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Texture and antioxidant evolution of naturally green table olives as affected by different sodium chloride brine concentrations

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SUMMARY: The results on the evolution of the texture and antioxidant activity during the processing of naturally fermented green olives are reported in the present study. The olives were brined with two different NaCl concentrations, 4 and 7%. A puncture test and a texture profile analysis (TPA) test were carried out to evaluate texture, while the antioxidant activity was assessed in consideration of the loss of absorbance of a stable radical upon reaction with a polyphenolic olive extract. The puncture test revealed both an increase and decrease in peel and flesh hardness, respectively. The olives processed with 7% NaCl concentration showed higher peel hardness than olives brined with 4% salt. The texture profile analysis did not reveal any difference between the two salt concentrations used, but showed a decrease in the value of all parameters after 30 days of brining. The antioxidant activity of the polyphenolic extract decreased significantly during processing, with a major loss after the first 30 days of processing and was higher in the 7% brined olives, than the sample treated with 4% NaCl.

KEYWORDS: Antioxidant activity; Natural Fermentation; Sensory analysis; Texture; Texture profile analysis; Tonda di Cagliari

RESUMEN: *Evolución de la textura y capacidad antioxidante de aceitunas de mesa procesadas al natural con diferentes concentraciones de cloruro de sodio.* En este trabajo se presentan los resultados sobre la evolución de la textura y la actividad antioxidante durante el proceso de fermentación natural de las aceitunas verdes. Los frutos se colocaron en salmueras con dos concentraciones de NaCl del 4% y 7%. Se evaluó la textura mediante ensayo de perforación y mediante análisis de perfil de textura (TPA), mientras que la actividad antioxidante se evaluó considerando la pérdida de absorbancia de un radical estable tras la reacción con un extracto polifenólico de las aceitunas. El ensayo de perforación reveló un aumento de la dureza de la piel y una reducción en la dureza de la pulpa. Las aceitunas procesadas con una concentración de NaCl del 7% mostraron una dureza mayor que aquellas tratadas con sal al 4%. El análisis del perfil de la textura no reveló diferencias entre las dos concentraciones usadas de sal, pero mostró una disminución en el valor de todos los parámetros después del primer mes de salmuera. La actividad antioxidante del extracto polifenólico disminuyó significativamente durante el tratamiento, con una pérdida importante después de los primeros 30 días de tratamiento y fue más alto en las aceitunas del 7% en salmuera respecto a las muestras tratadas con el 4% de NaCl.

PALABRAS CLAVE: Actividad antioxidante; Análisis de perfil de textura; Análisis sensorial; Fermentación natural; Textura; Tonda di Cagliari

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1. INTRODUCTION

According to International Olive Oil Council (2012), table olives represent about 10% of the total olive processing industry. Table olive processing includes the removal of the bitter taste of olives and fermentation. The main commercial preparations are the green Spanish style (green olives alkali treated and fermented), the Greek style (black olives naturally fermented) and the Californian style (turning color or black olives alkali treated and oxidized). In addition to these, there are many other processing systems in the world and each country has its specialties. Italy, for example, produces about 20% of naturally fermented green olives, which are prepared by directly placing fruits in brine, as reported by the International Olive Oil Council (2004). This type of olives is partially debittered by diffusion of the bitter polyphenolic compounds from the flesh to the brine over a time ranging from 5 to 8 months. Fermentation is mainly carried out by yeasts (Brenes-Balbuena *et al.*, 1986; Farris *et al.*, 1989; Marquina *et al.*, 1992; Borcackli *et al.*, 1993), although lactobacilli growth has been reported (Durán-Quintana and González-Cancho, 1973; González-Cancho *et al.*, 1975; Abriouel *et al.*, 2012). The presence of lactobacilli is, however, very sporadic and depends on the release of toxic secoiridoid derivatives from the flesh into the brine (Medina *et al.*, 2010). The chemical-physical evolution of brines and olives during processing has been deeply investigated in our lab and by other authors (Piga and Agabio, 2003; Poiana and Romeo, 2006; Romeo *et al.*, 2012), but no study on intact green olives has been undertaken so far concerning the instrumental measurements of texture, which is one of the attributes that most affects the consumer acceptance of table olives and may be strongly influenced during processing. The only paper focuses on cracked green olives (Bautista-Gallego *et al.*, 2011). Texture loss is mainly caused by the enzymatic activity of microorganisms that may result in softening, primarily due to the degradation of pectic substances of the cell wall and middle lamella (Araujo *et al.*, 1994; Jimenez *et al.*, 1997; Fernández-Bolaños *et al.*, 2001). Factors influencing the activity of hydrolytic enzymes are the pH and NaCl concentrations of brines (Fernández-Bolaños *et al.*, 2001).

An instrumental texture analysis has been applied to Spanish style olives (Jimenez *et al.*, 1997; Morales-Sillero *et al.*, 2008; Rejano-Navarro *et al.*, 2008), Greek style olives (Kanavouras *et al.*, 2005; Tassou *et al.*, 2007; Lanza *et al.*, 2010; Romeo *et al.*, 2012), Californian style (Georget *et al.*, 2003; Romeo *et al.*, 2012) and other styles (Marsilio *et al.*, 2000; Arroyo-López *et al.*, 2007; Romeo *et al.*, 2012). In a recent paper Romeo *et al.* (2012) evaluated the effect of post-processing treatments on the texture of fermented green olives, but no study

has been undertaken so far on naturally fermented green table olives. Recently, due to the association of sodium intake with hypertension, different authors have reported on the reduction or partial substitution of sodium chloride in brines (Bautista-Gallego *et al.*, 2010; Bautista-Gallego *et al.*, 2011; Panagou *et al.*, 2011; Moreno-Baquero *et al.*, 2012; Rodríguez-Gómez *et al.*, 2012). In this context we demonstrated that it is possible to successfully process green table olives in the natural style using a low sodium chloride brine concentration (2–4%), assuring that the brines are acidified at pH 4.0 (Piga and Agabio, 2003).

As stated before, debittering is essential to making table olives edible. Whatever the method used, a significant loss in polyphenols occurs during the various processing steps and if we consider that polyphenols not only affect the sensory properties, but also the health promoting capacity, we can understand that differences in health properties can be found among the processing technologies used. Therefore, the health promoting capacity can be indirectly evaluated by measuring the *in vitro* antioxidant activity, as reported for many foods, including table olives (Pereira *et al.*, 2006; Sousa *et al.*, 2008; Malheiro *et al.*, 2011).

For the above cited reason we investigated the evolution of the texture and antioxidant activity of the polyphenolic extract during the processing of naturally fermented green olives brined at two different sodium chloride brine concentrations. The chemical-physical parameters of brine and olives were also determined.

2. MATERIALS AND METHODS

2.1. Plant material

Fruits of the “Tonda di Cagliari” cultivar were handpicked from an irrigated orchard in north Sardinia in the last decade of October at a green ripe stage and soon transferred at our lab for processing. The cultivar has been chosen because it proved to be very suitable for this kind of preparation in previous trials carried out at our lab. Selection before brining was done by discarding olives with blemishes, cuts and insect punctures as well as under or over ripe fruits. Calibration was performed with laboratory callipers and olives ranging from 17 to 21 mm in transversal diameter were considered. Replicates of 12 kg were prepared for further processing.

2.2. Processing

After careful washing with tap water to remove field dust, the olives were transferred to 20 L previously sterilized plastic containers and then processed by brining, which was carried out by preparing sterile 4 and 7% (w/w) sodium chloride solutions which were

used to completely fill the containers which were finally closed after a perforated cap was placed between the olives and the top part of the container to submerge the olives in the brine. The pH of the brine was previously adjusted to 4 with lactic acid to prevent the growth of gas pockets producing *Enterobacteriaceae* (Garrido-Fernández *et al.*, 1997). Three containers per sodium chloride concentration were used. The containers were kept at 20°C. The sodium chloride concentration and pH were inspected at 5, 10, 15, 20, 30, 45 and 60 days and afterwards at monthly intervals up to 180 days in order to make corrections when necessary. The growth of aerobic oxidative yeasts and moulds on the brine surface was avoided by adjusting the brine level with freshly prepared sterile brine. We stopped determinations at 180 days as most of the chemical parameters had reached a steady state and the olives were ready for consumption as revealed by a sensory test.

2.3. Chemical-physical analysis

Triplicate analyses of the following parameters for each container were carried out in the brine: pH with a glass electrode, free acidity (g of lactic acid per 100 mL of brine), sodium chloride (g 100 mL⁻¹), reducing sugars (g 100 mL⁻¹ of brine) and phenols (mg gallic acid 100 g⁻¹ of brine), according to Garrido-Fernández *et al.* (1997). The free acidity value obtained was corrected by subtracting the initial and following amounts of lactic acid added. Determinations on homogenised flesh were pH, sodium chloride, reducing sugars and total phenols. Total phenols were extracted according to Amiot *et al.* (1986) and determined as described by Garrido-Fernández *et al.* (1997). Sodium chloride was determined as for brines after extraction from flesh: in brief, 10 g of flesh were diluted with 30 mL of water and homogenized with an Ultra Turrax for 60 seconds, the resulting slurry was brought to 50 mL and centrifuged for 10 min at 5000 rpm. An aliquot of 1 mL was taken to make the analysis following the method proposed by Garrido-Fernández *et al.* (1997). Reducing sugars and polyphenol analysis in brine and flesh and sodium chloride in flesh were made at 0, 30, 60, 120 and 180 days.

2.4. Antioxidant activity

The antioxidant activity was determined on the polyphenolic extract using the DPPH stable radical, following the method proposed by Ramadan and Morsel (2006). Briefly, 50 µL of methanolic extract were dissolved in 1950 µL of toluene and after vortexing for 1 minute an amount of 200 µL was made to react in a 1cm path cuvette at 22°C under continuous stirring with 1.8 mL of a 10⁻⁴ M toluenic solution of DPPH. The decrease in absorbance at 515 nm was taken at 1, 5, 15, 30 and 60 minutes

against a blank of pure toluene using a spectrophotometer (mod. 8453, Hewlett-Packard, Palo Alto, California). The antioxidant activity was considered as the difference in absorbance between each sample and a toluenic DPPH solution without the sample (control) and calculated as percentage of inhibition according to the following equation:

$$\% \text{ of inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample} \times 100}{\text{absorbance of control}}$$

2.5. Texture analysis

Texture analyses in fresh olives and changes during processing were monitored with a texture analyzer (mod. TA-XT2, Stable Microsystems, Surrey, UK) fitted with a 5 or 50 kg load cell. The Texture Expert program version 1.21 was used for processing data. A puncture test and a Texture Profile Analysis (TPA) were performed using twenty olives for each determination and brine concentration.

For the puncture test the olives were placed on an HDP/90 platform and punctured in the lateral face. The test was performed using an SMS P/2 needle probe and a 5 kg load cell. The test speed was 1.0 mm s⁻¹ and the penetration depth was limited to 3 mm. The force-time curve was graphically acquired and elaborated. Three parameters were taken into account to evaluate the evolution of texture during fermentation (Figure 1): the olive peel break force (yield point), as the maximum force (N) reached during puncturing (F1); the hardness of the peel, as the area under the curve (N mm⁻¹) up to the yield point (from point 0 to F1, that is A1); the hardness of the flesh (A2), as the area (N mm⁻¹) calculated starting from the point of minimum force after the probe penetration (F2) and the maximum force (F3).

The TPA was carried out on the lateral face of whole olives in compression mode using an SMS P/75 plate. The probe speed was set at 0.5 mm sec⁻¹, time between compressions was 5 sec and the test was stopped at 15% deformation after the auto triggering signal. A 50 kg load cell was used. Hardness, cohesiveness, gumminess, chewiness and springiness, as reported by Szczesniak (1963) and Friedman *et al.* (1963), were evaluated and calculated by the instrument software. Texture determinations were carried out at 0, 30, 60, 120 and 180 days.

2.6. Sensory evaluation

The table olives were assessed at the end of brining (180 days) by 30 untrained judges who were asked to express their preference on the two samples. We performed a paired preference test as reported by Stone and Sidel (2004a).

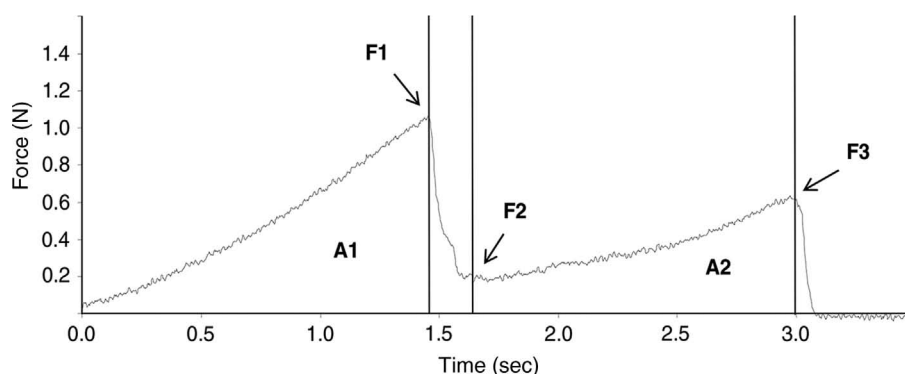


FIGURE 1. Representative graph of a puncture test.

2.7. Statistical analysis

Data on reducing sugars and polyphenol content on brines and olives and on the NaCl contents in the olives were subjected to one-way analysis of variance (ANOVA), with sodium chloride concentration as the group variable. Texture and antioxidant activity data were submitted to two-way ANOVA, where the factors were the sodium chloride and the days of storage. Data on sensory analysis were evaluated using the critical values table for duo-trio test and paired preference test (Stone and Sidel, 2004b). The software Statistica 6.0 for Windows was used to process data. Means, when required, were separated according to the Tukey test, with a significance level $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Physicochemical changes in brines and olives

An analysis of the chemical-physical parameters in the brines and olives were carried out mainly to check the appropriate evolution of processing and to correct, at each inspection time, pH and sodium chloride values. Data obtained, in fact, were as expected and as reported in the literature (Garrido-Fernández *et al.*, 1997; Piga *et al.*, 2001; Piga and Agabbio; 2003) (Table 1). The pH and sodium chloride concentration in brines showed a major shift from the fixed values during the first 60 days of processing, and after that were more stable (data not shown). The values for the corrected free acidity revealed the development of values not exceeding 0.5 g of lactic acid per 100 mL of brine, which is evidence of fermentative yeast growth and an absence of lactobacilli (data not shown). The diffusion of polyphenols and reducing sugars from the flesh into brines was noticeable and significantly higher in olives processed with a 4% sodium chloride concentration.

On the contrary and as expected, the sodium chloride content in the olives was significantly higher in the samples brined at a 7% salt concentration.

3.2. Texture evolution

As a result of the penetration test, the particular structure of the olive fruits generates a particular graph which shows a progressive increasing force during penetration up to the breaking of the peel. This maximum force (F1) has already been considered as a hardness index of the peel (Romeo *et al.*, 2012). This part of the curve looks like a type C one, as reported by Bourne (1979). In addition, the area under the curve up to F1, explains the work required to break the peel and thus represents its hardness. After this yield point a sudden force loss is registered due to the penetration of the first layers of flesh, which opposes a lower resistance to probe progress, and an increasing force up to a maximum value is registered. The area between the minimum force F2 and the maximum force F3 is a good index for flesh hardness. The evolution of the structure of the peel, as revealed by the puncture test, shows significant differences between olives brined with the two NaCl concentrations. In fact, the peel of olives brined with 7% NaCl showed higher values for the breaking force and hardness, with respect to the 4% processed olives (Table 2). These differences were significant after 60 days (Figures 2–3). Tassou *et al.* (2007) did not find differences in skin strength following brining with different sodium chloride concentrations, but they worked with black olives and performed the texture analysis on a peel strip and in tension mode. The most important result, however, is that peel break force and hardness increased during brining. Romeo *et al.* (2012) found similar results by brining turning color “Biancolilla” olives at 12%; while Georget *et al.* (2003) and Tassou *et al.* (2007) found a different behavior, but, as stated before, both the different degrees of ripening or

TABLE 1. Evolution of some chemical parameters of the brines and olives of Tonda di Cagliari olives during 180 days of brining

NaCl (%)	Sampling Period (days)	Brines		Olive flesh		
		Reducing sugars (%)	Total polyphenols (mg gallic acid 100 mL ⁻¹)	Sodium Chloride (%)	Reducing sugars (%)	Total polyphenols (mg gallic acid 100 mL ⁻¹)
4	0	–	–	0.12a	1.88a	359.2a
7	0	–	–	0.12a	1.88a	359.2a
4	30	0.210a*	62.6a	0.82b	1.68a	246.1b
7	30	0.144b	50.9b	1.87a	1.75a	267.9a
4	60	0.579a	92.7a	1.69b	1.46b	183.4b
7	60	0.406b	76.4b	2.81a	1.58a	228.2a
4	120	0.458a	110.9a	2.16b	1.32b	165.9b
7	120	0.298b	95.5b	3.04a	1.48a	197.8a
4	180	0.324a	135.8a	2.86b	1.02b	66.5b
7	180	0.224b	111.6b	3.74a	1.25a	80.5a

*Means followed by different letters within each column and sampling period are significantly different according to the Tukey test, significance level $P \leq 0.05$.

mechanical testing may have influenced the results. This peel hardening may be explained by the fact that the peel shrivelled due to the contemporary effects of sodium chloride absorption and water loss, thus resulting in the development of a crust.

On the other hand, the different NaCl concentrations did not influence the flesh hardness, which,

in turn, showed a significant decrease during the 180 days of brining (Table 2; Figure 4). This time, the results agree with those reported by Georget *et al.* (2003) and only in part with those reported by Romeo *et al.* (2012), and may be explained both by the loss of turgor and degradation of pectic polysaccharides during brining (Coimbra *et al.*, 1996).

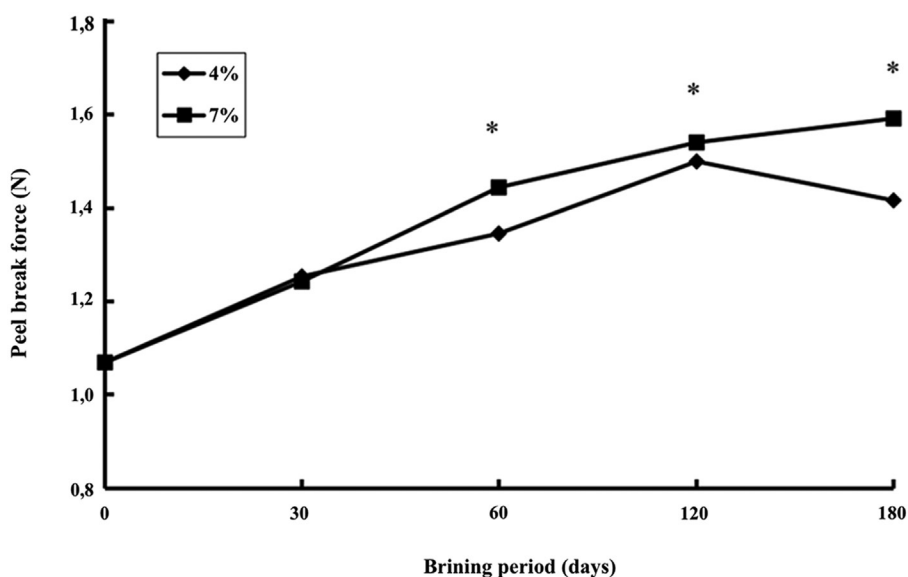


FIGURE 2. Evolution of peel break force as revealed by the puncture test during 180 days of brining of “Tonda di Cagliari” table olives. *Means statistically significant for the same period.

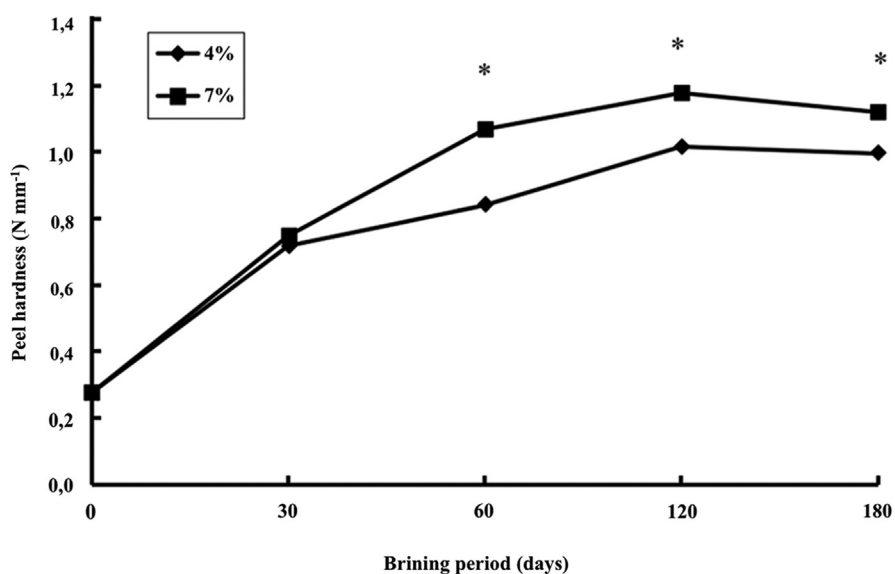


FIGURE 3. Evolution of peel hardness as revealed by the puncture test during 180 days of brining of “Tonda di Cagliari” table olives. *Means statistically significant for the same period.

Figure 5 shows a typical TPA graph where the two peaks of the two compression cycles can be seen. This kind of test was not able to show texture differences between the two sodium chloride concentrations used, but gave important information about the evolution of the different textural attributes during brining (Table 3). All the considered parameters, in fact, changed significantly at the first sampling time and then remained statistically constant. Hardness, which is the measurement of the maximum force of the first bite, decreased, thus indicating a softening of the flesh, as already

determined from the puncture test. The same trend was noticed for cohesiveness, which accounts for the strength of the bonds making up the body of the olive flesh, and for the springiness which explains the retained elasticity of the product. The decrease in these parameters influenced the evolution of chewiness and gumminess, calculated from the above first three cited parameters. The already cited work of Coimbra *et al.* (1996) may explain again the loss in texture of the product.

To our knowledge, these are the very first data on texture evolution using the TPA test during the

TABLE 2. Texture* of Tonda di Cagliari olives brined olives as affected by different brine salt concentrations and storage periods

Source of variation	Puncture test data		
	Peel break Force (N)	Peel hardness (N mm ⁻¹)	Flesh hardness (N mm ⁻¹)
NaCl (%)			
4	1.317b ^x	0.77b	0.846a
7	1.378a	0.88a	0.871a
Sampling period (days)			
0	1.069d	0.277d	1.475a
30	1.248c	0.733c	0.840b
60	1.396b	0.964b	0.731c
120	1.521a	1.098a	0.599d
180	1.504a	1.059a	0.645cd

*Texture has been determined with a puncture test (see the Materials and Methods section).

^xData with different letters within each source of variation, thesis and column differ significantly according to the Tukey test at $P \leq 0.05$.

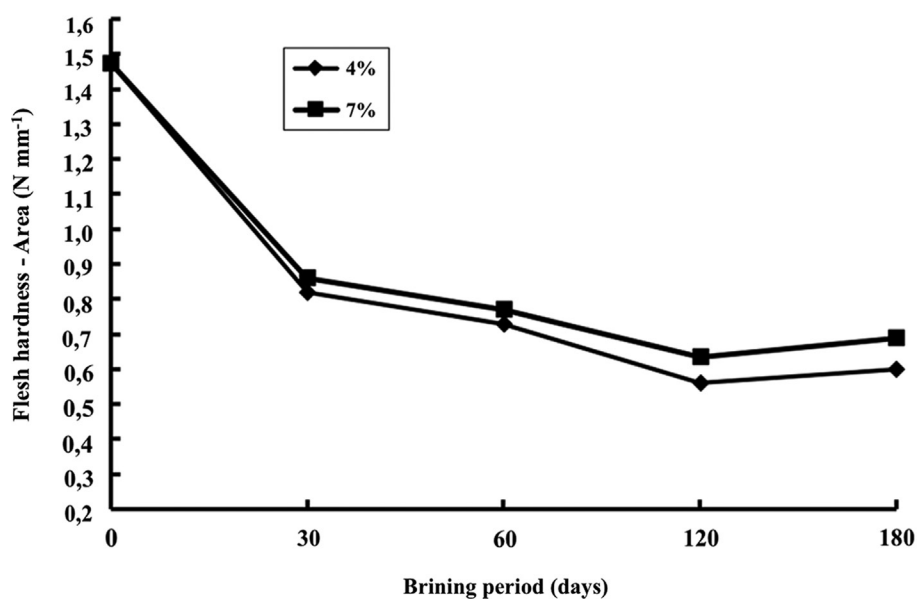


FIGURE 4. Evolution of flesh hardness as revealed by the puncture test during 180 days of brining of “Tonda di Cagliari” table olives.

brining of green table olives using two different sodium chloride concentrations.

3.3. Antioxidant activity evolution

The DPPH test is an *in vitro* radical scavenging (RS) measurement and its evolution during the 180 days of brining is reported in Figure 6 as percent absorbance decrease, with respect to a blank of DPPH without sample, after 60 minutes of reaction. It is worth pointing out that the major loss in RS activity has been recorded after the first month of brining (22–28%), thus mirroring the polyphenol loss, and that the total loss was close to 40% (34–42%) at the end of processing. A loss in antioxidant activity during brining has been already reported and was found to be well correlated to polyphenol

loss (Ben Othman *et al.*, 2009). In our case, the polyphenol loss was higher, accounting for 78–82% at the end of the brining period, with respect to the decrease in antioxidant activity reported before. Compounds other than polyphenols or polyphenols themselves in an intermediate state of oxidation may be present in the extract, thus explaining the lower decrease in RS, with respect to % loss in polyphenolic content. Olives brined with 7% of NaCl showed a higher RS activity, with respect to the 4% brined samples, probably due to the higher polyphenol content of the extract.

As stated for texture evolution, no other data are present in the literature concerning the antioxidant evolution during the brining of green table olives by means of two different sodium chloride concentrations.

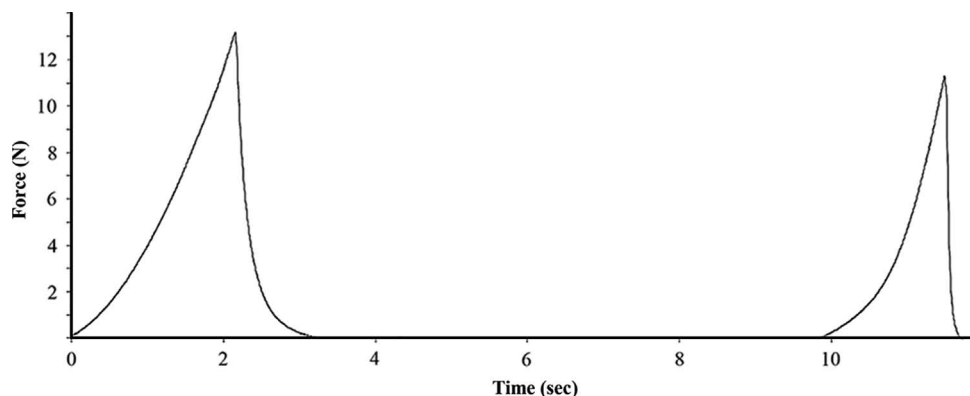


FIGURE 5. Representative graph of the TPA test.

TABLE 3. Texture* of Tonda di Cagliari brined olives as affected by different brine salt concentrations and storage periods

Source of variation	Texture Profile Analysis Parameters				
	Hardness (N)	Cohesiveness	Gumminess (N)	Chewiness (N mm ⁻¹)	Springiness
NaCl (%)					
4	29.79a	0.378a	11.74a	22.54a	0.622a
7	29.00a	0.379a	11.42a	21.50a	0.631a
Sampling period (days)					
0	56.11a	0.438a	24.69a	53.65a	0.732a
30	22.93b	0.345c	7.90b	13.28b	0.589b
60	22.97b	0.368bc	8.45b	14.58b	0.622b
120	21.94b	0.381b	8.31b	13.66b	0.595b
180	23.03b	0.362bc	8.54b	14.93b	0.593b

*Texture has been determined with a TPA test (see the Materials and Methods section).

[‡]Data with different letters within each source of variation, thesis and column differ significantly according to the Tukey test at $P \leq 0.05$.

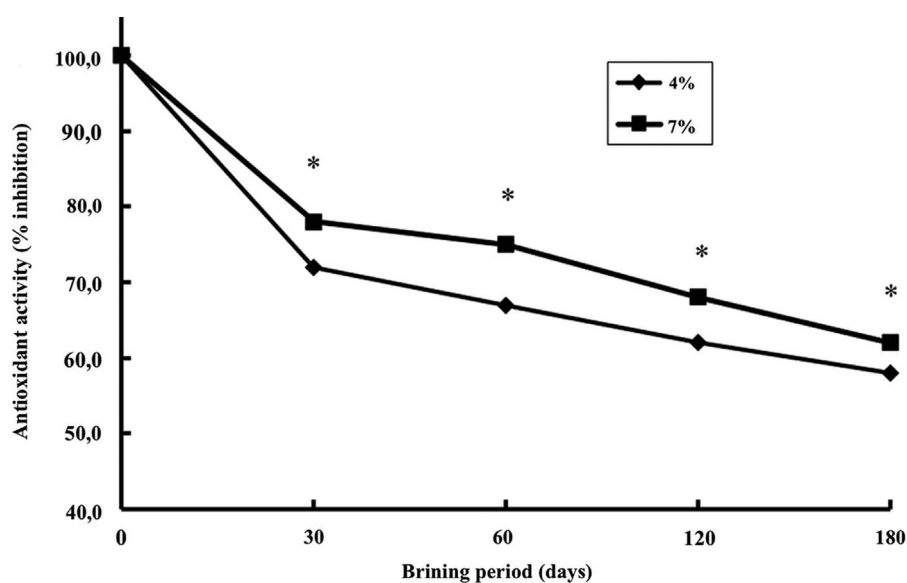


FIGURE 6. Evolution of antioxidant activity of the polyphenolic extract from the flesh of "Tonda di Cagliari" green olives during 180 days of brining. All the values have been normalized considering 100% as the value of fresh olives. *Means statistically significant means for the same period.

3.4. Sensory evaluation

The panellists positively judged both samples, however, 23 judges preferred the olives brined with 4% sodium chloride, as they found the olives less salty and bitter, with respect to the 7% brined ones. This result is significant at $P \leq 0.01$.

4. CONCLUSIONS

The use of different salt concentrations in the brining of naturally fermented green olives resulted in some chemical-physical, textural, antioxidant and sensory differences. Olives brined with the lower NaCl concentration maintained a lower polyphenol

content, antioxidant activity and peel hardness during and at the end of processing, but were preferred by panellists, with respect to the olives processed with the higher NaCl concentration. Both the texture tests used on the whole olives proved to be suitable to follow the texture evolution during brining of this table olive specialty.

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