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Effects of maturation and processing technologies on nutritional and sensory qualities of Itrana table olives

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RESUMEN

Efectos de la maduración y el procesamiento sobre la calidad nutricional y sensorial de las aceitunas de mesa Itrana

En el presente estudio se evalúan las características nutricionales y sensoriales de la variedad Itrana de aceituna (*Olea europaea* L.) de doble uso, elaborada en verde (Oliva Bianca di Itri) y en negro (Oliva di Gaeta), utilizando métodos griegos modificados al efecto. Un método consiste en una etapa inicial de inmersión en agua para favorecer el crecimiento de una flora específica que contribuye al endulzado de los frutos. Después de 15-45 días se añade sal a la solución en una cantidad que no exceda 8 kg por 100 g de fruto fresco. Otro procedimiento consiste en colocar directamente las aceitunas en salmuera utilizando un sistema de adición de la sal en dos etapas (la mitad de la sal se añade inmediatamente y el resto a los 15 días). Toda la información derivada de los análisis fisico-químicos, nutricionales y sensoriales ha permitido la separación de las muestras en cuatro grupos según la tecnología de elaboración, el estado de madurez y el almacenamiento. El proceso de adición de sal en dos etapas ("double salting") fue el procedimiento que dio mejores resultados.

PALABRAS CLAVE: Aceitunas de mesa – Etiqueta nutricional – Nutriente – Olea europaea L.

SUMMARY

Effects of maturation and processing technologies on nutritional and sensory qualities of Itrana table olives

In the present study, we evaluated the nutritional and sensory characteristics of table olives of the Italian double-aptitude olive cultivar (*Olea europaea* L. cv. Itrana) processed as green (Oliva bianca di Itri) and black (Oliva di Gaeta) table olives with modified Greek methods of preparation. One method provides an initial step of immersion in water to stimulate the growth of specific microflora which contributes to the debittering of the fruits. After 15-45 days, salt is added to the liquid in quantities not exceeding 8 kg per 100 kg of fresh olives. Another method entails the immersion of fruits directly in brine utilizing double-salting (half the amount of NaCl was added immediately and the remaining part after 15 days). All the information derived from chemico-physical, nutritional and sensory data have separated the samples into four groups according to techno-processings, ripening stage and storage. Double-salting is the method which assures the best results.

KEY-WORDS: Nutrient – Nutritional label – Olea europaea L – Table olives.

1. INTRODUCTION

Italy has a long tradition in producing "natural olives". In this process the olives are directly brined in 8-10% sodium chloride (Sánchez Gomez *et al.*, 2006). The brine stimulates the microbial activity for fermentation and reduces the bitterness of the fruits. The fermentation of these olives takes a long time because the diffusion of soluble components through the epidermis, in fruits not treated with alkali, is slow. A diverse microbiota grows in these brines, although yeasts are the microorganisms always present throughout the process. *Enterobacteriaceae* can be found during the first 7-15 days, but they disappear as the brine characteristics do not support their growth. The presence of lactic acid bacteria depends on the salt concentration and the polyphenol content of the variety used (Garrido Fernández *et al.*, 1997). These olives are popular because of their slightly bitter taste and aroma. When the bitterness has been sufficiently weakened the fruit can be marketed. To reduce the debittering phase, several researchers have evaluated the use of selected oleuropeinolytic lactic acid bacteria (LAB) as starter cultures in Greek-style olive processing. *Lactobacillus plantarum (*Ciafardini *et al.*, 1994; Marsilio *et al.*, 1996; Marsilio and Lanza, 1998; Marsilio *et al.*, 2005; Panagou *et al.*, 2008), *Lactobacillus pentosus* (Panagou *et al.*, 2003; Panagou *et al.*, 2008; Servili *et al.*, 2006) and other LAB species such as *Lactobacillus brevis* and *Pediococcus pentosaceus* (Ghabbour et al., 2011). In addition to developing rapid growth and good acidifying capacity even in the presence of high concentrations of salt (brine), they were able to grow in the presence of phenolic compounds (known for their antimicrobial action) and, thanks to the marked oleuropeinolytic activity, degraded the oleuropein in non-bitter compounds, thus considerably reducing the time required for debittering.

The "natural olives", according to the "Trade Standard Applying to Table Olives" (IOC, 2004) are "green olives, olives turning color or black olives placed directly in brine where they undergo complete or partial fermentation, preserved or not by the addition of acidifying agents". The most

important industrial preparation for natural black olives takes the name "Greek-style" because it is traditionally practiced in Greece using *Conservolea* cv. (Balatsouras, 1990).

The *Olea europaea* L. cv. *Itrana* (synonyms: Aitana, Aitanella, Aitanesca, Attanesca, Auliva a acqua, Cicerone, Esperiana, Gaetana, Gitana, Iatanella, Itana, Oliva di Esperia, Oliva di Gaeta, Oliva grossa, Olivacore, Raitana, Reitana, Strano, Tanella, Trana, Velletrana) (IOC, 2000) is an Italian double-aptitude olive cultivar that is grown mainly in the Lazio region and affects the hilly area of Ausoni, Lepini and Aurunci mountains. The production is concentrated in the Latina district (Itri, Cori, Rocca Massima and Sonnino). The olives destined to produce the famous "Oliva di Gaeta" are harvested at the stage of full maturity in the months of February-March (very late compared to most table olive cultivars). This ancient method (Serao, 1934) provides an initial step of immersion in water to stimulate the growth of specific microflora that contributes to the debittering of the fruits. After 15-45 days, salt is added to the liquid in quantities not exceeding 8 kg per 100 kg of fresh olives. According to a mythological legend, the sailors of Aeneas, sailing along the pontine coast before arriving at the port of Caieta (modern Gaeta), saw the dark fruits float on water (Aeneid, Virgil). They tasted them and found them delicious. Olives were falling overboard from trees grown near the sea, savory to stay in salt water: the first Gaeta olives in brine!

An alternative method provides that olives are processed directly in brine utilizing "double- salting" (half the amount of NaCl was added immediately and the remaining part after 15 days). After 4-6 months of storage in brine the olive flesh shows a typical red-wine color and acidic taste probably due to the contribution of heterofermentative bacteria and yeasts. In recent years another type of product named "Oliva bianca di Itri" has evolved. The processing system is basically the same but the Itrana fruits are collected at the beginning of ripening in the months of November-December, when they reach their final size and look green or turning-color, and immersed immediately in water. After 6-8 months, the product is ready to eat.

In the present study, we evaluated the nutritional and sensory characteristics of table olives of the Italian double-aptitude olive cultivar (*Olea europaea* L. cv. Itrana) processed as green (Oliva bianca di Itri) and black (Oliva di Gaeta) table olives in relation to modified Greek methods of preparation.

2. MATERIALS AND METHODS

2.1. Plant material and processing

To obtain "Oliva bianca di Itri" and "Oliva di Gaeta" table olives, olives from the cv. Itrana were hand-harvested at the green stage of ripening at the beginning of November and at the black stage in March, respectively. Olives, size-graded (16-18 mm), were processed as "modified natural olives" (IOC, 2004), by table olive firms from the cities of Rocca Massima, Cori, and Sonnino (Table 1). The olives were immediately immersed in potable water. After 15-45 days, salt was added to the liquid in quantities not exceeding 8 kg per 100 kg of fresh olives. One lot was processed directly in brine utilizing "double- salting" (half the amount of NaCl was added immediately and the remaining part after 15 days). A small lot of these olives were inoculated with a commercial non-specific starter of lactic acid bacteria. The flowchart of processing steps is shown in Figures 1 and 2. After 8-12 months of storage in brine the olives were ready to eat. Representative samples (n = 5) at different times of storage (8 and 12 months for "Oliva di Gaeta" olives and 12-16 months for "Oliva bianca di Itri") were taken out from different tanks by the growers.

Table 1
Code, type of product, origin and information on sampling

Code sample	Type	Origin	Sampling
G1 8M	Oliva di Gaeta	Rocca Massima (LT)	nov-10
G1i 8M	Oliva di Gaeta	Rocca Massima (LT)	nov-10
G5 8M	Oliva di Gaeta	Sonnino (LT)	nov-10
G6 8M	Oliva di Gaeta	Cori (LT)	nov-10
G1 12M	Oliva di Gaeta	Rocca Massima (LT)	mar-11
G6 12M	Oliva di Gaeta	Rocca Massima (LT)	mar-11
G1i 12M	Oliva di Gaeta	Cori (LT)	mar-11
B3 12M	Oliva bianca di Itri	Rocca Massima (LT)	nov-10
B4 12M	Oliva bianca di Itri	Sonnino (LT)	nov-10
B3 16M	Oliva bianca di Itri	Rocca Massima (LT)	mar-11

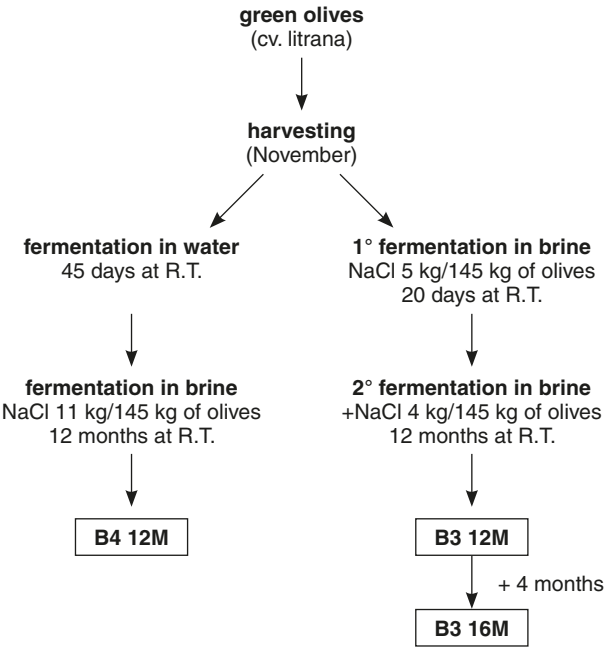

Figure 1
Flow diagram of processing steps for "Oliva bianca di Itri".

2.2. Nutritional analyses

All analyses in this section were carried out in duplicate for each sample. The pH of pulp and brine was measured with a Istek pH Meter 730P model (Istek, Inc. Seoul, South Korea).

The titratable acidity was determined by titrating 20 g of pulp or 30 mL of brine with NaOH 0.1 N solution using phenolphthalein as an indicator 1% (w/v) solution in ethanol. The results are expressed in grams of lactic acid per 100 g of fresh olive flesh or grams of lactic acid per 100 mL of brine.

The sodium chloride content was determined by titrating 20 g of pulp or 5 mL of brine with the standardized silver nitrate solution using potassium chromate as an indicator with 5% (w/v) solution in water (Garrido Fernández *et al.*, 1997). The results are expressed in grams of NaCl per 100 g of fresh olive flesh or grams of NaCl per 100 mL of brine.

Moisture was determined by the official gravimetric AOAC 925.40 method (AOAC, 2000). Samples were dried to constant weight in an air oven at 105 ± 5 °C and weight loss upon drying was expressed as moisture content (%).

Ash content was determined by the direct gravimetric AOAC 923.03 method (AOAC, 2000) which includes ashing of the samples in an oven at 550 °C until constant weight was attained.

Fat content was determined by extraction in a Soxhlet apparatus, according to the procedures described previously for olives (Lanza *et al.*, 2010).

Protein content was estimated based on the total nitrogen content of samples as determined by the Kjeldahl procedure according to the AOAC 955.04 method (AOAC, 2000), using an applicable converting factor (6.25) based on the total nitrogen contents of the proteins in the major compounds of vegetable foods.

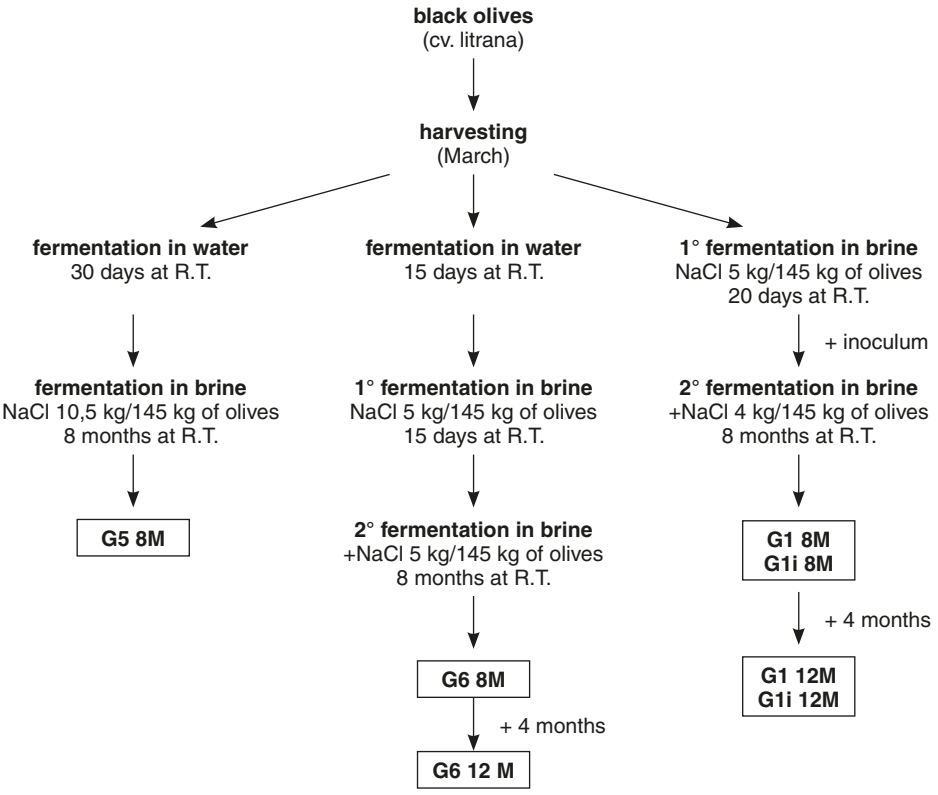

Figure 2
Flow diagram of processing steps for "Oliva di Gaeta".

Carbohydrates were calculated by subtracting the sum of the moisture, fat, protein and ash from 100, which was not reported because it may lead to an overestimation of the amounts of these compounds in table olives, resulting in an error for consumers. Reducing sugars were determined by Fehling's method and expressed as grams of glucose per 100 g of fresh olive flesh.

Total dietary fiber (TDF) was determined according to the enzymatic-gravimetric AOAC 985.29 method (AOAC, 2000) on samples of dried and defatted (because fat content is >10%) material.

Energy, expressed in kilocalories, was calculated as follow: Energy (kcal) = 9 × (g fat) + 4 × (g protein + g sugars) + 2 × (g fiber) (Regulation EU 1169/2011).

The total polyphenols were determined by the Folin-Ciocalteu's assay according to the procedures described previously for olives (Lanza *et al*., 2010). Five grams of olive flesh, pitted and triturated with a grinder, were extracted three times with a total of 100 mL of methanol. After 12 h at –20 °C, the methanolic extract was filtered and centrifuged (3500 rpm, 10 min). An aliquot (0.5 mL) of the methanolic extract was placed in a test tube and mixed with 0.5 mL of Folin-Ciocalteu's phenol reagent (Sigma Chemicals Co., St. Louis, MO, USA). After 3 min, 3 mL sodium carbonate saturated solution (22%, wt/vol) were added to the reaction mixture, which was finally mixed and diluted with water to 10 mL. The solution was allowed to stand for 30 min at room temperature in the dark and then centrifuged (3000 rpm, 10 min). The absorbance of the solution was measured against a blank sample by a Perkin-Elmer Lambda 2 UV/VIS Spectrophotometer (Norwalk, CT, USA) at a wavelength of 725 nm. The calibration curve was constructed using standard solutions of caffeic acid (Sigma Chemicals Co., St. Louis, MO, USA) within the range of 0.02-0.4 mg mL^{-1}. Total polyphenols were expressed as mg of caffeic acid × 100 g^{-1} of fresh pulp.

Sodium and calcium contents were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) according to EPA 6010C (EPA, 2007), after acid digestion according to EPA 3010A (EPA, 1992).

2.3. Analysis of oil fraction

2.3.1. Extraction of oil

To analyze the fat composition, the olive fruits (250 g) were pitted and triturated with a grinder. The olive paste was warmed in a water bath at 28 ± 2 °C for 30 min and the oil was extracted by centrifugation at 5000 rpm for 30 min in a refrigerated centrifuge ALC PK 120R (Thermo Electron Corporation, Waltham, Massachusetts, USA). The resulting surnatant oil, preleased with a pipette pasteur, was filtered in the presence of Na-sulphate anhydrous and stored at 4 °C in aluminum foil wrapped falcon tubes until analyses. This procedure simulates the extraction of olive oil in the olive mill (crushing, mixing and centrifugation) and was used to prevent changes in the oil quality as much as possible.

2.3.2. Fatty acid composition

Fatty acid composition was determined according to the method described in the European Union Commission Regulation EEC/2568/91 and its subsequent modifications Annex X.B (REG. EEC/2568, 1991), using a gas chromatography system (HRGC Mega 2 series 8560; Carlo Erba, Milano, Italy) equipped with a SP™-2380 (Supelco, Bellefonte, PA, USA) fused silica capillary column (60 m × 0.32 mm ID × 0.2 mm film thickness). The oven temperature program was programmed from 70 °C to 165 °C at 20 °C min^{-1} and held at 165 °C for 23 min, from 165 °C to 200 °C at 1.5 °C min^{-1} and held at 200 °C for 5 min, from 200 °C to 220 °C at 2 °C min^{-1} and held at 220 °C for 5 min. The detector temperature was 230 °C. Hydrogen was used as the carrier gas at a column head pressure of 60 kPa. The sample (0.4 µL) was injected *on-column*. The analysis was carried out in duplicate for each sample.

2.3.3. Sterol composition

The sterol profile and contents of the olive oil samples were determined according to the European Union Commission Regulation EEC/2568/91 and its subsequent modifications Annexes V and XIX (REG. EEC/2568, 1991). The olive oil, with added α-cholestanol as internal standards, was saponified with 2 N ethanolic potassium hydroxide, and the unsaponifiable matter was extracted with ethyl ether. The sterol fractions were separated from the extract by thin-layer chromatography on a basic gel plate, then recovered from the plate and transformed into trimethylsilyl ethers, and the mixture was analyzed by an HRGC 5160 Mega series (Carlo Erba, Milano, Italy) equipped with a *Zebron Phenomenex* ZB-5 capillary column (30 m × 0.32 mm ID × 0.25 µm film thickness). The gas chromatographic conditions for sterols were as follows: column temperature 265 °C; hydrogen was used as the carrier gas at a column head pressure of 50 kPa; split ratio 1:50 and substance amount injected into the split system 1 µL; injector and detector temperatures were respectively 280 °C and 290 °C. The gas-chromatographic conditions for alcoholic fractions were as follows: the initial isotherm was set at 180°C for 8 min and then programmed at 5 °C min^{-1} to 265 °C and a further 15 min at 265 °C; the injector and detector temperatures were 280 °C and 290 °C, respectively. The analysis was carried out in duplicate for each sample.

2.4. Determination of colour

The surface color of the fruits was measured using a Color-view spectrophotometer (Konica Minolta

Optics, 2970 Ishikawa-machi, Hachioji, Tokyo, Japan; Model CM-2600D). Color was expressed in terms of CIE (Commission Internationale de l'Eclairage) L* (lightness), a* (redness/greenness), b* (yellowness/blueness) and their derivates Chroma ($C = \sqrt{a^{*2} + b^{*2}}$). Data are the mean of 10 olives.

2.5. Sensory evaluation of end product

The organoleptic characteristics of table olives were evaluated by tasters of the CRA-OLI Città Sant'Angelo Panel, according to the COI/OT/MO No 1/Rev. 2. *Method for the sensory analysis of table olives* (IOC, 2011). The attributes evaluated were: negative sensations (abnormal fermentation, cooking effect, rancid, winey-vinegary, musty and other defects eventually present), gustatory sensations (salty, bitter, acidic) and kinaesthetic sensations (hardness, fibrousness and crunchiness). The Table Olive Profile Sheet uses a ten-point intensity scale ranging from 1 (no perception) to 11 (extreme). All analyses were carried out in duplicate for each sample. To elaborate sensory data, the method for calculating the median and the confidence intervals contained in Annex 1 (COI/OT/MO/n°1/Rev.2 Annex 1 *Method for calculating the median and the confidence intervals*) (IOC, 2011) was applied, taking into account those attributes with a robust coefficient of variation of 20% or less. The computer program for carrying out the calculations is presented in Annex 3 (COI/OT/MO/n°1/Rev.2 Annex 3 *Sensory analysis of table olives computer program*) (IOC, 2011). For classification purposes, only the median of the defect predominantly perceived (DPP) i.e. perceived with the greatest intensity was considered. According to the intensity of DPP, the samples shall be classified into four categories:

- Extra or Fancy: DPP ≤ 3
- First, 1st, Choice or Select: 3 < DPP ≤ 4.5
- Second, 2nd or Standard: 4.5 < DPP ≤ 7.0
- Olives that may not be sold as table olives: DPP > 7.0

2.6. Statistical analysis

Chemical, physical, nutritional and sensorial data were subjected to principal component analysis (PCA) and cluster analysis (CA). Both data elaborations were carried out by the software Past PAleontological STatistics (Version 2.12, Øyvind Hammer, Natural History Museum, University of Oslo).

3. RESULTS AND DISCUSSION

Table olives are a complete food from a nutritional point of view (Garrido-Fernández, 2008). It is a drupe consisting primarily of water, fat, carbohydrates, protein, fiber, pectin, biophenols, vitamins, organic acids and mineral elements (Montano *et al*., 2010). The quality of this product is linked to the combined effect of various factors, such as the suitability of raw materials, processing technologies, nutritional composition and, in no small measure, sensory properties (Lanza *et al*., 2010, Lanza, 2012).

The different organoleptic characteristics (abnormal fermentation, other defects, salty, bitter, acid, hardness, fibrousness and crunchiness) evaluated by the tasters of the CRA-OLI Città Sant'Angelo Panel, according to the COI/OT/MO No 1/Rev. 2. *Method for the sensory analysis of table olives* (IOC, 2011), have contributed to define a sensory profile of the different products (Figure 3 a-f). Unpleasant sensations are caused by the production of substances responsible for off-odors, which are not present in the fresh fruit or formed during well-performed processing treatments. The term "*abnormal fermentation*" includes all those olfactory sensations perceived directly or retronasally, reminiscent of the odor of decomposing organic matter, cheese, butter, rotten eggs, muddy sediment, sewer, rotten leather, caused by the development of contaminating microorganisms (butyric fermentation, putrid fermentation and zapateria). Defected samples G5 8M and B4 12M show, through the occurrence of the defect "abnormal fermentation", a decrease in kinaesthetic properties. *Winey-vinegary* is an olfactory'gustatory sensation reminiscent of wine or vinegar. The defect winey-vinegary is due to alcoholic fermentation by yeasts, while the feeling of acid defines the taste associated with acids naturally present or produced during the lactic fermentation by homo and hetero fermentative lactic acid bacteria. Defected sample G1i 8M and G1i 12M show the occurrence of the defect "winey-vinegary". Taking into account the median of the defect perceived with the greatest intensity (defect predominantly perceived or DPP) the "Oliva di Gaeta" G1i 8M, G1i 12M and "Oliva Bianca di Itri" B4 12M remained in the "Extra or Fancy" category (median value of DPP ≤ 3), whereas "Oliva di Gaeta" G5 8M is classified into the "First, 1st, Choice or Select" category (3 < median value of DPP ≤ 4.5).

The nutritional data, reported in Tables 2 and 3, have referred to an average value deriving only of un-defected samples.

Currently the provision of a nutrition label is regulated by Regulation (EU) 1169/2011 of the European Parliament and of the Council on nutrition labelling for foodstuffs with regards to recommended daily allowances, energy conversion factors and definitions. The information written on the label should refer to 100 g of product: in the case of whole olives, reference should be made to 100 g of drained product (therefore considering the stone even if not edible) or 100 g of edible portion (in this case only the pulp of olives). For pitted olives and olive paste this problem does not arise. The nutritional information may also be referred to as a portion or "serving size", based on the amount

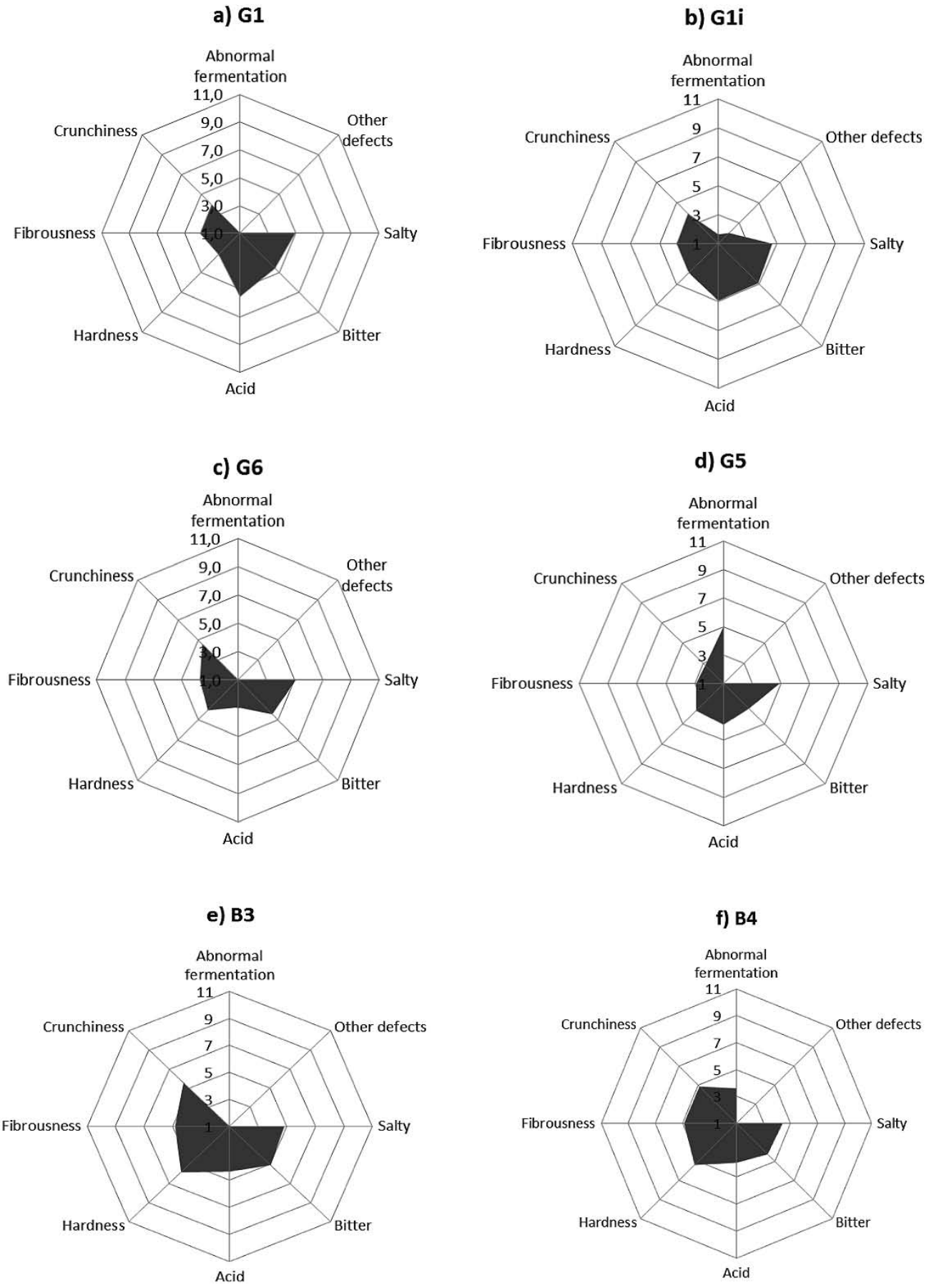

Figure 3
Sensory profiles of a) G1; b) G1i; c) G6; d) G5; e) B3 and f) B4.

of food consumed by a person. For table olives, a serving size could be formed by about 10 medium-sized olives and expressed in grams (taking into account the weight of the stone). It is also useful to relate the content of each nutrient with a daily reference value for a diet of 2000 kcal of an average weight adult performing limited physical activity. Percentages above 20% are considered significant, below 5%, modest.

The protein content is low (1.4-1.5 g 100 g^{-1} e.p.; Tables 2 and 3), but nutritional quality is high due to the presence of essential amino acids for adults (threonine, valine, leucine, isoleucine, phenylalanine and lysine), and for children (arginine, histidine and tyrosine) (Lanza *et al*., 2010; Lazovic *et al*., 1999; Lopez *et al*., 2007; López-López *et al*., 2010b).

The carbohydrate content in the olive fruit is, by itself, lower than any other edible fruit. However, table olives have even lower proportions of these compounds because during the fermentation processes or brine storage the microorganisms present in brines consume sugars. Therefore, table olives can be considered as practically free of sugar products. Therefore, the calculation of total carbohydrates as difference, as must be made for nutritional labeling in the US, may lead to an overestimation of the amounts of these compounds in table olives, resulting in an error for consumers.

These table olives are a good source of dietary fiber (Tables 2 and 3), which in addition, have a high digestibility rate (Jiménez *et al*., 2000; López-López *et al*., 2007). In Italy is possible to write the claim "source of fiber" on the label if the product contains at least 3g of fiber 100g^{-1} of e.p. The Italian recommended daily allowance (RDA) of fiber is 23 g day^{-1}, and Itrana table olives have a fiber content of 3.6-4.0 g 100 g^{-1} of e.p., so it can be considered as a source of fiber, representing about 16-17% of RDA.

It is worth highlighting its mineral content. Itrana olives contain discrete amounts of calcium (21.9-28.9 mg 100 g^{-1} e.p.) (Tables 2 and 3). The sodium (1.2-1.5 g 100 g^{-1} e.p.) content does not contrast with the sodium RDA of 2.4 g day^{-1} (considering the limit of 6 g of NaCl in Regulation EU 1169/2011 and a conversion factor = 2.5).

Both table olives are, despite debittering due to fermentation, still rich in natural antioxidants such as polyphenols, but "Oliva bianca di Itri" has a lower content (110 mg 100 g^{-1} in olive pulp; Tables 2) with respect to "Oliva di Gaeta" (239 mg 100 g^{-1} in olive pulp; Tables 3). The antioxidant capacity of biophenols and their functional effects on human wellbeing are ascertained (Baiano *et al*., 2009; Ben Othman *et al*., 2008; Boskou *et al*., 2006).

Table 4 shows the detailed fatty acid composition (relative percentage within the lipid fraction) of oil extracted from from Itrana olives. Oleic acid is the predominant one (76.5-80.7%), palmitic acid was the second most abundant fatty acid (10.1-13.6%), followed by linoleic acid (5.2-5.5%) and stearic acid (1.1-1.4%), a pattern common to most reported data (Lanza *et al*., 2010;

Table 2
Average nutritional and chemico-physical characteristics of cv. Itrana "Oliva bianca di Itri"

Nutrients/100g e.p.	Oliva bianca di Itri	Reference values Regulation EU 1169/2011	% RDA
Energy (kcal)	193 ± 11	2000	10
Ash (g)	4.9 ± 1.0		
pH	4.3 ± 0.6		
Titratable acidity (g)[a]	0.3 ± 0.1		
Proteins (g)	1.5 ± 0.0	50	3
Carbohydrates (g)	ND[d]	260	
Sugars (g)[b]	0.7 ± 0.3	90	1
Fat (g)	17.7 ± 1.4	70	25
• Saturated (g)	2.8 ± 0.1	20	14
• Mono-unsaturated (g)	14.0 ± 1.3	40[e]	35
• Poly-unsaturated (g)	0.9 ± 0.1	9[e]	10
Fibre (g)	3.6 ± 1.1	23[e]	16
Sodium (g)	1.2 ± 0.3	2.4	50
Calcium (mg)	21.9 ± 2.3	800	3
Total polyphenols (mg)[c]	110 ± 2		

[a] expressed as g lactic acid; [b] as reducing sugars; [c] expressed as mg caffeic acid; [d] not determined because the calculation of total carbohydrates as difference may lead to an overestimation of the amounts of these compounds in table olives, resulting in an error for consumers; [e] Larn, 1996.

Table 3
Average nutritional and chemico-physical characteristics of cv. Itrana "Oliva di Gaeta"

Nutrients 100g^{-1} e.p.	Oliva di Gaeta	Reference values Regulation EU 1169/2011	% RDA
Energy (kcal)	235 ± 55	2000	12
Ash (g)	4.7 ± 1.0		
pH	4.2 ± 0.1		
Titratable acidity (g) [a]	0.4 ± 0.1		
Proteins (g)	1.4 ± 0.0	50	3
Carbohydrates (g)	ND[d]	260	
Sugars (g) [b]	0.6 ± 0.0	90	1
Fat (g)	21.7 ± 6.3	70	31
• Saturated (g)	2.7 ± 0.9	20	14
• Mono-unsaturated (g)	17.7 ± 4.9	40 [e]	44
• Poly-unsaturated (g)	1.3 ± 0.4	9[e]	14
Fibre (g)	4.0 ± 0.8	23[e]	17
Sodium (g)	1.5 ± 0.0	2.4	63
Calcium (mg)	28.9 ± 3.6	800	4
Total polyphenols (mg) [c]	239 ± 32		

[a] expressed as g lactic acid; [b] as reducing sugars; [c] expressed as mg caffeic acid; [d] not determined because the calculation of total carbohydrates as difference may lead to an overestimation of the amounts of these compounds in table olives, resulting in an error for consumers; [e] Larn, 1996.

Sousa *et al*., 2011; Sakouhi *et al*., 2008; López *et al*., 2006; López-López *et al*., 2010a; Ünal and Nergiz, 2003; Issaoui *et al*., 2011). The composition of fatty acids in the analyzed samples, as expected, showed a similar composition to EVOO produced from the same cultivar (Poiana *et al*., 2001). In "Oliva di Gaeta" with respect to "Oliva bianca di Itri", MUFA were most abundant (82.1 *vs.* 78.3%), SFA less (12.2 vs. 15.8%) and PUFA represented about 6.0%. The intake of α-linolenic acid (C18:3 ω3), a precursor to the synthesis of long chain ω3 fatty acids, is appreciable (0.5-0.6%) but the ratio ω6:ω3 it is still too far towards ω6 and depends on the cultivar (9.1-11.6). Several sources of information suggest that human beings evolved on a diet with a ratio of ω6 to ω3 essential fatty acids of ~1 whereas in Western diets the ratio is 15-16.7. Western diets are deficient in omega-3 fatty acids, and have excessive amounts of omega-6 fatty acids compared with the diet on which human beings evolved and their genetic patterns were established (Simopoulos, 2008). The ratio of oleic to palmitic acid in dietary fats has a regulatory influence on certain thrombogenic and fibrinolytic markers during the postprandial state in healthy subjects (Pacheco *et al*., 2006). It has a recommended ratio of at least 5. The oleic/palmitic ratio for both products is > 5.0 (Table 5). The polyunsaturated/saturated fatty acid (PUFA/SFA) ratio is also used to assess the nutritional quality of the lipid fraction in foods. The consumption of saturated fatty acids has been associated with coronary heart disease (Serrano *et al*., 2005). Consequently, nutritional guidelines have recommended that the PUFA/SFA ratio should be above 0.4–0.5 (Wood *et al*., 2008). The PUFA/SFA ratio for Itrana table olives is about 0.4-0.5 (Table 4).

In regards to sterol composition, the main phytosterols and phytostanols found in Itrana table olives (Table 5) are β–sitosterol (87.8-88.7%) and Δ5-avenasterol (4.3-5.6%), followed by campesterol (2-4-2.6%), chlerosterol (1.0%), Δ7-campesterol (0.6-0.8%) and Δ5,24-stigmastadienol (0.4-0,6%). β-sitosterol (including Δ5,23-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol) is apparantly ≥93% the limit fixed by European Union Commission Regulation EEC/2568/91 and its subsequent modifications (REG. EEC/2568, 1991) for EVOO, for both the products. The composition of sterols in the analyzed samples, as expected, showed a similar composition to EVOO produced from the same cultivar (Poiana *et al*., 2001). Epidemiologic and experimental studies suggest that dietary phytosterols, and in particular β-sitosterol, may offer protection from the most common cancers in Western societies, such as colon, breast and prostate cancer and contribute to lowering the risk of cardiovascular disease. (Awad and Fink, 2000, Woyengo *et al*., 2009).

Table 4
Fatty acid composition percentage. Data are expressed as mean values ± standard deviation

Fatty acids	Range EVOO Regulation EEC/2568/91	Monovarietal EVOO from cv. Itrana[a]	Oliva bianca di Itri	Oliva di Gaeta
C14:0	≤ 0.05	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C16:0	7.5-20.0	14.0 ± 0.6	13.6 ± 0.9	10.1 ± 0.7
C16:1 ω7	0.3-3.5	2.1 ± 0.3	1.4 ± 0.2	1.1 ± 0.2
C17:0	≤ 0.3	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
C17:1 ω7	≤ 0.3	0.3 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
C18:0	0.5-5.0	1.4 ± 0.1	1.8 ± 0.1	1.7 ± 0.1
C18:1 ω9	55.0-83.0	77.6 ± 0.9	76.5 ± 1.6	80.7 ± 1.3
C18:2 ω6	3.5-21.0	3.1 ± 0.2	5.5 ± 1.2	5.2 ± 0.4
C20:0	≤ 0.6	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
C18:3 ω3	≤ 1.0	0.8 ± 0.1	0.5 ± 0.2	0.6 ± 0.1
C20:1 ω9	≤ 0.4	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
C22:0	≤ 0.2	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
C24:0	≤ 0.2	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
SFA		15.8 ± 0.2	15.8 ± 1.0	12.2 ± 0.7
MUFA		80.3 ± 0.4	78.3 ± 1.5	82.1 ± 1.1
PUFA		3.9 ± 0.1	6.0 ± 1.4	5.8 ± 0.4
Oleic/Palmitic		5.5	5.6	8.0
MUFA/SFA		4.9	5.0	6.8
ω6/ω3		3.9	11.6	9.1
PUFA/SFA		0.2	0.4	0.5
(MUFA + PUFA)/SFA		5.2	5.4	7.2

[a] source Poiana *et al.* (2001)

The skin color of "Oliva Bianca di Itri" is a pale pink whereas the color of "Oliva di Gaeta" olives is purple-black, due to the modifications in chlorophyll, carotenoid and anthocyanin contents occurring during treatments, taking into account the ripening stage (Table 6).

In the course of the fermentation lactic acid is produced from glucose. Analyzing conditioning brines, pH results in < 4.3 and the titratable acidity, expressed in grams of lactic acid per 100 mL^{-1} of brine, results in > 0.3 in all the samples. These limits are recommended by IOC standard (2004) for "natural olives". On the contrary, the safe limit of 6 g of NaCl per 100 mL^{-1} of brine, which is indicated by the IOC standard, is found in only 60% of the samples analyzed (Table 7).

The principal component analysis (PCA) of a matrix constructed considering all the information derived from the chemico-physical, nutritional and sensory data was carried out. PCA generates a set of new orthogonal variables (axes), the principal components (PC), linear combinations of the original variables, so that the maximum amount of variance contained in the starting data set (information) is concentrated in the first principal components. The scores of data plotted on the first two principal components showed quite distinctly "Oliva bianca di Itri" and "Oliva di Gaeta" samples (Fig. 4). Therefore, PCA is suitable for reducing the dimensionality of large data matrices by eliminating the non-significant principal components and facilitating successive analyses on the reduced data. The new data matrix of PCs was directly subjected to cluster analysis (CA). Only PCs contributing more than 0.1% of the total variance are retained. CA has separated the samples into four groups, related to techno-processing, ripening stage and storage (Figure 5).

4. CONCLUSIONS

Regarding the product "Oliva bianca di Itri", the olives processed according to the method that contemplates immersion in water for 45 days before brining (B4), show the occurrence of defects. The

Table 5
Sterolic composition percentage. Data are expressed as mean values ± standard deviation

Sterols	Range EVOO Regulation EEC/2568/91	Monovarietal EVOO from cv. Itrana[a]	Oliva bianca di Itri	Oliva di Gaeta
Cholesterol	≤ 0.5	0.2 ± 0.1	0.5 ± 0.3	0.4 ± 0.1
Brassicasterol	≤ 0.1	0.0 ± 0.0	0.1 ±0.1	0.0 ± 0.0
24-methylen cholesterol		0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Campesterol	≤ 4.0	2.6 ± 0.2	2.4 ± 0.2	2.6 ± 0.2
Campestanol		0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Stigmasterol	< campesterol	0.8 ± 0.2	0.7 ± 0.3	0.4 ± 0.1
Δ7-campesterol		0.0 ± 0.0	0.6 ± 0.6	0.8 ± 1.0
Δ5,23-stigmastadienol		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Clerosterol		1.2 ± 0.4	1.0 ± 0.2	1.0 ± 0.3
β-sitosterol		82.4 ± 1.9	88.7 ± 1.4	87.8 ± 1.4
Sitostanol		0.8 ± 0.2	0.7 ± 0.4	0.6 ± 0.1
Δ5-avenasterol		10.7 ± 1.7	4.3 ± 0.7	5.6 ± 0.4
Δ5,24-stigmastadienol		0.3 ± 0.1	0.6 ± 0.2	0.4 ± 0.3
Δ7-stigmastenol	≤ 0.5	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
Δ7-avenasterol		0.4 ± 0.2	0.2 ± 0.2	0.2 ± 0.1
β-sitosterol apparent[b]	≥ 93.0	95.3 ± 0.7	95.2 ± 1.3	95.3 ± 1.0

[a] source Poiana *et al.* (2001); [b] β-sitosterol apparently includes Δ5,23-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol.

Table 6
Skin color characteristics of the end products. Data are expressed as mean values ± standard deviation

Code sample	L	a*	b*	C
G1 8M	23.39 ± 5.04	16.72 ± 1.26	9.48 ± 4.54	19.59 ± 2.47
G1 12M	16.66 ± 1.22	21.43 ± 1.71	10.27 ± 1.17	24.15 ± 0.74
G1i 8M	23.30 ± 12.05	16.33 ± 4.22	6.93 ± 4.60	21.88 ± 4.45
G1i 12M	21.85 ± 4.05	18.51 ± 2.82	11.01 ± 2.32	21.60 ± 3.26
G5 8M	31.26 ± 2.28	20.50 ± 2.14	13.54 ± 2.66	24.61 ± 3.07
G6 8M	22.65 ± 2.63	19.91 ± 3.02	13.44 ± 2.47	24.07 ± 3.50
G6 12M	8.84 ± 2.38	17.53 ± 2.16	9.70 ± 0.57	20.39 ± 1.37
B3 12M	45.02 ± 3.73	11.51 ± 1.15	41.17 ± 5.73	42.77 ± 5.68
B3 16M	44.22 ± 2.01	40.82 ± 1.19	39.02 ± 2.46	39.61 ± 1.17
B4 12M	43.68 ± 1.28	13.48 ± 4.74	35.15 ± 8.22	38.03 ± 5.92

immediately double-salting method provides the best results (B3).

Regarding the product "Oliva di Gaeta", the olives processed according to the method that contemplates immersion in water for 30 days before brining (G5) show the occurrence of defects. The immediate double-salting (G1) method and salt addition after 15 days of immersion in water (G6), are the methods that assure the best results. The inoculum did not work and resulted in a product which was harder and more bitter with respect to the olives not inoculated.

The cluster analysis highlights the absolute correspondence of the methodology used during storage as well.

In the present study we evaluated the nutritional and sensory characteristics of these table olives in relation to the modified Greek methods of

Table 7
Chemico-physical characteristics of brines. Data are expressed as mean values ± standard deviation

Code sample	pH	Titratable acidity[a]	NaCl[b]
G1 8M	3.6 ± 0.1	1.5 ± 0.4	5.8 ± 0.3
G1 12M	3.5 ± 0.4	1.2 ± 0.1	5.8 ± 0.3
G1i 8M	3.9 ± 0.2	1.0 ± 0.3	6.0 ± 0.5
G1i 12M	4.0 ± 0.2	1.1 ± 0.5	6.4 ± 0.2
G5 8M	3.4 ± 0.1	1.0 ± 0.5	6.7 ± 0.4
G6 8M	3.8 ± 0.1	0.8 ± 0.2	6.3 ± 0.4
G6 12M	4.3 ± 0.1	0.5 ± 0.0	6.6 ± 0.1
B3 12M	4.0 ± 0.1	1.1 ± 0.4	5.1 ± 0.3
B3 16M	4.2 ± 0.1	0.9 ± 0.1	5.5 ± 0.1
B4 12M	3.9 ± 0.2	1.3 ± 0.5	6.2 ± 0.6

[a] expressed as g lactic acid 100 mL^{-1} of brine; [b] expressed as g NaCl 100mL^{-1} of brine.

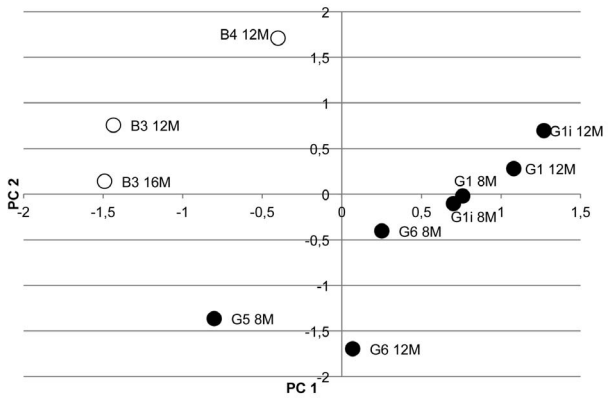

Figure 4
PCA scatter diagram of the first two principal components. White mark = "Oliva Bianca di Itri"; black mark = "Oliva di Gaeta".

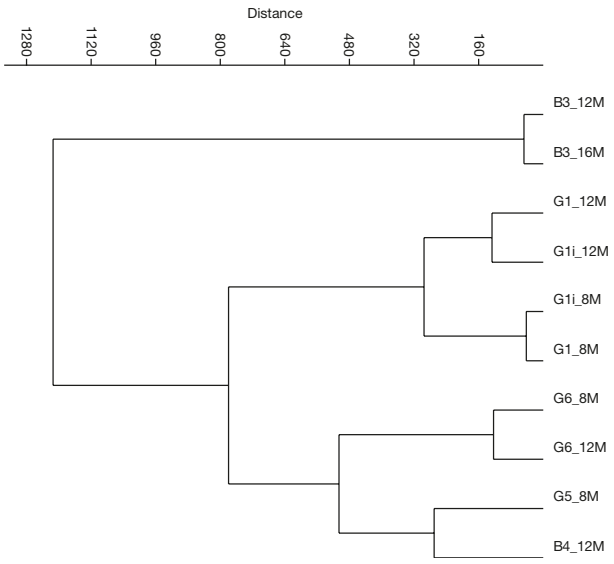

Figure 5
Dendrogram showing clusters of table olive samples.

preparation. In the future we will evaluate the microbiological aspects for choosing the best inoculum to recreate the optimal conditions of debittering and flavor producing.

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