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Three-factor approach for balancing the concentrations of phenols and volatiles in virgin olive oil from a late-ripening olive cultivar

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ABSTRACT

To investigate the possibilities of balancing the concentrations of phenols and volatiles in virgin olive oil from a late-ripening olive (*Olea europaea* L.) cultivar, the effects of ripening degree, malaxation duration, and temperature were investigated. Olives were harvested at three ripening degrees and processed by malaxation at 21/30 °C, for 30/60 min, respectively. The most important phenols and volatiles were generally found to decrease during ripening. The effect of higher malaxation temperature on phenols was dual, with a positive effect on 3,4-DHPEA-EDA and *p*-HPEA-EDA, and negative on other oleuropein and ligstroside aglycons. An unexpected increase of 1-penten-3-one and (*E*)-2-hexenal concentrations as a result of higher malaxation temperature correlated with the increase in key phenols and was a limiting factor in balancing their concentrations, which was confirmed by sensory analysis. Numerous interactions between the factors were established.

1. Introduction

The typical aroma of high quality virgin olive oil (VOO) consists of odoriferous C5 and C6 volatile compounds formed by lipoxygenase (LOX) and hydroperoxide lyase (HPL) enzymes during olive fruit milling and paste malaxation steps (Angerosa et al., 2004). C6 aldehydes are later partly reduced to C6 alcohols by alcohol dehydrogenase (ADH), and transformed to C6 esters by alcohol acyl transferase (AAT) (Angerosa, Mostallino, Basti, & Vito, 2001). Characteristic VOO bitterness and pungency, as well its oxidative stability, shelf life, and nutritional quality derive from phenols (Gómez-Rico, Inarejos-García, Salvador, & Fregapane, 2009; Servili & Montedoro, 2002). VOO phenols are formed mainly by cleavage of their glycosides by hydrolytic enzymes during olive fruit processing and their concentrations are further affected by oxidative degradation catalysed by polyphenoloxidases (PPO) and peroxidases (POD) (Amirante, Clodoveo, Tamborrino, Leone, & Dugo, 2012; Taticchi et al., 2013).

The quantity of substrates and the activity of enzymes in olives and VOO depend on many agronomic and processing parameters. Among these, ripening degree on one side and malaxation temperature and duration on the other are certainly among the most influential (Amirante, Clodoveo, Tamborrino, & Leone, 2012; Clodoveo et al., 2015; Mailer, Ayton, & Graham, 2010). The effects of these factors were widely studied and many contrasting data can be found in literature. It is generally considered that the activity of LOX enzymes and the availability of the main phenol precursor oleuropein decrease with the course of ripening, resulting in reduced content of volatiles and phenols (Gómez-Rico, Salvador, La Greca, & Fregapane, 2006; Morales, Aparicio, & Calvente, 1996). However, different results have also been published (Baccouri et al., 2008; Brkić Bubola, Koprivnjak, Sladonja, Škevin, & Belobrajić, 2012; Romero, Saavedra, Tapia, Sepúlveda, & Aparicio, 2016). Several authors observed a negative effect of higher malaxation temperatures on the concentration of both volatiles and phenols (Angerosa et al., 2001; Servili, Selvaggini, Taticchi, Esposto, & Montedoro, 2003), while others noted the contrary (Boselli, Di Lecce, Strabbioli, Pieralisi, & Frega, 2009; Ranalli, Contento, Schiavone, & Simone, 2001). A positive impact of longer malaxation durations on volatiles (Esposto et al., 2009; Gómez-Rico et al., 2009) and negative on phenols (Jiménez, Sánchez-Ortiz, & Rivas,

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2014) was noted. In other studies no significant effect of malaxation duration was established (Angerosa et al., 2001; Clodoveo, 2012; Kalua, Bedgood, Bishop, & Prenzler, 2006), while Reboredo-Rodríguez, González-Barreiro, Cancho-Grande, and Simal-Gándara (2014) observed contrasting effects with respect to cultivar.

A big part of the observed discrepancies has probably arisen from the large variations in olive ripening degree, but its influence on the effects of malaxation parameters was studied extremely rarely (Cevik, Ozkan, & Kıralan, 2016; Jiménez et al., 2014; Lukić et al., 2017). Malaxation results depend on olive ripeness (Cevik et al., 2016) and interactions between olive fruit ripening and paste malaxation parameters are multiple (Lukić et al., 2017). Although it turned out that these factors need to be considered in conjunction because of their interdependence, up to date this was the sole study in which their interactions were systematically evaluated. The mentioned reports encompassed rather different, but relatively standard ripening cycles with indexes from below 1 to 4 (Lukić et al., 2017) and from 3 to 6.63 (Cevik et al., 2016), respectively. On the other hand, the response of late-ripening cultivars has never been addressed in this context despite their economic importance for VOO producing countries. Finally, generally a rather limited number of olive ripening and malaxation studies were comprehensive in a way they focused simultaneously on phenols and volatiles, including sensory characteristics, which is another gap in the literature from this field that needs to be addressed.

The aim of this study was to investigate the effects and interactions of ripening degree, malaxation temperature, and malaxation duration on the content of phenols, volatiles, and sensory characteristics of a late-ripening cultivar VOO. The cultivar selected was Istarska bjelica, native and among the most widespread in Croatia and Slovenia (EU). Istarska bjelica VOO is generally appreciated for its sensory robustness and high nutritional value as a result of high concentration of phenols. However, it sometimes lacks harmony due to rather intense bitterness and especially pungency, which is in some years accompanied by only modest intensities of typical VOO *green* and fruity odours. It was considered that investigating the effects of the three crucial factors, as well as their interactions, would provide new knowledge useful for the better management of the balance between the concentrations of phenols and volatiles in VOOs from such late-ripening cultivar, as well as other cultivars. In the case of cultivars with high phenolic content such as Istarska bjelica, this would possibly enable obtaining milder, fruitier, and more balanced VOO, allowing producers to have a more diversified offer on the market.

2. Materials and methods

2.1. Olive fruits harvesting, processing, and oil extraction

Istarska bjelica olive fruits were harvested in 2015 in the experimental unirrigated olive orchard of the Institute of Agriculture and Tourism (Poreč, Croatia), located on the west coast of the region of Istria at 25 m above sea level, at the position 45°13′N, 13°36′E. Olives were harvested at three ripening degrees (RD): RD1 - October 12th, RD2 - November 18th, and RD3 - December 10th. Each replicate destined for further processing (1 kg) was taken from a corresponding homogenised batch of olive fruits handpicked from three marked trees. Ripening indexes (RI) were determined according to Beltrán, del Río, Sánchez, and Martínez (2004), and were as follows: 0.76 (RD1), 1.08 (RD2), and 1.27 (RD3).

Fruits were processed within 24 h after harvesting using a laboratory plant (Abencor, MC2 Ingeneria y Sistemas, Sevilla, Spain) consisting of a hammer crusher, vertical thermostated olive paste mixers, and a centrifuge. Olive pastes were malaxed in triplicates at 21 ± 1 °C and 30 ± 1 °C for 30 and 60 min, respectively. Boundary malaxation temperatures of 21 °C and 30 °C were selected for this study because were expected to produce extremely high concentrations of volatiles (at 21 °C) and phenols (at 30 °C). Produced olive oil samples were stored in dark-brown 125 mL bottles without headspace in the dark at 16–18 °C, were allowed to settle, and then decanted.

2.2. Chemicals

Methanol, water, and n-hexane were of HPLC grade purity (Sigma-Aldrich, St. Louis, MO, USA). Pure chemical standards of phenols and volatile compounds were purchased from Sigma-Aldrich, Alfa Aesar (Haverhill, MA, USA), Acros Organics (Geel, Belgium, EU), Cayman Chemical Co. (Ann Arbor, MI, USA), Fluka (Buchs, Switzerland), and Extrasynthèse (Genay, France, EU). A list of pure chemical standards used with details is reported in Table S1. Model standard solutions of phenols were prepared in pure methanol and solutions of volatiles in fresh refined sunflower oil.

2.3. Analysis of phenols

Extraction of phenols from VOO was performed according to the method based on ultrasound assisted liquid-liquid extraction with methanol proposed by Jerman Klen, Golc Wondra, Vrhovšek, and Mozetič Vodopivec (2015), with modifications reported in detail in our previous study (Lukić et al., 2017).

Analysis was performed using an Agilent Infinity 1260 HPLC-DAD system (Agilent Technologies, Palo Alto, CA, USA). A Kinetex PFP column (2.6 μm, 100 mm × 4.6 mm) with a PFP guard column was used (Phenomenex, Sydney, Australia) at 27 °C. Solvents were glacial acetic acid - water (50 mL/L) (A) and methanol (B), with a flow rate of 1 mL/min. Ten μL were injected. A 20-step solvent run was used as reported earlier (Lukić et al., 2017).

Identification was performed by comparing retention times and UV/Vis spectra with those of pure standards when available, and with UV/Vis spectra from the literature (Jerman Klen et al., 2015). Detection wavelengths were 280 nm (for simple phenols, vanillic acid, lignans, and secoiridoids), 320 nm (vanillin and *p*-coumaric acid), and 365 nm (flavonoids), while spectra were registered from 200 to 600 nm. Standard calibration curves were constructed for tyrosol, hydroxytyrosol, vanillic acid, vanillin, *p*-coumaric acid, luteolin, apigenin, pinoresinol, and oleuropein (Table S1). For other compounds semi-quantitative analysis was carried out: secoiridoids were expressed as oleuropein, and acetoxypinoresinol as pinoresinol equivalents.

2.4. Analysis of volatile compounds

Volatile compounds were isolated using headspace solid-phase microextraction (HS-SPME) with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (1 cm, 50/30 μm, Supelco, Bellefonte, PA, USA), according to the modified method proposed by Brkić Bubola et al. (2012). After 15 min equilibration at 40 °C, the extraction was carried out with 4 g of oil in 10 mL vials, at 40 °C for 40 min with stirring (800 rpm). GC injection at 245 °C was splitless for 3 min.

GC analyses were performed using a Varian 3350 gas chromatograph (Varian Inc., Harbor City, CA, USA) equipped with an injector operated at 245 °C, a flame ionization detector (FID) operated at 248 °C, and a capillary column Rtx-WAX (60 m × 0.25 mm i.d. × 0.25 μm film thickness; Restek, Bellefonte, PA, USA). Initial oven temperature was 40 °C, increased to 130 °C at 2 °C/min, increased to 245 °C at 20 °C/min, and kept for 20 min. The carrier gas was helium at the constant pressure of 138 kPa at the column head.