Liquid Smoke Effects on *Escherichia Coli* O157:H7, and its Antioxidant Properties in Beef Products

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ABSTRACT

Experiments were performed to evaluate the antibacterial properties of liquid smoke (LS), against *E. coli* O157:H7, in model (agar) and meat systems. The effects of 8% LS on growth of *E. coli* O157:H7 attached to ground beef, and 1.5% LS on warmed-over flavor (WOF) in precooked beef patties were also studied. *E. coli* O157:H7 growth was reduced ($p<0.05$) 2.3 $\log_{10}$ CFU/g in ground beef patties after 3d refrigerated storage. TBA numbers, aroma scores and pH values were lower ($p<0.05$) in LS treated beef patties. LS reduced undesirable flavor development and may help assure the safety of beef products.

Key Words: liquid smoke, *E. coli* O157:H7, beef, WOF

INTRODUCTION

OUTBREAKS OF FOODBORNE ILLNESS AND DEATHS ASSOCIATED with ground beef containing *E. coli* O157:H7 have been reported (Davis et al., 1993). Studies have demonstrated that *E. coli* O157:H7 is the third or fourth most common enteric pathogen re-covered from human stool samples (MacDonald et al., 1988; Marshall et al., 1990). *E. coli* O157:H7 has been recognized as a foodborne pathogen since 1982 and undercooked beef products, especially ground beef, have been implicated in outbreaks of *E. coli* O157:H7 infections (Anonymous, 1993; Belongia et al., 1991).

In addition to microbiological concerns, ground beef is susceptible to warmed-over flavor (WOF) or oxidative rancidity, which is a notable sensory change during freezer-storage (Bhattacharya et al., 1988). Lipid oxidation in meat products, however, has been effectively controlled or retarded by the use of antioxidants.

Smoking of food provides a desirable flavor and color, and contributes substantially to preservation by acting as an effective antioxidant and antimicrobial agent (Draudt 1963). As a food additive, the use of liquid smoke (LS) as an antioxidant and/or antimicrobial agent has the advantage of being labeled as "Smoked" or "Naturally Smoked" (USDA-FSIS, 1996).

The objectives of these studies were to evaluate the bactericidal effects of adding 8% LS to beef trimmings, inoculated with *E. coli* O157:H7 and allotted to the production of ground beef, and evaluate antioxidant properties of 1.5% LS in precooked beef patties.

MATERIALS & METHODS

Liquid smoke

A low flavor profile LS (Code V, Hickory Specialties, Brentwood, TN) was used for all experiments. The specifications of the LS fractions were titratable acidity, 6.8–7.8; staining level, none; phenol level, 1.4–4.0 mg/mL; carbonyl level, 2.0–7.0 g/100 mL; specific gravity at 25°C, 1.005–1.015; benzopyrene, <1 ppb; color, amber; and pH, 2.0.

Bacterial culture and inoculum

A strain of *E. coli* O157:H7 (Rifampicin resistant), from the Kansas State Univ. Food Microbiology culture collection, was used. The stock culture was subcultured monthly on a tryptic soy agar (TSA, DIFCO, Detroit, MI) slant at 4°C. Working cultures were transferred from the stock culture by inoculating brain heart infusion (BHI, DIFCO, Detroit, MI) broth. The culture was incubated at 37°C for 20h to obtain 10^9 colony forming units (CFU)/mL. After incubation, cells were harvested by centrifuging the culture at 5,500 × *g* for 10 min at 4°C, using a JA-14 rotor (Beckman J2-HS centrifuge, Beckman Instruments, Inc., Palo Alto, CA). The cell pellet was resuspended to original volume in 0.1% peptone (DIFCO, Detroit, MI), and used to inoculate beef trimmings. The suspension was plated on MacConkey sorbitol agar (MSA, DIFCO, Detroit, MI) containing Rifampicin (100 ppm; Sigma Chemical Co., St. Louis, MO) using a Spiral Plater™ (Model 500 D, Spiral Biotech Inc., Bethesda, MD) to provide an estimate of inoculum size.

Sample preparation for antimicrobial tests

Fresh beef trimmings (85% lean and 15% adipose; 4 days postmortem) were obtained from the Meat Lab of the Department of Animal Sciences and Industry, KSU. One treatment and two controls were used. *E. coli* O157:H7 was added to the beef trimmings to provide 10^7 CFU/g, and mixed for 4 min using a Hobart mixer (Hobart Corp., Troy, OH). LS and sterile water, treatment and control, respectively, were added to the inoculated trimmings for a final concentration of 8%, and mixed for 4 min. A second control, without LS, water or *E. coli* O157:H7, was mixed for 4 min and used for psychrotrophic counts. The treated sample and controls were coarsely ground (1.27 cm), followed by a fine grind (0.32 cm) using a sterile grinder (Hobart Corp., Troy, OH). Patties were made (70–90g), using a manual patty maker (Holly JR., Hollymatic Corp., Chicago, IL), packaged (Harbro Packaging Company, Chicago IL) aerobically, heat sealed (Multivac A300(16), Germany), and stored in the dark at 4°C for up to 3 days. The treated sample and both controls were obtained from the same batch of trimmings. Three replicates were performed for each treatment and control.

Microbial analysis

Immediately after inoculation, duplicate surface samples (25g) were taken each from inoculated, treated (LS or water), and non-inoculated beef trimmings to check initial *E. coli* O157:H7 populations, antibacterial effects of treated trimmings, and psychrotrophic counts, respectively. Surface samples (0.7 cm deep) were taken from the prepared trimmings with a sterile scalpel and tongs. Duplicate samples (25g) also were taken from the LS treated, inoculated controls, and non-inoculated control patties at day 0, 1, 2 and 3. Samples were placed in a filtered stomacher bag (Seward, London, UK), 225 mL of 0.1% peptone water was added, and stomached for 2 min (Model 400, Tekmar, Inc., Cincinnati, OH). Serial dilutions were prepared in peptone water (0.1%) and spiral plated on MSA containing 100 ppm Rifampicin for the inoculated samples, and on plate count agar (PCA, DIFCO, Detroit, MI) for the non-inoculated controls. The plates for inoculated samples were incubated at 37°C for 24h, and the plates for non-inoculated samples were incubated at 7°C for 10 d for psychrotrophic counts. A Laser Spiral System Bacterial Colony Counter (Spiral, Model 500A) set at 2.5 spot size, 10 cm plate size, 999 area limit, and with 3 degrees of sensitivity was used to automatically scan the petri dishes and count the colonies growing on or in the culture medium. The counts were reported as

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$\log_{10}$ CFU/g sample. The method for the detection of *E. coli* O157:H7 rifampicin resistant was a modification of the method used by Hardin et al. (1995). A higher peptone volume was used for our study.

Sample preparation for antioxidative tests

Fresh beef gooseneck round (9 kg, 2 wk postmortem), obtained from a commercial source was ground successively through 1.27, 0.48, and 0.32 cm plates, and formulated to give 20% fat content on a green weight basis. The meat block was split in half. One half was treated with 1.5% LS, and the other half (without water) served as a control. The treatment and the control were each blended in a mixer at speed 1 (Hobart Corp., Troy, OH), for 2 min. The ground beef was made into 113g patties (1.27 cm thick), using a patty maker machine (Hollymatic Corp., Countryside, IL). Patties were stored at 4°C for 15h prior to cooking. The study was repeated 3 times.

Cooked sample preparation

Patties made as described were cooked according to American Meat Science Association (AMSA) Cookery Guidelines (AMSA, 1978) on a preheated (163°C) electric skillet (Model TRG, Sunbeam Corp., Chicago, IL) to 71°C internal temperature. In order to achieve uniform heat distribution, patties were turned every 1.5 min. After 4 min cooking, patties were turned every 30s. Patties were removed from the grill when they reached an internal temperature of 69 to 71°C. The starting and endpoint temperatures were monitored by a hypodermic needle probe (Omega Engineering, Inc., Stanford, CT) connected to a Doric temperature recorder (Trendicator 410A, Vas Engineering, San Francisco, CA). After cooking, patties were individually and aerobically packed in heat sealed (Ultravac, Kansas City, MO) 20×15 cm plastic bags (Harbro Packaging Company, Chicago, IL), and immediately frozen at −15°C. Patties evaluated on day 0 were not frozen.

Cooked sample sensory evaluation

A 5-member, sensory panel from the KSU Department of Animal Sciences and Industry evaluated the warmed over flavor (WOF) profile of the beef patties. All panelists attended six-30 min training sessions over a period of 3 wk. Panel training consisted of members individually examining different intensities of WOF in reheated precooked beef patties, and discussing their opinions in open sessions to establish unanimity of WOF intensity.

Frozen (−15°C) precooked beef patties were thawed at 4°C for 24h. Samples were reheated to an internal temperature of 71°C, and kept warm in a dual flow gas convection oven (Model DFG-102 CH-3, The G.S. Blodgett Company, Inc., Burlington, VT) . The endpoint temperature was monitored by a hypodermic needle probe connected to a Doric temperature recorder. Sample evaluations were made on triangle shaped pieces of re-heated precooked beef patties (1/4 of patty) placed in glass petri dishes. Panelists used a 5-point scale (1=no WOF, 2=slight WOF, 3=moderate WOF, 4=very high WOF and 5=extreme WOF). Panel scores were based solely on the intensity of WOF aroma. Five sensory sessions (0, 30, 60, 75 and 90 day of frozen storage) were held in individual, three-sided booths under red and green lights located in a room free from outside noise and odor. Patties were evaluated individually, immediately following presentation and after 15 min of cooling. Twelve samples (4 samples/replica) were presented at each session.

TBA analysis procedure

Twelve patties (4 patties/replica) were used for each sampling day (0, 30, 60, 75 and 90). A modification of the thiobarbituric acid (TBA) method developed by Witte et al. (1970) was used. This method involves malonaldehyde extraction with aqueous acidic solutions followed by reaction with TBA. A 10g sample was mixed with 15 mL of 10% cold perchloric acid (Fisher Scientific, Pittsburgh, PA) and 20 mL of cold, distilled, deionized water, and blended using a homogenizer (PowerGen 35, Fisher Scientific, Pittsburgh, PA) for 1.5 min. The slurry was then filtered through Whatman # 2 filter paper (Whatman, England). The plastic container was rinsed with 5 mL distilled, deionized water and added to the slurry. Filtrate (5 mL) was transferred to a test tube containing 5 mL of 0.2M TBA reagent (Sigma Chemical Co., St. Louis, MO). Duplicate tubes were prepared for each sample. Test tubes were covered with Parafilm™ (American National Can, Neenah, WI), mixed with a Vortex mixer (Fisher Scientific Touch Mixer Model 232, Pittsburgh, PA) and stored in the dark in an incubator (Precision Scientific, GCA Corporation Chicago, Il) at 27.5°C for 18h to develop the color reaction. A spectrophotometer (Bausch & Lomb Optical Co., Rochester, NY) was used to measure absorbance of the resulting (pink) solution at 529.5 nm. Blanks prepared from 5 mL distilled, deionized water in 5 mL of 0.02M TBA reagent were held in the dark in the incubator along with the samples. TBA standards were prepared using 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO). Three replications were made.

pH determination

Each sample (10g) and 40 mL of deionized distilled water were combined in a stomacher bag (Seward, London, UK) and blended for 1 min at normal speed, before measuring pH. Duplicate samples were used for pH determinations. pH was determined using an Accumet Basic pH meter (Fisher Scientific, Pittsburgh, PA), and an AccuFet electrode (Fisher Scientific, Pittsburgh, PA). pH measurements were taken from the raw ground beef (day 0) and from thawed, cooked beef patties at each time of evaluation (days 0, 30, 60, 75, and 90). Three replications were made.

Proximate analysis

Raw and cooked beef patties were used for proximate analysis of antimicrobial and antioxidative treatments, respectively. Fat was determined by method 960.39, protein by Boric acid modification of Kjeldahl, method 984.13, moisture by method 950.46, and ash by burning at 600°C, method 942.05 (AOAC, 1990). Three replications were made.

Statistical analysis

The statistical design was a split-plot. The meat block was the whole plot, and the meat sample each day was the subplot. Significance was defined at $p \leq 0.05$. The ANOVA and LSD procedures were used for the analysis of variance (SAS Institute, Inc., 1995). The same statistical design was used in the experiment evaluating antioxidative properties of LS.

RESULTS & DISCUSSION

Antimicrobial effects of liquid smoke

Meat constituents of raw beef patties were determined by proximate analysis. Although fat content differences of control and treatment (8% LS) between replicas were significant (3.28, 8.71 and 9.50%), *E. coli* O157:H7 exhibited the same growth trend among replicates.

The addition of 8% LS to beef trimmings (which were later ground) inoculated with *E. coli* O157:H7 demonstrated some inhibition of growth (Fig. 1). In treated beef patties, *E. coli* O157:H7 counts were lower ($p<0.05$) from day 1 to day 3. While in untreated beef patties (control), growth did not change ($p>0.05$). *E. coli* O157:H7 growth was reduced ($p<0.05$) by 0.5, 1.2, 2.0, 1.6 and 2.3 $\log_{10}$ CFU/g after trimmings were treated with 8% LS, and in patties at day 0, 1, 2 and 3 of refrigerated storage, respectively. The psychrotrophic counts in beef trimmings and ground beef remained constant from day 0 to day 1, but increased rapidly from day 2 to day 3. Although counts increased, they did not impact *E. coli* O157:H7 growth (data not shown). Also, *E. coli* O157:H7 growth was not affected by meat fat content in treated or untreated patties. Thus, LS, at the concentration used, was effective in reducing *E. coli* O157:H7 growth in ground beef patties. LS has phenolic compounds and organic acids that inhibit growth of spoilage and pathogenic microorganisms. Phenolic

compounds such as 2.6-dimethoxyphenol, 2,6-dimethoxy-4-methylphenol and 2.6-dimethoxy-4-ethylphenol have high bactericidal activity. Among the organic acids such as formic, acetic, propionic, butyric, and isobutyric acids, acetic acid has the most bactericidal activity (Pearson and Gillett, 1996). Phenols inhibit the growth of bacteria by prolonging the lag phase proportionally to their concentration in the body or in products, whereas the growth rate in the exponential phase remains unchanged, unless the concentration of phenols is very high (Olsen, 1976).

Antioxidant effect of liquid smoke in precooked patties

Meat constituents of cooked beef patties were determined by proximate analysis. Fat content for control and treatment (1.5% LS) between replicate 1 (19.06%) and replicate 3 (16.44%) were different ($p<0.05$). Due to the fat content difference, TBA numbers and/or aroma scores were expected to differ among the two replicates. However, TBA numbers and/or aroma scores were not affected by fat content. The fat content difference probably was not large enough to cause different results.

pH results

The pH values of precooked beef patties treated with 1.5% (treatment) and 0% (control) LS were compared at day 0, 30, 60, 75 and 90 of storage at –15°C (Fig. 2). The pH values of the control were higher ($p<0.05$) than treated samples at all sampling days. These results were expected because the pH of the LS was 2.0. Randall and Bratzler (1970) and Kako (1968) also observed that smoking lowered the pH of meat products. Normal fresh muscle has a pH from 5.3 to 5.7 (Hedrick et al., 1994). In meat, the higher the pH, the longer the cooking time and/or higher the final internal temperature required for denaturation to be complete (Mendenhall, 1989). Schmidt and Trout (1984) showed that high pH inhibited the formation of brown cooked meat color. Muscle with higher pH is more susceptible to microbial problems and conversely, oxidation of meat pigment is favored by lower pH.

TBA results

LS treated precooked beef patties had lower ($p<0.05$) TBA numbers on all sampling days (Fig. 3). Watts and Faulkner (1954) found that liquid smoke at levels of 0.2 to 2% were effective at producing an antioxidant effect. In our study, TBA numbers clearly demonstrated that 1.5% LS in precooked beef patties exhibited antioxidative properties. TBA values for untreated, precooked beef patties increased during the initial 60d of frozen storage, then decreased after 60d and increased again after 75d. The TBA values for treated precooked beef patties decreased during the first 30d, then increased after 30d, decreasing more after 60d. The increase and/or decrease in TBA values at different storage times could be explained by the instability of the malonaldehyde produced, and/or to the oxidation of different lipid populations at different times. Although malonaldehyde can be used to indicate oxidative deterioration of meat, the relationship between oxidation and malonaldehyde production is not clear or direct (Arafa and Chen, 1976).

Aroma effects

Pearson and Gillett (1996) reported that the smoking of meat aided in the prevention of WOF development. WOF intensity, a type of fat rancidity, was evaluated in the precooked beef patties to correlate results with TBA results. Precooked beef patties were evaluated immediately after presentation (Fig. 4) and 15 min after presentation. LS treated beef patties had lower scores ($p<0.05$) compared to nontreated patties at both presentation times. LS treated beef patties evaluated immediately after presentation did not change ($p>0.05$) from day 0 to day 90. Nontreated beef patties scores evaluated immediately after presentation increased after day 0 and day 60. Beef patties scores after 15 min cooling were similar to scores immediately after presentation. However, LS treated beef patties scores increased ($p<0.05$) after day 75. The correlation coefficient between TBA numbers and WOF intensity scores was 0.84 ($p<0.05$). Tarladgis et al. (1960) reported that TBA numbers correlated highly with sensory scores of trained panelists for rancid odors in ground pork. Igene et al. (1985) reported a correlation coefficient of 0.87 ($p<0.01$) between

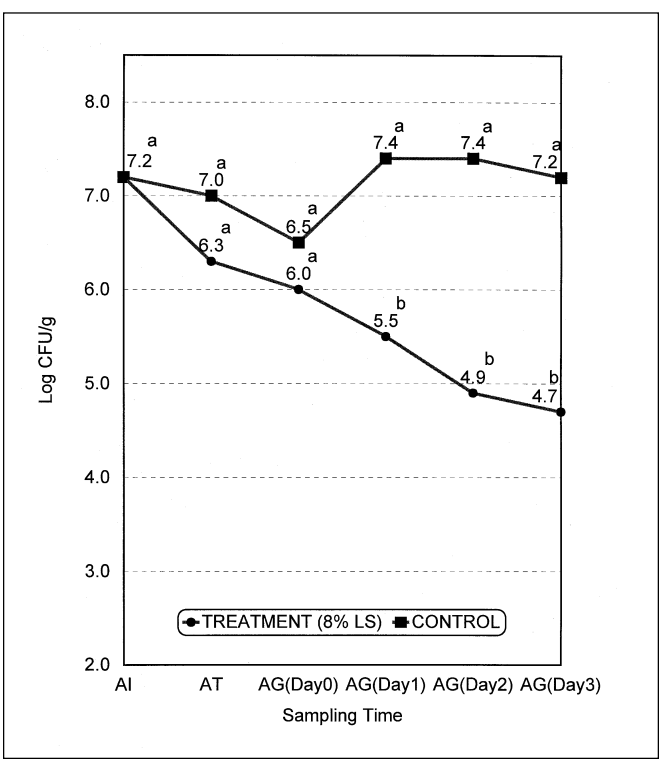

Fig. 1—Growth of *E. coli* 0157:H7 in inoculated beef trimmings and ground, treated with 8% liquid smoke and stored at 4°C. (Mean of 3 replicas) (AI=after inoculation, AT=after treatment, AG=after grinding). S.E.=0.15. a,b=Means having different letters are different ($p<0.05$).

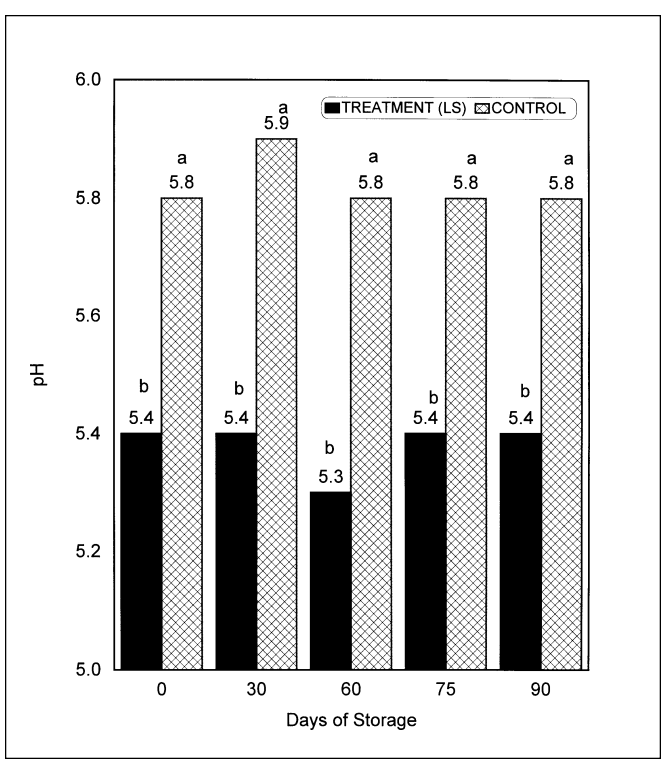

Fig. 2—pH of precooked beef patties treated with 1.5% (treatment) and 0% (control) liquid smoke at day 0, 30, 60, 75 and 90 of storage at -15°C. S.E.=0.22. a,b=Means having different letters are different ($p<0.05$).

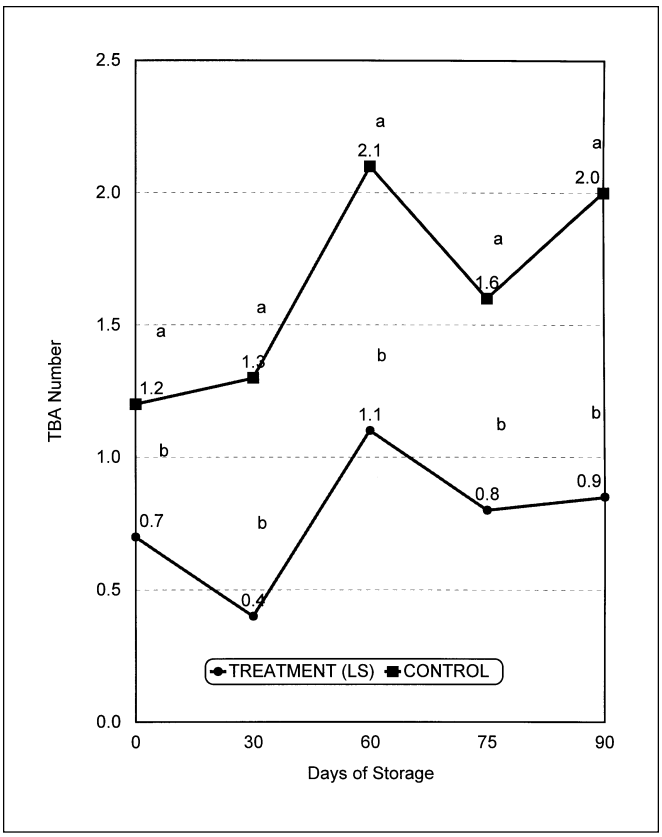

Fig. 3—TBA values for precooked beef patties treated with 1.5% (treatment) and 0% (control) liquid smoke at day 0, 30, 60, 75 and 90 of storage at -15°C. Fat rancidity was determined using the 2-thiobarbituric acid (TBA) method. S.E.=0.07. a,b=Means having different letters are different ($p<0.05$).

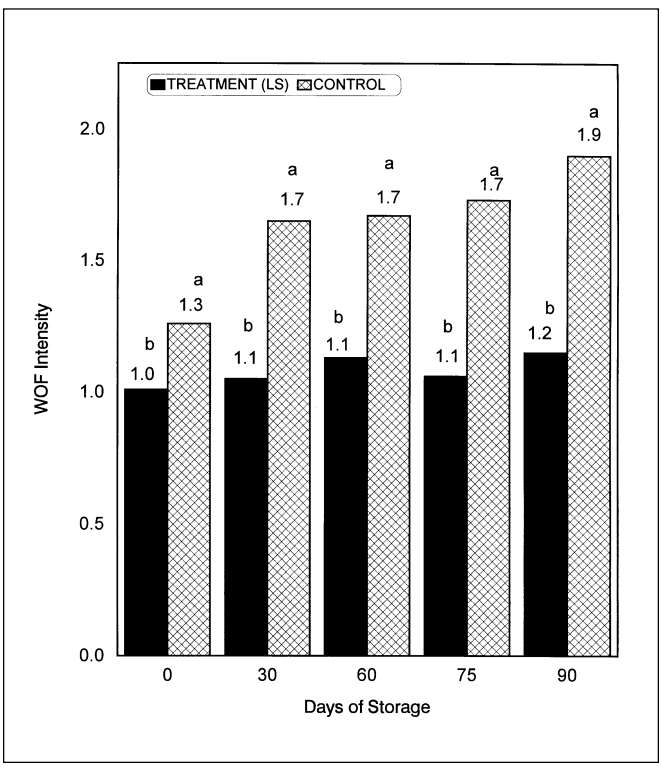

Fig. 4—Aroma evaluation (immediately after presentation) of precooked beef patties treated with 1.5% (treatment) and 0% (control) liquid smoke at day 0, 30, 60, 75 and 90 of storage at -15°C. (1=no WOF, 2=slight WOF, 3=moderate WOF, 4=very high WOF and 5=extreme WOF) S.E.=0.08. a,b=Means having different letters are different ($p<0.05$).

TBA numbers and panel scores for WOF in cooked chicken with and without chelators and/or antioxidants.

The level of LS used for evaluating antibacterial properties of LS against *E. coli* O157:H7 was higher than that recommended (1.5–2.0%) for meat products. However, addition of 8% LS to beef trimmings could be feasible as a food safety measure for sausage production if beef is added as a component of the product formulation. Beef trimmings treated with 8% LS used in a specific type sausage that required 25% beef and 75% pork would result in an LS level reduced to that normally used in an edible meat product.

CONCLUSIONS

LS (8%) EFFECTIVELY INHIBITED *E. COLI* 0157:H7 GROWTH IN beef. It was also effective in controlling WOF in stored beef. TBA numbers, aroma scores and pH values were lower ($p<0.05$) in treated beef patties. Thus, LS had antioxidative properties in cooked ground beef patties at the normally recommended percentage of 1.5%. LS is mainly used in meat to provide flavor and color. However, due to its antimicrobial and antioxidative characteristics, LS could be added to meat products to make them safer and extend product shelf-life.

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