Acid Hydrolysis of Secoiridoid Aglycons during Storage of Virgin Olive Oil

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The main change found in the phenolic composition of virgin olive oils of Arbequina, Hojiblanca, and Picual varieties during storage in darkness at 30 °C was the hydrolysis of the secoiridoid aglycons. This reaction gave rise to an increase in the free phenolics hydroxytyrosol and tyrosol in the oil. Filtration of oil and acidity influenced the hydrolysis to a large extent. Thus, the addition of commercial oleic acid to Hojiblanca and Picual oils increased the hydrolysis rate of the secoiridoid aglycons. In contrast, the concentration of lignans 1-acetoxypinoresinol and pinoresinol remained constant during storage. It must also be stressed that the total molar concentration of the phenolic compounds analyzed in the oils changed slightly (<20% reduction) after one year of storage, which is important from a nutritional point of view. However, the transformation of the secoiridoid aglycons into free phenolics may have consequences on oil taste and antioxidant capacity.

Keywords: *Olive oil; secoiridoid aglycon; hydrolysis; storage; phenolic*

INTRODUCTION

Olive oil polyphenols are receiving interest from consumers and food processors for several reasons. These compounds have been associated with the shelf life and bitter taste of oil (*1, 2*) and, recently, they have gained much more attention because of their potential beneficial health effects (*3*).

The main phenolic compounds in fresh virgin olive oil are the secoiridoid aglycons of oleuropein and ligstroside (*4*), and the lignans 1-acetoxypinoresinol and pinoresinol (*5*). On the other hand, limited and contradictory studies have been published on the evolution of all these substances during oil storage (*6, 7, 8*). Hydroxytyrosol and tyrosol tend to increase their concentration in oil with storage time (*9*) although a rise and fall behavior has also been observed (*4, 6*). Most researchers have reported a decrease in the total amount of polyphenols during storage, which could imply important consequences from a nutritional point of view. However, most of the data were obtained when keeping oils under light (*4, 8*). Oxygen and light are two well-known oxidation-producing factors (*10, 11*) that industries try to avoid during commercial activities. In contrast, olive oil acidity, one of the main chemical characteristics of olive oil, exerts a great influence on oil stability (*12*) and no data are available about the effect of this parameter on phenolic evolution during olive oil storage.

The objectives of this study were to (i) examine the effect of olive oil acidity on secoiridoid aglycons hydrolysis, and (ii) measure the changes in the amount of phenolic compounds during storage of virgin olive oil under the commercial conditions of darkness and no exposure to oxygen.

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MATERIALS AND METHODS

Samples. Evolution of phenolic compounds during storage was studied on virgin olive oils of the Picual, Hojiblanca, and Arbequina varieties obtained from local processors who used a two-phase extraction system. Oil (425 g) with and without drying over anhydrous sodium sulfate was bottled in 0.5-L amber glass jars which were closed with screw caps and kept in the dark for one year in a termostatically controlled chamber at 30 ± 1 °C.

Another experiment was run to study the influence of acidity on phenolic aglycons hydrolysis. In this case virgin olive oils of Picual and Hojiblanca varieties different from those of the former experiment, but obtained by the same way, were used. This time all oil samples were filtered through paper filters, and the storage experiments were performed in 0.125-L amber glass bottles. One kilogram of oil was distributed into 10 bottles and another one kilogram of oil with acidity increased 1% by adding oleic acid purchased from Sigma Chemical Co. (St. Louis, MO) was also distributed into another 10 bottles. Jars were sealed and stored in the dark at 35 ± 1 °C.

Samples of oil were taken from the bottles at various times throughout the storage experiment, frozen at −30 °C, and analyzed all at the same time.

Chemical Analyses. Determinations of humidity, oil acidity, peroxide value, and specific ultraviolet (UV) absorbance (K_{232} and K_{270}) were made following the analytical methods described in EC Regulations (*13*).

Tocopherols were evaluated following IUPAC Standard Method 2432 (*14*).

Polyphenols were determined as described recently (*15*). Briefly, 0.6 g of olive oil was extracted by using 3 × 0.6 mL of *N*,*N*-dimethylformamide (DMF). The extract was washed with hexane, and nitrogen was bubbled into the DMF extract to eliminate the residual hexane. Finally, the extract was filtered (0.45-μm pore-size) and injected into the liquid chromatograph. The HPLC system consisted of a Waters 717 plus autosampler, a Waters 600E pump, a Waters column heater module (Waters Inc., Milford, MA), and an ESA coulometric sytem (ESA Inc., Chelmsford, MA). A Spherisorb ODS-2 column was used, and separation was achieved by elution gradient using as solvents a 30 mM $LiClO_4$ solution (pH

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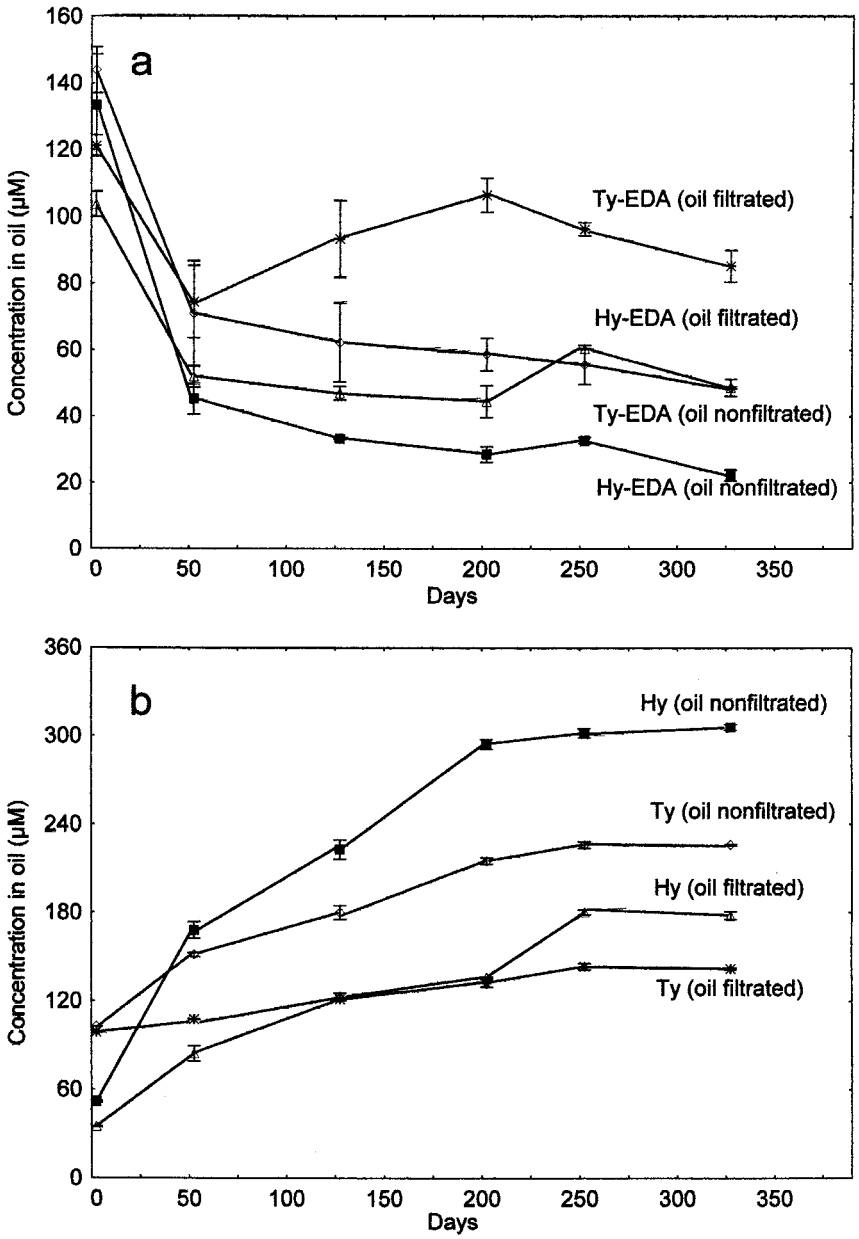

Figure 1. Influence of filtration on the evolution of hydroxytyrosol (Hy), tyrosol (Ty), and the dialdehydic form of elenolic acid linked to hydroxytyrosol (Hy-EDA) or tyrosol (Ty-EDA) during storage of Picual virgin olive oil at 30 °C. Mean values ± standard deviation. Where error bars are not visible, determinations were within range of symbols on graph.

adjusted to 3.1 with $HClO_4$) and methanol containing 30 mM $LiClO_4$. A flux of 1 mL/min and a temperature of 35 °C were also used.

Statistical Analysis. Statistical analysis of results was performed using the Statistica package software (Statistica for Windows, Tulsa, OK, 1996). Comparison between treatments were made by the multiple Duncan's range test. Significance of differences was defined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Figure 1 shows the evolution of the secoiridoid aglycons and the free phenolic hydroxytyrosol and tyrosol during storage of virgin olive oil in dark, 30 °C, and anaerobic conditions, which were chosen to mimic the commercial practice. Hydroxytyrosol and tyrosol concentration increased with time, as expected from previous experiments (*4*, *9*). However, in our experiments, these two compounds did not show a rise and fall trend (*6*) or a linear increase during storage (*9*). In contrast, hydroxytyrosol and tyrosol content rose rapidly during the first 200 days and then tended to equalize throughout the remainder of the year of storage. We believe that the differences found with other researchers were due to the different experimental conditions (*4*, *6*). In these cases, air, light, and humidity, which are oxidative variables, were present during the experiments and most likely caused consumption of these phenolic compounds during oil storage.

However, this is the first time that the molar concentration of the secoiridoid aglycons present in virgin olive oils have been quantified during storage of oil (Figures 1 and 2). All these compounds decreased their concentrations with time at the beginning of the storage. When comparing this behavior with that of the free phenolic hydroxytyrosol and tyrosol (Figure 1), it could be concluded that a hydrolysis reaction of the ester bound between the phenolic compound and the rest of the molecule occurred. In fact, that relationship was found

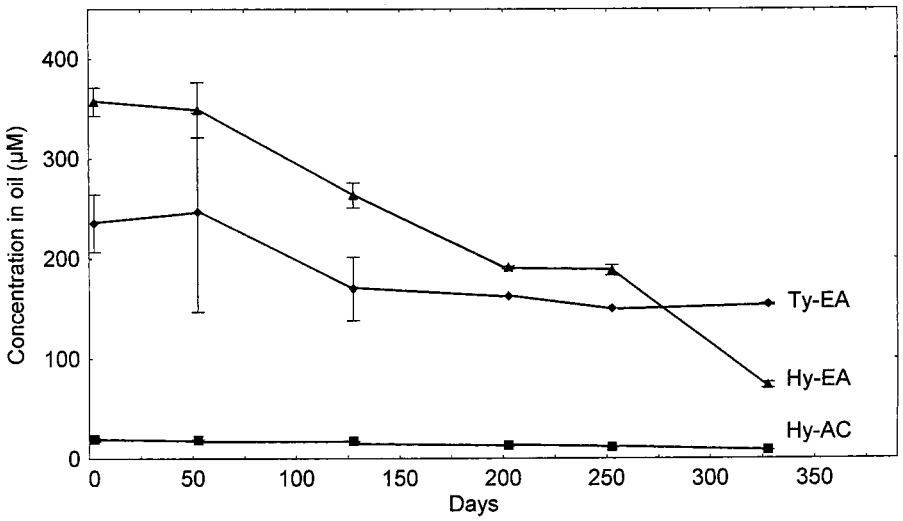

Figure 2. Evolution of 4-(acetoxyethyl)-1,2-dihidroxybenzene (Hy-AC) and the aglycons of oleuropein (Hy-EA) and ligstroside (Ty-EA) during storage of unfiltered Picual virgin olive oil at 30 °C. Mean values ± standard deviation. Where error bars are not visible, determinations were within range of symbols on graph.

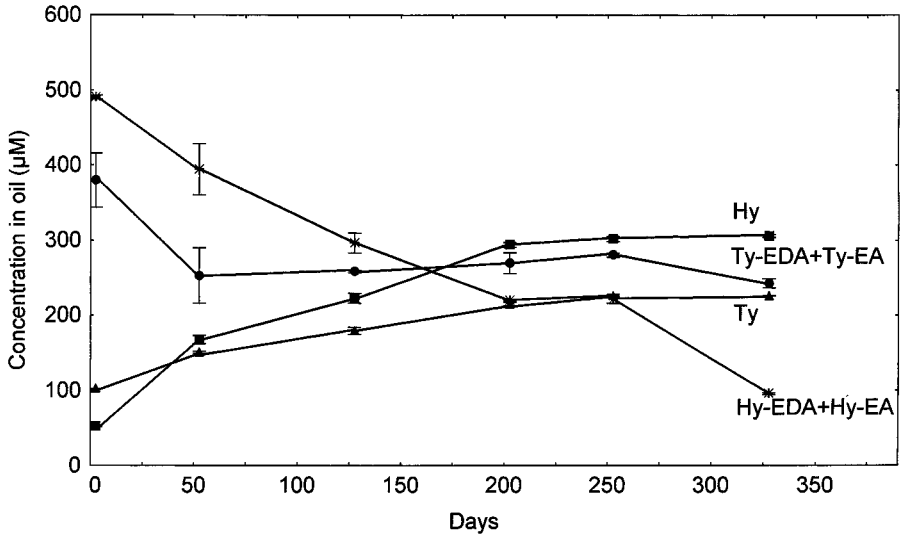

Figure 3. Relationship between hydroxytyrosol and tyrosol increase and aglycons decrease during storage of unfiltered Picual virgin olive oil at 30 °C. Mean values ± standard deviation. Where error bars are not visible, determinations were within range of symbols on graph.

between the increase in hydroxytyrosol and tyrosol and the decrease in secoiridoid aglycons (Figure 3). Also, it can be observed in this figure that the increase in hydroxytyrosol was higher than that in tyrosol, which was related to the higher decrease in hydroxytyrosol aglycons than that in tyrosol. An important conclusion from these data is that very little amount of hydroxytyrosol or tyrosol was consumed during storage, which contrasts with data reported by other researchers and is very important from a nutritional point of view.

It has been reported that total polyphenols decrease in a high proportion to their concentration during storage (*4, 6, 7, 10*). However, some of these experiments were run under light and air, and quantification of polyphenols was done colorimetrically. Table 1 illustrates the changes with storage time of all the phenolic compounds identified in virgin olive oils of Arbequina, Hojiblanca, and Picual under mimicked commercial conditions (dark, no air, 30 °C). Hydroxytyrosol and tyrosol increased their concentrations after one year of storage in all three olive oil varieties studied, albeit in the order Picual > Hojiblanca > Arbequina. Likewise, the concentrations of secoiridoid aglycons and 4-(acetoxyethyl)-1,2-dihidroxybenzene (Hy-AC) decreased, but the differences in content between 0 days and 340 days for all of them were statistically different only for Picual oils.

The lignans 1-acetoxypinoresinol and pinoresinol are compounds recently identified in olive oil (*5*) and found in a high amount in certain olive oil varieties such as Arbequina. Apart from their well-known health benefits, these compounds may be important for olive oil because their content in oil did not change with storage (Table 1). In fact, it has been reported that a peak, which most likely corresponded to lignans, was the most important finding detected after storage of olive oil under light and air conditions for 460 days (*4*).

Other minor compounds in olive oil, such as vanillin, vanillic and *p*-coumaric acids, and the flavonoids luteolin and apigenin, only decreased with time for Picual olive oil, and the storage effect was statistically significant for most of them.

Reduction in the total phenol content of the oils after one year of storage was significant for Hojiblanca and

Table 1. Influence of Storage and Filtration on the Concentration of Phenolic Compounds (μM) in Virgin Olive Oils of Arbequina, Hojiblanca, and Picual Varieties[a]

	Arbequina				Hojiblanca				Picual			
	nonfiltered		filtered		nonfiltered		filtered		nonfiltered		filtered	
analyte	0 days	340 days	0 days	340 days	0 days	340 days	0 days	340 days	0 days	340 days	0 days	340 days
Hy	12.9 a	85.9 b	8.3 a	45.3 c	34.9 a	106.5 b	18.8 a	74.6 c	52.0 a	305.9 b	34.8 c	178.0 d
Hy-AC	200.9 a	185.4 a	211.8 a	223.0 a	17.9 a	15.2 a	16.7 a	15.1 a	19.2 a	8.3 b	16.3 a	11.8 b
Hy-EDA	508.4 a	490.5 a	584.4 a	602.4 a	100.7 a	96.6 a	88.1 a	92.6 a	133.5 a	22.3 b	103.7 a	48.7 b
Hy-EA	18.1 a	16.0 a	28.9 a	29.2 a	206.9 a	104.4 b	185.5 a	124.5 b	357.5 a	73.7 b	337.6 a	117.9 b
Ty	8.7 a	17.3 b	8.5 a	13.9 c	40.5 a	60.7 b	40.2 a	58.5 b	102.8 a	226.1 b	98.9 a	141.9 b
Ty-EDA	85.9 a	120.5 a	92.2 a	115.1 a	104.7 a	61.6 b	92.7 a	59.3 b	143.9 a	48.7 b	121.2 a	85.3 b
Ty-EA	6.0 a	5.4 a	6.3 a	4.7 a	126.4 a	61.7 b	108.1 a	72.8 b	235.6 a	154.3 b	217.8 a	157.4 b
1-acetoxy pinoresinol	192.8 a	212.2 a	195.6 a	210.5 a	30.5 a	28.2 a	30.0 a	28.1 a	ND	ND	ND	ND
pinoresinol	104.4 a	116.0 a	105.1 a	115.7 a	33.4 a	32.9 a	34.4 a	33.6 a	130.6 a	121.3 a	128.2 a	120.2 a
luteolin	18.4 a	17.9 a	16.2 a	18.1 a	8.7 a	8.9 a	8.1 a	9.1 a	11.2 a	5.9 b	9.3 c	6.2 b
apegenin	4.4 a	4.6 a	4.2 a	4.9 a	3.4 a	4.5 a	3.4 a	4.8 a	3.1 a	1.2 b	3.0 a	1.4 b
vanillic acid	1.9 a	1.3 a	1.9 a	1.3 a	0.6 a	0.6 a	0.6 a	0.6 a	1.7 a	1.8 a	1.9 a	1.4 a
vanillin	1.1 a	1.4 a	1.1 a	1.3 a	4.7 a	4.8 a	4.5 a	4.7 a	1.8 a	1.1 b	1.7 a	1.2 a
p-coumaric	1.8 a	1.6 a	1.9 a	1.1 a	1.9 a	2.7 a	1.8 a	2.6 a	2.3 a	1.5 b	1.8 a	1.0 a
total	1165.7 a	1276.0 a	1266.0 a	1386.6 a	715.2 a	589.3 b	632.9 a	580.9 a	1195.2 a	972.1 b	1076.2 a	872.4 b

[a] Means in rows, within variety for each compound, followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level. ND = not detected.

Table 2. Influence of Storage and Filtration on the Analytical Characteristics of Virgin Olive Oils of Arbequina, Hojiblanca, and Picual Varieties[a]

variety	filtered?	time (days)	acidity (as % of oleic acid)	peroxide (meq O_2/kg)	K_{270}	K_{232}	α-tocopherol (mg/kg)
Arbequina	no	0	0.25a	7.4a	0.13a	2.1a	111.3a
Arbequina	no	340	0.34b	7.6a	0.17b	1.7a	112.6a
Arbequina	yes	0	0.25a	10.4b	0.12a	1.9a	115.7a
Arbequina	yes	340	0.26a	8.6a	0.16b	1.6b	105.6a
Hojiblanca	no	0	0.60a	8.1a	0.12a	1.6a	100.3a
Hojiblanca	no	340	0.98b	5.8b	0.18b	1.4a	97.4a
Hojiblanca	yes	0	0.58a	7.8a	0.11a	1.5a	103.2a
Hojiblanca	yes	340	0.76b	6.7b	0.16b	1.6a	103.3a
Picual	no	0	0.78a	11.4a	0.17a	2.1a	172.4a
Picual	no	340	1.06b	8.0b	0.22b	1.9a	115.6b
Picual	yes	0	0.73a	11.5a	0.14a	2.1a	160.6a
Picual	yes	340	0.90a	9.3b	0.20b	1.9a	134.5b

[a] Means in a column for the same variety followed by the same letter are not significantly different by Duncan's multiple test at the 5% level.

Picual oils, although the decrease was only around 15−20% of the initial content. Total polyphenol contents of Arbequina oils did not change statistically during storage. These differences between varieties may be attributed to the initial differences in oil acidity.

This result needs to be stressed, because, from a nutritional point of view, the content of the total phenolic antioxidants was rather changed with storage; and what it changed was the structure of the antioxidant molecule. Thus, fresh virgin olive oil retained a high proportion in lipophilic compounds (secoiridoid aglycons), and after storage the phenolic profile changed to hidrophilic compounds (hydroxytyrosol and tyrosol). These changes in the composition of the phenolic substances may imply important consequences for oils. First, hydroxytyrosol aglycons are bitter, and the free phenolic hydroxytyrosol is not (*16*). Therefore, oils are less bitter with increasing storage time. Second, the antioxidant activity of the phenolic compounds is strongly affected by the composition of the food (*17*). Thus, the antioxidant action of the dialdehydic form of elenolic acid linked to hydroxytyrosol (Hy-EDA) was the same as that of hydroxytyrosol (Hy) when evaluated in bulk oil (*18*) but very different when a micellar system was used (*19*). Finally, the absorption of olive polyphenols may depend on the type of phenolic molecule. There is no available information on the absorption of the secoiridoid aglycons in humans, although the presence of hydroxytyrosol and derivatives in urine has been reported after ingestion of olive polyphenols (*20*). However, it could be assumed that the absorption of these olive polyphenols may be different if they are free or bound to another molecule.

Table 2 displays the effect of storage on the chemical characteristics of olive oil as they are related to the oxidation process. Changes were in accordance with those reported previously for olive oil stored in darkness and under anaerobic conditions (*7, 8, 21*). A slight decrease in peroxide index and K_{232} was observed. In fact, a slight increase and decrease of the peroxide index was observed throughout the storage period (data not shown) which is in agreement with previous works (21). α-Tocopherol content decreased significantly only for Picual oils (as occurred for polyphenols (Table 1)) which also may be related to the high acidity content of this oil. On the contrary, K_{270} and acidity increased with storage. All these parameters indicate that there was no significant oxidation during storage, which is normal because the conditions during the experiment were intended to simulate those of commercial handling.

Virgin olive oil may be consumed fresh as obtained from olives, but it is normally stored in large containers in darkness and under a controlled temperature. Finally, oil is bottled in anaerobic conditions. Other pro-

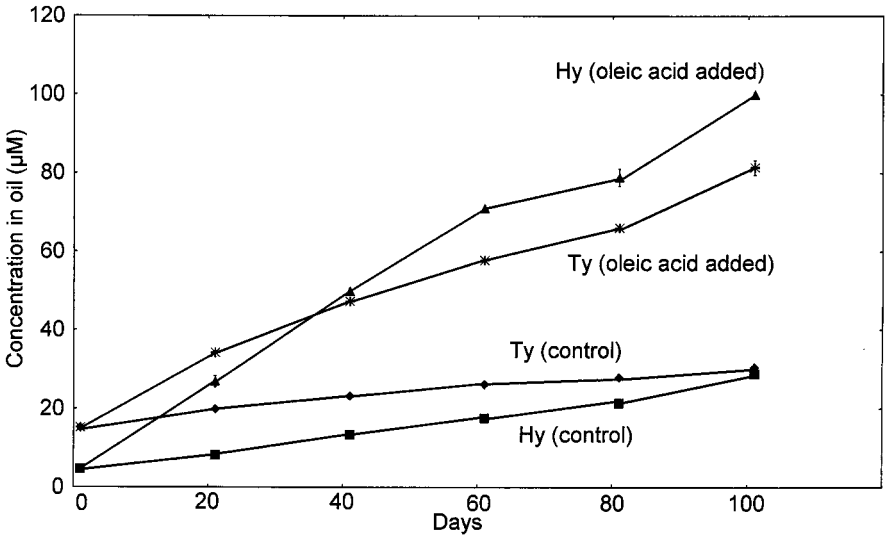

Figure 4. Effect of acidity (1% oleic acid aded) on the concentrations of hydroxytyrosol and tyrosol in Picual virgin olive oil during storage at 35 °C. Mean values ± standard deviation. Where error bars are not visible, determinations were within range of symbols on graph.

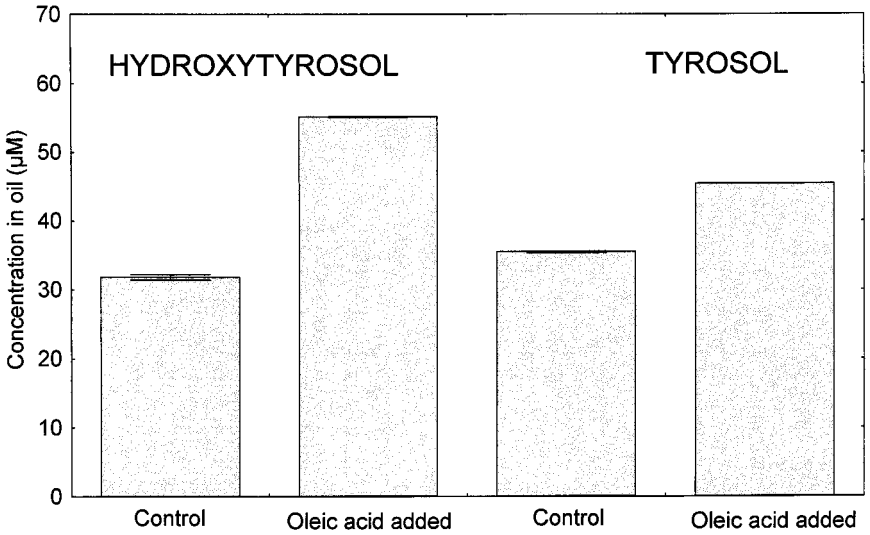

Figure 5. Effect of acidity (1% oleic acid added) on the concentrations of hydroxytyrosol and tyrosol in Hojiblanca virgin olive oil stored for 100 days at 35 °C. Mean values ± standard deviation. Where error bars are not visible, determinations were within range of graph lines.

oxidants such as metals and light are also avoided. However, there are two other intrinsic conditions in virgin olive oil that influence lipid oxidation and may also contribute to the phenolic changes observed during storage: i.e., turbidity and acidity.

Water is one of the main components of the virgin olive oil turbidity and it is well-known that the presence of this phase in oil accelerates the deterioration of the product, although results are contradictory (*12*). In fact, some industries often bottled their oil without filtration. In our experiments we found that storing olive oil without filtration gave rise to an increase in the chemical parameters related to the oxidation of the oil (no water was detected after filtration of oil) (Table 2). On the contrary, sensibly lower changes were observed in oils that were filtered. This was not the only effect observed: as can be seen in Figure 1 and Table 1 the filtration of oil had a great effect on the hydrolysis of secoiridoid aglycons for all three olive oil varieties studied. Free phenolics content (Hy and Ty) was always lower for filtered oils and, on the contrary, secoiridoid aglycons content was higher. Filtration did not affect the content of lignans.

Because secoiridoid aglycons decreased their concentration with time (with and without filtration of oil), and Hy and Ty increased, it can be assumed that the hydrolysis of the former compounds also may be influenced by the acidity of the oil. Alkaline and acid hydrolysis of oleuropein and its derivatives has been reported for table olives in brines (*22*). However, the acid hydrolysis of these compounds in a lipid medium has not been described. It was found that the hydrolysis of the aglycons was in the order Picual > Hojiblanca > Arbequina (Table 1) and it could be related to the oil acidity: Picual>Hojiblanca>Arbequina (Table 2). Although a relationship has been described between free fatty acids and oxidative stability of olive oil (*12*), no reports are available on the effect of oil acidity on the hydrolysis of phenolic esters. Pure oleic acid was added to Hojiblanca and Picual olive oils and the evolution of the phenolic compounds was followed during storage in darkness at 35 °C (Figures 4 and 5). An increase in

hydroxytyrosol and tyrosol content was observed in oils of both cultivars Hojiblanca and Picual when the fatty acid was added. Therefore, a strong relationship between oil acidity and hydrolysis of secoiridoid aglycons has been demonstrated.

The results of the above study show that the main changes in the phenolic compounds present in virgin olive oil during storage under mimicked commercial conditions were associated with the hydrolysis of the secoiridoid aglycons. These reactions were influenced by the acidity of the oil and the filtration step. This may have a practical application of interest for industries because these compounds participate in the bitter taste and stability of oil. In addition, this hydrolysis means that lypophilic compounds (secoiridoid aglycons) are transformed into hydrophilic substances (hydroxytyrosol and tyrosol), which is important for the antioxidant action of these compounds in micellar systems and human body absorption. The total molar content of the phenolic compounds in virgin olive oil slightly decreased after one year of storage, which must be accounted for when considering the nutritional aspects of an olive oil.

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