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Effect of Biophenols on Olive Oil Stability Evaluated by Thermogravimetric Analysis

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Virgin olive oil stability to autoxidation is mainly due to phenolic compounds naturally occurring in it, but contrasting data have been published on the effectiveness of the same antioxidant compounds. With thermogravimetric analysis (TGA) it is possible to have an estimation of oil resistance to oxidation, having a measure of weight gain percent due to reaction of sample with oxygen during the oxidation, and of initial and final oxidation temperatures. The following samples were examined: virgin olive oil, olive oil, and olive oil spiked with different amounts of some antioxidants. Tested phenols were p-HPEA, 3,4-DHPEA, 3,4-DHPEA-EA, caffeic acid, oleuropein, and, moreover, BHT and BHA. Data showed that natural antioxidant addition (especially oleuropein, 3,4-DHPEA, and 3,4-DHPEA-EA) could extend the olive oil shelf life and could protect oil from decomposition naturally occurring during thermal treatments (such as cooking process).

Keywords: *Biophenols; olive oil stability; lipid autoxidation; thermogravimetric analysis*

INTRODUCTION

Food products undergo a chain of changes in the natural matrix due to ripening, harvesting, primary processing, and storage. These changes are caused by several factors including browning reactions, microbial spoilage, and lipid autoxidation. Of the various factors, lipid autoxidation contributes significantly to the deterioration and reduction of the shelf life of many products. Lipid oxidation is a free-radical chain reaction that causes a total change in the sensory properties and nutritive value of food products. Changes in color, texture, odor, and flavor; loss of vitamins; and damage to proteins are some of the effects of lipid oxidation. The beginning of lipid oxidation can be delayed by the addition of antioxidants. Antioxidants are a group of chemicals effective in extending the shelf life of a wide variety of food products. Naturally occurring antioxidants impart a certain amount of protection against oxidation but they are often lost during processing or storage, necessitating the addition of exogenous antioxidants.

Virgin olive oil stability to autoxidation is mainly due to phenolic compounds naturally occurring in it or arising from the glycated precursors present in the olive fruit before extraction. The stability to oxidation has been correlated to the total amount of phenolic components as well as to the *o*-diphenols and to selected simple phenol components (Papadopoulos and Boskou, 1991; Tsimidou et al., 1992; Satue et al., 1995; Zunin et al., 1995). Complex phenols make up a part of the so-called "polar fraction" of virgin olive oil and little is known about the contribution of each single component to the stability of the oil (Papadopoulos and Boskou, 1991).

Contrasting data have been published on the effectiveness of the same antioxidant compound depending on the various conditions and methods used to

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evaluate the oxidation rate (Frankel, 1993; Frankel et al., 1994; Huang et al., 1996). Valid comparison of antioxidant activity depends on condition of oxidation and on which analytical method is used to determine the extent and endpoint of oxidation (Frankel et al., 1994). The aim of this study was to measure protection factors of some phenols, known to be present in virgin olive oil at higher concentrations, and to evaluate their single contribution to its stability using a method that employs a thermogravimetric balance; in this system changes in sample weight are monitored continuously while the sample is temperature programmed in a oxygen environment. The technique basically reproduces the well-known procedure of heating an oil in an oven and weighing it at intervals, but weight changes can be permanently recorded with this technique. It was proven that with thermogravimetric methods it is possible to have an estimation of oil resistance to oxidation, having a measure of weight gain percent due to oxidation, and an estimation of initial and final oxidation temperatures (Nieschlag et al., 1974).

Tested phenols were selected from among those present at higher concentration in virgin olive oil: caffeic acid (3,4-dihydroxycinnamic acid), *p*-HPEA (*p*-hydroxyphenylethanol), 3,4-DHPEA (3,4-dihydroxyphenylethanol), and 3,4-DHPEA-EA (elenolic acid linked to 3,4-dihydroxyphenylethanol, an isomer of oleuropein aglycon) (Papadopoulos and Boskou, 1991; Montedoro et al., 1992, 1993; Angerosa et al., 1995; Baldioli et al., 1996; Servili et al., 1996). Oleuropein, which is not very abundant in olive oil, was also tested because of its known high concentration in olive fruits and leaves and because it is the progenitor of some oil phenols such as 3,4-DHPEA and 3,4-DHPEA-EA (Gariboldi et al., 1986; Le Tutour and Guedon, 1992; Tranter et al., 1993; Visioli and Galli, 1994; Cortesi et al., 1995; Limiroli et al., 1995; Marsilio et al., 1996). In addition, two of the synthetic antioxidants used extensively in food industry, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), were analyzed, although in recent years there has been an enormous demand for natural antioxidant mainly because of adverse toxicological effects in various species of many widely used synthetic compounds (Aruoma et al., 1993; Madhavi and Salunkhe, 1996). In this context, it is worth mentioning that the Joint FAO/WHO Expert Committee on Food Additives (JECFA), on the basis of new toxicological evaluations, has reset the acceptable daily intake (ADI) of BHT (WHO, 1991) to lower values.

EXPERIMENTAL PROCEDURES

Samples. The following specimens were examined (i) olive oil and extra virgin olive oil, named respectively OO and OEVO (EEC, 1991), and (ii) OO spiked with different amounts of caffeic acid, oleuropein, *p*-HPEA, 3,4-DHPEA, 3,4-DHPEA-EA, BHA, and BHT. To reduce other interferences, each product was added separately to an OO known to have a very low phenol content.

Standard Materials. The *p*-HPEA was from Acros Organics N.V. (Geel, Belgium); caffeic acid, BHA, and BHT were purchased from Sigma (St. Louis, MO); oleuropein was from Extrasyntese (Genay, France). The 3,4-DHPEA and 3,4-DHPEA-EA were both synthesized in laboratory by means of acid hydrolysis and enzymatic hydrolysis of oleuropein, respectively (Walter et al., 1973). The β-glucosidase necessary for enzymatic hydrolysis was obtained from Sigma (St. Louis, MO).

Sample Preparation. Weighted quantities of each standard were dissolved in a 1:9 (v/v) mixture of methanol/chloroform. Small quantities of these solutions were added singularly to 1 mL of OO, to obtain the desired final concentrations, and the sample placed under a nitrogen flow for 30 min so as to remove the majority part of organic solvent carrier. Any residual trace evaporates during the initial phase of instrumental analysis. The choice of starting concentration was made on the basis of the average natural content of each phenol in extra virgin olive oil (Baldioli et al., 1996), and increasing or decreasing it to find the maximum experimental value of antioxidant activity.

Thermogravimetric Analysis. A thermobalance (TGA 7, Perkin-Elmer) coupled with a thermal analysis controller (TAC 7/DX, Perkin-Elmer) was employed. The instrument was calibrated with "alumel" alloy and nickel for temperature settings and with a 100-mg standard for weight accuracy. Approximately 3 mg of each sample were added to a tared aluminum balance pan. The pan was then placed in the room temperature furnace, and the exact sample weight was determined. The temperature was then increased to 70 °C at the rate of 10 °C min^{-1}, and the sample was held at this temperature for 15 min so as to remove any residual trace of solvent carrier. Thereafter, the sample was heated until 250 °C at the rate of 2 °C min^{-1}. To establish an environment suitable for the oxidation process, O_2 was chosen as sample purge gas (flow of 50 cm^3 min^{-1}), whereas N_2 was used as balance purge gas at the flow of 75 cm^3 min^{-1}. Sample weight variation was recorded on a plotter (ColorPro, Hewlett-Packard).

Statistical Analysis. To evaluate if the results relative to OO, OEVO, and OO spiked with biophenols were significantly different, analysis of variance (ANOVA test) was applied. Data processing was performed by means of Statgraphics software package (v. 7 for DOS, Manugistic).

RESULTS AND DISCUSSION

A typical thermogravimetric curve for olive oil is shown in Figure 1. The line AB indicates loss of solvent, if present, when the sample is held isothermally at 70 °C. Point B corresponds to the start of the temperature program. The line from B to T_i represents the static condition of oil resistance to oxidation when temperature is increasing. T_i is the "initiation temperature"; this is the temperature at which the rate of oxidation increases fast, as shown by a weight gain. T_f is the "final temperature", the point of maximum gain in sample weight (Δ). As temperature increases beyond T_f, the sample continually loses weight until the recorder pen is no longer on scale, and the run is finished. The ratio [(weight at T_f − weight at T_i)/(weight at T_i)] × 100 represents the sample weight gain percent (Δ %).

The results of thermogravimetric analysis for all samples taken into consideration in this study are shown in Table 1. Each mean value is accompanied by its standard deviation (SD). The values of these last prove that the precision of the method is more than satisfactory both for what concerns the weight gain percent and the temperatures (T_i and T_f). In the same table the significance level values (p) obtained by ANOVA testing are also indicated. All the comparisons have been made versus OO.

An assessment of data obtained so far makes it possible to point out some interesting outcomes. With regard to weight gain percent, the first consideration is related to the difference between OEVO and OO. This may be ascribed to the fact that the process of OO manufacture causes a more or less pronounced decrease in the phenols' concentration. In fact, the phenol content of OEVO, measured according to the method of Vázquez Roncero et al., (1973) was found to be 139.5 mg kg^{-1}, while for OO, it was 47.2 mg kg^{-1}.

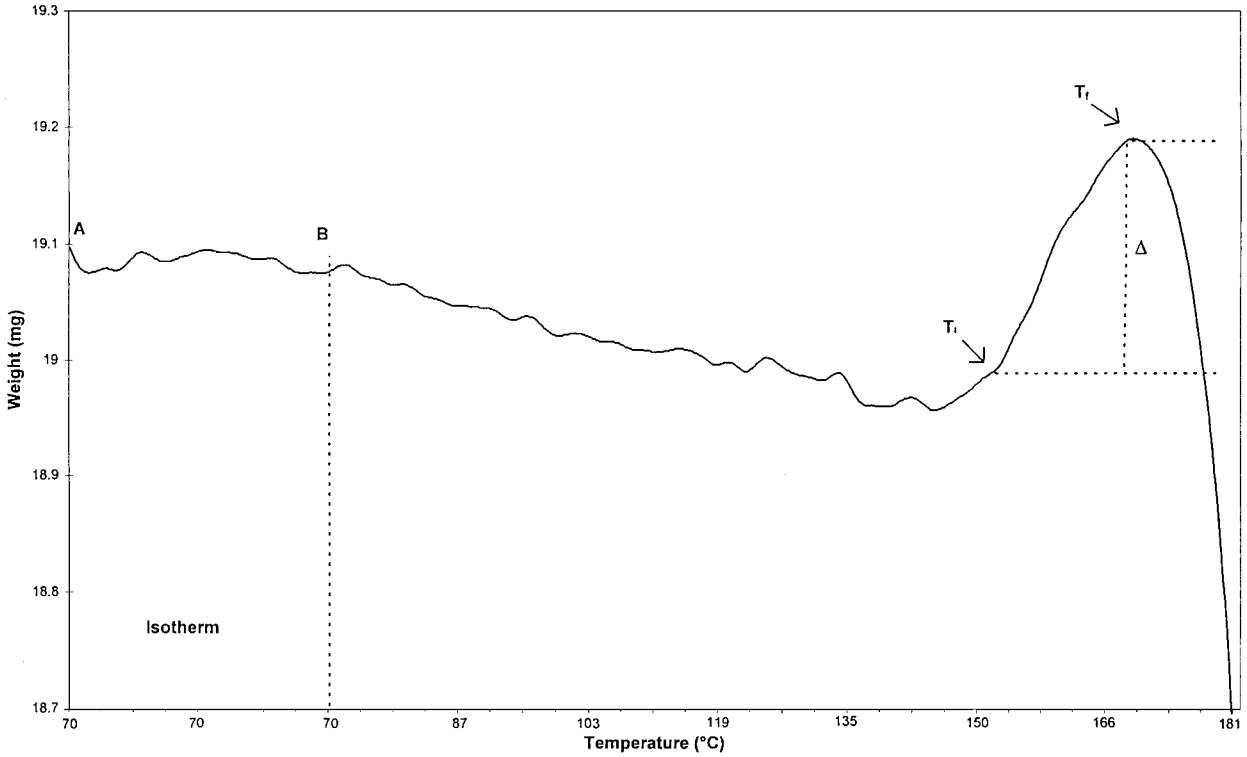

Figure 1. Typical thermogravimetric curve for an olive oil sample. See text for description.

Table 1. Figures of Merit for the Antioxidant Activity of Biophenols[a]

sample	concentration of addition (mg kg^{-1})	T_i[b] (°C) ± SD	T_f[b] (°C) ± SD	Δ %[b] ± SD
olive oil (OO)		152.1 ± 1.6	169.4 ± 1.5	0.22 ± 0.02
extra virgin olive oil		161.2 ± 1.8***	173.0 ± 1.4*	0.12 ± 0.03***
OO + 3,4-DHPEA-EA	100	151.3 ± 1.0	167.1 ± 1.4	0.20 ± 0.02
	200	157.2 ± 0.4**	169.4 ± 1.4	0.13 ± 0.03***
	300	151.1 ± 0.9	164.9 ± 1.7	0.16 ± 0.02**
OO + oleuropein	10	154.4 ± 1.5	169.4 ± 1.5	0.21 ± 0.02
	50	154.9 ± 1.5	166.0 ± 1.4	0.16 ± 0.03*
	100	160.5 ± 1.9***	171.0 ± 1.6	0.13 ± 0.02***
OO + 3,4-DHPEA	5	149.1 ± 1.8	167.4 ± 1.6	0.23 ± 0.01
	50	153.7 ± 0.5	169.5 ± 1.1	0.19 ± 0.02*
	100	160.1 ± 0.6***	174.5 ± 1.2*	0.16 ± 0.02**
OO + *p*-HPEA	10	148.1 ± 1.0	166.5 ± 1.5	0.23 ± 0.02
	50	149.7 ± 1.4	168.1 ± 1.2	0.26 ± 0.02
	100	143.9 ± 1.5	166.6 ± 1.8	0.30 ± 0.04
	500	149.2 ± 1.5	167.1 ± 0.8	0.28 ± 0.03
OO + caffeic acid	2	153.2 ± 0.7	170.2 ± 0.6	0.24 ± 0.02
	5	155.0 ± 0.5	170.2 ± 0.9	0.21 ± 0.02
	10	156.2 ± 0.4*	172.8 ± 1.0	0.22 ± 0.03
	50	160.5 ± 1.8***	174.6 ± 0.9*	0.20 ± 0.02
OO + BHT	100	158.1 ± 0.1**	175.0 ± 0.5*	0.19 ± 0.02
	200	160.2 ± 1.2***	176.3 ± 1.4**	0.23 ± 0.01
	300	158.0 ± 0.8**	172.3 ± 1.2	0.22 ± 0.02
	400	157.6 ± 1.3*	174.9 ± 1.7*	0.25 ± 0.01
OO + BHA	100	157.6 ± 0.5*	174.5 ± 0.6*	0.22 ± 0.03
	200	159.6 ± 0.6**	174.3 ± 0.7*	0.20 ± 0.02
	300	162.9 ± 1.0***	174.7 ± 0.5*	0.14 ± 0.02***

[a] (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$, compared to OO. [b] Each value of T_i, T_f and Δ % is the mean of 10 determinations.

Meticulous examination of all data relating to the OO samples spiked with phenols confirms that some phenols considerably influence oil stability. In particular, oleuropein, 3,4-DHPEA, and 3,4-DHPEA-EA addition strongly decreases the weight gain percent of OO and this means that lipid autoxidation process is reduced. It is interesting to note that the addition of only one of above-noted phenols attain the same antioxidant effect of the phenol mixture present in extra virgin olive oil.

The same considerations can be made for T_i and T_f since as their shift toward higher temperatures is correlated to weight gain percent decrease. Also in this case the greatest variations have been observed for oleuropein, 3,4-DHPEA, and 3,4-DHPEA-EA addition, but caffeic acid, BHT, and BHA have also shown higher T_i values. This rise of the temperature of oxidation starting point is of particular significance since the temperature, as is well-known, is one of the autoxidation lipid catalysts. The phenomenon is also particularly interesting from a technological point of view because it shows that the addition of phenols, in addition to extending the olive oil shelf life, could limit the extent of oil decomposition during cooking processes.

It is also worth mentioning that while the majority

of the tested phenols reached the minimum weight gain percent by giving rise to a plateau, 3,4-DHPEA-EA and BHT display a trend inversion when their concentration rise. In fact, it has been noted, relative to the concentrations tested in this study, that the above-noted two antioxidant compounds not only lost their protective action but also became weakly proxidants at higher concentrations. A possible explanation for this apparent discrepancy is that phenols have a multifunctional activity and can act as reducing agents, hydrogen donating antioxidant, and singlet oxygen quenchers. Furthermore, some of them also have the possibility of chelating metal ions and preventing iron- and copper-catalyzed formation of initiating radical species. Probably, the presence of small quantities of peroxyl radicals alter the relative weight of different action mechanisms so as to facilitate phenol autoxidation, especially in the presence of metal ions.

Finally, contrary to what was expected compounds considered as having high antioxidant activity, i.e. caffeic acid and BHT, have not been able to reduce lipid oxidation process in significant manner. In fact, these two antioxidants cause, as stated above, only an increase of T_i values, whereas Δ % values, which represent an index of lipid autoxidation extent, remain unchanged. No easy explanation can be given for this behavior, but it must be stated that the methodological approach adopted in this study differs completely from those applied in previous investigations that, in the majority of cases, were not be able to monitor the oxidation process in continuous manner. Thus, the difficulty, restricted to caffeic acid and BHT, lies in fully comprehending results, not their reliability and validity. On the other hand, with regard to BHT some authors have put in evidence that its antioxidant activity is limited by steric hindrance and, only, in the presence of tocopherols at high concentration does it effectively act (Boguth et al., 1970). It follows therefore that BHT activity will be greater in seed oil than in olive oil, the last being poor in tocopherols.

In conclusion, the experimental evidence gained in this work sheds further light on the antioxidant activity of the main phenols naturally present in virgin olive oil. This constitutes a notable starting point for sound and effective action in replacing artificial antioxidant additives with natural compounds so as to enhance the safety and quality of foodstuffs. With regard to this point of view, it must be considered that large quantities of biophenols are, also, present in olive oil mill wastewaters (WWs), which is the byproduct of olive oil production and is currently discarded at high running expenses (Ragazzi and Veronese, 1973; Camurati and Fedeli, 1982; Servili and Montedoro, 1989; Lo Scalzo and Scarpati, 1993; Visioli et al., 1995). The WWs disposal is a problem in olive oil producing countries, mainly due their toxicity; therefore, the possibility of finding methods to recycle WWs could be very interesting for two reasons. The first concerns the prospect of detoxifying a dangerous and polluting byproduct. The second one is related to the fact that WW recycling could simultaneously be a profitable operation if carried out to obtain natural antioxidants.

Finally, the experimental approach and analytical method developed in this study appear adequate for the purpose, and compared to older techniques as, for example, the AOM or the oxygen bomb method (Gearhart et al., 1957; Olcott and Einset, 1958a,b; Dahle et al., 1962) offers the advantages of (i) shorter analysis time (about 80 min); (ii) smaller sample amount (approximately 3 mg); (iii) good precision; and (iv) the possibility of following, in a continuous manner, the oxidation process.

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