Antioxidant Effect of Natural Phenols on Olive Oil

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The total polar fraction and individual phenols present in virgin olive oil were tested for their antioxidant effect in refined olive oil. Hydroxytyrosol and caffeic acid showed protection factors greater than BHT. Protocatechuic and syringic acid were also found to have antioxidant activity. Tyrosol, p-hydroxyphenylacetic acid, o-coumaric acid, p-coumaric acid, p-hydroxybenzoic acid and vanillic acid had very little or no effect, and their contribution to the stability of the oil is negligible.

KEY WORDS: Antioxidant activity, olive oil, phenols, polar fraction, protection factor.

Phenols make up a part of the so-called "polar fraction" of virgin olive oil, which is usually obtained by extraction with methanol/water mixtures (1). The presence of 3,4-dihydroxyphenylethanol (hydroxytyrosol), 4-hydroxyphenylethanol (tyrosol), 4-hydroxyphenylacetic acid, syringic acid, 4-hydroxybenzoic acid, vanillic acid, o-coumaric acid, p-coumaric acid and 3,4-dihydroxyphenylacetic acid have been reported previously (2–4); however, this fraction of the oil is very complex and many of its components remain unidentified (4,5).

There is an interest in the level of phenols in olives, olive oil and the so-called rape, a major by-product of the extraction process, because of the antioxidant activity of the total phenolic fraction (6–8). Little is known, however, about the contribution of each component to the stability of the oil. This information might be useful for the processing of the oil and it is also necessary for quality evaluation. The existing colorimetric method for phenol determination, based on the use of Folin-Ciocalteau reagent (1), is not specific for phenols and other compounds may interfere (9). High-performance liquid chromatography (HPLC) procedures, on the other hand, are very difficult to perform, due to the complexity of the phenolic fraction (5), as well as demanding rather sophisticated gradient elution separations.

If the more effective phenolic antioxidants of olive oil were known, HPLC methods could be modified to determine only these constituents. As a result, the information needed could be obtained by quicker determinations that are easier to carry out.

The purpose of the present study was to measure protection factors of major phenols known to be present in virgin olive oil and to evaluate their contribution to its stability.

EXPERIMENTAL PROCEDURES

Materials. Refined, bleached and deodorized (RBD) olive oil and virgin olive oil samples were obtained from a plant located in the area of Athens, Greece. Tyrosol (98%) was purchased from Aldrich Chemical Co. (Milwaukee, WI); caffeic acid (97%) from Fluka (Buchs, Switzerland). Protocatechuic acid, vanillic acid, 3,4-dihydroxyphenylacetic acid, p-hydroxyphenylacetic acid, o-coumaric acid, p-hydroxybenzoic acid, p-coumaric acid and syringic acid were all from Sigma Chemical Co. (St. Louis, MO).

Hydroxytyrosol (3,4-dihydroxyphenylethanol) was prepared in this laboratory as follows: 3,4-dihydroxyphenylacetic acid was methylated with methanol and sulphuric acid by refluxing for two hours (10). Methanol was removed in a rotary evaporator and the residue was extracted with diethylether. The ether extract was washed with a saturated solution of sodium bicarbonate. After evaporation of the solvent, the methylester was acetylated (11) in freshly distilled acetic acid anhydride and pyridine at room temperature for 18 hr. The mixture was diluted with water and extracted with ether. The combined extracts were washed with water, dried with sodium sulphate and the solvent was evaporated. Residual acetic acid was removed *in vacuo* over potassium hydroxide to give 3,4-diacetoxyphenylacetic acid methylester. This ester was finally reduced with lithium aluminum hydride in ether (12) in a three-necked flask by refluxing and stirring for three hours. The excess of lithium aluminum hydride was decomposed with the addition of ethylacetate and water in ether. The phenol was then freed from the alkali salts by acidification with dilute sulphuric acid. The ether layer was separated and the residue, after evaporation of the solvent, was purified by preparative thin-layer chromatography (TLC) on silica gel G plate. The final product was 94% pure as checked by HPLC. As reference material, in the high performance liquid chromatographic analysis, authentic hydroxytyrosol obtained by hydrolysis of oleouropein (13) was used.

Isolation of the phenol fraction. The polar fraction of virgin olive oil that contains the phenols was obtained by dissolving 50 g oil in 50 mL hexane and extracting three times with 30 mL of a methanol/water mixture (60:40, v/v) (1). The combined extracts were evaporated to dryness in a rotary evaporator at 40°C and the residue was dissolved in ethanol and brought to volume in a volumetric flask. After spectrophotometric determination of polyphenols (7), a suitable aliquot was added to RBD olive oil so that the final concentration was 200 ppm. Refined, bleached and deodorized olive oil was used as control. This oil is devoid of polyphenols because refining removes such polar compounds (1). After the removal of solvent in a rotavapor at 40°C, the olive oil was stirred for one hour to ensure complete dissolution of the antioxidant in the oil.

Stability. Special care was taken to use RBD olive oil without additives as the control. Samples of oil, 6 g each, were then transferred to a series of opened, transparent glass bottles of 8 cm^3 volume and 3 cm^2 cross-section, and the filled bottles were stored at 63°C in the dark. Peroxide values were determined periodically according to the AOCS method (14).

RESULTS AND DISCUSSION

Figure 1 presents the results obtained with refined olive oil that contained 200 ppm of the total polar fraction of virgin olive oil.

Refined olive oil deteriorated relatively rapidly, while the

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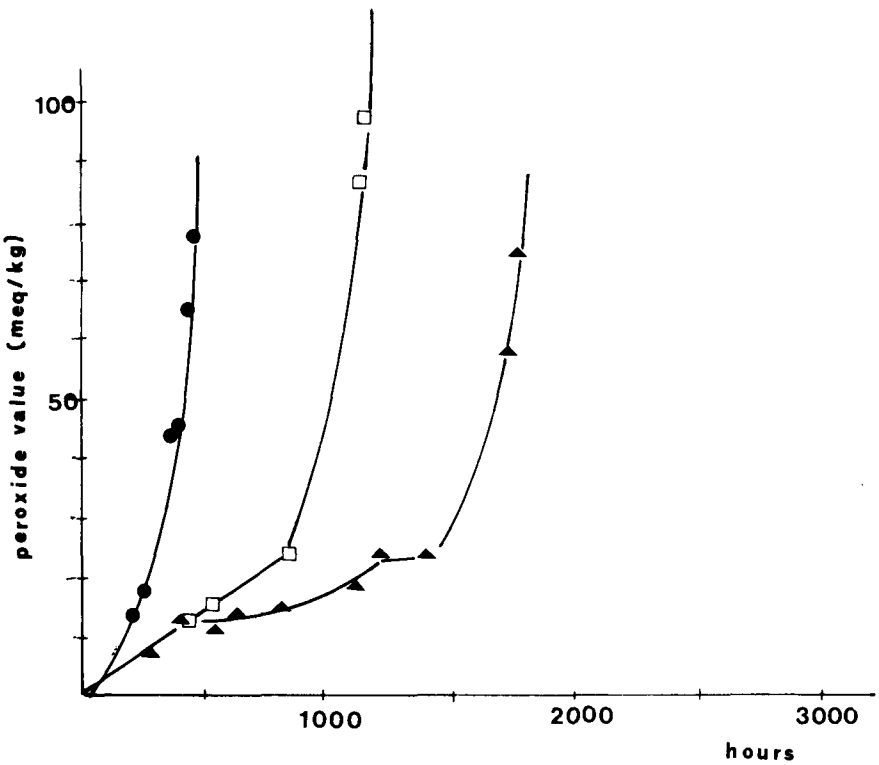

FIG. 1. Effect of the polar fraction on the autoxidation rate of RBD olive oil. ●, Control test; □, polar fraction; and ▲, BHT.

TABLE 1

Antioxidant Activity of Phenols

Phenols	PF_{20}[a]	PF_{70}
Vanillic acid	1.0	1.0
O-coumaric acid	1.0	1.1
p-Coumaric acid	1.2	1.1
Tyrosol	1.2	1.1
p-Hydroxybenzoic acid	1.3	1.1
p-Hydroxyphenylacetic acid	1.3	1.2
Syringic acid	1.5	1.4
Protocatechuic acid	2.7	2.3
BHT	4.4	3.8
Caffeic acid	5.7	5.2
Hydroxytyrosol	15.2	9.5
3,4-Dihydroxyphenylacetic acid[b]	>18.4	>10.6

[a]Protection factors are calculated from the times needed for POV of refined olive oil to attain n; T_{20}=264h, T_{70}=456h.

[b]The presence of this o-diphenol in olive oil is doubtful (3). Its effect, however, was studied because its structure is similar to other polyphenols, and mainly to hydroxytyrosol.

presence of the polar fraction under investigation provides the oil with a remarkable stability at 63°C. This effect is more pronounced in the case of hydroxytyrosol, caffeic acid and protocatechuic acid (Fig. 2), three of the main constituents of the polar fraction. Other phenolic compounds which may contribute to the stability of the oil, if present in considerable amounts, are 3,4-dihydrophenylacetic acid and syringic acid. These results are analytically presented in Table 1, which contains protection factors obtained from the curves of the peroxide value changes. As protection factor the ratio $PF_n = \frac{T_n}{T_n^\circ}$ is described (15), where T_n is the time for peroxide value of fats to attain the value n, and T_n° is the T_n in the control test.

It is clearly shown in Table 1 that tyrosol, the main phenol present in olive oil [in some cases it makes up 40% of the total phenolic fraction (2)], has a very low protection factor and has practically no antioxidant effect. This should be taken into consideration when total polyphenols are expressed as tyrosol (16).

It is important to know the concentration of the active

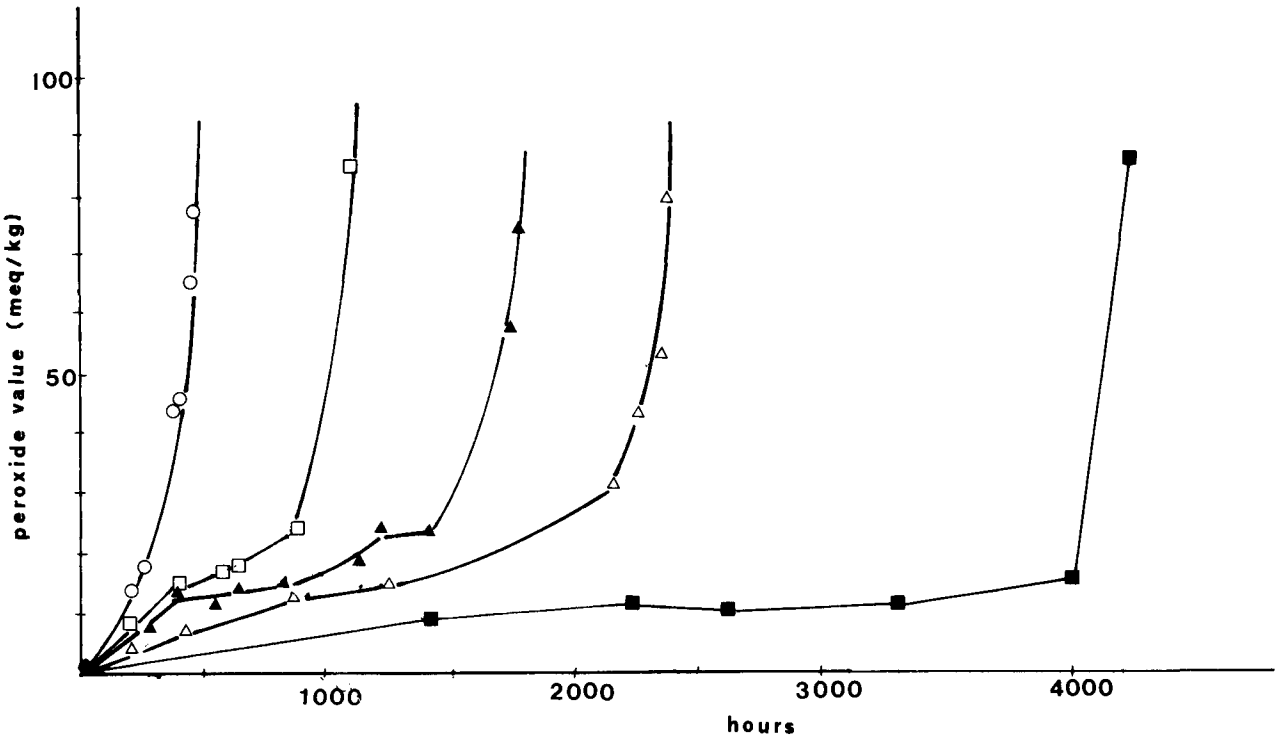

FIG. 2. Effect of individual polyphenols (200 ppm) on the autoxidation rate of refined olive oil at 63°C. ○, Control test; □ protocatechuic acid; ▲, BHT; △, caffeic acid; and ■, hydroxytyrosol.

phenolic compounds. These are mainly o-diphenols such as hydroxytyrosol, protocatechuic acid, caffeic acid and syringic acid. Analytical efforts should be focused on techniques which differentiate these o-diphenols and syringic acid from other constituents of the so-called polar fraction. If these constituents are also estimated in the total polyphenol content, the results can hardly be correlated with the stability of virgin olive oil.

Natural olive oil has a long shelflife compared to other vegetable oils. This should be attributed to its fatty acid composition as well as to the presence of antioxidants. When olives are processed for the production of virgin olive oil, the pulp is treated with warm water and a significant amount of valuable phenols are lost (17). It would be interesting to know if this loss is mainly due to the removal of o-diphenols, and if this depends on the extraction system applied and the temperature and amount of water used in the kneading stage. This is now under investigation in our laboratory.

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