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APPLICATION OF NISIN AND LACTOPEROXIDASE SYSTEM TO IMPROVE THE MICROBIOLOGICAL QUALITY OF MARINATED CHICKEN DRUMSTICKS

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Introduction

Currently, marination is practiced to improve poultry product's physical and sensory attributes (Young & Buhr, 2000; Zheng *et al*., 2000), but this process is usually not intended to improve the microbial quality of the product. Nisin, which is a natural, nontoxic, heat stable polypeptide produced by *Lactococcus lactis*, and has been shown to inhibit many microorganisms. 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, and has been shown to be inhibitory against some pathogenic and spoilage microorganisms, and has been mainly studied for the application in the milk and dairy products (Zapico *et al*., 1998). Limited information on the contribution of marination, nisin, and LPS to the microbial quality of treated products was available.

Objectives

The objective of this study was to evaluate the effects of nisin, LPS and storage time on the microbial quantity of marinated chicken drumsticks.

Methodology

A solution containing 1% acetic acid and 3% salt with pH adjusted to 4 was applied as a standard marinade. Adding nisin at levels of 0, 50 or 100 IU/ml with 20mM EDTA, the marinade solutions were adjusted to pH 4, autoclaved for 121°C for 15 min, and then stored at 4°C. A LPS consisted of 1 μg/ml of LP, 5.9mM KSCN, and 2.5mM H_2O_2. The LP and H_2O_2 were prepared in distilled water, and filter sterilized separately using a .45μm filter, and the KSCN solution was autoclaved. The individual components were then added to the marinade solution no earlier than 5 min before marinating. The contents of 1 and 2 units of LPS were 1, and 2 μg/ml LP, 5.9, and 11.8 mM KSCN, and 2.5, and 5.0 mM H_2O_2, respectively. Drumsticks were aseptically placed and marinated in a plastic bag with autoclaved marinade solution so that all the drumsticks could be covered

completely by the marinade solution and the drumsticks were marinated at 4°C for 18 hr. After marinating, the 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, and packaged individually in sterile plastic bags and storage under refrigeration at 4°C. At specified sampling times of 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 and manually shaken for 2 min. Duplicate plates using the pour plate method and plate count agar were prepared for enumeration of bacteria in each bacteria group. Total microflora and psychrotrophs were incubated at 35°C for 48 hours and 7°C for 10 days, respectively and were expressed as $\log_{10}$ CFU per ml of peptone rinse. Least square mean was analyzed using the GLM of SAS Procedures 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.

Results & Discussion

In this study, there was no significant three-way and two-way interactions among the three factors of nisin added level, LPS added level and storage time for the total microflora counts of the samples with different levels of nisin and LPS added during refrigerated storage at 4°C. Table 1 illustrates the nisin level effect on the microbial counts of the treated samples. Adding nisin at level of 50 IU/ml resulted in significantly lower total microflora counts of 7.09 log CFU/ml, when compared with the samples without adding any nisin, which had higher count of 7.46 log CFU/ml. Adding even more nisin to the level of 100 IU/ml resulted in a further significant lower microbial count of 6.83 log CFU/ml. The results imply that the more nisin added up to at level of 100 IU/ml the less total microflora counts obtained. The psychrotrophs counts of the samples exhibited similar patterns. A low concentration of nisin (either 50 or 100 IU/ml) was chosen in this study, because nisin alone was not intended to be the only hurdle treatment. In addition, even though higher levels of nisin may result in an increased effect, a lower concentration of nisin might be appropriate due the economic concern for the cost of nisin. Adding LPS at level of 1 unit resulted in significantly lower total microflora counts of 6.97 log CFU/ml, when compared with samples without adding any LPS which had a higher count of 7.71 log CFU/ml (Table 2). Adding even more LPS to the level of 2 units resulted in a further significant lower microbial count of 6.69 log CFU/ml. The results imply that the more LPS added up to at level of 2 units the less total microflora counts obtained. Similar patterns could be also observed for the psychrotrophs counts of the samples. No significant interactions among the nisin-added level, and LPS-added level on the microbial qualities of the treated samples were observed in this study. Table 3 illustrates the storage effect on the total microflora counts of the samples. The microbial counts significantly decreased from the 7.34 log CFU/ml of the day 0 samples to the 7.10 log CFU/ml of the day 2 samples. No significant difference of the microbial counts was found of the samples after 2 days refrigerated storage at 4°C up to 7 days. Typically, spoilage could be detected when bacterial numbers exceed 10^8 log CFU/g (Jay, 1996). In this study, even though a few total microflora counts of some samples exceeded

this “log 8 criteria”, no off-odors and slime formation was detected in any of the samples in this study when evaluated by sensory evaluation within 7 days of refrigerated storage.

Conclusions

In conclusion, adding nisin up to the level of 100 IU/ml, and adding LPS up to 2 units consisted of 2 μg/ml LP, 11.8 mM KSCN, and 5.0 mM H_2O_2, respectively, significantly decreased the total microflora and psychrotrophs counts of the marinated chicken drumsticks during refrigerated storage up to 7 days.

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Tables and Figures

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

Nisin added level (IU/ml)	Total microflora count[1] (log CFU/ml)	Psychrotrophs count[2] (log CFU/ml)
0	7.46[a]	8.09[a]
50	7.09[b]	7.68[b]
100	6.83[c]	7.49[c]

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

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

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

Table 2. Effects of LPS-added level on the total microflora and psychrotrophs counts of marinated chicken drumsticks

LPS added level (unit)[1]	Total microflora count (log CFU/ml)	Psychrotrophs count (log CFU/ml)
0	7.71[a]	8.42[a]
1	6.97[b]	7.63[b]
2	6.69[c]	7.21[c]

[abc] Means within a column with different superscript are significantly different ($p<0.05$).
[1]LPS unit: 1 unit = 1 μg/ml LP, 5.9 mM KSCN, and 2.5 mM H_2O_2; 2 unit = 2 μg/ml LP, 11.8 mM KSCN, and 5.0 mM H_2O_2.

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

Refrigerated storage time (day)	Total microflora count (log CFU/ml)	Psychrotrophs count (log CFU/ml)
0	7.34[a]	7.83[a]
2	7.10[b]	7.82[a]
4	6.94[b]	7.66[a]
7	7.12[b]	7.69[a]

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