Acceleration of Tenderization/Inhibition of Warmed-Over Flavor by Calcium Chloride-Antioxidant Infusion into Lamb Carcasses

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ABSTRACT

Infusion of 0.3M calcium chloride plus 1% sodium ascorbate or 0.25% maltol into freshly slaughtered lambs resulted in acceleration of post-mortem tenderization through activation of calcium dependent proteases, inhibition of lipid oxidation and retardation of warmed-over flavor on ground cooked patties from their meat. After storage 2 days at 4°C, patties from lamb carcasses treated with antioxidants retained more desirable flavor characteristics (meaty and mussy/herby) of lamb, and had less off-flavor (painty and cardboardy) intensities.

INTRODUCTION

RECENTLY, Kooomaraie et al. (1988a, 1989) reported that infusion of lamb carcasses with 0.3M calcium chloride resulted in acceleration of postmortem tenderization. They concluded that the observed postmortem proteolysis and subsequent tenderization was due to activation of Ca^{2+}-dependent proteases, and not due to the ionic strength of the calcium chloride solution.

Recently, scientists at the Southern Regional Research Center (SRRC) have been engaged in indepth research to elucidate the mechanism of action for development of warmed-over flavor (WOF) and to establish means of retarding or inhibiting the reaction without decreasing desirable meaty flavor characteristics. To this end, several reports were published. First, a new descriptive language was established to evaluate WOF in beef (Johnson and Civille, 1986), which was later modified (Love, 1988). Then, a direct gas chromatographic method to identify chemical marker volatile compounds was developed to assess meat quality, in particular WOF (St. Angelo et al., 1987; Dupuy et al., 1987; Liu et al., 1987; Verceilotti et al., 1987). The data from chemical, instrumental and sensory methodologies correlated very highly, and may be used in studies with various additives, such as free radical scavengers, chelators, or water activity regulators (St. Angelo et al. 1988, 1990).

In our current study, the objectives were to identify compounds that would dissolve in 0.3M calcium chloride, not bind or chelate calcium (needed to activate proteases that accelerate postmortem tenderization), retain desirable flavor characteristics and retard WOF development.

MATERIALS & METHODS

Animals

Twenty-five lambs (9 to 12 months of age, 36 to 45 kg, live weight) were slaughtered at the Meat Animal Research Center, Clay Center, NE, in groups of five; a group for each of five treatments, which included the following: (1) control (animals slaughtered by normal procedures); (2) electrically stimulated [ES] immediately after death [2 Hz, 100 volts, 360 pulses, 10 sec on, 10 sec off]; (3) ES and then infused with a volume equal to 10% live weight of 0.3M calcium chloride; (4) same as Treatment 3 but with 0.25% added maltol; (5) same as Treatment 3 but with 1.0% added sodium ascorbate (SA).

Infusion and sampling

Immediately after slaughter, lamb carcasses were transferred to a lamb cradle, the carotid artery was exteriorized, and solutions were pumped into the artery as previously described (Kooomaraie et al., 1989). Briefly, during infusion, one jugular vein remained intact while the other was cut and tied. The artery not used for infusion was clamped. When infusion was completed, carcasses were dressed and transferred to a holding cooler (1-2°C). Twenty-four hr after slaughter, the loin was removed, divided into two sections and assigned to sampling on day 1 or day 7 postmortem for determining shear force. The remaining loin was ground twice, frozen and shipped via air express to SRRC for evaluation by chemical, instrumental and sensory means.

Shear force determination

Shear force (Kg) of the cooked chops (2 chops x postmortem time^-1 x animal^-1) was determined according to the procedure of Kooomaraie et al., 1988b). Five animals were used on shear force determination per treatment.

Screening and identification of antioxidants

Many compounds previously shown (St. Angelo et al., 1988, 1990) to be active antioxidants for preventing development of WOF were screened for solubility in 0.3M calcium chloride. Three of these, maltol (3-hydroxy-2-methyl-4-pyrole), Aldrich Chemical Co., Milwaukee, WI, kojic acid (3-hydroxy-2-hydroxymethyl-4-pyrole) Aldrich Chemical Co., and sodium ascorbate (3-oxo-L-gulofuranolactone, vitamin C) Sigma Chemical Co., St. Louis, MO, were completely soluble. Maltol and sodium ascorbate were the selected antioxidants, kojic acid was not selected since it was neither on the Generally Regarded As Safe (GRAS) list nor FDA approved as a food additive.

Sensory evaluation of lamb and lamb-treated samples

An inhouse analytical sensory panel of 16 members was trained for descriptive analysis of lamb samples using ground lamb patties, freshly cooked and cooked/stored in the refrigerator for up to 3 days to develop the lexicon of descriptive terms. The patties were prepared from leg of lamb purchased from a local supermarket; the carcass fat was removed, and the remaining leg of lamb was ground twice (1.0 cm plate followed by 0.8 cm plate) and made into patties, 80g each. Standard patties were prepared from fresh leg of lamb ground as described above and stored frozen in glass petri plates until the morning of assay, at which time they were thawed, cooked, and assayed. The method used to train the panel was based on that previously described (Johnson and Civille, 1986: Love, 1988) but for lamb. The lexicon of descriptive terms included the following: meaty (the flavor associated with cooked muscle meat, such as beef), gamey or musty (the flavor associated with meat meat from wild game or older lambs), musty/herby (associated with wet soil or mulch and dried herbs, such as rosemary or thyme), browned/caramel (associated with the outside of grilled or broiled meat, scorched, but not burnt), grainy or powdery (associated with cow meat and/or meat in which grain or feed character was detectable), bloody/seerum (associated with raw lean meat), livery (associated with organ meats such as liver), fatty (associated with cooked lamb fat), pastry (similar to unseeded oil and associated with rancid fat or oil), and cardboardy (similar to wet cardboard and associated with refrigerated cooked meat). The four basic tastes were also used. These were: sweet (taste on tongue as with
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sugars), sour (as with acids), bitter (as with agents such as caffeine or quinine), and salty (as with sodium ions). The descriptor, asstringent (the chemical flavor on the tongue described as dry and as with tannin or alum) was also used. During tasting, the list was reduced to the most significant terms that described both desirable flavor of the lamb patties, i.e., meaty, fatty, mushy/herby, browned/caramel, and those that described undesirable flavors, i.e., pungent and cardboardy.

In preparation for the sensory panel session on patties made from the 5 sets of treated lamb prototypes, half the ground experimental samples were removed from the freezer and allowed to thaw overnight at 4°C. The next morning, they were made into 80g patties, cooked and stored in glass Petri plates at 4°C for 2 days, at which time they were rewarmed and evaluated. The day prior to the panel session, the remaining half was removed from the freezer and allowed to thaw overnight at 4°C. The following morning, they were made into 80g patties, cooked, and assayed as day zero samples. Sensory scores were presented as intensity values on a 15point scale with 0 indicating lowest intensity and 15 the highest (Melsen, 1987). After rewarining or cooking, each patty was cut into 8 wedges and served in glass petri plates (60mm x 15mm) with covers. Each sample was assigned a 3-digit random number to mask sample identity. Panelists were seated in partitioned booths, lighted with red lights to eliminate color bias. Panelists received one sample at a time, and were instructed to rinse with deionized water between samples, which were served 5min apart. All panel members were able to distinguish the lamb descriptors at a very high degree of proficiency and reproducibility. Standards were used for warm-up purposes and standardization of panelists.

Chemical and instrumental analyses

The effect of different treatments on lamb samples was evaluated chemically by determining the TBARS (2-thiobarbituric acid reactive substances) numbers by the method of Tarladgis et al. (1960), and by a direct gas chromatographic (DGC) method previously described (Dupuy et al., 1987; St. Angelo et al., 1987). A standard malonaldehyde (MDA) curve was prepared with 1,1,3,3-tetramethoxypropane (Aldrich Chem. Co.), a Hewlett-Packard 5450A, diode array spectrophotometer was used to measure absorbance at 532nm for calculating TBARS numbers reported as mg MDA/kg sample. The DGC method used an external closed inlet device (ECID, Scientific Instruments Service, Harahan, LA) interfaced with a Trace 222 GC. The method used the ECID for securing a small (0.84 cm x 8.5 cm) glass liner containing the ground lamb samples (ca.1.2g) directly into the injection port of a GC, heating the sample and eluting volatiles onto a packed column as described by Legnarde et al. (1979). From the GC profiles, the degree of lipid oxidation was measured as increase in integrator area counts for hexanal and for total volatiles. Duplicate samples were run and values were within 6% error. This method has been found highly repeatable at the 5-6% level (Dupuy et al., 1978; Vercellotti et al., 1988). Confirmation of hexanal was by standards and by a Finnigan-MAT 4000 GC/Mass Spectrometer/lnconos Data System previously described (Dupuy et al., 1987; St. Angelo et al., 1987).

Statistical analyses

Data were statistically analyzed by a two-way analysis of variance (ANOVA) method (SAS/STAT, 1985). Samples representing animals used under one of the five treatments at a given storage time were identified as replicates of an "animal" variable. All observations from each person on the panel were identified by a level of the variable, "PANEL." These classification variables were used as blocks in the analysis for the purpose of removing variability in the data caused by an individual panelist or animal. The remaining variables in the analysis, "TREATMENT" and "STORAGE TIME," were used as class variables in the two-way ANOVA. Statistical analyses were performed with 5 animals/treatment for 5 storage determinations and with 4 animals/treatment for chemical and sensory determinations.

Results of each of the 4 single degrees of freedom treatment contrasts were listed in ANOVA tables, and were presented in terms of P-values. These P-values result from the appropriate F-test for each contrast and corrected, where appropriate. In each contrast, as the probability that the observed difference in means occurred by chance alone, P-values were equivalent to the level of significance, α. Hence, contrasts (i.e., mean differences) resulting in a P-value less than 0.05 (*) or 0.01 (**) were significant at the 0.05 or 0.01 α-level of significance, respectively.

RESULTS & DISCUSSION

AS PREVIOUSLY REPORTED by Koohmaraie et al. (1988a, 1989), infusion of lamb carcasses with 0.3M calcium chloride reduced the shear force values at day 1 dramatically when compared with control carcasses or those that were electrically stimulated (ES) but not infused. Of several concentrations of calcium chloride solution examined, the 0.3M solution was most effective in reducing shear force at 24 hr postmortem. In our current study, maltol and SA, two antioxidants previously used to inhibit WOF in beef (St. Angelo et al., 1990) were found soluble in 0.3 M calcium chloride solution, which was infused into lambs immediately after electrical stimulation.

Mean comparisons and results of ANOVA for physical, chemical, and sensory markers by storage time are presented in Table 1. In the table, data from experiments were divided into separate days (1 and 7 for shear force, and 0 and 2 for chemical and sensory determinations) because there was a treatment interaction. Results indicated that shear forces of ES carcasses were not significantly different from those of controls. However, the shear force values of ES carcasses infused with calcium chloride were significantly reduced when compared to ES non-infused lambs or controls. Thus, tenderization was significantly increased. This was observed in both 1 day treated lambs and to a lesser degree, in 7 day treated animals. The slight differences observed in shear force between 1 and 7 days stored samples were within experimental error of the method of analysis. On adding either maltol or SA to the infusion medium, no differences (P>0.05) were observed in shear force from those of ES carcasses infused with calcium. This was apparent in both the 1 and 7 day postmortem lamb carcasses that were held in the chilled room. Therefore, the catalytic effect of calcium ions on proteolysis and tenderization of lamb carcasses was not impacted.

Results of mean comparisons for chemical and sensory markers for meat cooked and stored 0 and 2 days at 4°C showed no significant difference in hexanal or TBARS values for patties from controls or those from the ES treated, noninfused lambs. Two-day stored patties from samples from ES + calcium infused carcasses were significantly different (P<0.05) for hexanal and total volatiles, and very highly significant (P<0.01) for TBARS when compared to ES carcasses (no infusion) under the same conditions. In each case, mean values increased. When either maltol or SA were added to the ES + infusion medium, the 2-day patties showed highly significant differences when compared to ES + infusion medium. In those instances, mean values for hexanal, total volatiles, and TBARS were decreased by adding antioxidants. At 0-day storage, there were no significant contrasts for total volatiles, even though there was a decrease in mean values. However, at 0-day, samples that contained antioxidants had hexanal and TBARS mean values highly significantly different compared to samples that did not contain the antioxidants. Again, mean values had decreased in each comparable sample.

The linear trend analysis for chemical data, i.e., hexanal, total volatiles, and TBARS values, showed similar trends relative to treatments. For example, at 0 and 2 days, TBARS values for the control and ES treated lambs were essentially the same. When calcium chloride solution was infused, the mean was doubled, whereas it was more than doubled, whereas the highly significant differences observed were due to an increase in lipid oxidation catalyzed by calcium. When maltol was added to the calcium chloride solution, TBARS values decreased by 78% for the 0 day samples and 74% for samples stored 2 days, compared to the ES + CaCl2 control. Similar results were observed when SA was added to the calcium chloride solution. The decrease in TBARS values was 74% for 0 day samples and 92% for
Table 1—Means, standard errors, and P-values of treatment contrasts for responses with significant treatment by storage time interaction.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Means/standard error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>ES</td>
<td>ES + CaCl₂</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
</tr>
<tr>
<td>Shear Force</td>
<td>8.290/0.48</td>
<td>9.70/0.48</td>
</tr>
<tr>
<td></td>
<td>5.29/0.58</td>
<td>6.05/0.58</td>
</tr>
<tr>
<td>Hexanil</td>
<td>1.75/6.97</td>
<td>18.25/6.97</td>
</tr>
<tr>
<td></td>
<td>215.00/38.13</td>
<td>128.63/38.13</td>
</tr>
<tr>
<td></td>
<td>225.26/65.27</td>
<td>230.63/65.27</td>
</tr>
<tr>
<td>TBARS</td>
<td>0.010/0.004</td>
<td>0.0240/0.004</td>
</tr>
<tr>
<td>(mg MDA/kg sample)</td>
<td>0.011/0.004</td>
<td>0.0240/0.004</td>
</tr>
<tr>
<td>Mean*</td>
<td>0.45/0.10</td>
<td>0.48/0.11</td>
</tr>
<tr>
<td></td>
<td>3.56/0.13</td>
<td>3.74/0.15</td>
</tr>
<tr>
<td>Musty/Herby*</td>
<td>0.74/0.08</td>
<td>0.10/0.12</td>
</tr>
<tr>
<td>Carboxydo*</td>
<td>1.90/0.13</td>
<td>0.51/0.15</td>
</tr>
<tr>
<td></td>
<td>2.07/0.16</td>
<td>1.96/0.19</td>
</tr>
<tr>
<td>Painty*</td>
<td>1.90/0.13</td>
<td>0.31/0.15</td>
</tr>
<tr>
<td></td>
<td>2.25/0.17</td>
<td>0.80/0.20</td>
</tr>
<tr>
<td>Sweet*</td>
<td>0.69/0.09</td>
<td>1.32/0.10</td>
</tr>
<tr>
<td></td>
<td>1.85/0.12</td>
<td>1.29/0.14</td>
</tr>
</tbody>
</table>

* Sodium ascorbate.


* Mean differences significant at the 0.05 level.

** Mean differences significant at the 0.01 level.

those stored 2 days. These values were significantly less than the control and ES samples. Why calcium chloride caused an increase in TBARS values is not clear. However, that calcium can accelerate hydrolysis of fats by pancreatic lipase has been known for many years (Schonheyder and Volqvart, 1945; Borgstrom, 1954). Calcium ions have also been known to stimulate activity of soybean lipoygenase (Koch et al., 1971) and peanut lipoygenases 2 and 3 (Nelson et al., 1977). Perhaps, the increased lipid oxidation, as indicated by increased TBARS, related to the effects of calcium ions on those enzymes.

In addition, as WOF developed over 2 days at 4°C, the other chemical markers of lipid oxidation, hexanal and total volatiles, increased similarly to TBARS values in the control and ES samples. These changes came from lambs ES and infused with calcium chloride doubled lipid oxidation when compared to ES control without infusion. When either maltool or SA was added to the calcium chloride solution, lipid oxidation decreased significantly. In the 0 day samples, hexanal decreased from 47,300 area counts for ES + CaCl₂ control to 13,500 for maltool treated samples. This decrease was 71%. When calculations were made for 2-day samples, reduction in hexanal was 79%. Similar calculations for SA treated samples showed reductions in hexanal of 90% for both 0 and 2-day samples.

Adding maltol to calcium chloride treated samples decreased total volatiles by 41% in 0-day patties and by 64% in those stored 2 days. Adding SA to the calcium chloride infusion medium, decreased total volatiles by 47% for 0-day patties and by 74% for those stored 2 days. Thus, both maltol and SA retarded lipid oxidation of raw lamb ground meat when stored frozen at least 3 months, or thawed, made into patties, cooked, and then stored in the refrigerator two days.

In an attempt to determine whether lambs treated with calcium chloride infusion + antioxidants were different from those treated only by ES, comparisons were made from those patties. The ANOVA data showed no significant differences among those patties as judged by hexanal, total volatiles and TBARS. As shown above, calcium chloride significantly increased lipid oxidation (compare ES vs ES + CaCl₂). Addition of antioxidants to ES + infusion mixture significantly retarded WOF formation and thus overcome the calcium effect (compare ES - CaCl₂ vs ES + CaCl₂ + maltol or SA). Therefore, when one infuses calcium chloride solution into an animal to enhance tenderization, one should add to the medium an antioxidant, such as maltol or SA, which would not impede the desirable calcium effect. This would protect the meat from lipid oxidation and/or developing WOF.

Sensory data from the 5 experimental samples, means, and standard errors are presented as P-values in Table 1. Zero day samples from lambs not infused but ES showed highly significant differences (p<0.01) in WOF descriptors, cardboxydo, cardboxydo and painty, when compared to controls. However, no significant difference occurred among samples stored 2 days. When animals were infused with calcium chloride, no significant differences were observed in any of the flavor descriptors for zero or stored samples when compared to ES alone. However, by adding maltol or SA to the infusion medium, highly significant differences were observed in WOF descriptors (painty and cardboxydo) for fresh or stored patties.

As shown in the table, when calcium was infused into ES carcasses, the WOF sensory attributes, cardboxydo and painty, increased when the patties were stored 2 days compared to the ES treated carcasses. During storage, differences increased by 1.76 units for cardboxydo and 2.24 units for painty. On the other hand, increases observed in ES controls were 1.45 for cardboxydo and 1.74 for painty. These observations supported the chemical data that indicated lipid oxidation was promoted by calcium chloride infusion into ES carcasses. However, when maltol was added to the infusion mixture, the increase in 2-day sensory values from calcium chloride infused samples was greatly reduced. For example, the overall net increase was 0.26 for cardboxydo and 0.33 for painty. Likewise, when SA was added to the infusion mixture, the 2-day sensory values also were reduced when compared to the calcium infused carcasses. The net increase during storage was 0.12 for cardboxydo and 0.39 for painty.

Meaty, musty/herby, and sweet, were all sensory terms for desirable flavor notes. They showed a decrease of 1.01 units for meaty, and 0.15 units for sweet, and an increase of 0.05 units for musty/herby in the ES + calcium infused samples. When maltol was added to the infusion solution, the meaty descriptor decreased by 0.66 units but sweet taste increased 0.08 and musty/herby increased by 0.24 units. When sodium ascorbate was added to the patties, meaty decreased by 0.59, whereas sweet increased 0.20 and musty/herby increased 0.10 units. That is, adding maltol or SA to the calcium chloride infusion mixtures, not only reduced lipid oxidation, but also enhanced flavor.
retained desirable flavor characteristics and diminished formation of off-flavors. The flavor descriptors, gamey, browned/caramel, bloody/sour, fatty, sour, salty, bitter and astringent showed no significant treatment-storage interaction effects. However, by averaging responses from both storage times for each treatment, a significant treatment effect could be identified. These data are presented in Table 2 as P-values of treatment contrasts pooling storage times. That is, with any treatment, almost all these attributes showed significant change after being stored 2 days. When patties from ES + CaCl2 infused animals were compared to those with added maltol, the sensory attributes with the most significant differences were browned/caramel and sour. When ES was added to the SA + CaCl2 infusion mixture, the most significant difference in sensory descriptors was fatty, salty and bitter. A description of these differences as well as the means and standard error for all data are also presented in Table 2. For example, with maltol-treated samples, browned/caramel (a desirable flavor note) increased by 0.23 units, whereas sour (an undesirable flavor note associated with WOP) decreased by 0.17 units. In samples treated with SA, the flavor note, bitter, associated with WOP, decreased by 0.27 units.

In summary, our results indicated that infusion of lamb carcasses with calcium chloride accelerated postmortem tenderization such that storage of carcasses beyond 1 day to ensure tenderness was not necessary. Results also indicated that calcium chloride increased lipid oxidation and WOP development. Maltol and/or sodium ascorbate, co-infused with calcium chloride, overcame the prooxidant effect of calcium, reduced lipid oxidation and retarded development of WOP, but did not adversely interfere with tenderization effects of the calcium chloride. Samples treated with antioxidant had the lowest off-flavor (painty and cardboardy) intensities while maintaining high desirable (meaty, sweet and mushy/herby) intensities. Therefore, lamb carcasses treated with calcium chloride plus antioxidants were not only more tender, but patties made from them had more desirable flavor.

REFERENCES


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