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The Use of *Lactobacillus pentosus* 1MO To Shorten the Debittering Process Time of Black Table Olives (Cv. Itrana and Leccino): A Pilot-Scale Application

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Fifty lactobacilli isolated from black table olive brines were evaluated for their salt tolerance, resistance to oleuropein and verbascoside, and ability to grow in modified filter-sterilized brines. A strain of *Lactobacillus pentosus* was selected and used as a starter to ferment, in pilot plant, black olives (*Itrana* and *Leccino* cv.) in brines modified for pH, carbohydrate, and growth factor concentrations, at 28 °C. The temperature-controlled fermentation of *Leccino* cv. olives resulted in obtaining ready-to-eat, high-quality table olives in a reduced-time process. HPLC analysis of phenolic compounds from fermented olives showed a decrease of oleuropein, a glucoside secoiridoid responsible for the bitter taste of olive drupes, and an increase of the hydroxytyrosol concentration. The selected strain of *L. pentosus* (1MO) allowed the reduction of the debittering phase period to 8 days.

KEYWORDS: Debittering; fermentation; hydroxytyrosol; lactic acid bacteria; *Lactobacillus pentosus*; oleuropein; table olives; verbascoside

INTRODUCTION

Table olives represent one of the most popular fermented foods in southern Europe, especially in Greece, Italy, and Spain (*1*, *2*).

The olive drupe contains high concentrations of phenolic compounds, such as phenolic acids and phenolic alcohols, flavonoids, and secoiridoids, that can range between 1 and 3% of the fresh pulp weight (*3*) (**Figure 1**). While the phenolic acids and phenolic alcohols and flavonoids occur in many fruits and vegetables belonging to various botanical families, secoiridoids constitute a feature of *Olearaceae* that includes *Olea europaea* (L.) The phenolic compounds classified as secoiridoids are characterized by the presence of either elenolic acid or elenolic acid derivatives in their molecular structure (*3*). Oleuropein (**Figure 1**, structure **I**), demethyloleuropein, ligstroside, and nüzhenide are the most abundant secoiridoid glucosides in olive fruit (*3*−*5*). Olive fruit also contains the verbascoside (**Figure 1**, structure **II**), a derivative of the hydroxycinnamic acid. Oleuropein, demethyloleuropein, and verbascoside are mainly found in pulp, but also in the peel and seed, whereas nüzhenide has only been found in seeds (*5*). Several aglycon derivatives of oleuropein and demethyloleuropein such as the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (**Figure 1**, structure **III**) and the isomer of oleuropein aglycon are also commonly found in olive pulp (*5*−*7*). The main phenolic alcohols found in the olive drupe are hydroxytyrosol (**Figure 1**, structure **IV**) and tyrosol (**Figure 1**, structure **V**). Some of the above compounds are bitter tasting and/or inhibitory toward some microorganisms and have to be removed to convert the ripe fruit into a palatable product.

Two main methods are used to produce fermented table olives: the Spanish method (*8*) for green olives and the Greek method for black olives (*2*). Generally in both cases the fruits become edible after a spontaneous "natural" fermentation performed by a mixed population of indigenous microorganisms (*9*). For Spanish-style green olive production, the fermentation step is preceded by a treatment with sodium hydroxide (lye) (*8*) in order to hydrolyze the bitter-tasting oleuropein and demethyloleuropein into nonbitter compounds such as elenolic acid and hydroxytyrosol (*10*). In the Greek-style process the hydrolysis of secoiridoid glucosides is attributed to the enzymatic reactions of the spontaneous microorganisms by their glycosidases and esterase (*2*).

Rodríguez de la Borbolla y Alcalá and co-workers (*11*, *12*) reported that the spontaneous fermentation of Spanish-style green olives mainly depends on *Lactobacillus plantarum*, while both lactic acid bacteria (LAB) and yeasts dominate the brines

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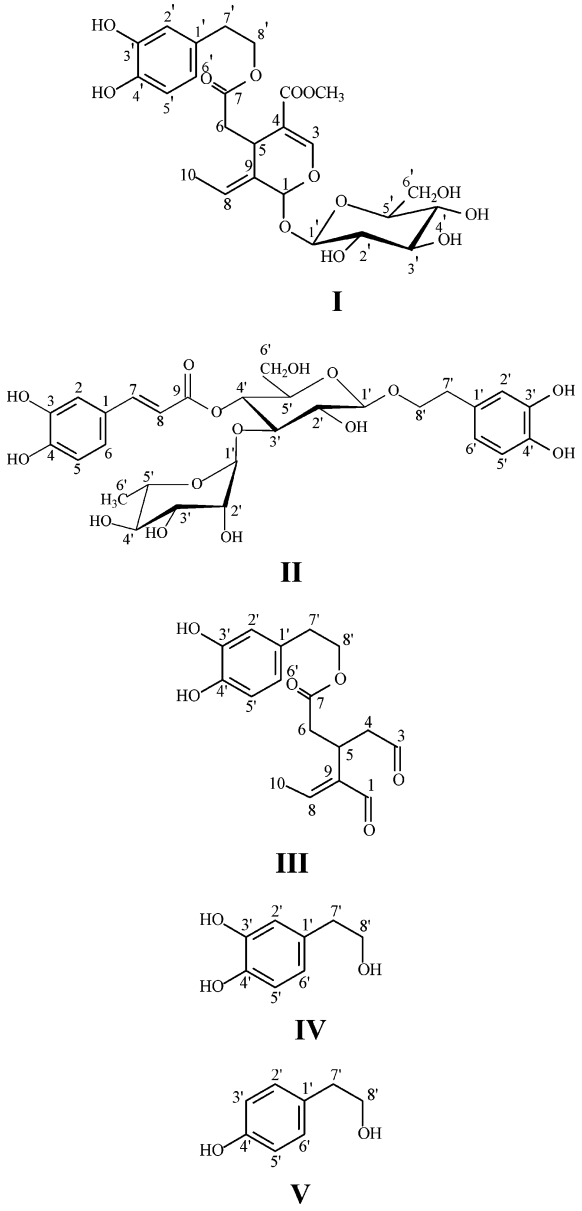

Figure 1. Chemical structures of the main phenolic compounds of the olive fruit: oleuropein (**I**), verbascoside (**II**), the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (**III**), hydroxytyrosol (**IV**), and tyrosol (**V**).

of naturally ripened olives brined directly after harvesting. Nevertheless, it is commonly recognized that the natural-style process leads to unpredictable and longer fermentation as well as low-quality products (*13*, *14*) with variable sensory characteristics. Many studies have focused on standardizing the quality of products by reducing the time period of the debittering process and the occurrence of spoilage, the selection of starter cultures (*15*), and/or the modification of physicochemical process parameters (*14*, *16*). As reported by Ruiz-Barba et al. (*17*), the basic characteristics of a starter culture for table olive fermentation include a rapid and predominant growth, homofermentative metabolism, tolerance to salt, acid, and polyphenols, and few growth factor requirements. On the basis of the above considerations the aims of the present study were (1) to isolate a large number of LAB from brines of naturally fermented table olives; (2) to select and identify a strain to be used as starter culture for fermentation of black table olives; (3) to apply the selected strain in pilot plant.

MATERIALS AND METHODS

Isolation and Physiological Characterization of LAB. LAB were isolated from the brines of naturally fermented black table olives (*Itrana* and *Leccino* cv.) after serial dilutions of samples and plating on MRS agar (Difco Laboratories, Detroit, MI). Plates were incubated at 30 °C for 48 h and, after growth, colonies of various forms were randomly picked from agar plates and transferred in MRS broth; the isolates were purified by successive subculturing, and their purity as well as cell morphology were checked microscopically. Gram-positive (Gram staining, Merck, Darmstadt, Germany) and catalase-negative (determined by transferring fresh colonies from MRS agar to a glass slide and adding 5% H_2O_2) isolates were stored at −80 °C in glycerol stocks until further experimentation was required.

Fifty rod-shaped LAB isolates were characterized for their NaCl tolerance. Isolates were evaluated using MRS broth with additional NaCl at concentrations of 0−8% (w/v). Resistance to oleuropein (0.5%) and verbascoside (0.1%) (both in MRS broth) and growth in modified (sterile filtered 0.3% glucose, 0.05% yeast extract and adjusted to pH 6.0 with 1 M NaOH) brine (6% NaCl, 0.05% oleuropein, and 0.15% verbascoside) were also evaluated. These cultures were grown at 30 °C, and the growth was monitored using a spectrophotometer (Lambda Bio 20, Perkin-Elmer, Boston, MA) (OD 600_{nm}) at 1 h intervals for 48 h.

Identification of LAB. In conditions with salt, oleuropein, and verbascoside and for growth in modified brine (mB), strain 1MO demonstrated the highest resistance, and thus, this strain was selected for further phenotypic and genotypic identification, by means of the API 50 CHL system (bioMérieux, Lyon, France) and 16SrRNA gene sequencing as described by Corsetti et al. (*18*), respectively.

Genomic DNA was extracted as reported by De Los Reyes-Gavilán et al. (*19*) from 2 mL samples of overnight cultures which had been incubated at 30 °C. The final concentration of lysozyme used for cell lysis was 2 mg/mL. The concentration and purity of DNA was assessed with a biophotometer (Eppendorf, Hamburg, Germany).

A *recA* gene-based multiplex PCR system was also used to distinguish between species of *L. plantarum* group (*L. plantarum*, *L. paraplantarum*, and *L. pentosus*) (*20*).

Olive Samples. Black olives of two cultivars, *Itrana* and *Leccino*, were supplied from "Madama Oliva"—Carsoli—L'Aquila, Italy. The samples were collected at the black stage of ripening. After discarding damaged olives and washing the remainder with water, fruits were immediately transferred in plastic vessels containing freshly prepared brine (8% NaCl) and stored at +4 °C until the start of fermentation trials.

Inoculation and Pilot Plant Fermentation. Freeze-dried preparations of *L. pentosus* 1MO, obtained by T. H. T. S. A. (Gembloux, Isnes, Belgium), represented the starter inoculum for olive fermentation. *L. pentosus* 1MO was inoculated at an initial cell concentration of about 10^8 cfu/mL. Fermentations were performed at pilot-scale for both *Itrana* and *Leccino* cv. at 28 °C in fermenters containing 100 kg of olives and 100 L of mB. Control, used for each trial, was represented by 100 kg of olives in 100 L of brine (6% NaCl), fermented by indigenous microflora.

Microbiological Analysis. Mesophilic lactobacilli were counted under microaerophilic conditions at 30 °C for 48 h onto MRS agar containing cycloheximide at a concentration of 170 mg/L, while coliforms were counted onto violet red bile agar (VRBA, Difco) at 37 °C for 18 h. Both enumerations were carried out before and during pilot-scale fermentations, at defined time intervals.

Phenolic Compounds. Oleuropein was obtained from Extrasynthese (Genay, France), while the verbascoside was isolated from the phenolic extract of industrial olive brines by semipreparative HPLC, according to the procedure previously reported by Montedoro et al. (*21*). The phenolic extract was obtained from the olive brines using liquid−liquid extraction by ethyl acetate: 100 mL of fresh brines filtered through a 0.45-μm CA syringe filter (Whatman, Clifton, NJ) were mixed with 100 mL of ethyl acetate and, after the two separation phases, the organic solvent was recovered. This procedure was performed in triplicate. The collected organic solvent was passed through a column filled with anhydrous sodium sulfate to remove residual water and evaporated in

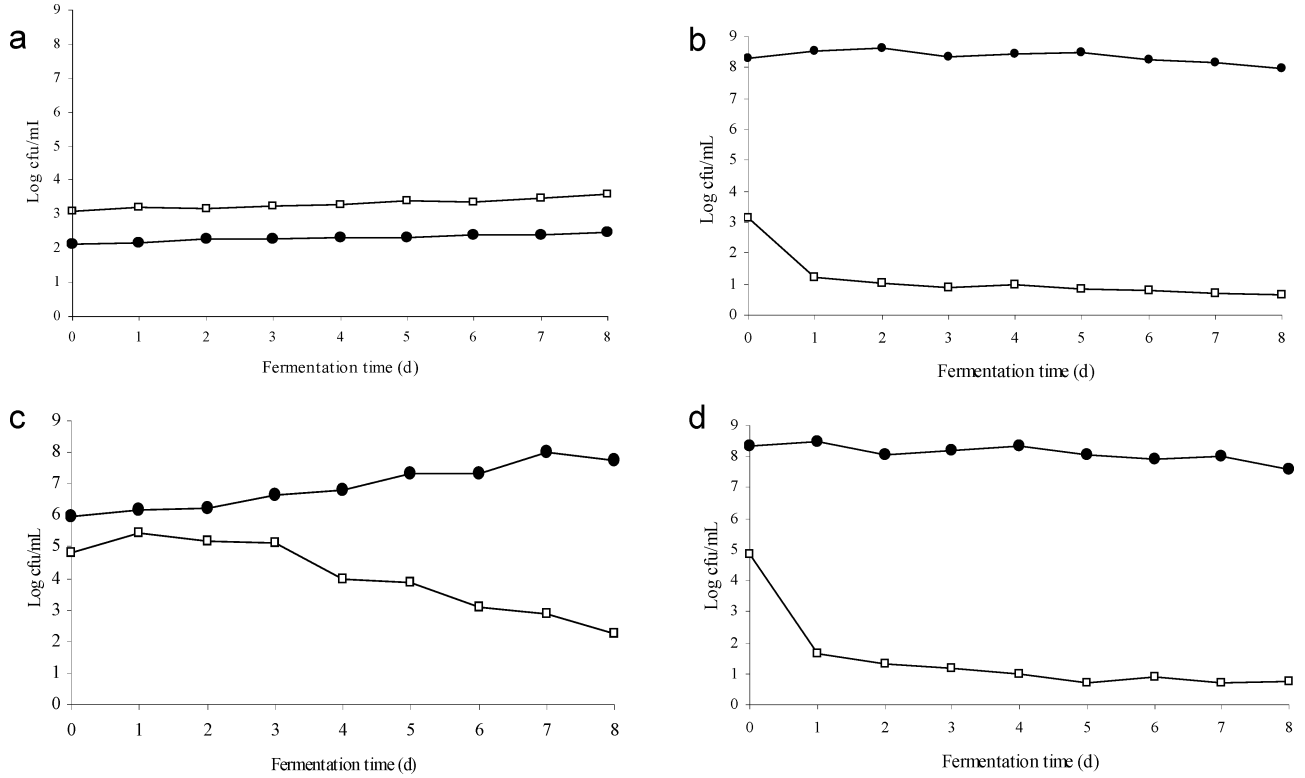

Figure 2. Evolution of LAB (●) and coliforms (□) during the fermentation of *Itrana* (**a** and **b**) and *Leccino* (**c** and **d**) olives by the indigenous flora (**a** and **c**) or by *L. pentosus* 1MO added as starter culture (**b** and **d**).

vacuum under nitrogen flow. The phenolic extract was recovered with 5 mL of methanol, and verbascoside was separated by semipreparative HPLC. The chemical structure was confirmed by NMR (*22*), and the level of purity (higher than 98%) was evaluated by HPLC.

Chemical Analysis. Concomitantly with microbiological analysis, brine samples were chemically assayed. The pH was measured with a Corning 140 pH meter (Corning, Halstead, England); the concentration of glucose as well as D- and L-lactic and acetic acid concentration were estimated using enzymatic methods (Boehringer-Mannheim, Milan, Italy) according to the manufacturer; phenolic compounds were analyzed by HPLC.

Phenolic compounds were evaluated from both olive and brine matrixes. Olive phenolic compound extraction and evaluation was carried out by HPLC as previously described by Servili et al. (*22*); the method was modified as follows: 10 g of fresh olives was homogenized with 100 mL of 80% methanol containing 20 mg/L of sodium diethyldithiocarbamate (DIECA). The extraction was performed in triplicate. After methanol removal, the aqueous extract was used for SPE phenol separation. Brines were first filtered through a 0.45-μm CA syringe filter (Whatman). The SPE procedure was followed for both olive extract and brine loading with 2 mL of sample a 5 g/25 mL Extraclean highload C_{18} cartridge (Alltech Italia S.r.l., Sedriano, Italy) using 200 mL of methanol as eluting solvent. An Inertsil ODS-3 column (150 mm × 4.6 mm i.d.) (Alltech) was employed for HPLC analysis.

Sensory Analysis. Descriptive−quantitative analysis of sensory attributes was carried out by a panel of eight people specialized to perform virgin olive oil sensory analysis after a training to familiarize with the specific descriptor for the bitter evaluation on table olives. Olive samples (three pieces) were presented to the assessors in duplicate in plastic glasses at room temperature. The samples were presented in balanced order to each assessor. The intensity of the bitter sensation was graded using a line scale and thus converted in numerical score by measuring the position of the placed mark along the 10 cm line. Results were calculated as averages among assessor sensory scores.

RESULTS

LAB Isolation, Characterization, Selection, and Identification. On the basis of preliminary assays (data not shown), bacteria isolated from brines were all considered to belong to the *Lactobacillus* genus. The 50 lactobacilli, based on their resistance to NaCl by evaluating their kinetics of growth in MRS broth containing various concentrations of salt, were divided into three groups: highly resistant to 8% NaCl (group A, $n = 23$); partially inhibited by the presence of NaCl (group B, $n = 12$); totally inhibited by salt (group C, $n = 15$).

Group A isolates were further investigated for their resistance to oleuropein and verbascoside, recognized as potent inhibitors of bacterial growth. The growth of only four isolates (1MO, 12MO, 35MO, and 37MO) were not significantly reduced by the presence of the inhibitory substances (data not shown). Therefore, to replicate the industrial conditions of brine fermentation, growth of the above four isolates was evaluated in a brine containing salt (6% NaCl), oleuropein (0.05%), verbascoside (0.15%), and growth factors (0.3% glucose and 0.05% yeast extract), which was adjusted to pH 6.0 and filter-sterilized. Strain 1MO demonstrated a greater aptitude to grow in mB over the other strains which showed almost the same growth characteristics (results not shown). On the basis of phenotypic and genotypic analysis (16S rRNA gene sequencing), strain 1MO was identified as a bacterium in the *L. plantarum* group. Further *recA* gene-based multiplex PCR identified this strain as a *L. pentosus* species.

Black Olive Fermentation. On the basis of the above results *L. pentosus* 1MO was selected to be used as a starter for the fermentation of black table olives at pilot-scale. All described results refer to a time period after 8 days of fermentation that represents the end of the debittering process, as indicated below by the chemical data. Moreover, besides LAB, microbiological data are also reported for coliforms of black table olives of both cultivars processed (**Figure 2**). After washing and prior to brining, microbial count values characterizing the two varieties [starting time (0)] indicated a different degree of fruit contamination. Values of log 3 cfu/mL and log 5 cfu/mL coliforms and

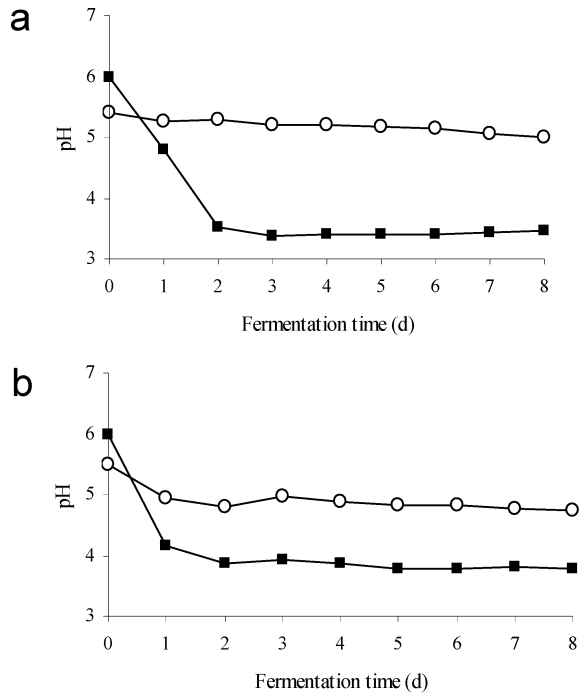

Figure 3. Evolution of pH during the fermentation of *Itrana* (**a**) and *Leccino* (**b**) olives by the indigenous flora (○) or by *L. pentosus* 1MO starter culture (■).

of log 2 cfu/mL and log 6 cfu/mL LAB were obtained for *Itrana* and *Leccino* cv., respectively (**Figure 2**, parts **a** and **c**). In both cases the brines inoculated with *L. pentosus* 1MO presented a significant increase (log 8 cfu/mL) in the number of LAB from the beginning of fermentation (**Figure 2**, parts **b** and **d**). *L. pentosus* remained stable at log 8 cfu/mL and dominated until the end of fermentation (8 days) (**Figure 2**, parts **b** and **d**). In control brine of *Itrana* cv., indigenous LAB, probably due to the low initial level, reached a value near log 3 cfu/mL after 8 days of fermentation (**Figure 2a**). However, in the most contaminated *Leccino* cv. control brine, the indigenous LAB reached a value of about log 8 cfu/mL after 8 days of fermentation (**Figure 2c**) and equaled the concentration of *L. pentosus* in started brine (**Figure 2d**). In control brines coliforms showed a small initial increase but soon thereafter, when LAB growth started, it decreased (see *Leccino* cv. in **Figure 2c**). The significant decrease of coliforms was particularly evident from the beginning of fermentation in brines inoculated with *L. pentosus* 1MO of both olive cultivars (**Figure 2**, parts **b** and **d**). In our study the pH value was used to monitor the fermentation process. The evolution of this parameter suggests a higher rate of fermentation when inoculation was performed. As reported in **Figure 3**, the pH of both inoculated processes dropped from pH 6.0 at the beginning of fermentation to values lower than 4.0 after 2 days of fermentation and reached a pH near to 3.6 after 8 days. Despite the high level of LAB throughout the control process of noninoculated *Leccino* cv. (**Figure 2c**), the final pH value was not lower than 4.8 (**Figure 3b**). This was probably due to a low glucose concentration, the absence of yeast extract, reduced acidifying activity of indigenous LAB with respect to that of the selected *L. pentosus* strain, and/or major competition for carbon sources with indigenous lactic acid-consuming yeasts. Even though similar results were obtained with *Leccino* cv. (data not shown), to have a better understanding of the role of the selected starter in the fermentation process and to eliminate the interference of indigenous LAB, only the data referring to the *Itrana* cv. (showing a low number of contaminating LAB) fermentation process are presented in our report.

Table 1. Glucose Consumption and Lactic and Acetic Acid Production during the Fermentation of *Itrana* Olives by the Indigenous Flora and *L. pentosus* 1MO

	glucose (g/L)[a]		lactic acid (g/L)		acetic acid (g/L)	
fermentation time (days)	indigenous flora	*L. pentosus* 1MO	indigenous flora	*L. pentosus* 1MO	indigenous flora	*L. pentosus* 1MO
0	0.30 ± 0.00	2.95 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	0.42 ± 0.02	2.30 ± 0.06	0.00 ± 0.00	1.21 ± 0.03	0.02 ± 0.01	0.03 ± 0.01
2	0.58 ± 0.01	0.47 ± 0.02	0.00 ± 0.00	2.98 ± 0.05	0.03 ± 0.01	0.08 ± 0.02
3	0.59 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	4.33 ± 0.04	0.03 ± 0.00	0.09 ± 0.03
4	ND[b]		0.05 ± 0.01	4.36 ± 0.06	0.05 ± 0.01	0.20 ± 0.03
5	ND[b]		0.09 ± 0.01	4.70 ± 0.04	0.12 ± 0.01	0.26 ± 0.01
6	ND[b]		0.12 ± 0.02	5.47 ± 0.05	0.13 ± 0.00	0.29 ± 0.02
7	ND[b]		0.17 ± 0.01	5.70 ± 0.04	0.18 ± 0.01	0.34 ± 0.01
8	ND[b]		0.22 ± 0.02	6.03 ± 0.04	0.21 ± 0.02	0.34 ± 0.04

[a] Results indicate mean ±SD of two independent experiments. [b] ND, not determined.

The relatively fast fermentation by *L. pentosus* 1MO in the inoculated brine is reflected by a fast consumption of the added glucose. During the pH decrease (**Figure 2b**), all the glucose was almost completely depleted within the second day of fermentation (**Table 1**). In the control brine there was an initial very low concentration of glucose which remained unchanged thereafter. This was probably due to limited microbial activity. To monitor the main products of LAB metabolism, lactic and acetic acids were measured during the fermentation process in both inoculated and noninoculated brines (**Table 1**). Lactic acid, the main product of glucose fermentation by *L. pentosus* 1MO, showed a fast increase within 3 days of fermentation, with respect to the rapid growth and fermentative activity of the starter culture (**Table 1**). At the end of fermentation 6 g/L of lactic acid was produced in brine by *L. pentosus* 1MO while a small amount of lactic acid (0.21 g/L) was detected in the control brine. An accelerated production of acetic acid was also observed, with nonsignificant differences between inoculated and control process (**Table 1**).

Phenolic Composition of Olives and Sensory Analysis. **Figures 4** and **5** illustrate that the fermentation of brined olives with *L. pentosus* 1MO modifies the phenolic composition of olives. Concentrations of oleuropein and the aglycon derivatives of oleuropein (the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol) significantly decrease in the olives after fermentation of both olive cultivars while hydroxytyrosol increases (**Figures 4a** and **5a**). With regard to the verbascoside, even though a reduced concentration was observed in the olives after fermentation, this was due to its partial release in the brine (**Figures 4b** and **5b**), where higher amounts were found after processing. Indeed, the concentration of caffeic acid, the phenolic acid obtained by verbascoside hydrolysis, remained unchanged both in the olives and in the brines (data not shown). An enzymatic activity on the secoiridoid compounds by the selected *L. pentosus* strain could be supposed on the basis of those results. The occurrence of the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol in the olives and in the brines seems to confirm that the oleuropein hydrolysis was an enzymatic reaction that included a β-glucosidase activity. In fact the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol should be hydrolyzed, as well as oleuropein, when a chemical hydrolysis takes place. Due to the release process, the hydroxytyrosol and the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol in the brine increase, consequently reducing the

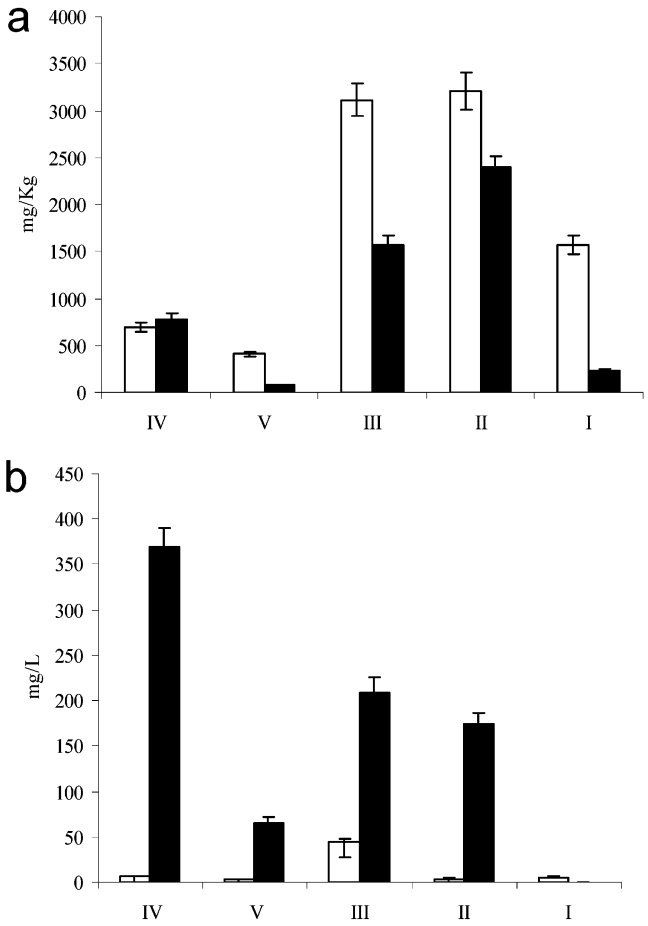

Figure 4. Effect of the fermentation by *L. pentosus* 1MO on the phenol concentration of *Itrana* cv. olives (**a**) and brines (**b**) evaluated by HPLC, before (□) and after (■) fermentation: I, oleuropein; II, verbascoside; III, the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol; IV, hydroxytyrosol; V, tyrosol.

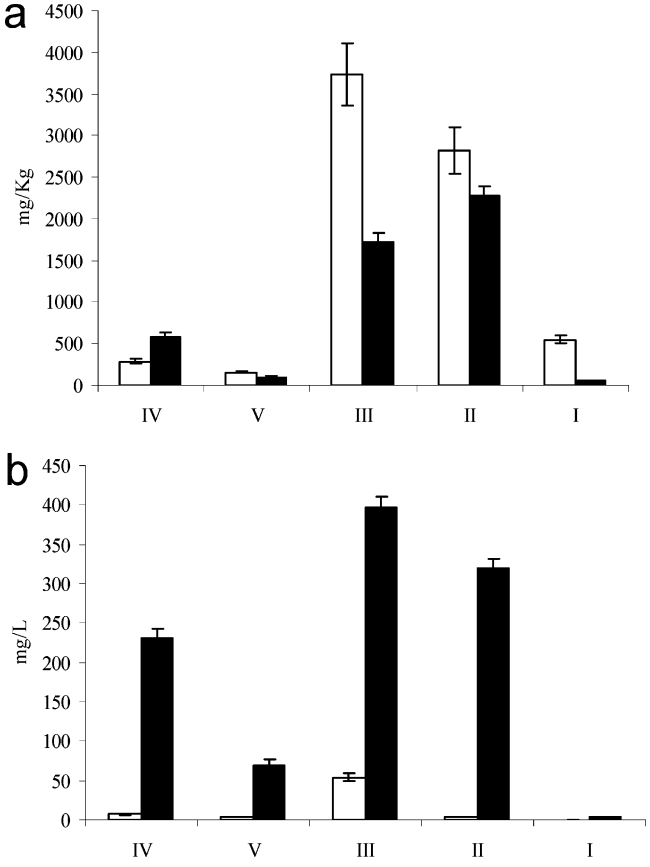

Figure 5. Effect of the fermentation by *L. pentosus* 1MO on the phenol concentration of *Leccino* cv. olives (**a**) and brines (**b**) evaluated by HPLC, before (□) and after (■) fermentation: I, oleuropein; II, verbascoside; III, the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol; IV, hydroxytyrosol; V, tyrosol.

phenolic concentration of fermented olives. The diffusion of hydrolyzed phenolic compounds in the brine after fermentation gave a significant reduction of bitter taste of brined olives, as shown by the sensory analysis results reported in **Figure 6**.

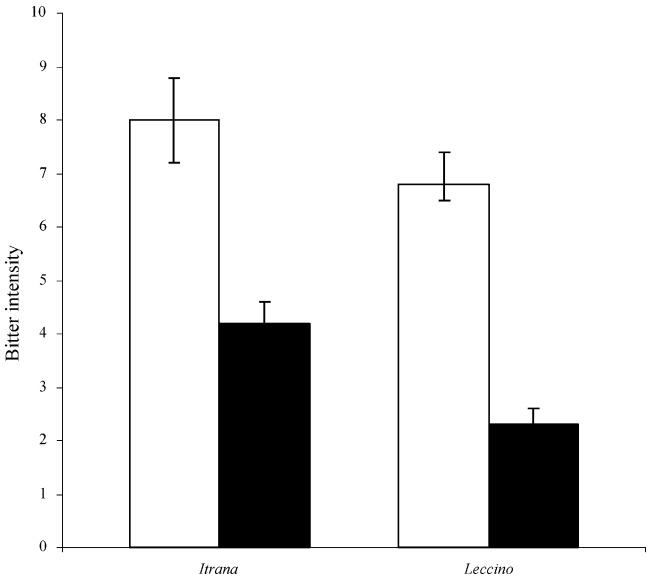

Figure 6. Bitter sensation intensity of *Leccino* and *Itrana* cv. olives tasted before (□) and after (■) fermentation by *L. pentosus* 1MO.

DISCUSSION

Successful table olive fermentation relies on the inoculation of optimal starter cultures, which generally belong to the LAB group. Tolerance to high NaCl concentration is of paramount importance when selecting LAB strains to be used as starter cultures as brines may contain up to 10−13% of salt (*23*). Among LAB, a species generally associated with olive fermentation is *L. plantarum* (*24*). Commonly reported advantages of the above species include the avoidance of serious olive defects (*25*) and the ability to hydrolyze oleuropein by means of β-glucosidase (*10*). The most inhibitory effect is caused by the presence of verbascoside contained in olive drupes, while oleuropein glucoside shows a slight inhibition of LAB. Verbascoside concentrations are generally high in olive cultivars subjected to biological debittering; thus, the resistance to this inhibitory compound is an essential parameter during the selection of debittering LAB strains.

In our study, to enhance the growth of resistant selected LAB over other strains, additional growth factors were present in brines: 0.3% glucose and 0.05% yeast extract. Our findings support evidence presented by previous reports which also found that it is during the first days of fermentation where the growth of non-LAB spoilage microorganisms are at their highest (*8*). Therefore, inoculation with LAB starter cultures, in particular lactobacilli, during these first days can improve the fermentation process, because lactic acid fermentation reduces the risks of spoilage occurring. Four strains (1MO, 12MO, 35MO, and 37MO) were selected based on their NaCl (8%) resistance and

capability to grow in the presence of oleuropein (0.05%) and verbascoside (0.15%); the latter trait relies, as already reported for *L. plantarum* strains (*10*), on their ability to hydrolyze phenolic compounds which partly contributes to the debittering process (*9*). It has been previously reported that the most important factors that could limit the adaptation of LAB to the brine environment are (1) the initial salt concentration, (2) nutrient availability, and (3) the presence of natural inhibitory compounds such as secoiridoids and verbascoside (*26*).

Among the four strains which demonstrated these favorable characteristics for their use in pilot plant, strain 1MO was employed as a starter culture for the fermentation process. This was because of its higher growth in MRS containing oleuropein or verbascoside and in mB (results not shown). Strain 1MO was identified as *L. pentosus*, which is both phenotypically and genotypically closely related to *L. plantarum*. *L. pentosus*, probably due to its misidentification based on the solely phenotypic characteristics and 16S rRNA gene sequence, is not as commonly associated with olive fermentation as *L. plantarum*. However, our pilot plant study firmly indicates the strong potential for the usage of *L. pentosus* 1MO for biological debittering during industrial black olive production.

The fermentation, as evaluated by chemical and sensory analysis, and the corresponding olive debittering were concluded 8 days after the strain addition to the olive brines, while 6 months or more are needed for biological debittering in spontaneous olive fermentation (*2*). Other studies have shown that more than 1 week is needed to obtain an acceptable product (*10*, *27*). Moreover, the addition of *L. pentosus* 1MO showed a strong control of the spontaneous bacterial strains, due to the fast pH reduction below pH 4.5. This aspect improves the safety of fermented olives, while avoiding the possible growth of spoilage and/or pathogenic strains. Besides the absence of defects and the improved microbial safety of the final product, fermentation by *L. pentosus* 1MO also improved the healthy aspects of ripened olives. In fact, in comparison to the phenol losses that may occur during a traditional Greek-style debittering, the quickly reached low pH of the brine and the shorter processing time can reduce the oxidative degradation of phenolic antioxidants, such as hydroxytyrosol and the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol, which occurs in brined olives. These compounds are very sensitive to oxidative reactions, although no data on the evolution of the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol during the spontaneous fermentation process are available in the literature. This lack of data may be related to the strong variability showed for this compound according to the cultivar and ripening stage of olives used for the natural debittering (*28*). These fermented olive-associated compounds, also found in virgin olive oil (*29*), are involved in the prevention of cancer and coronary diseases and fulfill a defining role in introducing bioactive natural compounds in the Mediterranean diet (*28*).

Four main conclusions can be draft from the present work: (1) the addition of *L. pentosus* represents a useful tool to decrease the possibility of spoilage and justifies the use of LAB as starter cultures in order to improve the safety and quality of black table olives; (2) the growth of LAB and the levels of the most important fermentation parameters (lactic acid production and pH) were significantly improved in the brines supplemented with glucose and yeast extract; (3) the pilot-scale trials showed that a rapid controlled fermentation process can be obtained by using brine modified for pH value, carbohydrate, and growth factor concentration in the presence of selected LAB; (4) *L. pentosus* 1MO affected hydrolysis of oleuropein and the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol, while verbascoside remained unchanged. The release of hydroxytyrosol and the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol in the brine reduced the phenolic concentration of fermented olives and, as a consequence, their bitter taste.

Due to the successful fermentation by *L. pentosus* 1MO (8 days are needed from harvesting to eating) the above process is now being applied at the industrial level.

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