Microbiological Changes of Marinated Broiler Drumsticks Treated with the Lactoperoxidase System and with or without Thermal Treatment

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ABSTRACT : The objective of this study was to evaluate the combined effects of lactoperoxidase system (LPS), thermal treatment and storage time on total microflora and psychrotrophs counts of the marinated broiler drumsticks. A marinade that contained acetic acid (1%) and salt (3%) with pH adjusted to 4 was developed as a standardized marinade. Drumsticks were marinated with various LPS levels, combined with thermal treatment (4 or 58°C for 2 min), and then stored at 4°C for 18 h. The microbial counts of the samples were measured after 0, 2, 4 and 7 days of storage for drumsticks held at 4°C. The results indicate that adding LPS at the level of 1 unit (1 μg/ml LP, 5.9 mM KSCN, and 2.5 mM H_2O_2) significantly ($p<0.05$) decreased the total microflora and psychrotrophs counts of the marinated broiler drumsticks. In addition, samples treated with a thermal treatment (58°C for 2 min) had significantly ($p<0.05$) lower microbial counts when compared with the control. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 1 : 109-112)***

Key Words : Microbial Quality, Marination, Lactoperoxidase System, Thermal Treatment

INTRODUCTION

Marination is a procedure of treating meat with an aqueous mixture of vinegar, salt and spices before cooking. This process is widely applied in the meat and poultry industry because it increases variety and adds value to products. Cannon et al. (1993) explained marinating as a process involving incorporation of acidic or alkaline solution into products to alter pH of the tissue. Currently, marination is practiced to improve poultry product's physical and sensory attributes, such as cooking yield, tenderness, water holding capacity, flavor and etc (Young and Lyon, 1997a,b; Lemos et al., 1999; Xiong and Kupski, 1999; Young and Buhr, 2000; Zheng et al., 2000), but the process is usually not intended to improve the microbial safety of the product.

The lactoperoxidase system (LPS), which consists of lactoperoxidase (LP), thiocyanate (SCN^-), and hydrogen peroxide (H_2O_2), is an inhibitory system that is present naturally in bovine milk. This system has been shown to be inhibitory against some spoilage and pathogenic microorganisms such as *Pseudomonas fluorescens*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella typhimurium* (Earnshaw et al., 1990; Kamau et al., 1990 a,b; Zapico et al., 1995). The LPS has been mainly studied for the application in the milk and dairy products (Reiter and Harnulv, 1984; Zapico et al., 1998), whereas only a few studies have been attempted to apply this LPS system in meat and poultry products (Wolfson et al., 1994; Kennedy et al., 2000). Thermal or heating treatment, which is one of the most commonly used physical methods to reduce the load of microorganisms, has been widely applied to preserve foods for years. Heating is involved in many food processes, such as cooking, scalding, pasteurizing, drying and etc.

Based on this information, thermal treatment could be used to inactive some microorganisms and extend shelf life of poultry products. In addition, LPS, which has been widely applied in milk and dairy products, should be valuable when applied to meat products. In this study, effects of LPS, thermal treatment and storage time on the microbiological changes on marinated broiler drumsticks were investigated.

MATERIALS AND METHODS

Development of the marinade solutions

After evaluating published marination recipes, acid, salt and some flavoring agents (such as black pepper and garlic powder) were recognized as the major components of most of these marinades. Based on the sensory results from preliminary experiments, a simplified water-base marinade that contains acetic acid (1%) and salt (3%) with pH adjusted to 4 (using HCl or NaOH solutions) was applied as the standardized marinade in this study.

Lactoperoxidase system preparation

The components of the LPS, including bovine milk lactoperoxidase (LP, EC 1.11.1.7; purity index 0.82 (A_{412}/A_{280}); Sigma Chemical Co., St. Louis, Missouri), hydrogen peroxide (H_2O_2, 30%, Fisher Scientific Co.; Pittsburgh, PA) and potassium thiocyanate (KSCN, Fisher Scientific Co.; Pittsburgh, PA), were added into the

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Table 1. Effect of LPS-added level on the total microflora and psychrotrophs counts of marinated chicken drumsticks

LPS addition (unit)[1]	Total microflora count[2] (log CFU/ml)	Psychrotrophs count[3] (log CFU/ml)
0	7.54±0.08[a]	7.04±0.08[a]
1	6.61±0.09[b]	6.22±0.07[b]
2	6.31±0.09[c]	5.89±0.07[c]

[a, b, c] Means within a column with different superscript are significantly different ($p<0.05$).

[1] LPS unit: 1 unit = LP (1 μg/ml), KSCN (5.9 mM), and H_2O_2 (2.5 mM); 2 unit = LP (2 μg/ml), KSCN (11.8 mM), and H_2O_2 (5.0 mM)

[2] Total microflora count: incubated at 35°C for 48 h.

[3] Psychrotrophs count: incubated at 7°C for 10 days.

standardized marinade solution. The LP and H_2O_2 were prepared in distilled water, and filter sterilized separately using a 0.45 μm filter (Gelman Sciences, Ann Arbor, MI), and the KSCN solution was autoclaved. The individual components were then added to the marinade solution no earlier than 5 min before marinating. The concentrations of 1 unit of LPS were: 1 μg/ml of LP, 5.9 mM of KSCN, and 2.5 mM of H_2O_2; the concentrations of 2 units of LPS were: 2 μg/ml of LP, 11.8 mM of KSCN, and 5.0 mM of H_2O_2.

Sample preparation

A total of 96 drumsticks in commercial packages were purchased from a local supermarket, placed in an insulated container to maintain sample temperature, transported to the laboratory within 30 min, and then stored in a 4°C walk-in cooler until experimental trials were conducted. Inside the walk-in cooler, drumsticks were initially mixed thoroughly within Scienceware heavyweight polyethylene bags (Fisher Scientific Co., Pittsburgh, PA) for 5 min to obtain even distribution of bacteria over the surfaces and to insure randomness in assigning drumsticks to the different treatments. After mixing, drumsticks were randomly chosen, equally assigned and labeled to the treatment groups. For those samples without thermal treatment, two drumsticks per treatment for each storage day were aseptically placed and marinated in a sterile plastic bag with 400 ml autoclaved marinade solution so that all the drumsticks could be covered completely by the marinade solution and stored at 4°C for 18 h.

Thermal marination preparation

For those samples with thermal-marinating treatment, the pre-autoclaved marinade solutions with various levels of LPS were added to sterile bags, which were heated in a water bath before the marinating process. Drumsticks were then aseptically added to the bags with the heated marinade solutions. After holding at 58°C for 2 min, the marinade-drumstick mixes in bags were cooled by immersing the bags in running tap water. When the marination mix was cooled to 25°C (approximately 10 min), the marination mix was then refrigerated at 4°C for 18 h (including the previous time of thermal marinating and cooling).

Microbial evaluation

After finishing marinating, the marinated drumsticks were aseptically removed and drained for 2.5 min, rotated, and drained an additional 2.5 min in a walk-in cooler maintained at 4°C. Following marinating and draining, the drumsticks were packaged individually in sterile plastic bags and storage under refrigeration at 4°C. At specified sampling times (0, 2, 4, or 7 day), using a rinse procedure**,** each drumstick was placed in a bag containing 20 ml of 0.1% peptone water (Difco Laboratories, Detroit, MI) and manually shaken for 2 min to facilitate removal of the microorganisms. Serial dilutions were then made with 0.1% peptone as the diluents. Duplicate plates using plate count agar (Difco Laboratories, Detroit, MI) and the pour plate method were prepared for enumeration of bacteria in each bacteria group. Total microflora and psychrotrophs were incubated at 35°C for 48 h and 7°C for 10 days, respectively. Microbial counts in this study were expressed as $\log_{10}$ colony forming units (CFU) per ml of peptone rinse.

Statistical analyses

The study was designed as a 3×2×4 factorial experiment, 3 LPS added levels (0, 1, and 2 units; 1 unit = 1 μg/ml LP, 5.9 mM KSCN, and 2.5 mM H_2O_2; 2 units = 2 μg/ml LP, 11.8 mM KSCN, and 5.0 mM H_2O_2), two thermal treatments (4 or 58°C for 2 min), and 4 storage times (day 0, 2, 4, and 7), and with two replicates. Least square mean (LSM) was analyzed using the general linear model (GLM) of Statistical Analysis System's Procedures (SAS Institute Inc., Cary, NC) at a 5% level of significance. A complete three-way GLM model was first used to analyze each measurement. Then, a new two-way GLM reduced model was conducted by SAS after the three-way interaction was removed from the model if the three-way interaction was not significant at the 0.05 level ($p>0.05$).

RESULTS AND DISCUSSION

In the current study, there was no significant ($p>0.05$) three-way and two-way interactions among the three factors of lactoperoxidase system (LPS) added level, thermal treatment and storage time for the total microflora counts for the samples.

As illustrated in Table 1, in the current study, as the LPS-added levels increased, less total microflora counts of the samples were obtained for the samples. Adding LPS at a level of 1 unit, which consisted of 1 μg/ml LP, 5.9 mM KSCN, and 2.5 mM H_2O_2 resulted in a significantly ($p<0.05$) lower total microflora counts of 6.61 log CFU/ml, when compared with samples without adding any LPS

Table 2. Effect of thermal treatment on the total microflora and psychrotrophs counts of marinated chicken drumsticks

Treatment	Total microflora count[2] (log CFU/ml)	Psychrotrophs count[3] (log CFU/ml)
Non-thermal treatment	7.11±0.07[a]	6.55±0.06[a]
Thermal treatment[1]	6.53±0.07[b]	6.22±0.06[b]

[a, b] Means within a column with different superscripts are significantly different ($p<0.05$).

[1] Thermal treatment: samples were aseptically placed in a pre-heated autoclaved marinade solution at 58°C for 2 min, cooled, and then refrigerated storage at 4°C for 18 h.

[2] Total microflora count: incubated at 35°C for 48 h.

[3] Psychrotrophs count: incubated at 7°C for 10 days.

Table 3. Effect of storage time on total microflora and psychrotrophs counts of marinated chicken drumsticks

Storage time (day)	Total microflora count[1] (log CFU/ml)	Psychrotrophs count[2] (log CFU/ml)
0	6.95±0.10[a]	6.14±0.09[a]
2	6.84±0.10[a]	6.60±0.08[b]
4	6.75±0.10[a]	6.48±0.08[bc]
7	6.74±0.10[a]	6.31±0.09[ac]

[a, b, c] Means within a column with different superscripts are significantly different ($p<0.05$).

[1] Total microflora count: incubated at 35°C for 48 h.

[2] Psychrotrophs count: incubated at 7°C for 10 days.

which had higher counts of 7.54 log CFU/ml. Adding even more LPS to the level of 2 units resulted in a further significantly ($p<0.05$) lower microbial count of 6.31 log CFU/ml. The result implies that the more LPS added up to the level of 2 units, the less total microflora counts were obtained.

The literature suggest at higher levels, either 20 or 40 ppm LP of the LPS, had resulted in a higher inhibitory effect against inoculated *Listeria monocytogenes* than at the lower level of 10 ppm LP after 9 and 16 h of incubation (Denis and Ramet, 1989). Kamau et al. (1990a, b) had explained that, in the presence of SCN^- and H_2O_2, the LPS generates the hypothiocyanite ($OSCN^-$) and hypothiocyanous acid (HOSCN), which are the main antimicrobial products, and strong oxidizing agents that can oxidize essential sulfhydryl groups in bacterial proteins and thus inhibits bacterial growth.

Table 2 illustrates the effect of thermal treatment on the total microflora counts of the treated samples. Samples treated with a thermal treatment, in which the samples were aseptically placed in a pre-heated autoclaved marinade solution at 58°C for 2 min, cooled, and then refrigerated storage at 4°C for 18 h, had a significantly ($p<0.05$) lower total microflora counts of 6.53 log CFU/ml, when compared with samples without a thermal treatment, which had a higher count of 7.11 log CFU/ml. This result implies that the thermal treatment significantly ($p<0.05$) retarded the growth of total microflora microorganisms of the marinated broiler drumsticks. Banwart (1989) pointed out that damage of the cytoplasmic membrane, leakage of cellular components, alternation of metabolic capabilities of the cell, impairment of enzyme activity, and degradation of ribosomes and ribonucleic acid, might be the reasons for inactivation of microorganisms due to the heat treatment. This also might explain in the current study the samples treated with 58°C for 2 min had significant lower total microflora and psychrotrophs counts because of cell damages due to thermal treatment.

Borch et al. (1989) investigated the antibacterial effect of LPS on *Campylobacter jejuni*, which was isolated from poultry. In their study, the survival of inoculated *C. jejuni* that was treated with LPS at different incubation temperature was evaluated. The authors reported that at higher temperature (37 and 52°C), the LPS had a more pronounced bactericidal effect against *C. jejuni* than the LPS at 20°C. Denis and Ramet (1989) investigated the temperature effect of the antibacterial activity of the LPS against *Listeria monocytogenes*. In their study, the survival of inoculated *L. monocytogenes* that was treated with LPS at different incubation temperatures was evaluated. The authors reported that at higher incubation temperature of 15°C, the LPS had a higher inhibitory effect against the inoculated *L. monocytogenes* than at the lower temperature of 4°C, and explained that this result might be related to the increase of the LPS activity. However, no significant ($p>0.05$) interactions among the LPS-added level and the thermal treatment on the microbial qualities of the treated samples were observed in the current study. The possible reason of this disagreement between the studies is probably that the thermal treatment (58°C for 2 min in this study) was much less than the time of more than 0.5 h in Borch's study.

In the current study, the total microflora counts of the samples remained stable during 7 days refrigerated storage (Table 3). Typically, spoilage can be detected when bacterial numbers exceed 8 log CFU/g (Jay, 1996; Gill and Newton, 1978). Similarly, Ayres (1955) reported that off odors and slime formation on broiler carcasses held at 4.4°C were first detectable when aerobic plate counts reach a population of log 8.0 CFU/cm^2. In the current study, none of the total microflora counts of the samples exceeded this "log 8 criteria", which was approximately the point when off odors and slime might be formed and detected. Moreover, no off-odors and slime formation was detected in any of the samples in the current study when evaluated by sensory evaluation within the 7 days of refrigerated storage at 4°C.

Similar to the total microflora counts, the psychrotrophs counts of the samples exhibited similar patterns. There was no significant ($p>0.05$) three-way or two-way interactions among the three factors of LPS-added level, thermal

treatment and storage time of the psychrotrophs counts for the samples with different levels of LPS added and thermal treatment or during the refrigerated storage at 4°C in the current study.

As illustrated in Table 1, in the current study, as the LPS-added levels increased, less psychrotrophs counts of the samples were obtained for the samples. Adding 1 unit of LPS resulted in a significantly ($p<0.05$) lower psychrotrophs counts of 6.22 log CFU/ml, when compared with samples without adding any LPS which had a higher count of 7.04 log CFU/ml. Adding even more LPS to the level of 2 units resulted in a further significant ($p<0.05$) lower microbial count of 5.89 log CFU/ml. The result implies that the more LPS added up to the level of 2 units, the less psychrotrophs counts were obtained.

Table 2 illustrates the effect of thermal treatment on the microbial counts of the treated samples. Samples treated with a thermal treatment (58°C for 2 min) had a significantly ($p<0.05$) lower psychrotrophs count of 6.22 log CFU/ml, when compared with samples without thermal treatment, which had a higher count of 6.55 log CFU/ml. This result implies that the thermal treatment significantly ($p<0.05$) retarded the growth of psychrotrophic microorganisms of the marinated broiler drumsticks.

In conclusion, adding LPS at the level of 1 unit (1 μg/ml LP, 5.9 mM KSCN, and 2.5 mM H_2O_2) significantly ($p<0.05$) decreased the total microflora and psychrotrophs counts of the marinated broiler drumsticks. In addition, a thermal treatment of heating the marinade solution at 58°C for 2 min, cooling, and then refrigerated storage at 4°C for 18 h, significantly ($p<0.05$) decreased the total microflora and psychrotrophs counts of the marinated broiler drumsticks. Further research to assess the changes in physical and sensory quality of marinated broiler drumsticks treated with LPS and thermal treatment is needed.

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