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Antioxidant Activity of γ-Oryzanol and Synthetic Phenolic Compounds in an Oil/Water (O/W) Emulsion System

-Research Note-

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Abstract

γ-Oryzanol is one of the chain breaking antioxidants. Both sterol (triterpene) and phenolic hydroxyl groups in the structure of γ-oryzanol may be responsible for its antioxidative function. We hypothesize that γ-oryzanol is more effective in preventing the autoxidation of polyunsaturated fatty acid (PUFA) than the synthetic phenolic compounds in an oil/water (O/W) emulsion system. The antioxidative effectiveness of different concentrations of γ-oryzanol and synthetic antioxidants was evaluated at different incubation times (0, 4, 8, 16, and 32 h) by measuring both the formation of hydroperoxides and the decomposition product of hydroperoxides (hexanal) in each emulsion system. Overall, the order of effectiveness of various antioxidants for inhibiting the formation of hydroperoxide in the O/W emulsion was: γ-oryzanol> *tert*-butylhydroquinone (TBHQ)> butylated hydroxytoluene (BHT)> butylated hydroxyanisole (BHA). O/W emulsion with selective lower concentrations of γ-oryzanol showed better effectiveness than that with higher concentration of synthetic antioxidants. However, the ability of both γ-oryzanol and synthetic antioxidants to decompose hydroperoxide was similar. γ-Oryzanol was more effective antioxidant than the synthetic phenolic compounds in preventing the formation of hydroperoxide in the O/W emulsion system.

Key words: γ-oryzanol, antioxidant, hydroperoxide, phenolic compound, emulsion

INTRODUCTION

Phenolic compounds are well-known chain-breaking antioxidants used to inhibit lipid radical chain reactions. However, their effectiveness is difficult to predict because there are several distinctly different mechanisms by which phenolic compounds influence the rate of lipid oxidation (1). Antioxidants have been evaluated by different methods of measuring oxidation in different lipid systems. The relative effectiveness of antioxidants is dependent on the lipid substrates, test system, concentration, oxidation time, and the method used to determine lipid oxidation (2,3). Evaluation of the effectiveness of antioxidants in emulsions has been difficult due in part to the complex interfacial phenomena (4). Hence, only limited data have been reported on the oxidative stability of antioxidants in different lipid emulsion systems.

γ-Oryzanol, an extract of rice bran, was reported to have potential antioxidant functions in foods and biological systems because it contains the ferulic acid structure (5). Also, γ-oryzanol is referred to as a free radical scavenger due to its capability of inhibiting lipid radical chain reactions. It is one of the natural phenolic chain-breaking antioxidants, which are associated with reducing the risk of a number of diseases caused by PUFA autoxidation in the cellular membrane of biological systems (5,6). Recently, the use of natural antioxidants has been reemphasized because of the increasing limitations on the use of synthetic antioxidants which have been suggested to have adverse health implications. In general, natural antioxidants are considered to be safer and are preferred by consumers.

The objective of this study was to systematically compare the effectiveness and the interactive effects of various parameters including concentration, physical state, and incubation time among the natural (γ-oryzanol) and the synthetic phenolic antioxidants (BHA, BHT, and TBHQ) in different O/W emulsions incubated at 80°C. The effectiveness of the phenolic antioxidants was evaluated at different oxidative stages by monitoring both the formation of hydroperoxides (conjugated dienes) and the decomposition of hydroperoxides (hexanal) in various emulsion systems.

MATERIAL AND METHODS

Materials

The following antioxidants were obtained from com-

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mercial sources: γ-oryzanol (Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan); BHT, TBHQ, and BHA (Sigma Co. Ltd., St. Louis, MO); Tween 20 (polyoxyethylene sorbitan monolaurate) (Sigma Co. Ltd., St. Louis, MO) and sepigel (polyacrylamide C13-14 isoparaffin laureth-7) (Seppic, Paris, France).

Preparation of emulsion samples

Mixtures of stripped soybean oil containing different amounts of γ-oryzanol (0, 25, 50, 100, and 200 ppm) or synthetic phenolic antioxidants (200 ppm BHT, TBHQ, and BHA) were prepared by direct addition. Ten percent (wt/vol) oil-in-water emulsion (100 mL) was prepared by mixing 10 g of the antioxidant-containing oil with 100 mL deionized water in a 100-mL Erlenmeyer flask and emulsifying with 10 g of Tween 20 and 10 g of Sepigel. The preparation of an emulsion was modified from the method of Frankel et al. (2).

Oxidation

Emulsion samples were incubated at 80°C in a shaker water bath. Oxidative stability was determined by measuring the conjugated diene hydroperoxide with spectrophotometer and hexanal by headspace Gas Chromatography (GC). All oxidation conditions and determinations were performed in duplicate.

Quantification of conjugated diene hydroperoxides

Measurements of the emulsion samples were carried out by a modified method described by Frankel et al. (2). Emulsion samples (5 mL) dispersed in methanol (7.5 mL) was first extracted with hexane (2.5 mL), and the extracts were evaporated to dryness under a stream of nitrogen, and redissolved in isooctane (25 mL). After the emulsions were incubated for 0, 4, 8, 16 and 32 hr, the absorbances of their conjugated diene were measured at 234 nm. Results were expressed as hydroperoxides in millimoles per kilogram of oil at absorptivity of 26,000 for linoleate hydroperoxides (7).

Quantification of hexanal by static headspace gas chromatography

Measurements of hexanal in the emulsion samples after incubation for 0, 4, 8, 16 and 32 hr were carried out according to the same procedures as described by Frankel et al. (2). Briefly, gas chromatography (Hewlett-Packard Co., Avondale, PA) with headspace autosampler (Tekmar Co., Cincinnati, OH) and a capillary DB-1701 column (30 m×0.32 mm×1-μm thickness, J&W, Folsom, CA) heated isothermally at 65°C was used. The gas chromatography conditions were as follows: helium linear gas velocity at 20 cm/s (helium head column pressure, 30 psi); splitless injector temperature at 180°C; and detector temperature at 200°C. All hexanal determinations of the emulsion samples were made after 0, 4, 8, 16, and 32 hr of incubation.

Statistical analysis

One-way analysis of variance (ANOVA) was calculated on the data obtained from the whole incubation period. Multiple comparisons between means were carried out by Tukey's studentized range test. Data were analyzed at the significance level of $p = 0.05$ (8).

RESULTS AND DISCUSSION

In the control emulsion, hydroperoxide formation increased sharply after 16 hr of incubation (Fig. 1). With the addition of γ-oryzanol, hydroperoxide formation was inhibited. This inhibition increased with the oxidation time at all levels of γ-oryzanol tested (Table 1). In the presence of 25 or 50 ppm of γ-oryzanol, the rate of hydroperoxide formation was not significantly different from the control until 16 hr of incubation. After 16 hr, significant differences were noted (Fig. 1). On the other hand, in the presence of 100 or 200 ppm of γ-oryzanol, the inhibition of hydroperoxide formation was significantly lower than the previous two treatments (Fig. 1). With the highest concentration (200 ppm) of γ-oryzanol used, the rate of hydroperoxide formation was quite stable (Fig. 1).

In the presence of γ-oryzanol, hexanal formation was inhibited at all levels in the emulsions (Fig. 2). In contrast to hydroperoxide formation, the inhibition of hexanal formation was not significantly different between 100 and 200 ppm of γ-oryzanol used (Table 1). The rate of hexanal formation decreased with an increase in the concentration of γ-oryzanol (Fig. 2). Overall, the inhibition of hexanal formation during incubation was

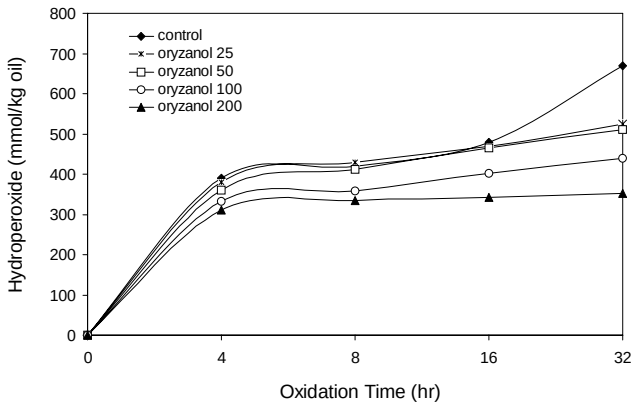

Fig. 1. Conjugated diene hydroperoxide detected in an oil/water emulsion system containing different concentrations of γ-oryzanol incubated at 80°C for 0, 4, 8, 16, and 32 hr.

Table 1. Percentage of inhibition of hydroperoxide and hexanal in an oil/water emulsion by γ-oryzanol and various synthetic phenolic compounds (% mean inhibition±SD)[1)]

Sample	Oil in Water (O/W) Emulsions			
	Hydroperoxide		Hexanal	
	4 hr	32 hr	4 hr	32 hr
Control	0.0±0.5[a2)]	0.0±0.8[a]	0.0±2.3[a]	0.0±1.1[a]
Oryzanol 25	2.6±0.3[ab]	21.7±1.2[b]	25.2±3.5[b]	36.1±3.2[b]
Oryzanol 50	7.7±0.4[b]	23.8±0.2[b]	33.6±3.5[b]	43.1±2.1[b]
Oryzanol 100	14.9±0.7[c]	34.4±0.4[c]	46.2±1.1[c]	64.5±1.5[c]
Oryzanol 200	20.5±0.3[d]	47.2±0.6[d]	50.0±0.5[c]	71.7±2.3[c]
Control	0.0±0.5[a]	0.0±0.8[a]	0.0±2.3[a]	0.0±1.1[a]
BHA	4.7±0.9[b]	18.5±0.6[bc]	36.1±1.2[b]	61.9±0.5[b]
BHT	7.9±1.4[b]	23.7±0.6[bc]	40.7±0.5[b]	64.5±1.5[b]
TBHQ	8.9±2.1[b]	28.5±0.2[c]	44.5±4.7[bc]	66.1±2.7[b]
Oryzanol	20.5±0.3[c]	47.2±0.6[d]	50.0±0.5[c]	69.7±2.3[b]

[1)]% inhibition = [(C − S)/C]×100; C = hydroperoxide or hexanal detected in control and S = hydroperoxide or hexanal detected in sample.
[2)]Values within each column followed by the same letter are not significantly different ($p<0.05$).

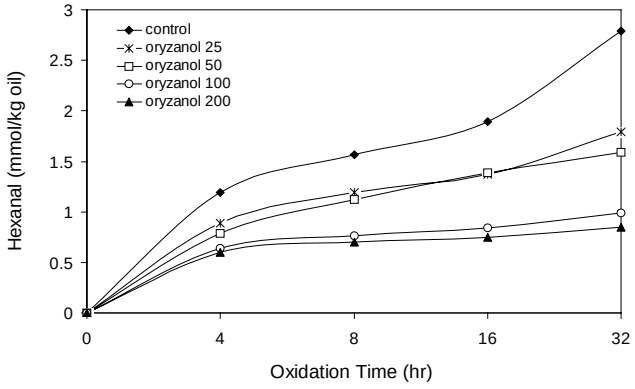

Fig. 2. Hexanal detected in an oil/water emulsion system containing different concentrations of γ-oryzanol incubated at 80°C for 0, 4, 8, 16, and 32 hr.

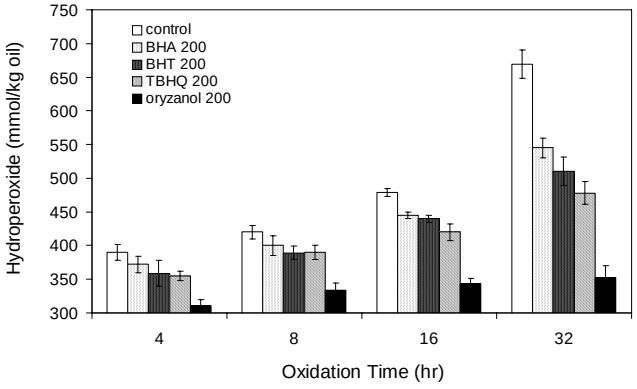

Fig. 3. Conjugated diene hydroperoxide detected in an oil/water emulsion system containing different phenolic compounds incubated at 80°C for 0, 4, 8, 16, and 32 hr.

more effective by increasing the concentration of γ-oryzanol.

On the basis of hydroperoxide formation, only γ-oryzanol and only at higher concentration levels had an antioxidant effect at the early stage of incubation. But on the basis of hexanal formation, γ-oryzanol showed antioxidant effect at all levels during the whole incubation period, and was more effective with increased concentration. Therefore, with hexanal as a marker of oxidative products, γ-oryzanol was shown to be an effective inhibitor of the decomposition of hydroperoxides to form volatile products. This can be explained by the fact that the γ-oryzanol may act as a potent hydrogen donor in inhibiting the formation of volatile thermal decomposition products from hydroperoxides (9).

In the control emulsion, the rate of hydroperoxide formation sharply increased after 16 hr of incubation (Fig. 3). But in the presence of 200 ppm of a synthetic phenolic compound such as BHA, BHT, or TBHQ, relatively slow increase in the formation of hydroperoxides was observed (Fig. 3). At 200 ppm of γ-oryzanol, the rate of hydroperoxide formation was quite stable during incubation. With emulsions containing 200 ppm (Table 1) and at 4 hr of incubation, the inhibition of hydroperoxide formation was not significantly different ($p>0.05$) among the synthetic phenolic compounds but was significant with γ-oryzanol ($p<0.05$). The inhibition was most effective with 200 ppm of γ-oryzanol. At 32 hr of incubation, the order of effectiveness among the antioxidants for inhibiting the formation of hydroperoxide was: γ-oryzanol>TBHQ>BHT>BHA (Table 1). According to the "polar paradox" theory, polar antioxidants (e.g. γ-oryzanol>TBHQ) are more effective in nonpolar lipid emulsions whereas nonpolar antioxidants (e.g. BHT>BHA) are more active in polar lipid emulsions (2). Also, the stability of an emulsion is maintained when its surface-to-volume ratio (e.g. in emulsified oils) and the hydrophilic-lipophilic balance (HLB) of a lip-

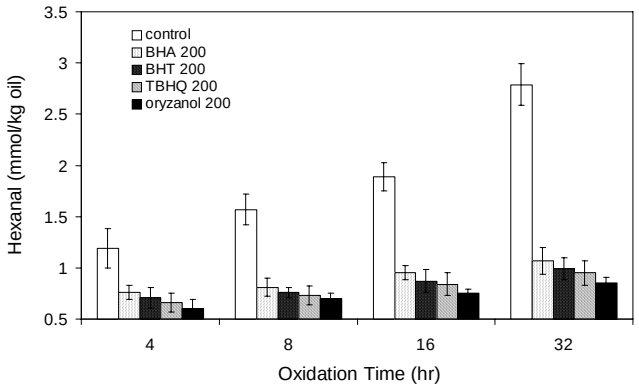

Fig. 4. Hexanal detected in an oil/water emulsion system containing different phenolic compounds incubated at 80°C for 0, 4, 8, 16, and 32 hr.

ophilic antioxidant (e.g. BHT, BHA) are high and low, respectively. However, the present results showed that γ-oryzanol, being relatively polar, was more effective as an antioxidant than the less polar antioxidants in the present model system. Contrary to the formation of hydroperoxide, the rate of hexanal formation was quite stable during incubation at all levels of antioxidant tested (Fig. 4). Furthermore, the inhibition of hexanal formation was not significantly different ($p>0.05$) among the emulsions treated with either phenolic antioxidants or γ-oryzanol at 32 hr of incubation (Table 1). Such variations in the effectiveness of the antioxidants may be related to their interfacial property and their hydrogen bonding with water near or at the interface of the emulsion system. In short, γ-oryzanol, a natural antioxidant, was strongly effective in an oil/water emulsion system to inhibit the formation of hydroperoxides and the decomposition of hydroperoxide than the conventional artificial antioxidants.

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