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Antioxidative activity of 3,4-dihydroxyphenylacetic acid and α-tocopherol on the triglyceride matrix of olive oil. Effect of acidity

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RESUMEN

Actividad antioxidante del ácido 3,4-dihidroxifenilacético y del α-tocoferol sobre la matriz de triglicéridos del aceite de oliva. Efecto de la acidez.

Los constituyentes menores del aceite de oliva virgen son importantes para la notable estabilidad del aceite en la autooxidación, pero el papel exacto y el alcance con que cada factor antioxidante contribuye al efecto antioxidante total no ha sido investigado a fondo. En este estudio el papel del α-tocoferol es examinado a varios niveles de acidez y a baja concentraciones de o-difenoles. Un sustrato de triacilgliceroles de aceite de oliva desprovisto de constituyentes prooxidantes o antioxidantes fue preparado a partir de aceite de oliva refinado mediante cromatografía en columna. A este sustrato, un poco oxidado, los aditivos (ácido oleico, ácido 3,4-dihidroxifenilacético y α-tocoferol) fueron añadidos y la estabilidad fue calculada mediante medidas periódicas del índice de peróxido. Se encontró que los ácidos grasos libres reducen principalmente la actividad protectora de los orto-difenoles. Se concluyó también que el α-tocoferol tiene un efecto sinergista con los orto-difenoles y contribuye significativamente al retraso de la formación de peróxidos. Esto es importante para los aceites pobres en orto-difenoles.

PALABRAS-CLAVE: Aceite de oliva virgen – Acidez – α-tocoferol – Efecto antioxidante – Orto-difenol.

SUMMARY

Antioxidative activity of 3,4-dihydroxyphenylacetic acid and α-tocopherol on the triglyceride matrix of olive oil. Effect of acidity.

Minor constituents of virgin olive oil are important for the remarkable stability of the oil in autoxidation, but the exact role and the extent to which each antioxidant factor contributes to the total antioxidant effect has not been thoroughly investigated. In this study the role of α-tocopherol is explored at various acidity levels and at low concentrations of ortho-diphenols. A substrate of olive oil triacylglycerols devoid of prooxidant or antioxidant constituents was prepared from refined olive oil by column chromatography. To this substrate, slightly oxidized, the additives (oleic acid, 3,4-dihydroxyphenylacetic acid and α-tocopherol) were added and the stability was assessed by periodical measurements of peroxide values. It was found that free fatty acids reduce mainly the protective activity of the ortho-diphenol. It is also concluded that α-tocopherol has a synergistic effect with the ortho-diphenols and contributes significantly to the retardation of peroxide formation. This is important for oils poor in ortho-diphenols.

KEY-WORDS: Acidity – α-tocopherol – Antioxidant effect – Ortho-diphenol – Virgin olive oil.

1. INTRODUCTION

Previous research on the stability of olive oil to autoxidation showed that there are many factors (fatty acid composition, natural antioxidants content, free acidity, peroxide value) which affect the shelf life of this valuable natural product. (Vázquez Roncero, 1978; Gutfinger, 1981; Papadopoulos *et al.*, 1993).

Among the antioxidants naturally occuring in olive oil, the most important are tocopherols and polar phenols. In the tocopherols fraction of olive oil α-tocopherol comprises about 90% of total (Sherwin, 1976; Baurfeind, 1980; Coors, 1991) while the fraction of polar phenols is a complex one and contains alcohols (4-hydroxyphenylethanol, 3,4-dihydroxyphenylethanol), vanillic, caffeic and other phenolic acids (Vázquez Roncero, 1976; Solinas and Cichelli, 1982; Cortesi and Fedeli, 1983) as well as a number of not fully identified aglycones of secoiridoid glycosides (Montedoro *et al.*, 1993; Angerosa, 1995; Cortesi, 1995).

Today it is recognised by all the investigators that the presence of naturally occurring antioxidants is important for the remarkable stability of virgin olive oil. However, the exact role and the extent to which each factor contributes to the total antioxidant effect is not clear. Thus, overall assessment of quality and prediction of stability based on chemical analysis remains a difficult problem to handle.

In a previous work (Blekas, *et al.*, 1995) an attempt was made to study the contribution of α-tocopherol to olive oil stability using a model system of purified olive oil triacylglycerols. It was found that although α-tocopherol acted as antioxidant at all levels of addition, the antioxidant effect was greater at low (100 mg/kg) than at higher concentrations (500 and 1000 mg/kg). In the presence of more effective antioxidants such as ortho-diphenols, α-tocopherol did not show any significant antioxidant activity during the period of low peroxide accumulation.

This present study was undertaken to investigate further the role of α-tocopherol at various levels of free fatty acids, which are a prooxidant factor, and at a low level of an ortho-diphenol which imply a reduced resistance to oxidative deterioration. Experiments were carried out to monitor the effect of the above antioxidants and prooxidants on peroxide formation.

As an ortho-diphenol 3,4-dihydroxyphenylacetic acid (3,4-DHPAA) was used. This phenol, which is not found in olive oil, is commercially available in a purified form and has a structure and antioxidant activity similar to that of hydroxytyrosol (Papadopoulos and Boskou, 1991). The latter, in its free or esterified form, is one of the most important antioxidants of olive oil but it is difficult to synthesise it or isolate it from natural sources. The significance of α-tocopherol was also studied at stages when the primary products of oxidation reach a critical point. The work is part of a larger project to study the shelf life of olive oil which is based on the principle of using a model system of purified triacylglycerols to which gradually more prooxidants and antioxidants present in olive oil are added.

2. EXPERIMENTAL

2.1. Samples and standards

Refined olive oil was donated by ELAIS S.A. (Athens, Greece), dl- α-tocopherol was from Merck (Darmstadt, Germany), 3,4-dihydroxyphenylacetic acid from Sigma Chemical Co. (St. Louis, Mo, USA) and oleic acid from Merck (Darmstadt, Germany).

2.2. Methods

Purification of refined olive oil

Refined olive oil was purified by the method of Lampi *et al.* (1992). Portions of 300 g of the oil diluted in 300 ml of n-hexane were passed through a chromatographic column (80 x 5 cm.) packed with a series of the following adsorbents suspended in n-hexane (a) 60 g of activated silicic acid (Sigma Chemical Co., St. Louis, Mo USA) as bottom layer, (b) 30 g of a 2:1 mixture of activated charcoal (Aldrich Chemical Co., Inc., Milwaukee, USA) and Kieselguhr (Riedel-de Haen AG,- Seelze, Germany), (c) 120 g of a 2:1 mixture of powdered sugar (Merck, Darmstadt, Germany) and Kieselguhr, and (d) 60 g of activated silicic acid as top layer. The eluent was collected until all the oil solution had been drawn into the column. The purified triacylglycerol fraction (approximately 150 g) obtained after evaporation of n-hexane in a rotary evaporator at 40°C and removal of the solvent traces by flushing with nitrogen was stored at -18°C. The purity of the produced triacylglycerol mixture was assessed by determination of free fatty acids, peroxide value, total tocopherols and pigments. (Blekas *et al.*, 1995).

Preparation of the samples for the experiments

300 g of purified olive oil triacylglycerols were mildly oxidized at 40°C in the dark to produce a more suitable substrate for the oxidative stability tests (peroxide value lower than 10 meq. O_2 /kg). 42 g-portions of the oxidized substrate containing antioxidants at various concentrations (100 mg/kg α-tocopherol, 5, 10 and 20 mg/kg ortho-diphenol, 100 mg/kg α-tocopherol in mixture with 5 and 10 mg/kg ortho-diphenol) or mixtures of an antioxidant (100mg/kg α-tocopherol or 10 mg/kg ortho-diphenol) and oleic acid (5 and 25 mg/g) were prepared. Antioxidants were added to the substrate in the form of a concentrated solution in absolute ethanol (0.1 ml/42 g substrate). The latter was removed by magnetic agitation of the sample at room temperature in the dark with nitrogen flushing.

Evaluation of peroxidation

The prepared samples were transferred in 3 g-portions to a series of open, transparent 8-ml glass bottles which measured 3 cm across, and the filled bottles were stored at 40°C in the dark. The rate of oxidation was followed by periodic determination of peroxide values, according to IUPAC method 2.201 (IUPAC, 1987), in duplicate. The method indicated good repeatability for the peroxide value meassurements ($x \pm CV\% = 9,6 \pm 4$).

3. RESULTS AND DISCUSSION

Figure 1 presents the rate of oxidation of purified olive oil triacylglycerols to which α-tocopherol, 3,4-DHPAA at three levels and two combinations of the α-tocopherol and the ortho-diphenol were added. The substrate was initially mildly oxidized at 40°C to a peroxide value of approximately 10 meq. O_2/kg. This condition was necessary because, as shown in our previous work (Blekas *et al.*, 1995), in the initial stages of peroxide formation α-tocopherol acts as prooxidant and the two phenols appear to have an antagonistic action. It should be stressed that peroxide values, ranging from 6 to 12 meq. O_2/kg oil, are the usual case in commercial virgin olive oils.

The level of addition of α-tocopherol (100 mg/kg) was selected because it is based on values usually reported for the concentration of this phenol in various natural olive oils. 3,4-DHPAA was added at relatively low levels (5-20 mg/kg). Such low levels of ortho-diphenols have been reported in the literature (Gutfinger, 1981; Zunin *et al.*, 1995).

As shown in the figure there is a synergistic effect between α-tocopherol and the ortho-diphenol. The role of α-tocopherol seems to be extremely important when the level of the ortho-diphenol is low.

It can be concluded from the curves in figure 1 that the protective effect of α-tocopherol is more pronounced when peroxide values reach numbers closed to the limit

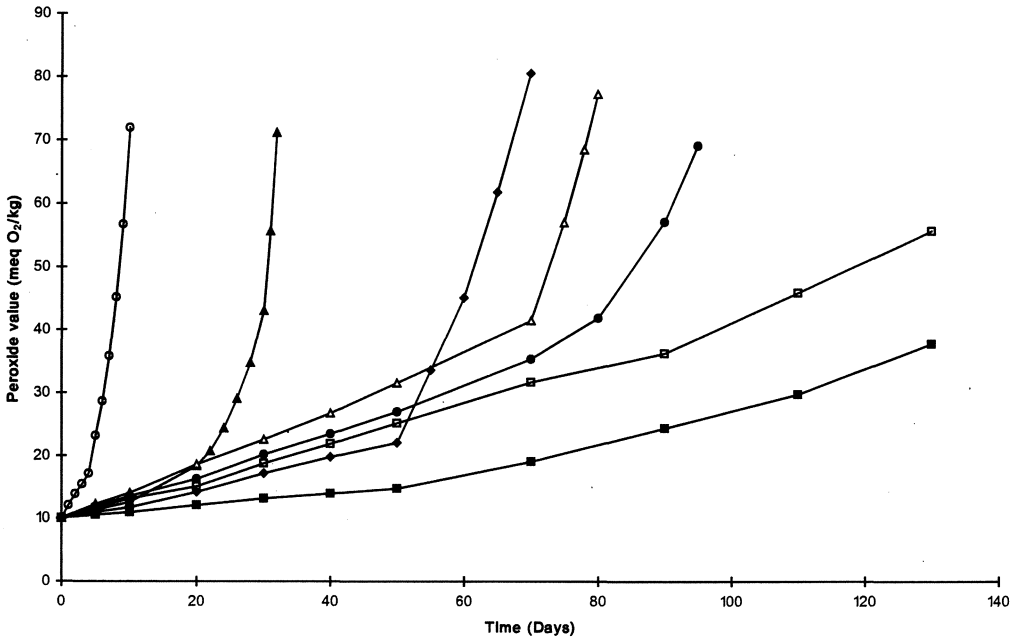

Figure 1
Effect of 3,4-DHPAA, α-tocopherol and mixtures of both compounds on the stability of mildly oxidized olive oil triacylglycerols (POV = 10,1 meq. O_2/kg). Control (o); plus 5 mg/kg 3,4-DHPAA (▲); plus 10 mg/kg 3,4-DHPAA (♦); plus 20 mg/kg 3,4-DHPAA (■) plus 100 mg/kg α-tocopherol (Δ); plus 5 mg/kg 3,4-DHPAA and 100 mg/kg α-tocopherol (●); plus 10 mg/kg 3,4-DHPAA and 100 mg/kg α-tocopherol (□)

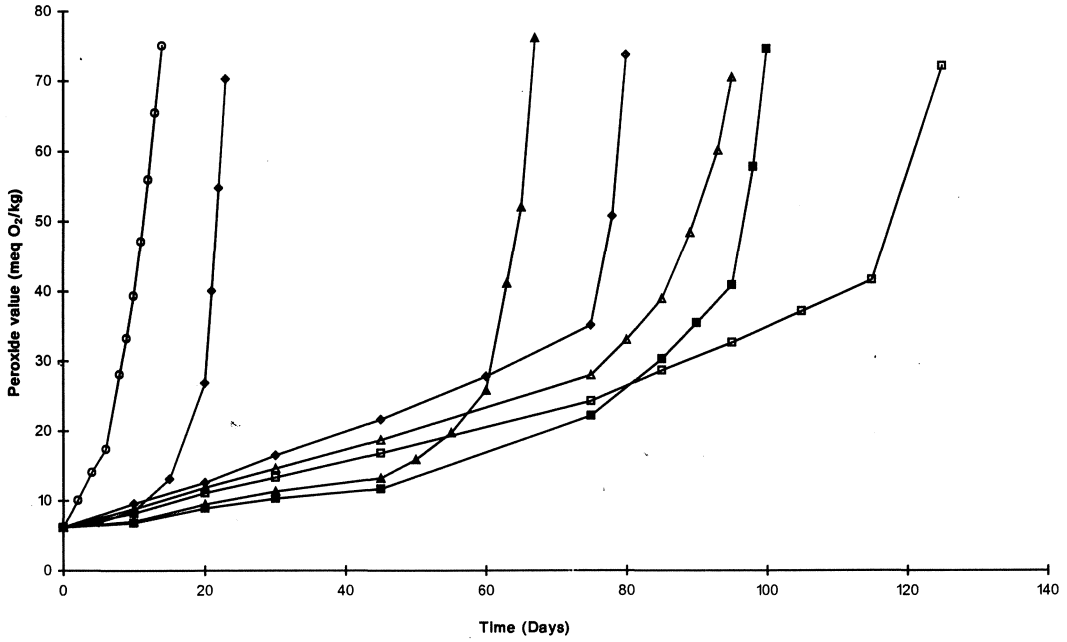

Figure 2
Effect of oleic acid on antioxidative activity of 3,4-DHPAA and α-tocopherol in slightly oxidized olive oil triacylglycerols (POV = 6,2 meq. O_2/kg). Control (o); plus 10 mg/kg 3,4-DHPAA (■); plus 10 mg/kg 3,4-DHPAA and 5 mg/g oleic acid (▲); plus 10 mg/kg 3,4-DHPAA and 25 mg/g oleic acid (♦); plus 100 mg/kg α-tocopherol (□); plus 100 mg/kg α-tocopherol and 5 mg/g oleic acid (Δ); plus 100 mg/kg α-tocopherol and 25 mg/g oleic acid (◇)

of 20 meq. O_2/kg set by the European Union (EC, 1991) and International Olive Oil Council (IOOC, 1995).

Figure 2 shows the results of the second experiment which was conducted with a slightly oxidized substrate (peroxide value 6,2 meq. O_2/kg) containing 10 mg/kg of the ortho-diphenol or 100 mg/kg of α-tocopherol and oleic acid at levels 0.5 and 2.5% w/w respectively. The results indicate that the effect of low levels of free acidity on the antioxidant activity of the ortho-diphenol is insignificant. However, at levels of acidity reaching the upper limit for edible natural olive oils (EC, 1991), the protective effect of the ortho-diphenol practically disappears. This does not happen in the case of α-tocopherol which seems to retain its protective effect even at high levels of acidity.

When stability tests are carried out in model systems it is difficult to extrapolate the results to actual natural oils because in the latter many other prooxidant or antioxidant factors may be involved. However, it can be concluded that there must be a critical concentration of primary products of autoxidation for the maximum effectiveness of both α-tocopherol and ortho-diphenol. At peroxide values near the regulatory limit, α-tocopherol seems to be significant for retarting of oxidation. This means that the conventional limit of 20 meq. peroxide oxygen per kg oil set by standarizing organisation only partly indicates the actual shelf life of the oil. In order to predict the stability properly and access better the overall quality, the role of prooxidant and antioxidant factors should be further studied and evaluated.

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