Do Antioxidants Improve the Oxidative Stability of Oil-in-Water Emulsions?

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ABSTRACT: Oil-in-water emulsions were prepared with 30% stripped sunflower oil, stabilized by 20 g/L BSA and homogenized under high pressure to obtain a mean droplet size near 0.5 µm. The emulsions were shown to be physically stable during storage in a shaker at 47°C for 5 d. Such a medium was suitable to test the efficiency of different types of antioxidants. Oxidation of control emulsions appeared rapidly without a lag phase, and the contents of conjugated dienes and hexanal reached a plateau after around 20 h. In the presence of EDTA, the oxidation was strongly inhibited, suggesting that some metallic ions present in the oil or the protein solution act as inducers. Ascorbic acid and ascorbyl palmitate were inactive. Isoeugenol was found to be a powerful antioxidant, better than eugenol, α-tocopherol, and Trolox.

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KEY WORDS: Antioxidant, ascorbic acid, EDTA, eugenol, isoeugenol, o/w emulsion, α-tocopherol, Trolox.

Food emulsions are structured foods that are commonly stabilized by surface-active agents such as proteins. In emulsified media, the lipid oxidation rate and antioxidant behavior are different from bulk oil systems because of the partitioning of components between the oil phase, the water phase, and the interfacial region (1). This region is particularly important in lipid oxidation since the initiation stages of lipid oxidation take place essentially at the interface (2). Some recent works report the effects of emulsifiers, pH, transition metals, and a few antioxidants on the oxidative stability of oil-in-water (o/w) emulsions (3–7). Free transition metals seem to play a key role owing to their ability to decompose lipid hydroperoxides into free radicals (5). Unfortunately, metals are ever-present in food systems as constituents of ingredients, water, or packaging. Their prooxidant activity is controlled by emulsifiers, which can facilitate in some cases or limit in other cases the contact between transition metals located in the aqueous phase and hydroperoxides located in the oil phase (3). Low pH makes metals more soluble in the water phase (3,6). Some antioxidants may chelate transition metal ions, hence reducing metal-induced oxidative reactions (6). In contrast, compounds able to reduce Fe^{3+} to the more active prooxidant Fe^{2+} (for example, ascorbic acid and some phenols) can increase the oxidation rate (4). Surfactants can protect lipids from oxidation either by their ability to form an interfacial film that reduces exchange between the reactive species or by concentrating antioxidants in the interfacial region (7).

In previous works (8,9), we examined the behavior of phenolic antioxidants in a micellar medium stabilized by Tween 20, where peroxidation of linoleic acid was induced by the Fe^{2+}/ascorbic acid system. In such a system, where the concentration of metallic ion and ascorbate was just sufficient to start the chain reactions, the chelator activity of the antioxidants seemed to play only a minor role. Both the polarity and the radical-scavenging capacity of antioxidants were shown to be related to their efficiency (9).

Rampon *et al.* (10), using an emulsion of stripped sunflower oil in water stabilized by purified BSA, found that small emulsion droplets favor a rapid development of oxidation. Lethuaut *et al.* (11) showed that in this system the greater the interfacial area, the faster the oxidation of lipids. Thus, the objective of the present work was to investigate the activity of antioxidants in such an emulsified system without added prooxidative metals; such a system is more representative of food media than the linoleic acid model system.

EXPERIMENTAL PROCEDURES

Chemicals. Pentane and isopropanol of analytical grade came from Carlo Erba (Val de Reuil, France). Commercial sunflower oil was stripped of tocopherols and minor compounds by using alumina adsorption chromatography according to the procedure described by Lethuaut *et al.* (11). Pentane used as the eluting solvent was evaporated from purified oil under vacuum and then traces were removed by flushing the oil with nitrogen gas. Tocopherol content in oil was less than 0.2 mg/kg oil as measured by HPLC analysis [procedure using silica column, hexane/isopropanol 98:2 (vol/vol) as eluting solvent; detection at 295 nm]. The stripped oil was stored under nitrogen gas at −20°C until used (within a few days). Oil composition was 60.36 ± 0.97% linoleic acid, 27.90 ± 0.98% oleic acid, 6.50 ± 0.14% palmitic acid, and 3.99 ± 0.48% stearic acid of the total FA ($n = 3$) as measured by GC analysis (procedure using methyl ester preparation, petroleum ether extraction, HP-WAX silica column separation, and C_{17}-TAG as internal standard).

Ultrapure water (resistivity 10–15 M Ω·cm, total organic compounds <30 mg/L) was prepared with Elix 3 system (Millipore, St Quentin en Yvelines, France). Powdered BSA

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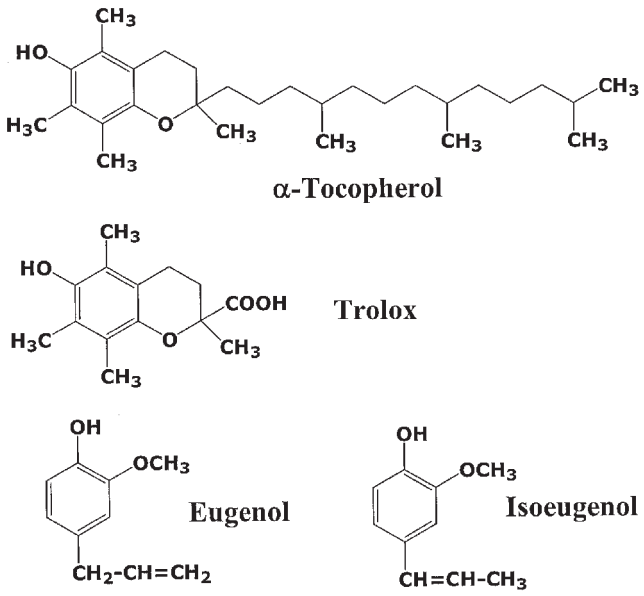
(fraction V, pH 5.2, purity 95–98%) was purchased from ICN Biomedicals Inc. (Aurora, OH). α-Tocopherol, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), eugenol, isoeugenol, EDTA, ascorbic acid, and ascorbyl palmitate were purchased from Sigma-Aldrich-Fluka (St Quentin Fallavier, France). Structures of phenolic antioxidants are shown in Scheme 1.

Preparation of emulsions. BSA solution (pH adjusted to 4.3 with HCl; 20 g/L; 175 mL) and stripped oil (75 mL) were emulsified for 2 min at 23,000 rpm with a rotor-stator homogenizer (Polytron PT 3100; Kinematica, Littau, Switzerland) fitted with a 12-mm-diameter head. The emulsion was then circulated through a two-stage (first and second valves set at 200 and 40 bar, respectively) high-pressure homogenizer (NIRO SOAVI 1001 Panda type; Parma, Italy). To obtain 0.5 μm droplet size, emulsions required 12 passes, i.e., 24 min of homogenization. As the temperature of the emulsion reached around 30°C at the end of the process, the emulsion was quick-cooled to ambient temperature by immersion in an iced water bath. Aliquots (3.5 mL) of emulsion were distributed in headspace vials (22.4 mL) and sealed with silicon/polytetrafluoroethylene septa and aluminum crimp seals. Before emulsification, methanolic solutions of apolar antioxidants (α-tocopherol, Trolox, eugenol, isoeugenol, ascorbyl palmitate) were added to stripped oil and polar antioxidants (ascorbic acid, EDTA) to BSA solution to achieve a 100 mg/kg concentration in the final emulsion.

Accelerated oxidation of emulsions. The emulsions were placed in an orbital shaking bath at 140 rpm and kept in the dark at 47°C. This temperature was chosen to accelerate the oxidation without changing the structure of the emulsion. In such conditions measurements were completed within 1 wk. At regular intervals, emulsion samples of each experiment were taken and oxidation status was determined. To obtain sufficient data over the initial 24 h, given that no analyses were conducted during the night, a few samples were stored at 4°C during the first day to minimize the oxidation reactions until the next morning. The physical and chemical state of these emulsions proved to be unchanged after refrigeration.



SCHEME 1

Characterization of droplet size of emulsions. Droplet size was determined with a laser granulometer Mastersizer (Malvern Instruments, Orsay, France) fitted with a 45-mm lens and a liquid sampler MS15 containing distilled water. Particle size distribution was presented as volume percentage vs. droplet diameter. Measurements were performed in triplicate on each emulsion and averaged.

Conjugated dienes (CD). CD were measured by spectrophotometry at 233 nm. Aliquots of emulsions were diluted three times in isopropanol to obtain a lipid concentration less than 500 mg/L. The isopropanol solutions were clarified by centrifugation for 4 min at 1200 × *g,* and the absorbance of the supernatants was measured. Measurements were performed at least in duplicate on each emulsion sample and averaged. Data were expressed in mM CD/kg of oil using 27,000 M^{-1} cm^{-1} as the molar extinction coefficient (12).

Hexanal measurement. The appearance of hexanal as a marker of oxidation was followed by static headspace GC. The vials containing emulsions were thermostated at 60°C for 30 min in a headspace sampler HS 40 XL (PerkinElmer, Norwalk, CT). After a 3-min pressurization with helium as the carrier gas, the headspace was injected during an interval of 0.10 min through the transfer line set at 115°C to the gas chromatograph (XL; PerkinElmer). The gas chromatograph was fitted with a DB-5 column (J&W Scientific, Folsom, CA) (30 m × 0.25 mm × 0.25 μm). The initial temperature of the oven was maintained at 35°C for 2 min, then increased to 80°C at a rate of 5°C/min, and to 220°C at a rate of 20°C/min. The FID was set at 250°C. Hexanal was identified by comparing the retention time of the peak at 6.5 min with that of standard hexanal and by GC–MS. Concentrations expressed in mg/L of emulsion were determined from peak areas using a standard curve made from standard hexanal added to the emulsion. Measurements were performed at least in duplicate on each emulsion sample and averaged.

RESULTS AND DISCUSSION

Droplet size distribution of o/w emulsions. The high-pressure homogenization at 240 bar gave a very small particle diameter (Fig. 1). The distribution was monomodal, and the mean droplet size was 0.42 ± 0.03 μm for the control emulsion (average of 10 emulsions). Droplet size and distribution were quite stable for at least a 5-d storage at 47°C. A small increase in the mean droplet size (0.47 μm) over the 5-d period was due to the presence of a few particles above 1-μm diameter (Fig. 1). No creaming was observed after 10 d, even in the absence of rotating agitation of the emulsions. Adding antioxidants did not change these characteristics. Thus, the medium appeared to be physically stable.

Oxidation of emulsions during accelerated aging. The production of CD and hexanal was followed for at least 80 h. The

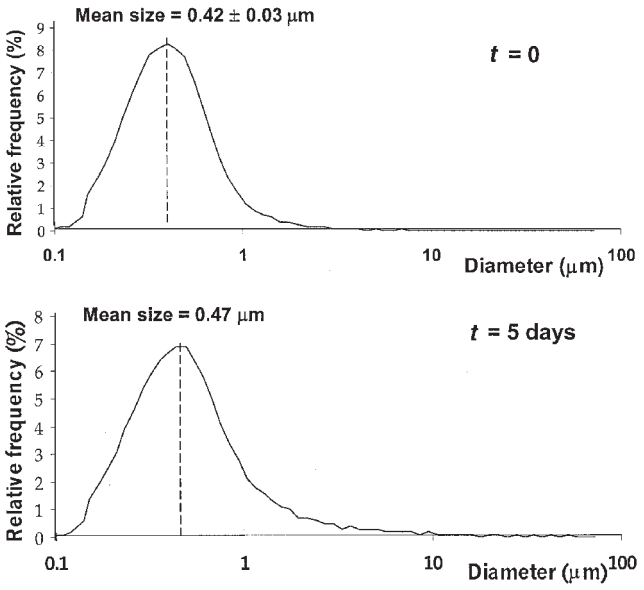


FIG. 1. Particle size distribution of oil-in-water (o/w) emulsions at $t = 0$ and after 5 d of aging.

curves in Figure 2 result from seven different emulsions. There are only 2–5 points per time because the interval between each measurement was not exactly the same from one experiment to another. The averages of the CV calculated at each time of the ascending part of the curves were 10.5% for CD and 10% for hexanal. These curves were used as control curves in all the antioxidant trials. Owing to the small size of the droplets, oxidation reactions took place rapidly without a lag phase, and CD and hexanal reached a plateau at about the same time (around 20 h). Table 1 gives the slope of the curves at the maximal rate of oxidation.

Role of metallic ions. EDTA (342 μM) delayed the oxidation for 20–30 h and decreased the oxidation rate for at least an additional 30 h, as indicated by both CD and headspace

TABLE 1
Maximal Rate of Oxidation, Calculated as the Slope of the Propagation Phase

	Maximal rate of oxidation (slope)	
	Conjugated dienes/h	Hexanal/h
Control	10.9	12.5
EDTA	3.8	7.1
Ascorbic acid	18.8	19.9
Ascorbyl palmitate	11.9	12.0
Trolox[a]	5.1	12.5
α-Tocopherol	2.5	7.1
Eugenol	2.8	2.3
Isoeugenol	0.8	1.5

[a]Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

hexanal (Fig. 3, Table 1). As EDTA is a metal chelator, the delay obtained in this case suggests that lipid oxidation of the emulsion is promoted by metallic ions that are naturally present in the oil, BSA, and/or water. In our system at pH 4.7, the prooxidative role of endogenous transition metals is favored, as the low pH increases the solubility of metallic ions, particularly of iron (5,6).

Mancuso *et al.* (3) found that 50 μM EDTA inhibited the oxidation of salmon o/w emulsions stabilized by synthetic surfactants such as Tween 20, SDS, or dodecyltrimethylammonium bromide for the entire incubation period of 150 h. Several hypotheses could explain the lower efficiency of EDTA in our system. First, a higher oxidation rate was reached in our conditions due to higher oil content (30 vs. 5%) and higher aging temperature (47 vs. 32°C). Second, the medium is more structured, a property that lowers the degree of freedom of the species. Finally, interactions of EDTA with BSA may decrease the ability of EDTA to chelate metals.

In test systems containing ascorbyl palmitate (241 μM), the presence of the antioxidant had no effect on the oxidation rate (Fig. 3, Table 1). A slight prooxidant effect was found in

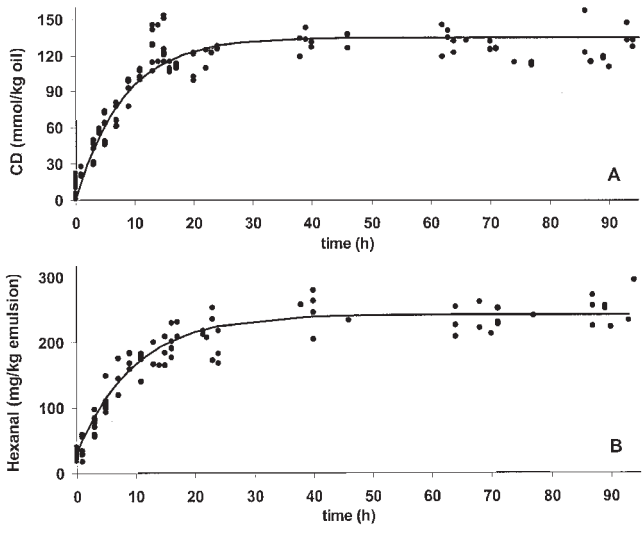


FIG. 2. Production of (A) conjugated dienes (CD) and (B) hexanal during oxidation of control o/w emulsions. See Figure 1 for other abbreviation.

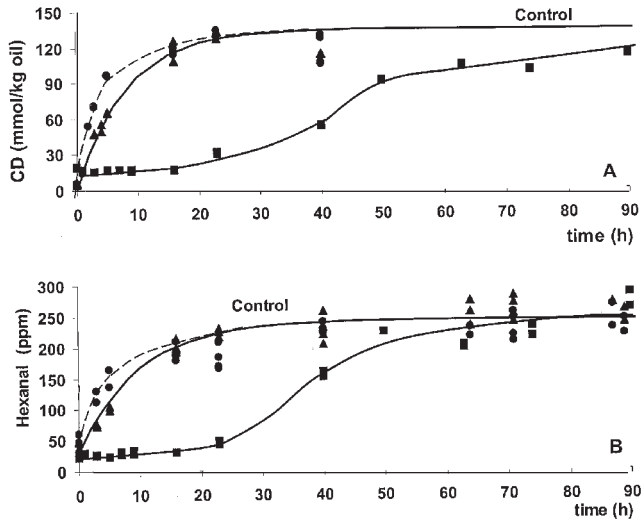


FIG. 3. Production of CD (A) and hexanal (B) during oxidation of o/w emulsions with EDTA (■), ascorbic acid (●), and ascorbyl palmitate (▲). For abbreviations see Figures 1 and 2.

the first five hours of oxidation in the ascorbic acid (568 μM) treatment (Fig. 3, Table 1). Although ascorbic acid is a well-recognized antioxidant, its scavenger effect on free radicals is somewhat limited (13). Paiva-Martins *et al.* (14) added 10 times more ascorbic acid than phenolic compounds to obtain an equivalent effect on liposome oxidation. In contrast, Van Ruth *et al.* (4) observed that 250 μM of ascorbic acid or ascorbyl palmitate had no effect on CD formation, but they did find a significant effect on the formation of hexanal and other odor-active compounds. In this case, the emulsions were prepared from nonstripped vegetable oil containing tocopherols, which are well-known antioxidants that can be regenerated by ascorbic acid.

Finally, as one would expect from the revelation of the presence of metallic ions through EDTA kinetics, ascorbic acid and ascorbyl palmitate also can be inducers of oxidation by regenerating prooxidant metallic ions in the same manner as in the Fe^{2+}/ascorbate-induced test (8). By reducing Fe^{3+} to Fe^{2+}, a stronger peroxide decomposer, ascorbic acid turns into a prooxidant. In our system, where there are no tocopherols to be regenerated, it is possible that the kinetics showed both pro- and antioxidant effects of ascorbic acid and ascorbyl palmitate.

Effect of phenolic antioxidants. The four phenolic compounds tested here are not known as metal chelators, so their efficiency can be directly related to their radical-scavenging activity. α-Tocopherol, Trolox, eugenol, and isoeugenol all presented a noticeable antioxidant effect against peroxide production and, except for α-tocopherol, against hexanal production as well (Figs. 4–6). Isoeugenol was the most efficient.

Trolox showed a classic antioxidant behavior. After a lag phase of about 20 h, the oxidation to hexanal occurred at exactly the same rate as for the control (Fig. 4, Table 1). Trolox acts by preventing peroxyl radical formation and thus prolongs the induction phase of oxidation. When all Trolox is consumed, the propagation phase starts in the same way as in the control.

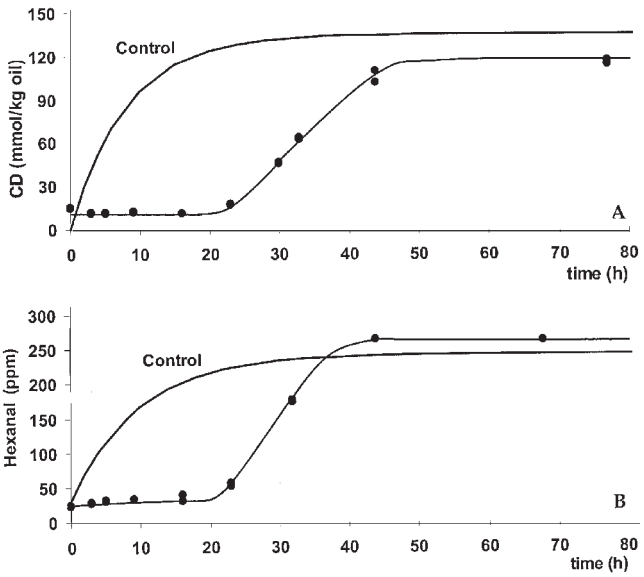


FIG. 4. Production of CD (A) and hexanal (B) during oxidation of o/w emulsions with Trolox (●). For abbreviations see Figures 1 and 2.

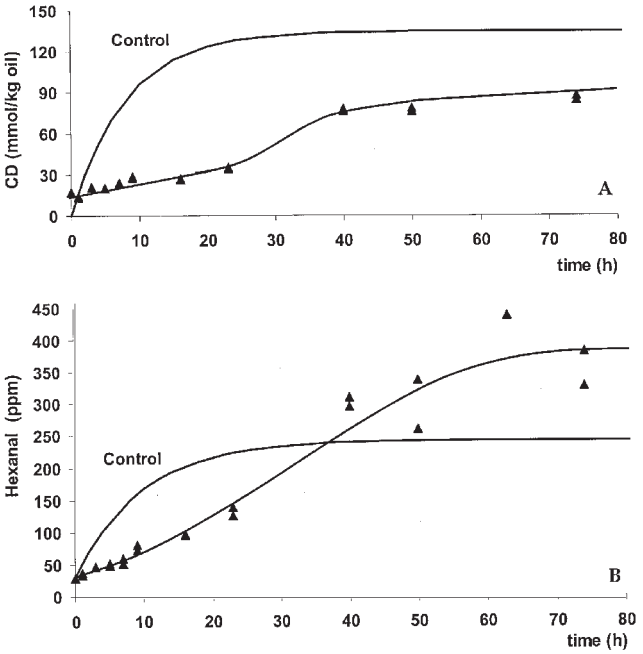


FIG. 5. Production of CD (A) and hexanal (B) during oxidation of o/w emulsions with α-tocopherol (▲). For abbreviations see Figures 1 and 2.

Trolox appears to be more powerful in this system than in biological systems. Indeed, at pH 4.3 in our system, Trolox, the p*K* of which is about 4, exists partly as nonionized free acid and may be located at the interface of the droplets. However, at the usual pH of 6–7 in biological systems, the acid group of Trolox is in the ionized form. On the one hand this form is more polar than the neutral form, but on the other hand electrostatic repulsion could occur with BSA, which is in the anionic form.

The behaviors of α-tocopherol, eugenol, and isoeugenol are different from that of Trolox. Overall, they acted by slowing the oxidation rate with no clear lag phase (Figs. 5,6;

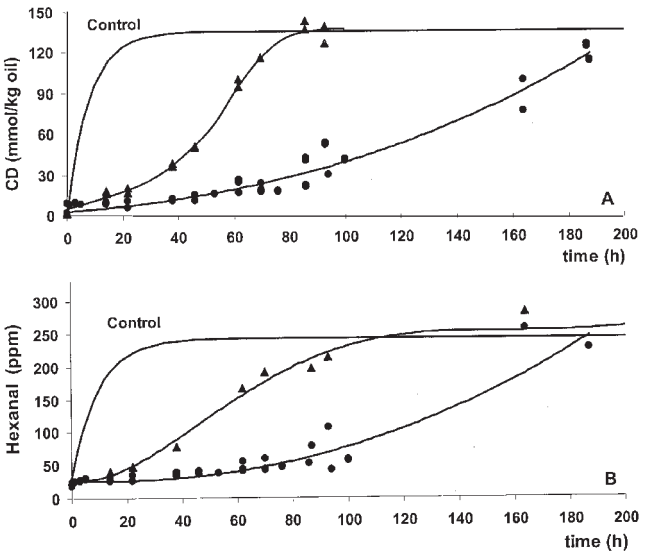


FIG. 6. Production of CD (A) and hexanal (B) during oxidation of o/w emulsions with eugenol (▲) and isoeugenol (●). For abbreviations see Figures 1 and 2.

Table 1). This is particularly evident for isoeugenol. Eugenol, isoeugenol, and α-tocopherol prevented the formation of CD from peroxyl radicals, thus slowing the propagation stage of oxidation.

The behavior of α-tocopherol is unexpected (Fig. 5). In the early stages of oxidation, α-tocopherol clearly acted as an antioxidant, and after 80 h the CD level was under control. But after only 40 h, hexanal production was higher than in the control, thus showing a later and selective prooxidant activity (Fig. 5). The prooxidant effect of α-tocopherol has already been observed (15,16); however, the concentrations used were higher than 500 ppm in emulsions and 250 ppm in bulk oil, whereas we used only 100 ppm. Moreover, the prooxidant effect was observed in these studies only at the early stages of oxidation and only vs. peroxides and not hexanal. In our study, the emulsions possibly were destabilized, favoring the transfer of hexanal to the aqueous phase and its release into the headspace. This result has to be verified.

The greater efficiency of isoeugenol vs. eugenol, as shown in Figure 6, may be related to the structural differences between the two molecules. That isoeugenol has an additional possibility of conjugation enhances the activity of the aromatic phenolic ring and improves the stability of the radical. The finding of high antioxidant activity of isoeugenol in emulsified media is interesting because it opens a new field of applications in cosmetic or food industries.

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