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EFFECT OF LACTIC ACID ON *Escherichia coli* O157:H7 AND ON COLOR STABILITY OF VACUUM-PACKAGED BEEF STEAKS UNDER HIGH STORAGE TEMPERATURE

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ABSTRACT

The effect of lactic acid (LA) addition on *Escherichia coli* O157:H7 survivability as well as the color stability was determined in vacuum-packaged beef steaks storage at 10°C for 50 days. *Longissimus dorsi* muscle was portioned into beef steaks and inoculated with *Escherichia coli* O157:H7. Afterwards, the samples were submitted at three treatments: without lactic acid addition or non-treated (NT); with 0.56 M (5%, v/v) (L5), and with 1.13 M (10%, v/v) (L10) of lactic acid addition. Same division was performed with samples non inoculated. All samples were package at vacuum and stored at 10ºC during 50 days. L10 demonstrated an efficient bacteriostatic effect ($P < 0.05$) against *E. coli* O157:H7 and total aerobic mesophilic bacteria. Nonetheless, LA induced a decrease ($P < 0.05$) in a^* values in samples after application and during storage, promoting discoloration of beef steaks. Therefore, L10 was efficient in controlling *E. coli* O157:H7 even at abusive storage temperatures. However, this decontamination treatment affects negatively the color stability of beef.

Keywords: Food preservation; Organic acid; *Longissimus dorsi*; Discoloration; EHEC

INTRODUCTION

Escherichia coli O157:H7 is an important foodborne pathogen, commonly harbored in cattle gut tract **(Bell, 2002; Caprioli *et al.*, 2005; Karmali *et al.*, 2010)** with high economic impact on industry and public health **(Scallan *et al.*, 2011)**. Thus, during cattle slaughter and carcass processing there is a considerable risk of fresh cut and processed product cross-contamination with *E. coli* O157:H7 (**Barkocy-Gallagher *et al.*, 2003; Huang and Sheen, 2011)** corroborating the link between outbreaks of this pathogen and beef **(Callaway *et al.*, 2009; Huang and Sheen, 2011)**.
In order to control microbial development, the meat industry usually uses organic acids on beef processing **(Buncic and Sofos, 2012)**. Lactic acid (LA) is a weak organic acid generally recognized as safe (GRAS) **(FDA, 2016)**, which is naturally present in the muscle tissue **(Siragusa, 1995; Muchenje *et al.*, 2009)** and is also produced by fermentative bacteria **(Martinez *et al.*, 2013; FDA, 2016)**. Acid decontamination with LA is considered a simple and valuable chemical strategy to improve food safety in meat products **(Ricke, 2003; Skandamis *et al.*, 2010)** due to the action immediately after application **(Anderson and Marshall, 2007)**, and also during storage, when the residual LA content exerts bacteriostatic effect on bacterial counts **(Dorsa *et al.*, 1998)**. However, despite LA bacteriological advantages, its application can promote surface color changes on meat (**Hunt *et al.*, 1999; Pipek *et al.*, 2005; Hosseini and Esfahani-Mehr, 2015)** resulting in economic losses **(Smith *et al.*, 2000)**.Beef color influences the consumer acceptability, which interfere on purchase decisions **(Suman *et al.*, 2010)**. This quality parameter is stated by myoglobin (Mb) redox state on meat: deoxymyoglobin (DMb; purplish-red color), oxymyoglobin (OMb; bright cherry-red color), and metamyoglobin (MMb; brown color). MMb results from the oxidation of myoglobin **(Suman *et al.*, 2007; Suman and Joseph, 2013)**, and the increase of MMb formation should be avoided in order to prevent the development of brown color on beef products surface. Therefore, it is important the use of package strategies, such as the vacuum package, in order to maintain meat color **(Kerry *et al.*, 2006; Zhou *et al.*, 2010)**, wholesome and safe **(Leistner, 2004; John *et al.*, 2005; Han, 2014)**, during storage.

Previous studies **(Youssef *et al.*, 2012; Harris *et al.*, 2012; Youssef *et al.*, 2013; Li *et al.*, 2015; Blagojevic *et al.*, 2015)** examined the effect of lactic acid concentrations up to 5% combined with temperature to evaluate the reduction of *E.coli* O157:H7 during storage, however, there is a little information about the inhibition effects of use lactic acid concentrations higher than 5% on vacuum package beef steaks, during prolonged storage at 10ºC.Therefore, the goals of the present research were 1) to evaluate the most efficient lactic acid concentration (0.56 M; 5%, v/v or 1.13 M;10%, v/v) for *E. coli* O157:H7 reduction and 2) the effect of lactic acid application on pH and surface color stability of vacuum packaged beef steaks (*Longissimus dorsi*) during 50 days of storage at 10ºC.

MATERIALS AND METHODS

Experimental design

Longissimus dorsi (LD) beef (21kg) was obtained between the 6th thoracic rib and the 6th lumbar vertebrae from a commercial processing facility inspected by the federal government (Colatina, Espírito Santo Brazil) after 36 h post-mortem. LD was transported under refrigeration (0°C) to the Meat Laboratory of Universidade Federal Fluminense where was portioned into beef steaks (100 g each; area: 256 cm²; 9.5 cm diameter and 2.0 cm thick approximately). Samples were equally divided in two groups (non-inoculated and inoculated). Both groups were randomly subjected to surface application of LA solution at either 0.56 M (5%, v/v) (L5) or 1.13 M (10%, v/v) (L10) or were not treated (NT). After LA treatment, samples were vacuum packed and stored for 50 days at 10°C. The abusive temperature model used to stimulate *E. coli* O157:H7 growth was performed follow the indication of **Hwang *et al.* (2014)**. Three trials (n = 3; 7 kg each trial) were carried out, totaling 70 unit samples (100g) per trial. Physicochemical and bacteriological analyses were performed at days 0, 3, 15, 30, and 50 of storage.

Raw beef inoculation

Escherichia coli O157:H7 strain (EDL 933) **(Gobert *et al.*, 2007)** was provided by the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil). The lyophilized

inoculum was added to brain heart infusion broth (BHI) (Difco®, Detroit, MI) and activated at 37°C for 24 h. Then, 1 ml aliquots were transferred to test tubes containing 9 ml of BHI, and incubated at 37ºC for 18 h **(Meng *et al*., 2001)**. The inoculum was calculated using an UV spectrophotometer (Smartspec Plus, BioRad, Hercules, CA) at 600 nm and a concentration of 10^8 cells/ml was detected **(Lázaro *et al*., 2014)**. The inoculum concentration was confirmed by spread plating on Plate Count Agar (PCA) (Merck®, Darmstadt, Germany) and O157:H7 Agar (Merck®, Darmstadt, Germany) **(Alegre *et al*., 2010)** achieving 6 log CFU/g on meat.

The inoculation was performed on beef steaks (100g), aseptically transferred to plastic packages BBL4 (Cryovac®, Saddle Brook, NJ). In this conditions 0.5 ml of *Escherichia coli* O157:H7 inoculum were pipetted on both sides of the beef surfaces (1ml per sample) **(Alegre *et al*., 2010)**. The inoculated samples were massaged for 1 min and remained 30 min at 22 ºC to allow the inoculum attachment to the meat surface **(Huang and Chen, 2011; Mahmoud, 2014)**. After inoculation, the samples were treated with different solutions of lactic acid.

Lactic acid treatment

Solutions of 0.56 M (5%, v/v) (L5) and 1.13 M (10%, v/v) (L10) of lactic acid were prepared from 85% lactic acid (Fisher Scientific, Pittsburgh, PA) using sterile deionized water. Non-inoculated and inoculated LD steaks were pipetted on each side with 2.5 ml of L5 or L10 solutions at room temperature, vacuum sealed, and stored at 10ºC.

Bacteriological evaluation of inoculated beef steaks

The inoculated steaks (25g) were aseptically transferred to sterile bags containing 225 ml of peptone saline (0.10% peptone in 0.85% NaCl) and homogenized utilizing a stomacher (Stomacher 80, Seward Ltd., London, United Kingdom) for 2 min. The homogenized samples were serially diluted in peptone saline and plated onto Petri dishes containing plate count agar (PCA) to evaluate total aerobic mesophilic bacteria (TAMB) **(APHA, 2001)**, and O157:H7 Agar (Merck® KGaA, Darmstadt, Germany) to determine *Escherichia coli* O157:H7 counts **(Alexa *et al*., 2011)**. Incubation was performed at 37°C for 24 h and results were expressed as log CFU/g.

Physicochemical evaluation of non-inoculated beef steaks

Non-inoculated steaks (NT, L5 and L10) were used to evaluate pH values and instrumental color parameters. The analysis of pH was performed using a Handylab 1 (SchottGlaswerke, Mainz, Germany) pH meter previously calibrated with buffer solutions at pH 4.0 and 7.0 **(AOAC, 2012)**.

Instrumental color parameters were evaluated using a Minolta CM-600d spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) with 8 mm diameter measuring aperture, illuminant A, and 10° standard observer **(Canto *et al*., 2015)**. Meat color was measured at the surface of non-inoculated steaks (NT, L5, and L10) after blooming for 10 min at 22°C. The parameters determined were: lightness (*L** value), redness (*a** value), yellowness (*b** value), and ratio of reflectance 630 to 580 nm (R630/580) **(AMSA, 2012)**.

Statistical analysis

A one-way analysis of variance was performed to evaluate the effect of LA application, LA concentration and days of storage on LD steaks on bacteriological (inoculated) and physicochemical (non-inoculated) parameters, using XLSTAT statistical software (Version 2014.5.03, Addinsoft, Inc., Brooklyn, NY, USA). Differences among means were tested using Tukey's test with 95% of confidence level.

RESULTS

Regarding the bacteriological evaluation, the inoculated LD steaks treated with LA solutions (L5 and L10) did not exhibit difference ($P > 0.05$) on *Escherichia coli* O157:H7 and total aerobic mesophilic bacteria (TAMB) counts when compared to non-treated (NT) samples on day 0 of storage (Table 1). However, from day 3 until day 50 of storage, group L10 showed a significant reduction ($P < 0.05$) of *E. coli* O157:H7 and TAMB counts compared to NT, suggesting the bacteriostatic effect of LA, even under abusive temperature.

Table 1 *Escherichia coli* O157:H7 and total aerobic mesophilic bacteria (TAMB) counts (log cfu/g) on *Longissimus dorsi* steaks treated with lactic acid storage at 10 ºC during 50 days.

Microorganism		Days of storage					
	Treatment *	0	3	15	30	50	*SEM*
Escherichia coli O157:H7	NT	6.0 ^{aB}	6.6 ^{aAB}	6.8 ^{aAB}	7.2 ^{aA}	7.7 ^{aA}	*0.07*
	L5	5.2 ^{aB}	5.6 ^{abAB}	5.7 ^{abAB}	6.2 ^{bAB}	6.6 ^{bA}	*0.07*
	L10	5.0 ^{aAB}	5.2 ^{bA}	4.9 ^{bAB}	4.9 ^{cAB}	4.1 ^{cB}	*0.07*
	SEM	*0.08*	*0.09*	*0.11*	*0.05*	*0.05*	
TAMB	NT	6.8 ^{aD}	7.4 ^{aCD}	8.4 ^{aBC}	9.5 ^{aAB}	10.4 ^{aA}	*0.16*
	L5	7.0 ^{aC}	7.5 ^{aABC}	7.2 ^{bBC}	8.7 ^{abA}	8.6 ^{bAB}	*0.14*
	L10	6.1 ^{aBC}	6.1 ^{bBC}	5.9 ^{cC}	7.2 ^{bAB}	6.9 ^{cABC}	*0.09*
	SEM	*0.20*	*0.01*	*0.08*	*0.19*	*0.17*	

Legend: $^{a—c}$ Means in a column without common superscripts are different ($P < 0.05$).
$^{A—D}$ Means in a row without common superscripts are different ($P < 0.05$).
* NT= Non-treated samples, L5= samples treated with 0.56 M (5%, v/v) LA solution, and L10= samples treated with 1.13 M (10%, v/v) LA solution.
SEM= standard error of the mean.

Lactic acid treatment decreased ($P < 0.05$) pH values (Table 2). In terms of LA concentration on days 0 and 15, NT exhibited the greatest ($P < 0.05$) pH values, followed by L5, and L10 which presented the lowest ($P < 0.05$) ones. On the other hand, on days 30 and 50 no difference ($P > 0.05$) was observed between pH values of L5 and L10. In addition, NT, L5, and L10 exhibited an increase ($P < 0.05$) pattern of pH, during the storage period.

Table 2 shows the instrumental color parameters. LA did not exhibit an immediate effect ($P > 0.05$) on *L** values. From day 15 to 50, L10 decreased ($P < 0.05$) sample lightness while L5 remained similar ($P > 0.05$) to NT samples. In addition, during storage L5 and L10 exhibited *L** value decrease ($P < 0.05$) whereas, NT samples did not exhibit ($P > 0.05$) variation. Regarding meat redness (Table 2), LA concentration affected ($P < 0.05$) *a** values. NT demonstrated the greatest ($P < 0.05$) *a** values, L5 intermediate, and L10 the lowest ones ($P < 0.05$) indicating a concentration-dependent pattern. From day 0 to 50, *a** values of L5 and L10 LD steaks decreased ($P < 0.05$), although NT samples did not demonstrated ($P > 0.05$) variation on redness during storage. In terms of yellowness (Table 2), LA treatment promoted an increase ($P < 0.05$) of *b** values after application, whereas on days 15, 30 and 50, L10 exhibited the lowest ($P < 0.05$) *b** values and no difference ($P > 0.05$) was observed on NT and L5 samples.

Regarding LD steak surface color stability (Table 2), LA concentration and storage period affected ($P < 0.05$) R630/580 values. During all storage days, NT exhibited the greatest ($P < 0.05$) R630/580 values. At days 0 and 15 of storage, L10 demonstrated the lowest ($P < 0.05$) R630/580 values, whereas at days 30 and 50 similar results were obtained for L5 and L10 ($P > 0.05$). In addition, NT, L5, and L10 demonstrated decrease ($P < 0.05$) of R630/580 values during storage.

Table 2 Means of pH and instrumental color parameters values on *Longissimus dorsi* steaks treated with lactic acid storage at 10 ºC during 50 days.

Treatment *	Days of storage				
	0	**15**	**30**	**50**	*SEM*
pH					
NT	5.38 ^{aBC}	5.33 ^{aC}	5.50 ^{aAB}	5.63 ^{aA}	*0.00*
L5	4.46 ^{bC}	4.84 ^{bB}	5.26 ^{bA}	5.37 ^{bA}	*0.01*
L10	4.05 ^{cC}	4.66 ^{cB}	5.19 ^{bA}	5.21 ^{bA}	*0.01*
SEM	*0.01*	*0.00*	*0.01*	*0.01*	
L* (lightness)					
NT	38.34 ^{aA}	37.76 ^{aA}	38.25 ^{aA}	37.83 ^{aA}	*1.89*
L5	40.97 ^{aA}	37.12 ^{aB}	36.83 ^{aB}	35.01 ^{aB}	*1.14*
L10	38.73 ^{aA}	29.72 ^{bB}	30.19 ^{bB}	30.00 ^{bB}	*1.04*
SEM	*1.60*	*0.84*	*1.39*	*1.60*	
a* (redness)					
NT	22.09 ^{aA}	21.32 ^{aA}	21.22 ^{aA}	21.85 ^{aA}	*0.16*
L5	20.77 ^{bA}	14.19 ^{bB}	13.27 ^{bB}	13.17 ^{bB}	*0.44*
L10	16.10 ^{cA}	11.43 ^{cB}	11.06 ^{cB}	9.18 ^{cC}	*0.40*
SEM	*0.18*	*0.28*	*0.34*	*0.54*	
b* (yellowness)					
NT	14.83 ^{bA}	13.73 ^{aAB}	13.67 ^{aB}	13.83 ^{aAB}	*0.18*
L5	15.79 ^{aA}	14.22 ^{aB}	13.21 ^{aB}	12.94 ^{aB}	*0.27*
L10	15.81 ^{aA}	9.76 ^{bB}	9.81 ^{bB}	7.84 ^{bC}	*0.47*
SEM	*0.12*	*0.13*	*0.49*	*0.49*	
R630/580 (ratio of reflectance)					
NT	4.48 ^{aA}	4.22 ^{aAB}	4.18 ^{aAB}	4.08 ^{aB}	*0.02*
L5	3.22 ^{bA}	1.76 ^{bB}	1.53 ^{bB}	1.53 ^{bB}	*0.04*
L10	1.82 ^{cA}	1.47 ^{cB}	1.41 ^{bBC}	1.34 ^{bC}	*0.00*
SEM	*0.05*	*0.00*	*0.00*	*0.02*	

Legend: $^{a—c}$ Means in a column without common superscripts within a attribute are different ($P < 0.05$).
$^{A—C}$ Means in a row without common superscripts are different ($P < 0.05$).
* NT= Non-treated samples, L5= samples treated with 0.56 M (5%, v/v) LA solution, and L10= samples treated with 1.13 M (10%, v/v) LA solution.
SEM= standard error of the mean.

DISCUSSION

The reduction of bacterial count in samples treated with LA solution is probably due to lactate anion accumulation **(Van Immerseel *et al*., 2006)** which promoting membrane damage, ATP depletion, and disrupting nutrient transport **(Cherrington *et al*., 1991)**. In addition, weak organic acids such as LA are considered lipophilic and capable of passing through cellular membrane, acidifying the bacterial cytoplasm, affecting homeostasis and influencing *E. coli* O157:H7 and TAMB growth **(John *et al*., 2005)**.

In agreement with present results, **Shrestha and Min (2006)** reported a concentration-dependent pattern on TAMB reduction from the 4th day of storage on fresh pork ham treated with solutions of LA at 1%, up to 6% (v/v). In partial agreement with present results, **Zeitoun and Debevere (1992)** observed that TAMB and *Enterobacteriaceae* counts remained constant in fresh chicken legs treated with lactic acid at 5% (v/v) and 10% (v/v) during at least 14 days of storage. On the other hand, **Harris *et al.* (2012)** observed a reduction of 0.8 log CFU/g on *E. coli* O157:H7 and TAMB counts in ground beef treated with lactic acid at 5% (v/v) on day 0. In addition, **Pittman *et al.* (2012)** reported *E. coli* O157:H7 reduction of 1.6 log CFU/g on beef carcasses after 24 h of decontamination using 5% (v/v) LA. Furthermore, **Mahmoud (2014)** observed reductions of 2.8 and 3.4 log CFU/g of gram negative bacteria in inoculated oysters treated with LA of 0.5 M and 1.1 M immediately after LA application, contrasting with our results. These contrasts could be attributed to differences on LA application methods and type of matrix.

In relation to pH values, the LA decreased them immediately after application, probably due to a proton imbalance in meat muscle caused by LA solutions **(Goli *et al*., 2011)**. Our results are in agreement with **Naveena *et al.* (2006)**, which observed a decrease in pH values in buffalo meat treated with 2% (v/v) of lactic acid, and with **Shrestha and Min (2006) and Grajales-Lagunes *et al.* (2012)** which treated pork meat with LA solutions ranging from 1% to 6% (v/v) at the beginning of the storage period. Moreover, the increase pattern in pH values observed during storage can be attributed to amino acids decarboxylation in response to an acid stress **(Halász *et al.*, 1994).** The decarboxylation is a cell mechanism to maintain the homeostasis, and the loss of a carboxylic group results in the formation of basic molecules, such as amines, that increases the pH of samples **(Pereira *et al.*, 2009)**.

Regarding surface color stability, *L** values decrease during storage on L5 and L10 can be attribute to denaturation of myofibrillar proteins promoted by LA application affecting the water-holding capacity. The water dispersed among the muscle fibers may influence meat surface reflectance **(Aktaş and Kaya, 2001)**. A decrease in *L** values was also observed in fresh pork ham treated with solutions of LA during storage **(Shrestha and Min, 2006)**.

Lactic acid affected *a** values probably due to heme pigment oxidation induced by pH shift **(Hunt *et al*., 1999; Pipek *et al*., 2005)**. The change on pH leads to premature browning of beef as a consequence of MMb accumulation **(Smulders and Greer, 1998)**. In addition, the decrease on *a** values during storage can be explained by myoglobin oxidation, promoting a decrease in redness **(Carlez *et al*., 1995)**. Meat redness decrease was reported in *Longissimus dorsi* beef **(Aktaş, and Kaya, 2001)**, beef carcasses, **(Pipek *et al*., 2005)**, beef trimmings **(Harris *et al*., 2012)** and pork ham **(Shrestha and Min, 2006)** treated with 1.5, 2.0, 5.0 and 6.0% (v/v) of LA solutions during storage, respectively.

The acid treatment can also affect the perception of *b** values **(Friedrich *et al*., 2008)**. LA application increase protein denaturation and exudate release, as function of pH drop, which potentially explains the variations observed on meat yellowness **(Greer and Dilts, 1995)**. The decrease in *b** values in beef carcasses treated with 2% (v/v) LA solutions during storage was also previously observed **(Pipek *et al*., 2005; Mohan *et al*., 2011)**.

Color stability can be estimated based on the ratio of reflectance at 630 nm to 580 nm (R630/580). High ratio values indicate greater redness reflected by the greater OMb than MMb content, and a ratio value of 1.0 represents a meat surface with essentially 100% of MMb **(Strange *et al*., 1974)**. In agreement with the present results, previous researches **(Stivarius *et al*., 2002; Pipek *et al*., 2005; Mohan *et al*., 2011)** observed a decrease in R630/580 values in beef treated with 2% (v/v), 5% (v/v) of LA, as well as organic acid solutions, respectively. In addition, a decrease in R630/580 values on *Longissimus lumborum* and *Psoas major* muscles was also observed due to storage period **(Joseph *et al*., 2012)**.

CONCLUSION

LA solution at 1.13 M (10%, v/v) promotes an efficient control on inoculated *E. coli* O157:H7 vacuum packaged steaks during storage at 10ºC. In spite of the technological potential of LA decontamination for meat industry, this method promoted meat discoloration with a premature browning on *Longissimus* beef surface.

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