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Effects of post-fermentation processing on the stabilisation of naturally fermented green table olives (cv Nocellara etnea)

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ABSTRACT

The effects of thermal treatment on green table olives were evaluated as a method to control enzymatic browning, to minimise the microorganism presence and to extend their shelf-life. However this treatment is often responsible for colour alterations, development of off-flavours and unfavourable texture changes. Moreover, the effect of different re-use of the natural brine of fermentation with or without different treatments was investigated. Calcium treatment was suggested to maintain firmness. Firming effects obtained from heat treatment combined with calcium treatment have been attributed to heat-activated pectin methylesterase and/or to increased calcium diffusion into tissues at higher temperatures. The results derived from this study will help in designing new processes which can be applied in table olive industry.

1. Introduction

Extending the shelf-life and safety of foodstuffs is of considerable importance to the food industry due to its significant economic impact. The use of heat treatment for prolonging shelf-life by destroying principally the pathogens, eliminating the total bacterial count and inactivating the enzymes, increases the food stability and lessens the oxidizing processes. The application of heat treatment is of particular importance for fermented vegetables, aimed at maintaining the original characteristics of the food, such as visual aspect which the consumer perceives as the quality of the product at the time of purchase (Luna-Guzmán & Barrett, 2000). Unfortunately, heat treatments have a negative effect on the qualitative characteristics of the end product in terms of negatively affecting polyphenolic, lipid, protein and vitamin fraction, alterations in consistency and colour (Calligaris, Manzocco, Anese, & Nicoli, 2004; Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001).

With regard to the firmness of olives, to preserve the original firmness as much as possible (Gras, Vidal, Betoret, Chiralt, & Fito, 2003), calcium was found to maintain the cell wall structure of vegetables by interacting with pectin to form calcium pectate. Calcium maintains firmness by crosslinking with cell wall and middle lamella pectins (Martin-Diana et al., 2005). Firming effects obtained from heat treatments alone or combined with calcium treatments have been attributed to the action of heat-activated pectin methylesterase and/or to increased calcium diffusion into tissues at higher temperature (García, Herrera, & Morilla, 1996).

In addition to firmness, colour is one of the parameters that most directly reflect the quality of vegetables subjected to heat treatment. In table olives, phenols have a significant influence on the colour as they play a crucial role in browning which occurs during processing, often caused by their polymerisation. Moreover, polyphenols represent the substrate for the activity of oxidative enzymes (Marsilio, Campestre, & Lanza, 2001). A number of methods have been adopted to inhibit polyphenoloxidase (PPO) including the addition of chemical substances (Fujita et al., 1995), pH correction (Siddiq, Sinha, & Cash, 1992), oxygen removal (Paulson, Vanderstoep, & Porritt, 1980), refrigeration (Lozano, Druids, & Ibarz, 1994) and heat treatment (Silva & Nogueira, 1983). Browning reactions are not noticed as long as the tissue remains healthy and intact in fruit and vegetables that are susceptible to browning. It is likely that the enzymes and substrates are located in different tissues or cell sections, separated by different membranes.

Also pigments play an important role in colour. In olives in particular, photosynthesis decreases and the concentration of chlorophyll and carotenoids progressively diminishes over time (Criado, Motilva, Goñi, & Romero, 2007). In chlorophyll heat causes in the coagulation of a lipoprotein that the chlorophyll would normally be fixed to and be protected by. Chlorophyll and carotenes also have an important role in the oxidative activity of processed foods due to their antioxidant nature under conditions of dark and

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pro-oxidant characteristics under lighting conditions (Fakourelis, Lee, & Minn, 1987) and so they influence the food quality.

The objective of the present work was to evaluate the effects of heat stabilisation process on naturally fermented olives (cv Nocellara etnea), packaged with different types of cover brine. A study was carried out into the possibility of reusing differently treated fermentation brine to cover the olives.

2. Materials and methods

2.1. Materials

Green olive fruits of the Nocellara Etnea *cultivar*, harvested in October, were immediately stored under anaerobic condition in 8% (w/v) NaCl for six months at 17 °C to permit a natural fermentation.

2.2. Experimental procedure

The samples were prepared as follows: 300 g of olives and 200 g of brine placed into glass containers (580 mL) were pasteurised in a thermostatic water bath at 75 °C and maintained the core of the sample at 67 °C for 4 min. Four replications for each thesis were performed. Following the sample was cooled rapidly in an ice bath. The parameters of the thermal treatment were established in accordance with International Olive Oil Council (2004) standards in order to obtain the highest requisites of hygienic quality. The treatments were monitored through the data logger (Escort Junior Data Logging Systems Ltd., New Zealand) and were carried out in quadruple. Two of the four samples of each treatment were stored in a temperature-controlled storage locker at 25 °C for 3 days up to the microbiological analysis, while the other two samples were immediately subjected to physicochemical characterisation and chemical analyses. The brines used to fill up the glass containers were: P1] brine of natural fermentation (FB); P2] filtered on paper (Whatman no. 42) FB; P3] reconstituted brine (NB) with 6% NaCl (w/v); P4] NB with 3% $CaCl_2$ and 3% NaCl; P5] FB warming up at 90 °C for 30 min and filtered; P6] FB deal in active coal (500 mg/L) at 70 °C for 30 min and filtered.

2.3. Sodium chloride, pH and acidity of brine and pulp

The NaCl, pH, free and combined acidity values were carried out by the routine methods (Fernàndez-Diez et al., 1985). pH by a pHmeter (Crison Basic 20), total acidity by titration with NaOH, chlorides by titration according to Mohr method with $AgNO_3$. Ten grams of each sample were mixed with 25 mL of distilled water three times with an Ultraturax and than the filtrated solution was collected and filled up to 100 mL in a graduated flask with distilled water. This solution was used to measure pH, free and combined acidity of flesh olives.

2.4. Total polyphenols of olives

Total polyphenols were extracted from olive flesh following the method reported by Amiot, Fleuriette, and Macheix (1986) and measured spectrophotometrically at 725 nm after reaction with the Folin-Ciocalteu's reagent, and expressed as mg/kg of gallic acid by means of a calibration plot using pure gallic acid as a standard at different concentrations.

2.5. Colour analysis

All samples were analysed before and next thermal treatment for colour by using a tristimulus colorimeter Minolta CR-300. For each sample, 10 olives were analysed on five points per fruit to evaluate the skin colour. L^*, a^* and b^* values were calculated using illuminant D65 according to the CIELAB scale. The ΔE variation of colour was calculated from the equation:

$$\Delta E = [(L_f - L_i)^2 + (a_f - a_i)^2 + (b_f - b_i)^2]^{1/2}$$

where L_i is the initial luminosity and L_f the final luminosity; a_f is the a^* value at the final time and the a_i is the a^* value at initial time; b_f the b^* value at final time and b_i is the b^* value at initial time.

2.6. Texture analysis

Hardness and its evolution in olives were determined through a texture analyser (TA.TX2, Stable Microsystems, Surrey, UK) equipped with a 2 mm diameter cylinder probe. Hardness measurement of samples by puncturing involved plotting force (N) versus distance (mm) and two parameters were calculated: (a) the force (F1) of the peak necessary to break the cuticle of olive; (b) the area (Area-FD 2:3) under the curve between F3 point (corresponding to half distance between F1 and F2) and the maximum applied force (corresponding to 2000 g) F2 (see Fig. 1 for graphical details).

The set parameters of each test were: pre-test speed 2 mm s^{-1}, test speed 0.5 mm s^{-1}, post-test speed 4 mm s^{-1} and force max 1500 g.

2.7. HPLC analyses

Preparation of olive extract, phenolic standards and HPLC analysis of phenols were carried out according to McDonald, Prenzler, Antolovich, and Robards (2001). Pure standards were purchased from Fluka (gallic acid, tyrosol, vanillic acid, chlorogenic acid, *o*-cumaric acid, *p*-cumaric acid, caffeic acid, 4-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, cinnamic acid, ferulic acid and apigenin-7-glucoside), Sigma (rutin, syringic acid, (−)cathechin and catechol) and Extrasynthese (oleuropein and verbascoside). HPLC analysis was conducted using a Knauer HPLC system (Smartline Pump 1000) equipped with Waters 486 UV detector set at 280 nm. A C18 monomeric 120 Å, 5 μm particle size, 4.6 × 250 mm column (Grace Vydac, Denali, USA) fitted with 4.6 mm guard column were used. The solvent flow rate was 1.0 ml/min. Separation was achieved by elution gradient using an initial composition of 90% of A solution (water acidified with 2% acetic acid) and 10% of B solution (methanol/acetonitrile 1:1, v/v). After 15 min of isocratic conditions, the concentration of B solution was increased to 30%, with further stepwise increases to 40% B at 25 min, 70% B at 35 min and 100% B at 40 min, hold for 5 min and return to initial conditions over 5 min.

2.8. Data analyses

Statistica software (version 6.0, StatSoft Inc.) was used for data processing. One-way analysis of variance was used to test the effects of the different treatments on the measured factors. Duncan's multiple range test was used to compare means when a significant variation was highlighted by analysis of variance.

3. Results and discussion

Table 1 shows the results regarding colour analysis of the epicarp and the consistency of the drupes before (P0) and after thermal treatment. The parameters examined for colour evidenced statistically significant differences among the samples treated. Indeed, it can be seen that the L^* values of the original (P1) and filtered (P2) brine samples were not significantly different from

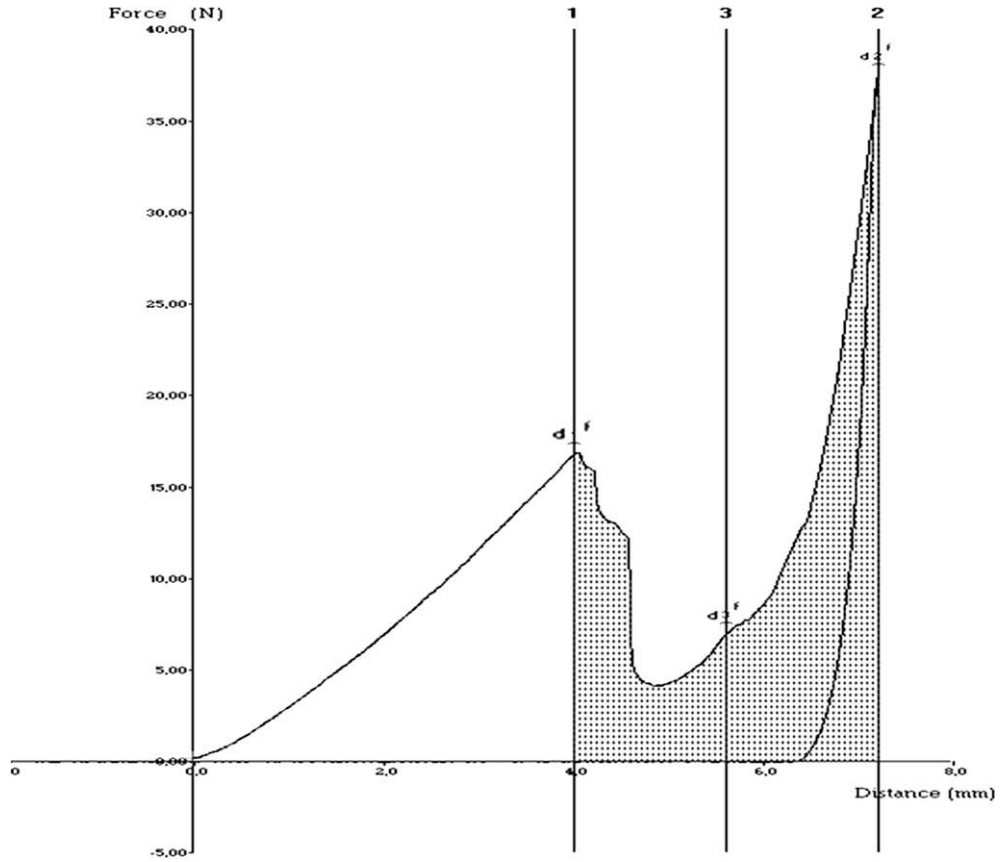

Fig. 1. Hardness measurement of samples by puncturing involved plotting force (N) versus distance (mm).

Table 1
Colorimetric and textural properties of olive pulp determined on different treated and untreated samples. For abbreviations see Section 2.

Samples	L^*	a^*	b^*	ΔE^*	Texture area-FD 2:3 (N mm)
P0	53.79 ± 3.01a	−1.75 ± 1.72d	36.32 ± 2.93a	0.00 ± 0.00d	21.67 ± 1.87ab
P1	54.39 ± 2.86a	0.17 ± 1.27c	35.63 ± 3.21ab	5.64 ± 2.20c	19.25 ± 1.30c
P2	52.90 ± 3.77ab	0.60 ± 1.73c	33.17 ± 5.14bc	7.95 ± 5.59bc	20.02 ± 1.27bc
P3	50.88 ± 2.70bc	2.02 ± 1.39b	32.95 ± 2.62bc	6.74 ± 4.11bc	23.12 ± 3.26a
P4	46.41 ± 4.25d	4.14 ± 1.81a	23.56 ± 4.94d	16.20 ± 7.01a	23.54 ± 1.20a
P5	49.17 ± 3.31c	2.57 ± 1.56b	31.52 ± 3.90c	9.25 ± 4.66b	20.03 ± 1.34bc
P6	48.76 ± 5.43c	2.19 ± 2.16b	33.59 ± 6.38abc	9.00 ± 6.88bc	20.95 ± 0.64bc
Sig.	**	**	**	**	**

Data followed by different letters are significantly different by Duncan's multiple range test.
** Significance at $P < 0.01$.

the untreated (P0) sample. In foods subjected to heat treatment, an increase in brightness can be attributed to the loss in activity of the PPO while a decrease in this parameter is associated with a greater degree of browning (King, Magnuson, Torok, & Godman, 1991). Instead, samples with a double heat treatment (P5–P6) and with brine reconstituted with 3% $CaCl_2$ (P4) showed the lowest L^* values. The a^* parameter displayed a distinct toning from green to red in all pasteurised samples, particularly in those with new brine and those subjected to double heat treatment. This increase may be associated with the breakdown of the chlorophyll, as reported by Bolin and Huxoll (1991) and the use of calcium-based solutions (Martin-Diana et al., 2005). Moreover, the increase of the a^* component may be attributable to reactions of browning, enzyme-catalysed or chemical oxidisation reactions which involve the oxidisation of *o*-diphenols of olives in quinones and their subsequent transformation into different dark compounds (Romero, Brenes, García, & Garrido, 1998). With regard to b^* parameter, it can be seen that single pasteurisation does not cause significant variations compared to the non-heat treated sample (P0); the same can also be said for filtration and the use of new brine with sodium chloride. The P4 sample was markedly different from the other samples and had the lowest value and, as a result of the presence of the calcium ion, may interfere in the colouring effect. In the ΔE parameter, which indicates the chromatic variation compared to P0, the P4 sample had the highest value and was again statistically different to all the other samples among which there was no significant difference.

Fig. 1 shows the results of texture analysis conducted on whole olives. Initially there is an increasing trend, due to the application of an ever rising force until breakage of the drupe occurs (F1); following the initial peak there was a waning phase caused by the elastic response of the same cuticle. After the breakage of the cuticle, a force is required which increases in proportion to the hardness of the pulp, up to the application of the maximum force (F2), reached in correspondence of the stone. The F3 force was calculated in correspondence of the mean point of the distance

between forces F1 and F2. The peak of force F1 was considered as the index of hardness of the cuticle (Lin & Chang, 2005) while the index of pulp consistency was considered as the area subtended from the curve between F2 and F3 to exclude the elastic effect of the cuticle, prevalent in the area between F1 and F3. The results obtained regarding the force necessary to break the cuticle do not show significant variations among all the samples analysed, varying from 16.8 to 18.6 N (data not shown), whereas variations were observed with regard to the consistency of the pulp (Table 1). The highest values among samples subjected to pasteurisation belong to those with brine reconstituted with sodium chloride and calcium with values statistically similar to the untreated sample (P0). Preservation of the original consistency of the pulp is attributable to the presence of added salts. It is known that vegetables subjected to similar processes have hardness similar to the untreated control sample and maintain this characteristic over time after heat treatment (Luna-Guzmán, Cantwell, & Barrett, 1999). The effects on consistency following the combination of heat treatment and the presence of calcium or sodium ions are attributable to the action of PME, activated by heat in the 55–70 °C range (Bartolome & Hoff, 1972) and the spread of salt inside the vegetal tissues (García et al., 1996). The application of calcium or sodium as fortifiers must however take into account the effective intercellular porosity, surface/volume ratios of the studied matrix as well as the solubility or dispersion limits of the salts (Gras et al., 2003). Moreover, calcium chloride is considered a good alternative to calcium lactate as the latter may lead to the appearance of a bitter taste and off-flavour in foods (Luna-Guzmán & Barrett, 2000).

Compared to the untreated sample (P0), an increase in pH value was observed in all the samples subjected to heat treatment, associated with a decrease in free acidity (Table 2). This probably occurs following the release of acids from the pulp to covering brine, a process facilitated by heat, and the beginning of new complex post-treatment osmotic balance mechanisms. The pasteurisation process causes micro-fractures in the tissues of the drupe thus encouraging the release of different substances from its interior into the surrounding liquid. The P4 sample, containing brine with calcium chloride, which has the highest value of pulp consistency, having suffered less damage to its vegetal tissues probably recorded a lower loss of acids; in support of this idea, it is possible to note that the value of free acidity, shown by P4, is greater among the samples that underwent heat treatment and closer to the untreated sample (P0) characterised by undamaged drupes.

The total polyphenols content of the drupes undergoes a decrease in all samples subjected to treatment following a partial release of the pulp into the brine as well as at the process of oxidisation of the same compounds, causing a browning of the olives, due to the activity of the PPO (Marsilio et al., 2001). The greatest loss was observed in the P5 sample (Table 2) which was subjected to double heat treatment. The P2 sample evidences a polyphenolic content lower than P1 which can be explained by the consideration that brine filtration may influence the spread of pulp compounds into the brine. It was found that the P3 sample, consisting of new brine with 6% salt, has a polyphenolic content statistically similar to P2 and P6. Instead, the presence of a calcium-based solution, characterised by the P4 sample, has no protective effect towards the polyphenolic substances of the pulp encouraging their diffusion in the covering brine compared to brine consisting only NaCl. The decrease in polyphenols conflicts with Martin-Diana et al. (2005) who recorded a decrease in PPO activity proportional to calcium concentration.

Individual phenolic compounds (Table 3) decreased in all the samples compared to the concentrations found in the sample that did not undergo heat treatment. The decrease was observed above all for *o*-diphenols, demonstrating that this process affected the phenolic profile reducing, in a different way, the contents. Indeed, during the fermentation period an increase in hydroxytyrosol to the detriment of oleuropein was observed (Amiot et al., 1986). The P1 sample, subjected to pasteurisation only and the P4 sample had an oleuropein concentration similar to the untreated sample, a result that may be attributable to a protective effect of calcium (Martin-Diana et al., 2005), even if this was not shown in the total polyphenols content. In this framework of results the role of temperature on the PPO needs to be considered. Indeed, at the temperature used for the pasteurisation of the brine (67 °C), the PPO may continue its activity as quoted by other authors (Aydemir, 2004), so that a decrease in phenolic compounds is likely.

Evaluation of the results of the brine analysis is important above all for that which regards the visual aspect that they acquire after heat treatment or after the different pre-treatments, considering that one of the objectives of the work is to test the possibility of reusing them. The results of colour analysis carried out on the brine of different samples (Table 4) revealed that heat treatment does not result in any variation of the brightness of the brine which is highlighted by the value of P1, statistically similar to that of P0, not subjected to pasteurisation. P2, instead, evidenced a slightly higher value; this phenomenon may be explained by the fact that following filtration pigments, polyphenols and various compounds which affect this parameter were separated from the brine. The highest L^* parameter values were attributed to samples with reconstituted brine and P4 in particular. The application of a dual heat treatment does not result in further browning of the brine which in the P5 and P6 samples shows values similar to P2. In addition, a variation was observed in the a^* colouring, tending towards red, of the brine in the P5 and P6 samples, subjected to dual treatment, in which the formation of brown compounds probably occurred on account of chemical-enzymatic processes caused by oxidative browning and the polymerisation of molecules, including phenols. Instead, lower values of the b^* colouring, the index of yellow colouration, were observed in samples with reconstituted brine (P3 and P4). Indeed, in the covering brine, formed initially only by added salts, those substances released during natural fermentation and subsequently subjected to browning during heat treatment, were not found as observed in the other samples. With

Table 2
Chemical analyses of olive pulp.

Samples	pH	Free acidity (mEq/L)	Combined acidity (mEq/L)	Phenols (ppm gallic ac.)
P0	4.70 ± 0.01f	20.66 ± 0.56a	25.98 ± 0.00c	2820.4 ± 20.20a
P1	5.06 ± 0.01a	17.99 ± 0.00c	27.47 ± 0.00b	2719.5 ± 19.90b
P2	5.02 ± 0.00b	17.35 ± 0.57c	21.19 ± 0.00g	2663.8 ± 48.57bc
P3	5.02 ± 0.01b	19.47 ± 0.57b	23.50 ± 0.00d	2630.4 ± 46.19c
P4	4.87 ± 0.01d	19.81 ± 0.00b	32.93 ± 0.00a	2497.4 ± 46.18d
P5	4.75 ± 0.01e	17.99 ± 0.00c	21.62 ± 0.00f	2424.6 ± 43.77e
P6	4.90 ± 0.00c	17.99 ± 0.00c	22.72 ± 0.00e	2645.1 ± 50.89c
Sig.	**	**	**	**

Data followed by different letters are significantly different by Duncan's multiple range test.
** Significance at $P < 0.01$.

Table 3
HPLC analysis of phenolic compounds performed on olive pulp. For abbreviations see Section 2.

Compounds[a]	Samples							
	P0	P1	P2	P3	P4	P5	P6	Sig.
Hydroxytyrosol	176.29 ± 1.25a	171.58 ± 0.35b	164.18 ± 0.35c	152.47 ± 0.34f	161.26 ± 1.04d	158.18 ± 1.24e	160.64 ± 0.57d	**
Tyrosol	6.52 ± 0.21a	2.45 ± 0.27b	2.45 ± 0.03b	2.52 ± 0.19b	1.11 ± 0.06c	2.81 ± 0.02b	2.43 ± 0.18b	**
4-Hydroxybenzoic ac.	15.12 ± 1.21a	15.01 ± 0.03a	12.74 ± 0.24b	12.14 ± 0.02b	10.16 ± 0.96c	12.36 ± 0.12b	11.35 ± 1.16bc	*
Chlorogenic ac.	9.66 ± 0.05a	8.52 ± 0.92bc	9.52 ± 0.11ab	8.51 ± 0.02bc	7.80 ± 0.28c	9.19 ± 0.53ab	5.91 ± 0.06d	**
Catechol	7.92 ± 0.51a	3.91 ± 0.01c	4.05 ± 0.05bc	4.35 ± 0.11bc	5.08 ± 0.10b	4.83 ± 0.03bc	4.07 ± 0.99bc	**
Syringic ac.	9.72 ± 0.18a	3.93 ± 0.02f	2.12 ± 0.02g	5.12 ± 0.13e	6.57 ± 0.17c	5.49 ± 0.06d	8.76 ± 0.25b	**
Vanillic ac.	2.61 ± 0.10a	1.36 ± 0.11bc	2.24 ± 0.18a	1.19 ± 0.22c	1.66 ± 0.02b	2.31 ± 0.01a	0.77 ± 0.28d	*
Caffeic ac.	15.29 ± 0.23a	10.64 ± 1.51c	15.25 ± 0.04a	12.53 ± 0.05b	13.61 ± 0.76ab	14.35 ± 1.11ab	14.06 ± 0.03ab	*
2,4-Dihydroxybenzoic ac.	5.43 ± 0.38a	4.59 ± 0.06bc	3.76 ± 0.13d	3.85 ± 0.09cd	4.71 ± 0.19ab	4.37 ± 0.68bcd	4.44 ± 0.02bcd	*
(−)Cathechin	3.59 ± 0.02a	3.42 ± 0.12a	2.10 ± 0.23b	3.23 ± 0.10a	1.68 ± 0.01c	1.47 ± 0.20c	2.10 ± 0.21b	**
Verbascoside	16.11 ± 0.84a	7.47 ± 1.20d	13.05 ± 0.20b	10.75 ± 0.05c	12.38 ± 0.05bc	12.40 ± 0.07bc	13.97 ± 1.76b	**
p-Coumaric ac.	20.36 ± 0.26a	11.94 ± 0.60cd	13.87 ± 1.31c	11.29 ± 0.39d	17.43 ± 0.68b	12.57 ± 1.36cd	19.36 ± 0.16a	**
Ferulic ac.	6.46 ± 0.81a	1.79 ± 1.17c	1.78 ± 0.11c	2.03 ± 0.15c	2.67 ± 1.19c	2.92 ± 0.07c	4.78 ± 0.99b	**
Rutin	20.68 ± 4.22a	14.69 ± 0.06bcd	13.89 ± 0.11cd	14.69 ± 0.31bcd	18.20 ± 0.10ab	11.61 ± 0.15d	16.95 ± 0.61abc	*
Apigenin-7-glucoside	84.47 ± 0.66a	51.07 ± 0.75e	83.41 ± 1.62a	67.20 ± 0.66d	71.99 ± 0.50c	78.03 ± 0.57b	77.76 ± 2.56b	**
o-Coumaric ac.	23.13 ± 0.69a	22.15 ± 0.21ab	17.92 ± 0.19d	20.00 ± 1.17c	22.19 ± 0.22ab	18.34 ± 0.17d	21.48 ± 0.83b	**
Oleuropein	95.09 ± 1.87a	95.38 ± 1.59a	61.64 ± 0.22e	87.76 ± 0.08c	95.04 ± 0.07a	80.17 ± 0.47d	91.18 ± 0.07b	**
Cinnamic ac.	8.33 ± 0.06a	7.82 ± 0.38abc	7.64 ± 0.07bcd	7.23 ± 0.09d	8.14 ± 0.08ab	7.63 ± 0.02bcd	7.56 ± 0.41cd	*

Data followed by different letters are significantly different by Duncan's multiple range test.
* Significance at $P < 0.05$.
** Significance at $P < 0.01$.
[a] The compounds are expressed as mg/kg of pulp.

Table 4
Colorimetric properties of olive brine determined on different treated and untreated samples. For abbreviations see Section 2.

Samples	L^*	a^*	b^*	ΔE^*
P0	27.07 ± 0.05d	−0.41 ± 0.06d	3.61 ± 0.06c	0.00 ± 0.00g
P1	27.13 ± 0.11d	−0.06 ± 0.09b	3.41 ± 0.17c	0.44 ± 0.11f
P2	27.84 ± 0.06c	−0.03 ± 0.05b	4.77 ± 0.13a	1.45 ± 0.10d
P3	30.90 ± 0.29b	−0.34 ± 0.07c	0.85 ± 0.05d	4.73 ± 0.23a
P4	31.84 ± 0.36a	−0.03 ± 0.09b	1.15 ± 0.05d	1.84 ± 0.17c
P5	27.74 ± 0.13c	0.33 ± 0.08a	3.95 ± 0.99b	2.70 ± 0.37b
P6	27.77 ± 0.33c	0.30 ± 0.12a	4.01 ± 0.03b	1.22 ± 0.37e
Sig.	**	**	**	**

Data followed by different letters are significantly different by Duncan's multiple range test.
** Significance at $P < 0.01$.

regard to the variation in the ΔE value of the brines, it was noticed that, as predicted, the greatest difference compared to P0 was shown by P3 with reconstituted brine, followed by P5 which had the highest temperature in the dual heat treatment.

As regards the chemical analysis of the brine (Table 5), the simple process of pasteurisation leads to an increase in the pH value. Samples with reconstituted brines (P3 and P4) recorded lower values of free acidity than those of the untreated product; acidity was affected only by the compounds released by the drupes. The samples with dual heat treatment evidenced a decrease in free and combined acidity which can be explained considering that the acidic substances were subjected to chemical-enzymatic modifications, caused by a greater exposure to heat. The pasteurisation process does not appear to have any significant effect on free acidity content and the chlorides present in the brine. Indeed, the P1 sample, which was subjected to heat treatment only, has values similar to P0. The transfer of polyphenols to the covering brine can easily be observed in samples P3 and P4. The P4 sample with brine containing calcium chloride has a higher polyphenolic content than the P3 sample, consisting of a brine based on only sodium chloride; this result is due to a greater chelating and protective capacity exerted by calcium compared to polyphenolic substances (Martin-Diana et al., 2005).

From the results obtained it may be concluded that to obtain a brighter and more limpid brine to use as covering liquid, it would be advisable to select products with reconstituted brine to introduce into the market. Considering the need to reduce the volume of wastes, the reusing of fermentation brines maintains the original composition of the product, rich in polyphenols, pigments, acids and other compounds that characterise aroma and nutritional value, even if subject to a slight browning following pasteurisation. Consistency similar to the initial product is maintained using regenerated and filtered brine.

From a sensory aspect, a product that has undergone heat treatment would be preferable as it reduces the content of glycosidic phenolic compounds (oleuropein and verbascoside), making olives obtained from a simple process of fermentation less bitter.

Table 5
Chemical analyses of brines.

Samples	pH	Free acidity (mEq/L)	Combined acidity (mEq/L)	Sodium chlorides (%)	Phenols (ppm gallic ac.)
P0	3.73 ± 0.01e	95.00 ± 0.00a	26.67 ± 0.01b	7.88 ± 0.00a	1390.1 ± 9.32c
P1	3.75 ± 0.01d	95.00 ± 0.00a	20.00 ± 0.00cd	7.83 ± 0.00a	1438.6 ± 18.65c
P2	3.76 ± 0.00d	94.17 ± 1.44a	35.02 ± 0.02a	7.57 ± 0.03c	1527.4 ± 16.80b
P3	3.80 ± 0.01b	8.17 ± 0.29d	21.17 ± 0.01c	6.07 ± 0.06f	386.2 ± 73.26e
P4	3.98 ± 0.01a	0.67 ± 0.03e	11.17 ± 0.00e	6.33 ± 0.07e	861.4 ± 22.36d
P5	3.78 ± 0.01c	86.67 ± 0.58c	22.00 ± 0.00c	7.03 ± 0.03d	1442.2 ± 33.81c
P6	3.78 ± 0.01g	91.67 ± 0.58b	19.04 ± 0.00d	7.71 ± 0.00b	1815.3 ± 55.91a
Sig.	**	**	**	**	**

Data followed by different letters are significantly different by Duncan's multiple range test.
** Significance at $P < 0.01$.

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