Suppression of Lipid Oxidation in Phosphatidylcholine Liposomes and Ground Pork by Spray-Dried Porcine Plasma†

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The ability of spray-dried porcine plasma to inhibit lipid oxidation in a model system and in ground pork was determined. At 2.5 mg of plasma protein/0.02 mg of phosphatidylcholine, the antioxidant activity of spray-dried plasma was 5.4-fold less than that of frozen–thawed plasma; however, 99% inhibition of lipid oxidation was achieved if spray-dried plasma concentration was increased to 12.5 mg of protein plasma/0.02 mg of phosphatidylcholine. Five percent (w/w) spray-dried plasma effectively inhibited lipid oxidation and color loss (change in *a* value) in salted ground pork stored at –15 °C for 120 days. These data suggest that spray-dried porcine plasma could be used to reduce lipid oxidation in pork and pork products.

INTRODUCTION

Lipid oxidation is a major cause of chemical deterioration in meats and meat products, especially during frozen storage (Asghar et al., 1988). Oxidative spoilage of meat can cause changes in color, texture, flavor, and the nutritive value of meat (Johns et al., 1989). Pork muscle shows a higher susceptibility to lipid oxidation than beef and lamb muscle during frozen storage due to its higher concentration of unsaturated fatty acids (Owen et al., 1975). Oxidative changes in meats increase when meats are subjected to processes such as grinding, flaking, and freeze–thaw abuse (Rhee, 1988). Addition of sodium chloride to meats also accelerates lipid oxidation (Rhee, 1983) and heme pigment oxidation (Fox, 1987).

Antioxidants are used by the food industry to inhibit lipid oxidation and associated flavor and color changes in meat and meat products (Dziezak, 1986). Although synthetic antioxidants are regarded as safe by the Food and Drug Administration (FDA), researchers have reported that butylated hydroxytoluene (BHT; Witchi, 1986), butylated hydroxyanisole (BHA; Grice, 1988), and tertiary butylhydroquinone (TBHQ; Giri et al., 1984) can cause cancer in laboratory animals. Concerns over the safety of synthetic antioxidants and increased consumer demand for "all natural" foods has resulted in an increased need for natural, nontoxic antioxidants in the food industry.

Currently, blood is an underutilized source of animal protein (Saito and Taira, 1987). Creating a market for animal blood would increase the value of meat animals as well as decrease expenses related to the disposal of blood. Blood plasma has the potential for utilization as a food additive because it contains several antioxidants. The antioxidants in plasma include superoxide dismutase, serum albumin, glutathione peroxidase, catalase, ceruloplasmin, transferrin, uric acid, and vitamin E (Halliwell and Gutteridge, 1986; Maddipati and Marnett, 1987; Avissar et al., 1989).

Recently our laboratory has found that porcine plasma can inhibit iron-catalyzed oxidation of phosphatidylcholine liposomes at the pH (5.5–7.0) common to muscle foods and storage temperatures of 4–37 °C (Decker and Faraji, 1991). Frozen–thawed porcine plasma (5.36 mg of protein/0.02 mg of phosphatidylcholine) inhibited 100% of iron-catalyzed lipid oxidation in vitro. In addition to inhibiting iron, frozen–thawed porcine plasma also inhibited hydrogen peroxide activated hemoglobin and singlet oxygen catalyzed lipid oxidation. Kanner and co-workers (Kanner et al., 1988) found that addition of bovine blood ceruloplasmin to minced turkey muscle resulted in the inhibition of lipid oxidation after 7 days of storage at 4 °C. These data suggest that blood plasma could be an effective antioxidant in meat.

The purpose of this study was to compare the antioxidant activity of spray-dried porcine plasma and frozen–thawed porcine plasma by measuring their ability to inhibit lipid oxidation in vitro. The ability of spray-dried plasma and BHT to inhibit lipid oxidation and color changes in salted ground pork was also investigated.

EXPERIMENTAL PROCEDURES

Materials. Frozen porcine plasma and spray-dried porcine plasma (AP 820) were donated by American Meat Protein Corp., Ames, IA. Soybean phosphatidylcholine (IV-S) was obtained from Sigma Chemical Co. Butylated hydroxytoluene (BHT) was purchased from Nutritional Biochemical Corp. *N,N*-Dimethyl-1,4-phenylenediamine and 4,6-dihydroxy-2-mercaptopyrimidine were obtained from Aldrich Chemical Co., Inc. All other chemicals were of reagent grade or better.

Methods. *Model System Study.* Liposomes were prepared from soybean phosphatidylcholine (PC) by homogenization and sonication and were quantitated by measuring phosphate (Decker and Hultin, 1990). In vitro lipid oxidation studies were performed in a model system containing 0.02 mg of phosphatidylcholine liposomes/mL of 0.12 M KCl, 5 mM histidine buffer (pH 7.0), and varying concentrations of spray-dried and frozen–thawed porcine plasma. Iron-catalyzed lipid oxidation was initiated by iron redox cycling using 15 μM $FeCl_3$ and 100 μM ascorbate (Decker and Hultin, 1990). Reactions were run at 37 °C for 30 min.

Lipid oxidation in the model system was monitored by measuring thiobarbituric acid reactive substances (TBARS) as described by (McDonald and Hultin, 1987). Inhibition of lipid oxidation was calculated as

$$\left(1 - \frac{\text{activity in presence of inhibitor}}{\text{activity in absence of inhibitor}}\right) \times 100$$

Frozen plasma was thawed under cold running tap water. Spray-dried plasma (stored at 5 °C) was reconstituted with water

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† This paper (90-5-202) is published with the approval of the Director of University of Kentucky Experimental Station.

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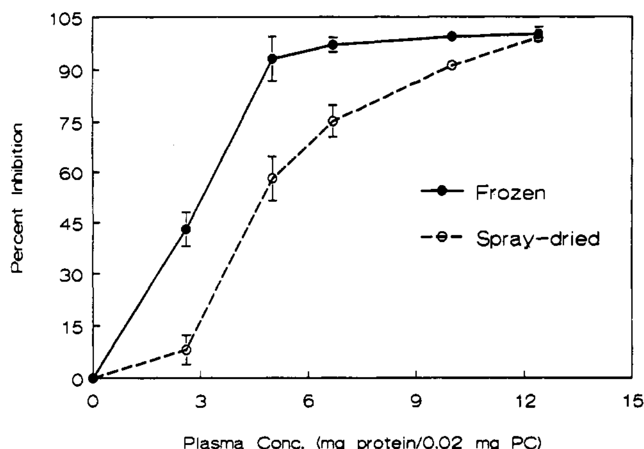

Figure 1. Effect of frozen-thawed and spray-dried porcine plasma on inhibition of iron-catalyzed oxidation of phosphatidylcholine (PC) liposomes. Experimental conditions are outlined under Methods.

to a protein concentration equal to that of frozen-thawed plasma immediately before use. Protein concentrations was determined by using the Biuret procedure as described by Torten and Whitaker (1964).

Ceruloplasmin (EC 1.16.3.1) activity was measured as described by Curzon (1960). Reconstituted spray-dried plasma and frozen-thawed plasma were diluted to 4.06 mg of protein/mL with 0.1 M NaCl and 1.0 mL was added to the assay.

Storage Study. Storage studies were conducted with pork Boston butts which had external fat removed and were ground twice through 3.2-mm plates at 4 °C. Fat content of the ground pork was measured by using the Soxhlet method as described in AOAC methods (Association of Official Analytical Chemists, 1980). Sodium chloride (2%) was added to the ground pork, and four separate batches were prepared: control, BHT (0.02% of the fat content, 13.8%), and spray-dried plasma (2.5% and 5.0% of the total weight). Salted ground pork from each treatment was divided into 25-g samples which were placed in Whirl-pak bags and stored at −15 °C. Samples were thawed under cold running tap water prior to analysis.

Lipid oxidation in stored ground pork was monitored by measuring TBARS in 0.2–0.4 g of meat as described by Sinnhuber and Yu (1977). TBARS were expressed as milligrams of malonaldehyde per kilogram of muscle, which was calculated by using the formula

$$(46 \times \text{abs}_{532\text{nm}})/\text{sample weight (g)}$$

The surface color of the salted ground pork was measured with a Hunterlab D 25M-2 tristimulus colorimeter (Rhee et al., 1983) standardized against a white standard plate ($L = 92.0$, $a = -20.0$, $b = 10.0$). Thawed pork (25 g) was placed in a Petri dish for analysis. Changes in a value were calculated as

$$(a \text{ value at 15, 30, 60, 90, or 120 days}) - (a \text{ value at 0 days})$$

Statistical Analysis. All experiments were performed on triplicate samples and were repeated a minimum of two times. Data were analyzed by assuming a mathematical model that included treatment, replicate (treatment), day and treatment × day interaction. Replicate (treatment) was used as the error term for testing differences among treatments. If the F test was significant, the least significant difference procedure was used to determine difference means at the 5% level of significance (Sendecor and Cochran, 1989).

RESULT AND DISCUSSION

The ability of spray-dried and frozen-thawed porcine plasma to inhibit iron-catalyzed lipid oxidation is shown in Figure 1. When 2.5 mg of plasma protein/0.02 mg of phosphatidylcholine was added to the model system, the antioxidative activity of spray-dried plasma was 5.4-fold less than that of frozen-thawed plasma. Decreased antioxidant activity of spray-dried plasma compared to that of frozen-thawed plasma might be due to denaturation and inactivation of plasma proteins by elevated temperatures and shear forces present during the spray-drying process. However, ceruloplasmin activity in spray-dried plasma was only 9% less than that of frozen-thawed plasma at plasma protein concentration of 4.06 mg/assay (data not shown). This indicates that the majority of ceruloplasmin remains active even after spray-drying and therefore could contribute to the antioxidant activity of spray-dried porcine plasma. Ceruloplasmin has been shown to be an effective antioxidant in minced turkey muscle (Kanner et al., 1988). The observed decrease in the antioxidative activity of spray-dried plasma could be due to inactivation of antioxidants such as catalase, glutathione peroxidase, superoxide dismutase, transferrin, and/or serum albumin. The decrease in antioxidant activity in spray-dried plasma could also be due to an increase in prooxidant concentration, which could occur by the release of protein-bound iron during the spray-drying process.

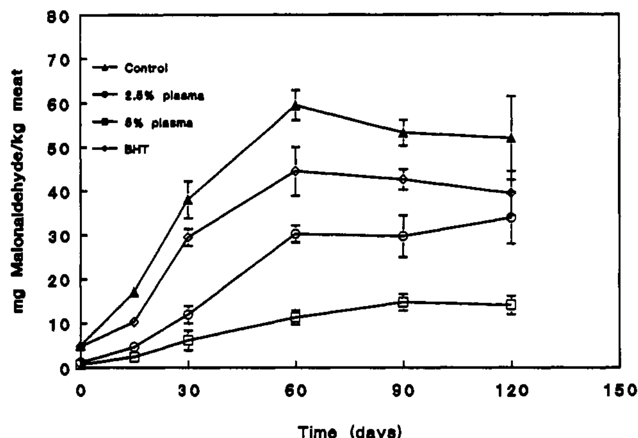

Figure 2. Effect of BHT (0.02% of fat) and 2.5% and 5% spray-dried porcine plasma on the inhibition of lipid oxidation in salted (2% NaCl) ground pork stored at −15 °C. Experimental conditions are outlined under Methods.

When the spray-dried plasma protein concentration in the model system was increased to 12.5 mg of plasma protein/0.02 mg of phosphatidylcholine, inhibition of lipid oxidation was 99% (compared to 97% inhibition for 6.7 mg of frozen-thawed protein plasma). These results suggests that spray-dried plasma can completely inhibit lipid oxidation if concentrations are increased. Use of spray-dried plasma as antioxidant in foods would be advantageous over frozen-thawed plasma since the concentration of protein (some of which are antioxidants) is approximately 10 times higher than in whole plasma.

As depicted in Figure 2, both 2.5% plasma and BHT effectively inhibited lipid oxidation compared to control samples over the entire storage study ($p < 0.05$). Plasma (2.5%) was a more effective antioxidant than BHT ($p < 0.01$) for the first 90 days of storage study. Inhibition of lipid oxidation by 5% spray-dried plasma was more effective than all other treatments (5% spray-dried plasma vs control, vs 2.5% spray-dried, and vs BHT have probability levels of $p < 0.0001$, $p < 0.05$, and $p < 0.001$, respectively) during the entire storage study. The decrease in antioxidant activity of 2.5% spray-dried plasma containing samples during storage could be due to the depletion of hydrogen donors such as serum albumin or inactivation of enzymes such as ceruloplasmin. However, increasing the concentration of spray-dried plasma to 5% effectively inhibited lipid oxidation over the entire storage study, suggesting that this level of plasma could be used to prevent oxidative deterioration of salted ground pork.

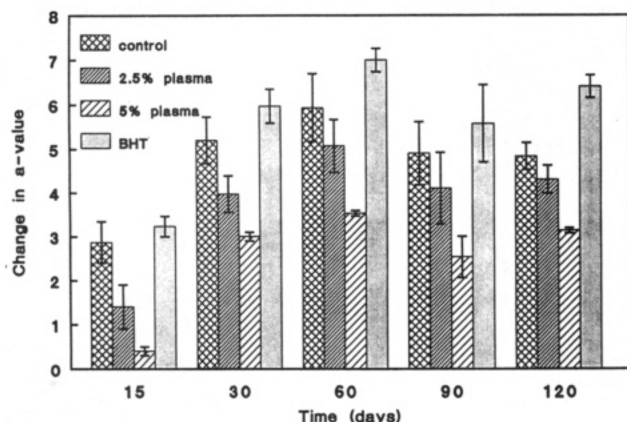

Figure 3. Evaluation of the changes in red color (*a* value) in salted (2% NaCl) ground pork containing BHT (0.02% of fat) and 2.5% and 5% spray-dried plasma stored at −15 °C. Experimental conditions are outlined under Methods.

Color is an important characteristic of meat that consumers use to judge meat products before purchase (Faustman and Cassens, 1990). Oxidation of the oxymyoglobin (red) to produce metmyoglobin (brown) is accelerated by lipid oxidation (Torres et al., 1988). As TBARS values of salted ground pork increased during storage, the redness of meat decreased (Figures 2 and 3). Loss of redness (increase in *a* value with time) in salted ground pork with 5% spray-dried plasma was less than in all other treatments during frozen storage ($p < 0.001$). During the first 30 days of storage, 2.5% spray-dried plasma significantly slowed color changes ($p < 0.005$), after which time color loss was not different from that in control samples. BHT did not effectively inhibit color loss over the entire storage study. The ability of plasma to prevent color changes could be due to its ability to inactivate lipid oxidation products which can oxidize myoglobin (Govindarajan et al., 1977) or to the presence of hydrogen donors which can reduce metmyoglobin (Ladikos and Wedzicha, 1988). Protection of color loss in salted ground pork by spray-dried plasma could be due in part to serum albumin which has been reported to inhibit oxymyoglobin oxidation (Koizumi et al., 1973).

Spray-drying porcine plasma decreased its antioxidant activity in vitro; however, complete inhibition of lipid oxidation could be achieved by increasing the spray-dried plasma concentration. Since spray-dried plasma contains approximately 10 times more protein than whole plasma, a lower concentration of spray-dried plasma than whole plasma could be used to inhibit lipid oxidation in foods. Inhibition of lipid oxidation in salted ground pork (−15 °C) was achieved when 2.5% plasma was added. Color protection of salted ground pork by plasma was not achieved unless 5% plasma was present. These data suggest that spray-dried porcine plasma could be used to help prevent lipid oxidation and color changes in pork.

LITERATURE CITED

Asghar, A.; Gray, J. I.; Buckley, D. J.; Pearson, A. M.; Booren, A. M. Perspectives on warmed-over flavor. *Food Technol.* **1988**, *46*, 102–108.

Association of Official Analytical Chemists. *Official Methods of Analysis*, 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1987.

Avissar, N.; Whitin, J. C.; Allen, P. Z.; Wagner, D. D.; Liegey, P.; Cohen, H. J. Plasma selenium-dependent glutathione peroxidase. *J. Biol. Chem.* **1989**, *264*, 15850–15855.

Curzon, G. The purification of human ceruloplasmin. *Biochem. J.* **1960**, *74*, 279–287.

Decker, E. A.; Hultin, H. O. Factors influencing the catalysis of lipid oxidation by the soluble fraction of mackerel ordinary muscle. *J. Food Sci.* **1990**, *55*, 947–950, 953.

Dziezak, D. J. Preservatives: Antioxidants, the ultimate answer to oxidation. *Food Technol.* **1986**, *40*, 94–102.

Faraji, H.; Decker, E. A. Inhibition of phosphatidylcholine liposome oxidation by porcine plasma. *J. Food Sci.* **1991**, in press.

Faustman, C.; Cassens, R. G. The biochemical basis for discoloration in fresh meat: a review. *J. Muscle Foods* **1990**, *1*, 217–243.

Fox, J. B., Jr. The pigments of meat. In *The Science of Meat and Meat Products*, 3rd ed.; Price, J. F., Schweigert, B. S., Eds.; Food & Nutrition Press: Trumbull, CT, 1987; p 193.

Giri, A. K.; Sen, S.; Talukder, G.; Shrma, A. Mutachromosomal effect of tert-butylhydroquinone in bone-marrow cells of mice. *Food Chem. Toxicol.* **1984**, *22*, 459–560.

Govindarajan, S.; Hultin, H. O.; Kotula, A. W. Myoglobin oxidation in ground beef: Mechanistic studies. *J. Food Sci.* **1977**, *42*, 571–577, 582.

Grice, H. C. Safety evaluation of butylated hydroxyanisole from the perspective of effects on forestomach and oesophageal squamous epithelium. *Food Chem. Toxicol.* **1988**, *26*, 717–723.

Halliwell, B.; Gutteridge, J. M. C. Iron and free radical reactions: two aspects of antioxidant protection. *Trends Biochem. Sci.* **1986**, *11*, 372–375.

Johns, A. M.; Birkinshaw, L. H.; Ledward, D. A. Catalysts of lipid oxidation in meat products. *Meat Sci.* **1989**, *25*, 209–220.

Kanner, J.; Sofer, F.; Harel, S.; Doll, L. Antioxidant activity of ceruloplasmin in muscle membrane and in situ lipid peroxidation. *J. Agric. Food Chem.* **1988**, *36*, 415–417.

Koizumi, C.; Nonaka, J.; Brown, W. D. Oxidative changes in oxymyoglobin during interaction with arginine linoleate. *J. Food Sci.* **1973**, *38*, 813–815.

Ladikos, D.; Wedzicha, B. L. The chemistry and stability of the haem-protein complex in relation to meat. *Food Chem.* **1988**, *29*, 143–155.

Maddipati, K. R.; Marnett, L. J. Characterization of the major hydroperoxide-reducing activity of human plasma. *J. Biol. Chem.* **1987**, *262*, 17398–17403.

McDonald, R. E.; Hultin, H. O. Some characteristics of the enzyme lipid peroxidation systems in microsomal fraction of flounder muscle. *J. Food Sci.* **1987**, *52*, 15–21, 27.

Owen, J. E.; Lawrie, R. A.; Hardy, B. Effect of dietary variation, with respect to energy and crude protein levels, on the oxidative rancidity exhibited by frozen porcine muscles. *J. Sci. Food Agric.* **1975**, *26*, 31–41.

Rhee, K. S. Enzymic and nonenzymic catalysis of lipid oxidation in muscle foods. *Food Technol.* **1988**, *42*, 127–132.

Rhee, K. S.; Smith, G. C.; Terrell, R. N. Effect of reduction and replacement of sodium chloride on rancidity development in raw and cooked ground pork. *J. Food Prot.* **1983**, *46*, 578–581.

Saito, M.; Taira, H. Heat denaturation and emulsifying properties of plasma protein. *Agric. Biol. Chem.* **1987**, *51*, 2787–2792.

Sendecor, G. W.; Cochran, W. G. *Statistical method*, 8th ed.; The Iowa State University Press: Ames, IA, 1989.

Sinnhuber, R. O.; Yu, T. C. The 2-thiobarbituric acid reaction, an objective measure of the oxidative detection occurring in fats and oils. *J. Jpn. Soc. Fish. Sci.* **1977**, *26*, 259–267.

Torres, E.; Pearson, A. M.; Gray, J. I.; Booren, A. M.; Shimokomaki, M. Effect of salt on oxidative changes in pre- and post-rigor ground beef. *Meat Sci.* **1988**, *23*, 151–163.

Torten, J.; Whitaker, J. R. Evaluation of Biuret and dye-binding methods for protein determination in meats. *J. Food Sci.* **1964**, *29*, 168–174.

Witschi, H. P. Enhanced tumor development by butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. *Food Chem. Toxicol.* **1986**, *24*, 1127–1130.

Received for review October 30, 1990. Revised manuscript received January 29, 1991. Accepted March 22, 1991.