

Research Note

# Commonly used food antioxidants: a comparative study in dispersed systems

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## Abstract

Some commonly used antioxidants, namely BHA, BHT, TBHQ,  $\alpha$ -tocopherol and caffeic acid were comparatively studied in phosphatidylcholine liposomes and in o/w emulsions. Trolox was included as reference. Oxidation was monitored at 37 °C by measuring peroxide formation. Low polarity and sometimes the molecular size seem to be the determining factors for an appreciable antioxidant performance. BHA and BHT were the most effective at the low levels of addition used.  $\alpha$ -Tocopherol had an intermediate activity. The pro-/antioxidant behaviour of caffeic acid was concentration dependent. TBHQ activity was slightly better than that of caffeic acid and comparable with that of Trolox. The results add to knowledge for structure antioxidant activity relationships in various systems and may also have a practical outcome concerning the optimum levels of use.

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*Keywords:* BHT; BHA; TBHQ; Trolox;  $\alpha$ -Tocopherol; Caffeic acid; Antioxidant activity; Liposomes; Emulsions

## 1. Introduction

Numerous compounds have been reported to possess antioxidant properties. Their use in foods, however, is limited for various reasons, and only a restricted number is accepted in the list of GRAS substances or permitted additives by international bodies (Miková, 2001). Among them, phenolic compounds, synthetic or natural, have been extensively examined as lipid oxidation retardants in an array of lipid substrates. A great number of articles cover past and current knowledge on the activity of food phenolic antioxidants mostly in bulk oils (Cuppett, Schnepf, & Hall III, 1997; Nakatani, 1997; Pratt & Hudson, 1990; Schuler, 1990; Yanishlieva, 2001). Moreover, interest in their activity in multiphase systems is increasing as actual food products are multicomponent matrices (e.g. Cuvelier, Bondet, & Bercet, 2000; Frankel, Huang, Kanner, & German, 1994; Gordon, Paiva-Martins, & Almeida, 2001; Pekkarinen, Stöckmann, Schwarz, Heinonen, & Hopia 1999; Satue-Garcia, Heinonen, & Frankel 1997).

The mechanism that explains the performance of antioxidants in multiphase systems differs from that of inhibited lipid oxidation in bulk oils due to more complex interfacial phenomena that are expected to affect the activity (McClements & Decker, 2000).

This work is a contribution on the comparative study of the activity of some commonly used antioxidants, namely BHA, BHT, TBHQ,  $\alpha$ -tocopherol and caffeic acid, in phosphatidylcholine liposomes and in o/w emulsions. Trolox was included as a reference compound. The results of such studies add to knowledge for structure antioxidant activity relationships in various systems. Moreover, they may have a practical outcome regarding the optimum levels of use of the examined compounds.

## 2. Materials and methods

### 2.1. Standards, reagents and solvents

Caffeic acid (97%) and Trolox (6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid) (97%) were from Riedel de Haën, (Seelze, Germany).  $\alpha$ -Tocopherol was from Merck (Darmstadt, Germany), BHT, BHA, cupric acetate and *L*- $\alpha$ -phosphatidylcholine (PC) ~40% from

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soybean were from Sigma Chemical Co. (St. Louis, MO). TBHQ (97%) was supplied by Aldrich Chemical Co. (Steinheim, Germany). Triolein ~65% was from Fluka (Buchs, Switzerland). Methanol (HPLC grade), chloroform, 1-octanol (for analysis),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  were from Panreac Quimica, S.A. (Barcelona, Spain). Absolute ethanol (HPLC grade),  $\text{NH}_4\text{SCN}$ , and  $\text{FeCl}_3$  were from Riedel de Haën. Tween 20 was from Merck and silicic acid (mesh size 100–200) was from Sigma.

## 2.2. Apparatus

A U-2000 Hitachi spectrophotometer (Tokyo, Japan) was used for all absorbance measurements. For preparation of emulsion samples an Ultra Turrax T25 (Janke & KunKel, Berlin, Germany) homogenizer was used. The particle size of emulsions was measured with a Mastersizer 2000 (Malvern Inst., Malvern, UK).

## 2.3. Estimation of partition coefficient ( $P$ )

A solution (0.1 mM) of each compound in 1-octanol was kept at 37 °C for 30 min, and the UV spectrum was then run. Absorbance value at the maximum wavelength was recorded ( $A_o$ ). Equal volumes of organic and aqueous phases were vortexed (2500 rpm) for 1 min. After separation of the layers, the UV spectrum of the organic layer was recorded every 30 min till the absorbance reached a constant value ( $A_x$ ). The partition

coefficient  $P$  was calculated according to the relationship,  $P = A_x / (A_o - A_x)$ . A solution of 1-octanol saturated with water was used as the blank.

## 2.4. Oxidation in phosphatidylcholine liposomes

Lecithin was suspended in double distilled water at a concentration of 8 mg/ml by stirring with a glass rod and sonication for approximately 5 min. Liposome formation was obtained through additional sonication with a rod (UP 200S, dr.Hielscher, GmbH, Berlin, Germany) (2.5 min for 10 ml aliquots of the liposome sample). Ethanol solutions of the antioxidants were added into Erlenmeyer flasks at a final concentration of approximately 15 and 30  $\mu\text{M}$  for BHA, BHT, TBHQ, Trolox and  $\alpha$ -tocopherol, while caffeic acid was added at 60  $\mu\text{M}$ . Liposome aliquots were weighed into the flasks and diluted with double distilled water to a final lecithin concentration of 0.8% (w/w). The samples were oxidized by adding cupric acetate (3  $\mu\text{M}$ ) and shaking at 37 °C in the dark. Liposome oxidation was monitored according to Yi, Meyer, and Frankel (1997).

## 2.5. Oxidation in o/w emulsion

Commercial triolein [triacylglycerol species expressed in equivalent carbon number (ECN,%): 50, 9.2%; 48, 65%; 46, 10%; 44, 7% and 42, 8.6%] was purified in the laboratory on three chromatographic columns in

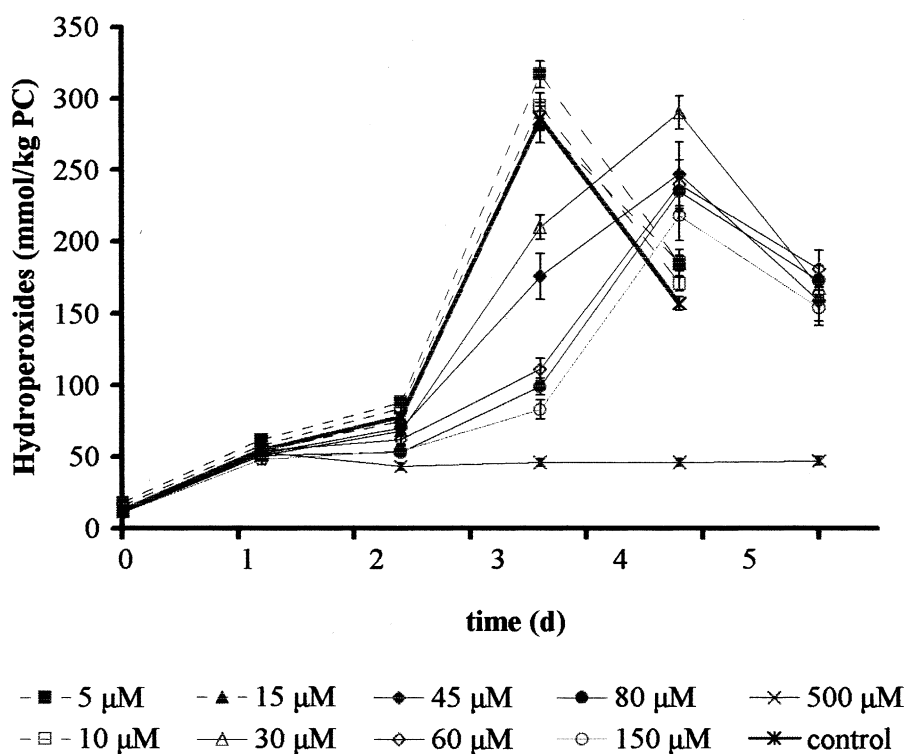


Fig. 1. Liposome oxidation with cupric acetate 3  $\mu\text{M}$  at 37 °C in the presence of caffeic acid at different levels of addition. Values of hydroperoxides are means of three measurements  $\pm$  standard deviation.

series. The first two were packed with activated carbon-Kieselguhr (1:2, w/w) while the third one was packed with silicic acid. Eluates were checked for their tocopherol content with HPLC (Psoimiadou & Tsimidou, 1998). After the third column the tocopherol content was null. Oil in water emulsions (10%, w/w) were prepared by homogenizing purified triolein (4.8 g), Tween 20 (0.55 g, 1%) and distilled water (44.5 g) (13 500 rpm, 1 min). Average particle size of micelles was 3.1  $\mu\text{m}$ . Antioxidants were then added in ethanol solutions and samples were purged with nitrogen. The final concentration for each antioxidant was 50  $\mu\text{M}$  (on oil weight basis). The initial pH of the emulsions was 5.6–5.9. Samples were placed in 100-ml Erlenmeyer flasks and incubated at 37 °C (120 rpm). The course of oxidation was monitored by measuring peroxide values (PV) using the ferric thiocyanate method (FTC) according to Shantha and Decker (1994) and conjugated diene formation at 234 nm ( $\epsilon=26\,000$  for methyl linoleate hydroperoxide) according to Chan and Levett (1977).

### 3. Results and discussion

Partitioning of an antioxidant between the lipid and aqueous phase defines its effective concentration in the former (McClements & Decker, 2000). A frequently

used descriptor for the estimation of the lipophilicity of phenolic compounds is the partition coefficient  $P$  between 1-octanol and water (Foti, Piattelli, Baratta, & Ruberto, 1996; Gordon et al., 2001; Vaes, Ramos, Verhaar, Cramer, & Hermens, 1998). Partition coefficient values should be interpreted with caution since the partitioning of antioxidant and consequently their efficacy may be influenced by factors such as charge, interactions with the emulsifier and pH (McClements & Decker, 2000). The  $P$  values (or the % partition in the organic phase,  $n=3$ ) estimated in this study were: caffeic acid,  $0.18\pm 0.01$  (or 10.5%); Trolox,  $0.49\pm 0.02$  (or 28.7%); TBHQ,  $0.54\pm 0.01$  (or 31.6%);  $\alpha$ -tocopherol,  $6.76\pm 0.39$  (or 87.1%); BHA,  $10.3\pm 0.35$  (or 91.7%) and BHT,  $12.6\pm 0.97$  (or 92.6%). Based on these values and if “the polar paradox” applies in the case of multiphase systems as it is suggested (McClements & Decker, 2000), caffeic acid should be the least active of the compounds under study.

Thus, it was considered useful to investigate first the behaviour of caffeic acid at different levels of addition (5–500  $\mu\text{M}$ ). In the selected range, caffeic acid showed antioxidant activity only above the concentration of 30  $\mu\text{M}$  (Fig. 1). It can be argued that its behaviour in a dispersed system is concentration dependent. At low levels of addition it seems to be ineffective or to promote oxidation in the presence of copper. Above a critical

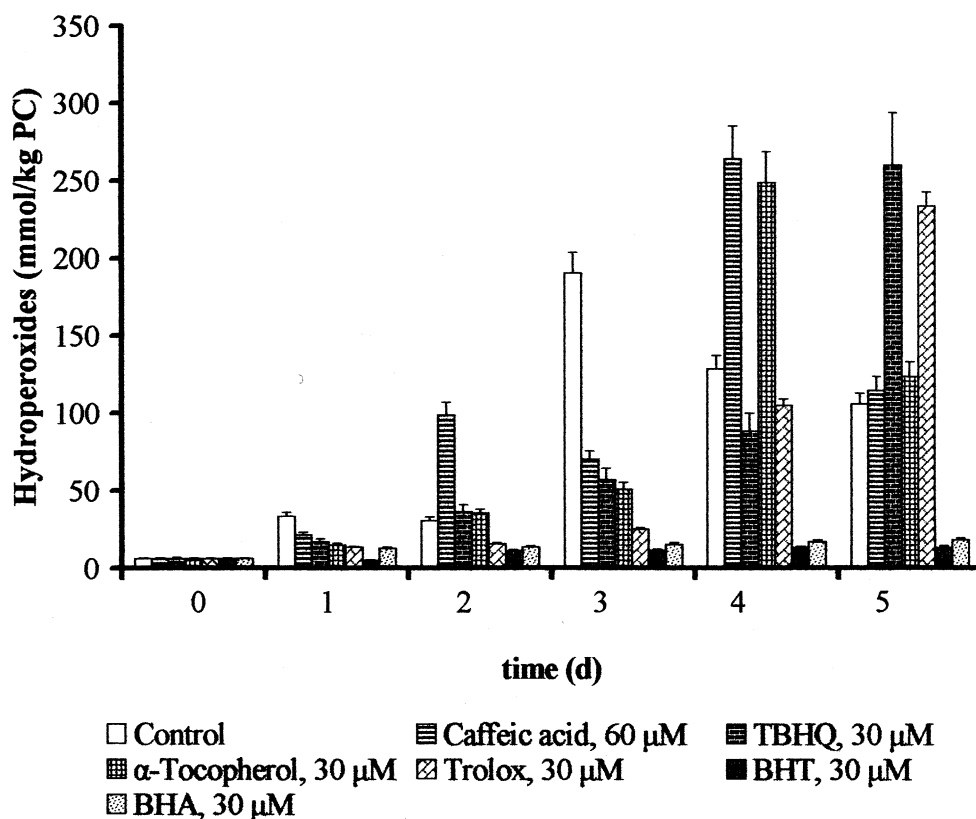


Fig. 2. Liposome oxidation with cupric acetate 3  $\mu\text{M}$  at 37 °C in the presence of phenolic compounds at 30  $\mu\text{M}$  and caffeic acid at 60  $\mu\text{M}$  level of addition. Values of hydroperoxides are means of three measurements  $\pm$  standard deviation.

level antioxidant capacity is prevailed. This result is also supported by the work of [Mei, McClements and Decker \(1999\)](#) who stated that “evaluation of the prooxidative/antioxidative balance of phenolics could provide useful information in predicting their antioxidant behaviour in lipid dispersions”. In subsequent experiments caffeic acid was used at the concentration of 60  $\mu\text{M}$  that ensured a clear antioxidant activity in the liposome system. To monitor oxidation within a reasonable length of time, the rest of the phenolic compounds were studied at 15 and 30  $\mu\text{M}$  levels, that is 2–4 times lower than that of caffeic acid. Oxidation experiments were carried out in triplicate. The profile of the course of oxidation was similar for all compounds and is illustrated selectively for the 30  $\mu\text{M}$  level of addition in [Fig. 2](#). Under the conditions of the present comparative study it was evidenced that all of the compounds were more effective

than caffeic acid though the latter was added at higher levels. TBHQ and Trolox had similar activity. These two compounds were stronger antioxidants than caffeic acid possibly due to their lower polarity. BHA and BHT retarded oxidation most efficiently during the monitoring period. The better activity of BHA and BHT in lecithin liposomes, in comparison to that of TBHQ and caffeic acid, has been also reported by [Porter, Black and Drolet \(1989\)](#).  $\alpha$ -Tocopherol was found to be less effective than its water analogue, Trolox. This implied that polarity might not always determine the antioxidant performance and that other factors should be also taken into consideration. The existence of the long side chain in  $\alpha$ -tocopherol leads to such a molecular conformation that may not allow the penetration of the liposome bilayers hampering, thus, the effective protection of lipids ([Castle & Perkins, 1986; Lucarini, Pedulli, & Valgimigli, 1998](#)).

In the oil/water emulsion system the antioxidants under investigation were studied at a relatively low level (50  $\mu\text{M}$ ). The level of addition was the same for all compounds based on data from preliminary studies. The results are illustrated in [Fig. 3a and b](#). BHA and BHT were proved to be the most potent in the triolein rich emulsion and inhibited almost completely the course of oxidation within the monitoring period. Both of them were better than  $\alpha$ -tocopherol in retarding autoxidation whereas Trolox was ineffective. Though the results found in the literature are sometimes contradictory ([Cort et al., 1975](#)), there is a general agreement for the better effectiveness of  $\alpha$ -tocopherol with comparison to that of Trolox in emulsions ([Cuvelier et al., 2000; Frankel et al., 1994; Huang, Hopia, Frankel, & German, 1996](#)). This activity is due to the presence of the phytol side chain that may impart better affinity toward oil particles in the interface or within the lipid droplets. Moreover, the lower performance of Trolox may be attributed to its partitioning into the water and Tween 20 micelles at the pH of our study (5.6–5.9) as was also noted for corn oil triglyceride emulsion ([Huang et al., 1996](#)). Caffeic acid, at the used level of addition, showed a clear prooxidative activity. This could not be directly related to reducing properties of caffeic acid ([Mei et al., 1999](#)) as no metal initiator was employed ([Bondet, Cuvelier, & Berset, 2000](#)). Conflicting results are presented in the literature concerning the relative activity of caffeic acid in o/w emulsions. [Chen and Ho \(1997\)](#) suggest that some polyphenols could induce the generation of hydrogen peroxide in aqueous solution, which then promote the oxidation of the emulsion. Others focus on the interactions of phenols with emulsifiers. In the presence of emulsifiers, increased solubility in the lipid phase does not necessarily ensure better performance of the phenol due to other factors such as hydrogen bonds ([Pekkarinen et al., 1999; Stöckmann, Schwarz, & Huynh-Ba, 2000](#)) though some opposing data can be also found ([Chen & Ho, 1997](#)). It is clear that more work is

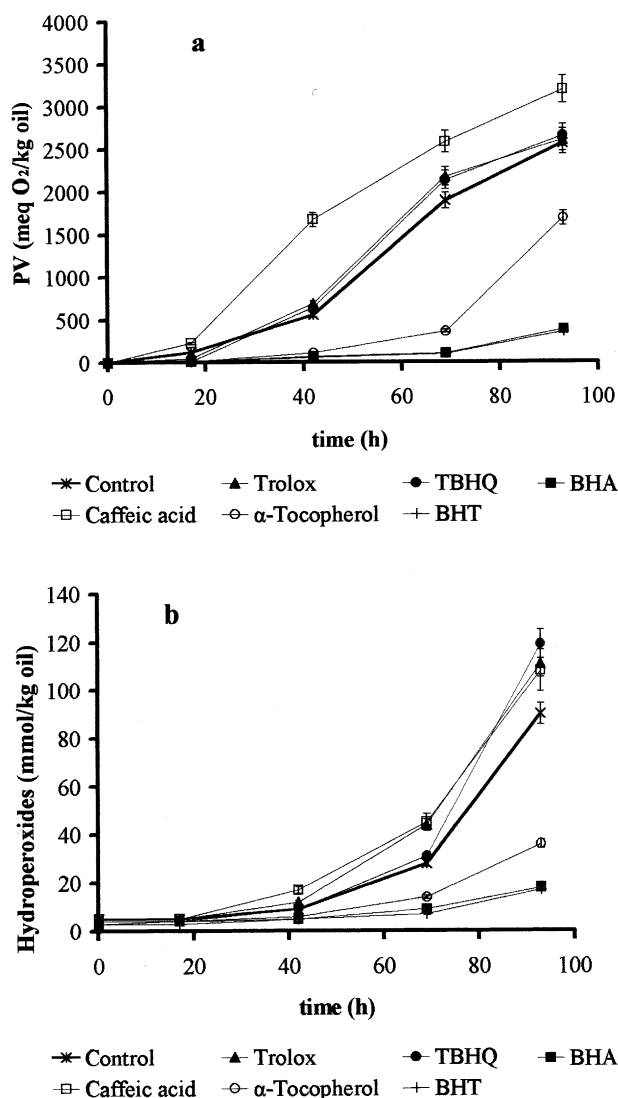


Fig. 3. Oxidation of a 10% o/w emulsion at 37 °C in the presence of phenolic compounds at 50  $\mu\text{M}$  level of addition (a) Peroxide values; (b) hydroperoxides formation at 234 nm. Values are means of three measurements  $\pm$  standard deviation.

needed to understand the behaviour of polar phenols in emulsions, especially of polyhydroxy ones. Indeed, poor effectiveness was also observed for the second diphenolic compound of our study, TBHQ that may be ascribed to the quinoid structure. The latter exhibited a similar to Trolox behaviour. Similarities in the effectiveness of TBHQ and Trolox have been also reported in the past (Cort et al., 1975) and coincide with closeness in the values of their partition coefficients ( $P$  values: 0.54 and 0.49, respectively). Our results are in accordance with those recently published by Cuvelier et al. (2000), who among other compounds, studied also BHA, BHT,  $\alpha$ -tocopherol, Trolox and caffeic acid in a linolenic acid emulsion.

Concluding, the low polarity of an antioxidant (e.g. BHA and BHT) and in certain cases the size of the molecule (e.g.  $\alpha$ -tocopherol–Trolox) seems to be crucial for a good performance in dispersed systems. The effectiveness of BHA and BHT was achieved at relatively low concentrations, a prerequisite in food applications. The discussion concerning the toxicity of synthetic antioxidants is still open but one should note that these compounds are very effective in multiphase systems where some natural antioxidants might be less effective or even pro-oxidative. On the other hand,  $\alpha$ -tocopherol is an antioxidant of average potency but free of disputes for its use. Our results may have a practical outcome concerning the optimum levels of addition.

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