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Combined effects of reduced malaxation oxygen levels and storage time on extra-virgin olive oil volatiles investigated by a novel chemometric approach

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ABSTRACT

Combined effects of oxygen level reduction in the malaxation headspace and storage time up to 6 months on the volatile composition of a monovarietal extra-virgin olive oil (EVOO), obtained from cv. Carboncella olives, were investigated by applying a full factorial design approach (4 oxygen levels × 4 storage times) on EVOOs extracted on an industrial scale in two mills, equipped with "two-phase" and "three-phase" centrifugation systems, respectively. The outcoming data were analysed by the chemometric technique called ANOVA-simultaneous component analysis (ASCA).

Both reduction of oxygen malaxation levels and storage time significantly affected the volatile profile of the extracted EVOOs. Reduction of oxygen malaxation levels hindered the formation of lipoxygenase derived volatiles (hexanal, 1-hexanol, (Z)-2-hexenal, (E)-2-hexen-1-ol, (Z)-2-penten-1-ol, 2,4-hexadienals), whereas prolonged storage times were associated with increased levels of autoxidation products (octane, hexanal, C10 hydrocarbons) and other compounds that could originate from exogenous microbial activity (1-octen-3-ol, 6-methyl-5-hepten-2-one, benzaldehyde, methyl salicylate).

1. Introduction

The worldwide growing appreciation of olive oil (International Olive Council, 2013) is promoted by its distinctive and highly valued flavour as well as by its nutritional and health-related properties, extra-virgin olive oil (EVOO) representing the top quality among all olive oil commercial grades (García-González & Aparicio, 2010). Volatile compounds, along with phenolic compounds, are the main determinants of sensory quality of EVOO (Angerosa et al., 2004; García-González, Morales, & Aparicio, 2010; Kalua et al., 2007). Most of the key odorants responsible for the positive aroma perceptions of olive oil are secondary volatiles not present in the intact fruit, which are quickly formed through reactions mediated by endogenous plant enzymes when the cell structures are disrupted during the extraction process. First and foremost are the reactions of the lipoxygenase (LOX) pathway, which in presence of oxygen catalyse the oxidative breakdown of unsaturated fatty acids, giving rise to the formation of several C6 and C5 straight-chain alcohols and aldehydes, and the corresponding esters, some of which have been recognised as key odorants of olive oil (Reiners & Grosch, 1998). Besides biochemical pathways, chemical autoxidation plays an important role, particularly during storage, promoting the formation of volatiles mainly associated with undesirable sensory defects (Kalua et al., 2007). Other compounds responsible for off-flavours originate from exogenous enzyme-mediated reactions, linked to the activity of spontaneous microbiota of fresh olives, whose growth is fostered by improper handling of harvested olive fruit before oil extraction (Angerosa et al., 2004; Vichi, Romero, Tous, & Caixach, 2011). However, it is worth mentioning that even though the main pathways of olive oil volatiles formation are known, it is not always straightforward to establish a clear link between a compound and a single formation pathway, because the same compound may potentially arise from several concurrent pathways (Angerosa et al., 2004; Kalua et al., 2007).

In research on olive oil increasing efforts have been recently devoted towards the optimisation of extraction conditions and equipment used in the production process, with the view of obtaining EVOOs of the highest sensory and nutritional quality (García-González et al., 2010; Servili, Taticchi, Esposto, Sordini, & Urbani, 2012). In this quest, investigations on the influence of malaxation conditions on phenolics and volatiles formation have led to the introduction of new top-covered malaxers (Servili

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et al., 2012). The use of these modified devices, in conjunction with the controlled flush of an inert gas into the malaxation chamber, has been proposed as a way to control oxygen contact with the olive paste during the malaxation step and thus to modulate the enzymatic activities that largely shape the final profile of volatiles and phenolics in the extracted oil (Angerosa et al., 2004; Frankel, 2010; Kalua et al., 2007). In particular, reduction of oxygen levels in the malaxation headspace has been shown to effectively minimise losses of phenolic compounds due to oxidation processes promoted by polyphenol oxidases and peroxidases (Servili et al., 2008). On the other hand, lower oxygen levels could, in principle, hinder the concurrent LOX-mediated formation of volatile compounds. Some studies investigating these possible adverse effects on volatiles formation, carried out under different experimental conditions, have provided somewhat contrasting results (Masella, Parenti, Spugnoli, & Calamai, 2011; Sánchez-Ortiz, Romero, Pérez, & Sanz, 2008; Servili, Selvaggini, Taticchi, Esposto, & Montedoro, 2003; Servili et al., 2008; Vezzaro et al., 2011).

Storage is another important factor to take into account, in order to preserve as much as possible the flavour of freshly extracted EVOO, because oxidative processes taking place during storage may markedly alter its volatile profile, giving rise to undesirable sensory defects (Angerosa et al., 2004; Bendini, Cerretani, Salvador, Fregapane, & Lercker, 2010; Frankel, 2010; Kalua et al., 2007). Sensory defects are generally perceived before changes are observed in quality parameters, such as peroxide value and spectrophotometric indices, and this stresses the importance of careful analysis of the volatile fraction to detect early stages of olive oil deterioration (Vichi et al., 2003). Several papers have identified some volatiles as potential markers of undesirable oxidative processes, but many of these compounds are characterised by high odour thresholds and their impact on off-flavour perception has not been clearly established (Kalua et al., 2007). In addition, most of these studies have been performed through high temperature accelerated tests, whose suitability in simulating the storage behaviour of olive oil at ambient temperature remains questionable (Frankel, 2010), and only few studies have been carried out by reproducing real-time shelf life conditions (Kalua, Bedgood, Bishop, & Prenzler, 2006; Kanavouras, Hernandez-Münoz, & Coutelieris, 2004; Stefanoudaki, Williams, & Harwood, 2010). A thorough understanding of factors that promote departure from freshness during storage at ambient temperature, as well as the identification of appropriate freshness markers, is of paramount importance to maintain the highest quality (Aparicio-Ruiz, Aparicio, & García-González, 2014).

The aim of this work was to study combined effects of oxygen level reduction in the malaxation headspace and storage time on volatile composition of a monovarietal EVOO, obtained from cv. Carboncella olives. Olive oil extraction was performed on an industrial scale in two distinct mills, equipped respectively with a continuous "two-phase" and "three-phase" centrifugation system, to explore the effects of the considered experimental factors when combined with different centrifugation systems. Moreover, the obtained EVOOs were bottled without any prior filtration. Even though most EVOOs available on the market are filtered, unfiltered EVOOs are also marketed and preferred by some consumers, who judge their cloudy appearance as an indicator of higher wholesomeness (Tsimidou, Georgiou, Koidis, & Boskou, 2005). The effects of oxygen level in the malaxer headspace, storage time and their interactions on the volatile profile of the extracted EVOOs were investigated by applying a balanced experimental design coupled to a multivariate exploratory data analytical technique called ANOVA–simultaneous component analysis (ASCA), a recently developed tool which overcomes the limitations in analysing multivariate datasets by multivariate ANOVA (Zwanenburg, Hoefsloot, Westerhuis, Jansen, & Smilde, 2011).

2. Materials and methods

2.1. Olive oil extraction process

Two tonnes of olives from cv. Carboncella, grown in the Sabina area of Latium region (Central Italy), were harvested on the same day and processed within 24 h in two distinct industrial olive mills (Pieralisi, Jesi, Italy), only differing in centrifugation system, based on a "two-phase" (P2) and on a "three-phase" (P3) decanter (M2 and M3 model, respectively, of the M series decanter centrifuges; Pieralisi, Jesi, Italy), respectively. In both olive mills the malaxer was modified to modulate oxygen concentration in the malaxation headspace: the top-covered chamber was sealed, connected to a laboratory nitrogen gas tank and equipped with an oxygen sensor. In each olive mill, four trials of malaxation (at 27 °C for 35 min) were performed, by using about 200 kg of olives in each trial. In the first trial (R1) no nitrogen was flushed into the chamber, whereas in the other trials (R2, R3 and R4), increasing amounts of nitrogen were flushed into the chamber, giving place inside it to the oxygen levels reported in Table 1S (Appendix A). In the four trials (from R1 to R4), oxygen levels detected at the end of the malaxation step amounted to 16.6, 4.6, 2.3 and 0.7 kPa, respectively. In the P3 mill a fixed amount of water was added to the olive paste (0.75:1 v:w) during malaxation to optimise olive oil extraction yield, whereas in the P2 mill neither water nor any technological co-adjuvants were added. The extracted olive oil was collected in 5-L tin containers without any prior filtering operation, and it was allowed to settle for 20 days. Then the oil was transferred into dark glass bottles, leaving no headspace, and stored afterwards in a dark closet at ambient temperature (17 ± 3 °C). The first sampling time was set at 35 days after extraction, and the following samplings were carried out after additional 1, 3 and 6 months of storage. At each sampling time one bottle for each malaxation condition (R1, R2, R3, R4) and each olive mill (P2, P3) was taken for analyses. In this way, for both olive oil mills a full factorial design of experiments (DoE) was performed, with four levels for both of the involved factors (oxygen concentration during malaxation and storage time).

2.2. Determination of olive oil quality parameters

Free acidity, peroxide value and spectrophotometric indices (K_{232}, K_{270}, ΔK) were determined according to EEC regulation No. 2568/91, and its successive modifications (EEC, 1991). Measured parameters were largely below the EU standards established for the EVOO grade in all the extracted olive oils and throughout the entire storage time (Table 2S, Appendix A). Fatty acid composition of the considered EVOO (palmitic 15.5%, palmitoleic 0.8%, stearic 1.9%, oleic 73.2%, linoleic 7.3%, linolenic 0.7%, arachidic 0.3%, gadoleic 0.2%) was characterised by a relatively high level of oleic acid.

2.3. Determination of volatile compounds

Volatile compounds were determined by following a method previously described in the literature (Vichi et al., 2003) with minor modifications, using headspace solid-phase microextraction (HS-SPME) for volatiles isolation and gas chromatography–mass spectrometry (GC–MS) for their analytical determination. Thirty grams of olive oil, sampled from a sealed bottle, were added with 100 μL of the internal standard solution (4-methyl-2-pentanol at 0.2 mg L^{-1} in methanol), and thoroughly stirred for homogenisation. Three aliquots of 2 g each were placed in 15-mL vials for HS-SPME, and hermetically closed with a silicon septum. Each vial was put in a water bath at 40 °C, and a DVB/CAR/PDMS fibre (50/30 μm, 2 cm long from Supelco, Bellefonte, PA) was exposed for