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Inactivation of Shiga toxin-producing *Escherichia coli* in fresh beef by electrolytically-generated hypochlorous acid, peroxyacetic acid, lactic acid and caprylic acid

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Abstract

Several studies have been conducted to verify the decontamination potential of electrolytically-generated hypochlorous acid, peroxyacetic acid, lactic acid and caprylic acid against Shiga toxin-producing *Escherichia coli* (STEC) in beef products. However, there is no consensus regarding their effectiveness. The aim of this study was to compare these four treatments under the same conditions and establish a ranking according to their effectiveness to inactivate STEC in fresh beef. Samples were inoculated with two levels of inoculum and rinsed for 15 s in 100 ml of antimicrobial solution treatment. Caprylic acid was the most effective treatment, followed by lactic acid and peroxyacetic acid. Electrolytically-generated hypochlorous acid had no effect. Sensory analysis showed no significant differences either in flavor or in color between samples treated with caprylic acid and reference samples. Caprylic acid appears to be an effective and viable alternative to conventional interventions frequently used for meat product decontamination.

Keywords: STEC, antimicrobial interventions

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens that can cause bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). In Argentina, STEC serogroups O157, O26, O103, O111, O145 and O121 are considered adulterant in ground beef. In the United States (US), the absence of detectable O157, O26, O45, O103, O111, O121 and O145 serogroups in ground beef and beef trimmings is mandatory (FSIS, 2012), the same as the six major STEC serogroups (O157, O26, O103, O111, O145 and O104:H4) in sprouts or ground beef and beef trimmings from the European Union (EFSA, 2013).

The cattle industry has sought to eliminate STEC and other pathogens, facing multiple hurdles in both preslaughter and postslaughter environments. In postslaughter interventions, different physical and chemical carcass decontamination procedures have been evaluated, such as electrolytically-generated hypochlorous acid (EGHA) to decontaminate beef samples (Ding, Rahman, Purev, & Oh, 2010). These authors evaluated the bactericidal effects of acidic electrolyzed oxidizing water (AcEOW) and slightly acidic electrolyzed oxidizing water (SAcEOW) against *E. coli* O157:H7 on beef and determined log reductions of 1.64 ± 0.13 or 1.72 ± 0.09, respectively. Nevertheless, years later Signorini et al. (2018) did not obtain significant differences in microbial counts between treated and control beef carcasses. In the case of organic acids, Ransom et al. (2003) and Kalchayanand et al. (2012) verified the decontamination potential of lactic acid (LA) and peroxyacetic acid (PA) and reported that they effectively reduced STEC strains more than 1 log CFU/g. However, Harris, Brashears, Garmyn, Brooks, & Miller (2012) informed that 2 and 5% LA treatment showed no measurable reduction of *E. coli* inoculated on the surface of beef trimmings. King et al. (2005) reported that bacterial populations on chilled beef carcass surfaces were not significantly different from those found on untreated control pieces after PA application.

It has been recently shown that new chemicals may be more effective against *E. coli* O157:H7 than the existent microbiological interventions. This is the case of caprylic acid (CA:

octanoic acid), a natural, 8-carbon, medium fatty acid present in breast milk, bovine milk and coconut oil (Jensen, 2002; Sprong, Hulstein, & Van der Meer, 2001). According to the joint FAO/WHO Expert Committee on Food Additives, CA is safe when used as flavor (JECFA, 2005). In the US, it has been approved for surface application on ready-to-eat meat and meat products (USDA, 2015). Mohan & Pohlman (2016) processed beef trimmings inoculated with *E. coli* O157:H7 with different organic acids and observed that 30g/L CA was highly effective.

The aim of this study was to compare the effectiveness of electrolytically-generated hypochlorous acid, peroxyacetic acid, lactic acid and caprylic acid to inactivate a pool of native STEC strains inoculated on fresh beef.

2. Materials and methods

2.1 Experimental design

Each treatment was carried out six times. Sample size was enough to detect differences of at least 0.8 log CFU/g with a standard deviation of 1.0 log CFU/g (95.0% confidence interval [CI]) among STEC counts in the high inoculum experiment, and a reduction of at least 20% in the prevalence of serogroups (95.0% CI) in the low inoculum.

2.2 Bacterial strains and inoculum preparation

The STEC strains used in this study were O26 (*stx*$_1$/*eae*) and O157 (*stx*$_2$/*eae*) isolated from beef products, O145 (*stx*$_2$/*eae*) isolated from a patient with HUS, and O103 (*stx*$_1$/*eae*) and O111 (*stx*$_2$/*eae*), both isolated from patients with diarrhea. All STEC strains used are included in IGEVET culture collection. The strains were kept in frozen culture at -80ºC until subcultures were prepared by inoculating a test tube containing 10 ml of tryptic soy broth (TSB, Biokar, France) with a single colony grown in MacConkey agar (MAC, Biokar), and individually incubated at 37 °C overnight. Cells were harvested by centrifugation at 12000 g for 5 min at room temperature and the pellets were washed twice with phosphate-buffered saline (PBS, pH 7.2, Oxoid, UK). In order to guarantee that all strains had similar concentrations, the optical

density was measured at 600 nm and fixed at the same value using a spectrophotometer (Metrolab 330, Metrolab, Argentina). The pool of strains was prepared by mixing equal volumes of each strain in PBS.

2.3 Sample preparation and inoculation procedure

Twenty pieces of eye of round beef cut (*Semitendinosus* muscles) were purchased from beef export-certified slaughterhouses. Absence of STEC in raw material was verified by analyzing the surface of each beef for the presence of *stx* and *eae* genes by real-time polymerase chain reaction (RT-PCR, Pall Corporation, USA). Each eye of round roast beef was sliced with a punch in order to obtain 25 cm^2 and 25 g samples. For the inoculation procedure, 50µl of a STEC pool was applied onto the sample surface and evenly spread with a drigalski spatula. Final concentration for high inoculum samples was of approximately 6-7 log CFU/g and for low inoculum samples was of approximately 0.5-1 log CFU/g. The inoculated slices were allowed to dry for 15 min at room temperature in a biological safety cabinet.

2.4 Antimicrobial treatment preparation

The antimicrobial treatments applied were 1) 50 ppm EGHA (Envirolife, Argentina); 2) an equilibrium mixture of PA, octanoic acid, acetic acid, hydrogen peroxide, peroxyoctanoic acid, and 1-hydroxyethylidene-1,1-diphosphonic acid, where PA (INSPEXX 200, Ecolab, USA) set at 200 ppm; 3) 2% LA at 50 ºC (Purac 85%, Netherlands); and 4) 3% CA at 50 ºC (Sigma Chemical Co, USA). While CA solutions were prepared in 98% ethanol, EGHA, PA and LA were prepared in distilled water. All antimicrobial compounds were prepared in accordance with the recommendations of the manufacturer and used within 1 week of preparation. The pH of the antimicrobial solutions was recorded with a pH meter (ThermoOrion model 710A+, Beverly MA, USA).

Samples were rinsed for 15 s in 100 ml of antimicrobial solution. Control samples were rinsed under the same conditions in distilled water and ethanol solution. After treatment,

samples were individually vacuum-packed (Cryovac BB2000CB, Sealed Air Co., Argentina) and kept at 4 ºC until analysis.

2.5 Microbiological analysis of high inoculum samples

Samples were transferred into sterile stomacher bags and 225 ml of 0.1% peptone water (PW, Biokar) were added. Immediately after, samples were stomached (easy Mix, AES, France) for 60 s and serial dilutions were prepared. The STEC counts were performed in TSA and MAC (Biokar) as non-selective and selective media, respectively. All plates were incubated in duplicate overnight at 37 °C.

Log reductions of EGHA, LA and PA were calculated by subtracting STEC counts in TSA of treated samples from STEC counts in TSA of water-treated samples, while CA log reductions were calculated by subtracting STEC counts in TSA of treated samples from STEC counts in TSA of ethanol-treated samples. Injured cells were calculated as the difference in microbial counts between TSA and MAC.

2.6 Microbiological analysis of low inoculum samples

Samples were transferred into sterile stomacher bags containing 225 ml of modified TSB (mTSB, Biokar); they were then incubated at 42 ºC for 20 h. After the enrichment step, samples were tested for the presence of *stx*$_1$, *stx*$_2$ and *eae* genes by RT-PCR (Pall Corporation). Samples positive for the *stx* and *eae* genes were tested for the presence of serogroup molecular markers by RT-PCR (Pall Corporation).

2.7 Sensory analysis

Sensory analysis was performed only on the two most effective treatments according to microbial results. A difference from control test (DFC) with two blind controls was developed to assess significant differences in color and flavor between treatment and control samples. This method is recommended when there is inherent product variability due to its components (Kilcast, 2010; Meilgaard, 2007; Muñoz, Civille, & Carr, 1992; Rogers, 2017). For the experiment, four complete eye rounds were randomly divided into similar weight pieces. Each

portion was randomly assigned to a treatment or became either the reference sample or a blind control (Figure 1). Samples were vacuum-packed and refrigerated (4 ± 0.5 °C) until assay.

2.7.1 Color assessment

Only one portion of each treatment, one reference sample and two blind controls (one from the same eye round as the reference and one from a different eye round) were selected to avoid too much heterogeneity, which is usually found in nonhomogeneous products like meat. Sensory color assessment was carried out under standard lightning conditions using a light cabinet (Verivide, CAC 120, United Kingdom) and illuminant D65. The panel was formed by 24 untrained judges recruited from the Food Research Institute (INTA, Argentina). The panelist viewing angle of 45° was kept constant to the light source to reduce glare from package film (Hunt et al., 2012). Samples were presented to the panelists in a sequential monadic order with a replication. A balanced blocked design was used to avoid presentation bias. Panelists were asked to compare the color of each codified sample in relation to a reference sample that was fixed. They had to record the magnitude of the difference perceived on a ballot paper in a 10 cm line scale, where 0 = no difference in color in comparison to the control sample, and 10 = completely different color in comparison to the control sample.

2.7.2 Overall flavor assessment

In order to assess differences in the overall flavor perception, steaks of eye rounds were cut into ≈1.27 cm long x 1.27 cm wide x 1.5 cm thick cubes. They were cooked by pairs on a preheated electric clamshell grill (Ingeniería Gastronómica, Argentina) for 2 min at 160 °C to an internal temperature of 70 °C in accordance to the guidelines provided by the American Meat Science Association (AMSA, 2015). The experiment was carried out in a sensory evaluation laboratory equipped with panel booths meeting ISO standards (ISO 8589/2007) . The panel was formed by eight well-trained judges. Each panelist received two cubes of each sample at ≈ 60 °C, disposed in closed thermal containers codified with a random three-digit number. A replication was performed. All samples were presented simultaneously using a balanced

blocked design to avoid presentation bias. Distilled water and unsalted soda crackers were provided to purge the palate of residual flavor notes between samples. The panelists were asked to taste each coded sample from left to right and to compare the coded samples against the reference sample. They had to complete a 6-point category scale in order to make their judgments on overall flavor differences in comparison with the reference sample, where 0 = no difference, 1 = very slight difference, 2 = slight difference, 3 = moderate difference, 4 = large difference and 5 = extremely large difference.

2.8 Statistical analysis

In the high inoculum experiment, the counts of STEC (both TSA and MAC) in all treatments were analyzed using ANOVA (control –water-, LA, PA and EGHA) and t-student test (control –ethanol- vs. CA). For the STEC count reduction variable, ANOVA was performed in order to compare all the treatments.

In the low inoculum experiment, the presence of *stx* and *eae* genes and molecular markers of serogroups O26, O103, O111, O145 and O157 was compared among treatments using the generalized linear model (GLM). The presence or absence of *stx* and *eae* genes and serogroups were the outcome variables in the GLM, using a binary logistic distribution as linked function.

In the sensory analysis, comparisons between the control sample and each of the treatment samples were done using two-way ANOVA (with Dunnet´s multiple comparison as post hoc test).

All statistical analyses were performed using InfoStat software (Universidad Nacional de Córdoba, Argentina).

3. Results

3.1 Samples with high inoculum level

The counts of STEC after treatments are shown in Table 1. No differences were found in bacterial counts of samples treated with EGHA and samples treated with water neither in TSA

nor in MAC (P>0.05). Survival (log CFU/g) of STEC in TSA of samples treated with LA and PA differed from the others and among themselves (P<0.05). Log reductions (log CFU/g) were 0.34 and 0.21 in LA and PA, respectively. Survival (log CFU/g) of STEC in MAC of samples treated with LA and PA differed from the others but not among themselves (P>0.05). No differences were found in bacterial counts of samples treated with ethanol or water neither in TSA nor in MAC (P>0.05). As to samples treated with CA, microbial counts in TSA and MAC were different from ethanol and all the other treatments analyzed; the log reduction was of 0.75 log CFU/g. Injured cell counts were 0.04, 0.09, 0.13, 0.27, 0.38 and 0.41 log CFU/g in samples treated with ethanol, water, EGHA, LA, CA and PA, respectively.

3.2 Samples with low inoculum level

Results of samples inoculated with low bacterial concentration are shown in Table 2. All samples (100%) treated with water, ethanol, EGHA, LA and PA were positive for both, *stx* and *eae* genes, by RT-PCR after the enrichment step. Only the CA treatment was effective in reducing the percentage of samples positive for *stx* and *eae* (47%). Regarding positivity for serogroups, no statistical differences were found between samples treated with water and samples treated with EGHA (P<0.05). In samples treated with LA and PA, the percentages of positive samples for O103, O111, O145 and O157 differed from samples treated with water but not between themselves (P<0.05). For O26, samples treated with PA did not differ from samples treated with water while samples treated with LA differed from samples treated with water, as well as, PA (P<0.05). The most effective treatment was CA, since percentages of samples testing positive for all the serogroups analyzed were statistically different from those of control samples and those treated with EGHA, LA and PA.

3.3 Sensory analysis

The sample averages for attribute differences of eye round samples are shown in Table 3. In order to adjust for the heterogeneity of the control product and to eliminate the placebo effect (the effect of asking the difference question) from DFC ratings, the average of the two hidden

control samples was computed and then this average subtracted from the average of each of the treated samples evaluated.

Fourteen answers from the analysis of color assessment were discarded, namely, those falling outside the limits of the test (X_1 being rated ≥ 2 points different from the reference sample or being assigned a higher grade of difference than the rest of the samples in comparison with the control) (AUST, GACULA, BEARD, & WASHAM II, 2006; D. Kilcast, 2010). Differences between $\overline{X_1}$ and $\overline{X_2}$ reflected product variation, which was greater than the treatment effects being measured. This within-control variability does not allow to establish significant differences between samples (AUST et al., 2006). On the other hand, the negative difference observed with CA samples reflected that the treatment effect was smaller than the variability between controls.

A significant difference was found between the reference and the treated samples in overall flavor data (P<0.05). The Dunnett's test found that DFC in LA samples was significantly different from mean DFC in the blind control (P<0.05), whereas DFC in CA samples was not significantly different from the blind control mean DFC (P<0.05). The difference in overall flavor in LA samples as compared with the reference sample was described as very slight to slight. The panel detected off-flavors in those samples, which were described as fish-like, cardboard, olive and intense pig flavor.

4. Discussion

The effectiveness of four chemical products to reduce native STEC strains on beef was evaluated under controlled conditions. Our results showed that EGHA was not effective in samples inoculated with either high or low inoculum level. This finding is in agreement with that reported by Signorini et al. (2018), who evaluated the antimicrobial effect of EGHA against STEC on beef carcasses at commercial abattoirs and did not obtain significant differences in microbial counts between treated and control samples. Our results also agree with those of

Kalchayanand et al. (2008), who demonstrated that EGHA was not effective for the reduction of *E. coli* O157:H7 in a beef head wash cabinet. Conversely, Ding et al. (2010) reported that after treatments with AcEOW or SAcEOW, 1.64 ± 0.13 and 1.72 ± 0.09 log reductions were observed. However, these authors considered control samples as samples inoculated and untreated while we considered control samples as inoculated water treated samples. In addition, contact time used by Ding et al. (2010) was of 3 min while we used 15 sec. These facts may explain the differences observed between assays. In this work, PA treatment reduced STEC counts in TSA from 6.81 to 6.61 log CFU/g. The difference between STEC counts in TSA and MAC was 0.41 log CFU/g, which represented the injured population. Treatment with PA was not effective at the low inoculum level, as no reduction in the percentage of samples positive for *stx* and *eae* was observed. In samples positive for serogroups O103, O111, O145 and O157, a 20 to 40% reduction was observed, which was expected since only 1/5 of STEC populations belonged to each serogroup. Regarding serogroup O26, no reduction was observed, probably because the strain may be resistant to PA treatment. Further studies should be performed to confirm this hypothesis. Some authors described that after PA application, microbial counts were not significantly different from those in untreated control pieces (King et al., 2005; Mohan & Pohlman, 2016; Signorini et al., 2018), while others described reductions of at least 1 log CFU/g (Kalchayanand et al., 2012; Ransom et al., 2003). Kalchayanand et al. (2012) evaluated the same product at the same concentration in fresh beef and reported 0.9 and 1.5 log CFU/g reductions depending on the serogroup analyzed. Ransom et al. (2003) evaluated a 0.02% PA solution on beef carcass tissue and reported a 1.4 log CFU/g reduction in the *E. coli* O157:H7 population. However, both authors calculated log reductions after subtracting STEC counts in selective media of treated samples from STEC counts in selective media of inoculated/untreated samples. In the present study, log reductions were calculated by subtracting STEC counts in non-selective media (TSA) of treated samples from STEC counts in non-selective media of inoculated water-treated samples. In the same study, Ransom et al. (2003) reported that survival

in TSA with yeast extract (TSAYE) was 6.4 log CFU/g in inoculated/untreated samples, 4.8 log CFU/g in samples inoculated and treated with water at 25 ºC, and 4.8 log CFU/g in samples inoculated and treated with PA. That is to say, samples treated with water had the same survival counts in TSA than samples treated with PA.

Treatment with LA resulted in a 0.34 log CFU/g reduction. The difference between STEC counts in TSA and MAC was 0.27 log CFU/g. No reduction was observed in samples with low inoculum as the percentage of samples positive for *stx* and *eae* after the enrichment step was 100%. The lack of efficacy observed in samples with low inoculum could be due to the fact that cells with higher resistance were able to survive and recover during the enrichment procedure. Nevertheless, a 20 to 30% reduction was observed in samples positive for serogroups O26, O103, O111, O145 and O157. Other authors evaluated LA treatment in beef products with variable results. On the one hand, Harris, Brashears, Garmyn, Brooks, & Miller (2012) informed that LA treatment at 2 and 5% showed no significant reduction of *E. coli* inoculated on the surface of beef trimmings. On the other hand, Kalchayanand et al. (2012) reported a 1.6 to 3.1 log reduction – depending on the serogroup analyzed – after treating fresh beef with 4% LA and Ransom et al. (2003) reported 3.3 log CFU/g reduction after treating beef carcass with 2% LA. However, it is important to consider which microbial counts were used to estimate log reductions. Ransom et al. (2003) reported survival in TSAYE as 6.4 log CFU/g in inoculated/untreated samples, 4.8 log CFU/g in samples inoculated and treated with water at 25 ºC, and 3.7 log CFU/g in samples inoculated and treated with LA, *i.e.*, 1.1 log CFU/g reduction. A recent publication of EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (EFSA, 2018) reinforced the importance of comparting treated samples with samples treated with water as concluded that lactic acid treatment was efficacious compared to untreated control but it could not be concluded if it was more efficacious than water treatment. The difference between the 0.34 log reduction observed in the present study and the 1.1 log reduction reported by Ransom et al. (2003) could be explained by the nature of the strains analyzed. Ransom et al.

(2003) inoculated *E. coli* O157:H7 non-pathogenic strains, while in our study O26, O103, O111, O145 and O157 native STEC strains were used. As to sensory analysis results, LA treatment did not produce perceptible differences of the color in eye round retail cuts. Nevertheless, these cuts imparted off-flavors described as fish-like, olive, cardboard and intense pig flavor, which are considered a disadvantage for LA application in beef products.

When comparing microbial counts of samples treated with water and samples treated with ethanol, we concluded that ethanol treatment had no bactericidal effect, which means that the reductions observed in samples treated with CA were caused exclusively by the acid and not by the solvent. This result is in agreement with that reported by Kim & Rhee (2015) and Choi, Kim, Lee, & Rhee (2013). Taking into consideration the six treatments analyzed, CA was the most effective: log reductions in TSA were 0.75 log CFU/g; the population of injured cells was 0.38 log CFU/g; samples positive for *stx* and *eae* genes were reduced by 53%; and the percentage of samples positive for the six serogroups analyzed was significantly reduced. Furthermore, CA effectiveness against *Cronobacter sakazakii* and *Salmonella* Typhimurium in an infant formula and against *E. coli* O157:H7 in carrot juice, beef trimmings and cattle hides has also been demonstrated by others authors (Choi, Kim, Lee, & Rhee, 2013; Kim & Rhee, 2015; Mohan & Pohlman, 2016; Baskaran et al., 2013). Mohan & Pohlman (2016) processed beef trimmings inoculated with *E. coli* O157:H7 with different organic acids and observed that 30 g/L CA was highly effective, showing a 4.8 log CFU/g reduction. Baskaran et al. (2013) studied the efficacy of 1% CA, 1% β-resorcylic acid (BR) and their combination CA+BR to reduce *E. coli* O157:H7 in cattle hides and reported that the three treatments were effective (3 to 4 log reductions CFU/g). The differences observed between our results and those reported by Mohan & Pohlman (2016) and Baskaran et al. (2013) could be explained by the variance in stress resistance of the inoculated strains, as well as by the food matrix evaluated. Concerning the sensory analysis, CA treatment did not produce perceptible differences either in color or in flavor between treated and reference samples.

Thus, CA appears as an interesting alternative to conventional interventions frequently used for meat product decontamination. Although this acid is generally recognized as a safe product for application in food stuff (Food Grade Statemente, Sigma-Aldrich), it could be potentially irritant at high concentrations (Safety data sheet, Sigma-Aldrich). In addition, it would be interesting to develop a strategy in order to dilute it in a non-alcoholic solution. Further studies should be conducted to determine whether CA treatment is feasible and effective for pathogen reduction in beef products.

5. Conclusions

The results obtained in the present study showed that CA was the most effective treatment against STEC in beef products, followed by LA and PA. Although significant differences were obtained, reductions were less than 1 log CFU/g. EHGA was not effective. Considering that interventions aimed at reducing STEC on beef are necessary and that there is no single strategy to ensure STEC reduction in commercial abattoirs, the combined use of several treatments could be the answer. The information generated in this study may contribute to the improvement of STEC control in beef.

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Table 1. Antimicrobial treatment effects on survival, reduction and injured bacterial counts (log CFU/g) on beef samples inoculated with STEC, by plating on Triptic Soy agar (TSA) and MacConkey agar (MAC).

Antimicrobial intervention	Survival TSA (log CFU /g)	Reduction TSA (log CFU/g)	Survival MAC (log CFU /g)	Injured cells (log CFU/g)
Water	6.81 (0.19) [a]	-	6.72 (0.22) a	0.09
Lactic acid (LA, 2%)	6.47 (0.22) [c]	0.34	6.20 (0.27) b	0.27
Electrolized water (EW, 50 ppm)	6.76 (0.22) [a]	0.05	6.63 (0.20) a	0.13
Peroxiacetic acid (PA, 200 ppm)	6.61 (0.22) [b]	0.21	6.20 (0.20) b	0.41
Ethanol (98%)	6.75 (0.09) [a]	0.06	6.68 (0.24) a	0.04
Caprylic acid (CA, 3%)	5.99 (0.24) [d]	0.76	5.61 (0.29) c	0.38

a, b, c, d Means within a column comparing antimicrobial treatment groups lacking common superscript differ ($P<0.05$)

Table 2. Percentage (%) of positive samples after an enrichment step for genes *stx*, *eae* and molecular markers of serogroups O26, O103, O111, O145 and O157.

Antimicrobial interventions	*stx* and *eae*	O26	O103	O111	O145	O157
Water	100 a	97 a	100 a	93 a	97 a	97 a
Lactic acid (LA, 2%)	100 a	70 b	77 b	83 b	80 b	73 b
Electrolized water (EW, 50 ppm)	100 a	100 a	100 a	100 a	100 a	90 a
Peroxiacetic acid (PA, 200 ppm)	100 a	97 a	77 b	77 b	80 b	60 b
Ethanol (98%)	100 a	100 a	100 a	100 a	100 a	100 a
Caprylic acid (CA, 3%)	47 b	17 c	7 c	7 c	13 c	3 c

a, b, c, d Means within a column comparing antimicrobial treatment groups lacking common superscript differ ($p < 0.05$)

Table 3. Difference-from-Control panel data for color and flavor of eye of round.

	Color				Overall flavor			
	X_1	X_2	*LA*	*CA*	X_1	X_2	*LA*	*CA*
Sample mean*	1.56	5.01	3.55	2.10	1.31	2.06	3.18	2.37
Control mean**	3.28				1.68			
Δ			0.26	-2.17			1.5	0.68

X*₁** – blind control from the same eye round that the reference sample; ***X*₂** – blind control from a different eye round from that of the reference sample, ***LA – lactic acid sample, ***CA*** – caprilic acid sample.

* X_1, X_2, X_{LA} and X_{CA}. N = 34 for color assessment (see text) and *N* = 16 for flavor assessment.

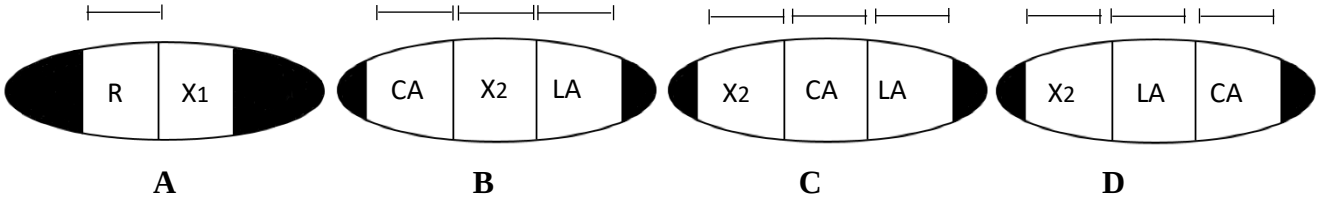
** Control mean was computed as $(\overline{X_1} + \overline{X_2})/2$

$\Delta = \bar{X}_{\text{treated sample}}$ - control mean

For color assessment, differences between $\overline{X_1}$ and $\overline{X_2}$ (1.56 and 5.01,respectively) reflected product variation, which was greater than the treatment effects being measured. This within-control variability does not allow to establish significant differences between samples. The negative difference observed with CA samples reflected that the treatment effect was smaller than the variability between controls.

For overall flavor data, a significant difference was found between the reference and the treated samples ($P<0.05$). The Dunnett's test found that DFC in LA samples was significantly different from mean DFC in the blind control ($P<0.05$), whereas DFC in CA samples was not significantly different from the blind control mean DFC ($P<0.05$).

Figure 1. Scheme of the 4 eye rounds used for color assessment and their assignment to a treatment, reference or blind control.



Being A, B, C y D different eye rounds, black parts of each eye round were discarded.
R = reference sample
X1 = blind control from the same eye round of the reference sample (check of internal validity of the panel and measurement of the effect of asking the difference question (placebo effect))
X2 = blind control from a different eye round from that of the reference sample (meat inherent variability)
LA = samples treated with lactic acid
CA = samples treated with caprylic acid

Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

- ✓ All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

- ✓ This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

- ✓ The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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