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MICROBIAL QUALITY OF GROUND BEEF WITH ADDED LACTIC ACID BACTERIA AT ABUSIVE AND REFRIGERATED TEMPERATURES PACKAGED IN MODIFED ATMOSPHERE AND TRADTIONAL PACKAGING

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Introduction

Currently, there are few food-safety interventions for ground beef processors. Research conducted has shown that lactic acid bacteria (LAB) reduced food-borne pathogens (3). LAB is GRAS (1) and can create a competitive environment through several different mechanisms including production of hydrogen peroxide, bacteriocins, and weak acids. However, for LAB to be approved by the FDA and USDA-FSIS, it must be determined if LAB addition would cause the product to be adulterated or misbranded by making the ground beef of greater value than untreated products or masking spoilage (2).

Objectives

The effect of LAB on the microflora of ground beef in traditional and MAP packaging displayed under refrigerated or abusive temperatures.

Methodology

Patty Preparation and Temperature Treatments

A total of 209.1 kg of coarse-grind ground beef was obtained from a commercial processing facility over a 6 week period, further processed under simulated industry conditions through a 0.32 cm grinder plate, and formed into 145.2 g patties. Treated ground beef was inoculated with 10^9 cfu LAB/g solution suspended in sterile distilled water. Control samples contained a similar volume (500 mL) of sterile distilled water to standardize added water. Equipment was cleaned thoroughly between treatments and rinsed with 180°C water. The LAB cocktail consisted of *Lactobacillus acidophilus* NP 51, *Lactobacillus crispatus* NP 35, *Pediococcus acidilactici*, and *Lactobacillus lactic* spp. *Lactic* (Culture Systems, Inc.; Mishawaka, IN). Samples were stored in retail display cases maintained at either 0°C or 10°C. Packages were randomly identified and assigned to coffin-style retail cases that were illuminated with fluorescent lighting specially designed to mimic retail grocery outlets. The intensity of lighting at the surface of the package averaged 1900 lux.

Packaging

Traditional Packaging. Ground beef was divided randomly into two treatment groups: control and LAB inoculated. Patties were placed on foam trays and wrapped with a gas permeable polyvinylchloride (PVC) film.

Modified Atmosphere Packaging. To mimic industry practice, the addition of rosemary oleoresin was included as an additional treatment in MAP portion of the study. Ground beef was divided randomly into four treatment groups: control; control + rosemary; LAB inoculated; and LAB + rosemary. Rosemary oleoresin was added at a level of 0.01% (w/w; 1000 ppm) using Herbalox® Type HT-W rosemary oleoresin (Kalsec, Inc.; Kalamazoo, MI). Beef patties were placed in #10 plastic lidded trays (Cryovac; Duncan, SC) and sealed with LID 1050 film (oxygen transmission rate: less than 20.0 cc, 24 h, m2 at 40°F and 100% RH). Packages were flushed with a targeted 80% O2 and 20% CO2. Headspace was analyzed on random packages during processing and all were monitored for leaks.

Microbial Analysis

For traditional replications, 2.5 g of 4 patties representing 1 treatment were combined (10 g total) in a sterile filtered homogenizer bag and 99 mL of buffered peptone water was added. For MAP replications, 5 g of 2 patties of 1 treatment were combined. The bag was processed at normal speed for 1 min in a laboratory blend stomacher. Serial dilutions were made and samples were automatically plated using the Spiral Biotech Autoplate® (Spiral Biotech, Norwood, MA). The following media were used to isolate and enumerate microorganisms present: Trypticase Soy Agar, Pseudomonas F Agar, YM Agar, Violet Red Bile Agar, Lactobacilli MRS Agar, and STAA Agar with supplement SR151E. Duplicate plates were incubated at appropriate temperatures for each media and were counted using the Spiral Biotech Q Count (Version 2.0, Spiral Biotech). Only colonies exhibiting a greenish-yellow fluorescent pigment on Pseudomonas F plates under short wavelength UV light (254 nm) were counted.

Statistical Analysis

All experiments were performed in triplicate for each of the packaging types. Microbial counts were transformed into log10 units for analysis. The Mixed Model of SAS (SAS Institute, Inc., Cary, NC) was used to evaluate the effect of treatments on microbial loads with replication as a random effect. The dependent variables were the population of bacteria (log transformed). Independent variables included treatment, presence of resin, and sampling time. Treatment*replication was a repeated measure and treatment and time were fixed effects. Significant main effects and interactions were analyzed using the least squares means (LSM) method and separated using the PDIFF option of SAS. All tests were considered significant at $P < 0.05$.

Results & Discussion

TRADITIONAL PACKAGING

At 0°C Temperatures

Microbial data can be seen in Tables 1 and 2. There was a significant interaction between treatment and time for total APC. APC counts for uninoculated patties significantly increased after 72 and 84 h of display. However, LAB-inoculated beef APC populations remained constant throughout display. Control patties had significantly lower APC counts at times 0, 24, and 48 h as compared to LAB-inoculated ground beef. This would be expected because the LAB would be counted as part of the aerobic plate count. After 72 and 84 h of display, there were no significant differences between APC counts for treatments indicating that the LAB inhibited some of the naturally occurring aerobic organisms. LAB counts were significantly higher in LAB-inoculated samples than controls. This was expected because LAB was added at approximately 10^9 cfu/g of meat so these differences were not a concern. Significant increases in LAB populations were observed during display (data not shown). YM counts in LAB-inoculated patties were significantly lower than controls, which indicated that LAB inhibited YM growth at refrigeration temperatures. YM populations significantly increased over time (data not shown). No significant differences were found in coliform, *B. thermosphacta*, and pseudomonad counts between treatments, but significant increases in populations over time were seen in both coliforms and *B. thermosphacta*, while pseudomonad counts did not significantly differ during display (data not shown).

At 10°C Temperatures

Microbial results can be seen in Tables 3 and 4. APC counts were significantly higher for treatment groups inoculated with LAB. This was expected as the TSA media would allow for growth of the LAB which was added to the ground beef. APC counts significantly increased over time (data not shown). The bacteria were present in an environment conducive to growth over time, which is a concern for product that has been temperature abused prior to or after the consumer has purchased the product. There was a significant interaction between treatment and time for LAB counts. The comtrols increased by approximately 3 log cfu/g after 36 h; however, LAB-treated samples showed no significant increase in growth and there were no differences between control and treated samples after 36 h. No significant differences were found in populations of yeast and molds, coliforms, *B. thermosphacta*, and pseudomonad counts between treatments, but significant increases in populations over time occurred for yeast and molds, *B. thermosphacta*, and coliforms. No significant differences were found over time for pseudomonad populations.

MAP PACKAGING

At 0°C Temperatures

Results can be seen in Table 5. Those treatments inoculated with LAB had significantly higher APC and LAB counts than those samples without added LAB. These results were expected as the medias used allowed the growth of LAB. Significant differences were also found in *B. thermosphacta* counts between treatments. However,

yeast and molds, coliform and Pseudomonad counts did not significantly differ between treatments. Over time, significant increases occurred for yeast and mold, coliform, *B. thermosphacta*, and Pseudomonad counts (data not shown).

At 10°C Temperatures

Microbial results can be seen in Table 6 and Table 7. APC counts were significantly higher for treatment groups inoculated with LAB. During display, APC counts significantly increased over time (data not shown). There was a significant interaction between treatment and time for LAB counts. No significant increases in LAB populations were observed in either LAB-inoculated treatment group over time. However, uninoculated treatment groups had significant increases in LAB counts as display progressed. At each interval, LAB treatment groups had significantly higher populations of LAB as compared to control groups. These results were expected since the ground beef was inoculated. No significant differences were observed between treatments for yeasts and molds, *B. thermosphacta*, coliforms, and Pseudomonads. Significant increases over time for yeasts and mold, *B. thermosphacta*, and coliforms counts occurred, while the pseudomonad population did not increase significantly (data not shown).

Conclusions

Previous research conducted has shown that the addition of certain strains of LAB to ground beef does inhibit the growth of *E. coli* O157:H7 and *Salmonella* spp. at a minimum inoculation level of 10^9 cfu/g of meat (3). It is important that proposed food additives not mask factors consumers associate with a spoiled product. While samples inoculated with LAB had significantly higher APC and LAB populations, microbiological and sensory data indicate the addition of LAB does not mask spoilage. Moreover, the populations of LAB from inoculated samples did not increase over time. Rosemary oleoresin, however, did not have a significant effect on growth of the microorganisms isolated in this study, but its addition did not interact with LAB and affect the spoilage characteristics or microbiological properties of the ground beef. Therefore, LAB can be added to ground beef as a processing intervention without masking spoilage and did not result in a misbranded or adulterated product as required by USDA-FSIS (2).

References

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Tables and Figures

Table 1 Aerobic plate counts (log cfu/g) for ground beef patties in traditional packaging at 0°C.

	Time (h)				
Treatment	0	24	48	72	84
Control	4.00^{az}	4.18^{az}	4.35^{az}	5.46^{bz}	5.74^{bz}
LAB	5.61^{ay}	5.49^{ay}	5.68^{ay}	5.75^{az}	6.14^{az}

^{a,b,c}Means within rows lacking same superscript differ ($P < 0.05$)
^{y,z}Means within columns lacking same superscript differ($P < 0.05$)

Table 2 Bacteria counts (log cfu/g) enumerated from ground beef patties in traditional packaging at 0ºC.

	Treatment	
Microorganisms	Control	Control w/resin
Lactic acid bacteria	4.10^{a}	6.88^{b}
Yeasts and molds	3.03^{a}	2.36^{b}
B. thermospatcha	2.04	2.02
Coliforms	4.13	4.18
Pseudomonads^{a}	1.22	1.07

^{a}Fluorescin producing pseudomonads
^{b,c,d,e}Means within rows lacking same superscript differ ($P < 0.05$)

Table 3 Bacteria counts (log cfu/g) enumerated from ground beef patties in traditional packaging at 10ºC.

	Treatment	
Microorganisms	Control	Control w/resin
Aerobic plate count	5.09^{a}	5.88^{b}
Yeasts and molds	3.16	3.27
B. thermospatcha	2.29	2.11
Coliforms	4.27	4.20
Pseudomonads^{a}	1.15	1.10

^{a}Fluorescin producing pseudomonads
^{b,c,d,e}Means within rows lacking same superscript differ ($P < 0.05$)

Table 4 Lactic acid bacteria counts (log cfu/g) for ground beef patties in traditional packaging at 10°C.

	Time (h)			
Treatment	0	12	24	36
Control	3.34^{az}	4.28^{abz}	4.85^{bcz}	6.23^{cz}
LAB	6.66^{ay}	6.88^{ay}	7.05^{ay}	7.10^{az}

^{a,b,c}Means within rows lacking same superscript differ ($P < 0.05$)
^{y,z}Means within columns lacking same superscript differ($P < 0.05$)

Table 5 Bacteria counts (log cfu/g) enumerated from ground beef patties in MAP packaging at 0ºC.

	Treatment			
Microorganisms	Control	Control w/resin	LAB	LAB w/resin
Aerobic plate count	3.92[b]	3.57[c]	5.41[d]	5.17[e]
Lactic acid bacteria	1.59[b]	3.12[c]	6.19[d]	6.70[d]
Yeasts and molds	2.27	1.86	1.85	2.21
B. thermospatcha	2.64[c]	2.00[b]	1.87[b]	2.36[bc]
Coliforms	3.83	3.54	3.64	3.60
Pseudomonads[a]	1.19	1.15	1.21	1.36

[a]Fluorescin producing pseudomonads

[b,c,d,e]Means within rows lacking same superscript differ ($P < 0.05$)

Table 6 Bacteria counts (log cfu/g) enumerated from ground beef patties in MAP packaging at 10ºC.

	Treatment			
Microorganisms	Control	Control w/resin	LAB	LAB w/resin
Aerobic plate count	4.26[b]	3.93[b]	5.26[c]	5.59[c]
Yeasts and molds	3.23	2.73	3.00	3.50
B. thermospatcha	3.01	2.34	2.52	2.70
Coliforms	4.20	3.86	3.98	3.94
Pseudomonads[a]	1.17	1.23	1.02	1.29

[a]Fluorescin producing pseudomonads

[b,c,d,e]Means within rows lacking same superscript differ ($P < 0.05$)

Table 7 Lactic acid bacteria counts (log cfu/g) for ground beef patties in MAP packaging at 10°C.

	Time (h)			
Treatment	0	12	24	36
Control	0.87[ax]	2.49[by]	3.90[cy]	4.48[cy]
Control w/resin	2.87[aby]	2.79[ay]	3.58[bcy]	4.33[cy]
LAB	3.40[z]	6.41[z]	6.35[z]	6.50[z]
LAB w/resin	6.85[z]	6.76[z]	6.75[z]	6.20[z]

[a,b,c]Means within rows lacking same superscript differ ($P < 0.05$)

[x,y,z]Means within columns lacking same superscript differ ($P < 0.05$)