

Quality evaluation of different typical table olive preparations (cv Nocellara del Belice)

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RESUMEN

Evaluación de la calidad de diferentes preparaciones típicas de aceitunas de mesa (cv Nocellara del Belice).

Aceitunas verdes de mesa de la variedad "Nocellara del Belice" disponibles en el mercado fueron procesadas en 26 empresas. Tres condiciones diferentes de procesamiento se utilizaron para obtener aceitunas sin amargor: fermentación natural, Castelvetro (un típico método del oeste de Sicilia) y aceitunas rajadas. Se analizaron las características fisicoquímicas de las aceitunas verdes en envases de vidrio de 200-500 mL que reveló diferencias significativas entre los tres métodos de eliminación del amargor y una gran diferencia entre las muestras, lo que refleja un bajo nivel de normalización conseguido en estos productos fermentados. Los resultados mostraron una influencia de los tratamientos en todos los parámetros físico-químicos, con la excepción del contenido de polifenoles totales y de luminosidad (L*) para los que no se encontraron diferencias significativas. El promedio de pH de las muestras de salmuera se encontró por debajo del límite sanitario de 4,5, con la excepción de las aceitunas de Castelvetro que mostró un valor de pH peligroso. La concentración de sal de las muestras de salmuera fue más baja que el límite comercial de acuerdo a las normas de la tabla del comercio de aceitunas. Algunos de los parámetros microbiológicos se analizaron también en las muestras de salmuera. No todas las muestras mostraron niveles aceptables de los valores comerciales establecidos, debido a que en algunas muestras se detectaron *Clostridium perfringens*, *Staphylococcus aureus* y bacterias coniformes.

PALABRAS CLAVE: Aceitunas de mesa – Estilo español – Fermentación natural – Método de Castelvetro – Nocellara del Belice – Proceso típico.

SUMMARY

Quality evaluation of different typical table olive preparations (cv Nocellara del Belice).

Commercially available green table olives of the Nocellara del Belice cultivar were processed in 26 companies. Three different processing conditions were used to obtain olive debittering: natural fermentation, "Castelvetro" (a typical west Sicilian method) and cracked olives. The green olives packed into 200-500 mL glass jars were analyzed for physico-chemical characteristics that revealed statistical differences among the three debittering methods and a great difference among the

samples, reflecting a low level of standardization achieved in these fermented products. The results showed an influence of the treatments on all physico-chemical parameters with the exception of total polyphenol content and lightness (L*) for which no significant differences were found. The average pH in the brine samples was under the hygienic limit of 4.5 with the exception of Castelvetro olives which showed a higher pH values. The salt concentration of the brine samples appeared lower than the commercial limit according to the table olive trade standards. The brine samples were also analyzed for some microbiological parameters. Not all the samples showed acceptable commercial standards, because *Clostridium perfringens*, *Staphylococcus aureus* and coliform bacteria were detected in a few samples.

KEY-WORDS: Castelvetro method – Natural fermentation – Nocellara del Belice – Spanish style – Table olives – Typical process.

1. INTRODUCTION

The olive fruit has a bitter component and low sugar content (2.6-6.0%) compared with other drupes along with a high oil content (12-30%) which depends on the growth and variety. Olive cultivars containing a low percentage of oil with a high sugar content are usually used as table olives; however, certain olive cultivars are suitable for both table olives and oil production (Kiritsakis, 1998). Oleuropein, a glycosidic phenol which is distinctive to the olive, has to be removed as it has a strong bitter taste. The main purpose of processing is, in fact, the removal of fruit bitterness by hydrolysis of the glycosidic phenols, especially oleuropein (Aponte *et al.*, 2010). Enzymatic hydrolysis of oleuropein, particularly by β -glucosidase, is well established in the literature for both bacteria and fungi (Ciardini *et al.*, 1994; Ciardini and Zullo, 2000). This kind of enzymatic activity is important in olive production when deriving from lactic acid bacteria, while fungal growth may negatively affect both the nutritive and aesthetic value of the product with the presence of mycelium on the surface and mycotoxin production (Panagou, 2006).

Depending on local methods and customs, the fruit could be treated in sodium hydroxide, put into brine and successively rinsed in water, or it can also be directly fermented in brine. Besides the most prominent preparations as Spanish-style, Greek-style and Californian-style, there are many other traditional table olive elaboration recipes that are less known in the international market (Panagou *et al.*, 2003). Some products are available only in local markets. Frequently they are named “typical” and no safety measures are respected. The quality of the final product may depend on the composition of the fresh fruits, production technology and the environmental conditions during the transformation process (Oliveira *et al.*, 2004; Kopsidas, 1995). The “trade standard applying to table olives” (IOOC, 2004) describes the type of preparation of table olives and quality factors of these products.

Generally, the Sicilian productions, as in other countries, are directly packed and the rest are stored in brine and packed throughout the year, according to demand. The product is commercialized in different glass jars or plastic containers. Often, this kind of production shows a typical instability and especially its freshness, which is highly appreciated by the consumers, is rapidly lost. In these cases, a complete stabilization of the final product must be achieved (Arroyo-López *et al.*, 2009).

The traditional production areas of table olives in Sicily generally enjoy good climatic conditions during fermentation. However, in most processing plants, fermenters are exposed to the open air and, hence, the fermentation temperature follows environmental fluctuations (Aponte *et al.*, 2010). Temperature control is a complex and expensive procedure not generally applicable, consequently, sodium chloride concentration and pH of the brine are the main control parameters acting during fermentation (Garrido-Fernández *et al.*, 1997).

Traditional olive productions are often unpacked but frequently are sold in local markets in glass jars or plastic pouches. In general, the final packing is achieved with fresh brine, but the re-use of fermentation brine is also possible for this operation (Sánchez Gómez *et al.*, 2006; Romeo *et al.*, 2009). The Nocellara del Belice variety of *Olea europaea* L. is widely used in the West Sicilian region because of its suitability for table olive production, mainly by traditional methods. In this area, different debittering methods are applied to table olives obtained by Spanish style, Castelvetro system, natural fermentation and other typical local preparations. These products are very heterogeneous and their quality changes according to seasonal variation, factory and processing methods. So it is difficult to characterize this kind of production.

The aim of this work was to evaluate the physicochemical and microbiological characteristics of commercial green table olives produced in Sicily with three different processing conditions: natural fermentation without a previous lye treatment, Castelvetro-method and Cracked olives.

2. MATERIALS AND METHODS

2.1. Sampling and preparation systems

The commercial table olives were treated and collected from 26 different Sicilian companies, where they were packed into 200-500 mL glass jars filled with the same brine of the preparation treatment used. The samplings were taken after different time periods depending on the method: after 7-10 months of brining for cracked and natural olive samples and after 1-2 months for Castelvetro olives. The number of samples was 99 of the same cultivar, Nocellara del Belice, which were separated according to the preparation method: 23 samples for natural fermentation (NF), 50 for “Castelvetro” system (CS) and 26 for cracked olives (CO).

The three methods are as follows:

The “Castelvetro” system (also called “dolcificata” in the local language) is typical of the west Sicily area. The process is very simple: fruits graded by size are placed in 200-220 L plastic drums, treated with lye overnight and after 12 hours 5-8 kg of salt are added. After 15 days of storage the product is ready, the olives are washed and sold in local markets before the temperature increases by the spring season.

Another type of preparation is cracked olives, which is diffused in the areas of Sicily and different olive cultivars are used. Whole olives are subjected to a process whereby the flesh is opened without breaking the stone, which remains whole and intact inside the fruit. The drupes are put into 200-220 L drums with a brine at 10-12% salt. After 2-3 months of fermentation the olives are ready.

In the traditional process of natural brining, olives are washed with tap water and put into containers and then filled with freshly prepared 8-12% NaCl brine. The olives are handled in a way so as to promote the growth of lactic acid bacteria in the fermentation brines, which is thought to be essential to providing the amount of lactic acid needed for preservation. During natural fermentation, an acidification occurs (Garrido Fernández *et al.*, 1997). In order to preserve foods with acidity, the practice requires the pH to be 4.5 or below.

All cracked and natural olive samples were collected after 7-10 months of brining, so that the fermentable sugars were already exhausted, except for the Castelvetro olives which were collected and packed just after 1-2 months of production.

2.2. Sodium chloride, pH and acidity of brine

The NaCl, pH, free and combined acidity values were determined using the routine methods (Fernández-Diez *et al.*, 1985). pH was measured with a pHmeter (Crison Basic 20), total acidity by titration with NaOH, combined acidity by titration with HCl up to pH 2.6 and expressed as mEq/L, chlorides by titration according to the Mohr method with AgNO₃.

2.3. Total polyphenols in olives

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

2.4. Water activity and dry matter determinations

The water activity (*aw*) was measured by an Aqua lab (3TE, Decagon devices Inc., Washington) apparatus which uses the chilled-mirror dew point technique to measure the *aw* of the homogenized pulp samples. The dry matter content was determined by oven drying at 105 °C up to constant weight. These analyses were performed on 20 homogenized olives.

2.5. Color determination

The color of the olives was measured using a reflection colorimeter (Minolta CR 300, Osaka, Japan). The CIE L*a*b* coordinates were measured using D65 illuminant. This analysis was assessed on two points of every olive and for ten olives randomly chosen from each jar. Chroma (C*) was calculated as $(a^{*2} + b^{*2})^{1/2}$.

2.6. Microbiological analyses

The different microbial populations were enumerated using the following selective media and conditions: total mesophilic bacteria in Plate Count Agar (Oxoid) incubated at 32 °C for 24 h, lactic acid

bacteria in MRS Agar (Oxoid) added with 50mg/L of Nystatin at 32 °C for 48 h in anaerobiosis, yeasts and moulds in OGYA (Oxoid) at 25 °C for 48 h, *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* in Mac Conkey MUG Agar (Liofilchem) at 37 °C for 24 h. The analyses were done in triplicate, and the plates were subjected to microbiological enumeration (CFU/mL).

2.7. Statistical analysis

SPSS software (version 17.0, Inc.) was used for data processing. One-way analysis of variance was used to test the effects of the different treatments on the measured factors. Duncan's multiple range test was used to compare means when a significant variation was highlighted by analysis of variance. Data subdivided by the different debittering methods were subjected to principal component analysis (PCA) in order to obtain major variation patterns.

3. RESULTS AND DISCUSSION

3.1. Physicochemical analyses

Several authors (Sánchez *et al.*, 2000; Montañó *et al.*, 2003) assumed that, once fermentation substrates are exhausted, an equilibrium between brine and olive juice is reached for physicochemical characteristics. In this condition, especially the water soluble substances of olive juice should not differ from that of the brine. At the end of fermentation the olives could be packed and are ready for consumption.

In Table 1, the one-way Anova results of the physicochemical analyses are shown. Physicochemical

Table 1
One-way Anova data of the effect of treatment on physicochemical analyses

	Natural fermentation	Castelvetro system	Cracked olives	Sig.
pH	4.34 ± 0.59 b	6.52 ± 0.70 a	4.23 ± .34 b	**
Chlorides (%)	6.01 ± 2.38 b	4.88 ± 2.45 b	7.57 ± .17 a	**
Free Acidity (mEq/L)	62.67 ± 17.52 a	27.50 ± 14.12 b	62.90 ± 15.31 a	**
Combined Acidity (mEq/L)	15.94 ± 7.2 b	41.80 ± 9.5 a	14.84 ± 4.0 b	**
Aw	0.964 ± 0.012 a	0.966 ± 0.017 a	0.945 ± 0.015 b	**
Dry matter (%)	42.84 ± 12.33 a	33.81 ± 2.82 c	38.44 ± 3.41 b	**
Total polyphenols (mg/Kg)	1850 ± 681	1677 ± 559	1581 ± 643	n. s.
Color parameters				
L*	48.86 ± 2.97	48.37 ± 4.03	48.03 ± 2.85	n. s.
a*	2.13 ± 3.48 a	-6.04 ± 2.84 b	2.27 ± 1.79 a	**
b*	30.26 ± 3.95 ab	31.77 ± 3.98 a	28.80 ± 3.72 b	**
C*	30.46 ± 3.33 ab	32.45 ± 4.14 a	28.95 ± 3.66 b	**

Data (means and standard deviations) followed by different letters are significantly different according to Duncan's multiple range test.

**Significance at $P < 0.01$, n. s. not significant.

characteristics were generally affected by the treatment with the exception of the total polyphenol contents and lightness (L^*) for which no significant differences ($P < 0.01$) were found. Also, the company where olives were produced had an influence on physicochemical results (data not shown) leading to a different qualitative levels among the commercial products.

The average pH in the brine samples was under the hygienic limit of 4.5 (only 3/23 of natural olives and 4/26 of cracked olive samples were over this limit) with the exception of Castelvetro olives which showed pH values ranging from 5.53 to 9.43 in all 50 samples. This is a frequent problem for this kind of treatment, in fact, the olives are often sold before the lye solution can be naturally neutralized reaching a safe pH value. The salt level was quite different among the samples, reaching the highest percentage in cracked olives. This was expected because in this production a higher level of salt is added in order to maintain the pulp consistency and therefore osmotic equilibrium is reached faster than in the other two productions.

The salt concentration of the brine samples appeared lower than the commercial limit, however, only 9/23 natural olives, 8/50 Castelvetro olives and 19/26 cracked olive samples followed the COI trade standard applying to table olives (2004).

Both free and combined acidity reflect the results of pH. The natural and cracked olives, which are processed without lye treatment, showed almost the same values while the Castelvetro olives presented the lowest free acidity value and the highest combined acidity value. This is a typical behavior with this kind of olive production because of the reaction between the lye solution and the organic acids in the drupes.

The water activity only revealed statistical differences in the cracked olives, probably because of the higher quantity of salt used for this kind of preparation. With regards to the dry matter of the samples, the natural olives showed a value clearly higher than the treated olives, in which the lye solution and the salt percentage extract a higher level of compounds from the pulp of the drupes. As expected, the lye treatment showed a higher osmosis action with respect to the salt.

Nocellara del Belice is a cultivar with a high polyphenol content of around 7000 mg/Kg (Cappello and Poiana, 2005), and the total polyphenols measured in the preparations reached the values reported in Table 1. The polyphenols of the different preparations revealed little variation in the mean values but without statistical differences.

Color parameters were measured in order to assess the variation among the three treatments, and only the lightness (L^*) was similar in each treatment. Whereas the other parameters were statistically different, the a^* parameter, which represents the redness/greenness had a negative value only in Castelvetro olives, which in fact are always a brilliant green as a consequence of the NaOH addition which blanches the olive pigments. The cracked olives showed the lowest b^* parameter, which represents the blueness/yellowness parameter. In this case the chroma (C^*), which represents the color saturation, depending on both a^* and b^* , is more informative. It followed the same behavior as the b^* parameter among the treatments, and so the Castelvetro olives were less subjected to a browning action.

3.2. Microbiological analyses

In Table 2, the one-way Anova results of the microbiological analyses are shown. Microbiological characteristics were also generally affected by treatment with the exception of the Lactic Acid Bacteria (LAB) for which there was no statistical difference.

The mesophilic aerobic count was different for $P < 0.05$ among the treatments and showed the highest value in natural fermented olives. Regarding the other bacterial populations, no statistical differences were shown between natural and cracked olives whereas the Castelvetro olives maintained the lowest value ($P < 0.01$). This last kind of treatment showed a bacteriostatic effect against all the bacteria counted with the exception of LAB but, on the other hand, the physicochemical analyses showed an unsafe pH value of the brine which can lead to a hygienic problems during storage. Sánchez *et al.* (2001) have reported that lactobacilli used as starter culture grew well at

Table 2
One-way Anova data of the effect of treatment on microbiological analyses

Media	Microorganism (Log UFC/mL)	Natural fermentation	Castelvetro system	Cracked olives	Sig.
MRS	Lactic acid bacteria	4.53 \pm 2.35	3.25 \pm 2.93	3.60 \pm 2.62	n. s.
PCA	Mesophilic aerobic bacteria	4.26 \pm 1.91 a	2.90 \pm 2.76 b	3.41 \pm 2.16 ab	*
OGYA	Yeasts and Moulds	2.27 \pm 1.85 a	1.23 \pm 1.54 b	2.84 \pm 2.18 a	**
MAC	Enterobacteriaceae	1.73 \pm 1.74 a	0.63 \pm 0.98 b	1.59 \pm 1.69 a	**
MSA	Staphylococci	0.86 \pm 1.20 a	0.06 \pm 0.23 b	0.93 \pm 1.16 a	**

Data followed by different letters are significantly different according to Duncan's multiple range test.

**Significance at $P < 0.01$, *significance at $P < 0.05$, n. s. not significant.

high pH despite the initial loss, while in our study the LAB count was poorly affected in Castelvetro olives with respect to the other without a lye treatment. Sabatini and Marsilio (2008), when analyzing the volatile compounds of different preparations of table olives, found the highest number of yeasts in Castelvetro olives (10^9 CFU/mL) with respect to Greek and Spanish-style olives. In the present work the microbiological results suggest that the Castelvetro-style can negatively influence the survival of microbial population after the end of fermentation.

In addition to the analyses shown in Table 2, *Clostridium perfringens*, staphylococci and coliforms were monitored in the brine of all the samples. The results highlighted poor hygienic standard throughout the handling and treatment of these Table olives, in fact, 7 positive samples for *C. perfringens* (4 in NF and 3 in CS samples), 14 for staphylococci (7 in NF and 7 in CO) and 25 for coliforms (11 in NF, 3 in CS and 11 in CO) were found (data not shown).

In Figures 1, 2 and 3, the score plot and loading values of the Principal Component Analysis of the

data are shown. In natural olives (Fig.1), the score plot shows that LAB and total mesophilic bacteria (on MRS and PCA respectively) are related to the free acidity and water activity, while staphylococci are related to dry matter and a^* . The other three color parameters, L^* , b^* and C^* , are strongly interrelated for cracked olives (Fig. 2), in which they are also linked to the water activity and total polyphenols. For natural olives as well as for cracked olives, staphylococci (on MSA) are always linked to dry matter and a^* while for Castelvetro olives it is only linked to a^* . In addition, for cracked olives, yeasts and moulds, LAB and total mesophilic bacteria (on OGYA, MRS and PCA respectively) are set apart from all analyzed chemical parameters while *Enterobacteriaceae* (on MAC medium) are related to the free acidity. This last result was expected because these bacteria are negatively influenced by a low pH value. In fact, also in Fig. 3, for Castelvetro-style, the MAC and free acidity show the same distance, and in this case, the LAB and total mesophilic bacteria are also related

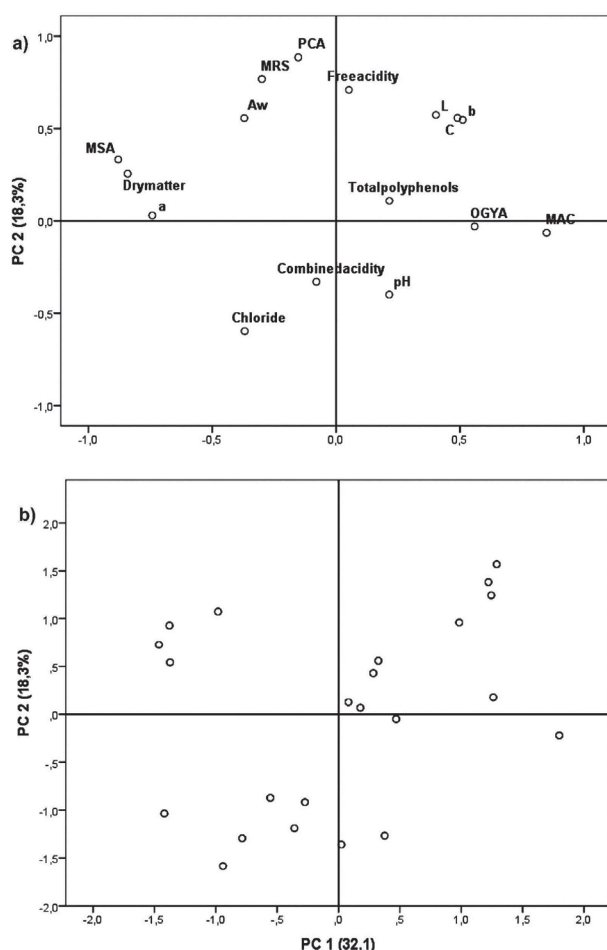


Figure 1

(a) Score plot of PCA showing separation of chemical and microbiological variables along principal components PC1 and PC2 for natural olives, and (b) loading values for natural fermented samples.

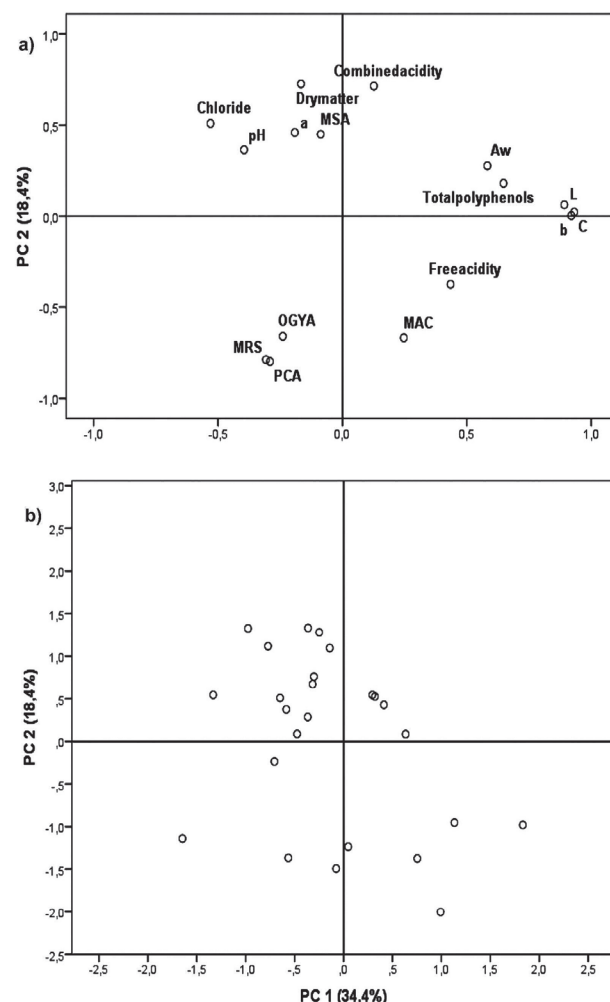


Figure 2

(a) Score plot of PCA showing separation of chemical and microbiological variables along principal components PC1 and PC2 for cracked olives, and (b) loading values for cracked olive samples.

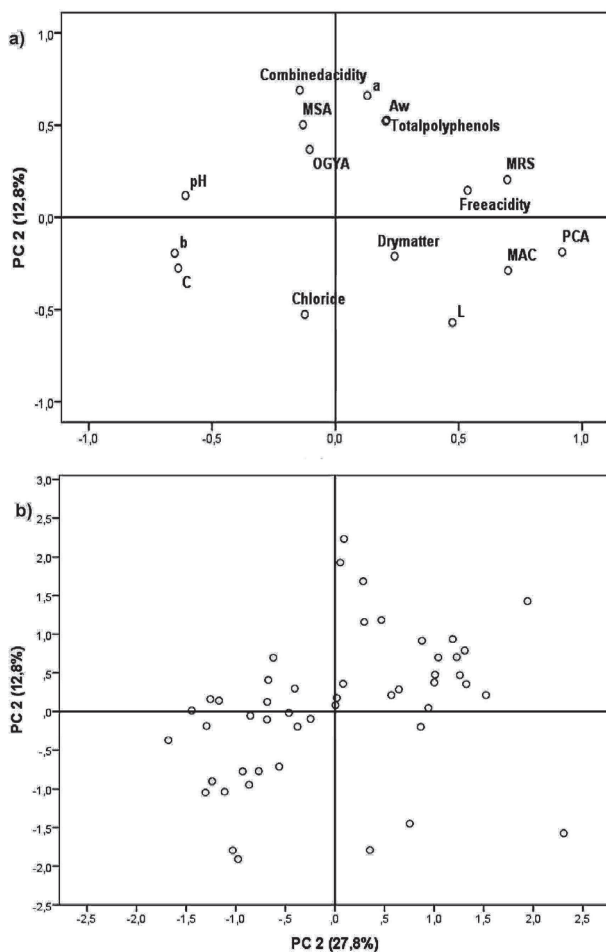


Figure 3
(a) Score plot of PCA showing separation of chemical and microbiological variables along principal components PC1 and PC2 for Castelvetrano olives, and (b) loading values for Castelvetrano samples.

to the free acidity. For this debittering method, staphylococci, yeasts and moulds are related to combined acidity, water activity, total polyphenols and the a^* parameter.

Comparing the microbiological results of the three figures, the dependence of most of the bacteria on aw and especially on free acidity is evident.

C^* and b^* are strongly related in all the three methods, but for the Castelvetrano-style, they are set apart from the brightness (L^*). The color parameters showed a behavior more similar in natural and cracked olives than in Castelvetrano. In fact, in NS and CO these parameters are related to polyphenols while in CS only a^* was near the total phenols. Probably, the color changes in NS and CO during treatments and storage resulted principally from phenol contents while in CS this change depended on the chemical (lye) treatment, in fact, b^* and C^* were related only to pH and chloride.

Moreover, analyzing all three loading plots, in which the points represent the individual samples, no correlation was found between the company and its products.

4. CONCLUSIONS

The 99 samples of Nocellara del Belice commercial olive preparations, treated with three different methods, showed great heterogeneity. The results suggest that there are great differences among the samples for the same kind of treatment and company. This fact reflects a low level of standardization achieved in this product. The score plots show that the cracked olives are more uniform with respect to the other two methods and that natural and cracked olives reveal more similarities than Castelvetrano. The microbiological results, in particular the presence of potential pathogens detected in all olives treatments, suggest the necessity to pH control and follow good hygienic practice throughout the process. Further research, concerning, for example, a correlation between each method and the chemical composition (for each cultivar) of the final product, or the influence of the treatment on the aroma compounds through a sensory analysis, is needed.

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