Food Chemistry 110 (2008) 932–937

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Inhibitors of lactic acid fermentation in Spanish-style green olive brines of the Manzanilla variety

Eduardo Medina, Concepción Romero, Antonio de Castro, Manuel Brenes, Aranzazu García *

Food Biotechnology Department, Instituto de la Grasa (CSIC), Avda. Padre García Tejero 4, 41012 Seville, Spain

ARTICLE INFO

Article history:
Received 20 December 2007
Received in revised form 27 February 2008
Accepted 28 February 2008

Keywords:
Lactobacillus pentosus
Fermentation
Antimicrobial
Phenolic
Oleoside
Elenolic acid

ABSTRACT

Frequently, a delay or lack of lactic acid fermentation occurs during the processing of Spanish-style green olives, in particular of the Manzanilla variety. Many variables can affect the progress of fermentation such as temperature, nutrients, salt concentration, antimicrobials in brines, and others. In this study, it was demonstrated that an inappropriate alkaline treatment (low NaOH strength and insufficient alkali penetration) allowed for the presence of several antimicrobial compounds in brines, which inhibited the growth of *Lactobacillus pentosus*. These substances were the dialdehydic form of decarboxymethyl elenolic acid either free or linked to hydroxytyrosol and an isomer of oleoside 11-methyl ester. Olive brines, from olives treated with a NaOH solution of low concentration up to 1/2 the distance to the pit, contained these antimicrobials, and no lactic acid fermentation took place in them. By contrast, a more intense alkaline treatment (2/3 lye depth penetration) gave rise to an abundant growth of lactic acid bacteria without any antimicrobial in brines. Therefore, the precise cause of stuck fermentation in Manzanilla olive brines was demonstrated for the first time and this finding will contribute to better understand the table olive fermentation process.

1. Introduction

Table olives have been a component of the Mediterranean diet for centuries and their consumption is increasing worldwide because of their nutritional and palatable characteristics. Among the different types of commercial table olives, Spanish-style green olives are the most popular, with Spain being the main producing country and the Manzanilla variety the most employed for this appreciated olive preparation (Garrido-Fernández, Fernández-Díaz, & Adams, 1997).

Spanish-style green olive processing consists of treating the fruits with a dilute NaOH solution (2–3%), followed by 1–2 washings with tap water and placing the olives in a brine (9–11%) where a spontaneous lactic acid fermentation takes place (Montaño, Sánchez, & de Castro, 1993). Inoculation of the brine with a lactic starter culture reduces the probability of spoilage and is currently employed by many processors (Marsilio et al., 2005; Ruiz-Barba, Cathcart, Warner, & Jiménez-Díaz, 1994). However, a delay or lack of fermentation of olives, especially those of the Manzanilla variety, often occurs and there is no explanation for this phenomenon. Many variables can affect the progress of lactic acid fermentation such as salt concentration (Tassou et al., 2007), temperature (Rodríguez-Borbolla & Rejano, 1979), nutrient content (Montaño, Sánchez, & de Castro, 2000), inhibitors (Fleming & Etchells, 1967) and others. The presence of a new polymer inhibitor of lactic acid bacteria (LAB) growth has recently been reported in acidified brines (de Castro, Romero, & Brenes, 2005) although stuck fermentation also occurs without any acidification of the medium.

Earlier investigations related the difficulties of LAB growth in olive brines with the presence of polyphenols (Juven & Henis, 1970). Oleuropein and its aglycon first (Fleming, Walter, & Etchells, 1973) and hydroxytyrosol later (Ruiz-Barba, Brenes, Jiménez, García, & Garrido, 1993) were attributed to antimicrobial activity. However, oleuropein is not found in the brines of Spanish-style green olives (Brenes, Rejano, García, Sánchez, & Garrido, 1995) because it is hydrolysed during the NaOH treatment (Brenes & de Castro, 1998), and hydroxytyrosol is found in both brines of treated and untreated olives with NaOH (Ruiz-Barba et al., 1993). Contradictory results are still being reported about antimicrobials in table olives (Landete, Curiel, Rodriguez, Rivas, & Muñoz, 2008; Pereira et al., 2006; Servili et al., 2006) although we have demonstrated that the main antimicrobials in brines of untreated olives with NaOH are an isomer of oleoside 11-methyl ester, the dialdehydic form of decarboxymethyl elenolic acid (EDA) and the latter substance linked to hydroxytyrosol (Hy-EDA) (Medina, Brenes, Romero, García, & de Castro, 2007). Whether the presence of these antimicrobials in Spanish-style green olive brines could provoke stuck fermentation is unsolved, in particular in the brines of olives treated with a low concentration of NaOH (Rodriguez-Borbolla, Fernández-Díaz, & González-Cancho, 1969).

* Corresponding author. Tel.: +34 954690850; fax: +34 954691262.
E-mail address: aranzag@cica.es (A. García).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.02.084

Therefore, the aim of this work was to demonstrate the effects of different alkaline treatments on the presence of several antimicrobial compounds, which inhibited LAB fermentation of Manzanilla olives.

2. Materials and methods

2.1. Preparation of Spanish-style green olives

Experiment A. Olives of the Manzanilla variety with a green–yellow colour on the surface were put into four PVC vessels. Five kilograms of fruits were covered with 4 L of 2% NaOH and maintained in the alkaline solution for 7 h until the lye had penetrated 2/3 the way to the pit of the olives. In another two vessels olives were maintained in the alkaline solution for only 3.5 h (lye penetrated 1/2 the way to the pit of the fruit). The temperature ranged between 22–24 °C. After the alkaline treatment, all olives were washed with tap water for 16 h and covered with brine (11% NaCl). When the pH of the brines was below 7 (5 days later), they were inoculated with a starter culture of *Lactobacillus pentosus* ATCC 8041 previously adapted to the saline environment and washed with saline. The initial population of the starter culture inoculated in the brines was 2.8×10^6 CFU/mL. Anaerobic conditions were reached by covering the surface of the brine with a floating cap.

Experiment B. Olives of the Manzanilla variety with a green–yellow colour on the surface were harvested in a different orchard than those of experiment A in order to confirm results because of the variability on olive fermentation. Also, two different concentration of NaOH were used to study its effect on olive fermentation. Fruits were put in 8 PVC vessels similar to those used for experiment A. Four different alkaline treatments were assayed: 2.2% NaOH for 6.5 h (lye penetrated 2/3 of the distance to the pit), 2.2% NaOH for 4.5 h (lye penetrated 1/2 of the distance to the pit), 1.7% NaOH for 9 h (lye penetrated 2/3 of the distance to the pit), and 1.7% NaOH for 6.5 h (lye penetrated 1/2 of the distance to the pit). The temperature ranged between 22–24 °C. The following steps were similar to those carried out in experiment A.

Two additional PVC vessels were filled with olives and covered with an acidified brine (5% NaCl, 0.5% acetic acid) without any alkaline treatment.

2.2. Testing oleoside 11-methyl ester for antimicrobial activity

This compound was isolated from three different brines of experiment B: (i) brine of olives untreated with NaOH; (ii) brine of olives treated with 1.7% NaOH until the lye penetrated two-thirds of the distance to the pit; and (iii) brine of olives treated with 1.7% NaOH until the lye penetrated one-half the distance to the pit. This compound was isolated from brines of the 1.7% NaOH treatment because lactic acid fermentation was inhibited to a large extent when the alkali penetrated one-half the distance to the pit. Samples were taken at 5 days after brining.

Oleoside 11-methyl ester was isolated by analytical HPLC. The chromatographic system consisted of a Waters 717 plus autosampler, a Waters 600E pump, a Waters 996 diode array detector and a Waters Fraction Collector II (Waters Inc. Mildford, MA). A Spherisorb ODS-2 (5 μm, 25 cm × 4.6 mm i.d., Waters Inc.) column was used. Separation was achieved using an elution gradient with an initial composition of 90% water (pH adjusted to pH 3.8 with 2 N HCl, 520 μL/L) and 10% methanol. The concentration of the latter solvent was increased to 30% over 10 min and maintained for 20 min. Subsequently, the methanol percentage was raised to 40% over 10 min, maintained for 5 min, and then increased to 50%. Finally, the methanol percentage was increased to 60%, 70%, and 100% in 5 min periods. A flow of 1 mL/min and a temperature of 35 °C were used. The compound was monitored at 240 nm.

Fractions from 80 HPLC runs were collected. The pooled extract was evaporated under vacuum to dryness and the residue was dissolved in 1 mL of deionized water. Finally, the purity and concentration of compounds was measured by HPLC. A control run was also performed by injecting deionized water and collecting all fractions of the run.

The antimicrobial activity of the isolated compounds was assessed in an aseptic brine obtained from olives of the Gordal variety preserved for two months in a sterile acidified brine, as described elsewhere (Medina et al., 2007). One hundred microliters of the Gordal brine were inoculated with a diluted overnight culture of *L. pentosus* to get, after the addition of 50 μL of the isolated compound, a population of 6.2 log CFU/mL. The mixture was incubated at 32 °C for 48 h and plated directly spreading both 50 μL and the 10^{-1} dilution (0.1% peptone) with a Spiral Plater (Don Whitley Sci. Ltd, model wasp 2, Shirpley, UK). The concentration of each isolated compound tested was 0.86 mM, and a control test was performed with the control HPLC run extract obtained.

2.3. Microbiological analyses

Brine samples and appropriate decimal dilutions (in sterile 1 g/L peptone water) 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, Darmstadt, Germany) incubated at 37 °C, lactobacilli on MRS agar (Oxoid) and yeasts on OGYE agar (Oxoid), both the latter two were incubated at 32 °C. Colonies were enumerated after 24, 48 or 72 h of incubation.

2.4. Chemical analyses

Titratable acidity, pH and combined acidity of brines were measured using a Metrohm 670 Titroprocessor (Herisau, Switzerland). Titratable 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.

Sugars (glucose, fructose, sucrose and mannitol), organic acids (lactic, acetic and propionic) and ethanol were analysed by HPLC, as described elsewhere (Brenes & de Castro, 1998).

Phenolic and oleosidic compounds were analysed by HPLC. A mixture of 250 μL of brine, 250 μL of internal standard (2 mM syringic acid) and 500 μL of deionized water was filtered through 0.45 μm pore size nylon filter. An aliquot (20 μL) was injected into the chromatograph. The analytical column, mobile phases, gradient and equipment were the same as those used for the oleoside 11-methyl ester isolation except the aqueous mobile phase, which was acidified with phosphoric acid to pH 3.0 (Medina et al., 2007).

3. Results and discussion

Phenolic and oleosidic compounds were analysed in the lyes and washing waters in order to look for the antimicrobials recently reported in table olives (Medina et al., 2007). In general, the phenolic and oleosidic substances were more concentrated in lyes which had penetrated 2/3 of the distance to the pit than 1/2, regardless of the NaOH concentration (Table 1). Obviously, these compounds diffused from the olives to the lye, and as the lye treatment prolonged the solutions were enriched. In contrast, slight differences were found among washing waters in relation to the lye penetration. Hydroxytyrosol and oleoside 11-methyl ester were detected in a very high concentration in both lyes and washing waters, followed by oleoside and hydroxytyrosol 4-glucoside. Hydroxytyrosol

Table 1
Phenolic and oleosidic compounds in the spent NaOH (lye) and washwater solutions of the Spanish-style green olive processing

Compound (mM)	Lye				Washwater			
	A[a]	B	C	D	A	B	C	D
Hydroxytyrosol	36.5 (2.2)[b]	27.8 (1.8)	36.0 (0.1)	23.1 (0.3)	28.2 (1.4)	29.2 (2.4)	28.3 (5.0)	32.8 (0.7)
Hydroxytyrosol 1-glucoside	7.1 (0.5)	5.4 (0.8)	7.4(0.6)	4.8 (0.6)	5.2 (0.1)	6.2 (0.4)	6.5 (0.3)	5.4 (1.0)
Hydroxytyrosol 4-glucoside	10.0 (0.5)	7.4 (0.6)	9.0 (0.1)	4.8 (0.4)	6.0 (0.6)	6.0 (1.0)	6.0 (1.0)	8.3 (2.4)
Tyrosol	2.1 (0.1)	1.5 (0.1)	2.2 (0.1)	1.2 (0.1)	2.2 (0.1)	2.0 (0.1)	2.7 (0.5)	2.8 (0.5)
Oleoside	15.7 (0.3)	12.5 (0.7)	17.9 (1.1)	11.2 (0.3)	4.9 (0.1)	4.1 (0.4)	11.8 (2.2)	11.8 (2.1)
Oleoside 11-methyl ester	22.8 (0.7)	18.1 (1.0)	21.0 (0.5)	12.5 (0.5)	23.4 (1.0)	24.8 (1.7)	25.5 (4.3)	29.0 (3.9)
Secologanoside	5.6 (0.2)	4.6 (0.2)	5.8 (0.2)	3.6 (0.1)	3.6 (0.2)	3.9 (0.2)	4.5 (0.9)	5.2 (0.7)

Data are from experiment B.
None secoxyloganin, Hy-EDA or EDA were detected in these solutions.
[a] A: 2/3 lye penetration (1.7% NaOH); B: 1/2 lye penetration (1.7% NaOH); C: 2/3 lye penetration (2.2% NaOH); D: 1/2 lye penetration (2.2% NaOH).
[b] Standard deviation.

and oleoside 11-methyl ester are formed during the alkaline hydrolysis of the oleuropein (Brenes & de Castro, 1998), which is the major phenolic compound in raw olive fruits, and it is predictable to find them in a very high concentration in lyes and washing waters. None of the new table olive antimicrobials such as EDA and Hy-EDA were detected in any of these solutions.

LAB grow in the washing waters of the Spanish-style green olive processing (de Castro & Brenes, 2001) unless a polymer is formed during their acidification (de Castro, Romero, & Brenes, 2005). However, this polymer can be easily eliminated by centrifugation and an abundant growth of LAB occurs, which means that no other anti-LAB compounds are currently present in these washing waters as confirmed by the data reported in Table 1.

Another two interesting findings were (i) the high content in oleoside of the washing waters obtained from the 2.2% NaOH treatment, and (ii) the absence of secoxyloganin in any solution. It could be assumed that a higher demethylation of oleoside 11-methyl ester occurred at high NaOH concentrations (Capozzi, Piperno, & Uccella, 2000). Furthermore, it is worthy to note that secoxyloganin was not found in any of the lyes, washing waters or brines analysed. This compound is usually present in the brines of olives which are not treated with NaOH (Medina et al., 2007). Hence, the analysis of this compound in olive brines could be useful to detect that a NaOH treatment of olives has not been carried out.

Hydroxytyrosol and tyrosol were the prevailing polyphenols in the brines of olives fermented for 7 months (Tables 2 and 3), the concentration of them being higher in brines of olives treated 1/2 depth lye penetration than 2/3 depth. These substances were also found in a higher concentration in brines from experiment A (Table 2) than from B (Table 3). In contrast, brines from experiment B had a higher concentration in hydroxytyrosol 4-glucoside and hydroxytyrosol 1-glucoside than those of experiment A. There are contradictory data about the inhibition of LAB growth by hydroxytyrosol (Landete et al., 2008; Ruiz-Barba et al., 1993) although we have recently been demonstrated that hydroxytyrosol is not the main antimicrobial compound in olive brines (Medina et al., 2007).

Table 2
Phenolic compounds in the olive brines fermented for 7 months

Compound (mM)	Treatment	
	2/3 Depth penetration	1/2 Depth penetration
Hydroxytyrosol	11.4 (0.4)[a]	15.1 (0.7)
Hydroxytyrosol 1-glucoside	<0.1	<0.1
Hydroxytyrosol 4-glucoside	<0.1	1.1 (0.1)
Tyrosol	1.4 (0.1)	1.6 (0.1)

Data are from experiment A.
[a] Standard deviation

Table 3
Phenolic compounds in the olive brines fermented for 7 months

Compound (mM)	Treatment			
	A[a]	B	C	D
Hydroxytyrosol	8.7 (0.3)[b]	9.7 (0.3)	9.9 (0.1)	10.1 (1.8)
Hydroxytyrosol 1-glucoside	5.7 (0.4)	6.7 (0.3)	5.8 (0.2)	6.5 (0.2)
Hydroxytyrosol 4-glucoside	0.4 (0.1)	0.4 (0.1)	<0.1	<0.1
Tyrosol	0.9 (0.2)	0.8 (0.1)	0.7 (0.1)	0.7 (0.1)

Data are from experiment B.
[a] A: 2/3 lye penetration (1.7% NaOH); B: 1/2 lye penetration (1.7% NaOH); C: 2/3 lye penetration (2.2% NaOH); D: 1/2 lye penetration (2.2% NaOH).
[b] Standard deviation

With regards to the LAB growth in the olive brines, it was confirmed for both experiment A and B that *L. pentosus* cells died in the brines of olives treated with NaOH up to 1/2 the distance to the pit (Figs. 1 and 2). Neither lactic acid nor LAB were detected during the first 15 days of brining whereas an abundant growth of LAB and formation of lactic acid was observed in the brines of olives treated with NaOH up to 2/3 the distance to the pit. In addition, glucose was consumed rapidly during the first 10–15 days in brines of the latter olives (Fig. 1).

Surprisingly, LAB grew in all olive brines treated with 2.2% NaOH regardless of the lye penetration depth but did not grow in the brines of olives treated with 1.7% NaOH with 1/2 depth lye penetration (Fig. 2). This phenomenon was observed during the first 15 days of brining along with a higher concentration of glucose in the latter brines than in the rest due to minimal fermentation. Furthermore, a higher population of *Enterobacteriaceae* was found in the brines of olives treated with NaOH only up to 1/2 the distance to the pit (Fig. 3). A similar trend was observed for yeasts (data not shown). This effect was observed for experiment A (2% NaOH) and only in brines of experiment B with olives treated with 1.7% NaOH. Additionally, the pH in fermented brines ranged from 4.3–4.5 after 30 days of brining and 5.0 in non-fermented brines. Titratable acidity was obviously higher in fermented than non-fermented brines and no significant differences were observed for combined acidity among treatments (data not shown), although the higher the NaOH concentration treatment, the higher the combined acidity in brines.

All these data together indicate that the alkaline treatment of Spanish-style green olives must be carried out until the lye penetrates 2/3 the distance to the pit, and the strength of the lye should be higher than 1.7% if a lactic acid fermentation is required. This conclusion is not new because Rodriguez-Borbolla et al. (1969) had similar results. However, the explanation for this phenomenon remained unsolved. On many occasions, processors detect stuck fermentation and they do not know the cause of it. Low tempera-

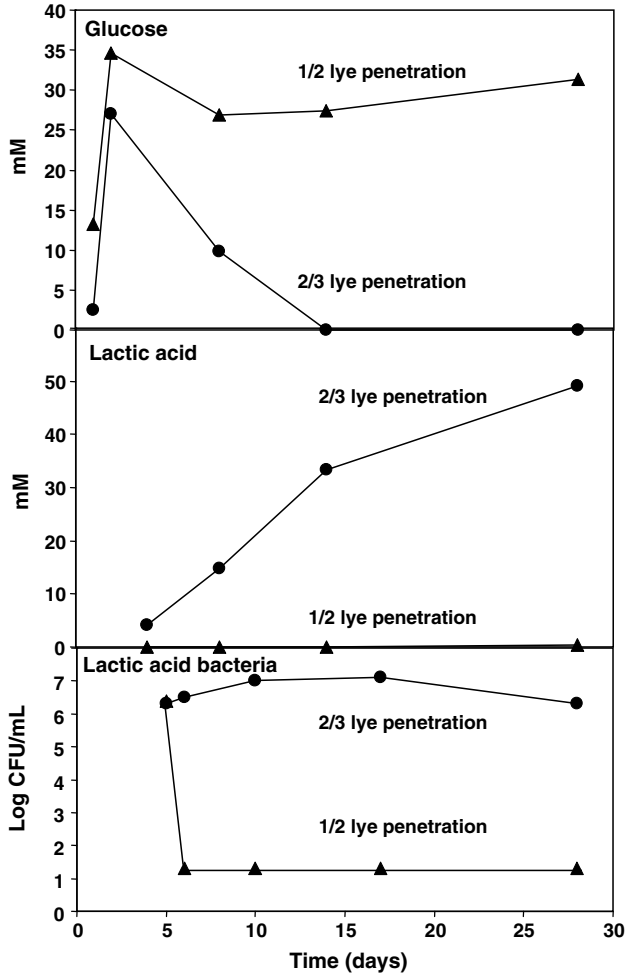

Fig. 1. Evolution of glucose, lactic acid and LAB in olive brines during the first month of fermentation. Data are from experiment A. Pooled standard deviation of glucose, lactic acid and lactic acid bacteria was 2.0, 5.6, and 0.4, respectively.

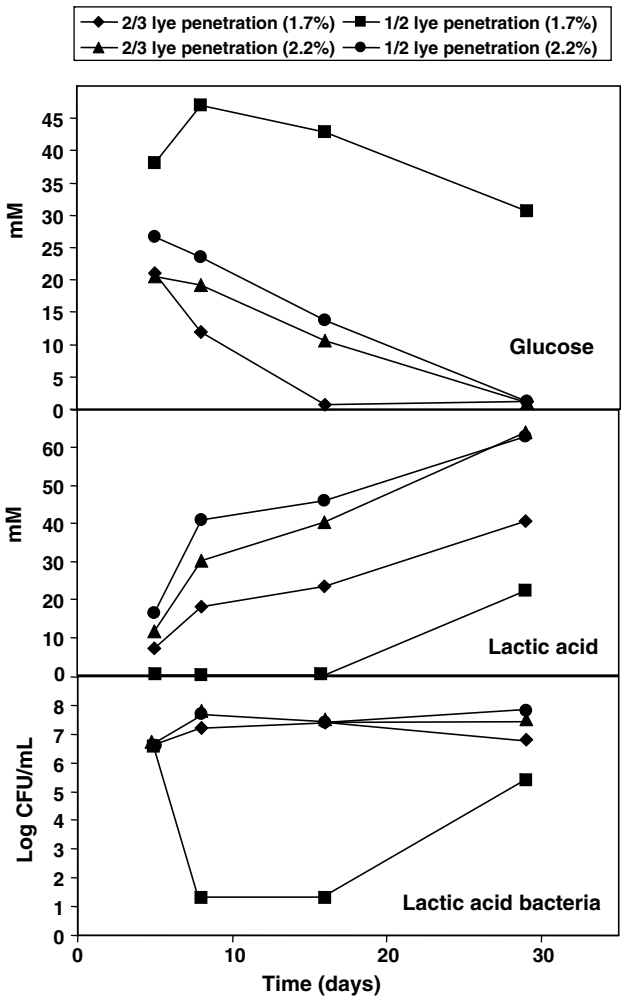

Fig. 2. Evolution of glucose, lactic acid and LAB in olive brines during the first month of fermentation. Data are from experiment B. Pooled standard deviation of glucose, lactic acid and lactic acid bacteria was 2.8, 6.1 and 0.3, respectively.

ture and low sugar concentration are good reasons for stuck fermentation but in our study brines were inoculated with LAB, the temperature was the same for all treatments (ambient temperature) and there was enough glucose in brines (Figs. 1 and 2).

Therefore, we focused our attention on the presence of antimicrobials in the olive brines, in particular Hy-EDA, EDA and oleoside 11-methyl ester (Medina et al., 2007). Thus, these substances were analysed in brines during the first 30 days of fermentation (Figs. 4 and 5). Hy-EDA and EDA were only found in brines where LAB did not grow, and they were not detected in brines of olives treated with 2.2% NaOH up to 2/3 the distance to the pit or even in brines of olives treated up to 1/2 the distance to the pit with a 2.2% NaOH solution (experiment B). Hence, there was a direct relationship between growth inhibition of LAB and the presence of these antimicrobials in brines. It must also be noted that Hy-EDA and EDA were not found in the brines of olives treated with a 2.2% NaOH up to 1/2 the distance to the pit. In this case, we could assume that the higher pH in olive flesh (Table 4) in comparison with 1.7% NaOH treated olives either did not allow for the formation of these substances or hydrolysed them during processing.

Another question remained unsolved: what was the contribution of oleoside 11-methyl ester to LAB growth inhibition? This compound was found in all brines (Figs. 4 and 5) and it produces anti-LAB activity (Medina et al., 2007), although it is only an isomer of oleoside 11-methyl ester which has antimicrobial properties. In order to clarify this point, compounds corresponding to the peak of oleoside 11-methyl ester were isolated by HPLC from the brines of olives treated with 1.7% NaOH up to 1/2 and 2/3 the distance to the pit. Then, these two compounds were added to a Gordal brine and inoculated with *L. pentosus*. The microorganism grew well in the brine without any added compound and in the brine with oleoside 11-methyl ester isolated from the brine of olives treated with NaOH up to 2/3 the distance to the pit. In contrast, the initial population was reduced 2 logs CFU/mL when oleoside 11-methyl ester was isolated from the brine of olives treated with NaOH up to 1/2 the distance to the pit or brines from olives which were not treated with NaOH (Fig. 6). Thus, it was demonstrated that oleoside 11-methyl ester was found, as its isomer with anti-LAB activity, in brines of olives non-treated or treated with an insufficient NaOH solution. Moreover, this anti-lactic isomer of oleoside 11-methyl ester was detected in brines where Hy-EDA and EDA were also found.

In summary, the results of this work have shown that an insufficient lye treatment of olives with an NaOH solution of low concentration gives rise to stuck lactic acid fermentation. This phenomenon is directly related to the presence of the antimicrobial compounds Hy-EDA, EDA in brines and an isomer of oleoside 11-methyl ester. Therefore, the information obtained in this study will be useful for processors to better understand the development of fermentation of Spanish-style green olives.

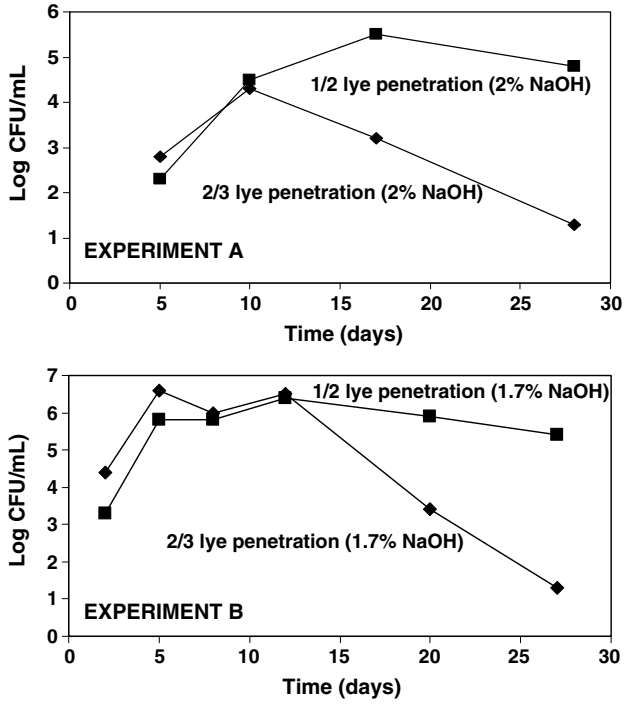

Fig. 3. Evolution of *Enterobacteriaceae* in olive brines during the first month of fermentation. Pooled standard deviation was 0.5.

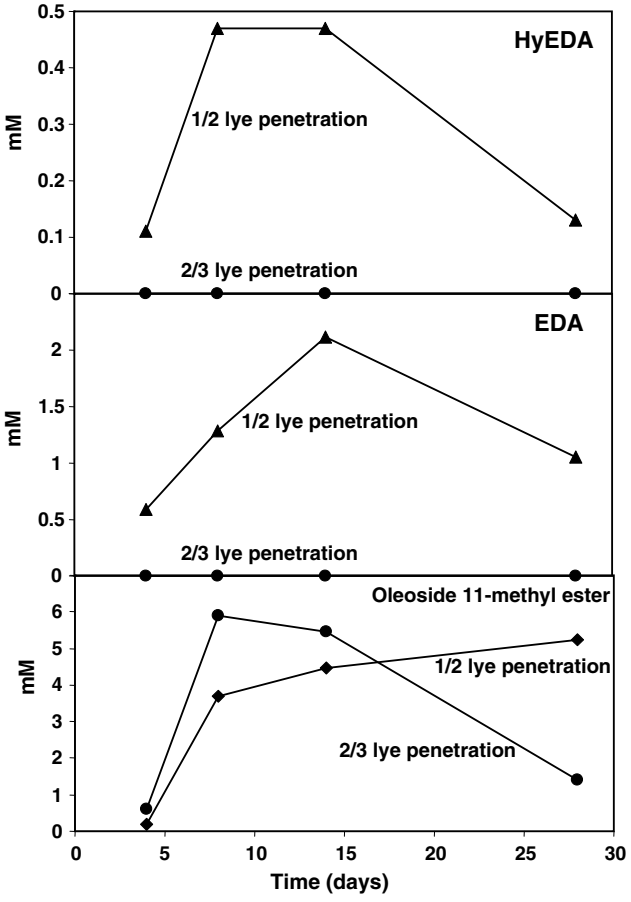

Fig. 4. Evolution of anti-LAB compounds in olive brines during the first month of fermentation. Data are from experiment A. Pooled standard deviation of Hy-EDA, EDA and oleoside 11-methyl ester was 0.07, 0.43 and 1.1, respectively.

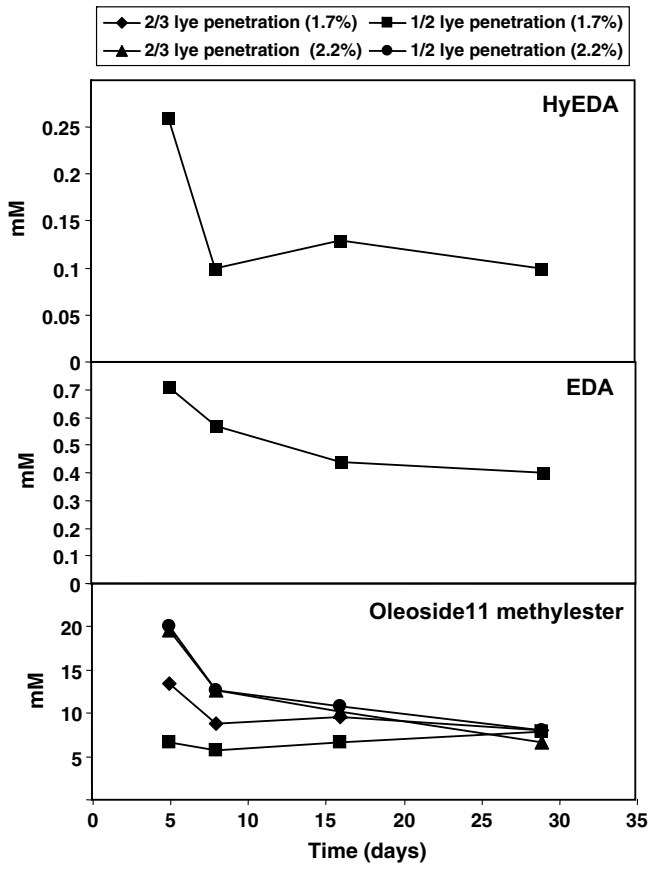

Fig. 5. Evolution of anti-LAB compounds during the first month of fermentation. Data are from experiment B. Hy-EDA and EDA were only detected in the 1.7% NaOH treatment and 1/2 lye penetration. Pooled standard deviation of Hy-EDA, EDA and oleoside 11-methyl ester was 0.06, 0.12 and 0.84 respectively.

Table 4
pH in the olive flesh and cover solutions at the beginning of processing

pH	Treatment			
	A[a]	B	C	D
Olive flesh after the washing step	10.1	9.6	11.4	10.8
Olive flesh after 1 day of brining	9.0	8.6	10.0	9.8
Washing water	9.9	9.7	10.8	10.7
Brine (1 day)	8.2	6.4	9.4	9.0

Data are from experiment B.
[a] A: 2/3 lye penetration (1.7% NaOH); B: 1/2 lye penetration (1.7% NaOH); C: 2/3 lye penetration (2.2% NaOH); D: 1/2 lye penetration (2.2% NaOH).

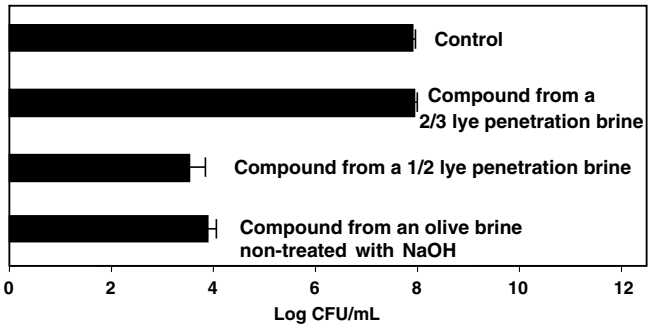

Fig. 6. Effect of oleoside 11-methyl ester isolated from different sources on the growth of LAB. Counts after 48 h incubation of a Gordal brine inoculated with 6.2 log CFU/mL. The Gordal brine was used as growth medium and different HPLC isolated isomers of oleoside 11-methyl ester were spiked in this medium. Bars mean standard deviation.

Acknowledgements

This study was supported by a grant from the Spanish Government and the European Union FEDER Funds (Project AGL-2003-001552.

References

Brenes, M., & de Castro, A. (1998). Transformation of oleuropein and its hydrolysis products during Spanish-style green olive fermentation. *Journal of the Science and Food Agriculture, 77*, 353–358.

Brenes, M., Rejano, L., García, P., Sánchez, A. H., & Garrido, A. (1995). Biochemical changes in phenolic compounds during Spanish-style green olive processing. *Journal of Agricultural and Food Chemistry, 43*, 2702–2706.

Capozzi, F., Piperno, A., & Ucella, N. (2000). Oleuropein site selective hydrolysis by technomimetic nuclear magnetic resonante experiments. *Journal of Agricultural and Food Chemistry, 48*, 1623–1629.

De Castro, A., & Brenes, M. (2001). Fermentation of washing waters of Spanish-style green olive processing. *Process Biochemistry, 36*, 797–802.

De Castro, A., Romero, C., & Brenes, M. (2005). A new polymer inhibitor of lactobacillus growth in table olives. *European Food Research and Technology, 221*, 192–196.

Fleming, H. P., & Etchells, J. L. (1967). Occurrence of an inhibitor of lactic acid bacteria in green olives. *Applied Microbiology, 15*, 1178–1184.

Fleming, H. P., Walter, W. M., & Etchells, J. L. (1973). Antimicrobial properties of oleuropein and products of its hydrolysis from green olives. *Applied Microbiology, 20*, 777–782.

Garrido-Fernández, A., Fernández-Díaz, M. J., & Adams, M. R. (1997). *Table olives: Production and processing*. London: Chapman and Hall.

Juven, B., & Henis, Y. (1970). Studies on the antimicrobial activity of olive phenolic compounds. *Journal Applied Bacteriology, 33*, 721–732.

Landete, J. M., Curiel, J. A., Rodriguez, H., Rivas, B., & Muñoz, R. (2008). Study of the inhibitory activity of phenolic compounds found in olive products and their degradation *Lactobacillus plantarum* strains. *Food Chemistry, 107*, 320–326.

Marsilio, V., Seghetti, L., Iannucci, E., Russi, F., Lanza, B., & Felicioni, M. (2005). Use of a lactic acid bacteria starter culture during green olive (Olea europaea L cv Ascolana ternera) processing. *Journal of the Science and Food Agriculture, 85*, 1084–1090.

Medina, E., Brenes, M., Romero, C., García, A., & de Castro, A. (2007). Main antimicrobials compounds in table olives. *Journal of Agricultural and Food Chemistry, 55*, 9817–9823.

Montaño, A., Sánchez, A. H., & de Castro, A. (1993). Controlled fermentation of Spanish-style green olives. *Journal of Food Science, 58*, 842–844.

Montaño, A., Sánchez, A. H., & de Castro, A. (2000). Changes in the amino acid composition of green olive brine due to fermentation by pure culture of bacteria. *Journal of Food Science, 65*, 1022–1027.

Pereira, J. A., Pereira, A. P. G., Ferreira, I. C. F. R., Valentao, P., Andrade, P. B., Reabra, R., et al. (2006). Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity. *Journal of Agricultural and Food Chemistry, 52*, 8425–8431.

Rodríguez-Borbolla, J. M., Fernández-Díaz, M. J., & González-Cancho, F. (1969). Influence of pasteurization and lye treatment on the fermentation of Spanish-style Manzanilla olives. *Applied Microbiology, 17*, 734–736.

Rodríguez-Borbolla, J. M., & Rejano, L. (1979). On the preparation of Sevillan style olives. The fermentation. I. *Grasas y Aceites, 30*, 175–185.

Ruiz-Barba, J. L., Brenes, M., Jiménez, R., García, P., & Garrido, A. (1993). Inhibition of *Lactobacillus plantarum* by polyphenols extracted from two different kinds of olive brine. *Journal Applied Bacteriology, 74*, 15–19.

Ruiz-Barba, J. L., Cathcart, D. P., Warner, P. J., & Jiménez-Díaz, R. (1994). Use of *Lactobacillus plantarum* LPCO10, a bacteriocin producer, as a starter culture in Spanish style green olive fermentation. *Applied Environmental Microbiology, 60*, 2059–2064.

Servili, M., Settanni, L., Veneziani, G., Esposto, S., Massiti, O., Taticchi, A., et al. (2006). The use of *Lactobacillus pentosus* 1MO to shorten the debittering process time of black table olives (Cv. Itrana and Lecchino):a pilot-scale application. *Journal of Agricultural and Food Chemistry, 54*, 3869–3875.

Tassou, C. C., Katsaboxakis, C. Z., Georget, D. M. R., Parker, M. L., Waldron, K. W., Smith, A. C., et al. (2007). Effect of calcium chloride on mechanical properties and microbial characteristics of cv. *Conservolea* naturally black olives fermented at different sodium chloride levels. *Journal of the Science and Food Agriculture, 87*, 1123–1131.