Reduction of Oil Bitterness by Heating of Olive (*Olea europaea*) Fruits

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Olives (*Olea europaea*) of the Manzanilla and Verdial varieties, harvested at the green mature stage of ripening, were heated at 30, 40, 45, and 50 °C during 24 h and at 40 °C during 24, 48, and 72 h, respectively. Just after treatments, oils were physically extracted from the olives. Olive heating promotes a reduction of oil bitterness in direct relationship to the time and temperature used. Fruit heating at ≤40 °C during 24 h did not produce significant changes of acidity, UV absorption, peroxide index, panel test score, or oxidative stability of the obtained oils. Both longer treatments at 40 °C and heating at >40 °C yielded oils with less oxidative stability. Oils obtained from olives heated at ≥40 °C showed higher concentrations of chlorophylls and carotenes. For each olive variety, a good correlation between oil bitterness and content of hydroxytyrosol secoiridoid derivatives was found.

Keywords: *Olea europaea; postharvest; olive oil bitterness; olive thermal treatment*

INTRODUCTION

Virgin olive oil, one of the main components of the "Mediterranean diet", is related to protection against cardiovascular diseases and cancer (*1*−*3*), due to its fatty acid profile and the presence of minor amounts of phenolic constituents. Virgin olive oil is obtained from crushed olives by press or centrifugation processes, preserving its sensory characteristics and nutritional value. Other vegetable oils have scarce odor and taste because they are obtained by solvent extraction with subsequent refining. Virgin olive oils with low or moderate level of bitterness are accepted by consumers, but very bitter ones are rejected; they must be blended with nonbitter virgin olive oil or with refined olive oil, constituting a "coupage" or an "olive oil", respectively. The development of a processing method to reduce the bitterness intensity, but not affecting other quality attributes, would facilitate the direct consumption of these virgin oils.

This technique could be applied to fruits with a lower degree of ripening. At the beginning of November, olives are hardly able to incorporate more oil in their cells (*4*), but the oil obtained from them is too bitter, astringent, and pungent. A reduction of the oil bitterness would allow an early harvesting of the olive crop, which would be presumed to have a series of additional advantages: a better amortization of the machinery; longer harvesting time; reduction of losses by fruit fall, parasitism, robbery, etc.; fruit more resistant to mechanical damage; and an improvement in the crop of the following season because the olive trees would be free of fruit for a longer time.

The intensity of the bitterness of olive oil has been related with the presence of phenolic compounds derived from the hydrolysis of oleuropein, a secoiridoid glucoside characteristic of the Oleaceae (*5*, *6*). These compounds, which are partially soluble in lipids, are conferred to the virgin oil during its extraction, through an unknown process in which at least two kinds of enzymes, such as glycosidases and estearases, should be involved (*7*). Glycosidases would break the glycosidic bond of the oleuropein, yielding secoiridoid derivatives of phenols, which are implied in oil bitterness. From oleouropein and/or secoiridoid derivatives the esterases would yield free phenols, which are not bitter. A physical treatment previously applied to the fruit might alter the activities of these enzymes, probably inhibiting them partially or totally, and, in consequence, changing the phenolic composition of the oil subsequently obtained. In the present paper, this hypothesis has been verified by analyzing the effects of previous heating of olive fruits on phenolic content, intensity of bitterness, and quality of the obtained oils.

MATERIALS AND METHODS

Olive fruits (*Olea europaea*), cvs. Manzanilla and Verdial, were harvested in two orchards of Andalusia (Spain) at the green mature stage, corresponding approximately to an average value of 1 of the maturation index (*8*) and transported the same day to the Instituto de la Grasa. There, healthy fruits (75 kg) were selected from each variety. The Manzanilla olives were randomly distributed in five treatment groups, each one in three 5 kg boxes. Four of these groups were maintained at 30, 40, 45, and 50 °C, respectively, in different rooms during 24 h. The fifth group was immediately processed without any treatment and was used as control. The Verdial olives were similarly distributed in four groups of 15 kg. Three of these groups were maintained at 40 °C during 24, 48, and 72 h, respectively. The fourth group was immediately processed and was considered as the treatment corresponding to time 0.

The oil from the olives of each 5 kg box of each treatment group was extracted separately, constituting triplicate samples, using an Abencor analyzer (Comercial Abengoa S.A., Sevilla, Spain). This unit, consisting of three basic elements, a mill, a thermobeater, and a pulp centrifuge, simulates at laboratory scale the industrial process of virgin olive oil production (*9*). The oil was decanted and subsequently filtered and stored at −20 °C under an N_2 atmosphere until its analysis.

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The titratable acidity, the peroxide index, and the coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) were determined from the extracted oils according to European Union standard methods (Anexes II and IX in European Community Regulation EEC/2568/91). Oxidative stability was measured according to the Rancimat method, which evaluates the time (hours) of resistance of 2 g oil samples exposed to a stream of dry air at a temperature of 100 °C to oxidation (*10*, *11*). 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 are expressed as milligrams per kilogram (*12*). The sensory quality of each oil sample was evaluated by a 12-member analytical panel of the Instituto de la Grasa, according to the method described in Annex XII of the European Union Regulation (EEC/2568/91). Each oil was graded according to a scale of nine points, 1 being the value for the poorest quality possible and 9 of the best. Quantitative descriptive analysis of the sensory attributes, including bitterness, were determined by the same analytical panel using a structured scale of five points, where 0 means the absence of attribute, 1 simple perception, 2 light presence, 3 middle presence, 4 strong intensity, and 5 the highest intensity. Bitterness intensity was also objectively estimated by the content in the oil of secoiridoid derivatives of hydroxytyrosol, which has been related with oil bitterness (*5*). For the determination of phenolic compounds, the phenolic fraction was isolated by solid phase extraction and analyzed by reversed phase HPLC using a diode array UV detector (*13*). Samples of virgin olive oil obtained in triplicate in each treatment (2.5 ± 0.001 g) were weighed, and 0.5 mL of standard solution (4.64×10^{-2} mg/mL of *p*-hydroxyphenylacetic acid) was added. The solvent was evaporated in a rotary evaporator at 40 °C under vacuum, and the oily residue was dissolved in 6 mL of hexane. A diol bonded phase cartridge was placed in a vacuum elution apparatus and conditioned by the consecutive passing of 6 mL of hexane. Then the vacuum was released to prevent drying of the column. The oil solution was applied to the column that was subsequently washed twice with 3 mL of hexane, which was run out of the cartridge. The sample container was washed again with 3 mL of the admixture hexane/ethyl acetate (90:10, v/v), which was run out of the cartridge and discarded. Finally, the column was eluted with 10 mL of methanol, and the solvent was evaporated in a rotary evaporator at room temperature under vacuum until dry. The residue was extracted with 500 μL of methanol/water (1:1, v/v) at 40 °C. An aliquot (20 μL) of the final colorless solution was injected into the HPLC system. HPLC was performed in a Hewlett-Packard series 1100 liquid chromatographic system equipped with a diode array UV detector and Rheodyne injection valve (20 μL loop) A Lichrospher 100RP-18 column (4.0 mm i.d. × 250 mm; particle size = 5 μm) (Merck, Darmstadt, Germany), maintained at 30 °C, was used. Elution was performed at a flow rate of 1.0 mL/min, using as mobile phase a mixture of water/acetic acid (97:3, v/v) (solvent A) and methanol/acetonitrile (50:50, v/v) (solvent B). The solvent gradient changed according to the following conditions: from 95% (A)−5% (B) to 70% (A)−30% (B) in 25 min, to 60% (A)−40% (B) in 5 min, and to 30% (A)−70% (B) in 10 min; 100% (B) was maintained for 5 min until the end of the run. Quantification of phenols was carried out at 280 nm, and the results are expressed in millimoles per kilogram. The sum of the contents of two secoiridoid derivatives of hydroxytyrosol, the dialdehydic form of decarboxymethyl oleuropein aglycon and the aldehydic form of oleuropein aglycon, was considered as an objective estimation of the oil bitterness.

Analysis of variance 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.

RESULTS AND DISCUSSION

In oils obtained from Manzanilla and Verdial olives a strong correlation was observed between the content of secoiridoid derivatives of hydroxytyrosol and bitterness intensity (Figure 1). Considering all of the data in one group only, the correlation between both variables reached a low value ($r = 0.854$), but it was very significant ($P_{r=0} \leq 0.01$). These results suggest that the content of hydroxytyrosol derivatives of the olive oils is related with their bitterness, but each variety presents a particular relationship. Thus, at the same level of bitterness, oils obtained from Manzanilla olives systematically showed a higher content of hydroxytyrosol derivatives than those extracted from Verdial olives.

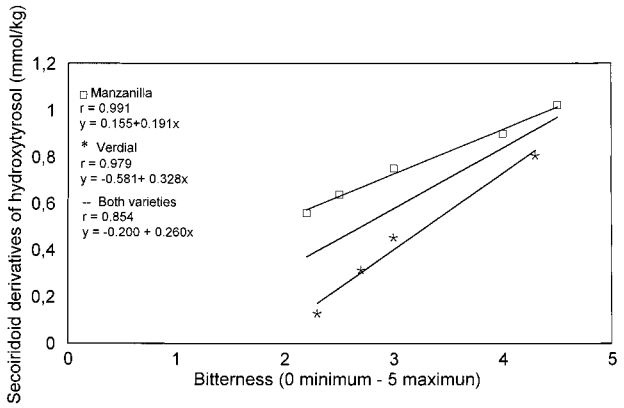

Figure 1. Correlation found between bitterness intensity and content of hydroxytyrosol secoiridoid derivatives on virgin olive oils obtained from Manzanilla and Verdial olives. Each point is the mean value of three replicates.

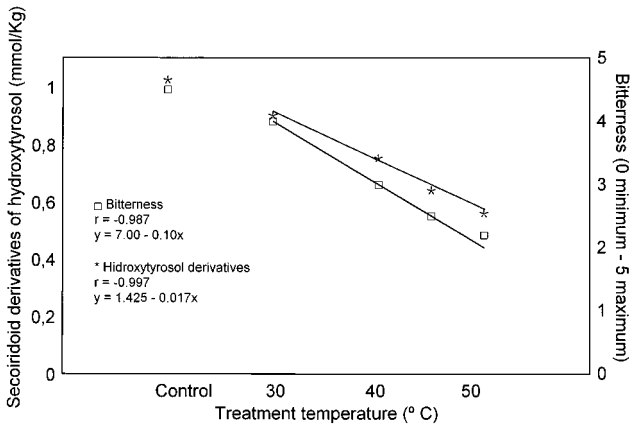

Figure 2. Changes in bitterness intensity and content of hydroxytyrosol secoiridoid derivatives of virgin olive oils obtained from nontreated (control) or previously heated at different temperatures during 24 h Manzanilla olives. Each point is the mean value of three replicates.

The bitterness of virgin olive oil, sensorially measured as well as chemically estimated by the content of hydroxytyrosol derivatives, decreased clearly according to the temperature used for heating the olives from which it was obtained (Figure 2). After 24 h at 40 °C, the heated olives yielded oils moderately bitter, whereas the oil extracted from nontreated fruits showed a bitterness level between strong and extreme. The results clearly demonstrate that a heat treatment applied to fruits can diminish the bitterness level of the obtained oil.

In general, the official parameters (acidity, peroxide index, UV absorption, and panel test) established to evaluate the quality level of virgin olive oils (EEC/2568/91 regulation) were not significantly affected by the heat treatment carried out on the fruits (Table 1). Only the

Table 1. Changes in the Quality Parameters of Virgin Olive Oils Obtained from Nontreated (Control) or Previously Heated at Different Temperatures during 24 h Manzanilla Olives[a]

temp (°C)	acidity (% oleic)	K_{232}	K_{270}	peroxide (mequiv of O_2/kg)	panel test (1−9)[b]	stability (h)	total phenols (mmol/kg)
control	0.17 a	1.48 a	0.12 a	7.73 b	8.0 a	69.35 a	1.89 a
30	0.20 a	1.54 a	0.13 a	7.65 b	8.0 a	70.75 a	1.70 b
40	0.18 a	1.51 a	0.13 a	8.21 a	8.0 a	67.15 a	1.48 c
45	0.18 a	1.40 a	0.12 a	8.25 a	7.8 a	57.00 b	1.31 d
50	0.11 a	1.43 a	0.11 a	8.40 a	6.8 b	55.00 b	1.23 e

[a] Each point is the mean value of three replicates. In each column, 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 the best one.

Table 2. Changes in the Sensory Attributes Determined by Quantitative Descriptive Analysis of Virgin Olive Oils Obtained from Nontreated (Control) or Previously Heated at Different Temperatures during 24 h Manzanilla Olives[a]

sensory attribute	temp of treatment				
	control	30 °C	40 °C	45 °C	50 °C
green olive fruit	2.5 ± 0.5	2.7 ± 0.9	2.6 ± 0.8	2.5 ± 0.6	2.6 ± 0.4
mature olive fruit	0.3 ± 0.6	0.3 ± 0.6	0.4 ± 0.9	0.6 ± 0.6	0.5 ± 0.5
apple fruit	0.6 ± 0.5	0.5 ± 0.6	0.6 ± 0.5	0.6 ± 0.5	0.6 ± 0.6
other fruits	0.6 ± 1.2	0.5 ± 1.0	0.6 ± 1.1	0.6 ± 0.8	0.6 ± 0.8
grass	2.3 ± 0.6	2.3 ± 0.8	2.1 ± 1.0	2.0 ± 0.9	2.0 ± 0.5
bitter	4.5 ± 0.4	4.0 ± 0.7	3.0 ± 0.8	2.5 ± 0.7	2.2 ± 0.6
pungent	4.0 ± 0.3	4.0 ± 0.4	3.2 ± 0.6	2.0 ± 0.3	2.0 ± 0.3
other tolerable	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.8
unacceptable	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

[a] Scale from 0, absence of the attribute, to 5, the highest intensity. Each point is the mean value ± SE of three replicates determined by a panel of 12 trained tasters.

oils obtained from olives treated at 50 °C showed a significant decrease in the overall grading of sensory quality of the oils. Despite this, the mean value obtained for this parameter was higher than the minimum value (6.5) established for the best quality category, named "extra". On the other hand, a slight increase in the peroxide value according to the temperature used was observed. However, this increment, although statistically significant, does not suppose a loss in the quality level of the oils, because the value remained considerably far from the established limit for the best commercial category of virgin olive oils (20 mequiv of O_2/kg). Furthermore, the values of K_{232} and K_{270} of these oils, indicating the progress of the oil oxidation, did not significantly differ from those shown by the oils obtained from nontreated olives. They achieve lower values than the limits established for these parameters (2.40 and 0.20, respectively) for the "extra" virgin olive oil. Otherwise, heat treatment did not induce triacylglycerol hydrolysis, because free fatty acids did not increase in the oils extracted from treated fruits. Nevertheless, other olive oil parameters, which are not included in the official regulations, were more clearly affected. Thus, a diminution of the phenolic content was observed for all oils obtained from heated olives, but the oxidative stability only decreased when olives were heated at ≥45 °C temperatures. This behavior suggests that oxidative stability depends not just on the phenol concentration.

Quantitative descriptive sensory analysis of the oils showed that the intensities of bitterness and pungency decreased as the temperature used in the treatment increased (Table 2). Unacceptable sensory attributes were not detected, but the oils obtained from olives treated at 50 °C showed a new tolerable sensory attribute, which was defined by three tasters as "cooked green beans".

The most important effect was the increase in oil pigmentation (Table 3). The oils obtained from fruits treated at ≥40 °C showed approximately double the content of carotenes and chlorophylls compared to that

Table 3. Changes in Pigment Contents of Virgin Olive Oils Obtained from Nontreated (Control) or Previously Heated at Different Temperatures during 24 h Manzanilla Olives[a]

temp (°C)	carotenes (mg/kg)	chlorophylls (mg/kg)
control	26.50 c	38.60 b
30	26.80 c	37.50 b
40	55.05 b	53.36 a
45	61.66 a	56.54 a
50	62.60 a	58.19 a

[a] Each point is the mean value of three replicates. In each column values followed by the same small letter are not statistically different ($P \leq 0.05$) according to Duncan's multiple-range test.

obtained from control olives. As a result, the oil acquired a more intense and darker green color, which increased with the temperature used in the treatment. Thus, a paradoxical case was found in the oils obtained after heat treatment: The greenest oils, traditionally related by the consumers with those obtained from unripe fruits and, therefore, with a high bitterness, actually presented the lowest intensity in this attribute.

In a second assay, using Verdial olives, decreases in bitterness and hydroxytyrosol derivative concentrations were observed in the oils, according to the time for which the fruits were treated at 40 °C before the oil extraction (Figure 3).

The oils obtained from the Verdial fruits, heated during different times at 40 °C, maintained the level of quality established for the extra virgin olive oil category (Table 4). Heating at 40 °C for 72 h provoked an increase in the peroxide index, although its value was lower than the limit. The phenolic content decreased with the heat treatments, but the oxidative stability remains unaffected in the oils obtained from fruit treated for 24 h. However, stability was significantly reduced in oils extracted from olives treated for 48 or 72 h. Despite this reduction, the stability of oil from treated olives was higher than the usual values shown by oils obtained

Table 4. Changes in the Quality Parameters of Virgin Olive Oils Obtained from Previously Heated at 40 °C during Different Times Verdial Olives[a]

time (h)	acidity (% oleic)	K_{232}	K_{270}	peroxides (mequiv of O_2/kg)	panel test (1−9)[b]	stability (h)	total phenols (mmol/kg)
0	0.19 a	1.56 a	0.15 a	2.78 b	8.3 a	46.3 a	2.14 a
24	0.20 a	1.54 a	0.14 a	2.53 b	8.0 a	46.5 a	1.60 b
48	0.23 a	1.50 a	0.13 a	2.90 b	7.7 a	34.6 b	1.22 c
72	0.22 a	1.40 a	0.14 a	4.35 a	7.8 a	29.6 c	0.75 d

[a] Each point is the mean value of three replicates. In each column, 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 the best one.

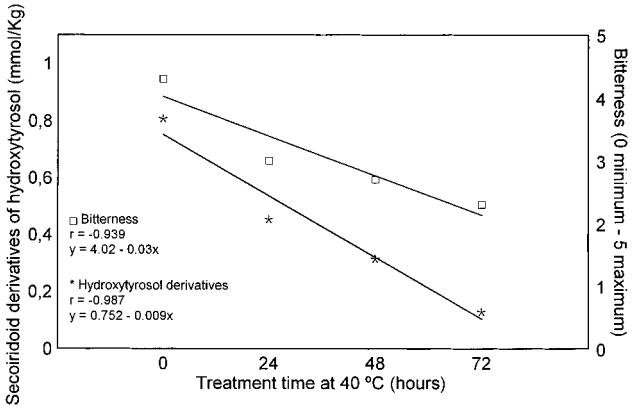

Figure 3. Changes in bitterness intensity and content of hydroxytyrosol secoiridoid derivatives of virgin olive oils obtained from nontreated (control) or previously heated at 40 °C during different times Verdial olives. Each point is the mean value of three replicates.

Table 5. Changes in the Sensory Attributes Determined by Quantitative Descriptive Analysis of Virgin Olive Oils Obtained from Nontreated (Control) or Previously Heated at 40 °C during Different Times Verdial Olives[a]

sensory attribute	time of treatment			
	control	24 h	48 h	72 h
green olive fruit	2.0 ± 0.7	2.0 ± 0.8	2.7 ± 0.9	3.3 ± 0.5
mature olive fruit	0.3 ± 0.5	0.3 ± 0.7	0.3 ± 0.9	0.3 ± 0.5
apple fruit	1.0 ± 0.6	0.7 ± 0.6	0.7 ± 0.8	0.7 ± 0.5
other fruits	0.6 ± 1.0	0.6 ± 1.0	0.6 ± 0.8	0.6 ± 0.7
grass	2.5 ± 0.7	2.7 ± 0.9	2.7 ± 1.0	2.3 ± 0.6
bitter	4.0 ± 0.4	3.0 ± 0.8	2.7 ± 0.8	2.3 ± 0.7
pungent	4.3 ± 0.5	3.8 ± 0.6	3.6 ± 0.5	3.3 ± 0.6
other tolerable	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
unacceptable	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

[a] Scale from 0, absence of the attribute, to 5, the highest intensity. Each point is the mean value ± SE of three replicates determined by a panel of 12 trained tasters.

Table 6. Changes in Pigment Contents of Virgin Olive Oils Obtained from Previously Heated at 40 °C during Different Times Verdial Olives[a]

time (h)	carotenes (mg/kg)	chlorophylls (mg/kg)
0	55.96 c	55.36 c
24	66.83 a	67.38 a
48	64.52 b	60.36 b
72	55.63 c	50.34 d

[a] Each point is the mean value of three replicates. In each column values followed by the same small letter are not statistically different ($P \leq 0.05$) according to Duncan's multiple-range test.

from other nonbitter varieties (*14*) and by oils (20 h) considered high for edible fats and oils (*10*).

The increase of the treatment time at 40 °C determined a clear decrease in bitterness and pungency and a slighter increase in the flavor of green olive fruit measured by quantitative descriptive sensory analysis (Table 5). No off-flavors were detected.

After 24 h at 40 °C, the fruits gave oils that showed a significant increase in photosynthetic pigments, similar to that observed in the assay previously carried out with Manzanilla olives (Table 6). Nevertheless, the content of carotenes and chlorophylls diminished as the treatment time increased, showing even lower values after 72 h than at the start.

Treatment for >24 h is not necessary because the bitterness reduction reached at this time could be considered enough.

Postharvest heat treatment of olives at 40 °C for 24 h promotes the reduction of bitterness in the obtained oils and could modulate their sensory characteristics toward a wide range of consumer acceptance.

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