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MICROBIOLOGICAL, PHYSICAL, AND SENSORY CHARACTERISTICS OF MARINATED CHICKEN DRUMSTICKS TREATED WITH NISIN, THERMAL TREATMENT, TUMBLING AND THE LACTOPEROXIDASE SYSTEM

DISSERTATION

Presented in Partial Fulfillment of the Requirements of
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
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*****

The Ohio State University
2002

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ABSTRACT

Currently meat marination is practiced to improve product's physical and sensory attributes, and usually not intended to improve the microbial quality of the product. Nisin, which is a natural, nontoxic, and heat stable polypeptide produced by Lactococcus lactis, has been shown to inhibit many microorganisms, and has been approved for use in some dairy products. Thermal treatment has been widely applied to control the growth of microorganisms in the food industry for years. Tumbling, which helps the distribution of marinade solution during processing should enhance the action of antimicrobial agents. The lactoperoxidase system (LPS) consists of lactoperoxidase (LP), thiocyanate (SCN'), and hydrogen peroxide (H_2O_2), is an inhibitory system that is present naturally in bovine milk, and had been showed to be inhibitory against some microorganisms. Limited information on the contribution of marination, nisin, thermal treatment, tumbling, and the lactoperoxidase system to the microbial quality of treated poultry products is available.

In the first part of the dissertation, four studies (experiments 1 to 4) focused on the microbial quality of the treated samples. The results showed that the combinations of nisin plus thermal treatment, nisin plus tumbling, nisin plus LPS, and LPS plus thermal treatment resulted in lower total microbial and psychrotrophs counts of the treated samples compared with the control samples during the 7 days of refrigerated storage at 4°C. Depending on the combinations of treatment, some interactions among treatments...
existed. Further studies were conducted to evaluate whether these combined treatments had any influence on the physical and sensory characteristics of the treated samples. In the second part of the dissertation, four studies (experiment 5 to 8) focused on the physical and sensory qualities of the treated samples. The results showed that the combinations of treatments did not impair most of the physical or sensory qualities of the treated samples. In conclusion, based on the microbial, physical and sensory qualities, marinated chicken drumsticks treated with the combinations of nisin plus thermal treatment, nisin plus tumbling, nisin plus LPS, or LPS plus thermal treatment were acceptable.
Dedicated to my parents, parents-in-law, and wife

In memory of my father – Mr. Tan Chang-Yang
ACKNOWLEDGMENTS

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Finally, I would like to express my deep memory for my father, Mr. Tan Chang-Yang, who loved, helped, and guided me since I was born, and unfortunately passed away this year. May God bless him in the heaven. I love you and miss you, Dad!
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PUBLICATIONS

Research Publication

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physical, and sensory characteristics of marinated chicken drumsticks. Proceedings of 


Assessment of fresh pork color with color machine vision. Proceedings of 50th Annual 

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FIELDS OF STUDY

Major Field: Animal Sciences (Meat Science)
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CHAPTER 1

INTRODUCTION

Marination, which is a procedure of treating meat with an aqueous mixture of vinegar, salt, and spices before cooking, is widely applied in the food industry because it increases variety and adds value to meat products. Currently, marination is practiced to improve product's physical and sensory attributes (e.g., tenderness, water holding, and flavor), but the process is usually not intended to improve the microbial quality of the product. Traditionally, chicken drumsticks are marinated and stored in a refrigerator for a period of time (e.g. overnight) before cooking. Limited information is available on the contribution of marination to the microbial quality of treated products.

Marination is an ideal system to incorporate preservative factors (or processing techniques) that may enhance the quality of treated meat. Sodium chloride has been widely applied to preserve food for years. Acetic acid, which is the predominant acid in vinegar, has been granted the category of “generally regarded as safe” (GRAS) for miscellaneous and general-purpose usage under FDA regulations (FDA, 1988). Used either as an acidulant to reduce pH, or as a flavoring agent to provide a sour flavor, acetic acid has also been used as an antimicrobial agent in the meat and poultry industries (Dickens and Whittemore, 1995).
Nisin, which is considered as a “biopreservative”, is a natural, nontoxic, and heat stable polypeptide produced by *Lactococcus lactis*. It has been shown that nisin can inhibit gram-positive microorganisms, such as *L. monocytogenes* (Ray, 2001). When combined with chelators (e.g. EDTA), nisin has also been shown to inhibit gram-negative microorganisms such as *Salmonella* and *Escherichia coli* (Cutter and Siragusa, 1995a,b).

Lactoperoxidase system (LPS), which consists of lactoperoxidase, thiocyanate and hydrogen peroxide, is a natural antimicrobial system that is present naturally in bovine milk (Reiter and Harnulv, 1984). This system has been shown to be inhibitory against many bacteria including *L. monocytogenes*, *S. aureus* and *E. coli* (Zapico et al., 1995; Kamau et al., 1990b), and is potentially useful as an effective antimicrobial factor in meat and poultry products.

Thermal treatment, which is one of the most common 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, bleaching, pasteurizing, drying, and etc. 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.

Tumbling, which has been showed to increase distribution of curing and marinade solution in some studies (Leak et al., 1984; Ockerman and Organisciak, 1978a,b), has been widely applied in the meat industry for years. Pieces of meat are placed inside a rotating drum-like tumbler. When the tumbler rotates and meat pieces drops from the top
to the bottom inside of the tumbler, a gravitation impact is produced and applied to the meat. This action plus abrasion against other meat pieces leads to extraction of the myofibrillar proteins to the surface, which are necessary 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, and decreasing curing time, and etc. (Huang et al. 2001; Nurmahmudi and Sams, 1997).

In addition to microbial quality, physical and sensory qualities of food products are important to consumers. In these studies, a water-base marinade that contained the basic ingredients was developed as a standardized marinade. Additional antimicrobial factors or processing techniques, such as nisin with EDTA, lactoperoxidase system, thermal treatment, and tumbling would be incorporated with the marinade to improve the microbial quality of marinated chicken drumsticks. The ultimate objective of these studies was to enhance the microbial quality of marinated chicken meat through development of a reduction in microbial numbers and to produce a physical and sensory desirable marinated product.

In the first part of the dissertation, four studies (experiments 1 to 4) would focus on the microbial quality of the treated samples, whereas in the second part of the dissertation, four studies (experiments 5 to 8) would focus on the physical and sensory qualities of the treated samples.
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attributes. In addition to acid, salt and some flavoring agents (such as black pepper, garlic powder, and etc.), which are recognized as the major components of most of the marinades, various ingredients can also be incorporated into marinade recipes. Depending on the components of the marinade contains, impact towards physical and sensory characteristics of the marinated samples, such as increasing moisture retention, water-holding capacity, yield, and tenderness, decreasing cooking loss, enhancing flavor, aroma, and color of products, decreasing warmed-over flavor development, preserving the products, increasing variety of products, and etc., have been reported in several studies.

In a study of evaluating the effect of marination on yield of poultry products. Chen (1982) pointed out that the frying yield of the chicken parts, which were previously marinated increased probably due to the increased water retention of the marinade. In the same study, by measuring salt concentration in the meat portions, the author also indicated that tumbling the meat with the marinade resulted in a greater marinade penetration for the chicken parts than those still-marinated samples.

Goodwin and Maness (1984) evaluated the influence of marination on yield of broilers. In their study, the broiler parts which were aged for 16 hrs in ice, then marinated in a solution (containing 92% water, 4% monosodium glutamate, and 4% sodium chloride) had significant (P<0.05) higher yield of 81.3%, whereas the samples which were only aged for 16 hrs but without marinating had lower yield of 79.7%.

Smith and Fletcher (1991) evaluated the effect of processing methods on duckling breast. In their study, Pekin duckling breast fillets were marinated with a commercial dry mix consisted of 8.6% NaCl, 5.4% sodium polyphosphate, and spices, and then cooked.
The authors reported that marinated ducking breast fillets had significant (p<0.01) higher cooking yield of 72.9% when compared with the controls (64.5%). In addition, marination resulted in the treated samples having a significant (p<0.01) lower shear value of 5.1 kg/g, which indicated more tenderness of the samples, when compared with the controls (6.9 kg/g).

Incorporated with salt, some phosphates, which can be used to increase the water retention of the products, have been widely applied for manufacturing many marinated products. Ockerman (1996) pointed out that some basic reacting phosphates increase the pH of meat, thus result in an increase in the water-holding capacity of muscle. Phosphates and salt have a synergistic effect on increasing water-holding capacity. This increase in water-holding capacity leads to further increase of juiciness and tenderness of the products, and decreases the cooking loss.

How to manufacture more tender products is always challenging the meat and poultry industry. Besides adding some enzymes or applying some technical tenderization, marinating with some food ingredients has also been evaluated. The purpose of adding some acids in meat and poultry products is not only to provide flavor, but also to tenderize the meat. Howat et al. (1983) demonstrated that marinating results in significantly more tender meat as determined by shear values and trained panel evaluations.

When evaluating the shear values of samples, the broiler parts which were aged for 16 hrs in ice, then marinated in a solution (containing 92% water, 4% monosodium glutamate, and 4% sodium chloride) had significant (P<0.05) lower shear value of 5.2 kg/g, whereas the samples which were only aged for 16 hrs but without marinating had
higher shear value of 6.8 kg/g (Goodwin and Maness, 1984). The authors explained that adding NaCl to chill water increases the water-holding capacity, thus increases the percentage yield and lowers the shear values of the samples.

By evaluating the myofibril fragment index, which is a measurement of the myofibril fragmentation. Chou et al. (1997) pointed out that Tsaiya duck breast muscles, which were marinated in lactic acid at 5°C resulted in an acceleration of post-mortem changes, and this might contribute to increasing the tenderness of meat during postmortem storage.

In a study of tenderizing spent fowl drumsticks, which were less tender and marinating them in a solution containing 2% NaCl with some weak acids (lactic acid or acetic acid) for 36 or 72 h, Kijowski and Mast (1993) reported that marination in acids resulted in tenderization of the treated samples as measured by shear force. These authors explained that acids and salt, which were contained in the solutions, change the structure of collagen, thus improve the tenderness of the samples.

In addition to improving some textural and physical characteristics, marination can also be used to enhancing flavor, aroma, and color of products. According to Post and Heath (1983), when samples were evaluated with skin, a significant (P<0.05) increase of flavor intensity rating for the marinated drumsticks was detected for the samples marinated in a marinade containing corn oil, vinegar, water, and other spices for 20 and 30 min, compared with the samples without marination.

Lyon and Lyon (2000) evaluated the influence of several factors including marination on the sensory characteristics of broiler breast muscle. In their study, chicken
breasts were injected with a commercial marinade solution containing phosphates, salts, and spices, cooked, and then analyzed. The authors reported that marination significantly (P<0.05) affected all the 16 attributes (flavor attributes: brothy, chicken/meaty, bloody/serumy/metallic, cardboardy, sweet, salty, sour, and bitter; textural attributes: springiness, hardness, moisture release, chewiness, particle size, ease of swallow, and residual particles; afterfeel/aftertaste: metallic) evaluated in the study.

Yang and Chen (1993) evaluated the influence of several factors including marination on color of chicken meat. In their study, color of the raw and microwave-cooked chicken fillets, which previously were marinated in a marinade solution containing water (12% of meat weight), salt (5% of meat weight), monosodium glutamate (2% of meat weight), citric acid (0, 0.5, or 1.0%), and Na₃PO₄ (0, 1, or 2%) altered by the concentrations of citric acid and Na₃PO₄.

In addition to increasing water-holding capacity of the meat and poultry products, Ockerman (1996) pointed out that some phosphates have antioxidant properties, which reduce oxidative changes, rancidity, and warmed over flavor of the products. Based on these unique characteristics, some phosphates are commonly added into the formulation of marinated meat and poultry products in order to decrease warmed-over flavor development.

Ang and Young (1987) evaluated the effect of marination with some polyphosphate salts on the oxidative stability of cooked broiler leg meat. By evaluating the thiobarbituric acid (TBA) values of the samples, the authors pointed out that oxidation of cooked broiler meat which were previously marinated with 6% sodium
pyrophosphate, or 3% sodium pyrophosphate plus 3% sodium chloride could retard TBA values during frozen storage and subsequent thawing and holding periods.

Ho (1990) reported that chicken parts, which were previously marinated with water (7.45%), salt (0.30%), and phosphate (0.25%), and tumbled (31.5 rpm) for 15 min had significantly (P<0.05) lower TBA values, when compared with the non-marinated samples. The author explained that the lower TBA values might be due to the presence of phosphate in the marinade.

Currently, how to manufacture various high quality products, which are strongly in high demand by consumers, becomes a critical issue for the meat and poultry industry. Since various ingredients can be incorporated into a marinade formulation during processing, marination can be one of the potential solutions to solve the problem.

Hashim et al. (1999a) investigated the effect of a honey marinade on the sensory characteristics of baked chicken breast. In their study, bone-in, skinless, chicken breast were injected with a lemon-pepper marinade containing 0.20 or 30% honey, baked, and then sensory analyzed. The authors reported that adding honey (20 or 30%) did not affect most of the physical characteristics, including marinade uptake, moisture content, fat content, and etc. Even though adding 20% honey significantly (P<0.05) increased shear force to 6.40 kg/g, when compared with the samples without honey, which had 3.20 kg/g, these authors, however, pointed out that the samples in this experiment were still considered as moderately tender to very tender. In the same study, samples marinated with 30% honey had significantly darker exterior (i.e. lower L* value) than the samples marinated with 0 or 20% honeys. The authors also mentioned that addition of honey to the marinade further enhanced some sensory characteristics of the baked chicken.
Marination on microbial quality

Marination is usually not intended to improve the microbial quality of the product. Marination of chicken has been the subject of several reports (Chen, 1982; Heath and Owens, 1991; Goodwin and Maness, 1984; Yang and Chen, 1993), but none were focusing on the microbial quality of the treated samples. Occasionally, some meat and poultry products are marinated for a comparatively longer period of time (e.g. overnight) before cooking. Limited information is available on the contribution of marination to the microbial quality of treated products. How to maintain a good microbial quality of the marinated products, or even extending the shelf life of products, has been continuously interesting for meat processors and researchers.

He (1996) evaluated the effect of marination on the microbiological quality of refrigerated shrimp. In that study, shrimp first were marinated with a commercial lemon pepper marinade, polyphosphates, and a whey protein concentrate, and then were tumbled at 4°C for 30 min, held for 30 min, packed and stored at 4°C. The author reported that marinated shrimp had significant (P<0.05) lower psychrotrophic and mesophilic counts than the unmarinated ones after 7 days of refrigerated storage at 4°C.

Feng (1998) evaluated the effect of marination on the microbial quality of channel catfish fillets. In that study, channel catfish fillets were marinated with 12 commercial marinades, which contained various low pH materials, such as fruit juice, vinegar, and citric acid for 30 min to 4 hrs, and then stored at 4°C for 20 days. The author reported that marination lowered the marinade solution pH, and the aerobic and psychrotrophic counts, and thus extended the shelf-life of marinated samples for 5 to 10 days, when
compared with control samples.

When incorporated with other ingredients, such as some preservatives, marination can be also applied to extend the shelf life of the products. Ho (1990) conducted a study to evaluate the effects of some preservatives including sorbic acid and sodium benzoate on refrigerated shelf life and spoilage microflora of marinated chicken. In that study, chicken parts were marinated with water (7.45%), salt (0.30%), and phosphate (0.25%), and tumbled (31.5 rpm) for 15 min. The author reported that addition of sorbic acid (0.5%) or sodium benzoate (0.5%) in the marinade mixture significantly (P<0.05) extended the shelf life of marinated chicken parts to approximately 8 more days when compared with the samples without the preservatives added.

Nisin

Introduction of nisin

Increasing demand for health, high quality, and convenient foods from consumers have made some naturally preserved methods become more desirable. Adding some "biopreservatives", for example bacteriocins, which are produced naturally by some microorganisms, has become common in the food industry. Bacteriocins are those antagonistic compounds that are produced by certain microorganisms and have bactericidal activities to inhibit closely related species of microorganisms. Among several bacteriocins, nisin is one of the most widely used bacteriocins especially in the cheese industry. Nisin has been granted a "generally recognized as safe" (GRAS) status and approved by the Food and Drug Administration (FDA) for the use in some elected foods in 1988 (FDA, 1988), and is allowed to be used as a food preservative in more than
50 countries (Delves-Broughton, 1993).

Nisin, which is considered as a "biopreservative", is a natural, nontoxic, and heat stable polypeptide produced by *Lactococcus lactis*, and has been shown to inhibit gram-positive microorganisms such as *L. monocytogenes* (Ray, 2001). El-Khateib *et al.* (1993) reported that nisin at a level of 4×10⁴ IU/ml decreased the counts of *L. monocytogenes* by 1.1 log₁₀ CFU/6 cm² in beef *Longissimus dorsi* during 48-h of storage.

When combining with some chelating agents such as EDTA and citrate, nisin can also be used to inhibit gram-negative bacteria such as *Salmonella* species and *Escherichia coli*. Cutter and Siragusa (1995a,b) reported that nisin in combination with chelating agents (EDTA, EGTA, citrate and phosphate) was effective in reducing populations of gram-negative bacteria. Liu (1997) reported that addition of nisin decreased *L. monocytogenes* Scott A count by 2-3 logs in less than an hour in roasted potatoes. Shefet *et al.* (1995) reported that when nisin was combined with chelating agents (EDTA, citric acid) and a surfactant (Tween 20), nisin might be used to reduce *S. typhimurium* on broiler drumstick skin by 3.1 to 4.9 log₁₀. A study done by Stevens *et al.* (1992) showed that a treatment consisting of 50 μg/ml nisin and 20 mM EDTA was able to effectively reduce several selected *Salmonella* species. Shefet *et al.* (1995) explained that binding of chelating agents with magnesium ions in the lipopolysaccharide (LPS) layer of the outer membrane of gram-negative bacteria results in an increase of permeability of the outer membrane to nisin. Jaquette and Beuchat (1998) suggested that nisin interacted with the phospholipid component of the cytoplasmic membrane results in leakage of ATP.
Other characteristics of nisin, such as being able to be destroyed by digestive enzymes, not contribute to off-flavors or off-odors, also make nisin widely applied in the food industry (Jay, 1996).

**Application of nisin in meat and poultry products**

Nisin, along with many other physical or chemical treatments, has been studied in extending the shelf life of the meat and poultry products.

Ariyapitipun et al. (1999) evaluated the effect of acid and nisin on the reduction of microbial counts of the raw beef samples. Their results showed that fresh beef samples dipped in a solution consisting of the combination of 2% lactic acid and 200 IU/ml nisin at 23°C for 5 min, resulted in significant (P<0.05) lower microbial counts for vacuum-packaged fresh beef stored at 4°C for up to 56 days. In addition, in the same study, the authors also mentioned that nisin alone at the level of 200 IU/ml did not effectively inhibit the growth of the microorganisms. The authors explained, in addition to a lower concentration (200 IU/ml) of nisin applied in this study, the ineffectiveness of nisin on the microorganisms might be due to the binding of nisin with protein particles on beef tissues.

Cosby et al. (1999) evaluated the effects of nisin-EDTA and package (aerobic, vacuum or modified atmosphere) on the shelf life of poultry. In their study, chicken drummettes were dipped with solutions consisted of various combinations of EDTA (10, 20, or 50 mM) and nisin (25, 50, or 100 μg/ml) with pH adjusted to 6.5 for 30 min at 15°C and stored at 4°C. Their results showed that there was no significant (P>0.01) difference of the total aerobic plate counts of the treated samples among the three levels.
of nisin (25, 50, and 100 μg/ml). In addition, by Day 18 all of the total viable cell counts of the samples treated with nisin-EDTA and packaged under aerobic conditions exceeded 10⁷ CFU/ml of rinse, which indicating spoilage, when the control samples without nisin and EDTA added became spoiled by Day 12. The authors concluded that the treatment, which combined nisin and EDTA and was packaged under aerobic condition, could approximately extend the shelf life for 4-6 days of the fresh poultry product over the control group.

Cutter and Siragusa (1994) evaluated the effect of spray washing of beef carcass surface tissue with nisin on inhibiting some inoculated bacteria. They found that nisin spray treatment (5000 AU/ml) significantly (P<0.05) reduced the inoculated *Brochothrix thermosphacta*, which was a meat spoilage organism, on lean beef tissue by 0.83 log CFU/cm² at storage day 0 when compared with the microbial count of 3.47 log CFU/cm² for the control samples. Similarly, the microbial counts of the treated adipose tissue were significantly (P<0.05) reduced by 1.77 log CFU/cm² when compared with 3.30 log CFU/cm² of the control samples. After stored at 4°C for 24 hours, the *B. thermosphacta* counts on both lean and adipose tissues of the treated samples were significantly lower than the control samples. In the same study, the authors reported that nisin spray treatment also resulted in a significantly reduction of *Carnobacteria divergens*, which is also a meat spoilage microorganism, on the treated beef samples (P<0.05). Therefore, the authors suggested that spraying with nisin on carcass might be used to inhibit spoilage bacteria and therefore, extend the shelf life of red meat.

Cutter and Siragusa (1996) evaluated the effect of a combination of nisin spray washes and followed by vacuum-packaged on inoculated *Brochothrix thermosphacta* in
lean and adipose beef tissues. They found that the treatment with a nisin spray wash (5000 AU/ml) and followed by vacuum-packaged resulted in lower numbers of *B. thermosphacta*, which is the predominant spoilage organism of vacuum-packaged or modified atmosphere packaged meat, when compared with the control samples stored under refrigerated condition for up to 28 days. The authors concluded that by suppressing or inhibiting the growth of undesirable bacteria, the shelf life of fresh beef could be extended by following a spray treatment with nisin, and vacuum packaging under refrigerated conditions.

Davies *et al.* (1999) reported that adding nisin (at the level of 25 µg/g) could inhibit the growth of the lactic acid bacteria spoilage organisms, which were inoculated into vacuum-packed pasteurized bologna-type sausages stored at 8°C. In that study, the samples treated with nisin remained unspoiled for more than 50 days of storage, which indicated that the LAB counts were less than $10^8$ CFU/g, when compared with the control samples, which spoiled by day 7.

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.* (1989b) pointed out that uneven distribution of added nisin was one of the reasons that resulted 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 resulted in the limited antibotulinal effectiveness of nisin in bacon.
Natrajan and Sheldon (2000) evaluated the efficacy of a nisin-based treatment formulation to extend the shelf life of refrigerated broiler drumsticks. In their study, drumsticks were immersed for 3 min in a solution consisting of 100 μg/ml nisin, 5.0 mM EDTA, 3.0% citric acid, and 0.5% Tween 80, with the pH adjusted to 3.5, followed by packaging in a foam traypack containing a nisin-treated PVC overwrap and absorbent tray pad, and refrigerated at 4°C. The authors estimated the shelf life of refrigerated broiler drumsticks was extended by 0.6 to 2.2 day when compared with the controls.

By evaluating the reduction of microbial counts of the drumsticks which were previously immersed in a solution containing nisin, Natrajan (1997) suggested that adding nisin in the chill or scald water or as a final carcass dip could help to reduce carcass bacterial populations.

When treated with a combination of lactic and nisin whey permeate, a significantly lower aerobic counts of the cold-smoked rainbow trout was obtained after 15 and 29 days of refrigerated storage (Nykanen et al., 1999).

The shelf life of refrigerated broiler drumsticks, which were previously immersed for 30 min in a solution consisting of 100 μg/ml nisin, 5.0 mM EDTA, 3% citric acid, 0.5% Tween 20, and pH adjusted to 3.5 for 30 min, was extended by 1.5 to 3 days when compared to those dipped in sterile distilled and deionized water (Shefet et al., 1995).

In a study done by Tsou (1995), fresh chicken drumsticks were treated with 5000 IU/ml of nisin, vacuum-packaged, and then refrigerated storage. The result showed that the samples with nisin added had significantly (P<0.05) lower total aerobic counts when compared with ones without nisin added during refrigerated storage for 9 days. In addition, after evaluating the odor scores, the author indicated that the fresh chicken...
drumsticks treated with 5000 IU/ml, vacuum-packaged, and refrigerated storage up to 9 days were still considered acceptable.

Wang (2000) reported that adding 100 mg/kg nisin to vacuum packaged Chinese-style sausage stored at 20°C did not retard the growth of microorganisms, including aerobe, anaerobe, lactic acid bacteria, and leuconostocs, and slime formation on the sausage during storage. Wang (2000) explained that this ineffectiveness of nisin added might be due to the higher pH (pH>6.6) of the products, high temperature storage (20°C), or resistance of natural microflora on this specific product to nisin. In addition, interference from lipids might also result in a poor preservative effect of nisin in this product.

Xu et al. (2000) reported that the total aerobic counts of the Nanjing salted duck samples, which were treated with nisin (200 or 400 mg/kg). sodium lactate (3.5%). vacuum-packaged, and microwave treated (915 MHz, 400 W, 3 min). remained at lower levels (2.16 and <1.0 log CFU/g, respectively for 200 or 400 mg/kg of nisin) after 20-days at room temperature (22 to 28°C) storage, whereas the counts of the controls already exceeded 4.57 log after 9 days of storage. In the same study, using a 9-point scale, after 20 days of storage, treated samples still obtained a score of 8, which was defined as very good for the sensory characteristics by the authors. Based on the microbiological, physicochemical (peroxide value), and sensory evaluation results. Xu et al. (2000) concluded that the treated salted duck had a longer shelf life of greater than 20 days, whereas the shelf life of the controls had only approximately 6 days.

Yang and Ray (1994) suggested that nisin could be added to the formulation of those low heat-processed vacuum-packaged meat products, thus inhibiting Leuconostocs.
strains, which frequently cause spoilage of these products, and then extend the shelf life of the products.

It was reported that a treatment combined of high hydrostatic pressure processing (350 Mpa), 1% glucono-delta-lactone (as an acidulant agent to lower the pH), and nisin (100 ppm) could extend the shelf life of mechanically recovered poultry meat, which was considered as highly perishable with a short shelf life, during 30 days of chilled storage (Yuste et al., 1998).

**Combined effect of nisin and thermal treatment**

Rather than applying each single treatment (or processing factor) at an extreme level, nowadays, it is more likely to apply several treatments together, which are at less extreme levels individually to function on foods. For example, even though processing food at higher temperatures may delay microbial growth or even kill microorganisms in the food, significant reduction in nutrition value and/or changes in organoleptic properties of food might be a great concern for manufacturers and consumers. Therefore, in conjunction with other preservative factors (or processing methods, e.g. nisin which is heat stable), relatively mild heat treatment is considered for controlling microorganisms in meat and poultry products.

Nisin is reported as heat-stable at 100°C for 100 min (Mahadeo, 1995; Jay, 1996). Also, Jeknic et al. (1996) conducted the structural analysis of thermal inactivated nisin and pointed out that, nisin at pH 4 retained activity after heating at 121°C for 7 h. Several studies involved in the effect of the combination of moderate and sublethal thermal treatment and nisin to microorganisms have been reported.
Budu-Amoako et al. (1999) evaluated the combined effect of nisin and moderate heat to kill inoculated *Listeria monocytogenes* in cold-pack lobster meat. Their result showed that nisin (at a level of 25 mg/kg of can contents of the brine surrounding the lobster), in combination with a reduced heat process (60°C for 5 min or 65°C for 2 min) resulted in greater killing of inoculated *L. monocytogenes* (3-5 logs) in cold-pack lobster meat, when comparing with either treatment with nisin or heat alone which only resulted in 1 to 3 logs of reduction. In addition, Budu-Amoako et al. (1999) explained that the higher residual level of nisin that was absorbed onto the surface of the lobster meat resulted in functioning against *L. monocytogenes* during process.

In a study done by Mahadeo and Tatini (1994), the scald water samples with a treatment which combined 100 IU/ml nisin and heat (52°C for 3 min) had lower total microflora counts (5.5 log CFU/ml) when compared with those with either nisin added (5.7 log CFU/ml) or heating (7.1 log CFU/ml). In the same study, the authors reported that the combined effect of 100 IU/ml nisin and heat (52°C for 3 min) had a greater log reduction of *L. monocytogenes*, which was previously inoculated in the scald water when compared with the samples with only either nisin-adding or heat treatment.

Modi et al. (2000) evaluated the combined effect of heat and nisin on the survival of inoculated *L. monocytogenes* and found that treatment combined with nisin (500 IU/ml) and heat treatment (55°C) resulted in a 3.7 log microbial reduction when compared with the individual treatments of nisin and heat only had a 1 log reduction for those nisin-resistant *L. monocytogenes* mutants.

Stevens et al. (1992) found that treatments which consisted of 50 μg/ml of nisin combined with temperature range between 30 and 42°C had a more significantly
(P<0.05) reduction of selected *Salmonella* species when compared with ones with lower temperature of either 4 or 20°C

Ueckert *et al.* (1998) showed that there was a synergistic antibacterial action of heat in combination with nisin. In their study, exposure to the treatment, which consisted of nisin (0.1-0.5μg/ml) and mild heat treatment (48-56°C for 5 min), resulted in a higher reduction of inoculated *Lactobacillus plantarum*, which is a food spoilage microorganism, when compared with the samples only receiving a single treatment.

**Thermal treatment**

**Introduction of thermal treatment**

Thermal treatment or heating, which is one of the most common physical methods used to reduce the load of microorganisms in foods, has been widely applied to preserve foods for years. Thermal treatment is involved in many food processes, such as cooking, scalding, pasteurizing, blanching, canning, drying, distillation, evaporation, concentration, and etc. Moisture heating, which is more common than dry heating, has been applied in the food industry to denature proteins of microorganisms during processing, thus leads to the death of microorganisms in foods. Banwart (1989) pointed out that the denaturation or coagulation of proteins, fractures deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and damages the cytoplasmic membrane, which results in the inactive of the microorganisms.
Many studies involving heat treatment, which might or might be not combined with other factors, in meat and poultry products have been reported. Kotrola et al. (1997) evaluated the survival of *E. coli* O157:H7 that was previously inoculated in various turkey products. The authors reported that it required 26, 3.1, or 0.37 min, respectively, to achieve a 5 D kill of *E. coli* O157:H7 in turkey franks cooked at 60, 65.6, or 71°C.

Murphy et al. (2001) evaluated the effects of thermal inactivation of inoculated *Salmonella senftenberg* and *Listeria innocua* in ground chicken breast patties processed in an air convection oven. In their study, before thermal processing, approximately 7 log CFU/g of *S. senftenberg* and *L. innocua* were inoculated into the chicken patties. The survival of microorganisms decreased with the increasing patty temperature. The authors reported that 1 to 4 and 3 to 5 log CFU/g of *S. senftenberg* and *L. innocua*, respectively, was detected in the cooked patties after reaching a final internal temperature of 70 to 80°C.

Murano et al. (1999) reported that incorporated with high hydrostatic pressure (414 megapascals, 60,000 lb/in² for up to 60 min), mild heat treatment (50°C for 6 min) could be used to achieve the highest log reduction for those most pressure-resistant strains of *L. monocytogenes*. This combined treatment consisted of high hydrostatic pressure and mild heat treatment could also extend the shelf life of the ground pork patties for 23 more days when compared with the controls. Similarly, Patterson and Kilpatrick (1998) evaluated the combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in poultry, and reported that the simultaneous
application of high pressure and mild heating was more lethal than either treatment alone for *E. coli* O157:H7 NCTC 12079 and *Staphylococcus aureus* NCTC 10652.

In addition, many mathematic models, which might include several preservative factors, have been developed to evaluate the effects of these factors on microorganisms. Among these factors, frequently, thermal treatment was one of the common factors to be included in the models. For example, with temperature (54.5-64.5°C), pH (4.2-9.6) and NaCl (0.5-8.5% w/w) as controlling factors, Blackburn *et al.* (1997) developed inactivation models for *Salmonella enteritidis* and *E. coli* O157:H7, further validated the models by comparing predictions with published data in foods. The authors mentioned that for *S. enteritidis*, 80% of the predicted D-values from their models were within the 95% confidence interval for different *Salmonella* in various foods.

Even though higher temperature can be applied to delay microbial growth or even kill microorganisms existing in the food, sensory or nutritional losses in the products due to too higher temperature treatment might be a great concern for manufacturers and consumers. Based on this reason, therefore, sometimes, in conjunction with other preservative methods, relatively mild heat treatments are preferred during food processing.

**Tumbling of meat and poultry products**

**Introduction of tumbling**

Tumbling has been widely applied in the meat and poultry industry for years. Pieces of meat are placed inside a drum-like container that rotates during processing. When meat pieces drops from the top to the bottom of the drum as it rotates, a gravitation
impact is produced and applied to the meat. Abrasion against other meat pieces leads to extraction of the myofibrillar proteins to the surface that are necessary for binding meat pieces together during further thermal processing.

Researches have showed that tumbling can improve some physical characteristics, such as increasing cure pickup, moisture retention, water-holding capacity, tenderization, cooking yield, and decreasing curing time as well as enhance some sensory characteristics. Several studies, mainly focused on physical and sensory characteristics of the products, have been conducted on the effects of tumbling on poultry products.

**Tumbling on physical, chemical and sensory qualities of poultry products**

Tumbling has been mainly used to make restructured meat product for several years. Some salt-soluble proteins were extracted during the tumbling process, thus acting as a binder during the further thermal processing. This technique has been widely applied to bind meat pieces together to produce a more solid and uniformly appearing meat and poultry products. Several studies have shown that tumbling could be applied to improve the products’ physical, chemical and sensory qualities, such as increasing moisture retention, water hold capacity, and cooking yield, tenderizing the products, increasing cure pickup, decreasing curing time, preventing oxidation, and etc.

Babji *et al.* (1982) investigated the effect of short-term tumbling on the quality of turkey breast muscle. In their study, the whole turkey carcasses were first tumbled for 30 min at 20 rpm, and then further analyzed. Babji *et al.* (1982) reported that short-term tumbling significantly reduced cooking loss of the tumbled breast muscle samples to 21% when compared with 24% for the non-tumbled samples. In the same study, they reported
that tumbling did not significantly affect chemical composition (protein, fat, moisture, and ash percentages of the raw samples and moisture of the cooked samples), pH of raw samples, and shear forces of the cooked samples.

Brauer (1992) conducted a study to evaluate the effects of applying liquid N\textsubscript{2} during tumbling of hams in order to compensate for excess heat generated during tumbling on the yield and quality of the finished product. In this study, samples were also tumbled without liquid N\textsubscript{2} as a control, or with temperature reduced to 0°C at the start of tumbling by a single dose of liquid N\textsubscript{2} or with intermittent cooling with liquid N\textsubscript{2}. The results show that the intermittent cooling process resulted in the highest cooking yield, color stability scores, and microbiological stability.

In the presence of salt and phosphate, Froning and Sackett (1985) reported that tumbling significantly reduced cooking losses of the turkey breast muscle. Also, in the presence of salt and phosphates, tumbling also improved binding ability, juiciness, and flavor of the breast samples.

Incorporation of adding either isolated soybean protein or carrageenan with the tumbling process, Huang et al. (2001) reported that restructured duck steak sustained good quality during 12 weeks storage at -18°C.

Moran and Bilgili (1996) conducted a study to evaluate the effect of tumbling, which was applied during the chilling process on water uptake and carcass yield of broilers. In their study, after eviscerating, chicken carcasses were either statically soaked in ice-water tank for 4 hr or tumbled in slush-ice for 45 min. The results show that paddle tumbling in slush-ice significantly increased the water uptake and carcass yield of the carcasses when comparing with the static chilling samples. The authors explained
that during tumbling, the additional water was absorbed by skin and entrapped between the skin and muscle surface, thus leading to the increase of water uptake.

Tumbling, which was incorporated with injection of CaCl₂ solution, has been applied to spent fowl meat in a series of studies (Nurmahmudi and Sams, 1997a,b; Nurmahmudi et al., 1997; Woods et al., 1997). Evaluating the effects of tumbling on the quality of the spent fowl meat, Nurmahmudi and Sams (1997a) reported that there was no significant (P>0.05) difference for cooking loss between samples treated with soaking in water for 1 hr or injecting with water, then tumbling for 2 hr. In addition, tumbled samples had significantly (P<0.05) lower shear values of 15.8 kg/g, whereas the water soaked samples had higher shear values of 22.1 kg/g. This result implies that tumbled samples were more tender than the water soaked samples. The authors explained that tumbling could improve penetration and distribution of brine; in addition, it induced mechanical damage to the myofibrillar integrity. Both of these results contribute to the increasing tenderness of samples. Also, in the same study, it was reported that tumbled samples had significantly (P<0.05) lower drip loss of 2.9%, whereas the water soaked samples had higher drip loss of 6.0%. By evaluating the reduction of the shear values of the testing samples, Nurmahmudi and Sams (1997a) pointed out that injecting 0.3 M CaCl₂ and followed by vacuum tumbling (vacuum of -635 mm Hg, 20 rpm at 20°C for 1 hr), could tenderize the spent fowl meat which was often considered as relatively tough in texture.

It is strongly believed that the sensory tenderness results have a high correlation with shear value measurements. Heat and Owens (1987) reported that tumbling for up to 20 min with a solution containing either 5% acetic acid or 1% NaCl did not
significantly (P>0.05) affected the shear values of broiler breast samples when comparing with the samples treated with water only. Further in the same study, they reported that a treatment containing 5% acetic acid and 0.5% NaCl with tumbling up to 20 min did not significantly (P>0.05) affected the shear values of broiler breast samples when comparing with the samples tumbled with water only.

Kijowski and Mast (1993) reported that vacuum tumbling of spent fowl drumsticks, which were less tender, in a solution containing 1.5% acetic acid and 2% NaCl for 2 hr significantly (P<0.05) reduced the shear force values of drumsticks by 32%. In addition, the authors also pointed out instead of marination for 36 hr, the tenderization result obtained for the samples could also be achieved by using a 8-10 hr tumbling treatment.

Maki and Froning (1987) evaluated the effect of tumbling on the quality characteristics of turkey breast muscle. In their study, the whole turkey carcasses were injected with 0 or 7% sodium chloride solution in the presence or absence of 12% sodium tripolyphosphates at 3% of the tissue weight. The authors reported that tumbling for one hour had significantly (P<0.05) lower cooking losses of 21.24% for the turkey breast muscles when compared with the samples without tumbling that had 24.97%.

By evaluating the reduction of the shear values of the tested samples, Nurmahmudi and Sams (1997a) pointed out that injecting 0.3 M CaCl$_2$ and followed by vacuum tumbling (vacuum of −635 mm Hg, 20 rpm at 20°C for 1 hr), could tenderize the spent fowl meat which was often considered as relatively tough in texture.
Tumbling on diffusion of curing agents, brine or marinade solution

In addition to improve some physical and sensory characteristics of the products, tumbling has also been applied to help the distribution of curing agents, brine, and marinade solution during processing.

By monitoring the diffusion rate of the curing agents, tumbling either as intermittent tumbling (10 min per hour for 18 hr of tumbling) or continuous tumbling (3 hr) was reported to increase migration of curing agents and brine (Ockerman and Organisciak, 1978 a,b). In a study of evaluating the effect of tumbling on distribution and content of sodium nitrite and sodium chloride in bacon, Ockerman and Dowiercial (1980) reported that tumbling result in a more even distribution of nitrite and chloride. Krause et al. (1978) found tumbling for 10 min out of each hour for 18 hr resulted in increased migration of salt and nitrite into semimembranosus muscles when compared with the non-tumbled samples. Similarly, Leak et al. (1984) found that tumbling significantly accelerated salt and nitrite penetration for the dry-cured hams during the first 2 weeks of curing.

Using a thin-slicing and dye-tracing method, Xiong and Kupski (1999a) monitored the phosphate marinade penetration in tumbled chicken fillets. Chicken breast fillets were tumbled for 0, 5, 15, and 30 mm in marinades containing 1.6 or 3.2% phosphates with or without 8% NaCl. By using a spectrophotometric measurement (absorbance at 627 nm) to trace FD&C Blue No. 1 dye migrating into different layers of the fillets, and marinade penetration was monitored. The authors pointed out that marinades with phosphates at lower concentration of 1.6% penetrated most rapidly in the initial 5 min. Similarly, Chen (1982) pointed out that marinating and tumbling in a
hexagonal-shaped drum rotated at 31.5 rpm at room temp had an increase in the rate of penetration of a salt solution when compared to the still-marinating at refrigerator temperature. The author stated that little increase of marinade penetration was found after 10 min of marinating and tumbling treatment.

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.

**Effect of tumbling on microbiological quality of meat products**

Currently the major function of tumbling applied to meat products focuses on improving the physical and sensory attributes of the products. Little information is available on the effect of tumbling on the microbiological quality of the products.

Knipe *et al.* (1981) studied the effect of tumbling on the total aerobic counts of hams. They found that intermittently tumbling for 10 min of every hour for 18 hrs significantly (p<0.01) lowered the total aerobic plate counts of the boneless cured ham. Yetim *et al.* (1996) reported that intermittently tumbling (40 min rest, 20 min of work for 12 hr) with curing ingredients slightly decreased the aerobic plate count (APC). *Pseudomonas*, lipolytic and proteolytic bacteria counts in catfish compared to non-tumbled samples. The authors explained that this microbial reduction might be due to better cure distribution by tumbling and faster cure penetration. In addition, the authors indicated that there was no negative effect on microbiological quality of tumbled catfish.

Leak *et al.* (1984) found that cry-cured hams treated with either 1 hr continuous tumbling, 3 hr intermittent tumbling (10 min/hr), or 6 hr intermittent tumbling (10
min/hr), generally had lower microbial counts when compared with the samples without tumbling.

Leblanc et al. (1996) evaluated the microbiological quality of rainbow trout fillet, which were previously vacuum tumbled in sodium lactate solution then stored under CO₂ at -2, 0 or 4°C for up to 56 days. The authors concluded that prior to storage under CO₂, vacuum tumbling (12 rpm, 20 min) in a sodium lactate solution followed by CO₂ atmosphere packaged could improve the shelf life of rainbow trout fillet.

Ramos and Lyon (2000) conducted a study showing that a treatment which consisted of 0.4% ascorbic acid, 0.4% NaCl, with vacuum tumbling (28 in. Hg, 8 rpm for 8 min) could result in a significant reduction (P<0.05) of the total aerobic counts of catfish fillets, when compared with treatments without tumbling. The authors pointed out that this process combination of tumbling, ascorbic acid, and salt could be applied to reduce the endogenous bacteria associated with catfish fillets.

**Lactoperoxidase system (LPS)**

**Introduction of lactoperoxidase system**

The lactoperoxidase system (LPS), which consists of lactoperoxidase (LP), thiocyanate (SCN⁻), and hydrogen peroxide (H₂O₂), is an inhibitory system that is present naturally in bovine milk. This system has been shown to be inhibitory against some pathogenic and spoilage microorganisms such as *P. fluorescens*, *L. monocytogenes*, *S. typhimurium* and *E. coli* (Zapico et al., 1995; Kamau et al., 1990a,b; Earnshaw et al., 1990). In the presence of SCN⁻ and H₂O₂, Kamau et al. (1990a,b) explained, the LPS generates the hypothiocyanite (OSC⁻) and hypothiocyanous acid (HOSCN), which are
the main antimicrobial products, and strong oxidizing agents that can oxidize essential sulfhydryl groups in bacterial proteins thus causing bactericidal effects. Ray (1992) explained that the LPS has antimicrobial characteristic because of the production of toxic, short-lived, oxidation products when lactoperoxidase catalyzes the oxidation of thiocyanate by hydrogen peroxide. Wolfson and Sumner (1993) explained that the LPS could alter many cellular systems, including the outer membrane, cell wall, cytoplasmic membrane, transport systems, glycolytic enzymes, and nucleic acids.

**Application of LPS in meat and poultry products**

The lactoperoxidase system has been mainly studied in the application for the milk and dairy products (Zapico et al., 1998). Only a few studies have been attempted to apply this lactoperoxidase system in meat and poultry products.

*Campylobacter jejuni*, which often contaminates poultry carcasses through fecal material during the slaughter process, is one of the major causes of bacterial diarrhea illness in the United States. Consumption of improperly cooked chicken and poor hygiene may cause a risk of *Campylobacter* infection. Borch et al. (1989) investigated the sensitivity of *C. jejuni* and *C. coli* isolated from poultry to the LPS. Their results showed that at 37°C the LPS had a more rapid bactericidal effect against *C. jejuni* and *C. coli* than the LPS at 20°C. Also, with decreasing pH values, the effect of the LPS decreased. The fastest reduction in viable numbers was obtained at pH 6.6 and 37°C. The authors concluded that the LPS might be utilized to reduce contamination of *C. jejuni* from poultry during the slaughter process.
Kennedy et al. (2000) evaluated the inhibitory effect of the LPS on the growth of selected microorganisms in a minced beef system. Sterilized ground beef was inoculated with *E. coli* O157:H7, *L. monocytogenes* L45 and *Staphylococcus aureus* R37, added LPS, and then stored at 0, 6 and 12°C. The authors reported that the inhibitory effect of LPS against the selected strains was temperature dependent. It was found that *L. monocytogenes* was the most sensitive to LPS among the selected microorganisms, and followed by *S. aureus* and *E. coli*. In addition, in the same study, the inhibitory effect of LPS against the naturally present bacterial populations in 8 retail beef mince samples was also evaluated. Except for one faecal coliform count, the authors reported that the total aerobic and faecal coliform counts in the LPS treated samples were significantly lower than those of the controls when the samples were incubated at room temperature for 4 hrs.

Wolfson et al. (1994) evaluated the inhibitory effect of LPS and heat treatment on *Salmonella typhimurium* on poultry. Inoculated chicken legs were immersed in a solution containing the LPS (1μg/ml lactoperoxidase, 5.9 mM potassium thiocyanate, and 2.5 mM hydrogen peroxide) at 25, 50, 55, or 60°C for 5, 15 or 30 min, and the microbial quality of the samples were then analyzed. Depending on the treatment time and temperature, the authors reported that the treatment of 60°C for 15 min resulted in the largest microbial reduction of 80.6% in this study.

In the same study, chicken legs and thighs were immersed in a 50°C bath containing the LPS (1μg/ml lactoperoxidase, 5.9 mM potassium thiocyanate, and 2.5 mM hydrogen peroxide) for 5 min, stored at 4°C for 2 days, and then analyzed for microbial quality (Wolfson et al., 1994). The authors reported that 2.15 log aerobic plate counts

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increased for the treated sample whereas 5.13 log increase was found for the control samples. In addition, 1.14 log psychrotrophic counts decreased for the treated samples whereas the counts for the control samples increased by 0.77 log. There were no significant (p<0.01) difference in Hunterlab color values (L, a, b) between the LPS-treated chicken thigh skin and controls. As measured by TBA values, no significant difference (P<0.05) in oxidative deterioration existed between the treated chicken thigh meat and controls. The authors concluded that the treatment combination of LPS and thermal treatment resulted in the reduction of Salmonellae on poultry, and the inhibition of psychrotrophs growth, thus leading to extended samples’ shelf life. In addition, there was no negative effect of LPS treated on the Hunter color and TBA values.

Heuvelink et al. (1999) evaluated the effects of several additives including LPS on the survival of verocytotoxin-producing E. coli O157:H7 (O157 VTEC) on meats obtained from retail outlets in the Netherlands. In their study, filet americain, which was a product of minced beef with a fat content of less than 10% and mixed with a mayonnaise-based sauce, was first mixed with 1 or 2 units of LPS (1 unit consisted of 18.5 μg/ml LP, 0.3% glucose, 0.1 U/ml glucose-oxidase, and 0.26 mM sodium thiocyanate), then inoculated with 10⁷ CFU/g of E. coli O157:H7. After incubation, the total number of viable E. coli O157:H7 VTEC cells was analyzed. The authors pointed out that addition of LPS did not result in a reduction of viable E. coli O157:H7 O157 VTEC cells, which were previously inoculated on the filet americain during storage at 7 or 15°C.
Combined effect of LPS and nisin

Boussouel et al. (2000) evaluated the effects of LPS and nisin against inoculated *L. monocytogenes* ATCC 15313 in skim milk. Adding nisin induced an immediate bactericidal effect whereas adding LPS resulted in a 48 h bacteriostatic phase. Also, *L. monocytogenes* would re-grow when adding either nisin or LPS alone. However, adding LPS and nisin simultaneously resulted in a synergistic and lasting bactericidal effect after the 8th day, and *L. monocytogenes* was not detectable until the 15th day.

Furthermore, in the same study, Boussouel et al. (2000) evaluated the inhibitory effect when one antimicrobial agent was added to the *L. monocytogenes*, which was previously treated with another agent. The authors reported that when LPS was added to the *L. monocytogenes*, which was already treated with 100 or 200 IU/ml nisin for 4 hr, the inhibitory activity was enhanced with no *L. monocytogenes* detectable after 72 or 48 hr, respectively, and *L. monocytogenes* was not detectable until 15 days. When LPS was added after 12 h of *L. monocytogenes*, which was previously treated with nisin, the bactericidal effect was followed by regrowth of *L. monocytogenes*. On the other hand, when 100 or 200 IU/ml of nisin was added to *L. monocytogenes*, which were already treated with LPS over 24 h, *L. monocytogenes* was not detectable after 196 and 244 h, respectively, and no regrowth of *L. monocytogenes* were detected. When nisin was added after 72 h of previously treated with LPS, *L. monocytogenes* counts were 8 log_{10} cycles lower than the control samples after 196 hr, but the microbial levels were similar to the control after 15th days. The authors concluded that adding nisin followed by the addition of LPS 4 hr later resulted in the maximum inhibition of *L. monocytogenes* ATCC 15313.
Zapico et al. (1998) evaluated the synergistic effect of nisin and LPS on *Listeria monocytogenes* in skim milk. In this study, adding nisin at either 10 or 100 IU/ml to UHT skim milk only slightly reduced the *L. monocytogenes* counts, and the microbial growth resumed again after 24 h at 30°C, whereas addition of the LPS resulted in a 3-log reduction when compared with the control milk after 24 h at 30°C. However, addition of nisin and LPS showed a synergistic antimicrobial effect and resulted in 5.6 log reduction lower than the control samples.

Furthermore, in the same study, Zapico et al. (1998) reported that the synergistic effect was enhanced when these two antimicrobial agents were added in two steps at different times on a two-hour interval. In their study, *L. monocytogenes* was first inoculated into skim milk, 3 hrs later followed by adding the first antimicrobial agent. Five hours later (2 h after the first agent added) the second agent was added. In their study, *L. monocytogenes* was first inoculated into skim milk. 3 hrs later followed by adding the first antimicrobial agent, and 5 hrs later (2 h after the first agent added) adding the second agent. Adding nisin and LPS together after 3 hr of *L. monocytogenes* Ohio inoculation lowered the microbial counts by 5.7 log unit compared with the control sample. However, adding two agents in two steps (LPS after 3 hr of inoculation, and nisin after 5 h inoculation) resulted in a 7.4 log reduction when compared with the controls. The authors concluded that LPS and nisin had a synergistic bactericidal effect on *L. monocytogenes*, and this effect was further enhanced by the addition of the two agents in two steps with a two-hr interval between addition times.
**Combined effect of LPS and thermal treatment**

It was reported that lactoperoxidase (LP), which is part of the lactoperoxidase system, was relatively heat-stable and it retained its activity after milk pasteurization at 63°C for 30 min or 72°C for 15 sec (Korhonen, 1980).

A study done by Kamau *et al.* (1990a) showed that LPS enhanced the thermal destruction of *L. monocytogenes* and *S. aureus*. D value (decimal reduction time), which is defined as the time required to destroy 90% of the microorganisms, is often used to indicate the ability of destruction of microorganisms. The smaller the D value for the specific microorganism, the less time was required to kill this microorganism at the given temperature. In their study, for *L. monocytogenes*, a D$_{52.2\,^\circ\text{C}}$ value for the LPS treated milk sample was 10.7 min, which was less than the 30.2 min of the controls. Similarly, the D$_{55.2\,^\circ\text{C}}$ and D$_{55.8\,^\circ\text{C}}$ values of the LPS treated samples were both less than the values of the controls. Similar reduction of thermal D values was also observed for *S. aureus*. The authors explained that by damaging bacterial membranes, LPS might enhance the thermal destruction of microorganisms. Also, the authors concluded that combination of LPS with thermal processing could increase safety of milk with respect to milkborne pathogens.

Wolfson and Sumner (1994) evaluated the effect of LPS and heat treatment against *Salmonella typhimurium* in trypticase soy broth. In their study, the LPS that was consisted of lactoperoxidase (1 µg/ml), potassium thiocyanate (5.9 mM) and H$_2$O$_2$ (2.5 mM), was found to enhance the thermal inactivation of *S. typhimurium*. The D$_{50\,^\circ\text{C}}$ value of the LPS activated broth was 12 min whereas the value of the control samples was greater than 60 min. Similarly, the D values of the treated samples at 52, 55, 58 and
60°C (7.5, 4, 2.5, 0.20 min, respectively) were smaller than the corresponding D values of the control samples (20, 14, 5, and 0.31 min, respectively) at the same temperatures.

Wolfson et al. (1994) reported that immersion of the inoculated chicken legs and wings in a 50°C solution containing the LPS (1µg/ml lactoperoxidase, 5.9 mM potassium thiocyanate, and 2.5 mM hydrogen peroxide) for 5 min, and stored at 4°C for 2 days resulted in a 2.15 log aerobic plate counts increase for the treated sample but a 5.13 log increase for the control samples. Also, psychrotrophic count decreased by 1.14-log for the treated samples whereas the control samples counts increased by 0.77 log.

**LPS on sensory quality**

Since most of the studies involving the application of LPS have been mainly focused on milk and dairy products, very limited information about the influence of the LPS on the sensory characteristics of treated meat and poultry products are available. Wolfson et al. (1994) pointed out that the Hunterlab color values (L, a, b) for the chicken thigh skin, and oxidative deterioration as measured by TBA values for the chicken thigh meat, did not significantly differ between the control and treated samples.

**Application of LPS to extend the shelf life of products**

Most of the efforts reported on LPS were to extend the shelf life of milk and dairy products. For example, a study done by Zajac et al. (1983) showed that adding NaSCN and percarbonate which were further decompose into carbonate and H₂O₂ in raw milk, at 4°C resulted in a standard plate count of LP-activated milk remained fairly unchanged for
at least 104 h, whereas bacterial multiplication in the controls started after 48h.

The lactoperoxidase system (LPS), which consists of lactoperoxidase (LP), thiocyanate (SCN'), and hydrogen peroxide (H₂O₂), is an inhibitory system that is present naturally in bovine milk. Various studies of this system have been shown to be inhibitory against hazardous and spoilage microorganisms. However, most of the studies were applied or focused on the preservation of dairy product especially raw milk. Similarly, Huss et al. (1995) also suggested even though there was no published report on the activity of LP on fish products, the LPS should have a potential to apply in the biopreservation of fish products. How to apply this valuable natural antimicrobial system in other food systems, such as meat and poultry products, might be considerable in the future.

Acetic acid

Introduction of acetic acid

Acetic acid (M.W. 60.05, pHₐ 4.76), which is the predominant acid in vinegar, has been granted as the category of “generally regarded as safe” (GRAS) for miscellaneous and general-purpose usage under FDA regulation (FDA, 1993). Used either as an acidulant to reduce pH, or a flavoring agent to provide sour flavor, acetic acid has also been used as an antimicrobial agent in meat and poultry industry.

Acetic acid on physical and sensory qualities of meat and poultry products

Bell et al. (1986) evaluated the effect of acid on the sensory qualities of beef. In
their study, beef cubes were dipped in a solution contained various levels of acetic acid. After dipping in a 0.6% acetic acid solution for 1 min, no significant (P>0.05) subjective color scores for the treated and control samples existed, whereas significant (P<0.05) subjective color scores existed for the solution contained more than 1.2% acetic acid. On the other hand, significantly (P<0.05) different subjective color scores were observed for the samples dipped in a solution contained 0.6% acetic acid for 10 min. In the same study, by a triangle test, the authors reported that there was a significant flavor difference between the cooked samples, which were previously dipped in 1.2% acetic acid or distilled water.

Dickens et al. (1994) investigated the effects of acetic acid on chicken carcass appearance, and cooked breast meat texture and flavor. In their study, prechill broiler carcasses were treated with 0.6% acetic acid at 10°C for 10 min, which represented a simulated prechill in poultry processing. By subjective evaluation, the authors reported that the skin of the acid-treated carcasses turned a light yellow. In addition, there was no significant (P<0.05) difference of the Warner-Bratzler shear force values for the treated and control cooked Pectoralis major. By a triangle test, the authors indicated that there was no significant (P<0.05) difference in sensory characteristics of the acid-treated when compared to the control cooked Pectoralis major. The authors concluded that there were no adverse effects on the texture and sensory qualities of the cooked poultry meat by applying 0.6 % acetic acid in the prechill water.

In another study, Dickens and Whittemore (1997) reported that sprayed with 1% acetic acid solution on chicken carcasses for 30 sec during the defeathering process did
not result in differences in chicken’s skin appearance. The authors explained that the appearance change of the poultry carcasses might be determined by the contact time of the acid.

Dickens and Whittemore (1994) evaluated the effects of acetic acid on appearance and moisture pick-up of processed poultry carcasses. In their study, pre-chill broiler carcasses were chilled in a 10°C solution containing 0.3 or 0.6% acetic acid for 10 min, and then analyzed. The authors mentioned that acetic acid treated samples had darkened or yellow skin. Similarly, darkened skin of the acetic acid treated broiler carcasses were also observed in another study (Dickens and Whittemore, 1995).

In a study of evaluating the effect of acetic acid on the physical quality of refrigerated chicken legs, Kim et al. (1998) reported that chicken legs treated with 1.0% acetic acid did not significantly (P>0.05) affect the L*, a*, and b* color values of the samples during 12 days of refrigerated storage at 4°C when compared with the control samples. Due to acidic odor and discoloration, the authors also pointed out that acetic acid treated chicken legs were preferred less by sensory panels than the control samples.

In a study of evaluating the effects of organic acids on the physical, textural and organoleptic qualities of chicken breast meat during storage, Jacques (1990) reported that chicken breasts which were tumbled with 1% acetic acid for 30 min at 10°C, and stored at 2-4°C did not affect the Hunter “L”, “a”, and “b” color values, and juiciness of the samples.

In a study of investigating the application of acetic acid to inactive microorganisms on chicken carcasses during process, Sakhare et al. (1999) reported that
the appearance of the treated carcasses was not affected by scalding in acidified water that contained low concentration (0.5%) of acetic acid at 58°C for 2 min.

Tamblyn and Conner (1997) observed some undesirable characteristics such as skin leaching and off-odor for the chicken skin samples, which were treated with acetic acid with concentration higher than 2%.

**Acetic acid on microbial quality**

In a study of evaluating the effects of acetic acid on the microbiological quality of processed poultry carcasses, Dickens and Whittemore (1995) reported that treating with 0.6% acetic acid for 1 hour chilling resulted in significant (P<0.05) lower total aerobes and Enterobacteriaceae counts when compared with the control samples.

Dickens and Whittemore (1994) evaluated the effects of acetic acid on the microbiological quality of processed poultry carcasses. In their study, pre-chill broiler carcasses were chilled in a 10°C solution containing 0.3 or 0.6% acetic acid for 10 min. and then analyzed. The authors reported that acid treated samples had significant (P<0.05) higher Enterobacteriaceae reduction when compared with the control carcasses. In the same study, the authors mentioned that the incidence of *Salmonella*-positive carcasses, which were previously inoculated with *Salmonella*, then treated with acetic acid solution, was significantly (P<0.05) reduced.

Kim *et al.* (1998) evaluated the microbiological quality of refrigerated chicken legs treated with acetic acid. In their study, chicken legs were treated with 0.5-1.5% of acetic acid for 5 and 10 min, stored at 4°C, then analyzed. The authors reported that the
aerobic plate and gram-negative bacterial counts on the surface of chicken legs treated with acid were significantly (P<0.05) reduced during storage. Also, the authors pointed out that samples treated with 1.5% acetic acid for 10 min had an extended microbiological shelf life by 4 additional days when compared with the samples treated for only 5 min.

**Combined effect of acetic acid and thermal treatment**

Combined treatments of acetic acid and heat (thermal treatment) have been applied to inactive microorganisms in meat and poultry products.

Anderson et al. (1988) evaluated the sanitizing effectiveness of hot acetic acid applied to lamb carcass samples. In their study, after either spraying or dipping in 1.5 or 3.0% acetic acid at either 25 or 55°C for 10 sec, microbial qualities of the samples were evaluated. The author reported that at 55°C a 3% concentration of acetic acid resulted in a higher reduction of microbial counts for aerobes, psychrotrophs, gram-negatives, coliforms, *Bacillus thermosphacta* and lactobacilli, than 1.5% acetic acid, although the reduction in counts was not always significant (P>0.05).

Since scalding has been thought to be one of the most potential cross contamination steps in poultry carcasses processing, researchers have tried to incorporated some chemicals including acetic acid into the poultry scalding water to reduce microbial counts of the poultry carcasses. Okrend et al. (1986) reported that adding 0.1% acetic acid to scald water reduced the thermal death time D_{50*} values of *S. newport, S. typhimurium*, and *Campylobacter jejuni*. Similarly, Lillard et al. (1987) applied 0.5 % acetic acid in poultry scalding water and reported almost a 100% reduction.
in total aerobic plate count (5.46 to 3.21 log_{10}/ml water) in the water but no significant microbiological effects on the carcasses.

Sakhare et al. (1999) investigated the effects of acetic acid dipping or spraying on the microbial quality of chicken carcasses during processing. In their study, following scalding, chickens were either dipped in 0.5% acetic acid or were spray washed with water alone or water with added 0.50% acetic acid. Scalding (58°C for 2 min) was achieved in water containing either acetic acid, or no acid added; scald water was later collected for microbial analysis and the microbial counts were analyzed. Addition of acid to scald water lowered the microbial load, lessening the possibility of cross contamination of chicken internal organs and edible tissues. Dipping the defeathered and eviscerated birds in acid also lowered the microbial load, although this was not as effective as spray washing with an acid-water solution. Dipping or spraying the chickens in this way significantly improved their microbiological quality, and also enhanced their shelf life.

Evaluating the effect of immersing beef tissues in up to 3% acetic acid at 20 or 55°C on survival of E. coli, Greer and Dilts (1992) reported that recovery of E. coli was significantly reduced by acid temperature and/or concentration of acid.

**Sodium chloride**

Salt, which probably is one of the most common food additives in food formulations, has been widely applied in manufacturing food for years. In addition to provide some salty flavor, salt (mainly sodium chloride) also retards microbial growth. Ockerman (1996) mentioned that bacterial cell walls, which are semi-permeable, allow
water but not salt to pass through. Water will then pass from the less to higher dense concentration, thus causes shriveling of the bacterial cell. In addition, direct toxicity of chloride, reducing effectiveness of bacterial proteolytic enzymes, and etc. also contribute the antimicrobial function of salt. Even though higher quantities of salt can be applied to retard the microbial growth, sensory acceptance of the high-salt products might not be met. Also, worried that salt may be a health concern, current consumers prefer a low salt consumption.

When incorporated with other ingredients, such as some phosphates, salt can be applied to tenderize and increase water-holding capacity of meat and poultry products. Also, salt is needed for extracting salt-soluble proteins for emulsifying products.
CHAPTER 3

EXPERIMENT 1

EFFECT OF NISIN WITH THERMAL TREATMENT ON THE MICROBIAL QUALITY OF MARINATED CHICKEN DRUMSTICKS

ABSTRACT

Currently meat marination is practiced to improve product's physical and sensory attributes, and usually not intended to improve the microbial quality of the product. Nisin, which is a natural, nontoxic, and heat stable polypeptide produced by *Lactococcus lactis*, has been shown to inhibit many microorganisms, and has been approved for use in some dairy products. Thermal treatment has been widely applied to control the growth of microorganisms in the food industry for years. Limited information on the contribution of marination, nisin, and thermal treatment to the microbial quality of treated poultry products is available. 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 thermal treatment (4 or 58°C for 2 min), and then stored at 4°C for 18 hrs. After marinating, the microbial counts of the samples were measured at 0, 2, 4, and 7 days (i.e. 1, 3, 5 and 8 days after the sell-by date of the samples) of refrigerated storage. The results indicate that at a given nisin-added level, the samples with thermal treatment had significantly (P<0.05)
lower microbial counts when compared with the samples without thermal treatment. Samples given a thermal treatment with nisin added had significant (P<0.05) lower microbial counts when compared with the samples with the same thermal treatment but with less nisin added. Microbial counts significantly increased with storage time. In conclusion, adding 50 IU/ml of nisin with thermal treatment (58°C for 2 min) decreased the total microflora and psychrotrophs counts of the marinated chicken broiler drumsticks.

Keywords: nisin; thermal treatment; marination; microbial quality

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 food industry because it increases variety and adds value to products. Hoogenkamp (1987) described marinating as a diffusion or penetration of a solution into the meat structure. Similarly, 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 tenderness, water holding, flavor and etc. (Hashim. et al. 1999; Lemos et al. 1999; Xiong and Kupski, 1999; Young and Buhr, 2000; Young and Lyon, 1997a,b; Zheng et al. 2000). but the process is usually not intended to improve the 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 \textit{Lactococcus lactis} strains, and has been shown to inhibit gram-positive microorganisms (Ray, 2001), and gram-negative bacteria when combining with a chelating agent such as EDTA (Stevens \textit{et al.} 1992; Stevens \textit{et al.} 1991).

Nisin, along with many other physical or chemical treatments, has been studied in extending the shelf life of poultry products. Natrajan and Sheldon (2000) reported that drumsticks immersed in a solution consisting of 100\,\mu{g}/ml nisin, 5.0 mM EDTA, 3.0\% citric acid, and 0.5\% Tween 80, with the pH adjusted to 3.5 for 3 min, followed by packaging in a foam traypack containing a nisin-treated PVC overwrap and absorbent tray pad, and refrigerated at 4\,^\circ\text{C}, was estimated to extend the shelf life by 0.6 to 2.2 days while compared with the controls. Immersion in a solution consisting of 100 \mu{g}/ml nisin, 5.0 mM EDTA, 3\% citric acid, 0.50\% Tween 20, and pH adjusted to 3.5 for 30 min. extended the shelf life of refrigerated broiler drumsticks by 1.5 to 3 days when compared to those dipped in sterile distilled and deionized water (Shefet \textit{et al.}, 1995). Tsou (1995) reported that fresh chicken drumsticks treated with 5000 IU/ml of nisin, then vacuum-packaged and stored under refrigeration had significantly (P<0.05) lower total aerobic counts when compared with samples without nisin added during the refrigerated storage for 9 days. Xu \textit{et al.} (2000) reported that salted duck treated with 400 mg/kg of nisin with 3.5\% sodium lactate, vacuum packed and exposed to a microwave treatment (915 MHz, 400 W, 3 min) had a longer shelf life of greater than 20 days, whereas the shelf life
of the controls had only a 6 day shelf life based on the microbiological, physicochemical (peroxide value) and sensory evaluation results.

Thermal or heating treatment, which is one of the most common 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, bleaching, pasteurizing, drying, and etc. 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.

Nisin, which was reported as a heat-stable bacteriocin (Jeknic et al., 1996; Mahadeo, 1995; Jay, 1996), has been combined with mild heat treatment to inactive microorganisms in several studies. Budu-Amoako et al. (1999) reported that nisin (25 mg/kg), in combination with a heat process (60°C for 5 min or 65°C for 2 min) resulted in a greater killing of inoculated Listeria monocytogenes (3-5 logs reduction) in cold-pack lobster meat, when compared with either a treatment with nisin or with heat alone (1-3 logs reduction). Kalchayanan et al. (1992) reported that moderate heat, along with nisin, resulted in greater bactericidal effect against L. monocytogenes than either heat or nisin added alone. Mahadeo and Tatini (1994) reported that the scald water samples treated with combination of 100 IU/ml nisin and heat (52°C for 3 min) resulted in a lower total microflora count (5.5 log CFU/ml) when compared with either nisin added (5.7 log CFU/ml) or heating (7.1 log CFU/ml) alone.

Maisnier-Patin et al. (1995) found a dramatic increase of thermal destruction of L. monocytogenes in the presence of low levels of nisin (25 or 50 IU/ml). Modi et al.
(2000) found that treatment combining nisin (500 IU/ml) and heat treatment (55°C) resulted in a 3.7 log microbial reduction when compared with the individual treatment of nisin or heat which resulted in only 1 log reduction for those nisin-resistant L. monocytogenes mutants. Exposure to the treatment that consisted of nisin (0.1-0.5 μg/ml) and mild heat treatment (48-56°C for 5 min) resulted in a higher reduction of inoculated Lactobacillus plantarum, which is a food spoilage microorganism, when compared with the ones with a single (nisin or heat) treatment (Ueckert et al., 1998).

The objective of this study was to determine the effect of various factors including nisin-addition (levels of 0, 50, or 100 IU/ml), thermal treatment (4 or 58°C for 2 min), and storage time (day 0, 2, 4, and 7; i.e. 1, 3, 5 and 8 days after the sell-by date of the samples) on the total microflora and psychrotrophs counts of the marinated chicken drumsticks.

**MATERIALS AND METHODS**

Development of the marinade solution:

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 experiment, a simplified water-based marinade that contained 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 20mM disodium EDTA (Fisher Scientific Co., Kansas City, MO), were added to the standardized marinade based on the
results from a 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 0.02N HCl and held for 2h at 25°C. After adding pre-assigned levels of nisin and disodium EDTA, the marinade solutions were adjusted to pH 4, autoclaved for 121°C for 15 min, and then stored at 4°C before conducting the experiment.

Sample preparation

A total of ninety-six drumsticks which had the same sell-by date and 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. On the sell-by date which was indicated on the packages, inside the walk-in cooler, drumsticks were initially mixed thoroughly in 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 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 hr.
Thermal marination preparation

For those samples receiving thermal-marination treatment, the bags containing pre-autoclaved marinade solutions with various levels of nisin added were first 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-chicken 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 hours (including the previous time of thermal marinating and cooling).

Microbial evaluation

After 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 bags and stored under refrigeration at 4°C. In the current study, refrigerated storage day 0 was set as one day after the sell-by date shown on the packages of the drumsticks. At specified sampling times (0, 2, 4, or 7 day; i.e. 1, 3, 5 or 8 days after the sell-by date of the samples), 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 bacteria group. Total microflora and psychrotrophs were incubated at

50
35°C for 48 hours 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. The total microflora and psychrotrophs counts of the day 0 (i.e. one day after the sell-by date) samples without any treatment were also evaluated. In the current study, the microbial counts in log CFU/ml of the rinse solution were also calculated and expressed as log CFU/cm\(^2\) of the surface area of the dramsticks based on the average surface area of 174 cm\(^2\). Experimental outline is illustrated in Figure 3.1.

Statistical analyses

The study was designed as a 3 \( \times \) 2 \( \times \) 4 factorial experiment, 3 nisin-adding levels (0, 50, 100 IU/ml), thermal treatment (4 or 58°C for 2 min), and 4 storage times (day 0, 2, 4, and 7; i.e. 1, 3, 5 and 8 days after the sell-by date of the samples) and with two replicates. 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 (\( P > 0.05 \)).

RESULTS AND DISCUSSION

At day 0 (i.e. one day after the sell-by date), the samples without any treatment had total microflora counts of 8.3 CFU/ml (6.0 CFU/cm\(^2\)) and psychrotrophs counts of 8.8 CFU/ml (6.5 CFU/cm\(^2\)). There was no significant (\( P > 0.05 \)) three-way interaction
(thermal treatment × nisin-adding level × storage time) for the total microflora counts for the samples with or without thermal treatment, different levels of nisin added, and during refrigerated storage.

Only one two-way interaction (thermal treatment × nisin-added level) was significant (P<0.05). Also, as illustrated in Figure 3.2, when no nisin was added, the difference of the total microflora counts between the samples with or without the thermal treatment was 1.3 log, which was approximately double the amount in the samples with nisin added at either 50 or 100 IU/ml (0.6 and 0.7 log, respectively) with thermal treatment.

As illustrated in Figure 3.2, in the current study, as the nisin-added levels increased, less total microflora counts of the samples were obtained for the samples with thermal treatment (same pattern was observed for the samples without thermal treatment, even though the slopes were different). For example, samples with thermal treatment and 100 IU/ml nisin had a significant (P<0.05) lower total microflora count of 5.4 log CFU/ml (3.1 log CFU/cm²), when compared with the samples with thermal treatment but without nisin added (6.1 log CFU/ml; 3.4 log CFU/cm²). Similarly, samples with 50 IU/ml nisin but without thermal treatment had a significant (P<0.05) lower total microflora count of 6.2 log CFU/ml (4.0 log CFU/cm²), when compared with the samples without thermal treatment and nisin added (7.4 log CFU/ml; 5.2 log CFU/cm²). Samples with 100 IU/ml nisin but without thermal treatment had significant (P<0.05) lower total microflora count of 6.0 log IU/ml (5.8 log CFU/cm²), when compared with samples without adding nisin and without thermal treatment. No significance difference of total microflora counts was observed between the samples treated either 50 or 100 IU/ml when
no thermal treatment was applied.

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.

Delves-Broughton (1993) reported that more lethality of *Pseudomonas fluorescens* was observed when adding nisin at higher levels of 25 or 75 μg/ml with 0.5-10 mM EDTA, comparing with lower levels (0, 1.25, 2.5, 7.5 μg/ml) of nisin. In the same study, Delves-Broughton (1993) reported that nisin at higher concentrations of 6.25 and 12.5 μg/ml resulted in a greater lethality of *P. fluorescens* when compared with the treatments with lower concentration of 2.5 μg/ml of nisin at treatment temperatures of 20, 25, and 30°C. Less difference of lethality due to different concentrations of nisin was observed at lower temperatures of 6 and 12°C.

Fang and Lin (1994) reported that adding higher levels of nisin at 1000 or 2500 IU/g resulted in significant higher reduction of inoculated *L. monocytogenes* on plate count agar, when compared with the samples with lower levels of nisin added (0 or 250 IU/g). Murray and Richard (1997) evaluated the effect of different levels (300, 1500, and 3000 AU/ml) of nisin on the *Listeria innocua* Lin 11 numbers of the raw pork cube samples, which were previously inoculated with *L. innocua* Lin 11, and then soaked in nisin solution, ground and stored aerobically at 5°C. They found that the microbial reduction increased as nisin concentration increased.

Stevens *et al.* (1992) evaluated the different treatment conditions, including nisin concentration, chelating types, treatment incubation temperature, and protein interference
on the inactivation of selected gram-negative bacteria. In their study, Stevens et al. (1992) reported that higher concentration (50 to 100 μg/ml) of nisin added was generally more effective at inactivation of the selected Salmonella species when compared with ones with lower levels (10 to 25 μg/ml) of nisin added. However, there was no significant difference between levels of 50 and 100 μg/ml of nisin added in retarding some Salmonella species, such as S. hadar 3503-2 and S. Heidelberg.

As illustrated in Figure 3.2, the total microflora count of 6.1 log CFU/ml (3.9 log CFU/cm²) for the samples with thermal treatment but without nisin added was significantly (P<0.05) lower when compared with the count of the samples without nisin added and without thermal treatment, which was 7.4 log CFU/ml (5.2 log CFU/cm²). Similarly, combined thermal treatment and 50 IU/ml nisin added resulted in significantly (P<0.05) lower total microflora count of 5.7 log CFU/ml (3.4 log CFU/cm²) when the samples with 50 IU/ml nisin added but without thermal treatment, which was with 6.2 log CFU/ml (4.0 log CFU/cm²). Treatment that combined thermal treatment and 100 IU/ml nisin added resulted in significantly (P<0.05) lower total microflora count of 5.4 log CFU/ml (3.1 log CFU/cm²) when compared with the samples contained 100 IU/ml nisin added but without thermal treatment which was with 6.0 log CFU/ml (3.8 log CFU/cm²). This result indicates that for each nisin-adding level, the thermal treatment resulted in significant (P<0.05) lower total microflora counts when compared with the samples without thermal treatment at the same nisin level.

When evaluating the temperature effect on the lethality of P. fluorescens, Delves-Broughton (1993) reported that at higher temperatures of 20, 25 and 30°C, treatments combined with nisin and EDTA resulted in more marked lethality of P. fluorescens when

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compared with the same treatments at lower temperatures of 6 and 12°C. In the
temperature range of 20 to 30°C, similar lethality of P. fluorescens was observed.

With adding 50 μg/ml and 20 mM EDTA, Stevens et al. (1992) evaluated the
treatment temperature effect, including 4, 20, 30, 37, and 42°C, on the inactivation of
selected Salmonella species, and found that higher treatment temperature resulted in
higher microbial reduction. Except for higher microbial reduction for S. choleraesuis
ATCC 10708 at 37°C, they also reported that there was no significant (P>0.05) difference
between 37 and 42°C treatment for other selected Salmonella species in their study. The
authors concluded that the treatments with a temperature range of 30 and 42°C was the
most effective to inactive the selected Salmonella species. Moreover, the authors
suggested that treatment temperature effect needed to be considered when applying nisin
to inhibit microorganisms in foods.

In the current study, the total microflora counts of the samples increased with
storage time as was expected. Figure 3.3 shows that there was no significant (P>0.05)
difference of the total microflora counts for the samples between the storage time day 0
(one day after the sell-by date) and day 4 (5 days after the sell-by date). However, at the
storage day 7 (8 days after the sell-by date), a significantly (P<0.05) higher total
microflora count of the samples, which was 6.4 log CFU/ml (4.1 log CFU/cm²), was
obtained when compared with 6.0 log CFU/ml (3.7 log CFU/cm²) for the day 0 samples.

Typically, spoilage can be detected when bacterial numbers exceed 10^8 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 8.0 log CFU/cm². In the current study, only the total microflora counts of the samples without adding any nisin and without thermal treatment were close to this “8 log criteria”, which was approximately the point when off odors and slime might be formed and detected. However, no off-odors and slime formation was detected in any of the samples in this study when evaluated by sensory evaluation at the 7 days of refrigerated storage period.

Similar to the total microflora counts, the psychrotrophs counts of the samples exhibited similar patterns. No significant three-way interaction (thermal treatment × nisin-adding level × storage time), and only one two-way interaction (thermal treatment × nisin-adding level) was significant (P<0.05) for psychrotrophs in the current study. Combination of thermal treatment and 100 IU/ml nisin added resulted in significantly (P<0.05) lower psychrotrophs count of 6.1 log CFU/ml (3.9 log CFU/cm²) when compared with the samples with the same thermal treatment but without nisin added which had 6.5 log CFU/ml (4.2 log CFU/cm²)(Figure 3.4). Similarly, treatment without thermal treatment but with 100 IU/ml nisin resulted in a significant (P<0.05) lower count (6.7 log CFU/ml; 4.4 log CFU/cm²) when compared with the samples without both thermal and nisin treatment (7.6 log CFU/ml; 5.3 log CFU/cm²).

For each nisin added level, the thermal treatment resulted in significant (P<0.05) lower psychrotrophs counts, when compared with the samples without thermal treatment. The psychrotrophs count of 6.5 log CFU/ml (4.2 log CFU/cm²) for the samples with thermal treatment but without nisin added was significantly (P<0.05) lower compared with the count of 7.6 log CFU/ml (5.3 log CFU/cm²) for the samples without nisin added and without thermal treatment (Figure 3.4). Similarly, combination with thermal
treatment and either 50 or 100 IU/ml nisin added resulted in significantly (P<0.05) lower total microflora count than the samples with 50 or 100 IU/ml nisin added but without thermal treatment.

Similar to the total microflora, the psychrotrophs microflora counts of the samples increased with storage time as expected. Figure 3.3 indicates that psychrotrophs counts of the samples significantly (P<0.05) increased to 6.8 log CFU/ml at storage day 2 (i.e. 3 days after the sell-by date) compared to the count at day 0 (i.e. 1 day after the sell-by date), which was 6.3 log CFU/ml (4 1 log CFU/cm²). In the current study, only the psychrotrophs counts of 7.6 log CFU/ml (5.3 log CFU/cm²)(Figure 3.4) for the samples without nisin added and without thermal treatment were close to the “8 log criteria.” However, none of the samples in this study was detected with off odors and slime formation based on the sensory evaluation within 7 days of refrigerated storage.

CONCLUSION

In conclusion, adding nisin at the level of 50 IU/ml with thermal treatment of heating the marinade solution at 58°C for 2 min, cooling, and then refrigerated storage at 4°C for 18 hours, decreased the total microflora and psychrotrophs counts of the marinated chicken drumsticks. Based on the results of this study, the poultry industry would be able (after government approval) to apply this information to produce various high value-added and longer shelf life marinated products. Further research to assess the changes in physical and sensory quality of marinated chicken drumsticks treated with nisin and thermal treatment is needed.
REFERENCES


Standardized marinade solution\(^1\)  |  Pre-assigned\(^2\) antimicrobial components  |  Raw chicken drumsticks

Autoclaved marinade solution  |  Mixing in a sterile bag for 5 min

Marinating without thermal treatment at 4°C for total 18 hrs  |  Marinating with thermal treatment\(^4\) at 4°C for total 18 hrs

Assigning drumsticks to each treatment\(^3\)

Microbial quality evaluation at 0, 2, 4, and 7 days of storage at 4°C

Total microbial count  |  Psychrotrophs count

\(^1\)Standardized marinade solution: acetic acid (1%), salt (3%) in distilled water then adjusted to pH 4.

\(^2\)Pre-assigned antimicrobial components: nisin at 0, 50 or 100 IU/ml with 20 mM disodium EDTA.

\(^3\)Treatments: a 3×2×4 factorial design with 3 nisin adding levels (0, 50, 100 IU/ml), 2 thermal treatment (4 or 58°C for 2min), and 4 storage time (0, 2, 4, and 7 days).

\(^4\)For the thermal treatment group, total 18 hours included thermal treating, cooling and marinating under refrigeration.

Figure 3.1 Experimental procedure
Figure 3.2 Interaction of nisin added level and thermal treatment for total microflora counts (incubated at 35°C for 48 hrs) of marinated chicken drumsticks

Means with different superscripts are significantly different (P<0.05).

Figure 3.2 Interaction of nisin added level and thermal treatment for total microflora counts (incubated at 35°C for 48 hrs) of marinated chicken drumsticks.
Figure 3.3 Effect of storage time on total microflora (incubated at 35°C for 48 hrs) and psychrotrophs counts (incubated at 7°C for 10 days) of marinated chicken drumsticks.

**Means with different superscript are significantly different (P<0.05).**

Figure 3.3 Effect of storage time on total microflora (incubated at 35°C for 48 hrs) and psychrotrophs counts (incubated at 7°C for 10 days) of marinated chicken drumsticks.
Means with different superscripts are significantly different (P<0.05).

Figure 3.4 Interaction of nisin added level and thermal treatment for psychrotrophs counts (incubated at 7°C for 10 days) of marinated chicken drumsticks.
CHAPTER 4

EXPERIMENT 2

APPLICABILITY OF NISIN AND TUMBLING TREATMENT TO IMPROVE THE MICROBIAL QUALITY OF MARINATED CHICKEN DRUMSTICKS

ABSTRACT

Meat marination has been applied to improve product's physical and sensory attributes for years, but usually it is not intended to improve the microbial quality of the product. Tumbling, which helps the distribution of the 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 of 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 hours. The total microflora and psychrotrophs counts of the samples were measured after 0, 2, 4, and 7 days (i.e. 1, 3, 5 and 8 days after the sell-by date of the samples) of storage. The results indicate that at a given storage time, the samples tumbled for either 10 or 20 min had significant (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 nisin level was increased up to the level of 100 IU/ml. In conclusion, addition of nisin at the level of 50 IU/ml combined with tumbling for 10 min decreased the total microflora and psychrotrophs counts of the marinated chicken broiler drumsticks.

Keywords: 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. (Hashim, et al. 1999; Lemos et al. 1999; Xiong and Kupski, 1999; Young and Buhr, 2000; Young and Lyon, 1997a, b; Zheng et al. 2000). However, this process is usually not intended to improve the 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 (Stevens et al. 1992; Stevens et al. 1991). Nisin, along with many other physical or chemical treatments, has been studied in extending the shelf life of poultry products. After immersing for 3 min in a solution consisting of
100 μg/ml nisin, 5.0 mM EDTA, 3.0% citric acid, and 0.5% Tween 80, with the pH adjusted to 3.5, followed by packaging in a foam traypack containing a nisin-treated PVC overwrap and absorbent tray pad, and refrigerated at 4°C, Natrajan and Sheldon (2000) reported that the shelf life of the treated drumsticks was extended by 0.6 to 2.2 day when compared with the controls. The shelf life of refrigerated broiler drumsticks, which were previously immersed for 30 min in a solution consisting of 100 μg/ml nisin, 5.0 mM EDTA, 3% citric acid, 0.50% Tween 20, and pH adjusted to 3.5 for 30 min, was extended by 1.5 to 3 days when compared to those dipped in sterile distilled and deionized water (Shefet et al., 1995). Tsou (1995) reported that fresh chicken drumsticks treated with 5000 IU/ml of nisin, then vacuum-packaged and stored under refrigeration had significantly (P<0.05) lower total aerobic counts when compared with samples without added nisin during the refrigerated storage of 9 days. Xu et al. (2000) reported that salted duck treated with 400 mg/kg of nisin, with 3.5% sodium lactate, vacuum packing and microwave treatment (915 MHz, 400 W, 3 min) had longer shelf life of greater than 20 days, whereas the shelf life of the controls had only approximately 6 days based on the microbiological, physicochemical (peroxide value) and sensory evaluation results.

Tumbling has been widely applied in the meat industry for years. Pieces of meat are placed inside a rotating drum-like tumbler. 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. Abrasion against other meat pieces leads to extraction of the myofibrillar proteins to the surface, which are necessary 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, and decreasing curing time, and etc (Babji et al., 1982; Bater et al., 1993; Bater et al., 1992; Froning and Sackett, 1985; Heath and Owens, 1987; Huang et al. 2001; Kotula and Heath, 1986a,b; Maki and Froning, 1987; Nurmahmudi and Sams, 1997).

Effect of tumbling on the microbiological quality of the products has been reported in some studies. Knipe et al. (1981) reported that intermittently tumbling for 10 min of every hour for 18 hrs significantly (p<0.01) lowered the total aerobic plate counts of the boneless cured ham. Leak et al. (1984) found that dry-cured hams treated with either 1 hr continuous tumbling, 3 hr intermittent tumbling (10 min/hr), or 6 hr intermittent tumbling (10 min/hr), generally had lower microbial counts when compared with the samples without tumbling. Yetim et al. (1996) reported that intermittently tumbling (40 min rest, 20 min for 12 hr) with curing ingredients slightly decreased the counts of aerobic plate count, *Pseudomonas*, lipolytic and proteolytic bacteria in catfish compared to non-tumbled samples. Treatment combining 0.4% ascorbic acid, 0.4% NaCl, with vacuum tumbling (28 in. Hg, 8 rpm for 8 min) resulted in a significantly reduction (P<0.05) of the total aerobic counts of the catfish fillets, when compared with the treatments without tumbling (Ramos and Lyon, 2000). Leblanc et al. (1996) reported that vacuum tumbled for 20 min in 6% sodium lactate solution, and storage under CO₂ packaging extended the shelf life of rainbow trout fillet.

Also, tumbling has been shown to increase distribution of curing and marinade solution in some studies. By monitoring the diffusion rate of curing agents, tumbling either as intermittent tumbling (10 min per hour for 18 hr of tumbling) or continuous
tumbling (3 hr) has been reported to increase migration of curing agents and brine by (Ockerman and Organisciak, 1978a,b). Krause et al. (1978) found tumbling for 10 min out of each hour for 18 hr resulted in increased migration of salt and nitrite into semimembranosus muscles when compared with the non-tumbled samples. Similarly, Leak et al. (1984) found that tumbling significantly accelerated salt and nitrite penetration for the dry-cured hams during the first 2 weeks of curing.

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 resulted in the limited antibotulinal effectiveness of nisin in bacon.

Based on this information, nisin could be used to inactive some microorganisms on the poultry products, and extend the shelf life of the products. In addition, tumbling, which helps the distribution of the marinade solution during processing, should increase the distribution of antimicrobial agents, thus enhancing the action of antimicrobial agents. In this study, efficacy of tumbling (0, 10, or 20 min) in conjunction with nisin (0, 50, or 100 IU/ml) and storage time (day 0, 2, 4, and 7; i.e. 1, 3, 5 and 8 days after the sell-by date of the samples) 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 experiment, 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 20mM disodium EDTA (Fisher Scientific Co., Kansas City, MO) were added to the standardized marinade based on the results from the 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 0.02N HCl and held for 2h at 25°C. After adding pre-assigned levels of nisin and disodium EDTA, the marinade solutions were adjusted to pH 4, autoclaved for 121°C for 15 min, and then stored at 4°C before conducting the experiment.

Sample preparation

A total of 144 drumsticks which had the same sell-by date and 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. On the sell-by date
which was indicated on the packages, inside the walk-in cooler, drumsticks were initially mixed thoroughly in 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 not tumbled, two drumsticks per treatment for each storage day were aseptically placed and marinated in a 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 hr.

For the tumbled samples, drumsticks combined 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 raffles 7.6 cm. manufactured at OSU, Columbus, OH, USA) at 12 rpm rotational speed in a refrigerated (4°C) room. After tumbling for the pre-assigned time, drumsticks with the marinade solution were then marinated at 4°C for a total of 18 hr (including the previous tumbling time) storage.

Microbial evaluation

After 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 stored under refrigeration at 4°C. In the current study, refrigerated storage day 0 was set as one day after the sell-by date shown on the packages.
of the drumsticks. At specified sampling times (0, 2, 4, or 7 day; i.e. 1, 3, 5 or 8 days after sell-by date of the samples), 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 bacteria group. Total microflora and psychrotrophs were incubated at 35°C for 48 hours 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. The total microflora and psychrotrophs counts of the day 0 (i.e. one day after the sell-by date) samples without any treatment were also evaluated. In the current study, the microbial counts in log CFU/ml of the rinse solution were also calculated and expressed as log CFU/cm² of the surface area of the drumsticks based on the average surface area of 174 cm². Experimental outline is illustrated in Figure 4.1.

Statistical analyses

The study was designed as a 3 x 3 x 4 factorial experiment, 3 nisin-adding levels (0, 50, 100 IU/ml), tumbling treatments (0, 10, 20 min), and 4 storage times (day 0, 2, 4, and 7; i.e. 1, 3, 5 and 8 days after the sell-by date of the samples), and with two replicates. 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 (P>0.05).

RESULTS AND DISCUSSION

At day 0 (i.e. one day after the sell-by date), the samples without any treatment had total microflora counts of 9.4 CFU/ml (7.2 CFU/cm²) and psychrotrophs counts of 9.6 CFU/ml (7.4 CFU/cm²). There was no significant three-way interaction (tumbling time x nisin added level x storage time) on the total microflora counts for the samples with different tumbling time treatment and different levels of nisin added during refrigerated storage at the 0.05 level. Only one two-way interaction (tumbling time x storage time) was significant (P<0.05), which indicates that tumbling time along with storage time would affect the total microflora counts of the samples.

At a given storage time, the more tumbling time, the fewer total microorganisms were detected. As illustrated in Figure 4.2, at storage time day 0, samples tumbled for 10 or 20 min had significantly (P<0.05) lower total microflora counts of 5.2 and 4.6 log CFU/ml (3.0 and 2.4 log CFU/cm²), respectively, when compared with the samples without tumbling which had 7.6 log CFU/ml (5.4 log CFU/cm²) count at day 0. Similarly, at the same storage time of 2, 4, or 7 days (i.e. 3, 5, or 8 days after the sell-by date), the tumbled samples either for 10 or 20 min resulted in significant (P<0.05) lower total microflora counts when compared with the samples without the tumbling treatment. At a given storage time, there was no significance (P>0.05) 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.

Figure 4.2 illustrates that the total microflora counts of the samples without tumbling treatment remained steadily at approximately 7.5 log CFU/ml (5.3 log CFU/cm²) during 7 days refrigeration storage. The results showed that the total microflora counts of the tumbled samples increased as the storage time increased. At a given tumbling time of 10 min, the total microflora counts increased significantly (P<0.05) between storage day 0 with 5.2 log CFU/ml (3.0 log CFU/cm²) and day 4 with 5.9 log CFU/ml (3.6 log CFU/cm²). Similarly, samples tumbled for 20 min at storage day 2 had a significant (P<0.05) higher total microflora count of 5.2 log CFU/ml (3.0 log CFU/cm²), when compared with the samples tumbled for 20 min at storage day 0 which had 4.6 log CFU/ml (2.4 log CFU/cm²). As storage time increased, the total microflora count of the samples tumbled for 20 min significantly (P<0.05) increased further to 5.7 log CFU/ml (3.4 log CFU/cm²) at day 7, when compared with the samples tumbled for 20 min at day 0 with 4.6 log CFU/ml (2.4 log CFU/cm²). 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 (6.1 and 5.7 log CFU/ml for 10 and 20 min tumbling respectively) remain significantly (P<0.05) lower than the microbial count of 7.5 log CFU/ml of the samples without tumbling.

In the current study, at a given storage time, the more tumbling time, the fewer total microorganisms were detected. Tumbling, which has been showed to help the distribution of curing agents and marinade solution during processing in several studies (Krause et al., 1978; Ockerman and Organisciak, 1978a,b), should increase the
distribution of antimicrobial agents. Not only nisin, which has been shown to inactive some microorganisms, but also acetic acid which reduced the solution pH, should enhance the action of the antimicrobial agents.

Table 4.1 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 (P<0.05) lower total microflora counts of 6.1 log CFU/ml (3.8 log CFU/cm²), when compared with samples without adding any nisin, which had higher count of 6.5 log CFU/ml (4.2 log CFU/cm²). Adding even more nisin to the level of 100 IU/ml resulted in a further significant (P<0.05) lower microbial count of 5.8 log CFU/ml (3.6 log CFU/cm²). The result implies that the more nisin added up to the level of 100 IU/ml, the less total microflora counts were obtained.

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.

A previous study conducted in the same laboratory showed a similar nisin-added level effect on the total microflora counts of the treated marinated chicken drumsticks (Information has been submitted for publication). In that study, there was a significant (P<0.05) two-way interaction of thermal treatment x nisin-added level on the total microflora counts for the samples with or without thermal treatment (4 or 58°C for 2min) and different levels of nisin (0, 50, or 100 IU/ml) added during refrigerated storage. At a given thermal treatment, samples with nisin added had significant (P<0.05) lower
microbial counts when compared with the samples with the same thermal treatment but with less nisin added.

Delves-Broughton (1993) reported that more lethality of *Pseudomonas fluorescens* was observed when adding nisin at higher levels of 25 or 75 μg/ml with 0.5-10 mM EDTA, comparing with lower levels (0, 1.25, 2.5, 7.5 μg/ml) of nisin. In the same study, the authors reported that nisin at higher concentrations of 6.25 and 12.5 μg/ml resulted in a greater lethality of *P. fluorescens* when compared with the treatments with lower concentration of 2.5 μg/ml nisin at treatment temperatures of 20, 25, and 30°C. Less difference of lethality due to different concentrations of nisin was observed at lower temperatures of 6 and 12°C.

Fang and Lin (1995) reported that adding higher levels of nisin at 1000 or 2500 IU/g resulted in significantly higher reduction of inoculated *L. monocytogenes* on plate count agar, when compared with the samples with lower levels of nisin added (0 or 250 IU/g). Murray and Richard (1997) evaluated the effect of different levels (300, 1500, and 3000 AU/ml) of nisin on the *Listeria innocua* Lin 11 numbers of the raw pork cube samples, which were previously inoculated with *L. innocua* Lin 11, soaked in nisin solution, then ground and stored aerobically at 5°C. They found that the microbial reduction increased as nisin concentration increased.

Stevens et al. (1992) evaluated the different treatment conditions, including nisin concentration, chelating types, treatment incubation temperature, and protein interference on the inactivation of selected gram-negative bacteria. In this study, the authors reported that higher concentration (50 to 100 μg/ml) of nisin added was generally more effective.
at inactivation of the selected *Salmonella* species, when compared with ones with lower levels (10 to 25 μg/ml) of nisin added. However, there was no significant difference between levels of 50 and 100 μg/ml of nisin added in retarding the growth of some *Salmonella* species, such as *S. hadar 3503-2* and *S. Heidelberg*, when a level of 25 μg/ml of nisin functioned significantly (P<0.05).

Typically, spoilage can be detected when bacterial numbers exceed 10^8 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 reached a population of 8.0 log CFU/cm². In the current study, only the total microflora counts of the samples without tumbling treatment had approximately 7.5 log CFU/ml (5.3 log CFU/cm²), 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.

Similar to the total microflora counts, the psychrotrophs counts of the samples exhibited similar patterns. No significant (P>0.05) three-way interaction (tumbling time × nisin-added level × storage time), but one two-way interaction (tumbling time × storage time) was significant (P<0.05). At a given storage time, the more tumbling time, the fewer psychrotrophic microorganisms were detected. At storage time day 0 (i.e. one day after the sell-by date), samples tumbled for 10 or 20 min had significantly (P<0.05) lower psychrotrophs counts of 5.5 and 5.1 log CFU/ml (3.3 and 2.9 log CFU/cm²), respectively, when compared with the samples without tumbling which had 7.8 log CFU/ml (5.6 log CFU/cm²) count at day 0 (Figure 4.3). Similarly, at the same storage time of either day 2, 4, or 7, the tumbled samples for either 10 or 20 min resulted in significant (P<0.05) lower
microbial counts when compared with the samples without the tumbling treatment. There was no significance (P>0.05) for the psychrotrophs counts between the samples tumbled for 10 or 20 min at a given storage time. This result implies that tumbling for 10 min with the marinade solution containing nisin in this study sufficiently reduced the psychrotrophs counts of the samples during storage up to 7 days.

The psychrotrophs counts of the samples without tumbling treatment remained steadily at approximately 7.7 log CFU/ml (5.5 log CFU/cm²) during 7 days storage (Figure 4.3). The microbial counts of the tumbled samples increased with storage time. Similar to the total microflora counts, at a given tumbling time (either 10 or 20 min), the psychrotrophs counts increased as the storage time increased (Figure 4.3). Even though the microbial counts of the tumbled sample increased with storage time, the microbial counts of the tumbled samples (6.3 and 5.9 log CFU/ml for 10 and 20 min tumbling, respectively) remained significantly (P<0.05) lower than the microbial count of 7.7 log CFU/ml of the samples without tumbling after 7 days refrigeration storage.

Similar to the total microflora counts, the more nisin added up to the level of 100 IU/ml, the less psychrotrophs counts that were obtained (Table 4.1). Also, only the psychrotrophs counts of the samples without tumbling treatment were approximately 7.7 log CFU/ml, which were close to the “8 log criteria”, and 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 refrigeration storage.
CONCLUSION

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. In addition, effectiveness of this treatment combined with tumbling and nisin to the pathogens associated with the poultry products should be evaluated in the future.

REFERENCES


Standardized marinade solution: acetic acid (1%), salt (3%) in distilled water then adjusted to pH 4.

Pre-assigned antimicrobial components: nisin at 0, 50 or 100 IU/ml with 20 mM disodium EDTA.

Treatments are 3 x 3 x 4 factorial design with 3 nisin adding levels (0, 50, 100 IU/ml), 3 tumbling time (0, 10, 20 min), and 4 storage time (0, 2, 4, and 7 days).

For the tumbling treatment group, total 18 hours included tumbling and marinating.

Figure 4.1 Experimental procedure
All means with different superscripts are significantly different (P<0.05).

Interaction existed between tumbling time and storage time.

Figure 4.2 Total microflora counts of marinated chicken drumsticks with different tumbling time treatments during refrigerated storage at 4°C.

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Figure 4.3 Psychrotrophs counts of marinated chicken drumsticks with different tumbling time treatments during refrigerated storage at 4°C. * Interaction existed between tumbling time and storage time.

abcd All means with different superscripts are significantly different (P<0.05).
<table>
<thead>
<tr>
<th>Nisin added level (IU/ml)</th>
<th>Total microflora count (incubated at 35°C for 48 hrs)</th>
<th>Psychrotrophs count (incubated at 7°C for 10 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>5.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts are significantly different (P<0.05).

Table 4.1 Effects of nisin added level on the total microflora and psychrotrophs counts of marinated chicken drumsticks.
CHAPTER 5

EXPERIMENT 3

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

ABSTRACT

Meat marination has been applied to improve product’s physical and sensory attributes for years, but usually it is not intended to improve the microbial quality of the product. Nisin, which is a natural, nontoxic, heat stable polypeptide produced by Lactococcus lactis strains, has been shown to inhibit many microorganisms, and has been approved for use in some dairy products. The lactoperoxidase system (LPS) consists of lactoperoxidase (LP), thiocyanate (SCN⁻), and hydrogen peroxide (H₂O₂), is an inhibitory system that is present naturally in bovine milk, and had been showed to be inhibitory against some microorganisms. The objective of this study is to evaluate the combined effects of nisin, LPS 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 LPS (0, 1, or 2 units; 1 unit LPS consisted of 1 μg/ml LP, 5.9 mM KSCN, and 2.5 mM H₂O₂; 2 units LPS consisted of 2
μg/ml LP, 11.8 mM KSCN, and 5.0 mM H$_2$O$_2$), and then stored at 4°C for 18 hours. The microbial counts of the samples were measured after 0, 2, 4, and 7 days (i.e. 1, 3, 5 and 8 days after the sell-by date of the samples) of storage. The results indicate that adding nisin up to the level of 100 IU/ml, and LPS added up to 2 units, respectively, significantly (P<0.05) decreased the total microflora and psychrotrophs counts of the marinated chicken drumsticks.

Keywords: nisin; lactoperoxidase system; marination; microbiological quality

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 food 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 tenderness, water holding, flavor and etc. (Hashim, et al. 1999; Lemos et al. 1999; Xiong and Kupski, 1999; Young and Buhr. 2000; Young and Lyon, 1997a,b; Zheng et al. 2000), but the process is usually not intended to improve the 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, and has been shown to inhibit gram-positive microorganisms (Ray, 2001), and some gram-negative bacteria when combining
with a chelating agent such as EDTA (Stevens et al. 1992; Stevens et al. 1991). In addition, nisin, along with many other physical or chemical treatments, has been studied in extending the shelf life of poultry products.

Natrajan and Sheldon (2000) reported that drumsticks immersed in a solution consisting of 100μg/ml nisin, 5.0 mM EDTA, 3.0% citric acid, and 0.5% Tween 80, with the pH adjusted to 3.5 for 3 min, followed by packaging in a foam traypack containing a nisin-treated PVC overwrap and absorbent tray pad, and refrigerated at 4°C, was estimated to extend the shelf life by 0.6 to 2.2 days when compared with the controls. Immersion in a solution consisting of 100 μg/ml nisin, 5.0 mM EDTA, 3% citric acid, 0.5% Tween 20, and pH adjusted to 3.5 for 30 min, extended the shelf life of refrigerated broiler drumsticks by 1.5 to 3 days when compared to those dipped in sterile distilled and deionized water (Shefet et al., 1995). Tsou (1995) reported that fresh chicken drumsticks treated with 5000 IU/ml of nisin, then vacuum-packaged and stored under refrigeration had significantly (P<0.05) lower total aerobic counts when compared with samples without nisin added during the refrigerated storage for 9 days. Xu et al. (2000) reported that salted duck treated with 400 mg/kg of nisin with 3.5% sodium lactate, vacuum packed and exposed to a microwave treatment (915 MHz, 400 W, 3 min) had a longer shelf life of greater than 20 days, whereas the shelf life of the controls had only a 6 day shelf life based on the microbiological, physicochemical (peroxide value) and sensory evaluation results.

The lactoperoxidase system (LPS), which consists of lactoperoxidase (LP), thiocyanate (SCN⁻), and hydrogen peroxide (H₂O₂), is an inhibitory system that is present naturally in bovine milk. This system has been shown to be inhibitory against some
pathogenic and spoilage microorganisms such as *P. fluorescens*, *L. monocytogenes*, *S. typhimurium* and *E. coli* (Zapico et al., 1995; Kamau et al., 1990a,b; Earnshaw et al., 1990). In the presence of SCN⁻ and H₂O₂, Kamau et al. (1990a,b) explained, the LPS generates the hypothiocyanite (OSCN⁻) and hypothiocyanous acid (HOSCN), which are the main antimicrobial products, and strong oxidizing agents that can oxidize essential sulphydryl groups in bacterial proteins, thus causing bactericidal effects. The LPS has been mainly studied for the application in the milk and dairy products (Zapico et al., 1998). Only a few studies have been attempted to apply this LPS system in meat and poultry products.

Kennedy et al. (2000) evaluated the inhibitory effect of the LPS on the growth of selected microorganisms in a beef mince system, and reported that *Listeria monocytogenes* L45 was the most sensitive to LPS, followed by *Staphylococcus aureus* R37 and *Escherichia coli* O157:H7. Boussouel et al. (2000) reported that combination of LPS and nisin resulted in a synergistic and lasting bactericidal effect against inoculated *Listeria monocytogenes* ATCC 15313 in skim milk, when compared with adding nisin and LPS alone. Wolfson et al. (1994) reported that immersion of the inoculated chicken legs and wings in a 50°C solution containing the LPS (1µg/ml lactoperoxidase, 5.9 mM potassium thiocyanate, and 2.5 mM hydrogen peroxide) for 5 min, and stored at 4°C for 2 days resulted in a 2.2 log aerobic plate counts increase for the treated sample but a 5.1 log increase for the control samples. Also, psychrotrophic count decreased 1.1-log for the treated samples whereas the control samples counts increased by 0.8 log.

Based on this information, nisin could be used to inactive some microorganisms and extend shelf life of the poultry products. In addition, LPS, which has been widely

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applied in milk and dairy products, should be valuable when applied to meat products. In this study, effects of nisin, LPS and storage time on the microbial quantity found on marinated chicken 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 experiment, 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 20mM disodium EDTA (Fisher Scientific Co., Kansas City, MO) were added to the standardized marinade based on the results from 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 0.02N HCl and held for 2h at 25°C. After adding pre-assigned levels of nisin and disodium EDTA, the marinade solutions were adjusted to pH 4, autoclaved for 121°C for 15 min, and then stored at 4°C before conducting the experiment.
Lactoperoxidase system preparation

The components of the LPS, including bovine milk lactoperoxidase (LP, EC 1.11.1.7; purity index 0.82 ($A_{412}/A_{230}$); 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 standardized marinade solution. The LP and $H_2O_2$ were prepared in distilled water, and filter sterilized separately using a .45μm filter (Gelman Sciences, Ann Arbor, MI), when 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 ninety-six drumsticks which had the same sell-by date and 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. On the sell-by date which was indicated on the packages, inside the walk-in cooler, drumsticks were initially mixed thoroughly in 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. Two drumsticks per treatment for each storage day were aseptically
placed and marinated in a 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 hr.

Microbial evaluation

After 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. In the current study, refrigerated storage day 0 was set as one day after the sell-by date shown on the packages of the drumsticks. At specified sampling times (0, 2, 4, or 7 day; i.e. 1, 3, 5 or 8 days after sell-by date of the samples), 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 bacteria group. Total microflora and psychrotrophs were incubated at 35°C for 48 hours 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. The total microflora and psychrotrophs counts of the day 0 (i.e. one day after the sell-by date) samples without any treatment were also evaluated. In the current study, the microbial counts in log CFU/ml of the rinse solution were also calculated and expressed
as log CFU/cm² of the surface area of the drumsticks based on the average surface area of 174 cm². Experimental procedure is illustrated in Figure 5.1.

Statistical analyses

The study was designed as a 3 x 3 x 4 factorial experiment, 3 nisin-added levels (0, 50, 100 IU/ml), lactoperoxidase system (0, 1, 2 units; 1 unit = 1 µg/ml LP, 5.9 mM KSCN, and 2.5 mM H₂O₂; 2 units = 2 µg/ml LP, 11.8 mM KSCN, and 5.0 mM H₂O₂), and 4 storage times (day 0, 2, 4, and 7; i.e. 1, 3, 5 and 8 days after the sell-by date of the samples), 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.

RESULTS AND DISCUSSION

At day 0 (i.e. one day after the sell-by date), the samples without any treatment had total microflora counts of 8.6 CFU/ml (6.3 CFU/cm²) and psychrotrophs counts of 9.6 CFU/ml (7.4 CFU/cm²). There was no significant (P>0.05) three-way and two-way interactions among the three factors of nisin added level, lactoperoxidase system (LPS) added level and storage time of the total microflora counts for the samples with different levels of nisin and LPS added during refrigerated storage at 4°C in the current study.
Figure 5.2 illustrates the nisin level effect on the microbial counts of the treated samples. Adding nisin at level of 50 IU/ml resulted in significantly (P<0.05) lower total microflora counts of 7.1 log CFU/ml (4.9 log CFU/cm²), when compared with samples without adding any nisin, which had higher count of 7.5 log CFU/ml (5.2 log CFU/cm²). Adding even more nisin to the level of 100 IU/ml resulted in a further significant (P<0.05) lower microbial count of 6.8 log CFU/ml (4.6 log CFU/cm²). The result implies that the more nisin added up to at level of 100 IU/ml the less total microflora counts obtained.

A previous study conducted in the same laboratory showed a similar nisin-added level effect on the total microflora counts of the treated marinated chicken drumsticks (Information has been submitted for publication). In that study, there was a significant (P<0.05) two-way interaction of thermal treatment \times nisin-adding level on the total microflora counts for the samples with or without thermal treatment (4 or 58°C for 2min) and different levels of nisin (0, 50, or 100 IU/ml) added during refrigerated storage. At a given thermal treatment, samples with nisin added had significant (P<0.05) lower microbial counts when compared with the samples with the same thermal treatment but with less nisin added.

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 an increased effect, a lower concentration of nisin might be appropriate due the economic concern for the cost of nisin.

Delves-Broughton (1993) reported more lethality of *Pseudomonas fluorescens* was observed when nisin was added at higher levels of 25 or 75 μg/ml with 0.5-10 mM
EDTA, comparing with lower levels (0, 1.25, 2.5, 7.5 μg/ml) of nisin. In the same study, the authors reported that nisin at higher concentrations of 6.25 and 12.5 μg/ml resulted in a greater lethality of *P. fluorescens* when compared with the treatments with lower concentration of 2.5 μg/ml nisin at treatment temperatures of 20, 25, and 30°C. Less difference of lethality due to different concentrations of nisin was observed at lower temperatures of 6 and 12°C.

Fang and Lin (1995) reported that adding higher levels of nisin at 1000 or 2500 IU/g resulted in significant higher reduction of inoculated *L. monocytogenes* on plate count agar, when compared with the samples with lower levels of nisin added (0 or 250 IU/g). Murray and Richard (1997) evaluated the effect of different levels (300, 1500, and 3000 AU/ml) of nisin on *Listeria innocua* Lin 11 numbers in raw pork cube samples, which were previously inoculated with *L. innocua* Lin 11, soaked in nisin solution, then ground and stored aerobically at 5°C. They found that the microbial reduction increased as nisin concentration increased.

Stevens *et al.* (1992) evaluated the different treatment conditions, including nisin concentration, chelating types, treatment incubation temperature, and protein interference on the inactivation of selected gram-negative bacteria. In their study, the authors reported that higher concentration (50 to 100 μg/ml) of nisin added was generally more effective at inactivation of the selected *Salmonella* species, when compared with lower levels (10 to 25 μg/ml) of nisin added. However, there was no significant difference between levels of 50 and 100 μg/ml of nisin added in inhibiting some *Salmonella* species,
such as *S. hadar* 3503-2 and *S. Heidelberg*, when level of 25 μg/ml of nisin functioned significantly (*P*<0.05).

Figure 5.3 illustrates the LPS level effect on the total microflora counts of the treated samples. Adding LPS at level of 1 unit (LP, 1 μg/ml; KSCN, 5.9 mM; H$_2$O$_2$, 2.5 mM) resulted in significantly (*P*<0.05) lower total microflora counts of 7.0 log CFU/ml (4.7 log CFU/cm$^2$), when compared with samples without adding any LPS which had a higher count of 7.7 log CFU/ml (5.3 log CFU/cm$^2$). Adding even more LPS to the level of 2 units (LP, 2 μg/ml; KSCN, 11.8 mM; H$_2$O$_2$, 5.0 mM) resulted in a further significant (*P*<0.05) lower microbial count of 6.7 log CFU/ml (4.5 log CFU/cm$^2$). The result implies that the more LPS added up to at level of 2 units the less total microflora counts obtained.

Figure 5.4 illustrates the storage effect on the total microflora counts of the samples. The microbial counts significantly (*P*<0.05) decreased from the 7.3 log CFU/ml (5.1 log CFU/cm$^2$) of the day 0 (i.e. one day after the sell-by date) samples to the 7.1 log CFU/ml (4.9 log CFU/cm$^2$) of the day 2 (i.e. 3 days after the sell-by day) samples. No significant (*P*>0.05) difference of the microbial counts was found of the samples after 2 days storage.

Boussouel *et al.* (2000) reported that combination of LPS and nisin resulted in a synergistic and lasting bactericidal effect against inoculated *Listeria monocytogenes* ATCC 15313 in skim milk, when compared with adding nisin and LPS alone. In their study, adding nisin induced an immediate bactericidal effect whereas adding LPS resulted in a 48 h bacteriostatic phase. In both cases of adding of either nisin or LPS alone, *L. monocytogenes* would re-grow. However, adding LPS and nisin simultaneously resulted
in a synergistic and lasting bactericidal effect up to 8th days, and *L. monocytogenes* was not detectable until the 15th day.

Zapico *et al.* (1998) evaluated the synergistic effect of nisin and LPS on *Listeria monocytogenes* in skim milk. In their study, adding nisin at either 10 or 100 IU/ml to UHT skim milk only slightly reduced the *L. monocytogenes* counts, and the microbial growth resumed again after 24 h at 30°C, whereas addition of the LPS resulted in a 3-log reduction compared to those of control milk after 24 h at 30°C. On the other hand, addition of nisin and LPS showed a synergistic antimicrobial effect and resulted in 5.6 log reduction compared to the control samples.

Typically, spoilage could be detected when bacterial numbers exceed 10⁸ 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 8.0 log CFU/cm². In the current study, even though a few total microflora counts of some samples exceeded this "8 log 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 (4°C) storage.

Similar to the total microflora counts, the psychrotrophs counts of the samples exhibited similar patterns. No significant (P>0.05) three-way and two-way interaction was obtained in the current study. Similar to the total microflora counts, the more nisin added up to at level 100 IU/ml, the less psychrotrophs counts were obtained (Figure 5.3).

In addition, adding LPS at level of 1 unit (LP, 1 μg/ml; KSCN, 5.9 mM; H₂O₂, 2.5 mM) resulted in significantly (P<0.05) lower psychrotrophs counts of 7.6 log CFU/ml (5.4 log CFU/cm²), when compared with samples without adding any LPS which had
higher count of 8.4 log CFU/ml (6.2 log CFU/cm²) (Figure 5.3). Adding even more LPS to the level of 2 units (LP, 2 µg/ml; KSCN, 11.8 mM; H₂O₂, 5.0 mM) resulted in a further significantly (P<0.05) lower microbial count of 7.2 log CFU/ml (5.0 log CFU/cm²). The result implies that the more nisin added up to at level of 2 units, the less psychrotrophs counts obtained.

No significant (P>0.05) difference of the psychrotrophs counts was found with the samples during 7 days of refrigerated storage at 4°C (Figure 5.4). Also, even though a few psychrotrophs counts of individual sample were greater than the “8 log criteria”, 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 at 4°C.

CONCLUSION

In conclusion, adding nisin up to the level of 100 IU/ml, and LPS added up to 2 units (LP, 2 µg/ml; KSCN, 11.8 mM; H₂O₂, 5.0 mM), respectively, significantly (P<0.05) 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 nisin and LPS is needed.

REFERENCES


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Standardized marinade solution

Nisin + EDTA

Sterilized LPS component

Raw chicken drumsticks

Mixing in a sterile bag for 5 min

Assigning drumsticks to each treatment

Autoclaved marinade solution

Marinating at 4°C for 18 hrs

Microbial quality evaluation at 0, 2, 4, and 7 days of storage at 4°C

Total microbial count

Psychrotrophs count

1Standardized marinade solution: acetic acid (1%), salt (3%) in distilled water then adjusted to pH 4.

2Nisin: 0, 50 or 100 IU/ml with 20 mM disodium EDTA.

3Sterilized LPS component: 1 unit LPS = 1 µg/ml LP, 5.9 mM KSCN, and 2.5 mM H2O2; 2 units LPS = 2 µg/ml LP, 11.8 mM KSCN, and 5.0 mM H2O2. The LP and H2O2 solutions were filter sterilized using a 0.45µm filter (Gelman Sciences, Ann Arbor, MI), whereas the KSCN solution was autoclaved.

4Treatments: a 3x3x4 factorial design with 3 nisin adding levels (0, 50, 100 IU/ml), 3 levels of LPS (0, 1 and 2 units), and 4 storage time (0, 2, 4, and 7 days).

Figure 5.1 Experimental procedures
Figure 5.2 Effect of nisin-added level on total microflora (incubated at 35°C for 48 hrs) and psychrotrophs counts (incubated at 7°C for 10 days) of marinated chicken drumsticks.

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LPS-added level: 1 unit of LPS consisted of 1 μg/ml LP, 5.9 mM KSCN, and 2.5 mM H$_2$O$_2$; 2 units of LPS consisted of 2 μg/ml LP, 11.8 mM KSCN, and 5.0 mM H$_2$O$_2$.

Figure 5.3 Effect of LPS-added level on total microflora (incubated at 35°C for 48 hrs) and psychrotrophs counts (incubated at 7°C for 10 days) of marinated chicken drumsticks.
Figure 5.4 Effect of storage time on total microflora (incubated at 35°C for 48 hrs) and psychrotrophs counts (incubated at 7°C for 10 days) of marinated chicken drumsticks.

\[ \text{Microbial count (log CFU/ml)} \]

Storage time (day)

- abc Means with different subscript are significantly different (P<0.05).
- xyz Means with different subscript are significantly different (P<0.05).
CHAPTER 6

EXPERIMENT 4

MICROBIAL QUALITY OF MARINATED CHICKEN DRUMSTICKS TREATED WITH THE LACTOPEROXIDASE SYSTEM AND WITH OR WITHOUT THERMAL TREATMENT

ABSTRACT

Meat marination has been applied to improve product’s physical and sensory attributes for years, but usually not intended to improve the microbial quality of the product. The lactoperoxidase system (LPS) consists of lactoperoxidase (LP), thiocyanate (SCN⁻), and hydrogen peroxide (H₂O₂), is an inhibitory system that is naturally present in bovine milk, and had been showed to be inhibitory against some microorganisms. Thermal treatment has been widely applied to control the growth of microorganisms in the food industry for years. The objective of this study is to evaluate the combined effects of LPS, thermal treatment and storage time on total microflora and psychrotrophs counts of the marinated chicken 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 hours. The microbial counts of the samples were measured after 0, 2, 4, and 7 days (i.e. 1, 3, 5 and 8 days after the sell-by
date of the samples) of storage 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 H2O2) significantly (P<0.05) decreased the total microflora and psychrotrophs counts of the marinated chicken drumsticks. In addition, samples treated with a thermal treatment (58°C for 2 min) had significant (P<0.05) lower microbial counts when compared with the control.

Keywords: microbial quantity; 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, flavor and etc. (Lemos et al. 1999; Xiong and Kupski, 1999; Young and Buhr, 2000; Young and Lyon, 1997a,b; Zheng et al. 2000), but the process is usually not intended to improve the microbial quality of the product.

The lactoperoxidase system (LPS), which consists of lactoperoxidase (LP), thiocyanate (SCN\textsuperscript{-}), and hydrogen peroxide (H2O2), 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.
In the presence of $SCN^{-}$ and $H_{2}O_{2}$, Kamau et al. (1990a,b) explained, the LPS generates the hypothiocyanite ($OSCN^{-}$) and hypothiocyanous acid ($HOSCN$), which are the main antimicrobial products, and strong oxidizing agents that can oxidize essential sulphydryl groups in bacterial proteins thus inhibits bacterial growth. The LPS has been mainly studied for the application in the milk and dairy products (Zapico et al., 1998). Only a few studies have been attempted to apply this LPS system in meat and poultry products.

Kennedy et al. (2000) evaluated the inhibitory effect of the LPS on the growth of selected microorganisms in a beef mince system, and reported that *Listeria monocytogenes* L45 was the most sensitive to LPS, followed by *Staphylococcus aureus* R37 and *Escherichia coli* O157:H7. Boussouel et al. (2000) reported that combination of LPS and nisin resulted in a synergistic and lasting bactericidal effect against inoculated *Listeria monocytogenes* ATCC 15313 in skim milk, when compared with adding nisin and LPS alone. Wolfson et al. (1994) reported that immersion of the inoculated chicken legs and wings in a 50°C solution containing the LPS (1μg/ml lactoperoxidase, 5.9 mM potassium thiocyanate, and 2.5 mM hydrogen peroxide) for 5 min, and stored at 4°C for 2 days resulted in a 2.2 log aerobic plate counts increase for the treated sample but a 5.1 log increase for the control samples. Also, psychrotrophic count decreased 1.1-log for the treated samples whereas the control samples counts increased by 0.8 log.

Thermal or heating treatment, which is one of the most common 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. 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.

Lactoperoxidase (LP), which is one of the components of the LPS, is relatively heat-stable. Korhonen (1980) indicated that LP retained its activity after milk pasteurization of 63°C for 30 min or 72°C for 15 sec. Similarly, Kussendrager and van Hooijdonk (2000) mentioned that the heat denaturation of LP begins at temperatures from about 70°C. Reiter and Harnulv (1984) also mentioned that bovine milk LP only partially inactivated by short time pasteurization at 74°C.

Kamau et al. (1990a) reported that LPS enhanced the thermal destruction of *L. monocytogenes* and *S. aureus* in LPS treatment milk by decreasing the D values of the microorganisms. The authors explained that by damaging bacterial membranes, LPS might enhance the thermal destruction of microorganisms. Similarly, Wolfson and Sumner (1994) reported that the LPS consisted of lactoperoxidase (1 μg/ml), potassium thiocyanate (5.9 mM) and H₂O₂ (2.5 mM), was found to enhance the thermal inactivation of *S. typhimurium*.

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 microbial quantity found on marinated chicken drumsticks were investigated.

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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 experiment, 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 (A412/A230); Sigma Chemical Co., St. Louis, Missouri), hydrogen peroxide (H2O2, 30%, Fisher Scientific Co.; Pittsburgh, PA) and potassium thiocyanate (KSCN, Fisher Scientific Co.; Pittsburgh, PA), were added into the standardized marinade solution. The LP and H2O2 were prepared in distilled water, and filter sterilized separately using a .45μm filter (Gelman Sciences, Ann Arbor, MI), when 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 H2O2; the concentrations of 2 units of LPS were: 2 μg/ml of LP, 11.8 mM of KSCN, and 5.0 mM of H2O2.
Sample preparation

A total of ninety-six drumsticks which had the same sell-by date and 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. On the sell-by date which was indicated on the packages, inside the walk-in cooler, drumsticks were initially mixed thoroughly in 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 hr.

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 hours (including the previous time of thermal marinating and cooling).

Microbial evaluation

After 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 stored under refrigeration at 4°C. In the current study, refrigerated storage day 0 was set as one day after the sell-by date shown on the packages of the drumsticks. At specified sampling times (0, 2, 4, or 7 day; i.e. 1, 3, 5 or 8 days after the sell-by date of the samples), 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 hours 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. The total microflora and psychrotrophs counts of the day 0 (i.e. one day after the sell-by date) samples, which were without any treatment, were also evaluated. In the current study, the microbial counts in log CFU/ml of the rinse solution were also calculated and expressed as log CFU/cm² of the surface area of the drumsticks based on the average surface area of 174 cm². Experimental procedure is illustrated in Figure 6.1.
Statistical analyses

The study was designed as a $3 \times 2 \times 4$ 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$_2$O$_2$; 2 units = 2 µg/ml LP, 11.8 mM KSCN, and 5.0 mM H$_2$O$_2$), thermal treatment (4 or 58°C for 2 min), and 4 storage times (day 0, 2, 4, and 7; i.e. 1, 3, 5 and 8 days after the sell-by date of the samples), 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

At day 0 (i.e. one day after the sell-by date), the samples without any treatment had total microflora counts of 8.1 CFU/ml (5.8 CFU/cm$^2$) and psychrotrophs counts of 9.3 CFU/ml (7.0 CFU/cm$^2$). 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 of the total microflora counts for the samples with different levels of LPS added and thermal treatment applied during the refrigerated storage at 4°C.
As illustrated in Figure 6.2, in the current study, as the LPS-added levels increased, less total microflora counts of the samples were obtained from the samples. Adding LPS at a level of 1 unit, which consisted of LP (1 μg/ml), KSCN (5.9 mM), and H₂O₂ (2.5 mM) resulted in a significantly (P<0.05) lower total microflora counts of 6.2 log CFU/ml (4.0 log CFU/cm²), when compared with samples without adding any LPS which had higher counts of 7.0 log CFU/ml (4.8 log CFU/cm²). Adding even more LPS to the level of 2 units resulted in a further significantly (P<0.05) lower microbial count of 5.9 log CFU/ml (3.7 log CFU/cm²). The result implies that the more LPS added up to the level of 2 units, the less total microflora counts were obtained.

A previous study conducted in the same laboratory showed a similar LPS-added level effect on the total microflora counts of the treated marinated chicken drumsticks (Information has been submitted for publication). In that study, the effects of addition of LPS and nisin on the microbial quantity of marinated chicken drumsticks were evaluated. The results of that study showed that adding LPS at level of 1 unit (LP, 1 μg/ml; KSCN, 5.9 mM; H₂O₂, 2.5 mM) resulted in significantly (P<0.05) lower total microflora counts of 7.0 log CFU/ml, when compared with samples without adding any LPS which had a higher count of 7.7 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 6.7 log CFU/ml.

Denis and Ramet (1989) investigated the antibacterial activity of the LPS on *Listeria monocytogenes*. In their study, the survival of inoculated *L. monocytogenes* that was treated with LPS at different levels was evaluated. The authors reported that at higher levels (either 20 or 40 ppm of LP) of the LPS resulted in a higher inhibitory effect against the inoculated *L. monocytogenes* than at the lower level (10 ppm of LP) after 9
and 16 hours of incubation. In addition, the authors reported that 20 and 40 ppm of LP resulted in a similar decrease of \textit{L. monocytogenes}.

Haddadin \textit{et al.} (1995) evaluated the antimicrobial effect of LPS at different concentrations on sheep, cow, and goat milk. In their study, different levels of sodium thiocyanate and H\textsubscript{2}O\textsubscript{2} (the components of the LPS), were added into sheep, cow and goat milk, which already contained LP at a level above minimum necessary for antibacterial activity. After 6 days refrigerated storage at 4\textdegree{}C, it was found that addition of 15 mg/l of sodium thiocyanate and 10 mg/l of H\textsubscript{2}O\textsubscript{2} to milk resulted in significant (P<0.01) lower total colony and coliform counts compared with the controls. However, less difference in the microbial counts between a higher level (100 mg/l of sodium thiocyanate and 150 mg/l of H\textsubscript{2}O\textsubscript{2}) and a lower level was observed.

Figure 6.3 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\textdegree{}C for 2 min. cooled, and then refrigerated storage at 4\textdegree{}C for 18 hours, had a significantly (P<0.05) lower total microflora counts of 6.2 log CFU/ml (4.0 log CFU/cm\textsuperscript{2}), when compared with samples without a thermal treatment, which had a higher count of 6.6 log CFU/ml (4.3 log CFU/cm\textsuperscript{2}). This result implies that the thermal treatment significantly (P<0.05) retarded the growth of total microflora microorganisms of the marinated chicken drumsticks.

Borch \textit{et al.} (1989) investigated the antibacterial effect of the LPS on the \textit{Campylobacter jejuni}, which was isolated from poultry. In their study, the survival of inoculated \textit{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 rapid bactericidal effect against *C. jejuni* than the LPS at 20°C. Similarly, 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.

In the current study, the total microflora counts of the samples increased significantly (P<0.05) from day 0 to days 2 and 4 (i.e. 1, 3, and 5 days after the sell-by date) (Figure 6.4). Typically, spoilage can be detected when bacterial numbers exceed $10^8$ 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 8.0 log CFU/cm². In the current study, none of the total microflora counts of the samples exceeded this "8 log 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 applied during the refrigerated storage at 4°C in the current study.

As illustrated in Figure 6.2, 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 6.6 log CFU/ml (4.4 log CFU/cm²), when compared with samples without adding any LPS which had a higher count of 7.5 log CFU/ml (5.3 log CFU/cm²). Adding even more LPS to the level of 2 units resulted in a further significant (P<0.05) lower microbial count of 6.3 log CFU/ml (4.1 log CFU/cm²). The result implies that the more LPS added up to the level of 2 units, the less psychrotrophs counts were obtained.

Figure 6.3 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 6.5 log CFU/ml (4.3 log CFU/cm²), when compared with samples without thermal treatment, which had a higher count of 7.1 log CFU/ml (4.9 log CFU/cm²). This result implies that the thermal treatment significantly (P<0.05) retarded the growth of psychrotrophic microorganisms of the marinated chicken drumsticks. Also, in the current study, the chicken drumsticks stored at 4°C for 7 days did not result in a significant (P>0.05) difference in psychrotrophs counts (Figure 6.4).

**CONCLUSION**

In conclusion, adding LPS at the level of 1 unit (1 μg/ml LP, 5.9 mM KSCN, and 2.5 mM H₂O₂) significantly (P<0.05) decreased the total microflora and psychrotrophs.
counts of the marinated chicken 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 hours, significantly (P<0.05) decreased the total microflora and psychrotrophs counts of the marinated chicken broiler drumsticks. Further research to assess the changes in physical and sensory quality of marinated chicken drumsticks treated with LPS and thermal treatment is needed.

REFERENCES


Standardized marinade solution \(^1\)

**Before marinating**

- Autoclaved marinade solution

**Marinating**

- Marinating without thermal treatment at 4°C for total 18 hrs
- Marinating with thermal treatment \(^4\) at 4°C for total 18 hrs

**After marinating**

- Microbial quality evaluation at 0, 2, 4, and 7 days of storage at 4°C
- Total microbial count
- Psychrotrophs count

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\(^1\) Standardized marinade solution: acetic acid (1%), salt (3%) in distilled water then adjusted to pH 4.

\(^2\) Sterilized LPS component: 1 unit LPS = 1 μg/ml LP, 5.9 mM KSCN, and 2.5 mM H₂O₂; 2 units LPS = 2 μg/ml LP, 11.8 mM KSCN, and 5.0 mM H₂O₂. The LP and H₂O₂ solutions were filter sterilized using a 0.45μm filter (Gelman Sciences, Ann Arbor, MI), whereas the KSCN solution was autoclaved.

\(^3\) Treatments: a 3×2×4 factorial design with 3 levels of LPS (0, 1 and 2 units), 2 thermal treatment (4 or 58°C for 2 min), and 4 storage time (0, 2, 4, and 7 days).

\(^4\) Thermal treatment: 4 or 58°C for 2 min.

Figure 6.1 Experimental procedure
Figure 6.2 Effect of LPS-added level on the total microflora (incubated at 35°C for 48 hrs) and psychrotrophs counts (incubated at 7°C for 10 days) of marinated chicken drumsticks.

abc Means with different superscript are significantly different (P<0.05).
xyz Means with different superscript are significantly different (P<0.05).
*LPS unit: 1 unit = LP (1 µg/ml), KSCN (5.9 mM), and H₂O₂ (2.5 mM).
2 unit = LP (2 µg/ml), KSCN (11.8 mM), and H₂O₂ (5.0 mM).
Figure 6.3 Effect of thermal treatment on the total microflora (incubated at 35°C for 48 hrs) and psychrotrophs counts (incubated at 7°C for 10 days) of marinated chicken drumsticks.

Means with different superscript are significantly different (P<0.05).

Thermal treatment: 58°C for 2 min.
Figure 6.4 Effect of storage time on the total microflora (incubated at 35°C for 48 days) and psychrotrophs counts (incubated at 7°C for 10 days) of marinated chicken drumsticks.

Means with different superscript are significantly different (P<0.05).

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CHAPTER 7

EXPERIMENT 5

THE INFLUENCE OF NISIN WITH THERMAL TREATMENT ON THE PHYSICAL AND SENSORY CHARACTERISTICS OF MARINATED CHICKEN DRUMSTICKS

ABSTRACT

Nisin and thermal treatments have been shown to inactive some microorganisms in foods. However, further studies were needed to evaluate whether these treatments had any influence on the physical and sensory characteristics of the treated samples. In this study, a solution that contains acetic acid (1%), salt (3%), and EDTA (20mM) with pH adjusted to 4 was developed as a standardized marinade. For the nisin-added samples, a 100 IU/ml of nisin was added to the marinade before pH adjustment. For the thermal treatment, samples were heated with the marinade solution at 58°C for 2 min, and then stored at 4°C for 18 hrs, whereas no thermal-treated samples were stored at 4°C for 18 hrs. For sensory evaluation, some flavoring agents including 0.3% black pepper and 0.15% garlic powder were added when preparing the marinade solution. For physical evaluation, no flavoring agents were used. Physical and sensory evaluations were then conducted. The results showed that the treatment, which combined 100 IU/ml nisin and thermal treatment (58°C for 2 min), did not impair the physical or sensory qualities of the
treated samples. In conclusion, marinated chicken drumsticks treated with nisin and thermal treatment were acceptable based on the physical and sensory qualities.

Keywords: nisin; thermal treatment; physical characteristics; sensory characteristics; marination

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 the pH of the tissue. Marination of poultry is practiced to improve product's physical and sensory attributes, such as tenderness, water-holding capacity, flavor and etc. (Hashim, et al. 1999a,b; Lemos et al. 1999; Xiong and Kupski, 1999; Young and Buhr, 2000; Young and Lyon, 1997a,b; Zheng et al. 2000).

Nisin, which is considered a "biopreservative", is a natural, nontoxic, and heat stable polypeptide produced by Lactococcus lactis, and has been shown to inhibit gram-positive microorganisms (Ray, 2001), and gram-negative bacteria when combined with a chelating agent such as EDTA (Stevens et al. 1992; Stevens et al. 1991). Even though several studies involving nisin and mild heat treatment to inactive microorganisms in meat and poultry products have been reported (Budu-Amoako et al., 1999; Kalchayanan et al., 1992; Mahadeo and Tatini, 1994; Modi et al., 2000; Ueckert et al., 1998), most of these studies focused on the effect of nisin toward the microbial quality of the treated...
samples. Limited information concerning the physical and sensory qualities influenced by addition of nisin is available. Ro2beh et al. (1993) reported that fresh beef samples previous treated with nisin (500 IU/g) and CO₂ (10% for 60 min) had a similar color score when compared with the samples previously treated only with CO₂ during vacuum-package storage at 3°C for 8 weeks. Usborne et al. (1986) reported that there was no significant (P<0.05) difference of color and preference ratings between bacon cured either with nitrite only or cured with nitrite and nisin.

Several studies involving adding nisin on the sensory quality of treated fish products have been reported. Nilsson et al. (1997) found that a treatment which consisted of 1000 IU/g nisin and packed in 60% CO₂ and 40% N₂ did not negatively influence the sensory characteristics of cold-smoked salmon samples. Nykanen et al. (2000) reported that injection of nisin (4000-6000 IU/ml) did not affect the sensory quality of cold-smoked rainbow trout. Nykanen et al. (1999) reported that a treatment which combined lactic acid with nisin whey permeate significant (p<0.001) enhanced the characteristic fishy flavor of the cold-smoked rainbow trout fillets, when compared with the control samples after storage at 3°C for 8 days. Also, sensory qualities of the treated samples remained unchanged after 15 days of storage (Nykanen et al., 1999). Nykanen et al. (1998) reported that treatment with nisin whey permeate containing 3% nisin did not change the sensory characteristics of the minced rainbow trout.

Thermal or heat treatment, which is one of the most common physical methods to reduce the load of microorganisms, has been widely applied for years to preserve foods. Heating is involved in many food processes, such as cooking, scalding, pasteurizing, drying, and etc.
Previous studies conducted in this same laboratory showed that a treatment, which combined nisin and a thermal treatment (58°C for 2 min) decreased the total microflora and psychrotrophs counts of marinated chicken broiler drumsticks during refrigerated storage for up to 7 days (Information has been submitted for publication). Limited information on the contribution of marination, nisin, and thermal treatment to the physical and sensory characteristics of treated poultry products is available. Whether or not adding nisin and thermal treatment would affect the physical and sensory characteristics of the treated samples should be evaluated.

The objective of this study was to investigate the effects of added nisin and thermal treatment on some physical and sensory characteristics of the marinated chicken drumsticks. In addition, the sensory profiles of marinated chicken drumsticks using the descriptive analysis techniques were characterized in this study. The results of this study could be used to determine the acceptability of marinated chicken drumsticks that were previously treated with antimicrobial compounds used to reduce potential microbial contamination during processing and to extend chicken drumsticks’ shelf life.

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. In the current study, a solution that contains of 1% acetic acid, 3% salt, and 20mM disodium EDTA (Fisher Scientific Co., Kansas City, MO) was prepared as the standardized marinade. A 100 IU/ml of nisin, which was
prepared from a commercial nisin powder (Sigma Chemical Co., St. Louis, MO), was added to the standardized marinade for the nisin-added treatments. The pH of the marinade solutions was then adjusted to 4 (using HCl or NaOH solutions). For the sensory evaluation, some flavoring agents including 0.3% black pepper and 0.15% garlic powder were added to the standardized marinade solutions for the marinated treatments 1 through 4 (with or without thermal treatment based on the assigned groups), whereas no flavoring agents were added to the treatments 5 and 6 samples. No flavoring agents were added into the marinade solution for all 6 treatments for the physical evaluation.

Sample preparation

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 the experiment was conducted. Drumsticks were randomly chosen and equally assigned and labeled to the treatment groups. For the samples without thermal treatment (treatment 1 and treatment 3), drumsticks were placed and marinated in plastic containers (Sterilite Corporation, Townsend, MA) with marinade solution so that all the drumsticks were covered completely by the marinade solution and stored at 4°C for 18 hr. Instead of the marinate solution, the treatment 5 control samples were stored and submerged in distilled water. For those samples with thermal-marinating treatment (treatment 2 and treatment 4), drumsticks were placed inside a plastic bag with the heated marinade solutions, which was previously heated in a water bath. After holding at 58°C for 2 min, the marinade-drumstick mixes in the bags were cooled by immersing the bags in running tap water.
When the marination mix was cooled to 25°C (approximately 10 min), the marinade-drumstick mix was moved into plastic containers and then refrigerated at 4°C for 18 hours (including the previous time of thermal marinating and cooling). There was no water or marinade solution added to treatment 6 samples.

Physical evaluations

Drumsticks were weighted individually before conducting the experiment. Figure 7.1 illustrates the experimental procedure.

The pH values measurement of marinade solutions and drumsticks

The pH meter was calibrated with a two-point calibration method using pH 4 and 7 buffers before use. The pH values of the marinade solution were measured before and after marinating of the drumsticks using a Corning pH meter (Model 430, Corning Inc., Corning, NY). Muscles of the whole drumsticks without bone and skin were blended for 30 sec using a Waring Lab Blender (Model 31BL91, Dynamics Corporation of America, New Hartford, Connecticut). Ten grams of muscle sample was then blended with 100ml of distilled water in a polyethylene bag for 1 min using a Seward Stomacher (Model 400, Tekmar Company, Cincinnati, Ohio), and then the pH of the mixture was measured.

Marinade absorption measurement

After finishing marinating, the drumsticks were removed from the marinade solutions and drained for 5 min at 4°C before determining the marinade absorption. Marinade absorption (grams marinade absorbed/100g of drumstick) was calculated based
on the unmarinated weight (Wt before marinating) of the sample and its weight after marination with 5-min draining (Wt after marinating and draining) and was calculated as follows.

\[
\text{Marinade Absorption} = \left( \frac{\text{Wt after marinating and draining} - \text{Wt before marinating}}{\text{Wt before marinating}} \right) \times 100
\]

Instrumental color measurement

The Hunter “L*” (lightness), “a*” (redness-greenness), and “b*” (yellowness-blueness) values of drumstick were measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey). Color measurements were obtained on the surfaces of the skin, and also on the muscle without skin covering, respectively, before and after marinating, and also after cooking. Three measurements were made at different locations on the drumsticks and averaged. A standard plate (number 18433010) with “Y” = 92.60, “x” = 0.3140, and “y” = 0.3206 was used as a reference.

Marinade solution salt content measurement

The salt contents of the marinade solutions after marinating were measured with a Dicromat® salt analyzer (Model DSA-1000, Diamond Crystal Salt Company, St. Clair, Michigan). The marinade solutions were mixed, and then the salt contents were measured. The salt analyzer was rinsed with distilled water between measurements.

Cooking loss measurement

Pre-weighted drumsticks were cooked in a 177°C (350°F) preheated conventional oven (Type EF111, The G.S. Blodgett Co. Inc., Burlington, VT) to an internal temperature
of 70°C (158°F). After reaching the internal temperature of 70°C monitored by Fisher 
brand traceable alarm thermometer/timer (Fisher Scientific, Pittsburgh, PA), the samples 
were equilibrated to room temperature and then the cooking loss measurement and sensory evaluation were accomplished. Cooking loss was calculated based on the weight of the sample before cooking (Wt before cooking) and its weight after cooking (Wt after cooking) and was calculated as follows.

\[
\text{Cooking Loss} = \left\{ \frac{\text{Wt before cooking} - \text{Wt after cooking}}{\text{Wt before cooking}} \right\} \times 100
\]

Yield measurement

Yield was calculated based on the weight of the sample before marinating (Wt before marinating) and its weight after cooking (Wt after cooking) and was calculated as follows.

\[
\text{Yield} = \left( \text{Wt after cooking} - \text{Wt before marinating} \right) \times 100
\]

Sensory evaluation

The panel of seven members consisted of faculty and graduate students majoring in meat science. They evaluated the sensory characteristics of the marinated products made with the standardized marinade solution with flavoring agents (0.3% black pepper and 0.15% garlic powder) with or without thermal treatment and/or nisin added (Figure 7.1). Descriptive analysis was conducted to evaluate the intensities of sensory characteristics of the raw and cooked samples.
Before conducting the experiment, the panelists were first trained in the general sensory descriptive analysis and were led through the development of a lexicon of descriptors for marinated chicken samples. Most of the references used during training were selected in accordance with Hashim et al. (1999a,b) recommendations. Next, the panelists were requested to list the most descriptive terms for the product. Panelists with the help of the panel leader identified sensory properties important in marinated chicken. The panel agreed on all attributes considered necessary for evaluation of the marinated chicken and defined these attributes.

Attributes of skin color, muscle color, marinated chicken aroma (combined raw marinated chicken aroma consisted of chicken, sour-acid, peppery, and garlic aromas), and off-aroma (any aroma which was not expected from the raw marinated chicken aroma) were used to evaluate the raw samples. After cooking followed the procedures described in the cooking loss measurement, the samples were cooled to room temperature (approximately 25°C) and served to the sensory panel. Attributes for skin color, muscle color, marinated chicken flavor (combined cooked marinated chicken flavor consisted of chicken, sour-acid, saltiness, pepper, and garlic), off-flavor (any flavor which was not expected from the cooked marinated chicken flavor), juiciness, and tenderness were used to evaluate the cooked samples. Appropriate reference samples were selected and used during training to exhibit the attributes and to set the anchors. Reference samples were available at each test trial for panelists to assist in making evaluations. The Hunter color values of the skin and muscle for the raw and cooked reference samples were also measured.
The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1 = light color; 9 = dark color), juiciness (1 = not juicy; 9 = very juicy), and tenderness (1 = not tender; 9 = very tender). The samples were randomly presented in plastic plates coded with a randomly selected three-digit code. Water and unsalted crackers were provided to panelists. Each panelist evaluated the samples independently.

Statistical analyses

Three trials were conducted in this study. Data were analyzed using the general linear model (GLM) of Statistical Analysis System's Procedures (SAS Institute Inc., Cary, NC) with a 5% level of significance. Means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

Physical evaluation of marinated chicken drumsticks with or without thermal treatment and nisin added is shown in Table 7.1. In the current study, treatment 1 had no nisin added and no thermal treatment; treatment 2 had no nisin added but with thermal treatment; treatment 3 had nisin added but without thermal treatment; treatment 4 had both nisin added and thermal treatment; treatment 5 had distilled water (instead of the marinade solution) added; treatment 6 had no water and no marinade solution added.

Before marinating, raw drumstick muscle pH of treatments 1 through 6 were from 6.75 to 7.09 (Table 7.1). After marinating for 18 hours at 4°C, the pH values of raw drumsticks dropped approximately 1.5-pH units to pH 5.36-5.43 for the marinated
treatments 1 through 4 without significant (P>0.05) differences among these 1 through 4 marinated treatments. The pH values of the controls with and without distilled water added (treatments 5 and 6) increased to 6.99 and 6.98, respectively, which were significantly (P<0.05) higher than the samples of marinated treatments 1 through 4. After cooking, the pH values of the samples for marinated treatments 1 through 4 increased to 5.60-5.77, and no significant (P>0.05) difference for pH was detected among these 1 through 4 marinated treatments (Table 7.1). Cooked drumstick muscle pH values of both control samples with or without addition of distilled water (treatments 5 and 6) increased to 7.06 and 7.16, and were significantly higher (P<0.05) than the samples for marinated treatments 1 through 4.

After marinating, the marinade solution pH values for the marinated treatments 1 through 4 were all 4.35, and therefore no significant (P>0.05) difference was obtained among these 1 through 4 marinated treatments (Table 7.1). The solution of the control with addition of distilled water was significant (P<0.05) higher (7.19) than the marinated treatments 1 through 4.

The marinade absorptions of the marinated treatments 1 through 4 samples ranged from 1.12 to 1.50%, and no significant (P>0.05) difference was obtained among these 4 marinated treatments. The absorption of the control samples with addition of distilled water was 7.89%, which was significant (P<0.05) higher than the samples of the marinated treatments 1 through 4 (Table 7.1). The cooking loss of the marinated treatments 1 through 4 marinated samples ranged from 22.27 to 27.78%, and no significant (P>0.05) difference was detected among the 1 through 4 marinated treatment samples. The cooking loss of the control with distilled water added (treatment 5) was
20.29%, which was not significant (P>0.05) different than the marinated treatments 1 through 4 (Table 7.1). The yield of the marinated treatments 1 through 4 marinated samples ranged from 76.98 to 80.94%. The salt content of the marinade solution after marinating of the marinated treatments 1 through 4 samples was 1.19% and no significant (P>0.05) difference was obtained among these 4 marinated treatments, but the salt content of the control samples with distilled water (treatment 5) was 0.08%, which was significant (P<0.05) lower than the marinated treatments of 1 through 4 as expected (Table 7.1).

Table 7.2 illustrates the color evaluation of marinade chicken drumsticks with or without thermal treatment and addition of nisin. The skin L* values of the all treatments (1 through 6) before marinating were in the range of 68.11-74.73 without any significant differences (P>0.05) among treatments. After marinating, all the skin L* values increased to 76.53-82.64, except for the control without addition of distilled water (treatment 6) which decreased to 73.48. Also there was no significant (P>0.05) difference for the skin L* values among treatments 1 through 3 after marinating. After cooking, all the skin L* values of the samples decreased to 59.80-64.97, and no significant (P>0.05) difference was obtained among any of the treatments 1 through 6.

The skin a* values of the all treatments (1 through 6) before marinating were in the range of 4.96-6.55 without any significant differences (P>0.05) among treatments. After marinating, the skin a* values of the marinated treatments 1 through 4 decreased to 0.32-1.24 without any significant differences (P>0.05), but the skin a* values of the control with or without addition of distilled water (treatments 5 and 6) had significant (P<0.05) higher values of 2.95 and 4.97, respectively. After cooking, the skin a* values
of the marinated treatments 1 through 4 increased to 1.16-2.24 without any significant
differences (P>0.05), whereas the skin a* values of the control with or without addition
of distilled water significantly (P<0.05) decreased to 2.30 and 3.25, respectively. The
skin b* values of the all treatments (1 through 6) before marinating were in the range of
7.00-11.15 without any significant (P>0.05) differences (Table 7.2). After marinating,
the skin b* remained in the range of 8.09 to 11.89 without any significant differences
(P>0.05) among 6 treatments. After cooking, the skin b* increased to 21.00-26.97
without any significant differences (P>0.05) among 6 treatments.

The muscle L* values of all treatments (1 through 6) before marinating were in
the range of 57.93-61.76 without any significant (P>0.05) differences among 6 treatments
(Table 7.3). After marinating, there was no significant (P>0.05) differences for the
muscle L* values among the 1 through 4 marinated treatments, whereas both of the
control samples with and without water added (treatments 5 and 6) had significant
(P<0.05) lower muscle L* values. After cooking, there was no significance (P>0.05) of
the muscle L* values among any of the six treatments.

The muscle a* values of the all treatments (1 through 6) before marinating were in
the range of 13.55-14.23 without any significant (P>0.05) differences. After marinating,
the muscle a* values of the marinated treatments 1 through 4 decreased to 2.39-3.03
without any significant (P>0.05) difference due to treatment, but the controls with or
without addition of distilled water (treatments 5 and 6) had significantly (P<0.05) higher
muscle a* values. After cooking, there was no significant (P>0.05) difference for the
muscle a* values of marinated treatments 1 through 4, but the controls with or without
addition of distilled water (treatments 5 and 6) had significant (P<0.05) higher muscle a*
values of 4.56 and 7.25 respectively.

The muscle b* values of the all treatments (1 through 6) before marinating were in the range of 9.98-15.23. After marinating, the muscle b* values of marinated treatments 1 through 4 increased to 13.40-16.99 without significant (P>0.05) difference among treatment, but the controls with addition of distilled water (treatment 5) had significant (P<0.05) lower muscle b* value of 11.34. After cooking, the muscle b* values increased to 19.17-22.82, and there was no significant (P>0.05) difference for the muscle b* values for all treatments 1 through 6.

The results of the total color difference are shown in Table 7.4. Total color difference \( (\Delta E_{ab}^* ) \) is calculated by the following formula: Total color difference \( (\Delta E_{ab}^* ) \) = square root of \( [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2] \), where \( L^* \) = light and dark, \( a^* \) = red and green, \( b^* \) = yellow and blue, and \( \Delta \) representing the difference between the two processing steps, and it is commonly applied to evaluate the color changes of samples at the two different processing steps in food science studies. There was no significant (P>0.05) difference for total color difference of skin between before and after marination for the marinated treatments 1 through 4 samples, but the control samples without distilled water added (treatment 6) had a significantly (P<0.05) lower total color differences for skin color. There was no significant (P>0.05) difference for total color difference for skin color between before and after cooking for the all six treatment samples. Similarly, no significant (P>0.05) difference for total color difference for skin between before marination and after cooking for the all six treatment samples was obtained in the current study. As shown in Table 7.4, in the current study, there was no significant (P>0.05) difference for total color difference in muscle color between before
and after marination for the marinated treatments 1 through 4 samples, but the control samples with or without distilled water added (treatments 5 and 6) had significant (P<0.05) lower total color difference in muscle color. There was no significant (P>0.05) total color difference in muscle between before and after cooking, and between before marination and after cooking for all six treatment samples.

Sensory evaluation of marinated chicken drumsticks with or without addition of nisin and/or thermal treatment is shown in Table 7.5. In the current study, 0.3% black pepper and 0.15% garlic powder were added for the marinated treatments 1 through 4 for the sensory evaluation, whereas no flavoring agents were added to treatments 5 and 6 samples. Based on a 1-9 scale, in which 1 and 9 representing the lighter and darker color respectively, the marinated treatments 1 through 4 samples in the current study had lower sensory raw skin color scores between 2.0 and 2.7 without any significant (P>0.05) differences among the marinated treatments. The controls with and without distilled water added (treatments 5 and 6) had higher sensory raw skin color scores of 4.4 and 5.7, respectively, which were significantly (P<0.05) higher than the samples of marinated treatments 1 through 4. Similarly, the marinated treatments 1 through 4 samples had significant (P<0.05) lower sensory raw muscle color scores of 2.6-3.6, but the controls with or without water added (treatments 5 and 6) that had color scores of 5.4 and 6.9. The lightness of the skin and muscle colors for marinated treatments 1 through 4 when comparing to the samples of the control groups was probably due to the addition of acid.

The results of color evaluation of treated samples due to addition of nisin agreed with previous studies by other researchers. Rozbeh et al. (1993) evaluated the influence of Nisaplin, which is a commercial preparation of nisin, on the color of refrigerated
vacuum-packaged beef. In their study, fresh beef samples were treated with 10% CO$_2$ and 90% N$_2$ for 60 min, 500 IU/g Nisaplin, vacuum-packaged, and then refrigerated at 3°C. Using a 10-point scale (1 = brown; 10 = bright cherry red), and the authors reported that samples previously treated with Nisaplin and CO$_2$ had a close color score of 3 when compared with the samples that previously had only been treated with 10% CO$_2$ which had a color score of 2 during storage at 3°C for 8 weeks.

In the current study, a marinated chicken aroma was defined as a combined aroma, which consisted of fresh raw chicken, acidic, black peppery, and garlic aromas. The marinated treatments 1 through 4 samples had significant (P<0.05) higher marinated chicken aroma scores of 3.9 to 5.4 (without significant difference among these 1 through 4 marinated treatments), when compared with the controls that had 1.3 and 2.0 scores probably due to lack of flavoring agents added. Low (1.1 to 1.4 based on a 1-9 scale) and without significant (P>0.05) differences of the sensory off-aroma (an aroma that was not expected from the raw marinated chicken aroma, scores) of the raw samples for all treatments 1 through 6 were obtained in this study (Table 7.5).

After cooking, the sensory skin and muscle color scores of the marinated treatments 1 through 4 samples increased. There was no significant (P>0.05) difference for the sensory cooked skin color scores of all treatments 1 through 6. The control samples (treatments 5 and 6) had significantly (P<0.05) higher sensory cooked muscle color scores of 5.4 and 6.9, when compared with the marinated treatments 1 through 4 samples. Similar to the marinated chicken aroma for the raw samples, in the current study, a marinated chicken flavor was defined as a combined flavor, which consisted of cooked chicken, acidic, black peppery, and garlic flavors. The marinated treatments 1
through 4 samples had significant (P<0.05) higher marinated chicken flavor scores of 3.6-6.4 (without significant difference among these 1 through 4 marinated treatments), when compared with the controls that had scores of 2.0 and 1.9 probably due to lack of flavoring agents added. Low (1.1 to 1.7 based on a 1-9 scale) and without significant (P>0.05) differences among treatments for the sensory off-flavor scores of the cooked samples for all treatments 1 through 6 were obtained in the current study (Table 7.5).

Usborne et al. (1986) evaluated the influence of nisin on the sensory quality of the bacon treated with nisin. In their study, pork bellies were cured with either nitrite only, or with 2000 or 4000 IU/ml nisin, which partially substituted for nitrite. The results of a triangle test showed that there was no significant (P<0.05) difference between the samples either cured with nitrite only or cured with nitrite and nisin. In addition, in the same study, they reported that there was no significant (P<0.05) difference for color and preference ratings between the samples cured either with nitrite only or cured with nitrite and nisin. The authors concluded that bacons made from the curing brine containing nisin were acceptable with respect to color and palatability of the final product.

Nykanen et al. (2000) evaluated the effect of nisin on the sensory quality of cold-smoked rainbow trout, which were injected with 4000-6000 IU/ml nisin, smoked, and then stored under refrigeration. After evaluating intensities of several sensory attributes, including smoked odor, fishy odor, fishy flavor, sweet taste, salty taste, and elasticity of the samples, the authors reported that the treatment with nisin added did not affect the sensory characteristics of the treated samples. In addition, in the same study, by comparing samples stored for 2 days to those stored for 16 and 23 days using a triangle test, Nykanen et al. (2000) reported that the sensory quality of the treated samples
remained unchanged for 23 days of storage at 3°C, whereas the control samples without nisin added remained unchanged for 16 days. The authors concluded that the treatment of injecting 4000-6000 IU/ml nisin did not alter the sensory characteristics of the treated smoked rainbow trout samples.

Nykanen et al. (1999) evaluated the effect of nisin whey permeate on the sensory quality of cold-smoked rainbow trout. In their study, fish fillets were injected with a mixture of lactic acid, and whey permeate containing 4000-6000 IU/ml, and a salt solution, followed by smoking at 28°C for 6 h, sliced, vacuum packaged and storage at 3°C. Influence of the treatment on sensory characteristics was analyzed after 8 and 22 days by sensory profiling. Nykanen et al. (1999) reported that the treatment which combined lactic acid and nisin whey permeate significant (p<0.001) enhanced the characteristic fishy flavor of the product when compared with the control samples after stored at 3°C for 8 days. Sensory qualities of the treated samples remained unchanged after 15 days of storage based on triangle test results (Nykanen et al., 1999).

Nykanen et al. (1998) evaluated the effect of whey permeate containing 3% nisin on the sensory quality of minced rainbow trout. The authors reported that the treatment with nisin whey permeate did not change the sensory characteristics of the treated samples, when evaluating intensities of several sensory attributes, including fishy odor, metallic flavor, fishy flavor, astringency, sourness, sweetness, saltiness and meaty flavor. The treatment 5 samples (control samples with water added) and treatment 6 samples (control samples without water added) respectively had higher (more juiciness) and lower (less juiciness) sensory juiciness scores; when comparing with the marinated treatments 1 through 4 samples (Table 7.5). No significance (P>0.05) of sensory tenderness scores.
was detected for the treatments 1 through 6 samples in the current study. Nilsson et al. (1997) found that a treatment which consisted of 1000 IU/g nisin and packed in 60% CO₂ and 40% N₂ did not negatively influence the sensory characteristics of cold-smoked salmon samples.

In the current study, the control samples with water added (treatment 5) and without water added (treatment 6), respectively, had higher and lower sensory juiciness scores when compared with marinated treatments 1 through 4 samples (Table 7.5). In addition, no significant (P>0.05) difference for sensory tenderness scores of marinated treatments 1 through 4 was observed in the current study.

CONCLUSION

In conclusion, the treatment consisting of nisin added (100 IU/ml) and thermal treatment (58°C for 2 min), which was previously showed to decrease some microorganisms of some food, did not impair the physical and sensory characteristics tested in the current study for the treated samples.

REFERENCES


Before marinating:
- Standard marinade solution
- Pre-assigned antimicrobial components
- Flavors

Adjust marinade solution to pH 4.0

Marinating with or without thermal treatment (58°C for 2 min) at 4°C for total 18 hrs

Marinated solution

After marinating:
- pH measurement
- Salt content measurement

Marinated solution

Raw drumstick
- (before marinating)
- pH measurement
- Hunter color measurement

Raw drumsticks

Cooking in oven until internal temp. 70°C

Cooking loss measurement

Yield measurement

pH measurement (cooked samples)

Hunter color measurement (cooked samples)

Sensory evaluation (cooked samples)

1Standard marinade solution: acetic acid (1%), salt (3%), and disodium EDTA (20 mM) in distilled water.
2Pre-assigned antimicrobial components: with or without addition of nisin (100 IU/ml) based on the assigned groups.
3Flavoring agents: ground black pepper (0.3%) and garlic powder (0.15%) for the sensory evaluation of marinated treatments 1 through 4; no flavor agents were added for the physical evaluation.
4Total time of 18 hrs included thermal treatment, cooling and marinating for the thermal treatment samples.

Figure 7.1 Experimental procedure
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5 Control (water added)</th>
<th>6 Control (no water added)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thermal treatment (58°C, 2 min)</td>
<td></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Raw drumstick pH^1 (before marinating)</td>
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<td>6.91</td>
<td>7.01</td>
<td>7.09</td>
<td>7.06</td>
<td>6.75</td>
<td>6.86</td>
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<td>Raw drumstick pH^4 (after marinating)</td>
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<td>5.41^a</td>
<td>5.36^a</td>
<td>5.43^a</td>
<td>5.36^a</td>
<td>6.99^b</td>
<td>6.98^b</td>
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<td>Cooked drumstick pH^4</td>
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<td>5.75^a</td>
<td>5.60^a</td>
<td>5.77^a</td>
<td>5.69^a</td>
<td>7.06^b</td>
<td>7.16^b</td>
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<td>Marinade solution pH (after marinating)</td>
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<td>4.35^a</td>
<td>4.35^a</td>
<td>4.35^a</td>
<td>7.19^b</td>
<td>ND</td>
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<td>Marinade absorption (%)</td>
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<td>1.25^a</td>
<td>1.50^a</td>
<td>1.46^a</td>
<td>1.12^a</td>
<td>7.89^b</td>
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<tr>
<td>Cooking loss (%)</td>
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<td>22.27^ab</td>
<td>27.36^a</td>
<td>23.18^ab</td>
<td>27.78^a</td>
<td>20.29^ab</td>
<td>13.74^b</td>
</tr>
<tr>
<td>Yield (%)</td>
<td></td>
<td>80.94^a</td>
<td>77.67^ab</td>
<td>80.82^ab</td>
<td>76.98^b</td>
<td>88.53^c</td>
<td>86.73^c</td>
</tr>
<tr>
<td>Salt content of marinade solution after marinating (%)</td>
<td></td>
<td>1.19^a</td>
<td>1.19^a</td>
<td>1.19^a</td>
<td>1.19^a</td>
<td>0.08^b</td>
<td>ND</td>
</tr>
</tbody>
</table>

^a, b Means within a row without the same superscript are significantly different (P<0.05).

ND: not determined.

^1Marination: Chicken drumsticks were marinated with a solution contained with 1% acetic acid, 3% salt, and adjusted to pH 4.

^2Control (water added): Instead of the marinade solution, samples were dipped in the unheated distilled water.

^3Control (no water added): Samples were placed in bags without adding the marination solution or distilled water.

^4Drumstick pH was measured for muscle without skin.

Table 7.1 Physical evaluations of marinated chicken drumsticks with or without thermal treatment and/or nisin added
### Table 7.2 Skin color evaluation\(^1\) of marinated\(^2\) chicken drumsticks with or without thermal treatment and/or nisin added

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>Thermal treatment (58°C, 2 min)</td>
<td></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>control</td>
<td>control</td>
</tr>
<tr>
<td><em><em>L</em> value</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before marinating</td>
<td></td>
<td>70.32</td>
<td>68.11</td>
<td>71.24</td>
<td>70.20</td>
<td>71.17</td>
<td>74.73</td>
</tr>
<tr>
<td>After marinating</td>
<td></td>
<td>76.53(^{abc})</td>
<td>78.15(^{ab})</td>
<td>80.45(^{ab})</td>
<td>82.64(^{b})</td>
<td>77.23(^{abc})</td>
<td>73.48(^{c})</td>
</tr>
<tr>
<td>After cooking</td>
<td></td>
<td>64.97</td>
<td>59.80</td>
<td>63.24</td>
<td>63.09</td>
<td>61.60</td>
<td>64.72</td>
</tr>
<tr>
<td><em><em>a</em> value</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before marinating</td>
<td></td>
<td>5.71</td>
<td>6.45</td>
<td>4.96</td>
<td>6.55</td>
<td>5.55</td>
<td>5.50</td>
</tr>
<tr>
<td>After marinating</td>
<td></td>
<td>1.24(^{a})</td>
<td>0.83(^{a})</td>
<td>0.32(^{a})</td>
<td>0.55(^{a})</td>
<td>2.95(^{b})</td>
<td>4.97(^{c})</td>
</tr>
<tr>
<td>After cooking</td>
<td></td>
<td>2.24(^{ab})</td>
<td>2.03(^{ab})</td>
<td>1.21(^{a})</td>
<td>1.16(^{a})</td>
<td>2.30(^{ab})</td>
<td>3.25(^{b})</td>
</tr>
<tr>
<td><em><em>b</em> value</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before marinating</td>
<td></td>
<td>11.12</td>
<td>8.48</td>
<td>11.37</td>
<td>7.00</td>
<td>11.15</td>
<td>9.60</td>
</tr>
<tr>
<td>After marinating</td>
<td></td>
<td>10.89</td>
<td>9.97</td>
<td>11.89</td>
<td>9.11</td>
<td>8.97</td>
<td>8.09</td>
</tr>
<tr>
<td>After cooking</td>
<td></td>
<td>25.98</td>
<td>23.20</td>
<td>26.97</td>
<td>21.00</td>
<td>23.80</td>
<td>23.82</td>
</tr>
</tbody>
</table>

\(^{a,b,c}\)Means within a row without the same superscript are significantly different (P<0.05).

\(^1\)Color evaluation: L* = light and dark, a* = red and green, and b* = yellow and blue, measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

\(^2\)Marination: Chicken drumsticks were marinated with a solution contained with 1% acetic acid, 3% salt, and adjusted to pH 4.

\(^3\)Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

\(^4\)Control (no water added): Samples were placed in containers without adding the marination solution or distilled water.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Control (water added)³</td>
<td>Control (no water added)⁴</td>
<td></td>
</tr>
<tr>
<td>Thermal treatment (58°C, 2 min)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**L* value**

| Before marinating | 61.76 | 58.53 | 58.20 | 59.94 | 57.93 | 59.63 |
| After marinating | 88.95ᵃ | 94.38ᵃ | 96.41ᵃ | 89.49ᵃ | 67.18ᵇ | 58.95ᵇ |
| After cooking | 72.48 | 74.32 | 74.87 | 74.72 | 71.79 | 70.89 |

**a* value**

| After marinating | 2.81ᵃ | 2.68ᵃ | 3.03ᵃ | 2.39ᵃ | 11.34ᵇ | 14.47ᶜ |
| After cooking | 3.27ᵃᵇ | 2.47ᵃ | 2.46ᵃ | 2.00ᵃ | 4.56ᵇ | 7.25ᶜ |

**b* value**

| Before marinating | 14.70ᵃ | 11.74ᵇ | 11.89ᵇ | 15.23ᵃ | 10.60ᵇ | 9.98ᵇ |
| After marinating | 16.99ᵃ | 13.40ᵃᵇ | 16.21ᵃ | 15.44ᵃ | 11.34ᵇ | 14.47ᵃᵇ |
| After cooking | 20.89 | 19.17 | 18.97 | 20.89 | 22.82 | 22.76 |

ᵃᵇᶜ Means within a row without the same superscript are significantly different (P<0.05).

¹Color evaluation: L* = light and dark, a* = red and green, and b* = yellow and blue, measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

²Marination: Chicken drumsticks were marinated with a solution containing 1% acetic acid, 3% salt, and adjusted to pH 4.

³Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

⁴Control (no water added): Samples were placed in containers without adding the marination solution or distilled water.

Table 7.3 Muscle color evaluation¹ of marinated² chicken drumsticks with or without thermal treatment and/or nisin added
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Control</td>
<td>Control (water added)³</td>
</tr>
<tr>
<td>Thermal treatment (58°C, 2 min)</td>
<td></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>Control</td>
<td>Control (no water added)⁴</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison of before and after marination</td>
<td></td>
<td>7.96&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.35&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Comparison of before and after cooking</td>
<td></td>
<td>40.16</td>
<td>38.38</td>
<td>40.76</td>
<td>35.51</td>
<td>39.77</td>
<td>45.59</td>
</tr>
<tr>
<td>Comparison of before marination and after cooking</td>
<td></td>
<td>16.55</td>
<td>18.20</td>
<td>18.78</td>
<td>16.63</td>
<td>16.23</td>
<td>17.61</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison of before and after marination</td>
<td></td>
<td>29.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Comparison of before and after cooking</td>
<td></td>
<td>34.24</td>
<td>27.04</td>
<td>34.58</td>
<td>35.84</td>
<td>41.27</td>
<td>49.37</td>
</tr>
<tr>
<td>Comparison of before marination and after cooking</td>
<td></td>
<td>16.07</td>
<td>21.07</td>
<td>21.57</td>
<td>19.62</td>
<td>20.81</td>
<td>18.44</td>
</tr>
</tbody>
</table>

<sup>k</sup><sup>b</sup>Means within a row without the same superscript are significantly different (P<0.05).

<sup>1</sup>Total color difference ($\Delta E^*_{ab}$) = square root of $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]$, where $L^*$ = light and dark, $a^*$ = red and green, $b^*$ = yellow and blue, and $\Delta$ representing the difference between the two processing steps; $L^*$, $a^*$, and $b^*$ were measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

<sup>2</sup>Marination: Chicken drumsticks were marinated with a solution contained with 1% acetic acid, 3% salt, and adjusted to pH 4.

<sup>3</sup>Control (water added): Instead of the marinate solution, samples were stored in unheated distilled water.

<sup>4</sup>Control (no water added): Samples were placed in bags without adding the marination solution or distilled water.

Table 7.4 Total color differences of marinated chicken drumsticks with or without thermal treatment and nisin added
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>Control (water added)</td>
<td>Control (no water added)</td>
</tr>
<tr>
<td>Thermal treatment (58°C for 2 min)</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>Raw samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin color</td>
<td></td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscle color</td>
<td></td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Marinated chicken aroma&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td>3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Off-aroma&lt;sup&gt;5&lt;/sup&gt;</td>
<td></td>
<td>1.1</td>
<td>1.4</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Cooked samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin color</td>
<td></td>
<td>4.7</td>
<td>4.6</td>
<td>4.9</td>
<td>5.0</td>
<td>3.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Muscle color</td>
<td></td>
<td>4.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.1&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>5.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Marinated chicken flavor&lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
<td>5.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Off-flavor&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
<td>1.7</td>
<td>1.1</td>
<td>1.1</td>
<td>1.4</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Juiciness</td>
<td></td>
<td>4.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.3&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tenderness</td>
<td></td>
<td>4.3</td>
<td>4.1</td>
<td>4.7</td>
<td>3.9</td>
<td>4.7</td>
<td>4.6</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d</sup> Means within a row without the same superscript are significantly different (P<0.05).

1 The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1 = light, 9 = dark), juiciness (1 = not juicy, 9 = very juicy), and tenderness (1 = not tender, 9 = very tender).

2 Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

3 Control (no water added): Samples were placed in containers without adding the marination solution or distilled water.

4 Marinated chicken aroma: the combination aroma consisted of chicken, sour (acidic), salty, peppery and garlic aromas; flavoring agents (0.3% ground black pepper and 0.15% garlic powder) were added to treatments 1 through 4.

5 Off-aroma: any aroma that was not expected from the raw marinated chicken aroma.

6 Marinated chicken flavor: the combination flavor consisted of chicken, sour (acidic), salty, peppery, and garlic flavors; flavoring agents (0.3% ground black pepper and 0.15% garlic powder) were added to treatments 1 through 4.

7 Off-flavor: any flavor that was not expected from the cooked marinated chicken flavor.

Table 7.5 Sensory characteristic intensities of marinated chicken drumsticks with or without addition of nisin and/or thermal treatment.
CHAPTER 8

EXPERIMENT 6

EFFECT OF NISIN AND TUMBLING ON THE PHYSICAL AND SENSORY CHARACTERISTICS OF MARINATED CHICKEN DRUMSTICKS

ABSTRACT

Nisin has been shown to inactive some microorganisms in foods and tumbling has been widely applied in the meat and poultry industry. However, further studies were needed to evaluate whether these treatments had any influence on the physical and sensory characteristics of the treated samples. In this study, a solution that contains acetic acid (1%), salt (3%), and EDTA (20mM), with pH adjusted to 4 was developed as a standardized marinade. A 100 IU/ml of nisin was added to the marinade before pH adjustment for nisin-added samples. Tumbling-treated drumsticks with the marinade were tumbled for 10 min, and then stored at 4°C for 18 hours. For sensory evaluation, some flavoring agents including 0.3% black pepper and 0.15% garlic powder were added when preparing the marinade solution. For physical evaluation, no flavoring agents were used. Physical and sensory evaluations were then conducted. The results showed that the treatment, which combined 100 IU/ml nisin and tumbling for 10 min, did not impair the physical or sensory qualities of the treated samples. In conclusion, marinated chicken...
drumsticks treated with nisin and tumbled were acceptable based on the physical and sensory qualities.

Keywords: nisin; tumbling; physical characteristics; sensory characteristics; marination

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. Hoogenkamp (1987) described marinating as a diffusion or penetration of a solution into the meat structure. Similarly, Cannon et al. (1993) explained marinating as a process involving incorporation of acidic or alkaline solution into products to alter the pH of the tissue. Marination of poultry is practiced to improve product's physical and sensory attributes, such as tenderness, water holding capacity, flavor and etc. (Hashim, et al. 1999a,b; Lemos et al. 1999; Xiong and Kupski, 1999; Young and Buhr, 2000; Young and Lyon, 1997a,b; Zheng et al. 2000).

Nisin, which is considered a "biopreservative", is a natural, nontoxic, and heat stable polypeptide produced by Lactococcus lactis, and has been shown to inhibit gram-positive microorganisms (Ray, 2001) and gram-negative bacteria when combined with a chelating agent such as EDTA (Stevens et al. 1992; Stevens et al. 1991). Even though several studies involving nisin in poultry products have been reported (Budu-Amoako et al., 1999; Kalchayanan et al., 1992; Mahadeo and Tatini, 1994; Modi et al., 2000; Ueckert et al., 1998), most of these studies focused on the effect of nisin toward the
microbial quality of the treated samples. Limited information concerning the physical and sensory qualities influenced by addition of nisin is available. Rozbeh et al. (1993) reported that fresh beef samples treated with Nisaplin (500 IU/g) and CO₂ (10% for 60 min) had a similar color score when compared with the samples previously treated only with CO₂ during vacuum-package storage at 3°C for 8 weeks. Usborne et al. (1986) reported that there was no significant (P<0.05) difference of color and preference ratings between bacon cured either with nitrite only or cured with nitrite and nisin.

Several studies involving adding nisin on the sensory quality of treated fish products have been reported. Nilsson et al. (1997) found that a treatment which consisted of 1000 IU/g nisin and packed in 60% CO₂ and 40% N₂ did not negatively influence the sensory characteristics of cold-smoked salmon samples. Nykanen et al. (2000) reported that injection of nisin (4000-6000 IU/ml) did not affect the sensory quality of cold-smoked rainbow trout. Nykanen et al. (1999) reported that a treatment which combined lactic acid with nisin whey permeates significant (p<0.001) enhanced the characteristic fishy flavor of the cold-smoked rainbow trout fillets, when compared with the control samples after storage at 3°C for 8 days. Also, sensory qualities of the treated samples remained unchanged after 15 days of storage (Nykanen et al., 1999). Nykanen et al. (1998) reported that treatment with nisin whey permeate containing 3% nisin did not change the sensory characteristics of the minced rainbow trout.

Tumbling has been widely applied in the poultry industry for years to improve the physical and sensory attributes of the products, such as increasing cure pickup, moisture retention, water hold capacity, tenderizing effect, cooking yield, and decreasing curing time, and etc. (Babji et al., 1982; Froning et al., 1985; Heath and Owens, 1987; Kotula
and Heath, 1986a,b; Maki and Froning, 1987; Nurmahmudi and Sams, 1997). Pieces of meat are placed inside a rotating drum-like tumbler. As 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. Ab-asion against other meat pieces leads to extraction of the myofibrillar proteins to the surface, which are necessary for binding meat pieces together upon heating.

Limited information on the contribution of marination, nisin, and tumbling on the physical and sensory characteristics of treated poultry products is available. Before conducting a study to evaluate the antimicrobial effect of the treatments combined with nisin and tumbling, it is necessary to evaluate whether this treatment could affect the physical and sensory qualities of the treated samples. The objective of this study was to investigate the effects of added nisin and/or tumbling on some physical and sensory characteristics of the marinated chicken drumsticks. In addition, the sensory profiles of marinated chicken drumsticks using the descriptive analysis techniques were characterized in this study.

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. A solution that contains of 1% acetic acid, 3% salt, and 20mM disodium EDTA (Fisher Scientific Co., Kansas City, MO) was prepared as the standardized marinade in this study. A 100 IU/ml of nisin, which was prepared
from a commercial nisin powder (Sigma Chemical Co., St. Louis, MO), was added to the standardized marinade for the nisin-acd treatment. The pH of the marinade solutions was then adjusted to 4 (using HCl or NaOH solutions). For the sensory evaluation, some flavoring agents including 0.3% black pepper and 0.15% garlic powder were added into the standardized marinade solutions for the marinated treatments 1 through 4 (with or without tumbling based on the assigned groups) when preparing the marinade solution, whereas no flavoring agents were added to the treatments 5 and 6 samples. No flavoring agents were added to the marinade solution for all 6 treatments for the physical evaluation.

Sample preparation

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 the experiment was conducted. Drumsticks were randomly chosen and equally assigned and labeled to the treatment groups. For those samples without tumbling treatment (treatment 2 and treatment 4), drumsticks were placed and marinated in plastic containers (Sterilite Corporation, Townsend, MA) with marinade solution so that all the drumsticks could be covered completely and stored at 4°C for 18 hr. For those samples with tumbling treatment (treatment 1 and treatment 3), drumsticks with the marinade solutions in plastic bags were tumbled for 10 min in a pre-cleaned rotating stainless steel drum (81 cm long and 58 cm diameter; three 7.6 cm baffles, manufactured at OSU, Columbus, OH, USA) at 12-rpm rotational speed in a refrigerated (4°C) room. After tumbling for the 10 min,
Drumsticks with the marinade solution were then marinated at 4°C for total 18 hr (including the previous tumbling time) storage. Instead of the marinate solution, the treatment 5 control samples were stored with distilled water. There was no water or marinade solution added to treatment 6 samples.

Physical evaluations

Drumsticks were weighted individually before conducting the experiment. Figure 8.1 illustrates the experimental procedure.

The pH values measurement of marinade solutions and drumsticks

The pH meter was calibrated with a two-point calibration method using pH 4 and 7 buffers before use. The pH values of the marinade solution were measured before and after marinating of the drumsticks using a Corning pH meter (Model 430, Corning Inc., Corning, NY). Muscles of the whole drumsticks without bone and skin were blended for 30 sec using a Waring Lab Blender (Model 31BL91, Dynamics Corporation of America, New Hartford, Connecticut). Ten grams of muscle sample was then blended with 100 ml of distilled water in a polyethylene bag for 1 min using a Seward Stomacher (Model 400, Tekmar Company, Cincinnati, Ohio), and then the pH of the mixture was measured.

Marinade absorption measurement

After finishing marinating, the drumsticks were removed from the marinade solutions and drained for 5 min at 4°C before determining the marinade absorption. Marinade absorption (grams marinade absorbed/100g of drumstick) was calculated based
on the unmarinated weight (Wt before marinating) of the sample and its weight after marination with 5-min draining (Wt after marinating and draining) and was calculated as follows.

\[
\text{Marinade Absorption} = \left( \frac{\text{Wt after marinating and draining} - \text{Wt before marinating}}{\text{Wt before marinating}} \right) \times 100
\]

Instrumental color measurement

The Hunter “L*” (lightness), “a*” (redness-greenness), and “b*” (yellowness-blueness) values of drumstick were measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey). Color measurements were obtained on the surfaces of the skin, and also on the muscle without skin covering, respectively, before and after marinating, and also after cooking. Three measurements were made at different locations on the drumsticks and averaged. A standard plate (number 18433010) with “Y” = 92.60, “x” = 0.3140, and “y” = 0.3206 was used as a reference.

Marinade solution salt content measurement

The salt contents of the marinade solutions after marinating were measured with a Dicromat® salt analyzer (Model DSA-1000, Diamond Crystal Salt Company, St. Clair, Michigan). The marinade solutions were mixed, and then the salt contents were measured. The salt analyzer was rinsed with distilled water between measurements.

Cooking loss measurement

Pre-weighted drumsticks were cooked in a 177°C (350°F) preheated conventional oven (Type EF111, The G.S. Blodgett Co. Inc., Burlington, VT) to an internal temperature
of 70°C (158°F). After reaching the internal temperature of 70°C monitored by Fisher brand traceable alarm thermometer/timer (Fisher Scientific, Pittsburgh, PA), the samples were equilibrated to room temperature and then the cooking loss measurement and sensory evaluation were accomplished. Cooking loss was calculated based on the weight of the sample before cooking (Wt$_{\text{before cooking}}$) and its weight after cooking (Wt$_{\text{after cooking}}$) and was calculated as follows.

\[
\text{Cooking Loss} = \left( \frac{Wt_{\text{after cooking}} - Wt_{\text{before cooking}}}{Wt_{\text{before cooking}}} \right) \times 100
\]

Yield measurement

Yield was calculated based on the weight of the sample before marinating (Wt$_{\text{before marinating}}$) and its weight after cooking (Wt$_{\text{after cooking}}$) and was calculated as follows.

\[
\text{Yield} = \left( Wt_{\text{after cooking}} - Wt_{\text{before marinating}} \right) \times 100
\]

Sensory evaluation

The panel of seven members consisted of faculty and graduate students majoring in meat science. They evaluated the sensory characteristics of the marinated products made with the standardized marinade solution with flavoring agents (0.3% black pepper and 0.15% garlic powder) with or without tumbling treatment and/or nisin added (Figure 8.1). Descriptive analysis was conducted to evaluate the intensities of sensory characteristics of the raw and cooked samples.
Before conducting the experiment, the panelists were first trained in the general sensory descriptive analysis and were led through the development of a lexicon of descriptors for marinated chicken samples. Most of the references used during training were selected in accordance with Hashim et al. (1999a,b) recommendations. Next, the panelists were requested to list the most descriptive terms for the product. Panelists with the help of the panel leader identified sensory properties important in marinated chicken. The panel agreed on all attributes considered necessary for evaluation of the marinated chicken and defined these attributes.

Attributes of skin color, muscle color, firmness and separation of skin and muscle, marinated chicken aroma (combined raw marinated chicken aroma consisted of chicken, sour - acid, peppery, and garlic aromas), and off-aroma (any aroma which was not expected from the raw marinated chicken aroma) were used to evaluate the raw samples. After cooking followed the procedures described in the cooking loss measurement, the samples were cooled to room temperature (approximately 25°C) and served to the sensory panel. Attributes for skin color, muscle color, firmness and separation of skin and muscle, marinated chicken flavor (combined cooked marinated chicken flavor consisted of chicken, sour - acid, saltiness, pepper, and garlic), off-flavor (any flavor which was not expected from the cooked marinated chicken flavor), juiciness, and tenderness were used to evaluate the cooked samples. Appropriate reference samples were selected and used during training to exhibit the attributes and to set the anchors. Reference samples were available at each test trial for panelists to assist in making evaluations. The Hunter color values of the skin and muscle for the raw and cooked reference samples were also measured.
The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1 = light color; 9 = dark color), firmness and separation of skin and muscle (1 = soft and more separation; 9 = firm and less separation), juiciness (1 = not juicy; 9 = very juicy), and tenderness (1 = not tender; 9 = very tender). The samples were randomly presented in plastic plates coded with a randomly selected three-digit code. Water and unsalted crackers were provided to panelists. Each panelist evaluated the samples independently.

Statistical analyses

Three trials were conducted in this study. Data were analyzed using the general linear model (GLM) of Statistical Analysis System's Procedures (SAS Institute Inc., Cary, NC) with a 5% level of significance. Means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

Physical evaluation of marinated chicken drumsticks with or without tumbling treatment and nisin added is shown in Table 8.1. In the current study, treatment 1 had no nisin added and no tumbling treatment; treatment 2 had no nisin added but with tumbling treatment; treatment 3 had nisin added but without tumbling treatment; treatment 4 had both nisin added and tumbling treatment; treatment 5 had distilled water (instead of the marinade solution) added; treatment 6 had no water and no marinade solution added.
Before marinating, raw drumstick muscle pH of treatments 1 through 6 were from 6.85 to 7.02 (Table 8.1). After marinating for 18 hours at 4°C, the pH values of raw drumsticks dropped approximately 1.5-pH units to pH 5.41 through 5.63 for marinated treatments 1 through 4 without significant (p>0.05) difference among these 1 through 4 marinated treatments. The pH values of the controls with and without distilled water added (treatments 5 and 6) slightly increased to 7.07 and 7.00, respectively, which were significantly (p<0.05) higher than the samples of marinated treatments 1 through 4. Moreover, as shown in Table 8.1, in the current study, it was found that there was no significant (P>0.05) difference for the muscle pH for the raw marinated samples with or without tumbling treatments (e.g. treatment 1 vs. treatment 2; treatment 3 vs. treatment 4). This result agreed with the finding of Babji et al. (1982), where tumbling for 30 min at 20 rpm did not significantly (p>0.05) affect the pH values of raw turkey breast muscles.

After cooking, cooked drumstick muscle pH values of the both control samples with or without addition of distilled water (treatments 5 and 6) slightly increased to 7.09 and 7.08, and were significantly higher (p<0.05) than the pH values of the samples for marinated treatments 1 through 4 (Table 8.1). The treatments 2 and 4 (with tumbling treatment) had significant (p<0.05) lower cooked drumstick muscle pH values (5.45 and 5.44, respectively), when compared with the treatments 1 and 3 (without tumbling treatment) that had higher pH values of 5.72 and 5.69. However, this result in the current study was contrary to the result from Babji et al. (1982). In their study, cooked tumbled (30 min) turkey breast had significant (P<0.05) higher pH of 6.44, when compared with the cooked but non-tumbled samples that had pH of 6.34.

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After marinating, treatment 2 and treatment 4 (with tumbling treatment) had significant (p<0.05) higher marinade solution pH values of 4.40 and 4.41, respectively, when compared with the treatment 1 and treatment 3 (without tumbling treatment) that had lower marinade solution pH values of 4.33 and 4.36. These pH changes might be due to the fact that more proteins (e.g. myofibrillar proteins which are soluble in salt solutions with an isoelectric point of approximately 5.5) were extracted and released into the marinade solution during the tumbling process, thus leading to a more drastic upper movement of solution pH from 4 (before marinating) to a higher pH after tumbling. The control sample with addition of distilled water (treatment 5) had significantly (p<0.05) higher marinade solution pH of 7.38 as expected.

Treatment 2 and treatment 4, which contained a tumbling treatment, had significant (p<0.05) higher marinade absorptions of 10.68 and 13.02%, respectively, when compared with the treatment 1 and treatment 3, which contained no tumbling treatment that had lower marinade absorptions of 2.08 and 1.83% (Table 8.1). The control samples with addition of distilled water (treatment 5) had significant (p<0.05) higher absorption of 8.25% when compared with the samples without tumbling treatment.

Tumbling has been reported to decrease cooking loss of treated samples in some studies. For example, Maki and Froning (1987) reported that tumbling for one hour had significantly (P<0.05) lower cooking loss to 21.24% of turkey breast muscles when compared with the samples without tumbling that had 24.97%. Similarly, Babji et al. (1982) reported that tumbled for 30 min significantly (P<0.05) decreased cooking loss of the turkey breast muscle to 21% when comparing with the samples without tumbling that had cooking losses of 24%. However, in the current study, as shown in Table 8.1, there
was no significant (P>0.05) difference for cooking losses between the tumbled and non-tumbled treatment samples (1 vs. 2 and 3 vs. 4). This result is probably because that in the current study, tumbling was mainly functionally applied to increase the distribution of ingredients with marinade solution. Only comparatively short time duration of tumbling (10 min in the current study) was applied in the current study, when comparing with the longer tumbling time applying in other studies.

There was no significant (P>0.05) difference for yield for the marinated treatments 1 through 4 samples (Table 8.1). The salt content of marinade solution after marinating of the marinated treatments 1 through 4 samples was 1.20% without significant (p>0.05) difference among these 4 marinated treatments, but the salt content of the control samples with distilled water added (treatment 5) was 0.09%, which was significant (p<0.05) lower than the marinated treatments of 1 through 4 as expected (Table 8.1).

Table 8.2 illustrates the color evaluation of marinade chicken drumsticks with or without tumbling treatment and/or addition of nisin. The skin L* values of the all treatments (1 through 6) before marinating were among 69.16-75.77. After marinating, there was no significant (p>0.05) difference for the skin L* value was obtained among any of the 1 through 6 treatments. Also, after cooking, all the skin L* values of the samples decreased to 60.29-63.73, and no significant (p>0.05) difference was obtained among any of the treatments 1 through 6. The skin a* values of the all treatments (1 through 6) before marinating ranged from 4.94 to 6.81 without any significant (p>0.05) differences among 6 treatments (Table 8.2). After marinating, there was no significant (p>0.05) difference for skin a* values for the marinated treatments 1 through 4.
Similarly, after cooking, the marinated treatments 1 through 4 had skin \( a^* \) values of 1.14-1.40 without any significant (\( p > 0.05 \)) difference among these 4 marinated treatments, but the skin \( a^* \) values of the control with or without addition of distilled water (treatments 5 and 6) had significant (\( p < 0.05 \)) higher skin \( a^* \) values. Before marinating the skin \( b^* \) values of the all treatments (1 through 6) ranged from 8.30 to 11.14 without any significant (\( p > 0.05 \)) difference among 6 treatments. After marinating, the skin \( b^* \) ranged from 7.83 to 11.18 without any significant difference (\( p > 0.05 \)) among 6 treatments. After cooking, the skin \( b^* \) increased to 21.36-26.34 without any significant difference (\( p > 0.05 \)) among 6 treatments.

Before marinating the muscle \( L^* \) values of the all treatments (1 through 6) ranged from 56.88 to 61.85 without any significant (\( p > 0.05 \)) difference (Table 8.3). After marinating, the muscle \( L^* \) values increased to 63.17-78.18. Also there was no significant (\( p > 0.05 \)) difference for the muscle \( L^* \) value for marinated treatments for the 1 through 4 marinated treatments, but the both control samples had significant (\( p < 0.05 \)) lower muscle \( L^* \) value after marinating. After cooking, there was no significance (\( p > 0.05 \)) of the muscle \( L^* \) values among the 1 through 4 marinated treatment samples, but both the control samples had lower muscle \( L^* \) values. Before marinating the muscle \( a^* \) values of the treatments 1 through 6 ranged from 11.08 to 14.50. After marinating, the muscle \( a^* \) values of the marinated treatments 1 through 4 decreased to 2.18-4.49. In addition, the controls with or without addition of distilled water had significant (\( p < 0.05 \)) higher muscle \( a^* \) values after marinating probably because no acid was added to these two treatments. After cooking, there was no significant (\( p > 0.05 \)) difference for the muscle \( a^* \) values for the marinated treatments 1 through 4, but the controls with or without addition of distilled
water had significant (p<0.05) higher muscle a* values of 9.02 and 9.75 respectively. Before marinating the muscle b* values of the all treatments (1 through 6) ranged from 12.00 to 15.77 without significant (p>0.05) difference. After marinating, the muscle b* of the marinated treatments 1 through 4 increased to 13.60-14.95 without significant (p>0.05) among these 4 marinated treatments. After cooking, the muscle b* values of the marinated treatments 1 through 4 increased to 17.74-20.55 without significant (p>0.05) difference.

The results of the total color difference are shown in Table 8.4. Total color difference (ΔE*ab) is calculated by the following formula: Total color difference (ΔE*ab) = square root of [(ΔL*)² + (Δa*)² + (Δb*)²], where L* = light and dark, a* = red and green, b* = yellow and blue, and Δ representing the difference between the two processing steps, and it is commonly applied to evaluate the color changes of samples at the two different processing steps in food science studies. There was no significant (p>0.05) difference for the total color difference for skin between before and after marination for the treatments 1 through 6 samples. Similarly, there is no significant (p>0.05) difference for total color difference in skin between before and after cooking, and between before marination and after cooking for the all six treatment samples.

As shown in Table 8.4, in the current study, there was no significant (p>0.05) difference for the total color difference in muscle between before and after marination for the marinated treatments 1 through 4 samples, but the control samples with or without distilled water added had significant (p<0.05) lower total color difference in muscle. There was no significant (p>0.05) total color difference in muscle between before and after cooking for the treatment 1 through 4, but the control group with or without addition
of distilled water had higher total color difference between before and after cooking in muscle. Similarly, no significant (p>0.05) total color difference in muscle between before marination and after cooking for the marinated treatments 1 through 4 samples was obtained.

Sensory evaluation of marinated chicken drumsticks with or without addition of nisin and/or tumbling treatment is shown in Table 8.5. In the current study, 0.3% black pepper and 0.15% garlic powder were added for the marinated treatments 1 through 4 for the sensory evaluation, whereas no flavoring agents were added to the treatments 5 and 6 samples. Based on a 1-9 scale, in which 1 and 9 representing the lighter and darker color respectively, the marinated treatments 1 through 4 samples in the current study had lower skin sensory color scores between 2.0 and 2.1 without any significant difference (P>0.05). The controls with and without distilled water added (treatments 5 and 6) had higher sensory raw skin color scores of 3.9 and 4.7, respectively, which were significantly (p<0.05) higher than the samples of marinated treatment 1 through 4. Similarly, the marinated treatments 1 through 4 samples had significant (p<0.05) lower sensory muscle color scores of 2.1-2.2, but the controls (with and without water added) that had color scores of 4.7 and 6.0. The lightness of the skin and muscle colors for marinated treatments 1 through 4 when comparing to the samples of the control groups was probably due to the addition of acid.

In the current study, the addition of nisin did not significantly (P>0.05) affect the instrumental and sensory skin and muscle colors for both the raw and cooked samples (e.g. treatments 1 vs. 3, and treatment 2 vs. 4). These results obtained in the current study agreed with the previous studies by other researchers. Rozbeh et al. (1993) evaluated the
influence of Nisaplin, which is a commercial preparation of nisin, on the color of refrigerated vacuum-packaged beef. In their study, fresh beef samples were treated with 10% CO$_2$ and 90% N$_2$ for 60 min, 500 IU/g Nisaplin, vacuum-packaged, and then refrigerated at 3°C. Using a 10-point scale (1 = brown; 10 = bright cherry red), and the authors reported that samples previous treated with Nisaplin and CO$_2$ had a close color score of 3 when compared with the samples that previously had only been treated with 10% CO$_2$ which had a color score of 2 during storage at 3°C for 8 weeks.

In the current study, there was no significant (p>0.05) difference for the sensory firmness and separation of skin and muscle scores of the raw samples for treatments 1 through 6. Also, in the current study, a marinated chicken aroma was defined as a combined aroma, which consisted of fresh raw chicken, acidic, black peppery, and garlic aromas. The marinated treatments 1 through 4 samples had significant (p<0.05) higher marinated chicken aroma scores of 3.6 to 4.6, when compared with the controls that had 1.4 and 1.7 probably due to lack of flavoring agents added. Low (1.0-1.3 based on a 1-9 scale) and without significant (p>0.05) difference for the sensory off-aroma scores of the raw samples for all treatments 1 through 6 were obtained in this study (Table 8.5).

After cooking, the sensory skin and muscle color scores of the marinated treatments 1 through 4 samples increased. There was no significant (p>0.05) difference for the sensory skin color scores of all the treatments 1 through 6. The control samples (treatments 5 and 6) had significantly (p<0.05) higher sensory cooked muscle scores. There was no significant (p>0.05) difference for the sensory firmness and separation of skin and muscle scores of the cooked samples for treatments 1 through 6.
Similar to the marinated chicken aroma for the raw samples, in the current study, a marinated chicken flavor was defined as a combined flavor, which consisted of cooked chicken, acidic, black peppery, and garlic flavors. The marinated treatments 1 through 4 samples had significant ($p<0.05$) higher marinated chicken flavor scores of 4.7-5.4, when compared with the controls had 1.4 and 1.7 probably due to lack of flavoring agents added. Low (1.1-1.4 based on a 1-9 scale) and without significant ($p>0.05$) difference for the sensory off-aroma scores of the raw samples for all treatments 1 through 6 were obtained in this study (Table 8.5).

Usborne et al. (1986) evaluated the influence of nisin on the sensory quality of the bacon treated with nisin. In their study, pork bellies were cured with either nitrite only, or with 2000 or 4000 IU/ml nisin, which partially substituted for nitrite. The results of a triangle test showed that there was no significant ($P<0.05$) difference between the samples either cured with nitrite only or cured with nitrite and nisin. In addition, in the same study, they reported that there was no significant ($P<0.05$) difference for color and preference ratings between the samples cured either with nitrite only or cured with nitrite and nisin. Usborne et al. (1986) concluded that bacons made from the curing brine containing nisin were acceptable with respect to color and palatability of the final product.

Nykanen et al. (2000) evaluated the effect of nisin on the sensory quality of cold-smoked rainbow trout, which were injected with 4000-6000 IU/ml nisin, smoked, and then stored under refrigeration. After evaluating intensities of several sensory attributes, including smoked odor, fishy odor, fishy flavor, sweet taste, salty taste, and elasticity of the samples, the authors reported that the treatment with nisin added did not affect the
sensory characteristics of the treated samples. In addition, in the same study, by comparing samples stored for 2 days to those stored for 16 and 23 days using a triangle test, Nykanen et al. (2000) reported that the sensory quality of the treated samples remained unchanged for 23 days of storage at 3°C, whereas the control samples without nisin added remained unchanged for 16 days. Nykanen et al. (2000) concluded that the treatment of injecting 4000-6000 IU/ml nisin did not alter the sensory characteristics of the treated smoked rainbow trout samples.

Nykanen et al. (1999) evaluated the effect of nisin whey permeate on the sensory quality of cold-smoked rainbow trout. In their study, fish fillets were injected with a mixture of lactic acid, and whey permeate containing 4000-6000 IU/ml, and a salt solution, followed by smoking at 28°C for 6 h, sliced, vacuum packaged and stored at 3°C. Influence of the treatment on sensory characteristics was analyzed after 8 and 22 days by sensory profiling. Nykanen et al. (1999) reported that the treatment which combined lactic acid and nisin whey permeate significant (p<0.001) enhanced the characteristic fishy flavor of the product when compared with the control samples after storage at 3°C for 8 days. Sensory qualities of the treated samples remained unchanged after 15 days of storage based on triangle test results (Nykanen et al., 1999).

Nykanen et al. (1998) evaluated the effect of whey permeate containing 3% nisin on the sensory quality of minced rainbow trout. The authors reported that the treatment with nisin whey permeate did not change the sensory characteristics of the treated samples, when evaluating intensities of several sensory attributes, including fishy odor, metallic flavor, fishy flavor, astringency, sourness, sweetness, saltiness and meaty flavor.
In the current study, there was no significant (p>0.05) difference for sensory juiciness scores for all 1 through 6 treatments (Table 8.5). In addition, there was no significant (p>0.05) difference for sensory tenderness scores for all 1 through 4 treatments (Table 8.5). The result from the current study is similar to that reported by Maki and Froning (1987). They reported that tumbling the whole turkey carcasses for one hour (2 hr at 20 rpm with 10 min rest intervals for every 10 min of tumbling) did not significantly (P>0.05) affect sensory properties including juiciness, tenderness, and flavor of the breast muscle samples.

CONCLUSION

In conclusion, the treatment consisted of nisin added (100 IU/ml) and tumbling treatment (10 min), which was previously showed to inactive some microorganisms of some foods, did not impair the physical and sensory characteristics tested in the current study for the treated samples.

REFERENCES


Standard marinade solution: acetic acid (1%), salt (3%), and disodium EDTA (20 mM) in distilled water.

Pre-assigned antimicrobial components: with or without addition of nisin (100 IU/ml) based on the assigned groups.

Flavoring agents: ground black pepper (0.3%) and garlic powder (0.15%) for the sensory evaluation of marinated treatments 1 through 4; no flavor agents were added for the physical evaluation.

Total time of 18 hrs includes tumbling and marinating.

Figure 8.1 Experimental procedure
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
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<tbody>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control (water added)^1</td>
<td>Control (no water added)^2</td>
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<tr>
<td>Tumbling (10 min)</td>
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<td></td>
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</tr>
<tr>
<td>Raw drumstick pH^3 (before marinating)</td>
<td>7.02^a</td>
<td>6.85^b</td>
<td>6.96^ab</td>
<td>7.00^a</td>
<td>6.95^ab</td>
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<tr>
<td>Raw drumstick pH^3 (after marinating)</td>
<td>5.61^a</td>
<td>5.41^a</td>
<td>5.63^a</td>
<td>5.44^a</td>
<td>7.07^b</td>
<td>7.00^b</td>
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<td>Cooked drumstick pH^3</td>
<td>5.72^a</td>
<td>5.45^b</td>
<td>5.69^a</td>
<td>5.44^b</td>
<td>7.09^c</td>
<td>7.08^c</td>
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<td>Marinade solution pH (after marinating)</td>
<td>4.33^a</td>
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<td>7.38^c</td>
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<td>Marinade absorption (%)</td>
<td>2.08^a</td>
<td>10.68^b</td>
<td>1.83^a</td>
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<td>8.25^b</td>
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<td>Cooking loss (%)</td>
<td>17.98^ab</td>
<td>21.52^a</td>
<td>19.30^a</td>
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<td>14.66^bc</td>
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<td>Yield (%)</td>
<td>83.73^a</td>
<td>86.86^ab</td>
<td>82.17^a</td>
<td>88.59^ab</td>
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<td>87.58^ab</td>
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<td>Salt content of marinade solution after marinating (%)</td>
<td>1.20^a</td>
<td>1.20^a</td>
<td>1.20^a</td>
<td>1.20^a</td>
<td>0.09^b</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

^a,b,c^ Means within a row without the same superscript are significantly different (P<0.05).

ND: not determined.

^1^Control (water added): Instead of the marinade solution, samples were dipped in the unheated distilled water.

^2^Control (no water added): Samples were placed in bags without adding the marination solution or distilled water.

^3^Drumstick pH was measured for muscle without skin.

Table 8.1 Physical evaluations of marinated chicken drumsticks with or without tumbling treatment and/or nisin added
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
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<td>Control</td>
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<td>Nisin addition</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>(water added)</td>
<td>(no water added)</td>
</tr>
<tr>
<td>Tumbling (10 min)</td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| L* value                      | Before marinating | 72.07<sup>a,b</sup> | 70.55<sup>a,b</sup> | 71.28<sup>a,b</sup> | 69.16<sup>a</sup> | 73.55<sup>a,b</sup> | 75.77<sup>b</sup> |
|                               | After marinating  | 78.41 | 82.11 | 79.16 | 80.49 | 79.84  | 75.73  |
|                               | After cooking     | 63.73 | 63.27 | 62.46 | 63.42 | 61.95  | 60.29  |

| a* value                      | Before marinating | 4.94 | 5.41 | 6.36 | 6.81 | 6.54 | 5.13 |
|                               | After marinating  | 1.17<sup>a</sup> | 0.36<sup>a</sup> | 1.21<sup>a</sup> | 0.53<sup>a</sup> | 3.27<sup>ab</sup> | 4.31<sup>b</sup> |
|                               | After cooking     | 1.14<sup>a</sup> | 1.40<sup>a</sup> | 1.82<sup>ab</sup> | 1.20<sup>a</sup> | 2.99<sup>b</sup> | 2.63<sup>ab</sup> |

| b* value                      | Before marinating | 11.14 | 11.10 | 9.96 | 8.30 | 9.02 | 10.03 |
|                               | After marinating  | 9.21 | 11.18 | 10.80 | 9.04 | 7.83 | 10.42 |
|                               | After cooking     | 22.17 | 23.15 | 23.04 | 21.36 | 25.87 | 26.34 |

Means within a row without the same superscript are significantly different (P<0.05).

1. Color evaluation: L* = light and dark, a* = red and green, and b* = yellow and blue, measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

2. Marination: Chicken drumsticks were marinated with a solution contained with 1% acetic acid, 3% salt, and adjusted to pH 4.

3. Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

4. Control (no water added): Samples were placed in containers without adding the marination solution or distilled water.

Table 8.2 Skin color evaluation<sup>1</sup> of marinated<sup>2</sup> chicken drumsticks with or without tumbling treatment and/or nisin added
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nisin addition (100 IU/ml)</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Control (water added)³</td>
<td>Control (no water added)⁴</td>
</tr>
<tr>
<td></td>
<td>Tumbling (10 min)</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L* value</td>
<td>Before marinating</td>
<td>56.88</td>
<td>58.43</td>
<td>61.85</td>
<td>57.43</td>
<td>59.40</td>
<td>62.10</td>
</tr>
<tr>
<td></td>
<td>After marinating</td>
<td>77.88a</td>
<td>77.41a</td>
<td>78.18b</td>
<td>75.71a</td>
<td>67.42⁵</td>
<td>63.17b</td>
</tr>
<tr>
<td></td>
<td>After cooking</td>
<td>76.74a</td>
<td>80.43a</td>
<td>73.50⁷</td>
<td>73.91⁷</td>
<td>68.39b</td>
<td>69.11b</td>
</tr>
<tr>
<td>a* value</td>
<td>Before marinating</td>
<td>12.03a</td>
<td>12.11a</td>
<td>11.78a</td>
<td>14.50b</td>
<td>12.50⁷</td>
<td>11.08⁸</td>
</tr>
<tr>
<td></td>
<td>After marinating</td>
<td>2.49a</td>
<td>2.99ab</td>
<td>2.18a</td>
<td>4.49b</td>
<td>10.83c</td>
<td>11.38c</td>
</tr>
<tr>
<td></td>
<td>After cooking</td>
<td>2.83a</td>
<td>2.41a</td>
<td>2.75a</td>
<td>3.62a</td>
<td>9.02b</td>
<td>9.75b</td>
</tr>
<tr>
<td>b* value</td>
<td>Before marinating</td>
<td>12.00</td>
<td>13.33</td>
<td>12.60</td>
<td>13.67</td>
<td>13.82</td>
<td>15.77</td>
</tr>
<tr>
<td></td>
<td>After cooking</td>
<td>18.26⁷</td>
<td>20.20⁷</td>
<td>17.74⁷</td>
<td>20.55⁷</td>
<td>24.33⁷</td>
<td>27.04c</td>
</tr>
</tbody>
</table>

Means within a row without the same superscript are significantly different (P<0.05).

¹Color evaluation: L* = light and dark, a* = red and green, and b* = yellow and blue, measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

²Marination: Chicken drumsticks were marinated with a solution contained with 1% acetic acid, 3% salt, and adjusted to pH 4.

³Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

⁴Control (no water added): Samples were placed in containers without adding the marination solution or distilled water.

Table 8.3 Muscle color evaluation¹ of marinated² chicken drumsticks with or without tumbling treatment and/or nisin added
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumbling (10 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td>8.16</td>
<td>12.71</td>
<td>11.24</td>
<td>13.10</td>
<td>8.11</td>
<td>4.86</td>
</tr>
<tr>
<td>Comparison of before and after</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>marination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison of before and after</td>
<td></td>
<td>19.91</td>
<td>22.36</td>
<td>20.84</td>
<td>21.07</td>
<td>25.48</td>
<td>22.28</td>
</tr>
<tr>
<td>cooking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison of before marination</td>
<td></td>
<td>14.39</td>
<td>14.95</td>
<td>16.45</td>
<td>15.45</td>
<td>21.00</td>
<td>22.87</td>
</tr>
<tr>
<td>and after cooking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td>23.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.48&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Comparison of before and after</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>marination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison of before and after</td>
<td></td>
<td>5.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.19&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>5.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.48&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>13.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.36&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>cooking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison of before marination</td>
<td></td>
<td>22.78&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>25.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>21.24&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14.55&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>13.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>and after cooking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means within a row without the same superscript are significantly different (P<0.05).

1 Total color difference (ΔE<sup>ab</sup>) = square root of [(ΔL*)<sup>2</sup> + (Δa*)<sup>2</sup> + (Δb*)<sup>2</sup>], where L* = light and dark, a* = red and green, b* = yellow and blue, and Δ representing the difference between the two processing steps; L*, a*, and b* were measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

2 Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

3 Control (no water added): Samples were placed in bags without adding the marinade solution or distilled water.

Table 8.4 Total color differences<sup>1</sup> of marinated chicken drumsticks with or without tumbling treatment and nisin added
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control (water added)²</td>
<td>Control (no water added)³</td>
</tr>
<tr>
<td>Tumbling (10 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(water added)²</td>
<td>(no water added)³</td>
</tr>
<tr>
<td>Raw samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin color</td>
<td>2.0</td>
<td>2.1</td>
<td>2.0</td>
<td>2.0</td>
<td>3.9</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Muscle color</td>
<td>2.3</td>
<td>2.3</td>
<td>2.4</td>
<td>2.1</td>
<td>4.7</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Firmness and separation of skin and muscle</td>
<td>4.1</td>
<td>3.3</td>
<td>4.7</td>
<td>3.6</td>
<td>3.3</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Marinated chicken aroma°</td>
<td>4.6</td>
<td>4.6</td>
<td>3.6</td>
<td>4.1</td>
<td>1.4</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Off-aroma°</td>
<td>1.3</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.0</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Cooked samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin color</td>
<td>4.9</td>
<td>3.9</td>
<td>4.1</td>
<td>4.0</td>
<td>4.3</td>
<td>4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>Muscle color</td>
<td>4.0</td>
<td>2.7</td>
<td>2.9</td>
<td>3.0</td>
<td>5.0</td>
<td>4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>Firmness and separation of skin and muscle</td>
<td>5.7</td>
<td>4.9</td>
<td>5.6</td>
<td>5.1</td>
<td>4.9</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Marinated chicken flavor°</td>
<td>5.0</td>
<td>5.4</td>
<td>4.7</td>
<td>5.3</td>
<td>1.4</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Off-flavor°</td>
<td>1.3</td>
<td>1.1</td>
<td>1.1</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Juiciness</td>
<td>4.3</td>
<td>3.9</td>
<td>5.1</td>
<td>4.3</td>
<td>4.0</td>
<td>5.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Tenderness</td>
<td>4.9</td>
<td>3.7</td>
<td>5.0</td>
<td>3.8</td>
<td>4.6</td>
<td>5.9</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Means within a row without the same superscript are significantly different (P<0.05).

1 The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1 = light, 9 = dark), firmness and separation of skin and muscle (1 = soft, more separation between skin and muscle, 9 = firm, less separation between skin and muscle), juiciness (1 = not juicy, 9 = very juicy), and tenderness (1 = not tender, 9 = very tender).

2 Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

3 Control (no water added): Samples were placed in containers without adding the marination solution or distilled water.

4 Marinated chicken aroma: the combination aroma consisted of chicken, sour (acidic), salty, peppery and garlic aromas; flavoring agents (0.3% ground black pepper and 0.15% garlic powder) were added to treatments 1 through 4.

5 Off-aroma: any aroma that was not expected from the raw marinated chicken aroma.

6 Marinated chicken flavor: the combination flavor consisted of chicken, sour (acidic), salty, peppery, and garlic flavors; flavoring agents (0.3% ground black pepper and 0.15% garlic powder) were added to treatments 1 through 4.

7 Off-flavor: any flavor that was not expected from the cooked marinated chicken flavor.

Table 8.5 Sensory characteristic intensities of marinated chicken drumsticks with or without tumbling and nisin addition
CHAPTER 9

EXPERIMENT 7

CHANGES IN PHYSICAL AND SENSORY CHARACTERISTICS OF MARINATED CHICKEN DRUMSTICKS TREATED WITH NISIN AND LACTOPEROXIDASE SYSTEM

ABSTRACT

Nisin and the lactoperoxidase system (LPS) have been shown to inactive some microorganisms in foods. However, further studies were needed to evaluate whether these compounds had any influence on the physical and sensory characteristics of the treated samples. In this study, a solution that contains acetic acid (1%), salt (3%), and EDTA (20mM), with pH adjusted to 4 was developed as a standardized marinade. A 100 IU/ml of nisin was added to the marinade before pH adjustment for nisin-added samples. The LPS consisted of 1 μg/ml of lactoperoxidase (LP), 5.9 mM of KSCN, and 2.5 mM of H₂O₂ was added to the marinade for the LPS-added samples. For sensory evaluation, some flavoring agents including 0.3% black pepper and 0.15% garlic powder were added when preparing the marinade solution. For physical evaluation, no flavoring agents were used. Physical and sensory evaluations were then conducted. The results showed that the treatment, which combined 100 IU/ml nisin with LPS (1 μg/ml of LP, 5.9 mM of KSCN, and 2.5 mM of H₂O₂), did not impair the physical or sensory qualities of the treated samples.
Marinated chicken drumsticks treated with nisin and LPS were acceptable based on the physical and sensory qualities.

Keywords: physical characteristics; sensory characteristics; marination; nisin; lactoperoxidase system

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 the pH of the tissue. Marination of poultry is practiced to improve product’s physical and sensory attributes, such as tenderness, water-holding capacity, flavor and etc. (Hashim, et al. 1999a,b; Lemos et al. 1999; Xiong and Kupski, 1999; Young and Buhr, 2000; Young and Lyon, 1997a,b; Zheng et al. 2000).

Nisin, which is considered a “biopreservative”, is a natural, nontoxic, and heat stable polypeptide produced by Lactococcus lactis, and has been shown to inhibit gram-positive microorganisms (Ray, 2001) and gram-negative bacteria when combined with chelating agent such as EDTA (Stevens et al. 1992; Stevens et al. 1991). Even though several studies involving nisin in poultry products have been reported (Budu-Amoako et al., 1999; Kalchayanand et al., 1992; Mahadeo and Tatini, 1994; Modi et al., 2000; Ueckert et al., 1998), most of these studies focused on the effect of nisin toward the microbial quality of the treated samples.
Rozbeh et al. (1993) reported that fresh beef samples previously treated with nisin (500 IU/g) and CO₂ (10% for 60 min) had a similar color score when compared with the samples previously treated only with CO₂ during vacuum-package storage at 3°C for 8 weeks. Usborne et al. (1986) reported that there was no significant (P<0.05) difference of color and preference ratings between bacon cured either with nitrite only or cured with nitrite and nisin.

Several studies involving adding nisin on the sensory quality of treated fish products have been reported. Nilsson et al. (1997) found that a treatment which consisted of 1000 IU/g nisin and packed in 60% CO₂ and 40% N₂ did not negatively influence the sensory characteristics of cold-smoked salmon samples. Nykanen et al. (2000) reported that injection of nisin (4000-6000 IU/ml) did not affect the sensory quality of cold-smoked rainbow trout. Nykanen et al. (1999) reported that a treatment which combined lactic acid with nisin whey permeate significant (p<0.001) enhanced the characteristic fishy flavor of the cold-smoked rainbow trout fillets, when compared with the control samples after storage at 3°C for 8 days. Also, sensory qualities of the treated samples remained unchanged after 15 days of storage (Nykanen et al., 1999). Nykanen et al. (1998) reported that treatment with nisin whey permeate containing 3% nisin did not change the sensory characteristics of the minced rainbow trout.

The lactoperoxidase system (LPS), which consists of lactoperoxidase (LP), thiocyanate (SCN⁻), and hydrogen peroxide (H₂O₂), is an inhibitory system that is present naturally in bovine milk. This system has been shown to be inhibitory against some pathogenic and spoilage microorganisms such as *P. fluorescens*, *L. monocytogenes*, *S. typhimurium* and *E. coli* (Zapico et al., 1995; Kamau et al., 1990a,b; Earnshaw et al.,

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The LPS has been mainly studied for the application in the milk and dairy products (Zapico et al., 1998). Only a few studies have attempted to apply this LPS system in meat and poultry products.

Even though LPS has been reported to have little effect on the sensory and physical characteristics of the treated milk and dairy products (Ridley and Shalo, 1990; Martinez et al., 1988), limited studies involving addition of LPS on the physical and sensory qualities of treated meat and poultry products have been reported. Wolfson (1992) reported that there was no significant (p>0.01) difference of the Hunterlab (L, a, b) color values between the LPS treated (1μg/ml lactoperoxidase, 5.9 mM potassium thiocyanate, and 2.5 mM hydrogen peroxide for 5 min) chicken thigh skin and controls after 24 and 48 hours storage at 4°C. In addition, in the same study, they evaluated the 2-thiobarbituric acid (TBA) values, and found no significant difference (P<0.05) of oxidative deterioration between the LPS treated chicken thigh meat and the controls.

Previous study conducted in this same laboratory showed that the adding of nisin at the level of 100 IU/ml, and LPS added (consisted of 1 μg/ml LP, 5.9 mM KSCN. and 2.5 mM H₂O₂) significantly (P<0.05) decreased the total microflora and psychrotrophs counts of the marinated chicken drumsticks during refrigerated storage at 4°C for up to 7 days (Information has been submitted for publication). Limited information on the contribution of marination, nisin, and LPS to the physical and sensory characteristics of treated poultry products is available. Whether or not adding nisin and LPS would affect the physical and sensory characteristics of the treated samples should be evaluated.

The objective of this study was to investigate the effects of nisin and LPS adding on some physical and sensory characteristics of the marinated chicken drumsticks. In
addition, the sensory profiles of marinated chicken drumsticks using the descriptive analysis techniques were characterized in this study. The results of this study could be used to determine the acceptability of marinated chicken drumsticks that were previously treated with antimicrobial compounds used to reduce potential microbial contamination during processing and to extend the shelf life.

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. A solution that contains of 1% acetic acid, 3% salt, and 20mM disodium EDTA (Fisher Scientific Co., Kansas City, MO) was prepared as the standardized marinade in this study. A 100 IU/ml of nisin, which was prepared from a commercial nisin powder (Sigma Chemical Co., St. Louis, MO), was added to the standardized marinade for the nisin-added treatment. A LPS consisted of 1 µg/ml of lactoperoxidase (LP, EC 1.11.1.7; purity index 0.82 (A412/A280); Sigma Chemical Co., St. Louis, Missouri), 5.9 mM of potassium thiocyanate (KSCN, Fisher Scientific Co.; Pittsburgh, PA), and 2.5 mM of hydrogen peroxide (H2O2, 30%, Fisher Scientific Co.; Pittsburgh, PA) was added to the marinade for the LPS-added treatment. The pH of the marinade solutions was then adjusted to 4 (using HCl or NaOH solutions). For the sensory evaluation, some flavoring agents including 0.3% black pepper and 0.15% garlic powder were added to the standardized marinade solutions for the marinated treatments 1 through 4, whereas no flavoring agents were added to the treatments 5 and 6 samples. No
flavoring agents were added to the marinade solution for all 6 treatments for the physical evaluation.

Sample preparation

Drumsticks in commercial packages were purchased from a local supermarket, placed in an insulated container to maintain samples' temperature, transported to the laboratory within 30 min, and then stored in a 4°C walk-in cooler until the experiment was conducted. Drumsticks were randomly chosen and equally assigned and labeled to the treatment groups. For the marinated samples (treatments 1 through 4), drumsticks were placed and marinated in plastic containers (Sterilite Corporation, Townsend, MA) with marinade solution so that all the drumsticks could be covered completely by the marinade solution and stored at 4°C for 18 hr. Instead of the marinate solution, the treatment 5 control samples were stored with the distilled water. There was no water or marinade solution added to treatment 6 samples.

Physical evaluations

Drumsticks were weighted individually before conducting the experiment. Figure 9.1 illustrates the experimental procedure.

The pH values measurement of marinade solutions and drumsticks

The pH meter was calibrated with a two-point calibration method using pH 4 and 7 buffers before use. The pH values of the marinade solution were measured before and after marinating of the drumsticks using a Corning pH meter (Model 430, Corning Inc.,
Muscles of the whole drumsticks without bone and skin were blended for 30 sec using a Waring Lab Blender (Model 31BL91, Dynamics Corporation of America, New Hartford, Connecticut). Ten grams of muscle sample was then blended with 100ml of distilled water in a polyethylene bag for 1 min using a Seward Stomacher (Model 400, Tekmar Company, Cincinnati, Ohio), and then the pH of the mixture was measured.

**Marinade absorption measurement**

After finishing marinating, the drumsticks were removed from the marinade solutions and drained for 5 min at 4°C before determining the marinade absorption. Marinade absorption (grams marinade absorbed/100g of drumstick) was calculated based on the unmarinated weight (Wt before marinating) of the sample and its weight after marination with 5-min draining (Wt after marinating and draining) and was calculated as follows.

\[
\text{Marinade Absorption} = \left( \frac{\text{Wt after marinating and draining} - \text{Wt before marinating}}{\text{Wt before marinating}} \right) \times 100
\]

**Instrumental color measurement**

The Hunter “L*” (lightness), “a*” (redness-greenness), and “b*” (yellowness-blueness) values of drumstick were measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey). Color measurements were obtained on the surfaces of the skin, and also on the muscle without skin covering, respectively, before and after marinating, and also after cooking. Three measurements were made at different locations on the drumsticks and averaged. A standard plate (number 18433010) with “Y” = 92.60, “x” = 0.3140, and “y” = 0.3206 was used as a reference.
Marinade solution salt content measurement

The salt contents of the marinade solutions after marinating were measured with a Dicromat® salt analyzer (Model DSA-1000, Diamond Crystal Salt Company, St. Clair, Michigan). The marinade solutions were mixed, and then the salt contents were measured. The salt analyzer was rinsed with distilled water between measurements.

Cooking loss measurement

Pre-weighted drumsticks were cooked in a 177°C (350°F) preheated conventional oven (Type EF111, The G.S. Blodgett Co. Inc., Burlington, VT) to an internal temperature of 70°C (158°F). After reaching the internal temperature of 70°C monitored by Fisher brand traceable alarm thermometer/timer (Fisher Scientific, Pittsburgh, PA), the samples were equilibrated to room temperature and then the cooking loss measurement and sensory evaluation were accomplished. Cooking loss was calculated based on the weight of the sample before cooking (Wt before cooking) and its weight after cooking (Wt after cooking) and was calculated as follows.

\[
\text{Cooking Loss} = \left( \frac{\text{Wt before cooking} - \text{Wt after cooking}}{\text{Wt before cooking}} \right) \times 100
\]

Yield measurement

Yield was calculated based on the weight of the sample before marinating (Wt before marinating) and its weight after cooking (Wt after cooking) and was calculated as follows.
Yield = \((W_{after\ cooking} - W_{before\ marinating}) \times 100\)

Sensory evaluation

The panel of seven members consisted of faculty and graduate students majoring in meat science. They evaluated the sensory characteristics of the marinated products made with the standardized marinade solution with flavoring agents (0.3% black pepper and 0.15% garlic powder) with or without the addition of nisin and/or LPS (Figure 9.1). Descriptive analysis was conducted to evaluate the intensities of sensory characteristics of the raw and cooked samples.

Before conducting the experiment, the panelists were first trained in the general sensory descriptive analysis and were led through the development of a lexicon of descriptors for marinated chicken samples. Most of the references used during training were selected in accordance with Hashim et al. (1999a,b) recommendations. Next, the panelists were requested to list the most descriptive terms for the product. Panelists with the help of the panel leader identified sensory properties important in marinated chicken. The panel agreed on all attributes considered necessary for evaluation of the marinated chicken and defined these attributes.

Attributes of skin color, muscle color, marinated chicken aroma (combined raw marinated chicken aroma consisted of chicken, sour - acid, peppery, and garlic aromas) and off-aroma (any aroma which was not expected from the raw marinated chicken aroma) were used to evaluate the raw samples. After cooking followed the procedures described in the cooking loss measurement, the samples were cooled to room temperature (approximately 25°C) and served to the sensory panel. Attributes for skin color, muscle
color, marinated chicken flavor (combined cooked marinated chicken flavor consisted of chicken, sour-acid, saltiness, pepper, and garlic), off-flavor (any flavor which was not expected from the cooked marinated chicken flavor), juiciness, and tenderness were used to evaluate the cooked samples. Appropriate reference samples were selected and used during training to exhibit the attributes and to set the anchors. Reference samples were available at each test trial for panelists to assist in making evaluations. The Hunter color values of the skin and muscle for the raw and cooked reference samples were also measured.

The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1= light color; 9 = dark color), juiciness (1 = not juicy; 9 = very juicy), and tenderness (1 = not tender; 9 = very tender). The samples were randomly presented in plastic plates coded with a randomly selected three-digit code. Water and unsalted crackers were provided to panelists. Each panelist evaluated the samples independently.

Statistical analyses

Three trials were conducted in this study. Data were analyzed using the general linear model (GLM) of Statistical Analysis System’s Procedures (SAS Institute Inc., Cary, NC) with a 5% level of significance. Means were separated using Duncan’s multiple range test.

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RESULTS AND DISCUSSION

Physical evaluation of marinated chicken drumsticks with or without addition of nisin and/or LPS is shown in Table 9.1. In the current study, treatment 1 had no nisin added and no LPS added; treatment 2 had no nisin added but with LPS added; treatment 3 had nisin added but without LPS added; treatment 4 had both nisin added and LPS added; treatment 5 had distilled water (instead of the marinade solution) added; treatment 6 had no water and no marinade solution added.

Before marinating, raw drumstick muscle pH of treatments 1 through 6 were from 6.87 to 6.95 (Table 9.1). After marinating for 18 hours at 4°C, the pH values of raw drumsticks dropped approximately 1.5-pH units to pH 5.23-5.43 for marinated treatments 1 through 4. Also there were no significant (P>0.05) differences of muscle pH detected among these 1 through 4 marinated treatments. The pH values of the controls with and without distilled water added (treatment 5 and treatment 6) remained at 6.82 and 6.92, respectively, which were significantly (P<0.05) higher than the samples of marinated treatments 1 through 4.

After cooking, the pH values of the samples for marinated treatments 1 through 4 increased to 5.51 through 5.74 without significant (P>0.05) differences among the marinated treatments (Table 9.1). Cooked drumstick muscle pH values of both control samples with or without addition of distilled water (treatments 5 and 6) increased to 6.98 and 7.07, and were significantly higher (P<0.05) than the pH of the samples for marinated treatments 1 through 4. After marinating, the marinade solution pH values for marinated treatments 1 through 4 were approximately 4.39, without significant (P>0.05)
differences among treatments (Table 9.1). The solution pH of the control with addition of distilled water (treatment 5) was significant (P<0.05) higher (7.14) than the marinated treatments 1 through 4.

The marinade absorptions of marinated treatments 1 through 4 samples ranged from 0.48 to 1.48% without significant (P>0.05) difference among the marinated treatments. The absorption of the control samples with addition of distilled water (treatment 5) was 7.47%, which was significant (P<0.05) higher than the samples of the marinated treatments 1 through 4 (Table 9.1). The cooking loss of the marinated treatments 1 through 4 marinated samples ranged from 25.29 to 27.21% without a significant (P>0.05) difference among the marinated treatments. The cooking loss of the control with distilled water added (treatment 5) was 23.92%, which was not significant (P>0.05) different than the samples of the marinated treatments 1 through 4 (Table 9.1). However, the control sample without addition of water (treatment 6) had significant (P<0.05) lower cooking loss of 15.90% when compared with the samples of all other marinated and water added treatments of 1 through 5. The yield of the marinated treatments 1 through 4 marinated samples ranged from 73.41 to 75.41% without a significant (P>0.05) difference among the marinated treatments. The salt content of the marinade solution after marinating of the marinated treatments 1 through 4 samples was approximately 1.18% without significant (P>0.05) difference among treatments, but the salt content of the control samples with distilled water (treatment 5) was 0.09%, which was significant (P<0.05) lower than the marinated treatments 1 through 4 as expected (Table 9.1).
Table 9.2 illustrates the color evaluation of the marinade chicken drumsticks with or without addition of nisin and with/without LPS. Before marinating the skin L* values of the all treatments (1 through 6) ranged from 70.13 to 74.26. After marinating, all the skin L* values increased to 80.58-84.39 without significant (P>0.05) differences among the marinated and water added treatments, except for the control without addition of distilled water (treatment 6), which was 74.47, and was significant (P<0.05) smaller. After cooking, all the skin L* values of the samples decreased to 65.74-69.66, and no significant (P>0.05) difference was obtained among any of the treatments 1 through 6.

Before marinating the skin a* values of the all treatments 1 through 6 ranged from 4.83 to 5.76. After marinating, the skin a* values of the marinated treatments 1 through 4 decreased to -1.86-0.80 without any significant difference (P>0.05) among the marinated treatments 1 through 4, but the skin a* values of the control with or without addition of distilled water (treatments 5 and 6) had significant (P<0.05) higher values of 3.73 and 5.97, respectively. Samples with LPS added (treatments 2 and 4) had lower skin a* values, when compared with the samples without LPS added (treatments 1 and 3) probably because of the addition of $H_2O_2$ for the LPS-added treatments 2 and 4. This reduction of a* values of the LPS treated samples was probably due to the addition of hydrogen peroxide, which is one of the components of the LPS treatment, and is a strong oxidizing agent that is occasionally used as a bleaching agent in the food industry (Daeschel and Penner, 1992). After cooking, the skin a* values of the marinated treatments 1 through 4 increased to 0.42-1.40 without any significant difference (P>0.05) among the marinated treatments of 1 through 4, but the skin a* values of the control with or without addition of distilled water significantly (P<0.05) decreased to 3.62 and 4.09,
respectively. Before marinating the skin $b^*$ values of the all treatments (1 through 6) ranged from 7.97 to 9.58 without any significant ($P>0.05$) differences among treatments (Table 9.2). After marinating, the skin $b^*$ of the marinated treatments 1 through 4 increased to 10.78-12.96 without any significant difference ($P>0.05$) among marinated treatments, when compared with the control samples with or without addition of distilled water which had significant ($P<0.05$) lower $b^*$ values of 7.71 and 8.80, respectively. After cooking, the skin $b^*$ increased to 23.24-25.29 without any significant differences ($P>0.05$) among all 6 treatments.

Before marinating the muscle $L^*$ values of the all treatments (1 through 6) ranged from 58.00 to 62.09 (Table 9.3). After marinating, the muscle $L^*$ values of the marinated treatments 1 through 4 and treatment 5 (no water added) increased to 71.34-88.20. whereas the treatment 6 (no water added) remained approximately the same. After cooking, there was no significant ($P>0.05$) difference for the muscle $L^*$ values among the six treatments. Before marinating the muscle $a^*$ values of the all treatments (1 through 6) ranged from 11.65 to 13.95 without any significant ($P>0.05$) differences among six treatments. After marinating, samples with the LPS added (treatments 2 and 4) had significant ($P<0.05$) lower muscle $a^*$ values, when compared with the samples without LPS added (treatments 1 and 3) and also the control samples with and without distilled water added (treatments 5 and 6). After cooking, there was no significant ($P>0.05$) difference for the muscle $a^*$ values of the marinated treatments 1 through 4, but the controls with or without addition of distilled water had significant ($P<0.05$) higher muscle $a^*$ values of 4.92 and 5.83 respectively. Before marinating the muscle $b^*$ values of the all treatments (1 through 6) ranged from 10.21 to 10.33. After marinating, the
muscle $b^*$ values of the marinated treatments 1 through 4 increased to 11.61 to 15.11 without any significant ($P>0.05$) difference among the marinated treatments 1 through 4. After cooking, the muscle $b^*$ values of the marinated treatments 1 through 4 increased to 17.19-19.64 without any significant ($P>0.05$) differences among the marinated treatments 1 through 4.

The results of the total color difference are shown in Table 9.4. Total color difference ($\Delta E_{*ab}$) is calculated by the following formula: Total color difference ($\Delta E_{*ab}$) = square root of $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]$, where $L^*$ = light and dark, $a^*$ = red and green, $b^*$ = yellow and blue, and $\Delta$ representing the difference between the two processing steps, and it is commonly applied to evaluate the color changes of samples at the two different processing steps in food science studies. There was no significant ($P>0.05$) difference for the total color difference for skin between before and after marination for the marinated treatments 1 through 4 samples, but the control samples without distilled water added (treatment 6) had a significantly ($P<0.05$) smaller total color difference for skin color. There was no significant ($P>0.05$) difference for total color difference for skin between before and after cooking, and between before marination and after cooking for the all six treatment in the current study. As shown in Table 9.4, in the current study, there was no significant ($P>0.05$) difference for the total color difference for muscle between before and after marination for the treatments 1-3 samples, but the control samples with or without distilled water added had significant ($P<0.05$) lower total color differences between muscles. Higher muscle total color difference was obtained for the control sample without distilled water added (treatment 6).
There was no significant (P>0.05) total color difference in the muscle color between before marination and after cooking for the six treatment samples.

Sensory evaluation of marinated chicken drumsticks with or without addition of nisin and LPS is shown in Table 9.5. In the current study, 0.3% black pepper and 0.15% garlic powder were added for the marinated treatments 1 through 4 for the sensory evaluation, whereas no flavoring agents were added to treatments 5 and 6 samples. Based on a 1-9 scale, in which 1 and 9 representing the lighter and darker color respectively, the marinated treatments 1 through 4 samples in the current study had lower sensory raw skin color scores between 2.0 and 3.0 without any significant differences (P>0.05) among the marinated treatments. The controls with and without distilled water added had higher sensory raw skin color scores of 5.1 and 6.7, respectively, which were significantly (P<0.05) higher than the samples of marinated treatment 1 through 4. Similarly, the marinated treatments 1 through 4 samples had significant (P<0.05) lower sensory raw muscle color scores of 2.1-3.0, but the controls (with and without water added) that had color scores of 6.6 and 7.0. The lightness of the skin and muscle colors for marinated treatments 1 through 4 when comparing to the samples of the control groups was probably due to the addition of acid.

The results of instrumental and sensory color evaluation of treated samples due to addition of nisin with or without LPS agreed with previous studies by other researchers. Wolfson et al. (1994) investigated the LPS effect on some physical characteristics of LPS treated poultry, and reported that there was no significant (p>0.01) difference for the Hunterlab color values (L, a, b) between the LPS treated (1μg/ml lactoperoxidase, 5.9 mM potassium thiocyanate, and 2.5 mM hydrogen peroxide for 5 min) chicken thigh skin.
and controls after 24 and 48 hours storage. Rozbeh et al. (1993) evaluated the influence of Nisaplin, which was a commercial preparation of nisin on the color of refrigerated vacuum-packaged beef. In this study, fresh beef samples were treated with 10% CO2 and 90% N2 for 60 min, 500 IU/g Nisaplin, vacuum-packaged, and then refrigerated at 3°C. Using a 10-point scale (1 = brown; 10 = bright cherry red), and the authors reported that samples previous treated with Nisaplin and CO2 had a close color score of 3 when compared with the samples that previously had only been treated with 10% CO2 which had a color score of 2 during storage at 3°C for 8 weeks.

In the current study, a marinated chicken aroma was defined as a combined aroma, which consisted of fresh raw chicken, acidic, black peppery, and garlic aromas. The marinated treatments 1 through 4 samples had significant (P<0.05) higher marinated chicken aroma scores of 4.9 to 6.1, when compared with the controls that had 1.4 and 1.0 scores probably due to lack of flavoring agents added. Low (1.1-1.6 based on a 1-9 scale) and without significant (P>0.05) difference for the sensory off-aroma scores for the raw samples for all treatments 1 through 6 were obtained in this study (Table 9.5).

After cooking, the sensory skin and muscle color scores of the marinated treatments 1 through 4 samples increased. Also, there was no significant (P>0.05) difference for the sensory cooked skin and muscle color scores of all treatments 1 through 6. Similar to the marinated chicken aroma for the raw samples, in the current study, a marinated chicken flavor was defined as a combined flavor, which consisted of cooked chicken, acidic, black peppery, and garlic flavors. The marinated treatments 1 through 4 samples had significant (P<0.05) higher marinated chicken flavor scores of 4.0-5.7, when compared with the controls that had scores of 1.9 and 1.3 probably due to lack of
flavoring agents added. Low (1.1-1.6 based on a 1-9 scale) and without significant (P>0.05) difference for the sensory off-flavor scores of the cooked samples for all treatments 1 through 6 were obtained in the current study (Table 9.5). In the current study, no significance (P>0.05) difference for the sensory juiciness and tenderness scores were detected for the marinated treatments 1 through 4 samples (Table 9.5).

Usborne et al. (1986) evaluated the influence of nisin on the sensory quality of the bacon treated with nisin. In their study, bacons were cured with either nitrite only, or with 2000 or 4000 IU/ml nisin, which partially substituted for nitrite. The results of a triangle test showed that there was no significant (P<0.05) difference between the samples either cured with nitrite only or cured with nitrite and nisin. In addition, in the same study, the authors reported that there was no significant (P<0.05) difference for color and preference ratings between the samples either cured with nitrite only or cured with nitrite and nisin. Usborne et al. (1986) concluded that bacons made from the curing brine containing nisin were acceptable with respect to color and palatability of the final product.

Several studies involving nisin added on the sensory quality of treated fish products have been reported. Nilsson et al. (1997) reported that the treatment consisted of 1000 IU/g nisin and packed in 60% CO₂ and 40% N₂ did not negatively influence the sensory characteristics of the cold-smoked salmon samples.

Nykanen et al. (2000) evaluated the effect of nisin on sensory quality of cold-smoked rainbow trout, which was injected with 4000-6000 IU/ml nisin, smoked, and then stored under refrigeration. After evaluating intensities of several sensory attributes, including smoked odor, fishy odor, fishy flavor, sweet taste, salty taste, and elasticity of
the samples, the authors reported that the treatment with nisin added did not affect the sensory characteristics of the treated samples. In addition, in the same study, by comparing samples stored for 2 days to those stored for 16 and 23 days using a triangle test, Nykanen et al. (2000) reported that the sensory quality of the treated samples remained unchanged for 23 days of storage at 3°C, whereas the control samples without nisin added remained unchanged for 16 days. Nykanen et al. (2000) concluded that the treatment of injecting 4000-6000 IU/ml nisin did not alter the sensory characteristics of the treated smoked rainbow trout samples.

Nykanen et al. (1999) evaluated the effect of nisin whey permeate on sensory quality of cold-smoked rainbow trout. In this study, fish fillets were injected with mixture of lactic acid, and whey permeate containing 4000-6000 IU/ml and a salt solution, followed by smoking at 28°C for 6 h, sliced, vacuum packaged and storage at 3°C. Influence of the treatment on sensory characteristics was analyzed after 8 and 22 days by sensory profiling. Nykanen et al. (1999) reported that the treatment which combined lactic acid and nisin whey permeate significant (p<0.001) enhanced the characteristic fishy flavor of the product when compared with the control samples after stored at 3°C for 8 days. Sensory qualities of the treated samples remained unchanged after 15 days storage based on triangle test results (Nykanen et al., 1999).

Nykanen et al. (1998) evaluated the effect of whey permeate containing 3% nisin on the sensory quality of minced rainbow trout. The authors reported that the treatment with nisin whey permeate did not change the sensory characteristics of the treated samples, when evaluating intensities of several sensory attributes, including fishy odor, metallic flavor, fishy flavor, astringency, sourness, sweetness, saltiness and meaty flavor.
CONCLUSION

In conclusion, the treatment consisting of addition of nisin (100 IU/ml) and LPS (1 µg/ml of LP, 5.9 mM of KSCN, and 2.5 mM of H$_2$O$_2$), which was previously showed to inactive some microorganisms of some foods, did not impair the physical and sensory characteristics tested in the current study for the treated samples.

REFERENCES


Standard marinade solution: acetic acid (1%), salt (3%), and disodium EDTA (20 mM) in distilled water.

2 Pre-assigned antimicrobial components: 100 IU/ml of nisin and LPS (1 μg/ml of lactoperoxidase, 5.9 mM of KSCN, and 2.5 mM of H2O2) based on the assigned groups.

3 Flavoring agents: ground black pepper (0.3%) and garlic powder (0.15%) for the sensory evaluation of marinated treatments 1 through 4; no flavor agents were added for the physical evaluation.

Figure 9.1 Experimental procedure
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
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<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Control (water added)²</td>
<td>Control (no water added)³</td>
</tr>
<tr>
<td>LPS addition¹</td>
<td></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw drumstick pH⁴ (before marinating)</td>
<td></td>
<td>6.95</td>
<td>6.94</td>
<td>6.87</td>
<td>6.94</td>
<td>6.89</td>
<td>6.88</td>
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<tr>
<td>Raw drumstick pH⁴ (after marinating)</td>
<td></td>
<td>5.33a</td>
<td>5.28a</td>
<td>5.23a</td>
<td>5.43a</td>
<td>6.82b</td>
<td>6.92b</td>
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<td>Cooked drumstick pH⁴</td>
<td></td>
<td>5.64a</td>
<td>5.51a</td>
<td>5.53a</td>
<td>5.74a</td>
<td>6.98b</td>
<td>7.07b</td>
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<tr>
<td>Marinade solution pH (after marinating)</td>
<td></td>
<td>4.39a</td>
<td>4.39a</td>
<td>4.39a</td>
<td>4.38a</td>
<td>7.14b</td>
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<td>Marinade absorption (%)</td>
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<td>1.05a</td>
<td>0.86a</td>
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<td>0.48a</td>
<td>7.47b</td>
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<td>Cooking loss (%)</td>
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<td>Yield (%)</td>
<td></td>
<td>73.71a</td>
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<td>81.77b</td>
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<td>Salt content of marinade solution</td>
<td></td>
<td>1.18a</td>
<td>1.18a</td>
<td>1.17a</td>
<td>1.18a</td>
<td>0.09b</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Means within a row without the same superscript are significantly different (P<0.05).
ND: not determined.

¹Lactoperoxidase system (LPS) = Lactoperoxidase (LP, 1 μg/ml), KSCN (5.9 mM) and H₂O₂ (2.5 mM).
²Control (water added): Instead of the marinade solution, samples were dipped in the unheated distilled water.
³Control (no water added): Samples were placed in bags without adding the marination solution or distilled water.
⁴Drumstick pH was measured for muscle without skin.

Table 9.1 Physical evaluations of marinated chicken drumsticks with or without addition of nisin and/or lactoperoxidase system (LPS)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
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<td>–</td>
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<td>+</td>
<td>Control (water added)³</td>
<td>Control (no water added)³</td>
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<td>LPS addition ³</td>
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<td>–</td>
<td>+</td>
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<td></td>
</tr>
<tr>
<td>L* value</td>
<td>Before marinating</td>
<td>72.63&lt;sup&gt;a&lt;/sup&gt;²</td>
<td>70.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.16&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>70.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70.13&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>74.26&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>After marinating</td>
<td>84.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.47&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>After cooking</td>
<td>69.66</td>
<td>65.97</td>
<td>65.93</td>
<td>66.23</td>
<td>66.44</td>
<td>65.74</td>
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<tr>
<td>a* value</td>
<td>Before marinating</td>
<td>5.76</td>
<td>4.83</td>
<td>5.03</td>
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<td>5.42</td>
<td>5.50</td>
</tr>
<tr>
<td></td>
<td>After marinating</td>
<td>0.80&lt;sup&gt;a&lt;/sup&gt;²</td>
<td>-1.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.97&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>After cooking</td>
<td>1.32&lt;sup&gt;a&lt;/sup&gt;²</td>
<td>0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>b* value</td>
<td>Before marinating</td>
<td>8.67</td>
<td>9.49</td>
<td>9.58</td>
<td>8.49</td>
<td>8.61</td>
<td>7.97</td>
</tr>
<tr>
<td></td>
<td>After marinating</td>
<td>11.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.78&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.80&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>After cooking</td>
<td>23.24</td>
<td>24.41</td>
<td>23.43</td>
<td>24.57</td>
<td>24.78</td>
<td>25.29</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means within a row without the same superscript are significantly different (P<0.05).

¹Color evaluation: L* = light and dark, a* = red and green, and b* = yellow and blue, measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

²Marination: Chicken drumsticks were marinated with a solution contained with 1% acetic acid, 3% salt, and adjusted to pH 4.

³Lactoperoxidase system (LPS) = lactoperoxidase (LP, 1 µg/ml), KSCN (5.9 mM) and H₂O₂ (2.5 mM).

⁴Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

⁵Control (no water added): Samples were placed in containers without adding the marination solution or distilled water.

Table 9.2 Skin color evaluation¹ results of marinated² chicken drumsticks with or without addition of nisin and/or lactoperoxidase system (LPS)
### Table 9.3 Muscle color evaluation results of marinated chicken drumsticks with or without addition of nisin and/or lactoperoxidase system (LPS)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS addition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS addition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em><em>L</em> value</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before marinating</td>
<td></td>
<td>62.09</td>
<td>60.19</td>
<td>60.37</td>
<td>58.00</td>
<td>58.77</td>
<td>60.92</td>
</tr>
<tr>
<td>After marinating</td>
<td></td>
<td>79.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>77.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.95&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>88.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.34&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>62.77&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>After cooking</td>
<td></td>
<td>75.70</td>
<td>75.55</td>
<td>76.08</td>
<td>77.21</td>
<td>73.64</td>
<td>74.01</td>
</tr>
<tr>
<td><em><em>a</em> value</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before marinating</td>
<td></td>
<td>12.42</td>
<td>13.58</td>
<td>10.54</td>
<td>10.38</td>
<td>10.21</td>
<td>10.33</td>
</tr>
<tr>
<td>After marinating</td>
<td></td>
<td>3.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.57&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>After cooking</td>
<td></td>
<td>2.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em><em>b</em> value</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before marinating</td>
<td></td>
<td>12.42</td>
<td>13.58</td>
<td>10.54</td>
<td>10.38</td>
<td>10.21</td>
<td>10.33</td>
</tr>
<tr>
<td>After marinating</td>
<td></td>
<td>12.42&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14.51&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11.61&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.05&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>After cooking</td>
<td></td>
<td>17.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.99&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>18.26&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>22.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>Means within a row without the same superscript are significantly different (P<0.05).

1Color evaluation: L* = light and dark, a* = red and green, and b* = yellow and blue, measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

2Marination: Chicken drumsticks were marinated with a solution contained with 1% acetic acid, 3% salt, and adjusted to pH 4.

3Lactoperoxidase system (LPS) = lactoperoxidase (LP, 1 μg/ml), KSCN (5.9 mM) and H<sub>2</sub>O<sub>2</sub> (2.5 mM).

4Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

5Control (no water added): Samples were placed in containers without adding the marination solution or distilled water.

Table 9.3 Muscle color evaluation results of marinated chicken drumsticks with or without addition of nisin and/or lactoperoxidase system (LPS)
### Table 9.4 Total color differences\(^1\) of marinated chicken drumsticks with or without addition of nisin and lactoperoxidase system (LPS)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nisin addition (100 IU/ml)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Control (water added)(^3)</td>
</tr>
<tr>
<td>LPS addition(^2)</td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>Comparison of before and after marination</td>
<td>12.86(^a)</td>
<td>13.14(^a)</td>
<td>11.23(^a)</td>
<td>16.63(^a)</td>
<td>10.65(^a)</td>
<td>1.81(^b)</td>
</tr>
<tr>
<td></td>
<td>Comparison of before and after cooking</td>
<td>18.67</td>
<td>19.72</td>
<td>19.94</td>
<td>21.99</td>
<td>22.19</td>
<td>18.84</td>
</tr>
<tr>
<td></td>
<td>Comparison of before marination and after cooking</td>
<td>15.53</td>
<td>16.58</td>
<td>15.48</td>
<td>16.95</td>
<td>16.68</td>
<td>19.43</td>
</tr>
<tr>
<td>Muscle</td>
<td>Comparison of before and after marination</td>
<td>19.95(^ab)</td>
<td>21.06(^a)</td>
<td>22.91(^a)</td>
<td>34.53(^c)</td>
<td>13.12(^b)</td>
<td>5.14(^d)</td>
</tr>
<tr>
<td></td>
<td>Comparison of before and after cooking</td>
<td>6.27(^ab)</td>
<td>5.53(^b)</td>
<td>8.14(^ab)</td>
<td>12.20(^b)</td>
<td>10.36(^ab)</td>
<td>18.08(^c)</td>
</tr>
<tr>
<td></td>
<td>Comparison of before marination and after cooking</td>
<td>19.19</td>
<td>20.18</td>
<td>20.60</td>
<td>24.76</td>
<td>19.19</td>
<td>19.58</td>
</tr>
</tbody>
</table>

\(^{*}\) Means within a row without the same superscript are significantly different (P<0.05).

\(^1\) Total color difference (ΔE\(^a,b\)) = square root of [(ΔL\(^*\))\(^2\) + (Δa\(^*\))\(^2\) + (Δb\(^*\))\(^2\)], where L\(^*\) = light and dark, a\(^*\) = red and green, b\(^*\) = yellow and blue, and Δ representing the difference between the two processing steps; L\(^*\), a\(^*\), and b\(^*\) were measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

\(^2\) LPS = lactoperoxidase (LP, 1 μg/ml), KSCN (5.9 mM) and H\(_2\)O\(_2\) (2.5 mM).

\(^3\) Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

\(^4\) Control (no water added): Samples were placed in bags without adding the marinade solution or distilled water.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin addition (100 IU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>LPS addition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(water added)³</td>
<td>(no water added)⁴</td>
</tr>
<tr>
<td>Raw samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin color</td>
<td></td>
<td>2.7ᵃ</td>
<td>3.0ᵃ</td>
<td>2.0ᵃ</td>
<td>2.6ᵃ</td>
<td>5.1ᵇ</td>
<td>6.7ᶜ</td>
</tr>
<tr>
<td>Muscle color</td>
<td></td>
<td>3.0ᵃ</td>
<td>2.4ᵃ</td>
<td>2.4ᵃ</td>
<td>2.1ᵃ</td>
<td>6.6ᵇ</td>
<td>7.0ᵇ</td>
</tr>
<tr>
<td>Marinated chicken aroma⁵</td>
<td></td>
<td>5.6ᵃ</td>
<td>5.3ᵃ</td>
<td>4.9ᵃ</td>
<td>6.1ᵃ</td>
<td>1.4ᵇ</td>
<td>1.0ᵇ</td>
</tr>
<tr>
<td>Off-aroma⁶</td>
<td></td>
<td>1.6</td>
<td>1.1</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Cooked samples</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin color</td>
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<td>4.7</td>
<td>4.1</td>
<td>4.0</td>
<td>3.7</td>
<td>3.9</td>
<td>4.0</td>
</tr>
<tr>
<td>Muscle color</td>
<td></td>
<td>3.9</td>
<td>4.1</td>
<td>3.0</td>
<td>2.9</td>
<td>4.4</td>
<td>4.3</td>
</tr>
<tr>
<td>Marinated chicken flavor⁷</td>
<td></td>
<td>5.4ᵃ</td>
<td>4.0ᵇ</td>
<td>5.0ᵃᵇ</td>
<td>5.7ᵃ</td>
<td>1.3ᶜ</td>
<td>1.9ᶜ</td>
</tr>
<tr>
<td>Off-flavor⁸</td>
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<td>1.3</td>
<td>1.3</td>
<td>1.1</td>
<td>1.3</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Juiciness</td>
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<td>4.3ᵃᵇ</td>
<td>3.3ᵇ</td>
<td>4.3ᵃᵇ</td>
<td>3.9ᵃᵇ</td>
<td>5.1ᵃᵇ</td>
<td>5.7ᵃ</td>
</tr>
<tr>
<td>Tenderness</td>
<td></td>
<td>4.7ᵃᵇ</td>
<td>4.1ᵇ</td>
<td>4.4ᵇ</td>
<td>3.9ᵇ</td>
<td>4.7ᵃᵇ</td>
<td>6.3ᵃ</td>
</tr>
</tbody>
</table>

Means within a row without the same superscript are significantly different (P<0.05).

The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1 = light, 9 = dark), juiciness (1 = not juicy, 9 = very juicy), and tenderness (1 = not tender, 9 = very tender).

³LPS = lactoperoxidase (LP, 1 μg/ml), KSCN (5.9 mM) and H₂O₂ (2.5 mM).

⁴Control (water added): Instead of the marinade solution, samples were stored in unheated water.

⁵Control (no water added): Samples were placed in containers without adding the marinade solution or water.

⁶Marinated chicken aroma: the combination aroma consisted of chicken, sour (acidic), salty, peppery, and garlic aromas; flavoring agents (0.3% ground black pepper and 0.15% garlic powder) were added to treatments 1 through 4.

⁷Off-aroma: any aroma that was not expected from the raw marinated chicken aroma.

⁸Marinated chicken flavor: the combination flavor consisted of chicken, sour (acidic), salty, peppery, and garlic flavors; flavoring agents (0.3% ground black pepper and 0.15% garlic powder) were added to treatments 1 through 4.

Table 9.5 Sensory characteristic intensities¹ of marinated chicken drumsticks with or without addition of nisin and lactoperoxidase system (LPS)
CHAPTER 10

EXPERIMENT 8

PHYSICAL AND SENSORY CHARACTERISTICS OF MARINATED CHICKEN DRUMSTICKS TREATED WITH LACTOPEROXIDASE SYSTEM AND THERMAL TREATMENT

ABSTRACT

The lactoperoxidase system (LPS) and thermal treatments have been shown to inactivate some microorganisms in foods. However, further studies were needed to evaluate whether these treatments had any influence on the physical and sensory characteristics of the treated samples. In this study, a solution that contains acetic acid (1%) and salt (3%) with pH adjusted to 4 was developed as a standardized marinade. The LPS consisted of 1 μg/ml of lactoperoxidase (LP), 5.9 mM of KSCN, and 2.5 mM of H₂O₂ was added to the marinade for the LPS-added treatments. For the thermal treatment, samples were heated with the marinade solution at 58°C for 2 min, and then stored at 4°C for 18 hrs, whereas no thermal-treated samples were stored at 4°C for 18 hrs. For sensory evaluation, some flavoring agents including 0.3% black pepper and 0.15% garlic powder were added when preparing the marinade solution. For physical evaluation, no flavoring agents were used. Physical and sensory evaluations were then conducted. The results showed that the treatment, which combined LPS and thermal
treatment, did not impair the physical or sensory qualities of the treated samples. In conclusion, marinated chicken drumsticks treated with LPS and tumbling were acceptable based on the physical and sensory qualities.

Keywords: physical characteristics; sensory characteristics; 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. Hoogenkamp (1987) described marinating as a diffusion or penetration of a solution into the meat structure. Similarly, Cannon et al. (1993) explained marinating as a process involving incorporation of acidic or alkaline solution into products to alter the pH of the tissue. Marination of poultry is practiced to improve product's physical and sensory attributes, such as tenderness, water-holding capacity, flavor and etc. (Hashim, et al. 1999a,b; Lemos et al. 1999; Xiong and Kupski, 1999; Young and Buhr, 2000; Young and Lyon, 1997a,b; Zheng et al. 2000).

The lactoperoxidase system (LPS), which consists of lactoperoxidase (LP), thiocyanate (SCN'), and hydrogen peroxide (H₂O₂), is an inhibitory system that is present naturally in bovine milk. This system has been shown to be inhibitory against some pathogenic and spoilage microorganisms such as P. fluorescens, L. monocytogenes, S. typhimurium and E. coli (Zapico et al., 1995; Kamau et al., 1990a,b; Earnshaw et al.,
1990). The LPS has been mainly studied for the application in the milk and dairy products (Zapico et al., 1998). Only a few studies have attempted to apply this LPS system in meat and poultry products.

Even though LPS has been reported to have little effect on the sensory and physical characteristics of the treated milk and dairy products (Ridley and Shalo, 1990; Martinez et al., 1988), limited studies involving addition of LPS on the physical and sensory qualities of treated meat and poultry products have been reported. Wolfson et al. (1994) reported that there was no significant (p<0.01) difference of the Hunterlab color values (L, a, b) between the LPS (1μg/ml lactoperoxidase, 5.9 mM potassium thiocyanate, and 2.5 mM hydrogen peroxide for 5 min) treated chicken thigh skin and controls after 24 and 48 hours storage. In addition, in the same study, they evaluated the 2-thiobarbituric acid (TBA) values, and found no significant difference (P<0.05) of oxidative deterioration between the LPS treated chicken thigh meat and the control samples.

Thermal or heat treatment, which is one of the most common physical methods to reduce the load of microorganisms, has been widely applied to preserve foods for years. In addition, heating is also involved in many food processes, such as cooking, scalding, pasteurizing, drying, and etc.

Previous studies conducted in this same laboratory showed that a treatment, which combined LPS and a thermal treatment (58°C for 2 min) decreased the total microflora and psychrotrophs counts of marinated chicken broiler drumsticks during refrigerated storage for up to 7 days (Information has been submitted for publication). Limited information on the contribution of marination, LPS, and thermal treatment to the physical
and sensory characteristics of treated poultry products is available. Whether or not adding LPS and thermal treatment would affect the physical and sensory characteristics of the treated samples should be evaluated.

The objective of this study was to investigate the effects of LPS-adding and thermal treatment on some physical and sensory characteristics of the marinated chicken drumsticks. In addition, the sensory profiles of marinated chicken drumsticks using the descriptive analysis techniques were characterized in this study. The results of this study could be used to determine the acceptability of marinated chicken drumsticks that were previously treated with antimicrobial compounds used to reduce potential microbial contamination during processing and to extend the shelf life.

MATERIALS AND METHODS

Development of the marinade solution:

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. A solution that contains 1% acetic acid and 3% salt was prepared as the standardized marinade in this study. A LPS consisted of 1 μg/ml of lactoperoxidase (LP, EC 1.11.1.7; purity index 0.82 (A$_{412}$/A$_{280}$); Sigma Chemical Co., St. Louis, Missouri), 3.9 mM of potassium thiocyanate (KSCN, Fisher Scientific Co.; Pittsburgh, PA), and 2.5 mM of hydrogen peroxide (H$_2$O$_2$, 30%. Fisher Scientific Co.; Pittsburgh, PA) was added to the marinade for the LPS-added treatment. The pH of the marinade solutions was then adjusted to 4 (using HCl or NaOH solutions). For the sensory evaluation, some flavoring agents including 0.3% black pepper and
0.15% garlic powder were added into the standardized marinade solutions for the marinated treatments 1 through 4 (with or without tumbling based on the assigned groups), whereas no flavoring agents were added to the treatments 5 and 6 samples. No flavoring agents were added in the marinade solution for all 6 treatments for the physical evaluation.

Sample preparation

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 the experiment was conducted. Drumsticks were randomly chosen and equally assigned and labeled to the treatment groups. For the samples without thermal treatment (treatment 1 and treatment 3), drumsticks were placed and marinated in plastic containers (Sterilite Corporation, Townsend, MA) with marinade solution so that all the drumsticks were covered completely by the marinade solution and stored at 4°C for 18 hr. Instead of the marinate solution, the treatment 5 control samples were stored and submerged in distilled water. For those samples with thermal-maringating treatment (treatment 2 and treatment 4), drumsticks were placed inside a plastic bag with the heated marinade solutions, which was previously heated in a water bath. After holding at 58°C for 2 min, the marinade-drumstick mixes in the bags were cooled by immersing the bags in running tap water. When the marination mix was cooled to 25°C (approximately 10 min), the marinade-drumstick mix was moved into plastic containers and then refrigerated at 4°C for 18
hours (including the previous time of thermal marinating and cooling). There was no water or marinade solution added to treatment 6 samples.

Physical evaluations

Drumsticks were weighted individually before conducting the experiment. Figure 10.1 illustrates the experimental procedure.

The pH values measurement of marinade solutions and drumsticks

The pH meter was calibrated with a two-point calibration method using pH 4 and 7 buffers before use. The pH values of the marinade solution were measured before and after marinating of the drumsticks using a Corning pH meter (Model 430, Corning Inc., Corning, NY). Muscles of the whole drumsticks without bone and skin were blended for 30 sec using a Waring Lab Blender (Model 31BL91, Dynamics Corporation of America, New Hartford, Connecticut). Ten grams of muscle sample was then blended with 100ml of distilled water in a polyethylene bag for 1 min using a Seward Stomacher (Model 400, Tekmar Company, Cincinnati, Ohio), and then the pH of the mixture was measured.

Marinade absorption measurement

After finishing marinating, the drumsticks were removed from the marinade solutions and drained for 5 min at 4°C before determining the marinade absorption. Marinade absorption (grams marinade absorbed/100g of drumstick) was calculated based on the unmarinated weight (Wt_before marinating) of the sample and its weight after marination with 5-min draining (Wt_after marinating and draining) and was calculated as follows.
Marinade Absorption = \[ \left( \frac{W_{t \text{ after marinating and draining}} - W_{t \text{ before marinating}}}{W_{t \text{ before marinating}}} \right) \times 100 \]

Instrumental color measurement

The Hunter “L*” (lightness), “a*” (redness-greenness), and “b*” (yellowness-blueness) values of drumstick were measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey). Color measurements were obtained on the surfaces of the skin, and also on the muscle without skin covering, respectively, before and after marinating, and also after cooking. Three measurements were made at different locations on the drumsticks and averaged. A standard plate (number 18433010) with “Y” = 92.60, “x” = 0.3140, and “y” = 0.3206 was used as a reference.

Marinade solution salt content measurement

The salt contents of the marinade solutions after marinating were measured with a Dicromat® salt analyzer (Model DSA-1000, Diamond Crystal Salt Company, St. Clair, Michigan). The marinade solutions were mixed, and then the salt contents were measured. The salt analyzer was rinsed with distilled water between measurements.

Cooking loss measurement

Pre-weighted drumsticks were cooked in a 177°C (350°F) preheated conventional oven (Type EF111, The G.S. Blodgett Co. Inc., Burlington, VT) to an internal temperature of 70°C (158°F). After reaching the internal temperature of 70°C monitored by Fisher brand traceable alarm thermometer/timer (Fisher Scientific, Pittsburgh, PA), the samples

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were equilibrated to room temperature and then the cooking loss measurement and sensory evaluation were accomplished. Cooking loss was calculated based on the weight of the sample before cooking \((Wt_{\text{before cooking}})\) and its weight after cooking \((Wt_{\text{after cooking}})\) and was calculated as follows.

\[
\text{Cooking Loss} = \left( \frac{Wt_{\text{before cooking}} - Wt_{\text{after cooking}}}{Wt_{\text{before cooking}}} \right) \times 100
\]

Yield measurement

Yield was calculated based on the weight of the sample before marinating \((Wt_{\text{before marinating}})\) and its weight after cooking \((Wt_{\text{after cooking}})\) and was calculated as follows.

\[
\text{Yield} = (Wt_{\text{after cooking}} - Wt_{\text{before marinating}}) \times 100
\]

Sensory evaluation

The panel of seven members consisted of faculty and graduate students majoring in meat science. They evaluated the sensory characteristics of the marinated products made with the standardized marinade solution with flavoring agents (0.3% black pepper and 0.15% garlic powder) with or without thermal treatment and LPS added (Figure 10.1). Descriptive analysis was conducted to evaluate the intensities of sensory characteristics of the raw and cooked samples.

Before conducting the experiment, the panelists were first trained in the general sensory descriptive analysis and were led through the development of a lexicon of descriptors for marinated chicken samples. Most of the references used during training
were selected in accordance with Hashim et al. (1999a,b) recommendations. Next, the panelists were requested to list the most descriptive terms for the product. Panelists with the help of the panel leader identified sensory properties important in marinated chicken. The panel agreed on all attributes considered necessary for evaluation of the marinated chicken and defined these attributes.

Attributes of skin color, muscle color, marinated chicken aroma (combined raw marinated chicken aroma consisted of chicken, sour-acid, peppery, and garlic aromas), and off-aroma (any aroma which was not expected from the raw marinated chicken aroma) were used to evaluate the raw samples. After cooking followed the procedures described in the cooking loss measurement, the samples were cooled to room temperature (approximately 25°C) and served to the sensory panel. Attributes for skin color, muscle color, marinated chicken flavor (combined cooked marinated chicken flavor consisted of chicken, sour-acid, saltiness, pepper, and garlic), off-flavor (any flavor which was not expected from the cooked marinated chicken flavor), juiciness, and tenderness were used to evaluate the cooked samples. Appropriate reference samples were selected and used during training to exhibit the attributes and to set the anchors. Reference samples were available at each test trial for panelists to assist in making evaluations. The Hunter color values of the skin and muscle for the raw and cooked reference samples were also measured.

The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1 = light color; 9 = dark color), juiciness (1 = not juicy; 9 = very juicy), and tenderness (1 = not tender; 9 = very tender). The samples were randomly presented in
plastic plates coded with a randomly selected three-digit code. Water and unsalted crackers were provided to panelists. Each panelist evaluated the samples independently.

Statistical analyses

Data were analyzed using the general linear model (GLM) of Statistical Analysis System’s Procedures (SAS Institute Inc., Cary, NC) with a 5% level of significance. Means were separated using Duncan’s multiple range test.

RESULTS AND DISCUSSION

Physical evaluation of marinated chicken drumsticks with or without the lactoperoxidase system (LPS) added and/or thermal treatment is shown in Table 10.1. In the current study, treatment 1 had no LPS added and no thermal treatment; treatment 2 had no LPS added but with thermal treatment; treatment 3 had LPS added but without thermal treatment; treatment 4 had both LPS added and thermal treatment; treatment 5 had distilled water (instead of the marinade solution) added; treatment 6 had no water and no marinade solution added.

Before marinating, raw drumstick muscle pH values of treatments 1 through 6 were from 6.79 to 6.91 (Table 10.1). After marinating for 18 hours at 4°C, the pH values of raw drumsticks dropped approximately 1.3-pH units to pH 5.53-5.60 for the marinated treatments 1 through 4 without significant (P>0.05) differences among these 1 through 4 marinated treatments. The pH values of the controls with or without distilled water added (treatments 5 and 6) were 6.76 and 6.87, respectively, which were significantly (P<0.05) higher than the samples of marinated treatments 1 through 4. After cooking, the
pH values of the samples for marinated treatments 1 through 4 increased to 5.64-5.93, and no significant (P>0.05) difference for pH was detected among these 1 through 4 marinated treatments. Cooked drumstick muscle pH values of both control samples with or without addition of distilled water (treatments 5 and 6) increased to 6.91 and 6.97, and were significantly higher (P<0.05) than the samples for marinated treatments 1 through 4. After marinating, the marinade solution pH values for the marinated treatments 1 through 4 were among 4.37 to 4.42, which were significant (P<0.05) lower than the solution pH of the control with addition of distilled water samples (treatment 5) as expected.

The marinade absorptions of the marinated treatments 1 through 4 samples ranged from 1.62 to 2.48%, and no significant (P>0.05) difference was obtained among these 4 marinated treatments. The absorption of the control samples with addition of distilled water (treatment 5) was 6.19%, which was significant (P<0.05) higher than the samples of the marinated treatments 1 through 4 (Table 10.1). The cooking loss of the marinated treatments 1 through 4 samples ranged from 22.56 to 23.118%, and no significant (P>0.05) difference was detected among the 1 through 4 marinated treatment samples. The cooking loss of the control without distilled water added (treatment 6) was 16.34%, which was significant (P<0.05) lower than the samples of the marinated treatments 1 through 4 (Table 10.1). The yield of the marinated treatments 1 through 4 samples ranged from 78.33 to 79.36%, and no significant (P>0.05) difference was detected among the 1 through 4 marinated treatment samples. The salt content of the marinade solution after marinating of the marinated treatments 1 through 4 samples was 1.17% and no significant (P>0.05) difference was obtained among these 4 marinated treatments, but the salt content of the control samples with distilled water added (treatment 5) was 0.09%,
which was significant (P<0.05) lower than the marinated treatments of 1 through 4 as expected (Table 10.1).

Table 10.2 illustrates the color evaluation of marinade chicken drumsticks with or without thermal treatment and/or addition of LPS. The skin L* values of the all treatments (1 through 6) before marinating were in the range of 71.19 to 74.50 without any significant differences (P>0.05) among treatments. After marinating, all the skin L* values of treatments 1 through 5 increased to 76.53-82.64, whereas the control without addition of distilled water (treatment 6) remained approximately the same at 72.06, which was significant (P<0.05) lower than the samples of the 1 through 5 treatments. After cooking, all the skin L* values of the samples for treatments 1 through 6 decreased to 62.33-69.29 without significant (P>0.05) differences among all 6 treatments.

The skin a* values of the all treatments of 1 through 6 before marinating were in the range of 4.89-6.59 without any significant differences (P>0.05) among all 6 treatments (Table 10.2). After marinating, treatment 3 and treatment 4 (LPS added), had significantly (P<0.05) lower skin a* values than treatment 1 and 2 (no LPS added). Similarly, after cooking, the skin a* values of treatment 3 and treatment 4 were significantly (P<0.05) lower than the values of treatment 1 and 2. This significant reduction of a* values of the LPS treated samples was probably due to the addition of hydrogen peroxide, which is one of the components of the LPS treatment, and is a strong oxidizing agent that is occasionally used as a bleaching agent in the food industry (Daeschel and Penner, 1992). The skin b* values of the all treatments (1 through 6) before marinating were in the range of 6.67 to 8.68 without any significant (P>0.05) differences (Table 10.2). After marinating, the skin b* values were in the range of 5.96
to 8.05 without any significant differences (P>0.05) among all 6 treatments. After cooking, the skin b* increased to 18.51-23.69 without any significant differences (P>0.05) due to any of the 6 treatments.

The significant changes of skin a* values of the LPS treated samples (treatments 3 and 4) in the current study did not agree with the report by Wolfson (1992). Wolfson (1992) investigated the LPS effect on some physical characteristics of LPS treated poultry. In that study, treated chicken legs and thighs were immersed in a 50°C bath containing the LPS (1μg/ml lactoperoxidase, 5.9 mM potassium thiocyanate, and 2.5 mM hydrogen peroxide) for 5 min, and then stored at 4°C, and no LPS was added for the control samples. Wolfson (1992) reported that there was no significant (p<0.01) difference for the Hunterlab color values (L, a, b) between the LPS treated chicken thigh skin and controls after 24 and 48 hours storage at 4°C. The possible reason of this disagreement between the two studies is probably that the LPS-treated time (18 hours in the current study) was much longer than the time of 5 min in Wolfson's study.

The muscle L* values of all treatments (1 through 6) before marinating were in the range of 60.02 to 65.23 without any significant (P>0.05) differences among treatments (Table 10.3). After marinating, all the muscle L* values of the marinated treatments 1 through 4 increased and were significant (P<0.05) higher than the values of the two control samples (treatments 5 and 6). After cooking, the only significant (P>0.05) difference in all 6 treatments for muscle L* values was that treatment 1 (no LPS added and no thermal treatment) was significantly (P<0.05) lower than all other treatments (marinated treatments 2 through 4 and control treatments 5 and 6).

The muscle a* values of the all treatments (1 through 6) before marinating were in
the range of 11.69 to 13.93 (Table 10.3). After marinating, treatment 3 and treatment 4 (LPS added) had significantly (P<0.05) lower muscle a* values than the values of treatment 1 and 2 (no LPS added). Similarly, after cooking, the muscle a* values of treatment 3 and treatment 4 (LPS added) were significantly (P<0.05) lower than the values of treatment 1 and 2 (no LPS added). The muscle b* values of the all treatments (1 through 6) before marinating were in the range of 8.96 to 13.33. After marinating, the muscle b* values of treatments 1 through 6 increased to 9.70-13.18 without significant (P>0.05) difference among 6 treatment. After cooking, there was no significant (P>0.05) difference for the muscle b* values for marinated treatments 1 through 4.

The results of the total color difference are shown in Table 10.4. Total color difference (ΔE*ab) is calculated by the following formula: Total color difference (ΔE*ab) = square root of [(ΔL*)^2 + (Δa*)^2 + (Δb*)^2], where L* = light and dark, a* = red and green, b* = yellow and blue, and Δ representing the difference between the two processing steps, and it is commonly applied to evaluate the color changes of samples at the two different processing steps in food science studies. There was no significant (P>0.05) difference for total color difference of skin between before and after marination for the marinated treatments 1 through 4 samples, but the control samples without distilled water added (treatment 6) had a significantly (P<0.05) lower total color differences for skin color. There was no significant (P>0.05) difference for total color difference for skin color between before and after cooking for the all six treatment samples. Similarly, no significant (P>0.05) difference for total color difference for skin between before marination and after cooking for the all six treatment samples was obtained in the current study. As shown in Table 10.4, in the current study, the control
samples with or without distilled water added (treatments 5 and 6) had significant (P<0.05) lower total color difference in muscle color, when compared with the samples of the marinated treatments 1 through 3. There was no significant (P>0.05) total color difference in muscle between before and after cooking, and between before marination and after cooking for all six treatment samples.

Sensory evaluation of marinated chicken drumsticks with or without addition of LPS and/or thermal treatment is shown in Table 10.5. In the current study, 0.3% black pepper and 0.15% garlic powder were added for the marinated treatments 1 through 4 for the sensory evaluation, whereas no flavoring agents were added to treatments 5 and 6 samples. Based on a 1-9 scale, in which 1 and 9 representing the lighter and darker color respectively, the marinated treatments 1 through 4 samples in the current study had lower sensory raw skin color scores between 2.1 and 2.7 without any significant (P>0.05) differences among the marinated treatments. The controls with or without distilled water added (treatments 5 and 6) had higher sensory raw skin color scores of 5.1 and 6.1, respectively, which were significantly (P<0.05) higher than the samples of marinated treatments 1 through 4. Similarly, the marinated treatments 1 through 4 samples had significant (P<0.05) lower sensory raw muscle color scores of 2.3-2.7, compared to the controls (with or without water added), which had color scores of 5.0 and 6.4 for treatments 5 and 6 respectively. The lightness of the skin and muscle colors for marinated treatments 1 through 4 when comparing to the samples of the control groups was probably due to the addition of acid.

In the current study, a marinated chicken aroma was defined as a combined aroma, which consisted of fresh raw chicken, acidic, black peppery, and garlic aromas.
The marinated treatments 1 through 4 samples had significant (P<0.05) higher marinated chicken aroma scores of 4.3 to 5.1, when compared with the controls that had 1.6 and 1.3 scores probably due to lack of flavoring agents added. Low (1.0 to 1.3 based on a 1-9 scale) and without significant (P>0.05) difference for all treatments 1 through 6 for sensory off-aroma (an aroma that was not expected from the raw marinated chicken aroma) of the raw samples were obtained in this study (Table 10.5).

After cooking, the sensory skin and muscle color scores of the marinated treatments 1 through 4 samples increased. Also, there was no significant (P>0.05) difference for the sensory cooked skin color scores of all treatments 1 through 6. No significant (P>0.05) difference for cooked muscle color scores for marinated treatments 1 through 4 was observed (Table 10.5). Similar to the marinated chicken aroma for the raw samples, in the current study, a cooked marinated chicken flavor was defined as a combined flavor, which consisted of cooked chicken, acidic, black peppery, and garlic flavors. The marinated treatments 1 through 4 samples had significant (P<0.05) higher marinated chicken flavor scores of 4.6 to 6.0, when compared with the controls that had scores of 1.6 and 2.0 probably due to lack of flavoring agents added. Low (1.1 to 1.9 based on a 1-9 scale) and without significant (P>0.05) difference for all treatments 1 through 6 for sensory off-flavor (a flavor that was not expected from the cooked marinated chicken flavor) of the cooked samples were obtained in the current study (Table 10.5).

In the current study, there was no significant (P>0.05) difference for the sensory juiciness score of all treatments 1 through 6. In addition, no significant (P>0.05)
difference for the sensory tenderness scores of marinated treatments 1 through 4 was observed in the current study.

CONCLUSION

In conclusion, the treatment consisting of addition of LPS (1 µg/ml of LP, 5.9 mM of KSCN, and 2.5 mM of H2O2) and thermal treatment (58°C for 2 min), which was previously showed to inactive some microorganisms of some foods, did not impair the physical and sensory characteristics tested in the current study for the treated samples.

REFERENCES


Figure 10.1 Experimental procedure

1 Standard marinade solution: acetic acid (1%), and salt (3%) in distilled water.
2 Pre-assigned antimicrobial components: with or without addition of lactoperoxidase (LPS = 1 µg/ml of lactoperoxidase, 5.9 mM of KSCN, and 2.5 mM of H₂O₂) based on the assigned groups.
3 Flavoring agents: ground black pepper (0.3%) and garlic powder (0.15%) for the sensory evaluation of marinated treatments 1 through 4; no flavor agents were added for the physical evaluation.
4 Total time of 18 hrs includes thermal treatment, cooling, and marinating.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS addition¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control (no water added)⁵</td>
<td>Control (no water added)⁵</td>
</tr>
<tr>
<td>Thermal treatment (58°C, 2 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw drumstick pH⁴ (before marinating)</td>
<td></td>
<td>6.83</td>
<td>6.81</td>
<td>6.91</td>
<td>6.79</td>
<td>6.88</td>
<td>6.85</td>
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<td>Raw drumstick pH⁴ (after marinating)</td>
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<td>5.60⁴</td>
<td>5.59⁴</td>
<td>5.53⁴</td>
<td>5.54⁴</td>
<td>6.76⁴</td>
<td>6.87⁴</td>
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<td>Cooked drumstick pH⁴</td>
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<td>5.93¹</td>
<td>5.72¹</td>
<td>5.82¹</td>
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<td>Marinade absorption (%)</td>
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<td>1.88¹</td>
<td>1.72¹</td>
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<td>2.48¹</td>
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<td>22.70¹</td>
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<td>85.21¹</td>
<td>82.65¹</td>
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<td>Salt content of marinade solution after marinating (%)</td>
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<td>1.17¹</td>
<td>1.17¹</td>
<td>1.17¹</td>
<td>1.17¹</td>
<td>0.09⁴</td>
<td>ND</td>
</tr>
</tbody>
</table>

*a,b* Means within a row without the same superscript are significantly different (P<0.05).

ND: not determined.

¹Lactoperoxidase system (LPS) = lactoperoxidase (1 µg/ml), KSCN (5.9 mM) and H₂O₂ (2.5 mM).

²Control (water added): Instead of the marinade solution, samples were dipped in the unheated distilled water.

³Control (no water added): Samples were placed in bags without adding the marination solution or distilled water.

⁴Drumstick pH was measured for muscle without skin.

Table 10.1 Physical evaluations of marinated chicken drumsticks with or without thermal treatment and/or lactoperoxidase system (LPS) added.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Control (water added)</td>
<td>Control (no water added)</td>
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<td>Thermal treatment (58°C, 2 min)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| L* value | Before marinating | 71.19 | 72.44 | 71.92 | 74.03 | 72.57 | 74.50 |
|          | After marinating  | 79.69<sup>a</sup> | 84.53<sup>c</sup> | 83.10<sup>bc</sup> | 82.73<sup>bc</sup> | 77.93<sup>a</sup> | 72.06<sup>d</sup> |
|          | After cooking     | 63.45 | 69.29 | 63.26 | 62.33 | 62.73 | 65.02 |

| a* value | Before marinating | 5.87  | 6.17  | 5.80  | 6.59  | 5.86  | 4.89  |
|          | After marinating  | 1.55<sup>a</sup> | 1.03<sup>a</sup> | -1.73<sup>b</sup> | -1.44<sup>b</sup> | 3.60<sup>c</sup> | 5.81<sup>d</sup> |
|          | After cooking     | 2.06<sup>a</sup> | 1.96<sup>ab</sup> | 0.44<sup>c</sup> | 0.62<sup>bc</sup> | 2.95<sup>a</sup> | 2.39<sup>a</sup> |

| b* value | Before marinating | 6.67  | 7.29  | 6.84  | 4.59  | 8.21  | 8.68  |
|          | After marinating  | 5.96  | 7.88  | 7.40  | 6.20  | 7.80  | 8.05  |
|          | After cooking     | 19.70 | 22.30 | 18.51 | 19.80 | 21.72 | 23.69 |

<sup>a,b,c</sup>Means within a row without the same superscript are significantly different (P<0.05).

<sup>1</sup>Color evaluation: L* = light and dark, a* = red and green, and b* = yellow and blue, measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

<sup>2</sup>Marination: Chicken drumsticks were marinated with a solution contained with 1% acetic acid, 3% salt, and adjusted to pH 4.

<sup>3</sup>LPS = lactoperoxidase (LP, 1 µg/ml), KSCN (5.9 mM) and H<sub>2</sub>O<sub>2</sub> (2.5 mM).

<sup>4</sup>Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

<sup>5</sup>Control (no water added): Samples were placed in containers without adding the marination solution or distilled water.

Table 10.2 Skin color evaluation<sup>1</sup> results of marinated<sup>2</sup> chicken drumsticks with or without thermal treatment and/or addition of lactoperoxidase system (LPS).
<table>
<thead>
<tr>
<th>Parameter</th>
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<th>3</th>
<th>4</th>
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<td>60.02</td>
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<td></td>
<td>84.65ab</td>
<td>91.21ab</td>
<td>98.10b</td>
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<tr>
<td>Before marinating</td>
<td></td>
<td>8.96a</td>
<td>11.97ab</td>
<td>13.33b</td>
<td>9.21a</td>
<td>9.61a</td>
<td>11.48ab</td>
</tr>
<tr>
<td>After marinating</td>
<td></td>
<td>9.70</td>
<td>11.80</td>
<td>13.18</td>
<td>12.55</td>
<td>10.36c</td>
<td>13.13</td>
</tr>
<tr>
<td>After cooking</td>
<td></td>
<td>13.90a</td>
<td>15.57a</td>
<td>16.80ab</td>
<td>16.48ab</td>
<td>18.84b</td>
<td>26.25c</td>
</tr>
</tbody>
</table>

Means within a row without the same superscript are significantly different (P<0.05).

1Color evaluation: L* = light and dark, a* = red and green, and b* = yellow and blue, measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

2Marination: Chicken drumsticks were marinated with a solution contained with 1% acetic acid, 3% salt, and adjusted to pH 4.

3LPS = lactoperoxidase (LP, 1 μg/ml), KSCN (5.9 mM) and H₂O₂ (2.5 mM).

4Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

5Control (no water added): Samples were placed in containers without adding the marination solution or distilled water.

Table 10.3 Muscle color evaluation1 results of marinated2 chicken drumsticks with or without thermal treatment and/or addition of lactoperoxidase system (LPS).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS addition&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Control (water added)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Control (no water added)&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thermal treatment (58°C, 2 min)</td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>Comparison of before and after marination</td>
<td>9.88&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>13.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.85&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.80&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Comparison of before and after cooking</td>
<td>21.37</td>
<td>21.07</td>
<td>22.87</td>
<td>24.76</td>
<td>20.66</td>
<td>17.53</td>
</tr>
<tr>
<td></td>
<td>Comparison of before marination and after cooking</td>
<td>16.01</td>
<td>16.09</td>
<td>15.92</td>
<td>20.16</td>
<td>17.31</td>
<td>17.96</td>
</tr>
<tr>
<td>Muscle</td>
<td>Comparison of before and after marination</td>
<td>27.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>29.78&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>36.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.25&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Comparison of before and after cooking</td>
<td>18.29</td>
<td>20.33</td>
<td>25.07</td>
<td>14.80</td>
<td>12.11</td>
<td>17.94</td>
</tr>
<tr>
<td></td>
<td>Comparison of before marination and after cooking</td>
<td>13.12</td>
<td>13.85</td>
<td>16.24</td>
<td>17.50</td>
<td>14.16</td>
<td>16.71</td>
</tr>
</tbody>
</table>

<sup>k</sup>Means within a row without the same superscript are significantly different (P<0.05).

<sup>1</sup>Total color difference ($\Delta E_{ab}^*$) = square root of $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]$, where $L^*$ = light and dark, $a^*$ = red and green, $b^*$ = yellow and blue, and $\Delta$ representing the difference between the two processing steps; $L^*$, $a^*$, and $b^*$ were measured with a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey).

<sup>2</sup>LPS = lactoperoxidase (LP, 1 µg/ml), KSCN (5.9 mM) and H<sub>2</sub>O<sub>2</sub> (2.5 mM).

<sup>3</sup>Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

<sup>4</sup>Control (no water added): Samples were placed in bags without adding the marinade solution or distilled water.

Table 10.4 Total color differences<sup>1</sup> of marinated chicken drumsticks with or without thermal treatment and lactoperoxidase (LPS) added.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LPS addition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Control</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td><strong>Thermal treatment (58°C, 2 min)</strong></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>(water added)</td>
<td>(no water added)</td>
<td></td>
</tr>
<tr>
<td><strong>Raw samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin color</td>
<td></td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscle color</td>
<td></td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Marinated chicken aroma&lt;sup&gt;5&lt;/sup&gt;</td>
<td></td>
<td>4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Off-aroma&lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
<td>1.3</td>
<td>1.3</td>
<td>1.1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Cooked samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin color</td>
<td></td>
<td>5.6</td>
<td>5.3</td>
<td>5.3</td>
<td>4.7</td>
<td>5.4</td>
<td>5.6</td>
</tr>
<tr>
<td>Muscle color</td>
<td></td>
<td>4.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Marinated chicken flavor&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
<td>4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Off-flavor&lt;sup&gt;8&lt;/sup&gt;</td>
<td></td>
<td>1.1</td>
<td>1.9</td>
<td>1.3</td>
<td>1.3</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Juiciness</td>
<td></td>
<td>3.4</td>
<td>3.3</td>
<td>3.6</td>
<td>3.4</td>
<td>3.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Tenderness</td>
<td></td>
<td>4.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 10.5 Sensory characteristic and intensities<sup>1</sup> of marinated chicken drumsticks with or without addition of lactoperoxidase system (LPS) and/or thermal treatment

---

<sup>1</sup>Means within a row without the same superscript are significantly different (P<0.05).

<sup>2</sup>LPS = lactoperoxidase (LP, 1 µg/ml), KSCN (5.9 mM) and H<sub>2</sub>O<sub>2</sub> (2.5 mM).

<sup>3</sup>Control (water added): Instead of the marinade solution, samples were stored in unheated distilled water.

<sup>4</sup>Control (no water added): Samples were placed in containers without adding the marinade solution or distilled water.

<sup>5</sup>Marinated chicken aroma: the combination aroma consisted of chicken, sour (acidic), salty, peppery and garlic aromas; flavoring agents (0.3% ground black pepper and 0.15% garlic powder) were added to treatments 1 through 4.

<sup>6</sup>Off-aroma: any aroma that was not expected from the raw marinated chicken aroma.

<sup>7</sup>Marinated chicken flavor: the combination flavor consisted of chicken, sour (acidic), salty, peppery, and garlic flavors; flavoring agents (0.3% ground black pepper and 0.15% garlic powder) were added to treatments 1 through 4.

<sup>8</sup>Off-flavor: any flavor that was not expected from the cooked marinated chicken flavor.
Adding nisin at the level of 50 IU/ml with thermal treatment of heating the marinade solution at 58°C for 2 min, cooling, and then refrigerated storage at 4°C for 18 hours, decreased the total microflora and psychrotrophs counts of the marinated chicken drumsticks. 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. Adding nisin up to the level of 100 IU/ml, and LPS added up to 2 units (LP, 2 μg/ml; KSCN, 11.8 mM; H₂O₂, 5.0 mM), respectively, significantly (P<0.05) decreased the total microflora and psychrotrophs counts of the marinated chicken drumsticks. Adding LPS at the level of 1 unit (1 μg/ml LP, 5.9 mM KSCN, and 2.5 mM H₂O₂) significantly (P<0.05) decreased the total microflora and psychrotrophs counts of the marinated chicken 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 hours, significantly (P<0.05) decreased the total microflora and psychrotrophs counts of the marinated chicken broiler drumsticks.

The treatment consisting of nisin added (100 IU/ml) and thermal treatment (58°C for 2 min), which was previously showed to decrease some microorganisms of some food, did not impair the physical and sensory characteristics tested in the current study.
for the treated samples. The treatment consisted of nisin added (100 IU/ml) and tumbling treatment (10 min), which was previously showed to inactive some microorganisms of some foods, did not impair the physical and sensory characteristics tested in the current study for the treated samples. Treatment consisting of addition of nisin (100 IU/ml) and LPS (1 μg/ml of LP, 5.9 mM of KSCN, and 2.5 mM of H₂O₂), which was previously showed to inactive some microorganisms of some foods, did not impair the physical and sensory characteristics tested in the current study for the treated samples. The treatment consisting of addition of LPS (1 μg/ml of LP, 5.9 mM of KSCN, and 2.5 mM of H₂O₂) and thermal treatment (58°C for 2 min), which was previously showed to inactive some microorganisms of some foods, did not impair the physical and sensory characteristics tested in the current study for the treated samples.

Based on the results of this study, the poultry industry would be able (after government approval) to apply this information to produce various high value-added and longer shelf life marinated products.
<table>
<thead>
<tr>
<th>Item</th>
<th>Marinating time</th>
<th>Water</th>
<th>Oil</th>
<th>Acidic</th>
<th>Flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scratch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bill Knapp's</td>
<td>12 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dave Wilson</td>
<td>Overnight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scratch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinegar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy sauce</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Honey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onion Powder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garlic Powder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ginger</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daniel Dave</td>
<td>1-2 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margarita</td>
<td>2 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spicy Caribbean</td>
<td>2-6 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steak &amp; Chicken</td>
<td>Overnight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hawaiian</td>
<td>4-24 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grilled chicken marinade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken marinade II</td>
<td>2-3 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morten's</td>
<td>≥ 4 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken BBQ marinade</td>
<td>Overnight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>National Broiler Council</td>
<td>Overnight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broiler or grilled marinated chicken (Gai Yang)</td>
<td>1 ~ 8 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oriental grilled chicken</td>
<td>1 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marinade-Baste for chicken BBQ</td>
<td>Overnight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken Fajitas</td>
<td>Overnight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Appendix A. Selected published marination recipes (continuous)

<table>
<thead>
<tr>
<th>Item</th>
<th>Flavor</th>
<th>Resource</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scratch</strong></td>
<td>Black pepper</td>
<td>Oregano (or marjoram), mustard</td>
</tr>
<tr>
<td><strong>Kikkoman</strong></td>
<td></td>
<td>Papaya, red onion, jalapeno pepper, rosemary</td>
</tr>
<tr>
<td><strong>Margarita</strong></td>
<td></td>
<td>Tequila, Cointreau</td>
</tr>
<tr>
<td><strong>Spicy Caribbean</strong></td>
<td></td>
<td>Green onion, jalapenos, dried thyme, ground allspice, nutmeg</td>
</tr>
<tr>
<td><strong>Steak &amp; Chicken</strong></td>
<td></td>
<td>Red pepper flakes, brown suger</td>
</tr>
<tr>
<td><strong>Hawaiian</strong></td>
<td></td>
<td>Pineapple, mustard</td>
</tr>
<tr>
<td><strong>Grilled chicken marinade II</strong></td>
<td></td>
<td>Parsley, sesame seeds</td>
</tr>
<tr>
<td><strong>Chicken marinade II</strong></td>
<td></td>
<td>Brown sugar, Treikai marinade and sauce</td>
</tr>
<tr>
<td><strong>Morten's</strong></td>
<td></td>
<td>Green onion, brown sugar</td>
</tr>
<tr>
<td><strong>Broiler or grilled marinated chicken (Gai Yang)</strong></td>
<td>Dry white wine</td>
<td>Minced cilantro root, fish sauce,</td>
</tr>
<tr>
<td><strong>Chicken Fajitas</strong></td>
<td></td>
<td>Chili Powder</td>
</tr>
</tbody>
</table>
Appendix B. Reference sample preparation for the sensory evaluation

<table>
<thead>
<tr>
<th>Samples</th>
<th>Sensory characteristics</th>
<th>Low intensity preparation</th>
<th>Higher intensity preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>Raw skin color</td>
<td>The visually lightest raw drumstick skins which were available, stored in water at 4°C for 18 hr</td>
<td>The visually darkest raw drumstick skins which were available, stored at 4°C for 18 hr</td>
</tr>
<tr>
<td></td>
<td>Raw muscle color</td>
<td>The visually lightest raw drumstick muscles which were available, stored in water at 4°C for 18 hr</td>
<td>The visually darkest raw drumstick muscles which were available, stored at 4°C for 18 hr</td>
</tr>
<tr>
<td></td>
<td>Firmness and less separation of skin and muscle</td>
<td>Raw drumsticks tumbled with water for 20 min, then stored at 4°C for 17 hr 40 min, and had the lowest firmness and most separation of skin and muscle scores.</td>
<td>Raw drumsticks stored at 4°C for 18 hr without any tumbling treatment, and had the highest firmness and least separation of skin and muscle scores.</td>
</tr>
<tr>
<td></td>
<td>Raw marinated chicken aroma&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Raw drumstick stored in water at 4°C for 18 hr, and had the lowest intensity of marinated chicken aroma</td>
<td>Drumstick marinated in the std. marinated solution&lt;sup&gt;3&lt;/sup&gt; at 4°C for 18 hr, and had the highest intensity of marinated chicken aroma.</td>
</tr>
<tr>
<td>Cooked</td>
<td>Cooked skin color</td>
<td>The visually lightest raw drumstick skins which were available, stored in water at 4°C for 18 hr, then cooked by the std. cooking method&lt;sup&gt;4&lt;/sup&gt;</td>
<td>The visually darkest raw drumstick skins which were available, stored at 4°C for 18 hr, then cooked by the std. cooking method&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cooked muscle color</td>
<td>The visually lightest raw drumstick muscles which were available, stored in water at 4°C for 18 hr, then cooked by the std. cooking method&lt;sup&gt;4&lt;/sup&gt;</td>
<td>The visually darkest raw drumstick muscles which were available, stored at 4°C for 18 hr, then cooked by the std. cooking method&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Firmness and less separation of skin and muscle</td>
<td>Raw drumsticks tumbled with water for 20 min, then cooked at 4°C, then cooked by the std. cooking method&lt;sup&gt;4&lt;/sup&gt;, and had the lowest firmness and most separation of skin and muscle scores.</td>
<td>Raw drumstick without any tumbling treatment, cooked by the std. cooking method&lt;sup&gt;4&lt;/sup&gt;, and had the highest firmness and least separation of skin and muscle scores.</td>
</tr>
<tr>
<td></td>
<td>Cooked marinated chicken flavor&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Raw drumstick stored in water at 4°C for 18 hr, then cooked by the std. cooking method&lt;sup&gt;4&lt;/sup&gt;, and had the lowest intensity of marinated chicken flavor</td>
<td>Drumstick marinated in the std. marinated solution&lt;sup&gt;3&lt;/sup&gt; at 4°C for 18 hr, then cooked by the std cooking method&lt;sup&gt;4&lt;/sup&gt;, and had the highest intensity of marinated chicken flavor</td>
</tr>
<tr>
<td></td>
<td>Juiciness</td>
<td>Raw drumstick cooked until the internal temp of 85°C, and had the lowest juiciness scores</td>
<td>Raw drumstick without any treatment, cooked by the std. cooking method&lt;sup&gt;4&lt;/sup&gt; (until the internal temp of 70°C), and had the highest juiciness scores</td>
</tr>
<tr>
<td></td>
<td>Tenderness</td>
<td>Raw drumstick cooked until the internal temp of 85°C, and had the lowest tenderness scores</td>
<td>Raw drumstick without any treatment, cooked by the std. cooking method&lt;sup&gt;4&lt;/sup&gt; (until the internal temp of 70°C), and had the highest tenderness scores</td>
</tr>
</tbody>
</table>

<sup>1</sup>Raw marinated chicken aroma: the combined aroma consisted of fresh chicken + salt + acid + spices (ground black pepper & garlic powder)

<sup>2</sup>Cooked marinated chicken flavor: the combined flavor consisted of cooked chicken + salt + acid + spices (ground black pepper & garlic powder)

<sup>3</sup>Standardized marinated solution: adding 1% acetic acid and 3% salt, adjusted to pH 4, then adding 0.3% ground black pepper and 0.15% garlic pepper

<sup>4</sup>Standardized cooking method: drumsticks cooked in a preheat (temperature set at 350°F) oven until the internal temp of the samples achieves 70°C
Appendix C. Sensory evaluation form (for Experiments 5, 7, and 8)

Name: ________________  Date: ____________

- Please evaluate each sample and **score (1 to 9)** each sensory attribute that represents your response.

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Raw samples</th>
<th>Cooked samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin color</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle color</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marinated chicken aroma(^1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Off-aroma(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin color</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle color</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marinated chicken flavor(^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Off-flavor(^4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juiciness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenderness</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>1 = light</th>
<th>1 = light</th>
<th>1 = weak</th>
<th>1 = weak</th>
<th>1 = light</th>
<th>1 = light</th>
<th>1 = weak</th>
<th>1 = weak</th>
<th>1 = not juicy</th>
<th>1 = not tender</th>
<th>9 = dark</th>
<th>9 = dark</th>
<th>9 = strong</th>
<th>9 = strong</th>
<th>9 = dark</th>
<th>9 = dark</th>
<th>9 = strong</th>
<th>9 = strong</th>
<th>9 = very juicy</th>
<th>9 = very tender</th>
</tr>
</thead>
<tbody>
<tr>
<td>240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Marinated chicken aroma: the combination aroma consisted of chicken, sour (acidic), salty, peppery, and garlic aromas.

\(^2\)Off-aroma: any aroma that was not expected from the raw marinated chicken aroma.

\(^3\)Marinated chicken flavor: the combination flavor consisted of chicken, sour (acidic), salty, peppery, and garlic flavors.

\(^4\)Off-flavor: any flavor that was not expected from the cooked marinated chicken flavor.
Appendix D. Sensory evaluation form (for Experiment 6)

Name: __________________  Date: __________

- Please evaluate each sample and score (1 to 9) each sensory attribute that represents your response.

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Raw samples</th>
<th>Cooked samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin color</td>
<td>1 = light</td>
<td>1 = weak</td>
</tr>
<tr>
<td>Muscle color</td>
<td>1 = light</td>
<td>1 = light</td>
</tr>
<tr>
<td>Firmness and separation of skin &amp; muscle</td>
<td>1 = soft, more separation</td>
<td>1 = light</td>
</tr>
<tr>
<td>Marinated chicken aroma</td>
<td>1 = weak</td>
<td>1 = light</td>
</tr>
<tr>
<td>Off-aroma</td>
<td>1 = weak</td>
<td>1 = soft, more separation</td>
</tr>
<tr>
<td>Skin color</td>
<td>1 = light</td>
<td>1 = weak</td>
</tr>
<tr>
<td>Muscle color</td>
<td>1 = light</td>
<td>1 = light</td>
</tr>
<tr>
<td>Firmness and separation of skin &amp; muscle</td>
<td>1 = soft, more separation</td>
<td></td>
</tr>
<tr>
<td>Marinated chicken flavor</td>
<td>1 = weak</td>
<td>1 = weak</td>
</tr>
<tr>
<td>Off-flavor</td>
<td>1 = weak</td>
<td>1 = not &amp;&amp;</td>
</tr>
<tr>
<td>Juiciness</td>
<td>1 = not</td>
<td>1 = not</td>
</tr>
<tr>
<td>Tenderness</td>
<td>1 = not</td>
<td>1 = not</td>
</tr>
</tbody>
</table>

### Notes:

1. Marinated chicken aroma: the combination aroma consisted of chicken, sour (acidic), salty, peppery, and garlic aromas.
2. Off-aroma: any aroma that was not expected from the raw marinated chicken aroma.
3. Marinated chicken flavor: the combination flavor consisted of chicken, sour (acidic), salty, peppery, and garlic flavors.
4. Off-flavor: any flavor that was not expected from the cooked marinated chicken flavor.
Appendix E. Sensory evaluation results for the reference samples used in Experiment 5: nisin + thermal treatment

<table>
<thead>
<tr>
<th>Raw samples</th>
<th>Cooked samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin color</td>
<td>Skin color</td>
</tr>
<tr>
<td>Light</td>
<td>Dark</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

1The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1 = light, 9 = dark), juiciness (1 = not juicy, 9 = very juicy), and tenderness (1 = not tender, 9 = very tender) by a panel of seven members that was consisted of faculty and graduate students majoring in meat science. The scores were then averaged.

2Reference samples: Preparation of each individual reference sample followed the description in Appendix B.

3Skin color: visually evaluated by the sensory panel

4Muscle color: visually evaluated by the sensory panel

5Marinated chicken aroma: the combination aroma consisted of chicken, sour (acidic), salty, peppery and garlic aromas.

6Marinated chicken flavor: the combination flavor consisted of chicken, sour (acidic), salty, peppery, and garlic flavors.

7Light: visually light (in color) sample used for references.

8Dark: visually dark (in color) sample used for references.

9Weak: sensory weak (in aroma or flavor) sample used for references.

10Strong: sensory strong (in aroma or flavor) sample used for references.

11Not juicy: sensory not juicy sample used for references.

12Very juicy: sensory very juicy sample used for references.

13Not tender: sensory not tender sample used for references.

14Very tender: sensory very tender sample used for references.
Appendix F. Sensory evaluation\(^1\) results for the reference samples\(^2\) used in Experiment 6: nisin + tumbling

### Raw samples

<table>
<thead>
<tr>
<th></th>
<th>Skin color(^7)</th>
<th>Muscle color(^8)</th>
<th>Firmness and separation of skin and muscle(^9)</th>
<th>Marinated chicken aroma(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light(^7)</td>
<td>Dark(^8)</td>
<td>Soft, more separation between skin and muscle</td>
<td>Weak(^9)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

|                  | Light\(^7\)     | Dark\(^8\)        | Firm, less separation between skin and muscle  | Strong\(^10\)               |
|                  | 7               | 2                  | 5                                              | 5                           |

### Cooked samples

<table>
<thead>
<tr>
<th></th>
<th>Skin color(^7)</th>
<th>Muscle color(^8)</th>
<th>Firmness and separation of skin and muscle(^9)</th>
<th>Marinated chicken flavor(^11)</th>
<th>Juiciness</th>
<th>Tenderness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light(^7)</td>
<td>Dark(^8)</td>
<td>Soft, more separation between skin and muscle</td>
<td>Weak(^9)</td>
<td>Not juicy(^12)</td>
<td>Not tender(^14)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

|                  | Light\(^7\)     | Dark\(^8\)        | Firm, less separation between skin and muscle  | Strong\(^10\)               | Very juicy\(^13\) | Very tender\(^15\) |
|                  | 6               | 3                  | 6                                              | 4                            | 6         | 4          |

\(^1\)The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1 = light, 9 = dark), juiciness (1 = not juicy, 9 = very juicy), and tenderness (1 = not tender, 9 = very tender) by a panel of seven members that was consisted of faculty and graduate students majoring in meat science. The scores were then averaged.

\(^2\)Reference samples: Preparation of each individual reference sample followed the description in Appendix B.

\(^3\)Skin color: visually evaluated by the sensory panel.

\(^4\)Muscle color: visually evaluated by the sensory panel.

\(^5\)Firmness and separation of skin and muscle: visually evaluated by the sensory panel.

\(^6\)Marinated chicken aroma: the combination aroma consisted of chicken, sour (acidic), salty, peppery and garlic aromas.

\(^7\)Light: visually light (in color) sample used for references.

\(^8\)Dark: visually dark (in color) sample used for references.

\(^9\)Weak: sensory weak (in aroma or flavor) sample used for references.

\(^10\)Strong: sensory strong (in aroma or flavor) sample used for references.

\(^11\)Marinated chicken flavor: the combination flavor consisted of chicken, sour (acidic), salty, peppery, and garlic flavors.

\(^12\)Not juicy: sensory not juicy sample used for references.

\(^13\)Very juicy: sensory very juicy sample used for references.

\(^14\)Not tender: sensory not tender sample used for references.

\(^15\)Very tender: sensory very tender sample used for references.
Appendix G. Sensory evaluation\(^1\) results for the reference samples\(^2\) used in Experiment 7: nisin + lactoperoxidase system

<table>
<thead>
<tr>
<th>Raw samples</th>
<th>Cooked samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin color(^3)</td>
<td>Muscle color(^4)</td>
</tr>
<tr>
<td>Light(^7)</td>
<td>Dark(^8)</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^1\)The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1 = light, 9 = dark), juiciness (1 = not juicy, 9 = very juicy), and tenderness (1 = not tender, 9 = very tender) by a panel of seven members that was consisted of faculty and graduate students majoring in meat science. The scores were then averaged.

\(^2\)Reference samples: Preparation of each individual reference sample followed the description in Appendix B.

\(^3\)Skin color: visually evaluated by the sensory panel

\(^4\)Muscle color: visually evaluated by the sensory panel

\(^5\)Marinated chicken aroma: the combination aroma consisted of chicken, sour (acidic), salty, peppery and garlic aromas.

\(^6\)Marinated chicken flavor: the combination flavor consisted of chicken, sour (acidic), salty, peppery, and garlic flavors.

\(^7\)Light: visually light (in color) sample used for references.

\(^8\)Dark: visually dark (in color) sample used for references.

\(^9\)Weak: sensory weak (in aroma or flavor) sample used for references.

\(^10\)Strong: sensory strong (in aroma or flavor) sample used for references.

\(^11\)Not juicy: sensory not juicy sample used for references.

\(^12\)Very juicy: sensory very juicy sample used for references.

\(^13\)Not tender: sensory not tender sample used for references.

\(^14\)Very tender: sensory very tender sample used for references.
Appendix H. Sensory evaluation results for the reference samples used in Experiment 8: lactoperoxidase + thermal treatment

<table>
<thead>
<tr>
<th>Raw samples</th>
<th></th>
<th>Cooked samples</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skin color$^3$</td>
<td>Muscle color$^4$</td>
<td>Marinated chicken aroma$^5$</td>
</tr>
<tr>
<td></td>
<td>Light$^7$</td>
<td>Dark$^8$</td>
<td>Light$^7$</td>
</tr>
<tr>
<td>Marinated chicken</td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

1The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity, respectively, for all attributes except for color (1 = light, 9 = dark), juiciness (1 = not juicy, 9 = very juicy), and tenderness (1 = not tender, 9 = very tender) by a panel of seven members that was consisted of faculty and graduate students majoring in meat science. The scores were then averaged.

2Reference samples: Preparation of each individual reference sample followed the description in Appendix B.

3Skin color: visually evaluated by the sensory panel.

4Muscle color: visually evaluated by the sensory panel.

5Marinated chicken aroma: the combination aroma consisted of chicken, sour (acidic), salty, peppery and garlic aromas.

6Marinated chicken flavor: the combination flavor consisted of chicken, sour (acidic), salty, peppery, and garlic flavors.

7Light: visually light (in color) sample used for references.

8Dark: visually dark (in color) sample used for references.

9Weak: sensory weak (in aroma or flavor) sample used for references.

10Strong: sensory strong (in aroma or flavor) sample used for references.

11Not juicy: sensory not juicy sample used for references.

12Very juicy: sensory very juicy sample used for references.

13Not tender: sensory not tender sample used for references.

14Very tender: sensory very tender sample used for references.
Appendix I. Instrumental and sensory color evaluation results for the reference samples used in Experiment 5: nisin + thermal treatment

<table>
<thead>
<tr>
<th>Skin</th>
<th>Instrumental evaluation</th>
<th>Sensory evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw samples</td>
<td>Cooked samples</td>
</tr>
<tr>
<td>Light¹</td>
<td>Dark¹</td>
<td>Light¹</td>
</tr>
<tr>
<td>L*</td>
<td>a*</td>
<td>b*</td>
</tr>
<tr>
<td>76.10</td>
<td>3.68</td>
<td>5.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Instrumental evaluation</th>
<th>Sensory evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw samples</td>
<td>Cooked samples</td>
</tr>
<tr>
<td>Light¹</td>
<td>Dark¹</td>
<td>Light¹</td>
</tr>
<tr>
<td>L*</td>
<td>a*</td>
<td>B*</td>
</tr>
<tr>
<td>75.93</td>
<td>8.75</td>
<td>9.47</td>
</tr>
</tbody>
</table>

¹Instrumental evaluation: The Hunter "L*" (lightness), "a*" (redness-greenness), and "b*" (yellowness-blueness) values of drumsticks were measured using a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey). Three measurements were made at different locations on the drumsticks and averaged. A standard plate (number 18433010) with "Y" = 92.60, "x" = 0.3140, and "y" = 0.3206 was used as a reference.

²Sensory evaluation: The panel of seven members consisted of faculty and graduate students majoring in meat science used a 1-9 scale (1 = light, 9 = dark) to evaluate the colors of the samples. The scores were then averaged.

³Light: Visually light (in color) sample used for references.

⁴Dark: Visually dark (in color) sample used for references.
Appendix J. Instrumental and sensory color evaluation results for the reference samples used in Experiment 6: nisin + tumbling

<table>
<thead>
<tr>
<th>Skin</th>
<th>Instrumental evaluation</th>
<th>Sensory evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light¹</td>
<td>Dark¹</td>
</tr>
<tr>
<td>Raw samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>L* a* b*</td>
<td>L* a* b*</td>
</tr>
<tr>
<td></td>
<td>79.44 3.00 5.21</td>
<td>69.93 5.36 11.76</td>
</tr>
<tr>
<td>Cooked samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>L* a* b*</td>
<td>L* a* b*</td>
</tr>
<tr>
<td></td>
<td>80.27 7.95 11.03</td>
<td>63.58 17.77 12.37</td>
</tr>
</tbody>
</table>

¹Instrumental evaluation: The Hunter "L*" (lightness), "a*" (redness-greenness), and "b*" (yellowness-blueness) values of drumsticks were measured using a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey). Three measurements were made at different locations on the drumsticks and averaged. A standard plate (number 18433010) with "Y" = 92.60, "x" = 0.3140, and "y" = 0.3206 was used as a reference.

²Sensory evaluation: The panel of seven members consisted of faculty and graduate students majoring in meat science used a 1-9 scale (1 = light, 9 = dark) to evaluate the colors of the samples. The scores were then averaged.

³Light: Visually light (in color) sample used for references.

⁴Dark: Visually dark (in color) sample used for references.
Appendix K. Instrumental and sensory color evaluation results for the reference samples used in Experiment 7: nisin + lactoperoxidase system

<table>
<thead>
<tr>
<th></th>
<th>Skin</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Instrumental evaluation</td>
<td>Instrumental evaluation</td>
</tr>
<tr>
<td></td>
<td>Raw samples</td>
<td>Cooked samples</td>
</tr>
<tr>
<td></td>
<td>Light¹</td>
<td>Dark¹</td>
</tr>
<tr>
<td>L*</td>
<td>a*</td>
<td>b*</td>
</tr>
<tr>
<td>79.30</td>
<td>2.72</td>
<td>5.36</td>
</tr>
<tr>
<td>L*</td>
<td>69.90</td>
<td>5.63</td>
</tr>
<tr>
<td>a*</td>
<td>2.98</td>
<td>23.55</td>
</tr>
<tr>
<td>b*</td>
<td>61.53</td>
<td>3.32</td>
</tr>
<tr>
<td>24.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>62.90</td>
<td>12.24</td>
</tr>
<tr>
<td>a*</td>
<td>2.98</td>
<td>6.80</td>
</tr>
<tr>
<td>b*</td>
<td>59.47</td>
<td>17.84</td>
</tr>
<tr>
<td>L*</td>
<td>71.99</td>
<td>4.20</td>
</tr>
<tr>
<td>a*</td>
<td>59.47</td>
<td>17.84</td>
</tr>
<tr>
<td>b*</td>
<td>71.99</td>
<td>4.20</td>
</tr>
<tr>
<td>26.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Instrumental evaluation: The Hunter "L*" (lightness), "a*" (redness-greenness), and "b*" (yellowness-blueness) values of drumsticks were measured using a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey). Three measurements were made at different locations on the drumsticks and averaged. A standard plate (number 18433010) with "Y" = 92.60, "x" = 0.3140, and "y" = 0.3206 was used as a reference.

²Sensory evaluation: The panel of seven members consisted of faculty and graduate students majoring in meat science used a 1-9 scale (1 = light, 9 = dark) to evaluate the colors of the samples. The scores were then averaged.

³Light: Visually light (in color) sample used for references.

⁴Dark: Visually dark (in color) sample used for references.
Appendix L. Instrumental and sensory color evaluation results for the reference samples used in Experiment 8: lactoperoxidase + thermal treatment

<table>
<thead>
<tr>
<th>Skin</th>
<th>Instrumental evaluation</th>
<th>Sensory evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw samples</td>
<td>Cooked samples</td>
</tr>
<tr>
<td></td>
<td>Light&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Dark&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>L*</td>
<td>A*</td>
<td>b*</td>
</tr>
<tr>
<td>75.74</td>
<td>3.71</td>
<td>7.69</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Instrumental evaluation</th>
<th>Sensory evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw samples</td>
<td>Cooked samples</td>
</tr>
<tr>
<td></td>
<td>Light&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Dark&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>L*</td>
<td>a*</td>
<td>b*</td>
</tr>
<tr>
<td>72.90</td>
<td>8.87</td>
<td>7.01</td>
</tr>
</tbody>
</table>

<sup>1</sup>Instrumental evaluation: The Hunter "L*" (lightness), "a*" (redness-greenness), and "b*" (yellowness-blueness) values of drumsticks were measured using a Chroma Meter (model CR-300, Minolta Co., Ltd. Ramsey, New Jersey). Three measurements were made at different locations on the drumsticks and averaged. A standard plate (number 18433010) with "Y" = 92.60, "x" = 0.3140, and "y" = 0.3206 was used as a reference.

<sup>2</sup>Sensory evaluation: The panel of seven members consisted of faculty and graduate students majoring in meat science used a 1-9 scale (1 = light, 9 = dark) to evaluate the colors of the samples. The scores were then averaged.

<sup>3</sup>Light: Visually light (in color) sample used for references.

<sup>4</sup>Dark: Visually dark (in color) sample used for references.
REFERENCES


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