of PVC and had a total capacity of 100 L. They were provided with an internal column through which air was bubbled from the bottom (Brenes et al., 1986).

Inoculum

Lb. plantarum 331H, isolated from nonalkali treated table olive brines, was used in all treatments. The bacterium was identified according to criteria in *Bergy's Manual of Systematic Bacteriology* (Kandler and Weiss, 1986). It was maintained as frozen stock at −80°C in MRS (Oxoid) plus 20% glycerol and subcultured twice in MRS medium (Oxoid) before use. Fermentors were inoculated with 1% (v/v) inoculum, using an overnight culture ($3.0*10^8$) grown in MRS broth (Oxoid) incubated at 30°C.

Physicochemical analysis

The pH was measured with a Model 901 Orion pH meter (Orion, USA). Concentration of sodium chloride was analyzed by titration with 0.1N silver nitrate solution, using potassium chromate solution as indicator, and titratable acidity, expressed as g lactic acid/100 mL brine, by titration with 0.1N NaOH solution, using phenolphthalein as indicator. Details about these analyses can be found in Fernández Díez et al. (1985). Sucrose, glucose, fructose and mannitol were determined by HPLC in a Hewlett-Packard Series 1050 liquid chromatograph, equipped with a Rheodyne 7125 injector and a column heater, a Perkin Elmer Model LC-25 refractive index detector and a Hewlett-Packard Model 3396 Series II integrator. An Aminex HPX-87 fixed ion resin column in the Ca^{++} form (Bio Rad Labs) held at 85°C and protected by a guard column with a Carbon-C cartridge (Bio Rad Labs) was used. Aliquots of 50 µL of previous purified brines (Durán et al., 1993) were injected into the chromatograph. Deionized water was used as eluent at 0.9 mL/min. Concentrations were calculated by comparison of sample peak areas with those of external standards for each compound.

Microbial analysis

Violet red bile dextrose agar (Merck) and nutrient agar (ICMSF, 1983) were used to detect Gram-negative bacteria. Plates were incubated at 30°C for 24 hr. Lactic acid bacteria were grown on MRS agar (Oxoid) containing 0.02% sodium azide (Sigma) and 0.05% cycloheximide (Sigma) to improve selectivity (Harrigan and McCance, 1966) and in Lemco-yeast-glucose broth (Naylor and Sharpe, 1958). Plates were incubated at 30°C for 48 hr. Tests on isolated pure cultures of lactobacilli were performed according to criteria in Bergey's Manual of Systematic Bacteriology (Kandler and Weis, 1986). Carbohydrate fermentation was checked by the API CLH system (Saint Nom La Breteche, France).

Table 1—Relationships between the physicochemical and coded values of the factors

Factor	Original levels		Coded levels	
	High	Low	High	Low
Temperature (°C)	30 ± 1	20 ± 1	1	−1
Salt (NaCl %, W/V)	3	0	1	−1
Initial pH	4.5	3.6	1	−1
Inoculation date (period after brining, days)	5	1	1	−1

Table 2—Structure of the fractional factorial design (2^{4-1}) used for the experiment

Number of treatment	Design matrix			
	T	S	pH	I
1	−1	−1	−1	−1
2	1	−1	−1	1
3	−1	1	−1	1
4	1	1	−1	−1
5	−1	−1	1	1
6	1	−1	1	−1
7	−1	1	1	−1
8	1	1	1	1

Note: Defining equation I = T.S.pH.I where T = Temperature; S = Salt; pH = Initial pH of liquid; I = Inoculation date (period after brining).

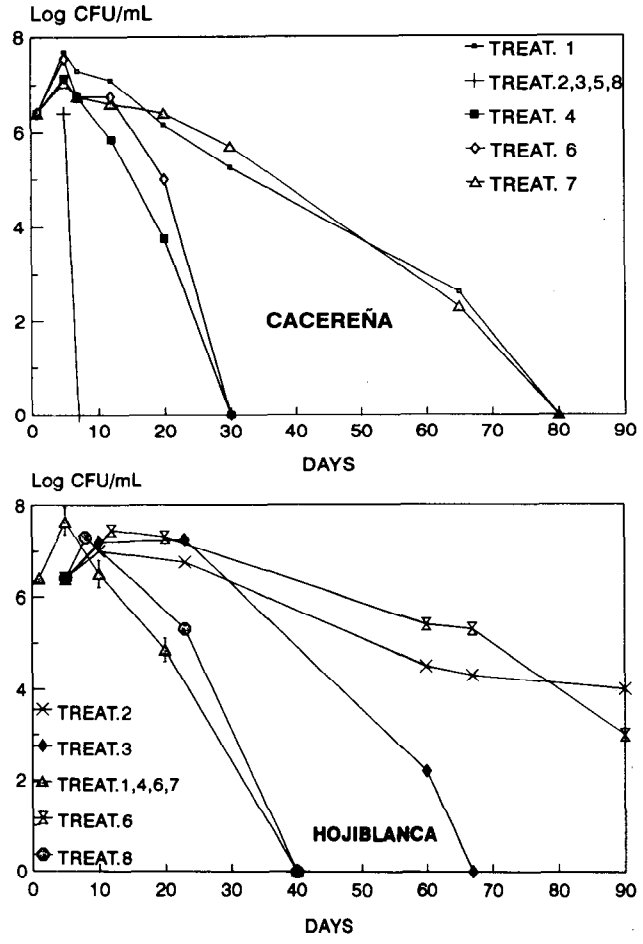

Fig. 1—Changes in the *Lb. plantarum* population throughout different treatments for storage of ripe olives in brine. Hojiblanca and Cacereña cultivar. Variations within grouped treatments are indicated by average and range. (See Table 2 for treatments)

Yeast growth was studied on oxytetracycline-glucose-yeast extract agar (Oxoid). Plates were incubated at 25°C for 48 hr. To obtain pure cultures of yeasts, selected colonies were plated onto yeast extract-glucose plates until complete purification was achieved. Isolated cultures were then identified taxonomically following criteria and techniques described by Lodder (1970). Morphological characteristics of vegetative reproduction and morphology of vegetative cells were studied in liquid malt extract. Sweet potato glucose agar (Wickerham, 1951) was used for formation of pseudomycelium. The culture characteristics were observed in both liquid malt extract and yeast infusion glucose agar. For sporulation tests, the medium of Starkey (1964) was used, and the ascospores were observed microscopically by the acid colorants method with methylene blue and eosin yellow (Jorgensen, 1948). To test the fermentative utilization of carbohydrates, a basal medium containing 1% yeast extract and, respectively, 2% glucose, galactose, maltose, sucrose, or lactose, or 4% raffinose with Durham's tubes was used (Lodder and Kreger Van Rij, 1952). The auxonographic method of Lodder and Kreger Van Rij (1952) was also used for oxidative utilization of carbohydrates and nitrogenated compounds. The following carbohydrates were tested with the API AUX tests: cellobiose, trehalose, xylose, inositol, glycerol and sorbitol. Enumeration of viable microorganisms in samples was carried out using a Spiral Plate System (Interscience, France) according to the manufacturer's recommended procedure.

Experimental design

The effects of different factors were studied by means of a resolution IV 2^{4-1} fractional factorial design, which did not confound main effects and two factor interactions (Box et al., 1978). Relationships between the physico-chemical and coded factors, as well as their levels, are shown in Table 1 and the structure of the fractional factorial design in Table 2. It was applied to both Hojiblanca and Cacereña cvs. A total of 16 runs were thus performed. Treatments were randomly assigned. Each treatment was carried out with 50 kg of whole olives, well washed in tap water (Hojiblanca and Cacereña cvs). They were put into the PVC fermentors and covered with 50L of brine. Later, fermentors were subjected to other conditions (incubation temperature and inoculation date) to complete the required combination of levels.

Initial pH was adjusted with food grade acetic acid. Initial NaCl concentration was increased, as in industrial practice, by 1% (W/V) every 14 days to reach 6% in all treatments. Concentration was then maintained during the rest of the experiment. Throughout the whole storage period, air was bubbled, from the bottom of the interior column, through each fermentor at a rate of 30 L/h for 8 h/day (Brenes et al., 1986). All treatments were inoculated with *Lb plantarum*. This bacterium was used because of its normal presence in green table olive fermentations (Fernández Díez et al., 1985), its identification in spontaneous processes of directly brined olives (García García et al., 1992, Durán Quintana et al., 1993), and its relative resistance to olive brine (Durán et al., 1993). Except for the studied factors, brining conditions were similar to those applied in industry, to facilitate later scale up of the process.

Fermentation was followed by measuring microbial growth, NaCl concentration, titratable acidity, pH, and sugars (sucrose, glucose, fructose and mannitol) at predetermined periods after brining.

RESULTS & DISCUSSION

Microbial analysis

Before inoculations, microbial analysis of brines from all treatments showed the growth of only a small population of Gram-negative bacteria (ranging from $2*10^1$ to $6.4*10^3$) (which were not detected in later samplings) and of yeasts, which counts were monitored throughout the whole storage. After *Lb. plantarum* inoculation, only one morphological type was observed on plates from some treatments which were also rod-shaped (by microscope). The isolated pure cultures belonged to the *Lb. plantarum* species, with a similar taxonomic profile to that of the inoculated strain. Two different survival patterns were observed: those corresponding to treatments in which *Lb. plantarum* was inhibited and those in which there was an initial growth from 15 to 30 days, followed by a variable declining rate of population (Fig. 1). Optimization of storage conditions should expand both periods to provide best physico-chemical characteristics for good olive preservation. In the Hojiblanca cultivar, *Lb. plantarum* was present throughout the period of storage in only two treatments, which corresponded to those which initially used a salt-free solution and were inoculated 5 days after brining. In the other treatments this bacterium practically disappeared after 1 mo. In Cacereña, *Lb. plantarum* presence was limited to only the 4 treatments inoculated on the first

Table 3—Estimated contrasts for log of the *Lactobacillus plantarum* population in the fermentation of Hojiblanca and Cacereña cvs at some predetermined brining periods

	Hojiblanca			Cacereña		
	13[a]	30	60	12	30	64
Effects:						
Temperature (T)	−0.25	−0.95*	−0.69*	−0.57*	−0.89*	−1.26*
Salt (S)	−0.34	−0.26	−1.96*	−0.11	−0.28	−0.01
Inoculation date (I)	−0.11	0.58*	2.96*	−6.32*	−5.42*	−1.26*
Interactions:						
T.S	−0.18	−0.35	−0.31	0.15	−0.42	0.01
T.I	0.23	0.64*	−0.69*	0.57*	0.89*	1.26*
S.I	0.35	0.41	−1.96*	0.11	0.28	0.01
T.S.I.	0.22	0.63	−0.31	−0.15	0.42	0.01

[a] Days after brining.
* Significant at $p < 0.05$. (SD effects = 0,25 f.d. = 6, estimated from the three order interactions of the six responses).

day. Then, its counts decreased and the organism disappeared after 2 and 1 month in treatments held at 20 and 30°C, respectively. Statistical analysis of the fractional factorial design showed that the effect of initial pH was not significant. Consequently, this factor was eliminated from the initial design that was thus transformed into a complete factorial for 3 factors (temperature, salt concentration and inoculation date). The lack of initial pH effect must be an effect of the tendency for pH values around 4.3-4.5 to be reached after a few days of brining. This was independent of the initial value, probably because of the spontaneous growth of a microflora (Durán Quintana et al., 1993) or to the solubilization of substances from olive flesh (Fernández González et al., 1992). Furthermore, *Lb. plantarum* was able to maintain a pH gradient between the brine and the interior of its cell even at high organic acid concentrations. This ability was the characteristic that permitted this bacterium to persist throughout most of the fermentation (McDonald et al., 1990). Thus the hypothesis of an initial pH adjustment to the covering solution still holds. This practice does not sensibly interfere with *Lb. plantarum* inoculation and, on the contrary, is important for obtaining spontaneous microfloral growth in directly brined olives, especially when salt-free solutions are used to cover the olives initially. The use of 0.2-0.4% acetic acid is recommended to reduce the Gram-negative bacteria and *Bacillus* population in such processes as well as to diminish formation of gas-pockets and softening (Fernández Díez et al., 1985).

A systematic negative effect of temperature was appreciable for both cvs. when the transformed complete factorial for the remaining 3 factors was studied (standard deviation was calculated from the 3 order interactions of the six responses, sampling dates for both cvs) (Table 3). In Hojiblanca, this effect was significant at 30 and 60 days. In Cacereña, it was always significant. This unexpected negative influence of the *Lb. plantarum* optimum incubation temperature was due to the influence of the same factor on the polyphenol content in brines. As was demonstrated by Brenes et al. (1993), an increase in temperature produced remarkably higher concentrations of both oleuropein and hydroxytyrosol in brines. The inhibitory effect of these compounds on *Lb. plantarum* (Juvens and Henis, 1970; Walter et al., 1973; Ruíz Barba et al., 1990 and 1992) probably thus exceeded the enhancing effect of temperature on growth. Differences in phenols concentrations due to temperature were very evident during the first days of brining and were reduced very slowly during storage (Brenes et al., 1993). This appeared to support the persistence of the temperature effect.

Initial salt concentration (Table 3) on *Lb. plantarum* counts was significant in Hojiblanca at 60 days. In Cacereña, possibly due to the fact that *Lb. plantarum* survival was so limited, this effect was not appreciable. Changes in NaCl concentrations during storage were those derived from the additions. In synthetic culture media, salt at 6% and 8% retarded the exponential phase of growth and reduced the density of the cultures, but the effect did not occur at lower levels (Bobillo and Marshall, 1991). However, when the culture media are brines, the effect of salt may be increased by other adverse factors. Thus, growth of *Lb. plantarum* was never observed in the fermentation of Hojiblanca cultivar during its storage at salt percentages from 3% to 6% (Fernández González et al., 1992). Spontaneous growth of *Lb. plantarum* has been found only when Hojiblanca olives were covered initially with a salt-free solution (Durán Quintana et al., 1994). This effect was reported in detail by Durán Quintana et al. (1994). They showed that the presence of 3% salt always made inoculum survival more difficult during the first days of brining and was critical when the level reached 6%. Thus, in general, the salt percentage should be maintained as low as possible to favor *Lb. plantarum* colonization. Nevertheless, total absence of salt during the initial phase of storage may be very risky due to the numerous extraneous microorganisms (molds, yeasts, bacilli, gram-negative bacteria, etc.) with the raw material (Vaughn, 1985). Only if a massive colonization of *Lb. plantarum* could be obtained from the very beginning of the process, should a salt-free solution be used initially. As shown, the use of up to 3% salt, when properly combined with other brining conditions, may help control this undesirable microflora.

The inoculation date was critical and depended on the cultivar. In Hojiblanca, inoculation after 5 days brining was preferable and significant in the long run (30 and 60 days). However, in Cacereña, only those treatments inoculated on the first day showed *Lb. plantarum* presence. This must be due to the fact that by the fifth day the polyphenol concentrations in Cacereña, especially that of oleuropein, were markedly higher than those in Hojiblanca and by that date the level could have been high enough to completely inhibit *Lb. plantarum* growth in Cacereña. The higher concentration of phenols in Cacereña during storage (Brenes et al., 1993) could also have been the cause of inhibition of *Lb. plantarum* after the second month of brining. In previous work, survival of *Lb. plantarum* in the storage brines of olives held at room temperature was also dependent on this factor and at least 3 days were needed to colonize such solutions and 6 when salt was present (Durán Quintana et al., 1994). A certain solubilization of nutrients from the olives is necessary to support the bacterium. So, diminution of the time period required for *Lb. plantarum* survival in Cacereña, while keeping the temperature constant, indicated a rapid osmotic exchange that may be attributed to slight differences in skin permeability and physiological characteristics.

The only consistent significant interaction (T*I) was found in the Cacereña cultivar. For the inoculation after 1 day of brining higher *Lb. plantarum* counts occurred at lower temperatures. The explanation was again that the polyphenol content was lower at 20°C (Brenes et al., 1993). In Hojiblanca, this interaction was contradictory.

In both cultivars a yeast population developed during the first 20–30 days and was present throughout the process, ranging counts from 10^5 to 10^7 (Fig. 2). In Hojiblanca, the species corresponded to *Pichia membranaefaciens* and *Pichia fermentans*, which had already been described in the aerobic fermentation of that cultivar (Fernández González et al., 1992). In Cacereña, whose fermentation has not been previously described, the most abundant species were: *Pichia minuta* (47%), *Pichia fermentans*

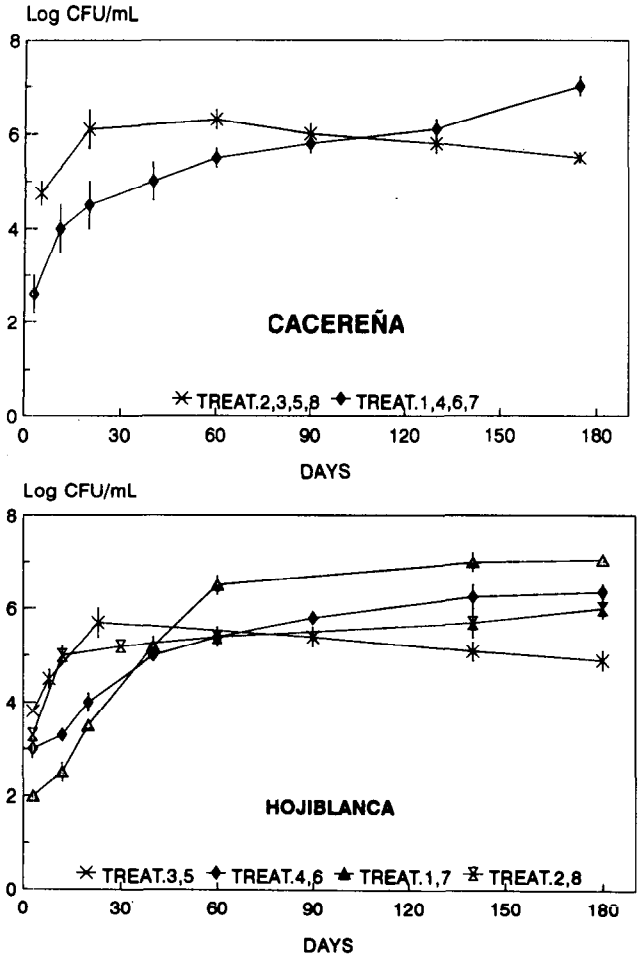

Fig. 2—Changes in the yeast population throughout different treatments for storage of ripe olives in brine. Hojiblanca and Cacereña cvs variations within grouped treatments are indicated by average and range. (See Table 2 for treatments)

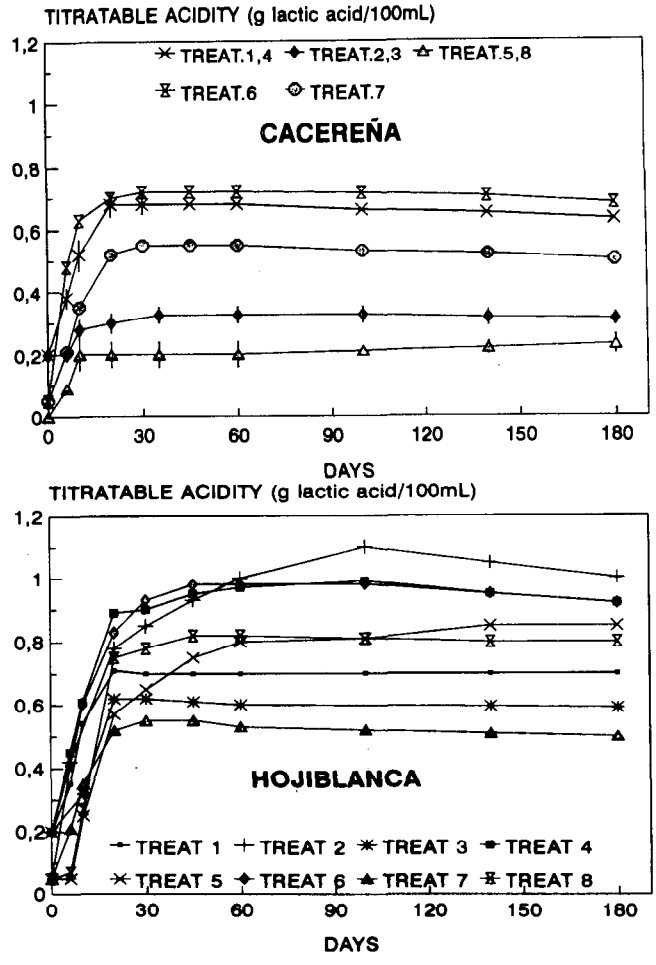

Fig. 3—Changes of free acidity throughout different treatments for storage of ripe olives in brine. Hojiblanca and Cacereña cvs variations within grouped treatments are indicated by average and range. (See Table 2 for treatments)

(21%), *Pichia membranaefaciens* (17%) and *Rodotonula spp* (9%). In general, growth of lactobacilli caused a diminution of yeast population proportional to *Lb. plantarum* counts. However, after disappearance of this bacterium, the yeast populations in such treatments increased progressively up to the end of storage and reached the highest values in most of them. The coexistence of *Lb. plantarum* and yeasts was not a deficiency. It is normal in most table olive fermentations (Fernández Díez et al., 1985) and other fermented vegetables (Vaughn, 1985), in which both types of microorganisms contribute to final flavor. A mixed culture can be beneficial and lead to more complete exhaustion of sugars (Daeschel et al., 1988). Additionally, the species found are not related to gas-pocket and other spoilage (Durán et al., 1979) and thus their presence during storage did not represent any risk.

Titratable acidity changes

The titratable acidities (and pH changes) observed were proportional to *Lb. plantarum* counts (Fig. 3). The highest titratable acidities were reached in those treatments in which the bacterium was present during the entire storage period (Hojiblanca). In that cultivar, the treatments with lower acidity were those inoculated on the first day of brining (treat. 7) or those with higher levels of salt (treat. 3) because of the negative effect of both factors on *Lb. plantarum*. Titratable acidity was higher than 0.5% in those treatments. The other treatments reached values between 0.8% and 1.0%. Thus, acid production in the storage phase was marked and reached values, in the most favorable conditions, that could guarantee safe preservation of olive fruits in brine before oxidation. In Cacereña, titratable acidity values were lower (never above 0.8%), but reached acceptable levels in treatments inoculated on the first day either without salt (treats. 6 and 1) or with 3% NaCl (treats. 4 and 7). The lower levels corresponded to treatments inoculated on the fifth day. In the former cases, the produced acidity could guarantee a safe preservation, but, in the others, it would be doubtful. A further improvement of lactic fermentation of this cultivar is needed.

Changes in fermentable carbohydrates

Amounts of sucrose were always negligible. Fructose and glucose maintained low concentrations ranging from 0.5 to 1.5 mM and 1.0 to 3.0 mM, respectively, with a slight declining tendency during storage. These concentrations were due to an equilibrium between sugar utilization by the microflora in each treatment and the diffusion from fruits, which in directly brined olives was very slow. Mannitol contents were higher (up to 5.5 mM in Cacereña and 11 mM in Hojiblanca) and showed a tendency to increase in those treatments in which *Lb. plantarum* was absent. This mannitol came from the fruits and its accumulation in those treatments without *Lb. plantarum* indicated that the yeast population was not able to use it while it was at least partially metabolized by the bacterium. The same behavior had been reported found by Garrido et al. (1993).

REFERENCES

Bobillo, M.P. and Marshall, V. 1991. Effect of salt and culture aeration in lactate acid acetate production by *Lactobacillus plantarum*. Food Microbiol. 8: 153–160.

Box, G.E.P., Hunter, W.G., and Hunter, J.S. 1978. *Statistics for Experimenters*. John Wiley and Sons, Inc., New York.
Brenes Balbuena, M., García García, P., Durán Quintana, M.C., and Garrido Fernández, A. 1986. Estudio comparativo de sistemas de conservación de aceitunas tipo negras. Grasas y Aceites 37: 123–128.
Brenes Balbuena, M., Durán Quintana, M.C. and Garrido Fernández, A. 1993. Concentration of phenolic compounds change in storage brines of ripe olives. J. Food Sci. 58: 347–350.
Daeschel, M.A., Fleming, H.P., and McFeeters, R.F. 1988. Mixed cultures fermentation of cucumber juice with *Lactobacillus plantarum* and yeasts. J. Food Sci. 53: 862–864.
Durán Quintana, M.C., González Cancho, F. and Garrido Fernández, A. 1979. Aceitunas negras al natural en salmuera. IX. Ensayos de producción de alambrado por inoculación de diversos microorganismos aislados de salmueras de fermentación. Grasas y Aceites 30: 361–367.
Durán Quintana, M.C., Brenes Balbuena, M., and Garrido Fernández, A. 1993. Lactobacillus plantarum survival during the first days of ripe olive brining. System. Appl. Microbiol. 16: 153–158.
Durán Quintana, M.C., García García, P., Brenes Balbuena, M., and Garrido Fernández, A. 1994. Induced lactic acid fermentation during the preservation stage of ripe olives from Hojiblanca cultivar. J. Appl. Bacteriol. 76: 377–382.
Fernández Díez, M.J., Castro Ramos, R., Garrido Fernández, A., Heredia Moreno, A., Mínguez Mosquera, M.I., Rejano Navarro, L., García García, P., Nosti Vega, M., and Castro Gómez Millán, A. 1985. Biotecnología de la aceituna de mesa. Consejo Superior de Investigaciones Científicas. Madrid.
Fernández González, J., Garrido Fernández, A., García García, P., Brenes Balbuena, M. and Durán Quintana, M.C. 1992. Características del proceso fermentativo durante la conservación de aceitunas de la variedad Hojiblanca destinadas a la elaboración del tipo negras. Grasas y Aceites 43: 212–218.
García García, P., Durán Quintana, M.C., Brenes Balbuena, M. and Garrido Fernández, A. 1992. Lactic fermentation during the storage of "Aloreña" cultivar untreated green table olives. J. Appl. Bacteriol. 73: 324–330.
Garrido Fernández, A., Durán Quintana, M.C., García García, P., Sánchez Roldán, F., and Brenes Balbuena, M. 1990. Fermentation of olives in aerobic conditions. In *Processing and Quality of Foods: Avenues to Healthy and Nutritional Products. Food Biotechnology*. Vol. 2, 219–230. P. Zeuten, J.C. Chefted, C. Eriksson, T.R. Cormley, P. Linko, and K. Paulus (Eds). Elsevier Applied Science, London.
Garrido Fernández, A., García García, P., Montaño Asquerino, A., Brenes Balbuena, M., and Durán Quintana, M.C. 1993. Biochemical changes during the preservation stage of ripe olive processing. Die-Nahrung 37: 583–591.
Harrigan, W.F. and McCance, M.E. 1966. Culture media composition. In *Laboratory Methods in Food and Dairy Microbiology*, p. 46–54. Academy Press, London.
ICMSF (International Commission on Microbiological Specifications for Foods). 1983. Microorganismos de los alimentos. Volume 2, 113–120. Ed. Acribia, Zaragoza.
Jorgensen, A. 1948. Yeast and yeast like organisms. In *Microorganisms and Fermentation*, p. 10–550. Charles Griffin, London.
Juven, B. and Henis, Y. 1970. Studies on the antimicrobial activity of olive phenolic compounds. J. Appl. Bacteriol. 33: 721–723.
Kandler, O. and Weis, N. 1986. Genus Lactobacillus Beijerinck 1901–212. In *Bergey's Manual of Systematic Bacteriology*. Vol. 2. P.M.A. Sneath, N.S. Mair, M.E. Sharpe, and J.G. Holt (Ed.), p. 1209–1224. Williams and Wilkins. Baltimore.
Lodder, J. 1970. Criteria and methods used in classification. In *The Yeast. A. Taxonomic Study*, p. 1–1385. North Holland Publishing, Amsterdam.
Lodder, J. and Kreger Van Rij. J.W. 1952. Criteria and methods used in classification. In *The Yeast. A Taxonomic Study*, p. 2–713. North Holland Publishing. Amsterdam.
McDonald, M.R., Fleming, H.P. and Hassan, H.M. 1990. Acid tolerance of *Leuconostoc mensenteroides* and *Lactobacillus plantarum*. Appl. Environ. Microbiol. 56: 2120–2124.
Naylor, J. and Sharpe, M.E. 1958. Lactobacilli in cheddar cheese. I. The use of selective media for isolation and of serological typing identification. J. Dairy Res. 25: 92–98.
Ruiz Barba, J.L., Brenes Balbuena, M., Jiménez Díaz, R., García García, P., and Garrido Fernández, A. 1992. Inhibition of *Lactobacillus plantarum* by polyphenols extracted from two different kinds of olive brines. J. Appl. Bacteriol. 73: 502–505.
Ruiz Barba, J.L., Ríos Sánchez, R.M., Fedriani Iriso, C., Ríos, J.C., Olías, J.M., and Jiménez Díaz, R. 1990. Bacteriocidal effect of phenolic compounds from green olives in *Lactobacillus plantarum*. Syst. Appl. Microbiol. 13: 199–205.
Starkey, R.L. 1964. Lipid production by soil yeast. J. Bacteriol. 51: 33–50.
Vaughn, R.M., Martin, M.H., Stevenson, K.E., Johnson, M.G. and Crampton, V.M. 1969. Salt-free storage of olives and produce for future processing. Food Technol. 23: 832–834.
Vaughn, R.M. 1985. The microbiology of vegetable fermentations. In *Microbiology of Fermented Foods*. Vol. 1. B.J.B. Wood (Ed.), p. 49–109. Elsevier Applied Science Publishers.
Walter, W.M. Jr., Fleming, H.P., and Etchells, J.L. 1973. Preparation of antimicrobial compounds by hydrolysis of oleuropein from green olives. J. Appl. Microbiol. 26: 773–776.
Wickerham, L.J. 1951. Taxonomy of Yeast. Techn. Bull. nº 1029. U.S. Dept. of Agriculture, Washington, DC.
Ms received 1/24/94; revised 6/7/94; accepted 6/20/94.

We thank CICYT (Spanish Government) for financial support of this research (ALI-91-1166-C03-01 project).

*OLIVE CELL WALL COMPOSITION. . .*From page 1196

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, D.A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350–356.
Durán, M. and Izquierdo, A. 1964. Estudios sobre la estructura histológica del fruto de OLEA EUROPAEA L. I. Variedad Zorzaleña (*O.E. Argentata*). Grasas y Aceites 15: 72–86.
Düsterhöft, E.M., Voragen, A.G.J., and Engels, F.M. 1991. Non-starch polysaccharides from sunflower (*Helianthus annuus*) meal and palm kernel (*Elaeis guineenis*) meal - Preparation of cell wall material and extraction of polysaccharide fractions. J. Sci. Food Agric. 55: 411–422.
Englyst, H.N. and Cumming, J.H. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-chromatography of constituent sugars as alditol acetates. Analyst 109: 937–942.
FAO. 1991. Elaboración de Aceitunas de Mesa. Bulletin of Agricultural Services. Rome.
Gil-Serrano, A., Mateos-Mato, M.I., and Tejero-Mateo, M.P. 1986. Acidic xylan from olive pulp. Phytochemistry 25: 2653–2654.
Guillén, R., Heredia, A., Felizón, B., Jiménez, A., and Fernández-Bolaños, J. 1991. Preparación y caracterización de fracciones de fibra en aceitunas (Variedad Hojiblanca). Grasas y Aceites 42: 334–338.
Guillén, R., Heredia, A., Felizón, B., Jiménez, A., Montaño, A., and Fernández-Bolaños, J. 1992. Fibre fraction carbohydrates in *Olea europaea* (Gordal and Manzanilla var.). Food Chem. 44: 173–178.
Heredia, A., Fernández-Bolaños, J., and Guillén, R. 1990. Cellulase inhibition by polyphenols in olive fruits. Food Chem. 38: 69–73.
Heredia, A., Ruiz-Gutierrez, V., Felizón, B., Guillén, R., Jiménez, A., and Fernández-Bolaños, J. 1993. Apparent digestibility of dietary fibre and other components in table olives. Die Nahrung 37: 226–233.
Ishii, T. and Hiroi, T. 1990. Linkage of phenolic acids to cell-wall polysaccharides of bamboo shoot. Carbohydr. Res. 206: 297–310.
Jiménez, A., Guillén, R., Fernández-Bolaños, J., and Heredia. A. 1994a. Pared celular de aceitunas. Influencia de la temperatura inicial de extracción en la composición de polisacáridos. Anales de Bromatología. In press.
Jiménez, A., Labavitch, J.M., and Heredia, A. 1994b. Changes in the cell wall of olive fruit during processing. J. Agric. Food Chem. 42: 1194–1199.
Lomax, J.A., Gordon, A.H., and Chesson, A. 1983. Methylation of unfractionated, primary and secondary cell-walls of plant, and the location of alkali-labile substituents. Carbohydr. Res. 122: 11–22.
Nahar, N., Mosihuzzaman, M., and Theander, O. 1990. Analysis of phenolic acids and carbohydrates in pigeon pea (*Cajanus cajan*) plant. J. Sci. Food Agric. 50: 45–53.
Ruiter, J.M. and Burns, J.C. 1987. Characterization of trifluoroacetic acid hydrolyzed of subtropical forage grass cell walls. J. Agric. Food Chem. 35: 308–316.
Selvendran, R.R. 1975. Analysis of cell wall material from plant tissues: extraction and purification. Phytochem. 14: 1011–1017.
Seymour, G.B., Colquhoun, I.J., DuPont, M.S., Parsley, K.R., and Selvendran, R.R. 1990. Composition and structural features of cell wall polysaccharides from tomato fruits. Phytochemistry 29: 725–731.
Siddiqui, I.R. 1990. Studies on vegetables. Investigation of water, oxalate, and sodium hydroxide soluble celery (*Apium graveolens*) polysaccharides: celery arabinogalactan. J. Agric. Food Chem. 38: 38–41.
Southgate, D.A.T. 1976. The chemistry of dietary fibre. In *Fiber in Human Nutrition*, G.A. Spiller and R.J. Amen (Ed.), p. 31–72. Plenum Press, New York.
Stevens, B.J.H. and Selvendran, R.R. 1980. The isolation and analysis of cell wall material from the alcohol-insoluble residue of cabbage (*Brassica oleracea* var. *capitata*). J. Sci. Food Agric. 31: 1257–1267.
Talbott, L.D. and Ray, P.M. 1992. Molecular size and separability features of pea cell wall polysaccharides. Implications for models of primary wall structure. Plant Physiol. 98: 357–368.
Trowell, H.C. 1976. Definition of dietary fiber and hypothesis that it is a protective factor in certain diseases. Amer. J. Clin. Nutr. 29: 417–427.
Vázquez, A., Graciani, E., and Maestro, R. 1974. Componentes fenólicos de la aceituna. I. Polifenoles de la pulpa. Grasas y Aceites 25: 269–279.
Wakabayashi, K., Sakurai, N., and Kuraishi, S. 1990. Sugar composition and molecular weight distribution of cell wall polysaccharides in outer and inner tissues from segments of dark grown squash (*Cucurbita maxima* Duch.) hypocotyls. Plant Physiol. 93: 998–1004.
Ms received 1/24/94; revised 5/13/94; accepted 7/31/94.

We acknowledge the financial support of C.I.C.Y.T. (ALI94-0980-C02-02).