

**The Effectiveness of Potassium Lactate and Lactic Acid Against
Campylobacter Species and Psychrotrophic Bacteria**

by

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
Master of Science
in
Food Science and Technology

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September 16, 1999

Blacksburg, Virginia

Keywords: Lactate, Lactic Acid, Chicken, *Campylobacter* sp., Psychrotrophic, Sensory

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ABSTRACT

This study examined the efficacy of potassium lactate and lactic acid to control *Campylobacter* sp. and psychrotrophic bacteria on chicken. The objectives of the two studies conducted were to determine the optimal combination of potassium lactate and lactic acid to inhibit *Campylobacter* sp. in a challenge study and to inhibit naturally occurring *Campylobacter* sp. and psychrotrophic bacteria in a shelf life study.

Boneless, skinless chicken breasts were injected with three levels of potassium lactate (0,1.5,2%), in conjunction with four levels of lactic acid. Lactic acid was injected (0, 0.1, 0.2, 0.3%) as well as applied directly to the surface (0.1% of weight of chicken breast). The chicken breasts were surface inoculated with a mixture of *Campylobacter* sp. and sampled over a period of 28 days at 11°C. The greatest inhibition was found using 2% potassium lactate in conjunction with any level of lactic acid (injected) or 0.1% lactic acid (surface application). Results of this study indicate that potassium lactate and lactic acid can be used to control the growth and/or survival of *Campylobacter* sp. on boneless chicken breasts.

The second study eliminated the 1.5% potassium lactate and 0.2% and 0.3% lactic acid treatments and chicken breasts were not inoculated with *Campylobacter* sp.. This 4°C shelf life study occurred over 32 days, testing for *Campylobacter* species, psychrotrophic bacteria, as well as testing for sensory perceptions of color and odor changes in the chicken. The most effective treatment was the 2% potassium lactate-0.1% lactic acid surface treatment, demonstrating the most inhibition against both target

populations. This treatment also had the greatest impact upon the odor of the chicken breasts. This treatment had the greatest difference from control samples, which was achieved by the inhibition of spoilage organisms on the chicken breasts.

ACKNOWLEDGMENTS

I would like to thank Dr. Susan Sumner for her support, encouragement, and faith in me. I would not be in the position I am today if it was not for your fighting for me. I would also like to express my gratitude to my committee members, Cameron R. Hackney and Joseph Eifert for their insight, concern and efforts over the past year. I'm also grateful to the staff for all of their assistance and friendship, especially LuAnn Oliver, Brian Smith and Harriet Williams. A special thanks to Dr. Wang for translating a Chinese journal article for me. I am also thankful for the great hospitality of Susan Duncan and Joseph Marcy for providing an office for me, as well as being available for numerous technical questions that I sought them for.

I also like to thank all of the graduate students for their assistance in my thesis and class work, as well as for their friendship, which has been of great value to me. I am also in debt to the faculty for answering questions, sharing equipment and for making my educational experience full and enriching. I also would like to thank the undergraduate students who have assisted in this research: Shawn Amann, Michele Eckhoff, Carrie Groseclose, Dorothea Kerber, Gabriel Sanglay and Alicia Stevans.

Lastly, I would like to thank my family, roommates (Brian, Greg, and Nate), and friends at Blacksburg Christian Fellowship for the loving support, faithful prayers, assistance and fellowship that I've been blessed with over the past two years. Lastly, I must thank my God, Jesus Christ, for all he is in my life and how he has guided and carried me through these past few years.

TABLE OF CONTENTS

ABSTRACT.....	ii.
ACKNOWLEDGEMENTS	iv.
TABLE OF CONTENTS	v.
LIST OF FIGURES	ix.
LIST OF TABLES	x.
INTRODUCTION.....	1
SECTION I: REVIEW OF LITERATURE	
A. <i>Campylobacter</i> species.....	3
1. Characteristics	3
2. Pathogenesis	3
3. Incidence of <i>Campylobacteriosis</i>	6
4. Sources of <i>Campylobacter</i>	7
B. Lactates.....	8
1. History of use	8
2. Antimicrobial Effects	9
3. Mode of Action	10
C. Lactic Acid.....	11
1. History of use	11
2. Antimicrobial Properties	12
D. REFERENCES	14

SECTION II: THE EFFECTIVENESS OF POTASSIUM LACTATE AND	
LACTIC ACID AGAINST <i>CAMPYLOBACTER</i> SPECIES: A	
CHALLENGE STUDY.....	20
ABSTRACT	20
INTRODUCTION.....	21
MATERIALS AND METHODS	22
Test Organism and Culture Maintenance	22
Determination of Inoculum Level.....	23
Inoculation of Samples	23
Samples Preparation	23
Samples Treatment	25
Storage of Samples	26
Microbial Evaluation of Samples	26
Evaluation of pH.....	27
Experimental Design	28
Statistical Analysis.....	29
RESULTS AND DISCUSSION.....	29
CONCLUSIONS.....	34
ACKNOWLEDGMENTS.....	35
REFERENCES.....	35

SECTION III THE EFFECTIVENESS OF POTASSIUM LACTATE AND	
LACTIC ACID AGAINST <i>CAMPYLOBACTER</i> SPECIES: A SHELF	
LIFE STUDY	46
ABSTRACT	46
INTRODUCTION.....	47
MATERIALS AND METHODS	48
Sample Preparation.....	48
Sample Treatment.....	50
Storage of Samples	50

Microbial Evaluation of Samples	50
Evaluation of pH.....	51
Sensory Evaluation of Potassium Lactate and Lactic Acid	52
Quantitative Color Analysis.....	52
Experimental Design	52
Statistical Analysis.....	53
RESULTS AND DISCUSSION.....	54
CONCLUSIONS.....	60
ACKNOWLEDGMENTS.....	60
REFERENCES.....	61
APPENDICES.....	70
APPENDIX A: Treatment Protocol	71
APPENDIX B: Sensory score sheets and information.....	74
APPENDIX C: Supplemental Figures for Section II	80
Figure 1. Mean pH of rinse of raw boneless, skinless chicken breasts inoculated with <i>Campylobacter</i> sp. over 28 days, treated with different levels of lactic acid (LA) at the 0% potassium lactate (KL) level.....	81
Figure 2. Mean pH of rinse of raw boneless, skinless chicken breasts inoculated with <i>Campylobacter</i> sp. over 28 days, treated with different levels of lactic acid (LA) at the 2% potassium lactate (KL) level.....	82
Figure 3. Mean pH of rinse of raw boneless, skinless chicken breasts over 32 days, treated with different levels of lactic acid (LA) at the 0% and 2% potassium lactate (KL) level.	83
VITAE.....	84

LIST OF FIGURES

Section II:

- Figure 1. Overall summation of the presence of *Campylobacter* sp. on inoculated raw boneless, skinless chicken breasts per level of potassium lactate. Values by different letter are significantly different ($p < 0.05$) (Fishers LSD).....39
- Figure 2. The two percent potassium lactate treatment block expressing the average presence of *Campylobacter* sp. throughout the duration of shelf life. Values by different letter are significantly different ($p < 0.05$) (Fisher LSD).....40
- Figure 3. Linear representation of the growth and/or survival trend of *Campylobacter* sp. to different treatments within the 2% potassium lactate treatment block...S41
- Figure 4. Trend analysis of the 2% potassium lactate-0.1%-surface lactic acid treatment, providing a line equation and R-squared value. The connected-dots shown represent the amount of *Campylobacter* sp. (logs CFU/ml)42
- Figure 5. Two percent potassium lactate treatment block, containing all the lactic acid surface treated samples. Individual points represent the presence of *Campylobacter* sp., exposed to a specific treatment43
- Figure 6. The Log CFU/ml of *Campylobacter* sp. of different treatment combinations of treatments. Values by different letter are significantly different ($p < 0.05$)44

Section III:

- Figure 1. Average presence of *Campylobacter* sp. on raw boneless, skinless chicken breasts for 2% potassium lactate treatments.....67
- Figure 2. Growth and/or recovery of *Campylobacter* sp. over the duration of the shelf life in response to each combination of potassium lactate (KL) and lactic acid (LA).....68
- Figure 3. Growth and/or recovery of psychrotrophic (Ps) and *Campylobacter* sp. (C) in response to the applied treatments during the 4°C shelf life study of raw boneless, skinless chicken breasts.....69

LIST OF TABLES

Section II:

Table 1. Mean <i>Campylobacter</i> sp. count on treated raw boneless, skinless chicken breasts, inoculated with <i>Campylobacter</i> sp.....	36
Table 2. Analysis per treatment combination against <i>Campylobacter</i> sp. on inoculated raw boneless, skinless chicken breasts	37
Table 3. Mean pH of rinse treated raw boneless, skinless chicken breasts inoculated with <i>Campylobacter</i> sp.....	38

Section III:

Table 1. Mean bacterial count of <i>Campylobacter</i> sp. on treated raw boneless, skinless chicken breasts taken on day 16 and 24.....	62
Table 2. Mean psychrotrophic count on treated raw boneless, skinless chicken breasts taken on day 8.	63
Table 3. Mean pH of treated raw boneless, skinless chicken breasts taken on day 16 and 24.	64
Table 4. Mean L,a,b, values of treated raw boneless, skinless chicken breasts over 35 days	65
Table 5. Mean difference in visual and odor observations between treated and control raw boneless, skinless chicken breasts.....	66

INTRODUCTION

The presence of *Campylobacter* sp. on chicken is well established. The processing of boneless, skinless chicken breasts entails greater handling by workers, as it is a further processed food. The potential for contamination with *Campylobacter* sp. is high.

The incidence of isolated cases of *Campylobacter* related illness is 19.7 per 100,000 individuals in the United States of America (USA). Although this figure is lower than the 1997 figure (24.7), campylobacteriosis is by far the leading cause of foodborne illness (CDC, 1999).

Standard thermal processing of raw food products is sufficient to kill *Campylobacter*. Inadequate cooking or cross contamination of foods that will not undergo a terminal heat processing step are at greater risk to cause food-borne illness. Given the prevalence of this pathogen, consumers and restaurant personnel must provide adequate thermal processing and not cross-contaminate foods that will not receive a final thermal process.

Research is needed to discover effective methods for inhibiting the growth of this pathogen on chicken. Temperature abuse of this product is of concern, since the infective dose can be reached on chicken. Bacteriostatic and bacteriocidal treatments are possible solutions if sensory characteristics are not negatively affected. The extension of the shelf life of the chicken would also be beneficial, by inhibiting the growth of psychrotrophic and other spoilage microorganisms.

One possible solution is the use of potassium lactate, which has been shown to enhance flavor and extend shelf life by acting as a bacteriostatic agent. Lactic acid treatments are also currently used in decontamination steps during processing, imparting bacteriocidal activity. The combination of these bacteriostatic and bacteriocidal treatments may prove beneficial to inhibit *Campylobacter* species and extend the shelf life of boneless, skinless chicken breast several days. The objectives of this study were to determine the effect of potassium lactate and lactic acid upon the survival and/or recovery of *Campylobacter* species and psychrotrophic bacteria on boneless, skinless chicken breasts.

SECTION I: REVIEW OF LITERATURE

A. *Campylobacter* species

1. Characteristics

Campylobacter was originally categorized as a *Vibrio* sp., yet due to its inability to ferment sugars and lack of DNA homology, it would not fit in this family, and was classified separately. The motile behavior helped classify the microorganism. This gram-negative, small (as small as 0.2 x 0.5 μm) curved rod exhibits a “cork-screw” like motion when it is motile (Litton, 1996). The name of this organism comes from “campylo” which is Greek for curved (Joklik et al., 1992). Motility is brought about by the presence of either an unipolar or bipolar flagellum (Litton, 1996). The ability to be motile is one of the primary reasons for the pathogenesis of this bacteria.

Despite the early discovery of *Campylobacter* in sheep in 1913, this pathogen has only received serious attention as a human pathogen since the early 1980s. The development of selective media has provided a means for laboratory isolation of this bacteria (Skirrow and Benjamin, 1980). *Campylobacter* also exhibits several other physiological characteristics that make it unique.

The microaerophilic nature is accompanied by being capnophilic, which is a preference of 10% carbon dioxide for growth. *Campylobacter* sp. are non-fermentative and non-oxidative, deriving energy from glycolytic derivatives, such as pyruvic acid. Most recommended microbial media for the recovery of *Campylobacter* sp. include sodium bisulfite, acting as an antioxidant (Doyle and Roman, 1982). *Campylobacter* sp. are mostly thermophilic, preferring 42°C for optimal growth (Park et al., 1983), yet a few strains prefer temperatures between 25 and 37°C, such as *C. fetus*.

Campylobacter jejuni is sensitive to drying, however lower temperatures increase the amount of viable cells present (Doyle and Roman, 1982). Stern et al (1984) report that *C. jejuni* is able to survive freezing, but cells are injured or more difficult to recover. Only one-third of the frozen samples were positive for its presence as compared to fresh, unfrozen samples.

It has long been thought that *Campylobacter* sp. would not grow in environments less than ambient room temperature due to their thermophilic nature (Bryan and Doyle, 1995). Lee et al. (1998) have shown that *C. jejuni* replicates at 4°C, as well as at room temperature. Given this, additional hurdles are needed to inhibit its growth. *Campylobacter fetus* subsp. *jejuni* can survive at 4°C on different parts of a poultry carcasses and microbial media (Grant et al., 1980).

2. Pathogenesis

Current understanding of the pathogenesis of *Campylobacter* centers on its motility. The presence of the flagella is the key to its invasive nature (Grant et al., 1993). Enterotoxin production is evident with different strains of *Campylobacter*, yet no clear correlation has been made concerning the induction of diarrhea in infected subjects (Lindblom, et al, 1989). Cytotoxic hemolysins have been shown to be produced by both *C. jejuni* and *C. coli* during the stationary phase of growth, indicating it is not a primary metabolite (Hossain et al., 1993). It was also found that adult chickens were resistant to these hemolysins, indicating a possible correlation between hemolysins and the pathogenesis of *Campylobacter* sp. in humans.

The invasive behavior and production of toxins by *Campylobacter* sp. provide the needed tools for inducing numerous complications in the infected host. Cases of bacteremias from *Campylobacter* sp. infections are rare. One of the reasons for this occurrence is linked to the observation that complement from human serum was crucial to clinical bacteriocidal activity. This observation suggests that *Campylobacter* sp. may directly activate the production of complement specific to its presence with minimal exposure to the immune system, stimulating production of complement via alternative pathways. The complement-saturated serum helps restrain the initial infection from becoming more systemic or overwhelming (Fernandez et al., 1995).

This immunological response of subjects does not inhibit the symptoms known for *Campylobacter* infection, such as diarrhea, nausea, cramping, and possible fever. Given the infective dose is as low as 500 cells, the probability of experiencing sequelae is quite high with exposure to this pathogen (Linton, 1996 and Park et al., 1983).

Although cases of campylobacteriosis are rarely fatal, infection may result in more serious health side effects, as well as expense to consumers, employers and the food industry (Bryan and Doyle, 1995). Severe complications, such as Guillain-Barre syndrome (GBS) and Reiter syndrome, can occur. GBS exhibits a demyelination resulting in acute neuromuscular paralysis (Altekruse, 1999). GBS is seen as often as 1 in 1,000 cases of campylobacteriosis, with 20% of the victims having residual effects in the future. Reiter syndrome is a reactive arthropathy, a joint disease leading to chronic pain and possible immobility. Both cases are thought to be autoimmune in nature, yet this has not been proven (Altekruse, 1999).

3. Incidence of Campylobacteriosis

The Centers for Disease Control and Prevention (CDC) provides evidence that *Campylobacter* is the clear cause of foodborne illness in the USA. Although the number of cases per 100,000 people decreased from 25.2 (1997) to 21.7 (1998), *Campylobacter* sp. are still the greatest overall cause of food borne illness, with *Salmonella* sp. a distant second (12.4 in 1998) (CDC, 1998). These are estimates and probably underestimate the true number of infections (CDC, 1998).

The CDC also reported for 1998 that the two most susceptible age groups for infection with *Campylobacter* sp. were the 0-10 year old and 20-30 year old age groups. The hospitalization rate for persons infected with *Campylobacter* sp. is 11%, which is one of the lowest reported rates for pathogens, tied with *Shigella* sp.. The mortality rate for *Campylobacter* sp. is also very low (CDC, 1998).

Numerous foods are implicated as sources of *Campylobacter*, including milk, pork, beef, lamb and poultry. Poultry is the primary sources of most recent outbreaks and isolations. *Campylobacter* is present on approximately 90% of all industrially processed chicken carcasses (USDA, 1997).

Campylobacter jejuni subsp. *jejuni* is the most prevalent species found in food sources and is the most common source of *Campylobacter* induced enteritis. Epidemiological studies in England reveal that almost 90% of isolates from subjects infected with a food borne illness, are *C. jejuni* (Litton, 1996). *Campylobacter coli*

makes up 10.3% of all isolates and has also is a causative agent in outbreaks of campylobacteriosis (Litton, 1996). *Campylobacter hyointestinalis* is pathogenic to humans, yet is still rare amongst *Campylobacter* sp. despite increased prevalence (Litton, 1996).

4. Sources of *Campylobacter*

Poultry is the most prevalent source for *Campylobacter*, followed by contaminated milk and water (Litton, 1996). Surveys of poultry indicate that 96% of all serotypes of *Campylobacter* sp. found on poultry are of the same serotypes that infect humans (Munroe et al., 1983). The infective dose has been estimated to be as low as 500 cells (Litton, 1996). Therefore, the probability of campylobacteriosis is high with ingestion of a contaminated food product.

The source of *Campylobacter* sp. is on the farm, where animals are infected and carry this pathogen into processing facilities. The presence of a few infected poultry may contaminate more carcasses in the slaughter process. Epidemiological data provides evidence that farms are not the direct cause of the high frequency of *Campylobacter*-positive poultry carcasses seen in retail markets (Bryan and Doyle, 1995).

The mass production of poultry carcasses increases the probability of contamination of *Campylobacter*, especially during the de-feathering of the carcasses (Bryan and Doyle, 1995). The feather follicles are enlarged during scalding, allowing the pathogens to find crevices for attachment and becoming a shield from other decontamination steps (Bryan and Doyle, 1995). Contamination of carcasses is almost always the intestines, which release fecal matter during defeathering and evisceration (Oosterom et al., 1982).

Contamination is the result of a lack of varied normal flora in poultry raised on large-scale farms. The sterile environments that accompany chicken hatcheries, without exposure to the parental normal flora, the chicks are not exposed to indigenous bacteria (via faeces). Hence, when later exposed in grow up facilities, colonization of *Campylobacter* sp. is rapid and highly probable (Smitherman et al., 1984). Vertical transmission in flocks is a primary mode of contamination, indicating contamination has

its origins from specific hatcheries and contamination stems from that point (Pearson et al., 1996).

B. Lactates

1. History of use

Lactates are the sodium, potassium or calcium salt of lactic acid, usually in the optically active form. Lactates also tend to be of a neutral pH, providing an advantage over acidic additives that could potentially impart negative sensory attributes to a food system. Lactates also enjoy “Generally Recognized As Safe” (GRAS) status in the USA, which provides for unregulated use in food products, until sensory characteristics are negatively influenced (Anonymous, 1987). The intensity of chicken flavor may be maintained using sodium lactate when solutions are kept near neutral. Metallic or sodium off-flavor in chicken treated with 2% sodium lactate is possible (Williams and Phillips, 1998). Cooked chickens treated with 2% sodium lactate increased in the amount of yellow color present when compared to controls. The tenderness of the chicken was not affected by the addition of the sodium lactate (Williams and Phillips, 1998).

The initial use for sodium/potassium lactate was to enhance the flavor by buffering pH changes in meat products. Lactates are commonly used as humectants in confectionery products (i.e., cakes), as well as in meats (Reid, 1969). Lactates are also used as flavor additives for savory meat products (Duxbury, 1990). Turner and Larick (1996) reported that sodium lactate enhanced the fresh roasted/meaty and saltiness sensory scores for sous vide processed chicken breasts.

2. Antimicrobial Effects

Lactates have also been proven to inhibit bacteria in both broth solutions, as well as foods (De Koos, 1993). Potassium lactate was found to be equally effective in inhibiting bacteria as sodium lactate (Shelef, 1994). The synergistic effects of sodium chloride with lactates may be reduced if the concentration of sodium chloride is too high in proportion to the lactate which is present (Shelef, 1994).

Egbert et al. (1992) observed that 2% and 3% potassium lactate in ground beef inhibited both mesophilic aerobes and psychrotrophic bacteria, while having no significant effect upon sensory perception of beef. Shelef et al. (1997) tested the effect of 2% sodium lactate against spoilage microflora on ground beef, finding at least a one log decrease versus the control samples. Sodium lactate (3%) reduced the mesophilic bacteria counts and enhanced the cooked roast beef color for beef rounds (Maca et al., 1997). Brewer et al. (1991) discovered that the addition of 2% or 3% sodium lactate helped to inhibit mesophilic bacterial counts in pork sausage by almost one log cycle, while not affecting the color or pH of the samples.

A study by Rozum and Maurer (1997) indicates that sodium lactate (2%) provides a 1 to 2 log decrease in mesophilic aerobic bacterial counts from control samples for at least three weeks on chicken breast meat, yet losing that effect after 5 weeks (Rozum and Maurer, 1997). Sodium lactate (2%) is effective against psychrotrophic bacteria, providing an average 2 log decrease in counts from control samples of broiler chicken breast meat, with the sodium lactate adjusted to a pH of 7.3 (Williams and Phillips, 1998). Chicken breasts dipped for 15s in a 10% sodium lactate solution resulted in decreased aerobic bacterial counts, especially when samples were stored at higher temperatures (i.e., 7°C) (Chow et al., 1996). Sodium lactate (in the presence of sodium chloride) produced a 1.4 to 2.4 log reduction in the presence of *Escherichia coli* O157:H7, recovered from frozen chicken meat (Conner and Hall, 1993).

The addition of 1% or 2% sodium chloride to turkey treated with 2% or 3% sodium lactate resulted in an increase in the delay of *Clostridium botulinum* toxigenesis (Meng and Genigeorgis, 1993). Delayed toxigenesis was also seen for *C. botulinum* in sous-vide processed chicken that was treated with at least 1.8% sodium lactate (Meng and Genigeorgis, 1994), with additional delays seen in fish and turkey.

Sodium lactate exhibits bacteriostatic tendencies against *Listeria monocytogenes* (Shelef, 1991). *Listeria monocytogenes* was inhibited by the presence of at least 2% potassium lactate (Miller and Acuff, 1994). The effectiveness of lactates has not been extensively studied on gram negative pathogens. Conner and Hall found that *E. coli* O157:H7 survival was enhanced on chicken breasts stored at 10°C or in tryptic soy broth

at 4°C by the presence of sodium lactate. This is contradicted by an earlier study where *E. coli* O157:H7 was inhibited one log by the presence of 2% sodium lactate with higher levels of sodium lactate (Miller and Acuff, 1994). *Escherichia coli* O157:H7 and *Salmonella typhimurium* are the only gram negative pathogens that sodium or potassium lactate has been tested against.

3. Mode of action

The two proposed mechanisms for the antibacterial action of lactates are the lowering of the water activity of the food product and the intracellular acidification of the bacteria. The mechanism for inhibition is most likely related to the intracellular acidification of bacterial cells (Shelef, 1994). Although the water activity might be lowered slightly, this was not found to be not the primary mechanism for the reported bacteriostatic effects of lactates (Chirife and Fontan, 1980). This factor also might be more food dependent, as foods with higher water activities were found to be less affected by the addition of sodium lactate than lower water activity foods (Shelef, 1991).

The main bacteriostatic effect lactates have upon bacterial cells is based upon the cell's ability to expend energy in maintaining a constant internal pH, which takes away energy from the replication of the bacterial cells (Shelef, 1994). The presence of lactate anions may contribute to the cellular conversion of pyruvate to lactate, thereby inhibiting aerobic energy metabolism (i.e., kreb cycle), which is the primary source of energy of most organisms (Shelef, 1994). This theory is more probable than the water activity mechanism, given salts do not equal the effectiveness of lactates at inhibiting microbial growth, yet salts lower the water activity more than lactates. There has been no research done concerning the effectiveness of lactates on *Campylobacter* species.

C. Lactic Acids

1. History of Use

Chemical treatments are used in the poultry industry, especially with carcass rinses, as they have been shown to be effective for decontamination (Hwang and Beuchat, 1994). Lactic acid rinses (buffered to pH 3) have been shown to be effective in decreasing psychrotrophic-aerobic plate counts on chicken legs, increasing their shelf life (Zeitoun and Debevere, 1990). The use of lactic acid along with a lactate salt provides a synergistic effect. Also, the buffering capacity provides help to counteract the potential negative sensory characteristics, while adding bacteriocidal activity to the bacteriostatic influence the lactate salt already has. Using lactic acid as a surface treatment along with a lactate salt injection could also prove to be beneficial by maintaining the individual main effects, while eliminating any sensory problems (Zeitoun and Debevere, 1990). This was shown by a lack of negative sensory responses with rising levels of a lactic acid (buffered with sodium lactate), even up to 10% (Zeitoun and Debevere, 1992). The use of a (3%) lactic acid solution has resulted in decreased sensory scores on chicken, when not buffered (Kolsarici and Candogan, 1995).

2. Antimicrobial Properties

The decontamination of meats has been accomplished to some extent by using a 2% lactic acid solution, resulting in a 0.5-2.0 log reduction in the presence of various surface contaminants (Van Netten and Huis In't Veld, 1994). Gram-negative bacteria, especially psychrotrophic bacteria, were the most sensitive to the lactic acid spray rinse, mostly due to the decrease in pH and not to changes in the undissociated acid (Gill and Newton, 1982). Those most resistant were yeast, lactobacilli, and other gram-positive (non-pathogenic) bacteria (Van Netten and Huis In't Veld, 1994).

Lactic acid decontamination has also been tested against pathogens. *Campylobacter jejuni* was the most affected by the 2% lactic acid rinse, followed by *S. typhimurium*, and *L. monocytogenes*. *Campylobacter jejuni* is a good candidate for lactic acid decontamination, as well as other lactic acid treatments, given the effectiveness shown in previous research (Van Netten and Huis In't Veld, 1994). Lactic acid sprays need to be

kept under 1.5% solutions to avoid bleaching effects upon the surface pigments of the poultry or other meats (Ellerbroek et al., 1998). This study also indicated that this type of application would be effective (around 1% level of lactic acid solution) against lactobacilli, when compared to trisodium phosphate.

A 0.5% lactic acid/0.5% benzoic acid mixture also was effective (at least one log reduction) against *C. jejuni* and psychrotrophic bacteria, having more of an effect on these bacteria than other pathogens that were tested (i.e., *Listeria monocytogenes* and *E. coli* O157:H7) (Hwang and Beuchat, 1994). A buffered lactic acid spray was effective against *L. monocytogenes*. Successively increased concentrations of lactic acid (0 to 10%) showed a greater bacterial reduction, thereby increasing the time at which 7 log CFU/ml was reached. This was shown by increased days of testing (which halted when readings reached over 7 log CFU/cm² of growth (Zeitoun and Debevere, 1991). This relationship has been seen elsewhere as well, expressed specifically with gram-positive microflora in ground beef (Nassos et al., 1985). Lactic acid decontamination was also effective against *S. typhimurium*, however skin decolorization occurred. The lactic acid treatment was more effective when the solution temperature was 37°C versus 4.4°C during application (Izat et al., 1990).

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**Section II: The Effectiveness of Potassium Lactate and Lactic Acid
Against *Campylobacter* Species: A Challenge Study**

by

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Keywords: Lactate, Lactic Acid, Chicken, *Campylobacter* sp.

**SECTION II: The Effectiveness of Potassium Lactate and Lactic Acid Against
Campylobacter species: a Challenge Study**

ABSTRACT

Campylobacter is the most frequently associated foodborne pathogen on poultry. This study examined the efficacy of potassium lactate and lactic acid to control *Campylobacter*. Boneless, skinless chicken breasts were injected with three levels of potassium lactate (0,1.5,2%), in conjunction with four levels of lactic acid injected (0, 0.1, 0.2, 0.3%) as well as applied directly to the surface (0.1%). The chicken fillets were surface inoculated with a mixture of *Campylobacter* species to obtain 3.0×10^3 CFU / breast. The greatest inhibition was found using 2% potassium lactate in conjunction with either 0.3% lactic acid (injected) or 0.1% lactic acid (surface application), exhibiting greater than 1.0 log difference from the positive controls which received no treatment. The 2% potassium lactate and 0.1% lactic acid-surface treatment was significantly different from all treatments containing 0% and 1.5% potassium lactate with less than 0.3% lactic acid ($P < 0.05$). The 2% potassium lactate and 0.3% lactic acid treatment was significantly different than all treatments containing 1.5% potassium lactate with less than 0.2% lactic acid, except for the 0.1% surface application of lactic acid ($P < 0.05$). Results of this study indicate that potassium lactate and lactic acid can be used to inhibit the growth of *Campylobacter* on boneless chicken breasts.

INTRODUCTION

The Centers for Disease Control and prevention (CDC) has reported in their 1998 survey using FoodNet, that *Campylobacter* sp. are by far the leading cause of foodborne illness in the United States of America (USA) (CDC, 1998). Although mortality rates are very low, a small percentage of the population may experience severe reactions, such as Guillain-Barre Syndrome (Altekruse, 1999).

Standard thermal processing of raw food products decreases the population of *Campylobacter* sp. to non detectable levels. Inadequate cooking or cross contamination of foods that will not undergo a terminal processing step (i.e., ready to eat) are at risk for foodborne illness. The probability that boneless, skinless chicken breast would be contaminated with *Campylobacter* sp. is high, given that the production of boneless, skinless chicken breasts entails greater handling by workers or machinery.

Bacteriostatic (potassium lactate) and bacteriocidal (lactic acid) treatments are possible solutions to the presence of *Campylobacter*, if sensory characteristics are not negatively affected. Treatments may extend the shelf life of chicken, by inhibiting the growth of psychrotrophic bacteria and other spoilage microorganisms, thus allowing the chicken maintain acceptable quality longer (Shelef, 1994).

The use of potassium lactate in rinses and injected, has been shown to enhance flavor and extend shelf life (Shelef, 1994). Lactic acid treatments are currently used in decontamination steps during processing, usually of whole bird carcasses (Hwang and Beuchat, 1994). The combination of lactates (injected) and lactic acid (injected or surface applied) may prove effective to inhibit *Campylobacter* sp., thereby reducing the risk of Campylobacteriosis from boneless, skinless chicken breast. The first research objective of this study was to determine the effectiveness of injected potassium lactate and injected (and surface treated) lactic acid for inhibiting the growth and/or survival of *Campylobacter* species. The next objective was to determine the optimal combination of

potassium lactate and lactic acid to inhibit growth and/or survival of *Campylobacter* species.

MATERIALS AND METHODS

Test organism and culture maintenance

Three species of *Campylobacter*, *C. jejuni* subspecies *jejuni* (ATCC 33560), *C. coli* (ATCC 33559) and *C. hyointestinalis* (ATCC 35217), were received from Noel Krieg, Department of Biology at Virginia Tech. The cultures were stored in Brucella semi-solid agar tubes at 18°C for short-term use and at 4°C for longer storage. Given the thermophilic nature of *C. jejuni* and *C. coli* any temperature below 30°C will retard growth. To ensure the viability of each microorganism, samples were observed under phase contrast microscopy to detect motility and the absence of coccoidal forms, which indicates viable, non-culturable cells. The cultures were also plated and tested for, nalidixic acid sensitivity, hippurate hydrolysis and catalase production.

Determination of Inoculum Level

Two 10 µL loops of each culture were transferred from the storage tubes containing 10 ml of FBP (ferrous sulfate, sodium bisulfite, and sodium pyruvate, in Brucella broth) broth (USDA, 1998). Tubes were incubated at 42°C in microaerophilic conditions, 5% oxygen/10% carbon dioxide/85% nitrogen. One ml from each culture was transferred to 297 ml of FBP broth and incubated in a 42°C shaker incubator (55 rpm), under microaerophilic conditions, until the concentration level reached approximately 1×10^5 CFU/ml of broth.

Inoculation of Samples

Loosely bagged boneless, skinless chicken breasts, which had been held at 4°C for approximately 2 hr, were each inoculated with 1 ml of the inoculum on the surface of the chicken breast. Attachment was allowed for 4 min, then the bags were sealed using a Koch Ultravac vacuum packer (Kansas City, Mo.).

Sample Preparation

Potassium lactate, PURASAL[®] P HiPure, and lactic acid samples were donated by Purac America, Inc. (Lincolnshire, IL). The potassium lactate was provided in a 60% solution and was stored at 4°C, under dark conditions. The lactic acid was provided as an 88% food-grade solution and stored at 18°C, under dark conditions. Top flow[®] salt was provided as a gift from Cargill, Inc. (Minneapolis, MN.). Sodium phosphate (Albriphos 602[®]) was donated by Albright and Wilson Americas (Richmond, VA.)

Treatments were prepared in glass beakers using (non-sterile) distilled water as the carrying agent. Three levels of potassium lactate solutions were evaluated (injected), 0%, 1.5% and 2.0%, each level combined with four (injected) levels of lactic acid solutions, 0%, 0.1%, 0.2%, 0.3%. Added to the lactic acid treatments was a surface treatment of 0.1% lactic acid, based on the average weight of the chicken breasts. This lactic acid treatment was applied with a 30% starch solution (N-Tack[®], donated by National Starch and Chemical Co., Bridgewater, NJ.). The potassium lactate was used at a 3.3% level for the desired 2% level and 2.5% for the desired 1.5% level, given the supplied source solution of 60% (wt/vol). The lactic acid was used at a 0.34% level for the desired 0.3% level, given the supplied source solution was 88% (wt/vol). The other lactic acid levels were also similarly adjusted.

The salt added was at a 1% (wt/vol) level and the sodium phosphate at a 0.45% (wt/vol) level. These levels were established by a survey of the levels used by different poultry processors. The components were dissolved into water, gaining full solubility for all components. All treatment solutions were chilled overnight to 4°C and held at 4°C until application. See Appendix A for a complete description of treatment composition.

Eight-ounce butterfly boneless skinless chicken breasts were donated by a commercial poultry processor. The samples were processed on the day of delivery and were held at 4°C until the next day. The samples were processed in the muscle foods processing facility (10°C) at the Department of Food Science and Technology at Virginia Tech. Each sample consisted of a 4-ounce chicken breast, which was made by splitting a whole chicken breast.

Sample Treatment

The chicken samples were pumped at a 17% pump weight to gain a 12% pump yield (based upon the weight of the chicken) (Rozum and Maurer, 1997). The samples were injected into three places on the chicken breast. The yield was determined by overall weight as measured by an electronic balance. The samples were randomly chosen for each treatment. The order of injection was randomized to control for natural contamination of *Campylobacter* species on the chicken breasts. Samples were separately bagged and stored at 4°C while the other samples were being treated.

Samples were placed in 2P trays (with soaker pads) and packaged in SES320 wrapping material. These materials were donated by Cryovac North America, Inc, a division the Sealed Air Corporation (Duncan, SC.). Before the samples were received the SES320 material was sliced into sections and sealed on two sides. Chicken breasts were injected while on the trays. Following injection the trays were placed inside the SES320 bags, inoculated, and sealed.

Storage of Samples

Samples were stored at 11°C to test the effectiveness of treatments with reduced temperature influence, thereby bypassing a low temperature hurdle for *Campylobacter*, while avoiding temperatures that would lead to even more growth of spoilage microorganisms (Miller and Acuff, 1994). A pathogen-modeling program was used to select 11°C and not 10°C, as the modeling indicated a significantly lower temperature effect with the higher temperature while maintaining the previously stated principles (USDA, 1998). Thirty-six samples were placed per shelf in a low temperature incubator. All of the 256 samples were assigned to a random location in the incubator. The trays were spaced within the incubator using ¾” cardboard strips to aid in air circulation within the incubator.

Microbial Evaluation of Samples

The chicken breasts were removed from the incubator, opened and placed in a filter stomacher bag. The samples were manually rinsed with an equivalent weight of a 2% buffered peptone solution, at a pH of 6.9 (Difco, Detroit, Mi.). The breasts were rubbed for approximately 2 min and then shaken for 30 additional seconds. The rinse solution was plated with a modified *Campylobacter*-CEFEX agar. Serial dilutions were distributed in duplicate pour plates. The plates were then placed in one gallon food storage bags, which were then filled with a microaerophilic gas (5% O₂, 10% CO₂, & 85% N₂). The inverted plates were incubated at 42°C for 48 hours (USDA, 1998). After incubation, plates were evaluated by finding a countable range of colony forming units (CFU) for each set of plates. The CFU/ml were then determined from those plates within a countable range. The day 0 samples were evaluated approximately 5 minutes after inoculation with *Campylobacter* sp.

Representative colonies were observed to confirm if samples were *Campylobacter* sp.. Colonies were tested for susceptibility for naldixic acid, catalase and hippurate hydrolysis. Some data points needed to be estimated, given a high amount of colonies, too great to count in entirety. To count these plates, representative squares (Quebec Counter) were used to estimate the growth for the entire plate. The reason for this clumping of cells that don't appear in higher dilutions is probably due to cell density-dependent gene expression, otherwise known as Quorum Sensing (Fuqua, 1994). Bacteria produce chemical signals that trigger genetic promoters, assisting the growth of colonies from nearby bacterial cells. No known research has been published concerning *Campylobacter* species, in how it exhibits this behavior.

Evaluation of pH

Once samples had been removed from the rinse solution for microbial evaluation, the pH of this solution was determined. A pH probe was placed into the stomacher bags to measure the pH. The pH probe was standardized at a pH of 7.0, given the pH of the chicken was close to pH of 7.0. This standardization was performed several times per sampling event.

Experimental Design

The effectiveness of potassium lactate and lactic acid to inhibit the growth and/or survival of *Campylobacter* species on boneless, skinless chicken breasts was tested over a period of 28 days. This challenge study was replicated three separate times, using separate incubators for the process. The incubators represent a statistical block, in which randomization occurs to control variation of temperature during the shelf life test. The treatments were 0%, 1.5% and 2.0% potassium lactate (injected), each combined with 4 levels of lactic acid, 0%, 0.1%, 0.2%, and 0.3% (injected). An additional application of lactic acid was also used, 0.1% (percent weight of chicken breast) on the surface delivered with a starch (N-Tack[®], from National Starch, Inc., Bridgewater, NJ.). The addition of a negative control, without potassium lactate or lactic acid and without inoculation of the pathogen, resulted in 16 total treatments tested. Two chicken breasts were assigned to each treatment per day.

Statistical Analysis

The microbial and pH data was analyzed using SAS (Statistical Analysis Software) (SAS Institute, 1990). Means were compared to test for any significant differences using Analysis of Variance (ANOVA) and Fisher's-LSD (least significant difference), for multiple comparisons, with a significance value of 0.05. A repeated measures analysis was utilized to analyze each day separately, which also included multiple comparisons of the means (Fishers-LSD) with a significance value of 0.05. The repeated-measures design ensures the appropriate error terms are assigned to each comparison, yet allowing for the analysis of relationship between treatments that may vary over time.

RESULTS AND DISCUSSION

Microbial Analysis

Each potassium lactate treatment block was compared, including all levels of lactic acid. The 2% potassium lactate level was found to be significantly different from the 1.5% and 0% potassium lactate levels ($p < 0.05$). The 1.5% potassium lactate level was

not significantly different than the 0% level ($p>0.05$). The analysis of the microbiological data indicates that the 2% potassium lactate treatment block exhibited the greatest inhibition across the entire shelf life, followed by the 1.5% and 0% potassium lactate treatment blocks, respectively (Figure 1). Within the 2% potassium lactate treatment block, the addition of lactic acid was significant ($p<0.05$), yet additional amounts of lactic acid were not significant ($p>0.05$) (Figure 2).

The lowest overall mean presence of *Campylobacter* species occurred using the combination of 2% potassium lactate and 0.1% surface-lactic acid treatment. The next lowest mean observed was the combination of 2% potassium lactate and 0.3% lactic acid. Treatment means are displayed in Table 1, including multiple comparisons of means. The 2% potassium lactate, 0.1% surface-lactic acid treatment was significantly different from all treatments, except those at the 2% potassium lactate level with any lactic acid combination and the other lactic acid-surface treatments. The 2% potassium lactate, 0.3% lactic acid treatment combination was only significantly different than the 1.5% potassium lactate treatments with 0.1% and 0% lactic acid, as well as 0% potassium lactate treatments with 0.3%, 0.2%, and 0% lactic acid.

In this study potassium lactate inhibited the growth and/or survival of *Campylobacter* sp., especially in the presence of lactic acid. Although the inhibition was not as dramatic as previously seen with *Listeria monocytogenes* (Shelef, 1991). Growth and/or survival inhibition is evidenced by the difference seen in the treated samples and the controls.

The 2% potassium lactate-0.1% lactic acid-surface treatment had the greatest effect at the beginning of the shelf-life study (day 0), as it exhibited the lowest initial counts (at least half of a log lower than the other treatments). Potassium lactate treatments did not improve the ability of the *Campylobacter* species to survive, as had been reported with *E. coli* O157:H7 (Conner and Hall, 1996).

The 2% potassium lactate-0.1% lactic acid-surface treatment lost its effect upon *Campylobacter* sp. as the storage time increased (Figure 3). This observation also indicates that this treatment had an immediate effect upon *Campylobacter* sp., which indicates that the surface treatment of lactic acid had the primary effect (Van Netten and

Huis In't Veld, 1994). The only other treatment with a positive slope was the negative control (no treatments and no inoculation). This positive slope indicates growth or increased recovery of *Campylobacter* species at 11°C, which could contradict reported minimal growth temperatures for *Campylobacter* sp. (Bryan, F.L & M.P. Doyle, 1995), yet agrees with more current research that growth may occur as low as 4°C (Lee et al., 1998). Trend analysis is done for each treatment and is found in Appendix C.

The results from day 0 provide evidence that the 2% potassium lactate treatment had the greatest initial inhibitory effect upon *Campylobacter* sp.. The average y-intercept for the 2% potassium lactate block was the lowest (3.12) (Figure 4), as opposed to the 0% and 1.5% treatment blocks (3.41 and 3.47, respectively). Each of the samples that were surface treated with 0.1% lactic acid exhibited slopes which were less negative than the average of its respective treatment block. The effect of the surface treatment with lactic acid upon the growth and survival of *Campylobacter* species was more pronounced during the first 16 days of the shelf life. The presence of higher levels of potassium lactate increased this inhibitory effect by the lactic acid (surface) for *Campylobacter* sp. (Figure 5).

The treatment exhibiting the next greatest amount of inhibition was the 2% potassium lactate & 0.3% lactic acid treatment (Figure 6). However, this treatment was only statistically significantly different than the non (lactic acid) surface treated 0% potassium lactate treated samples, 1.5% potassium lactate treatments with less than 0.2% lactic acid, 2% potassium lactate treatments without injected lactic acid. This treatment was also not significantly different from any of the lactic acid surface treatments. Higher levels of lactic acid for the 2% potassium lactate treatment block are unnecessary, as there was no statistically significant difference between the different levels of injected lactic acid.

The mean presence of *Campylobacter* sp. on the negative control indicates that the chicken received from the poultry plant had a fairly high level of contamination. This higher level affected the study, as the target level of attachment (including pre-existing) of *Campylobacter* sp. was three logs and the uninoculated treatments exhibited a mean presence of *Campylobacter* sp. greater than two logs. The mean attachment of

Campylobacter species was almost 3.3 logs on the inoculated samples, which includes natural contamination.

The only samples significantly different from the positive control (0% potassium lactate and 0% lactic acid, with inoculation) were those with 2% potassium lactate and at least 0.1% lactic acid. Given this observation, any addition of potassium lactate less than 2% and a lack of lactic acid was shown to be ineffective for the purpose of inhibiting *Campylobacter* sp., under the growth conditions provided. The significance of any effect that these treatments upon the growth and/or survival of *Campylobacter* sp. might be seen in shelf life studies where the storage temperature is lower, hence allowing for this hurdle to have a greater and more noticeable effect.

The overall model for this project was found to be highly statistically significant ($p < 0.0001$), with the degrees of freedom (DF) being 514. The model for this project included the following variables: replication, potassium lactate treatment and the lactic acid treatment. This model explained 63.8% of the variation in this project. The potassium lactate treatment effect was found to be significant ($p < 0.001$), as well its effect over time ($p = 0.016$). The lactic acid effect was not found to be significant ($p = 0.1825$), yet its effect over time was significant ($p = 0.036$). The interaction of the main effects was not significant ($p = 0.1825$), indicating a possible lack of synergistic effects.

Analysis of pH

The mean pH of each treatment for the entire shelf life study, including multiple comparisons, is located in Table 3. The pH of the samples ranged from 6.86 to 6.96, with an overall average pH (across all samples) of 6.91. The model for this analysis was the same as for the microbial analysis, including the day, study, potassium lactate and lactic acid as variables. The overall model was shown to be highly significant ($p < 0.0001$). This model accounted for 88.5% of the variation seen in the data.

The 0% and 1.5% potassium lactate treatment blocks were significantly different (6.89 and 6.92, respectively). The 2% potassium lactate block exhibited an average pH of 6.91 and was not statistically different from the other two treatment blocks. Each

treatment effect (potassium lactate & lactic acid) was significant concerning its influence upon the resulting pH ($p=0.001$ and $p<0.0001$, respectively), yet the interaction was not significant ($p=0.22$). The storage time also not significant (both with $p>0.7$).

The average pH of the carcass rinse for each treatment did not correlate with the mean presence of *Campylobacter* sp. for each treatment. Although internal acidification of the bacterial cells is the most likely bacteriostatic mode of action for potassium lactate, the pH of the rinse solution was not clearly correlated with decreases in the presence of the pathogen (Shelef, 1994).

The difference between the largest and smallest overall pH readings is less than 0.2 indicating that the chicken acted as a buffer for the given treatments. Between day 8 and 16, a dramatic rise in the pH occurred. After day 16, the pH stabilized between 7.1 and 7.2, rising at least 0.4 pH units from day 8. This increase in pH helps account for why there wasn't a significant difference with increasing amounts of lactic acid. Given the rise in pH, the lactic acid starts to convert to its salt, which is either potassium or sodium lactate. The average percent of undissociated acid (in the carcass rinse) at day 0 is 0.14%, yet at day 16 this percentage decreases to an average of 0.05%. Given the rise of the pH of overtime, the addition of more than 0.1% lactic acid is not necessary. The differences in pH might be greater than reported given the use of buffered peptone as the rinsing agent.

CONCLUSIONS

Given the rise in pH overtime and the clear evidence that at least 2% potassium lactate is needed, it would be beneficial to use 0.1% lactic acid with the 2% potassium lactate level. The surface treatment of the lactic acid is the best application of this chemical treatment, which reinforces the findings that *C. jejuni* is very susceptible to direct contact with lactic acid. Additional research to find a suitable carrier or methods of application to gain optimal contact of the lactic acid on the surface of the chicken is highly recommended, especially to avoid visual defects.

ACKNOWLEDGMENTS

This research was supported by a grant from Purac America, Inc. (Lincolnshire, Il.). The authors wish to thank Greg Steeno and Bob Noble for assistance with the statistical analysis. The authors wish to also thank Shawn Amann, Carrie Groseclose and Dorothea Kerber for assistance in the evaluation of the samples.

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Table 1. Mean *Campylobacter* sp. count on treated raw boneless, skinless chicken breasts, inoculated with *Campylobacter* sp.

Treatments	Log CFU/ml ^z
1.5% potassium lactate & 0.1% lactic acid	3.34 ^a
0% potassium lactate & 0.3% lactic acid	3.27 ^a
0% potassium lactate & 0% lactic acid	3.19 ^a
0% potassium lactate & 0.2% lactic acid	3.19 ^a
1.5% potassium lactate & 0% lactic acid	3.17 ^a
0% potassium lactate & 0.1% lactic acid	3.11 ^a
1.5% potassium lactate & 0.2% lactic acid	3.08 ^a
2% potassium lactate & 0% lactic acid	3.04 ^{a b}
1.5% potassium lactate & 0.3% lactic acid	3.03 ^{a b}
0% potassium lactate & 0.1% lactic acid-surface	3.02 ^{a b}
1.5% potassium lactate & 0.1% lactic acid-surface	3.00 ^b
2% potassium lactate & 0.1% lactic acid	2.92 ^{b c}
2% potassium lactate & 0.2% lactic acid	2.92 ^{b c}
2% potassium lactate & 0.3% lactic acid	2.83 ^{b c}
0% potassium lactate & 0% lactic acid-no inoculation	2.71 ^c
2% potassium lactate & 0.1% lactic acid-surface	2.65 ^c

^zValues by different letters are significantly different (p<0.05)

Table 2. Trend analysis per treatment combination against *Campylobacter* sp. on inoculated raw boneless, skinless chicken breasts

Treatments	Slopes	R ² Values	Y-intercept
2.0% potassium lactate & 0.1% lactic acid-surface	0.0192	0.2146	2.4919
0% potassium lactate & 0% lactic acid-no inoculation	0.0132	0.1125	2.5401
0% potassium lactate & 0.1% lactic acid-surface	-0.0017	0.0011	3.0498
1.5% potassium lactate & 0.2% lactic acid	-0.0045	0.0074	3.295
2% potassium lactate & 0% lactic acid	-0.0045	0.0101	3.3022
0% potassium lactate & 0.2% lactic acid	-0.0108	0.1163	3.3388
2.0% potassium lactate & 0.3% lactic acid	-0.0132	0.0693	3.0304
1.5% potassium lactate & 0.1% lactic acid-surface	-0.0188	0.1143	3.2133
0% potassium lactate & 0% lactic acid	-0.02	0.2852	3.4673
1.5% potassium lactate & 0% lactic acid	-0.0202	0.3057	3.4698
0% potassium lactate & 0.1% lactic acid	-0.0302	0.3533	3.5114
1.5% potassium lactate & 0.3% lactic acid	-0.0315	0.3674	3.4083
2% potassium lactate & 0.1% lactic acid	-0.0338	0.7841	3.334
0% potassium lactate & 0.3% lactic acid	-0.0355	0.4244	3.6855
2% potassium lactate & 0.2% lactic acid	-0.0424	0.7199	3.4323
1.5% potassium lactate & 0.1% lactic acid	-0.0497	0.6673	3.9885

Table 3. Mean pH of rinse of treated raw boneless, skinless chicken breasts inoculated with *Campylobacter* sp.

Treatments	Values (with mean comparisons) ^z
1.5% potassium lactate & 0% lactic acid	6.962 ^a
0% potassium lactate & 0% lactic acid-no inoculation	6.956 ^a
1.5% potassium lactate & 0.1% lactic acid	6.943 ^a
2% potassium lactate & 0.2% lactic acid	6.940 ^{a b}
0% potassium lactate & 0% lactic acid	6.934 ^b
1.5% potassium lactate & 0.3% lactic acid	6.920 ^b
2% potassium lactate & 0% lactic acid	6.919 ^b
1.5% potassium lactate & 0.2% lactic acid	6.907 ^{b c}
2% potassium lactate & 0.1% lactic acid	6.898 ^{c d}
0% potassium lactate & 0.1% lactic acid	6.897 ^{c d}
0% potassium lactate & 0.3% lactic acid	6.895 ^d
2% potassium lactate & 0.1% lactic acid-surface	6.891 ^d
2% potassium lactate & 0.3% lactic acid	6.891 ^{d e}
0% potassium lactate & 0.2% lactic acid	6.878 ^e
1.5% potassium lactate & 0.1% lactic acid-surface	6.874 ^{e f}
0% potassium lactate & 0.1% lactic acid-surface	6.861 ^f

^zValues with the same letter are significantly different (p<0.05)

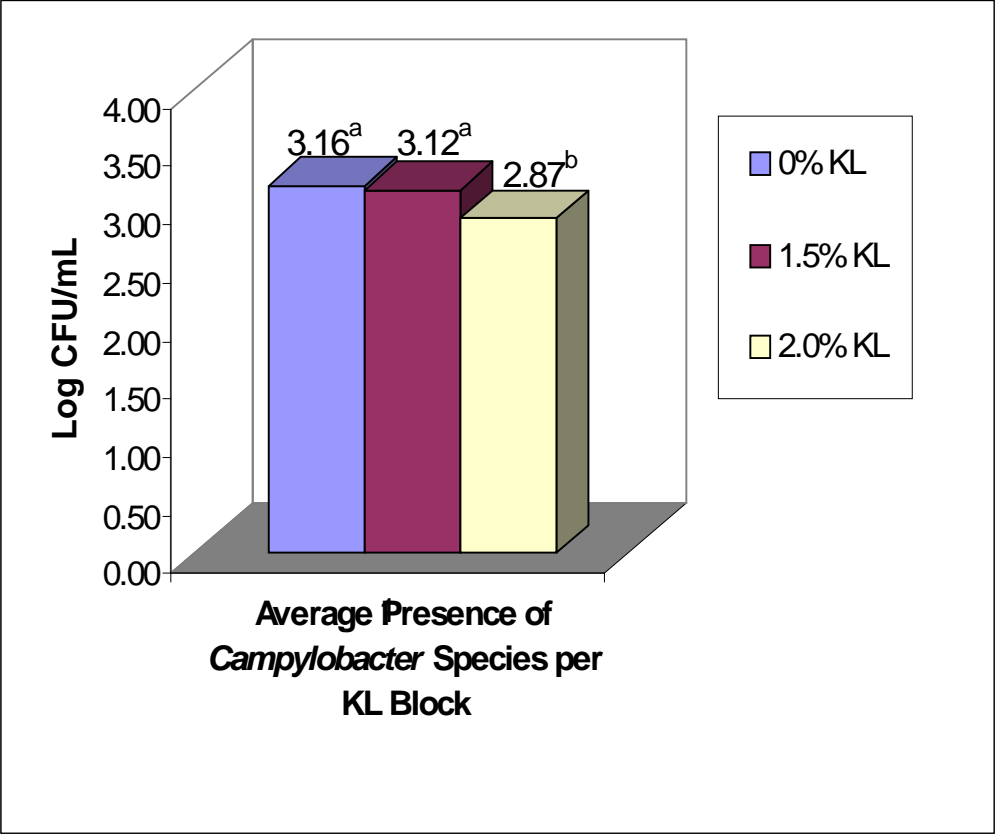


Figure 1. Overall summation of the presence of *Campylobacter* sp. on inoculated raw boneless, skinless chicken breasts per level of potassium lactate. Values by different letter are significantly different ($p < 0.05$) (Fishers LSD)

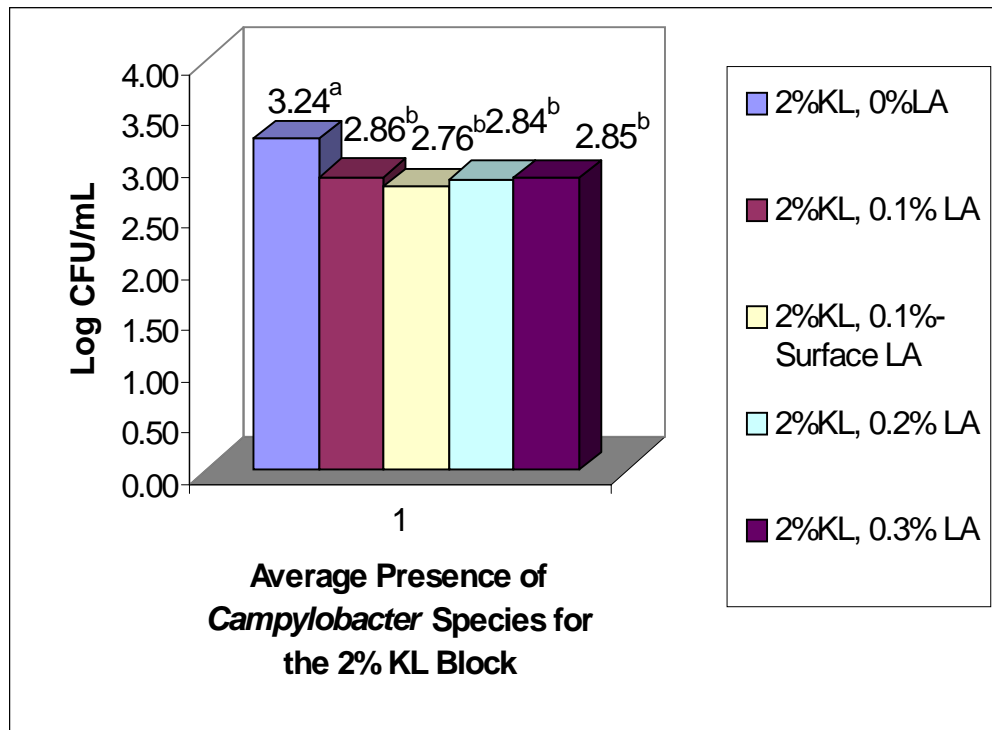


Figure 2. Two percent potassium lactate treatment block expressing the average presence of *Campylobacter* sp. throughout the duration of shelf life. Values by different letter are significantly different ($p < 0.05$) (Fishers LSD).

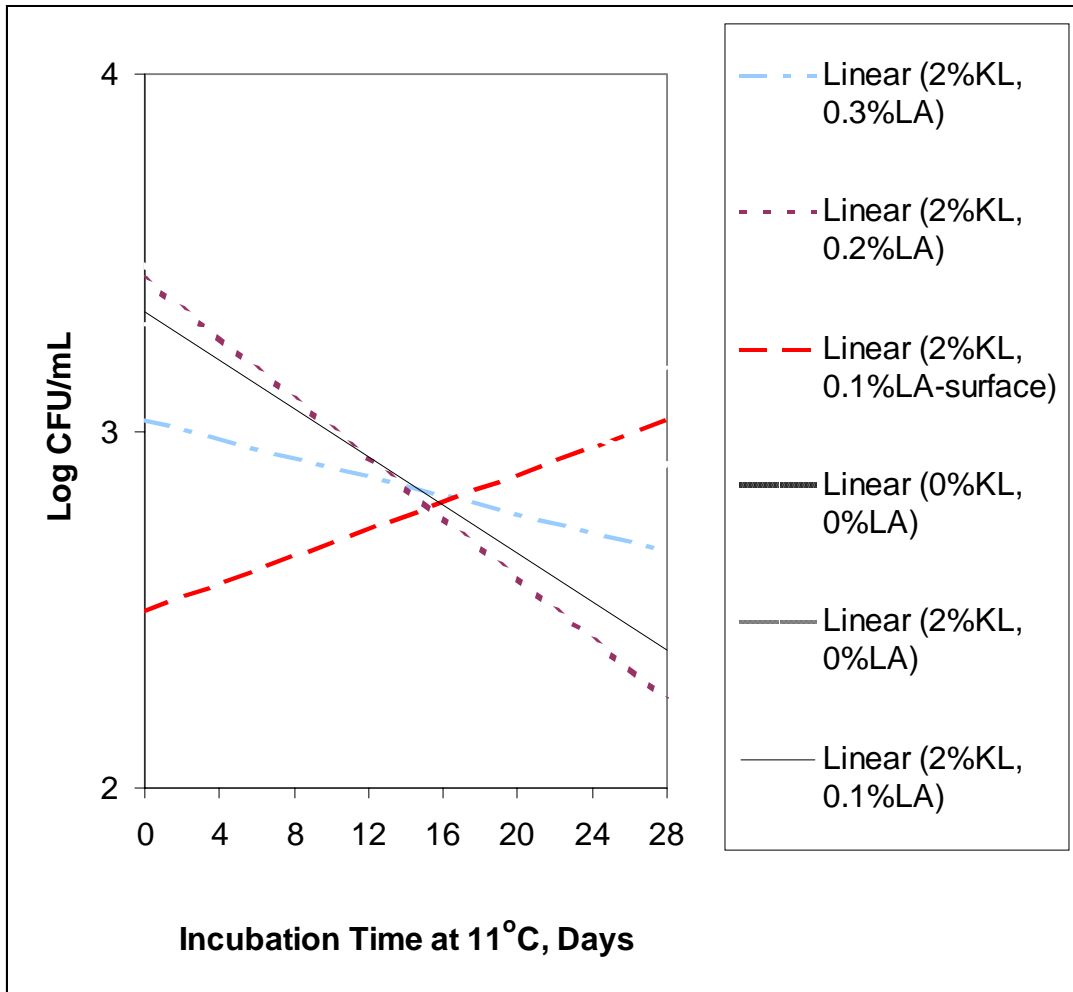


Figure 3. Linear representation of the growth and/or survival trend response of *Campylobacter* sp. to different treatments within the 2% potassium lactate treatment block.

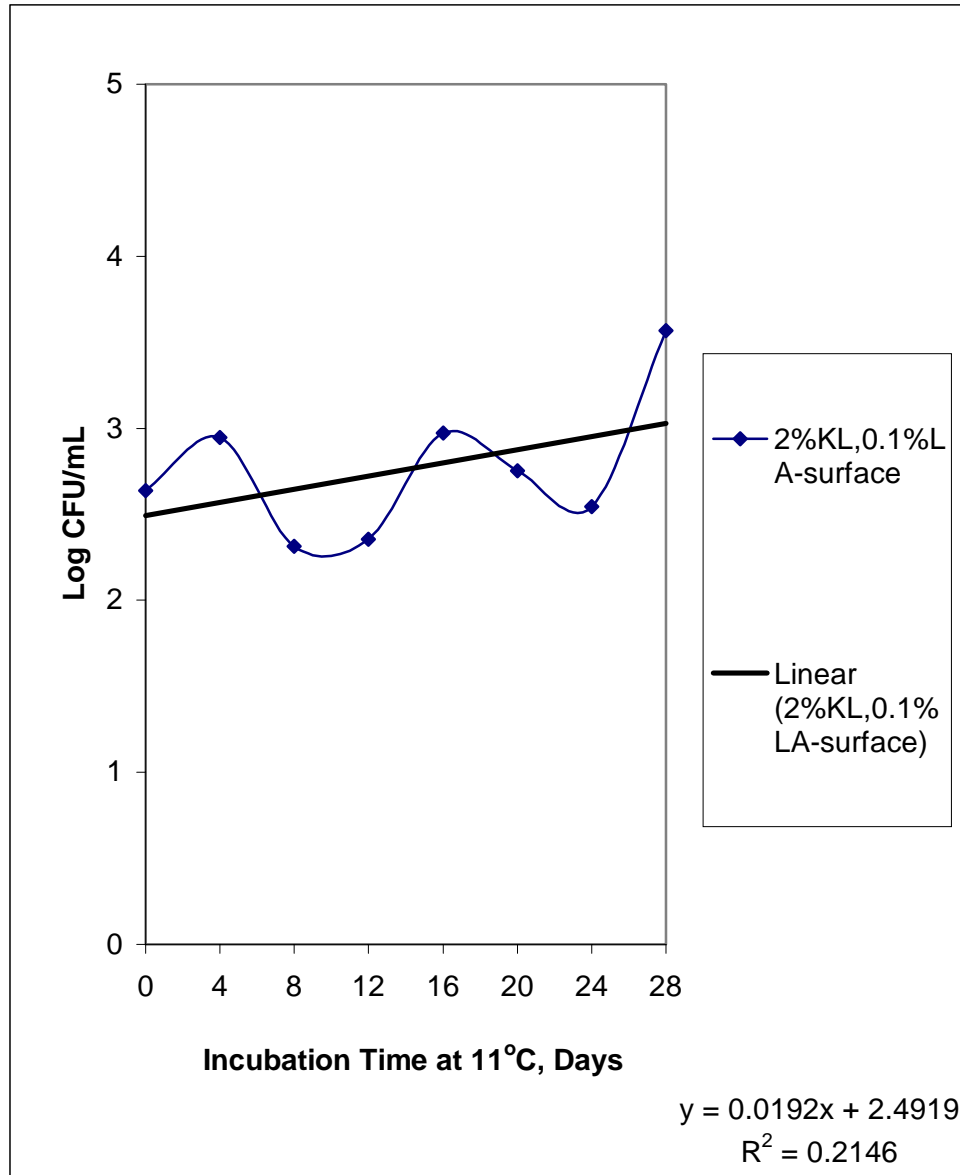


Figure 4. The trend analysis of the 2% potassium lactate-0.1%-surface lactic acid treatment, providing a line equation and R-squared value. The connected-dots shown represent the amount of *Campylobacter* sp. present (log CFU/ml).

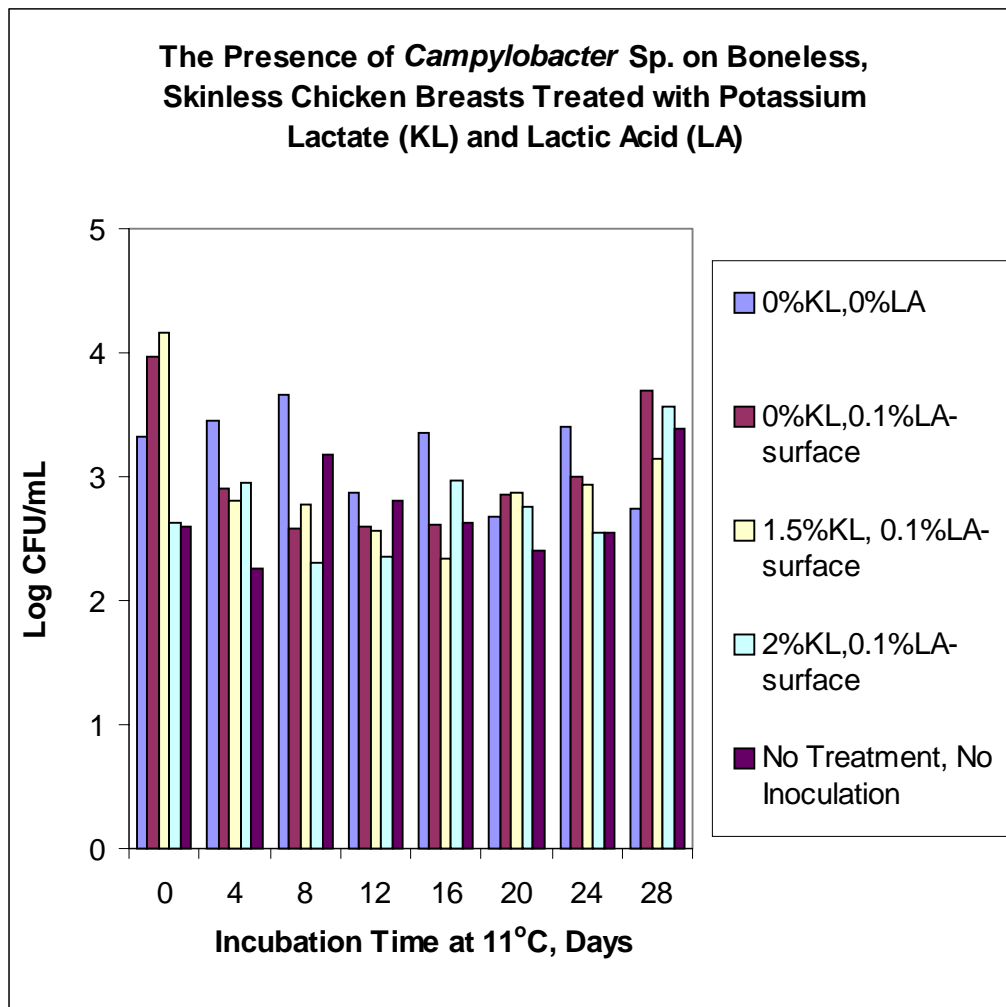


Figure 5. The 2% potassium lactate treatment block, containing the lactic acid surface treated samples. The individual points represent the presence of *Campylobacter* sp. at each day.

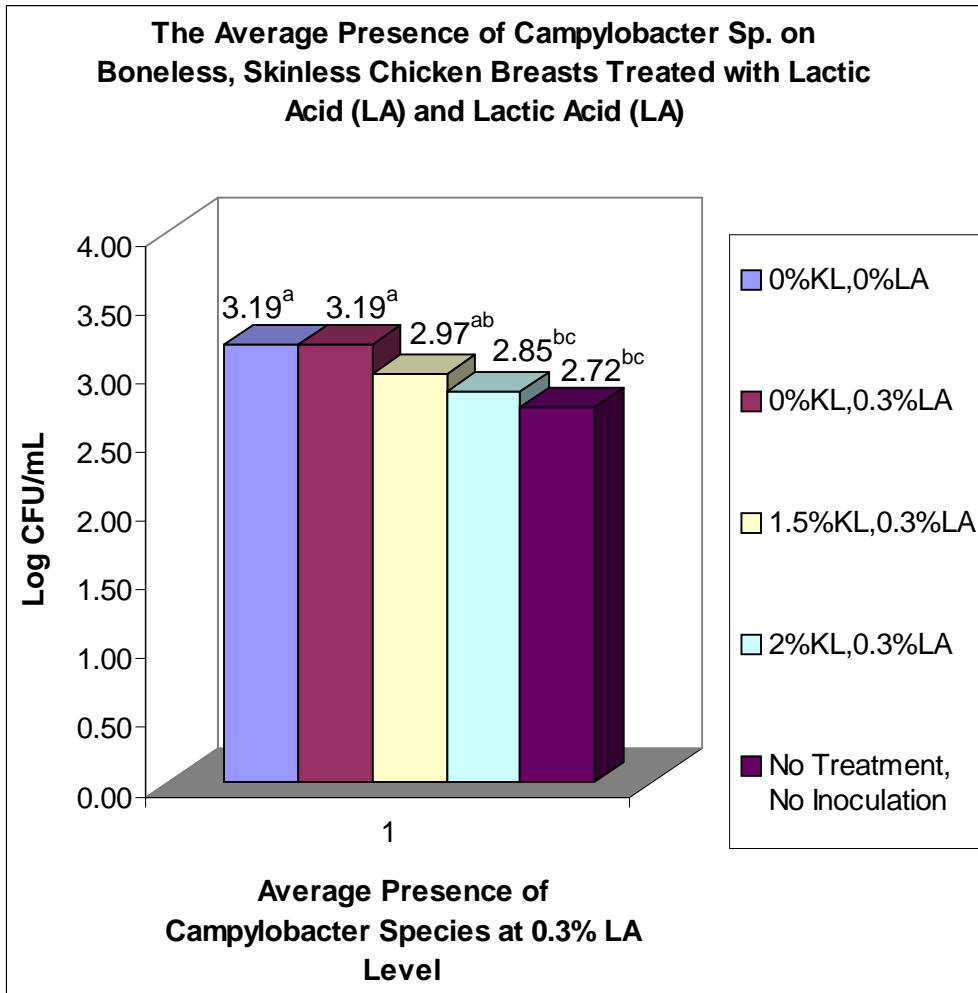


Figure 6. The log CFU/ml of *Campylobacter* sp. of all the different combinations of Treatments. Values by different letter are significantly different ($p < 0.05$) (Fishers LSD).

**Section III: The Effectiveness of Potassium Lactate and Lactic Acid
Against *Campylobacter* Species and Psychrotrophic Bacteria:
A Shelf Life Study**

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**SECTION III: The Effectiveness of Potassium Lactate and Lactic Acid Verses
Campylobacter Species and Psychrotrophic Bacteria:
A Shelf Life Study**

ABSTRACT

Campylobacter is the most frequently associated food-borne pathogen on poultry. This study examined the efficacy of potassium lactate and lactic acid to control naturally occurring *Campylobacter* sp. and psychrotrophic bacteria. Boneless, skinless chicken breasts were injected with two levels of potassium lactate (0%, 2%), in conjunction with two levels of lactic acid. The lactic acid was injected (0%, 0.1%) as well as applied directly to the surface (0.1%). The samples were stored at 4°C for 32 days, with sampling every 8 days.

Two percent potassium lactate in conjunction with the 0.1% lactic acid-surface application demonstrated the greatest inhibition of *Campylobacter* sp. and psychrotrophic bacteria was found using. The 2% potassium lactate and 0.1% lactic acid-surface treatment was significantly different than all treatments containing 0% potassium lactate and any level of injected lactic acid ($P < 0.05$). Results of this study indicate that potassium lactate and surface application of lactic acid can be used to control the growth and/or recovery of *Campylobacter* sp. and psychrotrophic bacteria on boneless chicken breasts.

INTRODUCTION

The Center for Disease Control and prevention (CDC) reported in a 1998 survey using FoodNet that the incidence of isolated cases of *Campylobacter* related illness is 21.7 per 100,000 individuals in the United States of America (USA). Although this figure is lower than the 1997 figure (25.2), campylobacteriosis is by far the leading cause of food-borne illness in the United States of America (USA).

Campylobacter sp. are been reported to be on 27% of pre-processed broiler chickens (Pearson et al, 1996). This same study indicated that transmission rates between flocks was low. Eighty-eight percent of post-processed poultry carcasses contain *Campylobacter* sp. (USDA, 1998). Processing of poultry carcasses, especially defeathering and evisceration, greatly increases fecal contamination on carcasses, thereby increasing the incidence of *Campylobacter* sp. (Oosterom et al., 1983).

Research is therefore necessary to discover effective methods for inhibiting the growth of this naturally pathogen on chicken. Temperature abuse of this product is especially of concern, where the infective dose is reached on the chicken (Bryan and Doyle, 1995). Lactates (bacteriostatic) and lactic acid (bacteriocidal) treatments may effectively inhibit *Campylobacter* sp., if sensory characteristics are not negatively affected.

The extension of the shelf life of the chicken would also prove beneficial, by inhibiting the growth of psychrotrophic bacteria and other spoilage microorganisms, which have been shown to produce strong odors (i.e., *Pseudomonas* sp.) (Barnes and Impey, 1968). Sodium lactate increased the yellow color in sous-vide processed chicken breast meat (Turner and Larick, 1996). Two percent sodium lactate extended the shelf life of cooked chicken breasts by one week when compared to control samples (Rozum and Maurer, 1997). Shelef (1991) reported sodium lactate to effectively inhibit the growth and survival of *Listeria monocytogenes*. Sodium lactate is reported to inhibit *Escherichia coli* O157:H7 on frozen chicken meat (Conner and Hall, 1994).

Lactic Acid decontamination of poultry carcasses has proven effective for extension of the shelf life of poultry (Zeitoun and Debevere, 1990). Lactic acid surface applications exhibited bacteriostatic action against *Campylobacter jejuni* and *Listeria monocytogenes* (Van Netten and Huis In't Veld, 1994). Psychrotrophs were inhibited by lactic acid surface treatments (Gill and Newton, 1982).

This study was designed to investigate the effects of potassium lactate and lactic acid treatments on naturally occurring *Campylobacter* sp. and psychrotrophic bacteria on boneless, skinless chicken breasts. Treatment effects upon *Campylobacter* sp. were tested in respect to the possible inhibitory effect by the presence of psychrotrophic bacteria. Sensory panels were conducted to determine effects on color and odor of uncooked chicken breasts.

MATERIALS AND METHODS

Sample Preparation

Potassium lactate, PURASAL[®] P HiPure, and lactic acid samples were donated from Purac America, Inc. (Lincolnshire, IL). The potassium lactate was provided in a 60% solution and was stored at 4°C, under dark conditions. The lactic acid was provided as an 88% food-grade solution and stored at 18°C, under dark conditions. Top flow[®] salt donated from Cargill, Inc. (Minneapolis, MN.) was used at a 1% level. Sodium phosphate (Albriphos[®] 602) donated from Albright and Wilson Americas, Inc. (Richmond, VA) was used at a 0.45% level.

Solutions were prepared in glass beakers using non-sterile distilled water as the carrying agent. Two levels of potassium lactate were evaluated, 0% and 2.0%, each containing two levels of lactic acid, 0% and 0.1%. The potassium lactate was used at a 3.3% level for the desired 2% level, given the supplied source solution of 60%. The lactic acid was used at a 0.114% level for the desired 0.1% level, given the supplied source solution was 88%. The potassium lactate and lactic acid were in liquid form and to deliver the solutions, equivalent volumes were determined for the required weight of poultry. A surface lactic acid treatment at 0.1% (wt/wt) lactic acid was also conducted. This lactic acid treatment was applied with a 30% starch solution (N-Tack[™], donated by

National Starch and Chemical Co, Bridgewater, NJ.). All solutions were chilled overnight to 4°C until application of the treatment. See Appendix A for a complete description of treatment composition.

A local commercial poultry processor donated eight-ounce butterfly boneless, skinless chicken breasts. The samples were processed on the day of delivery and held at 4°C. The samples were processed in the meats processing facility (10°C) at Cryovac, Inc. (Sealed Air Corporation) in Duncan, S.C. and transported back to Blacksburg, VA (Virginia Tech) in ice-packed insulated coolers for incubation and sampling. Each sample consisted of two 4-ounce pieces, which was made by splitting the 8-ounce chicken breast.

Sample Treatment

The chicken breast samples were injected at a 17% pump weight to yield a 12% pump weight (Rozum and Maurer, 1997). The samples were treated in a random order. After samples were injected, they were packaged using 2P trays and SES340 packaging material. A SES340 film was wrapped around the tray and sealed on the bottom of the tray with an “T” seal, which is typically used by poultry processors.

Storage of Samples

Samples were kept at 4°C for the duration of the shelf life. The samples were randomly assigned a specific location in the incubator to control for any variation of temperature of within the incubator. Samples were spaced on ¾” cardboard strips to ensure equal treatment of the temperature.

Microbial Evaluation of Samples

Samples were manually rinsed in an equivalent amount of 2% buffered peptone solution (Difco, Detroit, Mi.), in filtered stomacher bags. The chicken breasts were rubbed for approximately 2 min, then shaken for 30 additional seconds. The rinse solution was plated on modified Abeyta-Hunt-Bark (AHB) agar (FDA, 1998), supplemented with 5 grams of sodium pyruvate per liter. A modified psychrotrophic

count was done using 3M Aerobic Plate Count Petrifilm, incubated at 21°C for 4 days (Marshall, 1992 and Williams & Phillips, 1998).

Representative colonies were observed to confirm if samples were *Campylobacter* sp.. Colonies were tested for susceptibility for naldixic acid, catalase and hippurate hydrolysis. Some data points may need to be estimated, given a high amount of colonies, too great to count in entirety. To count these plates, representative squares (Quebec Counter) were used to estimate the growth for the entire plate. The reason for this clumping of cells that don't appear in higher dilutions is probably due to cell density-dependent gene expression, otherwise known as Quorum Sensing (Fuqua, 1994). Bacteria produce chemical signals that trigger genetic promoters, assisting the growth of colonies from nearby bacterial cells. No known research has been published concerning *Campylobacter* species, in how it exhibits this behavior.

Evaluation of pH

The pH of the rinse solution was measured by placing the pH probe directly into the rinse solution. The pH probe was calibrated at a pH of 7.0 using a buffer solution of 7.0 given the pH of the chicken was close to the pH of 7 versus pH 4 or 10, providing a more accurate result. Standardization was performed several times per sampling event.

Sensory Evaluation of Potassium Lactate and Lactic Acid

Visual and olfactory (aroma) tests were conducted on separate samples. A Difference-From-Control test ($\alpha=0.05$) was utilized to determine if the treatments had an affect upon the appearance and odor of the chicken breasts. Subjects evaluated a fresh control and an age-parallel control versus the entire 2% potassium lactate block. A total of 14 comparisons were made every 7 days beyond the initial test (done on day 2).

Samples were randomized per station. A 7-point scale was provided for each subject to evaluate each pair of samples. Each station represented a statistical block, resulting in an incomplete randomized block design. See Appendix B for randomization of samples, score sheets and work sheets.

Quantitative Color Analysis

To quantify the visual characteristics of the chicken samples, color analysis was done. Samples used in the sensory testing were analyzed using a hand-held Minolta colorimeter (Minolta Corporation, Ramsey, NJ).

Experimental Design

The effectiveness of potassium lactate and lactic acid to inhibit the growth of naturally present *Campylobacter* and psychrotrophic bacterial species on boneless, skinless chicken breasts was evaluated over 32 days. Samples were measured every eight days. There were two separate samples per treatment. The shelf life test was replicated two separate times during different time periods. The incubator represents a statistical block, in which randomization occurs to control variation of temperature during the shelf life test.

Experimental design changes from the challenge study were the removal of the 1.5% potassium lactate treatment block and the lack of a need for a negative control, as the samples were not inoculated. The increase in the pH of the chicken breasts resulted in the lactic acid being dissociated into a salt of lactic acid. The lack of a significant difference between the different levels of lactic acid coupled with the increasing pH indicated there was no need to test the higher concentrations of lactic acid (0.2% & 0.3%) that were used in the challenge study.

Statistical Analysis

The microbial and pH data was analyzed using SAS (Statistical Analysis Software) (SAS, 1990). Means were compared to test for any significant differences by using a repeated-measures design and Fisher's-LSD (least significant difference), for multiple comparisons, with a significance value of 0.05. The repeated-measures design ensures the appropriate error terms are assigned to each comparison, yet allowing for the analysis of relationship between treatments that may vary over time.

The sensory and colorimeter (lactic acid) data was analyzed using JMP 3.2.1 statistical software. Means were compared to test for any significant difference using Analysis of Variance (ANOVA), with a significance value of 0.05.

RESULTS AND DISCUSSION

Microbial Analysis: Campylobacter

The overall presence of *Campylobacter* species for the 0% potassium lactate level was 1.71 log CFU/ml of rinse, which was slightly higher than the 2% potassium lactate level, which was 1.41 log CFU/ml of rinse. The lowest overall mean (1.03 Log CFU/ml of rinse) was exhibited by the 2% potassium lactate-0.1% lactic acid-surface treatment, where the positive control (0% potassium lactate-0% lactic acid) was 1.75 log CFU/ml of rinse. The next lowest overall mean was exhibited by the 0% potassium lactate-0.1% lactic acid-surface treatment, 1.47 Log CFU/ml.

Treatments with 0.1%-injected-lactic acid, for the 0% and 2% potassium lactate treatment blocks, exhibited higher overall averages than the (positive) control, 1.90 and 1.65 log CFU/ml of rinse, respectively (Figure 1). The 2% potassium lactate-0% lactic acid treatment exhibited lower overall growth and/or recovery than the control, 1.54 and 1.75 log CFU/ml of rinse, respectively. The individual treatments did not show any significant differences until Day 16 (Figure 2). Day 16 is the typical industry end of shelf life for boneless, skinless chicken breasts.

Differences between the means for *Campylobacter* on day 16 are shown in Table 1. The lowest mean for this day was the 2% potassium lactate-0.1% lactic acid-surface treatment, which was significantly different than the 2% potassium lactate-0.1% lactic acid treatment, the 0% potassium lactate-0.1% lactic acid treatment and the control ($p < 0.05$). The effectiveness of direct contact of lactic acid with *Campylobacter* sp. reinforces the finding of Van Netten and Huis In't Veld (1994), surface application of lactic acid is effective to inhibit *Campylobacter jejuni*. The statistical model for this day was shown to account for 87% of the variation. The potassium lactate and lactic acid main effects were also shown to be significant ($p < 0.05$), yet their interaction was not

significant ($p>0.05$). The model (replication, potassium lactate and lactic acid) was significant for this day (16) ($p=0.04$).

For day 24 samples, the 2% potassium lactate-0.1% lactic acid-surface treatment was only significantly different than the positive control ($p<0.05$). The decrease in the range of values for each day is the most probably cause of this decrease, where day 16 exhibited a range of 2.13 log CFU/ml of rinse, day 24 samples exhibited a range of 1.78 Log CFU/ml of rinse (Figure 2). The model for this day accounted for 66.7% of the variance, yet the model was not significant ($p>0.2$). The interactions and main effects were also not significant for day 24 ($p>0.05$).

The increasing bacterial counts of *Campylobacter* sp. at 4°C over time was evident from this data, indicating either growth and/or increased recovery due to other factors (i.e., increased growth of spoilage bacteria assisting growth and/or recovery of *Campylobacter* sp.). This data could contradict previous assertions if growth of *C. jejuni* is occurring (Bryan and Doyle, 1995), and supports more current research (Lee et al., 1998).

Microbial Analysis-Psychrotrophic Bacteria

The analysis of the psychrotroph data provided only one day with significance, day 8. There were no significant differences between the means for day 0 or day 16. The listing of mean values for day 8 are found in Table 1, including multiple comparisons of the means. The 2% potassium lactate-0.1% lactic acid-surface mean exhibited the lowest mean for day 8. This mean was significantly different than the 2% potassium lactate-0% lactic acid treatment, 0% potassium lactate-0.1% lactic acid treatment and the control ($p<0.05$). The next closest mean value was the 0% potassium lactate-0.1% lactic acid-surface treatment, which was approximately 0.6 logs higher than the lowest mean value. Surface treatment with the lactic acid has proven to be highly effective in providing additional inhibition of psychrotrophic bacteria (Gill and Newton, 1982). The surface treated samples clearly exhibited the lowest mean for the duration of the shelf life.

The statistical model for day 8 accounted for 89.1% of the variation in the data. The model consisted of the following variables: replication, potassium lactate, and lactic acid.

The lactic acid main effect was close to being statistically significant ($p=0.06$), yet the potassium lactate main effect and the interaction of potassium lactate and lactic acid were not significant ($p>0.1$).

Throughout the duration of the shelf life the addition of the potassium lactate had an effect, although not statistically significant (Figure 3). The combination of lactic acid with 2% potassium lactate resulted in a lower, yet non-significant difference when compared to the control. The combination of the two treatments provided a synergistic effect, given that in the absence of potassium lactate the lactic acid addition resulted in a higher, yet non-significant difference when compared to the control.

After day 16, the overall mean for all treatments did not change. The only difference observed can be accounted for in normal variation of the growth of the psychrotrophic bacteria. Despite this high, almost saturated level of psychrotrophic bacteria the naturally occurring *Campylobacter* sp. continued to exhibit increased growth and/or recovery. A definite lack of competitive inhibition of the *Campylobacter* sp. was seen in reference to the high presence of the psychrotrophic bacteria. The increased presence of psychrotrophic bacteria may have assisted the rate of recovery of *Campylobacter* sp., yet the treatments minimized this recovery or growth, which may have occurred as well.

Analysis of pH-Modified Carcass Rinse

The mean pH value for each treatment, over the duration of the shelf life, was determined (Figure 5). The values of significance are from day 16 and day 24. The mean pH values for day 16 and 24 are found in Table 3. Two separate trends developed in both day 16 and 24 (Figure 6). The pH of the lactic acid-surface treated samples did not increase over time until after day 24. There was no significant difference between the two different surface treatments, having either 0% or 2% potassium lactate ($p>0.05$). The other treatments were also not significantly different, yet consistently increased after day 8 ($p>0.05$).

The model for both day 16 and 24 exhibited almost identical patterns, by accounting for 82% of the variation and being nearly statistically significantly different ($p=0.05$). For day 16 data, there was a moderate correlation between the increase in pH values and the

increase in presence of *Campylobacter* sp.. This was not the case for day 24 data, where the pH values continued to increase, yet the level of *Campylobacter* sp. was maintained (Figure 2.). There was no apparent overall relationship between the growth of psychrotrophic bacteria and their respective pH values.

Colorimetric Analysis of Chicken

The L-values measure were for a fresh control (FC), an aged control (AC), 2% potassium lactate-0% lactic acid treatment and a 2% potassium lactate-0.1% lactic acid treatment for each day, including multiple samples of each type per day. The degrees of freedom (DF) for the shelf life were 90 and the test was shown to be statistically significant ($p < 0.0001$). The overall means (including multiple comparisons) for the L-values are found in Table 4.

The L-values generated indicate that both treatments helped darken the chicken, slightly beyond what the fresh control was and were shown to be statistically significantly different than both the AC and FC ($p < 0.05$). The L-value range is from 1 to 100, with the lower numbers indicating darkness. The average of the L-values in this study were near 50, which indicates an appearance that is neither dark nor bright. The addition of the treatments have at least some effect upon the darkness of the chicken breasts.

The A-values produced values were close to zero (from a possible range of -60 (green) to +60 (red)). The A-values, including multiple comparisons are found in Table 4. The treated samples tended to be slightly more red, yet really a mixture of the two colors. Only the 2% potassium lactate-0.1% lactic acid treated samples and the FC were significantly different. The DF of this test was 90 and the test was not statistically significant ($p > 0.19$). The treatments and the age seemed to have little (if any) effect upon the redness of the chicken breasts.

The B-values produced (from a possible range of -60 (blue) to +60 (yellow)) values that were overall slightly more yellow, with an overall average being approximately 4.64 (Table 4). None of the values were significantly different from each other.

Sensory Testing of Chicken Over-Time

The mean difference-from-control values for visual and olfactory evaluation are found in Table 5. The differences in appearance were found more in the 2% potassium lactate-0.1% lactic acid combination, as compared to the 2% potassium lactate treatment without lactic acid. The lactic acid surface treated samples were the only samples to have a greater difference than the comparisons of like controls (i.e., AC vs. AC), indicating only that this treatment made it past the baseline measurement. The test was shown to be statistically significant ($p < 0.001$), with degrees of freedom of 437. With no hedonic feedback, conclusions were not made about the direction of the difference. The lack of treatment in the AC provided the largest difference from the FC.

The olfactory evaluation of the sensory samples also produced differences similar to the visual based tests. The surface treated samples exhibited the largest difference from control, when compared to the 2% potassium lactate, 0% lactic acid treatment. The surface treated samples were also the only treatment to exhibit a greater difference than both like controls (i.e., AC vs. AC and FC vs. FC). This test was shown to be statistically significant ($p = 0.0025$), with degrees of freedom of 436. No hedonic measures were taken as the samples were uncooked and would never score well given the lack of appeal that would exist for raw chicken. The differences found may be related to inhibition of psychrotrophic bacteria, such as (non-pigments) *Pseudomonas* sp. which may produce strong odors (Barnes and Impey, 1968). The differences seen in the different comparisons definitely lead us to conclude that both potassium lactate and lactic acid have an affect upon the color of chicken breasts, which contradicts previous ideas about lactates (Duxbury, 1988).

CONCLUSIONS

Overall, the most effective treatment in producing a higher quality chicken breast that exhibits a lesser probability of inducing campylobacteriosis is the 2% potassium lactate-0.1% lactic acid-surface treatment. If surface treatment is not utilized, then the 2% potassium lactate-0.1% lactic acid treatment is recommended, especially to lengthen

the shelf life. Although the pH of the chicken has some significance, it doesn't directly correlate with the inhibition of either *Campylobacter* sp. or psychrotrophic bacteria.

ACKNOWLEDGMENTS

This research was supported by a grant from Purac America, Inc. (Lincolnshire, Il.). The authors wish to thank Greg Steeno and Bob Noble for assistance with statistical analysis. The authors also wish to thank Dorothea Kerber, Gabriel Sanglay, and Alicia Stevans for their assistance in the evaluation of the samples.

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Table 1. Mean bacterial count of *Campylobacter* sp. on raw boneless, skinless chicken breasts taken on day 16 and 24^z

Treatments	Day 16 - Log CFU/ml	Day 24 - Log CFU/ml
0% potassium lactate, 0.1% lactic acid	2.97 _e ^a	2.49 _e ^{a b}
0% potassium lactate, 0% lactic acid	2.57 _e ^{a b}	2.86 _e ^a
2% potassium lactate, 0.1% lactic acid	2.07 ^{a b}	2.58 _e ^{a b}
2% potassium lactate, 0% lactic acid	1.70 ^{b c}	1.70 ^{a b}
0% potassium lactate, 0.1% lactic acid-surface	1.45 ^{b c}	2.29 ^{a b}
2% potassium lactate, 0.1% lactic acid-surface	0.84 ^c	1.08 ^b

^zValues by different letter are significantly different (p<0.05); n=24

^eEstimated values (counting representative square)

Table 2. Mean psychrotrophic count on treated raw boneless, skinless chicken breasts taken on day 8^z

Treatments	Log CFU/ml
0% potassium lactate, 0.1% lactic acid	8.49 ^a
0% potassium lactate, 0% lactic acid	8.27 ^a
2% potassium lactate, 0% lactic acid	8.26 ^a
2% potassium lactate, 0.1% lactic acid	7.77 ^{a b}
0% potassium lactate, 0.1% lactic acid-surface	7.56 ^{a b}
2% potassium lactate, 0.1% lactic acid-surface	6.99 ^b

^zValues by different letter are significantly different ($p < 0.05$); n=24

Table 3. Mean pH of treated raw boneless, skinless chicken breasts taken on day 16 and 24^z

Treatments	Day 16-Mean pH Values	Day 24-Mean pH Values
0% potassium lactate, 0% lactic acid	6.98 ^a	7.08 ^a
2% potassium lactate, 0% lactic acid	6.97 ^a	7.03 ^a
0% potassium lactate, 0.1% lactic acid	6.93 ^{a b}	7.02 ^{a b}
2% potassium lactate, 0.1% lactic acid	6.86 ^{a b c}	7.05 ^a
0% potassium lactate, 0.1% lactic acid-surface	6.73 ^{b c}	6.63 ^c
2% potassium lactate, 0.1% lactic acid-surface	6.71 ^c	6.69 ^{b c}

^zValues by different letter are significantly different ($p < 0.05$); n=24

Table 4. Mean L,a,b, values of raw boneless, skinless chicken breasts over 35 days^{yz}.

Treatments	L-Values	a-Values	b-Values
0% potassium lactate, 0% lactic acid-Aged Control	52.35 ^a	0.708 ^b	4.73 ^a
0% potassium lactate, 0% lactic acid-Fresh Control	51.92 ^a	0.306 ^c	4.65 ^a
2% potassium lactate, 0% lactic acid	49.54 ^b	0.892 ^a	4.64 ^a
2% potassium lactate, 0.1% lactic acid	47.77 ^c	0.984 ^a	4.55 ^a

^zValues by different letter are significantly different (p<0.05); n=91

^yObservations made every 7 days for 35 days and averaged.

Table 5. Mean difference in visual and odor observations between treated and control raw boneless, skinless chicken breasts^{yz}

Treatments	Mean Difference in Appearance [*]	Mean Difference in Odor [*]
Fresh vs. aged controls	3.93 ^a	3.13 ^{a b}
Aged control vs. 2% potassium lactate-0.1% lactic acid	3.84 ^{a b}	3.42 ^a
Fresh control vs. 2% potassium lactate-0.1% lactic acid	3.74 ^{a b}	2.93 ^{a b}
Aged control vs. aged control	3.27 ^{b c}	2.35 ^{c d}
Fresh control vs. 2% potassium lactate-0% lactic acid	2.93 ^c	2.64 ^{b c d}
Fresh control vs. fresh Control	2.87 ^c	2.73 ^{b c}
2% potassium lactate-0%lactic acid vs. 2% potassium lactate-0.1% lactic acid	2.84 ^c	2.64 ^{b c d}
Aged control vs. 2% potassium lactate-0% lactic acid	2.76 ^c	2.29 ^d

^zValues by different letter are significantly different (p<0.05); n=437

^yValues are listed on a scale from 1-7, with 0=no difference and 7=very large difference, using a difference-from-control method.

^{*} The mean standard error for each test was 0.21

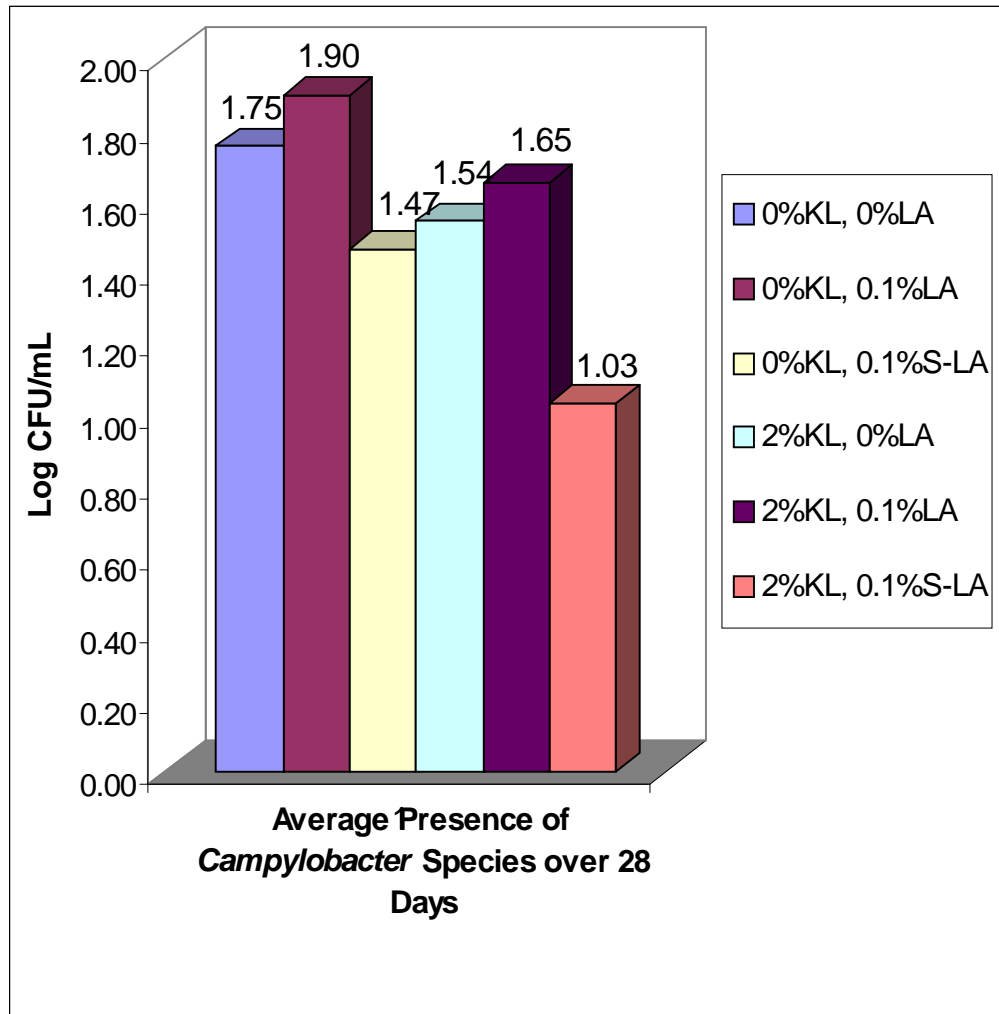


Figure 1. Average presence of *Campylobacter* sp. on raw boneless, skinless chicken breasts for 2% potassium lactate (KL) treatments.

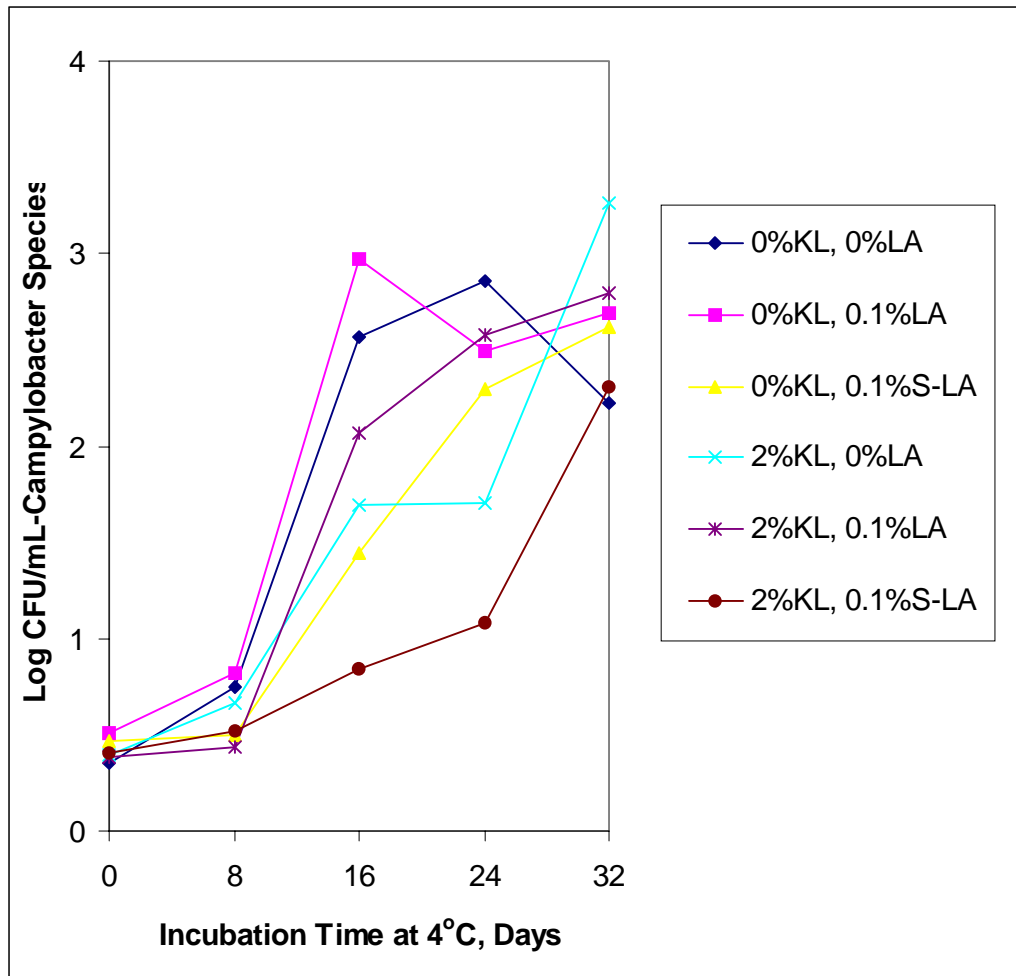


Figure 2. Growth and/or recovery of *Campylobacter* sp. over the duration of the shelf life in response to each combination of potassium lactate (KL) and lactic acid (LA).

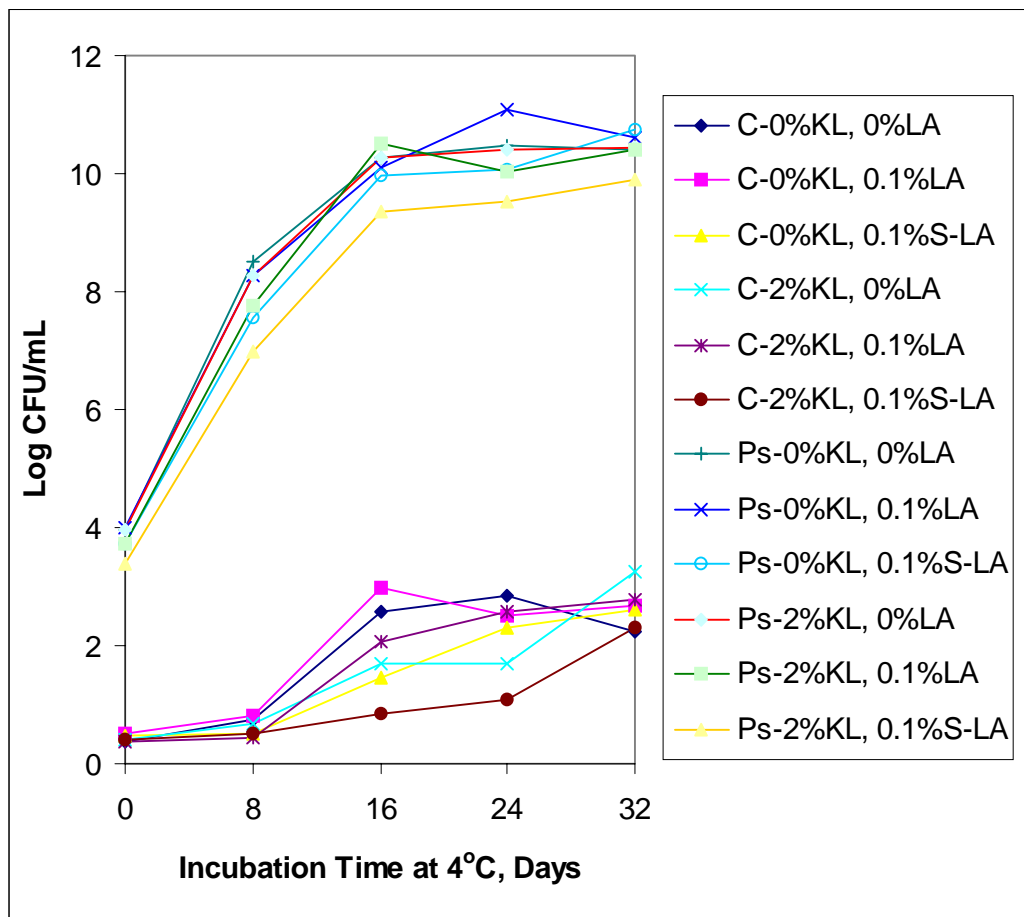


Figure 3. Growth and/or recovery of psychrotrophic bacteria and *Campylobacter* sp. (C) in response to the applied treatments during the 4°C shelf life study of raw boneless, skinless chicken breasts.

APPENDICES

APPENDIX A:

Pump %	17	Weight (g)	Weight (lbs)	To brine %	To meat %
water		12.94297	0.03	67.08	9.75
salt		1.32795	0.00	6.88	1
sodium phosphate		0.597578	0.00	3.10	0.45
potassium lactate		4.4265	0.01	23	3.33
lactic acid		0	0.00	0.00	0
Total Brine		19.295	0.0425	100.00	14.53
water		12.79211	0.03	66.30	9.63
salt		1.327942	0.00	6.88	1
sodium phosphate		0.597574	0.00	3.10	0.45
potassium lactate		4.426474	0.01	23	3.33
lactic acid		0.150903	0.00	0.78	0.11
Total Brine		19.295	0.0425	100.00	14.53
water		12.6412	0.03	65.52	9.52
salt		1.327942	0.00	6.88	1
sodium phosphate		0.597574	0.00	3.10	0.45
potassium lactate		4.426474	0.01	23	3.33
lactic acid		0.301805	0.00	1.56	0.23
Total Brine		19.295	0.0425	100.00	14.53
water		12.4903	0.03	64.73	9.41
salt		1.327942	0.00	6.88	1
sodium phosphate		0.597574	0.00	3.10	0.45
potassium lactate		4.426474	0.01	23	3.33
lactic acid		0.452708	0.00	2.35	0.34
Total Brine		19.295	0.0425	100.00	14.53

Pump %	17	Weight (g)	Weight (lbs)	To brine %	To meat %
water		14.0496	0.03	72.81	10.58
salt		1.32795	0.00	6.88	1
sodium phosphate		0.597578	0.00	3.10	0.45
potassium lactate		3.319875	0.01	17	2.5
lactic acid		0	0.00	0.00	0
Total Brine		19.295	0.0425	100.00	14.53
water		13.89873	0.03	72.03	10.47
salt		1.327942	0.00	6.88	1
sodium phosphate		0.597574	0.00	3.10	0.45
potassium lactate		3.319855	0.01	17	2.5
lactic acid		0.150903	0.00	0.78	0.11
Total Brine		19.295	0.0425	100.00	14.53
water		13.74782	0.03	71.25	10.35
salt		1.327942	0.00	6.88	1
sodium phosphate		0.597574	0.00	3.10	0.45
potassium lactate		3.319855	0.01	17	2.5
lactic acid		0.301805	0.00	1.56	0.23
Total Brine		19.295	0.0425	100.00	14.53
water		13.59692	0.03	70.47	10.24
salt		1.327942	0.00	6.88	1
sodium phosphate		0.597574	0.00	3.10	0.45
potassium lactate		3.319855	0.01	17	2.5
lactic acid		0.452708	0.00	2.35	0.34
Total Brine		19.295	0.0425	100.00	14.53

Pump %	17	Weight (g)	Weight (lbs)	To brine %	To meat %
water		17.36947	0.04	90.02	13.08
salt		1.32795	0.00	6.88	1
sodium phosphate		0.597578	0.00	3.10	0.45
potassium lactate		0	0.00	0	0
lactic acid		0	0.00	0.00	0
Total Brine		19.295	0.0425	100.00	14.53
water		17.21858	0.04	89.24	12.97
salt		1.327942	0.00	6.88	1
sodium phosphate		0.597574	0.00	3.10	0.45
potassium lactate		0	0.00	0	0
lactic acid		0.150903	0.00	0.78	0.11
Total Brine		19.295	0.0425	100.00	14.53
water		17.06768	0.04	88.46	12.85
salt		1.327942	0.00	6.88	1
sodium phosphate		0.597574	0.00	3.10	0.45
potassium lactate		0	0.00	0	0
lactic acid		0.301805	0.00	1.56	0.23
Total Brine		19.295	0.0425	100.00	14.53
water		16.91678	0.04	87.67	12.74
salt		1.327942	0.00	6.88	1
sodium phosphate		0.597574	0.00	3.10	0.45
potassium lactate		0	0.00	0	0
lactic acid		0.452708	0.00	2.35	0.34
Total Brine		19.295	0.0425	100.00	14.53

APPENDIX B.

III. Sensory Evaluation Worksheets and Randomization

Judge# _____

Date: _____

Boneless, Skinless Chicken Breasts:

Instructions: Please, do not touch the samples.

Please visually observe sample # _____ first and then sample # _____ second.

Circle one response per comparison. You must select only one answer per observation

VISUAL

No difference

Very Slight difference

Slight/moderate difference

Moderate difference

Moderate/large difference

Large difference

Very large difference

Secondly, smell sample # _____ first and then smell sample # _____ second.

Circle one response per comparison. You must select only one answer per observation

SMELL

No difference

Very Slight difference

Slight/moderate difference

Moderate difference

Moderate/large difference

Large difference

Very large difference

REMEMBER THAT A DUPLICATE CONTROL IS THE SAMPLE SOME OF THE TIME.

Comments: _____

Questions:

1.) Gender: Female _____ Male _____ (check one)

2.) Age:

18-24 _____

25-31 _____

32-38 _____

39-45 _____

46-52 _____

53-59 _____

60-66 _____

>67 _____

3.) How often do you purchase chicken from the grocery store

Never _____

Rarely (3 per year) _____

Sometimes (several times a month) _____

Often (weekly) _____

Always (Daily) _____

4.) Are you color blind (any degree)? YES _____ NO _____

5.) Do you have trouble detecting odors? YES _____ NO _____



Human Subjects Forms for Sensory Evaluation

Protocol for Projects of Sensory Evaluation

If the project involves sensory evaluation, please complete the following questions about the project to assist you and the Institutional Review Board in determining the risk level of the project.

Definition: Sensory evaluation is the evaluation of food or other substances by the senses including taste, touch, smell, sight and hearing.

Check all that apply:

1. The procedure for sensory evaluation in this project involves:
 - Tasting in the mouth (includes tests where the panelist is instructed to spit it out)
 - Substances applied to the skin
 - Substances smelled for odor components
 - Substances evaluated by sound when chewed
 - Substances evaluated by visual senses

2. The product/s to be evaluated are:
 - Made entirely of ingredients approved by FDA for consumption or application under approved conditions of processing
 - Made of ingredients approved by FDA but not approved for the use in the project (e.g. heating of aspartame, fat substitutes approved only as an emulsifier).
 - Made partially or entirely of experimental ingredients pending FDA approval.
 - Made partially or entirely of experimental ingredients not approved for human consumption or topical use
 - Made from materials from or altered by biotechnology

3. The processing or preparation of the product is:
 - By usual approved good manufacturing or preparation practices for that food or topical product.
 - By experimental procedures including non-good manufacturing practices. Briefly describe the procedures.

4. The packaging of the product includes:
 - Processing or storage in FDA-approved packaging materials.
 - Processing or storage in packaging materials not approved by FDA.

5. Describe the storage protocols for the product that are necessary to maintain the product in safe condition.

The chicken breasts are packaged and stored in a 4°C incubator to maintain the safety and freshness of the product.

6. If microbiological cultures are a part of the food processing or preparation procedure, describe what cultures will be used, if they will be active on consumption, and give evidence that these cultures are known to be safe for human consumption.

There will be no microbial cultures used in this experiment.

7. Allergies
 - Are any ingredients to be used potentially allergenic as consumed or by topical application? If yes, describe. Have panelists been made aware of these ingredients?

When you have completed this form, indicate the risk level to the panelists of this project. Complete the appropriate form; for "not at risk", the Certificate of Exemption form; for "at minimal risk", the Request for Approval form.

Virginia Polytechnic Institute and State University
Informed Consent for Participation in Sensory Evaluation

Title of Project: Sensory Evaluation of Boneless, Skinless Chicken Breasts

Principal Investigator: David D. Rasmussen

I. THE PURPOSE OF THIS PROJECT

You are invited to participate on a sensory evaluation panel about chicken. This purpose of this project is test if Potassium lactate and/or lactic acid have an effect upon the visual and olfactory characteristics of chicken, concerning a difference form control methodology.

II. PROCEDURES

There will be 6 sessions over a period of 7 weeks involving about 10 minutes at each session. You will be presented with approximately 14 comparisons at each session.

III. BENEFITS/RISKS OF THE PROJECT

Your participation in the project will provide the following information that may be helpful. (Benefits to science or the individual such as thresholds, etc.) You may receive the results or summary of the panel when the project is completed.

IV. EXTENT OF ANONYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. COMPENSATION

For participation in the project, you will receive food (candy) for each session completed. For completion of the entire project, cookies or candy will be offered.

Course Credit

You may not receive extra credit for any classes in which you are enrolled.

VI. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology.

Please provide address and phone number so investigator may reach you in case of emergency or schedule changes.

Address _____

Phone _____

IX. SUBJECT'S PERMISSION

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason why I cannot participate in this study

Signature

Date

Detach This Page and Take It With You:

Should I have any questions about this research or its conduct, I should contact:

David D. Rasmussen (540) 231-8845

Investigator/Phone

Department of Food Science and Technology

Susan Sumner, Ph.D. (540) 231-5280

Faculty/Phone

Department of Food Science and Technology

Cameron Hackney, Ph.D. (540) 231-5247

Faculty/Phone

Department of Food Science and Technology

Joseph Eifert, Ph.D. (540) 231-3658

Faculty/Phone

Department of Food Science and Technology

Tom Hurd (540) 231-5281

Director, Sponsored Programs

Outline for Protocol to Accompany IRB Request

JUSTIFICATION OF PROJECT

The purpose of this project is determine whether Potassium Lactate and Lactic acid have a detrimental effect upon the visual and olfactory aspects of boneless, skinless chicken breasts, which requires human subjects to provide the sensory evaluation, given they are the principle consumers of the chicken. Potassium Lactate is an additive used for its flavor improvement and humectant properties. Lactic Acid is also a food additive used for its bacteriocidal properties. The findings of this study will help understand whether the treatments will significantly change the odor and appearance of boneless, skinless chicken breasts. Since human subjects are the consumers of this product, it is necessary to determine if they can sense differences in the chicken concerning odor and appearance.

PROCEDURES

The subjects allowed to participate will be 18 years of age or older and either male or female. The only other restriction for those allowed to participate are those who aren't able to see or smell . Subjects will be solicited by the investigator of this experiment to volunteer for the experiment.

There will be six sessions that will last for approximately 15 min, each session separated by a week. The human subjects will be involved in the visual and odor evaluation of the fourteen different comparisons concerning the degree of difference each sample has from the labeled control.

David Rasmussen, Dorothea Kerber, Gabriel Sanglay and Alicia Stevans will have direct contact with the subjects.

RISKS AND BENEFITS

The only risk is for those who accidentally touch the samples and then ingest bacteria from the chicken breasts.

CONFIDENTIALITY/ANONYMITY.

The subjects will be provided with a judge number that only the investigators of for this experiment will have knowledge of. The participation and results of the experiments will be kept confidential, where names of the subjects will not be revealed to anyone. The principle investigator and other students assisting in the investigation will have access to the data, including the advising committee.

CONSENT

These forms are incorporated into this report on the previous pages.

APPENDIX C

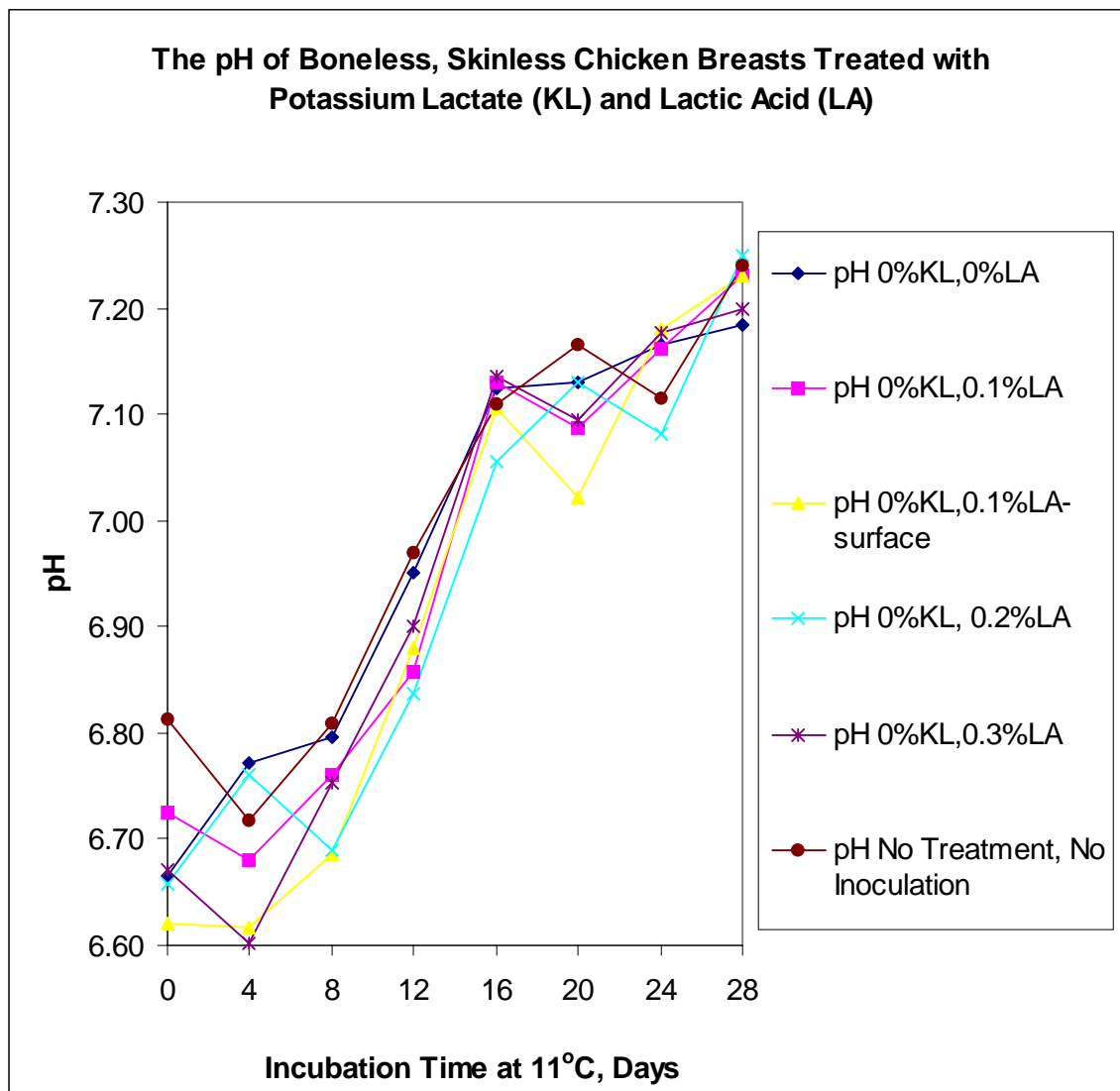


Figure 1. Mean pH of rinse solutions from raw boneless, skinless chicken breasts inoculated with *Campylobacter* sp. over 28 days, treated with different levels of lactic acid (LA) at the 0% potassium lactate (KL) level.

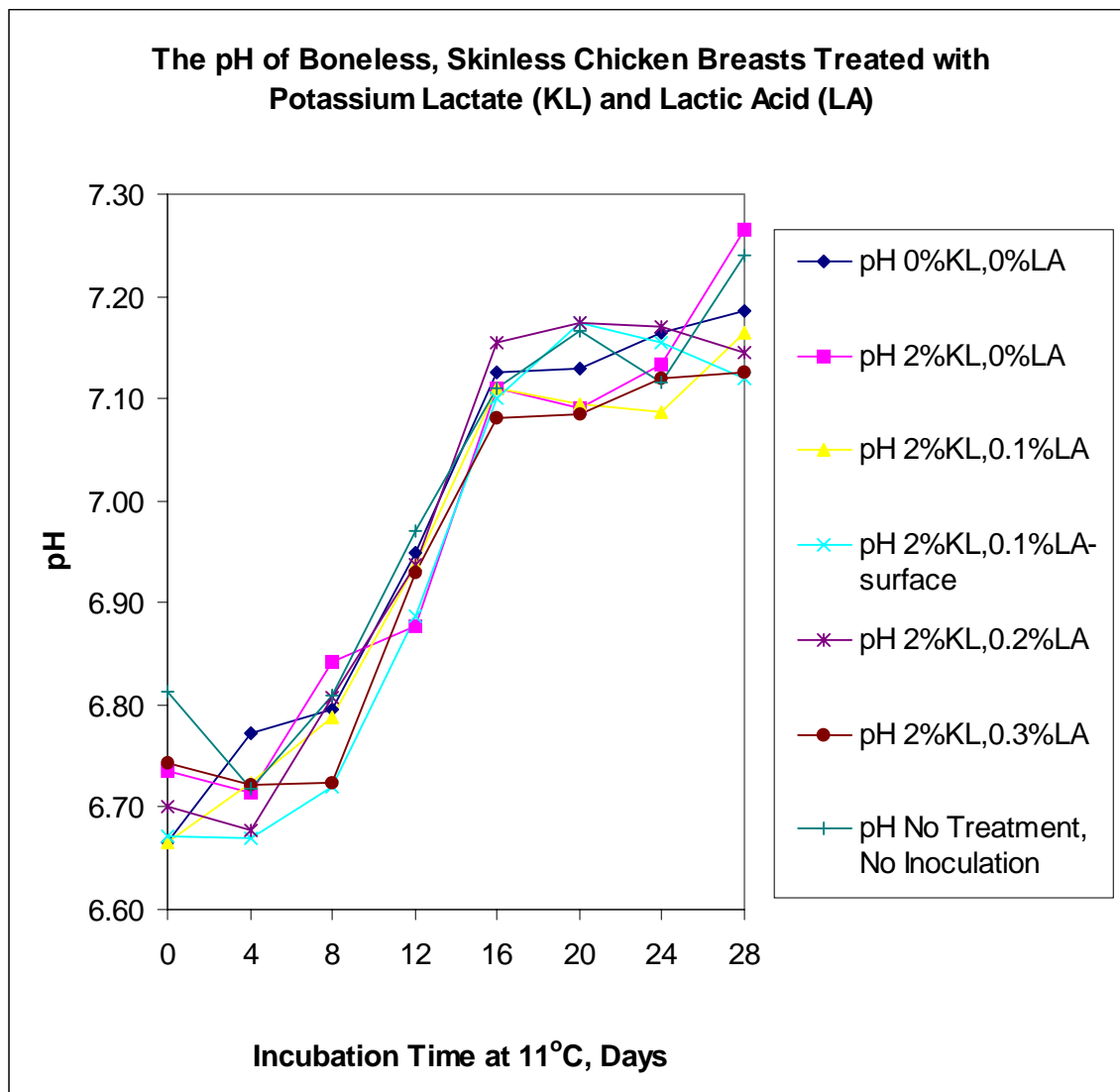


Figure 2. Mean pH of rinse solutions from raw boneless, skinless chicken breasts inoculated with *Campylobacter* sp. over 28 days, treated with different levels of lactic acid (LA) at the 2% potassium lactate (KL) level.

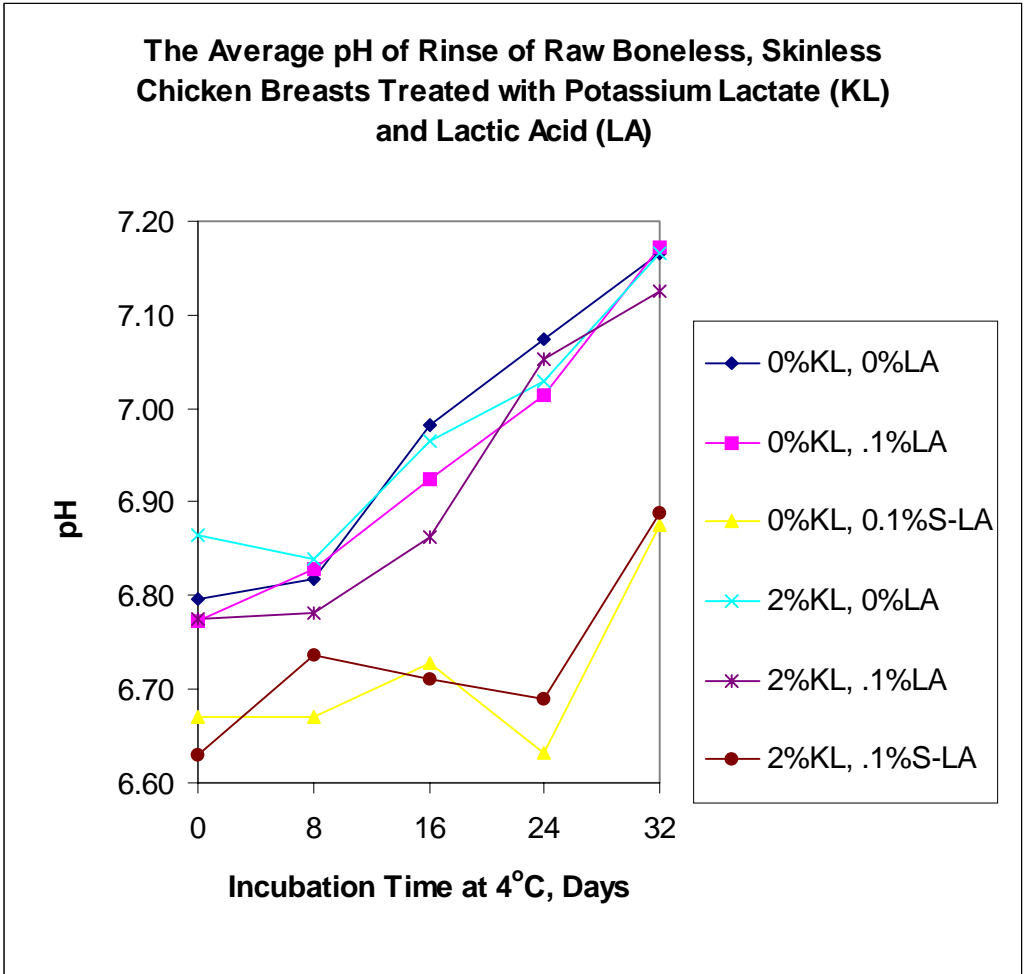


Figure 3. Mean pH of rinse solutions from raw boneless, skinless chicken breasts over 32 days, treated with different levels of lactic acid (LA) at the 0% and 2% potassium lactate (KL) level.

APPENDIX D

VITAE

David Rasmussen was born in Manchester, Connecticut and was raised nearby in Broad Brook, Connecticut. He graduated from East Windsor High School in June of 1991. He continued on to Virginia Tech where he received a Bachelor of Science degree in Biology, completing an option in Microbiology and Immunology and a minor in Chemistry in May of 1996. He also received a second Bachelor of Science degree in Psychology in May of 1996. During his time at Virginia Tech, David was a member of the American Society for Microbiology.

After taking a year and half off to work, David returned to Virginia Tech to pursue a Masters of Science degree in Food Science and Technology in January of 1998. While at Virginia Tech during this time, he was a member of the Institute of Food Technologists; the International Association of Milk, Food, and Environmental Sanitarians; and Phi Tau Sigma.