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INHIBITION OF *Salmonella* ON POULTRY AS INFLUENCED BY FLASH PASTEURIZATION, LACTIC ACID BUFFERED SYSTEM AND MODIFIED ATMOSPHERE PACKAGING.

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ABSTRACT

The effect of the treatment with flash pasteurization (FP) for 15 seconds; decontamination with 10% lactic acid buffered system pH3 (LABS); Modified Atmosphere Packaging (MAP) (90% CO_2 + 10% O_2); combination of FP+LABS; FP+MAP; LABS+MAP and finally FP+LABS+MAP on *Salmonella typhimurium* of chicken legs stored at 5°C was investigated. The initial artificial contamination level of *Salmonella typhimurium* on the chicken legs surface was 4.76 log_{10} cfu/cm^2 of skin. A reduction of 2.42 and 2.65 log10 cfu/cm^2 units of *Salmonella typhimurium* were obtained by the treatments with flash pasteurization (FP) and 10% lactic acid buffer system pH3 (LABS), respectively as compared with untreated sample. Treatment with; FP+LABS+ MAP and LABS+ MAP eliminated 4.76log_{10} cfu/cm^2 of *Salmonella typhimurium* from poultry within 10 and 14days of storage at 5 °C, respectively. Such elimination would improve the safety of poultry. After 6, 8 and 10 days of storage at 5 °C , there was significant decrease ($P<0.05$) in the log_{10} cfu/cm^2 of *Salmonella typhimurium* for all samples treated with LABS+ MAP compared with legs only packed in modified atmosphere MAP. Likewise a similar significant decrease ($P<0.05$) was observed between samples treated with FP+ MAP and legs only packed in modified atmosphere MAP. This can be explained by a synergistic effect between lactic acid buffer system pH3 (LABS) and MAP in one hand; and synergistic effect between flash pasteurization (FP) and modified atmosphere packaging (MAP) on the other hand. Results also revealed that FP+MAP efficacy against *Salmonella typhimurium* was enhanced by 10% lactic acid buffer system (LABS). Results showed that the total viable bacteria, *Enterobacteriaceae,* and lactic acid bacteria were inhibited by all treatments used as compared with the untreated sample. The buffering capacity of the buffered system LABS seemed to be sufficient to maintain a low pH of the skin during storage at 5°C. These synergistic effects between FP; LABS and MAP would improve the safety against pathogenic bacteria on fresh poultry during storage at 5°C.

Keywords: *Salmonella typhimurium*; Inoculation; Poultry; Flash pasteurization; Lactic acid buffer system; Modified atmosphere packaging.

INTRODUCTION

Food-borne illness is a major international public health concern and a significant cause of reduced economic growth (Bryan and Doyle, 1995; White *et al.*, 1997). The *Salmonella* is a leading cause of bacterial food borne disease outbreaks in developed countries (FAO/WHO, 2002 ; Sumner *et al.*, 2004; Kramer *et al.*, 2005; Francis *et al.*, 2006) and is also a public health concern in developing countries (Sow *et al.*, 2000; Medeiros *et al.*, 2001; Antunes *et al.*, 2003), which causes diarrhea, fever, headache, nausea, abdominal pain, vomiting, and less frequently, blood in the stool (White *et al.*, 1997; Schmidt, 1998; Bacumler *et al.*, 2000). Diarrhea, a common symptom of human salmonellosis, kills three million children each year in developing countries (White *et al.*, 1997; Medeiros *et al.*, 2001). Poultry products are frequent vehicles in the transmission of *Salmonella*, dominating other foods of

animal origin as potential source of infection (Mulder, 1999; Bacumler *et al.*, 2000; Capita *et al.*, 2003; Mike *et al.*, 2005; Mead, 2006). The pathogenicity of *Salmonella* is very high because a single *Salmonella* cell may constitute a human infectious dose (FAO/WHO, 2002 ; D'Aoust, 2005). This development has stimulated the interest of researchers and the food industry in determining the potential of the organism to contaminate various foods and survive processing procedures (Carramina *et al.*, 1997; WHO, 2000; Sumner *et al.*, 2004; Mead, 2006).

The objective of this investigation was to evaluate the effect of the treatment with flash pasteurization; lactic acid buffered system and modified atmosphere packaging on the inhibition of *Salmonella typhimurium* of fresh poultry stored at 5°C.

MATERIALS AND METHODS

Preparation of bacterial inoculum:

Salmonella typhimurium was kindly provided by prof.J.M.Debevere (Faculty of Bioscience Engineering, University of Gent, Belgium). Culture was activated by transferring a loop from frozen (-20 °C) stock culture in 10 ml Tryptone Soya Broth (TSB) (Oxoid CM 129), and incubated for 24 h at 35°C. One drop of this TSB culture was transferred into a second tube of sterile TSB, which was again incubated for 24 h at 35°C. One loop of this culture was streaked onto plate count agar (PCA) (Oxoid CM 325) and incubated for 24 h at 35 °C. A colony of the PCA culture was transferred into 100 ml of sterile BHI and incubated for 24 h at 35 °C. The working culture of *Salmonella typhimurium* was prepared by diluting the BHI culture in 25 liter sterile physiological saline containing 0.1% peptone so that the number of *Salmonella typhimurium* was 68× 105 cfu/ml. The working cultures were made in duplicate (each 25 liter).

Chicken legs inoculation and treatment application: Frech chicken legs were obtained immediately after slaughter from a local commercial poultry processing plant (In Alexandria). The samples were transported under refrigeration to the laboratory of Faculty of Agric. Saba Bacha, within two hours. Legs were used for practical reasons, instead of whole carcasses.

One hundred and sixty two of fresh chicken legs were submerged in the working culture of *Salmonella typhimurium* (Each 81 in 25 liter of working cultures) for 2 min. After this artificial contamination the legs were then kept at 5 °C for 2 h to drain and to allow the attachment of the *Salmonella typhimurium* cells on the skin. The contamination level was 57×10^3 cfu/cm^2 (None treated Controls). Non treated samples (30 samples) (Controls) were packed separately in Sidamil plastic bags (permeability: 6ccO_2 /m^2 /24h, 15cc CO_2 /m^2/ 24h, 2ccN_2 /m^2 /24h, at 25 °C and 100% RH) and stored at 5 °C. Twenty one samples of non treated samples were packed in modified atmosphere (MAP).

Flash pasteurization: Fresh chicken legs (87 chiken legs) were pasteurized by immersion in boiling water (1:2 w/v) at 95°C for 15 seconds, chicken legs were allowed to cool and drain at 5°Cfor two hrs, then packed in Sidamil plastic bags (permeability: 6ccO_2 /m^2 /24h, 15cc CO_2 /m^2/ 24h, 2ccN_2 /m^2 /24h, at 25 °C and 100% RH) and stored at 5°C and 96% RH. Twelve

samples were treated with (FP), twenty four samples treated with FP+MAP; twenty one samples treated with FP+LABS and Thirty chicken legs were treated with FP+LABS+MAP.

Lactic acid buffered system treatment: Chicken legs (45 chicken legs) were decontaminated by spraying with lactic acid /sodium lactate buffered system pH3 according to Zeitoun and Debever, 1990. Spraying was performed uniformly over the surface on both sides of the legs by using spray gun. After this treatment, the chicken legs were allowed to drain at 5°C for two hrs, packed separately in Sidamil plastic bags and stored at 5°C. 18 samples were treated only with LABS; 27 Samples of LABS were packed in modified atmosphere (LABS+MAP).

Gas packaging: Samples of flash pasteurized; flash pasteurized and decontaminated with 10% lactic acid buffered system; decontaminated with 10% lactic acid buffered and untreated samples were packed in Sidamil plastic bags. All bags were totally evacuated from air and completely flushed with gas mixture of 90% CO_2 and 10% O_2 (International Co. for Air and Gases Products, El-Sadat city) and then heat sealed. The bags were stored at 5°C.

Microbiological sampling and analysis: At each sampling time, three legs were sampled aseptically taken by means of excision of surface areas of 15 cm^2 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 physiological saline containing 0.1% peptone and were plated. The *Salmonella* Chromogenic Agar Base medium (Oxoid CM 1007) was used for selective enumeration of *Salmonella typhimurium* (Antunes *et al.*, 2003; Capita *et al.*, 2003). The plates were incubated at 35°C for 24 hr, red colonies with black centers were consider as presumptive *Salmonella typhimurium*. All presumptive colonies of *Salmonella typhimurium* grown on Chromogenic Agar Base medium were streaked onto Brilliant Green Agar (BGA) (Oxoid CM329) and incubated for 24 hr at 35°C. Red colonies surrounded by bright red were subjected to the following identification tests: Gram stain, catalase, cultured onto triple sugar iron Agar (TSIA) (Merck No. 3915), Lysine Decarboxylase Broth (Oxoid CM 308) and Urea Agar Base (Oxoid CM53)(Antunes *et al.*, 2003; Capita *et al.*, 2003; Dawn *et al.*, 2007). Total viable bacteria 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). *Enterobacteriaceae* were determined as colony forming units on Violet Red Bile Glucose Agar (VRBG) (Oxoid CM 485), overlaid with the same medium and incubated at 37 °C for 24 h. (Zeitoun *et al.*, 1994; Panagiotis and George, 2002). Lactic acid bacteria were assessed as colony forming units on MRS agar (Oxoid 361) with an over lay of the same agar incubated for 3 days at 30°C (Jimenez *et al.*, 1999; Panagiotis and George, 2002).

The 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: Data were analyzed using two ways analysis of variances (ANOVA) and means were compared by least significant difference (LSD) test, at a significance level of P=0.05 (Waller and Duncan, 1969). Computations were done using SAS (1996). Bacterial counts are expressed as $\log_{10}$ /cm^2. Values of zero count were recorded as $\log_{10}$ of 1 for purposes of statistical analysis.

RESULTS AND DISCUSSION

The effect of treatment with flash pasteurization (FP), 10% lactic acid buffered system (pH3) (LABS) and Modified Atmosphere Packaging (MAP) on pH of the skin of chicken legs artificially contaminated with *Salmonella typhimurium* at 57 × 10^3 cfu/cm^2 are shown in Table 1. The Initial pH value for the skin of chicken legs used in this study was 6.34. A reduction of 2 pH units is obtained by the treatment with 10% lactic acid buffered system pH3 (LABS). The pH values of chicken legs treated with LABS; modified atmosphere packaging (MAP); LABS+ MAP; FP + LABS; FP+ MAP and FP +LABS +MAP were significantly decreased (P< 0.05) when stored for 2 and 3 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; Zeitoun *et al.*, 1994; Panagiotis and George, 2002). In contrast, the buffering capacity of the buffered system LABS seemed to be sufficient to maintain a pH of the skin lower than the initial pH of the skin for 8 days of storage at 5°C. Similar trend was obtained by Zeitoun and Debever, (1990).

Salmonella is the most common food poisoning bacteria associated with refrigerated poultry (Mulder, 1999; Bacumler *et al.*, 2000; FAO/WHO, 2002; Capita *et al.*, 2003; Mike *et al.*, 2005; Mead, 2006). Thus, reducing *Salmonella* contamination of poultry products will reduce the risk of foodborne disease to consumers (Bryan and Doyle, 1995; FAO/WHO, 2002; Mead, 2006). The antimicrobial activity of flash pasteurization (FP), lactic acid buffer system (LABS) and Modified Atmosphere packaging (MAP) against *Salmonella typhimurium* is shown in Table 2. The initial contamination level of *Salmonella typhimurium* on chicken legs surface was 4.76 $\log_{10}$ cfu/cm^2 of skin. A reduction of 2.65 $\log_{10}$ units were obtained by the treatments with 10% lactic acid buffer system pH3 (LABS). These results are contradictory to those obtained by Anang *et al.*, (2006). The antimicrobial activity of lactic acid is the result of decrease in pH and a specific antimicrobial effect of the undissociated molecule (Smulders *et al.*, 1986; Van der Marel *et al.*, 1988; Zeitoun and Debevere, 1993). The number of *Salmonella typhimurium* on samples treated with flash pasteurization (FP) was reduced by 2.42 $\log_{10}$ cfu/cm^2. This means that the probability of its survival under this condition is 1/anti-log of 2.42 or 1/263.

Table (1). Effect of treatment with flash pasteurization (FP), lactic acid buffer system (LABS) (pH3) and Modified Atmosphere Packaging (MAP) on pH of the skin of chicken legs artificially contaminated with *Salmonella typhimurium*.

Treatments	Days of storage at 5 °C									
	0	2	3	6	8	10	12	14	16	18
Blank	6.34Ca	6.67Ba	7.02Aa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Flash pasteurization (FP)	6.38Da	6.53Cb	6.71Bb	6.93Aa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10% LABS (pH3)	4.37Eb	4.34Ed	4.61Dd	5.13Cd	5.65Bc	6.42Ab	n.d.	n.d.	n.d.	n.d.
MAP (90% CO_2 + 10% O_2)	6.34Ca	6.11Dc	6.19Dc	6.30Cb	6.38Ca	6.56Ba	6.70Aa	n.d.	n.d.	n.d.
FP + LABS	4.35Eb	4.32Ed	4.30Ee	4.57De	4.98Cd	5.67Bd	6.79Aa	n.d.	n.d.	n.d.
FP+ MAP	6.38BCa	6.05Gc	6.12FGc	6.17EFc	6.24Db	6.31CDc	6.43Bb	6.57Aa	n.d.	n.d.
LABS + MAP	4.37Gb	4.18He	4.21Hef	4.54Fe	4.77Ee	5.16Df	5.58Cc	5.83Bb	6.35Aa	n.d.
FP + LABS + MAP	4.35GHb	4.16Ie	4.12If	4.31Hf	4.48Ff	4.75Eg	4.92Dd	5.28Cc	5.72Bb	6.21A

1. Values with the same superscripts in the same horizontal row (A-G) or vertical column (a-h) are not significantly different ($p \geq 0.05$).
2. n.d. = not determined because of spoilage.
3. FP= Flash pasteurization.
4. LABS= 10% Lactic acid buffer system pH3.
5. MAP = 90% CO_2 + 10% O_2.

Table (2). Effect of treatment with flash pasteurization (FP), lactic acid buffer system (LABS) (pH3) and Modified Atmosphere Packaging (MAP) on *Salmonella typhimurium* count of chicken legs stored at 5°C.

Treatments	Log_{10}CFU of *Salmonella* count at n days of storage at 5°C									
	0	2	3	6	8	10	12	14	16	18
Blank	4.76Ca	5.38Ba	6.17Aa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Flash pasteurization (FP)	2.34Cb	2.41Cc	2.85Bc	3.92Aa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10% LABS (pH3)	2.11Dc	1.83Ee	2.08Dd	2.34Cb	2.75Bb	3.25Ab	n.d.	n.d.	n.d.	n.d.
MAP (90% CO_2 + 10% O_2)	4.76Aa	4.02Bb	4.10Bb	3.90Ca	3.85Ca	3.52Da	3.23Ea	n.d.	n.d.	n.d.
FP + LABS	1.73Dd	1.61Ef	1.65Ef	1.83Dc	2.08Cc	2.43Bc	2.98Ab	n.d.	n.d.	n.d.
FP+ MAP	2.34Ab	2.03Bd	1.83Ce	1.91Cc	1.71Dd	1.65Dd	1.42Ec	1.38Ea	n.d.	n.d.
LABS + MAP	2. 11Ac	1.67Bf	1.45Cg	1.33Dd	1.25De	1.11Ee	0.85Fd	0.00Gb	0.00Ga	n.d.
FP + LABS + MAP	1.73Ad	1.32Bg	1.11Ch	0.85De	0.50Ef	0.00Ff	0.00Ff	0.00Fb	0.00Fa	0.00F

1. Values with the same superscripts in the same horizontal row (A-G) or vertical column (a-h) are not significantly different ($p \geq 0.05$).
2. n.d. = not determined because of spoilage.
3. FP= Flash pasteurization.
4. LABS= 10% Lactic acid buffer system pH3.
5. MAP = 90% CO_2 + 10% O_2.

This also means that in order to have survivors of *Salmonella typhimurium* the contamination level on chicken legs should be > 263 cells/cm^2 present initially. Several investigators have been observed that flash pasteurization was effective against *Salmonella* and spoilage bacteria (Purnell *et al.*, 2004; Corry *et al.*, 2006). In contrast, the number of *Salmonella typhimurium* on untreated samples increased rapidly and rich to 6.17 log_{10} cfu/cm^2 after 3 days of storage at 5°C. Populations of *Salmonella typhimurium* on all treated

samples were significantly lower ($P< 0.05$) than those counted on untreated samples (artificially contaminated) on day 2 and day 3 of storage at 5 °C. These results are contradictory to those obtained for lactic acid bacteria (Table.3), *Enterobacteriaceae* (Table.4) and total viable count (Table.5). The antimicrobial activity of MAP against *Salmonella typhimurium* is due to the bacteriostatic effect of CO_2 which inhibits the growth of *Salmonella typhimurium*, as a result of an extension of lag phase of growth and a decrease in the growth rate during the logarithmic phase (Dixon and Kell, 1989; Farber, 1991; Church and Parsons, 1995) and also due to the antimicrobial effect produced by Lactic acid bacteria (Michael *et al.*, 1999; Singh *et al.*, 2006). Treatment with; FP+LABS+ MAP and LABS+ MAP eliminated 4.76 $\log_{10}$ cfu/cm^2 of *Salmonella typhimurium* from poultry within 10 and 14 days of storage at 5 °C, respectively. Such elimination would improve the safety of poultry. After 6, 8 and 10 days of storage at 5 °C , there was significant decrease ($P<0.05$) in the $\log_{10}$ cfu/cm^2 of *Salmonella typhimurium* for all samples treated with LABS+ MAP compared with legs only packed in modified atmosphere MAP. Likewise a similar significant decrease ($P<0.05$) was observed between samples treated with FP+ MAP and legs only packed in modified atmospherе MAP. This can be explained by a synergistic effect between lactic acid buffer system pH3 (LABS) and MAP in one hand; and synergistic effect between flash pasteurization (FP) and modified atmosphere packaging (MAP) on the other hand. Results also revealed that FP+MAP efficacy against *Salmonella typhimurium* is enhanced by 10% lactic acid buffer system (LABS).

Lactic Acid Bacteria are constituted of a heterogeneous group of Gram positive bacteria with a strictly fermentative metabolism from which lactic acid is the key metabolite (Singh *et al.*, 2006; Topisirovic *et al.*, 2006). Moreover Bacteriocins of lactic acid bacteria have the potential to prevent microbial food spoilage and to inhibit growth of pathogens (Singh *et al.*, 2006; Topisirovic *et al.*, 2006).In Food matrices the bacteriocin activity may be affected by (i) changes in solubility and charge of the bacteriocins, (ii) binding of the bacteriocins to food components, (iii) inactivation by proteases, and (iv) changes in the cell envelope of the target organisms (Michael *et al.*, 1999). Moreover their long history of safe use commonly referred to as the GRAS (Generally Recognized As Safe) Status, combined with a variety of interesting metabolic characteristics have led to a wide range of industrial applications (Michael *et al.*, 1999; Singh *et al.*, 2006; Topisirovic *et al.*, 2006). Changes in lactic acid bacteria are presented in Table 3. The initial number of lactic acid bacteria was 3.84 $\log_{10}$ CFU/cm^2 on blank samples. A reduction of 1.09 $\log_{10}$ CFU/cm^2 of lactic acid bacteria was obtained for treatment with LABS as compared with blank samples. The data indicate that there was a significant decrease ($P< 0.05$) in $\log_{10}$ CFU/cm^2 of lactic acid bacteria on legs treated with LABS + MAP compared with legs only packed in modified atmosphere (MAP), after 2, 3, 6, 8, 10 and 12 days of storage 5°C. This difference is due to the inhibitory effect of LABS. On the day of spoilage, on all samples packed in modified atmosphere (MAP), irrespective of the treatment received, lactic acid bacteria were found to be the dominating flora. This observation is supported by the work of other investigators (Dixon and

Kell, 1989; Farber, 1991; Church and Parsons, 1995; Bjorn *et al.*, 2006) who have demonstrated that lactic acid bacteria are less inhibited by CO_2 than Gram-negative bacteria.

The assessment of *Enterobacteriaceae* commonly formed a part of the microbiological quality monitoring of foods processed for safety, and their presence in numbers of CFU exceeding carefully established levels is traditionally related to hygiene and safety (Mossel, 1975; Zeitoun, *et al.*, 1994).The ANOVA indicates that storage time and treatment effects were significant ($P< 0.05$) in *Enterobacteriaceae* counts (Table.4). The growth of *Enterobacteriaceae* on all samples packed in modified atmospheres was strongly inhibited. This fact is mainly due to the inhibitory effect of the higher concentration of CO_2 (90%) on *Enterobacteriaceae* growth (Zeitoun and Debever, 1992; Panagiotis and George, 2002). In contrast the $\log_{10}$ cfu/cm^2 of *Enterobacteriaceae* on blank samples (control) increased rapidly and reached to 6.73 $\log_{10}$ cfu/cm^2 after 3 days of storage at 5°C. Meainwhile the number of *Enterobacteriaceae* on samples treated with FP + LABS + MAP was Lower than the initial number by 1.16 $\log_{10}$ CFU/cm^2 after 17 days of storage at 5°C. A reduction of 2.67, 2.88 and 3.43 $\log_{10}$ CFU/cm^2 was obtained for treatment with FP, LABS and FP+LABS, respectively as compared with blank. Such reduction would enhance the safety of the poultry (Mossel, 1975; Van der Marel *et al.*, 1988; Corry *et al.*, 2006).

The effect of storage time X treatment interaction on total viable bacteria was significant ($P<0.05$), as shown in Table 5. The initial number of total viable bacteria was 5.84 $\log_{10}$ CFU/cm^2 on fresh chicken legs artificially contaminated with *Salmonella typhimurium*. The number of total viable bacteria was reduced by 1.50, 1.97 and 2.59 $\log_{10}$ cfu/cm^2 for treatment with FP; LABS and FP+ LABS respectively, as compared with untreated sample. After 3 days of storage at 5°C, the number of total viable bacteria on samples packed in modified atmosphere packaging (MAP) was significantly lower ($P< 0.05$) than that counted on untreated samples (artificially contaminated). This due to the inhibiting effect of CO_2 (Jimenez *et al.*, 1999; Bjorn *et al.*, 2006). 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 (Smulders *et al.*, 1986; Van der Marel *et al.*, 1988; Zeitoun and Debevere, 1992). All samples at the end of storage periods were below the critical marginal quality, followed by off odour next day. Samples treated with flash pasteurization (FP); 10% lactic acid buffer system (LABS); Modified Atmosphere Packaging (MAP); FP+LABS; FP+MAP; LABS+MAP and FP+LABS+MAP showed shelf life of 6, 10, 12, 12, 14, 16 and 18 days of storage at 5 °C, respectively. However, the untreated artificially contaminated legs (control) had 3 days.

Table (3). Effect of treatment with flash pasteurization (FP), lactic acid buffer system (LABS) (pH3) and Modified Atmosphere Packaging (MAP) on lactic acid bacteria count of chicken legs stored at 5°C.

Treatments	Log_{10} CFU of lactic acid bacteria count at n days of storage at 5°C									
	0	2	3	6	8	10	12	14	16	18
Blank	3.84Ca	4.37Ba	4.93Aa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Flash pasteurization (FP)	3.09Db	3.47Cc	3.84Bc	4.68Aa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10% LABS (pH3)	2.75Ec	2.97Dfg	3.12Dd	3.54Cb	4.03Bb	4.86Ab	n.d.	n.d.	n.d.	n.d.
MAP (90% CO_2 + 10% O_2)	3.84Fa	3.91Fb	4.35Eb	4.64Da	4.97Ca	5.87Ba	6.62Aa	n.d.	n.d.	n.d.
FP + LABS	2.32Fd	2.41Fh	2.57Ee	2.84Dc	3.24Cc	3.86Bd	4.79Ac	n.d.	n.d.	n.d.
FP+ MAP	3.09Fb	3.17Fdf	3.13Fd	3.52Eb	3.87Db	4.65Cc	5.27Bb	6.56Aa	n.d.	n.d.
LABS + MAP	2.75Hc	2.82GHg	2.76He	2.94Gc	3.17Ec	3.57De	3.96Cd	5.62Bb	6.76Aa	n.d.
FP + LABS + MAP	2.32Fd	2.17Fi	2.25Ff	2.30Fd	2.84Ed	2.98Ef	3.65De	4.61Cc	5.72Bb	6.64A

1. Values with the same superscripts in the same horizontal row (A-G) or vertical column (a-h) are not significantly different ($p \geq 0.05$).
2. n.d. = not determined because of spoilage.
3. FP= Flash pasteurization.
4. LABS= 10% Lactic acid buffer system pH3.
5. MAP = 90% CO_2 + 10% O_2.

Table (4). Effect of treatment with flash pasteurization (FP), lactic acid buffer system (LABS) (pH3) and Modified Atmosphere Packaging (MAP) on *Enterobacteriaceae* count of chicken legs stored at 5°C.

Treatments	Log_{10} CFU of *Enterobacteriaceae* count at n days of storage at 5°C									
	0	2	3	6	8	10	12	14	16	18
Blank	5.61Ca	6.14Ba	6.73Aa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Flash pasteurization (FP)	2.94Db	3.87Cc	4.68Bb	6.25Aa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10% LABS (pH3)	2.73Ec	2.82Ed	3.04Dc	3.95Cc	5.02Ba	6.13Aa	n.d.	n.d.	n.d.	n.d.
MAP (90% CO_2 + 10% O_2)	5.61Ba	4.93Db	4.75Fb	4.87EFb	5.17Ca	5.68Bb	6.14Aa	n.d.	n.d.	n.d.
FP + LABS	2.18Ed	2.31Eef	2.17Ee	3.24Dd	4.15Cb	5.07Bc	5.92Ab	n.d.	n.d.	n.d.
FP+ MAP	2.94Eb	2.47Fe	2.36Fd	3.02Ee	3.91Dc	4.74Cd	5.25Bc	5.84Aa	n.d.	n.d.
LABS + MAP	2.73Fc	2.15Gf	1.87Hf	2.24Gf	2.95Ed	3.37De	3.98Cd	4.25Bb	5.38Aa	n.d.
FP + LABS + MAP	2.18Fd	1.74Hg	1.25Ig	1.37Ig	1.98Ge	2.44Ef	2.85De	3.18Cc	3.62Bb	4.45A

1. Values with the same superscripts in the same horizontal row (A-G) or vertical column (a-h) are not significantly different ($p \geq 0.05$).
2. n.d. = not determined because of spoilage.
3. FP= Flash pasteurization.
4. LABS= 10% Lactic acid buffer system pH3.
5. MAP = 90% CO_2 + 10% O_2.

Table (5). Effect of treatment with flash pasteurization (FP), lactic acid buffer system (LABS) (pH3) and Modified Atmosphere Packaging (MAP) on total viable count of chicken legs stored at 5°C.

Treatments	Log_{10} CFU of total viable count at n days of storage at 5°C									
	0	2	3	6	8	10	12	14	16	18
Blank	5.84Ca	6.37Ba	6.89Aa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Flash pasteurization (FP)	4.34Db	4.75Cb	5.12Bb	6.85Aa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10% LABS (pH3)	3.87Ec	3.92Ed	4.08Dd	4.82Cb	5.75Ba	6.92Aa	n.d.	n.d.	n.d.	n.d.
MAP (90% CO_2 + 10% O_2)	5.84Ca	5.08Ec	4.92Fc	4.86Fb	5.38Db	6.17Bb	7.05Aa	n.d.	n.d.	n.d.
FP + LABS	3.25Ed	3.18Ef	3.27Ef	3.85Dd	4.46Cd	5.37Bc	6.78Ab	n.d.	n.d.	n.d.
FP+ MAP	4.34Eb	3.82Fd	3.65Ge	4.27Ec	4.69Dc	5.08Cd	5.85Bc	6.96Aa	n.d.	n.d.
LABS + MAP	3.87Ec	3.41Ge	3.18Hf	3.57Fe	3.92Ee	4.27De	4.71Cd	5.76Bb	6.94Aa	n.d.
FP + LABS + MAP	3.25Fd	2.94Gg	2.75Hg	2.96Gf	3.17Ff	3.42Ef	3.65De	4.01Cc	5.13Bb	6.67Aa

1. Values with the same superscripts in the same horizontal row (A-G) or vertical column (a-h) are not significantly different ($p \geq 0.05$).
2. n.d. = not determined because of spoilage.
3. FP= Flash pasteurization.
4. LABS= 10% Lactic acid buffer system pH3.
5. MAP = 90% CO_2 + 10% O_2.

Conclusion

a synergistic effect between flash pasteurization (FP), lactic acid buffer system (LABS) and modified atmosphere packaging (MAP) was evidence which improves safety against pathogenic bacteria.

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تثبيط نشاط الـ *Salmonella typhimurium* في الدجاج المبرد باستخدام البسترة السريعة وحامض اللاكتيك المنظم والتعبئة في جو غازي معدل.

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تم إجراء المعاملات المنفردة المختلفة التالية علي أفخاذ الدجاج الطازج :

البسترة السريعة لمدة ١٥ ثانية علي درجة حرارة ٩٥°م أو الرش بمحلول حامض اللاكتيك المنظم ١٠% أو التعبئة في جو غازي معدل مكون من خليط ٩٠% ثاني أكسيد كربون و١٠% أكسيجين . وكذلك المعاملات المشتركة التالية البسترة السريعة + الرش بمحلول حامض اللاكتيك المنظم ١٠% كمعاملة واحدة،، البسترة السريعة + التعبئة في جو غازي معدل كمعاملة مستقلة ، ثم الرش بمحلول حامض اللاكتيك المنظم ١٠% + التعبئة في جو غازي معدل، وأخيراً تم عمل البسترة السريعة + الرش بمحلول حامض اللاكتيك المنظم ١٠% + التعبئة في جو غازي معدل معا كمعاملة واحدة وذلك لدراسة تأثير هذه المعاملات علي *Salmonella typhimurium* الملقحة علي أفخاذ الدجاج الطازج المخزن علي درجة حرارة ٥°م. كان مستوي العدد البكتيري من السالمنيلا الملقحه صناعيا على سطح جلد افخاذ الدجاج هو $4.76 \log_{10} cfu/cm^2$.

أدت المعاملة بالبسترة السريعة و المعاملة بالرش بالحامض الي انخفاض العدد من السالمونيلا الملقحة بمقدار $2.42 \log_{10} cfu/cm^2$ و $2.65 \log_{10} cfu/cm^2$ علي التوالي. ولقد أدت المعاملة بالبسترة السريعة +الرش بالحامض + التعبئة في جو غازي معدل الي إزالة $4.76 \log_{10} cfu/cm^2$ من السالمونيلا في ١٠ أيام في الدجاج المخزن علي درجة حرارة ٥°م بينما المعاملة بكل من الحامض والتعبئة في الجو الغازي أدت الى نفس النتيجة بعد ١٤ يوم. هذا التخلص من السالمونيلا سوف يحسن ويؤكد سلامة الدجاج المبرد. وقد أوضحت النتائج انخفاض معنوي في عدد السالمونيلا للمعاملات بالحامض + الغاز المعدل مقارنة بالمعاملة بالغاز فقط بعد ٦و ٨ و١٠ يوم من التخزين علي درجة ٥°م. وأيضا وجد انخفاض معنوي ما بين العينات المعاملة بالبسترة السريعة + الغاز المعدل مع العينات المعاملة بالغاز المعدل فقط. هذه النتيجه يمكن أن ترجع إلي التأثير المحفز بين نظام الحامض والجو الغازي المعدل من جهة وأيضا التأثير المحفز بين البسترة والجو الغازي المعدل من جهة أخري .أيضا أظهرت النتائج أن كفاءة المعاملة بالبسترة السريعة والجو الغازي المعدل ضد السالمونيلا تم زيادتها بواسطة المعاملة بحامض الاكتيك. أدت هذه المعاملات الي تثبيط واضح لكل من العدد الكلي البكتيري والـ *Enterobacteriaceae* وبكتريا حامض اللاكتيك اذا ما قورنت بالعينة الغير معاملة .ولقد كانت السعة التنظيمية للحامض كافية لإبقاء الـ pH منخفض أثناء التخزين المبرد. هذا التأثير الحفزي بين البسترة السريعة والمعاملة بحامض اللاكتيك والتعبئة في جو غازي معدل سوف يحسن من سلامة الدجاج المبرد ضد البكتريا الممرضة.