Final Report to AMIF

Project Title: Validation of Levulinic Acid for Topical Decontamination of Meat Surfaces

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EXECUTIVE SUMMARY

Objectives

1. Investigate the reduction of pathogenic bacteria inoculated onto meat surfaces that results from washing with water and organic acids (2% lactic, acetic, or levulinic).

2. Look at the reduction of *E. coli* O157:H7 inoculated on beef plate that results from washing with levulinic acid at various concentrations and temperatures.

3. Study the residual protection against growth of pathogenic bacteria inoculated on meat surfaces previously washed with water and organic acids.

4. Evaluate the organoleptic implications from spraying slices of turkey roll or beef trim with water and organic acids.

Conclusions

1. Acid washes did not reduce numbers of *E. coli* O157:H7 or *L. monocytogenes* on beef plate and turkey roll slices, respectively Acid washes reduced numbers of Salmonella on pork belly and chicken skin by about 1 log/sq cm, but the effect was no more than attained using only a water wash.

2. Washing with 2% levulinic acid did not reduce numbers of *E. coli* O157:H7 on beef plate even when applied at elevated temperatures.

3. Washing with the organic acids did not provide residual protection against growth of *E. coli* O157:H7 and *L. monocytogenes.* Only acetic acid protected against growth of *Salmonella*, and only on chicken skin.

4. Spraying slices of turkey roll and beef trim with 2% lactic, acetic, or levulinic acid did not impact consumers' overall liking of the turkey roll or cooked patties, respectively. There were

some small effects on instrumental measures of color, but these appeared to be of little practical significance.

Deliverable

Washing with organic acids was no more effective than water at reducing numbers of pathogenic bacteria on meat surfaces regardless of organic acid, bacterial species, or meat tissue type. Thus, it was not possible to establish the potential for levulinic acid as a substitute for lactic and acetic acids employed for surface decontamination of meat. This may be related to the 2% concentration of acids used in this research that was chosen based on industry practice at the time our proposal was submitted. It is interesting to note that the meat industry has since gone to using lactic and acetic acids at higher concentrations. However, we cannot find a sound validation for their use at concentrations greater than 2%.

Recommendations for Future Research

The meat industry has widely adopted the use of organic acid washes based on research that reports 2-3 log reduction in bacterial numbers. In contrast, our results are consistent with other research indicating that washes with organic acids are not effective for decontamination of pathogens from meat tissues. This highlights the need for the meat industry to reconsider the effectiveness of organic acid washes for decontamination of meat tissues, especially the extent to which washing with organic acids contributes to a total food safety program.

Presentations and Publications

Smith, J. Validation of Levulinic Acid for Topical Decontamination of Meat Surfaces
MS thesis, Utah State University, In preparation.

Smith, J., Carpenter, C.E., Broadbent, J.B. Washing with organic acids at 2% concentration does not reduce pathogens on meat tissues more than a water wash alone. J. Food Science or Meat Science. In preparation.

TECHNICAL ABSTRACT

Objective 1: Investigate the reduction of pathogenic bacteria (*E. coli* O157:H7, *Listeria monocytogenes, and Salmonella)* inoculated onto meat surfaces (beef plate, turkey roll slices, chicken skin and pork belly, respectively) that results from washing with water and organic acids (2% lactic, acetic, or levulinic).

Procedure: Five strains of *Listeria monocytogenes*, twelve strains of *Escherichia coli* O157:H7, and eight strains of *Salmonella* were used in this study. Cocktails of each pathogen were prepared after growing individual strains overnight at 37ºC in Brain-Heart Infusion broth. Pieces of meat measuring 5cm x 5cm were placed side by side on stainless steel trays, and 0.1ml of cocktail was pipetted onto one of a pair of meat samples. The second piece was placed on top of the inoculated piece and briefly rubbed together to spread the inoculum. Pieces were allowed to dry for 20 minutes and then sprayed for 20s with decontamination washes (water, 2% acetic acid, 2% lactic acid, and 2% levulinic acid, all at 54ºC). Additional inoculated samples were not washed, serving as a positive control from which to calculate the extent of decontamination. Samples were allowed to dry for 20 minutes after washing, and subsequently sealed in vacuum packages and stored overnight at 4ºC. Samples were removed from storage and 100ml of sterile PBS were added to a sample, which was then agitated in a stomacher (Seward 400) for 30 seconds on medium speed. Stomached suspension was serially diluted with sterile PBS and plated (0.1ml) in duplicate on selective and differential media (Rapid'L.Mono agar for *Listeria monocytogenes*, and BBL ChromagarO157 for *E. coli* O157:H7. Colonies were counted after incubation at 37ºC for 48 hours. The experiment was replicated a second time.

Results:

Table 1: Numbers of *L. monocytogenes* recovered from turkey slices. Means in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Treatment	Mean Log CFU/cm^2	Log Reduction (Treatment vs. control)	Log Reduction (Acid vs. Water)
Untreated	6.14^a	---	---
Water	5.88^{ab}	NS	---
Acetic	5.96^a	NS	NS
Lactic	5.56^b	0.58	NS
Levulinic	5.86^{ab}	NS	NS

NS=No significant log reduction

Table 2: Numbers of *E. coli* O157:H7 recovered from beef plate. Means in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Treatment	Mean Log CFU/cm^2	Log Reduction (Treatment vs. control)	Log Reduction (Acid vs. Water)
Untreated	5.85^a	---	---
Water	5.34^a	NS	---
Acetic	5.17^a	NS	NS
Lactic	4.79^a	NS	NS
Levulinic	4.97^a	NS	NS

NS=No significant log reduction

Table 3: Numbers of *Salmonella* spp. recovered from skin-on pork belly. Means in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Treatment	Mean Log CFU/cm^2	Log Reduction (Treatment vs. control)	Log Reduction (Acid vs. Water)
Untreated	5.49^a	---	---
Water	4.74^{ab}	NS	---
Acetic	4.68^b	0.81	NS
Lactic	4.14^b	1.35	NS
Levulinic	4.47^b	1.02	NS

NS=No significant log reduction

Table 4: Numbers of *Salmonella* spp. recovered from chicken skin. Means in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Treatment	Mean Log CFU/cm^2	Log Reduction (Treatment vs. control)	Log Reduction (Acid vs. Water)
Untreated	5.96^a	---	---
Water	5.55^{ab}	NS	---
Acetic	4.88^b	1.08	NS
Lactic	5.04^b	0.92	NS
Levulinic	4.76^b	1.2	NS

NS=No significant log reduction

Table 5: Pathogen recovery pooled over all model systems. Means in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Treatment	Mean Log CFU/cm^2	Log Reduction (Treatment vs. Control)	Log Reduction (Acid vs. Water)
Untreated	5.86^{a}	---	---
Water	5.38^{ab}	NS	---
Acetic	5.25^{bc}	0.61	NS
Lactic	4.81^{c}	1.05	0.57
Levulinic	5.02^{bc}	0.84	NS

NS=No significant log reduction

Discussion and Conclusions: Only lactic acid wash reduced the numbers of *L. monocytogenes* recovered from turkey roll slices as compared to the no-wash controls (Table 1). The water and acid washes did not reduce numbers of *E. coli* O157:H7 recovered from beef as compared to the no-wash controls (Table 2). All acid washes lowered numbers of *Salmonella* recovered from pork and chicken skin as compared to the no-wash controls, although acid washes were generally no more effective for decontamination than water washes (Tables 3-4). Over all model systems, acid washes resulted in about a 0.6 to 1.0 log reduction in numbers of pathogen recovered from meat surfaces, but only the decontamination using lactic acids was greater than that achieved by a water wash alone (Table 5).

Objective 2: Look at the reduction of *E. coli* O157:H7 inoculated on beef plate that results from washing with levulinic acid at various concentrations (0.5%, 1.0%, and 2.0%) and temperatures (55.4ºC, 68.3ºC, 76.7ºC).

Procedure: Pathogen culture and wash procedure as outlined in Objective 1, with washes administered at 55.4, 68.3, and 76.7ºC.

Results:

Table 6: Numbers of E. *coli* O157:H7 recovered from beef plate after washing with 2% levulinic acid at various temperatures. Means in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Temperature (ºC)	Treatment	Mean Log CFU/cm^2	Log Reduction (Treated vs. Untreated)	Log Reduction (Acid vs. Water)
	Untreated	5.45^{a}	---	---
55.4	Water	5.14^{ab}	NS	---
	0.5% Levulinic	5.32^{ab}	NS	NS
	1.0% Levulinic	5.32^{ab}	NS	NS
	2.0% Levulinic	5.38^{ab}	NS	NS
68.3	Water	5.35^{ab}	NS	---
	0.5% Levulinic	5.23^{ab}	NS	NS
	1.0% Levulinic	5.21^{ab}	NS	NS
	2.0% Levulinic	5.34^{ab}	NS	NS
76.7	Water	6.37^{ab}	NS	---
	0.5% Levulinic	6.06^{ab}	NS	NS
	1.0% Levulinic	5.89^{ab}	NS	NS
	2.0% Levulinic	5.03^{ac}	NS	1.34

NS=No significant log reduction

Discussion and Conclusions: The treatments were not effective for decontamination of *E. coli* O157:H7 on beef surface (Table 6). There was significant decontamination using 2% levulinic acid at 76.7˚C as compared to the water wash, but not as compared to the no-intervention control. That suggests that the reduction in bacterial counts resulted from other than the treatment, and it is an anomaly that we cannot explain.

Objective 3: Evaluate the residual protection against growth of pathogenic bacteria inoculated on meat surfaces previously washed with organic acids (model systems same as for objective 1). **Procedure:** Meat samples were washed and inoculated as described in previous objectives, except that wash treatment occurred prior to inoculation, and pathogen cocktail was diluted 100-fold. Turkey slices inoculated with *L. monocytogenes* were stored at 4ºC, and pathogen counts determined at 0, 2, 4, 8, 12, and 16 weeks. All other samples were stored at 8ºC and pathogen counts determined at 0, 2, 4, 6, and 8 weeks.

Results:

Figure 1: Growth of *L. monocytogenes* recovered on sliced turkey roll during 16 weeks storage at 4ºC.

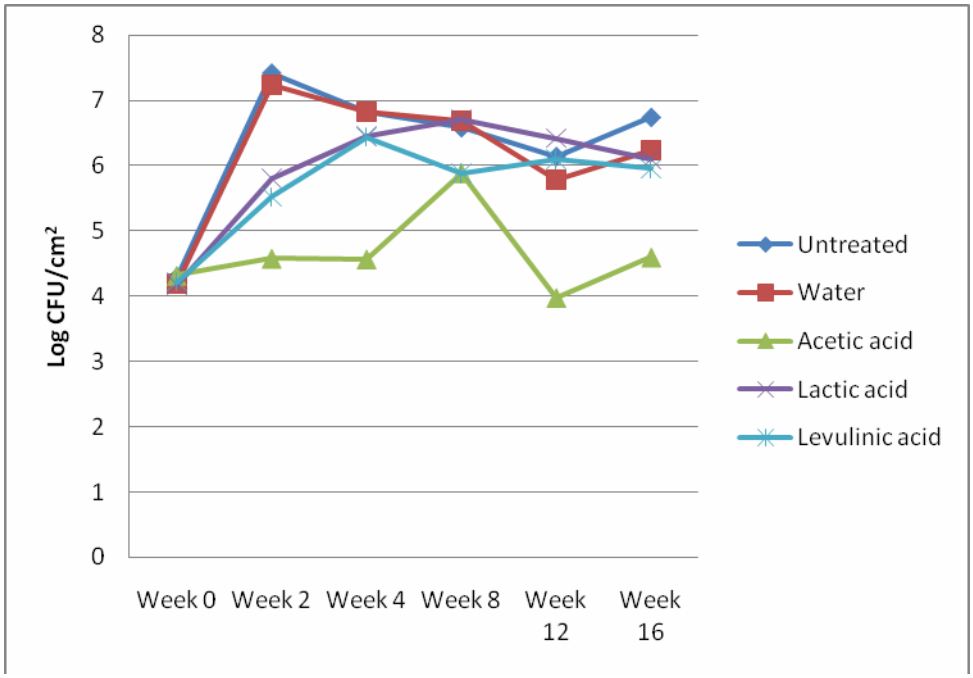

Table 7: Numbers of *L. monocytogenes* recovered from sliced turkey roll during 16 weeks of storage at 4ºC. Results are given as log CFU/cm^2. Values in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Treatment	Week 0	Week 2	Week 4	Week 8	Week 12	Week 16	Pooled Mean	SEM
Untreated	4.28^{a}	7.42^{a}*	6.83^{a}	6.58^{a}	6.14^{a}	6.74^{a}	6.33^{a}	0.44
Water	4.19^{a}	7.24^{a}*	6.82^{a}	6.69^{a}	5.78^{a}	6.23^{a}	6.16^{a}	0.44
Acetic acid	4.32^{a}	4.58^{b}	4.57^{b}	5.89^{a}	3.98^{a}	4.60^{a}	4.66^{a}	0.27
Lactic acid	4.17^{a}	5.8^{ab}	6.45^{ab}	6.71^{a}	6.42^{a}	6.10^{a}	5.94^{a}	0.38
Levulinic acid	4.22^{a}	5.52^{ab}	6.44^{ab}	5.88^{a}	6.10^{a}	5.95^{a}	5.69^{a}	0.32

Figure 2: Growth of *E. coli* O157:H7 on beef plate during 8 weeks storage at 8ºC.

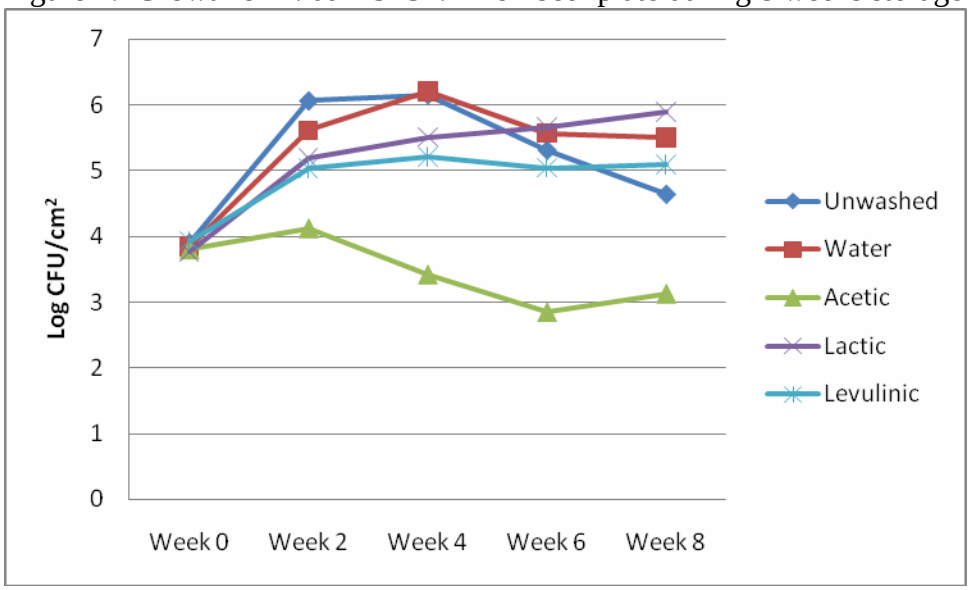

Table 8: Numbers of *E. coli* O157:H7 recovered from beef plate during 8 weeks storage at 8ºC. Results are given as log CFU/cm^2. Values in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Treatment	Week 0	Week 2	Week 4	Week 6	Week 8	Pooled Mean	SEM
Unwashed	3.90^a	6.06^a	6.15^a	5.31^a	4.64^a	5.21^a	0.43
Water	3.85^a	5.61^a	6.20^a	5.57^a	5.50^a	5.35^a	0.39
Acetic	3.80^a	4.12^a	3.42^a	2.85^a	3.13^a	3.46^a	0.23
Lactic	3.77^a	5.19^a	5.51^a	5.66^a	5.89^a	5.20^a	0.38
Levulinic	3.93^a	5.03^a	5.21^a	5.04^a	5.09^a	4.86^a	0.23

Figure 3: Growth of *Salmonella* spp. on chicken skin during 8 weeks storage at 8ºC.

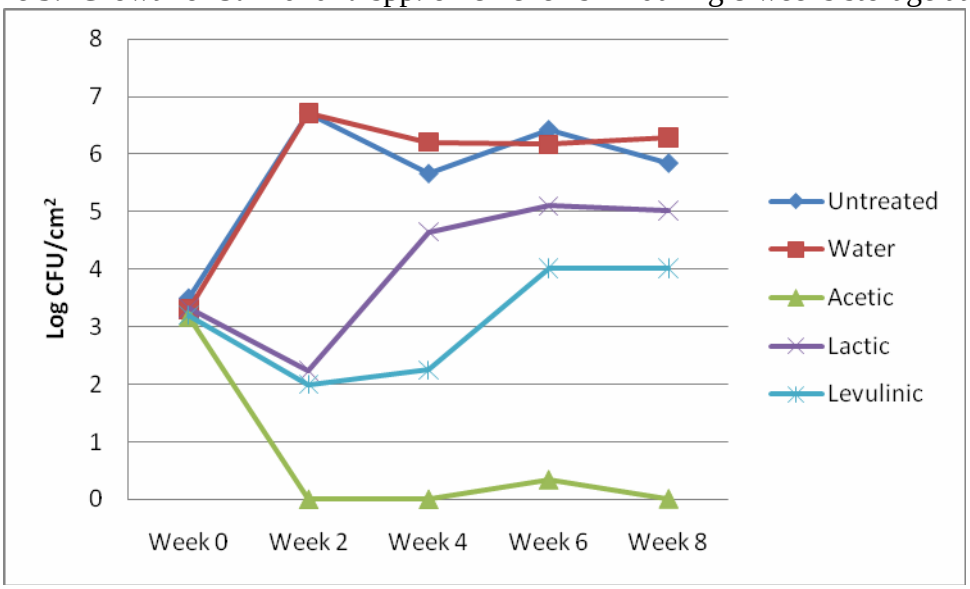

Table 9: Numbers of *Salmonella* spp. recovered from chicken skin during 8 weeks storage at 8ºC. Results are given as log CFU/cm^2. Values in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Treatment	Week 0	Week 2	Week 4	Week 6	Week 8	Pooled Mean	SEM
Untreated	3.50^{a}	6.71^{a}	5.66^{a}	6.42^{a}	5.84^{ab}	5.63^{a}	0.56
Water	3.30^{a}	6.71^{a}	6.21^{a}	6.17^{a}	6.29^{a}	5.74^{a}	0.62
Acetic	3.17^{a}	0.00^{b}	0.00^{b}	0.34^{b}	0.01^{b}	0.70^{b}	0.62
Lactic	3.32^{a}	2.24^{ab}	4.65a^{b}	5.10^{ab}	5.02^{ab}	4.07^{ab}	0.56
Levulinic	3.20^{a}	2.00^{ab}	2.25^{ab}	4.01^{ab}	4.01^{ab}	3.09^{ab}	0.42

Figure 4: Growth of *Salmonella* spp. on pork skin during 8 weeks storage at 8ºC.

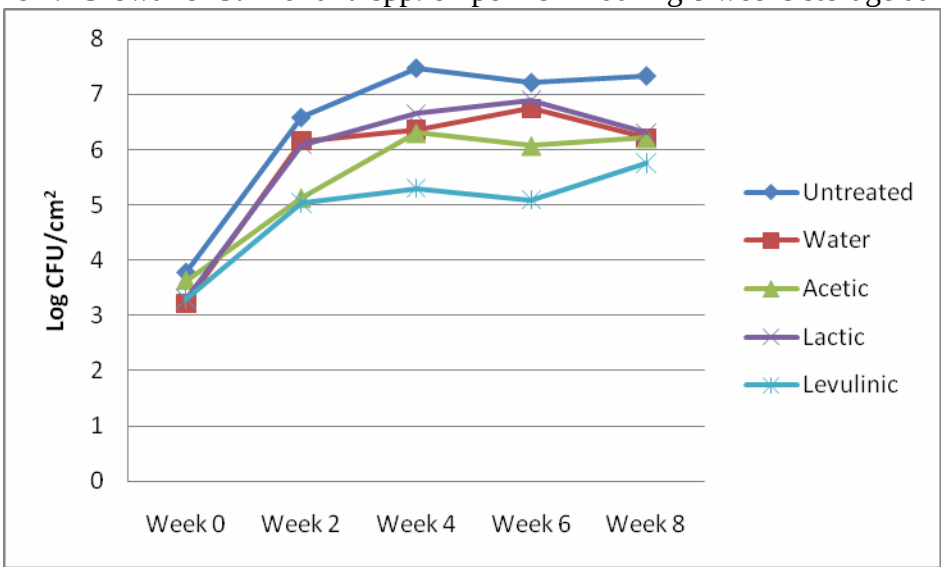

Table 10: Numbers of *Salmonella* spp. recovered from pork skin during 8 weeks storage at 8ºC. Results are given as log CFU/cm^2. Values in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Treatment	Week 0	Week 2	Week 4	Week 6	Week 8	Pooled Mean	SEM
Untreated	3.79^a	6.58^a	7.47^a*	7.21^a	7.33^a	6.48^a	0.69
Water	3.22^a	6.16^a	6.36^{ab}	6.74^a*	6.21^a	5.74^a	0.64
Acetic	3.63^a	5.12^a	6.3^{ab}	6.07^a	6.21^a	5.47^a	0.51
Lactic	3.30^a	6.09^a	6.65^{ab}	6.89^a	6.30^a	5.85^a	0.65
Levulinic	3.30^a	5.03^a	5.30^b	5.09^a	5.75^a	4.89^a	0.42

Discussion and Conclusions: Only acetic acid provided residual protection and only against Salmonella on chicken skin based on comparison of the Pooled Means in Tables 7-10.

Objective 4: Evaluate the organoleptic implications from spraying slices of turkey roll or beef trim with organic acids.

Procedure: Slices of turkey roll and beef trim were sprayed with water or 2% lactic, acetic or levulinic acids at 55.4ºC. Beef trim was ground and formed into patties. Samples were over-wrapped with PVC and stored for seven days at 4ºC. Color was instrumentally monitored each day during storage. Additional samples were served (beef after cooking) to a consumer panel for evaluation of overall liking.

Results:

Table 11: Mean sensory scores for turkey and hamburger patty samples. Values in same column sharing a superscript letter are not significantly different ($p > 0.05$).

Sample Type	Water	2% Acetic acid	2% Lactic Acid	2% Levulinic acid	P-value
Turkey	6.25[a]	6.20[a]	6.58[a]	6.55[a]	0.089
Hamburger Patty	6.34[a]	6.05[a]	6.07[a]	6.19[a]	0.220

Figure 5: Lightness of sliced turkey roll during 7 days storage at 4ºC.

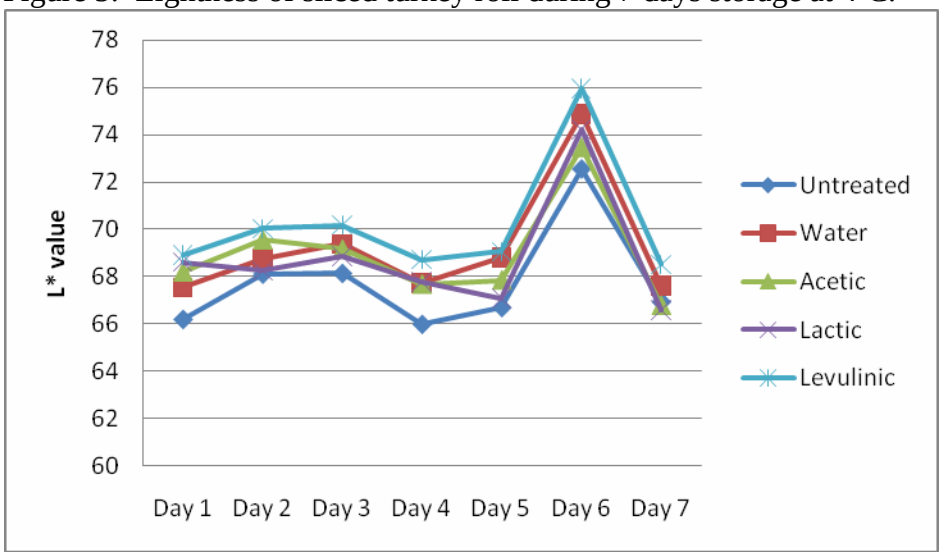

Table 12: L* values of sliced turkey roll during 7 days storage at 4ºC. Pooled mean values sharing a superscript letter are not significantly different ($p > 0.05$).

L* value	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pooled Mean	SEM
Untreated	66.2	68.1	68.1	66.0	66.7	72.5	66.9	67.8[a]	0.9
Water	67.5	68.8	69.4	67.7	68.8	74.9	67.6	69.2[b]	1.0
Acetic	68.2	69.6	69.2	67.7	67.9	73.5	66.8	69.0[b]	0.8
Lactic	68.6	68.3	68.9	67.8	67.1	74.2	66.6	68.8[b]	1.0
Levulinic	68.9	70.0	70.2	68.7	69.1	76.0	68.5	70.2[c]	1.0

Figure 6: Color of sliced turkey roll during 7 days storage at 4ºC.

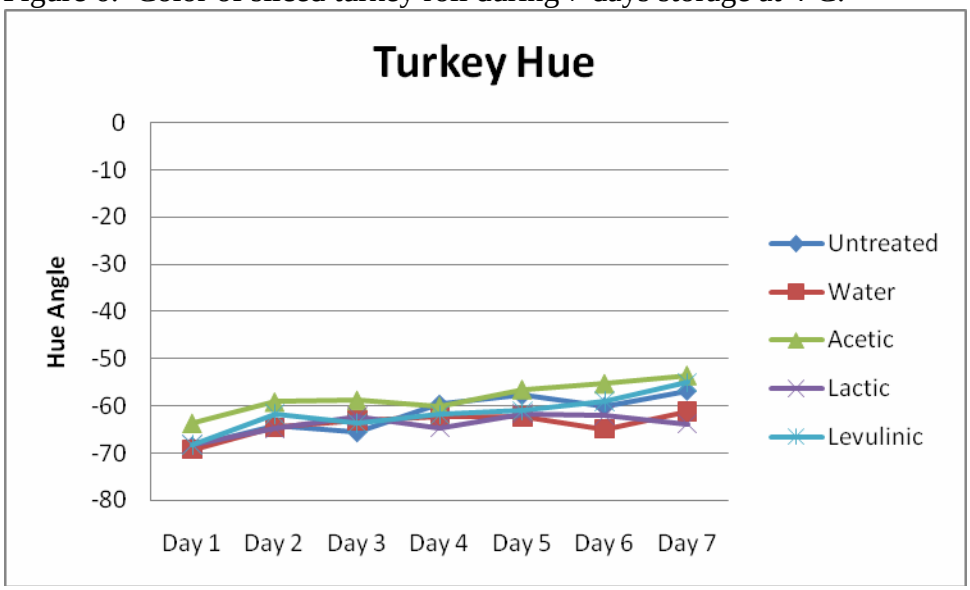

Table 13: Hue angle of turkey slices during 7 days storage at 4ºC. Pooled mean values sharing a superscript letter are not significantly different ($p > 0.05$).

Hue Angle	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pooled Mean	SEM
Untreated	-68.4	-64.1	-65.5	-59.6	-57.7	-60.1	-56.8	-61.7^{b}	1.6
Water	-69.2	-64.5	-63.1	-62.3	-62.2	-64.9	-61.2	-63.9^{b}	1.0
Acetic	-63.6	-59.1	-58.8	-60.0	-56.5	-55.3	-53.5	-58.1^{a}	1.3
Lactic	-68.1	-64.7	-62.3	-64.6	-61.6	-62.0	-63.8	-63.9^{b}	0.9
Levulinic	-68.3	-61.8	-63.6	-61.8	-61.0	-59.0	-55.0	-61.5^{ab}	1.5

Figure 7: Lightness of ground beef during 7 days storage at 4ºC.

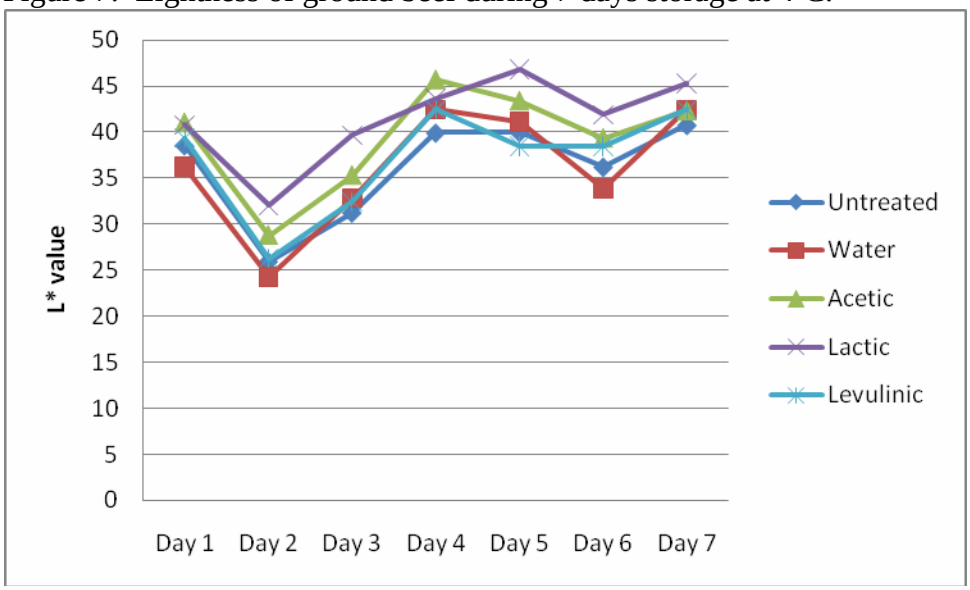

Table 14: L* values of ground beef during 7 days storage at 4ºC. Pooled mean values sharing a superscript letter are not significantly different ($p > 0.05$).

Lightness	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pooled Mean	SEM
Untreated	38.6	25.9	31.2	39.9	40.0	36.2	40.8	36.1^{c}	2.1
Water	36.2	24.3	32.8	42.4	41.1	33.8	42.4	36.2^{c}	2.5
Acetic	41.0	28.8	35.3	45.7	43.4	39.3	42.3	39.4^{b}	2.2
Lactic	40.8	32.0	39.7	43.7	46.8	41.9	45.3	41.4^{a}	1.8
Levulinic	39.4	26.3	32.4	42.5	38.4	38.4	42.4	37.1^{ab}	2.2

Figure 8: Color of ground beef during 7 days storage at 4ºC.

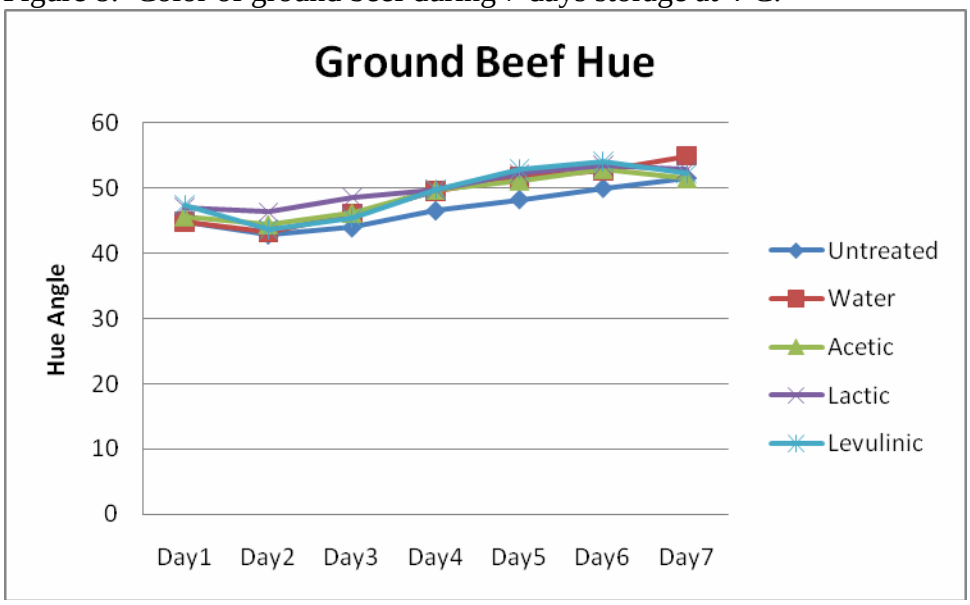

Table 15: Hue angle of ground beef patties during 7 days storage at 4ºC. Pooled mean values sharing a superscript letter are not significantly different ($p > 0.05$).

Hue	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Pooled Mean	SEM
Untreated	44.9	42.9	44.0	46.5	48.2	49.9	51.5	47.8^{c}	1.2
Water	44.8	43.2	46.1	49.5	51.7	52.6	54.9	49.0^{ab}	1.6
Acetic	45.6	44.3	46.1	49.7	51.1	52.9	51.5	48.7^{b}	1.3
Lactic	47.0	46.3	48.5	49.7	52.2	53.4	52.8	50.0^{a}	1.1
Levulinic	47.5	43.6	45.4	49.8	52.9	54.1	52.2	49.3^{ab}	1.5

Discussion and Conclusions: Topical application of 2% lactic, acetic, or levulinic acid to slices of turkey roll and to beef trim did not impact the overall liking the turkey roll or hamburger patties, respectively (Table 11). Samples treated with levulinic acid were just as well-liked as the other treatments tested. Spraying with water or acid had some variable effects on instrumental measure of lightness and color (as measured by hue angle) (Table 12-16). The differences were small in magnitude, and our experience suggests that they would be of little practical significance.