GRASAS Y ACEITES, 61 (3),
JULIO-SEPTIEMBRE, 251-260, 2010,
ISSN: 0017-3495
DOI: 10.3989/gya.108809

Effect of acidification and salt concentration on two black brined olives from Sicily (cv moresca and giarraffa)

By **Flora V. Romeo,* Amalia Piscopo and Marco Poiana**

Department of Biotechnologies for Agricultural Food and Environmental Monitoring, (BIOMAA).
Mediterranean University of Reggio Calabria. C.da Melissari, 89060 Reggio Calabria, Italy
(*Corresponding author: flora.romeo@unirc.it)

RESUMEN

Efecto de la acidificación y concentración salina en dos variedades de aceitunas negras Sicilianas en salmuera (cv. Moresca y Giarrafa).

En el presente trabajo, los efectos de diferentes tratamientos con salmuera en aceitunas de mesa maduras durante su fermentación natural fueron evaluados. Los cultivos de aceitunas considerados son típicos de Sicilia: Moresca y Giarraffa. Ellas son cosechadas en su estado maduro. Los datos carpológicos revelan su buena calidad como aceituna de mesa. La fermentación natural fue realizada con y sin acidificación hasta pH 4, y a una concentración de sal del 8% y 15%. Cambios físicos, químicos y microbiológicos de las aceitunas y salmueras a través de todo el proceso fue monitorizado. La acidificación afectó y seleccionó la población microbiana y mantuvo el pH bajo, necesario para la seguridad higiénica del producto. De hecho, en la salmuera de Moresca las bacterias lácticas desaparecieron totalmente después de 60 días de fermentación mientras que en Giarraffa, ellas mantuvieron su presencia en la salmuera hasta 180 días con un valor entre 10^4 UFC/mL y 10^6 UFC/mL, dependiendo de la concentración salina. La población microbiana fue afectada también por el contenido de fenoles, que fue diferente en los distintos cultivos. El color de los frutos de aceituna fue mayormente influenciado por la acidificación y menos por la concentración salina. La adición de sal mostro una influencia diferente en los cultivos estudiados; de hecho, solamente el análisis químico de Giarraffa mostro una diferencia significativa entre los dos niveles de concentración salina.

PALABRAS CLAVE: Aceitunas maduradas naturalmente – Acidez – Crecimiento microbiano – Sal – Salmuera.

SUMMARY

Effect of acidification and salt concentration on two black brined olives from Sicily olives (cv. moresca and giarraffa).

In the present work the effects of different brining treatments on mature table olives during natural fermentation were evaluated. The considered olive cultivars are typical of Sicily: Moresca and Giarraffa. They were harvested at pigmented state. The carpological data revealed their good quality as table olives. Natural fermentation was performed with or without acidification up to pH 4, and at 8% and 15% salt concentrations. The physical, chemical and microbiological changes in olives and brines were monitored throughout the processing period. The acidification affected and selected the microbial population and maintained the low pH necessary for the hygienic safety of the product. In fact, in Moresca brines,the lactic acid bacteria totally disappeared after 60 days of fermentation while in Giarraffa they maintained their presence in the brines up to 180 days with a value between 10^4 UFC/mL and 10^6 UFC/mL, depending on the salt concentration. The microbial population was also affected by the polyphenol content, which was different between the cultivars. The color of olive fruits was greatly influenced by acidification and less by salt concentration. The addition of salt showed a different influence on the studied cultivars, in fact only the chemical analyses of Giarraffa showed a significant difference between the two levels of salt concentration.

KEY-WORD: Acidity – Brining – Naturally mature olives – Microbial growth – Salt.

1. INTRODUCTION

The fermentation of brined olives is a preservation process which leads to a product with different characteristics with respect to that obtained through lye treatment. In fact, sensorial, textural and microbiological profiles are very different. Fermentation, instead of adding preservatives, offers the advantages of acid formation and removal of fermentable sugars which serve to prevent the growth of pathogenic microorganisms and to stabilize the products. Moreover, fermentation offers the potential for flavor enhancement to the products (Fleming *et al*., 1983). There is more bibliographic information about Spanish-style and natural-style green table olives than naturally black olives. Fermenting naturally black olives in brine is the traditional type of olive processing in Turkey and Greece (Fernandez Diez, 1983). In this process, olives are placed in brine with a high salt concentration (10-14 g of NaCl /100 mL) and the diffusion of the soluble components from the fruits into the brine solution is very slow. This fermentation process usually lasts up to 10-12 months even though the bitterness does not disappear completely from the product (Özay and Borcakli, 1996) because no debittering treatment is carried out. Oleuropein, which is the major component responsible of the unacceptable bitter taste of unprocessed olives, is only partially removed through diffusion from flesh to brine (Brenes Balbuena *et al*., 1993).

The successful fermentation of brined vegetables is influenced by numerous chemical and physical factors, including the concentration and type of fermentable carbohydrates of the raw product and buffering capacity of the vegetable (Fleming, 1982). In olive fermentation, the cultivar is also an important factor affecting the final result. There is a great deal of variation in storage capacity between cultivars even when grown in the same area (Nanos *et al*., 2002). The quality of the processed product depends on the skin color and the flesh firmness of the drupe at the time of processing.

As for green table olives, as well as for naturally black olives, the fermentable material and other nutrients diffuse from the olives into the brine during brining, while NaCl diffuses into the olives. Equilibration of fermentable substrates and NaCl may affect the fermentation rate (Sànchez *et al*., 2000). Besides the use of starter cultures and different salt levels, the application of a highly acidified solution, with acetic or lactic acid, is an alternative to preserve the product. There is extensive research on the acidification of brining vegetables, for Durán Quintana *et al*. (2005), in a study of yeasts from table olives in brine at a low temperature, the type of acid used is more effective than the NaCl concentration against the growth of the main yeast species related to table olives. The acetic acid showed a strong effect with respect to the use of lactic acid. Also, Fernández Gonzalez *et al.* (1993) obtained good results using acetic acid and an initially high salt concentration to prevent alterative fermentations.

Naturally black olives suffer some of the same processing problems as naturally fermented green table olives when brined. The pH of brines are almost always higher than 4.5 which can lead to a microbiological risk; the salt concentration is always too high at the beginning of the process, resulting in the shrivelling of the skin (Piga and Agabbio, 2003), and too low at the end to prevent spoilage. In fact, the salt concentration is rarely checked during fermentation. Naturally black olives, when brined at a high NaCl concentration, loose part of their dark color, becoming unacceptable to consumers. Moreover, when the olives are harvested at a fully ripe state, the final product can be affected by textural problems having slightly softer flesh.

The aim of the present study was to determine the influence of different treatments such as acidification and salt addition on the quality of brined black olives. The purpose was to improve the sensorial, textural and microbiological quality of the final product.

2. MATERIAL AND METHODS

2.1. Plant material

Moresca and Giarraffa olive cultivars were used for the experiments. The cultivars were collected at a pigmented state with the typical color of full black. Fruits were harvested at a maturity stage suitable for processing, and immediately transported to the laboratory, where only fruits without peel defects were selected. Calibration by weight was performed in order to have uniform fruit callipers.

2.2. Olive processing

After washing with tap water, the olives were put into 25 litre PVC containers (three fermentation vessels for each thesis). The experimental plan adopted two levels of salt concentration and two levels of acidification. The containers were filled with fresh prepared brine and the trials were performed as reported below:

– 15% NaCl brine and acidified with lactic acid (90%, Fluka) up to pH 4.0;
– 15% NaCl brine and not acidified;
– 8% NaCl brine and acidified as reported above;
– 8% NaCl brine and not acidified.

Olives were brined with a fruit/brine ratio of 1. During the period of fermentation, salt concentration was adjusted up to the initial value with salt powder. The acidified containers were also monitored to correct the pH to the decided value with lactic acid. The olives were maintained at room temperature.

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

The NaCl, pH, free and combined acidity values were determined by the routine methods (Fernández-Diez *et al*., 1985): the pH was measured by a pH meter (Crison Basic 20), total acidity by titration with NaOH, chlorides by titration with $AgNO_3$ according to the Mohr method. About 10 g 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 to perform the pH, free and total acidity analyses on the pulp.

2.4. Water activity, dry matter and ash determinations

The water activity was measured by an Aqua lab (3TE, Decagon devices Inc., Washington) apparatus which uses the chilled-mirror dew point technique to measure the a_w of a sample. The dry matter content was determined after oven drying at 105°C up to constant weight. These analyses were carried out on 10g of homogenized samples. The ash quantity of the drupes was determined after the samples were burnt at 500°C in an oven (AOAC, 1990).

2.5. Total polyphenols of olives

Total polyphenols were extracted from olive flesh following the method reported by Amiot, Fleuriette &

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 standard at different concentrations.

2.6. Color analysis

All samples were analyzed for color using a tristimulus colorimeter Minolta CR-300. For each sample, 10 olives were analyzed on 5 points per fruit to evaluate the skin color. L* a* b* values were calculated using an illuminant D65 according to the CIELab scale. The chroma (C*) variation of color was calculated from the equation:

$$C^* = (a^2+b^2)^{1/2}.$$

2.7. Texture analysis

Hardness and its evolution in olives were determined through a texture analyzer (TA.TX2, Stable Microsystems, Surrey, UK) equipped with a 2 mm diameter cylinder probe. A measurement of the hardness 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 the distance between F1 and F2) and the maximum applied force (corresponding to 1500 g) F2.

The set parameters of each test were: pre-test speed 1 mm sec-1, test speed 0,5 mm sec-1, post-test speed 4 mm sec-1 and force max 1500 g.

2.8. Microbiological analyses

During fermentation, the numbering of different microbial populations in brine using selective media was carried out. The viable mesophilic counts in brine were estimated on Plate Count Agar Standard (Oxoid) incubated at 32°C for 48 h. The lactic acid bacteria amount was estimated on MRS Agar (Oxoid) charged with 50 mg/L of Nystatin (Sigma) at 32°C for 48 h in anaerobic conditions. The population of yeasts and moulds was estimated on Glucose Chloramphenicol Agar (YGC, BioMérieux) at 25°C for 48 hrs. The presence of bacterial pathogens of brine were examined as follows: *Clostridium perfringens* in OPSPA (Oxoid) at 37°C for 3-5 days in anaerobiosis, *Staphylococcus* spp. in MSA (Liofilchem) at 32°C for 72 h, Enterobacteriaceae and coliform bacteria in Mac Conkey MUG Agar (Liofilchem). The analyses were done in triplicate and the plates were subjected to microbiological numbering by CFU counting.

2.9. Data analyses

SPSS software (version 13.0, Inc.) was used for data processing. Two-way analysis of variance was used to test the effects of the different treatments on the final values of the measured factors. The data reported inside the tables are means, marginal means and significance.

3. RESULTS AND DISCUSSION

The carpological parameters reveal that the two cultivars are suitable for processing as table olives according to the IOOC (2000). In fact, both cultivars were classified as high weight fruits because "Moresca" was more than 5 g in weight and "Giarraffa" was considerably greater than this (9 g in mean weight). Other technological parameters, such as the flesh to pit ratio, was very interesting based on the classification proposed by Brighigna (1998), because the cultivars had a flesh/pit >5 (flesh percentage higher then 84-86%) and so they can be considered very good for table olives. Very interesting was "Giarraffa" sample which showed a flesh to pit ratio higher than 7. This large size could be due to the specialized and irrigated orchard where they were picked, but these two cultivars are well known as table olives.

The influence of acidification and salt percentage on the analyses done on the flesh of olives after 240 days in brine are reported in tables 1 and 2, with the related statistical analysis. We can note that the Giarraffa olives were influenced by the two treatments more than the Moresca, probably because of the different drupe size and composition. For both cultivars the ash content and water activity were obviously affected by salt concentration, according to water loss and salt absorption, depending on the osmotic mechanism caused by NaCl. With respect to salt addition, acidification seems to have had no effect on chemical analyses for Moresca and Giarraffa olives, with the only exception of water activity of Moresca.

The Moresca samples had a higher fat percentage than Giarraffa olives (respectively 25 and 18% in the starting material), but the latter were affected by time spent in the brine, with a significant decrease in fat in the flesh. Moreover, the fat of Giarraffa olives was also lowered by the higher 15% salt concentration (Table 2).

Regarding the total polyphenols, the two varieties showed different value from the start of processing. In fact, Moresca olives had twice the content with respect to Giarraffa olives (6338 vs 3744 mg/Kg). Our prediction was that the two processing variables, salt content and acidification, could be influential on the behaviour of polyphenols, but only Giarraffa olives showed a different effect that probably depended on the cuticle barrier effect, which was different between the cultivars. The NaCl – acidified samples showed the highest amount of flesh in Giarraffa olives and the lowest in the 8% unacidified ones.

In tables 3 and 4, the color analyses performed on the skin of olives expressed as L*, a*, b* and Chroma (C*) are also reported. Moresca olives

Table 1
Influence of different treatments on final values of the main analyzed parameters of "Moresca" olives

		Acidified	Not acidified		Sig.	
Free acidity (% lactic acid)	8% NaCl	0.260±0.066	0.317±0.018	0.289	Acidif.	n. s.
	15% NaCl	0.400±0.068	0.304±0.005	0.352	Salt	n. s.
		0.330	0.311		Acid.*salt	*
Fat (%)	8% NaCl	22.26±1.70	21.91±2.20	22.08	Acidif.	n. s.
	15% NaCl	23.16±1.29	24.75±0.27	23.95	Salt	n. s.
		22.71	23.33		Acid.*salt	n. s.
Total polyphenols (mg/Kg)	8% NaCl	3502±90.6	3473±243.9	3488	Acidif.	n. s.
	15% NaCl	2844±164.0	3669±277.4	3256	Salt	n. s.
		3173	3571		Acid.*salt	n. s.
Dry matter (%)	8% NaCl	36.90±1.98	37.39±0.13	37.14	Acidif.	n. s.
	15% NaCl	39.93±0.42	38.00±1.60	38.97	Salt	n. s.
		38.42	37.69		Acid.*salt	n. s.
Ash (%)	8% NaCl	3.56±0.45	4.10±0.14	3.83	Acidif.	n. s.
	15% NaCl	7.20±0.71	6.80±0.64	7.00	Salt	**
		5.38	5.45		Acid.*salt	n. s.
Aw	8% NaCl	0.947±0.0012	0.952±0.0016	0.949	Acidif.	*
	15% NaCl	0.903±0.0028	0.906±0.0077	0.905	Salt	**
		0.925	0.929		Acid.*salt	n. s.

Data are shown as means, standard deviations and marginal means. * Significance at $p < 0.05$. ** Significance at $p < 0.01$, n. s. not significant.

proved to be more strongly affected by the combined salt and acidification variables with respect to the a* and C* parameters than the Giarraffa samples where all the color components were influenced by these variables, above all by acidification.

The influence of processing variables on the pH and free acidity of brine samples is shown in figures 1 and 2. As expected, the acidified samples (fig. 1) reached their lowest value but the Giarraffa 8% unacidified samples, after 30 days, showed a rapid decrease. In fact, for Moresca and Giarraffa brines the pH and free acidity trend had a correlation with the lactic acid bacteria growth and death. For Moresca the maximum value corresponded to 60 days of fermentation, after which the lactic flora disappeared. Evidently the acid production was still active up to this day and remained higher to decrease immediately after that. This behavior could depend on Moresca's polyphenol content which was substantially higher than the other cultivar. It is well known that olive polyphenols, in particular the oleuropein and its derived products, have an antimicrobial effect (Brenes & De Castro, 1998). For Giarraffa brines the free acidity (fig. 2) had an increasing trend to the end of fermentation, in fact the lactic acid bacteria of this cv, shown in figure 3, reached a plateau between 60 and 90 days and remained alive also after the end of monitoring, with values between 10^4 UFC/mL and 10^6 UFC/mL, depending on salt concentration (respectively 15% and 8%).

Table 2
Influence of different treatments on final values of main analyzed parameters of "Giarraffa" olives

		Acidified	Not acidified		Sig.	
Free acidity (% lactic acid)	8% NaCl	0.358±0.639	0.359±0.625	0.359	Acidif.	n. s.
	15% NaCl	0.332±0.034	0.284±0.086	0.308	Salt	n. s.
		0.345	0.322		Acid.*salt	n. s.
Fat (%)	8% NaCl	14.76±0.34	13.53±1.13	14.14	Acidif.	n. s.
	15% NaCl	11.84±0.03	11.16±0.66	11.50	Salt	**
		13.30	12.34		Acid.*salt	n. s.
Total polyphenols (mg/Kg)	8% NaCl	1665±239.1	1808±226.2	1737	Acidif.	n. s.
	15% NaCl	2101±74.1	1766±78.1	1934	Salt	*
		1883	1787		Acid.*salt	*
Dry matter (%)	8% NaCl	27.04±0.80	25.95±0.12	26.50	Acidif.	n. s.
	15% NaCl	29.86±1.61	31.45±1.88	30.65	Salt	**
		28.45	28.70		Acid.*salt	n. s.
Ash (%)	8% NaCl	4.18±0.18	4.39±0.12	4.29	Acidif.	n. s.
	15% NaCl	8.13±0.02	8.06±0.73	8.10	Salt	**
		6.16	6.23		Acid.*salt	n. s.
Aw	8% NaCl	0.948±0.002	0.951±0.003	0.950	Acidif.	n. s.
	15% NaCl	0.905±0.003	0.905±0.004	0.905	Salt	**
		0.927	0.928		Acid.*salt	n. s.

Data are shown as means, standard deviations and marginal means. * Significance at $p < 0.05$. ** Significance at $p < 0.01$, n. s. N

The effect of the cultivar characteristics on the final values of several chemical and physical analyses performed on olive pulps and brines is summarized in Table 5.

Figure 4 shows the graph obtained from the texture analysis conducted on whole olives (this is a Giarraffa sample). Initially there was an increasing trend, due to the application of a rising force until breakage of the skin drupe occurred (F1); following the initial peak there was a waning phase caused by the elastic response of the same cuticle. After breakage of the cuticle, a force was required which increased in proportion to the hardness of the pulp, up to the application of the maximum force (F2) to reach the pit. F3 force was calculated in relation to correspondence of the mean point of the distance between forces F1 and F2. The peak of force F1 was considered the index of the hardness of the cuticle (Lin & Chang, 2005) while the index of pulp consistency was considered as the area extending from the curve between F2 and F3 to exclude the elastic effect of the cuticle, prevalent in the area between F1 and F3. The obtained results regarding the force necessary to break the cuticle showed significant variations between the tested cultivars ($p<0.05$), while there were no differences determined by the acidification and salt percentage (data not shown). Regarding the area between F2 and F3, Giarraffa samples showed a starting value of 12 N*mm and decreased until 10

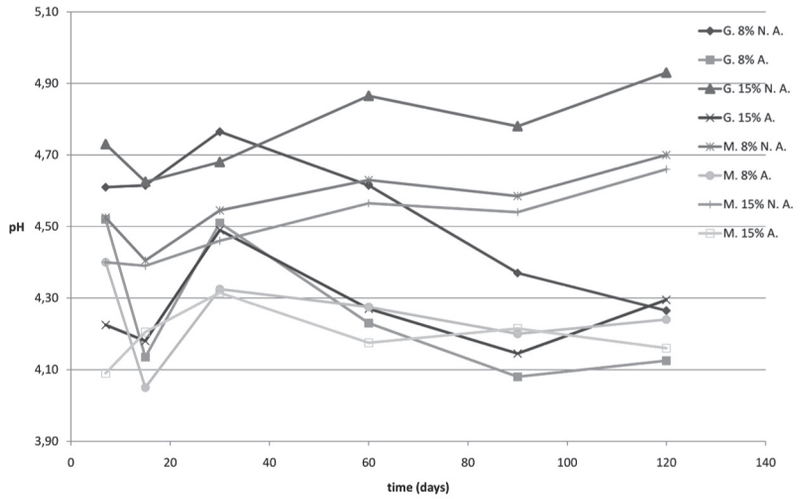

Figure 1
Changes in pH value in brine during fermentation of Moresca and Giarraffa olives.
Legenda: G. = Giarraffa; M. = Moresca; A. = acidified; N.A. = not acidified.

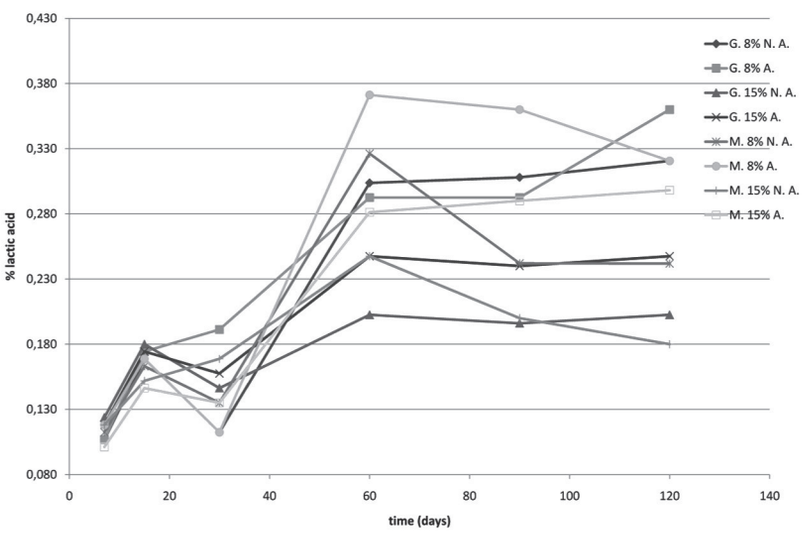

Figure 2
Changes in free acidity in brine during fermentation of Moresca and Giarraffa olives.
Legenda: G. = Giarraffa; M. = Moresca; A. = acidified; N.A. = not acidified.

N*mm at 90 days, remaining stable till the end of the period. Moresca samples started at 16 N*mm and remained at 9 N*mm from 90 days to the last sampling (data not shown). These results suggest that the texture of Moresca olives suffered the brining process more than Giarraffa because, with respect to the starting material, they became softer than the Giarraffa olives.

In figures 5 and 6, the F1 trends of Giarraffa and Moresca are reported. The cultivars reached their highest values at 120 days of storage in brine. Giarraffa samples showed an F1 force higher than Moresca and appeared more uniform in trend, with no substantial differences throughout the analyses. All the Giarraffa samples, except the 15% NaCl – acidified sample, started to decrease at 120 days, showing a harder cuticle, above all the 15% NaCl – acidified sample which maintained its level at about 7 N. In Moresca olives, the samples with the highest salt concentration reached their greatest values and all the samples started to decrease at 120 days. At the end of the observation time the Moresca samples with the highest values were those acidified. Therefore, regarding the texture results we can say that Giarraffa olives were more suitable than Moresca for these processing conditions as table olives.

In Figure 3 the lactic acid bacteria growth in Giarraffa brines is reported. From 7 days of fermentation the bacterial activity began with different rates, increasing up to 90 days in samples with 8% NaCl and up to 60 days in samples with 15% NaCl. At the end of monitoring period, the first samples showed a 10^6 UFC/mL count while the second maintained 10^4

Table 3
Influence of different treatments on final values of color parameters of "Moresca" olives

		Acidified	Not acidified		Sig.	
L*	8% NaCl	38.48±7.67	39.59±6.37	39.03	Acidif.	n. s.
	15% NaCl	37.89±8.47	39.90±9.87	38.90	Salt	n. s.
		38.18	39.75		Acid.*salt	n. s.
a*	8% NaCl	24.29±5.23	12.04±2.55	18.17	Acidif.	**
	15% NaCl	11.03±2.97	9.52±4.37	10.28	Salt	**
		17.66	10.78		Acid.*salt	**
b*	8% NaCl	9.02±6.08	8.85±5.49	8.93	Acidif.	n. s.
	15% NaCl	8.54±6.87	9.89±6.40	9.22	Salt	n. s.
		8.78	9.37		Acid.*salt	n. s.
Chroma	8% NaCl	26.94±4.60	15.76±3.30	21.35	Acidif.	**
	15% NaCl	15.14±4.55	15.35±6.43	15.24	Salt	**
		21.04	15.55		Acid.*salt	**

Data are shown as means, standard deviations and marginal means.
* Significance at $p < 0.05$. ** Significance at $p < 0.01$, n. s. not significant.

Table 4
Influence of different treatments on color parameters of "Giarraffa" olives

		Acidified	Not acidified		Sig.	
L*	8% NaCl	47.97±8.05	52.45±7.46	50.21	Acidif.	**
	15% NaCl	46.40±7.22	48.31±9.04	47.35	Salt	*
		47.18	50.38		Acid.*salt	n. s.
a*	8% NaCl	7.73±3.61	5.01±2.53	6.37	Acidif.	**
	15% NaCl	8.42±2.71	7.17±3.30	7.80	Salt	*
		8.07	6.09		Acid.*salt	n. s.
b*	8% NaCl	21.39±7.85	24.92±8.17	23.16	Acidif.	**
	15% NaCl	18.55±6.70	22.12±7.71	20.33	Salt	*
		19.97	23.52		Acid.*salt	n. s.
Chroma	8% NaCl	23.87±5.41	26.17±6.89	25.02	Acidif.	**
	15% NaCl	20.94±5.31	23.85±6.42	22.40	Salt	**
		22.40	25.01		Acid.*salt	n. s.

Data are shown as means, standard deviations and marginal means.
* Significance at $p < 0.05$. ** Significance at $p < 0.01$, n. s. not significant.

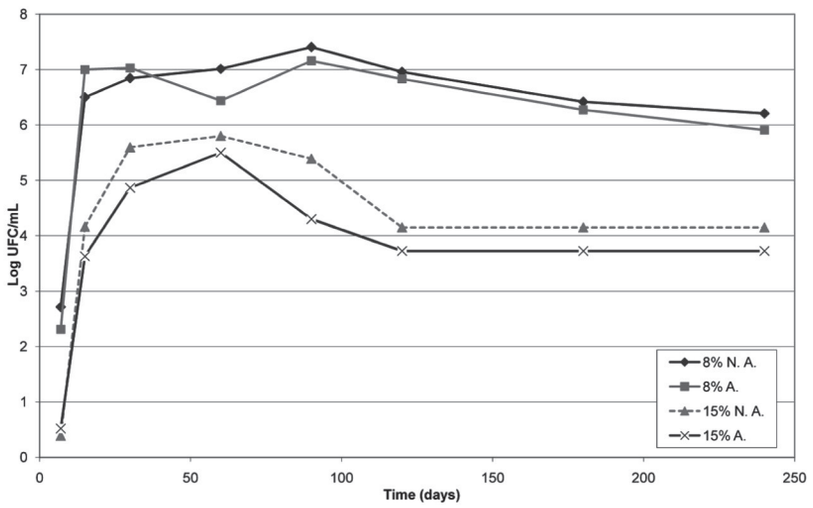

Figure 3
Changes in population of Lactic Acid Bacteria in brine (log_{10} CFU/mL) during natural fermentation of Giarraffa mature olives. Legenda: A. = acidified; N.A. = not acidified

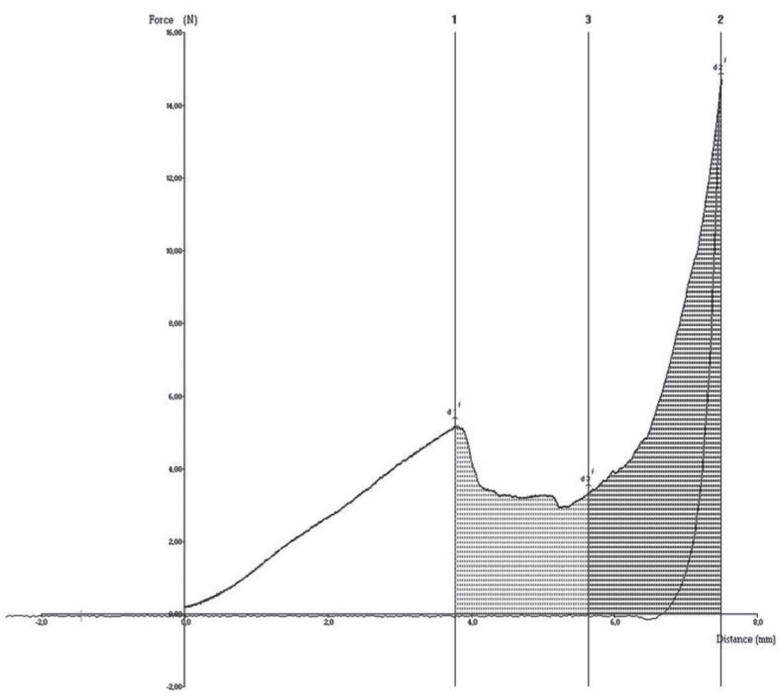

Figure 4
Hardness measurement of samples by puncturing involved plotting force (N) versus distance (mm).

UFC/mL at 120 days to the end of the period. Besides the lactic acid bacteria monitoring, the mesophilic viable count and yeast and mould population counts were also carried out. The mesophilic viable count of Moresca olives at the first control performed after 7 days of brining started from 10^3 UFC/mL in 15% NaCl - samples and from 10^5 UFC/mL in 8% NaCl - samples and maintained their trend after 90 days. Then it decreased slowly till the end of period and the value reached about 10^4 UFC/mL. The same analysis showed a different trend for Giarraffa olives. At first the control started from 10^2 UFC/mL in 15% NaCl - samples and from 10^3 UFC/mL in 8% NaCl – samples to reach the maximum growth at 30 days ($5*10^5$ UFC/mL in the highest salt content and 10^7 UFC/mL in the others). After this fermentation time, the 8% NaCl – samples held steady while the 15% NaCl ones decreased to a value of $5*10^5$ UFC/mL. The difference between the first value and the final sampling value agreed with the lactic acid bacteria results, showing an inhibition effect of the Moresca cultivar probably because of its polyphenol fraction. The same conclusions arose from the yeasts and moulds concentration trends in the brines.

Table 5
Influence of the two cultivar on final values of analyzed parameters of olive brine and flesh

	Giarraffa	Moresca	Sig.
on olive:			
Free acidity (% lactic acid)	0.333	0.320	n. s.
Dry matter (%)	28.58	38.06	**
Ash (%)	6.19	5.42	n. s.
Fat (%)	12.82	23.02	**
Aw	0.927	0.927	n. s.
Total polyphenols (mg/Kg)	1835	3372	**
L*	48.78	38.96	**
a*	7.08	14.22	**
b*	21.75	9.07	**
C*	23.71	18.30	**
on olive brine:			
pH	4.40	4.44	n. s.
Free acidity (% lactic acid w/v)	0.267	0.260	n. s.

** Significance at $p < 0.01$, n. s. not significa

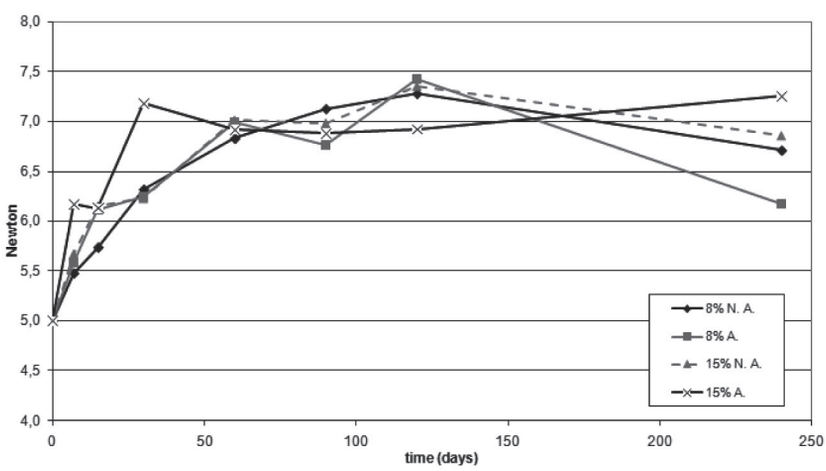

Figure 5
Evolution of cuticle hardness in Giarraffa olives during fermentation. Legenda: A. = acidified; N.A. = not acidified.

Regarding the pathogen monitoring, among the above mentioned species, only in one sample of Giarraffa cv, which was at 8% NaCl and acidified, 10^3 UFC/mL coliform bacteria was detected after up to 30 days of fermentation. However they disappeared after only a few days probably because of the increase in acidity which was more marked than the interactions of competition among the species. In fact, it is known that this kind of bacteria generally die as soon as the pH decreases in brine to a value of less than 4 and in the presence of bacteriocine producer lactic acid bacteria (Vescovo *et al.,* 1995).

CONCLUSIONS

The two analyzed cultivars had different behaviors when brined because of the different drupe dimension and physicochemical characteristics. The effect of acidification maintained the low pH required for hygienic safety in both cultivar samples, reaching the highest value in only 15% NaCl unacidified samples. The salt concentration influenced the chemical analyses of the two cultivars in a different way and had a strong effect on the skin color of the drupes. Acidification mainly affected the color and the microbial population. The absence of pathogens, or their disappearance, as in the case of coliforms, is

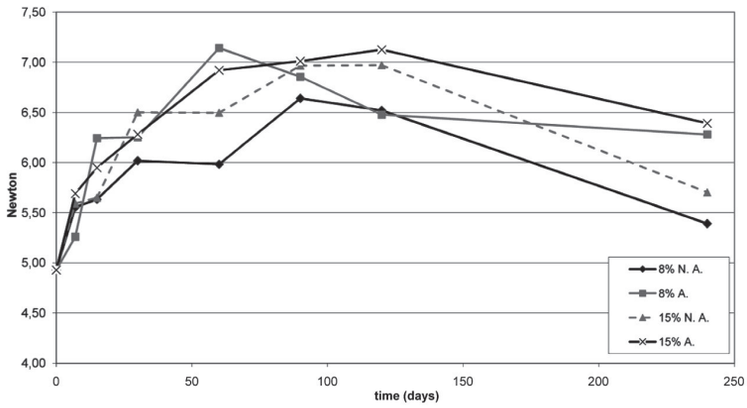

Figure 6
Evolution of cuticle hardness in Moresca olives during fermentation. Legenda: A. = acidified; N.A. = not acidified.

the most interesting result because it underlines that a careful monitoring of the processing parameters is determinant for quality and safety.

Moresca is traditionally processed as dehydrated olives preserved with salt. The textural results confirmed that this cultivar is unsuitable for brining, in fact its skin became softer than the Giarraffa cuticles during the brining process.

Giarraffa olives maintained a better texture and were more suitable for brining and for achieving a successful fermentation by lactic acid bacteria because of its lower total polyphenol content.

The typical problems of table olives such as shrivelling, incomplete fermentation and salty taste due to a high salt concentration, could be resolved by using an initial low salt concentration to be increased in the storage brine, or using starter cultures to improve the desired lactic fermentation. In our case, the 8% NaCl conditions produced better results than the 15% brine.

REFERENCES

Amiot MJ, Fleuriette A, Macheix JJ. 1986. Importance and Evolution of Phenolic Compounds in Olive during growth and maturation. *J. Agric. and Food Chem.* **3**, 823-826.

AOAC 1990. *Official Methods of Analysis*, 15th Ed., Association of Official Analytical Chemists, Arlington VA.

Borcakli M, Özay G, Alperden I, Özsan E, Erdek Y. 1993. Changes in chemical and microbiological composition of two varieties of olive during fermentation. *Grasas y Aceites* **44**, 253-258.

Brenes Balbuena M, García García P, Duran Quintana MC, Garrido Fernández A. 1993. Concentration of phenolic compounds change in storage brines of ripe olives. *J. Food Sci.* **58**, 347-350.

Brenes M, de Castro A. 1998. Transformation of Oleuropein and its Hydrolysis products during Spanish-style green olive processing. *J. Sci. Food Agric.* **77**, 353-358.

Brighigna A. 1998. Valutazione commerciale delle olive da tavola, in Brighigna A. (Ed.) *Le olive da tavola: Varietà, lavorazioni, legislazione, impiantistica e analitica di controllo* (Table olives: cultivars, processing, law, plants and analytical control), Edagricole, Bologna, Italy, pp. 55-60.

Durán Quintana MC, Noé Arroyo F, García García P, Garrido Fernández A. 2005. Evolución del crecimiento en salmuera, a bajas temperaturas y diferentes acidulantes, de levaduras aisladas de aceitunas de mesa. *Grasas y Aceites* **56**, 9-15.

Fernández Diez MJ. 1983. Olives, in *Biotechnology*, vol. 5, eds Rehm HJ & Reed G. Chemie, Weinheim, pp. 380-397.

Fernández Gonzalez MJ, García García P, Garrido Fernández A, Durán Quintana MC. 1993. *J. Appl. Bacteriol.* **75**, 226-233.

Fernández-Diez MJ, Castro Ramos R, Garrido Fernández A, Heredia Moreno A, Minguez Mosquera A, Rejano Navarro L, Nosti Vega M, García García A, Castro Ramos A. 1985. *Biotecnologia de la aceituna de mesa*. Instituto de la Grasa y sus Derivados, Sevilla, Spain.

Fleming HP, McFeeters RF, Thompson RL, Sanders DC. 1983. Storage stability of vegetables fermented with pH control. *J. Food Sci.* **48**, 975-981.

Fleming HP. 1982. Vegetable fermentations, in *Economic Microbiology*, vol. 7, Academic Press, Inc., London, England.

García García P, Durán Quintana MC, Brenes Balbuena M, Garrido Fernández A. 1992. Lactic fermentation during the storage of "Alorena" cultivar untreated green table olives. *J. Appl. Bacteriol.* **73**, 324-330.

Lin CH, Chang CY. 2005. Textural change and antioxidant properties of broccoli under different cooking treatments. *Food Chem.* **90**, 9-15.

Nanos GD, Kiritsakis AK, Sfakiotakis EM. 2002. Processing storage conditions for green "Conservolea" and "Chondrolia" table olives. *Postharvest Biol. and Technol.* **25**, 109-115.

Özay G, Borcakli M. 1996. Effect of brine replacement and salt concentration on the fermentation of naturally black olives. *Food Res. Int.* **28**, 553-559.

Piga A, Agabbio M. 2003. Quality improvement of naturally green table olives by controlling some processing parameters. *Ital. J. Food Sci.*, **15**, 259-268.

Sànchez AH, de Castro A, Rejano L, Montaño A. 2000. Comparative study on chemical changes in olive juice and brine during green olive fermentation. *J. Agric. Food Chem.* **48**, 5975-5980.

Vescovo M, Orsi C, Scolari G, Torriani S. 1995. Inhibitory effect of selected lactic acid bacteria on microflora associated with ready-to-use vegetables. *Letters in Appl. Microbiol.* **21**, 121-125.

Recibido: 6/10/09
Aceptado: 22/12/09