Evaluation of Milk Mineral Antioxidant Activity in Beef Meatballs and Nitrite-cured Sausage

MIHIR N. VASAVADA AND DAREN P. CORNFORTH

ABSTRACT: The objective of this study was to determine the antioxidant activity of 1.5% milk mineral (MM) added to uncured cooked beef meatballs and to evaluate possible synergistic effects of MM in combination with 20-ppm or 40-ppm sodium nitrite in beef sausages. All treatments were also formulated with 1.5% salt and 10% added water. Thiobarbituric acid (TBA) values and Hunter color values were determined at 1 d, 8 d, and 15 d of storage at 2 °C. Meatball cooked yield was also measured. Cooked yield was not different (P < 0.05) between control meatballs and those containing MM. As expected, treatments containing nitrite had higher redness (CIE a*) than samples without nitrite. Redness values increased with storage time in sausages containing 40-ppm nitrite. However, redness values decreased (P < 0.05) during storage for control meatballs, associated with increased lipid oxidation (higher TBA values). Lipid oxidation was lower (P < 0.05) in samples containing 1.5% MM with TBA values <1.2 after 15 d of storage compared with 6.1 for control samples. There was no synergistic inhibition of lipid oxidation in samples containing 20-ppm or 40-ppm sodium nitrite plus 1.5% MM. Milk mineral alone at 1.5% of meat weight was sufficient for inhibition of lipid oxidation in cooked beef samples.

Keywords: milk mineral, nitrite, antioxidant, meatballs

Introduction

Lipid oxidation is a major cause of deterioration in the quality of meat and meat products (Asghar and others 1988; Ladikos and Lougovois 1990). Lipid oxidation leads to production of malonaldehyde, a potent mutagen and/or carcinogen (Shamberger and others 1974). Lipid oxidation is faster in heated meat than in raw meat tissues (Tichivangana and Morrissey 1985). The rate and degree of autoxidation degradation has been directly related to the degree of unsaturation of the lipids present (Igene and Pearson 1979; Tichivangana and Morrissey 1985) and degree of oxygen exposure (O’Grady and others 2000; Jayasingh and others 2002). Oxidation of unsaturated fatty acids in cooked meats during storage and reheating results in stale or rancid flavors known as warmed-over flavor (WOF) (Sato and Hegarty 1971).

The greater propensity of WOF in cooked and comminuted products is due to the release of non-heme iron during cooking and grinding (Igene and others 1979). Unsaturated lipids, especially those of the membrane phospholipids fraction, are the compounds undergoing autoxidation (Younathan and Watts 1960; Igene and Pearson 1979). The development of WOF in cooked meat is generally accepted to be the result of autoxidation of tissue lipids (Younathan and Watts 1960; Ruenger and others 1978).

Cooked meat develops rancid flavor more rapidly than uncooked meat during refrigerated storage, resulting in WOF (Tims and Watts 1958). The thiobarbituric acid test (TBA) is the most frequently used test to assess lipid oxidation in meat. Sensory panelists describe the extent of lipid oxidation in terms of rancid odor or taste. Tarladgis and others (1960) found that TBA numbers (milligrams of TBA reactive substances/kilogram of tissue) were highly correlated with trained sensory panel scores for rancid odor in ground pork. The TBA number at which a rancid odor was 1st perceived was between 0.5 and 1.0. This “threshold” has served as a guide for interpreting TBA test results. According to Greene and Cumuze (1981), the range of oxidized flavor detection for inexperienced panelists was within a range of TBA numbers similar to the previously determined threshold level for trained panelists.

Nitrates and nitrites function as antioxidants by binding to heme iron, which upon reduction form NO-heme complexes that stabilize the heme group during cooking. The ionic iron released by cooking is the primary prooxidant in cooked meats (Igene and others 1979). Milk mineral (MM) is the mineral fraction of skin milk. It works as an antioxidant in cooked meats by iron-chelation to colloidal calcium phosphate (Cornforth and West 2002). The objective of this study was to evaluate possible synergistic effects of MM and sodium nitrite to reduce TBA values of cooked beef samples during storage at 2 °C for 15 d.

Materials and Methods

Experimental design and Statistics

The study was a factorial design with 4 treatments (control, 1.5% MM, 1.5% MM + 20 ppm sodium nitrite, 1.5% MM + 40 ppm sodium nitrite), 3 cooked meat storage times (1, 8, and 15 d), and 3 replicates of the experiment. The treatment means were calculated by analysis of variance (ANOVA) using STATISTICA™ software (Statsoft Inc., Tulsa, Okla., U.S.A.). Significant differences among means were determined by calculation of Fisher’s least significant difference (LSD) values. Significance was defined at P < 0.05.

Sample preparation

Milk mineral (MM) is a dried, white, free-flowing powder obtained from Glanbia Foods (Twin Falls, Idaho, U.S.A.). The composition of MM is shown in Table 1. The treatments were formulated as described in Table 2. All 4 treatments had 10% water and 1.5% salt, based on meat weight. The samples (500 g each) were formulated by...
manually mixing the ingredients in the amounts listed. Meatballs (treatments 1 and 2) were cooked in a boiling water bath to an internal temperature of 85 °C as measured with a Versatuff 396 digital thermometer with micro-needle probe (Atkins Technical Inc., Gainesville, Fla., U.S.A.). Nitrite cured sausages (treatments 3 and 4) in fibrous cellulose casings were cooked to an internal temperature of 74 °C. After cooking, products were placed in resealable plastic bags (S.C. Johnson and Son, Inc., Racine, Wis., U.S.A.), cooled for 10 to 15 min at room temperature, and stored for 1, 8, or 15 d at 2 °C.

Cooked yield

Raw meatballs were weighed. After cooking, meatballs were held at room temperature for 10 min. The fluid exudate (drip) was drained off, and the samples were re-weighed. Cooked yield was calculated as follows:

\[
\text{Cooked yield} (\%) = \frac{[(\text{drained weight after cooking}) / (\text{weight before cooking})] \times 100}
\]

Hunter color measurement

Hunter color lightness, redness, and yellowness (CIE \(L^*, a^*, b^*\)) values were measured on the meatballs and sausage samples using a Hunter Lab Miniscan portable colorimeter with a 5-mm aperture (Reston, Va., U.S.A.). The instrument was set for illuminant D-65 and 10° observer angle, and standardized using black and white standard plates.

TBA value

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978). Duplicate meat samples (0.5 g) for all the treatments were mixed with 2.5 mL of stock solution containing 0.375% TBA (Sigma Chemical Co., St. Louis, Mo., U.S.A.), 15% TCA (Mallinkrodt Baker, Inc., Paris, Ky., U.S.A.) and 0.25% HCl. The mixture was heated for 10 min in a boiling water bath (100 °C) to develop a pink color, cooled in tap water, and then centrifuged (Sorvall Instruments, Model RC 5C, DuPont, Wilmington, Del., U.S.A.) at 6000 rpm for 10 min. The absorbance of the supernatant was measured spectrophotometrically (Spectronic 21D, Milton Roy, Rochester, N.Y., U.S.A.) at 532 nm (Sinnhuber and Yu 1958). The MDA concentration was converted to TBA number (milligrams TBA chromagen/kilogram of meat sample) as follows:

\[
\text{TBA nr (mg/kg)} = \frac{A_{532} \times (1 \text{ M TBA Chromagen/}156000) \times [(1 \text{ mole/L}) / M] \times (0.003 L / 0.5 \text{ g meat}) \times (72.07 \text{ g MDA/mole MDA}) \times (1000 \text{ g/kg})}{(1)}
\]

or

\[
\text{TBA nr (ppm)} = A_{532} \times 2.77
\]

Results and Discussion

Control meatballs had a cooked yield of 65.8%, which was not different \((P < 0.05)\) from the mean cooked yield of 68.7% for the meatballs containing 1.5% MM. Treatments (control, 1.5% MM, 1.5% MM + 20 ppm sodium nitrite, 1.5% MM + 40 ppm sodium nitrite) significantly affected the Hunter color redness \((a^*\)\) and yellowness \((b^*\)\) values but had no effect on lightness \((L^*\)\) values (Table 3). The redness values (pooled over storage time) from highest to lowest were 1.5% MM + 40 ppm nitrite > 1.5% MM + 20 ppm nitrite > 1.5% MM > control (Table 4). Storage days after cooking significantly affected Hunter color \(b^*\) values but had no effect on \(L^*\) or \(a^*\) values (Table 3). Yellowness \((b^*\)\) values were higher \((P < 0.05)\) after 8 d or 15 d storage compared with day 1 samples (Table 4). The treatment \(\times\) storage time interaction was significant \((P < 0.05)\) for Hunter color \(a^*\) values but not for \(L^*\) or \(b^*\) values (Table 3). Control samples (without MM or nitrite) had a significant decrease in redness \((a^*\)\) values during storage, from 4.6 on day 1 to 1.3 on day 15 (Table 4). The MM and both treatments with sodium nitrite had a protective effect on color during storage, and no change was observed in cooked samples during storage (Table 4). As expected, nitrite cured samples had a pink color and higher redness values than control or MM samples. Redness values exhibited a dose response with higher redness values for samples with the higher level of added nitrite (40 ppm; Table 4). It was also noted that redness values significantly increased during 15 d of storage for samples treated with 1.5% MM + 40-ppm nitrite (Table 4).

With regard to TBA values, treatment main effects and the 2-way interaction of treatment \(\times\) storage time were highly significant (Table 3). The main effect of storage time (days) did not affect TBA values because TBA values did not change significantly with time for 3 of the 4 treatments (those containing MM; Table 3).

Figure 1 shows the 2-way interaction for treatment \(\times\) day effects on TBA values of cooked products. TBA values of control meatballs increased to >6.0 during 15 d of refrigerated storage (Figure 1). Meatballs with 1.5% MM had lower \((P < 0.05)\) TBA values than the control meatballs. Sausages with 1.5% MM and 20-ppm or 40-ppm sodium nitrite also had TBA values lower than control samples but not significantly different from the treatment with MM alone (Figure 1).

Cornforth and West (2002) previously reported that cooked ground beef and pork required 2% MM to maintain TBARS values <1.0 after 14 d of storage, compared with 1% MM for ground turkey. TBARS 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, Ca, and citrate), polyphosphates most effectively maintained low TBA values during

Table 1—Composition of milk mineral

<table>
<thead>
<tr>
<th>Constituent</th>
<th>% Total weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral</td>
<td>80.2%</td>
</tr>
<tr>
<td>Inorganic mineral (ash)</td>
<td>71.2%</td>
</tr>
<tr>
<td>Organic mineral (citrate)</td>
<td>9.0%</td>
</tr>
<tr>
<td>Calcium</td>
<td>24.0%</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>13.5%</td>
</tr>
<tr>
<td>Water</td>
<td>4.0%</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0%</td>
</tr>
<tr>
<td>Protein</td>
<td>5.0%</td>
</tr>
<tr>
<td>Fat</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

Typical particle size <7-μm dia


Table 2—Formulation of beef meatballs and beef sausage

<table>
<thead>
<tr>
<th>Treatment Constituents (% meat weight)</th>
<th>Constituents (% meat weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Ground beef, 10.0% water, 1.5% salt, made into meatballs</td>
<td>Ground beef, 10.0% water, 1.5% salt, made into meatballs</td>
</tr>
<tr>
<td>Milk mineral</td>
<td>Milk mineral + sodium nitrite 20 ppm</td>
</tr>
<tr>
<td>Milk mineral + sodium nitrite 40 ppm</td>
<td>Ground beef, 10.0% water, 1.5% salt, made into meatballs</td>
</tr>
</tbody>
</table>

Table 3—Results for Hunter color lightness, redness, and yellowness (CIE \(L^*, a^*, b^*\)) values for samples with the higher level of added nitrite (40 ppm; Table 4). It was also noted that redness values significantly increased during 15 d of storage for samples treated with 1.5% MM + 40-ppm nitrite (Table 4).

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Milk mineral in cooked or cured meats . . .

Table 3—Summary of significance (P < 0.05) as determined by analysis of variance (ANOVA)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>mm</td>
<td>*</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>mm + nit 20 ppm</td>
<td>*</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>mm + nit 40 ppm</td>
<td>*</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>LSD0.05</td>
<td>NS</td>
<td>0.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* = significant at P < 0.05; NS = not significant at P > 0.05.

storage. The authors concluded that MM chelates soluble iron to colloidal calcium phosphate particles, thus removing iron as a catalyst for lipid oxidation (Cornforth and West 2002). Lactoferrin is a milk protein that binds iron and thus may possibly contribute to antioxidant effects of MM. However, the antioxidant contribution of lactoferrin in MM is small. Lactoferrin in TruCal™ MM is non-detectable by immunoassay. MM contains only 5% protein consisting entirely of alpha-lactalbumin (MW 14000) and beta-lactoglobulin (MW 18500) (Bastian 2005).

Jayasingh and Cornforth (2003) compared the antioxidative activity of 0.5% to 2.0% MM with that of BHT and sodium tripolyphosphate (STPP) in raw and cooked pork mince during frozen (–20 °C) or cold (2 °C) storage. In addition, effects of holding time before serving were investigated on the TBA values of pork patties, and the impact of TBA values on sensory acceptability was determined. The different treatments had no effect on the oxidative stability of raw meat (Jayasingh and Cornforth 2003). However, cooked samples with MM or STPP had significantly lower TBA values than were observed for the treatment with butylated hydroxytoluene (BHT). TBA values of cooked patties did not significantly increase during 0 to 60 min of holding time, but TBA values were significantly higher after 90 or 120 min. Sensory panelists preferred patties with TBA values <0.5, compared with patties that had TBA values >1.4 (Jayasingh and Cornforth 2003).

In the United States, sausages are typically formulated with 156-ppm sodium nitrite. However, cured pink color development occurs with as little as 14-ppm sodium nitrite in beef rounds or 4 ppm in pork shoulder cuts (Heaton and others 2000). The USDA-FSIS permits nitrite levels as low as 40 ppm in bacon, in combination with sugar and starter cultures, so that fermentation occurs (USDA 1999). Inhibition of Clostridium botulinum is achieved by product acidification during fermentation.

In the present study, sausages with 20-ppm or 40-ppm sodium nitrite were both pink, but pink color was most intense in sausages with 40-ppm nitrite after 15 d of storage. There were no synergistic effects of 1.5% MM with sodium nitrite on TBA values during storage of beef sausages. MM (1.5%) alone was sufficient to maintain low TBA values during storage. Addition of 20 or 40 ppm nitrite to samples containing 1.5% MM did not decrease the TBA values during storage, compared with samples with MM alone.

Conclusions

1.5% MM was very effective for inhibition of oxidation in cooked meatballs during 15 d of refrigerated storage. Thus, MM has a potential application as an antioxidant for addition to ground meats before cooking. Addition of 20-ppm or 40-ppm sodium nitrite to sausages containing 1.5% MM did not result in lower TBA values. Thus, there was no synergistic effect between 1.5% MM and sodium nitrite for improving oxidative stability of cooked beef sausages.

Acknowledgments

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References


URLs and E-mail addresses are active links at www.ift.org
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