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A comparison of the antioxidant properties of steryl ferulates with tocopherol at high temperatures

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Abstract

Steryl ferulates (esters of phytosterols and ferulic acid) have long been studied for their health-promoting properties, partially owing to their capacity to inhibit oxidation. The good heat-stability of rice bran oil has been attributed to its high content of steryl ferulates and tocopherols. It has been suggested that these compounds have a synergistic effect as antioxidants. In this model experiment, we determined the capacity of sitostanyl ferulate and α-tocopherol (alone or as a mixture) to prevent polymerization of high oleic sunflower oil at 100 and 180 °C. The formation of polymers was significantly reduced at both temperatures and by both antioxidants, as well as their mixture, though no synergistic effect was seen. Further, we followed the decrease in antioxidant levels and found that sitostanyl ferulate was degraded at a lower rate than α-tocopherol, indicating that sitostanyl ferulate is a promising antioxidant for high temperature applications.
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Keywords: Steryl ferulate; Phytosterol; Ferulic acid ester; Antioxidant; Oryzanol

1. Introduction

Steryl ferulates (ferulic acid esters of sterols) are bioactive compounds that have been studied for their cholesterol-lowering properties (reviewed by Orthoefer & Eastman, 2004) as well as for their antioxidant capacity (Kim, Godber, & Prinyawiwatkul, 2001; Nyström, Mäkinen, Lampi, & Piironen, 2005; Wang, Hicks, & Moreau, 2002; Yagi & Ohishi, 1979). Other properties, such as anti-inflammatory activity (Akihisa et al., 2000) and antitumor activity (Iwatsuki et al., 2003), have also been reported, but not studied as extensively. The mixture of steryl ferulates in rice, commonly known as γ-oryzanol, has been studied from various viewpoints over the past century. Over the past decade, the study of steryl ferulates has been extended to other cereal sources, such as corn, rye and wheat (Hakala et al., 2002; Moreau, Powell, Hicks, & Norton, 1998). The key difference in the composition of steryl ferulates in rice and other cereals is the dominance of dimethylsterol moieties (cycloartenol and 24-methylenecycloartanol) in rice compared to desmethylsterols (sitosterol, campesterol and their respective saturated forms, stanols) in other cereal sources (Fig. 1).

The antioxidant activity of steryl ferulates is primarily based on hydrogen donation from the ferulic acid hydroxyl group. Ferulic acid, like various other common antioxidants used in food applications (BHA, BHT, and TBHQ), is a small and polar compound with only a

Abbreviations: HOSO, high oleic sunflower oil; SF, sitostanyl ferulate; α-T, α-tocopherol.

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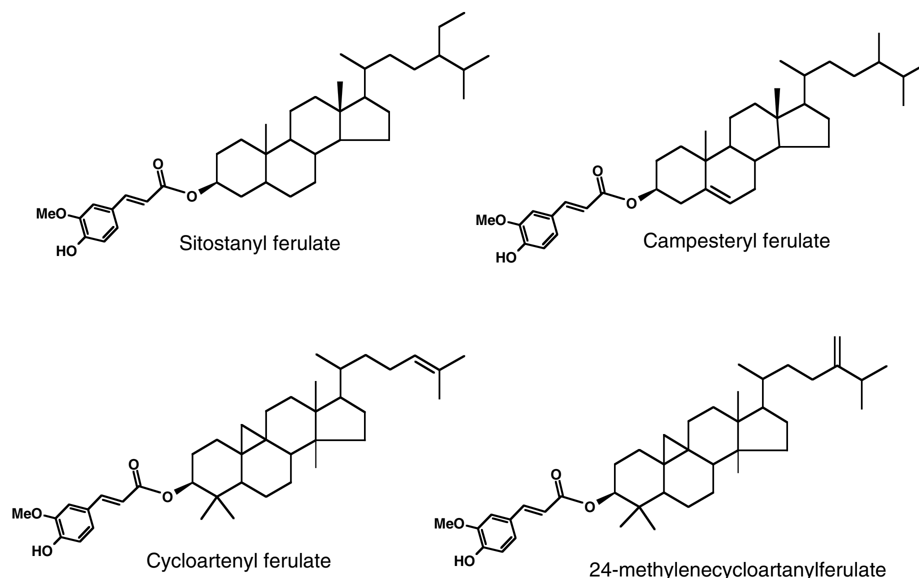

Fig. 1. Structural formulae of sitostanyl ferulate (**1**), campesteryl ferulate (**2**), and principal components of γ-oryzanol: cycloartenyl ferulate (**3**) and 24-methylenecycloartanyl ferulate (**4**). Compounds **1** and **2** are desmethylsterols with no methyl groups at C-4, whereas **3** and **4** are dimethylsterols with two methyl groups.

limited solubility in oils. Further, these antioxidants are relatively easily volatilized and thus cannot inhibit oxidation at high temperatures for long periods of time. Once esterified to a nonpolar compound like phytosterol, the polarity of the molecule is decreased and solubility in oils is improved. Esterification of ferulic acid to a sterol also improves the heat stability of the larger compound as has been demonstrated by Shopova and Milkova (2000) in studies analysing the thermal decomposition of these compounds. The intensive phase of thermal decomposition of cholesteryl ferulate was observed in a temperature range of 245–415 °C, whereas the same range for non-esterified ferulic acid was 125–230 °C. Therefore, steryl ferulates could be considered better antioxidants than free ferulic acid in high temperature food applications, such as boiling and frying.

Rice bran oil, which may contain up to 1.5% of oryzanol is well known for its good stability as a frying oil (Norton, 1995). Addition of rice bran oil has been shown to inhibit oxidation in a number of food systems, such as whole milk powder (Nanua, McGregor, & Godber, 2000), restructured beef roasts (Kim & Godber, 2001), refrigerated cooked beef (Kim, Suh, Yang, & Lee, 2003) and french fries frying oil (Kamal-Eldin, Appelqvist, Gertz, & Stier, 1998). Rice bran oil, however, also contains significant quantities of other antioxidants, such as tocopherols and tocotrienols, so that the net effect is not attributable only to γ-oryzanol. Depending on the analytical system, the capacity of γ-oryzanol to inhibit oxidation has been either greater than (Gertz, Klostermann, & Kochhar, 2000; Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002) or less than (Juliano, Cossu, Alamanni, & Piu, 2005; Nyström et al., 2005) that of α-tocopherol. The overall concentration of steryl ferulates in many cereal brans is, however, nearly tenfold higher than the concentration of the total tocopherols and thus steryl ferulates are very important natural antioxidants from cereals. There are also some differences in antioxidant capacities between steryl ferulates with different sterol moieties. Desmethylsteryl ferulates were shown to be better antioxidants than was γ-oryzanol (principally comprised of dimethylsteryl ferulates) (Nyström et al., 2005; Wang et al., 2002). In his review of frying oils and natural antioxidants, Kochhar (2000) stated that γ-oryzanol has substantial synergistic effects with tocopherols. Kalitchin and co-workers (1997) also suggested possible synergism between steryl ferulates and tocopherols, but neither of these studies had experimental data to show that steryl ferulates would protect tocopherols from degradation and to our knowledge there are no studies, so far, to demonstrate the possible synergistic effect of steryl ferulates and tocopherols.

At high temperatures, there are certain requirements for antioxidants and specialized methods are required for their analysis. Antioxidants, such as BHA, BHT and phenolic components, are only sparingly soluble in oils and they evaporate relatively easily (Pokorný & Schmidt, 2001). When oils are heated at high temperatures, oxygen below the surface becomes limited and reactions involving oxygen are exceeded by addition reactions forming oligomers and polymers of the triacylglyceryl radicals. Further, if the concentration of an antioxidant, such as tocopherol is high, the concentration of antioxidant free radicals may also increase and dimers or trimers may be formed (however, they may still possess some antioxidant activity). As polymerization is a major oxidation reaction at high temperatures, the extent of oxidation is best monitored by analyzing the products of polymerization or by analyzing changes in oil viscosity caused by them.

In this study, the antioxidant capacity of steryl ferulates was studied at boiling and frying temperatures, either alone or combined with α-tocopherol. The antioxidant action of these compounds together in oils at high temperatures has been suggested in the literature without any supporting data. Further, most of the earlier studies of antioxidant activity of steryl ferulates at high temperatures were conducted by adding rice bran oil, not steryl ferulate alone, thus also adding a number of other compounds to the system. This study was done to evaluate the antioxidant activity of steryl ferulate and α-tocopherol in a model system at two temperatures. Sitostanyl ferulate was chosen as the model compound owing to the earlier studies suggesting better heat-stability of saturated stanols compared to sterols (Soupas, Juntunen, Lampi, & Piironen, 2004). The capacity of sitostanyl ferulate to inhibit oil polymerization was compared to that of α-tocopherol. Further, the possible synergistic effect of these two antioxidants was studied, as well as the consumption of antioxidants in the oil during heating.

2. Materials and methods

2.1. Materials

Sitostanyl ferulate was synthesized by the method of Condo, Baker, Moreau, and Hicks (2001) and its purity was >99% using the HPLC methods described in a later section. DL-α-tocopherol (98–102%) and aluminium oxide (90 activity neutral, activity I) were purchased from Merck (Darmstadt, Germany). High oleic sunflower oil (RBD, refined, bleached and deodorized) was obtained from Raisio plc (Raisio, Finland). All solvents used in this study were of HPLC grade and were supplied by Rathburn Chemicals (Walkerburn, Scotland). Acetic acid was purchased from J.T. Baker (Deventer, The Netherlands).

2.2. Stripping of high oleic sunflower oil to remove pro- and antioxidants

The high oleic sunflower oil (HOSO) was stripped to remove natural pro- and antioxidants, applying the method of Lampi, Dimberg, and Kamal-Eldin (1999) with some minor modifications. A slurry of 180 g of aluminium oxide (activated at 100 °C for 16 h and 200 °C for 8 h) and 320 ml of heptane was applied to a glass chromatography column (520×28 mm i.d.) and heptane was allowed to flow through the column, until the solid phase was evenly packed. A mixture of 100 g high oleic sunflower oil and 100 ml of heptane was added to the column and eluted with heptane. The first 150 ml of the eluting heptane was discarded and the following 300 ml were collected in a flask covered with aluminium foil to prevent light-induced oxidation of the purified oil. The stripped oil was analyzed to confirm that the concentration of tocopherols was less than 1 μg/g of oil. The oil was stored as a heptane solution at −20 °C until used in the experiment.

2.3. Addition of antioxidants to oil and heating of the samples

Sitostanyl ferulate and α-tocopherol were added to the purified oil at two different levels, 0.5% and 1.0% (w/w), and as a mixture at 0.5% of both antioxidants. Further, a control sample without antioxidant additions and a sample of unstripped high oleic sunflower oil in its natural state were analyzed. Solid compounds were weighed in a round-bottom flask; high oleic sunflower oil in heptane was added and the solvent was evaporated with a rotary evaporator at +30 °C. Samples (1 g) were weighed in open glass vials (10 ml) with a 3 cm^2 surface area and thus a 3:1 surface to oil volume ratio. A vial of each antioxidant sample and a control were removed from the oven at sampling points. Samples were heated in a dark oven at 100 or 180 °C. At the lower temperature, samples were heated for 0, 24, 72 and 96 h and at the higher temperature for 0, 1, 3, 6 h. After heating, each of the samples was cooled in a desiccator, then dissolved in 10 ml of heptane and stored at −20 °C until analyzed. Each heating experiment was replicated three times.

2.4. Analysis of lipid polymers

The levels of polymerized lipids in samples (measured as wt% of total lipids) were analyzed with high performance size exclusion chromatography (HPSEC) with three size exclusion chromatography columns (100 Å and 2×50 Å, 5 μm, 300×7.5 mm (Polymer Laboratories Inc., Amherst, MA)) connected in series, with dichloromethane as the eluent at 0.6 ml/min and column heating at 35 °C. The liquid chromatograph used was HP 1090 II and the polymers were detected with a refractive index detector (Hewlett–Packard 1047A) with an A/D-converter (Hewlett–Packard 35900). The system separated triacylglycerol monomers (retention time = 29 min), dimers (retention time = 27 min) and polymers (retention time = 26 min) that were quantified on the basis of peak areas. The extent of oxidation of the high oleic sunflower oil was determined by adding up the percentages of dimers and polymers of total lipid. Later, total dimers and polymers are referred to as merely polymers. Repeatability of the analytical system was monitored by a daily analysis of a control sample (purified high oleic sunflower oil heated for 3 h at 180 °C). The percentage of di- and polymers in the control sample was $17.6 \pm 0.7\%$ ($n = 46$).

2.5. NP-HPLC analysis of antioxidants in oil

A rapid method to analyze sitostanyl ferulate and α-tocopherol in a single chromatographic run was developed on the basis of the method by Moreau and co-workers (1996). The method was changed to an isocratic system with a shorter time of analysis. Sitostanyl ferulate and α-tocopherol in oil samples were analyzed with normal phase

high performance liquid chromatography (NP-HPLC), using a diol column (LiChrosorb Diol 5 μm, 100 × 3.0 mm, VDS Optilab, Berlin, Germany) and an isocratic elution of heptane–isopropanol–acetic acid (99:1:0.1) at a flow rate of 0.5 ml/min with a Hewlett–Packard 1090 II liquid chromatograph (Waldbronn, Germany). Sitostanyl ferulate was detected at 315 nm with the diode array detector of the HP1090 and α-tocopherol was detected with a fluorescence detector (Hewlett–Packard 1046 A) using an excitation wavelength of 290 nm and emission wavelength 325 nm. The linear range of response, limit of detection and limit of determination were analyzed to validate the chromatographic method. For sitostanyl ferulate, the linear range of response was 0.01–10 μg/injection ($R^2 = 0.9999$) and, for α-tocopherol, 0.01–0.1 μg/injection ($R^2 = 0.9995$). Limit of detection was determined with analysis of dilute standard solutions as the mass that gave a peak height that was three times greater than the detector noise. This result was used to deduce the limit of determination that is three times the limit of detection. Limits of detection and determination were 2 and 6 ng for sitostanyl ferulate and 2.5 and 7.5 ng for α-tocopherol, respectively. The performance of the chromatographic system was monitored with a daily analysis of a control sample that was a mixture containing 0.5% sitostanyl ferulate and 0.5% α-tocopherol in 20 ml of heptane. For sitostanyl ferulate, the concentration of the control sample was 7.3 ± 1.5 mg/g ($n = 19$) and, for α-tocopherol, 4.1 ± 0.4 mg/g ($n = 14$).

2.6. Data analysis

Statistical analysis of the results was conducted using analysis of variance (ANOVA) and Fisher's LSD-test or the Kruskall–Wallis test at 95% confidence level. Statgraphichs Plus-software (STTC Inc., Rockville, ML) was used for the statistical analyses. For the analysis of synergism, the inhibition percentage (INH) of a compound to prevent polymerization was calculated using the polymer contents (POL) and the following formula: $INH = (POL_{control} - POL_{antioxidant})/POL_{control} \times 100$ and these results were further used to determine the percentage of synergism: $Synergism\% = [INH_{mixture} - (INH_{antioxidant1} + INH_{antioxidant2})]/(INH_{antioxidant1} + INH_{antioxidant2}) \times 100$.

3. Results and discussion

3.1. The effect of the sitostanyl ferulate and α-tocopherol addition on the formation of polymers in high oleic sunflower oil

All of the added compounds were able to act as antioxidants that significantly inhibited polymerization under the conditions applied (Table 1). At the lower temperature of heating (100 °C), the proportion of polymers in the control sample began to increase rapidly after 24 h and by 72 h the content of polymerized lipid was already 25% (Table 1). The regulatory limit of polymers for frying oils varies between 10% and 16% in different countries and the recommendation confirmed by the German Society for Fat Sciences is 12% (Anonymous, 2000). Reflecting to the limit, the control oil had already become unacceptable, whereas all the other oils were still quite low in polymer content, with no significant differences between different antioxidants. After 3 days of heating (72 h), the formation of polymers began to increase in other samples and, by the 4th day (96 h), the polymer content had significantly increased above the acceptable level in unstripped HOSO and both sitostanyl ferulate and α-tocopherol at the lower addition level (0.5%). The samples containing 1.0% sitostanyl ferulate or 0.5% of both antioxidants polymerized only mildly during the heating, with polymer contents of 3.9% and 2.8%, respectively, at the end of heating. The induction period for 1% α-tocopherol was clearly reaching the end and polymerization was beginning to increase at the end of heating at 96 h. One may see that some of the α-tocopherol (1.0%) samples had already polymerized much more than the others, as indicated by the greater standard deviation in the polymer contents. At the turning point of oxidation, from the induction period to the log-phase, there may be significant deviation in the results of individual samples as they begin to polymerize rapidly at slightly different time points. The endpoint of the experiment was chosen on the basis of polymerization in the control sample in such a way

Table 1
A time-course study of the increase in the concentrations of dimers and polymers (wt% of total lipids) in high oleic sunflower oil (HOSO) and di- and polymers of total lipid at different time points

Temperature (°C)	Time (h)	Control stripped HOSO	Unpurified HOSO	SF 0.5%	SF 1.0%	α-T 0.5%	α-T 1.0%	SF 0.5% + α-T 0.5%
100	0	0.2 ± 0.0^{a}	0.4 ± 0.2^{a}	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}	0.3 ± 0.0^{a}	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}
	24	2.2 ± 1.4^{a}	0.3 ± 0.2^{a}	0.4 ± 0.1^{a}	0.4 ± 0.2^{a}	0.5 ± 0.2^{a}	0.5 ± 0.2^{a}	0.5 ± 0.2^{a}
	72	24.7 ± 0.4^{a}	4.5 ± 2.6^{b}	3.9 ± 2.4^{b}	2.7 ± 1.4^{b}	2.3 ± 1.4^{b}	1.8 ± 1.1^{b}	2.4 ± 1.5^{b}
	96	32.4 ± 0.8^{a}	18.4 ± 1.7^{b}	15.4 ± 0.8^{c}	3.9 ± 1.2^{d}	11.1 ± 6.0^{cd}	6.1 ± 5.2^{d}	2.8 ± 0.9^{d}
180	0	0.2 ± 0.1^{a}	0.5 ± 0.1^{a}	0.2 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}
	1	4.9 ± 0.8^{a}	1.3 ± 0.1^{c}	2.6 ± 0.6^{b}	2.3 ± 0.6^{b}	1.8 ± 0.5^{bc}	2.1 ± 0.4^{bc}	1.8 ± 0.3^{bc}
	3	21.3 ± 1.0^{a}	8.5 ± 0.5^{c}	12.8 ± 1.6^{b}	8.6 ± 1.4^{c}	5.6 ± 1.2^{d}	6.4 ± 1.2^{d}	5.3 ± 1.0^{d}
	6	37.8 ± 1.7^{a}	22.1 ± 3.2^{c}	27.5 ± 1.8^{b}	21.5 ± 1.5^{c}	18.8 ± 2.7^{c}	11.2 ± 1.2^{d}	13.3 ± 2.4^{d}

Different superscript letters within a row (within one time point) indicate a statistically significant difference.

that the polymer content was about the same (~35%) at the end of the experiment at both temperatures. Therefore, the possible differences in the antioxidant capacities of 1% sitostanyl ferulate and the antioxidant mixture could not be seen.

At the higher temperature (180 °C), polymerization was much more rapid, as expected. Similar or even higher polymer levels were reached in 6 h of heating compared to 4d at 100 °C (Table 1). The control sample had began to polymerize significantly faster than others already after 1 h of heating and reached the unacceptable level of polymers between 1 and 3 h. Though both of the antioxidants showed a positive dose response, sitostanyl ferulate was not such an effective antioxidant at this higher temperature as was α-tocopherol. At the end of heating, the ability of sitostanyl ferulate (1.0%) to inhibit polymerization was comparable to the lower level of α-tocopherol (0.5%) and the natural antioxidant levels present in unstripped HOSO, with polymer contents of 21.5%, 18.8% and 22.1%, respectively. The lower level of sitostanyl ferulate had somewhat higher polymer content (27.5%) at the end of heating. The formation of polymers was best inhibited by 1.0% of α-tocopherol and the antioxidant mixture that contained 0.5% of both antioxidants.

3.2. A comparison of the rates of degradation of antioxidants during the heating of high oleic sunflower oil

The degradation of antioxidants in controls and antioxidant-containing samples was monitored at all time points and both temperatures. The error between the added antioxidant concentration and analytical result at the zero time point is most likely caused by some residual solvent that did not evaporate and thus diluted the sample (Figs. 2 and 3). At 100 °C, the degradation of α-tocopherol was faster than that of sitostanyl ferulate (Fig. 2). The degradation of α-tocopherol could be seen already after 24 h when the content of sitostanyl ferulate had remained at the original level. After 72 h of heating, about half of the 1% sitostanyl ferulate addition was still remaining in the sample, whereas all of the α-tocopherol in the 1% α-tocopherol was degraded during that time. In a similar manner, at the lower antioxidant addition (0.5%), half of the sitostanyl ferulate still remained after 72 h, whereas all the α-tocopherol had disappeared. At the end of heating (96 h), no α-tocopherol could be detected in the samples, but some sitostanyl ferulate still remained in the 1% sitostanyl ferulate and the antioxidant mixture. In the antioxidant mixture, the levels of α-tocopherol decreased faster than the levels of sitostanyl ferulate. Furthermore, the rate of decrease in the levels of α-tocopherol in the mixture were the same as in the 0.5% α-tocopherol alone. A more rapid degradation of α-tocopherol than γ-oryzanol was also seen when the nonsaponifiable fraction of rice bran oil was used to prevent cholesterol oxidation (Kim et al., 2001). These findings do not support the view suggested by Kochhar (2000) that the steryl ferulate possibly could depress the degradation of tocopherol at high temperature.

At 180 °C, the rates of degradation were quite similar for both sitostanyl ferulate and α-tocopherol (Fig. 3). The 1% samples of either antioxidant immediately began to be degraded and about a half of either antioxidant

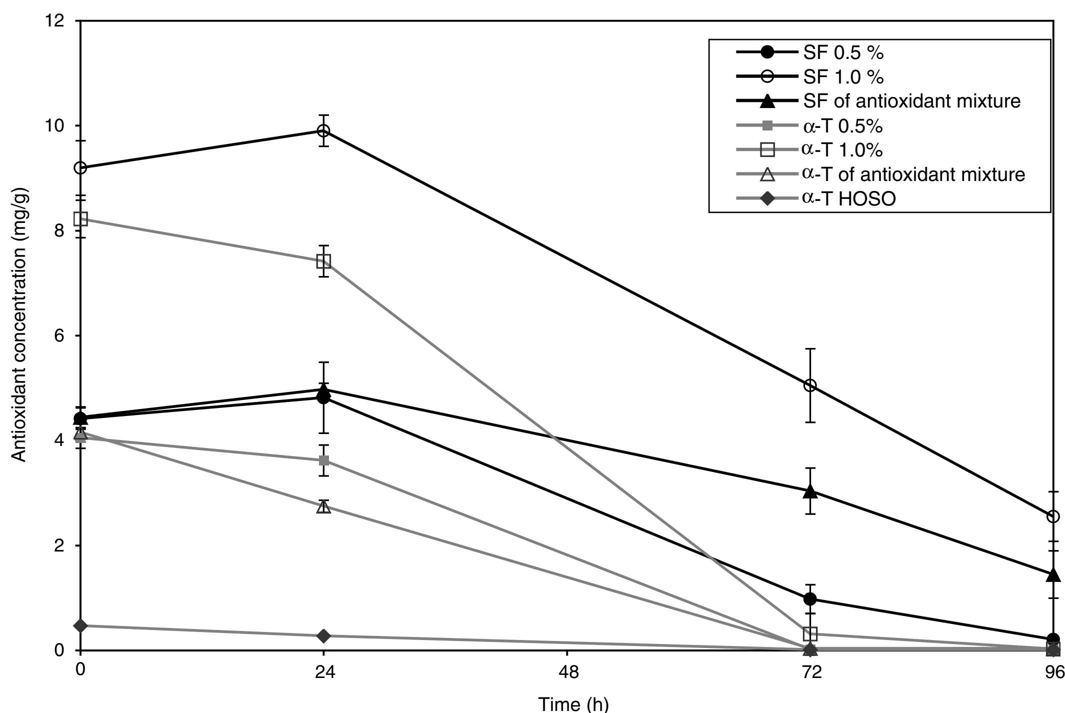

Fig. 2. A time-course study of the concentrations of antioxidants during heating of high oleic sunflower oil at 100 °C.

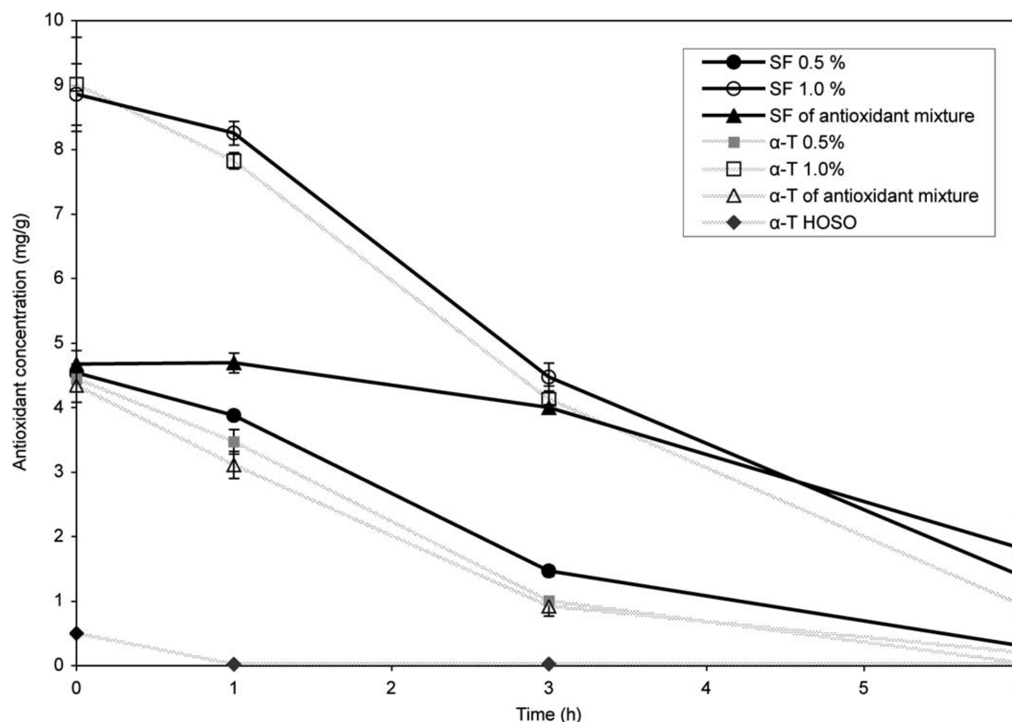

Fig. 3. A time-course study of the concentrations of antioxidants during heating of high oleic sunflower oil at 180 °C.

was still present halfway through the heating (3 h). The lower level samples (0.5%) of sitostanyl ferulate and α-tocopherol exhibited nearly equal degradation rates and were virtually all consumed by the end of 6 h oxidation. As was observed with the antioxidant mixture at the lower temperature, also at 180 °C α-tocopherol in the mixture was degraded much faster than sitostanyl ferulate and the rate of degradation was no slower than that of 0.5% α-tocopherol alone. Further, at this higher temperature, sitostanyl ferulate in the mixture degraded much slower than sitostanyl ferulate (0.5%) alone. Again one may conclude that, under these oxidation conditions, steryl ferulate does not protect α-tocopherol, but rather vice versa.

It is possible that the differences in the degradation rates of different antioxidants are not only caused by their actual actions as antioxidants possibly regenerating the other compound, but rather their general properties of heat stability. Theoretical, calculated value for the enthalpy of vaporization for sitosteryl ferulate is 100.54 ± 3.0 kJ/mol, whereas the respective value for α-tocopherol is only 83.48 ± 3.0 kJ/mol, indicating that α-tocopherol is more easily evaporated than sitosteryl ferulate. (These were calculated using Advanced Chemistry Development (ACD/Labs) Software Solaris V4.67, information gained from SciFinder Scholar-program supplied by the American Chemical Society). (Similar value for sitostanyl ferulate is not available, but the values for free sitosterol and sitostanol are nearly equal). Therefore, it is possible that the ability of sitostanyl ferulate to inhibit polymerization at high temperatures is not only caused by the ability to donate a hydrogen to a radical, but also due to good heat stability which ensures that the antioxidant is available for a longer period of time.

The formation of polymers in the unstripped high oleic sunflower oil was very slow if one only considers the content of α-tocopherol as the polymerization-preventing agent. The α-tocopherol concentration of the unstripped oil was 0.05% at the start of the experiment, which is one-tenth of the lower addition to the purified oil. α-Tocopherol of the unstripped oil was consumed fairly early in the experiment: at 180 °C, all of the tocopherol was used up after 1 h heating and still the rate of polymerization increased moderately, but not rapidly after this. A study by Barrera-Arellano and co-workers (2002) also shows that natural tocopherols in monounsaturated oil matrices are depleted relatively quickly and thus the oils are not protected by tocopherols over as long a time as polyunsaturated oils are. In their study, the natural levels of tocopherols (0.06% content) were entirely lost after a 6 h heating at 180 °C, which is much slower than in our experiment. However, the heating system was different and so the two studies cannot be strictly compared. α-Tocopherol is the major tocopherol in HOSO (contributing 98% of tocopherols) and so the good heat stability of the unstripped oil cannot be explained by significant concentrations of other tocopherols. The stripping of the oil may have also removed other minor components that affect the heat stability of natural oil. According to Yanishlieva and Marinova (2001), the composition of a natural oil is often close to ideal, so addition of any compound already naturally present in the oil does not necessarily enhance its heat stability. However, as steryl ferulates are not naturally present in common oils, with

the exception of rice bran oil, they could be added as natural antioxidants to enhance oil stability. Juliano and coworkers (2005) demonstrated that 10 mmol/kg addition of γ-oryzanol had an equal effect as the same concentration of BHT, indicating that the addition level of steryl ferulates need not necessarily be higher than those of other antioxidants.

The addition levels of antioxidants (0.5% and 1.0%) used in this study were relatively high, but it can still be argued that they were minor components in the system and were in line with the natural steryl ferulate concentration in rice bran oil. It is also noteworthy that, as these compounds were added on a percent basis, their molar concentrations were not equal. The molar concentration of sitostanyl ferulate was about 30% less than that of α-tocopherol, owing to their molecular weights being 592 and 431 g/mol, respectively. Even if the antioxidant concentration does not always positively correlate with antioxidant capacity, one may assume that the difference between antioxidant capacities of these compounds could have been greater if they were added on the basis of concentration and not weight.

3.3. A possible synergism between steryl ferulate and tocopherol

One aim of this study was to investigate whether steryl ferulates and tocopherols do in fact have synergistic antioxidant effects at high temperatures, as has been suggested in the literature. Synergism of two antioxidants is a condition in which two antioxidants as a mixture inhibit oxidation better than should be expected by adding together the effects of the individual compounds. The percentage of synergism was calculated at the endpoint of oxidation using the inhibition percentages of the antioxidant additions of 0.5% and the mixture that contained 0.5% of both antioxidants. The percentages of synergism were −22.7% and 3.2% at 100 °C (96 h) and 180 °C (6 h), respectively, which indicates that, at the concentration levels used in this study, there is no synergistic effect at 100 °C and only a very mild effect at 180 °C. A possible explanation for this could be that most significant synergistic effects have been demonstrated with a combination of antioxidants that act with a different antioxidant mechanism and thus prevent oxidation in different ways. For sitostanyl ferulate and α-tocopherol, however, the principal mechanism for inhibiting oxidation is hydrogen donation to a radical and therefore they do not significantly enhance the action of each other. The results gained with the conditions used in this study do show that the mixture of sitostanyl ferulate and α-tocopherol does effectively inhibit polymerization, but not in a synergistic manner.

4. Conclusion

This study demonstrates that sitostanyl ferulate can inhibit polymerization of high oleic sunflower oil at 100 and 180 °C at 0.5% and 1.0% addition levels. At the lower temperature, the capacity is somewhat less than that of α-tocopherol, but at the higher temperature their activities are similar. When combined, the overall effect of tocopherol and sitostanyl ferulate was nearly a sum of two individual components with no obvious synergistic effect. Further, sitostanyl ferulate did not protect α-tocopherol from degradation in the mixture samples, as has previously been suggested in the literature. Sitostanyl ferulate was more heat-stable than was α-tocopherol and thus it can be concluded that, at high temperatures, under the conditions applied in this study, sitostanyl ferulate is able to inhibit oxidation for a longer period of time, as it is present in the system for longer.

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