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# MODE OF ACTION OF MILK MINERAL IN PREVENTING OXIDATIVE RANCIDITY IN COOKED MEAT SYSTEMS

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## Introduction

Milk mineral (MM) is a by-product of the production of whey protein concentrates. It is a fine powder (particle size <7 µm), obtained by purifying and drying the ultra-filtration permeate of whey. MM has been shown to significantly reduce the rate of oxidative rancidity in cooked meat systems (Cornforth and West, 2002, Jayasingh and Cornforth, 2003). It has been suggested that the calcium phosphate fraction of MM may function as a Type II antioxidant, as does sodium tripolyphosphate (STP), by binding iron and effectively preventing it from catalyzing lipid oxidation (Cornforth and West, 2002).

## Objectives

To study the mode of action by which MM functions to prevent lipid oxidation.

## Methodology

**Iron Binding Column Preparation:** Columns were prepared using small (14.5 cm length) disposable borosilicate Pasteur-type pipettes (Scientific Products, McGraw Park, IL). Columns were plugged with glass wool, then filled with test material to a depth of 2.5 cm. The amount of test material added to each column was determined by weight difference. MM (Glanbia, Monroe, WI), STP (Fisher, Fairlawn, NJ), calcium phosphate monobasic (CM; JT Baker, Phillipsburg, NJ), and calcium pyrophosphate (CP; Aldrich, St. Louis, MO) were used as test materials. Columns were pre-wetted with 1 ml of distilled water (DI), then 0.5 ml of 1 mg/ml iron standard (ferrous chloride, JT Baker, Phillipsburg, NJ) and 0.5 ml DI were added. Eight additional 1 ml DI rinses were added, for a total wash volume of 10 ml. At least 9.75 ml of filtrate was recovered in all cases. Ten replicates (n=10) were performed for each test material.

**Percent Packing Loss:** Spent iron binding columns were dried overnight at 90° C, then cooled in a dessicator. Columns were weighed to determine the amount of packing solubilized. Results were expressed as a percent of the original packing weight for each column.

**Iron Retention:** Total iron content of the filtrates was determined using the Ferrozine assay (Carter, 1971). Samples were read at 562 nm using a Shimadzu UV2100U spectrophotometer (Columbia, MD) as a measure of total iron concentration. Each filtrate was assayed in duplicate. Iron retention, in mg iron / g packing compound, was

calculated based on a target value of 0.05 mg iron / ml filtrate, the expected concentration where no iron is retained by the column.

**Microscopy:** Lean ground beef (90%) was obtained from the USU Meat Lab. Samples were prepared by adding MM or STP at 0.75% and 1.5% levels to 50 g of meat. Samples were mixed thoroughly (kneading 25 times), wrapped in plastic film, then placed in resealable sandwich bags and held under refrigeration for three days. Samples were prepared for light microscopy by dehydrating, embedding in paraffin, sectioning, and re-hydrating. Von Kossa staining (Sheehan and Hrapchak, 1980) was performed on the re-hydrated sections to test for the presence of undissolved calcium. Slides were viewed at 40x magnification.

**Ascorbate Test:** To test the catalytic activity of iron and MM or STP solutions, the ascorbate test of Buettner (1988) was used. Briefly, 0.3 g MM or STP was spiked with 25 µl of 0.1 mg/ml iron standard (ferrous chloride), then 3.25 ml distilled deionized water (DDI) was added (3.5 ml for unspiked MM and STP controls). Samples were vortexed for 3 seconds every 10 minutes for one hour, then allowed to sit overnight. To 3 ml aliquots of samples and controls, 3.5 µl of 0.1 M ascorbic acid solution (Fisher, Fair Lawn, NJ) was added, and loss of ascorbate was monitored at 265 nm for 15 minutes. Controls (DDI, DDI + iron, and STP or MM only) were assayed once. Samples (STP or MM + iron) were prepared and assayed in triplicate and graphed as an average.

**Experimental Design:** Statistical analysis was performed for Iron Retention and Percent Packing Loss values. Analysis of variance (ANOVA) was used to identify significant differences at the 95% confidence level. Least significant difference (LSD) tests were used to separate means of treatments (packing compound type).

## Results & Discussion

**Iron Binding and Packing Loss:** The type of packing compound used significantly affected the amount of iron bound by the columns ($p < 0.0001$) and the percent of the packing that was solubilized ($p < 0.0001$). MM was found to bind more iron per gram than any of the other three compounds (Table 1), and was less soluble than STP and CM (Table 2). On the average, 20% of the MM column packing was lost. MM contains approximately 80% mineral, 10% lactose, and 4 – 5% protein and moisture; it is likely the portion lost from the column was solubilized lactose and protein, though some small fraction of the finest mineral particles may have been lost as well. This is in contrast to STP, which lost 70% of its original weight. Some STP columns drained more slowly than others, allowing the packing to be in contact with DI for a longer period of time. In these cases, almost 100% of the packing was lost, suggesting that over time, STP will completely dissolve in aqueous systems (such as meat). Neither of the calcium phosphate forms (CP and CM) bound as much iron as did MM. However, once corrected for mineral content, the solubility of MM was closer to that of CP, suggesting that the form of calcium phosphate present in MM is more similar to CP than CM.

**Microscopic Examination for Undissolved Calcium:** The presence of insoluble calcium in ground beef + MM samples is verified by the black spots seen in Fig. 1. The absence of such spots in the ground beef + STP samples is not surprising, as no calcium was added. However, evidence supporting the solubility of STP can also be seen. Compared to the control, the 0.75% STP sample shows much less muscle fiber shrinkage

(white gaps between red muscle fibers). In the 1.5% STP sample, this difference is even more pronounced. In addition to iron, soluble phosphates will bind water. This series of slides shows the effect of increasing levels of STP; as the percentage increases, so does the water binding capacity, and less cell shrinkage is observed.

**Catalytic Activity of Chelated Iron:** In systems without added iron (Fig. 2), ascorbate loss was minimal, and did not vary greatly between treatments. In systems with added ferrous iron (Fig. 3), greater differences were observed. Ascorbate loss was greater with STP-iron complexes than with those of MM-iron. In sample preparation for this assay, STP was observed to be highly water soluble, while MM formed a suspension that settled over time. This observation may partially explain the results seen in Fig. 3.

Although STP is recognized as an effective iron chelator and inhibitor of lipid oxidation, it allowed measurable oxidation of ascorbate in this study. As free iron is not highly soluble in water, it must be bound to some water-soluble chelator to become catalytically active in an aqueous system (Kanner, Hazan, and Doll, 1988). Polyphosphate-type iron chelators are believed to promote the autoxidation of bound ferrous iron, stabilizing the ferric state (Aust, Morehouse, and Thomas, 1985). STP-iron mixtures may be forming soluble ferric iron-phosphate complexes with the ability to interact with and oxidize ascorbate. STP would not render the iron completely catalytically inert. Iron-MM complexes were not seen to dissolve, and in fact they settled out relatively quickly. Thus, MM could act to remove iron from solution, reducing the loss of ascorbate by limiting the catalytic activity of iron.

## Conclusions

MM is an effective Type II, iron-chelating antioxidant. It was found to bind significantly more iron per gram than STP, despite containing only 80% mineral. The ability of MM to bind iron and remain insoluble may account for its antioxidant effect.

## References


Aust, S. D., Morehouse, A. M., and Thomas, C. E. (1985). Role of metals in oxygen radical reactions. Journal of Free Radicals in Biology and Medicine, 1, 3–25.

Buettner, G. R. (1988). In the absence of catalytic metals ascorbate does not autoxidize at pH 7: ascorbate as a test for catalytic metals. Journal of Biochemical and Biophysical Methods, 16, 27–40.

Carter, P. (1971). Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). Analytical Biochemistry, 40, 450–458.

Cornforth, D. P. and West, E. M. (2002). Evaluation of the antioxidant effects of dried milk mineral in cooked beef, pork, and poultry. Journal of Food Science, 67(2), 615–618.

Jayasingh, P. and Cornforth, D. P. (2003). Comparison of antioxidant effects of milk mineral, butylated hydroxytoluene and sodium tripolyphosphate in raw and cooked ground pork. Meat Science, 66, 83–89.

Kanner, J., Hazan, B., and Doll, L. (1988). Catalytic “free” iron ions in muscle foods. J Agric Food Chem. 36:412–415.

Sheehan, D. C., and Hrapchak, B. B. (1980). Theory and Practice of Histotechnology, 2nd Ed. Battelle Press, Columbus, OH.

## Tables and Figures

Table 1. Iron Binding Capacity (mg iron bound / g column packing). Means sharing a letter are not different at $p > 0.05$.

| | Mean ± SD | |
|---|---|---|
| Milk mineral | 1.71 ± 0.24 | a |
| Calcium pyrophosphate | 1.16 ± 0.15 | b |
| Sodium tripolyphosphate | 1.11 ± 0.08 | b |
| Calcium phosphate monobasic | 0.44 ± 0.45 | c |

Table 2. Column Packing Loss (% initial weight). Means sharing a letter are not different at $p > 0.05$.

| | Mean ± SD | |
|---|---|---|
| Calcium phosphate monobasic | 75.2 ± 9.4 | a |
| Sodium tripolyphosphate | 69.4 ± 25.3 | a |
| Milk mineral | 20.7 ± 0.6 | b |
| Calcium pyrophosphate | 0.9 ± 0.6 | c |

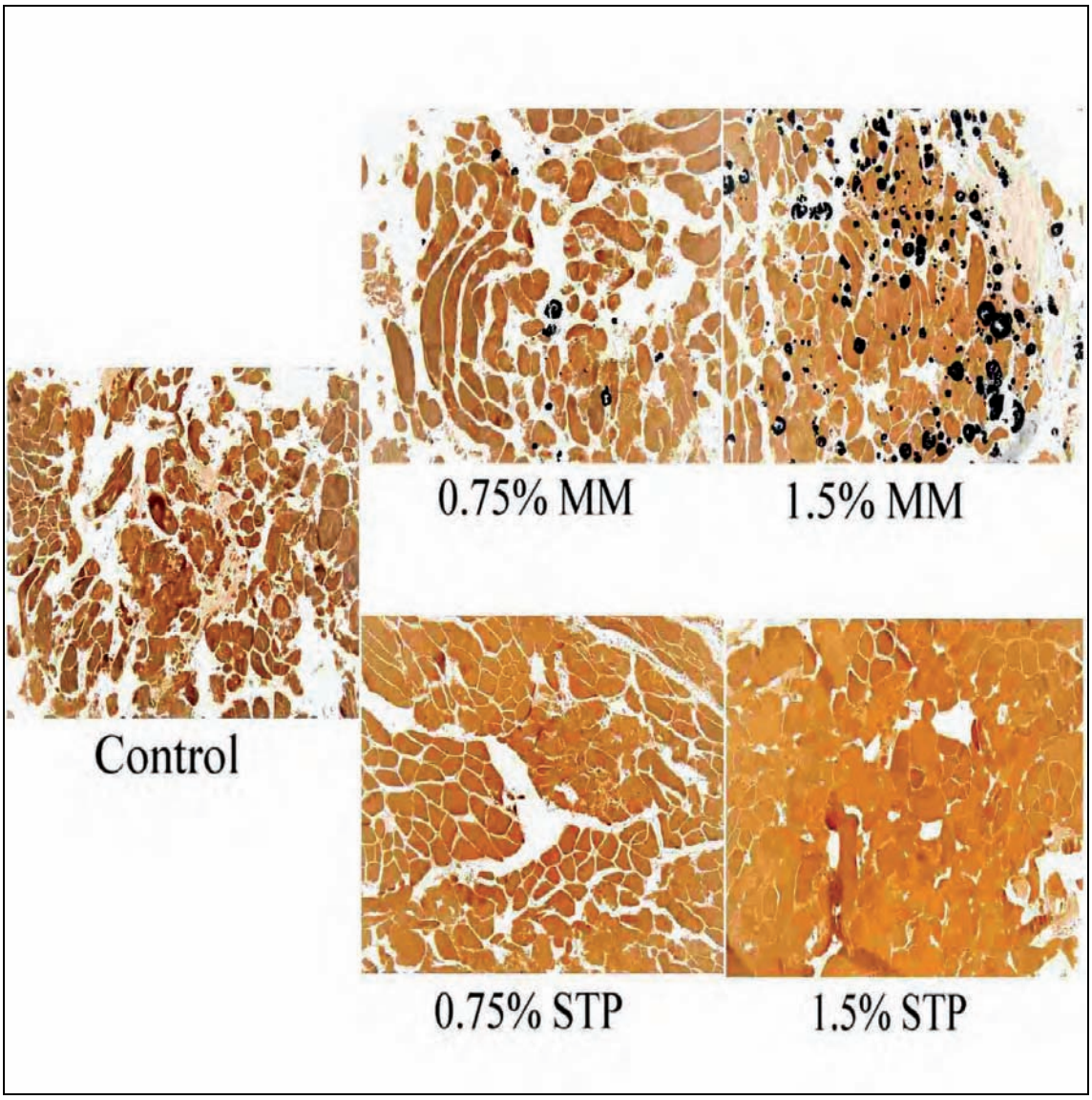


**Fig. 1. Light microscopy of lean ground beef samples containing milk mineral (MM) or sodium tripolyphosphate (STP). Samples were treated with the Von Kossa stain; calcium-containing particles are black. Cell shrinkage during fixation is indicated by white gaps between muscle fibers. (40x magnification)**

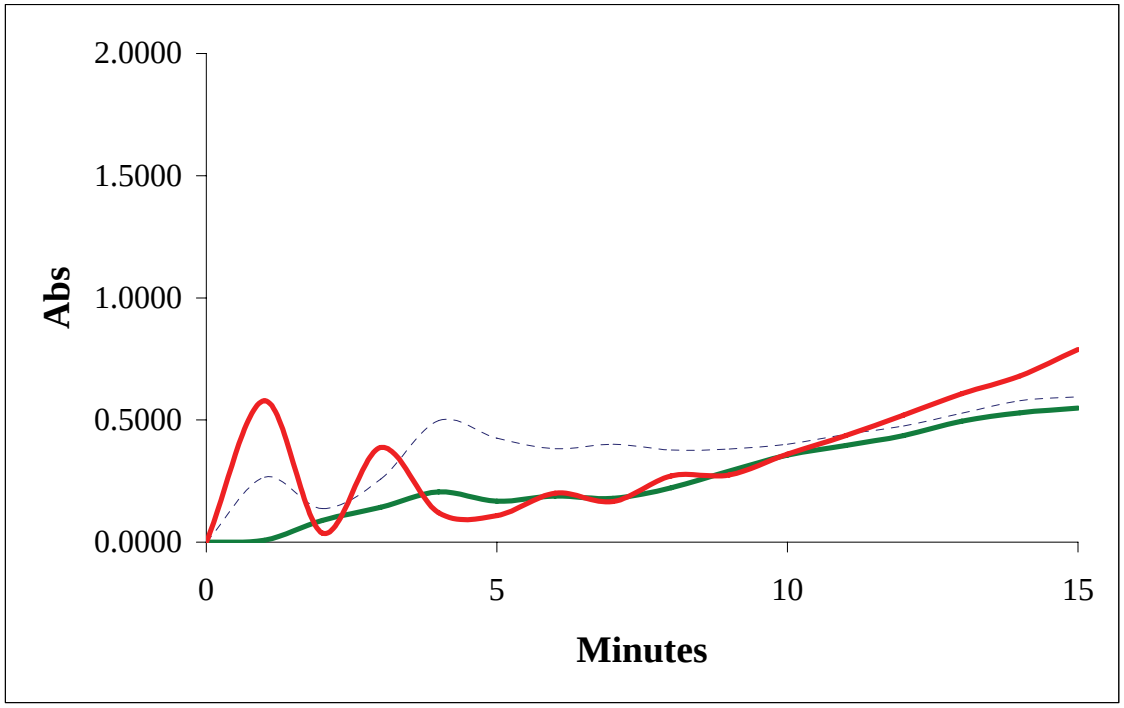


**Fig. 2. Ascorbate degradation (increase @ $A_{265}$) in iron-free systems (- -, distilled deionized water; —, milk mineral; —, sodium tripolyphosphate).**

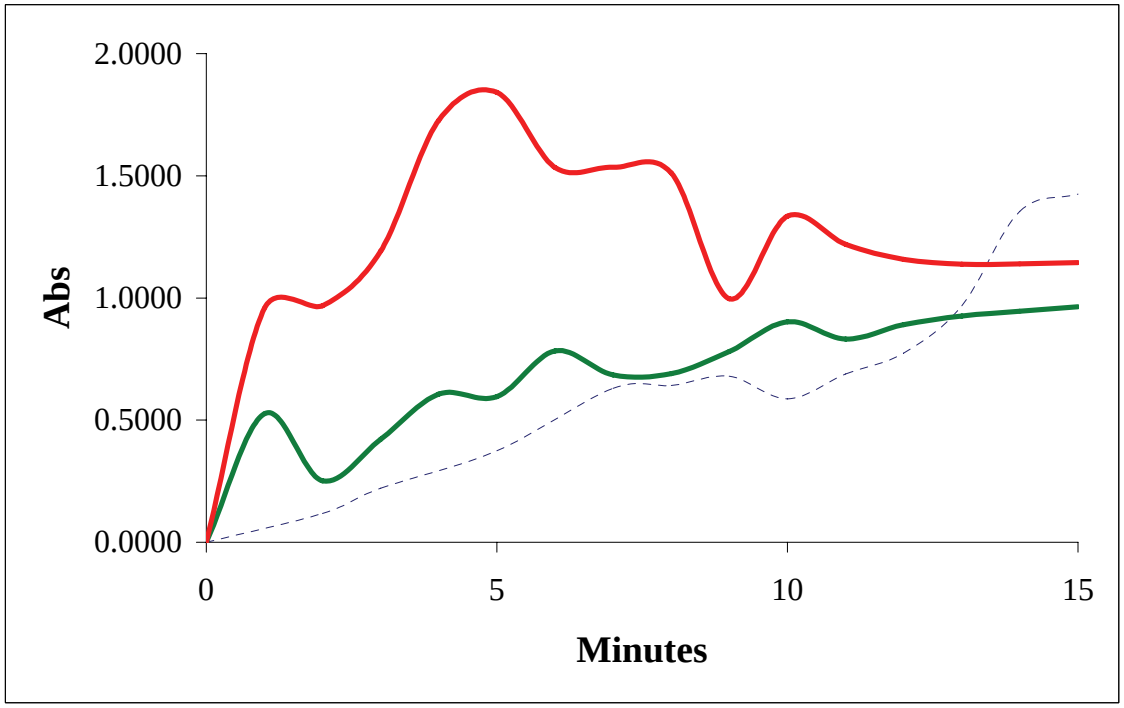


**Fig. 3 Ascorbate degradation (increase @ $A_{265}$) in iron-added systems (- -, distilled deionized water + Fe; —, milk mineral + Fe; —, sodium tripolyphosphate + Fe).**