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Debittering of Olives by Polyphenol Oxidation

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In this study, green olives preserved in acidified brine were debittered by subjecting them to an overpressure of oxygen or air for 1−3 days. It was demonstrated that fruits lost their bitter taste due to the enzymatic oxidation of the phenolic compounds, in particular, the glucoside oleuropein. Hence, the concentrations of both *o*-diphenols and, to a lesser extent, monophenols decreased in the olives with oxidation. This process also gave rise to a darkening effect on the superficial and interior color of the olives, which turned from yellow-brown to brown. Likewise, the effect of several variables on the oxidation rate of the olives, such as type of gas (oxygen, air), temperature, overpressure level, and size of the olives, was also studied. Results indicate that a new debittering method which could be a promising alternative to the treatment of fruits with NaOH is available to the industry. In addition, a new product with different color and texture from the traditional table olives is presented.

KEYWORDS: Table olives; phenolic; bitter; oxidation; enzyme

INTRODUCTION

The raw olive drup is inedible because of its high content in a bitter glucoside named oleuropein (*1*), which is formed by glucose, elenolic acid, and the *o*-diphenol hydroxytyrosol (*2*) (**Figure 1**). The concentration of this secoiridoid glucoside in fruits depends on many factors such as variety, irrigation, and degree of ripening. Indeed, the level of oleuropein in olives decreases significantly with maturation (*3*).

There are many methods to prepare edible olives, although from an economic point of view, there are three main types of commercial table olives: green, black, and natural black olives. Fruits intended for Spanish green and California black olive types are harvested before full ripeness is attained, with a green-yellow color on the surface, and possess a strong bitter taste. By contrast, mature olives, which are black in color and less bitter, are used for natural black olives (*4*). The current method used to debitter green and black olives is based on a treatment of fruits with a dilute NaOH solution. The alkali breaks down the ester bond of the oleuropein molecule in a few hours (*5*), and none of its hydrolysis products (hydroxytyrosol and oleoside 11-methyl ester) are bitter (*6*). This chemical debittering method produces a high volume of heavily contaminated wastewaters, not only the alkaline solutions but also the further washwaters needed to remove the excess alkali from the olive flesh. Besides, the use of NaOH is not allowed to debitter organic table olives in many countries.

García et al. (*7*) have suggested heating the raw olives to reduce bitterness in virgin olive oil. The enzyme β-glucosidase is inactivated, and the bitter secoiridoid aglycons are not formed. In the case of table olives, this method cannot be applied because a very high concentration of oleuropein remains in the fruit after heating (*8*).

The microbial debittering of table olives has also been proposed as a natural and promising technology. Researchers have indicated that yeasts (*9*−*11*) and lactic acid bacteria (*12*−*14*) are able to degrade oleuropein during the brining of fruits and, therefore, debitter the product. However, the predominant microorganism in brines is not always the inoculated starter, and the growth of lactic acid bacteria is often inhibited by phenolic and oleosidic antimicrobial compounds (*13, 8*).

The binding of phenolic hydroxytyrosol to the rest of the oleuropein molecule is essential for the bitter taste of the substance (**Figure 1**), and the rupture of the ester bond is very rapid under alkaline conditions but very slow in acid conditions (*15*). An alternative to the breaking down of the ester bond could be to modify the hydroxytyrosol moiety and, consequently, that of oleuropein. Hydroxytyrosol is an *o*-diphenol and is very sensitive to chemical and enzymatic oxidation. It is well-known that the chemical and enzymatic oxidation of *o*-diphenols is rapid at alkaline and acidic pH, respectively. Olives are preserved in acidified brines at a pH below 4.5, and the enzymatic oxidation of phenolic compounds could occur under these conditions if oxygen is available, although there are no previous reports. Shasha et al. (*16*) passed an air-stream through a suspension of olive pulp in water, and the bitter taste was abolished. A method has recently been patented to debitter olives by keeping the fruits under an overpressure of oxygen (*17*). The presence of oxidases in fresh olives is well-documented (*18*−*20*), although most of this research has been carried out to study the browning of fruits after harvesting (*21*−*23*). To our knowledge, there are no data

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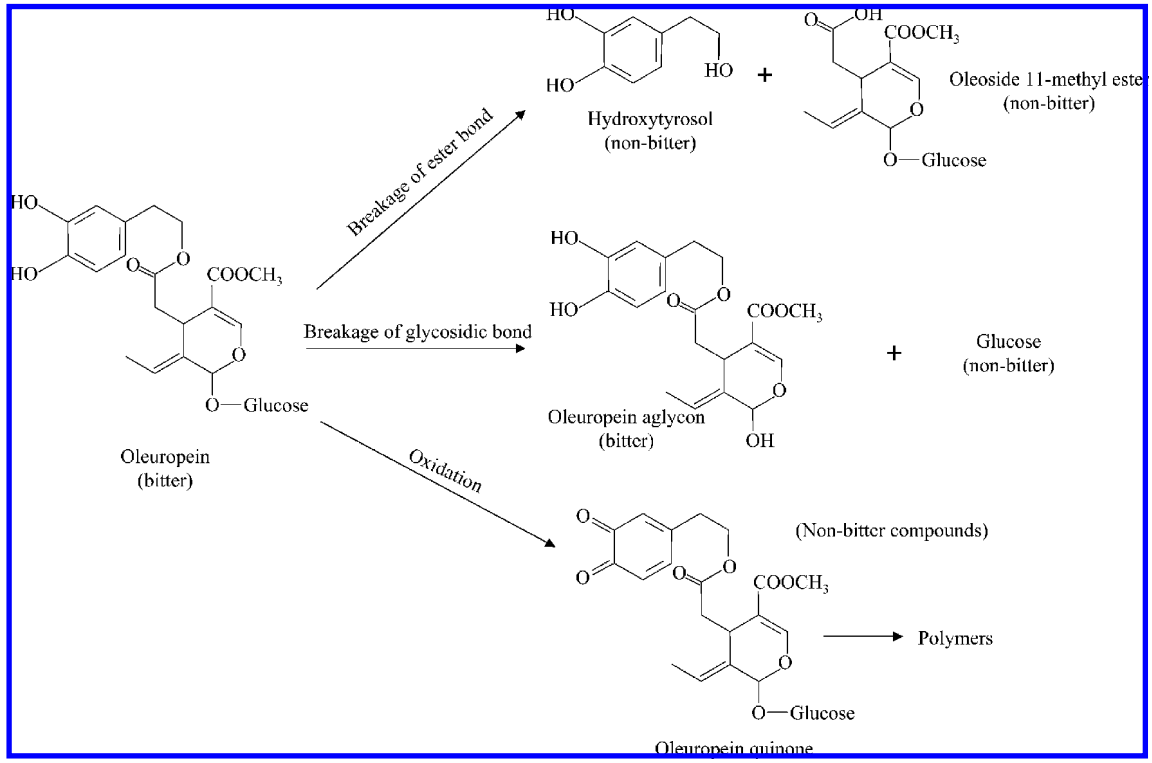

Figure 1. Bitterness of oleuropein and products of its chemical transformations.

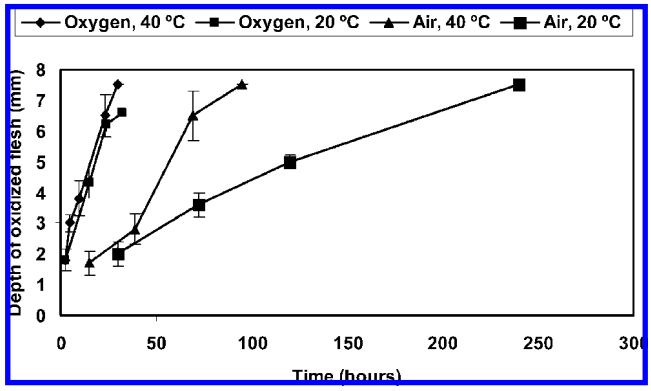

Figure 2. Oxidation of plain olives of the Manzanilla variety under an overpressure of 0.7 bar.

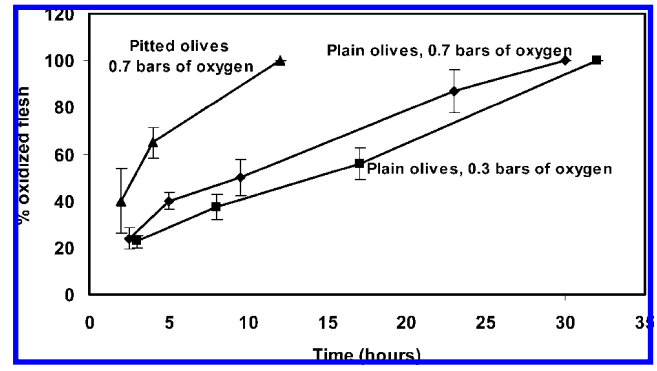

Figure 3. Oxidation of Manzanilla olives at 40 °C and oxygen overpressure.

on polyphenol oxidase (PPO) in preserved olives and its action under these acidic conditions.

The purpose of this work was to investigate a new olive debittering method based on the oxidation of the bitter glucoside oleuropein in situ, without breaking the tissues, by means of an overpressure of oxygen applied to diffuse the gas into the olive flesh.

MATERIALS AND METHODS

Olive Samples. Fruits of the Manzanilla variety with a green-yellow color on the surface were put into an acidified brine (5% NaCl, 0.7% acetic acid) and stored under anaerobic conditions for 4 months. Most of the oxidation experiments were carried out with these olives. Another four samples of preserved olives were purchased from a table olive factory, two of the Manzanilla variety and another two of the Hojiblanca variety. All of these olives were harvested with a green-yellow color and stored in acidified brine similar to that used at laboratory scale for 6 months. However, in this case, fruits were maintained under aerobic conditions in underground industrial tanks of 10000 kg of capacity (*24*).

Oxidation Experiments. An Oxoid anaerobic jar of 3.5 L of capacity with a pressure gauge used for microorganism culture was employed (Oxoid, Ltd., Basingstoke, U.K.). The safety valve of the jar was substituted by an inlet gas valve. Three hundred grams of olives was put in the bottom of the jar, which was hermetically sealed. Subsequently, gas (oxygen or air) was introduced through the inlet valve and expulsed through the outlet valve for 5 min. The outlet valve was closed, and an overpressure was obtained in the jar. Finally, the jar was incubated in a thermostatic chamber at 20 or 40 °C.

The oxidation rate of olives was checked visually because the olive flesh darkened progressively from the skin to the pit. In the case of pitted olives, darkening started from both the skin and the side of olives that was in contact with the pit. The progress of oxidation was analyzed by measuring the darkening of flesh with a micrometer on 10 olives of 18 mm diameter.

All experiments were run in duplicate.

Color Analysis. Colorimetric measurements on olives were performed using a BYK-Gardner model 9000 Color-view spectrophotometer, equipped with computer software to calculate the CIE L^* (lightness), a^* (redness), and b^* (yellowness) parameters. Interference by stray light was minimized by covering samples with a box that had a matte black interior. The data of each measurement are the average of 20 olives.

Texture Analysis. Firmness of olives was measured using a Kramer shear compression cell coupled to an Instron Universal Testing Machine

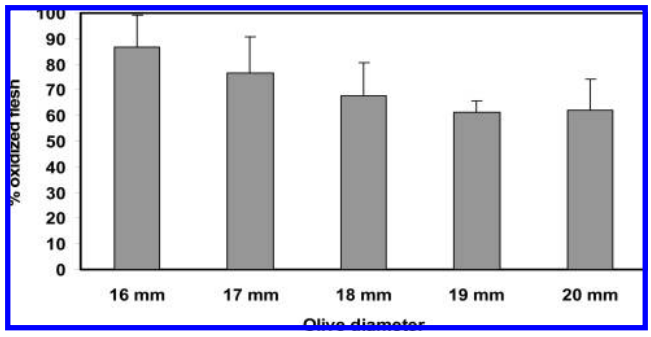

Figure 4. Influence of olive size on the oxidation rate. Fruits were of the Manzanilla variety, and they were oxidized under an overpressure of oxygen (0.7 bar) at 40 °C for 6 h.

(Canton, MA). The firmness of the olives was expressed as the mean of 10 measurements, each of which was performed on two pitted olives. Shear compression force was expressed as kN/100 g of pitted olives.

Analysis of Phenolic Compounds. Phenolic compounds were analyzed in both the aqueous (juice) and lipidic phases (oil) of olive pulp (*4*). Olives refrigerated at 5 °C were pitted, and 100 g was blended in a commercial mill. The paste was centrifuged at 6000 rpm for 5 min, and the oil was separated from the juice with a pipet. One milliliter of the juice was centrifuged at 10000 rpm for 8 min, and the supernatant was diluted 1:1 with distilled water. Subsequently, the mixture was centrifuged at 10000 rpm for 8 min and passed through a 0.45 μm nylon filter. Finally, 20 μL was directly injected into the chromatograph.

The phenolic compounds were extracted from the oil phase following the procedure described elsewhere (*4*). Briefly, 0.6 mL of olive oil was extracted by using 3 × 0.6 mL of *N*,*N*-dimethylformamide (DMF), the extract was washed with hexane, and N_2 was bubbled into the DMF extract to eliminate the residual hexane. Finally, the extract was filtered through 0.45 μm pore size and injected into the chromatograph.

The chromatographic system consisted of a Waters 717 Plus autosampler, a Waters 600E pump, a Waters column heater module, and a Waters 996 diode array detector (Waters Inc., Milford, MA). A Spherisorb ODS-2 (5 μm, 25 × 4.6 mm i.d., Waters Inc.) column was used. Separation was achieved using an elution gradient with an initial composition of 90% water (adjusted to pH 3.0 with phosphoric acid) and 10% methanol. The concentration of the latter solvent was increased to 30% over 10 min and maintained for 20 min. Subsequently, the methanol percentage was raised to 40% over 10 min, was maintained for 5 min, and was then increased to 50%. Finally, the methanol percentage was increased to 60, 70, and 100% in 5-min periods. Initial conditions were reached in 15 min. A flow rate of 1 mL/min and a temperature of 35 °C were used in all experiments. Phenolic compounds were monitored at 280 nm.

RESULTS AND DISCUSSION

Olives of the Manzanilla variety preserved for 4 months in acidified brine at laboratory scale possessed a strong bitter taste. When they were left in open air for 5 days at 20 and 40 °C, their surface slightly darkened, they shriveled, and a strong bitter taste remained (informal sensory evaluation of olives was carried out by a five-member, trained panel). On the contrary, fruits subjected to an overpressure of both oxygen and air darkened from a yellow-brown to a brown color, and the bitter taste was completely removed. It was observed that the interior of the fruits also darkened with time (**Figure 2**), and a uniform oxidation front progressed from the skin to the pit. Thus, the course of the debittering and oxidation of olives was run in parallel and controlled visually in a similar manner to that currently employed during NaOH treatment. It must be pointed out that the fruits oxidized under an overpressure of gas were debittered but those exposed to open air were not. This overpressure was necessary to allow the diffusion of oxygen through the olive flesh.

The concentration of oxygen in the oxidation jar was determinant for the rate of the process; a faster oxidation rate was found for the experiments carried out with pure oxygen than with air (**Figure 2**). Also, temperature had little influence on the oxidation rate when oxygen was employed and, by contrast, an increase in temperature from 20 to 40 °C supposed a reduction in the complete oxidation process from 250 to 92 h when air was employed.

The skin of the fruits, in particular that of olives not treated with NaOH, is a barrier for the diffusion of compounds (*25*), and it could also be expected for gas diffusion. Indeed, pitted olives were completely oxidized in just only 12 h, whereas plain olives needed 30 h (**Figure 3**). Oxygen diffused through both the skin and the interior of the olives, and oxidation progressed from both sides in pitted olives. An overpressure of gas in the jar was necessary to oxidize the olives, but a level of 0.3 bar was sufficient because a small difference in the oxidation rate was found between 0.3 and 0.7 bar of overpressure.

Another variable that influenced the oxidation rate was the size of the olives: the higher the size, the lower the oxidation rate was (**Figure 4**). It must be stressed that olives did not shrivel at any of the overpressure levels studied.

As mentioned above, olives darkened during the oxidation process, but they did not reach the black color that occurs during the processing of black ripe olives (*4*). In the latter case, fruits are treated with NaOH and oxidized at alkaline conditions until the olives have a shiny black surface color. In our case, the olives changed in color from yellow-brown to brown (**Table**

Table 1. Influence of Oxidation Debittering Conditions on Color and Firmness of Plain and Pitted Manzanilla Fruits[a]

	raw olives	plain olives, O_2, 20 °C (32.5 h)	plain olives, O_2, 40 °C (30 h)	plain olives, air, 40 °C (240 h)	pitted olives, O_2, 40 °C (15 h)	plain olives, pasteurized O_2, 40 °C (145 h)
			superficial color			
L	52.0 (1.5)[b]	31.6 (0.8)	27.8 (0.2)	31.9 (0.1)	33.8 (0.1)	32.9 (0.7)
*a**	7.7 (0.5)	7.2 (0.1)	6.5 (0.3)	7.3 (0.1)	7.7 (0.1)	7.4 (0.1)
*b**	34.0 (0.6)	10.7 (0.5)	8.0 (0.5)	9.8 (0.1)	14.3 (0.4)	9.9 (0.8)
			internal color			
L	61.2 (0.7)	39.2 (1.2)	32.7 (1.5)	35.4 (0.6)	34.1 (1.8)	35.7 (0.2)
*a**	4.1 (0.2)	6.8 (0.1)	8.5 (0.7)	9.7 (0.5)	8.7 (0.5)	10.7 (0.1)
*b**	32.4 (0.7)	12.7 (1.4)	10.4 (0.5)	12.4 (0.6)	15.0 (0.9)	15.2 (0.2)
			firmness			
kN/100 g of pitted fruits	6.8 (0.8)	5.8 (1.0)	6.1 (0.3)	6.0 (0.9)	6.4 (0.8)	3.3 (0.9)

[a] Experiments were carried out at 0.7 bar of overpressure. [b] Standard deviation of duplicates.

Table 2. Color of Industrial Olives Debittered under 0.7 bar of Oxygen Pressure at 40 °C

	olive samples							
	Manzanilla 1		Manzanilla 2		Hojiblanca 1		Hojiblanca 2	
	initial	15 h	initial	40 h	initial	15 h	initial	15 h
				superficial color				
L	47.2 (0.1)[a]	33.6 (0.2)	35.6 (0.7)	33.8 (0.7)	42.0 (1.1)	35.4 (0.2)	42.0 (0.7)	37.0 (0.2)
*a**	6.0 (0.4)	6.8 (0.3)	8.3 (0.3)	6.2 (0.4)	5.4 (0.1)	6.0 (0.1)	5.5 (0.2)	5.6 (0.2)
*b**	26.4 (0.4)	11.2 (0.2)	14.2 (0.6)	9.0 (0.8)	20.1 (0.3)	12.8 (0.9)	21.1 (0.4)	14.4 (0.2)
				internal color				
L	55.1 (7.0)	35.3 (0.4)	53.8 (0.6)	42.0 (0.3)	52.4 (0.2)	37.0 (0.6)	51.4 (0.2)	36.1 (0.7)
*a**	3.4 (0.1)	8.6 (0.1)	4.7 (0.4)	7.9 (0.1)	5.3 (0.3)	8.7 (0.1)	6.0 (0.1)	8.9 (0.2)
*b**	30.3 (0.3)	11.0 (0.1)	20.0 (2.1)	10.8 (0.6)	26.1 (0.7)	12.4 (1.0)	25.2 (0.4)	12.5 (0.5)

[a] Standard deviation of duplicates.

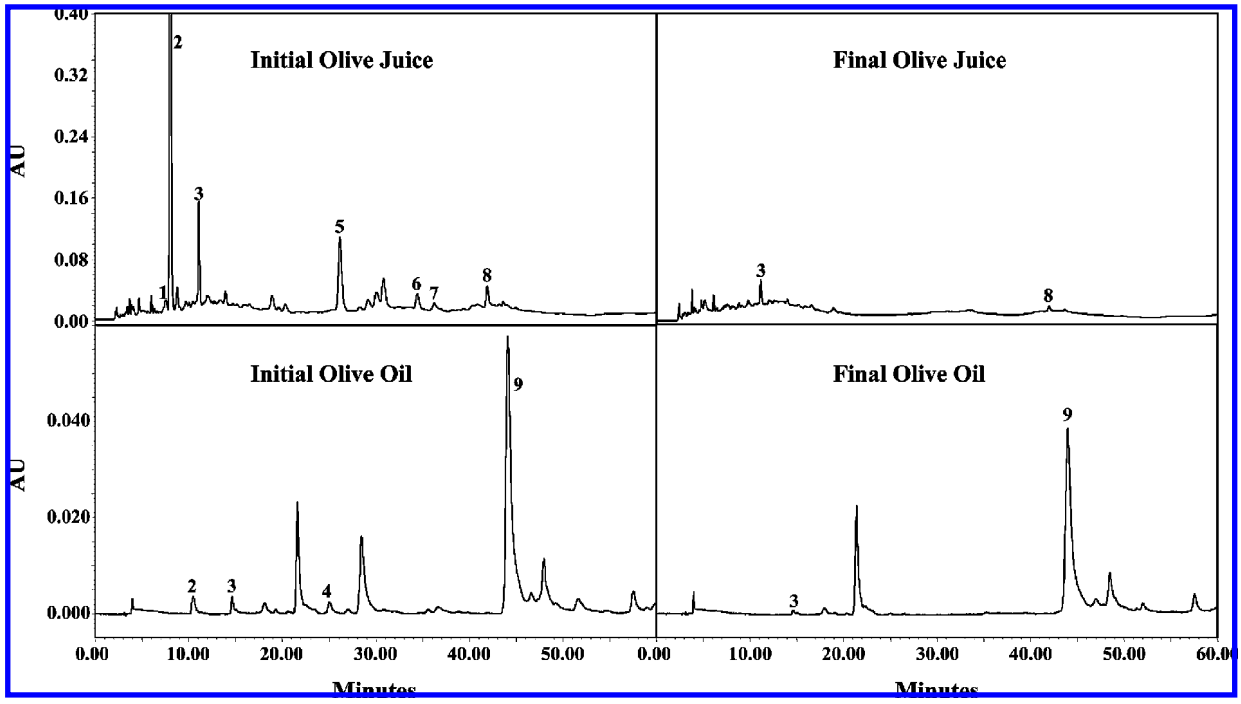

Figure 5. HPLC chromatograms of the phenolic compounds in the juice and oily phases of Manzanilla olives before and after the oxidation process. Peaks: 1, hydroxytyrosol 4-glucoside; 2, hydroxytyrosol; 3, tyrosol; 4, hydroxytyrosol acetate (HyAC); 5, verbascoside; 6, oleuropein; 7, caffeoyl ester of secologanoside; 8, comselogoside; 9, dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol (TyEDA).

Table 3. Concentration of Phenolic Compounds (Millimolar) in Olives of the Manzanilla Variety Oxidized under an Overpressure of Oxygen at 40° C

	olive juice				olive oil			
	hydroxytyrosol	HyGlu4[a]	tyrosol	oleuropein	hydroxytyrosol	tyrosol	HyAC	TyEDA
raw olives	18.1 (0.8)[d]	0.5 (0.1)	3.3 (0.2)	0.3 (0.1)	0.3 (0.1)	0.2 (0.1)	0.6 (0.1)	4.5 (0.2)
NPO[b], plain, 30 h	nd[c]	nd	0.8 (0)	nd	nd	0.1 (0)	nd	3.2 (0.1)
PO, plain, 145 h	8.5 (0.3)	0.3 (0.2)	2.1 (0.1)	0.1 (0)	0.1 (0)	0.1 (0)	0.9 (0.2)	3.3 (0.1)
PO, pitted, 42 h	12.7 (0.4)	0.4 (0.3)	2.2 (0.2)	0.1 (0)	0.1 (0)	0.1 (0.1)	1.1 (0.1)	3.5 (0.1)

[a] HyGlu4, hydroxytyrosol 4-glucoside; HyAC, acetylated hydroxytyrosol; TyEDA, dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol. [b] NPO, nonpasteurized olives; PO, pasteurized olives. [c] Not detected. [d] Standard deviation of duplicates. Data for verbascoside, cafffeoyl ester of secologanoside and comselogoside were not included because their concentrations in the olive drup were low (<0.1 mM).

1). The lightness parameter (*L**) of the fruits was reduced from 52 to around 30 and the yellowness parameter (*b**) from 34 to around 10, regardless of the type of oxidation process applied. Therefore, these olives cannot commercially be classified as green or black, and a new product is presented.

Four samples of industrial olives were also oxidized in the jars, and similar results were found as reported for olives preserved at laboratory scale. Fruits darkened and lost their bitter taste. In this case, the superficial color of olives (**Table 2**) was darker than that of Manzanilla olives preserved at laboratory scale (**Table 1**), which can be attributed to the use of aerobic conditions in the industry to preserve the product. In particular, the sample Manzanilla 2 had the lowest initial values for *L** and *b**, which related to a higher degree of maturation. These olives were debittered in 40 h, whereas the other industrial samples required only 15 h. Also, the texture was not significantly influenced by the oxidation treatment.

Although it is difficult to distinguish between the chemical and enzymatic oxidation of polyphenols, it can be supposed that the olive polyphenols were oxidized mainly via enzymatic reaction. The pH of the olive flesh was around 4, which is low for a rapid chemical oxidation, whereas olive PPO acts in a pH

Table 4. Concentration of Phenolic Compounds (Millimolar) in Industrial Olives Oxidized under an Overpressure of Oxygen at 40° C

	olive juice				olive oil			
	hydroxytyrosol	HyGlu4[a]	tyrosol	oleuropein	hydroxytyrosol	tyrosol	HyAC	TyAC
raw Manzanilla 1	9.0 (0.1)[b]	3.5 (0.1)	1.4 (0.2)	0.2 (0.1)	0.1 (0)	0.1 (0)	1.2 (0.1)	0.1 (0)
oxidized Manzanilla 1	0.6 (0.1)	1.9 (0.1)	0.7 (0.1)	nd[c]	nd	0.1 (0)	nd	0.1 (0)
raw Manzanilla 2	8.5 (0.1)	0.7 (0.1)	1.4 (0.1)	0.1 (0)	0.1 (0)	0.1 (0)	0.6 (0.1)	0.5 (0.1)
oxidized Manzanilla 2	6.6 (0.1)	0.3 (0.1)	1.3 (0.1)	nd	nd	0.1 (0)	nd	0.5 (0.1)
raw Hojiblanca 1	9.1 (0.1)	4.1 (0.1)	1.2 (0.1)	0.5 (0.1)	0.1 (0)	0.1 (0)	0.7 (0.1)	0.1 (0)
oxidized Hojiblanca 1	0.9 (0.1)	2.9 (0.1)	0.8 (0.1)	nd	nd	0.1 (0)	nd	0.1 (0)
raw Hojiblanca 2	9.1 (0.1)	3.8 (0.2)	1.2 (0.1)	0.3 (0.1)	0.1 (0)	0.1 (0)	0.3 (0.2)	0.1 (0)
oxidized Hojiblanca 2	1.8 (0.1)	2.7 (0.1)	0.7 (0.1)	nd	nd	0.1 (0)	nd	0.1 (0)

[a] HyGlu4, hydroxytyrosol 4-glucoside; HyAC, acetylated hydroxytyrosol; TyAC, acetylated tyrosol. [b] Standard deviation of duplicates. [c] Not detected, below 0.01 mM. All of the olive varieties were oxidized for 15 h, except Manzanilla 2, which was oxidized for 40 h. Data for verbascoside, cafffeoyl ester of secologanoside, and comselogoside were not included because their concentrations in the olive drup were low (<0.1 mM).

range of 3−7 (*21, 23*). In addition, PPO activity decreases with olive maturation (*22, 26*), and this can explain the slower oxidation rate found for the mature olives of the Manzanilla 2 sample. Nevertheless, Ortega-García et al. (*20*) have recently found PPO activity mainly in the epidermis of olives of the Picual variety, and we observed oxidation in the whole fruit. Also, there are contradictory results about a direct relationship between PPO activity and browning (*19, 22*).

Hence, a new experiment was undertaken to clarify this point. Manzanilla olives preserved at laboratory scale were heated at 90 °C for 30 min and subsequently oxidized under an overpressure of oxygen or air. The phenolic compounds were measured in the juice and oily phases of the olives (**Figure 5**), and they were lost after 30 h of oxidation in the case of nonpasteurized olives, which was checked visually by cutting the fruits and confirmed by the very low concentration of phenolic compounds found in the flesh (**Table 3**). By contrast, pasteurized olives did not show a uniform or complete browning of the flesh even after 145 h of oxidation. Indeed, a low decrease in phenolic compounds was detected in comparison with nonpasteurized fruits, and the bitter taste remained in them, as well as a residual concentration of the bitter glucoside oleuropein. These results confirmed the low contribution of the chemical oxidation of polyphenols to olive debittering in comparison with the enzymatic reaction. On the other hand, the concentration of oleuropein is very high in fresh fruits (*3*), but it decreases during the preservation of olives in acidified brines (*27*). However, this glucoside possesses a strong bitter taste (*6*) that was detected in the experimental olives even at the low concentration of oleuropein found (**Tables 3** and **4**).

The high concentration of the dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol (TyEDA) in the oily phase of these Manzanilla olives must be highlighted (**Table 3**). A significant amount of this substance was retained in the olives after oxidation, and the slightly pungent taste detected in fruits was attributed to its presence because it has been confirmed to be responsible for this sensation in virgin olive oil (*28*). However, neither a pungent taste nor TyEDA was detected in any of the industrial samples (**Table 4**).

Both Manzanilla and Hojiblanca olive varieties were debittered in <40 h, and oleuropein was not detected in their olive flesh after oxidation. Overall, the concentration of *o*-diphenols decreased in a higher proportion than that of monophenols during oxidation. Despite the fact that some researchers have not found monophenolase activity in olives (*21*), tyrosol concentration diminished in olive flesh with oxidation. However, diphenolase activity was higher than monophenolase because hydroxytyrosol and hydroxytyrosol-like compounds almost disappeared after oxidation, whereas a residual concentration of tyrosol remained.

The enzymatic oxidation of polyphenols is a major concern in the food industry because of the darkening consequences. However, this reaction is also required during processing of many food products such as black tea, cocoa, prunes, raisins, and others. Likewise, oxidation and polymerization of olive polyphenols occurred during elaboration of the commercial black ripe olives (*29*) and natural black olives (*30*).

In conclusion, a new method of debittering olives has been discovered, which is based on the enzymatic oxidation of phenolic compounds, in particular, the bitter glucoside oleuropein. Likewise, an overpressure of gas (oxygen, air) is needed to perform the debittering process on whole, preserved olives.

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