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# Evaluation of black table olives in different brines

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**RESUMEN**

**Evaluación de aceitunas negras estilo griego utilizando salmueras de diferentes concentraciones.**

Se han ensayado tres procesos para la elaboración de aceitunas negras de mesa. Los frutos se colocaron en: a) una salmuera con una concentración de sal del 16% (w/w), proceso tradicional; b) en una solución tampón compuesta de $CH_3COOH$ (0.05M) y $Ca(OH)_2$ (0.025M), sin NaCl y con un pH inicial de 4.3; c) una solución tampón compuesta de $CH_3COOH$ (0.05 M) y $Ca(OH)_2$ (0.025M),conteniendo 12.8% (w/w) NaCl y un pH de 4.3. Se realizó el aislamiento, identificación y el recuento de los micoorganismos predominantes, tanto del fruto como de las salmueras, durante la fermentación. Asimismo, se estudió el color, textura y las características organolépticas de los productos finales. El tercer tipo de proceso fermentativo, tipo c, dio lugar a un producto con baja sal, ausencia de microorganismos alterantes o de cualquier otra alteración, dando una textura y un color significativamente mejor y resultando con una mayor aceptación entre los consumidores ($p<0.05$) en comparación con los otros dos tipos ensayados. Como consecuencia, se propone el mismo como una modificación del proceso tradicional de elaboración de aceitunas negras naturales de mesa.

*PALABRAS-CLAVE: Aceitunas negras - Análisis microbiológicos -Fermentación - Preferencias - Propiedades físico-química.*

**SUMMARY**

**Evaluation of greek-style black table olives in salt varying brines.**

Three fermentation processes with black table-olives were tested. Olives were placed in: a) 16%(w/v) concentration of NaCl, (traditional treatment), b) a buffer of $CH_3COOH$ (0.05M) and $Ca(OH)_2$, (0.025M) without any NaCl and initial pH 4.7, and c) a buffer of $CH_3COOH$ (0.05M) and $Ca(OH)_2$ (0.025M) containing 12.8% (w/v) NaCl, and pH 4.3. Isolation, identification and enumeration of predominant microorganisms from fruits and brines sampled during the fermentation periods as well as color, intensity, texture and sensory evaluation tests of the final products were conducted. The third fermentation process, (c), yielded a product with low salt content no presence of spoilage microflora or other alterations during the fermentation period, with significantly better final texture and color, and higher acceptability among the consumers ($P<0.05$) compared to the other two. Potential use of a low-salt modification of the traditional black-table olives' fermentation process was proposed.

*KEY-WORDS: Black olives - Chemico-physical properties- Feeding preferences - Fermentation - Microbiological analysis.*

## 1. INTRODUCTION

Greek-style table olives have been traditionally produced in Greece and Turkey-and make up 31% of the world production, thus meeting increasing demands in local and international markets. Processing, with or without the addition of different products or aromatic substances, leads to a product of high nutritional value (Aligizakis,1982), through a spontaneous process mainly characterized by the domination of yeasts (Gonzalez Cancho et al., 1975; Garrido Fernandez et al., 1997).

A pre-treatment for removing phenols, the addition of starter cultures, control over salt concentration, anaerobic conditions and temperature, to prevent spoilage and economic losses, have been suggested (Vaughn, 1985; Özay and Borcakli, 1996; Kivanc and Akgul, 1990). The ratio of olive fruits to brine solution, soluble sugars; temperature, salt concentration; and development and composition of the micro-flora control the fermentation process (Borcakli et al., 1993).

Initially, Gram+ bacteria of the *Bacillus* and *Clostridium* species dominate although gradually disappear in 10-14 days; the lactic acid bacteria are then predominant. *Leuconostoc mesenteroides* and *Pediococcus cerevisiae* are the first to appear followed by the lactobacilli, mainly *L. plantarum* and *L. brevis*. Final pH in the product commonly reaches 3.8-4.0. *Candida diddensii, Hansenula anomala, Pichia membranaefaciens, Saccharomyces oleaginosus, Torulopsis candida Trichosporon, Saccharomyces, Kloechera, Debariomyces, Kluiveromycews* and *Cryptococcus* were identified (Balatsouras, 1966a; Gonzalez Cancho et al., 1975; Durán Quintana and Gonzalez Cancho, 1977; Kotzekidou, 1997). Isolates of Basidiomycetous

found were allocated to *Rhodotorula mucilaginosa* (Marquina et al., 1992).

Serious problems occurring in fermentation are: the fruits' softening by oxidative yeast species of *Rhodotorula* (*R. glutinis var. glutinis, R. minuta var. minuta and R. rubra*), and fermenting species (*Saccharomyces oleaginosus, S. kluiveri and Hansenula anomala var. anomala*), plant or microbial pectinolytic enzymes, *Bacillus subtilis, B. pumilis, B. mesentaricus, B. polymixa, B. megatherium and B. macerans*, and fungi (*Verticillium* and *Streptomyces*), fermentative molds (*Fusarium, Penicillium* and *Aspergilus*), as well as low salt concentrations (Balatsouras and Vaughn, 1958; Vaughn 1954; Minquez-Mosquera et al., 1987; Fleming et al., 1992). Additional problems might be attributed to butyric acid forming bacteria (*Clostridium botulinum*) (Aligizakis, 1982; Fleming et al., 1989.). Also propionic, butyric, and valerienic or caprilic acids, were found to be produced during the "zapatera" spoilage by *Propionibacterium pentosaneum, Propionobacterium zeae, Desulfovibrio desulfuricans*, (Balatsouras 1966a).

Due to market demand for low-salt and sodium-reduced food products, new processing techniques and modifications have been tested (Özay and Borcakli, 1996). In this study a modification of the traditional olives fermentation, as performed on the island of Crete, southern Greece, was tested to obtain a higher quality product, acceptable by the consumers and of extended shelf life. Results could be used to further understand the process in order to identify the critical points that could either be altered or enhanced towards improving quality.

## 2. MATERIALS AND METHODS

### 2.1. Olive samples

Olives of uniform size, harvested from October-November 1995, provided by A.V.E.A., (a black-table olive processing operation in the city of Chania, Greece). Untreated fruits were placed for 12 days in 5 tons water-containing tanks. 60kgr of these fruits were divided into 3 batches and placed in 24 plastic containers, 5L each, (2.5kg fruits/1.4L brine solution). A plastic net was placed above the olives to keep them submerged in the brine. Containers were sealed and placed in a well-aerated room with an average temperature during the experiment of around 15$^{o}$C. For sampling one container from each batch was opened, olives and brine were randomly sampled and the content was not further used. Sampling took place every week for the first 15 days and thereupon every 3 weeks, for a period of 21 weeks. Sampling was performed in duplicates and analysis in triplicates.

### 2.2. Brine solutions

The following brine solutions were used: 1$^{st}$ batch: the traditionally used method, NaCl 16.8% (14$^{o}$Be); 2$^{nd}$ batch: Acetic acid and $Ca(OH)_2$ of 0.05M and 0.025M final concentrations respectively. Acetic acid was used to adjust the pH to 4.7; 3$^{rd}$ experimental batch: buffer as previously described plus 12.8% NaCl initially.

### 2.3. Reagents and solutions

NaOH, glyserol, acetic acid glacial, NaCl, ethanol, $AgNO_3$, $K_2CrO_4$, phenolphthalein, iso-butyric acid, propionic acid, and valeric acid were purchased from Merck, (Darmstadt, Germany). n-pentane was obtained from Farmitalia Carlo-Erba S.P.A. (Milano,Italy). Bacto 3-step gram satin procedure by DIFCO, (Detroit, MI, USA). ROGOSA agar, BK 033, Violet Red Bile Glucose Agar BK 011, Malt Agar BK 045, were all from Biokar diagnostics, (UK), while Plate Count Agar (Tryptone Glucose Yeast Agar) CM 325 from OXOID. All API systems were from bioMerieux, (France).

### 2.4. Chemical and physical analysis

Brines' pH was directly measured by a pH-meter, (ORION, 920A). Titratable acidity and NaCl % content were tested according to standard AOAC (1990) method. Primarily to GC analysis of acids, samples were filtered through a 22μm pore size filter (MILLIPORE), and 1μL was injected into the GC. Reference compounds were used and retention times recorded for identification. Quantification was performed by comparing the measured samples from the brines to standard mixtures prepared by mixing 258mg acetic acid, 253mg iso-butyric acid, 264mg propionic acid, 277mg valeric acid and 230mg ethanol, each in 50mL of distilled water. Two more concentrations were prepared by diluting each of the above solutions to equal amount of distilled water (1:1) and the resulting solutions in the same way in water (1:1). A Hewlett Packard, model 5890 series II, GC apparatus was used, equipped with a flame ionization detector adjusted at 280$^{o}$C, and controlled by the HP 3365 series II Chemstation software. An auto injector HP 7673 was used. The injector, equipped with a split liner with cup and a plug of silanized glass wool, was heated at 250$^{o}$C. Helium was used as a carrier gas at 200kPa pressure and a split vent flow of 15mL/min. The oven temperature was initially kept at 85$^{o}$C for 3 min, subsequently increased by 5$^{o}$C/min up to 220$^{o}$C and retained there for 10min. A FFAP, Fused Silica Column, 50m long, 0.20mm i.d. and 0.3μm of film thickness were used.

### 2.5. Color

Color characterized by a MINOLTA chromatometer (Model CR-300) with a data processor MINOLTA DP-301. Chromatic values L*, a*, b*, C*, h, $E^*_{ab}$, $H_{ab}$, C* and H* provided either from the equipment or by applying proper equations, in order to describe the differences in color among the olive samples. L* value indicates lightness, a*, b* move from red (+a*) to green (-a*) and yellow (+b*) to blue (-b*). E*ab marks the size of the color difference; C* is chroma, H is the hue angle and C* describes the saturation of the color.

### 2.6. Firmness

The SHATILLON DPP 5kg with a 6mm, depth and diameter, conical tip was used. Approximately 100 olives were tested form each batch. Results were in Kg of force applied to penetrate into the skin till the 6mm depth.

### 2.7. Sensory evaluation

For this study 39 untrained randomly selected persons performed a sensory assessment of the final product from the three batches. Appearance, taste and the level of liking on a 9 points scale (dislike extremely to like extremely), were in question. After re-tasting the samples the intensity of salt, vinegar, pungency, level of fermentation and unpleasant characteristics were evaluated on a 3-points scale (too light to too strong). Their preferences were also recorded on a 3-point scale (like only a little to like very much) (Meilgard, 1991).

### 2.8. Microbiological analysis

Approximately 100gr of olive fruits were aseptically drawn from each container. 20gr of flesh were mixed with 180mL NaOH solution (0.9%) in a sterile Waring blender for 2 min. 50mL of the homogenized sample was placed in a sterilized bottle, (FALCON), with 20% v/v glycerol. Brine samples of 30mL each were mixed with 20% v/v glycerol in a sterilized bottle. All samples were stored at –80$^o$C till analysis. Samples were in triplicate for each sampling date and batch. Appropriate dilutions were plated on PCA agar and in various selective media, namely Sabouraugh, McConkey, Rogosa, or Chapman, and incubated for 1-3 days at 37oC and 3-5 days at 30$^o$C before enumeration (Priego et al., 2000; Jordano et al., 1995). Different colonies grown in every plate were isolated and examined for GRAM stain. Yeast and mould were also properly stained (green of malachite, safranin and/or sinic ink). Bacteria colonies were re-grown in the same medium from which they had been isolated from, or in Sabouraugh, McConkey, Rogosa, or Chapman media. Incubation at 37$^o$C for 1 day for bacteria, or 26$^o$C for 5 days for molds was applied (Mossel et al., 1995). Antibiograms and API tests, (API 20 C AUX used for yeasts, API 50 CHL Medium for *Lactobacillus* species and API 20 E for *Enterobacteriaceae* family, were also implemented for further identification. Results obtained were 70-99% confident.

### 2.9. Statistical analysis

The SAS® program was used for the analysis of the results obtained throughout the fermentation process for the brines and the olives of the three treatments. ANOVA was used and the Least Significance Difference Test (LSD0.05 test) was implemented for separating the means of significant main effects or interactions. Simple correlation was determined between selected response variables. Results form the taste panel test were analyzed by SPSS® program. One-Way-ANOVA was performed on ranked panel scores to analyze the data. Dunkan's Multiple range test was implemented to determine significant differences among means. The level of statistical significance was 0.05.

## 3. RESULTS AND DISCUSSION

Microorganisms ferment the soluble sugars leached from fruits to products that influence pH and acidity, while salt from brines enters the fruits' flesh till equilibrium is established, (Balatsouras, 1966b; Gonzalez et al., 1975; Borkali et. al., 1993, Garrido Fernandez et al. 1997). Similar trends in pH, NaCl and acidity changes observed in the first, (traditional) and the third, (proposed low salt) batches. Acidity around 0.1 – 0.8% and pH around 4.5 indicate the absence of lactic acid bacteria activity which was more obvious in the second (no-salt) batch. Along with volatile fatty acids, they are considered to be responsible for a product's quality, organoleptic characteristics and shelf life (Balatsouras, 1985). Fluctuations observed might be due to the different buffering capacities of the brines, mainly depending on the organic acids formed by the microflora, which substantially differed among the batches (Figure IV) (Borkali et al., 1993).

Volatile fatty acids analyzed by GC further were used to understand, evaluate and compare the three fermentation treatments. Acetic acid although in higher initial amounts in the second and third batches seems to change differently, diminishing after the 8$^{th}$ week for the second batch, a point were a profound increase of lactic acid bacteria and a stabilization of the yeast population were recorded. Acetic acid content remained almost stable (approx. 100ppm) in the first batch throughout the tested period (Table IV). Ethanol and propionic acid were

Table I

**$LSD_{0.05}$ values for the batches, according to chemical characteristics**

| Batch | pH | NaCl in brine (%) | Acidity in brine (%) | Acidity in olives (%) | NaCl in olives (%) |
|---|---|---|---|---|---|
| 1 | 0.38 | 1.48 | 0.12 | 0.24 | 1.56 |
| 2 | 0.3 | - | 0.12 | 0.24 | - |
| 3 | 0.09 | 0.49 | 0.07 | 0.11 | 0.12 |

Table II

**$LSD_{0.05}$ values for days of fermentation, according chemical characteristics**

| Days | pH | Acidity (%) | NaCl (%) | Acidity 2 (%) | NaCl 2 (%) |
|---|---|---|---|---|---|
| 1 | 0.163 | 0.08 | 0.016 | 0.058 | 0 |
| 2 | 0.11 | 0.06 | 0.016 | 0.10 | 0.519 |
| 3 | 24.16 | 0.02 | 0.016 | 0.02 | 0.036 |
| 4 | 0.163 | 0.02 | 0.025 | 0.03 | 0.025 |
| 5 | 0.36 | 0.07 | 0.058 | 0.23 | 0.05 |
| 6 | 0.11 | 0.01 | 0.12 | 0.52 | 11.94 |
| 7 | 0.198 | 0.072 | 0.11 | 0.02 | 0.30 |
| 8 | 0.163 | 0.12 | 0.016 | 0.5 | 0.07 |
| 9 | 0.163 | 0.021 | 0.11 | 0.024 | 0.062 |

Table III

**$LSD_{0.05}$ test and $F_{prob.}$ values for treatment, according to date and chemical characteristics (p =0.05)**

| | pH | Acidity in brine | Acidity in olives | NaCl in brines | NaCl in olives |
|---|---|---|---|---|---|
| **LSD** | **0.074** | **0.026** | **0.091** | **0.025** | **1.41** |
| **$F_{prob}$** | **0.000** | **0.000** | **0.000** | **0.000** | **0.0068** |

changing in similar patterns (Table IV), probably as a result of a similarity in the microflora present in the brines of the three batches during fermentation, (*Propionibacterium pentosaneum, Propionobacterium zeae, Desulfovibrio desulfuricans*) (Balatsouras 1966a). Increased quantities of propionic, butyric and iso-butyric and valeric acids, which might be conceived as indicators of a deviation from the normal process occurred in the second batch (Garrido Fernandez et al., 1997). Acids fairly increased after the $8^{th}$ week of treatment. Butyric acid increased form $15.10^3$ to$15.10^5$ ppm (Table IV).

Table IV
**Organic acids and ethanol (in ppm) changes with time in the brines of the three batches**

| WEEKS | Ethanol | | | Acetic acid | | | Propionic acid | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1[a] | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 1 | 146 | 37 | 63.94 | 66.51 | 247.93 | 316.65 | 477.7 | 156.59 | 403.37 |
| 2 | 132 | 288 | 288.6 | 99.97 | 282.43 | 290.48 | 870.23 | 185.9 | 458.78 |
| 5 | 185 | 127 | 91.7 | 124.36 | 415.6 | 510.62 | 306.18 | 319.71 | 355.44 |
| 8 | 136 | 169 | 169 | 110.57 | 552. 9 | 436.85 | 500.67 | 358.31 | 520.62 |
| 11 | 157 | 184 | 197 | 176.56 | 383.07 | 426.32 | 583.3 | 460.26 | 367.31 |
| 14 | 150.7 | 151 | 138.26 | 80.53 | 184.94 | 663.81 | 324.74 | 264.71 | 544.61 |
| 17 | 133.6 | 149 | 148.2 | 111.58 | 355.25 | 385.99 | 463.58 | 350.43 | 296.42 |
| 21 | 118.38 | 160 | 159 | 109.92 | 203.31 | 732.11 | 344.15 | 451.74 | 630.29 |
| | **Butyric acid** | | | **Iso-butyric acid** | | | **Valeric acid** | | |
| 1 | 6285 | 15466 | 3905 | 2.73 | 2.399 | 2.05 | 69.46 | 1 | 49.86 |
| 2 | 1617 | 12727 | 4681 | 2.83 | 1.3 | 1.31 | 178.52 | 2 | 83.12 |
| 5 | 6462 | 14602 | 4240 | 1.12 | 1.66 | 1.21 | 12.32 | 2.4 | 18.23 |
| 8 | 4961 | 15394 | 7826 | 1.8 | 1.03 | 1.024 | 30.81 | 2.23 | 39.57 |
| 11 | 6226 | 59916 | 6587 | 2.27 | 7.55 | 0.2 | 4.51 | 0 | 18.81 |
| 14 | 6618 | 57675 | 6618 | 0.98 | 5.3 | 1.1 | 5.33 | 0 | 9.33 |
| 17 | 6024 | 62348 | 4652 | 0.78 | 4.8 | 0.1 | 5.8 | 0 | 5.64 |
| 21 | 4787 | 153048 | 6800 | 0.77 | 28.35 | 0.41 | 1.2 | 0 | 3.15 |

[a] Bach number

Valeric acid, although initially present in the first and third batches (74 and 53 ppm respectively), almost disappeared at the end of the tested period,(21 weeks later), while traces were detected in samples taken from the second batch (Table IV).

When main effects or interactions were significant, means were separated by using the $LSD_{0.05}$ test. Simple correlations were determined between selected response variables. Interaction between chemical characteristics values and the corresponding days of fermentation (Tables I, II &III).

Color changes of olives, as measured by MINOLTA CR-300, were recorded and analyzed by MINOLTA DP-301 data processor at the end of the testing period. Results (Tables V & VI) indicated that fruits from the first batch were lighter in color than the other two, but more close to the third batch. The third batch gave darker olives than the second one, (both $\Delta L^*$ and $\Delta C^*$ were negative). Olives from the first batch were more vivid compared to the second, and those of the third batch were slighter more vivid than those of the first batch. The second batch gave a duller olive, (negative $\Delta C^*$ values). The discoloration process could be related to either the addition of acids or low pH.

Olives from the first and the third batches had higher values i.e. less soft, when measured by the Penetrometer compared to olives from the second batch. The olives in the second batch had been exposed to high amounts of acids either initially present, acetic acid, or formed during the fermentation process (Table VII). One-Way-ANOVA

Table V
**Colorimetric values for olives at the end of the tested period, according to batches**

| | Batch 1 | | | Batch 2 | | | Batch 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | L* | α* | b* | L* | α* | b* | L* | α* | b* |
| Max | 52.79 | 22.52 | 48.21 | 48.21 | 17.65 | 22.15 | 45.57 | 18.85 | 23.03 |
| Min | 28.27 | 8.74 | 23.46 | 23.46 | 3.22 | 1.09 | 25.03 | 7.64 | 2 |
| Mean | 39.19 | 15.31 | 16.81 | 35.93 | 10.21 | 11.93 | 36.46 | 13.24 | 12.54 |
| SD | 6.02 | 3.46 | 5.72 | 6.48 | 3.17 | 6.08 | 5.35 | 2.93 | 5.39 |

Table VI
**Color comparison of olives at the of the process**

| Batches compared | 1 & 2 | 1 & 3 | 2 & 3 |
|---|---|---|---|
| ΔL* | 10.72 | 7.45 | -0.28 |
| ΔE*ab | 7.7 | 5.4 | 3.23 |
| ΔC* | 0.9216 | 0.59 | -0.35 |
| ΔH* | 6.99 | 4.7 | 3.169 |

Table VII
**Firmness evaluation (Kg of force) by SHATILLON penetrometer**

| | Batch 1 | Batch 2 | Batch 3 |
|---|---|---|---|
| Sum | 56.55 | 36 | 48.95 |
| Maximum | 1.35 | 1.05 | 1.2 |
| Minimum | 0.15 | 0.05 | 0.1 |
| Mean | 0.8078 | 0.51428 | 0.69928 |
| SD | 0.268602 | 0.26582 | 0.234905 |

indicated significant differences in olives from the three batches. Enzymes might also be involved when produced from fungi (*Verticillium* and *Streptomyces*) usually at low pH conditions. *Saccharomyces, Rhodotorula* and *Hansenula, Bacillus* species have been associated with gassy fermentation spoilage and softening of olives (Fleming et al., 1992; Vaugh et al., 1972) and have been isolated here as well.

The results of the questionnaire provided to 39 untrained people mainly form the Mediterranean basin area and EU, showed some biases mainly due to the composition of the panel (traditional vs. potential consumers). Significant differences among olives from the three batches were found when Dunkan's test was applied at significance level 0.05. Olives from the third batch were described as more

Table VIII
**Olives' overall acceptance on a 9 points scale**

| Batch | Minimum | Maximum | Mean |
|---|---|---|---|
| 1 | 2 | 9 | 6.1 |
| 2 | 1 | 6 | 1.8 |
| 3 | 3 | 0 | 6.7 |

fermented, highly pungent, not too salty, with the least unpleasant flavor, and a mild vinegary taste. Panelists liked them the most. Olives from the first batch got lower grades concerning the fermentation stage, and pungency, higher grades for the salty and vinegary tastes (probably describing acidity) but there were some unpleasant tastes recorded that made the product less favorable. Olives from the second treatment got the worst evaluation as had been expected form the chemical and physical characteristics recorded (Table VIII).

Cultivation on PCA growth media was initially used for a rough estimation of the load and types of microorganisms present in the brines and olives. *Staphylococcus aureus* species isolated during the first days of the fermentation in all the batches, could be related to the hygienic conditions occurring in the process plant. Population decreased rapidly after storage in NaCl and low pH brines. Few colonies of Enterobacteria (most probably *Pseudomonas fluorescense/putida*), counted on VRBDG agar from brine samples of the first batch during the first week of the treatment. No colonies from olive samples appeared after up to three days of media incubation. LAB were found to be present in higher or lower populations and to coexist with the yeast population (Figure IV) at relatively low salt concentrations (<10%) (Gonzalez Cancho et al., 1975). In general, the total population of microorganisms identified in the first batch had the lowest numbers compared to the other two during the first period, as neither the salt nor the temperature are appropriate for the growth of these species (Garrido Fernandez et al., 1997). Their increase towards the end of the time tested could be due to resulting lower salt in the brines and higher temperatures recorded.

Higher numbers of yeast colonies was recorded for the second batch throughout the entire period while the other two batches showed much lower numbers (Figure IV). Oleuropein from olives that had not been pre-treated by alkali could be hydrolyzed yielding products that favor the predominance of yeast species over the lactic acid bacteria in the brines (Ciafardini et al., 1994). That might be the case at the initial stages of the treatments, declining and ending after the 3rd week (Figure IV).

In brines of the first and third batches, Gram+ cocci were predominant, followed by the Gram+ bacilli, until the Gram- rod-shaped bacteria reached higher populations. In third batch Lactobacilli were in greater populations than the Leuconostoc and Pediococci species, also showing an increase towards the 11th week followed by a small reduction in population till the 21st week tested, due to lower salt and their higher tolerance to low pH and acidity (Garrido Fernandez et al., 1997).

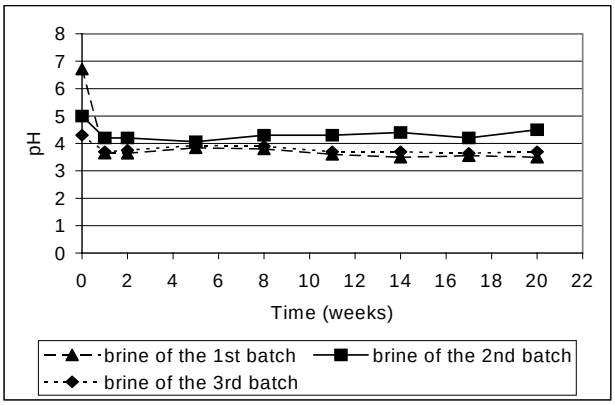


Figure 1
Changes in pH of the brines from the three batches.

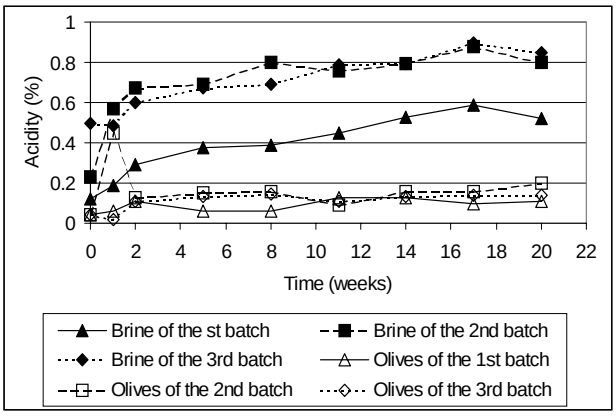


Figure 2
Changes in titratable acidity in brines and olives from the three batches.

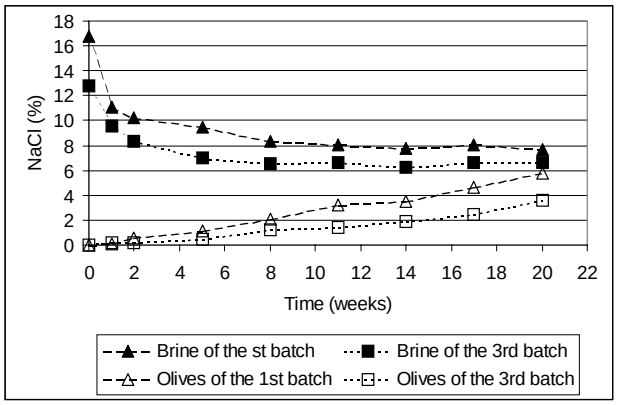


Figure 3
Changes of NaCl content in brines and olives from the first and second batches.

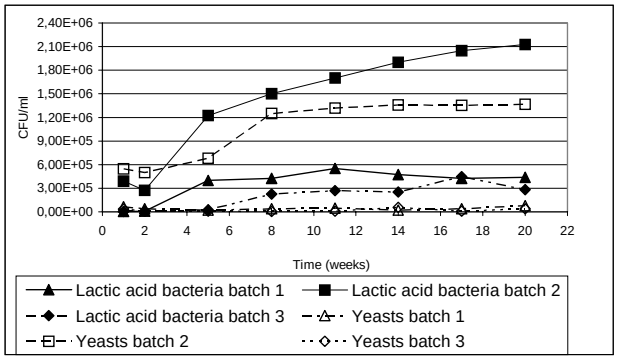


Figure 4
Population of lactic acid bacteria and yeast species in brines from the three treatments.

In the second batch (no salt) the Gram+ rod-shaped were predominant followed by the Gram+ cocci, with *Leuconostoc mesenteroides* being the most abounding species, followed by *Pediococci. Leuconostoc* colonies were initially found in samples at the 12$^{th}$ and 18$^{th}$ week of treatment, continuously increasing afterwards (Balatsouras, 1966b). Lactobacilli, a high acidity tolerant species, appeared after the 10$^{th}$ week when the acidity level was higher, while the identified *Lactobacilus fermentum* and *L. fructivorans,* had previously been related to spoilage by Giafardini et al. (1994), isolated from the later stages, mainly due to the absence of salt and high pH (pH>4.5). Results were similar for both the brines and fruits tested. *Propionibacterium* species, although samples not tested for, were most probably present, I due to the increase in butyric acid after the 8$^{th}$ week of treatment. Domination of oxidative microorganisms most probably leads to a spoiled product. The higher levels of butyric and iso-butyric acids (Table IV), recorded for the second batch after the first 7-8 weeks may be due to higher pH and no salt (Garrido Fernandez et al., 1997). Lactobacilli species dominated after that point, most probably consumed the rest of the sugars producing organic acids that could explain the increase in acidity recorded during the second half of the fermentation period. No-salt brines enhanced the growth of lactic acid bacteria, while they remained notably low in the first batch (higher amount of salt) (Figure IV), Another possible reason for this might be the amounts of phenols leached out of the different brines. The lower the salt concentration the more the phenols the higher the higher the retardation (Özay and Borcakli, 1996). Sugars leached out of the fruits, initially present in higher amounts in the brine solutions, could be fermented by microorganisms and consequently alter the acidity and the pH of the brines. Following that initial period, a reduction in available sugars was recorded, by Özay and Borcakli (1996) which might introduce the domination of less acid tolerant bacteria probably responsible for product spoilage and unpleasant odors, (second batch). Both fermentative and/or oxidative yeast species were isolated from the initial brines, high in soluble sugars, or later fermentation stages. No correlation could be established between the stage of fermentation and the occurrence of fermentative yeast species (Manquina et al., 1992). Yeast species were isolated during the whole fermentation period from the first batch, with higher population numbers counted in the first 5-10 days. *Pichia membranaefaciens*, a high salt tolerant yeast, was identified throughout the fermentation period (Manquina et al.,1992), while *Candida curvette*, *Rhodotorula slutinis* and colonies of *Sacharomycetaceae* family (most probably *S. kluveri*), were isolated from samples from the 5$^{th}$ week although in rather low populations. *Schizosacharomyces versatilis* was dominating in brine samples from the second batch during the whole period and yeast species had the highest loads. *Rhodotorula slutinis* was present in continuously raising rates from the first week, while *Hansenula anomala* was also present but in low populations. *Trichorporum beiselii* was isolated and identified from samples taken on the 1$^{st}$ and 13$^{th}$ weeks of fermentation, with higher numbers during the first week. *Saccharomyces spp., Hansenla anomala, and Rhodotorula slutinis* could be applied allied to the softness and/or spoilage of the olives. Degradation of acetic acid, as well as other organic acids, might also be in accordance to the existence of these species. (Nout and Rombousts, 1992). High salt, low pH tolerant species of *Torulaspora* and *Zyggosacharomyces*, typically occurring in black table olives (Pitt and Hocking, 1985) were also identified. Additionally, the low pH, high salt, low temperature tolerance and producer of extra-cellular lipase, *Debaryomyces hansenii* (Marguina et al.,1992, Davenport, 1980, Viljoen and Greyling, 1994) was also described mainly in the first

batch samples. *Penicillium* species were present in the 20th week and *Fusarium oxisporum* sporadically isolated from the first week samples of the third batch (Balatsouras, 1966; Durán Quintana and González Cancho, 1977; Morquina et al., 1992). Olive fruits gave similar qualitative results, with the addition of *Candida humicola*, appeared on the second (no salt) batch from samples taken at the 17th and 20th week of the treatment.

## 4. CONCLUSIONS

This work attempted to propose a modification of the traditional way that black-table olives have been processed by a great part of the Greek industry. Batch with no salt but only buffer appeared technically difficult in its application, as it requires continuous monitoring and probably external applications to maintain the proper conditions for the fermentation to proceed. Olives treated with reduced salt brines seemed to be a suitable product for further investigation. This study showed that such improvements can be quite promising, since potential and habitual consumers seem to prefer the olives low salt as part of a more healthy diet. New or modified brines with or without starting cultures of yeast species and/or bacteria, could standardize the fermentation and thus final products.

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