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PAPER

QUALITY IMPROVEMENT OF NATURALLY GREEN TABLE OLIVES BY CONTROLLING SOME PROCESSING PARAMETERS

CONTROLLO DI ALCUNI PARAMETRI DI PROCESSO
PER IL MIGLIORAMENTO DELLE OLIVE DA TAVOLA VERDI AL NATURALE

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ABSTRACT

The results of making some technological corrections, designed to avoid the main processing problems of naturally processed olives are reported. The following processing parameters were kept constant throughout time: salt (2 and 4%), pH (acidification with lactic acid to pH=4), fermenting temperature (25°C) and brine level. Results indicate that pH did not exceed the threshold of 4.5, while there was a weak free, combined and volatile acidity. Sugar and polyphenol diffusion into the brines was

RIASSUNTO

Vengono riportati i risultati di uno studio sull'efficacia di alcuni interventi correttivi con l'intento di cercare di superare i principali problemi durante la trasformazione di olive verdi al naturale. I parametri di processo delle salamoie sono stati tenuti costanti durante tutto il processo per ciò che riguarda: sale (2 e 4%), pH (acidificazione con acido lattico a pH=4), temperatura di fermentazione (25°C) e livello della salamoia. I risultati indicano che il pH non ha superato il valore soglia di 4,5, men-

- Key words: brines, naturally green olives, polyphenols, processing -

higher in the 2% brined olives than in the 4% ones. Gas pockets were not recorded, while a low incidence of irreversible shrivelling was noted only in the 4% brined olives. Panelists rated the olives as excellent for firmness and for residual bitter taste, and expressed a slight preference for 4% olives, because they were saltier.

tre si è avuto uno sviluppo modesto dell'acidità libera, combinata e volatile. Le olive al 2% hanno fatto registrare una maggiore diffusione di zuccheri e polifenoli nelle salamoie, rispetto a quelle al 4%. In entrambe le tesi non si è avuta presenza di "gas-pockets", mentre si è registrata una lieve incidenza di raggrinzimento irreversibile nella tesi al 4%. Gli assaggiatori hanno giudicato eccellenti entrambe le tesi sia per la consistenza, sia per la presenza di un sapore amaro residuo, anche se hanno leggermente preferito le olive trasformate al 4%, in quanto maggiormente salate.

INTRODUCTION

Naturally processed olives are one of the most popular table olive varieties in southern Italy. The olives are harvested at the green or black stage, sorted, size-graded and then placed in an 8 to 14% NaCl brine. This differs from Spanish-style processing, in which the bitterness is totally removed in a very short time (8-12 h) by hydrolysis with a dilute NaOH solution (lye) (BORBOLLA y ALCALÁ and REJANO NAVARRO 1981; FERNANDEZ DIEZ, 1971). In naturally green olive processing there is no debittering treatment. Hence, oleuropein, which is primarily responsible for the unacceptable bitter taste of unprocessed olives, is only partially removed by diffusion from the flesh to the brine or to a lesser extent, by acid hydrolysis (BRENES BALBUENA *et al.*, 1992) over a period of 6 months to one year.

Fermentation is also somewhat different in the two processes, as lactic acid bacteria develop in the brines of Spanish-style olives, while anaerobic yeasts predominate in the brines of natural-style olives (FARRIS *et al.*, 1989). There are also some sensorial differences, compared with the Spanish-style processing, as naturally processed olives retain some bitterness and develop a more acidic taste due to a higher acetic acid content.

The processing of naturally green olives is normally carried out by small-scale producers or, more rarely, on an industrial scale. After years of observation at the processing plants and in our laboratories, the following problems have been noted: a) the pH value of brines and olives is generally higher than 4.5. Since the product does not normally receive any sterilisation treatment, there could be serious safety risks if the sodium chloride concentration (≥7%) and pH values (≤4.5) are not respected. Moreover, high pH values at the beginning of the process favour the growth of Gram-negative bacteria (from contamination of industrial devices in contact with the brine and olives), which cause gas-pockets in the olives and putrid fermentation. b) The salt concentration is always so high at the beginning of the process that it causes shrivelling of the peel and inhibits the growth of lactic acid bacteria, which in turn, does not allow the pH to go below 4.5. Furthermore, the salt concentration is rarely checked during processing. c) Since evaporated brine is rarely replaced, the surface soon develops oxidative

yeasts and moulds. The yeasts consume lactic acid, which results in a higher brine pH, while the moulds could pose risks due to aflatoxin production, even though brines, in general, strongly inhibit mycotoxin-producing fungi (PASTER *et al.*, 1988; GOURAMA *et al.*, 1989). d) Since there is rarely temperature control during processing, severe loss can occur from gas-pockets due to yeasts, even if the pH and salt concentrations are controlled (personal observations). This is true especially when the brine is exposed to sunlight or high temperatures in concrete or fibre-glass tanks.

The lack of scientific data on naturally processed green olives has stimulated our department to conduct research on the technological processing, that could lead to improvement of the quality and safety of the product. Therefore, this study was carried out to determine the effect of: a) acidifying of brines; b) using brines at low salt concentration (<4%); c) controlling brine level; d) controlling temperature.

MATERIALS AND METHODS

Plant material

Olives were selected according to marketing (n. olives/kg, mean weight, % flesh and pit, flesh to pit mean ratio) and technological (percentage distribution in each calliper class) parameters, as well as the suitability of the cultivar for this processing method. For several decades only certain cultivars have been used for processing olives processed in the region of Sardinia; of theses "Tonda di Cagliari" olives were chosen for this investigation. This cultivar has a flesh to pit ratio of 3.65 (the minimum required is 3 for table olives) a mean weight of 4.6 g (216 olives per kg) and a good distribution in the various callipers (80% of the olives were in the 16-17 and 18-19 mm range).

Harvesting and sampling

Ripe green olives were hand-harvested during the first ten days of October in an irrigated grove in southern Sardinia and were immediately transported to the laboratory. Only olives free of blemishes, cuts and insect punctures were selected. The olives were then size-graded with laboratory callipers ranging from 16 to 21 mm in transversal diameter, and were equally divided into three 11 kg replications.

Processing

The olives were washed with tap water to remove dust, placed in 20 L sterilised plastic containers (three per trial) and brined with freshly prepared 2 and 4% (w/w) NaCl brine. The brine was acidified with lactic acid to pH 4.0. Processing was carried out at a temperature not exceeding 25°C. The brine concentration and pH were kept constant throughout the fermentation process. Corrections for NaCl concentration and acidity were made at 5, 9, 12, 16, 22, 30, 45 and 60 days and afterwards at monthly intervals. A perforated cap was used to submerge olives in the brine. Containers were not tightly sealed during the first 10 days, in order to allow the large amount of CO_2 evolving from fermentation and fruit respiration to escape. After this time, the containers were filled to the top with fresh brine and carefully closed, so that air could not enter (to avoid growth of oxidative yeasts and moulds on the brine surface). The brine level was adjusted whenever necessary. The measurements were discontinued at 210 days as most of the assessed parameters had reached a steady state. It must be noted that all four processing parameters (pH, NaCl, temperature, lactic acid) were modified simultaneously, because the results were inconsistent, if one parameter was changed at a time.

Determinations

The following determinations were carried out on the brine: pH with a glass electrode (Orion mod. 420, Beverly, USA), free (g of lactic acid per 100 mL of brine), combined (mEq/L) and volatile acidity (g of lactic acid per 100 mL of brine), and reducing sugars (g of glucose/100 mL of brine), according to GARRIDO FERNANDEZ *et al.* (1997). The free acidity value obtained was corrected by subtracting the initial and subsequent amounts of lactic acid added. Polyphenols were extracted according to BRENES *et al.* (1990) and determined spectrophotometrically at 760 nm (HP 8453, Palo Alto, California), after reaction with the Folin-Ciocalteu reagent (expressed as mg gallic acid/100 g of olive flesh). For pH and free acidity determinations were carried out at the time of each correction time and monthly for the others. The parameters pH, salt, reducing sugars and total polyphenols were determined on homogenised flesh. Sugars, were extracted by centrifuging twice ten grams of flesh with 40 mL of water at 6,000 rpm for 15 min. They were determined using the Fehling method on the supernatant. Polyphenols were extracted according to AMIOT *et al.* (1986) and determined as previously described for brines. Salt concentration was determined as reported previously (PIGA *et al.*, 2001).

Laboratory personnel (10 people) performed an informal taste test at 180 days of brining. They were asked to detect off-flavours and to indicate the trial they preferred. They also judged saltiness, consistency and crispness of the olives. The assessments were reported as written comments and not as ratings. The incidence of gas-pockets and shrivelling was calculated as the percentage of affected fruits per 1,000 olives from each container. Shrivelling was specified as either reversible or irreversible, by placing the olives in water and checking for irreversible (permanence) or reversible (disappearance) shrivelling after 24 hours.

When appropriate, data were subjected to analysis of variance, where brine concentration was the group variable, and means were separated by Duncan's Multiple Range Test at $P<0.01$ level. The tests were replicated three times over three consecutive years and no significant differences were found between the three years. Thus the data from only one year are presented as representative of the three-year study.

RESULTS AND DISCUSSION

Chemical changes in brines and olives

Processing green olives by the traditional method implies using a high salt concentration simply to avoid the growth of putrefactive microorganisms. This may inhibit lactic acid bacteria, cause shrivelling of the peel and limit diffusion of sugar from flesh to brine, thus slowing fermentation and subjecting the olive flesh to attacks by polygalacturonase-forming yeasts. Therefore, monitoring of the pH and salt concentration of the brines is of paramount importance from the technological and safety point of view.

In this study, diffusion of water-soluble compounds from the olives to the brine and salt uptake occurred, until equilibrium was reached. Microorganisms fermented reducing sugars, diffusing from olives, to other compounds (mainly lactic acid). Salt concentration and pH triggered the development of the different microorganisms (bacteria and yeasts). Data related to the pH and corrected free acidity values are reported in Figs. 1 and 2. The correction of pH with lactic acid was beneficial for maintaining safe values, with the exception of the first nine days, when the pH values of the brines reached 4.6, as a result of compounds diffusing from the olive flesh. The pH values ranged from 4 to 4.1 during the first four months and from 3.8 to 3.9 afterwards. The rise in pH during

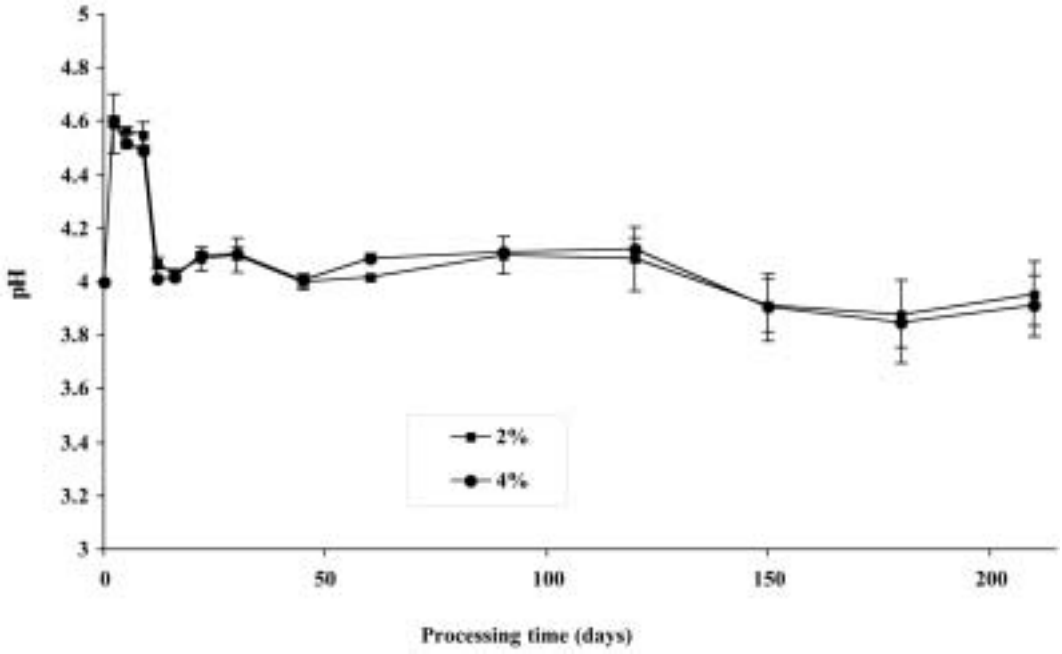

Fig. 1 - Change in the pH of brines of fermented "Tonda di Cagliari" table olives during 210 days of brining. Each value is the mean of nine determinations. Vertical bars indicate standard deviation.

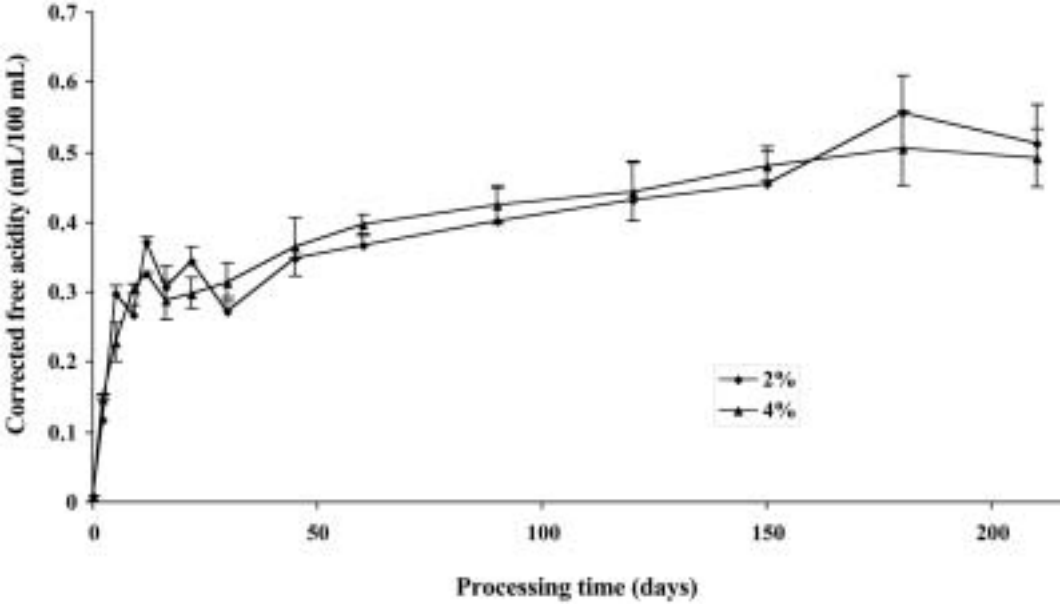

Fig. 2 - Change in free corrected acidity of brines during 210 days of fermentation of "Tonda di Cagliari" table olives. Each value is the mean of nine determinations. Vertical bars indicate standard deviation.

the first 120 days may be ascribed to the increase in combined acidity (Table 1), while the decrease was probably caused by the concomitant reduction in combined acidity and the increase in free acidity (Fig. 2). It should be pointed out that the simple addition of lactic acid, which did not exceed 4 mL/L of brine during the 210 days of processing, was sufficient to adjust the pH in the brines. Natural acidification in the brines, sufficient enough to save further pH corrections, did not occur. This was surely due to the microorganisms that were able to grow in the brines. Green olives, are rich in polyphenols, whose inhibitory effect on lactic acid bacteria (LAB) has been demonstrated (JUVEN and HENIS, 1970; RUIZ-BARBA *et al.*, 1990). Since diffusion of fermentable substrates in these untreated olives is very slow (as will be seen below), LAB do not find a favourable substrate on which to grow. Fermentation was therefore probably caused by fermentative yeasts, which are tolerant of high polyphenol levels (BALATSOURAS *et al.*, 1983), but produce small amounts of lactic acid, in contrast to LAB. Analysis of the data concerning corrected free acidity, further demonstrates this hypothesis (Fig. 2). In fact, the brines attained a weak acidity (not higher than 0.5 g of lactic acid per 100 mL of brine), which is evidence of fermentative yeast activity (BRENES *et al.*, 1986; FARRIS *et al.*, 1989; BALATSOURAS, 1990; MARQUINA *et al.*, 1992; BORCACKLI *et al.*, 1993). Since the focus of

Table 1 - Changes in combined acidity, volatile acidity, reducing sugars and polyphenols in brines at different salt concentrations of naturally processed green "Tonda di Cagliari" olives.

Salt concentration (%)	Sampling (days)	Combined acidity (mEq/L)	Volatile acidity (%)	Reducing sugars (%)	Polyphenols (mg/100 mL)
2	15	16.1±0.42[a]	0.018±0.001	<0.1	39.5±4.48
4		14.7±0.42	0.018±0.002	<0.1	39.4±1.18
2	30	22.0±1.69	0.027±0.001	<0.1	69.2±6.40
4		20.4±0.56	0.027±0.001	<0.1	69.0±1.97
2	60	25.5±0.49	0.0315±0.006	<0.1	96.8±8.11
4		22.6±1.69	0.036±0.001	<0.1	97.7±2.28
2	90	31.1±1.41	0.065±0.02	0.11±0.01	110.9±4.24
4		32.3±0.28	0.04±0.02	0.12±0.001	118.2±4.17
2	120	42.5±2.56	0.065±0.02	0.15±0.01	122.0±13.25
4		40.2±1.23	0.07±0.02	0.14±0.05	165.0±34.60
2	150	40.8±4.52	0.08±0.04	0.14±0.05	171.1±11.40
4		36.8±0.98	0.085±0.04	0.13±0.04	161.8±5.27
2	180	31.2±2.82	0.095±0.04	0.13±0.04	162.5±3.51
4		32.56±0.68	0.155±0.001	0.11±0.001	161.6±5.48
2	210	35.2±4.52	0.115±0.05	0.13±0.04	165.1±5.64
4		36.2±1.02	0.15±0.06	0.11±0.04	166.7±21.74

[a] Each value is the mean of nine determinations (three per each container) plus or minus standard deviation.

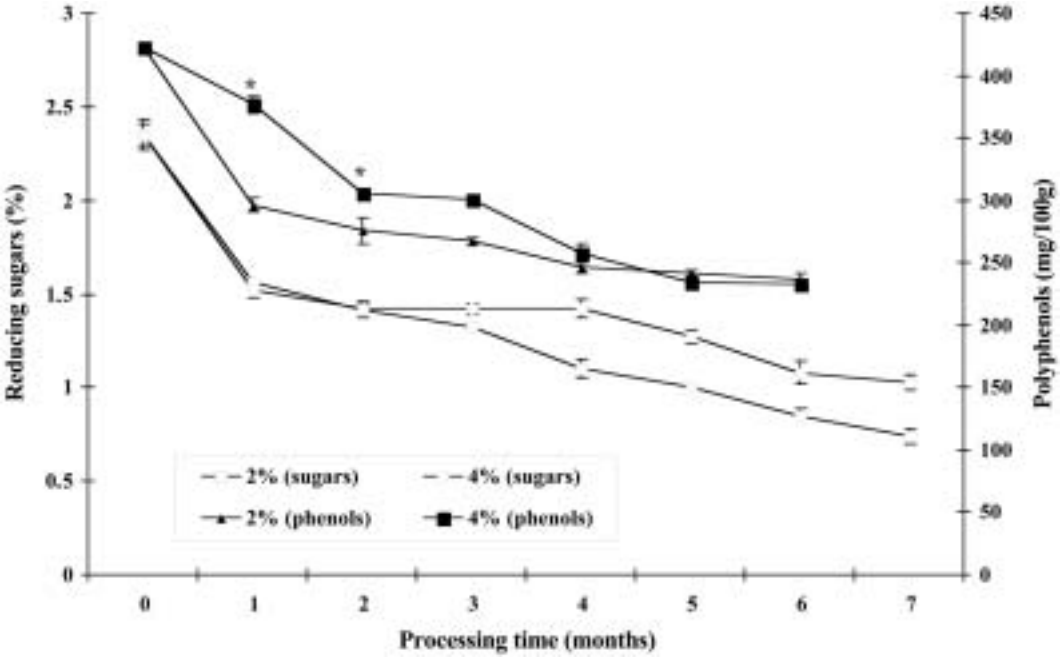

Fig. 3 - Changes in the reducing sugars and polyphenol content in the flesh of fermented "Tonda di Cagliari" table olives during 210 days of brining. Each value is the mean of nine determinations. Vertical bars indicate standard deviation. * Significantly different means within each period.

this study was mainly on technological aspects, microbiological analyses were not conducted, but are projected in future studies. The pH values and corrected free acidity showed no significant differences between the two trials.

As expected, the 2% brined olives exhibited a very slow salt uptake, while salt uptake was more pronounced in the 4% trial and equilibrium was never reached (data not shown). DRUSAS *et al.* (1988) found that the diffusion coefficients of untreated olives were about one fifth of those of alkali-treated ones. At the end of the process, the total amount of salt diffused inside the olive flesh was 0.33 and 1.71 g/100 g for the 2% and 4% trials, respectively. These values are much lower than those encountered with normal processing.

The combined and volatile acidity evolved as expected. The former was about 35 mEq/L at the end of the 210 days for the two trials, while the latter ranged from 0.09 to 0.15 g lactic acid per 100 mL of brine (Table 1). The combined acidity in this kind of process is derived from organic acids in the olives (mainly polyphenolic) as well as from the fermentation process, and reaches the values observed in our experiment and in turning colour and black olives (BORBOLLA y ALCALÁ *et al.*, 1971; FERNANDEZ DIEZ and GARRIDO FERNANDEZ, 1969; PIGA *et al.*, 2001). The volatile acidity was low (Table 1).

The sugar content in the brines was always very low during processing, less than 0.1% during the first two months, which indicates that fermentation proceeded very slowly. Fig. 3 shows that the reducing sugars in the olive flesh decreased. This indicates that fermentable substrates were used by microorganisms as soon as they diffused into the brine. This confirms the hy-

pothesis that reducing sugars were the limiting factor of fermentation. Diffusion of sugars depends on several factors (GARRIDO FERNANDEZ *et al.*, 1997). Skin permeability, salt concentration, temperature and olive-to-brine ratio probably affected the rate of the osmotic process. As expected, the amount of sugars in the flesh was significantly lower from the fourth month of fermentation in the 2% trial (GARRIDO FERNANDEZ *et al.*, 1997), compared to the 4% concentration. The 0.1–0.2% sugar content in the brine after 210 days of processing, and above all the large residual amount in the flesh, may pose problems in storage brines during marketing.

The polyphenol contents in the brine and flesh are shown in Table 1 and Fig. 3, respectively. An equilibrium in the polyphenol concentration between brines and flesh was reached at the fourth month for the 4% brines and at the fifth month for the 2% brines. Statistical analysis showed that the diffusion of polyphenols from the flesh was significantly higher in the 2% brines until the second month of processing, as previously observed by GARRIDO FERNANDEZ *at al.* (1997). In contrast, the same behaviour was not found in the brines. This was probably due to a slightly higher brine-to-olive ratio, which was the result of continuous brine replacement, which led to the dilution of polyphenols.

The main problem encountered by processors of this table olive variety is product loss due to gas pockets and shrivelling. Gas-pockets, or what the Spanish call "afarolado", occur as pressurised gas between the peel and the flesh and result in a transparent pocket. The data in Table 2 demonstrate that gas pocket development was completely inhibited by accurately controlling the temperature and pH of the brines. Shrivelling, was very low and totally reversible in olives brined at 2%, while a slight but significantly higher incidence of shrivelling was found in 4% brined olives. Processors may have up to 30% incidence of irreversible shrivelling when the same olive cultivar is brined at 8%. The results of fermentation with brines of less than 4% salt concentration would appear to be very beneficial for reducing shrivelling loss.

Table 2 - Incidence of gas pockets and shrivelling in "Tonda di Cagliari" olives brined with different salt concentrations after 210 days of fermentation.

Brine (%)	Gas pockets (%)	Peel shrivelling	
		reversible	irreversible[z]
2	0a[y]	0.5b	0b
4	0a	7.8a	3.5a

[z] Irreversible shrivelling was estimated by placing olives in water and checking for permanence of the shrivelling after 24 hours.
[y] Values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.01$.

Good results were also obtained in the sensorial evaluation, as the panelists did not report any off-flavour or off-odour and judged all olives to be excellent for firmness and residual bitter taste. They showed a slight preference for 4% olives, due to a more pronounced saltiness (data not shown).

CONCLUSIONS

Good results were obtained by correcting some process parameters (pH, salt, brine level and temperature) in the processing of naturally green table olives. In particular, safe pH values, a correct fermentation pattern and very good sensorial properties were obtained, with no microbial alterations.

Problems may arise when olives are transferred from the fermentation to the storage brines, where no adjust-

ment of pH is possible after packaging. Since there is a low combined acidity, brines have a weak buffering capacity, so the pH may exceed 4.5, the threshold for pathogen growth. Moreover, sugar residues in the flesh may be a source of microorganisms in storage brines. It is also necessary to shorten the processing time, the length of which is determined by loss of the bitter taste. Pasteurisation can solve the problem of brine stabilisation. Initial olive washing or the oleuropeinolytic bacteria can be used instead of chemicals for fast debittering. These alternatives may be too weak due to the slow diffusion of oleuropein in brines, which, in turn, may not be depleted with washing nor be available for bacterial (CIAFARDINI *et al.*, 1994) or yeast metabolism (BALLONI *et al.*, 1977).

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