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Investigation of a tunnel pasteurizer for "Nocellara del Belice" table olives processed according to the "Castelvetrano method"

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SUMMARY: The influence of pasteurization temperature and time of treatment on the flesh firmness and the evolution of microbial communities was studied for table olives Cv. Nocellara del Belice, packed in glass jars and processed with a tunnel pasteurizer. The experiment was first carried out on the laboratory level in order to select the optimal combination of pasteurization time/temperature so as to obtain the proper balance between the consistency of the pulp and the microbiological quality of the final product. Pasteurization at industrial scale was then carried out in a tunnel pasteurizer applying the treatment at 75 °C for 8 min in the thermal center of the jars. Besides flesh firmness and microbial evolutions, the pH, total titratable acidity (TTA) and color were evaluated for the table olives during storage at 6, 12 and 15 months from packing. The table olives showed a high stability and acceptable flesh firmness for the entire period under observation. Specifically, olive pulp texture decreased during the storage period, but the softening was most evident in the deeper layers of the pulp. The results indicated that the storage period should not exceed 6 months. Although the hygiene is preserved, after this period the firmness might not be acceptable to consumers.

KEYWORDS: *Compression force; Dynamometer; Microbiological analysis; Table olives; Texture; Tunnel pasteurizer*

RESUMEN: ***Investigación sobre un pasteurizador de túnel para las aceitunas de mesa "Nocellara del Belice" procesadas mediante el "Método Castelvetrano"***. En el presente trabajo se estudió la influencia de la temperatura y del tiempo de pasteurización en la firmeza de la pulpa y la evolución de las comunidades microbianas para la aceituna de mesa "Nocellara del Belice" procesadas con un pasteurizador de túnel. El experimento se llevó a cabo preliminarmente a nivel de laboratorio con el fin de seleccionar la combinación óptima de tiempo/temperatura de pasteurización para obtener el compromiso adecuado entre la consistencia de la pulpa y la calidad microbiológica del producto final. A continuación se llevó a cabo la pasteurización en escala industrial dentro de un pasteurizador de túnel aplicando el tratamiento a 75 ° C durante 8 minutos. Fueron evaluados pH, acidez total titulable (ATT) y el color para las aceitunas de mesa durante el almacenamiento a los 6, 12 y 15 meses de envasado. Las aceitunas de mesa mostraron una alta estabilidad y una firmeza de pulpa aceptable para todo el período de observación; en particular, la textura de pulpa disminuyó durante el período de almacenamiento, pero el ablandamiento fue más evidente en las capas más profundas de la pulpa. Los resultados indicaron que el período de almacenamiento no debe ser superior a 6 meses.

PALABRAS CLAVE: *Aceitunas de mesa; Análisis microbiológico; Dinamómetro; Fuerza de compresión; Pasteurizador de túnel; Textura*

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1. INTRODUCTION

Industrial pasteurization must guarantee the safety of foods. A prolonged shelf-life is not only possible thanks to the hygienic stability of foods, but also by interrupting the oxidative processes. This depends on the treatment conditions and the monitoring of the temperature profile is of utmost importance (Plazl *et al*., 2006).

Pasteurization is based on a partial thermal degradation of microorganisms and the denaturation of the enzymes responsible for the spoilage of foods (Buckenhskes *et al*., 1988). If on the one hand heat treatments are particularly important for the microbial quality, on the other hand they can be the cause of negative effects on the chemical characteristics of the foods in terms of polyphenols, lipids, proteins and vitamins, thereby altering texture and color (Manzocco *et al*., 2001).

Many studies on the textural attributes of fruits and vegetables have considered the relationships between texture and maturity processes. Several authors have studied the influence of humidity and temperature, water status, and apoplastic and symplastic water on changes in texture (Herppich *et al*. 2003). Storage time is also a relevant factor in response to changes in temperature, although this response does not appear to be correlated significantly with orchard or harvest maturity (Johnston *et al*., 2001).

Firmness is one of the most common physicochemical parameters used to evaluate fruit quality but does not take into account all the aspects of fruit texture. Texture is a difficult concept to define; it is related to the mechanical quality of fruit and is influenced by a combination of properties (Diezma, 2004) that relate to the fruit's ability to resist mechanical deformation. The instrumental estimation of fruit textural properties is based on the mechanical response of fruit tissues. Many studies of single or multidimensional approaches for the sensory evaluation of fresh fruit and vegetable texture have been performed. The most extensive research has been carried out on apple (Harker *et al*., 2002; Mehinagic *et al*., 2003), pear (Ozturk *et al*., 2009), peach (Ortiz *et al*., 2000), mango (Valente *et al*., 2009), orange (Topuz *et al*., 2005), cactus pear (Kabas and Ozmerzi, 2008), and also on table olives (Cardoso *et al*., 2008; Catania *et al*. 2014; Kiliçkan and Güner, 2008).

The pasteurization of table olives on an industrial scale is mostly carried out using a tunnel pasteurizer where the continuous pasteurization of the glass jars occurs because of their movement through the tunnel. It consists of progressively hotter, holding and progressively cooler zones (Engelman and Sani, 1983). The process temperature is controlled by the temperature of the water spray on the product inside the tunnel. Dilay *et al*. (2006) developed a simplified mathematical model to obtain the energetic behavior of a pasteurization tunnel used for bottled beer production which is capable of performing a geometric optimization of typical pasteurization tunnels. The authors found that a slightly rectangular tunnel is the optimal geometric configuration considering total power consumption. The heat transfer inside a tunnel pasteurizer was studied by Zheng and Amano (1999) in order to optimize the pasteurization design; the models they developed were useful to predict the operation status of the pasteurization process.

Due to the presence of oleuropein, a glucoside composed of glucose, elenolic acid and o-diphenol hydroxytyrosol (Servili *et al*., 2006), olive drupes cannot be eaten just after harvesting because of their unpleasant bitter taste. Hence, olive drupes undergo a transformation process before consumption and the resulting products, namely "table olives", are classified in treated olives, natural olives, olives darkened by oxidation, dehydrated and/or shriveled olives and specialties (IOCC, 2004).

Table olives are the major fermented edible vegetable of the Mediterranean basin. Within this area, Sicily (south Italy) represents an important area of production in southern Europe (Poiana and Romeo, 2006). Among the autochthonous Sicilian cultivars, Nocellara del Belice is quantitatively the most represented one and its table olives enjoy the protected denomination of origin (PDO) status (Regulation EC No 134/1998).

In Sicily, table olives are usually processed with different methods to eliminate bitterness, mainly the "Spanish-style" system, the most economically relevant (Aponte *et al*., 2012), the "natural" system without the use of chemicals and the typical "Castelvetrano" system (Romeo *et al*., 2012). The last transformation process is traditionally applied in western Sicily and consists of a treatment of drupes with lye overnight followed by the addition of salt after 12 hours. The resulting table olives are ready for consumption after 15 days (Romeo *et al*., 2012).

Table olive fermentation is driven by lactic acid bacteria (LAB) and yeasts (Aponte *et al*., 2010). However, other undesired microorganisms, such as *Enterobacteriaceae*, *Pseudomonas* spp., *Clostridium* spp. and *Staphylococcus* spp., may develop during olive transformation. Specifically, they are present at the beginning of olive fermentation but at the end of process, when the pH decreases, they are not generally detected (Randazzo *et al*., 2012). Since the microbial dynamics during table olive fermentations are, to a certain extent, unpredictable, a thermal treatment might be relevant for productions characterized by high microbial hygiene and stability (Romeo *et al*., 2012). In this regard, the main treatment processes the drupes undergo are pasteurization and sterilization. However, the thermal

treatments negatively affect the consistency and color of the final product (Romeo *et al*., 2009).

Today, process monitoring and control are fundamental requirements in the modern food industry. The automatic control of the main process parameters is essential for the rationalization of the process (Aiello *et al*., 2012; Carrara *et al*., 2005; Carrara *et al*., 2008; Catania *et al*., 2013a; Catania *et al*., 2013b). This requirement can also be considered valid in the tunnel pasteurizer regulation controlling pasteurization time and temperature.

The aim of this study was to evaluate the influence of temperature and time of pasteurization on several quality parameters of table olives of the Cv. Nocellara del Belice, packaged in glass jars and processed with a tunnel pasteurizer. After the selection of the right parameters for pasteurization which determine a good consistency of the pulp and the microbiological safety of the olives, the study continued at industrial scale applying that treatment in a tunnel pasteurizer and the table olives were evaluated during storage at 6, 12 and 15 months from packing.

2. MATERIALS AND METHODS

2.1. Plant material

The experimental tests were performed on Nocellara del Belice table olives harvested in October 2012; drupe characteristics (fruit and pit mass, fruit and pit longitudinal diameter, mesocarp mass and thickness) are summarized in Table 1. The fruit and pit mass were measured by an electronic balance to an accuracy of 0.01 g (ORMA, BC4000S, Italy); the fruit and pit diameter and the flesh thickness were measured by a digital micrometer caliper to an accuracy of 0.01 mm. The mesocarp thickness and mass represented 55% and 86% of the drupe, respectively; the consumer highly appreciates this kind of fruit flesh whose consistency, in terms of hardness, represents a primary qualitative aspect for this table olive.

Fruits were processed according to the "Castelvetrano" system, also called "dolcificata" in the local language. This method allows for a rapid debittering of the fruits that maintains good texture, green color and a distinctive taste of soda due to the NaOH solution that penetrates into the pulp. According to this method, olives, graded by size, are placed in plastic drums and treated with a 1.5–2.2% NaOH aqueous solution. The NaOH percentage mainly depends on olive size and degree of maturation. The immersion in the alkaline solution lasts a few hours and, after that dry salt is added in order to create a brine with a concentration of 6–7% (corresponding to 5–6 kg of NaCl in a 220 l volume drum). After 15 days of storage the product is ready for consumption.

The fruits processed in this study were packed in glass jars with 488 g gross weight, 300 g net weight and 160 g drained weight.

2.2. Experimental tests

The tests were carried out using a tunnel pasteurizer included in a plant for table olive processing and packing in glass jars.

The experiment was preliminarily carried out at laboratory scale in order to determine the optimal conditions of temperature and time for pasteurization that obtain the best results concerning consistency of the pulp and the microbiological safety of the table olives processed. These conditions were then applied at the industrial level using a tunnel pasteurizer.

2.2.1. Pasteurization at laboratory scale

In the first part of our work, the pasteurization was carried out in a thermostatic bath using an automatic vertical autoclave model Alfa-Plus-10 (VWR International PBI, Italy). Four different treatments were applied, one for each of the pasteurization temperatures adopted: 65 °C, 75 °C, 85 °C and 95 °C. A control test was also carried out (test Tc, not pasteurized). Each test was conducted at 4, 8 and 12 min (Table 2).

2.2.2. Pasteurization at industrial scale

Pasteurization at industrial scale was carried out with a one level tunnel pasteurizer (model Atlantic, TMCI Padovan, Italy) at a temperature of 75 °C for 8 min in the thermal center of the product (jars).

Table 1. Olive fruit characteristics

Character	Value[a]
Fruit longitudinal diameter [mm]	18.96 ± 0.59
Pit longitudinal diameter [mm]	10.90 ± 0.49
Mesocarp thickness [mm]	8.06 ± 0.94
Fruit mass [g]	5.47 ± 0.61
Pit mass [g]	0.86 ± 0.17
Mesocarp mass [g]	4.61 ± 0.54

[a]Numeric values are means ± standard error of thirty replicates.

Table 2. Tests of the pasteurization at laboratory scale

	Pasteurization time		
Temperature	**4 min**	**8 min**	**12 min**
T_C (control)	–	–	–
65 °C	$T_{65°-4}$	$T_{65°-8}$	$T_{65°-12}$
75 °C	$T_{75°-4}$	$T_{75°-8}$	$T_{75°-12}$
85 °C	$T_{85°-4}$	$T_{85°-8}$	$T_{85°-12}$
95 °C	$T_{95°-4}$	$T_{95°-8}$	$T_{95°-12}$

The machine is a "rain type pasteurizer". During their advancement through the appropriate mechanical conveyor inside the tunnel, the glass jars are gradually brought to the maximum temperature of pasteurization with nebulized hot water at increasing thermal levels in succession. After this treatment, the jars are gradually cooled through a series of water sprays at decreasing thermal levels, and then they come out of the tunnel to go to the labeling machine. Typically there is a heating phase, with gradually increasing temperatures, as well as a longer step where the pasteurization at the desired conditions occurs; then the cooling steps, with gradually decreasing temperatures. The system allows for the application of uniform temperatures with a gradual transition of temperature without sudden changes that may cause thermal shock and consequent breakage of the jars. The total duration of the cycle of pasteurization adopted is 55 min including the time for heating and cooling.

The machine is made up of stainless steel modular elements, with a working capacity of about 1000 jars/h and 14 m^2 effective surface. The single module is made up of a supporting frame on which the tunnel, representing the fixed structure, and the moving unit for the jar transport are set. The tunnel is insulated to reduce heat loss and is equipped with large doors that are also insulated and facilitate inspection and cleaning.

The module structure is designed to collect the dripping water into a stainless steel tank placed under it, for recycling. The centrifugal pump is placed in the lower part of the machine, outside the tanks; it provides water for the nozzles located at the top of the tunnel and ensures a uniform heating and cooling of the jars.

The advancement of the jars inside the tunnel occurs by means of a fixed and mobile system of grids on the entire base of the tunnel, where the jars are placed. The fixed grids are made up of groups of stainless steel parallel plates, arranged in the advancement direction of the jars, fixed to the structure of the machine and uniformly spaced. The mobile grids are placed in parallel with the fixed grids and interspersed among them; they are made up of a series of plates fixed to a movable structure which gives them progressive vertical-horizontal movements determining their uplift, advancement and moving down via eccentrics connected to a hydraulic cylinder.

The jars are introduced into the tunnel through a conveyor belt, then they are transferred onto the fixed grids; the upward movement of the plates raises the jars that advance from a given portion of the tunnel through the subsequent horizontal movement. Thanks to the movable plates that return to their original position, the jars are rested a little further on the fixed grids; then a new cycle of lifting, advancement and lowering starts with the next row of jars.

Water recycling is adopted to limit heat consumption, since the preheating and cooling zones at the same temperature are connected. In this way, the heat recovered is transferred from the jars outgoing to the water that will be used for preheating the incoming jars. Thus, the water from the first preheating stage is derived from the tank of the last cooling stage, and so on. Steam pipes connected with a steam generator are used to heat the water and to make up for the inevitable heat loss.

The pasteurizing machine is equipped with a central electrical control panel to regulate and control the cycle.

Temperature values during pasteurization were acquired through the mini-temperature logger EBI 11 T230 (Tectronik, Italy) located at the geometric center of the glass pot (Table 3). The detection time of 55 min, the maximum temperature (75 °C) and the range of data acquisition to 15 sec were set during the tests. The data sets were then transferred to a personal computer (PC) using the software Winlog.pro.

After performing pasteurization at industrial scale, the pulp texture, microbiological characteristics, color, pH and total titratable acidity of the table olives were evaluated. Samples were stored at room temperature and tested 6, 12 and 15 months after treatment; the tests were respectively named TP6, TP12 and TP15. The control was represented by olives just after pasteurization (TPC).

2.3. Mechanical tests for texture evaluation of olive fruits

The mechanical tests to evaluate the olive fruits' texture were performed by compressing the olives using a mechanical dynamometer (Imada DPS 5R, USA) connected to an electronic stand (Imada MX2-500N–L, USA) and a PC for data download. Half of the pulp was removed through a blade dissecting the fruit according to the longest side and the drupe was placed on a steel plate. The fruit compression was obtained by means of a cylindrical

TABLE 3. Temperature data logger technical features

Characteristic	Value
Temperature	0°–150 °C
Accuracy	± 0.1 °C
Resolution	0.01 °C
Data acquisition speed	1s–24h
Memory	15.000 values
Operating temperature	0°–150 °C
Sensor	Pt 1000
Dimensions (without probe)	Ø 16.5 × 22 mm
Probe dimensions	Ø 3 × 20 mm

steel plate of 16 mm in diameter, whose surface was disposed orthogonally to the minor axis of the olive. The test speed was set at 0.125 mm s^{-1} keeping it constant during the tests. The compression force was recorded continuously along the entire olive pulp. In our analysis we considered the values corresponding to 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mm depth going from the skin to the pit.

Compression tests were performed two months after laboratory pasteurization (time called t_1) and after 6, 12 and 15 months after pasteurization at industrial scale. The tests were performed using twenty olives for each treatment.

2.4. Microbiological analysis

Microbiological analyses were performed on 10 g of olives, diluted in a 90 ml physiological solution (0.9% NaCl) and homogenized for 2 min using a Stomacher (BagMixer 400, Interscience, Saint Nom, France). After decimal serial dilution, the microbial suspensions were plated and incubated as follows: total mesophilic count (TMC) on Plate Count Agar (PCA) incubated aerobically at 30 °C for 72 h; *Enterobacteriaceae* on Violet Red Bile Glucose Agar (VRBGA) incubated anaerobically at 37 °C for 24 h; yeasts and moulds on Dichloran Rose-Bengal Chloramphenicol agar base (DRBC) with the addition of Chloramphenicol Selective Supplement incubated aerobically at 30 °C for 48 h. All media and supplements were purchased from Oxoid. Analyses were carried out in triplicate.

2.5. Color determination

Color was measured on four points of the olive surface of four drupes of each sample by means of a colorimeter (Chroma Meter CR-400C, Minolta, Osaka, Japan). The Hunter's scale parameters were determined: Chroma, Hue°, L*, a* and b*.

2.6. pH and total titratable acidity (TTA)

Values of pH were determined electrometrically using the pH meter BASIC 20 + (Crison Instrument S.A., Barcelona, Spain) and the total titratable acidity (TTA) was determined by titration with 0.1 N NaOH and expressed in terms of mmol/kg of flesh. Five grams of olives were diluted with 45 ml of distilled H_2O and homogenized with a Sorvall Omni-Mixer (Dupont Instruments, Newtown, CT) at the maximum speed for 4 min. Analyses were carried out in triplicate.

2.7. Statistical analysis

Compression test data were analyzed by a general linear model (GLM) in order to build a statistical model describing the impact of more factors on one dependent variable. The effects of olive sample, depth into the pulp and test and their interactions on the compression force values were analyzed; it was assumed that the errors follow a normal distribution. Compression force values obtained at different depths by compression tests on the olives were included in the model as the dependent variable, always considering olive sample, pulp depth and test as independent variables. The variable "olive sample" represents each of the twenty randomized samples (numbered 1 to 20) identified inside a glass jar. The variable "depth" is the depth inside the olive pulp at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mm from the skin to the pit. The variable "test" represents the test performed, corresponding to the different treatments applied at laboratory level (pasteurization temperatures of 65 °C, 75 °C, 85 °C and 95 °C and time of the treatment for 4, 8 and 12 min plus the control trial corresponding to non-pasteurized olives) or the different time of analysis after pasteurization (control, TP6, TP12 and TP15). Therefore, three input factors (olive sample, depth, and test) were included in the model and a response factor corresponding to compression force. The Durbin-Watson test was applied to determine significant correlations from the regression analysis. These statistical analyses were carried out by means of the statistical software package Statgraphics centurion, version XV, Statpoint Inc. (Warrenton, Va., 2005).

The microbiological, pH, TTA and color data were subjected to one-way analysis of variance to evaluate the effects of time and temperature of pasteurization. The Student "t" test was used for mean comparison. The post-hoc Tukey method was applied for pairwise comparison. The program used for these statistical analyses was Statistica 10 (StartSoft Inc. 1984–2011).

3. RESULTS AND DISCUSSION

3.1. Pasteurization at laboratory scale

3.1.1. Compression tests

Compression curves for the pasteurization lasting 4 min are reported in Fig. 1; tests $T_{85°-4}$ and $T_{75°-4}$ showed the highest compression force values (79.37 and 72.63 N, respectively), while tests $T_{65°-4}$ and $T_{95°-4}$ displayed values between 55 and 50 N. The results of the statistical analysis are reported in Table 4. The R-squared statistic indicated that the model fit the data well (90.6%); the standard deviation of the residuals was 7.4667, while the mean absolute error of 4.94996 is the average value of the residuals. The Durbin-Watson statistic tests did not indicate serial autocorrelation in the residuals ($p>0.05$).

With reference to the tests where pasteurization lasted 8 min (Fig. 2), the highest compression force value was 80 N for test $T_{75°-8}$; then we have tests $T_{95°-8}$

TABLE 4. Results of the analysis of variance (GLM) performed for compression force for pasteurization at laboratory scale lasting 4 min

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Olive sample	1663.22	19	184.802	3.31	0.0008
Depth	122223	5	24444.5	438.45	0.0000
Test	10421.7	4	2605.43	46.73	0.0000
Depth*Test	5876.43	20	293.821	5.27	0.0000
Residual	14551.2	261	55.7515		
Statistical Parameter	Value				
R-squared	90.5961%				
Standard error of est	7.4667				
Mean absolute error	4.94996				
Durbin-Watson statistic	2.20557 (P = 0.9625)				

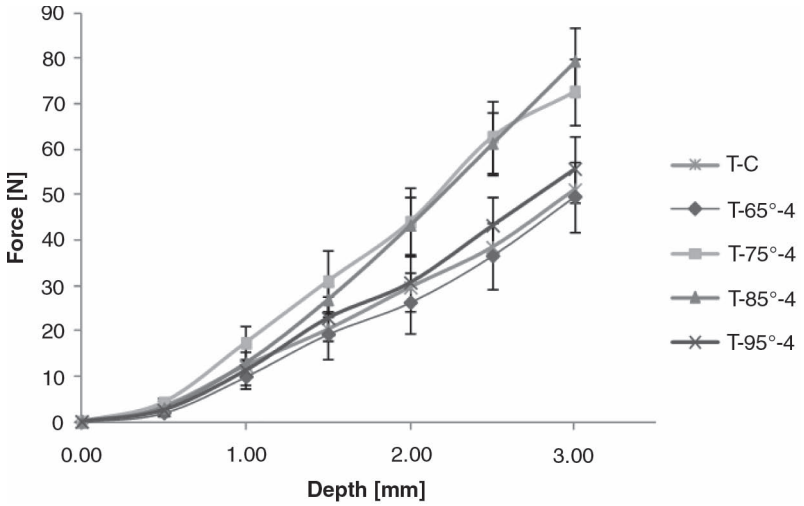

FIGURE 1. Compression curves for pasteurization lasting 4 minutes and different temperatures. Data are reported as means of twenty replicates. Bars represent standard deviation of the mean.

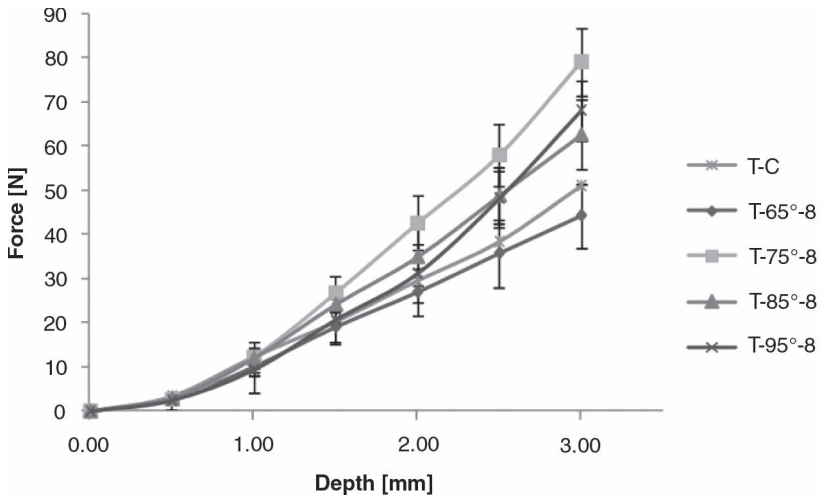

FIGURE 2. Compression curves for pasteurization lasting 8 minutes and different temperatures. Data are reported as means of twenty replicates. Bars represent standard deviation of the mean.

and $T_{85°-8}$ with values between 60 and 70 N and tests $T_{65°-8}$ and Tc (not pasteurized) with values lower than 50 N. The R-Squared statistic indicates that the model as fit explained 87.9% of the variability in compression force values (Table 5); the standard error of the estimate showed the standard deviation of the residuals to be 8.25141 and the mean absolute error of 5.33732 was the average value of the residuals. With reference to the Durbin-Watson statistic, since the p-value was greater than 0.05, there was no indication of serial autocorrelation in the residuals.

The results presented in Fig. 3 show the compression curves for the pasteurization lasting 12 min; tests $T_{75°-12}$ and $T_{85°-12}$ showed the highest compression force values (75.53 and 71.11 N, respectively), while values between 60 and 50 N were registered for tests $T_{95°-12}$ and Tc. As shown in Table 6, the R-Squared statistic points out that the model as fit explained 89.6% of the variability in compression force values, the standard error of the estimate showed the standard deviation of the residuals to be 7.82298 and the mean absolute error of 4.80556 was the average value of the residuals. The Durbin-Watson statistic indicated no serial autocorrelation in the residuals.

The values of the compression force agreed with those obtained by Kiliçkan and Güner (2008), who reported a mean value of 72.00 N on olives with physical characteristics similar to those used in our study.

Overall, the values of the compression force observed at 0–1.5 mm pulp layer were similar in all the tests, whereas the variability in the layer from 1.5 to 3.0 mm was higher (Table 7).

The heat treatment for 8 min allowed for obtaining drupes with compression force values greater than those registered at 4 and 12 min; in particular, statistically significant differences were found in the layer of the pulp from 1.5 to 3 mm between the test performed at 75 °C and the others.

TABLE 5. Results of the analysis of variance (GLM) performed for compression force for pasteurization at laboratory scale lasting 8 min

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Olive sample	1415.4	19	157.267	2.31	0.0163
Depth	115564	5	23112.8	339.47	0.0000
Test	6458.18	4	1614.54	23.71	0.0000
Depth*Test	5865.64	20	293.282	4.31	0.0000
Residual	17770.4	261	68.0857		
Statistical Parameter	Value				
R-squared	87.9173%				
Standard error of est	8.25141				
Mean absolute error	5.33732				
Durbin-Watson statistic	1.91113 (P = 0.2212)				

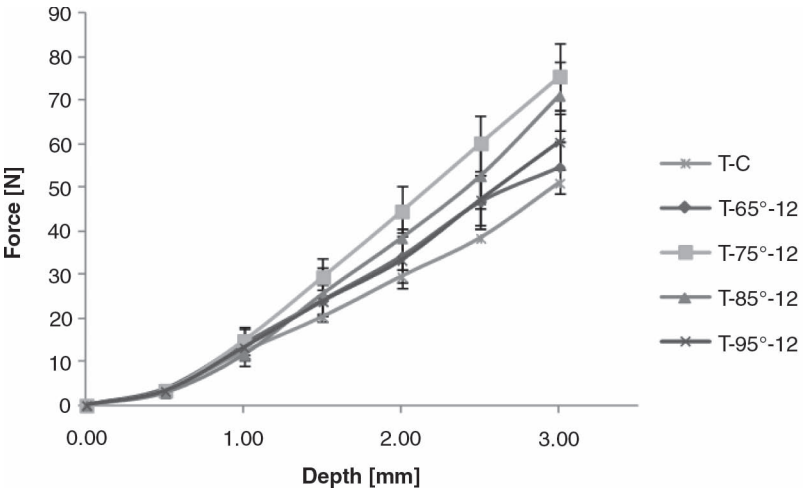

FIGURE 3. Compression curves for pasteurization lasting 12 minutes and different temperatures. Data are reported as means of twenty replicates. Bars represent standard deviation of the mean.

Table 6. Results of the analysis of variance (GLM) performed for compression force for pasteurization at laboratory scale lasting 12 min

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Olive sample	1094.91	19	121.657	1.99	0.0410
Depth	127923	5	25584.7	418.06	0.0000
Test	4914.24	4	1228.56	20.07	0.0000
Depth*Test	3690.62	20	184.531	3.02	0.0000
Residual	15972.9	261	61.1989		
Statistical Parameter	Value				
R-squared	89.6007%				
Standard error of est	7.82298				
Mean absolute error	4.80556				
Durbin-Watson statistic	2.43837 (P = 0.9999)				

Table 7. Compression force at different depths of the mesocarp for pasteurization lasting 4, 8 and 12 min at 65 °C, 75 °C, 85 °C and 95 °C

Pasteurization time [min]	Depth [mm]	Force [N]			
		65 °C	75 °C	85 °C	95 °C
4	0.50	1.95 ± 0.68^{a}	4.18 ± 1.17^{c}	2.94 ± 0.61^{b}	2.43 ± 0.58^{ab}
	1.00	9.92 ± 2.43^{a}	17.31 ± 3.77^{b}	12.82 ± 2.66^{a}	10.13 ± 2.87^{a}
	1.50	19.28 ± 5.30^{a}	30.89 ± 6.90^{c}	26.84 ± 3.40^{bc}	20.28 ± 4.92^{a}b
	2.00	26.26 ± 6.63^{a}	43.97 ± 7.52^{b}	43.26 ± 6.14^{b}	26.98 ± 6.14^{a}
	2.50	36.52 ± 7.33^{a}	62.67 ± 7.85^{b}	61.27 ± 6.87^{b}	38.20 ± 6.26^{a}
	3.00	49.52 ± 7.81^{a}	72.63 ± 7.38^{bc}	79.37 ± 7.60^{c}	49.02 ± 7.31^{ab}
8	0.50	2.66 ± 0.94^{a}	2.61 ± 0.70^{a}	2.91 ± 0.68^{a}	2.52 ± 1.90^{a}
	1.00	10.04 ± 2.23^{a}	12.21 ± 3.41^{a}	11.95 ± 2.17^{a}	11.21 ± 5.01^{a}
	1.50	19.07 ± 3.75^{a}	26.71 ± 4.03^{b}	24.16 ± 3.85^{a}	22.78 ± 4.98^{a}
	2.00	26.98 ± 5.42^{a}	42.50 ± 6.18^{b}	34.93 ± 6.32^{a}	30.59 ± 6.70^{a}
	2.50	35.81 ± 7.68^{a}	57.95 ± 6.95^{b}	48.82 ± 6.31^{a}	43.18 ± 6.39^{a}
	3.00	44.32 ± 7.24^{a}	79.08 ± 7.64^{b}	62.64 ± 7.88^{a}	55.64 ± 6.83^{a}
12	0.50	3.76 ± 1.10^{a}	3.37 ± 1.02^{a}	2.78 ± 0.57^{a}	2.43 ± 1.01^{a}
	1.00	14.13 ± 3.49^{a}	14.81 ± 3.38^{a}	11.55 ± 2.38^{a}	9.36 ± 2.71^{a}
	1.50	24.16 ± 3.81^{a}	29.45 ± 4.38^{a}	25.54 ± 6.16^{a}	20.67 ± 2.81^{a}
	2.00	34.25 ± 6.20^{a}	44.52 ± 5.65^{b}	38.37 ± 6.98^{ab}	31.17 ± 6.30^{a}
	2.50	46.80 ± 6.29^{a}	60.19 ± 6.41^{b}	52.63 ± 7. 21^{ab}	48.20 ± 5.61^{a}
	3.00	54.70 ± 6.03^{a}	75.53 ± 7.55^{b}	71.11 ± 7.95^{ab}	68.14 ± 6.44^{ab}

Data are reported as means of twenty replicates ± standard deviation. Values in each row having different letters (a–c) are significantly different from one another at p<0.05 (Tukey's test).

3.1.2. Microbiological analysis

Enterobacteriaceae were not detected at t_0, while yeasts and molds showed values below the detection threshold (<1 Log CFU g^{-1}). TMC reached its maximum value in the control trial TC (6.0 Log CFU g^{-1}) and decreased according to the increase in temperature and time of treatment (1.70 Log CFU g^{-1} at $T_{85°-4}$). The exposure of samples at temperature/time over $T_{85°-8}$ showed microbial loads below the detection threshold (Table 8).

Enterobacteriaceae were not present after 2 months of storage (t_1). Yeasts and molds reached their maximum concentrations in TC (6.48 Log CFU g^{-1}) and the values decreased following the increase in temperature and time (2.70 Log CFU g^{-1} at $T_{75°-4}$). Values below the detection level were recorded with treatments over $T_{75°-8}$. TMC at t_1 reached the

TABLE 8. Microbial (Log CFU g^{-1}) and chemical characteristics of Nocellara del Belice olives subjected to different laboratory scale pasteurization treatments at t_0

Test	Total mesophilic count (TMC)	Yeasts and moulds	*Enterobacteriaceae*	pH	TTA
T_C	6.00 ± 0.13^a	$<1^a$	0^a	4.51 ± 0.01^a	8.00 ± 1.00^a
$T_{65°-4}$	5.84 ± 0.25^a	$<1^a$	0^a	4.47 ± 0.03^a	6.00 ± 1.00^a
$T_{65°-8}$	5.74 ± 0.34^a	$<1^a$	0^a	4.52 ± 0.01^a	6.00 ± 1.00^a
$T_{65°-12}$	5.18 ± 0.11^b	$<1^a$	0^a	4.52 ± 0.01^a	6.00 ± 1.00^a
$T_{75°-4}$	4.70 ± 0.27^b	$<1^a$	0^a	4.51 ± 0.04^a	7.00 ± 0.60^a
$T_{75°-8}$	3.83 ± 0.43^b	$<1^a$	0^a	4.50 ± 0.02^a	7.00 ± 0.60^a
$T_{75°-12}$	3.48 ± 0.36^b	$<1^a$	0^a	4.47 ± 0.01^a	6.00 ± 0.60^a
$T_{85°-4}$	1.70 ± 0.28^b	$<1^a$	0^a	4.46 ± 0.01^a	6.00 ± 0.60^a
$T_{85°-8}$	$<1^b$	$<1^a$	0^a	4.52 ± 0.02^a	6.00 ± 0.60^a
$T_{85°-12}$	$<1^b$	$<1^a$	0^a	4.61 ± 0.01^b	6.00 ± 0.60^a
$T_{95°-4}$	$<1^b$	$<1^a$	0^a	4.54 ± 0.00^a	6.00 ± 0.60^a
$T_{95°-8}$	$<1^b$	$<1^a$	0^a	4.49 ± 0.01^a	7.00 ± 0.60^a
$T_{95°-12}$	$<1^b$	$<1^a$	0^a	4.59 ± 0.03^a	6.00 ± 1.00^a
Statistical significance	*	NS	NS	*	*

Lowercase (a, b) letters indicates different statistical significances according to Tukey's test at P values of 0.05. [a]P value: *P≤0.05; NS not significant.

TABLE 9. Microbial (Log CFU g^{-1}) and chemical characteristics of Nocellara del Belice olives subjected to different laboratory scale pasteurization treatments at t_1

Test	Total mesophilic count (TMC)	Yeasts and moulds	*Enterobacteriaceae*	pH	TTA
T_C	7.49 ± 0.13^a	6.48 ± 0.24^a	0^a	4.80 ± 0.00^a	20.00 ± 0.60^a
$T_{65°-4}$	7.04 ± 0.25^a	5.34 ± 0.11^b	0^a	4.71 ± 0.01^a	22.00 ± 0.60^a
$T_{65°-8}$	6.65 ± 0.34^a	4.87 ± 0.15^b	0^a	4.69 ± 0.01^a	22.00 ± 0.60^a
$T_{65°-12}$	6.11 ± 0.11^b	3.67 ± 0.36^b	0^a	4.72 ± 0.01^a	24.00 ± 0.40a
$T_{75°-4}$	5.93 ± 0.27^b	2.70 ± 0.29^b	0^a	4.70 ± 0.01^b	28.00 ± 1.00^b
$T_{75°-8}$	5.81 ± 0.43^b	$<1^b$	0^a	4.63 ± 0.00^a	26.00 ± 0.60^b
$T_{75°-12}$	5.11 ± 0.36^b	$<1^b$	0^a	4.72 ± 0.02^a	36.00 ± 0.00^b
$T_{85°-4}$	4.81 ± 0.28^b	$<1^b$	0^a	4.74 ± 0.01^a	32.00 ± 0.60^b
$T_{85°-8}$	4.25 ± 0.17^b	$<1^b$	0^a	4.74 ± 0.00^b	32.00 ± 0.00^b
$T_{85°-12}$	3.90 ± 0.10^b	$<1^b$	0^a	4.68 ± 0.02^a	31.00 ± 1.00^b
$T_{95°-4}$	3.00 ± 0.20^b	$<1^b$	0^a	4.74 ± 0.00^a	28.00 ± 0.60^b
$T_{95°-8}$	1.71 ± 0.23^b	$<1^b$	0^a	4.57 ± 0.00^b	28.00 ± 1.00^b
$T_{95°-12}$	$<1^b$	$<1^b$	0^a	4.61 ± 0.00^b	26.00 ± 0.00^b
Statistical significance	*	*	NS	*	*

Lowercase (a, b) letters indicates different statistical significances according to Tukey's test at P values of 0.05. [a]P value: *P≤0.05; NS not significant.

highest level in the control trial (7.49 Log CFU g^{-1}); the increase in temperature and time of treatment determined a reduction in the levels until 1.71 Log CFU g^{-1} at $T_{95°-8}$. Values below the detection threshold were observed after exposure at $T_{95°-12}$ (Table 9). Mesophilic bacteria, yeasts and molds decreased until values below the detection threshold by increasing the temperature. The results obtained showed a direct correlation between temperature and microbiological load. No previous studies evaluated the effects of different temperature/time combinations on the microbiological characteristics of table olives. However, Sánchez-Gómez *et al*. (2013), who treated olive paté at 80 °C for 20 min,

observed a concentration of 3.8 Log CFU g^{-1} mesophilic bacteria after one day from treatment, while *Enterobacteriaceae*, yeasts and molds were below the detection level; but after 30 days of refrigerated storage, mesophilic bacteria decreased and yeasts and molds increased, confirming that a monitoring of the microbial populations over time is necessary.

3.1.3. pH and TTA

The thermal treatment did not determine significant variations in pH or TTA at t_0 (Table 8). However, after 2 months' storage (Table 9), relevant changes were registered for both parameters. In general, pH values were inversely correlated with TTA data, because pH decreased with increasing temperatures and treatment time exposure, and, on the contrary, TTA increased. The most significant variations in TTA were obtained after applying the treatment at 75 °C.

3.2. Pasteurization at industrial scale

3.2.1. Tunnel pasteurizer control

Figure 4 shows the experimental time-temperature data of the table olives packed in jars and submitted to pasteurization treatment at 75 °C for a total time of 55 minutes. During the first five min the temperature gradually reached 72 °C, followed by the effective pasteurization phase lasting 25 min. The temperature remained stable at 75 °C for 8 min at the thermal center of the product; then the temperature was decreased to 58 °C until the cooling phase started. Cooling was divided into two sub-phases: the first step with a 7.8 °C temperature decrease and the second step with temperature reaching about 18 °C at the end of the cycle.

3.2.2. Compression tests

The compression force values monitored during storage (Fig. 5) showed an increase from the skin to the central part of the olive pulp; till 2.0 mm the compression force values did not differ among the tests, while the curves began to differentiate deeper into the pulp. In particular, statistically significant differences were found among the four tests from 3.0 mm of depth.

The curves showed a decrease in flesh firmness during storage, and these results are in accordance with those reported by Sanchez-Gomez et al. (2013). The maximum value was 98.7 N for the control at 4.00 mm; at the same depth, the value 84.2 N was registered for test TP6 with a 14% reduction, 72.5 N for test TP12 with a 26% reduction, and 64.2 N for test TP15 with a 35% reduction compared to the control test. The results of the statistical analysis are reported in table 10. The highest p-value was 0.2775, belonging to depth-test interaction. The R-squared statistic indicated that the model fit the data well; the standard deviation of the residuals was 5.62376,while the average value was 3.65868. The Durbin-Watson statistic showed no serial autocorrelation in the residuals.

3.2.3. Microbiological aspects

The application of the treatment 75 °C for 8 min, carried out in the tunnel pasteurizer, determined an effective reduction in all the microbial groups monitored in this study (results not shown). Although TMC before treatment was around 10^6 CFU g^{-1}, no detectable levels of any bacterial or fungal population were found after pasteurization. These results were observed also during storage at 6, 12 and

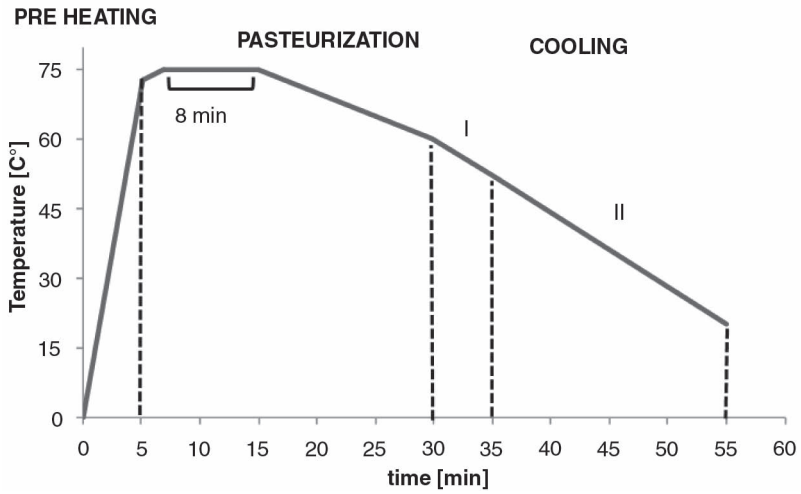

FIGURE 4. Time–temperature data acquired during the industrial pasteurization (interval of time 15 s) with the data logger. Temperature course in the center of the table olive jars.

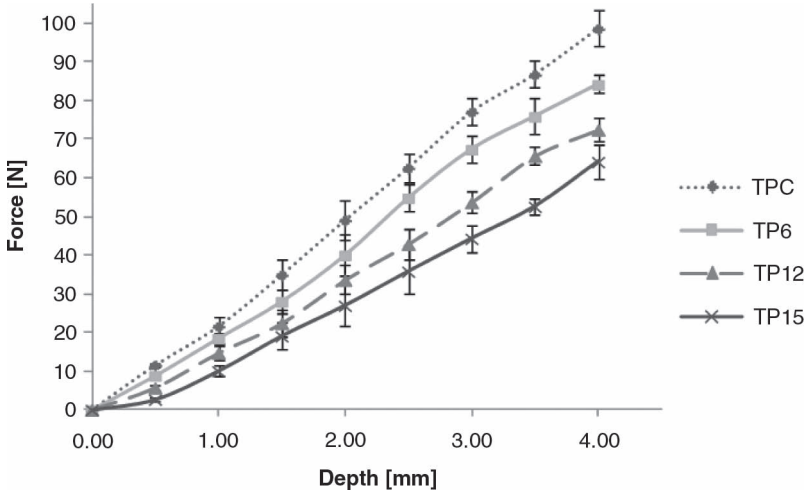

FIGURE 5. Compression force values measured in the monitoring period for the four tests.

15 months, confirming the efficacy of the treatment applied for the hygiene of the table olives processed in this study; in fact, detectable levels of yeasts have been reported for Nocellara del Belice table olives obtained with the "Castelvetrano" method (Romeo *et al*., 2012).

3.2.4. pH and TTA

Table 11 reports the data registered for pH and TTA during the industrial experimentation. The pH decreased significantly during storage, reaching the lowest value (4.21) after 15 months (TP15). Based on the observations of the laboratory scale pasteurization, as expected, TTA increased with storage time. The pH decrease can be due to a re-equilibration of acid compounds between olives and brines (Pradas *et al*., 2012), even though Romeo *et al*. (2009) stated that the pH of table olives pasteurized at 75 °C changed depending on the type of cover brine.

3.2.5. Color

Color is a quality parameter well considered by consumers. Thus, unlike the pasteurization carried out at laboratory scale the one performed with the industrial pasteurizer was also evaluated for its effect on the olive color. Regardless of storage time, the parameters b* and Chroma were almost comparable for all samples, while the L* and a* values displayed soon after treatment were different from those registered during storage, even though the values registered at TP6, TP12 and TP15 were not statistically different from one another. Regarding the Hue° parameter, only the values at T_{P6} and T_{P12} months

TABLE 10. Results of the analysis of variance (GLM) performed for compression force of olive treated with the tunnel pasteurizer

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Olive sample	2970.56	19	156.345	4.94	0.0000
Depth	84002.2	7	16800.4	531.21	0.0000
Test	1600.4	3	533.467	16.87	0.0000
Depth*Test	563.559	21	37.5706	1.19	0.2775
Residual	13820.9	437	31.6267		
Total (corrected)	102958	479			
Statistical Parameter	Value				
R-squared	86.5762 %				
Standard error of est	5.62376				
Mean absolute error	3.65868				
Durbin-Watson statistic	2.47537 (P = 1.0000)				

Table 11. Chemical characteristics of Nocellara del Belice olives treated with the tunnel pasteurizer

Test	pH	TTA
TPC	4.69 ± 0.01^{a}	20.00 ± 1.00^{a}
TP6	4.52 ± 0.02^{b}	24.00 ± 0.00^{b}
TP12	4.43 ± 0.03^{b}	32.00 ± 1.00^{b}
TP15	4.21 ± 0.01^{b}	42.00 ± 1.00^{b}
Statistical significance	*	*

Lowercase (a, b) letters indicates different statistical significances according to Tukey's test at P values of 0.05. [a]P value: *P≤0.05; NS not significant.

Table 12. Color parameters of Nocellara del Belice olives treated with the tunnel pasteurizer

Test	L*(C)	a*(C)	b*(C)	Chroma	Hue°
TPC	53.7^{a}	-6.2^{a}	39.8^{a}	40.3^{a}	98.8^{a}
TP6	56.6^{b}	-3.7^{b}	41.9^{a}	42.0^{a}	95.1^{c}
TP12	56.9^{b}	-3.5^{b}	39.6^{a}	39.8^{a}	95.0^{c}
TP15	56.5^{b}	-4.1^{b}	39.6^{a}	38.9^{a}	100.5^{b}

Lowercase (a.b.c) letters indicates different statistical significances according to Tukey's test at P values of 0.05.

were almost superimposable (Table 12). Escudero-Gilete *et al*., (2009), who studied the effects of different temperatures of pasteurization (62.5, 75, 85, and 95 °C for 15 min) on the color of olive pastes from the cultivar Manzanilla, found no differences among the several treatments applied. Since Romeo *et al*. (2012) reported that Nocellara del Belice olives processed with the "Castelvetrano" method were less subjected to a browning action than olives naturally fermented, based on our results, it can be stated that pasteurization does not greatly affect the color of Nocellara del Belice table olives.

4. CONCLUSIONS

The pasteurization performed at laboratory scale allowed for the selection of the optimal time-temperature combination to warrant the microbial safety of table olives without compromising consistency. In particular, from the microbiological point of view, it is appropriate to employ pasteurization temperatures of at least 75 °C for 8 min; regarding the olive fruits' mechanical properties, the results obtained at 4 min were almost superimposable with those shown at 8 min.

The subsequent application of these pasteurization conditions by means of the tunnel pasteurizer for the table olives processed following the "Castelvetrano method", determined a high stability and an acceptable flesh firmness for the entire period under observation, which lasted 15 months. As expected, olive pulp texture decreased during storage. However, this behavior was more evident in the deeper layers of the pulp while the external part of the mesocarp better preserves its mechanical properties. The reduction in flesh firmness during storage, in fact, is more evident from 3 mm depth where there are statistically significant differences among all the tests. As shown by the results, an advisable storage period for this product should not exceed 6 months; although the hygiene is preserved, after this period, the firmness might compromise its acceptability by consumers.

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