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Effect of natural-style processing on the oxidative and hydrolytic degradation of the lipid fraction of table olives

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ABSTRACT

The aim of this work was to determine the effect of natural-style processing, by using three different cultivars, on the oxidative and hydrolytic degradation of the lipid fraction of table olives. Samplings were made at harvest and after 4 and 8 months of processing. The Italian table olive cultivars Bella di Cerignola, Termite di Bitetto, and Peranzana, diffused in the Apulia region, were considered. During processing, the total phenols content of the lipid fraction decreased from 381–417 mg/kg oil to 106–125 mg/kg oil, depending on the cultivar. The level of primary oxidation at the end of processing was relatively low, as compared to high quality lipids such as extra virgin olive oil: peroxide value and oxidized triacylglycerols accounted for 11.7–13.1 meq O_2/kg oil and 3.3–7.1 g/kg respectively, depending on the cultivar. A very modest secondary oxidation occurred, as shown by final amounts of triacylglycerol oligopolymers ranging from 0.6 to 0.9 g/kg. On the contrary, a relevant hydrolytic degradation was observed, with free fatty acids accounting for 2.04–2.25 g/100 g oleic acid and diacylglycerols in the range 45.4–48.0 g/kg, at the end of processing. The polar compounds, mainly represented by diacylglycerols, reached values in the ranges 41.1–55.9 g/kg after 4 months and 58.0–62.0 g/kg after 8 months.

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1. Introduction

Table olives are one of the main fermented food products, with an important economic role in the Mediterranean countries. Apulia region is the second producer in Italy, after Sicily, accounting for 24.7% of the total production (UNAPROL, 2010). Some Apulian table olive cultivars have been grown since ancient times in restricted areas of origin, that is a pre-requisite to be awarded by Protected Designation of Origin (PDO) mark, according to the requirements of the EC Commission Reg. no. 510/06. In particular, the "Bella della Daunia" PDO is referred to olives of cv. Bella di Cerignola harvested in the area of Foggia (Apulia) (EC Commission Reg. no. 1904/2000), and the olives of Termite di Bitetto cultivar have been included in the list of the Italian traditional agri-food products (Decree of the Italian Agricultural Ministry of June 7th 2012, sub-list Apulian products).

The main purpose of table olive processing is to remove the natural bitterness of fruits, mainly due to oleuropein, and to preserve them from microbiological spoilage (Garrido, Fernández, & Adams, 1997). Debittering involves hydrolysis of oleuropein into less bitter forms that can leach out of the olive and be removed. Hydrolysis can be either chemical or enzymatic (Charoenprasert & Mitchell, 2012). Various studies point out the role played by the fermenting brine microflora: some lactic acid bacteria strains and yeasts are able to degrade oleuropein during the brining of fruits (Marsilio, Lanza, & Pozzi, 1996; Servili et al., 2006).

Table olive processing is mainly conduced according to three methods, called Spanish (or Seville), Californian, and Greek, the latter also referred to as "natural-style" (Sánchez, García, & Rejano, 2006). Among them, the most time-consuming is the natural-style method because debittering is achieved by in-brine treatment only (NaCl 8–10% w/v), without the help of preliminary alkaline hydrolysis. The elimination of bitterness is due to diffusion of a portion of the phenolic compounds into the brine, and equilibrium is reached in 8–12 months (Sánchez et al., 2006). Indeed, the final product usually retains a slight bitter taste (Romero, Brenes, García, García, & Garrido, 2004).

Many studies have been carried out about the influence of different processing methods of table olives on the levels of: total and single phenolics (Brenes, Rejano, García, Sánchez, & Garrido Fernández, 1995; Marsilio, Campestre, & Lanza, 2001; Piga, Gambella, Vacca, & Agabbio, 2001; Pereira et al., 2006; Poiana &

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Romeo, 2006; Romero, Brenes, García, et al., 2004; Romero, Brenes, Yousfi, et al., 2004); α-tocopherol (Sakouhi et al., 2008); fatty acids (Sakouhi et al., 2008; Ünal & Nergiz, 2003); sugars (Marsilio, Campestre, Lanza, & De Angelis, 2001); fiber and minerals (Ünal & Nergiz, 2003); polyols (Marsilio, Campestre, Lanza, & De Angelis, 2001); volatile compounds (Sabatini & Marsilio, 2008); antioxidant potential and antimicrobial activity (Pereira et al., 2006). On the contrary, few studies have been aimed to assess if process affects the quality of the lipid fraction: López, Rodríguez, Cortés, Montaño, and Garrido (2009) studied the influence of Californian-style processing on oil characteristics and composition, and López, Cortés, and Garrido (2011) characterized the lipids released during the conditioning processes of table olives, such as pitting and stuffing.

Due to prolonged processing according to natural-style method, the lipid fraction could undergo oxidative and hydrolytic degradations. Moreover, some intermediate and final products of oxidative degradation of lipids may have harmful effects on consumers health (Billek, 2000; Saguy & Dana, 2003). However, at our best knowledge, no studies have been carried out about the variations induced on the lipid fraction of table olives by natural-style processing.

The aim of this work was to assess the hydrolytic and oxidative degradation level of the lipid fraction induced by natural-style processing of table olives. Three Italian table olive cultivars diffused in the Apulia region were considered: Bella di Cerignola, Termite di Bitetto, and Peranzana.

2. Materials and methods

2.1. Samples

Olives of the Italian cultivars Bella di Cerignola, Peranzana, and Termite di Bitetto were collected at the completely black-ripe stage (a lot of about 30 kg per trial, for each cultivar) at the experimental field of the Olive Pre-multiplication Centre Concadoro at Palagiano (Italy). Two processing trials were performed per each cultivar by collecting the olives from different trees. Olives (three sub-lots of about 10 kg per trial so as to make three replicates per trial) were processed separately with 8% (w/v) NaCl brine, according to the so-called "natural-style" method, at a local olive-processing factory (Puglia Conserve, Modugno, Italy). Plastic tanks having the capacity of 50 L were filled with the olives, then with brine, and kept at ambient temperature for 8 months. The olives were maintained submerged in the brine by a perforated cap. During the first 10 days, the lids of the containers were not hermetically closed to allow the CO_2 derived from fermentation and fruit respiration to escape. After that, the tanks were closed. The brine concentration was kept constant throughout the fermentation process by means of regular corrections: weekly during the first month and monthly afterwards. Samplings were effected at 4 and 8 months.

2.2. Analyses of physical and chemical characteristics of the olives

Physical characteristics of olives at harvest (mean weight, flesh-to-pit mean ratio and main caliper class of transversal diameter) were determined on 50 fruits per cultivar, randomly sampled in the whole batch. The chemical parameters were determined on olive pulp, after manual de-pitting and homogenization (Omni Mixer 17108, Sorvall Omni International, Kennesaw, GA), both at harvest and after 4 and 8 months of in-brine processing. For the determination of titratable acidity and pH, 50 g of homogenized pulp were diluted in 100 mL of distilled water to obtain a fluid slurry. The titratable acidity (expressed as lactic acid) was measured with 0.1 N NaOH up to pH 8, while for pH was used a pH-meter (Orion, Boston, MA). The reducing sugars were determined by Fehling method according to AOAC no. 925.02 (AOAC, 2003). The oil content was determined, after drying the olive pulp at 105 °C for 24 h, by Soxhlet extraction with 40–60 °C petroleum ether for 6 h, followed by rotary evaporation of the solvent (IUPAC method 1.122). All analyses were carried out in duplicate.

2.3. Analyses of chemical characteristics of the oil fraction

Samples of 1 kg of olives were manually de-pitted, finely minced for 10 min with a HR 2881 Storemaster electronic blender (Philips, Eindhoven, the Netherlands) and centrifuged at 9000 *g* for 15 min to recover the oil fraction. The determinations of free fatty acids (expressed as g oleic acid per 100 g oil) and peroxide value (expressed as meq O_2 per kg oil) of the oil extracted were carried out as described in the EC Commission Reg. no. 1989/2003, Annexes II and III, respectively (European Commission, 2003). The polar compounds were separated by silica gel column chromatography from the oil extracted, according to the AOAC method no. 982.27 (AOAC, 2003). After elution of the non-polar components with 150 mL of petroleum ether–diethyl ether (87:13, v/v), the polar compounds were recovered with 150 mL of diethyl ether. The efficacy of separation was checked by thin layer chromatography as recommended by the same method. Then, the polar compounds, recovered in dichloromethane, were analyzed by means of High Performance Size-Exclusion Chromatography (HPSEC) using dichloromethane as eluant at flow rate of 1 mL/min. The HPSEC system consisted of a pump (series 200, Perkin–Elmer, Norwalk, CT, USA) with Rheodyne injector, a 50 μL loop, a PL-gel guard column (Perkin–Elmer, Norwalk, CT, USA) of 5 cm length × 7.5 mm i.d., and a series of three PL-gel columns (Perkin–Elmer, Norwalk, CT, USA) of 30 cm length × 7.5 mm i.d. each. The columns were packed with highly cross-linked styrene-divinylbenzene copolymer with particle size of 5 μm and a pore diameter of 500, 500 and 100 Å, respectively. The refractive index detector was a series 200 (Perkin–Elmer, Norwalk, CT, USA) connected to an integrator. Peak identification and quantification were carried out as described in Gomes (1992). To determine the total phenols content, 15 g of oil were mixed with 30 mL of *n*-hexane, placed into a separating funnel and extracted three times by vigorous shaking with 20 mL methanol:water 60:40 (v/v) per time. The methanol:water phases were recovered, reunited (about 60 mL), and washed with 30 mL of *n*-hexane. The clean methanol:water phase was then submitted to rotary evaporation. The residue was dissolved in 5 mL methanol and submitted to quantitative determination of total phenols by spectrophotometric measure at 765 nm after reaction with Folin–Ciocalteu reagent (Sigma–Aldrich, Buchs, Switzerland). The results were expressed as mg of gallic acid per kg of oil. All the analyses were carried out in duplicate.

2.4. Statistical analyses

Data were submitted to statistical analysis by XLStat software (Addinsoft SARL, New York, NY, USA). One-way analysis of variance (ANOVA) was performed, followed by the Tukey HSD test for multiple comparisons.

3. Results and discussion

The main quality characteristics of olives at harvest (Table 1) evidenced good attitudes to table processing for all the cultivars, according to the trade standard of table olives (IOOC, 2004). In particular, the flesh-to-pit ratio was above 5 and the main caliper class of the transversal diameter ranged from 15–16 mm to 22–23 mm. The highest weight was observed in Bella di Cerignola,

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