

Applicability of Nisin and Tumbling to Improve the Microbiological Quality of Marinated Chicken Drumsticks

F. J. Tan* and H. W. Ockerman¹

Department of Animal Science, National Pingtung University of Science and Technology
Pingtung, 91201, Taiwan, ROC

ABSTRACT : Meat marination has been applied to improve product's physical and sensory attributes for years, but usually it is not intended to improve microbial quality of the product. Tumbling, which helps the distribution of marinade solution during processing, should enhance the action of antimicrobial agents. The objective of this study is to evaluate the combined effects of nisin, tumbling and storage time on total microflora and psychrotrophs counts on poultry. 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 nisin levels (0, 50, or 100 IU/ml) combined with tumbling (0, 10, or 20 min), and then stored at 4°C for 18 h. The total microflora and psychrotrophs counts of the samples were evaluated after 0, 2, 4, and 7 days of storage. The results indicated that at a given storage time, the samples tumbled for either 10 or 20 min had significantly ($p < 0.05$) lower microbial counts when compared with the samples without the tumbling treatment. The microbial counts of the tumbled samples increased as storage time increased. Microbial counts significantly ($p < 0.05$) decreased when more nisin was increased up to the level of 100 IU/ml. In conclusion, adding of nisin at the level of 50 IU/ml with tumbling for 10 min decreased the total microflora and psychrotrophs counts of the marinated chicken broiler drumsticks. (*Asian-Aust. J. Anim. Sci.* 2006. Vol 19, No. 2 : 292-296)

Key Words : Nisin, Tumbling, Marination, Microbial Quality

INTRODUCTION

Marination is a procedure of treating meat with an aqueous mixture of vinegar, salt and spices before cooking. It has been widely applied in the poultry industry to improve product's physical and sensory attributes, such as cooking yield, tenderness, water holding, flavor and etc. (Chou et al., 1997; Young and Lyon, 1997a, b; Li, 1998; Perez et al., 1998; Hashim et al., 1999; Lemos et al., 1999; Xiong and Kupski, 1999a, b; Young et al., 1999; Young and Buhr, 2000; Zheng et al., 2000). However, this marinating process is usually not intended to improve microbial quality of the product. Limited information is available on the contribution of marination to the microbial quality of treated products.

Nisin, which is considered as a "biopreservative", is a natural, nontoxic, and heat stable polypeptide produced by *Lactococcus lactis* strains, and has been shown to inhibit gram-positive microorganisms (Ray, 2001), and gram-negative bacteria when combined with chelating agents such as EDTA (Cutter and Siragusa, 1995). Nisin, along with many other physical or chemical treatments, has been studied in extending the shelf life of poultry products (Shefet et al., 1995; Tsou, 1995; Natrajan and Sheldon, 2000; Xu et al., 2000).

Tumbling has been widely applied in the meat industry for years. Pieces of meat are placed inside a rotating drum-like cylinder. When the tumbler rotates and meat pieces drops from the top to the bottom inside the tumbler, a gravitation impact is produced and applied to the meat. This impact plus abrasion against other meat pieces leads to extraction of some of the myofibrillar proteins to the surface, which is useful for binding meat pieces together upon heating. Currently the major function of tumbling applied to poultry products focuses on improving the physical and sensory attributes of the products, such as increasing cure pickup, moisture retention, water hold capacity, tenderizing effect, cooking yield, decreasing curing time, and etc (Bater et al., 1992; Bater et al., 1993; Nurmahmudi and Sams, 1997; Huang et al., 2001). Several studies concerning the effect of effect of tumbling on the microbiological quality of the products have been reported (Leak et al., 1984; Leblanc et al., 1996; Yetim et al., 1996; Ramos and Lyon, 2000). Also, tumbling has been showed to increase distribution of curing and marinade solution in some studies (Ockerman and Organisciak, 1978a, b; Leak et al., 1984; Taylor and Somers, 1985; Chung et al., 1989; Davies et al., 1999).

Based on this information, nisin could be used to inactivate some microorganisms on the poultry products, and extend shelf life of the products. In addition, tumbling, which helps the distribution of marinade solution during processing, should increase the distribution of antimicrobial agents, thus enhancing the action of the antimicrobial agents. In this study, the efficacy of tumbling (0, 10, or 20

* Corresponding Author: F. J. Tan. Tel: +886-8-770-3202 (6197), Fax: +886-8-774-0148, E-mail: tanfj@mail.npust.edu.tw

¹ Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, USA.

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min) in conjunction with nisin (0, 50, or 100 IU/ml) and storage time (day 0, 2, 4, and 7) to the microbiological quality of chicken drumsticks was 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.

Nisin at levels of 0, 50 or 100 IU/ml with 20 mM disodium EDTA (Fisher Scientific Co., Kansas City, MO) were added to the standardized marinade based on the results from other preliminary experiment (data not shown). Commercial nisin powder (Sigma Chemical Co., St. Louis, MO), which contains 2.5% nisin and denatured milk solids, were first dissolved in 0.02 N HCl and held for 2 h at 25°C. After adding pre-assigned levels of nisin and disodium EDTA of 0, 50 or 100 IU/ml, the marinade solutions were adjusted to a pH of 4, autoclaved for 121°C for 15 min, and then stored at 4°C before conducting the experiment.

Sample preparation

A total of 216 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 tumbling treatment, two drumsticks per treatment for each storage day were aseptically placed with 400 ml of autoclaved marinade in a plastic bag. All the drumsticks were covered by the marinade solution, and stored at 4°C for 18 h.

For those samples with tumbling treatment, drumsticks with the marinade solutions in plastic bags were tumbled for either 0, 10, or 20 min in a pre-cleaned and sanitized rotating stainless steel drum (81 cm long and 58 cm diameter, three 7.6 cm baffles 7.6 cm, manufactured at OSU, Columbus, OH, USA) at 12 rpm in a refrigerated (4°C) room. After tumbling for the pre-assigned time of either 0, 10, or 20 min, drumsticks with the marinade solution were

then marinated at 4°C for a total of 18 h (including the previous tumbling time) storage.

Microbial evaluation

After 18 h of 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. 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 the pour plate method and plate count agar (Difco Laboratories, Detroit, MI) were prepared for enumeration of bacteria in each treatment 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 log₁₀ colony forming units (CFU) per ml of peptone rinse.

Statistical analyses

The study was designed as a 3×3×4 factorial experiment, 3 nisin-adding levels (0, 50, 100 IU/ml), tumbling treatment (0, 10, 20 min), and 4 storage times (day 0, 2, 4, and 7), and the experiment was replicated 3 times. Least square means (LSM) were 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. Means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

In the current study, there was no significant three-way interaction on the total microflora counts. Only one two-way interaction (tumbling time×storage time) was significant ($p < 0.05$), which indicated that tumbling time along with storage time would affect the total microflora counts of the treated samples.

At a given storage time, the more tumbling time, the fewer total microorganisms were detected (Table 1). At storage time day 0, samples tumbled for 10 or 20 min had significantly lower total microflora counts of 5.24 and 4.59 log CFU/ml, respectively, when compared with the samples without tumbling which had 7.61 log CFU/ml count at day 0. Similarly, at the same storage time of either 2, 4, or 7

Table 1. Total microflora counts and psychrotrophs counts of marinated chicken drumsticks with different tumbling time during refrigeration storage at 4°C

Tumbling time (min)	Storage time (day)			
	0	2	4	7
Total microflora counts (log CFU/ml)				
0	7.61 ^a	7.57 ^a	7.50 ^a	7.48 ^a
10	5.24 ^d	5.20 ^d	5.88 ^b	6.11 ^b
20	4.59 ^e	5.16 ^d	5.37 ^{cd}	5.67 ^{bc}
Psychrotrophs counts (log CFU/ml)				
0	7.79 ^a	7.69 ^a	7.68 ^a	7.72 ^a
10	5.52 ^d	5.53 ^d	5.96 ^{bc}	6.28 ^b
20	5.14 ^d	5.55 ^{cd}	5.67 ^{bcd}	5.90 ^{bcd}

^{a, b, c, d, e} All means with different superscripts for each microbial test are significantly different ($p < 0.05$).

days, the tumbled samples either for 10 or 20 min resulted in significant lower total microflora counts when compared with the samples without tumbling treatment. At a given storage time, there was no significance for the total microbial counts between the samples tumbled for 10 or 20 min after 2 days of storage except at day 4. This result implies that tumbling for 10 min with the marinade solution containing nisin in this study sufficiently reduced the total microflora counts of the samples during refrigeration storage up to 7 days.

In an investigation of the effectiveness of nisin for the control of lactic acid bacteria spoilage in refrigerated, vacuum-packaged bologna sausages, Davies et al. (1999) mentioned that even distribution of nisin throughout the products is important when applying nisin as a preservative in meat products, which are often heterogeneous in composition. Similarly, Chung et al. (1989) pointed out that uneven distribution of added nisin was one of the reasons which resulting in a loss of nisin activity on meat. Taylor and Somers (1985) evaluated the antibotulinal effectiveness of nisin in bacon. In addition to binding to some meat particles, they explained that uneven distribution of nisin might be one of the reasons resulting in the limited antibotulinal effectiveness of nisin in bacon. Not only nisin, which has been shown to inactivate some microorganisms, but also acetic acid which reduced the solution pH, should enhance the action of the antimicrobial agents. Tumbling, which has been showed to help the distribution of curing agents and marinade solution during processing in several studies (Ockerman and Organisciak, 1978a, b; Leak et al., 1984), should increase the distribution of antimicrobial agents. In the current study, at a given storage time, the more tumbling time, the fewer total microorganisms were detected.

Table 1 illustrates that the total microflora counts of the samples without tumbling treatment remained steadily at approximately 7.50 log CFU/ml during 7 days refrigeration storage. The results showed that the total microflora counts

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

Nisin added level (IU/ml)	Total microflora count (incubated at 35°C for 48 h)	Psychrotrophs count (incubated at 7°C for 10 days)
	0	6.48 ^a
50	6.08 ^b	6.32 ^b
100	5.79 ^c	6.02 ^c

^{a, b, c} Means within a column with different superscript are significantly different ($p < 0.05$).

of the tumbled samples increased as the storage time increased. At a given tumbling time of 10 min, the total microflora counts increased significantly between storage day 0 with 5.24 log CFU/ml and day 4 with 5.88 log CFU/ml. Similar result could be also seen for the samples tumbled for 20 min. Even though the microbial counts of the tumbled sample increased with the storage time, after 7 days refrigeration storage, the microbial counts of the tumbled samples remain significantly lower than the microbial count for the samples without tumbling.

Similar to the total microflora counts, the psychrotrophs counts of the samples exhibited similar patterns (Table 1). No significant three-way interaction, but one two-way interaction (tumbling time × storage time) was significant. At a given storage time, samples tumbled for 10 or 20 min had significantly lower psychrotrophs counts, when compared with the samples without tumbling. There was no significance for the psychrotrophs counts between the samples tumbled for 10 or 20 min at a given storage time.

Table 2 illustrates the nisin level effect on the microbial counts of the treated samples. Adding nisin at a level of 50 IU/ml resulted in a significantly lower total microflora counts of 6.08 log CFU/ml, when compared with samples without adding nisin, which had higher count of 6.48 log CFU/ml. Adding even more nisin to the level of 100 IU/ml resulted in a further significantly lower microbial count of 5.79 log CFU/ml. Also, the more nisin added up to the level of 100 IU/ml, the less psychrotrophs counts that were obtained. Similar results were also reported by other researchers (Delves-Broughton, 1993; Fang and Lin, 1994; Murray and Richard, 1997). A low concentration of nisin (either 50 or 100 IU/ml) was chosen in the current study, because nisin alone was not intended to be the only hurdle treatment. In addition, even though higher levels of nisin may result in increased effect, a lower concentration of nisin might be appropriate due the economic concern for the cost of nisin.

Typically, spoilage can be detected when bacterial numbers exceed 10^8 CFU/g (Jay, 1996). In the current study, only the total microflora counts of the samples without tumbling treatment had approximately 7.50 log CFU/ml, which were close to this "8 log criteria". However, no off-odors and slime formation was detected in any of the

samples in this study when evaluated by sensory evaluation within the 7 days of refrigerated storage.

In conclusion, adding nisin at the level of 50 IU/ml with tumbling treatment for 10 min decreased the total microflora and psychrotrophs counts of the marinated chicken drumsticks. Further research to assess the changes in physical and sensory quality of marinated chicken drumsticks treated with tumbling and nisin is needed.

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