

INACTIVATION OF *Escherichia coli* O157:H7 ON POULTRY AS INFLUENCED BY BUFFERED LACTIC ACID AND MODIFIED ATMOSPHERE PACKAGING

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ABSTRACT

The effect of the treatment with various concentrations of lactic acid (2 %, 5 %, 7.5 % and 10 % w/v) buffer system pH3 (LABS), Modified atmosphere packaging (MAP) (90 % CO₂ + 10 % O₂); combination of 2 % LABS + MAP; 5 % LABS + MAP; 7.5 % LABS + MAP and 10 % LABS + MAP on *Escherichia coli* O157:H7 and on the shelf life of chicken legs stored at 5 °C was investigated. The initial contamination level of *Escherichia coli* O157:H7 on chicken legs surface was 3.59 log₁₀ cfu / cm² of skin. Reduction of 0.92, 1.23, 1.39, 1.81 log₁₀ units of *Escherichia coli* O157:H7 were obtained by the treatments with 2 % lactic acid buffer system pH3 (LABS), 5 % (LABS), 7.5 % (LABS) and 10 % (LABS), respectively. The antimicrobial effect increased with increasing concentrations of lactic acid in the buffer system. On day 3 there was a significant difference ($p < 0.05$) for the number of *Escherichia coli* O157:H7 between all the treatments compared with untreated samples (control). Treatment with 10 % LABS + MAP, 7.5 % LABS + MAP, 5 % LABS + MAP and 2 % LABS + MAP eliminated 3.59 log₁₀ cfu/cm² of *Escherichia coli* O157:H7 from poultry within 7, 8, 10 and 11 days of storage at 5 °C, respectively. Lactic acid buffer system pH3 (LABS) and Modified Atmosphere Packaging provides a natural means of killing *Escherichia coli* O157:H7 in poultry and can be used in other food products. Results revealed that the total viable bacteria and lactic acid bacteria were inhibited by all treatments used as compared with the untreated samples. The highest inhibition was observed with 10 % LABS + MAP treatment, followed by 7.5 % LABS + MAP. The buffering capacity of the buffer systems (LABS) seem to be sufficient to maintain a low pH of the skin during storage. Legs treated with 2 % LABS; 5 % LABS; 7.5 % LABS; 10 % LABS; Modified atmosphere packaging (MAP); 2 % LABS + MAP; 5 % LABS + MAP; 7.5 % LABS + MAP and 10 % LABS + MAP have a shelf life at 5 °C of 7, 8, 10, 11, 12, 13, 14, 15 and 16 days respectively. This signifies a prolongation of shelf life at 5 °C of 2, 3, 5, 6, 7, 8, 9, 10 and 11 days respectively, as compared with untreated samples. A synergistic effect between lactic acid buffer system (LABS) and modified atmosphere packaging (MAP) was evidence.

INTRODUCTION

Escherichia coli O157:H7 is a major food-borne pathogen, which causes life threatening hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura in the young, old and immuno-compromised (Doyle, 1991; Rowe, 1995; Acheson *et al.*, 1996). An estimated 73,480 cases of *Escherichia coli* O157:H7 related illness are reported each year in the United States with approximately 85 % due to food-borne transmission (Mead *et al.*, 1999; Campbell *et al.*, 2004). Many food vehicles have been responsible for the transport of *Escherichia coli* O157:H7, including poultry (Doyle, 1991; Chapman *et al.*, 1997; Heuvelink *et al.*, 1999; Chandler *et al.*, 2001; Ganiere *et al.*, 2001), meat (Okrend *et al.*, 1990;

Chapman *et al.*, 2001), fruits (Besser *et al.*, 1993; Nguyen-the and Carlin, 1994; Cheng-hsin and Cheng-chun,2001), vegetables(Nguyen-the and Carlin, 1994; Francis and Berine,2001;) and water (Sargeant *et al.*, 2003; Ibekwe and Grieve, 2004; Shelton *et al.*, 2004). The infection risk for *Escherichia coli* O157:H7 is high because the infective dose in food is low as 10 cfu/g (Griffin and Tauxe 1991; Coia, 1998; Rasmussen and Casey, 2001). This development has stimulated the interest of researchers, regulatory authorities, and the food industry in determining the potential of the organism to contaminate various foods and survive processing procedures (Doyle, 1991; Coia, 1998; Rasmussen and Casey, 2001).

One way to retard microbial growth is through antimicrobial agents. Lactic acid has bactericidal properties (Zeitoun and Debevere, 1990; Sawaya *et al.*, 1995; Dorsa and Marshall, 1995) and can be used as a natural antimicrobial agent for poultry and meat products (Smulders *et al.*, 1986; Zeitoun *et al.*, 1994; Sawaya *et al.*, 1995).

Modified atmosphere packaging of fresh food has been used increasingly in recent years (Jayas and Jeyamkondan, 2002; Panagiotis and George, 2002; Khawla *et al.*, 2005) and has been shown to be effective against pathogenic bacteria and for extension of the shelf life of poultry (Zeitoun and Debevere, 1993b; Sawaya *et al.*, 1995; Jimenez *et al.*, 1999; Khawla *et al.*, 2005).

The objectives of the present investigations were to evaluate the effect of the treatment with different concentrations of lactic acid buffered system pH3 or/and modified atmosphere packaging on inactivation of *Escherichia coli* O157:H7 and on the shelf life of poultry stored at 5 °C.

MATERIALS AND METHODS

Materials:

Fresh chicken legs were obtained from a local commercial poultry processing plant (Al-Hausa, Saudi Arabia). They were taken from the production line and transported under refrigeration to the laboratory of College of Agricultural and food sciences, King Faisal University of Saudi Arabia within an hour. Legs were used for practical reasons, instead of whole carcasses.

***Escherichia coli*:** *Escherichia coli* O157:H7 was kindly provided by prof.J.M.Debevere (Faculty of Agriculture Sciences, University of Gent, Belgium). Intermediate cultures were prepared by inoculating a loopful from the slant into liquid brain heart infusion (BHI) (Oxoid CM 225) which was then incubated aerobically at 37 °C for 24 h One drop of this BHI culture was transferred into a second tube of sterile BHI, which was again incubated for 24 h at 37 °C. One loop of this culture was streaked onto plate count agar (PCA) (Oxoid CM 325) and incubated for 24 h at 37 °C. A colony of the PCA culture was transferred into 100 ml of sterile BHI and incubated for 24 h at 37 °C. The working culture of *Escherichia coli* O157:H7 was prepared by diluting the BHI culture in 25 liter sterile physiological saline containing 0.1% peptone

so that the number of *Escherichia coli* O157:H7 was 21×10^5 cfu/ml. The working cultures were made in duplicate (each 25 liter).

Artificial contamination of the chicken legs: Two hundred and thirty of fresh chicken legs were submerged in the working culture of *Escherichia coli* O157:H7 (Each 115 in 25 liter of working cultures) for 15 s. After this artificial contamination the legs were then kept at 5 °C for 2 h to drain and to allow the attachment of the *Escherichia coli* O157:H7 cells on the skin. The contamination level was 39×10^2 cfu/cm² (None treated Controls). Non treated samples (Controls) were packed separately in polyethylene bags (70µm thickness, with oxygen permeability of 750 ml / m² /24 h at 1 atmosphere and 23 °C) and stored at 5 °C.

Lactic acid buffer systems pH3 (LABS) treatment: After artificial contamination, chicken legs were decontaminated by spraying with lactic acid buffer systems pH3 (LABS) at four concentrations, 2%, 5%, 7.5% and 10%(w/v). After treatment the chicken legs were allowed to drain at 5 °C for 2 hours (Zeitoun and Debevere, 1990). A half of the treated samples were packed separately in polyethylene bags and stored at 5 °C.

Gas packaging: The second half of the treated samples were packed separately under modified atmosphere (90%CO₂ + 10% O₂) in Sidamil plastic bags (permeability : 6ml O₂ /m² /24 h, 15 ml CO₂ /m² /24 h, 2ml N₂ /m² /24 h, at 1 atmosphere and 23 °C) and stored at 5 °C (Zeitoun and Debevere, 1992).

Microbiological analysis: At each sampling time, three legs were sampled aseptically taken by means of excision of surface areas of 15 cm² of skin. A sterile filter paper (6×2.5 cm) was used to outline the area. Filter paper and skin were homogenized for 2 min in 150 ml sterile physiological saline supplemented by 0.1% peptone, using a stomacher (Lab Blender 400, Seward Medical, London). From this homogenate, decimal dilutions were prepared in duplicate in physiological saline containing 0.1% peptone and were plated in duplicated.

Total viable count of organisms were determined by the pour-plated method in plate count agar (PCA; Oxoid CM 325), incubated at 25 °C for 72 h (Jimenez *et al.*, 1999; Panagiotis and George, 2002). The sorbitol MacConkey agar (SMAC) (Oxoid CM981) medium was used for selective enumeration of *Escherichia coli* O157:H7, incubated for 24 h at 37 °C (Heuvelink *et al.*, 1999; Rodriguez *et al.*, 2005). Lactic acid bacteria were assessed as colony forming units on MRS agar (Oxoid CM 361) with an overlay of the same agar incubated for 3 days at 30 °C (Jimenez *et al.*, 1999; Panagiotis and George, 2002).

pH measurement: After sampling for microbiological analysis, the rest of the skin was removed, macerated (skin only) in a blender for 10s (Zeitoun and Debevere, 1990) and the pH was measured using a digital pH meter (Thermo Orion, model 260A) (USA).

Statistical analysis: Obtained data were analyzed using analysis of variances two ways (ANOVA) and subjected least significant difference (LSD) at 0.05% level of significant was used to compare the treatment means (Waller and Duncan, 1969). Computations were done using SAS (1996).

RESULTS AND DISCUSSION

The effect of lactic acid Buffer system pH3 (LABS) and Modified Atmosphere Packaging (MAP) on the total viable count when the *Escherichia coli* O157:H7 was inoculated at $3.59 \log_{10} \text{ cfu/cm}^2$ of the skin of chicken legs are shown in Table 1. The initial total viable bacteria on chicken legs was $5.02 \log_{10} \text{ cfu/cm}^2$. The decrease in total viable count immediately after decontamination was more pronounced with increasing the concentration of lactic acid buffer system (LABS). According to Zeitoun and Debevere, (1990), increasing the concentration of lactic acid to 10% in the buffer system (LABS) appeared not to influence the sensory quality of the chicken legs. Several considerations have led to the use of lactic acid as a decontamination agent because of the excellent bactericidal properties (Smulders *et al.*, 1986; Zeitoun *et al.*, 1994; Sawaya *et al.*, 1995). At day 5 and 7 of storage at 5 °C, marked decrease in $\log_{10} \text{ cfu/cm}^2$ of total viable count was evident for all samples treated with different concentrations of lactic acid buffer system (LABS) in combination with modified atmosphere packaging (MAP) compared with $\log_{10} \text{ cfu/cm}^2$ on legs only treated with MAP. This can be explained by the antimicrobial effect of lactic acid buffer system (LABS) and a synergistic effect between (LABS) and modified atmosphere packaging (MAP). On the day of spoilage, corresponding with the critical spoilage level of $\log_{10} \text{ CFU} = 7.0-8.0$, followed by typical off-odours on the next day (Van der Marel *et al.*, 1988; Zeitoun *et al.*, 1994). All samples at the end of storage periods were below the critical marginal quality, followed by off odour next day. Samples treated with 2% LABS; 5% LABS; 7.5% LABS; 10% LABS; Modified atmosphere packaging (MAP); MAP+ 2% LABS; MAP+ 5% LABS; MAP+ 7.5% LABS and MAP+ 10% LABS have a shelf life at 5 °C of 7, 8, 10, 11, 12, 13, 14, 15 and 16 days respectively. This signifies a prolongation of shelf life at 5 °C of 2, 3, 5, 6, 7, 8, 9, 10 and 11 days respectively, as compared with untreated samples. The data revealed that decontamination of poultry with buffer lactic acid pH3 (LABS) is particularly suitable in combination with MAP.

The pH of foods influences their susceptibility to microbial growth, and acidification by the addition of acids or by fermentation, is used in preservation of many types of food (Sawaya *et al.*, 1995). In situations where the pH must be altered, it is usually desirable to stabilize the pH at the desired level through a buffer system. Also, in some foods where substantial amounts of acid are used, the resulting sour, tart taste will be perceived by many consumers as flavour detriment. This problem can be solved by establishing a buffer system of weak organic acids and their salts, which not only lowers and stabilizes the pH, but also increases the antimicrobial effect (Zeitoun and Debevere, 1990; 1993_a). The evolution of the pH of the chicken legs treated with various concentrations of lactic acid buffer system pH3 (LABS) and modified atmosphere packaging (MAP) during storage at 5 °C is illustrated in Table 2.

Table (1): Effect of treatment with Lactic acid buffer system and Modified Atmosphere Packaging (MAP) on Total viable count.

Treatments	Days of storage at 5 °C															
	0	3	5	7	8	10	11	12	13	14	15	16				
Untreated (artificially contaminated)	5.02 ^{Ca}	6.01 ^{Ba}	7.05 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
2% Lactic acid buffer system pH3(LABS)	4.19 ^{Db}	5.01 ^{Cb}	5.92 ^{Bb}	6.88 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
5% LABS	4.12 ^{Eb}	4.77 ^{Dc}	5.40 ^{Cc}	6.04 ^{Bb}	6.82 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
7.50%LABS	3.81 ^{Ec}	3.88 ^{Ef}	4.48 ^{De}	5.14 ^{Ccd}	5.88 ^{Bb}	6.92 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
10% LABS	3.72 ^{Fc}	3.79 ^{Ff}	4.29 ^{Ef}	4.86 ^{Dde}	5.24 ^{Cd}	6.11 ^{Bb}	6.75 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.				
MAP 90% CO ₂ + 10% O ₂	5.02 ^{Fa}	4.52 ^{Gd}	4.98 ^{Fd}	5.25 ^{Fc}	5.56 ^{Dc}	5.98 ^{Cc}	6.34 ^{Bb}	6.90 ^{Aa}	n.d.	n.d.	n.d.	n.d.				
MAP+ 2% LABS	4.19 ^{Gb}	4.07 ^{Hc}	4.24 ^{Gg}	4.56 ^{Fef}	4.88 ^{Ee}	5.22 ^{Dd}	5.67 ^{Cc}	6.30 ^{Bb}	6.84 ^{Aa}	n.d.	n.d.	n.d.				
MAP+ 5% LABS	4.12 ^{Hb}	4.02 ^{Ie}	4.18 ^{Hg}	4.32 ^{Gf}	4.51 ^{Ff}	4.90 ^{Ee}	5.32 ^{Dd}	5.74 ^{Cc}	6.17 ^{Bb}	6.98 ^{Aa}	n.d.	n.d.				
MAP+7.5%LABS	3.81 ^{Ic}	3.78 ^{Ii}	3.68 ^{Jh}	3.90 ^{Hg}	4.12 ^{Gg}	4.55 ^{Ff}	4.80 ^{Ee}	5.34 ^{Dd}	5.78 ^{Cc}	6.12 ^{Bb}	6.78 ^{Aa}	n.d.				
MAP+ 10%LABS	3.72 ^{Ic}	3.53 ^{Jg}	3.55 ^{Ji}	3.64 ^{Ig}	3.90 ^{Hh}	4.13 ^{Gg}	4.48 ^{Ff}	4.77 ^{Ee}	5.10 ^{Dd}	5.55 ^{Cc}	5.95 ^{Bb}	6.69 ^{Aa}				

1. Values with the same superscripts in the same horizontal row (A-J) or vertical column (a-i) are not significantly different (p< 0.05).
 2. The log colony forming units (C.F.U.) values stated refer to three samples.
 3. n.d. = not determined because of spoilage.
 4. LABS= Lactic acid buffer system pH3.
 5. MAP= 90% CO₂ + 10% O₂.

Table (2): Effect of lactic acid buffer system pH3 and Modified Atmosphere Packaging on pH of the skin of poultry artificially contaminated with *Escherichia coli* O157:H7.

Treatments	Days of storage at 5 °C															
	0	3	5	7	8	10	11	12	13	14	15	16				
Untreated (artificially contaminated)	6.54 ^{Ca}	6.71 ^{Ba}	6.95 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
2% Lactic acid buffer system pH3(LABS)	5.72 ^{Db}	5.84 ^{Cc}	6.18 ^{Bc}	6.50 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
5% LABS	5.31 ^{Ec}	5.72 ^{Dd}	5.98 ^{Cd}	6.17 ^{Bc}	6.42 ^{Ca}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
7.50%LABS	4.63 ^{Fd}	4.78 ^{Eg}	5.30 ^{Df}	5.82 ^{Cd}	5.90 ^{Bc}	6.28 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
10% LABS	4.57 ^{Fd}	4.62 ^{Fh}	4.98 ^{Eg}	5.28 ^{Df}	5.57 ^{Cd}	5.86 ^{Bb}	6.12 ^{Ab}	n.d.	n.d.	n.d.	n.d.	n.d.				
MAP 90% CO ₂ + 10% O ₂	6.54 ^{Aa}	6.28 ^{Cb}	6.37 ^{Cbb}	6.35B ^{Cb}	6.29 ^{Cb}	6.34 ^{Cba}	6.30 ^{Ca}	6.41 ^{Ba}	n.d.	n.d.	n.d.	n.d.				
MAP+ 2% LABS	5.72 ^{Fb}	5.60 ^{Ee}	5.71 ^{Fe}	5.80 ^{Ed}	5.88 ^{Dc}	5.92 ^{Dcb}	5.95 ^{Cc}	6.05 ^{Bb}	6.18 ^{Aa}	n.d.	n.d.	n.d.				
MAP+ 5% LABS	5.31 ^{Fc}	5.12 ^{Gf}	5.26 ^{Ff}	5.41 ^{Ee}	5.47 ^{Edc}	5.54 ^{Dc}	5.50 ^{Dd}	5.62 ^{Cc}	5.84 ^{Bb}	6.02 ^{Aa}	n.d.	n.d.				
MAP+7.5%LABS	4.63 ^{Gd}	4.51 ^{Hi}	4.64 ^{Gh}	4.85 ^{Fg}	4.92 ^{Ff}	5.10 ^{Eg}	5.08 ^{Eg}	5.20 ^{Dd}	5.41 ^{Cc}	5.60 ^{Bb}	5.97 ^{Aa}	n.d.				
MAP+ 10%LABS	4.57 ^{Hid}	4.38 ^J	4.41 ^{Jl}	4.52 ^h	4.61 ^{HGg}	4.67 ^{FGe}	4.72 ^{Ff}	4.81 ^{Ee}	4.95 ^{Dd}	5.18 ^{Cc}	5.55 ^{Bb}	5.88 ^A				

1. Values with the same superscripts in the same horizontal row (A-J) or vertical column (a-j) are not significantly different (p< 0.05).

2. The pH values stated refer to three samples.

3. n.d. = not determined because of spoilage.

4. LABS= Lactic acid buffer system pH3.

5. MAP= 90% CO₂ + 10% O₂.

The initial pH value of chicken legs used in this study was 6.54. A reduction of 0.82, 1.23, 1.91 and 1.97 pH units were obtained by treatment with 2% LABS; 5% LABS; 7.5% LABS and 10% LABS, respectively as compared with untreated samples. The pH values of chicken legs treated with 2% LABS; 5% LABS; 7.5% LABS; 10% LABS; modified atmosphere packaging (MAP); 2%LABS+ MAP; 5%LABS+ MAP; 7.5%LABS+ MAP and 10%LABS+ MAP were significantly decreased ($P < 0.05$) when stored for 3 and 5 days at 5 °C as compared with untreated chicken legs. The pH value of untreated samples increased rapidly, as the microbial population increased (Van der Marel *et al.*, 1988; Sawaya *et al.*, 1995). These results are contradictory to those obtained for pH and total viable count (Table 1). After 5, 7, 8 and 10 days of storage at 5 °C, there was significant decrease ($P < 0.05$) in the pH value for all samples treated with LABS+ MAP compared with legs only treated with LABS. This can be explained by a synergistic effect between lactic acid buffer system pH3 (LABS) and modified atmosphere packaging (MAP). Similar results were obtained for total viable bacteria (Table 1). On the day of spoilage, corresponding with the critical spoilage level of \log_{10} CFU= 7.0-8.0, followed by typical off-odours on the next day (Van der Marel *et al.*, 1988; Zeitoun *et al.*, 1994), the pH values of all poultry samples treated with various concentrations of lactic acid buffer system pH3 (LABS) were still lower than the initial pH. The buffering capacity of the buffer system seemed to be sufficient to maintain a low pH of the chicken legs.

The effect of treatment with various concentrations of lactic acid buffer system (LABS) and modified atmosphere packaging (MAP) on the growth of lactic acid bacteria are illustrated in Table 3. The numbers of lactic acid bacteria were reduced by 0.66, 0.70, 0.96 and 1.03 \log_{10} cfu/cm² for treatment with 2%LABS, 5%LABS, 7.5%LABS and 10%LABS, respectively. A similar trend was observed for total viable count (Table 1). The ANOVA indicates that storage time and treatment effects were significant ($P < 0.05$) in lactic acid bacteria counts. By day 3, populations of lactic acid bacteria on all treated samples were significantly lower ($P < 0.05$) than those counted on untreated samples (artificially contaminated). This bactericidal effect, increased with increasing concentration of lactic acid in the buffer system (LABS) combined with modified atmosphere packaging (MAP). As expected, on the day of spoilage, on all samples packed in modified atmosphere (MAP) or treated with lactic acid buffer system and packed in modified atmosphere (LABS+ MAP), lactic acid bacteria were found to be dominating flora. The data also indicated a synergistic effect between LABS and MAP, which increase with increasing the concentrations of LABS.

Escherichia coli O157: H7 is the most notorious serogroup of verotoxigenic *E.coli* (VTEC) and belongs to subgroup of VTEC that is associated with human disease and referred to as enterohemorrhagic *E.coli* (EHEC) (Griffin and Tauxe 1991). EHEC can produce asymptomatic infections, cause non-bloody diarrhea, bloody diarrhea or hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombocytic thrombocytopenic purpura (TTP) (Griffin, 1995). Hemorrhagic colitis caused by EHEC is characterized by severe abdominal cramps, bloody stools, little or no fever and evidence of colonic mucosal oedema (Griffin and Tauxe 1991).

Table (3): The growth of Lactic acid bacteria on poultry treated with Lactic acid buffer system and Modified Atmosphere Packaging (MAP).

Treatments	Days of storage at 5 °C															
	0	3	5	7	8	10	11	12	13	14	15	16				
Untreated (artificially contaminated)	4.01 ^{Ca}	4.92 ^{Bc}	5.64 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
2% Lactic acid buffer system pH3(LABS)	3.35 ^{Db}	4.01 ^{Cb}	4.50 ^{Bb}	5.17 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
5% LABS	3.31 ^{Eb}	3.82 ^{Dc}	4.16 ^{Cc}	4.63 ^{Bb}	5.08 ^{Aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
7.50%LABS	3.05 ^{Ec}	3.33 ^{Ee}	3.58 ^{De}	4.18 ^{Cd}	4.41 ^{Bc}	5.19 ^{Ab}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
10% LABS	2.98 ^{Fc}	2.92 ^{Fh}	3.43 ^{Ef}	3.74 ^{Dg}	4.19 ^{Ce}	4.58 ^{Bc}	4.95 ^{Ac}	n.d.	n.d.	n.d.	n.d.	n.d.				
MAP 90% CO ₂ + 10% O ₂	4.01 ^{Ga}	3.98 ^{Gb}	4.15 ^{Fc}	4.42 ^{Ec}	4.70 ^{Db}	5.29 ^{Ca}	5.78 ^{Ba}	6.34 ^{Aa}	n.d.	n.d.	n.d.	n.d.				
MAP+ 2% LABS	3.35 ^{Ib}	3.54 ^{Hd}	3.69 ^{Hd}	4.05 ^{Fe}	4.29 ^{Ed}	4.75 ^{Dd}	5.21 ^{Cb}	5.73 ^{Bb}	6.38 ^{Aa}	n.d.	n.d.	n.d.				
MAP+ 5% LABS	3.31 ^{Jb}	3.51 ^{Id}	3.72 ^{Hd}	3.84 ^{Gf}	4.01 ^{Fi}	4.41 ^{Ed}	4.84 ^{Dd}	5.22 ^{Cc}	5.67 ^{Bb}	6.49 ^{Aa}	n.d.	n.d.				
MAP+7.5%LABS	3.05 ^{Jc}	3.33 ^{Ie}	3.25 ^{Ig}	3.47 ^{fh}	3.71 ^{Gg}	4.02 ^{Fe}	4.37 ^{Ee}	4.75 ^{Dd}	5.26 ^{Cc}	5.57 ^{Bb}	6.24 ^{Aa}	n.d.				
MAP+ 10%LABS	2.98 ^{Kc}	3.14 ^{Jf}	3.20 ^{Ig}	3.28 ^{li}	3.43 ^{Hh}	3.63 ^{Gf}	3.99 ^{Fi}	4.21 ^{Ee}	4.59 ^{Dd}	5.05 ^{Cc}	5.61 ^{Bb}	6.29 ^A				

1. Values with the same superscripts in the same horizontal row (A-K) or vertical column (a-i) are not significantly different (p<0.05).

2. The log colony forming units (C.F.U.) values stated refer to three samples.

3. n.d. = not determined because of spoilage.

4. LABS= Lactic acid buffer system pH3.

5. MAP= 90% CO₂ + 10% O₂.

Approximately 7% of patients affected by VTEC develop systemic complications, most commonly HUS, which is characterized by microangiopathic hemolytic anemia, thrombocytopenia, renal failure and central nervous system symptoms. TTP is considered to be a manifestation of HUS where renal failure is normally mild but neurological involvement is greater (Griffin, 1995). *Escherichia coli* O157:H7 requires a very low infection dose in food to cause severe manifestations (Griffin and Tauxe 1991). The pathogen has become a major public health concern all over the world (Coia, 1998). Therefore many investigations about the different properties and the availability of this organism in different foods have been performed continuously (Nguyen-the and Carlin, 1994; Heuvelink *et al.*, 1999; Chandler *et al.*, 2001; Chapman *et al.*, 2001; Ganiere *et al.*, 2001; Rodriguez *et al.*, 2005). The antimicrobial effect of lactic acid buffer system pH3 (LABS) and modified atmosphere packaging (MAP) on *Escherichia coli* O157:H7 are shown in Table 4. The initial contamination level of *Escherichia coli* O157:H7 on chicken legs surface was $3.59 \log_{10} \text{ cfu/cm}^2$ of skin. The data indicate the decontaminating effect of the lactic acid buffer system pH3 (LABS). It was seemed that a reduction of 0.92, 1.23, 1.39, 1.81 \log_{10} units were obtained by the treatments with 2% lactic acid buffer system pH3 (LABS), 5% (LABS), 7.5% (LABS) and 10% (LABS), respectively. The antimicrobial effect increased with increasing concentrations of lactic acid in the buffer system. Jordan *et al.*, (1999) also reported that *Escherichia coli* O157:H7 was sensitive to lactate at pH3. On day 3 there was a significant difference ($P < 0.05$) for the number of *Escherichia coli* O157:H7 between all the treatments compared with untreated samples. These results are contradictory to those obtained for total viable count (Table.1), pH (Table.2) and lactic acid bacteria (Table.3). The number of *Escherichia coli* O157: H of the untreated artificially contaminated skin remained constant during 5 days of storage at 5 °C. Coleman *et al.*, (2003) also reported that *Escherichia coli* O157: H does not grow at 5 °C. At 10% lactic acid (LABS), *Escherichia coli* O157:H7 was reduced by $2.24 \log_{10} \text{ cfu/cm}^2$ within 5 days from an initial inoculum of $3.59 \log_{10} \text{ cfu/cm}^2$. This means that the probability of its survival under this condition is $1/\text{anti-log of } 2.24$ or $1/174$. This also means that in order to have survivors up to day5, there should be $> 174 \text{ cells/cm}^2$ present initially. On day 7, there was significant decrease ($P < 0.05$) in the number of *Escherichia coli* O157: H for all samples treated with LABS+ MAP compared with legs only treated with LABS. This can be explained by a synergistic effect between lactic acid buffer system (LABS) and modified atmosphere packaging (MAP). Treatment with; 10%LABS+ MAP, 7.5%LABS+ MAP, 5% LABS+ MAP and 2% LABS+ MAP eliminated $3.59 \log_{10} \text{ cfu/cm}^2$ of *Escherichia coli* O157:H7 from poultry within 7, 8, 10 and 11 days of storage at 5 °C, respectively. Such elimination would improve the safety of poultry.

In conclusion, the effect of lactic acid buffer system (LABS) on the inhibition of *Escherichia coli* O157:H7 and on shelf life increases with increasing concentrations of lactic acid in the buffer system. A synergistic effect between lactic acid buffer system (LABS) and modified atmosphere packaging (MAP) was evidence. Lactic acid buffer system pH3 and Modified Atmosphere Packaging provides a natural means of killing *Escherichia coli* O157:H7 in poultry and can be used in other food products.

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وقف نشاط بكتيريا الـ *Escherichia coli* O157:H7 باستخدام حامض اللاكتيك المنظم والتعبئة في جو غازي معدل في الدجاج المبرد
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تم عمل التلقيح الصناعي لسبقان الدجاج الطازج ببكتيريا الـ *Escherichia coli* O157:H7 الممرضة وذلك بتركيز $3.59 \log_{10} \text{ cfu/cm}^2$. ثم أجريت المعاملات التالية المختلفة: الرش بتركيزات مختلفة (٢%، ٥%، ٧.٥%، ١٠%) من حامض اللاكتيك المنظم pH3 أو التعبئة في جو غازي معدل (٩٠% ثاني أكسيد الكربون + ١٠% أوكسجين) كل منهم علي حدة . كما تم إجراء الرش بتركيزات مختلفة (٢%، ٥%، ٧.٥%، ١٠%) من حامض اللاكتيك المنظم مع التعبئة في جو غازي معدل كالتالي : (٢% محلول حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) و (٥% محلول حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) و (٧.٥% محلول حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) و (١٠% محلول حامض اللاكتيك المنظم + التعبئة في جو غازي معدل). وذلك لدراسة تأثير هذه المعاملات علي نشاط الـ *Escherichia coli* O157:H7 ومدة الصلاحية لسبقان الدجاج الطازجة المخزنة علي درجة حرارة ٥°م . أوضحت النتائج أن التأثير المضاد لحامض اللاكتيك المنظم علي بكتيريا *Escherichia coli* O157:H7 الممرضة ازداد بزيادة تركيز الحامض . بعد ثلاثة أيام من التخزين ، أدت جميع المعاملات إلي الإنخفاض المعنوي لأعداد بكتيريا *Escherichia coli* O157:H7. أوضحت النتائج أن المعاملة (١٠% حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) والمعاملة (٧.٥% حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) و المعاملة (٥% حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) و المعاملة (٢% حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) قد أدت إلي القضاء علي $3.59 \log_{10} \text{ cfu/cm}^2$ من بكتيريا الـ *Escherichia coli* O157:H7 الممرضة خلال ٧، ٨، ١٠، ١١ يوم من التخزين علي درجة حرارة ٥°م، علي التوالي. هذه المعاملات أدت للقضاء علي المخاطر التي يمكن أن تحدث نتيجة لتلوث الدجاج الطازج بهذه البكتيريا الممرضة. أشارت النتائج أن الرش بتركيزات مختلفة (٢%، ٥%، ٧.٥%، ١٠%) من حامض اللاكتيك المنظم جعل الـ pH للعينات المعاملة منخفضا معنويا إذا ما قورنت بالعينة غير المعاملة. كذلك أدت جميع المعاملات لانخفاض معنوي لأعداد بكتيريا العدد الكلي وبكتيريا حامض اللاكتيك بالمقارنة مع العينة الغير معاملة. وقد أوضحت النتائج أن معاملة الدواجن بحامض اللاكتيك المنظم عند تركيزات ٢%، ٥%، ٧.٥%، ١٠% والتعبئة في جو غازي معدل كل علي حدة قد أعطي عمر تخزيني ٧، ٨، ١٠، ١١، ١٢ يوما علي التوالي . بينما أعطت المعاملة لكل من (٢% حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) و (٥% حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) و (٧.٥% حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) و (١٠% حامض اللاكتيك المنظم + التعبئة في جو غازي معدل) عمر تخزين ١٣، ١٤، ١٥، ١٦ يوما علي التوالي. بينما العينة الغير معاملة قد أعطت عمر تخزيني ٥ أيام فقط علي درجة حرارة ٥°م . أوضحت الدراسة أن المعاملة بحامض اللاكتيك المنظم مع التعبئة في جو غازي معدل تؤدي إلي التخلص من المخاطر التي تنتج نتيجة لتلوث الدجاج الطازج ببكتيريا *Escherichia coli* O157:H7 الممرضة وكذلك تؤدي لإطالة مدة الصلاحية.