Ability of Phenolic Acids to Protect α-Tocopherol

Zuzana RÉBLOVÁ and Petra OKROUHLÁ

Department of Food Chemistry and Analysis, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic

Abstract

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The ability of phenolic acids (gallic, gentisic, protocatechuic, syringic, vanillic, ferulic, caffeic, and sinapic; 600 mg/kg) to protect α-tocopherol was tested during the heating of sunflower oil on a hot plate set at 180°C and was compared with the ability of these phenolic acids to slow down the formation of polymerised triacylglycerols (TAG) in the same conditions. The half-life of α-tocopherol (calculated as the time needed for the α-tocopherol content to decrease to 50% of the original value) was extended significantly by gentisic, caffeic, and gallic acids (from 1.16 h to 1.77 h, 1.78 h, and 2.26 h, respectively), while the formation of polymerised TAG was slightly suppressed only by gallic acid.

Keywords: phenolic acids; frying; α-tocopherol; polymerised triacylglycerols

In the testing of antioxidants for their application in the food industry (for the stabilisation of fats, oils, and fatty foods), only the ability of these compounds to protect fatty acids is usually tested (in terms of their ability to slow down the increases in peroxide value, to extend the induction period or to slow down the formation of polymerised triacylglycerols). However, the ability of the tested compounds to protect tocopherols (i.e. compounds with vitamin E activity) should also be tested. For example, during frying tocopherols are often fully destroyed before the point at which the frying oil should be replaced, based on the contents of polymerised triacylglycerols (i.e. 12% (Anonymous 2000)) or polar compounds (i.e. 24% (Anonymous 2000)) (Normand *et al.* 2001; Barrera-Arellano *et al.* 2002). Furthermore, the protection of tocopherols is one way in which antioxidants act *in vivo* in the prevention of cardiovascular diseases (Lapointe *et al.* 2006).

Many compounds have already been tested for their ability to protect tocopherols. Able to protect tocopherols during frying were ascorbyl-palmitate (Gordon & Kouřimská 1995a, b), catechin (Kajimoto *et al.* 1991a), gallic acid (Kajimoto *et al.* 1990a, b, 1991a), phospholipids (tested as soybean lecithin) (Kajimoto *et al.* 1987, 1990a, 1991a), quercetin, BHA, TBHQ (Kajimoto *et al.* 1992), eugenol (Kajimoto *et al.* 1992; Tomaino *et al.* 2005), acylated sterylglycosides (Murui & Nakanishi 1994), thiodipropionic acid (Kajimoto *et al.* 1990b), retinyl-palmitate (Simonne & Eitenmiller 1998), polyphenols isolated from virgin olive oil (Pellegrini *et al.* 2001), rosemary extracts (Gordon & Kouřimská 1995a, b), and essential oils of some spices (essential oil of sweet basil containing linalool as the main component, oregano containing carvacrol, clove containing eugenol, cinnamon containing cinnamaldehyde and thyme containing thymol) (Tomaino *et al.* 2005).

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In the same conditions, controversial results were obtained with sesamol (Kajimoto *et al.* 1991b; Kajimoto *et al.* 1992), while flavone, β-carotene (Kajimoto *et al.* 1992), BHT, oryzanol (Kajimoto *et al.* 1991b), ascorbyl-stearate (Kajimoto *et al.* 1991a, b), and essential oil of nutmeg (containing α- and β-pinene and sabinene) (Tomaino *et al.* 2005) did not protect tocopherols (or their ability to protect tocopherols was very small). However, ascorbyl-stearate increased the effects of gallic acid and soybean lecithin (Kajimoto *et al.* 1991a) and was effective in a mixture with BHT or oryzanol, which did not protect tocopherols on its own (Kajimoto *et al.* 1991b).

Although many studies have tested the ability of different compounds to protect tocopherols during frying and in similar conditions, they have not generally compared the ability of antioxidants to protect tocopherols and their ability to slow down the undesirable reactions of fatty acids. Therefore, the ability of eight phenolic acids (gallic, gentisic, protocatechuic, syringic, vanillic, ferulic, caffeic, and sinapic) to protect α-tocopherol was studied during the heating of sunflower oil on a hot plate set at 180°C and was compared with their ability to slow down the formation of polymerised triacylglycerols (TAG).

MATERIAL AND METHODS

Material. The sunflower oil was purchased in an ordinary shop. The initial content of α-tocopherol was 603 ± 8 mg/kg (the total initial content of the other tocopherols was 43 ± 3 mg/kg) and the initial content of polymerised TAG was 0.81 ± 0.05%.

The phenolic acids tested (Figure 1) were purchased from Sigma-Aldrich (St. Luis, USA) with the following purities: gallic acid (Fluka, Buxchs, Switzerland; purity higher than 98%), gentisic acid (Sigma-Aldrich; purity 98%), protocatechuic acid (Fluka; purity higher than 97%), syringic acid (Fluka; purity higher than 97%), vanillic acid (Fluka; purity higher than 97%), ferulic acid (Fluka; purity higher than 98%), caffeic acid (Sigma; purity higher than 98%); sinapic acid (Fluka; purity higher than 97%).

Experiments. The phenolic acids were added to the sunflower oil separately, at 600 mg/kg each, using the following procedure: 10 ml of the solution of the studied acid in acetone (150 mg/100 ml) were pipetted into 25 g ± 1% of sunflower oil in a 100 ml beaker with the internal diameter of 43 mm. The oil and the acetone solution were mixed and the beakers were placed in a fume cupboard in which acetone evaporated at room temperature overnight.

On the following day, the beakers were heated on a hot plate (Präzitherm PZ 28-2, Harry Gestigkeit GMBH, Düsseldorf, Germany with a steel adapter with outlets; internal diameter 60 mm and depth 28 mm) set at 180 ± 1°C for 1–8 hours. Under these conditions, the temperature of the oil increased in the course of 20 min and then fluctuated between 154°C and 156°C. For sampling, the whole beaker was removed from the hot plate. Thus, the oil volume in the beakers was constant during the whole heating period. Three experiments were performed with each phenolic acid and three control experiments were performed with acetone only.

Determination of tocopherols. Tocopherols were determined using reverse-phase HPLC with

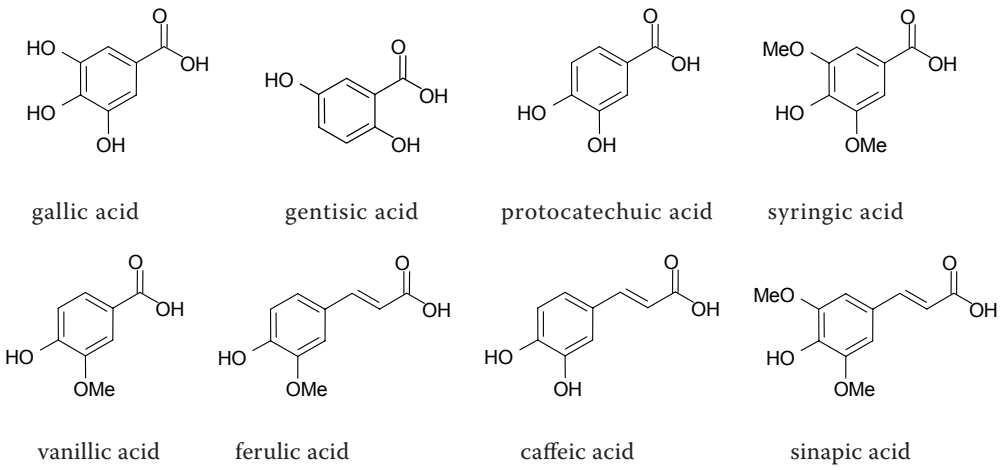

Figure 1: The tested phenolic acids

amperometric detection under the following conditions: mobile phase – methanol/acetonitrile (1:1, v/v) mixture with $LiClO_4$ (0.02 mol/l) and NaCl (0.005 mol/l); flow rate – 1 ml/min (LCP 4020.31 non-steel pump; Ecom, Prague, Czech Republic); injected volume 20 µl; sample preparation – solution in acetone; column Hypersil ODS, 200 × 4.6 mm, particle size 5 µm (Hewlett-Packard, Palo Alto, USA); column temperature 28°C (LCO 101 column heater; Ecom); detection potential +1.05 V (HP 1049A amperometric detector equipped with a glassy-carbon working electrode; Hewlett-Packard, Palo Alto, USA).

Determination of polymerised TAG. Polymerised TAG were determined using HP-SEC under the following conditions: mobile phase – tetrahydrofuran; flow rate – 0.6 ml/min (LCP 4000.11 pump; Ecom); injected volume – 5 µl; sample preparation – solution in tetrahydrofuran; column – PL-gel Mixed-E, 300 × 7.5 mm, particle size 3 µm (Agilent Technologies, Santa Clara, USA); detection – refractometric (HP 1047A; temperature 30°C; Hewlett Packard, Palo Alto, USA).

Data processing. The half-life of α-tocopherol in the individual oils was calculated as the time needed for its content to decrease to 50% of the original value, and the half-life of the oils was calculated as the time taken for the content of polymerised triacylglycerols to reach 6%. The differences between the oils were tested using *t*-test at the 0.05 level of probability.

RESULTS AND DISCUSSION

Ability of phenolic acids to protect α-tocopherol

Figure 2 shows a typical course of α-tocopherol degradation (in the control oil and in the oil with gallic acid). Under the conditions studied, gallic, gentisic, and caffeic acids significantly protected α-tocopherol (Table 1). Gallic acid was the most active, almost doubling the half-life of α-tocopherol. Gentisic and caffeic acids had almost identical effects, both of them extending the half-life of α-tocopherol by one half.

The results obtained are generally consistent with the previously published results. The ability of gallic acid to protect tocopherols has been documented in many papers (Kajimoto *et al.* 1988a,b, 1990a,b, 1991a), under various conditions, including frying (Kajimoto *et al.* 1988a, 1990a,b, 1991a). The ability of caffeic acid to protect tocopherols has also been reported previously (Ochi *et al.* 1994; Raneva *et al.* 2001, 2002; Pazos *et al.* 2005a), although the ability of this phenolic acid to protect tocopherols has not yet been studied during frying. However, it has been reported that protocatechuic acid is able to protect α-tocopherol (during oxidation of human LDL lipoproteins *in vitro*) (Zhang *et al.* 2001), although it did not protect α-tocopherol in the present study. This phenolic acid was also able to regenerate α-tocopherol from the tocopheroxyl radical (Pazos *et al.* 2007), which is one of the mechanisms that act in α-tocopherol protection (Segawa *et al.* 1994; Facino *et al.* 1998; Jia *et al.* 1998; Zhu *et al.* 1999, 2000; Pazos *et al.* 2005b).

With the given contradictory results regarding the protection of tocopherols by protocatechuic acid, it is possible that the ability of antioxidants to protect tocopherols depends on the experimental conditions (as does antioxidant activity (Kamal-Eldin & Appelqvist 1996; Yanishlieva 2001)). The ability to protect tocopherols only in some cases and not in other ones has previously been reported for phospholipids (Koga & Terao 1995) and for epicatechine and epicatechine-gallate (Zhu *et al.* 1999; Hashimoto *et al.* 2000; Hiramoto *et al.* 2002; Murakami *et al.* 2002; Raneva *et al.* 2004) (while epigallocatechine and epigallocatechine-gallate protected tocopherols under all conditions studied (Zhu *et al.* 1999; Hashimoto *et al.* 2000; Hiramoto *et al.* 2002; Murakami *et al.* 2002; Raneva *et al.* 2004).

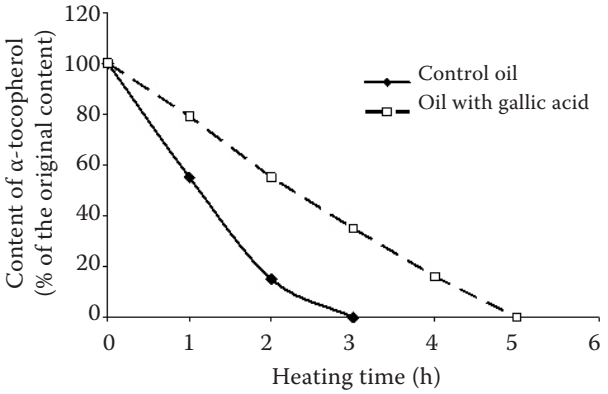

Figure 2: Degradation of α-tocopherol during heating of sunflower oil with and without gallic acid (600 mg/kg) on a hot plate set at 180°C

Table 1. Ability of phenolic acids to protect α-tocopherol (based on time taken for content of α-tocopherol to decrease to 50% of the original content, $t_{\alpha T}$) and their ability to slow down the formation of polymerised TAG (based on time taken for content of polymerised TAG to reach 6%, t_{pTAG}) during heating of sunflower oil on a hot plate set at 180°C

Phenolic acid	$t_{\alpha T}$ (h; mean ± S.D.)	t_{pTAG} (h; mean ± S.D.)
–	1.16 ± 0.04	3.13 ± 0.25
Gallic	2.26 ± 0.07*	3.83 ± 0.09*
Gentisic	1.77 ± 0.14*	3.39 ± 0.15
Protocatechuic	1.14 ± 0.10	3.22 ± 0.18
Syringic	1.27 ± 0.04	3.18 ± 0.13
Vanillic	1.20 ± 0.04	3.18 ± 0.12
Ferulic	1.13 ± 0.03	3.00 ± 0.10
Caffeic	1.78 ± 0.01*	3.47 ± 0.24
Sinapic	1.25 ± 0.04	3.35 ± 0.07

*marked results are significantly different from the control values ($P < 0.05$)

The factors that are known to affect antioxidant activity are the concentration of antioxidants, temperature, light, type of substrate, physical state of the system, as well as numerous microcomponents acting as pro-oxidants or synergists (Kamal-Eldin & Appelqvist 1996; Yanishlieva 2001). The main factor enabling protocatechuic acid to protect α-tocopherol during oxidation of LDL lipoproteins (Zhang *et al.* 2001) (and preventing it to protect α-tocopherol in the present study) may be that the oxidation of LDL lipoproteins was initiated by a hydrophilic source of free radicals (2,2'-azo-bis(2-amidinopropane) dihydrochloride, AAPH). Consequently, protocatechuic acid (present in the water phase (Stöckmann *et al.* 2000)) reacted with free radicals more easily than α-tocopherol, which is localised in lipoproteins. This hypothesis is supported by the results obtained with caffeic acid. This phenolic acid spared α-tocopherol consumption more effectively in rat plasma oxidation induced by a hydrophilic initiator (AAPH) than in rat plasma oxidation induced by a lipophilic initiator (Raneva *et al.* 2001). Similarly, the ability of selenium-containing antioxidants to protect α-tocopherol during the oxidation of multilamellar vesicle membranes dependeds on the location of these antioxidants in relation to the source of free radicals (Raneva *et al.* 2002).

Futhermore, the detected ability of protocatechuic acid to regenerate α-tocopherol from the tocopheroxyl radical (Pazos *et al.* 2007) is probably due to the high molar protocatechuic acid–tocopheroxyl radical ratio. This ratio was approximately 400:0.6 (while, in the present study, the molar protocatechuic acid–α-tocopherol ratio was approximately only 3.9:1.4). However, the ability of protocatechuic acid to regenerate α-tocopherol was only small and the other results on the ability of phenolic acids to regenerate α-tocopherol are consistent with the present results. Protocatechuic acid was able to reduce the concentration of α-tocopheroxyl radical by 16%. Gallic acid had a greater ability to regenerate α-tocopherol than protocatechuic acid and vanillic and syringic acids were not able to regenerate α-tocopherol at all (Pazos *et al.* 2007).

Ability of phenolic acids to slow down the formation of polymerised TAG and the relationship between the ability to protect α-tocopherol and to slow down the formation of polymerised TAG

Under the conditions studied, the formation of polymerised TAG was significantly suppressed by gallic acid only (Table 1). This phenolic acid prolonged the time taken for the content of polymerised TAG to reach 6% by 24%.

There is a lack of data in the literature on the antioxidant activity of phenolic acids during frying. Three phenolic acids (caffeic, ferulic, and vanillic) were tested in two studies (Shahina *et al.* 2004, 2005). The authors reported rather a

Table 2. Ability of phenolic acids to slow down the formation of polymerised TAG (F_{pTAG}) and to reduce the loss of α-tocopherol ($L_{\alpha T}$) during one hour of heating of sunflower oil on a hot plate set at 180°C

Phenolic acid	F_{pTAG} (%; mean ± SD)	$L_{\alpha T}$ (% of the original content; mean ± SD)
–	0.93 ± 0.23	43.7 ± 1.7
Gallic	0.57 ± 0.06	21.1 ± 0.7*
Gentisic	0.83 ± 0.06	27.3 ± 1.3*
Protocatechuic	1.07 ± 0.15	44.6 ± 4.2
Syringic	0.87 ± 0.06	39.7 ± 1.4
Vanillic	1.00 ± 0.10	42.5 ± 1.9
Ferulic	1.30 ± 0.70	44.0 ± 1.8
Caffeic	0.67 ± 0.15	28.3 ± 1.6*
Sinapic	0.80 ± 0.26	40.4 ± 2.0

*marked results are significantly different from the control value ($P < 0.05$)

good antioxidant activity of these phenolic acids based on the changes in peroxide values. However, the peroxide value is not a suitable parameter for following the undesirable reactions of fatty acids that arise during frying (DGF 2008). In two other papers, the antioxidant activity of ferulic acid during frying was tested using a method for total polar compound determination (Warner & Laszlo 2005) or a method for polymerised TAG determination (Gertz *et al.* 2000) (both of which are suitable parameters for following the undesirable reactions of fatty acids arising during frying (DGF 2008)). In these studies, ferulic acid had no antioxidant activity (Warner & Laszlo 2005) or this activity was very small (when a large amount of ferulic acid (2500 mg/kg) was used) and the activity of gallic acid esters was higher than that of ferulic acid (Gertz *et al.* 2000).

The fact that α-tocopherol was preserved (during heating of sunflower oil on a hot plate set at 180°C) by three phenolic acids (gallic, caffeic, and gentisic) with a marked protective effect (approximately 100% or 50%, see earlier), while the formation of polymerised TAG was inhibited only by gallic acid with a mild protective effect (approximately 24%), is an important finding of this study. These two properties (i.e. the ability to protect tocopherols and the antioxidant activity) are often considered to be contextual (Beddows *et al.* 2001; Pazos *et al.* 2005a) and most studies (comparing the ability to protect tocopherols and the antioxidant activity under the same conditions) acknowledged this finding (Beddows *et al.* 2000, 2001; Zhu *et al.* 2000; Wong *et al.* 2001; Huang *et al.* 2004). However, different results were obtained in some studies (Ochi *et al.* 1989; Murui & Nakanishi 1994; Lampi *et al.* 1999). Typically, γ- and δ-tocopherols are able to slow down the formation of hydroperoxides (in oils containing α-tocopherol) but they are not able to protect α-tocopherol (Ochi *et al.* 1989; Lampi *et al.* 1999). By contrast, during frying, some antioxidants are able to protect α-tocopherol but they do not slow down the undesirable reactions of fatty acids (based on the formation of polymerised TAG, for example) (Murui & Nakanishi 1994), as in the present study.

The fact that phenolic acids (gallic, caffeic, and gentisic) are able to protect tocopherols (α-tocopherol) during frying but are not able to protect fatty acids (i.e. they are not able to slow down the formation of polymerised TAG, or their ability to do this is negligible), can also have a practical impact. These phenolic acids cannot prolong (or only slightly prolong) the lifetime of plant oils during frying (i.e. the duration of time before the frying oil should be replaced), but they can increase the content of tocopherols in fried foods. This effect would be the most remarkable in food prepared by one-shot frying, where the loss of tocopherols is tens of percent (Steinhart & Rathjen 2003).

After one hour of sunflower oil heating on a hot plate set at 180°C, the content of polymerised TAG increased by 0.9% and this increase was not significantly affected by any phenolic acid (Table 2). The loss of α-tocopherol was higher than 40% under these conditions and was significantly reduced

by gallic, caffeic, and gentisic acids. Therefore, the use of these phenolic acids (and/or different antioxidants with similar properties) could contribute to a higher intake of tocopherols, which is currently not sufficient according to several studies (Maras *et al.* 2004; Gao *et al.* 2006).

CONCLUSIONS

In the present study, the ability of phenolic acids (gallic, gentisic, protocatechuic, syringic, vanillic, ferulic, caffeic, and sinapic; 600 mg/kg) to protect α-tocopherol during sunflower oil heating was studied and compared with the ability of these phenolic acids to slow down the formation of polymerised TAG in the same conditions. α-Tocopherol was protected by three phenolic acids (gallic, caffeic, and gentisic) with a marked protective effect, while the formation of polymerised TAG was inhibited only by gallic acid with a mild protective effect. The use of the effective phenolic acids could increase the content of tocopherols in fried foods and thus vitamin E intake.

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Corresponding author:

Ing. Zuzana Réblová, Ph.D., Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav chemie a analýzy potravin, Technická 5, 166 28 Praha 6, Česká Republika
tel.: + 420-220 443 181, e-mail: zuzana.reblova@vscht.cz