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Modification of Volatile Compound Profile of Virgin Olive Oil Due to Hot-Water Treatment of Olive Fruit

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The effect of hot-water treatments of olive fruits before processing on the biosynthesis of virgin olive oil aroma was investigated by quantifying the variation within the major classes of volatile compounds. Data showed that hot-water treatments gave rise to changes in the volatile aroma profile of virgin olive oil from the three olive cultivars under study, Manzanilla, Picual, and Verdial. Different effects by thermal treatments were observed according to cultivar. In general, these changes are mainly due to a decrease in the contents of C_6 aldehydes and C_5 compounds. Contents of C_6 alcohols and esters remained constant or decreased slightly when the temperature of the treatment was increased. Thus, heat treatments seemed to promote a partial deactivation of the lipoxygenase/hydroperoxide lyase enzyme system, whereas other enzymatic activities, within the lipoxygenase pathway, such as alcohol dehydrogenase and alcohol acyltransferase, remained apparently unaffected as a consequence of heat treatments.

KEYWORDS: Volatiles; olive oil; hot-water treatment; aroma

INTRODUCTION

Virgin olive 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 (*1*−*3*). 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 is being developed for modulation of the bitterness intensity of olive oil. Bitterness is a common and desirable attribute of virgin olive oil flavor when present at low to moderate intensity. However, it is rejected by consumers when present at high intensity. García et al. (*4*) demonstrated that air-heating of olive fruit promoted a reduction of olive oil bitterness directly related to the time and temperature of treatment. 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. A strong correlation was observed in that study between bitterness intensity and the content of secoiridoid derivatives of hydroxytyrosol in the oil. However, this heat treatment also affected other quality traits such as oxidative stability and color and could produce a change in the aroma profile of the olive oil as well.

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, and also for maintenance of fruit quality throughout chilled storage. Postharvest exposure to moderate temperatures often increases storage life and improves the flavor of a number of fruits. The impact on flavor varies with species, temperature, and duration of the heat treatment (*5*). Physical treatments applied to the fruits might alter the enzymatic systems by partial or complete inhibition of their activities, causing changes in the aroma profile. It has been observed that heat treatment of apple fruits markedly inhibited the emission of volatiles, but the emission recovered slowly afterward due to a temporary inhibition of the enzymes or by deactivation and resynthesis (*6*). On the other hand, McDonald et al. (*7*) reported that tomato volatile aroma level and profile are altered as a function of the temperature used in the heat treatment.

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, hot-water treatments of olive fruits in the temperature range of 60−68 °C have proved to be quite effective in reducing olive oil bitterness. However, this emerging technology should be checked for its effect on other quality attributes of olive oil.

Aldehydes and alcohols of six straight-chain carbons (C_6) and the corresponding esters are the most important compounds in the aroma of virgin olive oil, from either a qualitative or quantitative point of view (*8*, *9*). The participation of the lipoxygenase (LOX) pathway in the biosynthesis of these C_6

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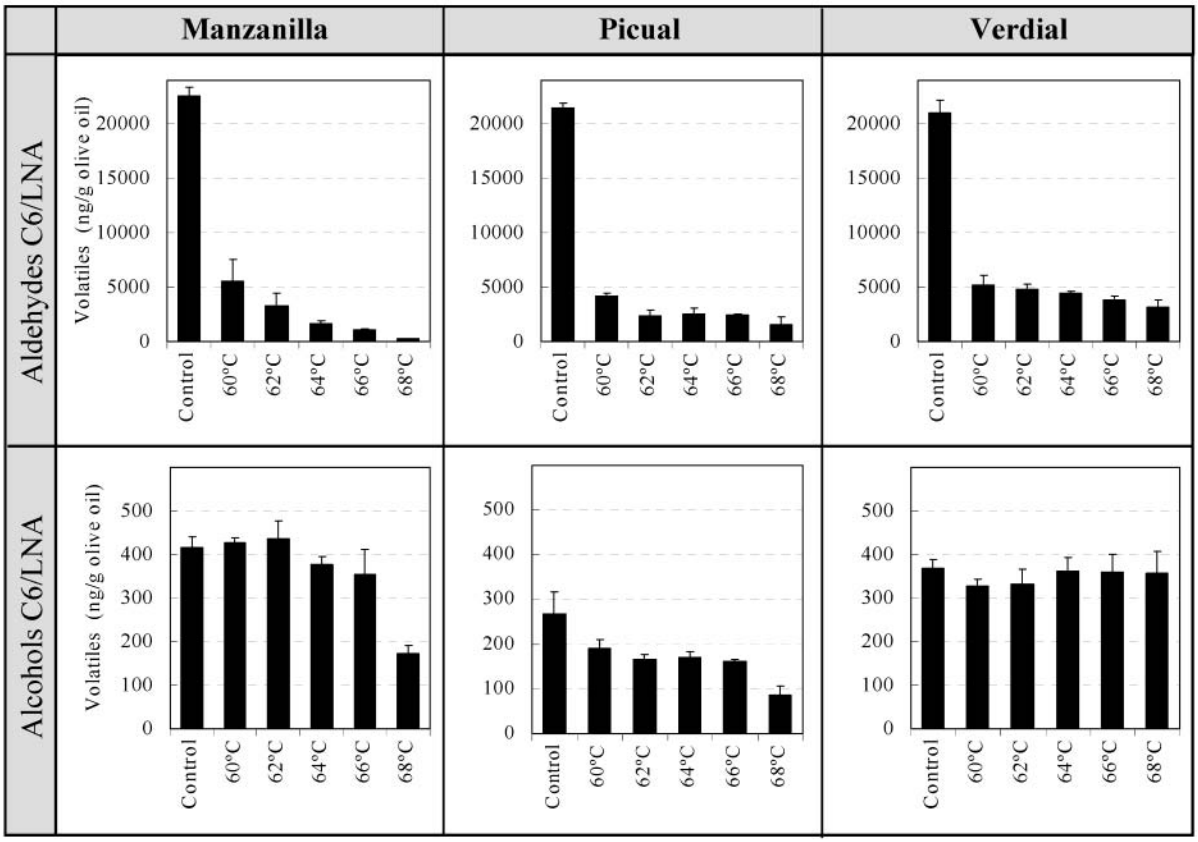

Figure 1. Contents of C_6 compounds derived from linolenic acid (LNA) in olive oils from fruits previously dipped in water at different temperatures. Volatile contents of aldehydes C_6/LNA are the sum of (*E*)-hex-2-enal, (*Z*)-hex-3-enal, (*Z*)-hex-2-enal, and (*E*)-hex-3-enal. Volatile contents of alcohols C_6/LNA are the sum of (*E*)-hex-2-enol, (*Z*)-hex-3-enol, and (*E*)-hex-3-enol.

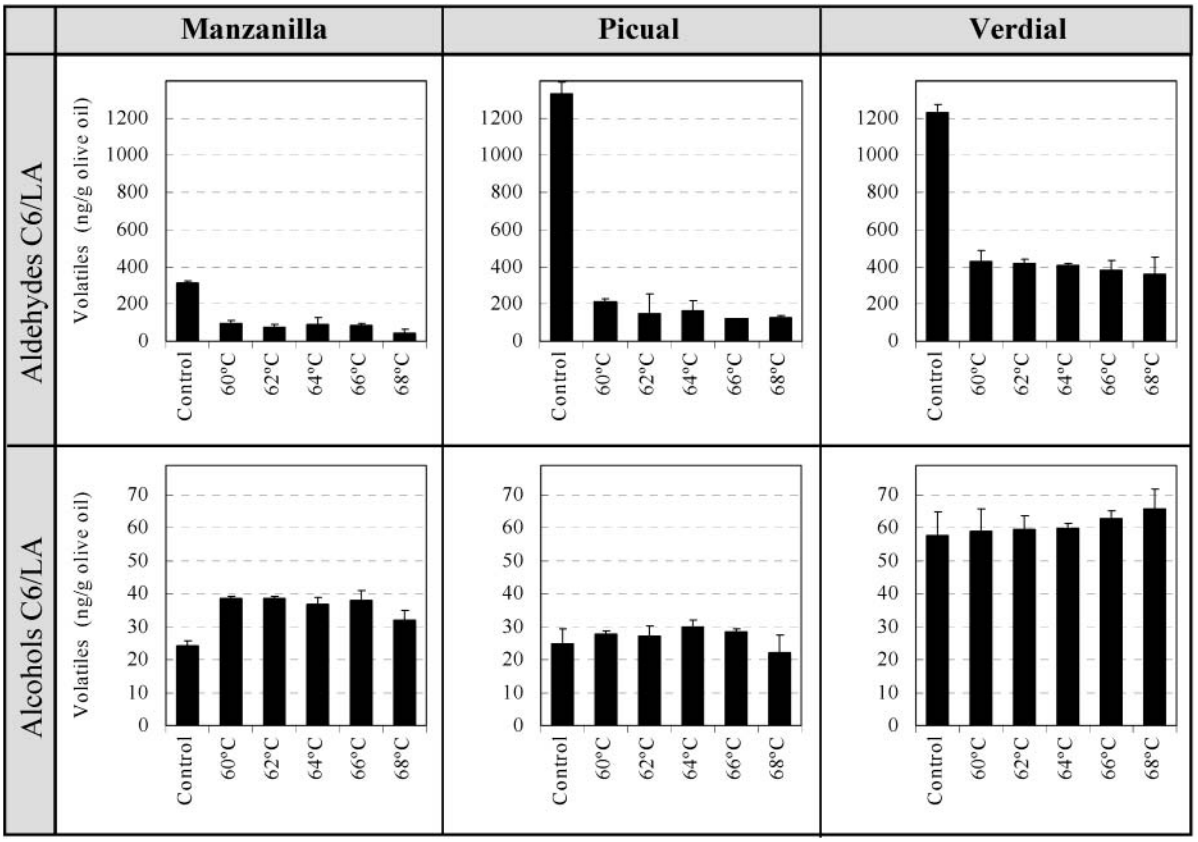

Figure 2. Contents of C_6 compounds derived from linoleic acid (LA) in olive oils from fruits previously dipped in water at different temperatures. Volatile contents of aldehydes C_6/LA represent the contents of hexanal. Volatile contents of alcohols C_6/LNA represent the contents of hexan-1-ol.

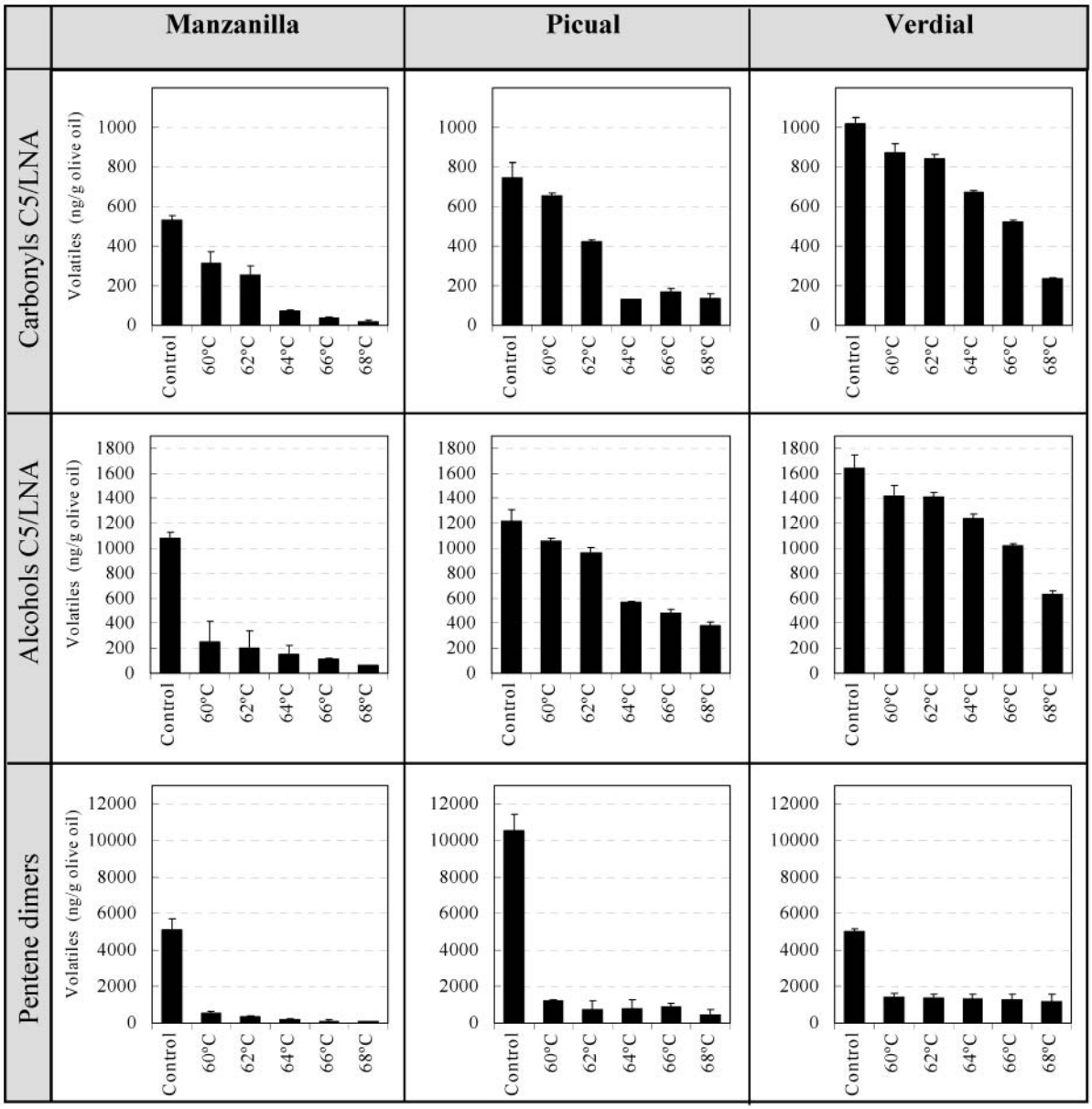

Figure 3. Contents of C_5 compounds derived from linolenic acid (LNA) in olive oils from fruits previously dipped in water at different temperatures. Volatile contents of carbonyls C_5/LNA are the sum of pent-1-en-3-one, (*E*)-pent-2-enal, and (*Z*)-pent-2-enal. Volatile contents of alcohols C_5/LNA are the sum of pent-1-en-3-ol, (*E*)-pent-2-en-1-ol, and (*Z*)-pent-2-en-1-ol.

compounds was established a decade ago (*10*). These compounds are synthesized from polyunsaturated fatty acids containing a *Z*,*Z*-1,4-pentadiene structure such as linoleic (LA) and linolenic (LNA) acids. In a first step of this pathway LOX produces the 13-hydroperoxide derivative that is subsequently cleaved by hydroperoxide lyase (HPL) to C_6 aldehydes (*10*−*12*). C_6 aldehydes can then undergo reduction by alcohol dehydrogenases (ADH) to form C_6 alcohols. Finally, by means of an alcohol acyltransferase (AAT) activity, C_6 alcohols can be transformed into the corresponding esters. Work by Angerosa et al. (*13*) has also demonstrated the relevance of C_5 compounds in the aroma of olive oil. C_5 compounds would be generated through an additional branch of the LOX pathway from LNA that would involve the production of a 13-alkoxyl radical by LOX as demonstrated in soybean seeds by Gardner's group (*14*, *15*). Studies on the LOX pathway in olive fruits have been restricted so far to fruit pulp. However, we have recently found that olive seed plays an important role in the biosynthesis of virgin olive oil aroma through this pathway (*16*).

In the scope of our current search for hot-water treatments on olive fruit to modulate bitterness intensity in virgin olive oils being compatible with an industrial use, the aim of the present work was to study the effect on the biosynthesis of virgin olive oil aroma of the temperature range demonstrated (unpublished results) with regard to bitterness reduction in hot-water treatments of olive fruits.

MATERIALS AND METHODS

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, color index 1 according to the guidelines of García et al. (*17*).

Heat Treatment of Fruits. Fruits were randomly distributed in 3.5 kg batches for every treatment in triplicate. Range of temperature for treatment (60−68 °C) was selected on the basis of its effectiveness in the modulation of bitterness intensity of the oils (unpublished results).

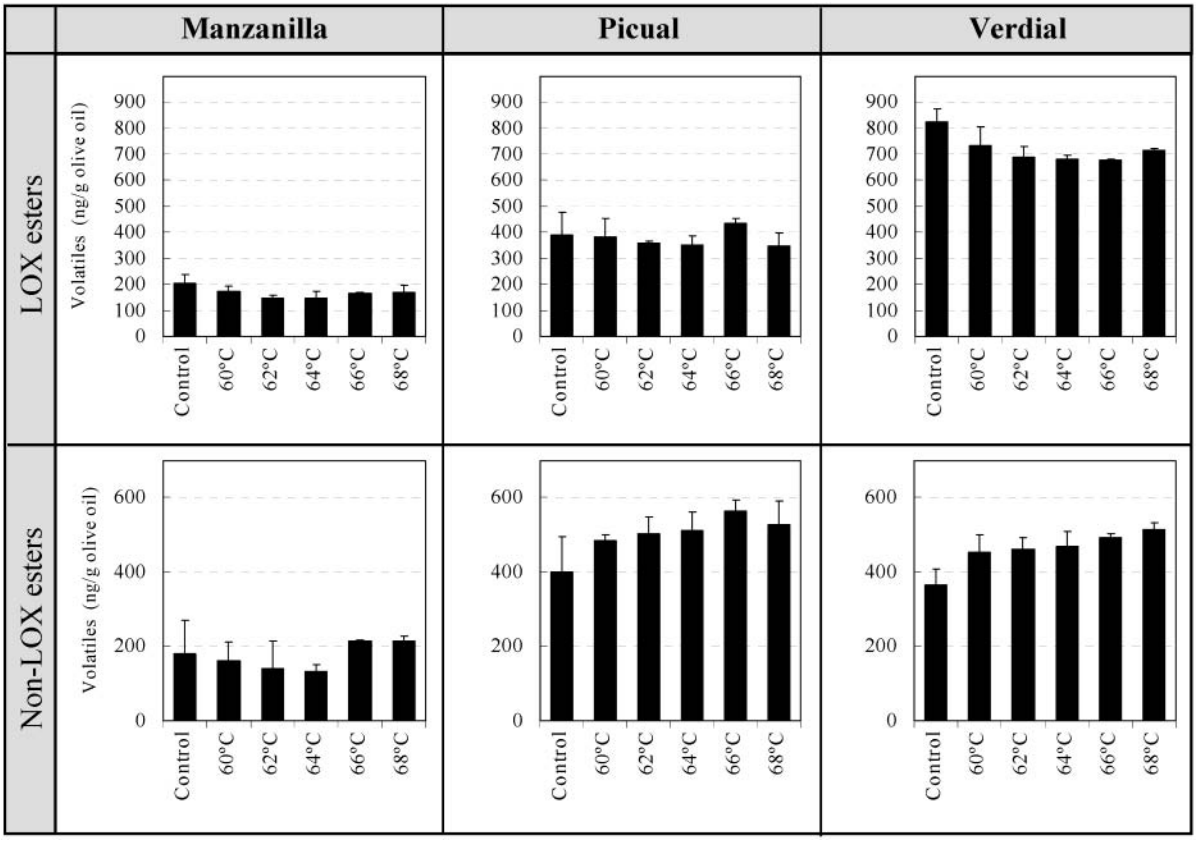

Figure 4. Contents of esters in olive oils from fruits previously dipped in water at different temperatures. Contents of LOX esters are the sum of hexyl acetate and (*Z*)-hex-3-en-1-yl acetate. Contents of non-LOX esters are the sum of methyl acetate, ethyl acetate, 3-methylbut-1-yl acetate, methyl hexanoate, and ethyl hexanoate.

Every batch of olive fruits was dipped in a 90 L thermostatic water bath for 3 min at different temperatures and processed immediately for olive oil extraction.

Olive Oil Extraction. After hot-water treatment of olive fruits, olive oil was immediately extracted using an Abencor analyzer (Comercial Abengoa, S.A., Seville, Spain) that simulates at laboratory scale the industrial process of virgin olive oil production (*18*). Malaxation was carried out for 30 min with the Abencor thermobeater operating at 30 °C. After centrifugation, oils were decanted and paper-filtered. Samples for volatile analysis (0.5 g) were taken in 10-mL sealed vials and stored at −18 °C until analysis.

Analysis of Volatile Compounds. Olive oil samples were conditioned to room temperature and then placed in a vial heater at 40 °C. After 10 min of equilibrium time, volatile compounds from the headspace (HS) were adsorbed on a solid phase microextraction (SPME) fiber DVB/Carboxen/PDMS 50/30 μm (Supelco Co., Bellefonte, PA). Sampling time was 50 min at 40 °C and carried out in duplicate. Desorption of volatile compounds trapped in the SPME fiber was done directly into the gas chromatograph (GC) injector. Volatiles were analyzed using an HP-5890 GC equipped with a fused silica capillary column DB-Wax (30 m × 0.25 mm; J&W Scientific, Folsom, CA). Operating conditions were as follows: N_2 as carrier gas; injector and detector at 250 °C; column held for 6 min at 40 °C and then programmed at 2 °C/min to 120 °C. Quantification was performed using individual calibration curves for each identified compound performed by adding known amounts of different compounds to a re-deodorized high-oleic sunflower oil (*16*). Calibration curves were linear in the concentration range found in the aroma of olive oils. Compound identification was carried out on an HRGC-MS Fisons series 8000 equipped with a similar stationary phase column and two different lengths, 30 and 60 m, matching against the Wiley/NBS Library and by GC retention time against standards.

Chemicals and Reagents. Reference compounds used for volatile identification and quantification were supplied by Sigma-Aldrich (St. Louis, MO) except for (*Z*)-hex-3-enyl acetate, purchased from Givaudan Co. (Clifton, NJ), and (*Z*)-hex-3-enal, generously supplied by S. A. Perlarom (Louvaine-La-Neuve, Belgium). Compounds such as (*E*)-hex-3-enal, (*Z*)-hex-2-enal, (*Z*)-pent-2-enal, and pentene dimers were tentatively identified on the basis of mass spectra and their concentrations approximately quantified according to their available isomers (*16*).

RESULTS AND DISCUSSION

The effect of these hot-water treatments on the biosynthesis of aroma volatiles of virgin olive oil was investigated by using the HS-SPME technique and by quantifying the variation within the major classes of volatile compounds.

It is known that C_6 compounds from LNA are the most abundant class of volatiles present in the aroma of virgin olive oil (*8*, *9*). **Figure 1** shows the effect of hot-water dip treatments of olive fruits on the level of this class of compounds in olive oil aroma. The content of total C_6 aldehydes from LNA decreased as a consequence of hot-water treatments in the three cultivars under study. This decrease was a function of the water temperature and ranged on average between 77% for 60 °C and 93% when the water temperature was 68 °C. However, this decrease in C_6 aldehydes was not reflected in the content of C_6 alcohols from LNA. No significant changes in the content of these compounds were observed among the different treatments, although a slight decrease was displayed in 68 °C treatments in cultivars Manzanilla and Picual.

Similarly to C_6 aldehydes from LNA, hexanal (C_6 aldehyde from LA) contents experienced a decrease after hot-water treatments (**Figure 2**). The percentage of this decrease ranged between 73 and 83% for 60 and 68 °C, respectively, in the three cultivars under study. The hexanol content (C_6 alcohol from LA) increased slightly (in Manzanilla fruits) or remained

constant (in Picual and Verdial fruits) in any treatment temperature under study (**Figure 2**). Data seem to point out a deactivation of the LOX/HPL enzyme system as a consequence of hot-water treatment. In this sense, a higher thermal stability was reported for LOX than for HPL in olive pulp, showing an unusual optimum temperature for HPL of 15 °C (*19*). On the contrary, ADH activities would remain apparently unaltered at temperatures of up to 66−68 °C. These results are compatible with our earlier observations (*16*) of an important ADH activity load in the olive seed that might be protected against thermal treatments by the stone hull, thus fully contributing to the production of alcohols during the crushing−malaxation process. Moreover, no HPL activity seems to be present in olive seed but other enzymatic activities apparently metabolize 13-hydroperoxides, contributing to a reduction of the concentration of C_6 and C_5 compounds in virgin olive oil aroma.

C_5 volatile compounds contribute to sensory perceptions in ways quite similar to those of the C_6 volatile compounds (*13*, *20*). Hot-water treatments of olive fruit commonly reduced the levels of every class of C_5 compounds in the resulting olive oils (**Figure 3**). Thus, C_5 carbonyl derivative contents decreased an average of 23% in 60 °C treatments up to an average of 85% in 68 °C treatments. A decrease in the content of C_5 alcohols was also observed as a function of treatment temperature, this decrease being dependent on olive cultivar. Whereas treatments on Manzanilla fruits gave rise to a low level of this class of compounds, 23% in 60 °C treatments, the same treatment temperature decreased the level of C_5 alcohols to ∼87% in olive oils from Picual and Verdial fruits. Pentene dimers were found to be the major volatiles among C_5 compounds. Again, differences were found according to cultivar. The content of pentene dimers decreased more in Manzanilla and Picual fruits (89%) than in Verdial fruits (71%) as a consequence of the 60 °C treatments. Olive oils obtained after 68 °C treatments from the former cultivars presented an average 3% in the content of terpene dimers with respect to control fruits and an average 23% in the case of Verdial fruits. The quite similar pattern observed for the content of C_5 alcohols and carbonyl derivatives as a consequence of hot-water treatment points out the inactivation of, at least, the LOX isoform responsible for the synthesis of the 13-alkoxyl radical origin of the C_5 family of compounds. In this sense, several labile and resistant isoforms of LOX and HPL have been suggested to be present in tomato, giving rise to the superior flavor associated with tomato products obtained by the cold break process at 60 °C (*21*).

Concerning the contents of esters, it has been reported that hexyl and (*Z*)-hex-3-en-1-yl acetates contribute mainly to the green sensory perception of virgin olive oils (*20*). These esters are synthesized by AAT within the LOX pathway and, as shown in **Figure 4**, showed almost no variation as a consequence of the hot-water treatment of olive fruits. Similarly, contents of esters synthesized from other than LOX pathways seem not to be affected by the hot-water treatment. Even an increase in their contents could be observed when the temperature of treatment was increased for Picual and Verdial cultivars. This class of esters typically found in fresh fruits might contribute to the fruity sensory perception of virgin olive oils.

We have recently observed that olive seed is a major contributor of ester-forming activity (AAT) in olive fruits (*16*). As in the case of ADH discussed above, hot-water treatment is probably not so intense in the inner part of the fruit, the seed, so that AAT activity remains unaffected during the thermal treatment, producing esters either with a LOX or non-LOX origin during the crushing−malaxation process to produce virgin olive oil. In this sense, we have previously found that strawberry AAT is quite resistant to temperature deactivation, presenting an optimum temperature in the range of 35−40 °C (*22*).

In summary, results obtained in this study showed that hot-water treatments on olive fruits before processing, in the temperature interval useful to reduce oil bitterness, gave rise to changes in the volatile aroma profile of the resulting virgin olive oil. Hot-water treatments seem to promote a deactivation of the LOX/HPL enzyme system, commonly reducing the biosynthesis of C_6 aldehydes and C_5 compounds. On the contrary, other enzymatic systems within the LOX pathway such as the ADH and AAT enzymatic activities remained apparently unaffected as a consequence of hot-water treatment, so that C_6 alcohol and ester contents showed almost no variation. The reason for this behavior could be based on a protective effect of the olive hull on the seed enzymes during heat treatments of olive fruits.

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