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The influence of the malaxation temperature on the activity of polyphenoloxidase and peroxidase and on the phenolic composition of virgin olive oil

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ABSTRACT

The effect of the malaxation temperature under sealed conditions on the qualitative and quantitative composition of the phenolic compounds in virgin olive oils produced from four Italian cultivars was assessed for two atmospheric conditions. In both cases, the results show a positive relationship between temperature and the concentration of the derivatives of the secoiridoid aglycones; the effect of the temperature on the oxidoreductases that promote oxidation (polyphenoloxidase and peroxidase) was investigated to determine their optimal temperatures and thermal stability. While olive peroxidase (POD) showed the highest activity at 37 °C and high stability in the temperature range tested, polyphenoloxidase (PPO) exhibited the optimum activity at approximately 50 °C, but showed low stability at 40 °C, with a large variation in stability according to the olive cultivar. These results may contribute to an understanding of the increase in the phenol concentration found in virgin olive oils obtained following higher temperatures of malaxation.

1. Introduction

The concept of quality for virgin olive oil (VOO) currently goes beyond compliance with the analytical parameters listed in EU Reg. 61/2011 (EU, 2011), which is a modification of EU Reg. 2568/1991 that guarantees authenticity and, to a certain extent, quality. In fact, in recent years, more and more attention has been given to a superior concept of VOO quality that is based on the sensory and healthy properties of this vegetable fat, but this concept is not widely acknowledged by EU regulations. This aspect of the quality of VOO is strongly correlated to the presence of phenolic and volatile compounds. Indeed, the olfactory attributes arise mainly from the occurrence of C_5 and C_6 saturated and unsaturated aldehydes, alcohols and esters responsible for some typical sensory notes, such as "cut grass", "haylike" and "floral", whereas the bitter and pungent taste of VOO is attributed to the phenolic fraction (Angerosa et al., 2004; Servili et al., 2004). Furthermore, the health benefits of VOO (Bach-Faig et al., 2011; López-Miranda et al., 2010) are most likely related to several phenolic compounds occurring in VOO that are now considered to be the main bio-active molecules of extra-virgin olive oil; these compounds show high antioxidant activities and are also credited with the main role in the apparent relationship between the consumption of olive oil and the prevention of cardiovascular disease and cancer in humans (Covas, 2009; EFSA, 2011; Servili et al., 2009). The qualitative and quantitative composition of both the volatile and the phenolic fractions are affected by genetic and agronomic factors of the olive cultivars and by the enzymatic reactions occurring during the mechanical extraction of oil (Angerosa et al., 2004; Servili et al., 2004). In particular, the malaxation operating conditions are, among all the technological factors, the most important factor responsible for modifications to the phenolic and volatile fractions. During this operation, enzyme activities involving the transformation of volatile and phenolic compounds take place. For example, the lipoxygenase pathway (LPO) leads to the formation of the aforementioned C_5 and C_6 saturated and unsaturated aldehydes, alcohols and esters from the oxidative degradation of unsaturated C:18 fatty acids (Angerosa et al., 2004). In addition, the phenolic fraction is affected by the activity of the hydrolytic enzymes that catalyse the liberation of aglycone secoiridoids from their respective glucoside forms (Servili et al., 2004) and by the oxidative degradation activities catalysed by polyphenoloxidases (PPO) and peroxidases (POD) (Sciancalepore, 1985; Servili et al., 1998). The malaxation operating variables that may influence those enzymatic activities have been subject to investigation in recent years. Early experimental protocols were based on the traditional malaxation model without control of the atmosphere during the operation, as in the use of open-top malaxer machines, which have been mostly phased out recently. Under these conditions, the most

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important findings related to a negative correlation between the phenolic concentration in the VOO and the time and temperature of malaxation (Angerosa, Mostallino, Basti, & Vito, 2001; Di Giovacchino, Sestili, & Di Vincenzo, 2002; Kalua, Bedgood, Bishop, & Prenzler, 2006; Ranalli, Contento, Schiavone, & Simone, 2001), thus demonstrating that the oxidative enzymatic degradation of secoiridoid derivatives due to PPO and POD activities caused the loss of those compounds. Subsequently, the effect of reducing the oxygen concentration in the malaxer headspace on the inhibition of the activity of such oxidoreductases (Servili, Selvaggini, Taticchi, Esposto, & Montedoro, 2003a, 2003b) was demonstrated. New-generation malaxing machines can operate in a sealed state, allowing for the control of gaseous exchanges. Under these conditions, it has been observed that an initial natural atmosphere composition (30 kPa of O_2) results in only a slight loss of phenols in the final oil due to oxygen depletion during malaxation (Servili et al., 2008). The influence of the malaxation temperature on the concentration of phenolic compounds in VOO has recently been the object of new investigations (Boselli, Di Lecce, Strabbioli, Pieralisi, & Frega, 2009; Gómez-Rico, Inarejos-García, Salvador, & Fregapane, 2009) and a positive relationship between the malaxation temperature and the phenolic concentration has been shown. There is still a lack of data, however, concerning the direct influence of the malaxation temperature on PPO and POD activities in olive pastes and their specific role in modeling the final concentration of phenols in VOO. Therefore, the purpose of this paper was to investigate the effect of the temperature on the evolution of phenolic substances in olive pastes and in the corresponding VOOs under different atmospheric conditions during malaxation and to understand the influence of temperature on the activity of oxidoreductases responsible for the enzymatic degradation of oils from four Italian olive cultivars.

2. Materials and methods

2.1. Olives

Drupes of the Coratina, Ogliarola, Moraiolo and Peranzana cultivars, harvested during the year 2010, were used. The ripening stage of the olives [evaluated by the pigmentation index, according to Pannelli, Servili, Selvaggini, Baldioli, & Montedoro, 1994], was 0.95.

2.2. References compounds

(3,4-Dihydroxyphenyl)ethanol (3,4-DHPEA) was obtained from Cayman Chemicals LTD. (USA) and (p-hydroxyphenyl)ethanol (p-HPEA) was obtained from Janssen Chemical Co. (Beerse, Belgium). Oleuropein glucoside was purchased from Extrasynthèse (France). Demethyloleuropein and verbascoside were extracted from the olive fruit according to the procedure reported in a previous paper (Servili, Baldioli, Selvaggini, Macchioni, & Montedoro, 1999 GF). Briefly, the phenols were extracted from freeze-dried olive pulps (5 g) using an 80:20 v/v mixture of methanol:water (50 mL) at low temperature. The extraction procedure was performed three times. The dialdehydic forms of elenolic acid linked to 3,4-DHPEA and p-HPEA (3,4-DHPEA-EDA and p-HPEA-EDA, respectively), the isomer of oleuropein aglycon (3,4-DHPEA-EA), (+)-1-acetoxypinoresinol and (+)-pinoresinol were extracted from VOO using a procedure previously reported (Montedoro et al., 1993). In short, the phenols were extracted from the oil using 80:20 v/v methanol:water. After solvent evaporation and the partial purification of the crude extract obtained from the olive fruit and VOO, the phenols were separated by semi-preparative High Performance Liquid Chromatography (HPLC) analysis using a 9.4 mm i.d. × 500 mm Whatman Partisil 10 ODS-2 semi-preparative column. The mobile phase was 0.2% acetic acid in water at pH 3.1 (solvent A)/methanol (solvent B) at a flow rate of 6.5 mL/min. Phenol detection was performed using a Diode Array Detector (DAD) (Montedoro et al., 1993). The purity of all the substances obtained from direct extraction was determined by HPLC and their chemical structures were verified by NMR using the same operating conditions reported in previous papers by recording ^1H and ^{13}C spectra (Montedoro et al., 1993; Servili et al., 1999). Pure analytical standards of the volatile compounds were purchased from Fluka and Aldrich (Milan, Italy).

2.3. Virgin olive oil mechanical extraction process

The experiments were conducted on an industrial scale using a Rapanelli SPA industrial implant. Each extraction was performed on a sample of 150 kg of olives. For the crushing operation, a hammer crusher Model GR 32 (Rapanelli Fioravante S.p.A., Foligno, Italy) was employed. Malaxation was carried out for 40 min. The malaxing machine (Ravanelli S.p.A, Foligno, Italy) was top-covered and equipped with two valves for O_2 and N_2 entry and a sensor for the measurement of oxygen (Mettler Toledo mod. O_2 4100). Six trials were performed in duplicate to test 3 malaxation temperatures (20, 25 and 35 °C) and 2 initial oxygen partial pressures in the headspace of the malaxer chamber (30 kPa, as in the normal atmosphere composition, and 50 kPa). Oil separation was obtained by using a three phase decanter, Ramef Mod. 400 ECO-G (Rapanelli Fioravante S.p.A., Foligno, Italy) with low water addition (0.2:1 v:w). The VOO samples were filtered and stored in the dark at 13 °C until analysed. To evaluate the phenolic composition of the olive pastes and their modifications during malaxation, paste samples were collected at the beginning (after crushing), every 10 min and at the end of the malaxing process and were immediately frozen in liquid nitrogen and stored at −80 °C until analysed.

2.4. Analytical methods

2.4.1. Extraction and HPLC analysis of the phenolic compounds of olive pastes

The phenolic compounds were extracted from the crushed and malaxed olive pastes by modifying the procedure previously described by Servili et al. (1999). Five grams of olive paste were homogenised with 100 mL of 80% methanol containing 20 mg/L of sodium diethyldithiocarbamate (DIECA); the extraction was performed in triplicate. After removal of methanol, the aqueous extract underwent SPE phenol separation. The SPE procedure was performed by loading 2 mL of the aqueous extract into a 5 g/25 mL Extraclean highload C18 cartridge (Alltech Italia S.r.l., Sedriano, Italy). Methanol (200 mL) was used as the eluting solvent. After removing the solvent under vacuum at 30 °C, the phenolic extract was recovered and then dissolved in methanol (1 mL). Reversed-phase HPLC analyses of the phenolic extracts were conducted with an Agilent Technologies system Mod. 1100, which was composed of a vacuum degasser, a quaternary pump, an autosampler, a thermostated column compartment, a DAD and a FLD. To evaluate the phenolic compounds (Selvaggini et al., 2006), a Spherisorb column ODS-1 250 × 4.6 mm with a particle size of 5 μm (Phase Separation Ltd., Deeside, UK) maintained at 25 °C was employed. Sample volume was 20 μL. The mobile phase was composed of 0.2% acetic acid (pH 3.1) in water (solvent A)/methanol (solvent B) at a flow rate of 1 mL/min. The gradient was changed as follows: 95% A/5% B for 2 min, 75% A/25% B in 8 min, 60% A/40% B in 10 min, 50% A/50% B in 16 min, 0% A/100% B in 14 min. This composition was maintained for 10 min and was then returned to the initial conditions and equilibration in 13 min; the total running time was 73 min. Lignans were detected by using the FLD operated at an excitation wavelength of 280 nm and