Antioxidant Activity of Polar Lipids from Nitrite-Treated Cooked and Processed Meats

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Polar lipids having considerable antioxidant effect on linoleic acid have been isolated from nitrite-treated, laboratory-cooked ground pork and beef during and after four weeks' storage at 4 C. The antioxidant activity of these polar lipids is 1.5–3 times greater than that of untreated meats. Antioxidatively active polar lipids have also been found in commercially processed, nitrite-containing meats, including pepperoni, ham, frankfurters and bacon.

The antioxidant activity of active polar lipids was stable on storage and partially survived treatment with acids and bases and conversion to methyl esters. Separation of the active polar lipids or the active methyl esters by classes gave no fraction in which the antioxidant activity was highly concentrated; however, it seemed to be more highly associated with the polyunsaturates fraction of the methyl esters. Indications are that more than one antioxidant factor is involved and that at least one is associated with the acyl portion of the polar lipids.

Use of sodium nitrite in processing meats provides some protection against the early onset of oxidative rancidity (1,2), as measured by a number of standard analytical tests (3–5). Earlier work in our laboratory has demonstrated that sodium nitrite, which has no antioxidant activity, generates an antioxidant factor in uncooked meat (beef or pork) (6) and that this factor is associated with the polar lipid fraction when lipids are isolated by a dry column procedure. Further work showed that the application of the dry column method (7,8) to cooked and commercially processed meats required modification to eliminate pigments that interfered in the determination of antioxidant activity (9). The present work deals with an investigation of the antioxidant activity found in nitrite-treated cooked meat and in commercially processed meats. We also report the results of physical and chemical tests intended to provide more information on the nature and chemical reactivity of the antioxidant factor(s).

EXPERIMENTAL PROCEDURES

Material and reagents. Linoleic acid (>99%), methyl heptadecanoate (>99%), cytochrome c (type III from horse heart) and *trans* β-carotene (Type I) were purchased from Sigma Chemical Co. (St. Louis, Missouri); sodium methoxide (0.5 N) from Supelco Inc. (Bellefonte, Pennsylvania); hexanal (purum) from Fluka Chemical Corp. (Hauppauge, New York); Sep-pak C_{18} cartridges from Waters Associates (Milford, Massachusetts); and thin layer plates silica gel G, 250 μ and 500 μ, silica gel H, 500 μ with 10% $AgNO_3$ and silica gel H, 500 μ from Analtech, (Newark, Delaware). All solvents were distilled in glass grade (Burdick and Jackson, Muskegon, Michigan).

Meat preparation. The procedures used for preparing fresh and processed meat were the same as those described previously (6,9).

Lipid extraction. Neutral and polar lipids were extracted sequentially from 5 g of tissue samples by the modified dry column procedure (modification II, ref. 9).

Antioxidant activity. The carotene bleaching method (10) was used as described previously (6).

Transesterification. Active polar lipids (7 mg) were converted to the fatty acid methyl esters by the procedure of Christie (13). The final reaction mixture was evaporated under nitrogen, reconstituted with hexane to a concentration of 7 mg/ml and tested for antioxidant activity. For further purification the methyl ester solution in hexane was loaded onto a silica Sep-Pak and eluted with 6 ml hexane (fraction 1) and 10 ml hexane/ethyl ether (95:5, v/v) (fraction 2).

Thin layer chromatography (TLC). Analytical TLC was performed on silica gel G plates (250 μ), and samples were developed with $CHCl_3$, CH_3OH, H_2O (65:25:4, v/v/v). For isolation of lipid samples, silica gel G or H preparative plates (500 μ) were developed with the same solvent system. Bands were located by charring the edges of the plates. Located bands were scraped and extracted with $CHCl_3$, CH_3OH (2:1, v/v) 4 × 4 ml; $CHCl_3$, CH_3OH (1:1, v/v) 2 × 4 ml; and $CHCl_3$, CH_3OH, H_2O (5:5:0.1, v/v/v) 2 × 3 ml. All eluates were tested for antioxidant activity.

Argentation TLC was used for separating methyl esters derived from the extracted polar lipids. The plates were developed with hexane/ether (9:1,v/v), and elution of the visualized (2,7-dichlorofluorescein) bands was carried out with hexane/ether (1:1, v/v) 3 × 5 ml followed by 5 ml ether. Each eluate was treated with 1/3 its volume of aqueous NH_4OH (3 M) for removal of the visualization agent and silver oxide.

Gas chromatography (GC). Methyl esters were analyzed on a Sigma 3B gas chromatograph (Perkin-Elmer, Norwalk, Connecticut) equipped with a flame ionization detector and an on-column capillary injector (J. & W. Scientific, Inc., Folsom, California). Data were collected and integrated on a Hewlett-Packard 3390A integrator. The column was a 60 m × 0.32 mm I.D. FSOT bonded phase methyl silicone column with 0.25 mm film thickness. Helium was the carrier gas at 36 cm/sec. Initial oven temperature was 150 C and was programmed at 15°/min to 250 C. The detector temperature was 350 C. Quantitation was by peak area comparison to the internal standard methyl heptadecanoate.

Chemical treatment of polar lipids. (a) Reactions in solution. Antioxidatively active polar lipids (7 mg) derived from meat were dissolved in hexane (1 ml), and the solution was stirred with five vol. of 0.1 N or 0.01 N aqueous acid or base at room temperature for 15 min. The hexane layer was separated from the aqueous and washed with distilled water until neutral. The hexane solution was dried over anhydrous sodium sulfate, filtered,

evaporated to dryness, reconstituted with hexane to a concentration of 7 mg/ml and tested for antioxidant activity.

Active polar lipids (28 mg) were dissolved in methylene chloride (1 ml) and hexanal (13 μl) was added. The mixture was allowed to stand at room temperature overnight and then was evaporated under a stream of nitrogen. The residue was dissolved in methylene chloride (4 ml) and tested for antioxidant activity.

(b) Reactions with gaseous reagents. A column of Celite 545 in a disposable Pasteur pipette was prepared according to the method of Schwartz and Allen (11). Active polar lipid in hexane (2 ml, 7 mg/ml) was applied to the lower part of the Celite column, and the solvent was removed with nitrogen. The tip of the column was inserted into a 9-ml vial (11) containing concentrated hydrochloric acid or ammonium hydroxide. Vapors of the reagent were aspirated through the Celite bed for 10 min, and residual vapors were purged with a stream of nitrogen. Lipids were eluted from the column with methylene chloride/methanol (2:1, v/v, 4 × 3 ml). Solvent was removed from the eluate with a stream of nitrogen, the residue was reconstituted with hexane to a concentration of 7 mg/ml, and the solution was tested for antioxidant activity.

RESULTS AND DISCUSSION

Earlier work (6) has clearly demonstrated that polar lipids extracted from raw, nitrite-treated beef or pork exhibit substantial antioxidant activity compared to untreated controls. Examination for antioxidant activity of polar lipids from cooked beef or pork was precluded by the presence of pigments liberated during the heating process. Modification of the dry column extraction procedure (9) has now permitted the examination of pigment-free polar lipids from cooked beef and pork. The method of Ben-Aziz et al. (10) was used to measure antioxidant activity. In this procedure linoleic acid is allowed to oxidize in the presence of cytochrome C and β-carotene, and progress of the oxidation is followed spectrophotometrically by observation of the bleaching of β-carotene over four min after the reagents are mixed. Relative antioxidant activity, i.e., the ability to inhibit bleaching of β-carotene, was measured and compared with that of controls containing no antioxidant. The results are shown in Figures 1 and 2. The plots shown are average values from 8–10 samples tested over a 4-wk period while meat was kept at 4 C and are illustrative of a larger number of experiments carried out. On each of the sampling days, 5-g portions of the treated and untreated meat were removed from storage and extracted, and the antioxidant activity of the isolated polar lipids was determined along with that of a control. Statistical analysis of the daily values (at 2 and 4 min) indicated that daily values of each type of sample (treated, untreated and control) did not differ significantly ($p<0.05$), whereas the differences between sample types were significant. It is apparent that nitrite treatment imparts antioxidant activity on the polar lipids of cooked meat as it has been shown previously to impart on those of raw meat (6) and that it does so consistently. Calculations of data (not shown)

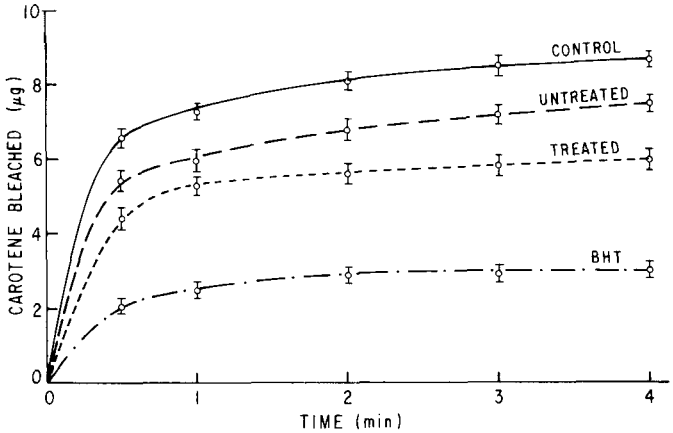

FIG. 1. Antioxidant effect on linoleic acid of polar lipid fraction from untreated and nitrite-treated laboratory-cooked beef sampled over four weeks and compared to control and to BHT. Vertical bars indicate standard error.

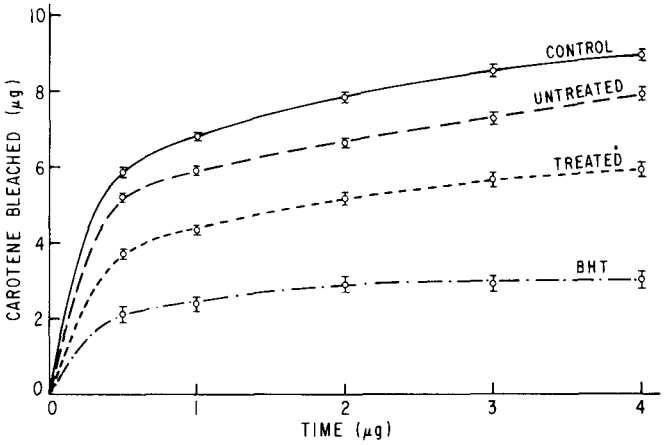

FIG. 2. Antioxidant effect on linoleic acid of polar lipid fractions from untreated and nitrite-treated laboratory-cooked pork sampled over four weeks and compared to control and to BHT. Vertical bars indicate standard error.

indicate that the polar lipids from nitrite-treated cooked meat are 1.5–3.0 times more effective as antioxidants than those from untreated meat.

Effects of changes in nitrite concentration in the meat on antioxidant activity were measured. The polar lipids of cooked pork treated with sodium nitrite at the 40-ppm level had less activity than those treated at the 150-ppm level. Concentrations of residual nitrite in these samples were not measured.

Polar lipids extracted from commercially processed meats whose label indicated past treatment with sodium nitrite were examined. The results shown in Figure 3 indicate that polar lipids isolated from pepperoni, beef frankfurters and bacon all had antioxidant activity. In these products comparison with untreated meat was not possible. Also examined were ham, poultry frankfurters and pork frankfurters that contained added nitrite; the polar lipids of these products had antioxidant activity as well.

Examination by analytical TLC of polar lipids from treated and untreated cooked meats (pork or beef) showed no noticeable differences attributable to the

nitrite-treated (active) and untreated pork is shown in Table 1. The GC traces were identical in that all peaks seen in one trace were also present in the other. There was some difference in the relative composition (Table 1) in that the active methyl esters (nitrite-treated pork) were somewhat higher in polyunsaturated fatty acids. This may be the result of the protection against oxidation afforded by the presence of the antioxidant factor(s).

The methyl ester mixtures derived from treated and untreated pork were subjected to preparative argentation TLC to separate them by degree of unsaturation. The highest antioxidant activity was noted in the slowest moving band containing the polyunsaturated methyl esters. In this band the esters derived from nitrite-treated meat had almost three times the antioxidant activity of the esters derived from untreated meat. However, the amount of material in this band was just sufficient to carry out the antioxidant test. Less activity was also associated with mono- and diunsaturated methyl esters. As observed in TLC of the polar lipids, activity was distributed through all of the methyl ester bands separated by degree of unsaturation and was not exclusively associated with any one band. Isolation and identification of the antioxidant factor(s) remains an elusive goal.

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