Comparative Study on Chemical Changes in Olive Juice and Brine during Green Olive Fermentation

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Changes in physicochemical characteristics, substrate depletion, and product formation during fermentation were followed in both brine and olive juice in order to achieve a complete knowledge of fermentation chemistry in Spanish-type green olives. Both spontaneous and controlled fermentations were investigated. Fermentation rate, irrespective of the type of fermentation, was lower in olive juice than in brine, but the main acid products eventually reached equilibrium. Final free acidity remained significantly ($p < 0.05$) higher, and combined acidity remained lower, in brine than in olive juice in both fermentations, but differences in final pH were not significant in controlled fermentation. Final concentrations of lactic and formic acids were significantly ($p < 0.05$) higher, and those of ethanol and succinic acid were lower, in controlled fermentation than in spontaneous fermentation. Butanediol, attributable to *Enterobacteriaceae* growth, was formed only in the latter case. Calculated carbon recoveries were not significantly ($p < 0.05$) different in any case, giving a mean of some 78%.

Keywords: *Olives; fermentation; Lactobacillus pentosus; acids; ethanol; 2,3-butanediol*

INTRODUCTION

Processing of Spanish-type green table olives consists of a treatment with an alkaline lye (1.8−2.5%, w/v, NaOH) to eliminate bitterness, followed by a washing step with water to remove the excess of alkali. Brine (10−13%, w/v, NaCl) is then added, and a spontaneous fermentation, mainly carried out by lactic acid bacteria (Borbolla y Alcalá and Rejano, 1979), takes place. Immediately after brining, fermentable material and other nutrients diffuse from the olives into the brine, while NaCl diffuses into the olives. Equilibration of fermentable substrates and NaCl may affect fermentation rate. The latter compound appears to approach equilibrium by 3−4 days (Borbolla y Alcalá, 1979), whereas sugars, organic acids, and volatile components (initially inside the olives) appear to equilibrate in 5−7 days at room temperature, in the absence of microbial activity (Montaño et al., 1993). Borbolla y Alcalá (1979) found that the equilibration rate for reducing sugars in olives increased as brine concentration decreased. In addition, the diffusion rate for sugars and NaCl increased as lye concentration and temperature increased during the lye treatment stage; this was attributed to the influence of these factors on skin permeability (Borbolla y Alcalá and Rejano, 1978). Studying the microbial and physicochemical changes during spontaneous fermentation of Spanish-type green olives, Borbolla y Alcalá and Rejano (1981) established three successive phases. In the first phase, which generally lasts from 2 to 3 days, the brine has initially a high pH which decreases to a value of approximately 6. The microorganisms that best characterize this phase are *Enterobacteriaceae*. During the second phase (up to 10−15 days), lactobacilli and yeasts generally develop quickly, and *Enterobacteriaceae* decrease until they disappear completely at a pH of around 4.5. During the third phase, which lasts until the fermentable material is exhausted, only species of *Lactobacillus* (mainly *Lb. plantarum*) abound and coexist with a yeast flora. The final pH reaches 4.0 or less. Substrate depletion and product formation during spontaneous green olive fermentation, however, have not been followed up to now. Montaño et al. (1993) studied the substrate and product evolution in Spanish-type green olive brine in response to *Lb. plantarum* pure culture fermentation.

We are not aware of any published work on the chemical changes inside the olive flesh during fermentation. However, lactic acid bacteria growth has been demonstrated in fermenting cucumbers (Daeschel et al., 1987), and thus, besides growing in brine, the bacteria may also grow within brined olives. Moreover, differences in the main chemical parameters between olives and the surrounding brine may significantly affect preservation of the final product. The aim of the present work was to investigate in both brine and olive juice the changes in the main physicochemical parameters, in the substrates, and in the metabolic products during green olive fermentation. The effect of a different fermentation rate was considered by studying both spontaneous and controlled fermentation.

MATERIALS AND METHODS

Olive Processing. Four fermentors (20 kg fruits plus 8.2 L brine capacity) were filled with olives (Manzanillo variety harvested in mid-October), and the steps of Spanish-type green olive preparation were carried out: (a) lye treatment, 8.2 L of 1.96% NaOH for 6.5 h at room temperature; (b) washing, 8.2 L of tap water for 17 h; and (c) brining, 8.2 L of 10.7% NaCl. After that, to accelerate the processes, all fermentors were placed in a thermostated room at 25 °C. Two fermentors were subjected to spontaneous fermentation by the environmental microbiota, and the other two were subjected to controlled fermentation using *Lactobacillus pentosus* CECT 5138, a

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strain originally isolated in our laboratory from a natural Spanish-type green olive fermentation. Propagation of inocula was carried out using MRS broth (Oxoid Ltd., Basingstoke, Hampshire, England), with 4.5% NaCl to allow adaptation to the saline environment. After incubation at 30 ± 2 °C for 24 h, the cultures were centrifuged and washed in saline, and finally, the volume calculated to get the desired population (ca. 10^8 cfu/mL) was added to the fermentor.

Sampling. Both brine and olive samples were taken immediately after brining and at 2, 4, 6, 8, 11, 14, 18, and 22 days of fermentation. Olive juice was prepared by pitting a 150 g random sample of olives and blending them in a homogenizer (Braun, Kronberg, Germany) without added liquid. The homogenate was squeezed through cheesecloth and then centrifuged for 30 min at 12 000g using a Sorvall RC-5 superspeed centrifuge (Du Pont Instruments, Newton, CT). One portion of the aqueous supernatant was used for physicochemical analyses, and the rest was frozen until analysis by HPLC and GC. Brine samples were also divided in two portions. One portion was used immediately for microbiological and physicochemical analyses, and the other was centrifuged at 11 600g for 15 min and the supernatant frozen until analysis by HPLC and CG.

Titrimetric Analyses. The pH, free acidity, and combined acidity of samples were measured using a Metrohm 670 Titroprocessor (Herisau, Switzerland). Free acidity was determined by titrating up to pH 8.3 with 0.2 N NaOH and expressed as percent (w/v) of lactic acid. Combined acidity was determined with 2 N HCl until the pH value reached 2.6 and was expressed as the equivalent of sodium hydroxide per liter (Fernández-Díez et al., 1985).

HPLC Analyses. Carbohydrates (sucrose, glucose, fructose, and mannitol) and organic acids (malic, citric, lactic, acetic, succinic, and formic acids) were determined using 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-87C column (300 × 7.8 mm i.d., Bio Rad Labs) held at 70 °C and deionized water as eluent at 0.7 mL/min were used for carbohydrates analysis. Samples (0.5 mL) were desalted by adding 1 g of a strongly acidic resin (Amberlite IR-120, Fluka Chemie AG, Buchs, Switzerland) plus 1 g of a weakly basic resin (Amberlite IRA-93, Fluka). An internal standard (1.5 mL of 0.1% sorbitol) was also added for quantification by the internal standard method. Samples were shaken occasionally during a 60 min desalting period. An aliquot ($\approx$1 mL) of the solution was centrifuged at 11 600g for 10 min, and 50 μL was injected into the chromatograph. Malic, citric, lactic, acetic, and succinic acids were analyzed using a Spherisorb ODS-2 (5 μm, 25 cm × 4 mm i.d., Teknokroma, Barcelona, Spain) column with deionized water (pH adjusted to 2.2 using concentrated H_3PO_4) as mobile phase. Flow rate was 1.0 mL/min. Samples (0.5 mL) were diluted 1:1 with deionized water and then 10 μL of concentrated H_3PO_4 was added. An aliquot (20 μL) was injected into the chromatograph after the aliquot was centrifuged at 11 600g for 10 min. Formic acid was analyzed using an Aminex HPX-87H (300 × 7.8 mm i.d., Bio Rad Labs) column, held at 65 °C, with 0.005 M H_2SO_4 as mobile phase at a flow rate of 0.7 mL/min. For this acid, sample preparation was conducted as before, except that an acidification step was not used. Concentrations were calculated by comparison of peak heights with those of external standards for each compound.

GC Analyses. Ethanol was analyzed using the headspace method described by Montaño et al. (1990). The system consisted of a Fisons HRGC Mega 2 series gas chromatograph, equipped with a flame ionization detector and a split-splitless injection port, a Hewlett-Packard 19395A headspace sampler, and a computer with Chrom-Card for Windows software (Fisons Instruments, Milan, Italy) for processing data.

Acetoin and 2,3-butanediol were analyzed using a Perkin-Elmer 3920B gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard model 3396 A integrator. The column was a PorapaK Q (mesh 80−100, 2 m × $^1/_4$ in. o.d.). The oven temperature was 220 °C and the
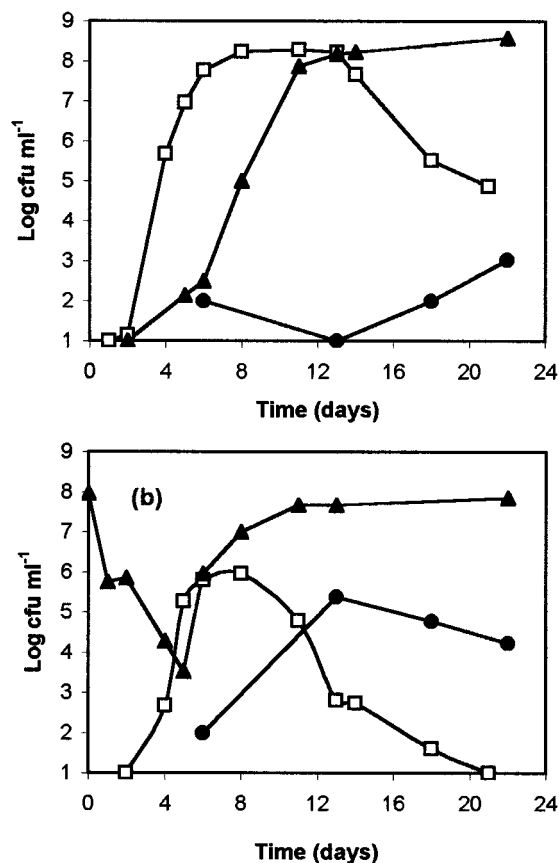

Figure 1. Microbiological changes in the brine of Spanish-type green olives during (a) spontaneous and (b) controlled fermentation: lactic acid bacteria (▲), *Enterobacteriaceae* (□), and yeasts (●). Coefficients of variation (%) were lactic acid bacteria, 33.0 and 45.5; *Enterobacteriaceae*, 69.6 and 116.4; and yeasts, 34.7 and 33.3, for spontaneous and controlled fermentation, respectively.

detector temperature was 250 °C. Nitrogen was used as the carrier gas at 20 mL/min. To 0.5 mL of sample were added 0.1 mL of 0.25% *n*-heptanol as an internal standard and 20 μL of 20% sodium carbonate. The mixture was clarified by centrifugation before injection.

Microbiological Analyses. Brine samples and appropriate decimal dilutions were plated using a Spiral System model DS (Interscience, Saint Nom La Breteche, France). *Enterobacteriaceae* were counted on crystal violet neutral-red bile dextrose agar (Merck), lactic acid bacteria were counted on MRS agar (Oxoid), and yeasts were counted on OGYE agar (Oxoid). Plates were incubated at 32 °C for 48 or 72 h.

Data Analysis. Mean values of the different parameters from duplicate fermentations were calculated and graphed, but, for clarity, error bars were not included. Instead, to give estimates of variability, coefficients of variation were obtained at each time, and an average value was given in the graph legend. After fermentation, the data were subjected to analysis of variance to identify significant (p < 0.05) differences between mean values.

RESULTS AND DISCUSSION

Microbiological Changes during Fermentation. Changes in microbial populations in the olive brines for spontaneous and controlled fermentation are summarized in Figure 1. Because of the addition of inoculum, the population of lactobacilli in the case of controlled fermentation exceeded that in the spontaneous fermentation during the first 8 days, despite the initial loss of viability observed until day 5. This low survival can be attributed to bacterial death due to alkaline pH
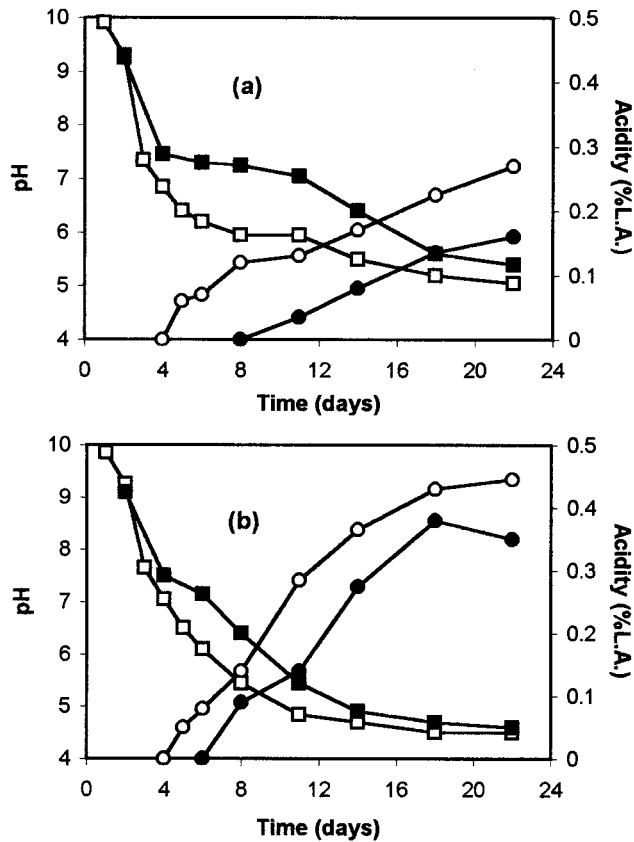
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Figure 2. Changes in pH and free acidity (% as lactic acid) of both brine and olive juice during (a) spontaneous and (b) controlled fermentation of Spanish-type green olives: pH of juice (■), pH of brine (□), acidity of juice (●), and acidity of brine (○). Coefficients of variation (%) were pH of brine, 2.2 and 0.6; pH of juice, 2.2 and 0.7; acidity of brine, 10.8 and 11.7; and acidity of juice, 18.3 and 0.7, for spontaneous and controlled fermentation, respectively.

of the medium during the first days. Lactobacilli reached stationary phase in both fermentations after 11 days. The use of the *Lb. pentosus* starter culture resulted in a clear decrease of *Enterobacteriaceae* growth (more than 2 log cycles) compared with spontaneous fermentation, thereby diminishing the risk of appearance of "gas pocket" spoilage, which has been related to growth of these microorganisms (Borbolla y Alcalá et al., 1960). Growth of yeast was affected by the starter culture, and occurred earlier and in higher numbers than in spontaneous fermentation. Differences were found during only the first 3 weeks of fermentation, but they were not significant from day 22 on (data not shown). These microorganisms do not appear to present any risk during the fermentation step of Spanish-type green olive and, on the contrary, their presence may be advantageous from a flavor standpoint (Garrido et al., 1995).

Changes in Physicochemical Characteristics. Production of acidity and decrease in pH occurred faster in brine than in olive juice, irrespective of the type of fermentation, and faster in controlled fermentation than in spontaneous fermentation (Figure 2). Throughout both fermentations, free acidity in brine was, on average, some 0.1% higher than that in olive juice, but the pH difference between brine and olive juice was lower in the case of controlled fermentation (0.5 units, on average, compared with 0.8 units for spontaneous fermentation). Significant ($p < 0.05$) differences between brine and juice were found for free acidity after 22 days brining in both fermentations, but for pH only in

Table 1. Physicochemical Characteristics in Fermented Green Olives after 22 days Brining[a]

characteristic	spontaneous fermentation		controlled fermentation	
	juice	brine	juice	brine
pH	5.37 a	5.04 b	4.60 c	4.49 c
free acidity (% lactic acid)	0.16 d	0.27 c	0.35 b	0.44 a
combined acidity (*N*)	0.138 a	0.109 c	0.121 b	0.113 c
combined/free acidity	7.82 a	3.65 b	3.13 c	2.29 d

[a] Values are means of duplicate fermentations. Means along a row with the same letter were not significantly different ($p < 0.05$).
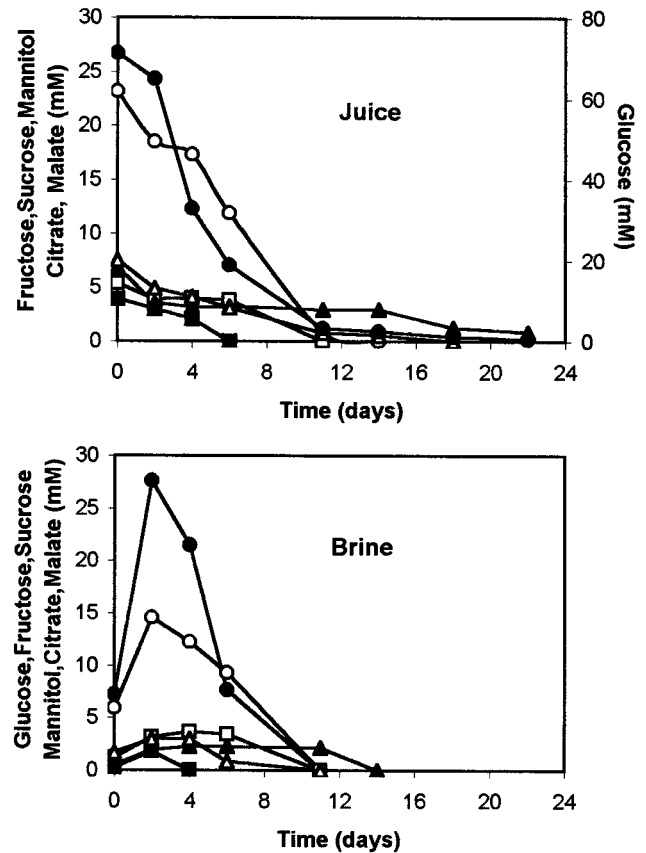

Figure 3. Substrate depletion in olive juice and brine during spontaneous fermentation of Spanish-type green olives: glucose (●), fructose (○), sucrose (■), mannitol (□), citric acid (▲), and malic acid (△). Coefficients of variation (%) were glucose, 19.4 and 48.6; fructose, 18.2 and 4.9; sucrose, 5.8 and 5.3; mannitol, 19.0 and 5.4; citric acid, 9.1 and 2.0; and malic acid, 17.8 and 3.1, for fermentation in juice and brine, respectively.

spontaneous fermentation (Table 1). Because preliminary operations (lye treatment and washing step) were identical in all assays, and the level of combined acidity is mainly dependent on these operations (Borbolla y Alcalá and Rejano, 1978), identical values of this parameter should be expected in both fermentations. This was true for brine, but significant ($p < 0.05$) differences were observed when comparing olive juice (Table 1). It is likely that equilibrium between brine and juice would be reached for free and combined acidity during the postfermentation stage for prolonged time, as found in other fermentation assays carried out in our department (data not shown). The final pH values were directly related to the combined/free acidity ratio, as expected according to the Henderson–Hasselbalch equation. It is noteworthy that a value of 4.4 or below was not reached in any case after 22 days, indicating that content of fermentable material in the fresh olives was
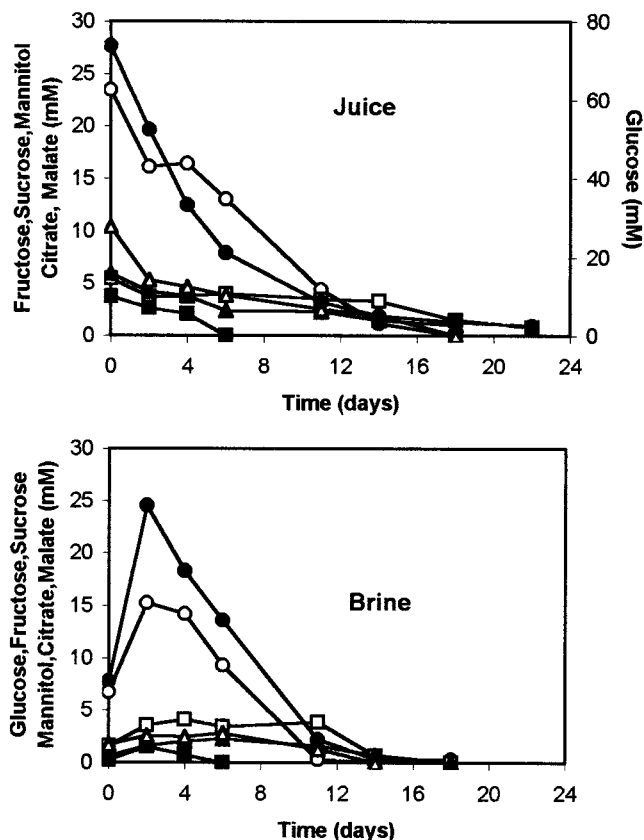
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Figure 4. Substrate depletion in olive juice and brine during controlled fermentation of Spanish-type green olives: glucose (●), fructose (○), sucrose (■), mannitol (□), citric acid (▲), and malic acid (△). Coefficients of variation (%) were glucose, 10.9 and 25.7; fructose, 7.8 and 13.8; sucrose, 14.4 and 8.2; mannitol, 6.1 and 11.0; citric acid, 11.3 and 22.1; and malic acid, 6.3 and 13.4, for fermentation in juice and brine, respectively.

relatively low. This is not surprising knowing that sugars in olive flesh show a continuous decline during ripening (Fernández-Díez, 1971), as is the case in the late harvest (middle of October) of the raw olives used in the present work. Industrially, sugar (glucose or sucrose) addition is recommended in such cases to continue the lactic acid fermentation and to achieve the proper level of acidity for preservation of olives (Fernández-Díez et al., 1985).

Changes in Fermentation Substrates and Products. Substrates present in the fruits diffused into the brines and were then degraded. In general, substrates were consumed faster in brine than in olive juice (Figures 3 and 4). No substrate was detected in brine after 22 days in either fermentation, but residual fermentable material (0.5 mM glucose and 0.9 mM citric acid in the spontaneous fermentation; 2.5 mM glucose, 0.8 mM mannitol, and 1.0 mM citric acid in the controlled fermentation) was detected in olive juice at that time. In both types of fermentation, consumption of sugars (glucose, fructose, and sucrose) started first, between 0 and 4 days of brining, before equilibrium between fruits and brine was approached, whereas degradation of mannitol, malic acid, and citric acid started after equilibrium.

Fermentation products measured were the major ones expected from sugar and organic acid metabolism by lactic acid bacteria, *Enterobacteriaceae*, and yeasts in vegetable products (Montaño et al., 1992). The final concentration of lactic acid—the main acid product in
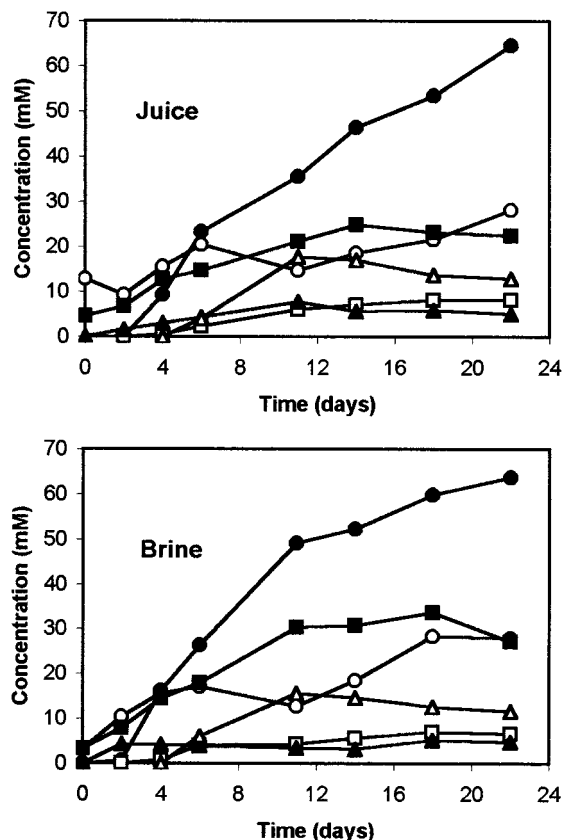
Figure 5. Product formation in olive juice and brine during spontaneous fermentation of Spanish-type green olives: lactic acid (●), acetic acid (○), ethanol (■), succinic acid (□), formic acid (▲), and 2,3-butanediol (△). Coefficients of variation (%) were lactic acid, 20.0 and 18.7; acetic acid, 11.5 and 11.4; ethanol, 16.0 and 18.2; succinic acid, 27.9 and 23.5; formic acid, 11.7 and 11.4; and 2,3-butanediol, 43.6 and 64.9, for fermentation in juice and brine, respectively.

Spanish-type green olive fermentation—was significantly ($p < 0.05$) higher in controlled fermentation than in spontaneous fermentation (ca. 100 mM against ca. 60 mM) (Figures 5 and 6, Table 2). During the first 10–14 days of brining, the lactic acid concentration was higher in brine than in olive juice, in agreement with the differences in substrate consumption rate, but thereafter, and once the substrates in brine were exhausted, the opposite occurred, and an equilibrium between brine and juice was eventually reached. Formation of volatile acidity during lye treatment in green olives was demonstrated by Borbolla y Alcalá et al. (1956). Acetic acid and ethanol were formed in olive juice during the lye treatment step (data not shown), probably from fragmentation by alkali of other compounds, such as sugars (Belitz and Grosch, 1999). Both compounds were also formed during fermentation, and, in the case of acetic acid, nonsignificant ($p < 0.05$) differences of concentration were found both between spontaneous and controlled fermentation and between olive juice and brine after 22 days of brining (Table 2). From the final concentrations of lactic and acetic acids, lactic to acetic ratios of 3.0 and 5.6 were obtained for spontaneous and controlled fermentation, respectively. A high ratio of volatile to nonvolatile acids appears to have a positive effect on flavor, as observed for sauerkraut (Trail et al., 1996), cucumbers (Chavasit et al., 1991), and kimchi (Fleming et al., 1995). However, this has not yet been demonstrated in olives. Ethanol was formed in a greater amount in the case of spontaneous fermentation. Equi-
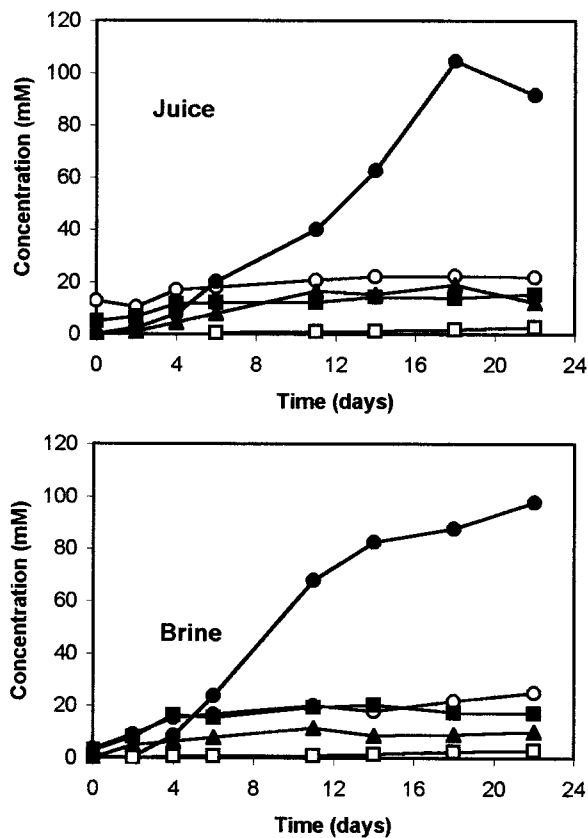
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Figure 6. Product formation in olive juice and brine during controlled fermentation of Spanish-type green olives: lactic acid (●), acetic acid (○), ethanol (■), succinic acid (□), and formic acid (▲). Coefficients of variation (%) were lactic acid, 8.3 and 14.4; acetic acid, 7.5 and 13.8; ethanol, 4.5 and 10.9; succinic acid, 15.5 and 17.6; and formic acid, 13.2 and 13.3, for fermentation in juice and brine, respectively.

librium for this compound was not reached after 22 days of brining in the case of spontaneous fermentation (Table 2). Succinic and formic acids were formed in both fermentations, but the equilibrium concentrations were significantly different: the level of succinic acid was higher in spontaneous fermentation, whereas that of formic acid was higher in controlled fermentation. However, the most noticeable difference between the two fermentations was found in relation to the level of 2,3-butanediol. This compound was detected only in spontaneous fermentation, and its origin can be attributed to *Enterobacteriaceae* activity, which was substantially lower in controlled fermentation (Figure 1). *Enterobacter cloacae*, the main species of *Enterobacteriaceae* found during Spanish-type green olive fermentation (Fernández-Díez et al., 1985), has been demonstrated to follow the butanediol fermentation pathway in olive brine (Montaño et al., 2000). Another fermentation product detected in both fermentations was acetoin, but it was found only in trace amounts (<1 mM) (data not shown).

An estimation of the substrate conversion to products was made for each type of fermentation both in brine and in olive juice (Table 2). For this, the initial substrate concentrations were calculated assuming equilibrium between olives and brine and were based on concentrations in the olive juice after the washing step. It was assumed that the volume of juice in the fermentor was 60% of the weight of the fruit (Fernández-Díez et al., 1985). Carbon recovery estimates from both spontaneous and controlled fermentation were not significantly

Table 2. Substrates Consumed, Products Formed, and % Carbon Recovery in Fermented Green Olives after 22 days Brining[a]

	spontaneous fermentation		controlled fermentation	
	juice	brine	juice	brine
substrate consumed (mM)[b]				
glucose	46.4 b	46.9 a	44.4 c	46.9 a
fructose	16.5 a	16.5 a	16.5 a	16.5 a
mannitol	3.9 a	3.9 a	3.1 b	3.9 a
sucrose	2.0 a	2.0 a	2.0 a	2.0 a
citric acid	2.5 b	3.4 a	2.5 b	3.4 a
malic acid	5.7 a	5.7 a	5.7 a	5.7 a
products formed (mM)[c]				
lactic acid	64.2 b	63.4 b	91.1 a	97.3 a
acetic acid	21.4 a	21.2 a	15.2 a	18.4 a
ethanol	18.8 b	23.5 a	11.4 c	13.4 c
succinic acid	8.1 a	6.6 a	2.7 b	3.0 b
formic acid	4.9 b	4.7 b	12.1 a	10.1 a
2,3-butanediol	12.8 a	11.6 a	ND[d]	ND
carbon recovery (%)	78.1 a	75.8 a	78.6 a	80.1 a

[a] Values are means of duplicate fermentations. Means along a row with the same letter were not significantly different (*p* < 0.05). [b] Initial concentrations of substrates were calculated assuming equilibrium between the olives and brine. Olive juice after washing step contained 78.9 mM glucose, 27.7 mM fructose, 6.6 mM mannitol, 3.3 mM sucrose, 5.8 mM citric acid, and 9.6 mM malic acid. [c] Concentrations represent the net increase over those initially present. Olive juice after washing step contained 11.3 mM acetic acid, 6.3 mM ethanol, and 0.6 mM lactic acid. [d] ND, not detected.

(*p* < 0.05) different, giving a mean of some 78%. This indicates that substrates were degraded to compounds not measured in this study, such as CO_2, as a result of growth of microorganisms other than homofermentative lactic acid bacteria. Montaño et al. (1993) obtained a carbon recovery slightly above 100% in the case of *Lb. plantarum* in green olive fermentation. Those authors were able to achieve pure culture fermentation by taking aseptic precautions in the olive preparation prior to inoculation with *Lb. plantarum* and using a suitable fermentor. However, this procedure is currently considered economically impractical for commercial use. In the present work, in the case of controlled fermentation, no effort was made to remove or eliminate the naturally occurring microflora present in the olives, fermentor, etc., as it is also not done in industry.

It can be concluded that, for the first time, a complete study on fermentation chemistry in Spanish-type green olives has been carried out. Lactic acid fermentation progresses more slowly in olive juice than in brine, but eventually most metabolic products approach equilibrium. However, in the case of physicochemical parameters, significant differences between juice and brine may occur when sugars are exhausted. Although these differences seem to disappear at prolonged time during the postfermentation stage, this finding should be borne in mind if olives are packed early. In such situations, olive juice should be analyzed instead of brine, to fix more exactly the amount of acid in the cover brine of packed olives.

LITERATURE CITED

Belitz, H.-D.; Grosch, W. *Food Chemistry*, 2nd ed.; Springer: Berlin, Germany, 1999.

Borbolla y Alcalá, J. M. R. Sobre la preparación de la aceituna estilo sevillano. La aceituna en salmuera. *Grasas Aceites* **1979**, *30*, 31−36.

Sánchez et al.

Borbolla y Alcalá, J. M. R.; Rejano, L. Sobre la preparación de la aceituna estilo sevillano. El lavado de los frutos tratados con lejía. *Grasas Aceites* **1978**, *29*, 281−291.

Borbolla y Alcalá, J. M. R.; Rejano, L. Sobre la preparación de la aceituna estilo sevillano. La fermentación I. *Grasas Aceites* **1979**, *30*, 175−185.

Borbolla y Alcalá, J. M. R.; Rejano, L. Sobre la preparación de la aceituna estilo sevillano. La fermentación II. *Grasas Aceites* **1981**, *32*, 103−113.

Borbolla y Alcalá, J. M. R.; Herrera, C. G.; Cancho, F. G.; Díez, M. J. F.; Quijano, R. G.; Tamayo, A. I.; Pellissó, F. G.; Ladrón, R. V.; García, R. G. *El aderezo de aceitunas verdes.* CSIC: Madrid, Spain, 1956.

Borbolla y Alcalá, J. M. R.; Díez, M. J. F.; Cancho, F. G. Estudios sobre el aderezo de aceitunas verdes. XIX. Nuevas experiencias sobre el "alambrado". *Grasas Aceites* **1960**, *11*, 256−260.

Chavasit, V.; Hudson, J. M.; Torres, J. A.; Daeschel, M. A. Evaluation of fermentative bacteria in a model low salt cucumber juice brine. *J. Food Sci.* **1991**, *56*, 462−465.

Daeschel, M. A.; Andersson, R. E.; Fleming, H. P. Microbial ecology of fermenting plant materials. *FEMS Microbiol. Rev.* **1987**, *46*, 357−367.

Fernández-Díez, M. J. The olive. In *The Biochemistry of Fruits and Their Products*; Hulme, A. C., Ed.; Academic Press: London, 1971; pp 255−279.

Fernández-Díez, M. J.; Castro, R.; Fernández, A. G.; Cancho, F. G.; Pellissó, F. G.; Vega, M. N.; Moreno, A. H.; Mosquera, I. M.; Navarro, L. R.; Quintana, M. C. D.; Roldán, F. S.; García, P. G.; Castro, A. *Biotecnología de la aceituna de mesa*; CSIC, Ed.; CSIC: Madrid, Spain, 1985.

Fleming, H. P.; Kyung, K. H.; Breidt, F. Vegetable fermentations. In *Biotechnology*; Rehm, H.-J., Reed, G., Eds.; VCH: Weinheim, Germany, 1995; pp 629−661.

Garrido, A.; García, P.; Brenes, M. Olive fermentations. In *Biotechnology*; Rehm, H.-J., Reed, G., Eds.; VCH: Weinheim, Germany, 1995; pp 593−627.

Montaño, A.; Sánchez, A. H.; Rejano, L. Rapid quantitative analysis of headspace components of green olive brine. *J. Chromatogr.* **1990**, *521*, 153−157.

Montaño, A.; Castro, A.; Rejano, L. Transformaciones bioquímicas durante la fermentación de productos vegetales. *Grasas Aceites (Sevilla)* **1992**, *43*, 352−360.

Montaño, A.; Sánchez, A. H.; Castro, A. Controlled fermentation of Spanish-type green olives. *J. Food Sci.* **1993**, *58*, 842−844, 852.

Montaño, A.; Sánchez, A. H.; Castro, A. Changes in the amino acid composition of green olive brine due to fermentation by pure culture of bacteria. *J. Food Sci.* **2000**, *65*, 1022−1027.

Trail, A. C.; Fleming, H. P.; Young, C. T.; McFeeters, R. F. Chemical and sensory characterization of commercial sauerkraut. *J. Food Qual.* **1996**, *19*, 15−30.

JF000563U