

Biotechnology of olive fermentation of 'Galega' Portuguese variety

By Manuela Oliveira¹, Dulce Brito¹, Luís Catulo¹, Fausto Leitão¹, Lucília Gomes², Sandra Silva², Luís Vilas-Boas², Amália Peito³, Isabel Fernandes³, Francisca Gordo⁴, Cidália Peres^{1*}

- (1) Instituto Nacional de Investigação Agrária e das Pescas/EAN, Oeiras, Portugal;
(2) Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal;
(3) Instituto Nacional de Engenharia e Tecnologia Industrial, Lisboa, Portugal;
(4) Associação de Agricultores Distrito Portalegre, Portalegre, Portugal.

*Corresponding author. Address mail: PO Box 127, Quinta do Marquês 2781-901 Oeiras, Portugal; Phone: 351 21 446 95 69; Fax: 351 21 441 12 77; Email: cperes@itqb.unl.pt

RESUMEN

Biotecnología de la fermentación de aceitunas de la variedad portuguesa 'Galega'.

La variedad de aceitunas más importante en Portugal es la 'Galega', que representa una gran porcentaje de la producción de aceitunas de mesa portuguesas por métodos caseros ó industriales.

La fermentación se produce por una compleja flora microbiana, principalmente levaduras y bacterias del ácido láctico (LAB), siendo la especie *Pichia membranaefaciens* la que domina en todo el proceso.

Las LAB desarrollan su actividad a lo largo de la segunda fase de la fermentación, en donde *Lactobacillus plantarum* y *Lactobacillus pentosus* fueran aislados y identificados, así como *Leuconostoc pentosaceus*.

Los resultados de los análisis químicos muestran la mejor eficiencia del método casero. Además, la composición química de la salmuera es más parecida entre las muestras caseras que entre las de la industria. Importantes diferencias se encuentran en el perfil de los compuestos fenólicos, principalmente en la fase final de la fermentación.

La cantidad de compuestos volátiles aumenta en la misma fase en ambos métodos y se encuentran algunas diferencias entre ellos.

El análisis sensorial muestra que los mejores resultados corresponden a las aceitunas obtenidas por el método casero.

PALABRAS-CLAVE: Calidad - Fermentación - Microbiota - Parámetros físico-químicos - Variedad 'Galega'.

SUMMARY

Biotechnology of olive fermentation of 'Galega' Portuguese variety.

'Galega' is the main Portuguese olive variety providing the greatest percentage of table olive production from homemade and industrial methods.

In this work a better understanding about the fermentation involved in both methods is intended.

Yeasts and lactic acid bacteria (LAB) constitute the microflora acting in olive fermentation, being *Pichia membranaefaciens* the dominant yeast specie present throughout the process.

LAB develop their activity mainly along the second fermentation stage where *Lactobacillus plantarum* and *Lactobacillus pentosus* were isolated and identified, as well as *Leuconostoc mesenteroides* and *Pediococcus pentosaceus*.

Results of a chemical analysis have shown the effectiveness of both homemade and industrial fermentation methods. Nevertheless, the chemical composition of the brines from homemade samples was more similar than those from the industrial ones. Remarkable differences were found in the phenolic compounds profile mainly on the final fermentation stage. The amount of volatile compounds has enhanced on the same phase in both methods and some differences were found between them.

Sensorial analysis has shown the best results obtained through the homemade method.

KEY-WORDS: Chemical parameters - Fermentation - 'Galega' variety - Microbiota - Quality.

1. INTRODUCTION

Traditional regional foods are a ribbon throughout multicultural Europe allowing the different nations to grow together while maintaining their regional individuality. Table olive production has a deep-rooted tradition in all Mediterranean countries. These typical products are mostly fermented foods produced by specific microbiota (Fernández-Díez et al., 1983, 1985). In Portugal olive growing represents an important agriculture area mainly located in unfavored regions. The 'Galega' variety of *Olea europaea* is widely used in Portugal for the production of olive oil and represents 90% of olive growing. In rural areas, this variety is also used for table olive production, mainly by traditional methods. Their fruits, which have been studied, produce an excellent black pickled olive although their average size is quite small. A high percentage of table olive production concerns this variety, though the bulk is prepared by homemade methods and consumed domestically. The quality of the final product may depend on the composition of the fresh fruits, production technology and the environmental conditions during the transformation process.

Fermentation is a spontaneous phenomenon in a traditional way, including several stages, depending on spontaneous colonisation by microbial strains, associated with the raw materials and local environmental conditions, which still takes place in olive producing regions. Several factors can affect the native microflora such as polyphenols, which inhibit LAB growth (Garrido-Fernandéz and Vaughn, 1978). The sodium chloride concentration and the pH of the brine are other control parameters acting during fermentation (Özay and Borcali, 1996).

In this process, black olives are placed in brine where a mixing native microbial population naturally ferments them and where an initially small population of lactic acid bacteria (LAB) becomes the predominating microbial flora. Yeasts acted during the first few days and remained until the end of the process keeping company with LAB.

LAB are known to produce a heterologous array of functional products, which include organic acids, ethanol, carbon dioxide, flavor compounds, proteases, and anti-microbial compounds, such as bacteriocins (Muriana et al., 1993) that regulate the microbial development. Bacteriocins are proteinaceous substances with bactericidal or bacteriostatic activity against sensitive bacterial species (Franz et al., 1997). In some cases they have the ability to kill completely unrelated species (Klaenhammer, 1998). The only bacteriocin produced by LAB and currently used in the food industry is nisin, which is produced by *Lactococcus lactis* ssp. *lactis*, and has limited application because of its instability at a neutral pH (Enan et al., 1995). This clearly suggests that there is a need to continue research in identifying lactic cultures, which could produce antimicrobial compounds active at neutral pH conditions leading to wider applications in food preservation (Enan et al., 1995). Many bacteriocins are rather small molecules so they can easily diffuse into the water phase of food products. Because of the thermostability of the majority of lactic acid bacterial bacteriocins some of them may survive the thermal processing cycle of foods. Other bacteriocins can be used in acid and cold processed foods or in stored food products, on account of their activity under low levels of pH and temperature (de Vuyst et al., 1996).

The aim of this work was to characterise the traditional fermentation processes of 'Galega' variety concerning the microbiological, physico-chemical and sensory aspect.

2. MATERIAL AND METHODS

2.1. Sampling and sample characterization

Fruits of 'Galega' variety from the east-region of Portugal were selected for this study. The technological processes studied were run according to the preparation of the type 'black natural in brine'.

Sampling was made periodically throughout the fermentation time and samples were submitted to physico-chemical, microbiological and sensorial analyses.

In the homemade processing method (A) plastic and/or earthen vessels were used, whose capacities can vary between 10 to 60 L. The fruits were washed and placed directly in water from 20 days to 2 months, and once or twice a changing of the water took place, depending on the producer, followed by placing the fruits in brine where an 8% (w/v) NaCl concentration was provided. The fermentation period was around 7-8 months.

In the industrial processing method (B) bulks with 2000 L were used. The fruits were washed and placed directly in water for 1.5 months, and the water was changed once. After 3 months the addition of 8% (w/v) NaCl took place and fermentation proceeded in this brine for 5-6 months.

2.2. Microbiological analysis

For microbial studies the microorganisms were isolated from the brines of 'Galega'. Brine samples were diluted in a saline aqueous solution and the appropriate dilutions were plated on MRS agar (Merck Mikrobiologie), acidified and kept at 30 °C for incubation for 72 hours. After this period LAB isolation and quantification were carried out. The number of colony forming units per millilitre (cfu/mL) of different LAB populations was determined by spreading the diluted samples on MRS agar (Merck Mikrobiologie). LAB were identified using kit API 50CH-50CHL.

Oxytetracycline yeast-extract-peptone-agar 2% (v/v) glucose (Merck Mikrobiologie) was used for yeast isolation and enumeration, aerobically at 25 °C. The isolation of yeasts was based on colonies morphology and the identification was made using the classical methods Kreger-van Rig (1984) and Barnett et al. (1990).

2.3. Characterisation of strains antagonistic activity

The bacteriocin-producing strains were stored at -80 °C in MRS broth plus 50% (v/v) glycerol before experimental use. Agar media was prepared by addition of 0.8% Agar (Merck Mikrobiologie) to the broth medium and covered by an overlay agar containing 0.8% Agar.

The strains were tested by the agar spot test (Schillinger et al., 1989) using overnight grown culture, and well diffusion test (Schillinger et al., 1989) using supernatant culture, obtained by centrifugation at 10000 g and pH adjusted to 6.5 of a 24 h culture grown aerobically at 28 °C. The cell-free supernatant (CFS) was then heated at 100 °C for 5

Table I
Source and designation of samples

Source	Home made	Industrial	Gordal	Bought at Markets								
Olives	OH	OI	O0	O1	O2	O3	O4*	O5	O6	O7	O8	O9*
Brines	BH	BI	B0	B1	B2	B3	B4*	B5	B6	B7	B8	B9*

(*) different batches from the same mark

min. *Weissella paramesenteroides* and some pathogens were used as indicators in their tests.

Effect of heat, enzymes and pH on bacteriocin activity – The heat effect on the activity of bacteriocin was determined by well diffusion test (Schillinger et al., 1989). The CFS was incubated at the following temperature: 121 °C (15 min and 1 h), 100 °C (20 min and 1 h), 65 °C (30 min), 37 °C (4 h), 25 °C (4 h) and 4 °C (1 week, 1 month) and the temperature effect were determined by well diffusion test (Schillinger et al., 1989). The effect of catalase (Sigma-Aldrich, Steinheim, Germany) addition to CFS was also tested. Catalase was treated according to manufacturers' instructions and resuspended in MRS broth. The effect of various enzymes, Proteinase K, Trypsin, α -Quimotrysin (Sigma-Aldrich, Steinheim, Germany), Pepsin (Calbiochem), Glucoamylase and α -Amilase (Merck) on bacteriocin activity was determined by the addition of 10 μ l of the appropriate enzyme with 990 μ l of each bacteriocin and incubating at 37 °C for 1 hour, except Glucoamylase (60 °C), Quimotrypsin (25 °C) and α -Amilase (25 °C). The activity was tested by the well diffusion test. The effect of pH was tested on CFS adjusted to a pH ranging from 2.0 to 8.0. The curing of plasmids was attempted by treatment of cultures with HCl-Acryflavine and SDS at various concentrations. The loss of bacteriocin phenotype was determined by spot test (Schillinger et al., 1989). Bacteriocin-negative mutants were isolated and CFS was/were tested by well diffusion test (Schillinger et al., 1989).

2.4. Chemical analysis

Samples - Fruits 'O' and final brines 'B' of processing 'Galega' variety was used in this study. Nine samples (1-9) were commercially available products and two samples were produced under controlled conditions in the Portalegre region by two different fermentations: a traditional (homemade - H) method and the other run on an industrial (I) scale: samples of fresh olives were collected before starting these two fermentations.

One sample of a different variety ('Gordal' – 0) has been included for comparison (Tab. 1).

Chromatographic analysis – Non-volatile components were analyzed by High Performance Liquid Chromatography (HPLC) with diode array

detector using a gradient program (Gomes et al., 1999). Fruit pulp (15 g) was mixed with ascorbic acid (0.5 g), crushed and extracted with methanol/water (4:1).

Volatile compounds were analyzed by Gas Chromatography coupled with mass spectrometry detector (GC-MS). Sample preparation was carried out by head space/solid phase micro-extraction (PDMS/DVB fiber, at 37 °C, 30 minutes).

Principal component analysis was done using NTSYS-pc program (Exeter Software).

2.5. Sensorial analysis

The sensorial analysis was performed using an official Panel for Table Olives. The Panel was comprised of 20 assessors who were selected and highly experienced in the characterization of sensorial table olive quality. A profile sheet, which was previously studied and adapted to Natural Black Table Olives, was used. The profile sheet uses a non-structured scale for scoring the intensity of the perception of sensory attributes. It includes olfactory, gustatory, visible, and mechanical, geometrical and surface attributes. The number of tests per sample was two replicates for each sensory evaluation. The results were analyzed statistically using the MSTAT program.

3. RESULTS AND DISCUSSION

3.1. Microbiological analysis

During the homemade process the growth of LAB only occurred at the beginning of fermentation and its disappearance coincided with the addition of NaCl 8% w/v. At the same time, in this technology the water was replaced twice, which could affect LAB growth (Fig. 1) as a consequence of nutrients leaching into the water. The LAB species identified were *Lb. plantarum* and *Lc. mesenteroides*.

Concerning the growth of lactic acid bacteria during fermentation in the industrial process it was possible to divide the process into three stages. LAB growth occurred at the beginning of fermentation (first stage) and in the last stage, and the level of LAB was similar in these two stages. In the middle stage no LAB growth was detected (Fig. 2). Differences

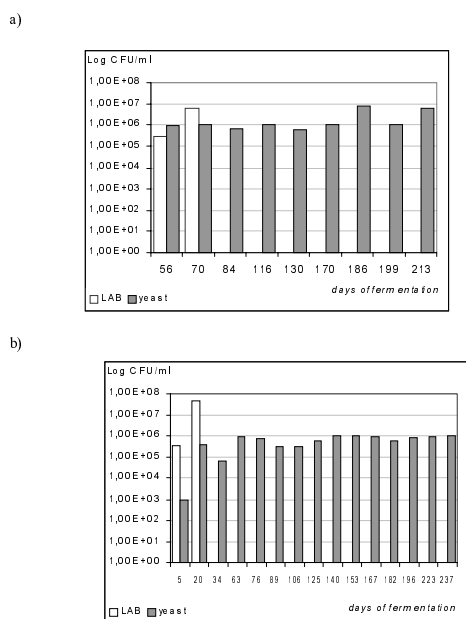


Figure 1

Homemade processing - 'Galega' variety. Yeast and Lactic Acid Bacteria (LAB) viable counts during fermentation. a) 1997/1998 season; b) 1998/1999 season.

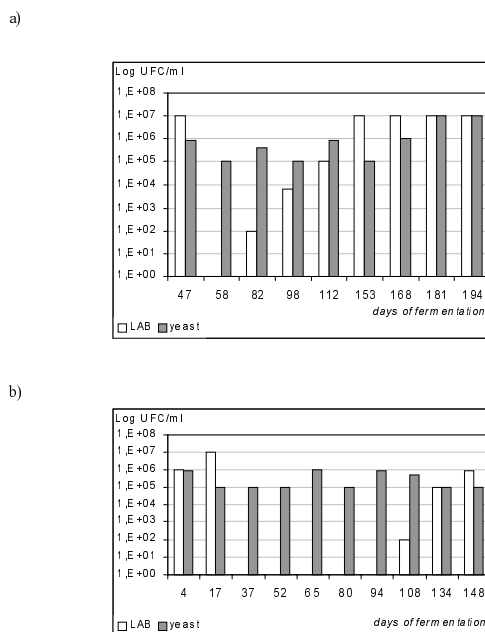


Figure 2

Industrial processing - 'Galega' variety. Yeast and Lactic Acid Bacteria (LAB) viable counts during fermentation. a) 1997/1998 season; b) 1998/1999 season

were observed in two years of trials. In the first year, only *Lactobacillus* species (*Lb. plantarum* and *Lb. pentosus*) were isolated and identified, while in the second year coccus species appeared. *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* were isolated and identified, as well as *Lb. plantarum* and *Lb. pentosus*.

Therefore differences were found between the two technological methods. In the industrial one the LAB are present throughout the fermentation period while in the homemade process they appear only at the beginning. In this case they disappear from the brine when NaCl is added which acts as an inhibiting agent due to his high concentration in an incipient phase of LAB growth.

A total of 80 strains of LAB were characterized for their antimicrobial activity and 8 of them (all were identified as *Lb. plantarum*) produced antimicrobial activity in MRS Agar against *W. paramesenteroides* and only 3 (*Lb. plantarum* Zn 42, *Lb. plantarum* Zn 50 and *Lb. plantarum* Zn 52) against *Listeria monocytogenes*, *Staphylococcus viridans*, 2 strains of *Escherichia coli*, and *Salmonella* sp. The study was only carried out with *Lb. plantarum* strains that showed antimicrobial activity against *Listeria* strain. All the culture supernatant fluids were considered to be heat stable as activity remained after heating at 121 °C for 15 min. They were inactivated by Pepsin, Trypsin and α -Quimotrysin but not by α -Amilase or β -Glucosidase. The antagonistic compounds were active at pH ranging from 2.0 to 8.0, but activity increased at pH 6.0 and pH 7.0.

The antimicrobial compounds detected are very resistant to environmental conditions under extreme values of temperature and pH. A bactericidal activity against both Gram-positive and Gram-negative bacteria was also observed. The results of enzymatic tests mean that along with the previously mentioned characteristics, the bacteriocin most probably belongs to the Class II bacteriocins (Klaenhammer, 1993). The general stability of the bacteriocin is extremely useful for possible food applications, and the results of enzymes mean that it is quite possibly safe for consumption. Some of the enzymes which were found to easily degrade the bacteriocin are related to the body. This means that along with the general understanding of these small proteins, but also the massive lack of specific knowledge, there is an enormous field to be investigated and exploited. A bacteriocin-negative mutant was isolated in plasmid curing experiments with HCl-Acryflavine and SDS at various concentrations; loss of the bacteriocin-production trait could be correlated with a loss of the plasmid. To confirm this result other studies have to be carried out. This metabolic property of the strains and its characteristics under stress conditions will be used for starter producing.

Yeast growth does not seem to be affected by any of the treatments and exhibited the same development pattern in all batches. The number of yeasts remained more or less constant throughout fermentation time ($10^4 - 10^5$ cfu/mL) in both processes (Fig. 1, 2).

Table II
Metabolic characteristics of technological interest of yeasts strains predominating in brines

Species/n° of tested strains	N° of positive strains			Growth in the presence of NaCl (% p/v)				
	Glucose fermentation	Lactate assimilation	Oleuropein assimilation	0	4	6	8	10
<i>Pichia membranaefaciens</i> / 71	0	0	0	+	+	+	+	+
<i>Candida boidinii</i> / 64	64	64	0	+	+	+	+	+
<i>Pichia fermentans</i> / 36	36	36	0	+	+	-	-	-
<i>Torulasporea delbrueckii</i> / 11	11	0	1	+	+	+	+	-
<i>Pichia Kluyveri</i> / 7	7	7	0	+	+	-	-	-

+ positive
- absence of growth

Table III
Evaluation of killer activity in predominant yeasts strains from brines, in the presence and absence of NaCl

Species/n° of tested strains	Sensitive strains				
	<i>Candida boidinii</i> with NaCl/without NaCl	<i>Candida valida</i> with NaCl/without NaCl	<i>Kluyveromyces lactis</i> with NaCl/without NaCl	<i>Saccharomyces cerevisiae</i> with NaCl/without NaCl	<i>Saccharomyces bayanus</i> with NaCl/without NaCl
<i>Pichia membranaefaciens</i> / 71	14+ / 0	69+ / 63+	10+ / 0	13+ / 0	70+ / 46+
<i>Candida boidinii</i> / 64	ND	0 / 0	ND	ND	0 / 0
<i>Pichia fermentans</i> / 36	ND	0 / 0	ND	ND	0 / 0
<i>Torulasporea delbrueckii</i> / 11	ND	0 / 0	ND	ND	0 / 0
<i>Pichia kluyveri</i> / 7	ND	0 / 0	ND	ND	0 / 0

with NaCl: tested inhibition in the presence of NaCl
without NaCl: tested inhibition in the absence of NaCl
+: positive (n° of killer strains that inhibited the correspondent sensitive)
ND: not determined

Pichia membranaefaciens predominated in both processes, followed by *Candida boidinii*, being *P. fermentans*, *P. kluyveri* and *Torulasporea delbrueckii* also present in the brines.

189 strains belonging to those 5 species were studied for their potentially relevant features for olive fermentation, such as glucose fermentation, lactate assimilation and salt tolerance (Table 2).

Killer activity was also studied and 20% of *P. membranaefaciens* strains displayed this skill against five sensitive strains (Table 3): *C. boidinii*, *C. valida*, *Kluyveromyces lactis*, *Saccharomyces bayanus* and *S. cerevisiae*, mainly when salt is present (Cansado

et al., 1993). This fact was also observed and referred by Marquina et al. (1992).

The incidence of killer activity and salt tolerance of *P. membranaefaciens* may be related to the dominance of this specie in olive brines and this ability would work against undesirable yeasts during the processes.

P. membranaefaciens and *T. delbrueckii* can have a beneficial effect in olive fermentation. These two species don't utilize lactate produced by LAB, which is good for maintaining low pH values. The second specie of yeasts utilizes the oleuropein, the most important and frequent phenolic compound found in

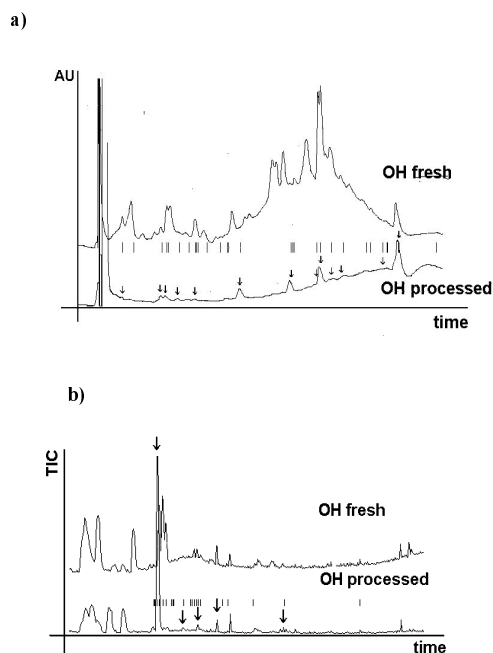


Figure 3

Chromatograms of fresh and homemade processed fruits: a) -HPLC (absorption at $\lambda=254\text{nm}$); b) -GC (Total Ion Current); the retention times of peaks selected for multivariate analysis are shown by vertical lines and arrows for peaks present in the samples

olives, which is highly toxic to LAB and needs to be removed. These two yeasts may be used as starter cultures.

3.2. Chemical analysis

There are great differences in the chemical composition of fresh and processed fruits illustrated by chromatograms presented in Fig. 3. A principal component analysis was used to study the large number of peaks detected in liquid and gas chromatography: the retention times of peaks selected for the multivariate analysis are shown by vertical lines and arrows for peaks present in the samples.

The HPLC chromatograms (Fig. 3 a) confirm that fresh fruits have higher concentrations of compounds than the corresponding homemade processed fruits as a result of a large fraction of soluble compounds present in fresh fruits, which have been transferred to the brines during the process (Catulo et al., 2002). Most of the peaks detected are phenolic compounds, which are responsible for the olives' astringency as well as the consumer's appreciation of their bitter taste.

The volatile compounds contribute to the flavor of the final product and the GC chromatograms presented in Fig. 3 b) show the higher content of volatile compounds in the homemade processed

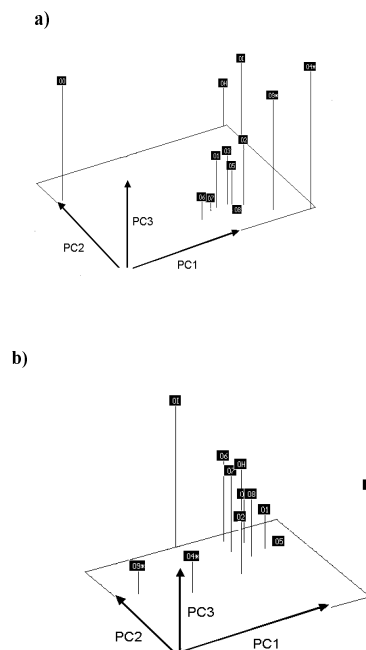


Figure 4

Representation of samples of processed olives projected onto the 3 first principal components: a) HPLC; b) GC data

fruits. In addition, the compounds detected in the fresh olives do not exist in the processed fruits.

HPLC chromatograms were compared using a principal component analysis and Fig. 4 a) presents a representation of processed fruit samples.

The sample O0 ('Gordal' variety included for comparison) is different from the samples of 'Galega' variety. In the group of 'Galega' variety olives, the samples O4*, O9* (* means different batches from the same product), OH and OI stand out. Their apparent differences from the others may be explained by the higher content of phenolic compounds present in these samples. This observation would suggest further studies with sensorial analysis in order to find out if consumers prefer the predicted stronger taste of these samples.

The volatile compounds are produced during the process and may be related to the microbial flora involved in the fermentation. The results of gas chromatography treated by principal component analysis led to the representation presented in the Fig. 4 b). The samples O0, O4*, O9*, OH and OI are again different from the others suggesting that the differences in composition of volatile compounds may be related to those factors conditioning the composition on phenolic compounds.

The overall results of HPLC and GC analysis were compared to principal component analysis (results not shown). We may distinguish the 'Gordal' sample and the 'Galega' samples. The distribution of samples OH and OI suggest a need for further study by sensorial analysis.

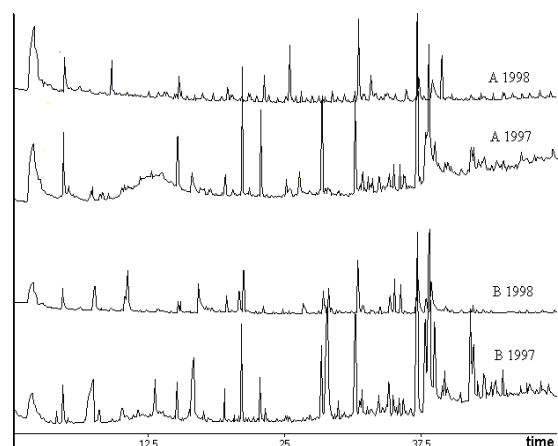


Figure 5
Chromatographic profiles of volatile compounds, by GC/MS from homemade (A) and industrial (B) brines at the end fermentation stage

The composition of processed olives was compared to the corresponding final brines analyzed by chromatography and treated by principal component analysis. It may be noticed that for most of the samples there is relationship between olives and their respective brines (results not shown).

During the fermentation process several changes in chemical composition occur and measurements of pH, sugar and lactic acid contents are very useful, allowing monitorisation.

Results from chemical analyses have shown the effectiveness of the homemade and industrial fermentation methods. Nevertheless, the chemical composition of brines was more similar between homemade samples than the industrial ones. Remarkable differences were found in the phenolic compound profile mainly in the final fermentation stage. The amount of volatiles had been enhanced on the same phase in both methods and some differences were found between them.

Results from chemical analyses of the total phenolic compounds and reducing sugars, have shown the effectiveness of homemade and industrial fermentation methods. Analysis performed in brine samples throughout fermentation processing has denoted some expected variations, since they coincided with previous water changes.

Phenolic compound profiles of brines, obtained by HPLC, exhibit similarities among samples from the same process in different years, mainly at the final fermentation stage (Fig. 5). The profile of phenolic compounds showed its metabolism throughout fermentation time by the involved microbiota (Rozès and Peres, 1998).

The volatile compounds profiles of brines, by GC/MS (Fig. 6), were compared throughout the fermentation processes and remarkable qualitative

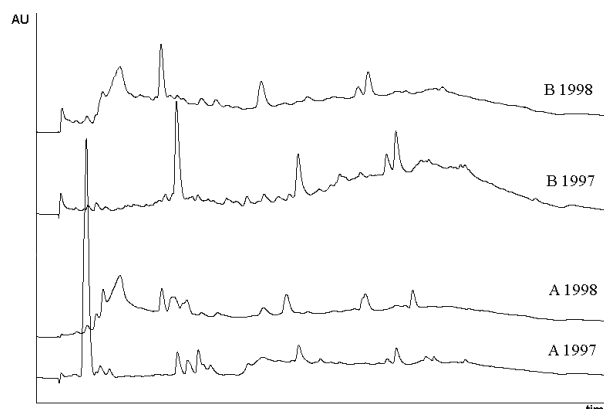


Figure 6
Chromatographic profiles of phenolic compounds, by HPLC, from homemade (A) and industrial (B) brines at the end fermentation stage

and quantitative differences were verified. Some components identified such as organic acids and alcohols revealed more than 85% similarity to the equipment's library program. The differences detected during the fermentation period indicated a high amount of compounds in the last stage. This fact may be related to the dominance of yeast growth, and their accumulation during the process. On the other hand, in the homemade type no growth of LAB was detected after the addition of salt. For this reason the production of volatile metabolites by yeasts may be confirmed. At the end of fermentation the amount of volatile compounds revealed to be higher for 1997 than 1998 in both processes being in agreement with different microbial development.

3.3. Sensorial analysis

Taste and flavor are important parameters in a sensorial analysis and therefore in the consumer's appreciation. The Sugar content of fermented fruits is not significant after the fermentation process and the salt and acid content of the final fruits may be adjusted before sending the products to the market. So, at this stage the phenolic compounds are determinant factors in the taste and the volatile ones are very important as contributors to flavor characteristics.

In a qualitative table, test samples from the homemade process achieved the classification of Good while the test samples from the industrial process were classified as Fair. The most important influential parameters for panel preferences were: size and colour homogeneity and salty and acid taste. This quality evaluation is in agreement with the fruit characteristics and the technology developed in the two processes.

The results suggest that a technological proceedings revision is required as well as the introduction of some new stages, improving the method efficiency and the final product quality.

A previous selection of fruits according to their size and uniformity, as well as the use of suitable starters will improve the organoleptic characteristics and the ensurance of final product homogeneity.

ACKNOWLEDGEMENTS

This study was supported by PAMAF/IED/INIA/6030 project.

REFERENCES

- Barnett, J. A., Payne, R. W., Yarrow, D. 1990. Yeasts: characteristics and identification. 2nd Ed., Cambridge, University Press, UK.
- Cansado J., Longo, E., Calo, P., Sieiro, C., Vélazquez, J. B., Villa, T. G. 1993. Role of killer character in spontaneous fermentations from NW Spain: ecology, distribution and significance. *Appl. Microbiol. Biotechnol.* **34**, 643-667.
- Catulo, L., Oliveira, M. M., Leitão, F., Silva, S., Gomes, M. L., Peito, A., Peres, C. 2002. Table olive fermentation of 'Galega' portuguese variety. Microbiological, physico-chemical and sensorial aspects. *Acta Hort. (ISHS)* **586**, 611-615.
- de Vuyst, L., Calleweert, R., Pot, B. 1996. Characterization of antagonistic activity of *Lactobacillus amylovorus* DCE 471 and large scale isolation of its bacteriocin amylovoryn L471. *System. Appl. Microbiol.* **19**, 9-20.
- Enan, G., El-Essawy, A. A., Uyttendaele, M., Debevere, J. 1995. Antibacterial activity of *Lactobacillus plantarum* UG1 isolated from dry sausage: Characterization, production and bactericidal action of plantaricin UG1. *Int. J. Food. Microbiol.* **30**, 189-215.
- Fernandéz-Díez, M. J. 1983. *Biotechnology*, Rehm H. J., Reed G. Ed. Vol. 5, Ch. 7, p. 379, Verlag Chemie, Weinheim.
- Fernandéz-Díez, M. J., Castro Ramos, R., Garrido-Fernandéz, A., Heredia Moreno, A., Mínguez Mosquera, I., Rejano Navarro, L., Durán Quintana, M. C., González Cancho, F., Castro Gomez-Millán, A., García García, P., Sánchez Roldan, F. 1985. *Biotechnología de la Aceituna de Mesa*. Edición del Consejo Superior de Investigaciones Científicas, CSIC. Madrid, Spain.
- Franz, C. M. A. P., Du Toit, M., Olasupo, N. A., Schillinger, U., Holzapfel, W. H. 1997. Plantaricin D, a bacteriocin produced by *Lactobacillus plantarum* BFE 905 from ready-to-eat salad. *Lett. Appl. Microbiol.* **26**, 231-235.
- Garrido-Fernandéz, A., Vaughn, R. H. 1978. Utilization of oleuropein by microorganisms associated with olive fermentation. *Can. J. Microbiol.* **34**, 680-684.
- Gomes, M. L., Catulo, L., Peres, C., Vilas Boas, L., Leitão, F. 1999. Composition of brines from olive fermentations with specific lactic starters. *Acta Hort. (ISHS)* **474**, 729-734.
- Klaenhammer, T. R. 1998. Bacteriocins of lactic acid bacteria, *Biochimie* **70**: 337-349.
- Klaenhammer, T. R. 1993. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol. Rev.* **12**, 39-86.
- Kreger-Van Rig, N. Y. W. 1984. The yeasts. a taxonomic study. 2nd Ed., Elsevier: Amesterdan, The Netherlands.
- Marquina, D., Peres, C., Caldas, F.V., Marques, J. F., Peinado, J. M. y Spencer-Martins, I. 1992. Characterization of the Yeast Population in Olive Brines. *Lett. Appl. Microbiol.* **14**, 279-283.
- Muriana, P. M. y Luchansky, J. B. 1993. *Biochemical Methods for Purification of Bacteriocins*. Bacteriocins of Lactic Acid Bacteria, Hoover G. Dallas. pp. 41-59 Stevenson Larry, R. Eds., Academic Press, Inc. San Diego, California.
- Özay, G. y Borcali, M. 1996. Effect of brine replacement and salt concentration of naturally black olives. *Food Res. Int.* **28**, 6.
- Rozès, N. y Peres, C. 1998. Effects of phenolic compounds on the fatty acid composition of *Lactobacillus plantarum*. *Appl. Microbiol. Biotechnol.* **49**, 108-111.
- Schillinger, U. y Lüke, F.-K. 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. *Appl. Environ. Microbiol.* **55**, 1901-1906.

Recibido: Mayo 2003
Aceptado: Octubre 2003