Grasas y Aceites
Vol. 51. Fasc. 6 (2000), 429-439

Naturally fermented black olives of Taggiasca variety (*Olea europaea* L.)

By **Mauro Amelio**[a*] and **Emilio DeMuro**[b]

[a] Laboratorio Controllo Qualitá, Fratelli Carli SpA, Via Garessio, 11/13, 18100 - Imperia, Italy. Tel.: +390183720000; Fax: +390183293236; e-mail: Lab@oliocarli.it.
[b] Laboratorio ARPAL, Dipartimento di Imperia, 18100 - Imperia, Italy.

RESUMEN

Aceitunas negras fermentadas al natural de la variedad Taggiasca (*Olea europaea* L.).

Por primera vez, se ha estudiado una fermentación natural de aceitunas negras de la variedad Taggiasca (*Olea europaea* L.). Esta variedad es típica del Oeste de Liguria (Noroeste de Italia), donde se usa principalmente para la producción de aceite de oliva. Además del proceso tradicional, tres procesos ligeramente diferentes fueron considerados. La fermentación fue llevada a cabo en barriles de 200 litros mantenidos a temperatura ambiente (7-25°C) y duró aproximadamente 6 meses. Al final las aceitunas fueron pasteurizadas y almacenadas durante otros 6 meses para equilibrarlas. A través de todo el proceso se analizaron: pH, NaCl, acidez, contenido en biofenoles y análisis microbiológico (recuento de colonias, coliformes (*Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Escherichia coli*), Lactobacilos (25°C, 36°C, 45°C), Pseudomonas (*P. fluorescens, P. cepacia*), Levaduras (*Candida* spp.), Mohos (*Penicillum* spp., *Clostridium* spp., *Vibrio* spp.) de salmueras. Los resultados mostraron que es recomendable una corrección inicial del pH y una inmersión no demasiado larga de las aceitunas en agua (3+3 días), excepto un corto lavado justo antes de ponerlas en salmuera.

PALABRAS-CLAVE: Aceituna negra fermentada al natural - Análisis microbiológico - Olea europaea L. - Taggiasca (variedad).

SUMMARY

Naturally fermented black olives of Taggiasca variety (*Olea europaea* L.).

For the first time, a natural fermentation of black Taggiasca variety (*Olea europaea* L.) olives was studied. This cultivar is typical of Western Liguria (NW of Italy), where it is mainly used for olive oil production. Beside the traditional process, three slightly different processes were taken into account. The fermentation was carried out in 200 litre barrels left at environmental temperature (7-25°C) and took about 6 months to be complete. At the end the olives were pasteurised and stored for a further 6 month period to equilibrate. Throughout the process, pH, NaCl, acidity, free biophenol content and microbiological analysis (Colony count, Coliforms (*Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Escherichia coli*), Lactobacilli (25°C, 36°C, 45°C), Pseudomonas (*P. fluorescens, P. cepacia*), Yeasts (*Candida* spp.), Moulds (*Penicillum* spp., *Clostridium* spp., *Vibrio* spp.) of brines were performed. The results showed that an initial pH correction is recommended and no long soaking of the olives in water (3+3 days) is advisable, except a short washing just before brining.

KEY-WORDS: Microbiological analysis - Naturally fermented black olive - Olea europaea L. - Taggiasca (variety).

1. INTRODUCTION

In the Mediterranean area, the olive tree has a long tradition that goes back to the IV millenium b.C. (Zohary D.,1975; Nisbet R. 1993). Nowadays, the olive oil trade is one of the most important items for the economies of several countries in the Mediterranean basin, whilst table olives are slowly gaining new markets thanks to the benefits of the so called «Mediterranean diet» which are becoming ever more evident. The present paper is devoted to the study of the traditional way of preparing table olives in brine of Taggiasca variety (*Olea europaea* L.). It grows on the western side of Liguria, a mountainous coastal region in the Northwest of Italy and it is the typical and almost the only variety present from the coast up to 500-600 m a.s.l., which is the local upper limit for the olive tree to be cultivated. Normally, the harvest time begins in late November and goes on up to April, sometimes later, depending on the altitude of the olive tree grove, on the weather, on the olive fly attacks etc. The olive fly attacks often severely damage the crops, mainly near the coast where the climate is milder. Most of the harvest is for the production of olive oil, while a smaller amount is used for table olives. For this purpose, people usually collect ripe but not overripe olives, normally not before February, best in March. The local home-made process consists in a previous debittering by water renewing for about 40 days (oleuropein diffusion) followed by brining. This continuous change of water probably does not allow the microbes to develop significantly but, on the other hand, it reduces the presence of useful strains, while yeasts and mould are common and sometimes well accepted. For industrial processes, it could be advisable to perform an olive washing just before brining. The olives are naturally fermented and a large scale process could be divided into six steps: collection of ripe fruits, water washing of drupes and sorting for size and soundness, brining in barrels (200 L) with a sodium chloride 12-14% (w/v) solution. The olives are stored in brine for 6-8 months and the salt level kept up throughout. At the end, the olives are sorted again, the brine renewed and aromatized

(e.g.: thyme, rosemary, bay) and thus they are ready for consuming or pasteurised for long term preservation. Minor differences in the process could be found from one place to another. The process via natural fermentation is typically practised in Greece and for an extensive description of it with references, refer to the book by Garrido Fernández A., 1997. In the present study, we chose this method and we tried three more slightly different ways described later. As far as we know, no previous papers on Taggiasca olive brining have been available until now. Thus, due to the industrial exploiting of the present work outcomes, the aim of the work was to collect a set of data of technological interest on the growth of the main microbes present in the different brines, the biophenolic content, both in the raw olives (free and alkali-hydrolizable biophenols, FHBP) and in the brines, and technological parameters such as temperature, pH, NaCl content, titrable, fixed and volatile acidities.

2. MATERIALS AND METHODS

2.1. Olives

During January 1998, about 1600 Kg of Taggiasca variety olives were bought all over the Diano S. Pietro area, near Imperia, where the cultivar Taggiasca (*Olea europaea* L.) is the only cultivated variety. Due to the bad crop (poor harvest, severe olive fly attacks etc.), the collection of the whole amount took about one month. The olives were divided in 14 barrels in accordance with the following treatments: Method 1 (5 barrels, 540 Kg), according to the traditional way described above; Method 2 (3 barrels, 355 Kg), as in method 1, but the pH was set at 4.0-4.5 with acetic acid; Method 3 (3 barrels, 330 Kg), the olives were stored in water 3 days and after water renewing, 3 more days. Then, they were brined as in method 1; Method 4 (3 barrels, 375 Kg), as in method 3, but the pH of the brine was set at 4.0-4.5 with acetic acid. Hereinafter, these methods will be respectively referred to as M1, M2, M3, M4. All the barrels were stopped and filled up with proper brine in order to reduce the air contact and aerobic microbial growth to a minimum. Throughout the fermentation, the barrels were stored in an open warehouse where the temperature was highly dependent on the outside values. The olives showed a mean equatorial diameter of about 15 mm and a mean polar diameter of about 22 mm. In the barrel, the ratio olive/brine (w/w) was, on average, about 0.9. Not all the fruits were at the same stage of ripeness: about 25% of the olives in M3 were still at the green stage. Due to the above mentioned reasons, about 40% of fruits had been bitten by olive fly. All the samplings were carried out from the brine surface after removing the yeast layer if any, then the barrels were stopped and filled up with the proper brine each time. The main drawback was the likely difference in chemical and microbiological composition of the deeper layers compared to the upper one. This was evident when the brine was discarded after fermentation: the nearer the bottom of the barrel, the darker the brine was. This way of sampling was not ideal, but it reduced to a minimum the possibility of contamination, olive damage and barrel handling.

2.2. Sampling

In order to investigate the microbial growth that is faster and more varied during the first days of fermentation, samplings were carried out about 2, 10, 14, 20, 30, 50 days after brining and then once a month, both for microbiological and chemical analyses.

2.3. Chemicals

Unless stated otherwise, reagents were of analytical grade, provided from: tyrosol, water (HPLC grade), methanol (HPLC grade), n-hexane, ethyl acetate (Fluka, Buchs, Switzerland); acetic acid (HPLC grade) (BDH, Poole, Dorset, UK); gallic acid, caffeic acid, hydrocaffeic acid, p-hydroxy-benzoic acid, sinapinic acid, trans-cinnamic acid, syringic acid, ferulic acid, p-coumaric acid, vanillic acid, protocatechuic acid (Sigma, St. Louis, MO); hydrochloric acid, phenolphthalein (Carlo Erba Reagenti, Rodano, Italy); sodium hydroxide 0.1N solution, potassium chromate (Riedel-de Haen, Seelze, Germany); ethanol, silver nitrate 0.1N solution (Merck, Darmstadt, Germany).

2.4. Solutions

Potassium chromate indicator: dissolve 50g of potassium chromate in a small quantity of water. Add silver nitrate 0.1N solution until a red precipitate appears. Leave to stand for 12 hours, then filter and bring up to 1 L with distilled water. Gallic acid standard solution: dissolve in methanol a suitable amount of gallic acid exactly weighed to get about 2 mg/mL solution. Biophenol standard solution: exactly weigh and dissolve in methanol a suitable amount of standard to get about 0.1 mg/mL solution of each biophenol.

2.5. Methods for chemical analysis of brine

pH. Potentiometric measurement.

Titratable acidity. Titrate 10 mL of filtered brine with NaOH 0.1N, using phenolphthalein as indicator. If the brine is highly coloured, dilute it before titrating.

Fixed acidity. Evaporate 10 mL of filtered brine on a water bath at 90°C, add few mL of water and

evaporate again three or four times to remove the volatile acidity. Transfer the residue into an erlenmeyer flask with some water, add two drops of phenolphthalein as indicator and titrate with NaOH 0.1N.

Volatile acidity. By difference between Titratable and Fixed acidity.

NaCl concentration. Weigh exactly about 2 g of brine and dilute it to 100 mL with distilled water. Set the pH between 7 and 10. Add 1 mL of potassium chromate as indicator and titrate with $AgNO_3$ 0.1N.

Free biophenol extraction. Add 4 µL of gallic acid standard solution to 1 mL of filtered brine. If the brine contains significant amounts of gallic acid, another internal standard must be chosen. Add about 20 µL of hydrochloric acid (2N) to get a sharply acid pH. Extract twice with 1 mL of n-hexane each time to remove the fatty material and separate the two phases by centrifugation. Discard the organic phase. Extract twice with 1 mL of ethyl acetate, centrifugate and mix the extracts. Evaporate under a gentle N_2 stream or under vacuum at room temperature. Dissolve the extract in 500 µL of the HPLC mobile phase at time=0 min (90% solvent A), then perform the HPLC separation.

HPLC free biophenol separation. The following were used: Series 200 Ic pump, Series 200 autosampler and Diode Array 235C detector from Perkin Elmer (San Jose, CA); chromatographic column: LiChroCART 250-4 Supersphere 100 RP-18 from Merck (Darmstadt, Germany). The mobile phase was made up with: water with 1% of acetic acid (solvent A); methanol (solvent B). The gradient was the following: solvent A, from 90% to 85% in 3 min; 9 min at 85% then to 71% in 7 min; 8 min at 71%; to 50% in 8 min; to 0% in 10 min; 10 min at 0%; to 90% in 0.2 min; 15 min at 90% before the next run. The flow was 1 mL/min; the loop size, 200 µL; the detector wave length, 280 nm. The chromatogram integration was performed with Turbochrom Navigator, Version 4.1 (Perkin Elmer, PE Nelson Division, San Jose, CA). Previous runs of biophenol standard solutions were performed to set the response factor with respect to the internal standard (gallic acid). The peak identification of unknown samples was performed comparing their relative retention time and spectra with those taken from standard runs.

2.6. Methods for microbiological analysis of raw olives and brine

The procedure described by Messer in order to obtain a «rinse» (Messer JW, 1992) from raw olives was adopted. Both the «rinse» and the brine were treated according to the ISO 6887 method (ISO 6887, 1999) and to the Oxoid Manual (Oxoid Manual, 1993). The following were performed: for Colony count, Plate Count Agar. For Coliforms (*Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Escherichia coli),* Violet Red Bile Agar and the API 20 E Identification System (bioMerieux, Marcy l'Etoile, France); for Lactobacilli (25°C, 36°C, 45°C), deMan Rogosa Sharpe Agar. For Pseudomonas *(P. fluorescens, P. cepacia)* and *Vibrio* spp., Pseudomonas Agar Base. For Yeasts (*Candida* spp.) and Moulds (*Penicillum* spp.), Rose-Bengal Chloramphenicol Agar. In particular, for *Candida* spp., the identification was performed using the ID 32 C Identification System (bioMerieux). For *Clostridium* spp., Sulphite Polimixin Sulphadiazine. Samplings were performed according to the usual microbiological techniques.

2.7. Methods for chemical analysis of raw olives

The free and alkali-hydrolizable biophenol (FHBP) composition only was investigated. It was adopted the method described by Bianco (Bianco A., 2000) as «Procedure B»: it allows the free and alkali-hydrolyzable biophenols to be extracted from fruits. It consists of a controlled alkali treatment of the drupes and in a further ethyl acetate extraction of the solution. The solute is then partitioned by a mixture made up of acetonitrile/hexane 1: 1. FHBP are extracted by the acetonitrile phase.

3. RESULTS

Temperature. At the beginning of the process, the temperature was about 10°C and reached its minimum (7°C) at the end of January, after few days of fermentation. At the beginning of April the temperature reached 15°C, then increased up to 25°C by the end of July (Fig. 1).

pH. The water used to prepare the brine was about 7.5-7.7. The pH decreased to 4.9-5.1 in 30 days for washed olives (M3), and in 50 days for the traditional method (M1). For washed olives with acidified brine the pH, set to 4.1, reached 4.9 in 30 days for M4, and in 40 days for M3. As shown in Fig.2, the values showed the greatest decrease during the first 2-3 days in M1, while in M3, pH took

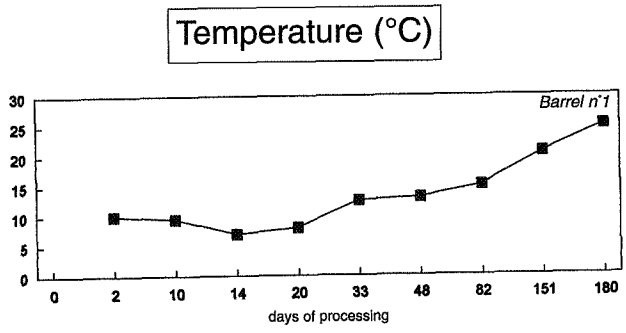

Figure 1

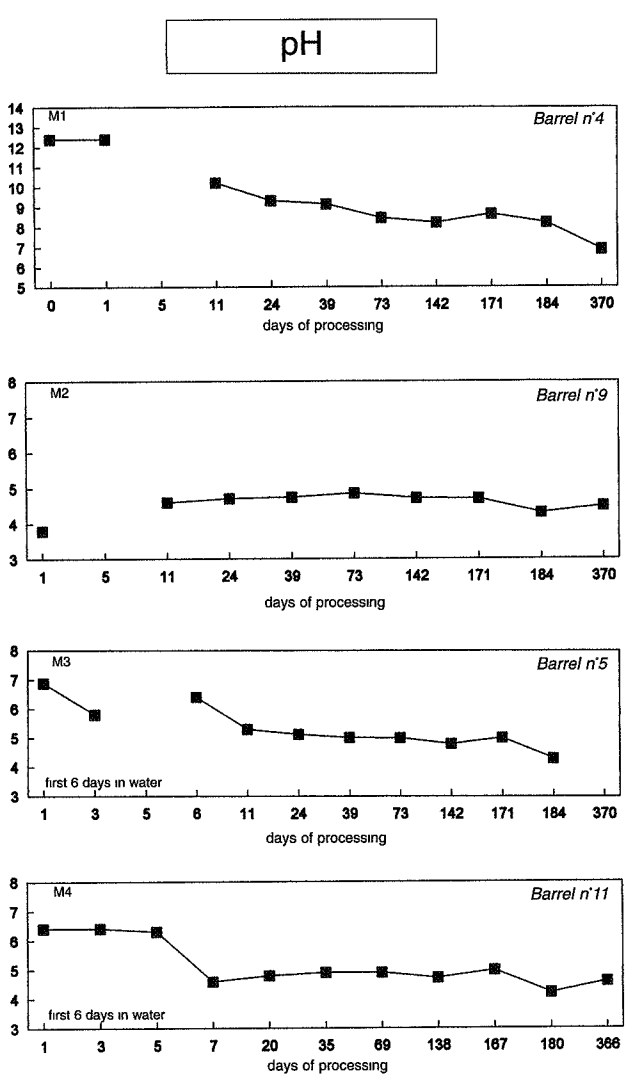

Figure 2

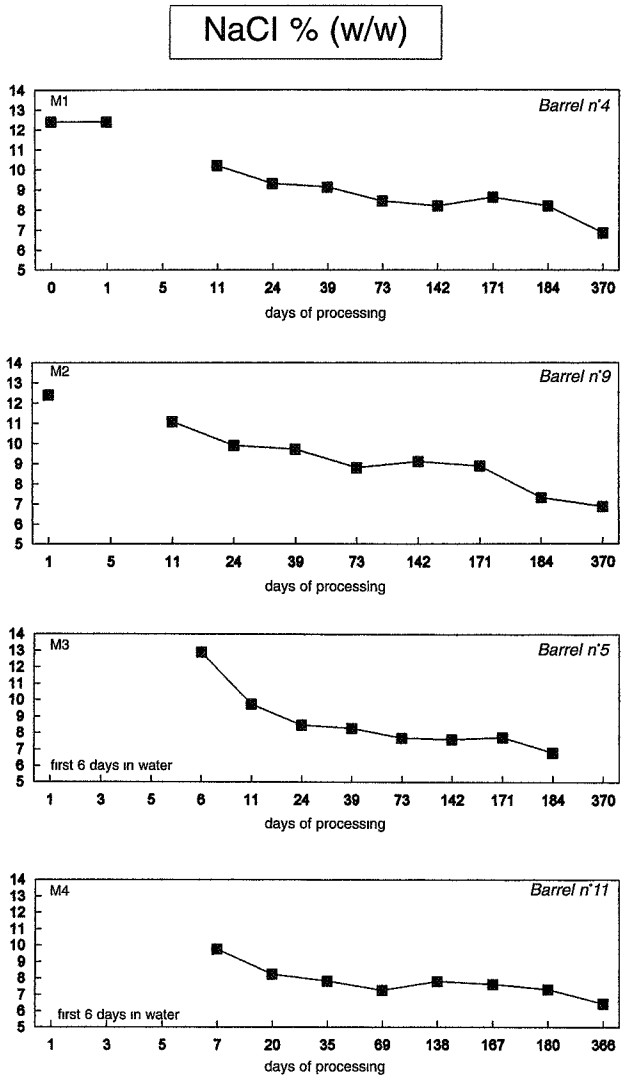

Figure 3

about 10 days to reach the same values. When the temperature reached about 20°C, the titrable acidity decreased and pH increased of 0.1-0.3. This pH change was less relevant where the pH of brine was corrected. The final pH values were between 4.5-5.0.

NaCl concentration (% w/v). The initial concentration was 12-13% (w/v). For M1 and M2, the salt level was equilibrated at 9% in about 40 (M2) and 50 days (M1), while M3, M4 reached 8% in about 30 days. This lower concentration may be due to a dilution effect because of the residual washing water still present in the barrels. If the pH was corrected, the equilibrium was reached faster (about 10 days sooner): more investigation is needed to ascertain a possible correlation. The final values are between 7.6-9.2% (Fig.3). They could be too low for a safe preservation, in particular with «high» pH. Nevertheless, olives have the brine to be renewed and pasteurised.

Titrable acidity (g lactic acid/100 mL brine). The acidity reached values 0.15-0.20 in about 50 days for M1, while it took 40 days to reach 0.15 for M3. The same data for M2 and M4 are not significant because of the initial pH correction with acetic acid. After about 150 days of fermentation, the acidity reached its maximum (0.45-0.50 for M1, M2 and 0.33-0.44 for M3, M4), then it decreased during the following 30 days (Fig.4). This behaviour was already observed for other variety as Lechín and Picual: after 66 days of fermentation the acidity (0.52) fell to 0.44 in 34 more days (Garrido-Fernández A., 1997).

Fixed acidity (g lactic acid/100 mL brine). In 35-40 days the acidity reached 0.05-0.08 for each method. After 150 days of fermentation, the values were 0.17-0.23 for M1, M3, and 0.17-0.28 for M2, M4. Fixed acidity shows the same trend of titrable acidity in the further 30 days, a fall of about 0.05-0.11. The higher levels were reached in M2 with the least

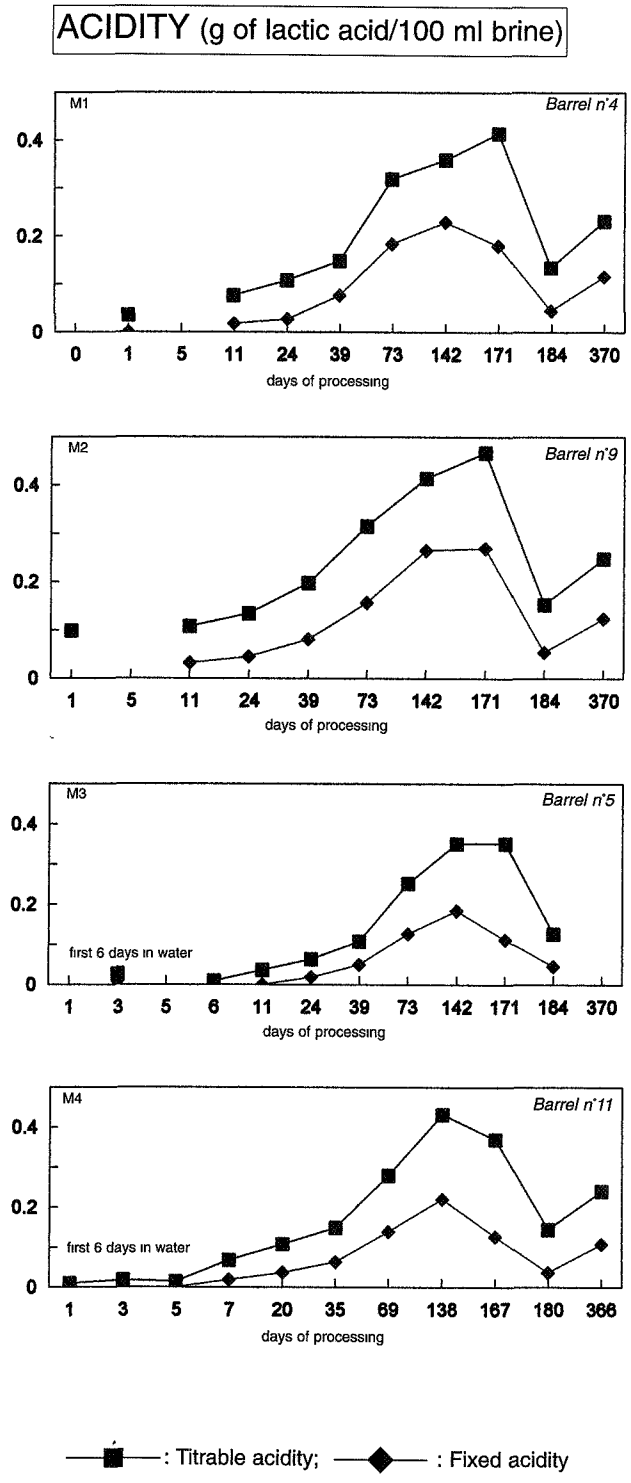

Figure 4

subsequent drop (0.28 to 0.23) (Fig.4). One barrel belonging to M1 had an higher value (0.29) if compared with similar barrels (about 0.23).

Volatile acidity (g lactic acid/100 mL brine). For M2, M4 the presence of acetic acid to correct pH enhanced the volatile content (0.17-0.24), even if at the end of the process M1, M3 showed higher values (0.22-0.28). This parameter increased continuously throughout the fermentation, even when the titrable acidity was decreasing. The same barrel as above showed the highest volatile acidity level (0.31).

Olive free and alkali-hydrolizable biophenol (FHBP) composition (mg/100g olives). In the first processed olive stock, about 610 mg/100g of total FHBP were found, 81% being hydroxytyrosol+tyrosol. Among the others, 20 mg/100g of p-coumaric acid and no hydrocaffeic acid were found. Furthermore, because of the alkaline extraction method, no oleuropein could be found: an equivalent amount of hydroxytyrosol was instead produced (Garrido-Fernández A., 1997). Other FHBP were: protocatechuic acid (5.0), vanillic acid (1.3), syringic acid (0.3), ferulic acid (2.7).

Brine free biophenol (FBP) evolution (mg/L). The previous water soaking (M3, M4) makes the FBP diffuse into the water up to low levels (about 55 mg/L), while the brine makes it more effective, even if the olives already lost FBP amounts in water. Within about 20 days, the brines reached 280 mg/L of total FBP. At the end of fermentation the total FBP concentrations ranged between 800-1700 mg/L, the most abundant being hydroxytyrosol and tyrosol (>90%). The highest levels (about further 100-200 mg FBP/L) were found in M3. The other FBP were <50 mg/L each, the most abundant being caffeic acid and, in few cases vanillic acid. It was noticed that when the most abundant FBP (normally caffeic acid) was about >40 mg/L, the count of colonies+yeasts+moulds was $>10^6$. Furthermore, it is worthwhile to notice the caffeic acid behaviour: it showed increasing values (up to 52 mg/L) until the temperature was below 20-22°C, then there was a sharp fall (down to<12mg/L). At the same time, hydrocaffeic acid, the reduced form of caffeic acid with a saturated side chain, that was not found in fresh olives, suddenly increased up to >200 mg/L in M1, M3 and <170mg/L in M2, M4. The caffeic acid and, to a lesser extent, the disappearance of hydroxytyrosol could be also related to an oxidative polymerization during the darkening process, which, however, is not relevant in this type of process (Garrido-Fernández A., 1997). The hydrocaffeic acid increased 3 to 10 times (M2, M4) and 5 to 20 times (M1, M3) in 30 days. In M1, M3 oleuropein was never found, whilst in M2, M4 small amounts were found (up to 6 mg/L): after about 20 days of fermentation, oleuropein was zeroed. This difference among methods could be explained if one recalls that the higher pH makes the oleuropein hydrolize, while the pH correction in M2, M4 slowed down this reaction. p-Coumaric acid was absent in brines while the temperature was below 15°C (first 70-80 days), then it was revealed up to 9 mg/L.

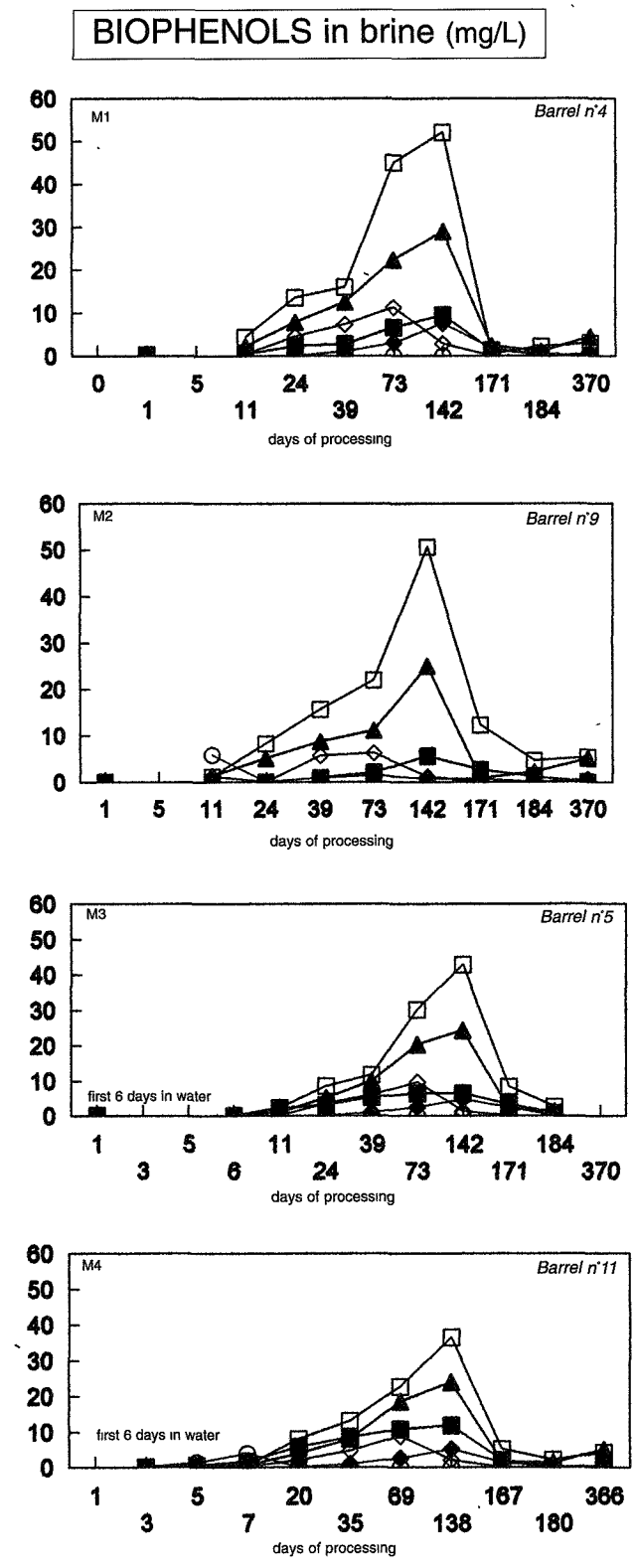

Figure 5

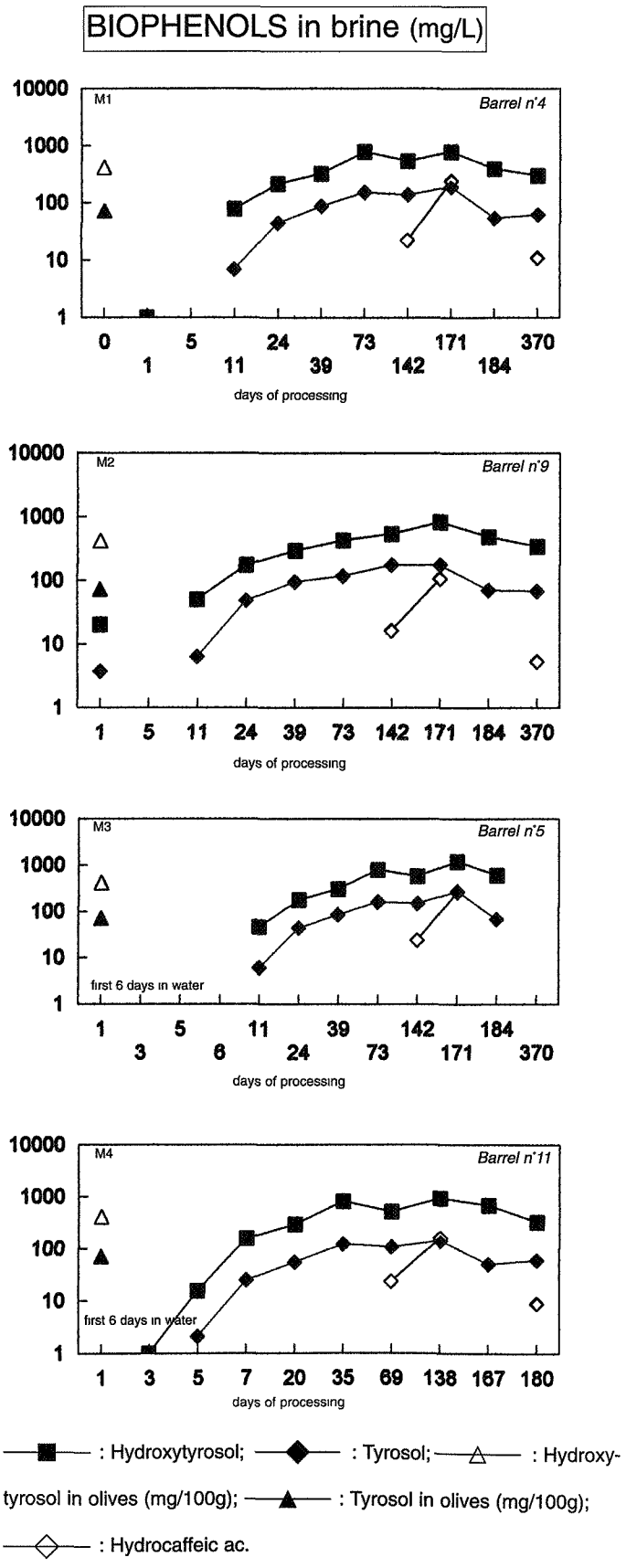

Figure 6

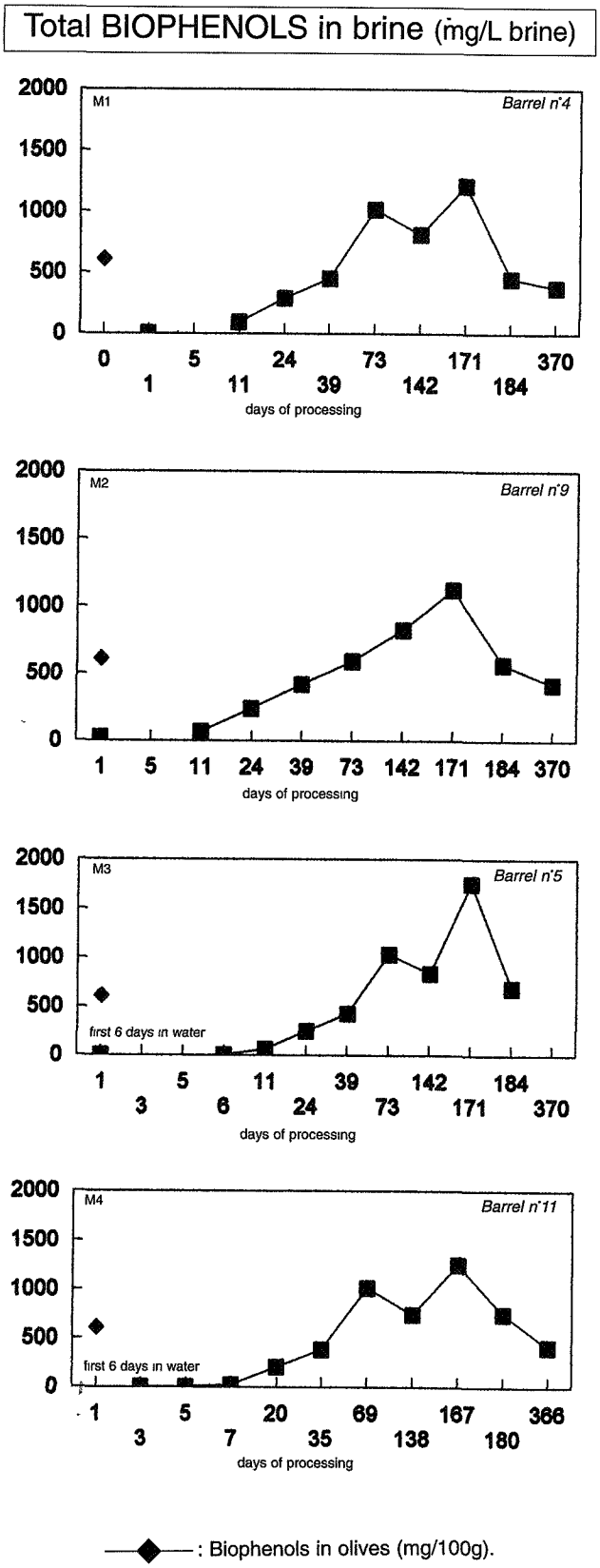

Figure 7

p-Coumaric acid was present in olives (20 mg/100gr), but due to its low solubility, no significant diffusion into brine occurred at lower temperature (Garrido-Fernández A., 1997). On the whole, 10-20% of FBP contained in the fresh olives were transferred into the brine, but the loss of FBP could have been increased by further reactions that gave unidentified products (Fig. 5, 6, 7).

Brine colony count (CFU/g). There was a sharp difference between M1, M2 and M3, M4 during the first days of processing. The brines with olives that underwent 3+3 days in water showed a relevant microbial growth, both for Colony count and for Coliforms (few billions CFU/g). On the contrary, M1, M2 reached, with a regular decreasing trend, about 10-20 CFU/g in 25 days. When water soaked olives were put into the brine, they reached the same microbial concentration as in M1, M2, in about 18-20 days. This period was independent of the colony count at the beginning of the process. The concentration showed a relevant decreasing pattern until about 70 days of process. When the environmental temperature reached 15°C, there was a decimal growth per month in M1 (up to 7000), while it was slower in the other cases (up to 1000) (Fig. 8).

Brine coliforms (Klebsiella spp., *Enterobacter* spp., *Citrobacter* spp., *E. coli; CFU/*g). During the first 3+3 days in water, the total count reached as high a concentration as about $3x10^8$. On the other hand, after 20-30 days from brining, coliforms were eliminated. This evident growth is dangerous because it could cause, for example, the gas-pocket spoilage (e.g.: «alambrado»), which is an irreversible defect (Garrido-Fernández A., 1987; Garrido-Fernández A., 1997; IOOC, 1990). *Klebsiella* spp. was found ($2x10^4$) in only one barrel belonging to M3 and was rapidly zeroed after brining (5 days). *Enterobacter* spp. and *Citrobacter* spp., when present, reached 10^7 (E.) and $14x10^6$ (C.). *Citrobacter* spp. appeared not to be correlated to a particular process method. E. coli was met in few samples up to $3x10^6$ (M3). The water renewing after the first 3 days, decreased its level of 1 order of magnitude and the brining rapidly zeroed its presence. As already said, all these Gram-negative strains which are due to environmental contamination and typical of the first days of fermentation, have to be avoided because they are mainly responsible for gas spoilages (Fernández-Díez M.J., 1985; Garrido-Fernández A., 1997).

Brine Lactobacilli spp.*(CFU/g)* The presence of *Lactobacilli* was very different between M1, M2 and M3, M4. The previous water soaking made the *Lactobacilli* develop more abundantly, also in the washing water where strains growing at 36°C and 45°C were found: at 25°C, up to $1.4x10^6$; at 36°C, up to $1.6x10^6$; at 45°C, up to $4.7x10^4$. It was noticed that after brining the *Lactobacilli* concentration often

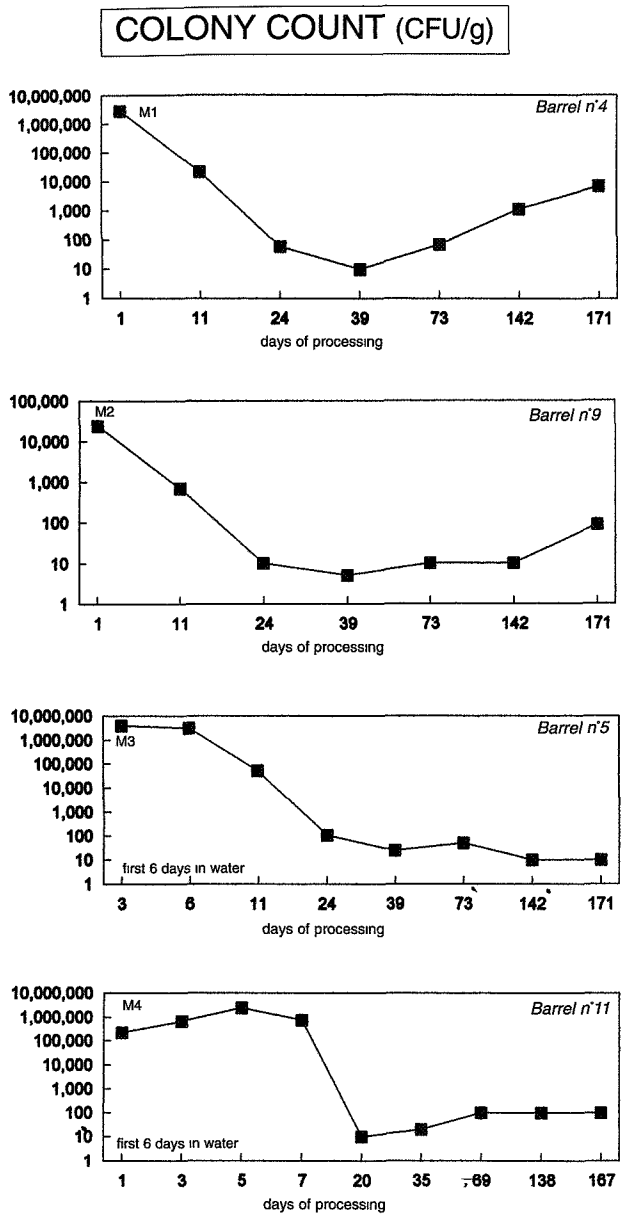

Figure 8

decreased at very low levels (few tens CFU/g), while other samples showed a regular increase up to few thousand CFU/g. In particular, where the pH was corrected, they reached a level of about $3x10^3$. For all the barrels, when the temperature reached 15°C, there was a sudden growth of *Lactobacilli*, followed by a decrease, probably due to the lowered sugar content in brine. The highest levels were reached in M1 ($2.5x10^5$) at temperatures >15°C. Thermophilic *Lactobacilli* (36°C, 45°C) were found ($3x10^3$) in samples taken from M3, M4. It is advisable to promote the *Lactobacilli* growth, mainly at the process beginning to rapidly lower the pH (3.5-4.0) and set a prevailing homolactic fermentation. The initial pH correction is useful to avoid the growth of undesired strains, but *Lactobacilli* must dominate as soon as possible (Fig. 9).

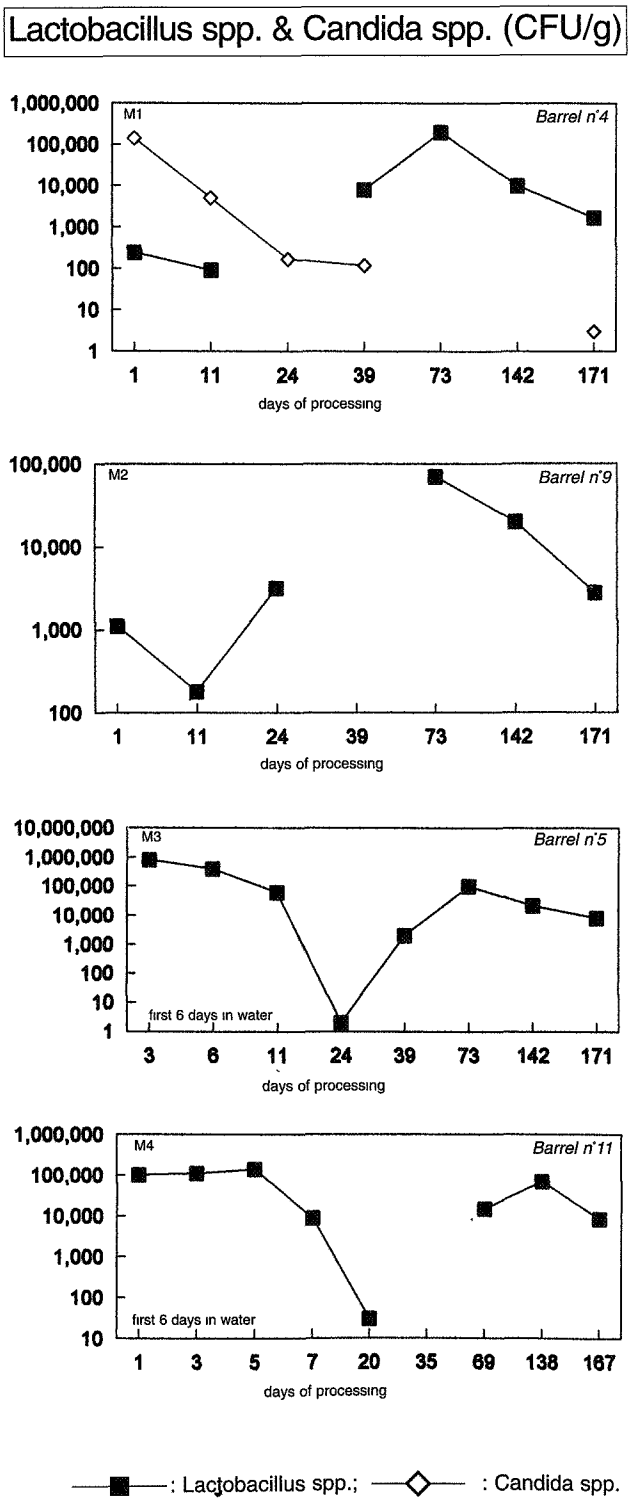

Figure 9

Brine Pseudomonas spp. (*P. Fluorescens; CFU/g).* The highest levels ($6x10^8$) were reached in the washing water (M3, M4), then this microbe grew until the temperature was within 15°C. As *Lactobacilli* increased and dominated, Pseudomonas was rapidly zeroed. In general, no significant development was observed after brining.

Brine Yeasts (Candida spp., *CFU/g).* An irregular growth was observed barrel by barrel. *Candida* spp. was mainly found in M1 (up to $2.5x10^5$), but showed a decreasing trend. A few dozen CFU/g were found in M2. In some cases not strictly dependent on the temperature, up to $3.2x10^6$ CFU/g were found (Fig. 9). Even if yeasts are usually accepted in the traditional process, we think that it is advisable to reduce them by choosing an anaerobic condition. Furthermore, it frequently occurs that moulds grow along with yeasts in aerobic conditions and aflatoxins could reach dangerous levels.

Brine Moulds (Penicillum spp.; *CFU/g).* An irregular development of moulds was observed, with a greater vital activity as the temperature went above 15-20°C. The highest levels ($9x10^4$) were reached (M4) at the beginning of the process, perhaps due to fresh olive contamination. Not strictly anaerobic conditions facilitated their growth (e.g.: barrels not tightly closed).

Brine Clostridium spp.*(CFU/g).* This microbe is responsible for malodorous spoilages. No significant growth was observed and the original content was zeroed in a couple of weeks after brining.

Debittering. At the time of samplings, a few olives taken from each barrel were tasted for bitterness. After about 60-65 days they were debittered, even if, in some case, a delay (10-15 more days) was observed, probably due to the original state of ripeness.

4. DISCUSSION

Barrels processed in the same way showed remarkable chemical and microbiological differences that are not the best result for industrially processed table olives. The olive soaking in water for six days appeared inadvisable because of the great development of Gram-negative microbes *(Coliforms)* that are responsible for irreversible spoilages. The typical Taggiasca variety table olives do not undergo an important lactic fermentation, and the low temperature throughout the first 60-70 days of process does not promote it to a relevant extent. It was evident also in the pH evolution that was not so low as it should be. Thus, even if the pH correction does not belong to the local tradition, it is advisable to do it to prevent undesired microbial growth. When the temperature went above 15°C, an increase of the volatile acidity was observed. It is possible that other heterolactic strains grew or had more vital activity like yeasts and moulds, with acetic acid production. High salt levels in brine, far from making the Taggiasca olives shrivel, speed up the microbe selection *(Lactobacilli)* and, perhaps, make the fermentation safer. On the other hand, it does not promote the *Lactobacilli* development to the extent advisable (Ozay G., 1996) and in the shortest time. Furthermore, higher free biophenol contents were found in brine without pH correction: on the contrary, it could be better to preserve the highest biophenol content due to their antioxidant activity (Shahidi F., 1995), in order to keep the olive quality at its best. The pasteurization of fermented Taggiasca olives, sealed in glass jars (720 mL) with a renewed and aromatised brine (rosemary, bay, laurel), takes 45 min (overall process), 12-15 min of which are spent at about 85°C. After cooling, the olives are not at their best: many of them are shrivelled and too soft. Within 4-6 months, the olives gain a satisfying toughness and a smooth skin. Furthermore, this process causes the water soluble matter contained in the olive flesh to diffuse into the brine. Thus, the olives become less rich, not only in biophenols, but also in acids and the pH increases. This is why the new brine should be corrected with acid (usually, lactic or acetic). As a main outcome of this first year study, we can state that it is advisable to choose the Method 2, because of the more selective environment respect to the strain growth, and for the least loss of olive biophenols. From a sensory point of view, no relevant differences were found. Nevertheless, measurements of the percentage of sound olives at the end of fermentation should be performed in order to depict a more precise evaluation of the whole process, also from an economical point of view: this is one of the next year study targets.

Abreviations used

FBP = free biophenols.
FHBP = free and alkali-hydrolizable biophenol.
M1, M2, M3, M4 = Method 1, Method 2, Method 3, Method 4.

ACKNOWLEDGEMENTS

The authors thank Dr. R. Rizzo, Mr. F. Varazini, Mr. G. De Andreis, Mr M. Zat, Mr M. Porro (Fratelli Carli SpA) and Dr. L. Maxwell for their invaluable cooperation. Furthermore, Prof. A. Bianco (Dept. of Chemistry, «La Sapienza» University, Roma) for the manuscript revision and Program FAIR CT97-3053 (Olitext) supported by the European Community, are also gratefully acknowledged.

BIBLIOGRAPHY

Bianco A., Uccella N. (2000).—«Biophenolic components of olives».—*Food Res. Int.,* (in press).

Fernández-Díez M.J., Castro-Ramos R., Garrido-Fernández A., González-Cancho F., González-Pellissó F., Nosti-Vega M., Heredia-Moreno A., Mínguez-Mosquera M.I., Rejano-Navarro L., Durán-Quintana M.C., Sánchez-Roldán F., García-García P. and Castro-Gómez-Millán A. (1985).—«Biotecnología de la aceituna de mesa».—*Consejo Superior de Investigaciones Científicas,* Instituto de la Grasa, Madrid-Sevilla, Spain.

Garrido-Fernández A., Durán-Quintana C., García-García P. (1987).—«Fermentación en medio aeróbico de aceitunas negras al natural en Salmuera. Efecto de algunas variables sobre la formación de "alambrado"». —*Grasas y Aceites,* **38,** 27-32.

Garrido-Fernández A., Fernández-Díez M.J., Adams, M.R.(1997).—«Table olives».—Chapman & Hall Eds., Publisher: London, UK.

IOOC (1990).—«Table Olive Processing».—International Olive Oil Council, p.1-109, Madrid, Spain.

ISO 6887 (1999).—«General rules for the preparation of the initial suspension and decimal dilutions».—Publisher: International Organization for Standardization, Geneva, Switzerland.

Messer J.W., Midura T.F., Peeler J.T. (1992).—«Sampling plans, sample collection, shipment, and preparation for analysis, in *Compendium of methods for the microbiological examination of foods*».—C. Vanderzant, D.F. Splittstoesser Eds., Publisher: *Am-Public Health Ass.,* Washington, DC 20005.

Nisbet R. (1993).—«La diffusione dell'olivo in *Catalogo del Museo dell'Olivo*»— p. 26-27, Museo dell'Olivo (info@,museodellolivo.com): Imperia, Italy. Oxoid Manual. Unipath Limited Eds., Publisher: Hampshire, UK, 1993.

Ozay G., Borcakli M. (1996).—«Effect of brine replacement and salt concentration on the fermentation of naturally black olives».—*Food Res. Int.,* **28,** 553-559.

Shahidi F. and Naczk M. (1995).—«Food phenolics»—. Technomic Pub., Lancaster, PE, USA.

Zohary D. and Spiegel-Roy P. (1975).—«Beginning of fruit growing in the old world».—*Science,* **187,** 319-327.

Recibido: Noviembre 1999
Aceptado: Junio 2000