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Effects of heat-treatments of olive fruit on pigment composition of virgin olive oil

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Abstract

The effects of heat-treatments of olive fruits on olive oil colour were investigated by quantification of the main pigments present in virgin olive oil. Contents of lutein and β-carotene increased as a consequence of heat-treatments. Lutein content increased at least 2.2-fold compared to control olive oil. β-Carotene contents also increased due to heat-treatments although not as much as lutein contents. Chlorophyllic compounds, such as chlorophylls *a* and *b* and pheophytins *a* and *b*, also increased significantly. Thus, chlorophyll *a* contents increased in a range of 2.0 to 7.7-fold compared to the amount found in control olive oil, while pheophytin *a* contents did so over a wider range (1.4 to 17.5-fold). The temperature of fruits when entering the crusher is the only factor responsible for this effect. The involvement of LOX activity in pigment degradation is considered.

Keywords: Virgin olive oil; Pigments; Heat-treatment; Lipoxygenase

1. Introduction

Olive oil is one of the oldest known plant oils and it is unique among them since it can be consumed as a fruit juice called virgin olive oil. This oil, one of the main components of the Mediterranean diet, is related to protection against cardiovascular diseases and cancer, due to its fatty acid profile and the presence of minor amounts of phenolic constituents (Ruíz-Gutiérrez, Muriana, & Villar, 1998; Visioli, Bellomo, & Galli, 1998; Visioli & Galli, 1998). A large increase in the demand for high quality virgin olive oil during recent years can be attributed, not only to its potential health benefits, but also to its particular organoleptic properties. The aim of increasing the quality standards for virgin olive oil is continuously stimulating the search for new technologies. In this sense, a new technological procedure, involving heat-treatment of olive fruit, has been developed by our research group for modulation of bitterness intensity of olive oil. Bitterness is a common and desirable attribute of virgin olive oil flavour when present at low to moderate intensity. However, it is rejected by consumers when present at high intensity. Previous studies have demonstrated that air-heating of olive fruit promoted a reduction of olive oil bitterness directly related to time and temperature of treatment (García, Yousfi, Mateos, Olmo, & Cert, 2001). This reduction is probably due to a partial inhibition of glycosidases and esterases involved in the release of secoiridoid derivatives from oleuropein during the crushing-malaxation process to obtain virgin olive oil. However, heat-treatments also affect other quality traits, such as oxidative stability and aroma volatile composition (Pérez, Luaces, Ríos, García, & Sanz, 2003), and could also produce a change in the pigment contents of the virgin olive oil.

Postharvest heat-treatments of fruits are currently used commercially in several countries for disease control, as a quarantine technique, to modify fruit responses to other stresses, but also for maintenance of fruit quality throughout chilling storage. Postharvest exposure to moderate temperatures often increases

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storage life and has different effects on colour. The impact on pigment content varies with species, temperature, and duration of heat-treatment (Paull & Chen, 2000). Physical treatments applied to the fruits might alter the enzymatic systems by partial or complete inhibition of their activities, causing changes in the pigment profile. In this sense, an increase in the content of the carotene and chlorophyll pigment fractions of virgin olive oil was observed after 24 h of air-heat treatments of olive fruits (García et al., 2001).

The development of a postharvest technology for olive fruits, able to reduce bitterness intensity in the resulting oils, would facilitate, and in some cases allow, the marketability of the virgin olive oils. In this sense, water-heat treatments of olive fruits have proved to be quite effective in reducing olive oil bitterness. However, this emerging technology should be checked in order to assess its effect on other quality attributes of olive oil.

Colour is a major factor affecting consumer acceptance of plant products in general and of virgin olive oil in particular, so there is a commercial interest in studying the modifications of pigment contents as a consequence of processing. Chlorophylls and carotenoids are the main pigments in virgin olive oil (Gandul-Rojas & Mínguez-Mosquera, 1996a; Mínguez-Mosquera, Rejano-Navarro, Gandul-Rojas, Sánchez-Gómez, & Garrido-Fernández, 1991) and it was found that about 80% and 40% of chlorophylls and carotenoids, respectively, are lost during olive oil extraction (Mínguez-Mosquera, Gandul-Rojas, Garrido-Fernández, & Gallardo-Guerrero, 1990). In this sense, Jarén-Galán, Carmona-Ramón, and Mínguez-Mosquera (1999) suggested that lipoxygenase (LOX) activity could be the factor affecting olive oil quality through its effect on oil colour, since it is not possible to attribute the losses of chlorophyll pigments to activation of chlorophyllase during oil processing (Gandul-Rojas & Mínguez-Mosquera, 1996b). This process would allow the possibility of oxidizing non-specifically compounds, such as chlorophylls and carotenoids, through a suggested co-oxidation mechanism involving free radicals (Cohen, Grossman, Klein, & Pinski, 1985; Hildebrand & Hymowitz, 1982; Sanz, Pérez, & Olías, 1994). The presence of the LOX pathway enzyme system in olive fruit was established by our group a decade ago in relation to the biosynthesis of C_6 volatile compounds in virgin olive oil (Olías, Pérez, Rios, & Sanz, 1993) and, more recently, we have found that the heat-treatments seemed to promote a partial deactivation of this enzyme system, leading to a net decrease in the contents of C_6 and C_5 volatile compounds (Pérez et al., 2003).

The aim of the present work was to study the effect of the temperature, used for bitterness reduction in water-heat treatments of olive fruits, on the contents of the main pigments of virgin olive oil.

2. Materials and methods

2.1. Plant material

Olive fruits (*Olea europaea* L.) from Spanish cultivars Verdial, Picual and Manzanilla were harvested in Opracol Huelva orchards (Villarrasa, Huelva, Spain) and in experimental fields of the Instituto de la Grasa (Seville, Spain), during the 2002–2003 season, at green stage, colour index 1 according to García, Gutiérrez, Barrera, and Albi (1996).

2.2. Heat-treatment of fruits

Fruits were randomly distributed in 3.5 kg batches for every treatment in triplicate. Range of temperature for treatment was selected based on its effectiveness in the modulation of bitterness intensity of the oils (unpublished results). Every batch of olive fruits was dipped in a 90 l thermostatic water bath for 3 min at different temperatures and processed for olive oil extraction.

2.3. Olive oil extraction

After heat-treatment of olive fruits, olive oil was immediately extracted using an Abencor analyser (Comercial Abengoa, S.A., Seville, Spain) that simulates, on the laboratory scale, the industrial process of virgin olive oil production (Martínez, Muñoz, Alba, & Lanzón, 1975). Malaxation was carried out for 30 min with the Abencor thermobeater, operating at 30 °C. After centrifugation, oils were decanted, paper-filtered, and stored at −18 °C nitrogen prior to analysis.

Olive fruits (cultivar Manzanilla) used for studying the effect of fruit temperature at milling were heated as above and immediately crushed in a hammer mill at the appropriate temperature or after being cooled to 0–4 °C in an ice-water bath. Once the olive paste was obtained, it was immediately conditioned at 30 °C before the malaxation process took place.

2.4. Pigment analysis

Contents of chlorophyllic compounds and major carotenoids in olive oil samples were determined in triplicate by dissolving 0.1 g of olive oil in 370 μl of ethyl acetate and resolving the sample by HPLC on a Beckman System Gold Programmable Solvent Module 126 coupled to a diode array detector Module 168, according to the method of Pérez, Sanz, Richardson, and Olías (1993). The column was a Beckman Ultrasphere ODS (C18) (250 × 2 mm), 5 μm, operating at 30 °C, fitted with a 20 μl injection loop in a Rheodyne valve. A two-step gradient elution utilized solvent A: acetonitrile/H_2O (90:10) and B: ethyl acetate, programmed at 0.5 ml/min and detection at 430 nm.

2.5. Fruit temperature measurements

Temperatures in the different parts of the olive fruit were recorded every 8 s using 1 mm thermocouples and DeltaTrack data loggers (Modesto, Canada) by setting the thermocouples, respectively under the fruit skin (outer pulp), close to the stone (inner pulp) and in the kernel by drilling a 1 mm hole through the stone hull.

2.6. Extraction and measurement of LOX activity

Fresh fruits were thoroughly rinsed with water, wiped with paper tissue, and then pitted. The fruit pulp (10 g) was ground in 4 volumes of grinding buffer, 100 mM phosphate buffer, pH 6.7, containing 0.1% Triton X-100, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 5 mM α-aminocaproic acid, and 2 g PVPP. Grinding was carried out over two 1 min periods with an Ultraturrax homogenizer. The homogenate was filtered under vacuum through Miracloth and centrifuged for 20 min at 27,000*g*. The supernatant was centrifuged again for 10 min at 10,000*g*.

Lipoxygenase activity was determined spectrophotometrically at 25 °C, with linolenic acid as substrate, by monitoring the increase in absorbance at 234 nm arising from the conjugated double bonds formed by hydroperoxidation of this acid (Olías et al., 1993).

3. Results and discussion

Results on modulation of bitterness intensity of virgin olive oil by means of water-heat treatments of olive fruit, prior to olive oil extraction, showed that a range of temperature 60–68 °C was the most effective for this purpose. The effect of these treatments of olive fruits on the olive oil colour was by quantification of the main pigments present in virgin olive oil (Fig. 1). Quantitatively, the main carotenoids of virgin olive oil are lutein and β-carotene, conferring the yellow colour. Contents of both compounds increased as a consequence of heat-treatments, especially in the case of lutein, but no obvious correlation was found between carotenoid contents and the temperature used in the treatment. Lutein content increased at least 2.2-fold compared to control olive oil. β-Carotene content also increased due to heat-treatments although not as much as the lutein content.

Chlorophyllic compounds, such as chlorophylls *a* and *b*, and pheophytins *a* and *b* are responsible for the green colour of virgin olive oil, the *a* forms being the main components. As in the case of carotenoids, significant increases of the contents of chlorophyllic compounds were observed in the olive oils due to heat-treatments of the olive fruits. Thus, chlorophyll *a* contents increased in a range of 2.0 to 7.7-fold the amount found in control olive oil, while pheophytin *a* contents did so over wider interval range (1.4 to 17.5-fold). Again, in general, no relationship was found between contents of chlorophyllic compounds and the treatment temperatures.

Since fruits after heat-treatments were processed over a variable period of time, ranging from 5–10 min, simulating the industrial process, doubts were raised about whether the effect on oil colour was due to the heat-treatment itself or the temperature of fruits undergoing crushing in the hammer mill. In order to investigate this further, the temperature–time course was first studied in different parts of the fruit during treatment at 60 °C and afterwards when fruits were placed at room temperature (20 °C). Fig. 2 shows that, as expected, the outer pulp temperature increased more quickly than the inner pulp and the kernel temperatures. At the end of the 3 min treatment, the average temperature in the whole fruit was 59.4 ± 1.8 °C. After heat-treatment, fruit temperature lowered, more rapidly in the outer pulp than in the other parts of the fruit, although deviation from the average temperature was not more than 5.9%. The average temperatures in the fruit, after 5 and 10 min from the end of the 3 min, treatment were 41 and 33 °C, respectively. These differences in fruit temperature in the hammer mill, found in a simulated industrial process, could explain the random increases in the pigment contents shown in Fig. 1.

In order to study the effect of the milling temperature on pigment content of the resulting olive oils, an experimental trial was carried out to evaluate the effect of crushing temperature on pigment profile of virgin olive oil, overcoming the variable of time after treatment. Fig. 3 shows the contents of carotenoids and chlorophyllic compounds in virgin olive oil as a result of the different temperatures of fruits entering the hammer mill. A significant increase in pigment content was found when fruits were crushed at increasing temperatures, more marked for fruit temperatures above 20 °C, and especially in the case of chlorophyll and pheophytin *a* whose contents increased around 10× at 60 °C. Caponio, Pasqualone, Gomes, and Catalano (2002) recently observed that the quality of olive oils was influenced by the crushing temperature in terms of susceptibility to oxidation and the level of compounds related to oxidative and hydrolytic degradation. That work was carried out after the observation that, during mechanical crushing of olive fruits the temperature of outgoing paste increased by 4–10 °C compared to olive fruits, entering the hammer crusher (Amirante, Di Renzo, Di Giovacchino, Bianchi, & Catalano, 1993).

Similarly, our data suggest that temperature of olive fruit during the hammer crushing is the only factor affecting virgin olive oil colour. No effect on the pigment profile was observed when fruits were heated at 60 °C for 3 min and then their temperature lowered immediately at 0–4 °C in an ice-water bath for 10 min prior to the oil extraction (Fig. 3, 60 + 4 °C bars). Resulting oils

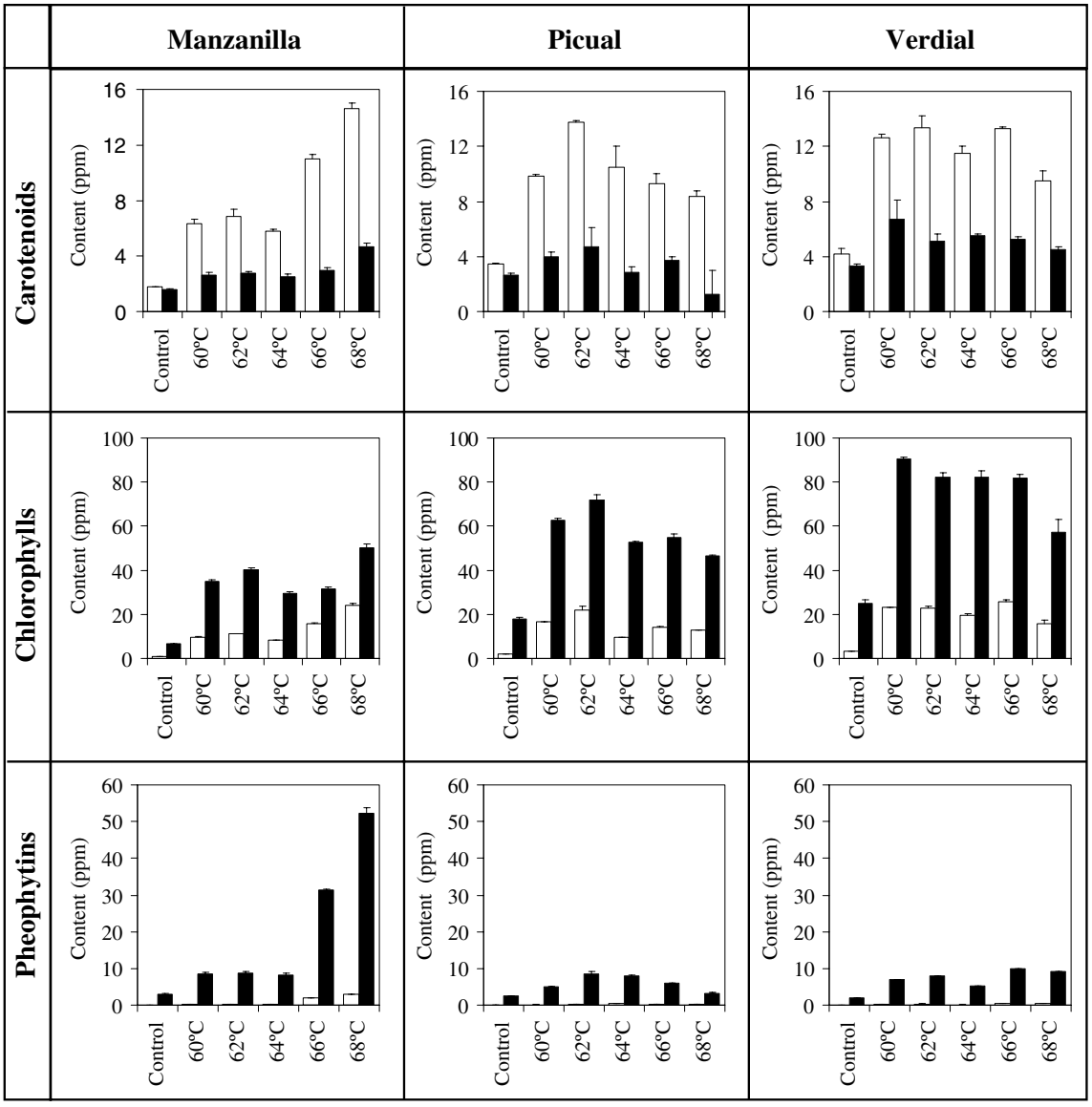

Fig. 1. Contents of carotenoids (lutein, white bars; β-carotene, black bars), chlorophylls (chlorophyll *b*, white bars; chlorophyll *a*, black bars), and pheophytins (pheophytin *b*, white bars; pheophytin *a*, black bars) in olive oils from fruits of three olive cultivars dipped in water at different temperatures prior to oil extraction.

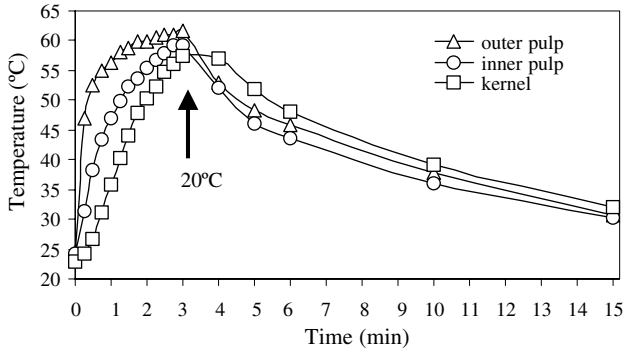

Fig. 2. Temperature–time course of different parts of olive fruit during heat-treatment and afterwards when placed at 20 °C (arrow).

showed quite similar pigment compositions to those from fruits crushed at 0–4 °C (Fig. 3, 0–4 °C bars).

Taking into account the possible involvement of LOX on pigment degradation during the olive crushing process to obtain virgin olive oil as suggested by Jarén-Galán et al. (1999), the thermal stability of this enzymatic activity in olive fruits was assessed. LOX extracts from olive fruits showed that, after 3 min of heating, increasing temperatures promoted an increasing deactivation of this enzyme activity (Fig. 4). Heating at 60 °C almost completely deactivated LOX enzyme, while heating at 40 °C lowered LOX activity to 82.5% with respect to heating at 20 °C. These differences in thermal stability of LOX activity could well explain the different

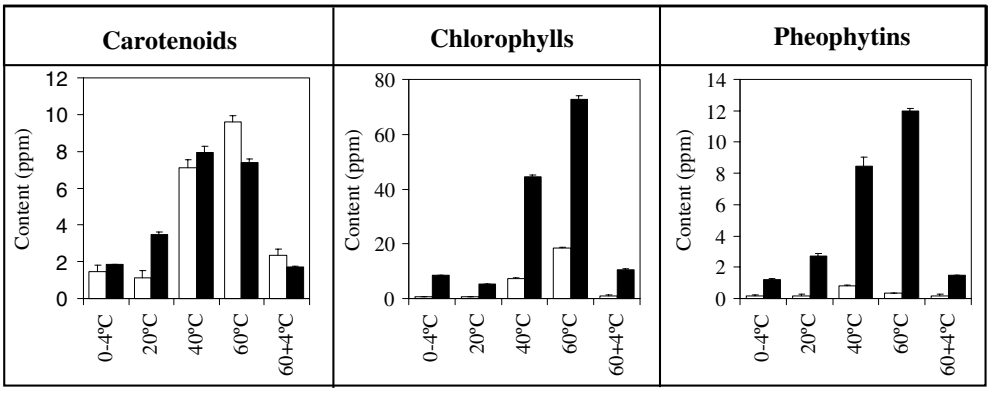

Fig. 3. Contents of carotenoids (lutein, white bars; β-carotene, black bars), chlorophylls (chlorophyll *b*, white bars; chlorophyll *a*, black bars), and pheophytins (pheophytin *b*, white bars; pheophytin *a*, black bars) in olive oils from Manzanilla olive fruits crushed in a hammer mill at different temperatures. Oils at 60 + 4 °C were produced by dipping the fruits in water at 60 °C for 3 min and immediately lowering their temperature to 0–4 °C in an ice-water bath for 10 min prior to oil extraction.

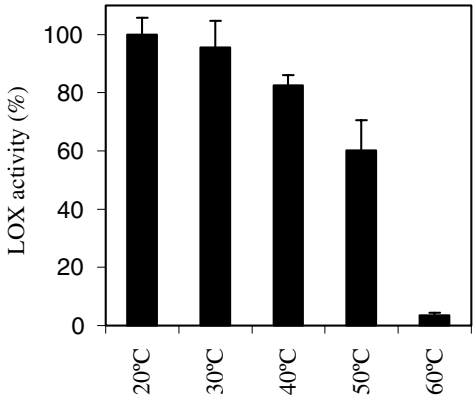

Fig. 4. Level of LOX activity of olive extracts after heating during 3 min at different temperatures.

levels of pigments in virgin olive oil in relation to fruit temperature at crushing.

In conclusion, the emerging technology developed for modulating the bitterness intensity of virgin olive oil by means of heat-treatments of olive fruits prior to olive extraction seems also to affect the colour of the oils, increasing the contents of both the chlorophyllic compounds and the carotenoids. Data suggest that the temperature of fruit when entering the crusher is the only factor responsible for this effect, not the treatment per se, and that increasing fruit temperature might lower the content of chemical species able to non-specifically oxidize olive fruit pigments by reducing the level of LOX activity during crushing. From an industrial point of view, it would be interesting to achieve a better control of olive fruit temperature at crushing, by regulating the water temperature for treatment and time after treatment, in order to modulate the colour of virgin olive oil to satisfy consumer demands.

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