Evaluation of the Antioxidant Effects of Dried Milk Mineral in Cooked Beef, Pork, and Turkey

D.P. CORNFORTH AND E.M. WEST

ABSTRACT: This study was done to determine the optimum level of dried milk mineral (MM) to inhibit lipid oxidation in various ground meats. Cooked ground beef and pork required 2% MM to maintain thiobarbituric acid (TBA) values < 1.0 after 14 d refrigerated storage, compared to 1% MM for ground turkey. TBA values of cooked ground beef were lower ($p < 0.05$) when MM was added in water suspension, rather than as a dry powder. Among MM components (phosphate, calcium, and citrate), polyphosphates most effectively maintained low TBA levels during storage. MM probably chelates soluble iron to colloidal calcium phosphate particles, thus removing iron as a catalyst for lipid oxidation.

Keywords: antioxidant, TBA, milk, phosphate, meat

Introduction

IN COOKED MEATS, OFF-FLAVOR DEVELOPMENT ASSOCIATED WITH lipid oxidation is one of the major limitations to extended shelf life. Warmed-over flavor (WOF) is the term used to describe the stale or rancid flavor and odor that develops in cooked meats during storage and reheating (Tims and Watts 1958; Younathan 1985). Unsaturated fatty acids in the membrane phospholipid fraction are particularly susceptible to oxidation (Igene and Pearson 1979). Thus, even lean meats or poultry will develop off-flavors during storage after cooking. A major pro-oxidant factor is soluble ionic iron (Igene and others 1979; Miller and Aust 1989), derived from the heat degradation of heme pigments (Igene and others 1979; Buchowski and others 1988). Metal chelating agents, sodium nitrite, and various reductants inhibit lipid oxidation when added to cooked ground beef (Sato and Hegarty 1971; Igene and others 1979).

Barley flour and wild rice have been shown to reduce oxidation and extend shelf life of cooked ground beef patties (Katsanidis and others 1997). The active antioxidant fraction in barley flour is vitamin E and related tocotrienols, which can reduce lipid peroxy radicals and interrupt the propagation step of lipid oxidation. Wild rice contains phytate (Wu and others 1994), a type II antioxidant capable of chelating metal ions and thus inhibiting metal catalysis of lipid oxidation (Katsanidis and others 1997). Phytate has strong antioxidant activity in cooked chicken (Empson and others 1991) or beef (Lee and others 1998), but it is too expensive in its purified form for commercial use at present.

Whey is another natural food ingredient with antioxidant activity (Colbert and Decker 1991; Browdy and Harris 1997), due to the presence of protein sulfhydryl groups with reducing abilities, and also due to iron chelation by whey proteins (Tong and others 2000). Interestingly, calcium ions reduce WOF in cooked chicken, apparently by displacing iron from phospholipid binding sites (Graf and Panter 1991). Thus, this study was done to investigate the possible antioxidant effects of dried milk mineral (MM), a natural calcium source derived from whey, when added at various levels to ground beef, pork, and turkey.

Materials and Methods

Experimental design and statistics

Dried milk mineral (MM) was added to raw ground beef, pork, or turkey at 0% (control), 0.25%, 0.50%, 1.0%, and 2.0% of meat weight. After cooking, lipid stability was measured using the thiobarbituric acid (TBA) test described by Buege and Aust (1978). TBA measurements were taken after 1, 4, 7, and 14 d of storage at 2 °C. Three replications were done on separate days for each meat. All measurements were performed in duplicate. A second experiment was done to compare TBA values of cooked ground beef as affected by the method of MM addition (dry powder or a 10% suspension in water). A third experiment was done to compare antioxidant activity of MM components (phosphate, calcium, and citrate) when added to ground beef at levels equivalent to those found in 1% MM. Experiments 2 and 3 were replicated twice on separate days, and TBA values were measured in duplicate after 1, 5, 9, and 14 d of storage.

Treatment means were calculated by analysis of variance (ANOVA), using Statistica™ (Statsoft Inc., Tulsa, Okla., U.S.A.). Significant differences between means were determined by calculation of Fisher's least significant difference (LSD) values, when appropriate. Significance was defined at $p < 0.05$. Figures were prepared using the curve-smoothing feature of the Cricket™ 1.01 graphics program (Computer Associates International, Islandia, N.Y., U.S.A.).

Sample preparation

Raw ground beef, pork, and turkey (7%, 23%, and 20% fat, respectively, as indicated on the label) were purchased locally and cooked the same day. Dried milk mineral (Lactoval Q™ and TruCal™) were obtained from DMV Intl. (Fraser, N.Y., U.S.A.) and Glanbia Foods (Twin Falls, Ida., U.S.A.) respectively. Dried MM was a white, free-flowing powder that was 92.1% mineral, 5.5% water, 1.0% lactose, 1% protein, and 0.4% fat (DMV International, Fraser, N.Y., U.S.A.). The anhydrous mineral complex consisted of phosphate (36.2%), calcium (24.5%), and citrate (18.6%). The MM was commercially prepared by drying the permeate obtained from ultrafiltration of whey.

Meat samples were prepared by addition of 0.25% to 2.0% MM to 100 g of raw meat. The MM was added either as a dry powder, or as a suspension with 10% added de-ionized water (20 °C), based on meat weight. The MM was manually mixed with meat; the samples were then grilled at 163 °C for 5 min. The fat was drained off, and the well-done crumbles were placed in resealable Zip-Lock™ plastic bags (S.C. Johnson and Son, Racine, Wis., U.S.A.) and held at 2 °C for 14 d.

TBA analysis

Thiobarbituric acid-reactive substances (TBARS) were measured as described by Buege and Aust (1978). Cooked meat samples (2 g) were blended 20 sec at medium speed with 10 mL final volume of 50 mM Hepes buffer, pH 7.4, using a probe-type homogenizer (Ultra-Turrax T25, Janke and Kunkel, Staufen, Germany). The homogenate was centrifuged 10 min at 5000 × g to obtain a clear supernatant. A mixture of 0.8 mL of sample supernatant and 0.2 mL of de-ionized water was incubated for 30 min at 40 °C. To measure the TBA number, 2 mL of 0.375% TBA-15% trichloroacetic acid – 0.25 N HCL stock solution was added to the incubation solution and heated for 10 min in a boiling water bath to develop pink color. Sample tubes were then cooled with tap water and centrifuged for 10 min at 1000 × g. Sample absorbance was measured at 535 nm. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ (Sinnhuber and Yu 1958). The MDA concentration was converted to TBA number (mg MDA/kg meat sample) as follows:

(1) TBA No. = sample A_{535} × (1 M MDA / 1.56×10^5) × [(1 mole / L) / M] × (0.003 L / 0.16 g meat) × (72.07 g MDA / mole MDA) × (1000 mg / g) × (1000 g / kg), or

(2) TBA No. (ppm) = sample A_{535} × (1 / 1.56) × 13.5 mg MDA / kg meat, or

(3) TBA No. = sample A_{535} × 8.66

Results and Discussion

THE TBA NUMBER VALUES OF COOKED GROUND BEEF CONtrols increased ($p < 0.05$) from 3.0 at 1 d refrigerated storage to 4.95 after 14 d storage (Figure 1), with noticeable rancid odor and flavor. Addition of 0.25% to 2.0% dry MM reduced ($p < 0.05$) TBA number values in a dose-dependent manner. With 2% MM, TBA numbers were < 1.0 during 14 d storage at 2 °C. According to Igene and Pearson (1979), TBA number values of 1 to 2 corresponded to slight warmed-over flavor (WOF). Thus, TBA numbers < 1.0 would indicate little or no WOF. The TBA number values of cooked ground pork controls were 4.9 or more for all storage times (Figure 2). TBA number values of all cooked pork samples with MM were lower ($p < 0.05$) than controls. The addition of 1% or 2% MM maintained TBA numbers < 2.0 throughout the 14-d storage period. The TBA number of cooked ground turkey control samples increased ($p < 0.05$) from 1.7 at 1 d to 5.1 after 14 d of storage (Figure 3). MM addition at either 1% or 2% maintained TBA numbers at very low levels (< 0.6) throughout the storage period. Thus, MM very effectively maintained low TBA numbers of cooked samples when added at the 2% level to raw ground beef, or at the 1% level to ground pork or turkey.

Addition of 1% MM as a 10% suspension in water was slightly but significantly ($p < 0.05$) more effective than addition of dry MM powder to the raw meat. Both methods of MM addition maintained TBA levels < 2 during storage of cooked beef, compared to TBA numbers of 4.5 to 7.1 for controls without MM (Figure 4). Either method of MM addition would be commercially feasible for cooked meats, but

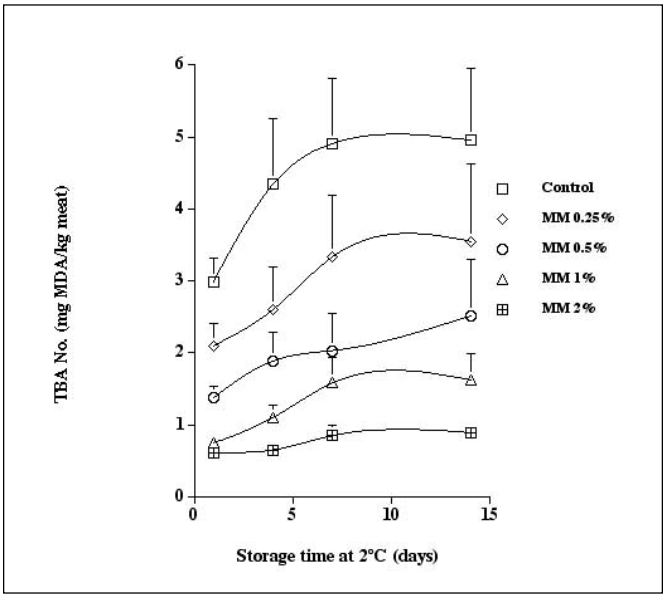

Figure 1—TBA number of cooked ground beef crumbles made with various levels of dried milk mineral (MM) and stored at 2 °C for 14 d. TBA No. = thiobarbituric acid number, expressed as milligrams malonaldehyde (MDA) per kilogram sample. Data points represent means, and error bars represent positive standard error. Some error bars lie within data points.

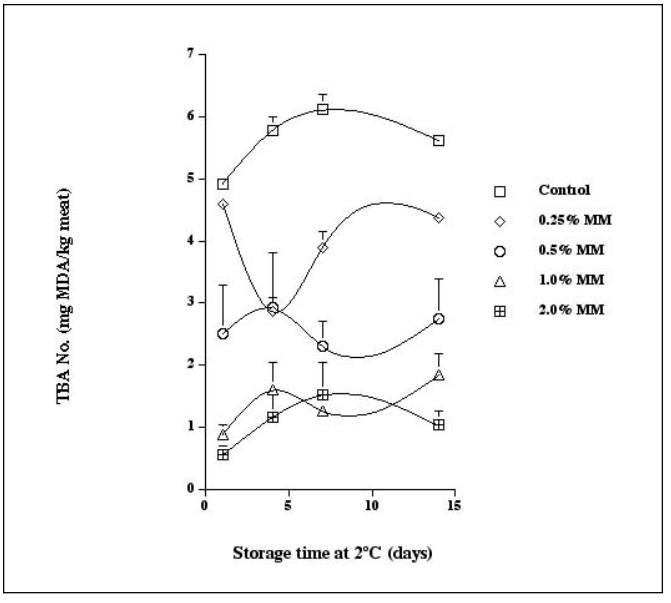

Figure 2—TBA number of cooked ground pork crumbles made with various levels of dried milk mineral (MM) and stored at 2 °C for 14 d. TBA No. = thiobarbituric acid number, expressed as milligrams malonaldehyde (MDA) per kilogram sample. Data points represent means, and error bars represent positive standard error. Some error bars lie within data points.

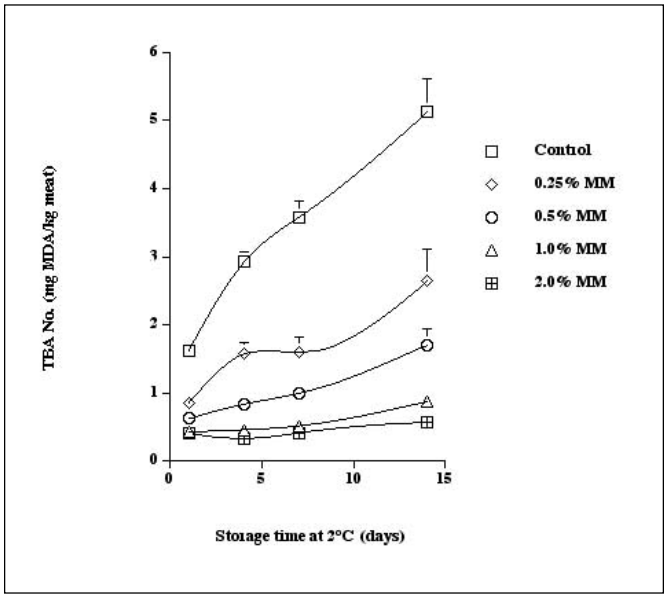

Figure 3—TBA number of cooked ground turkey crumbles made with various levels of dried milk mineral (MM) and stored at 2 °C for 14 d. TBA No. = thiobarbituric acid number, expressed as milligrams malonaldehyde (MDA) per kilogram sample. Data points represent means, and error bars represent positive standard error. Some error bars lie within data points.

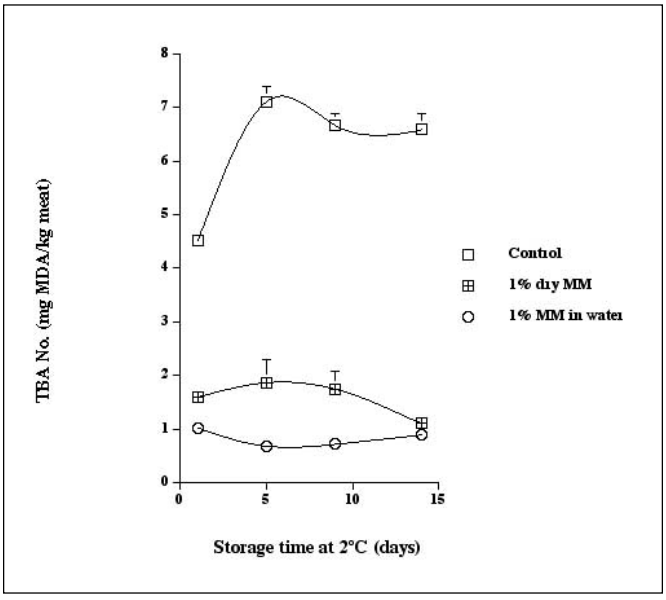

Figure 4—Effect of method of addition of milk mineral (MM), either as a dry powder or a water suspension, on TBA number of cooked ground beef crumbles stored at 2 °C for 14 d. Dry MM was added at 1% of raw meat weight. The MM suspension in water was prepared by adding dry MM to 10% added de-ionized water (20 °C), based on raw meat weight. TBA No. = thiobarbituric acid number, expressed as milligrams malonaldehyde (MDA) per kilogram sample. Data points represent means, and error bars represent positive standard error. Some error bars lie within data points.

probably not for fresh ground meats or sausages. White MM particles were visible in the meat after addition of MM powder, but particles were less noticeable after addition of MM suspension. After cooking, MM particles were not visible, regardless of method of MM addition. Addition of water or other substances is not permitted to products labeled as ground beef, pork, or other meat (de Holl 1981). However, added substances are permitted to products labeled as patties, so long as the added substances are listed on the ingredient statement. Water may also be added to cooked meat products, although the product name may require a statement in prominent letters indicating the level of added water remaining after cooking, as for example, ham with 20% added water (USDA 1984).

Dried MM is 92.1% mineral, 5.5% water, 1.0% lactose, 1% protein, and 0.4% fat (DMV International, Fraser, N.Y., U.S.A.). The anhydrous mineral complex consisted of phosphate (36.2%), calcium (24.5%), and citrate (18.6%). The antioxidant capabilities of the MM components (calcium, phosphate, and citrate) were compared when added to ground beef at levels equivalent to addition of 1% dry MM as a percentage of raw beef weight (Figure 5). Sodium monophosphate and calcium chloride had little antioxidant capability in cooked ground beef, as indicated by high TBA numbers ranging from 3.9 to 5.1 after 5 d of storage (Figure 5). TBA numbers of cooked beef with added citrate or sodium tripolyphosphate (STPP) were much lower ($p < 0.05$) than controls. STTP was particularly effective at maintaining TBA numbers < 1.0 during 14-d storage, while TBA numbers of samples with added citrate were < 2.0 during storage (Figure 5). STPP is a more effective chelator of metal ions than is sodium monophosphate, accounting for the higher antioxidant effect of tripolyphosphate, compared to monophosphate. Although STPP had high antioxidant capability in cooked

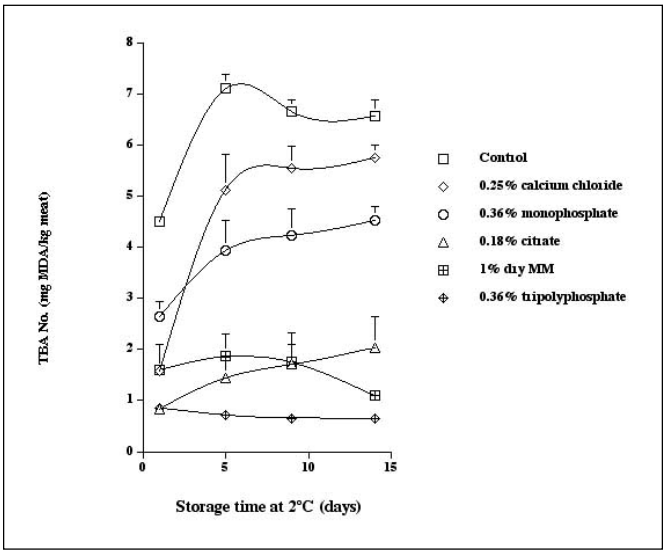

Figure 5—Comparison of milk mineral (MM) components on TBA number of cooked ground beef crumbles stored at 2 °C for 14 d. Each component (phosphate, calcium, citrate) was added at a level equivalent to that found in 1% MM. TBA No. = thiobarbituric acid number, expressed as milligrams malonaldehyde (MDA) per kilogram sample. Data points represent means, and error bars represent positive standard error. Some error bars lie within data points.

ground beef (Figure 5), it is generally thought to be less effective in fresh meats, due to hydrolysis of STPP to orthophosphate (monophosphate) by meat phosphatases (Awad 1968; Lee and others 1998). Since polyphosphates and citrate

were more effective inhibitors of TBA formation than were calcium or monophosphate, it appears that iron chelation is the most important mechanism by which MM components inhibit the TBA reaction, rather than calcium displacement of iron binding sites on phospholipids, as suggested by Graf and Panter (1991). MM probably chelates soluble iron in the cooked meat system to colloidal calcium phosphate particles, thus removing iron as a catalyst for lipid oxidation.

Conclusions

DRIED MM INHIBITS LIPID OXIDATION IN COOKED GROUND beef, pork, and turkey crumbles in a dose-dependent manner. Among MM components, polyphosphates had much more antioxidant activity in cooked ground beef than did monophosphate, calcium chloride, or sodium citrate. Thus, the mechanism of MM inhibition is probably the chelation of soluble iron to colloidal calcium phosphate particles, removing iron as a catalyst for lipid oxidation.

References

Awad MK. 1968. Hydrolysis of polyphosphates added to meat [MS thesis]. Edmonton, Canada: Univ of Alberta. 74 p. Available from: Univ of Alberta Science Library or Dept of Agricultural, Food, and Nutritional Sciences Library, Univ of Alberta, Edmonton, Alberta, Canada T6G 2P5.

Browdy AA, Harris ND. 1997. Whey improves oxidative stability of soybean oil. J Food Sci 62(2):348-350, 376.

Buchowski MS, Mahoney AW, Carpenter CE, Cornforth DP. 1988. Cooking and the distribution of total and heme iron between meat and broth. J Food Sci 53(1):43-45.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. Meth Enzymol 52:306.

Colbert LB, Decker EA. 1991. Antioxidant activity of an ultrafiltration permeate from acid whey. J Food Sci 56(5):1248-50.

de Holl JC. 1981. Encyclopedia of labeling meat and poultry products. 5th ed. St. Louis, Mo.: Meat Plant Magazine.

Empson KL, Labuza TP, Graf E. 1991. Phytic acid as a food antioxidant. J Food Sci 56(2):560-563.

Graf E, Panter SS. 1991. Inhibition of warmed-over flavor development by polyvalent cations. J Food Sci 56(4):1055-58, 1067.

Igene JO, King JA, Pearson AM, Gray AI. 1979. Influence of heme pigments, nitrite, and non-heme iron on the development of warmed-over flavor (WOF) in cooked meat. J Agric Food Chem 27(4):838-42.

Igene JO, Pearson AM. 1979. Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. J Food Sci 44(5):1285-1290.

Katsanidis E, Addis PB, Epley RJ, Fulcher RG. 1997. Evaluation of the antioxidant properties of barley flour and wild rice in uncooked and precooked ground beef patties. J Foodservice Systems 10(1):9-22.

Lee BJ, Hendricks DG, Cornforth DP. 1998. Effect of sodium phytate, sodium pyrophosphate and sodium tripolyphosphate on physico-chemical characteristics of restructured beef. Meat Sci 50(3):273-283.

Miller DM, Aust SD. 1989. Studies of ascorbate-dependent, iron-catalyzed lipid peroxidation. Arch Biochem Biophys 271(1):113-119.

Sato K, Hegarty GR. 1971. Warmed-over flavor in cooked meats. J Food Sci 36(6):1098-1102.

Sinnhuber RO, Yu TC. 1958. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technol 12(1):9-12.

Tims MJ, Watts BM. 1958. Protection of cooked meats with phosphates. Food Technol 12(5):240-243.

Tong LM, Sasaki S, McClements DJ, Decker EA. 2000. Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. J Agric Food Chem 48(5):1473-1478.

USDA 1984. Introduction to PFF (Protein Fat-Free) in cured pork products. Bulletin published by the United States Dept Agric, Food Safety and Inspection Service, Meat and Poultry Inspection, Washington, D.C., Program Training Division. 21 p. Available from: USDA FSIS, 300 12th St. SW, Washington DC 20250

Wu K, Zhang W, Addis PB, Epley RJ, Salih AM, Lehrfeld J. 1994. Antioxidant properties of wild rice. J Agric Food Chem 42(1):34-37.

Younathan MT. 1985. Causes and prevention of warmed-over flavor. Recip Meat Conf Proc 38:74-79.

MS 20010092 Submitted 2/26/01, Revised 8/9/01, Accepted 8/9/01, Received 8/9/01

This research was supported by Glanbia Foods (Twin Falls, Id., U.S.A.), Dairy Management Inc., the Western Dairy Center (Logan, Utah), and the Utah Agricultural Experiment Station (Utah State Univ, Logan). UAES journal paper no. 7377. The authors also thank Ayako Sasaki and Nani Wijaya for their valuable technical assistance.

Authors are with the Dept of Nutrition and Food Sciences, Utah State Univ, Logan, UT 84322-8700. Direct correspondence to author Cornforth (E-mail: darenc@cc.usu.edu).