

Hot Water Dipping of Olives (*Olea europaea*) for Virgin Oil Debittering

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Olives (*Olea europaea* L.) of the Manzanilla, Picual, and Verdial varieties harvested at the green mature stage of ripening were dipped in hot water at a range of temperatures between 60 and 72 °C for 3 min. Immediately after treatment, oils were physically extracted from the olives. Olive heating promotes a reduction of oil bitterness in direct relationship to the temperature used. Fruit heating at ≥ 60 °C for 3 min did not cause significant changes in acidity, UV absorption, peroxide index, and panel test score of the oils obtained but decreased its oxidative stability. Oils extracted from heated fruit showed higher concentrations of chlorophylls and carotenes and lower total phenol content.

KEYWORDS: *Olea europaea*; postharvest; heat treatment; oil quality; phenolic compounds

INTRODUCTION

Bitter taste is a sensory attribute characteristic of virgin olive oil. However, its excessive presence may mean the rejection by consumers accustomed to the milder taste of refined oils, such as the important markets of Japan, Canada, U.S.A., Australia, China, or Northern European countries. "Picual" virgin oils, obtained from the most cultivated olive variety in Spain, habitually show a high level of this attribute. In consequence, for the Spanish olive oil industry to develop a method to control the level of bitterness should be very interesting. This method should be physical, because, by definition, virgin olive oil must be obtained exclusively by physical systems, and chemical treatments should not be involved in the extraction process. In our laboratory, a treatment based on the heating of the fruits in a thermostated room at 40 °C for 24–72 h was successfully tested (1). A heat treatment, previously applied to the fruit, might alter the activities of enzymes during the oil extraction process. These enzymes, such as glycosidases and esterases, are involved in the hydrolysis of a water-soluble highly bitter secoiridoid glycoside characteristic of the *Oleaceae*, named oleuropein. The lipophilic phenolic compounds derived from this hydrolysis are responsible for the oil bitterness (2, 3). Probably inhibiting these enzyme activities, partially or totally, heat treatment achieves a decrease in the content of bitter compounds in the oil. Mateos et al. (4) demonstrated that the hydroxytyrosyl elenolate was the phenolic compound of the olive oil most strongly related to bitter taste. This kind of treatment is difficult to adapt to the production lines of the olive oil extraction industry. It requires an equipped room, a relatively long treatment time, the use of boxes, and is a discontinuous process that requires loading and unloading of

the treated material. Dipping in water allows a more rapid and efficient transmission of the heat to the fruits. Using this new method, the heat treatment could be carried out in only a few minutes, and its adaptation to the industrial production lines would be relatively easier. It would only be necessary to add a heating system to the fruit washing process normally used in the oil extraction lines of the industry.

In the present work, the use of heat treatment by dipping olives in hot water to control the presence of bitterness in the oil subsequently extracted has been tested, using three different Spanish cultivars: Manzanilla, Picual, and Verdial.

MATERIAL AND METHODS

Plant Material. Olive fruits (*Olea europaea*) cvs. Manzanilla, Picual, and Verdial were harvested during the 2002–2003 season in two orchards of Andalusia (Spain) at the green mature stage, corresponding approximately to the average value "1" of the ripening index habitually used in the oil industry (5) and transported the same day to the Instituto de la Grasa. There, 100 kg of healthy fruits were selected from each variety. The olives were randomly distributed in six treatment groups, with each one in three 3.5 kg batches. Five of these groups were dipped in a 400 L thermostatic water bath at temperatures between 60 and 72 °C during 3 min. The sixth group was immediately processed without any treatment and was used as a control.

Oil Extraction. The oil from the olives of each 3.5 kg batches of each group of treatment was extracted separately, constituting triplicate samples, using an Abencor analyzer (Comercial Abengoa S.A., Seville, Spain). This unit, consisting of three basic elements, a mill, a thermobearer, and a pulp centrifuge, simulates at laboratory scale the industrial process of virgin olive oil production (6). After centrifugation, the oil was decanted in a graduated tube to measure the volume obtained to calculate the oil yield, which was expressed as a percentage of the fresh weight. Subsequently, the extracted oil was filtered and stored at -20 °C under N_2 atmosphere until its analysis.

Oil Analysis. The titratable acidity, the peroxide index, the coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}), and

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Table 1. Changes in Quality Parameters of Virgin Olive Oils Obtained from Nontreated (Control) or Previously Treated at Different Temperatures during 3 Min Manzanilla Olives^a

| | control | 60 °C | 62 °C | 64 °C | 66 °C | 68 °C |
|---|---------|---------|---------|---------|---------|---------|
| oil yield (%) | 10.3 b | 14.2 a | 14.0 a | 13.0 a | 10.4 b | 10.5 b |
| acidity (% oleic acid) | 0.50 a | 0.43 b | 0.42 b | 0.26 c | 0.39 b | 0.22 c |
| peroxide index (meq O ₂ /kg) | 7.2 c | 5.6 d | 7.6 c | 7.5 c | 8.6 b | 10.3 a |
| K ₂₃₂ | 2.28 a | 2.04 b | 1.72 c | 1.71 c | 1.62 c | 1.12 d |
| K ₂₇₀ | 0.26 a | 0.23 a | 0.17 b | 0.16 b | 0.16 b | 0.10 c |
| carotenes (mg/kg) | 12.65 d | 24.45 c | 26.39 c | 23.01 c | 33.16 b | 49.3 a |
| chlorophylls (mg/kg) | 20.91 e | 45.78 d | 63.85 c | 48.68 d | 123.6 b | 137.8 a |
| stability (h) | 186.2 a | 168.2 b | 160.5 b | 154.8 b | 159.4 b | 126.2 c |
| panel test (1–9) ^b | 7.4 | 7.5 | 7.4 | 7.6 | 7.5 | 7.7 |
| bitterness (0–5) ^c | 4.9a | 4.5 b | 4.3 b | 3.7 c | 3.5 c | 2.7 d |

^a Each point is the mean value of three replicates. In each file, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA, and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. ^b 1 indicates the worst sensory quality possible, and 9 indicates the best sensory quality possible. ^c 0 indicates the absence of the attribute, and 5 indicates the highest intensity.

Table 2. Changes in Quality Parameters of Virgin Olive Oils Obtained from Nontreated (Control) or Previously Treated at Different Temperatures during 3 Min Picual Olives^a

| | control | 60 °C | 62 °C | 64 °C | 66 °C | 68 °C |
|---|---------|---------|---------|---------|---------|----------|
| oil yield (%) | 18.2 | 18.5 | 17.7 | 17.6 | 18.0 | 17.8 |
| acidity (% oleic acid) | 0.18 | 0.22 | 0.23 | 0.23 | 0.20 | 0.16 |
| peroxide index (meq O ₂ /kg) | 6.4 | 5.7 | 7.3 | 5.5 | 6.9 | 4.7 |
| K ₂₃₂ | 1.27 b | 1.64 a | 1.21 b | 1.34 b | 1.61 a | 1.16 c |
| K ₂₇₀ | 0.15 | 0.16 | 0.15 | 0.11 | 0.15 | 0.10 |
| carotenes (mg/kg) | 14.44 d | 30.1 b | 35.7 a | 21.38 c | 29.50 b | 25.65 bc |
| chlorophylls (mg/kg) | 14.26 e | 77.50 b | 98.18 a | 50.95 d | 76.25 b | 63.33 c |
| stability (h) | 174.0 a | 164.0 b | 173.0 a | 156.0 c | 120.6 d | 76.5 e |
| panel test (1–9) ^b | 7.7 | 7.5 | 7.7 | 7.4 | 7.6 | 7.9 |
| bitterness (0–5) ^c | 4.7 a | 4.2 b | 4.0 b | 3.6 c | 3.3 d | 2.8 e |

^a Each point is the mean value of three replicates. In each file, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA, and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. ^b 1 indicates the worst sensory quality possible, and 9 indicates the sensory quality possible. ^c 0 indicates the absence of the attribute, and 5 indicates the highest intensity.

the panel test were determined from the extracted oils according to the European Union standard methods (Annexes II and IX in European Community Regulation EEC/2568/91). For the panel test, the overall sensory quality of each oil sample was graded by a panel of eight trained (≥ 5 years experience) tasters according to a scale of nine points, with "1" being the value for the poorest quality possible and "9" being the value for the best. Bitterness intensity was determined by the same analytical panel using a structured scale of five points, where "0" means the absence of the attribute, "1" means simple perception, "2" means light presence, "3" means middle presence, "4" means strong intensity, and "5" means the highest intensity. Oxidative stability was measured by the Rancimat method, which evaluates the time (h) of resistance of 3 g of oil samples exposed to a stream of dry air at a temperature of 100 °C to oxidation (7, 8). The content of pigments in the oils was evaluated by their absorbances at 470 and 670 nm for carotenoids and chlorophylls, respectively, and the results expressed as mg/kg (9). Phenolic fraction was isolated by solid-phase extraction and analyzed by reversed-phase HPLC using a diode array UV detector (10). Quantification of phenolic compounds (except ferulic acid) was carried out at 280 nm using *p*-hydroxyphenylacetic acid as an internal standard, whereas quantification of flavones and ferulic acid was done at 335 nm using *o*-coumaric acid as an internal standard. The results were expressed in millimoles per kilogram.

Statistical Analysis. Analysis of variance (ANOVA) was carried out on all data. A 5% level of least significant difference (Lsd), calculated by Duncan's multiple range test, was used to establish differences between the mean values, when ANOVA detected a significant ($p \leq 0.05$) effect because of the treatments.

RESULTS AND DISCUSSION

The intensity of bitterness of virgin olive oils obtained from the three tested varieties decreased significantly depending on the dipping temperature (Tables 1, 2, and 3). Manzanilla and

Picual oils exhibited a similar response of bitterness intensity at the same treatment temperatures. Olives of these cultivars dipped at 68 °C produced oils with, approximately, a score of 3 of this attribute, whereas the oils extracted from nontreated fruits exhibited almost the highest perceivable intensity of bitterness (a score of 5). However, for Verdial olives, the use of 68 °C only reduced oil bitterness to a level of score 3.8, and the use of 72 °C was necessary to obtain an, approximately, score of 2 of this attribute. Heat applied to Manzanilla and Verdial olives in a thermostated room at 40 °C obtained similar results after 24 and 48 h of treatment, respectively (1). Heating by dips in water clearly reduced the time of treatment in relation to the transmission of heat by air, allowing the use of higher temperatures. This finding is especially interesting for the adaptation of this system to industry. The time of 3 min was considered as optimal for the industrial application of the method. The reduction of this time would imply the use of too high temperatures, and the effective use of lower temperatures would involve an excessive increase of the time of treatment (data not shown).

The heat treatment by water dipping affected the oil yield obtained from the treated fruits, in different ways. Thus, heating at 60, 62, or 64 °C produced a significant increase in the oil yield from Manzanilla olives. No effect was detected on Picual fruits, and a significant decrease on this parameter was observed, when the Verdial variety was treated. Oil yield depends on very different variables of the fruit structure, such as variety, age, texture, oil content, humidity, relationship between flesh/stone weights, etc., and they can dramatically change from season to season, depending on the climatic conditions. The use of dipping

Table 3. Changes in Quality Parameters of Virgin Olive Oils Obtained from Nontreated (Control) or Previously Treated at Different Temperatures during 3 Min Verdial Olives^a

| | control | 60 °C | 64 °C | 68 °C | 70 °C | 72 °C |
|---|---------|----------|----------|--------|----------|----------|
| oil yield (%) | 20.0 a | 18.8 b | 18.4 b | 18.5 b | 18.6 b | 17.0 c |
| acidity (% oleic acid) | 0.25 | 0.25 | 0.24 | 0.18 | 0.21 | 0.16 |
| peroxide index (meq O ₂ /kg) | 5.4 c | 6.9 b | 5.0 c | 9.8 a | 10.1 a | 6.6 b |
| K ₂₃₂ | 1.51 | 1.57 | 1.62 | 1.6 | 1.48 | 1.37 |
| K ₂₇₀ | 0.15 | 0.16 | 0.16 | 0.14 | 0.14 | 0.12 |
| carotenes (mg/kg) | 22.07 c | 44.19 a | 43.12 a | 36.9 b | 45.65 a | 43.48 a |
| chlorophylls (mg/kg) | 40.26 d | 116.82 a | 111.24 a | 83.4 c | 108.25 b | 100.53 b |
| stability (h) | 74.7 a | 69.4 b | 76.0 a | 54.8 c | 54.0 c | 49.0 d |
| panel test (1–9) ^b | 7.5 | 7.7 | 7.5 | 7.6 | 7.7 | 7.6 |
| bitterness (0–5) ^c | 4.4 a | 4.1 b | 4.0 b | 3.8 b | 3.4 c | 2.1 d |

^a Each point is the mean value of three replicates. In each file, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA, and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. ^b 1 indicates the worst sensory quality possible, and 9 indicates the best sensory quality possible. ^c 0 indicates the absence of the attribute, and 5 indicates the highest intensity.

Table 4. Changes in Phenolic Compound Content of Virgin Olive Oils Obtained from Nontreated (Control) or Previously Treated at Different Temperatures during 3 Min Manzanilla Olives^a

| compound (mmol/kg) | control | 60 °C | 62 °C | 64 °C | 66 °C | 68 °C |
|---------------------------------|---------|---------|---------|---------|---------|---------|
| hydroxytyrosol | 0.040 b | 0.045 b | 0.055 a | 0.032 c | 0.028 c | 0.034 c |
| tyrosol | 0.031 c | 0.034 c | 0.045 a | 0.031 c | 0.045 a | 0.039 b |
| vanillic acid | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| vanilline | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| <i>p</i> -cumarinic acid | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| hydroxytyrosol acetate | 0.015 | 0.013 | 0.012 | 0.014 | 0.016 | 0.012 |
| 3,4-DHPEA-EDA ^b | 0.430 | 0.612 c | 0.759 b | 0.588 c | 0.800 a | 0.724 b |
| tyrosol acetate | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| <i>p</i> -HPEA-EDA ^c | 0.303 f | 0.486 e | 0.642 c | 0.520 d | 0.684 b | 0.756 a |
| pinosresinol | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.009 |
| cinamic acid | 0.008 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 |
| acetoxypinosresinol | 0.024 a | 0.001 c | 0.000 c | 0.000 c | 0.000 c | 0.009 b |
| 3,4-DHPEA-EA ^d | 1.963 a | 1.456 b | 1.218 c | 1.023 d | 0.783 e | 0.781 e |
| <i>p</i> -HPEA-EA ^e | 1.091 a | 1.012 a | 1.036 a | 0.867 b | 0.558 c | 0.586 c |
| ferulic acid | 0.003 | 0.003 | 0.002 | 0.002 | 0.001 | 0.002 |
| luteoline | 0.015 a | 0.006 b | 0.002 c | 0.001 c | 0.000 c | 0.000 c |
| apigenine | 0.008 a | 0.003 b | 0.001 c | 0.001 c | 0.000 c | 0.000 c |
| total phenols | 3.932 a | 3.670 b | 3.771 b | 3.079 c | 2.915 c | 2.953 c |
| total orthodiphenols | 2.463 a | 2.132 b | 2.046 b | 1.658 c | 1.627 c | 1.551 c |
| total secoiridoid derivatives | 3.787 a | 3.566 b | 3.655 b | 2.998 c | 2.825 d | 2.847 d |

^a Each point is the mean value of three replicates. In each file, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA, and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. ^b Dialdehydic form of the decarboxymethyl oleuropein aglycone. ^c Dialdehydic form of the decarboxymethyl ligstroside aglycone. ^d Hydroxytyrosyl-elenolate. ^e Tyrosyl-elenolate.

at high temperatures could produce emulsion of the oil during the subsequent extraction process (data not shown).

Heat treatments produced changes in the values of the official parameters (acidity, peroxide index, UV absorption, and panel test), established to evaluate the quality level of virgin olive oils (EEC/1989/03 regulations), but these changes did not affect the quality category ("extra virgin") of any of the oil samples analyzed. For instance, free acidity of Manzanilla oils was even significantly reduced as a consequence of the treatments, whereas no effect was observed on the oils of the other two varieties. Probably, these facts could be related to a reduction of the lipase activity during the extraction process of the Manzanilla olives, because of the previous action of the heating (11). In Picual and Verdial olives, this activity could be initially inactivated. For this reason, they exhibited very low values of free fatty acid in their control oils. In general, the peroxide index values increased significantly in the oils extracted from treated Manzanilla and Verdial olives. Nevertheless, the values obtained are considerably far from the established limit for losing the best commercial category of quality (20 meq O₂/kg). Absorbances at 232 and at 270 nm clearly decreased in the oil extracted from treated Manzanilla olives, and they were practically not affected by the treatments applied to Picual and Verdial

olives. Probably, enzymes such as lipoxygenase and hydroperoxide liase should also be active in Manzanilla paste and may be inhibited by heating. Farag et al. (11) observed that lipoxygenase activity of ripe Picual olives decreased linearly with the intensity of microwave heating applied. The sensory quality of the oil samples (panel test) did not change significantly because of the heating in any of the varieties tested.

Other quality parameters, which are not included in the official regulations, were more affected by the treatments. Thus, the stability to oxidation was reduced as a consequence of the heating on the three varieties studied. As a general rule but with some exceptions, this parameter decreased as the temperature of the treatment increased in a parallel way, with a reduction observed on bitterness intensity. However, the remaining values of this parameter after the heat treatment of the varieties Manzanilla and Picual were very high, absolutely considered. In the case of Verdial olives, the lowest stability value shown (49.0 h) is higher than those normally exhibited by virgin oils extracted from nontreated Arbequina olives or even from nontreated Verdial olives with a higher level of maturity (12). Carotene and chlorophyll contents significantly increased as a consequence of the treatments in all of the varieties assayed. This is probably due to the heat-induced inactivation of the

Table 5. Changes in Phenolic Compound Content of Virgin Olive Oils Obtained from Nontreated (Control) or Previously Treated at Different Temperatures during 3 Min Picual Olives^a

| compound (mmol/kg) | control | 60 °C | 62 °C | 64 °C | 66 °C | 68 °C |
|---------------------------------|---------|---------|---------|----------|----------|---------|
| hydroxytyrosol | 0.015 b | 0.022 a | 0.011 b | 0.014 b | 0.012 b | 0.010 b |
| tyrosol | 0.017 a | 0.017 a | 0.008 b | 0.008 b | 0.005 c | 0.005 c |
| vanillic acid | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| vanilline | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| <i>p</i> -cumaric acid | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| hydroxytyrosol acetate | 0.000 b | 0.008 a | 0.005 a | 0.005 a | 0.006 a | 0.005 a |
| 3,4-DHPEA-EDA ^b | 0.206 f | 0.764 a | 0.544 b | 0.350 e | 0.411 d | 0.459 c |
| tyrosol acetate | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| <i>p</i> -HPEA-EDA ^c | 0.235 b | 0.402 a | 0.222 b | 0.180 c | 0.171 c | 0.133 d |
| pinoselinol | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| cinamic acid | 0.005 a | 0.004 a | 0.002 b | 0.002 b | 0.001 bc | 0.000 c |
| acetoxypinoselinol | 0.035 a | 0.027 b | 0.018 c | 0.005 de | 0.009 d | 0.002 e |
| 3,4-DHPEA-EA ^d | 1.601 a | 1.372 b | 1.252 c | 0.964 d | 0.951 d | 0.995 d |
| <i>p</i> -HPEA-EA ^e | 1.332 a | 0.806 b | 0.561 c | 0.371 d | 0.323 d | 0.113 e |
| ferulic acid | 0.003 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 |
| luteoline | 0.021 a | 0.006 b | 0.002 b | 0.003 b | 0.004 b | 0.003 b |
| apigenine | 0.012 a | 0.003 b | 0.001 b | 0.002 b | 0.001 b | 0.002 b |
| total phenols | 3.484 a | 3.433 a | 2.627 b | 1.905 c | 1.895 c | 1.728 d |
| total orthodiphenols | 1.843 b | 2.172 a | 1.814 b | 1.336 d | 1.384 d | 1.472 c |
| total secoiridoid derivatives | 3.374 a | 3.344 a | 2.579 b | 1.865 c | 1.856 c | 1.700 d |

^a Each point is the mean value of three replicates. In each file, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA, and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. ^b Dialdehydic form of the decarboxymethyl oleuropein aglycone. ^c Dialdehydic form of the decarboxymethyl ligstroside aglycone. ^d Hydroxytyrosyl-elenolate. ^e Tyrosyl-elenolate.

Table 6. Changes in Phenolic Compound Content of Virgin Olive Oils Obtained from Nontreated (Control) or Previously Treated at Different Temperatures during 3 Min Verdial Olives^a

| compound (mmol/kg) | control | 60 °C | 64 °C | 68 °C | 70 °C | 72 °C |
|---------------------------------|---------|----------|---------|---------|---------|---------|
| hydroxytyrosol | 0.029 a | 0.017 c | 0.023 b | 0.008 d | 0.017 c | 0.013 c |
| tyrosol | 0.052 a | 0.016 c | 0.022 b | 0.021 b | 0.014 c | 0.014 c |
| vanillic acid | 0.004 a | 0.000 b | 0.000 b | 0.000 b | 0.000 b | 0.000 b |
| vanilline | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| <i>p</i> -cumaric acid | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| hydroxytyrosol acetate | 0.014 b | 0.012 b | 0.013 b | 0.016 b | 0.033 a | 0.026 a |
| 3,4-DHPEA-EDA ^b | 0.606 a | 0.530 b | 0.509 b | 0.260 c | 0.322 c | 0.245 c |
| tyrosol acetate | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| <i>p</i> -HPEA-EDA ^c | 0.565 a | 0.529 a | 0.582 a | 0.327 b | 0.386 b | 0.326 b |
| pinoselinol | 0.000 c | 0.000 c | 0.000 c | 0.009 a | 0.000 c | 0.004 b |
| Cinamic acid | 0.003 a | 0.004 a | 0.004 a | 0.001 b | 0.000 b | 0.000 b |
| Acetoxypinoselinol | 0.019 a | 0.012 bc | 0.015 b | 0.009 c | 0.009 c | 0.004 d |
| 3,4-DHPEA-EA ^d | 1.084 a | 0.694 b | 0.723 b | 0.467 c | 0.456 c | 0.281 d |
| <i>p</i> -HPEA-EA ^e | 0.994 a | 0.596 b | 0.670 b | 0.464 c | 0.357 d | 0.224 e |
| ferulic acid | 0.004 | 0.002 | 0.002 | 0.000 | 0.002 | 0.001 |
| luteoline | 0.006 a | 0.003 b | 0.002 b | 0.002 b | 0.002 b | 0.001 b |
| apigenine | 0.004 a | 0.001 b | 0.002 b | 0.002 b | 0.002 b | 0.001 b |
| total phenols | 3.384 a | 2.416 b | 2.567 b | 1.586 c | 1.600 c | 1.140 d |
| total orthodiphenols | 1.739 a | 1.256 b | 1.270 b | 0.753 c | 0.830 c | 0.566 d |
| total secoiridoid derivatives | 3.249 a | 2.349 b | 2.484 b | 1.518 c | 1.521 c | 1.076 d |

^a Each point is the mean value of three replicates. In each file, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA, and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. ^b Dialdehydic form of the decarboxymethyl oleuropein aglycone. ^c Dialdehydic form of the decarboxymethyl ligstroside aglycone. ^d Hydroxytyrosyl-elenolate. ^e Tyrosyl-elenolate.

enzymes responsible for the pigment degradation during the oil extraction process. A similar effect was observed using heating by air transmission (1). Luaces et al. (13) published a more detailed description of this phenomenon. The production of highly pigmented oils should be very interesting for the industry. Carotenes have vitamin characteristics and are antioxidants, whereas chlorophylls are oxidants in the presence of light and antioxidants in darkness (14). Maintaining these oils in darkness should improve their stability. Furthermore, to have these kinds of oils available, greener and softer, should mean many different mixing possibilities ("coupages") with other virgin olive oils for improving its color without modifying its taste.

Total phenolic compounds, total orthodiphenols, and total secoiridoid derivatives were reduced by olive heating in the three

varieties tested (Tables 4, 5, and 6). However, after each phenolic compound was analyzed separately, the behavior of the three varieties differed. Thus, in Manzanilla oil, the decrease of the different groups of phenolic compounds is mainly due to the reduction in the content of the hydroxytyrosyl-elenolate and the tyrosyl-elenolate (3,4-DHPEA-EA and *p*-HPEA-EA, respectively), whereas the dialdehydic forms of the decarboxymethyl oleuropein aglycone and of the decarboxymethyl ligstroside aglycone (3,4-DHPEA-EDA and *p*-HPEA-EDA, respectively) showed a clear increase related with the fruit heating, and the other compounds did not play a role because of their low concentration. In Picual oils, a similar tendency was observed, but the content on *p*-HPEA-EDA increased only after the treatment at 60 °C and decreased significantly after

≥ 64 °C treatments. Finally, in Verdial oils, the contents on these four different secoiridoid derivatives clearly decreased as a consequence of the increasing treatment temperature. These results coincided with the decrease in the orthodiphenol content obtained by García et al. (1), using heat treatment in a thermostated room. The decrease observed on the *p*-HPEA-EA and 3,4-DHPEA-EA contents in the oils of the three varieties coincided with the reduction of bitterness intensity observed, confirming the findings obtained by Gutierrez-Rosales et al. (15), who identified these compounds as the main ones responsible for the bitter taste of virgin olive oil. However, the concentrations of 3,4-DHPEA-EA found in the oils after the treatment were higher than expected by the level of bitterness evaluated sensory, according to the equation proposed by Mateos et al. (4). Heat treatments have resulted in oils with different bitterness intensity using the same original fruit, facilitating the identification of the compounds responsible for this sensory attribute. In the same way, these treatments allow us to obtain oils with different stability against oxidation from the same fruit, facilitating the identification of the compounds responsible for this quality parameter. Our results do not support the findings of Morello et al. (16), who identified the molecules of 3,4-DHPEA-EDA as the main molecules responsible for the antioxidative activity of the Arbequina olive oil, because the increase of temperature treatment determined a decrease on the stability coinciding with the increase on the 3,4-DHPEA-EDA concentration. This result does not necessary imply that this molecule has not antioxidant activity. Probably the decrease on stability is due to the decrease of other molecules related with this parameter despite the 3,4-DHPEA-EDA increase. In contrast, our results agree with the ones obtained by del Carlo et al. (17), who found that the total polyphenol determination was better correlated to oxidative stability than individual polyphenols.

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