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Organic Acids and Applications used for Reduction of *E. coli* on Beef Shoulder Clods used for Ground Beef

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Summary with Implications

Small processors normally grind beef shoulder clods for ground beef that have not been previously tested for shiga toxin-producing E. coli. Three antimicrobial solutions were applied using three application methods to beef sub-primals to evaluate the effectiveness of reducing E. coli and the effects on quality attributes. Antimicrobials effectively reduced Rifampicin resistant E. coli. However, none of the treatments changed color attributes or total plate counts compared to a control. These results suggest that an appropriate antimicrobial solution and application method can be selected for use by small meat processors without affecting quality attributes.

Introduction

Small meat processing operations often purchase beef shoulder clods for grinding that have not been tested for shiga toxin-producing *E. coli* (STEC). However, *E. coli* O157:H7 and other STEC are considered to be adulterants in raw, non-intact beef products due to significant health risks. The use of antimicrobial interventions applied to the surface of beef shoulder clods may offer small processors a method to reduce the risk of STEC in ground beef. The objectives of this research were to evaluate the effect of organic acid type and application method applied to the surface of beef shoulder clods as a means to reduce the risk of STEC and the effects on color and shelf life of the ground beef produced.

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Procedure

Rifampicin Resistant E. coli

Eleven beef shoulder clods were cut in half prior to inoculation to form clod roasts. Since fat and lean tissue have different buffering capabilities, one half was used as the outer fat surface and the other half was used as the inner lean surface of clod roasts for inoculation and application of an organic acid. Each half was inoculated with a five strain cocktail (~5.6 log CFU/cm^2) of Rifampicin resistant *E. coli* (*E. coli^{Rif}*). After inoculation, five core samples (3.92 in^2) were taken from the inoculated surface for initial inoculation concentrations. Then 4.5% lactic acid (LA), 2.5% Beefxide™ (BX, lactic acid + citric acid), or 380 ppm peroxyacetic acid (PAA) was applied at 67–74˚F to each clod roasts using spray (5 sec, 20 psi), dip (15 sec), or electrostatic spray (ESS, 10 sec). Additionally, a non-inoculated control was used to assure that there were no *E. coli^{Rif}* naturally present and inoculated control was used to compare treatments to assure that the antimicrobial was effective. After antimicrobial treatment, five core samples (3.92 in^2) were taken from the treated surface to determine the reduction of *E. coli^{Rif}* concentrations. Each clod roast was then ground and a 25 gram sample was collected for microbial analysis. All samples were extracted in peptone water containing Rifampicin and then enumerated on ACP and *E. coli*/coliform Petrifilm. This process was replicated three times.

Color and Total Plate Counts

Beef shoulder clods were treated with the same concentrations of LA, BX and PAA using spray (11 sec/side, 20 psi), dip (15 sec), or ESS (10 sec/side). Beef shoulder clods were ground and a 25 gram sample was collected for microbial analysis using ACP Petrifilm. Microbial analysis was done on days 0, 1, 3, 5, and 7. Approximately one pound portions were formed using a Colosimo press and were placed in simulated retail display where a subjective color panel (8–10 panelists) evaluated discoloration daily. In addition, L*, a*, and b* values were measured daily using a Minolta colorimeter. Delta E values were then calculated from the L*, a*, and b* values using the following formula: $\Delta E = \sqrt{[(L_1-L_0)^2 + (a_1-a_0)^2 + (b_1-b_0)^2]}$. Day 0 was used as the initial value from each sample to compare the rate of discoloration. Six independent replicates were conducted.

Statistical Analysis

The PROC GLIMMIX of SAS 9.4 (SAS Inst., Inc., Cary, NC) was used to determine the effects of organic acids and application methods on the reduction of *E. coli^{Rif}* and ground beef color. All means were separated with the LS-means (LSM) statement and the Tukey adjustment with an alpha of 0.05.

Results

All treatments reduced (P <0.01) *E. coli^{Rif}*counts when compared to the inoculated control (0.39–1.13 log CFU/cm^2 reduction) when using *E. coli*/coliform petrifilm. When using ACP petrifilm, all treatments reduced (P < 0.01) *E. coli^{Rif}* counts except BX ESS (P = 0.423 log CFU/cm2), LA ESS (P = 0.328 log CFU/cm2) and PAA ESS (P = 0.088 log CFU/cm2). There were no interactions between organic acids and application methods, however, dip and spray applications were more effective (P < 0.001) at reducing *E. coli^{Rif}* when compared to the ESS method (Table 1) using both ACP and *E. coli*/coliform petrifilm. Additionally, LA had the greatest reduction while BX had the smallest reduction for organic acid type on *E. coli^{Rif}* (Table 2) using both ACP and *E. coli*/coliform petrifilm. Reductions of *E. coli^{Rif}* on the outer fat surface (0.85 log CFU/cm^2) was greater (P < 0.01) than reduction of *E. coli^{Rif}* on the inner lean surface (0.59 log CFU/cm^2) of the clod roast. This may be due to the buffering capabilities of the lean tissue versus fat tissue. Microbial

Table 1. Effect of application method on the reduction of *E. coli*^{Rif} (log CFU/cm^2) on beef shoulder clods using 15 s dip, 10 s ESS at 6–12 inches, and 5 s spray at 6–12 inches.

	Application Method			SEM	*P*-value
	Dip	Electrostatic Spray	Spray		
E. coli/coliform Petrifilm	0.875[a]	0.466[b]	0.830[a]	0.075	< 0.001
ACP Petrifilm	0.621[a]	0.115[b]	0.608[a]	0.071	< 0.001

[a,b] Means within a row without a common superscript are significantly different

Table 2: Effect of organic acid type on the reduction in *E. coli*^{Rif} (log CFU/cm^2) on beef shoulder clods using 2.5% Beefxide™, 4.5% lactic acid, and 380 ppm peroxyacetic acid.

	Organic Acid Type			SEM	*P*-value
	Beefxide™	Lactic Acid	Peroxyacetic Acid		
E. coli/coliform Petrifilm	0.547[b]	0.863[a]	0.762[ab]	0.075	< 0.001
ACP Petrifilm	0.289[b]	0.493[ab]	0.563[ab]	0.071	< 0.05

[a,b] Means within a row without a common superscript are significantly different

samples of ground beef produced from the clod roasts showed that *E. coli*^{Rif} counts for only the spray and dip treatments were different than the inoculated control ($P < 0.001$). Additionally, ground beef *E. coli*^{Rif} counts were greater ($P < 0.001$) from roasts treated on the lean surface (3.74 log CFU/g) than roasts treated on the fat surface (3.32 log CFU/g). It is possible that the application time or the application distance used for ESS from the meat surface reduced the amount of organic acid that adhered to the surface of the clod roasts to reduce the impact of the organic acid on *E. coli*^{Rif}.

In an organic acid by application method interaction ($P < 0.001$) for L* values of ground beef, PAA Spray (LSM = 48.23) resulted in a darker colored surface area than LA spray (LSM = 49.88), BX spray (LSM = 49.91), and PAA dip (LSM = 49.96). An organic acid by application method interaction ($P < 0.01$) showed BX dip and BX spray increased in b* values (yellowness) while LA ESS decreased in b* values. As expected, L*, a*, and b* values all decreased ($P < 0.001$) with increasing days of display. Delta E values, a measure of color change, showed an organic acid by application method interaction ($P < 0.05$) but no means separation occurred after applying Tukey's adjustment. There was no organic acid type or application method effect on discoloration ($P > 0.23$). Delta E values and discoloration percentages both increased ($P < 0.001$) with increased days of display.

Total plate counts of ground beef in display exhibited an organic acid by application method interaction ($P <0.01$) showing that LA ESS had more aerobic growth than all other treatments. Total plate counts increased growth ($P < 0.001$) from day 0 (LSM = 2.03 log CFU/g) to day 7 (LSM = 4.11 log CFU/g).

Conclusions

Small meat processors can select an antimicrobial treatment to reduce the risk of STEC on the surface of beef sub-primals and in ground beef. Processors should consider either the LA or PAA organic acids as these were more effective at reducing *E. coli*^{Rif} counts on the surface of beef shoulder clods. In addition, when looking at the shelf life and color of the ground beef, a small meat processor can consider the use of any of the organic acids or application treatments as the impacts on ground beef quality as measured by L*, a*, or b* values, Delta E values, or discoloration percentages were minimal. The use of antimicrobials to minimize the risk of STEC may be applied to beef sub-primals by small meat processors without impacting the color characteristics of ground beef in retail display.

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