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ORIGINAL PAPER

Postharvest Heat Treatment for Olive Oil Debittering at the Industrial Scale

Khaled Yousfi · Maria J. Moyano · Fernando Martinez · José A. Cayuela · José M. Garcia

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Abstract To enhance the debittering of olive oil, 500-kg olive fruit (*Olea europaea* L.) samples in duplicate from different olive cultivars and orchard locations in Spain (Manzanilla olive fruits from Villarrasa during the 2002/2003, 2004/2005 and 2005/2006 seasons, or from Dos Hermanas during the 2004/2005 and 2005/2006 seasons, Picual olive fruits from Cabra during the 2004/2005 season and Verdial olives from Villarrasa during the 2004/2005 and 2005/2006 seasons) were treated by dipping in hot water under different conditions (50–68 °C for 3 or 5 min), which had been previously determined based on laboratory-scale experiments, and subsequently processed for virgin olive oil extraction. Heat treatment produced a change in the intensity of the oil bitterness in all cases, increased the pigment content, decreased stability and reduced the sensory freshness of the oil. Although heat treatment reduced the phenolic content of the oil, this effect was not uniform among the different phenolic compounds and depended on the crop season and olive variety. Therefore, the determination of debittering conditions will require a series of preliminary laboratory-scale experiments.

Keywords Cultivation origin · Oil quality · Olea europaea · Phenolic compounds · Ripening · Stability

K. Yousfi · M. J. Moyano · F. Martinez · J. A. Cayuela · J. M. Garcia (✉)
Departamento de Fisiología y Tecnología de Productos Vegetales, Instituto de la Grasa (CSIC), Avda. Padre García Tejero 4, 41012 Seville, Spain
e-mail: jmgarcia@cica.es

Introduction

Although bitter taste is considered a characteristic attribute of virgin olive oil, excessive levels of bitterness are undesirable for consumers. Consequently, a method that reduces the level of bitterness would improve olive oil marketability. Such a method should be physical, because, by definition, virgin olive oil must be obtained exclusively by physical means, and chemical treatments should be avoided not only during the extraction process, but even during the postharvest handling of the fruit. Thus, a chemically debittered olive oil cannot be marketed as virgin olive oil. Storage of olive fruits at 40 °C for 24–72 h reduced the level of secoiridoid derivatives produced by the hydrolysis of oleuropein, thus reducing the level of oil bitterness [1, 2]. Hydroxytyrosyl elenolate has been identified as the phenolic compound most related to this sensory attribute [3]. However, additional research is required before this treatment can be employed. Garcia et al. [4] demonstrated that dipping olive fruits in warm (60–70 °C) water for 3 min successfully achieved debittering. This approach can be adapted to industrial olive oil production by adding a heating system to the fruit-washing process. However, this approach requires additional research before it can be employed on a larger scale. For example, if a too high temperature or too long residence time is employed, the cellular structure of olive fruits can be denatured, inducing emulsion formation and thus reducing oil yield. Furthermore, the effect of variation of olives due to differences in cultivation conditions and origin, olive variety and the level of fruit ripening is not well understood.

In this work, dipping olive fruits in hot water was tested to control the oil bitterness in an oil mill with fruit from three different crop seasons, using three different varieties from three different cultivation origins, and three different

fruit ripening levels, in order to understand the problems that may arise in its industrial application.

Experimental Procedures

Olives

Five hundred-gram samples of olive fruit (*Olea europaea* L.) in duplicate from different olive cultivars and orchard locations in Spain (Manzanilla olive fruits from Villarrasa during the 2002/2003, 2004/2005 and 2005/2006 seasons, or from Dos Hermanas during the 2004/2005 and 2005/2006 seasons, Picual olive fruits from Cabra during the 2004/2005 season and Verdial olive fruits from Villarrasa during the 2004/2005 and 2005/2006 seasons), grown under different climatic conditions (Table 1) at different levels of ripening [5], were harvested and transported in 25-kg plastic boxes on the same day to the Instituto de la Grasa in Seville. The boxes of each variety were randomly distributed into groups of 20 boxes, constituting two replicate samples of 500 kg for industrial assays. Another two boxes (50 kg) were reserved to be used previously at the laboratory scale.

Evaluation of Maturity Level

Two hundred fruits were taken from each replicate sample for objectively assessing their level of maturity and evaluating their epidermis color and fruit firmness [6]. The color was determined on the equatorial zone, using a Minolta CR200 (Minolta Camera Co., Osaka, Japan) chromameter with a measuring area of 8 mm diameter, diffuse illumination and a viewing angle of 0°. The International Commission on Illumination color notation system (CIE L*a*b*) was applied to determine the parameters L*, a* and b*, where L* indicates the lightness, a* means the color axis from green to red and b* means the color axis from blue to yellow [7]. By means of these parameters a color index (CI) was calculated according to the formula:

Table 1 Climatic variables during the period of olive fruit growing and maturation (April to October)

Season	Location (Spain)	Rainfall[a] (Lm^{-2})	Temperature[b] (°C)
2002/2003	Dos Hermanas	215.8	20.6 ± 3.8
2004/2005	Dos Hermanas	312.9	20.8 ± 5.1
2004/2005	Cabra	210.2	22.2 ± 5.2
2004/2005	Villarrasa	140.7	20.8 ± 4.8
2005/2006	Villarrasa	32.0	20.5 ± 4.1

[a] Total rainfall in the season

[b] Mean ± SD temperature during the season

$$CI = L^*(b^* - a^*)/100 \quad (1)$$

This equation has been previously used to monitor the changes in skin color during olive cold storage [8]. Fruit firmness was also evaluated on the equatorial zone of the same fruits using a Zwick 3300 hand densimeter (Zwick GmbH & Co., Ulm, Germany). The consistency of the fruit was measured without rupture by the pressure of a 5-mm-diameter disk. The results were expressed in N/cm^2. The traditional subjective system to evaluate the ripening index (RI) was calculated using the same fruits and the same evaluator to determine the color of the olive skin and flesh [5]. The procedure consists of distributing the same 100 olives into eight groups, according to the following characteristics: group 0, bright-green skin; group 1, green-yellowish skin; group 2, green skin with reddish spots; group 3, reddish-brown skin; group 4, black skin with white flesh; group 5, black skin with <50% purple flesh; group 6, black skin with ≥50% and >100% purple flesh; group 7, black skin and purple flesh. The RI is determined by the equation:

$$RI = \sum_{i=0}^{i=7} (in_i)/100 \quad (2)$$

where i is the number representative of each maturity group (0, 1, 2, 3, 4, 5, 6 or 7) and n_i the number of olives in it.

Laboratory-Scale Heat Treatments

All the tests applied at the industrial scale using 500-kg samples of olive fruit were preceded by experiments at the laboratory scale, which allowed for establishing the appropriate treatment conditions. For this purpose, in each season, 50 kg of the initial fruits of each variety, origin and level of ripening were randomly distributed into seven treatment groups, with each one in two 3.5-kg batches. Six of these groups were dipped in a 400-l thermostatic water bath at temperatures between 50 and 70 °C for 3 min. The seventh group was immediately processed without any treatment and was used as a control.

The oil from the olives of each 3.5-kg batch of each treatment group was extracted separately, constituting duplicate samples, using an Abencor analyzer (Comercial Abengoa S.A., Seville, Spain). This unit, consisting of three basic elements, a hammer mill, a thermobeater and a pulp centrifuge, simulates the industrial process of virgin olive oil production at laboratory scale [9]. The samples were crushed in a hammer mill with a speed of 3,000 rpm (radius 47.5 mm) and a 5.0-mm-hole diameter sieve. The resulting olive paste was introduced into 1-l stainless steel jars and malaxated for 30 min in the thermobeater at 30 °C using four stainless steel crossed blades at 54.5 rpm (radius 53 mm). Subsequently, the paste was centrifuged in the pulp centrifuge for 1 min at 3,500 rpm (radius 100 mm) to

separate the liquid phase (oil and waste water) from the solid waste. Then, the oil was decanted into a graduated tube to measure the volume obtained in order to calculate the oil yield, which was expressed as the percentage of fresh weight, using 0.916 kg l^{-1} the olive oil density at ambient temperature. Subsequently, the extracted oil was paper filtered and immediately analyzed to evaluate its overall grading of sensory quality and its bitterness intensity.

Industrial-Scale Heat Treatment

An industrial pasteurizer of table olives, PAP1 (Desarrollos Tecnológicos Bando S. L., Dos Hermanas, Spain), was used for applying the heat treatments in the oil mill. The apparatus consists of a drum equipped with a rotator in its interior that drags the olive fruits, keeping them submerged in a thermostatized water bath. The speed of the treatment was regulated by means of a mechanical speed reducer so that the total treatment time would be from 3 to 5 min. The temperature of the water was adjusted by means of vapor injection coming from a heat boiler with an adjustable valve. The production capacity was adjusted based on the entrance of the fruit into the pasteurizer at the rate of 500 kg/h. The treated olives were collected in plastic boxes of 25-kg capacity and were processed immediately in the oil mill. In the assays carried out with Picual and Verdial olives (0.5 RI), after heat treatment they were immediately processed or kept at room temperature for 24 h before processing. In each assay a control was carried out with a similar amount of olives, which was processed without a previous heat treatment. Subsequently, a two-phase continuous system was employed (Pieralisi Model SC-45; Jesi, Ancona, Italy) to extract the virgin olive oil. Each duplicate of 500 kg of olives from each treatment was crushed using a stainless steel hammer mill at ambient temperature, operating at 3,000 rpm (radius 195 mm), which was equipped with a 5.0-mm-hole sieve. Malaxation of pastes was done in a mixer at 14 rpm (radius 320 mm) at 30 °C for 1 h. Separation of the paste into oily must and pomace was performed using a two-phase centrifugal decanter working at 3,500 rpm (radius 175 mm). Finally, a vertical centrifuge operating at 6,500 rpm (radius 125 mm) and fed with 0.25 l tap water/kg oily must at 40 °C was used to remove the remaining solids from the must. The oil extracted was weighed, and the industrial oil yield was calculated according to the initial weight of the fruits before heat treatment. A 1.5-l bottle was filled from the oil extracted and stored at −20 °C under N_2 atmosphere until analysis.

Oil Analysis

Free acidity, peroxide index value, coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) and the overall grading of the sensory quality of the oils were evaluated according to the European Union Standard Methods (Annexes II and IX 153 in European Community Regulation EEC/2568/91) [10]. Specifically, the overall sensory quality of each oil sample was graded by a panel of eight trained tasters (6 years' experience) according to a 9-point scale, with 1 being the value for the poorest quality possible and 9 for the best. The presence of any negative attribute (rancid, fusty, winey, musty, etc.) causes the oil to be evaluated below 6.5, the limit value established for the best commercial category ("extra"). The intensity of bitterness was determined by the same panel of testers using a structured scale of five points where 0 means the absence of this attribute, 1 means its simple detection, 2 its light presence, 3 its middle presence, 4 its strong intensity and 5 the highest possible intensity of bitterness.

Oxidative stability was measured by the Rancimat method, which evaluates the time (h) of resistance to oxidation of 3 g of oil sample exposed to a stream of dry air at a temperature of 100 °C [11]. The content of pigments in the oils was evaluated by their absorbance at 470 and 670 nm for carotenoids and chlorophylls, respectively, and the results were expressed as mg/kg [12]. The tocopherol content of the oil samples was measured by HPLC using the IUPAC method [13]. Phenolic fraction was isolated by solid-phase extraction and analyzed by reversed-phase HPLC using a diode array UV detector [14]. The quantification of phenolic compounds (except ferulic acid) was carried out at 280 nm using *p*-hydroxyphenylacetic acid as an internal standard, whereas the quantification of flavones and ferulic acid was done at 335 nm using *o*-coumaric acid as an internal standard. The results were expressed in millimol per kilogram.

Statistical Analysis

An analysis of variance (ANOVA) was carried out on the data of the parameter used to evaluate the level of fruit ripening. 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. All the other data have been presented as the mean of two replicates ± SD. In each assay, the results obtained from the oils extracted from fruit that was not treated (control) or heat treated were compared. A third treatment (24 h of processing delay after dipping) was considered in the assay carried out during the 2004/2005 season using Picual olives. The different cultivation origin was compared using the results obtained from the assays carried out during the 2004/2005 season with Manzanilla olives from two different locations. The different crop season was compared separately with the Manzanilla olives from

Dos Hermanas, harvested during the 2002/2003 and the 2004/2005 seasons, and with the Manzanilla olives from Villarrasa, harvested during the 2004/2005 and the 2005/2006 seasons. Finally, the different ripening levels were compared using the results obtained from the Verdial olives with two different ripening levels during the 2005/2006 season.

Results and Discussion

Ripening Level of the Fruit Used

The fruit used displayed three significantly different levels of maturity, according to RI (Table 2). A first group of the least ripe fruit was formed with the Manzanilla olives from different origins used in the three seasons tested and the Verdial fruits used in one of the assays carried out in the 2005/2006 season (RI 0.5 ± 0.4). A second group of significantly riper fruit was made up of the Verdial olives used in the second assay during the 2005/2006 season (RI 1.5 ± 0.5); finally, a third group of the ripest fruit was formed by the Picual olives used during the 2004/2005 season (RI 3.5 ± 0.8). This subjective determination coincided with the objective discrimination carried out by the CI, which also distinguished the same three significantly different groups of olives (Table 2). Fruit firmness was less effective in differentiating the ripening level of the olives (Table 2). It failed to discriminate a significant difference between the fruits of the first and the second group of ripening. The results obtained confirm that all the Manzanilla olives used in the assays displayed the same level of maturity, allowing the examination of the effect of different seasons or origins in the treatment development, or that the two groups of Verdial olives harvested during the 2005/2006 season can be used to test the effect of the maturity level.

Influence of Heat Treatment, Cultivation Origin and Crop Season on the Quality of the Oil Extracted from Manzanilla Olives Processed at Industrial Scale

In all the assays carried out with Manzanilla olives from different origins during three different seasons, the oils extracted from heat-treated fruit exhibited a lower bitterness (Table 3). However, the temperature of treatment necessary for oil debittering varied according to the season considered. Thus, in the 2002/2003 and 2005/2006 seasons temperatures of 68 and 65 °C, respectively, were required to reduce oil bitterness intensity from "extreme" and "strong" (average 4.6) to a level between "strong" and "medium" (average 3.7), whereas olives from the 2004/2005 season showed a higher reduction of the same attribute at a lower temperature (56 °C), regardless of the fruit origin. Therefore, the selection of treatment conditions depends more on the particular climatic parameters of each season than on the variability because of a different cultivation origin and points to the necessity to establish new treatment conditions for each season by previous assays at the small scale.

The oil yield obtained from the Manzanilla olives of Villarrasa was clearly lower in the 2004/2005 season (Table 3). These olives were especially prone to oil emulsion. Only in this case did heat treatment induce a higher oil yield. Probably in the other cases the oil yield was so high that it could not be increased by the use of heat treatment. A similar effect was previously observed with Picual olives by Garcia et al. [4].

Sensory quality of the oil was deteriorated by heat treatment (Table 3). Pérez et al. [15] observed that this treatment partially deactivated the lipoxygenase-hydroperoxide lyase enzyme system, resulting in a decrease of volatile compounds of 5 or 6 carbon atoms in the oils. The presence of these volatiles is important to evaluate the

Table 2 Parameters (means ± SD) for evaluating the maturity level of olives used for heat treatments at industrial scale

Season	Variety (Spain origin)	Color index[a]	Firmness (N cm^{-2})[a]	Ripening index[b]
2002/2003	'Manzanilla' (Dos Hermanas)	29.01 ± 1.72a	46.82 ± 2.85ab	0.5 ± 0.3c
2004/2005	'Manzanilla' (Villarrasa)	27.83 ± 1.65a	49.45 ± 2.65a	0.5 ± 0.3c
2004/2005	'Manzanilla' (Dos Hermanas)	28.98 ± 1.84a	45.13 ± 3.01ab	0.6 ± 0.3c
2005/2006	'Manzanilla' (Villarrasa)	27.93 ± 1.84a	49.44 ± 2.59a	0.5 ± 0.3c
2005/2006	'Verdial' (Villarrasa)	28.43 ± 1.65a	47.55 ± 2.45ab	0.5 ± 0.4c
2005/2006	'Verdial' (Villarrasa)	23.21 ± 1.02b	44.28 ± 3.01b	1.5 ± 0.5b
2004/2005	'Picual' (Cabra)	0.06 ± 1.45c	37.83 ± 2.75c	3.5 ± 0.8a

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

[a] Each value is the mean value of 400 determinations

[b] Each value is the mean value of 4 determinations

Table 3 Quality parameters of virgin olive oils obtained from Manzanilla olives processed at industrial scale

Season	2002/2003		2004/2005		2004/2005		2005/2006	
Cultivation origin	Spain		Dos Hermanas		Villarrasa		Villarrasa	
Treatment parameter	Dos Hermanas							
	Control	68 °C	Control	56 °C	Control	56 °C	Control	65 °C
Bitterness intensity[a]	4.8 ± 0.2	3.8 ± 0.3	4.5 ± 0.2	3.0 ± 0.4	4.5 ± 0.3	3.2 ± 0.4	4.4 ± 0.3	3.6 ± 0.3
Yield (%)	15.2 ± 1.3	16.6 ± 1.1	16.9 ± 1.2	16.7 ± 1.3	4.7 ± 0.8	7.7 ± 1.1	15.9 ± 1.0	16.9 ± 0.8
Acidity (% oleic)	0.41 ± 0.18	0.38 ± 0.22	0.36 ± 0.20	0.34 ± 0.23	0.19 ± 0.14	0.22 ± 0.12	0.26 ± 0.11	0.25 ± 0.09
Peroxide value (mEq of O_2 kg^{-1})	6.2 ± 1.3	5.8 ± 1.5	8.4 ± 1.2	9.2 ± 1.3	7.7 ± 1.8	6.3 ± 1.4	10.75 ± 1.6	12.84 ± 1.5
K^{b}_{232}	1.67 ± 0.15	1.56 ± 0.14	1.57 ± 0.21	1.47 ± 0.22	1.40 ± 0.22	1.44 ± 0.20	1.87 ± 0.19	1.85 ± 0.17
K^{c}_{270}	0.19 ± 0.04	0.15 ± 0.03	0.16 ± 0.03	0.14 ± 0.04	0.17 ± 0.03	0.17 ± 0.04	0.20 ± 0.04	0.18 ± 0.05
Sensory quality[d]	8.1 ± 0.3	7.3 ± 0.4	8.0 ± 0.2	7.0 ± 0.4	8.0 ± 0.3	8.2 ± 0.4	7.0 ± 0.2	6.5 ± 0.2
Carotenoids (mg kg^{-1})	18.6 ± 3.5	34.4 ± 5.6	15.8 ± 3.4	36.4 ± 5.1	17.4 ± 3.2	34.5 ± 4.5	25.65 ± 6.7	50.56 ± 6.9
Chlorophylls (mg kg^{-1})	30.4 ± 4.5	102.6 ± 9.3	35.6 ± 5.9	108.8 ± 10.1	29.7 ± 4.7	112.6 ± 7.9	14.34 ± 3.8	56.46 ± 7.9
Tocopherols (mg kg^{-1})	160.7 ± 8.4	138.6 ± 7.2	157.6 ± 8.9	156.4 ± 9.3	230.9 ± 7.5	212.5 ± 8.3	201.4 ± 8.4	185.5 ± 8.2
Stability (h)[e]	145.4 ± 5.3	121.6 ± 5.5	124.2 ± 5.2	98.0 ± 4.6	89.2 ± 3.9	62.1 ± 3.2	155 ± 5.6	127 ± 6.4

Manzanilla olives of different origins during different seasons were not treated (control) or previously dipped in hot water at different temperatures for 3 min at industrial scale. Treatment conditions were previously selected as suitable in each case at laboratory scale. Each value is the mean value of two replicates. All the olives used for these assays had the same ripening index ($\leq$1)

[a] Bitterness intensity sensory evaluation: 0 indicates the absence of the attribute, and 5 indicates the highest intensity

[b] Coefficient of specific extinction at 232 nm

[c] Coefficient of specific extinction at 270 nm

[d] Overall grading of sensory quality, 1 indicates the worst sensory quality possible and 9 indicates the best sensory quality possible

[e] Stability of the oil against oxidation using the Rancimat method, expressed in hours

sensory "freshness" of the oil, because it is related to attributes such as "green" or "cut grass" [16].

The peroxide value of the oils varied with both the crop season and the cultivation origin, but was not changed by heat treatment (Table 3). The oils extracted from olives of the 2004/2005 and the 2005/2006 seasons showed higher values than those extracted from the olives of the same locations (Dos Hermanas and Villarrasa) harvested during the 2002/2003 and the 2004/2005 seasons, respectively. The peroxide values of the oils extracted from Villarrasa fruit were higher than those obtained from olives grown in Dos Hermanas in the same season (2004/2005). Probably, the interaction between the meteorological conditions of each season and the cultivation characteristics of each location induced different effects on the oxidative reactions of the oil during the extraction process.

Heat treatment systematically induced a significant increase in the chlorophyll and carotenoid contents of the oil, a fact that has been described previously in other papers (Table 3) [17, 18]. Probably, heat treatment reduced the enzyme activity responsible for pigment degradation and/or allowed for a better accessibility of the oil to dissolve these compounds during the process of oil extraction.

In general, tocopherol oil content was reduced as a result of the previous heat treatment (Table 3). Oils extracted from Dos Hermanas olives during the 2004/2005 season showed lower values of this parameter than the oils extracted from the olives grown in Villarrasa during the same crop season, although the difference was not substantial.

Oil stability against oxidation systematically deteriorated with the heat treatment (Table 3). This fact, previously observed at laboratory scale in other works [1, 5, 18], is related with the decrease of phenolic compounds that was also due to this treatment [19]. Furthermore, this parameter was affected by the location and the crop season. Thus, during the same crop season (2004/2005), the oil extracted from Dos Hermanas olives exhibited higher stability than that obtained from Villarrasa fruit. However, the highest values were shown by the oil from Villarrasa olives during the following season.

Heat treatment of olives mainly reduced the concentration of hydroxytyrosyl elenolate (3,4 DHPA-EA) and tyrosyl elenolate (p-HPEA-EA), consistent with the decrease of bitterness and stability (Tables 3, 4). The concentration of other phenolic compounds showed a less

Table 4 Contents of different phenolic compounds (mmol kg^{-1}) of virgin olive oils obtained from Manzanilla olives processed at industrial scale

Season	2002/2003		2004/2005		2004/2005		2005/2006	
Cultivation Spain origin	Dos Hermanas		Dos Hermanas		Villarrasa		Villarrasa	
Phenolic compounds (mmol kg^{-1})	Control	68 °C	Control	56 °C	Control	56 °C	Control	65 °C
Hydroxytyrosol	0.042 ± 0.006	0.038 ± 0.007	0.169 ± 0.018	0.088 ± 0.012	0.048 ± 0.008	0.074 ± 0.009	0.121 ± 0.012	0.059 ± 0.008
Tyrosol	0.033 ± 0.005	0.034 ± 0.007	0.122 ± 0.014	0.068 ± 0.009	0.038 ± 0.007	0.042 ± 0.008	0.062 ± 0.007	0.043 ± 0.008
Vanillic acid	0.001 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.002 ± 0.001	0.000 ± 0.000	0.001 ± 0.000	0.000 ± 0.000
Vanilline	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.000 ± 0.000	0.001 ± 0.001
p-Cumaric acid	0.000 ± 0.000	0.000 ± 0.000	0.005 ± 0.001	0.002 ± 0.001	0.005 ± 0.001	0.004 ± 0.001	0.004 ± 0.002	0.002 ± 0.001
Hydroxytyrosol Acetate	0.013 ± 0.003	0.012 ± 0.004	0.041 ± 0.007	0.040 ± 0.005	0.025 ± 0.004	0.053 ± 0.005	0.017 ± 0.003	0.018 ± 0.004
3,4 DHPA-EDA[a]	0.465 ± 0.023	0.683 ± 0.027	0.659 ± 0.030	0.430 ± 0.020	0.486 ± 0.022	0.367 ± 0.018	0.889 ± 0.042	0.739 ± 0.032
Tyrosol Acetate	0.000 ± 0.000	0.000 ± 0.000	0.118 ± 0.022	0.029 ± 0.008	0.028 ± 0.009	0.018 ± 0.007	0.044 ± 0.012	0.024 ± 0.009
p-HPEA-EDA[b]	0.344 ± 0.022	0.560 ± 0.032	0.481 ± 0.028	0.301 ± 0.035	0.507 ± 0.026	0.376 ± 0.022	0.757 ± 0.031	0.749 ± 0.029
Pinoresinol	0.000 ± 0.000	0.001 ± 0.000	0.010 ± 0.002	0.007 ± 0.002	0.008 ± 0.001	0.005 ± 0.001	0.039 ± 0.007	0.011 ± 0.00
Cinamic acid	0.007 ± 0.002	0.004 ± 0.001	0.006 ± 0.002	0.004 ± 0.001	0.002 ± 0.000	0.001 ± 0.000	0.005 ± 0.001	0.005 ± 0.002
Acetoxy-pinoresinol	0.022 ± 0.003	0.019 ± 0.002	0.055 ± 0.007	0.015 ± 0.002	0.027 ± 0.008	0.013 ± 0.003	0.063 ± 0.007	0.044 ± 0.005
3,4 DHPA-EA[c]	1.943 ± 0.156	0.699 ± 0.042	0.576 ± 0.039	0.243 ± 0.032	0.264 ± 0.035	0.182 ± 0.032	1.097 ± 0.112	0.523 ± 0.032
p-HPEA-EA[d]	1.100 ± 0.042	0.506 ± 0.032	0.328 ± 0.035	0.085 ± 0.018	0.133 ± 0.019	0.079 ± 0.016	0.360 ± 0.022	0.346 ± 0.015
Ferulic acid	0.003 ± 0.001	0.002 ± 0.001	0.001 ± 0.000	0.001 ± 0.000	0.002 ± 0.001	0.002 ± 0.001	0.001 ± 0.000	0.001 ± 0.000
Luteoline	0.013 ± 0.012	0.014 ± 0.012	0.005 ± 0.012	0.001 ± 0.012	0.003 ± 0.012	0.002 ± 0.012	0.006 ± 0.012	0.002 ± 0.012
Apigenine	0.007 ± 0.002	0.006 ± 0.001	0.003 ± 0.002	0.001 ± 0.001	0.002 ± 0.001	0.001 ± 0.000	0.004 ± 0.002	0.001 ± 0.000
Total phenols	3.993 ± 0.192	2.578 ± 0.112	2.581 ± 0.127	1.317 ± 0.096	1.537 ± 0.092	1.220 ± 0.082	3.470 ± 0.172	2.568 ± 0.112
Total orthodiphenols	2.476 ± 0.106	1.446 ± 0.097	1.450 ± 0.094	0.802 ± 0.076	0.826 ± 0.075	0.678 ± 0.070	2.130 ± 0.101	1.341 ± 0.089
Total secoiridoids	3.852 ± 0.178	2.448 ± 0.102	2.044 ± 0.112	1.059 ± 0.085	1.390 ± 0.089	1.004 ± 0.075	3.103 ± 0.151	2.357 ± 0.097

Manzanilla olives of different origins during different seasons were not treated (control) or had previously been dipped in hot water at different temperatures for 3 min at industrial scale. Treatment conditions had been previously selected as suitable in each case at laboratory scale. Each value is the mean value of two replicates. All the olives used for these assays had the same ripening index (≤1)

[a] Dialdehydic form of the decarboxymethyl oleuropein aglycone

[b] Dialdehydic form of the decarboxymethyl ligstroside aglycone

[c] Hydroxytyrosyl elenolate

[d] Tyrosyl elenolate

Table 5 Quality parameters of virgin olive oils obtained from Picual and Verdial olives processed at industrial scale

Variety (ripening index)[a]	Picual (3.5)			Verdial (0.5)		Verdial (1.5)	
Parameters	Control	50 °C	50 °C (+24 h)[b]	Control	57 °C	Control	57 °C
Bitterness intensity[c]	4.5 ± 0.3	3.2 ± 0.4	3.0 ± 0.3	4.5 ± 0.3	3.5 ± 0.4	4.1 ± 0.3	3.1 ± 0.4
Yield (%)	24.4 ± 1.8	24.1 ± 1.4	24.5 ± 1.5	18.5 ± 1.6	19.7 ± 1.4	21.8 ± 1.5	23.0 ± 1.3
Acidity (% oleic)	0.15 ± 0.11	0.16 ± 0.12	0.14 ± 0.13	0.26 ± 0.12	0.27 ± 0.14	0.32 ± 0.16	0.30 ± 0.14
Peroxide value (mEq of O_2 kg^{-1})	7.8 ± 1.3	8.0 ± 1.0	8.2 ± 1.2	11.33 ± 1.9	12.80 ± 1.8	12.70 ± 1.7	12.91 ± 2.3
K^{d}_{232}	1.48 ± 0.20	1.49 ± 0.18	1.44 ± 0.19	1.87 ± 0.18	1.73 ± 0.18	1.93 ± 0.18	1.66 ± 0.18
K^{e}_{270}	0.15 ± 0.04	0.14 ± 0.03	0.12 ± 0.04	0.22 ± 0.04	0.17 ± 0.03	0.21 ± 0.02	0.15 ± 0.03
Sensory quality[f]	8.2 ± 0.3	7.3 ± 0.4	7.4 ± 0.3	8.0 ± 0.4	7.0 ± 0.4	8.0 ± 0.4	7.0 ± 0.4
Carotenoids (mg kg^{-1})	12.2 ± 3.5	27.0 ± 4.8	26.7 ± 5.1	17.90 ± 5.5	36.18 ± 6.5	18.12 ± 4.5	29.49 ± 3.9
Chlorophylls (mg kg^{-1})	20.8 ± 4.2	84.5 ± 6.9	86.1 ± 6.2	31.79 ± 5.2	81.57 ± 6.5	36.76 ± 4.8	86.14 ± 5.9
Tocopherols (mg kg^{-1})	208.9 ± 10.8	219.8 ± 9.8	206.6 ± 9.5	211.5 ± 9.7	209.4 ± 9.2	208.5 ± 9.1	218.7 ± 9.9
Stability (h)[g]	150.0 ± 5.4	136.0 ± 4.2	121.6 ± 4.8	80 ± 3.6	61 ± 3.8	82 ± 4.1	63 ± 3.5

Non-treated Picual and Verdial olives (control) harvested in Cabra and Villarrasa, respectively, or previously submerged in hot water at different temperatures for 3 min (Picual) or for 5 min (Verdial) in the 2004/2005 and the 2005/2006 seasons, respectively. Treatment conditions were previously selected in each case as suitable at laboratory scale. Each value is the mean value of two replicates

[a] Mean values of four replicates

[b] The oils were extracted immediately after treatment or after a delay of 24 h (+24 h)

[c] Bitterness intensity sensory evaluation: 0 indicates the absence of the attribute, and 5 indicates the highest intensity

[d] Coefficient of specific extinction at 232 nm

[e] Coefficient of specific extinction at 270 nm

[f] Overall grading of sensory quality, 1 indicates the worst sensory quality possible, and 9 indicates the best sensory quality possible

[g] Stability of the oil against oxidation using the Rancimat method, expressed in hours

consistent variation in relation to heat treatment, with changes highly dependent on the cultivation origin and growing season. In each location the decrease in total rainfall (Table 1) coincided with an increase of the phenolic compounds in the oils (Table 4), which is in agreement with previous reports [20]. However, oils from Dos Hermanas olives always showed higher phenol content than those obtained from Villarrasa fruits (Table 4), in spite of the fact that the total rainfall had been considerably higher in Dos Hermanas (Table 1).

Influence of Heat Treatment in Relation to Processing Delay on the Quality of the Oils Extracted from Picual Olives Processed at Industrial Scale

Heat treatment applied to middle-ripe Picual olives induced a reduction in the intensity of oil bitterness (Table 5). A 24-h delay in fruit processing after the heat treatment did not have any additional effect on this parameter. Consequently, the reduction of oil bitterness depended directly on the temperature used in the treatment, with the milling temperature being less relevant, because after a delay of 24 h the fruit was crushed at ambient temperature (20 °C).

Heat treatment did not increase the oil yield (Table 5). The value obtained from non-treated fruit was very high (24.4%), considering that the oil yield obtained from Picual olives normally does not surpass 25% of its fresh weight. In other similar experiments with unripe Verdial olives (0.5 RI), the 24-h delay produced emulsification of the oil, making its physical extraction impossible (data not shown). After the fruit was removed from the heat source, it retained a high temperature for several minutes, slowly cooling from 40 °C to room temperature. This prolongation of the treatment induced the emulsification of the oil.

The decrease of intensity of sensory attributes such as "grass," "green" or "immature fruit" because of the heat treatment resulted in a reduction of the sensory quality grading overall (Table 5). In spite of this, the values obtained for these oils reached an acceptable level, regardless of whether the olives were processed immediately or after a delay of 24 h. Heat treatments did not induce any deterioration of acidity, peroxide value, K_{232}, K_{270} and tocopherol content (Table 5). Furthermore, the pigment content increased notably, doubling the carotenoid content and quadrupling the presence of chlorophylls (Table 5). The processing delay of 24 h did not change this effect. This result does not agree with the findings of Luaces et al. [17], who suggested that the milling temperature is the only factor responsible for the increase of pigments in the oil. The oil extracted from the fruits after a

Table 6 Content of different phenolic compounds (mmol kg^{-1}) of virgin olive oils obtained from Picual and Verdial olives processed at industrial scale

Variety (ripening index)[a]	Picual (3.5)			Verdial (0.5)		Verdial (1.5)	
Phenolic compounds ($mmolkg^{-1}$)	Control	50 °C	50 °C (+24 h)[b]	Control	57 °C	Control	57 °C
Hydroxytyrosol	0.089 ± 0.008	0.074 ± 0.009	0.053 ± 0.010	0.062 ± 0.011	0.075 ± 0.007	0.128 ± 0.009	0.042 ± 0.010
Tyrosol	0.043 ± 0.007	0.036 ± 0.010	0.047 ± 0.012	0.061 ± 0.012	0.140 ± 0.014	0.113 ± 0.015	0.074 ± 0.013
Vanillic acid	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.001	0.001 ± 0.000	0.000 ± 0.000
Vanilline	0.001 ± 0.000	0.003 ± 0.001	0.003 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.002 ± 0.000
P-Cumaric acid	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.003 ± 0.002	0.002 ± 0.000	0.002 ± 0.001	0.002 ± 0.001
Hydroxytyrosol acetate	0.009 ± 0.003	0.010 ± 0.004	0.008 ± 0.003	0.010 ± 0.003	0.015 ± 0.004	0.009 ± 0.003	0.011 ± 0.004
3,4 DHPA-EDA[c]	0.241 ± 0.038	0.250 ± 0.033	0.157 ± 0.032	0.460 ± 0.036	0.289 ± 0.032	0.445 ± 0.032	0.282 ± 0.036
Tyrosol acetate	0.029 ± 0.009	0.027 ± 0.009	0.016 ± 0.006	0.013 ± 0.002	0.011 ± 0.008	0.029 ± 0.009	0.012 ± 0.005
p-HPEA-EDA[d]	0.283 ± 0.038	0.299 ± 0.042	0.261 ± 0.042	0.650 ± 0.041	0.620 ± 0.048	0.571 ± 0.042	0.416 ± 0.036
Pinoresinol	0.012 ± 0.005	0.012 ± 0.006	0.011 ± 0.004	0.008 ± 0.002	0.005 ± 0.004	0.010 ± 0.004	0.006 ± 0.003
Cinamic acid	0.011 ± 0.005	0.010 ± 0.004	0.008 ± 0.004	0.006 ± 0.002	0.004 ± 0.002	0.003 ± 0.002	0.003 ± 0.002
Acetoxy-pinoresinol	0.055 ± 0.010	0.050 ± 0.008	0.036 ± 0.007	0.100 ± 0.025	0.043 ± 0.010	0.081 ± 0.017	0.032 ± 0.012
3,4 DHPA-EA[e]	0.856 ± 0.045	0.584 ± 0.045	0.544 ± 0.042	0.657 ± 0.042	0.355 ± 0.032	0.499 ± 0.045	0.274 ± 0.026
p-HPEA-EA[f]	0.222 ± 0.034	0.239 ± 0.032	0.205 ± 0.033	0.323 ± 0.032	0.255 ± 0.027	0.261 ± 0.032	0.156 ± 0.021
Ferulic acid	0.001 ± 0.000	0.002 ± 0.001	0.002 ± 0.001	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.001	0.001 ± 0.000
Luteoline	0.002 ± 0.001	0.002 ± 0.000	0.003 ± 0.001	0.004 ± 0.001	0.001 ± 0.000	0.005 ± 0.001	0.001 ± 0.000
Apigenine	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.002 ± 0.001	0.001 ± 0.001	0.002 ± 0.001	0.001 ± 0.000
Total phenols	1.857 ± 0.171	1.601 ± 0.145	1.358 ± 0.126	2.360 ± 0.175	1.818 ± 0.185	2.160 ± 0.184	1.315 ± 0.120
Total orthodiphenols	1.197 ± 0.112	0.920 ± 0.098	0.765 ± 0.085	1.193 ± 0.106	0.735 ± 0.075	1.086 ± 0.087	0.610 ± 0.062
Total secoiridoids	1.602 ± 0.135	1.372 ± 0.102	1.167 ± 0.116	2.090 ± 0.164	1.519 ± 0.122	1.776 ± 0.106	1.128 ± 0.107

Non-treated Picual and Verdial olives (control) harvested in Cabra and Villarrasa, respectively, or previously submerged in hot water at different temperatures for 3 min (Picual) or for 5 min (Verdial) in the 2004/2005 and the 2005/2006 seasons, respectively. Treatment conditions were previously selected as suitable in each case at laboratory scale. Each value is the mean value of two replicates

[a] Mean values of four replicates

[b] The oils were extracted immediately after treatment or after a delay of 24 h (+24 h)

[c] Dialdehydic form of the decarboxymethyl oleuropein aglycone

[d] Dialdehydic form of the decarboxymethyl ligstroside aglycone

[e] Hydroxytyrosyl elenolate

[f] Tyrosyl elenolate

24-h delay of the heat treatment showed a similar increase in pigment content to those obtained from olives crushed immediately after dipping at 50 °C.

Heat treatment induced a notable loss in stability against oxidation in the oils, which was more evident when the oil was extracted after a 24-h delay (Table 5). The prolongation of treatment time, which the 24 h of processing delay implied, could explain this fact.

Heat treatment induced a clear reduction of the total content of phenolic compounds in the oil (Table 6). The contents of orthodiphenols and secoridoid derivatives also decreased, mainly because of the reduction of 3,4 DHPA-EA, coinciding with the reduction of both stability and bitterness in the oils (Table 5). The 24-h processing delay caused an additional reduction of the 3,4 DHPA-EDA (mainly), hydroxytirosol, acetoxy-pinoresinol and tyrosol-acetate contents, coinciding with an additional loss of oil stability (Table 5). These facts suggest that 3,4 DHPA-EA plays a more important role in the intensity of oil bitterness than 3,4 DHPA-EDA and confirms the antioxidant nature of both.

Influence of Heat Treatment and Fruit Ripening Level on the Quality of the Oil Extracted from Verdial Olives Processed at Industrial Scale

Heat treatment (57 °C) induced a clear reduction in the bitterness intensity of the subsequently extracted oils, regardless of the ripening level of the Verdial olives (Table 5). In contrast, for reducing the bitterness level of the oil extracted from Manzanilla olives of the same ripening level, from the same location and during the same season (2005/2006), a temperature of 65 °C was necessary (Table 3). Therefore, the treatment conditions for one olive variety cannot be extrapolated to another variety. Heat treatment did not increase oil yield values (Table 5).

However, ripening clearly increased this parameter. Thus, a delay of harvesting date from October (0.5 RI) to November (1.5 RI) will increase the oil yield. Only the overall grading of the sensory quality and the oxidative stability of the oil were deteriorated by heat treatment, regardless of the ripening level of the fruit (Table 5). This treatment also induced an increase of pigments, but caused no changes in the tocopherol content of the oil. The increase of fruit-ripening level did not deteriorate any of the oil quality parameters.

Heat treatments reduced the 3,4 DHPA-EDA, 3,4 DHPA-EA and p-HPEA-EA contents and the increase of hydroxytyrosol and tyrosol contents in the oil extracted from the less ripe olives (0.5 RI), or a generalized decrease in the content of the different phenolic compounds in the oil extracted from the riper fruits (1.5 RI) (Table 6). The progress of ripening induced an increase of hydroxytyrosol and tyrosol contents in the oils and a decrease of the rest of the phenolic compounds with the exception of 3,4 DHPA-EDA, which maintained its initial content (Table 6). Nevertheless, this reduction in phenolic compound contents did not imply a parallel reduction in the bitterness intensity or in the stability of the oil (Table 5).

Conclusions

In summary, the heat treatments carried out at industrial scale with three different varieties, regardless of their origin and season of harvest, fulfilled the primary objective proposed: to modify the intensity of the oil bitterness until reaching an appropriate level for a wide range of consumers. Nevertheless, to select suitable treatment conditions to achieve this objective, regardless of the fruit-ripening level, origin, crop season and variety, is not possible. In each case, a previous selection of the suitable treatment conditions at a small scale will be necessary before applying them at the industrial scale.

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