Food Chemistry 49 (1994) 61–65

Antioxidant activity of plant leaves in relation to their alpha-tocopherol content

J. F. Mallet, C. Cerrati, E. Ucciani, J. Gamisans & M. Gruber

Applied Organic Chemistry and Ecological Botany, CNRS, Box 412, University of Aix-Marseille, F-13397 Marseille Cedex 13, France

(Received 11 November 1992; revised version received and accepted 22 March 1993)

The leaf alpha-tocopherol content of fifteen plant species was determined by a gas-chromatographic method with alpha-tocopherol acetate used as internal standard. Highest amounts were found in leaves of *Pelargonium* sp. (412 ppm) and *Thalictrum flavum* (371 ppm), which could represent an interesting source of alpha-tocopherol for different purposes, such as the stabilization of food products.

Antioxidant activity of leaf extracts was evaluated spectrophotometrically by coupled oxidation of beta-carotene and linoleic acid. The species could thus be ranked on the basis of an antioxidant-activity coefficient. Correlation established between the alpha-tocopherol content and antioxidant activity yielded a coefficient of 0·93, suggesting that alpha-tocopherol is the major liposoluble antioxidant found in leaves.

INTRODUCTION

Phenolic antioxidants are widely used to prevent deterioration of oxidizable goods, such as food, cosmetics, pharmaceuticals, and plastics. Despite the fact that synthetic antioxidants account for many of them, there is an increasing tendency to search for new natural sources that could provide safe additives to the food industry. The plant kingdom offers a large range of phenolic compounds, among which alpha-tocopherol is best known as one of the most efficient naturally occurring liposoluble antioxidants.

Tocopherol biosynthesis takes place inside the chloroplast membranes of the plant (Booth, 1963; Schultz, 1990), and, though no evidence has yet been brought, it seems that photosynthesis is influenced by the ratio tocopherol/chlorophyll. The antioxidant activity of alpha-tocopherol is thus quite useful in plant leaves as a means of protection against metabolic disorders.

Quantitative determination of leaf tocopherols is less common than seed oils for which numerous methods have been used (Müller-Mulot, 1976; Hartman, 1977; Podlaha *et al.*, 1978; Deldime *et al.*, 1980; Wong *et al.*, 1988; Warner & Mounts, 1990). Booth (1963) investigated the leaf tocopherol content of several plants by the Emmerie–Engel colorimetric method. More recently (Gapor *et al.*, 1986), alpha-tocopherol was determined in palm leaves by HPLC.

Numerous investigations have been devoted to the antioxidant activity of alpha-tocopherol, whether alone (Cillard & Cillard, 1980; Burton *et al.*, 1980; Burton & Ingold, 1981, 1986; Koskas *et al.*, 1984) or in association with synergists such as the common naturally occurring ascorbic acid (Cort, 1974; Han *et al.*, 1991). Much is known about its antioxidant mechanism and considerable attention is given to its *in-vivo* activity (Burton & Ingold, 1986). It is an accepted fact that alpha-tocopherol shows excellent biological activity as a free-radical scavenger, and for this reason there is agreement that it could serve as a therapeutic drug against free-radical-involved diseases (Kappus, 1991).

Since the early work of Chipault *et al.* (1952, 1955, 1956), the antioxidant properties of crude plant extracts have been studied in view of their possible utilization as protective agents for foods (Manganari & Oreopoulou, 1991; Economou *et al.*, 1991), but also to characterize new efficient natural antioxidants (Wu *et al.*, 1982; Taga *et al.*, 1984; Whittern *et al.*, 1984).

The present paper reports a chromatographic method for the determination of alpha-tocopherol in leaves of fifteen plant species. After examination of the antioxidant properties of these species, a correlation will be established between alpha-tocopherol content and antioxidant activity.

MATERIALS AND METHODS

Preparation of extracts

Air-dried leaves (10 g) were ground to a fine powder and then extracted with 300 ml of hexane by percola-

Food Chemistry 0308-8146/93/$06.00 © 1993 Elsevier Science Publishers Ltd, England. Printed in Great Britain

tion on a glass column (2 cm × 55 cm). The crude extracts obtained after vacuum evaporation to dryness were stored under argon at −10°C.

Thin-layer chromatography (TLC)

TLC analysis was performed on silica-gel 60 pre-coated aluminium sheets (Merck) by using hexane:diethyl ether (4:1, v/v). Plates were dipped in a 3% sulfuric acid ether solution and then charred at 200°C.

Column chromatography

A mixture of 120 mg of hexane extract and 5 mg of alpha-tocopherol acetate (Fluka) was added to the top of a chromatographic column (1·5 cm × 40 cm) containing 12 g of 70–230 mesh Silicagel 60 (Merck) in hexane:diethyl ether (4:1, v/v). Elution was carried out with 200 ml of the above-mentioned solvent mixture and was monitored by TLC. A sample of alpha-tocopherol (Fluka) was used as reference standard. Fractions (5 ml each) containing alpha-tocopherol and alpha-tocopherol acetate were assembled, and a purified tocopherol extract was obtained after solvent removal.

Gas chromatography

Tocopherol extracts were analysed on a Girdel 300 chromatograph equipped with a flame-ionization detector and linked to an Enica 21 integrator. Separation was achieved on a fused-silica capillary column (OV-17 25 m, internal diameter 0·25 mm, film thickness 0·2 μm). The oven temperature was programmed from 200 to 320°C at 15°C/min. Split-injector and detector temperatures were 300 and 320°C, respectively. Helium (80 kPa) was used as carrier gas.

Gas chromatography–mass spectrometry (GC–MS)

Tocopherol extracts were analysed by GC–MS on a Delsi-Nermag Automass. GC conditions were the same as those previously described. The source temperature was kept at 120°C. and mass fragmentation was obtained from an emission current of 0·329 mA and an ionization energy (EI) of 70 eV.

Measurement of antioxidant activity

Antioxidant activity of leaf extracts was determined by a spectrophotometric method as described by Taga *et al.* (1984) and Chevolleau *et al.* (1992), this being based on the ability of the different extracts to decrease oxidative losses of beta-carotene in a beta-carotene–linoleic acid emulsion. Methanol solutions of the extracts (0·01%) were used to evaluate their antioxidant properties.

RESULTS AND DISCUSSION

Identification of alpha-tocopherol

Because of its liposoluble property, alpha-tocopherol is understandably present in hexane extracts of plant leaves, and this is evidenced by TLC comparison with an authentic sample whose R_F value is 0·45. We looked for further proofs by GC and GC–MS analyses of purified extracts obtained after fractionation on Silicagel. GC analysis of pure alpha-tocopherol yielded an identical retention time to that of the expected compound. MS analysis finally confirmed an alpha-tocopherol structure, since the characteristic fragment ions (Schepple et al., 1972) were at *m/z* 430 (M^+), *m/z* 205, and *m/z* 165.

Quantitative determination of alpha-tocopherol

We selected a GC method previously developed in our laboratory (Chevolleau, 1990) to determine the alpha-tocopherol content in the leaves. It was recently shown (Ulberth *et al.*, 1992) that capillary GC, as well as HPLC, was a suitable method for the quantitative analysis of tocopherols. In a preliminary step, extracts containing alpha-tocopherol acetate as internal standard were purified on Silicagel. Combined fractions of alpha-tocopherol and its acetate, referred to as the tocopherol extract, were directly analysed by GC. Figure 1 shows

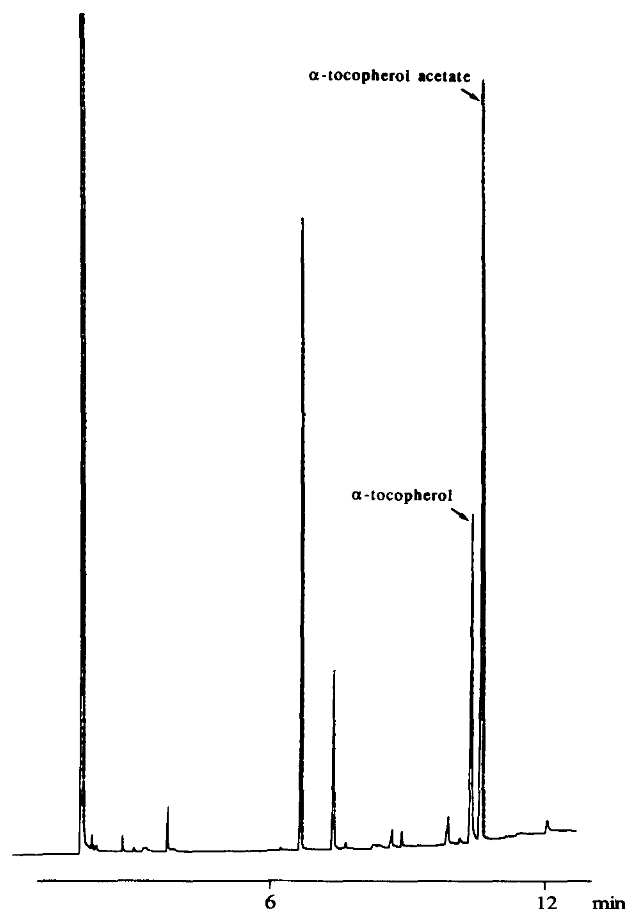

Fig. 1. Gas chromatogram of tocopherol extract *Pelargonium* sp.

Table 1. Leaf alpha-tocopherol content of plant species

Species	Extract yield (%)	α-Tocopherol content	
		Extract (%)	Leaves (ppm)
Berberis stenophylla L. (Berberidaceae)	1·7	0·1	17
Buxus sempervirens L. (Buxaceae)	2·1	0·5	105
Helleborus corsicus (Willd) Tutin (Ranunculaceae)	1·9	0·9	169
Ligustrum lucidum Ait. fil. in Ait. (Oleaceae)	1·2	0·2	25
Nerium oleander L. (Apocynaceae)	2·4	0·9	221
Pallenis spinosa (L.) Cass. (Asteraceae)	1·2	0·3	36
Parthenocissus quinquefolia (Koehne) Planch. (Vitaceae)	0·5	1·1	55
Pelargonium sp. (Geraniaceae)	2·1	2·0	412
Platanus hybrida Brot. (Platanaceae)	1·3	0·1	14
Populus alba L. (Salicaceae)	1·6	<0·1	2
Prunus cerasus L. (Rosaceae)	1·1	0·5	55
Prunus laurocerasus L. (Rosaceae)	1·4	0·5	70
Simmondsia chinensis Link. (Buxaceae)	1·3	1·1	143
Thalictrum flavum L. (Ranunculaceae)	3·9	1·0	371
Thuja occidentalis L. (Cupressaceae)	4·6	<0·1	5

the chromatogram of one of these tocopherol extracts. Most of them gave similar chromatograms with few unidentified peaks. The chromatographic conditions used were acceptable for no interference of alpha-tocopherol with other tocopherols or tocotrienols that could be present in the extracts (Mordret *et al.*, 1978). Confirmation was obtained from mass-spectral data.

The alpha-tocopherol content of each extract was calculated from the following well-known equation:

$$M_T = K(A_T/A_S) \times M_S$$

where M_T and A_T are, respectively, the mass and peak area of alpha-tocopherol, M_S and A_S are, respectively, the mass and peak area of the internal standard (alpha-tocopherol acetate), and K is the response factor corresponding to the ratio K_T/K_S where K_T and K_S are, respectively, the correlation coefficients of alpha-tocopherol and alpha-tocopherol acetate. Different synthetic mixtures of alpha-tocopherol and its acetate were subjected to GC analysis for the determination of K. By a linear regression, we found a K value of 1·1. Results of alpha-tocopherol content expressed as percentage of hexane extracts and as ppm of dry leaves are given in Table 1.

As expected from TLC analysis, alpha-tocopherol occurred in all the species. Levels in hexane extracts were rarely higher than 1%. A 2% content was found for *Pelargonium* sp., and trace amounts were detected in *Populus alba* and *Thuja occidentalis*. Leaves of *Pelargonium* sp. and to a lesser extent those of *Thalictrum flavum* presented considerably greater amounts of alpha-tocopherol (412 and 371 ppm, respectively) than the other species.

If we compare our data with those for common oleaginous seeds, whose total tocopherol content rarely reaches 500 ppm, it seems that leaves of such species as *Pelargonium* sp. and *Thalictrum flavum* could constitute an interesting source of alpha-tocopherol. Further work is in progress in order to search for other species containing higher amounts of this compound.

Table 2. Antioxidant-activity coefficient (AAC) of extracts and dry leaves

Species	Antioxidant-activity coefficient (AAC)	
	Extracts	Leaves
Berberis stenophylla	31	0·5
Buxus sempervirens	90	1·9
Helleborus corsicus	117	2·2
Ligustrum lucidum	13	0·2
Nerium oleander	177	2·2
Pallenis spinosa	30	0·4
Parthenocissus quinquefolia	165	0·8
Pelargonium sp.	395	8·3
Platanus hybrida	17	0·2
Populus alba	70	1·1
Prunus cerasus	31	0·3
Prunus laurocerasus	133	1·9
Simmondsia chinensis	290	3·8
Thalictrum flavum	251	9·8
Thuja occidentalis	35	1·6

Antioxidant activity of extracts

Methanol-extract solutions were added to an aqueous oxygenated emulsion of beta-carotene and linoleic acid and absorbance at 470 nm was read at regular intervals until complete decoloration of the control occurred. Antioxidant activity was determined by the following expression (Chevolleau *et al.*, 1992):

$$AAC = (A_{E(120)} - A_{C(120)}/(A_{C(0)} - A_{C(120)}) \times 1000$$

where AAC is an antioxidant-activity coefficient ranging from 0 to 1000, $A_E(120)$ is the absorbance at t = 120 min for the extract sample, and $A_C(0)$ and $A_C(120)$ are the absorbance of the control at $t = 0$ and $t = 120$ min, respectively. Calculated values of the antioxidant-activity coefficient of the leaf extracts are given in Table 2.

AAC values above 400 were not found, the most active extracts being those of *Pelargonium* sp. (AAC 395) and, to a lesser extent, *Simmondsia chinensis* (AAC 290) and *Thalictrum flavum* (AAC 251). The other extracts were much less efficient, since their AAC values were rarely higher than 100.

Table 2 also provides AAC data relative to dry

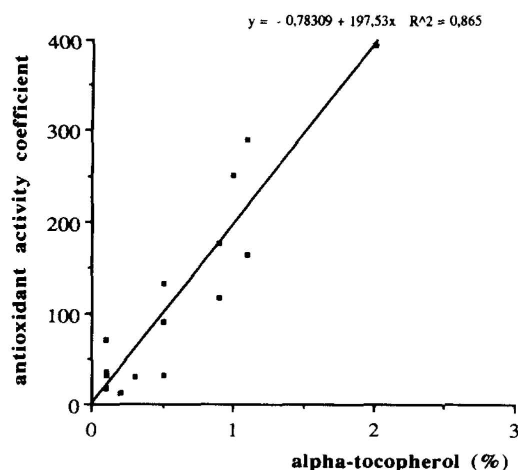

Fig. 2. Graph showing alpha-tocopherol content in relation to antioxidant activity of leaf extracts.

leaves and corresponding to the product-extract yield (%) × AAC. These low-AAC values ($P < 10$) are more meaningful to compare the antioxidant properties of the plant species. Hence *Thalictrum flavum* presented the highest antioxidant activity (AAC 9.8), this being followed by *Pelargonium* sp. (AAC 8.3) and *Simmondsia chinensis* (AAC 3.8).

Relation between alpha-tocopherol content and antioxidant activity

The high antioxidant activity of alpha-tocopherol suggests that this compound could account for much of the observed activity. In order to confirm this hypothesis, we searched for a correlation between alpha-tocopherol content and antioxidant activity. Plotted data are shown in the graphs of Figs 2 and 3, corresponding to extracts and dry leaves, respectively.

The correlation coefficient determined by linear-regression analysis was 0·93 for the extracts and 0·92 on a dry-leaf basis. Such results constitute additional proof of the outstanding antioxidant activity of alpha-tocopherol. Though other antioxidants are probably present in hexane extracts of leaves, there is no doubt

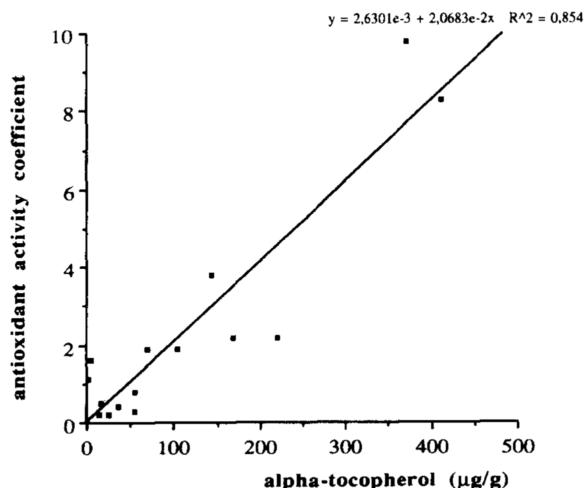

Fig. 3. Graph showing alpha-tocopherol content in relation to antioxidant activity of dry leaves.

that alpha-tocopherol is responsible, to a great extent, for the antioxidant activity of these extracts. Work in our laboratory is being carried out to characterize other liposoluble plant antioxidants.

REFERENCES

Booth, V. H. (1963). α-Tocopherol, its occurrence with chlorophyll in chloroplasts. *Phytochemistry*, **2**, 421–7.

Burton, G. W. & Ingold, K. U. (1981). Autoxidation of biological molecules. 1: The antioxidant activity of Vitamin E and related chain-breaking phenolic antioxidants *in vitro*. *J. Am. Chem. Soc.*, **103**, 6472–7.

Burton, G. W. & Ingold, K. U. (1986). Vitamin E: Application of the principles of physical organic chemistry to the exploration of its structure and function. *Acc. Chem. Res.*, **19**, 194–201.

Burton, G. W., Le Page, Y., Gabe, E. J. & Ingold, K. U. (1980). Antioxidant activity of Vitamin E and related phenols: Importance of stereoelectronic factors. *J. Am. Chem. Soc.*, **102**, 7791–2.

Chevolleau, S. (1990). Etude de l'activité antioxydante des plantes. Importance de l'α-tocopherol. Thesis, University of Aix-Marseille III, Marseille, France.

Chevolleau, S., Debal, A. & Ucciani, E. (1992). Détermination de l'activité antioxydante des extraits végétaux. *Rev. Fr. Corps Gras*, **39**, 3–8.

Chipault, J. R., Mizuno, G. R., Hawkins, J. M. & Lundberg, W. O. (1952). Antioxidant properties of natural spices. *Food Res.*, **17**, 46–55.

Chipault, J. R., Mizuno, G. R. & Lundberg, W. O. (1955). Antioxidant properties of spices in oil-in-water emulsions. *Food Res.*, **20**, 443–8.

Chipault, J. R., Mizuno, G. R. & Lundberg, W. O. (1956). The antioxidant properties of spices in foods. *Food Technol.*, **10**, 209–11.

Cillard, J. & Cillard, P. (1980). Behaviour of alpha, gamma and delta tocopherols with linoleic acid in aqueous media. *J. Am. Oil Chem. Soc.*, **57**, 39–42.

Cort, W. M. (1974). Antioxidant activity of tocopherols, ascorbyl palmitate and ascorbic acid and their mode of action. *J. Am. Oil Chem. Soc.*, **51**, 321–5.

Deldime, P., Lefebvre, G., Sadin, Y. & Wybauw, M. (1980). L'analyse des tocopherols dans les huiles végétales par la CLHP. *Rev Fr. Corps Gras.*, **27**, 279–83.

Economou, K. D., Oreopoulou, V. & Thomopoulos, C. D. (1991). Antioxidant activity of some plant extracts of the family *Labiateae*. *J. Am. Oil Chem. Soc.*, **68**, 109–13.

Gapor, A., Kato, A. & Ong, A. S. H. (1986). α-Tocopherol content in oil palm leaflet. *J. Am. Oil Chem. Soc.*, **63**, 330–1.

Han, D., Yi, O. S. & Shin, H. K. (1991). Solubilization of Vitamin C in fish oil and synergistic effect with Vitamin E in retarding oxidation *J. Am. Oil Chem. Soc.*, **68**, 740–3.

Hartman, K. T. (1977). A simplified gas liquid chromatographic determination for Vitamin E in vegetable oils. *J. Am. Oil Chem. Soc.*, **54**, 421–3.

Kappus, H. (1991). Toxikologie freier Radikale und Anti-oxidantien unter besonderer Berücksichtigung von Vitamin E. *Fat. Sci. Technol.*, **93**, 128–31.

Koskas, J. P., Cillard, J. & Cillard, P. (1984). Autoxidation of linoleic acid and behaviour of its hydroperoxides with and without tocopherols. *J. Am. Oil Chem. Soc.*, **61**, 1466–9.

Manganari, G. & Oreopoulou, V. (1991). Combined effect of some plant extracts and BHA on lipid oxidation. *Riv. Ital. Sost. Grasse.*, **68**, 305–8.

Mordret, F. & Laurent, A. M. (1978). Application de la chromatographie en phase gazeuse sur colonne capillaire de

verre à l'analyse des tocopherols. *Rev. Fr. Corps Gras*, **25**, 245–50.
Müller-Mulot, W. (1976). Rapid method for the quantitative determination of individual tocopherols in oils and fats. *J. Am. Oil Chem. Soc.*, **53**, 732–6.
Podlaha, P., Eriksson, A. & Toregard, B. (1978). An investigation of the basic conditions for tocopherol determination in vegetable oils and fats by differential pulse polarography. *J. Am. Oil Chem. Soc.*, **55**, 530–2.
Scheppele, S. E., Mitchum, R. K., Rudolph, C. J., Kinneberg, K. F. & Odell, G. V. (1972). Mass spectra of tocopherols. *Lipids*, **7**, 297–304.
Schultz, G. (1990). Biosynthesis of α-tocopherol in chloroplasts of higher plants. *Fat. Sci. Technol.*, **92**, 86–90.
Taga, M. S., Miller, E. E. & Pratt, D. E. (1984). Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc.*, **61**, 928–31.
Ulberth, F., Reich, H. & Kneifel, W. (1992). Zur Analytik von Tocopherolen. Ein Methodenvergleich zwischen HPLC und GC. *Fat. Sci. Technol.*, **94**, 51–4.
Warner, K. & Mounts, T. L. (1990). Analysis of tocopherols and phytosterols in vegetable oils by HPLC with evaporative light-scattering detection. *J. Am. Oil Chem. Soc.*, **67**, 827–31.
Whittern, C. C., Miller, E. E. & Pratt, D. E. (1984). Cottonseed flavonoids as lipid antioxidants. *J. Am. Oil Chem. Soc.*, **61**, 1075–8.
Wong, M. L., Timms, R. E. & Goh, E. M. (1988). Colorimetric determination of total tocopherols in palm oil, olein and stearin. *J. Am. Oil Chem. Soc.*, **65**, 258–61.
Wu, J. W., Lee, M. H., Ho, C. T. & Chang, S. S. (1982). Elucidation of the chemical structure of natural antioxidants isolated from rosemary. *J. Am. Oil Chem. Soc.*, **59**, 339–45.