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Influence of Thermal Treatments Simulating Cooking Processes on the Polyphenol Content in Virgin Olive Oil

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Virgin olive oils were subjected to simulated common domestic processing, including frying, microwave heating, and boiling with water in a pressure cooker. The impact of these processes on polyphenol content and physicochemical characteristics of oils was assessed. Thermal oxidation of oils at 180 °C caused a significant decrease in hydroxytyrosol- and tyrosol-like substances. In contrast, oils heated for 25 h still retained a high proportion of the lignans 1-acetoxypinoresinol and pinoresinol. Thermal oxidation also resulted in a rapid degradation of α-tocopherol and the glyceridic fraction of oils. Microwave heating of oils for 10 min caused only minor losses in polyphenols, and the oil degradation was lower than that in thermoxidation assays. Again, lignans were the least affected polyphenols and did not change during microwave heating. Boiling a mixture of virgin olive oil and water in a pressure cooker for 30 min provoked the hydrolysis of the secoiridoid aglycons and the diffusion of the free phenolics hydroxytyrosol and tyrosol from the oil to the water phase. Losses of polyphenols were detected only at pH lower than 6. Moreover, α-tocopherol and the glyceridic fraction of oils were not modified during this process. It is worth noting that all the heating methods assayed resulted in more severe polyphenols losses and oil degradation for Arbequina than for Picual oil, which could be related to the lower content in polyunsaturated fatty acids of the latter olive cultivar. These findings may be relevant to the choice of cooking method and olive oil cultivar to increase the intake of olive polyphenols.

KEYWORDS: Olive oil; microwave heating; boiling; frying; polyphenols; lignans

INTRODUCTION

Epidemiological investigations support the relationship between the Mediterranean diet and low incidence of cardiovascular disease. Usual components of the Mediterranean diet are vegetables, fruits, and legumes, and olive oil as the main source of fat, and their consumption has been associated with lower incidence of coronary heart disease, primarily due to the diet's high content of monounsaturated fatty acids. However, virgin olive oil is also rich in phenolic compounds with strong antioxidant properties (*1*), such as secoiridoid aglycons and lignans (*2*, *3*). Hence, there has been an increasing interest in these substances during the past few years. Even though many researchers have studied polyphenols in raw and stored virgin olive oils (2, *4*), their influence on the stability and organoleptic properties of the oil (*5*, *6*), and nutritional benefits (*1*), scant data have been reported on the availability of these compounds after domestic heating of the oil (*7*, *8*). In addition, phenolic compounds have been determined colorimetrically which did not show the different predictable behavior of them, as happened during oil storage (*4*).

Virgin olive oil may be consumed raw in toasts, salads, and other foodstuffs, but it is also consumed after domestic heating, such as frying, boiling, and microwave heating.

Frying is one of the most popular methods for preparation of foods worldwide, and during this process hydrolytic, oxidative, and thermal reactions occur that lead to the formation of oxidized and polymerized products that could be undesirable from a nutritional point of view (*9*). There are numerous studies that report changes in olive oils after heating or frying procedures (*10*−*12*) and the effect of these processes on the antioxidant and nutritional properties of oil (*8*, *13*−*15*). Few recent studies have also shown a decrease in total polyphenols during frying (*8*, *15*, *16*). However, changes occurring in each individual phenolic compound during frying of virgin olive oil have never been quantified.

The effect of microwave heating on the thermoxidative degradation of olive oils has also been determined (*17*, *18*) and a reduction of 96% in total polyphenols has been reported (*7*), although the assays were prolonged too long and no characterization of individual polyphenols was performed.

Virgin olive oil is also commonly used in typical recipes of the Mediterranean diet by boiling a mixture of oil, water, and other foodstuffs in a pressure cooker for 30−60 min. Boiling vegetables in water gives rise to two phenomena: a diffusion

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of compounds from the food to the water phase and a total loss of nutritional components such as polyphenols (*19*−*22*). Tuna can be packed with virgin olive oil and a diffusion of polyphenols from the oil to the water−muscle interphase phase has been suggested (*23*), and simulated oil−brine canned experiments have also recently been performed (*24*).

The aim of this work was to study the effects of various heating methods on the polyphenols changes in virgin olive oils and their relationship to the degradation of oils. Virgin olive oils may differ widely in chemical, organoleptic, and nutritional properties which depend to a large extent on the olive cultivar. Thus, virgin olive oils of the Picual and Arbequina cultivars with different compositions of fatty acids were used for the experiments.

MATERIALS AND METHODS

Samples. Virgin olive oils of the Picual and Arbequina cultivars were obtained using dual-phase decanter centrifugation by industrial processors.

Microwave Heating of Bulk Oil. A domestic Moulinex microwave oven (model B945 GS, France), capable of generating 0.5 kW power at 2450 MHz, was used. Virgin olive oil (15 ± 0.1 g) was placed in a Petri dish (diameter 9.5 cm), covered with commercial plastic food wrap, and microwaved at the maximum power for 5 and 10 min, respectively. All assays were run in triplicate.

Simulated Frying. Thermal oxidation was carried out in triplicate under strictly controlled conditions using a Rancimat (Metrohm Lts., Herisau, Siwtzerland) apparatus. Briefly, 8 ± 0.1 g of virgin olive oils were weighed out in Rancimat vessels and inserted in the heating block previously heated at 180 ± 1 °C. Samples were heated for 1.5, 3, 5, 10, 15, 20, and 25 h. Rancimat instructions were carefully observed for temperature correction. No bubbling of air was applied during heating and the tubes were left open. This procedure simulated the discontinuous frying process as it has been described elsewhere (*25*).

Boiling of Water−Oil Mixtures. Distilled water (2.5 L) with the pH adjusted to 4, 5 (0.2 M acetic acid/sodium acetate buffer), and 6 (0.2 M phosphate buffer), and virgin olive oil (60 g), were put into a domestic pressure cooker (4-L capacity). The oil/water mixture was heated in a domestic electric heater for 10 min until a temperature of 109 ± 1 °C was reached and then maintained for 30 min. This temperature was monitored with a thermistor introduced into the pressure cooker (Datatrace, Lakewood, CO). After the mixture was boiled, the pressure cooker was cooled at ambient temperature and opened. The oil and water phases were separated in a graduated cylinder. Their volumes were recorded because steam was released from the pressure cooker during boiling. All assays were run in triplicate.

Tocopherol Determination. A solution of oil in hexane was analyzed by HPLC on a silica gel column (Merck, Superspher Si 60, particle size 4 μm, 250 mm × 4 mm i.d.), eluting with hexane/2-propanol 99.3:0.7 at a flow rate of 1 mL/min. A fluorescence detector (Jasco 821-FP) with excitation wavelength at 290 nm and emission wavelength at 330 nm was used (*25*).

Fatty Acids Composition. Fatty acids were analyzed by GLC after derivatization to fatty acid methyl esters with KOH 2 N in methanol, according to the IUPAC standard method (*26*). A HP 6890 chromatograph on a HP Innowax capillary column (poly(ethylene glycol), 30 m × 0.25 mm i.d., film thickness 0.25 μm) (Hewlett-Packard, Palo Alto, CA) was under the following temperature program: 180 °C (4 min), and 4 °C/min up to 230 °C (15 min). Samples were introduced to the column via a split injector (split ratio 1:40) at 250 °C, and the flow rate of hydrogen, used as carrier gas, was 1 mL/min. Temperature of both split injector and flame ionization detector was 250 °C.

Induction Time. Stability was evaluated by measuring the oxidation induction time, with the use of a Methrohm 679 Rancimat apparatus (Methrohm S. A., Herisau, Switzerland). A flow of air (15 L/h) was bubbled through the oil heated at 110 °C, and the volatile compounds were collected in cold water, increasing the water conductivity (*27*).

Total Polar Compounds Content. Total polar compounds were determined in oil samples by silica column chromatography, following the method proposed by the IUPAC (*26*). Distribution of polar compounds was performed by high-performance size-exclusion chromotography (HPSEC). Conditions applied were as follows: a Rheodyne 7725i injector with a 10-μL sample loop and a Waters 510 HPLC pump (Waters Inc., Milford, MA), two 100 and 500 Å Ultrastyragel columns (Waters), 25 cm × 0.77 cm i.d., packed with a porous, highly cross-linked styrenedivinylbenzene copolymer (<10 μm), connected in series, and a refractive index detector (Hewlett-Packard). HPLC grade tetrahydrofuran served as the mobile phase with a flow of 1 mL/min, and the sample concentration was between 15 and 20 mg/mL in tetrahydrofuran (*28*).

HPLC Analysis of Phenolic Compounds. The phenolic extracts of olive oil were obtained following the procedure described elsewhere (*29*). 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 a 0.45-μm pore size filter and injected into the HPLC chromatograph. Phenolic compounds were also analyzed in the aqueous phase of the boiling assays. Aqueous samples were only filtered through a 0.45-μm filter, and 40 μL was injected directly into the chromatograph. The HPLC system consisted of a Waters 717 plus autosampler, a Waters 600E pump, and a Waters column heater module. A Spherisorb ODS-2 (5 μm, 25 cm × 4.6 mm i.d., Technokroma, Barcelona, Spain) column was used. Separation was achieved with an elution gradient by using an initial composition of 90% water (pH adjusted to 3.1 with 0.2% of acetic acid) and 10% methanol. The concentration of the latter solvent was increased to 30% in 10 min and maintained for 20 min. Subsequently, the methanol percentage was raised to 40% in 10 min, maintained for 5 min, and then increased to 50% in 5 min, and maintained for another 5 min. Finally, the methanol percentage was increased to 60, 70, and 100% in 5-min periods. Initial conditions were reached in 15 min. Phenolic compounds were analyzed by fluorescence detection (Jasco FP-920 fluorescence detector, Jasco Corp., Tokyo, Japan). An excitation wavelength of 280 nm and an emission wavelength of 320 nm were used. Phenolic compounds were quantified by using the reference compounds from commercial suppliers of semipreparative HPLC as described elsewhere (*3*).

HPLC−MS was also used to confirm the assignment of phenolic compounds to peak chromatograms. A 600E pump and a ZMD4 mass spectrometer (Waters) equipped with an electrospray ionization ion source (ESI) were used. The ionspray mass spectra in the negative-ion mode were obtained under the following conditions: capillary voltage, 3 kV; cone voltage, 10 V; extractor voltage 12 V; desolvation temperature, 250 °C; and source temperature, 120 °C.

RESULTS AND DISCUSSION

The HPLC analysis of virgin olive oils heated at 180 °C revealed a decrease of the phenolic compounds with heating time, but this decline depended on each compound. Hydroxytyrosol (Hy) and compounds containing this molecule, such as 4-(acetoxyethyl)-1,2-dihydroxybenzene (Hy-AC), dialdehydic form of elenolic acid linked to hydroxytyrosol (Hy-EDA), and oleuropein aglycon (Hy-EA), rapidly decreased their content in oil with heating time (**Figure 1**), although this behavior was more pronounced for Arbequina than for Picual oil. The reduction of orthodiphenols in oils during heating can be attributed to thermal destruction or oxidative degradation because of their contribution to oil stability. These compounds are lost during oil storage at ambient temperature and contribute to oil stability (*4*, *5*). In contrast, tyrosol (Ty) and tyrosol-like species, such as the dialdehydic form of elenolic acid linked to tyrosol (Ty-EDA) and ligustroside aglycon (Ty-EA), do not participate in oil stability, but they also decreased in concentration with heating time (**Figure 2**). The strange behavior of Ty-EDA must also be noted. Apparently, the content of this compound increased significantly in oils during the first 5 h of heating. However, the phenolic extracts were also analyzed by

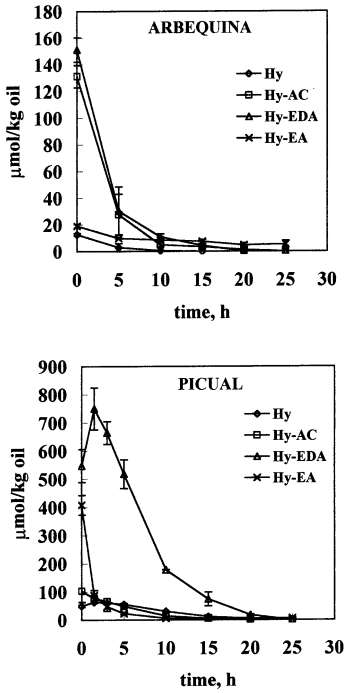

Figure 1. Changes in hydroxytyrosol (Hy), 4-(acetoxyethyl)-1,2-dihydroxybenzene (Hy-AC), dialdehydic form of elenolic acid linked to hydroxytyrosol (Hy-EDA), and oleuropein aglycon (Hy-EA) in virgin olive oils of the Arbequina and Picual cultivars during heating at 180 °C. Values are the means ± standard deviation of 3 replicate analyses.

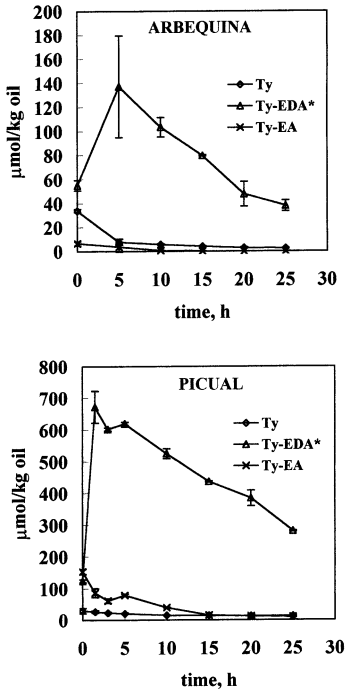

Figure 2. Changes in tyrosol (Ty), mixture of dialdehydic form of elenolic acid linked to tyrosol (Ty-EDA) and Ty-EDA-oxidized, and ligustroside aglycon (Ty-EA) in virgin olive oils of Arbequina and Picual cultivars during heating at 180 °C. Values are the means ± standard deviation of 3 replicate analyses.

HPLC−MS, and the peak corresponding to Ty-EDA was, in fact, a mixture of two compounds coeluting: Ty-EDA and a new compound with molecular mass of 320 amu. We isolated the mixture and hydrolyzed it with HCl, and tyrosol was the only simple phenolic compound liberated. Recent research has found in stored olive oils a new compound that coelutes with Ty-EDA and 320 amu (*30*), which has been characterized by HPLC−MS as Ty-EDA-oxidized. Thus, it seems that the peak formed during heating, and coeluting with Ty-EDA, must be Ty-EDA-oxidized.

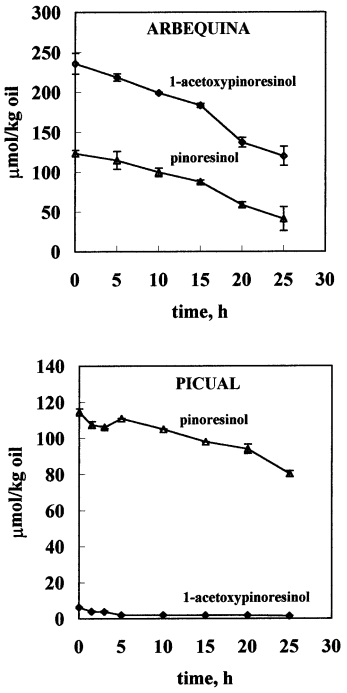

Figure 3. Changes in 1-acetoxypinoresinol and pinoresinol in virgin olive oils of the Arbequina and Picual cultivars during heating at 180 °C. Values are the means ± standard deviation of 3 replicate analyses.

Minor losses were found for the recently identified lignans 1-acetoxypinoresinol and pinoresinol (*3*) during heating of both Arbequina and Picual oils (**Figure 3**). Arbequina oil heated for 25 h surpassed the limit of polar compounds (25%) established in most official regulations for discarding frying fats and about 50% of lignans still remained in oil, whereas other phenolic compounds were not detected at this time (**Table 1**). Lignans are also the two phenolic compounds that remained unchanged during storage of olive oil for one year (*4*). This is of great importance because in recent years much attention has been paid to the beneficial effects of these phytoestrogen substances (*31*).

Overall, there was a drastic decrease in olive oil polyphenols during simulated frying but the disappearance rate depended on each individual compound and the olive cultivar. Heating Picual oils resulted in minor loss of polyphenols compared to that in Arbequina oils. This effect could be related to the lower amount of polyphenols in Arbequina oil than in Picual and, therefore, to a lower stability of the former oil. In fact, the oxidative stability (induction time) of Picual oil was higher than that of Arbequina oil (**Table 1**). However, the differences in fatty acids of the two oils could be another important reason for this different behavior, as it is well-known that thermal degradation is closely related to the unsaturation of fatty acids (*10*). Arbequina oil had 66% and 12% of oleic and linoleic acid, respectively, and Picual oil had 80% and 4%. Thus, it has been reported that Arbequina oils showed lower oxidative stability than Picual, even though Arbequina oil contains a higher amount of polyphenols than Picual (*32*). The analysis of the glyceridic fraction of each oil also demonstrated the higher degradation rate of Arbequina oil compared to that of Picual oil. The limit of 25% in polar compounds was surpassed for Arbequina oil after 25 h of heating, whereas Picual oil did not reach this limit.

Table 1. Changes in Physicochemical Characteristics of Virgin Olive Oils Heated at 180 °C

	induction time (h)	α-tocopherol (mg/kg)	polar compounds (%)	polymers (%)	oxidized triglycerides (%)	diglycerides (%)	free fatty acids (%)
			Picual Oil				
0 h	47.7 (1.0)[a]	172 (2)	2.5 (0.2)	tr[b]	0.4 (0.1)	1.5 (0.1)	0.5 (0.1)
5 h	42.7 (0.8)	111 (5)	4.8 (0.1)	1.3 (0.1)	1.6 (0.1)	1.5 (0.1)	0.5 (0.1)
10 h	35.9 (0.5)	72 (20)	7.0 (0.2)	2.3 (0.1)	2.6 (0.1)	1.6 (0.1)	0.5 (0.1)
15 h	27.0 (2.3)	39 (19)	8.9 (0.4)	6.2 (3.2)	3.5 (0.2)	1.6 (0.1)	0.5 (0.1)
20 h	16.7 (4.7)	13 (7)	10.5 (0.6)	4.3 (0.3)	4.1 (0.4)	1.6 (0.1)	0.5 (0.1)
25 h	6.9 (1.3)	tr	14.8 (0.6)	6.5 (0.3)	6.2 (0.3)	1.7 (0.1)	0.4 (0.1)
			Arbequina Oil				
0 h	11.7 (0.1)	141 (2)	3.5 (0.2)	tr	0.8 (0.1)	2.1 (0.1)	0.5 (0.1)
5 h	5.5 (1.1)	38 (28)	8.4 (2.5)	3.2 (1.3)	2.9 (1.3)	2.2 (0.1)	0.4 (0.1)
10 h	3.1 (0.8)	3 (1)	12.3 (1.7)	5.2 (0.8)	4.5 (0.8)	3.2 (0.1)	0.4 (0.1)
15 h	1.6 (0.1)	tr	16.6 (0.8)	7.8 (0.4)	6.1 (0.3)	2.3 (0.1)	0.4 (0.1)
20 h	1.5 (0.1)	tr	24.2 (1.9)	12.4 (1.4)	9.1 (0.6)	2.2 (0.1)	0.5 (0.1)
25 h	1.4 (0.1)	tr	28.1 (0.8)	14.9 (0.2)	10.6 (0.3)	2.3 (0.2)	0.4 (0.1)

[a] Mean and standard deviation ($n = 3$). [b] Traces.

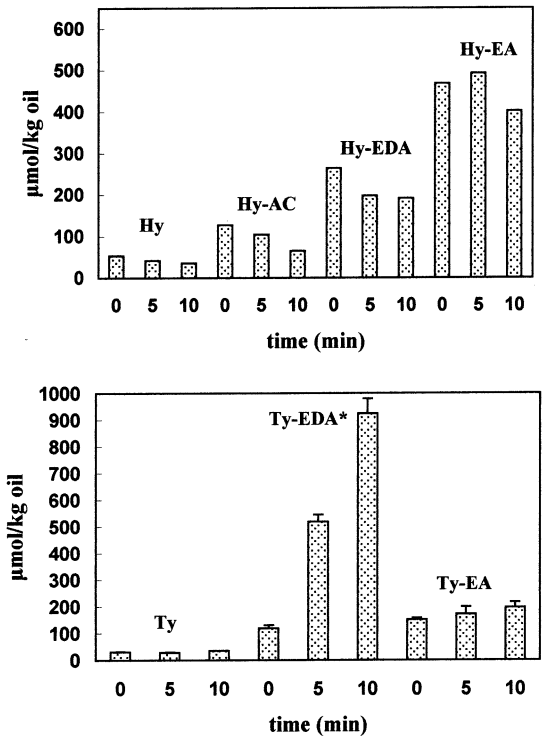

Figure 4. Effect of microwave heating on the polyphenols content of virgin olive oil of the Picual cultivar. Values are the means ± standard deviation of 3 replicate analyses. (More details are provided in captions of **Figures 1** and **2**).

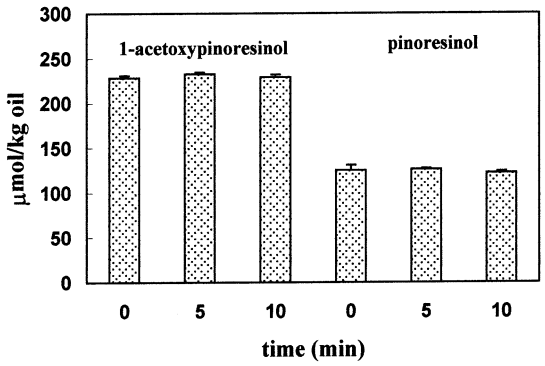

Figure 5. Effect of microwave heating on the lignans content of virgin olive oil of the Arbequina cultivar. Values are the means ± standard deviation of 3 replicate analyses.

Increase in polymers and oxidized triglycerides was also higher for Arbequina than Picual oil.

As expected from previous works (*8*, *15*), the concentration of α-tocopherol, the predominant tocopherol in olive oil, also decreased rapidly in oil with heating time, with this disappearance rate being more pronounced for Arbequina oil than Picual oil. However, as can be observed in **Table 1**, α-tocopherol was exhausted for similar levels of polar compounds.

Microwave heating provoked only minor changes to oil polyphenols. Hydroxytyrosol, its oleosidic forms (Hy-EDA and Hy-EA), and Hy-AC slightly diminished their concentration (20−30%) in oils of both Picual and Arbequina cultivars after microwave heating for 5 and 10 min (**Figure 4**). In contrast, Ty-EDA increased, again, significantly with heating time, but, as it has been mentioned for frying, this was because the new compound coeluted with Ty-EDA. Thus, both frying and microwave heating gave rise to the formation of Ty-EDA-oxidized, which in the case of the microwave heating did not disappear after heating for 10 min. It has been reported that most of the phenolic compounds determined colorimetrically were lost when olive oils were microwave heated for 120 min (*7*); but this condition does not represent habitual domestic conditions, and, therefore, from our results, microwave heating may be considered a less degradative heating process than frying, which is also in agreement with reports on heating onions (*21*). Lignans did not change after 10 min of microwave heating (**Figure 5**), which confirmed the high stability of these substances to microwave heating (*33*).

In contrast, α-tocopherol was lost in both Arbequina and Picual oils in a very high amount (**Table 2**), which is in agreement with findings of a previous work (*18*). Again, the loss was higher for Arbequina (80%) than for Picual oil (60%). It has been postulated that polyphenols are stabilizers of α-tocopherol during olive oil heating (*8*), and differences in polyphenols could explain the different behavior of α-tocopherol, although the different content in fatty acids must also be considered.

As happened with polyphenols, oil degradation was very low when the oils were heated by microwave (**Table 2**). Even for the less stable Arbequina oil, polar compounds increased only up to 6% after heating for 10 min.

Changes in polyphenols during boiling are presented in **Figures 6** and **7**. To better understand the overall losses of these compounds, we expressed the content of each one as the total amount (μmol) in both the oil and the water phase. First, it has

Table 2. Effect of Microwave Heating on Physicochemical Characteristics of Virgin Olive Oils

	induction time (h)	α-tocopherol (mg/kg)	polar compounds (%)	polymers (%)	oxidized triglycerides (%)	diglycerides (%)	free fatty acids (%)
			Picual				
0 h	47.7 (1.0)[a]	172 (2)	2.5 (0.2)	tr[b]	0.4 (0.1)	1.5 (0.1)	0.5 (0.1)
5 min	39.9 (2.6)	127 (2)	3.1 (0.2)	tr	1.0 (0.1)	1.6 (0.1)	0.6 (0.1)
10 min	38.2 (1.4)	65 (9)	3.8 (0.2)	0.5 (0.1)	1.3 (0.1)	1.5 (0.1)	0.6 (0.1)
			Arbequina				
0 h	11.7 (0.1)	141 (2)	3.5 (0.2)	tr	0.8 (0.1)	2.1 (0.1)	0.5 (0.1)
5 min	9.6 (0.1)	92 (25)	3.8 (0.4)	tr	1.0 (0.2)	2.0 (0.1)	0.6 (0.1)
10 min	6.0 (0.4)	27 (3)	6.0 (0.4)	1.2 (0.2)	2.0 (0.1)	2.2 (0.1)	0.7 (0.1)

[a] Mean and standard deviation ($n = 3$). [b] Traces.

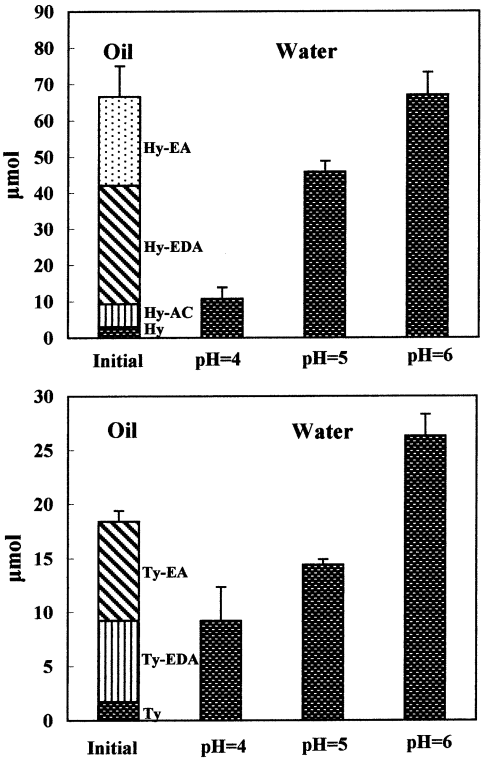

Figure 6. Effect of the pH on the contents of olive oil polyphenols (Hy, Hy-AC, Hy-EDA, Hy-EA, Ty, Ty-EDA, and Ty-EA) in the water phase of an oil/water mixture heated in a pressure cooker for 30 min. Phenolic compounds in nonheated oil are also shown, and they were not detected in heated oil. Virgin olive oil was of the Picual cultivar. Values are the means ± standard deviation of 3 replicate analyses. Total amount of each phenolic compound in nonheated oil and heated water was displayed in order to better present the hydrolysis and diffusion processes.

to be noted that neither simple nor combined hydroxytyrosol or tyrosol were detected in the oil phase after boiling, which did not occurr when a mixture of oil/brine was heated to simulate fish canning (*24*); although in the latter case the ratio oil/water was much higher than that in our experiments. Second, hydroxytyrosol and tyrosol aglycons were completely hydrolyzed at the pH interval studied, and the hydrophilic free hydroxytyrosol and tyrosol were completely diffused from the oil to the water phase (**Figure 6**). The pH also had a marked effect on the final amount of hydroxytyrosol and tyrosol in water. The lower the pH that was used, the higher the loss in polyphenols that was found. It is noteworthy that boiling vegetables always gave rise to an overall loss of polyphenols (*19*, *22*, *34*) even when performing the assays at pH 7 (*22*). However, in our experiments the total amount of hydroxytyrosol and tyrosol (sum of free and combined) remained constant after

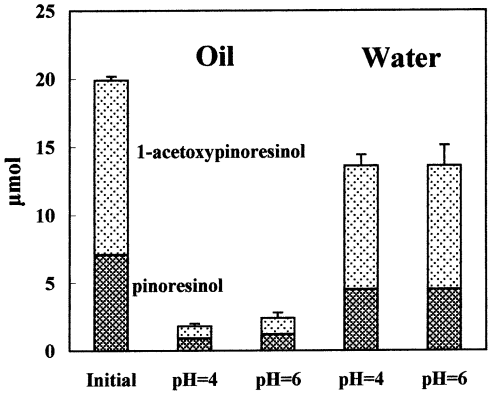

Figure 7. Effect of pH on the contents of olive oil lignans in the water and oil phase of a boiled oil/water mixture in a pressure cooker for 30 min. Virgin olive oil was of the Arbequina cultivar. Values are the means ± standard deviation of 3 replicate analyses.

boiling at pH 6. The total amount of tyrosol was higher in the heated water phase than in nonheated oil, which could be due to nonquantification of tyrosol-like compounds such as tyrosol acetate (*30*). Likewise, the steam generated during boiling was analyzed and polyphenols were not detected in it; as the evaporation rate of this compounds is higher at pH 4 than at pH 6, pH was, therefore, ruled out as the cause of their losses at this pH (data not shown). We chose the interval of pH 4−6 because most vegetables and cooked food have their pH in this zone. Boiling onions at a very acid pH, lower than 2, caused a hydrolysis of the quercetin glycosides but not a decrease in the total amount of flavonoids (*21*). Also, it has been reported that phenolic compounds were more stable during boiling if present in the vegetable tissue than in the water phase (*19*). In our experiments, however, the pH of water was the critical factor for the degradation of the phenolic compounds. In contrast, pH did not influence lignan degradation (**Figure 7**). As lignans are more lypophilic compounds than are hydroxytyrosol and tyrosol, a certain amount of 1-acetoxypinoresinol and pinoresinol still remained in oil after boiling, although a high proportion diffused to the water phase. A reduction of 30% was observed in the total amount irrespective of the pH.

Our data confirm that the hydrolysis of the secoiridoid aglycons to give simple phenols occurs during thermal processing of an oil/water mixture, and a migration of hydrophilic polyphenols from the oil to the water phase took place (*24*). Moreover, a degradation of these compounds at pH 4 and 5 also occurred. In contrast, concentration of α-tocopherol did not change in Arbequina nor in Picual oils after boiling with water at any of the pH levels assayed. As for α-tocopherol, changes in the glyceridic fraction of oils were not observed (data not shown).

To our knowledge, this is the first report showing that olive oil is not oxidized when boiling with water in a pressure cooker. Moreover, it must be stressed also that after boiling most of the phenolic compounds were found as simple phenols in the water phase, and human absorption of these substances could be influenced positively (*35*).

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