

Effect of Oxidized Lipid/Amino Acid Reaction Products on the Antioxidative Activity of Common Antioxidants

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The antioxidative activity of mixtures of oxidized lipid/amino acid reaction products (OLAARPs) and common antioxidants were evaluated to determine the role of OLAARP formation in the antioxidative activity observed for common antioxidants in foods. 1-(5-Amino-1-carboxypentyl)pyrrole, 1-methyl-4-pentyl-1,4-dihydropyridine-3,5-dicarbaldehyde, *N*-(carbobenzyloxy)-1(3)-[1'-(formylmethyl)hexyl]-L-histidine dihydrate, 1-(*N*²-(carbobenzyloxy)-L-lysyl)-2-[3'-carboxy-2'(*E*)-propen-1'-yl]-4-pentylpyridinium betaine, butylated hydroxytoluene, α -tocopherol, and mixtures of them were added at 50–200 ppm to soybean oil which was oxidized under air in the dark at 60 °C. Oil peroxidation was evaluated by the thiobarbituric acid-reactive substances (TBARS) assay. Both OLAARPs and common antioxidants increased the induction period of the oil and exhibited an additive effect when tested in mixtures. For most samples the percentage of synergistic efficiency was within the $\pm 20\%$ range. These results suggest that the antioxidative activity observed for common antioxidants in protein-containing foods may be increased by the antioxidative activity of the OLAARPs produced during the oxidative process.

Keywords: Antioxidants; nonenzymatic browning; amino acid modification; lipid peroxidation; oil stability; BHT; α -tocopherol.

INTRODUCTION

As urbanization continues and the time between harvest and consumption of food increases, problems concerning a limited storage life of food products become more critical (Erickson, 1997). One of the major causes of loss of quality in foods is lipid peroxidation, which leads to deterioration, rancidity, or discoloration of these products (Nawar, 1996; Frankel, 1995). Since these are important factors in consumer acceptance, many methods are used to prevent oxidative damage. Thus, improvements in food preparation, refrigeration, and packaging have been developed to retard oxidation. These types of improvements, however, may not be sufficient to prevent oxidation. Therefore, processors prevent or delay oxidation by adding antioxidants directly into the food product. The most commonly used antioxidants include the phenolic compounds butylated hydroxyanisole and butylated hydroxytoluene (BHT) as synthetic antioxidants, and tocopherols as natural ones (Giese, 1996; Rice-Evans et al., 1997).

In addition to these antioxidants, foods may contain other compounds with antioxidant properties that also contribute to their stability. Among these compounds, the production of oxidized lipid/amino acid reaction products (OLAARPs) has been described as a final step in the lipid peroxidation process when it takes place in the presence of proteins (Chio and Tappel, 1969; Zamora and Hidalgo, 1995). These compounds, which are produced simultaneously with the lipid peroxidation process, exhibited antioxidant properties (Alaiz et al.,

1996) and delayed the oxidative process at the same time that they were produced (Zamora et al., 1997). As a continuation of those studies, the present investigation was undertaken to study the interactions among the antioxidative activities of compounds that are usually present or added to foods with the OLAARPs produced during the lipid peroxidation process. The study of the antioxidative activities of mixtures containing both types of antioxidants should provide a better knowledge of the antioxidative activities observed for the phenolic compounds that are usually used in food preservation.

EXPERIMENTAL PROCEDURES

Materials. Soybean oil was obtained from our Institute's Pilot Plant (Instituto de la Grasa, CSIC, Sevilla, Spain). α -Tocopherol (TOC) was purchased from Aldrich Chemical Co. (Milwaukee, WI). 2-Thiobarbituric acid monohydrate (TBA) was purchased from Merck (Darmstadt, Germany). BHT was purchased from Sigma Chemicals (St. Louis, MO). Other reagents and solvents used were of analytical grade and were purchased from reliable commercial sources.

OLAARP Synthesis. Four OLAARPs were selected to be studied in mixtures with BHT and TOC. The OLAARPs were representative of four major derivatives that are produced in oxidized lipid/amino acid reactions: pyrroles, produced in the reaction of amino groups with 4,5-epoxy-2-alkenals (Hidalgo and Zamora, 1993), 4-hydroxy-2-alkenals (Sayre et al., 1993), or unsaturated epoxyoxo fatty acids (Hidalgo and Zamora, 1995); dihydropyridines, produced between malondialdehyde and amino groups (Kikugawa and Ido, 1984); Michael adducts, produced between histidine and α,β -unsaturated aldehydes (Uchida and Stadtman, 1992); and pyridinium salts, produced between amino groups and aldehydes (Suyama and Adachi, 1979). The compounds prepared were 1-(5-amino-1-carboxypentyl)pyrrole (ACP), 1-methyl-4-pentyl-1,4-dihydropyridine-3,5-dicarbaldehyde (DHP), *N*-(carbobenzyloxy)-1(3)-[1'-(formylmethyl)hexyl]-L-histidine dihydrate (ZHO), and 1-(*N*²-(carbobenzyloxy)-L-lysyl)-2-[3'-carboxy-2'(*E*)-propen-1'-yl]-4-pentylpyridin-

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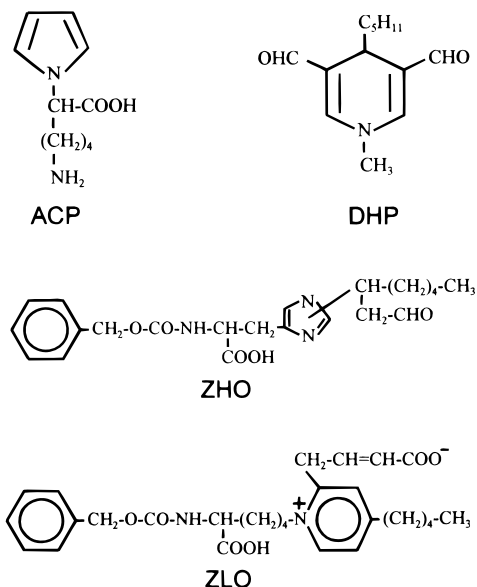


Figure 1. Chemical structures of OLAARPs prepared and tested for antioxidative activity in this study.

ium betaine (ZLO). Preparation of ACP, DHP, ZHO, and ZLO was carried out according previously described procedures (Alaiz et al., 1995a; Kikugawa et al., 1984; Alaiz et al., 1995b; for the synthesis of ACP, DHP, and ZHO and ZLO, respectively). Obtained compounds were chromatographically pure, and their structures were confirmed by ^1H and ^{13}C nuclear magnetic resonance spectroscopy and mass spectrometry. Structures for these compounds are given in Figure 1.

Measurement of Antioxidative Activity. Oxidative stability of refined soybean oil with no antioxidant added was compared with oil samples containing ACP, DHP, ZHO, ZLO, BHT, TOC, or mixtures of them added at 50–200 ppm. Oil samples (10 g) were weighed into 90 × 20 mm Petri dishes and oxidized for 20 days under air in the dark at 60 °C. Peroxidation was evaluated periodically by using the thiobarbituric acid-reactive substances (TBARS) assay as described by Kosugi et al. (1989). These determinations were carried out by using three replicates, and the results given are mean values of these determinations.

For comparison purposes, the obtained mean values were used to determine the induction periods (IPs) in the samples. IPs were determined (in hours) by the method of tangents to the two parts of the kinetic curve. The percentage of synergistic efficiency of the OLAARPs in the mixtures was determined, as described by Dziejdz and Hudson (1984), according to the following equation:

$$\% \text{ synergistic efficiency} = 100 \frac{[(IP_M - IP_O) - (IP_1 - IP_O) - (IP_2 - IP_O)]}{(IP_M - IP_O)}$$

where IP_M is the IP of the mixture of the tested compounds, IP_O is the IP of the oil, IP_1 is the IP of one of the tested compounds, and IP_2 is the IP of the other of the tested compounds.

RESULTS

Antioxidant Activities of BHT–ZLO Mixtures.

Figure 2A shows the TBARS production in oil samples treated with 50 ppm of ZLO, 50 ppm of BHT, and the mixture of 50 ppm of ZLO and 50 ppm of BHT. The IP of the oil (186.1 h) increased with the addition of both antioxidants and resulted 189.9 h after addition of ZLO and 203.8 h after BHT treatment. Addition of 50 ppm of ZLO also increased the IP of the oil treated with 50 ppm of BHT, and the IP of the oil treated with a mixture of both compounds added at 50 ppm was 232.3 h. The

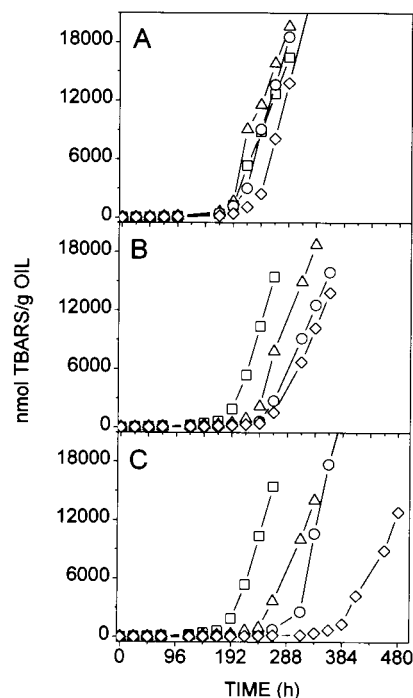


Figure 2. Effects of ZLO (Δ), BHT (\circ), and the mixture of the same quantity of ZLO and BHT (\diamond), added at A, 50; B, 100; and C, 200 ppm, on soybean oil oxidation (measured as thiobarbituric acid-reactive substances (TBARS) formation).

increase of the IP obtained for the mixture was a 53% higher than the sum of the IP increases obtained for the compounds tested separately and according to equation given in the Experimental Procedures section.

This synergistic effect was not related to the concentration of the tested compounds, and although the addition of the OLAARP always increased the IP of the oil treated with BHT, the synergistic effect observed at 50 ppm was not observed at 100 ppm. Figure 2B shows the TBARS production in a soybean oil treated with 100 ppm of the tested compounds, and the IPs obtained for the oil treated with 100 ppm of ZLO, 100 ppm of BHT, and the mixture of 100 ppm of ZLO and 100 ppm of BHT were 224.6, 246.0, and 268.8 h, respectively. These IPs gave a percentage of synergistic efficiency of –19%. This negative value suggested a slight antagonist effect between ZLO and BHT at this concentration.

Addition of 200 ppm of ZLO also increased the IP of the oil treated with BHT, and in contrast to the antagonist effect observed at 100 ppm, at 200 ppm a slight but positive synergistic effect was observed. Figure 2C collects the TBARS production in oil treated with 200 ppm of ZLO, 200 ppm of BHT, and the mixture of 200 ppm of ZLO and 200 ppm of BHT. The IPs obtained for these samples were 236.9, 301.3, and 373.6 h, respectively, and the percentage of synergistic efficiency was 11%.

Antioxidant Activities of BHT–ACP Mixtures.

Analogous to the results obtained for ZLO at the three concentrations assayed, addition of 50 ppm of ACP also increased the IP of the oil treated with BHT. Figure 3 shows the TBARS production in a soybean oil treated with 50 ppm of ACP, 50 ppm of BHT, and the mixture of 50 ppm of ACP and 50 ppm of BHT. The IPs obtained for these three treated oils were 199.2, 203.8, and 225.4 h, respectively. The synergistic efficiency between ACP and BHT at this concentration was 22%.

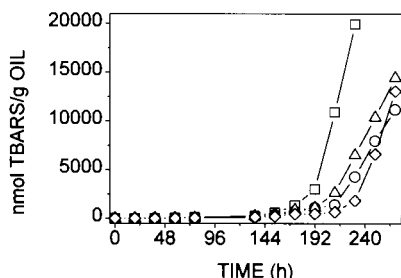


Figure 3. Effects of ACP at 50 ppm (Δ), BHT at 50 ppm (\circ), and the mixture of 50 ppm of ZLO and 50 ppm of BHT (\diamond), on soybean oil oxidation (\square) measured as thiobarbituric acid-reactive substances (TBARS) formation.

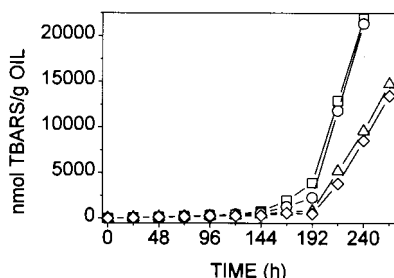


Figure 4. Effects of DHP at 50 ppm (Δ), TOC at 50 ppm (\circ), and the mixture of 50 ppm of DHP and 50 ppm of TOC (\diamond), on soybean oil oxidation (\square) measured as thiobarbituric acid-reactive substances (TBARS) formation.

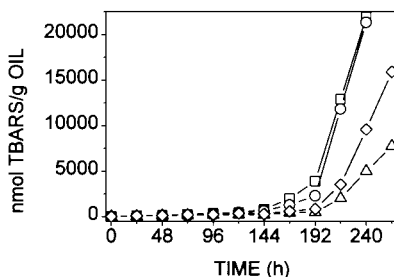


Figure 5. Effects of ZHO at 50 ppm (Δ), TOC at 50 ppm (\circ), and the mixture of 50 ppm of ZHO and 50 ppm of TOC (\diamond), on soybean oil oxidation (\square) measured as thiobarbituric acid-reactive substances (TBARS) formation.

Antioxidant Activities of TOC–OLAARP Mixtures. Analogous to the additive effects observed between BHT and the OLAARPs, which always increased the IPs of the oils treated with BHT, the same additive effects were also observed between TOC and the assayed OLAARPs. Figure 4 shows the TBARS production in oil samples treated with 50 ppm of DHP, 50 ppm of TOC, and a mixture of 50 ppm of DHP and 50 ppm of TOC. The IPs obtained for these three oil samples were 194.2, 190.4, and 197.9 h, respectively. Different from the addition of BHT, the addition of TOC increased very slightly the IP of the oil, which was a worse antioxidant than the tested OLAARP. The addition of DHP to the oil treated with TOC increased the IP of this oil in an amount similar to the increase obtained in the oil only treated with the OLAARP, and there was a very small synergistic efficiency, which was negative (–5%).

The effect observed in the antioxidative activities of TOC/DHP mixtures was very similar to the effect observed in TOC/ZHO mixtures. Figure 5 shows the TBARS produced in a soybean oil incubated at 60 ppm of TOC, and a mixture of 50 ppm of ZHO and 50 ppm of TOC. The induction periods obtained for these three

oil samples were 205.4, 190.4, and 207.7 h, and the synergistic efficiency obtained was –9%.

DISCUSSION

Lipid peroxidation is a very complex process in which unsaturated fatty acids are transformed, in a first step, into fatty acid hydroperoxides, and later, into secondary products. Among all these products, OLAARPs have been found to be produced as a final step in the lipid peroxidation process, when it takes place in the presence of proteins, and they have been shown to reduce lipid oxidation in different systems at the same time that they are being produced (Alaiz et al., 1995c, Zamora et al., 1997).

The results obtained in this study show that in oils treated with different phenolic antioxidants, the antioxidative activities exhibited by the OLAARPs in the absence of other compounds is added to the antioxidative activities of the other antioxidants. Therefore, mixtures of both types of antioxidants were more protective than if any of them was used alone, and this was a general conclusion for the different OLAARPs and antioxidants tested.

No large interactions were observed among the different antioxidants tested. Thus, for most experiments, the increases observed for the mixtures were similar to the sum of the increases observed for both antioxidants when they were tested separately, and the percentage of synergistic efficiency of these mixtures was within the $\pm 20\%$ range. Only in the mixture of 50 ppm of BHT and 50 ppm of ZLO was a synergistic effect of 53% observed.

The above results are in agreement with previous results that suggested that OLAARP formation is contributing to food stability (Alaiz et al., 1997) and suggest that the antioxidative activity observed for the artificial and natural antioxidants in protein-containing foods may be increased by the antioxidative activity of the OLAARPs produced during the oxidative process. This might be an additional factor to explain the differences observed in antioxidant activities of antioxidants in different foods.

ABBREVIATIONS USED

ACP, 1-(5-amino-1-carboxypentyl)pyrrole; BHT, butylated hydroxytoluene; DHP, 1-methyl-4-pentyl-1,4-dihydropyridine-3,5-dicarbaldehyde; IP, induction period; OLAARPs, oxidized lipid/amino acid reaction products; TBARS, thiobarbituric acid-reactive substances; TOC, α -tocopherol; ZHO, *N*-(carbobenzoyloxy)-1(3)-[1'-(formylmethyl)hexyl]-L-histidine dihydrate; ZLO, 1-(*N*²-(carbobenzoyloxy)-L-lysyl)-2-[3'-carboxy-2'(*E*)-propen-1'-yl]-4-pentylpyridinium betaine.

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