



Sensory, Physicochemical and Microbiological Changes in Water-cooked Salted Duck during Storage at 4°C*

Yanliang Li^{1,2}, Dongrui Yao¹, Daoying Wang¹, Weimin Xu^{1,**}, Yongzhi Zhu¹ and Bangquan Jin²

¹ Institute of Agricultural Products Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

ABSTRACT : Water-cooked salted ducks were tray-packaged and stored under refrigeration (4±1°C) in order to evaluate the quality changes during storage. pH, total volatile basic nitrogen (TVB-N), sensory and microbiological analysis were determined at 0, 2, 4, 6, 8, 9 and 10 days of storage. pH value and TVB-N (mg N/100 g) varied from 6.47±0.16 to 6.69±0.10 and from 5.90±0.93 to 13.42±2.46, respectively. Sensory results indicated that ducks were unacceptable at the 10th day of storage. The predominant spoilage bacteria at the end of the shelf-life were *Brochothrix thermosphacta*, lactic acid bacteria (LAB) and minor components were *Enterobacteriaceae*, members of *Micrococci*, yeasts and moulds. *Pseudomonads* were also detected. Both total bacteria and the various spoilage ones, overall, increased from the initial sampling to the final day. (**Key Words :** Sensory, pH, Total Volatile Basic Nitrogen, Spoilage Bacteria)

INTRODUCTION

Cooked duck products are popular in China for their delicate flavour and nutritional value. About thirty million ducks are consumed annually just in Nanjing city (Liu et al., 2007). Nanjing water-cooked salted duck, one of the low temperature meat products in China, is famous for its delicate flavor, tenderness and texture.

Meat quality is determined by using microbiological, chemical and sensory analyses (Dainty et al., 1996). Studies have revealed that meat spoilage at low temperatures conditions, is mainly the result of growth of Gram-negative, psychrotrophic, aerobic rods dominated by *Pseudomonas spp.* Other organisms, including *Brochothrix thermosphacta*, lactic acid bacteria (LAB) and cold tolerant *Enterobacteriaceae* can also grow but they usually account for a small proportion of the total flora (Dainty and Mackey, 1992; Garcia-lopez et al., 1998).

Studies on cooked duck products have been concerned largely on the flavour and odour (Liu et al., 2007; Xu et al.,

2008; Wang et al., 2009). Little literature, however, is focused on the microflora development during storage in Nanjing water-cooked salted duck. The present study sought to determine the microflora in different storage stage in traditional Chinese Nanjing water-cooked salted duck. The changes of pH, total volatile basic nitrogen (TVB-N, mg N/100 g) and sensory characteristics of duck meat during storage were also determined.

MATERIALS AND METHODS

Production and sample preparation

Water-cooked salted ducks were prepared by a local meat factory (Nanjing Sweet-scented Osmanthus Duck Ltd.) using the conventional method.

Cherry Valley ducks from a commercial feedlot were slaughtered humanly, each of which was about 1.5 kg. After chilling for 2 h, ducks were processed by dry-cured, brining, roasting and boiling. Each duck was dry-cured using 100 g stir-fried salt with *Illicium verum* Hook.f (Beijing Meiquan) for 2 h. The brining process was 4 h, roasting process was 1 h at 90°C for drying the carcass and decreasing the subcutaneous lipid. Low boiling temperature from 85-90°C was used for 40min for tender taste (Liu et al., 2006).

After being tray-packaged with polyethylene membrane (120 µm thickness), 21 ducks were randomly selected and transported to the laboratory in ice boxes within 2 h, and were stored under refrigeration (4±1°C). Three ducks were

* This study was funded by Natural Science Foundation Program of Jiangsu Province (BK2009329) and National Agricultural Transform Program of China (2009GB2 C100137).

** Corresponding Author: Weimin Xu. Tel: +86-25-84390065, Fax: +86-25-84390065, E-mail: weiminxu2002@yahoo.com.cn

² Food Science and Nutrition Department, Nanjing Normal University, Nanjing 210097, China.

Received October 22, 2009; Accepted December 31, 2009

selected as sample at every sampling day. Samples were analyzed at predetermined time intervals, namely at 0, 2, 4, 6, 8, 9 and 10 days of storage.

Physicochemical analysis

The pH value was recorded using a Hanna HI9025C pH meter (Italy). Ten grams of duck sample were homogenized in 20 ml distilled water and the homogenate was used for pH determination. TVB-N (total volatile basic nitrogen) was determined according to the method proposed by GB/T 5009.44-1996 (Ministry of Health of the P.R. China, 1996). TVB-N content was expressed as mg/100 g duck sample.

Sensory evaluation

Each sampling day, duck samples were evaluated for appearance of the muscle and skin, odour and taste by a panel of five judges, who were well trained according to standard ISO 8586-1 (1993). Acceptability as a composite of taste, appearance and odour was estimated using a scale ranging from 1 to 9. The scale points were: excellent, 9; very good, 8; good, 7; acceptable, 6; poor (first off-odour, off-taste development) <6; a score of 6 was taken as the low limit of acceptability. The product was defined as unacceptable after development of first off-odour or off-taste.

Microbiological analysis

Using aseptic techniques, 25 g sample was homogenized in 225 ml sterile peptone saline (0.1% peptone and 0.85% NaCl). After shaking at 230 rpm for 10 min in a stomacher, serial decimal dilutions were prepared and 1 or 0.1 ml samples of appropriate dilutions were poured or spread on agar plates.

One milliliter dilutions were inoculated onto plate count agar (PCA, LuQiao, Beijing, China) to obtain the total viable count, and onto de Man Rogosa Sharpe agar (MRS Agar, LuQiao, Beijing, China) to count lactic acid bacteria (LAB). Plates were then incubated for 48 h at 37°C and 30°C, respectively. For members of the family *Enterobacteriaceae*, 0.1 ml sample was inoculated into the molten (45°C) violet red bile dextrose agar (VRBDA, LuQiao, Beijing, China). After setting, a 10 ml overlay of molten medium was added and incubated at 37°C for 72 h. Pseudomonads numbers were determined on *pseudomonas* isolation agar (LuQiao, Beijing, China) after incubation at 25°C for 3 days. *Brochothrix thermosphacta* was determined on streptomycin sulphate-thallos acetate-cycloheximide (actidione) agar, prepared from basic ingredients in the laboratory after incubation at 25°C for 2 days. Yeasts and moulds were enumerated using potato dextrose agar (PDA, LuQiao, Beijing, China) after incubation at 25°C for 5 days. *Micrococci* were enumerated on mannitol salt agar (MSA, LuQiao, Beijing, China) after

incubation at 37°C for 48 h. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. In addition, the selectivity of each medium was checked routinely by Gram staining and microscopic examination of smears prepared from randomly selected colonies from all of the media (BAM, 1998).

Statistical analysis

Experiments were three replicated on different occasions with different duck samples. Using software SAS9.0 (2002, USA), physicochemical and microbiological data were statistically analysed, means and standard deviations were calculated, and, when F-values were significant at the $p < 0.05$ level, mean differences were separated by the least significant difference (LSD) procedure. Microbiological counts were converted to log cfu/g and were subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Physicochemical changes

Many physical and chemical methods have been suggested as indices of deterioration of meat quality during storage. Chemical tests usually measure the amounts of breakdown products derived from enzymatic, bacterial or oxidative activities. The assay of some of these substances usually provides useful data for the evaluation of meat freshness or quality. In the present work, the potential chemical quality indicators assessed to determine the chemical changes in water-cooked salted duck during refrigeration ($4 \pm 1^\circ\text{C}$) were pH and TVB-N. Figure 1 shows the average values for pH and TVB-N analyzed on each sampling day.

The pH varied between 6.47 and 6.68 without any significant differences ($p > 0.05$). As dissolved CO_2 is acidic, lower pH values in salted duck samples would be expected

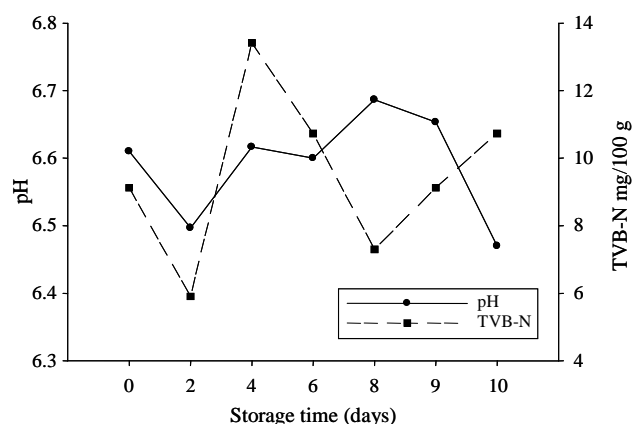


Figure 1. Changes in pH and TVB-N value of water-cooked salted duck during storage at $4 \pm 1^\circ\text{C}$.

Table 1. Sensory evaluation of water-cooked salted duck during storage at 4±1°C

Days of storage	0	2	4	6	8	9	10
Muscle appearance	9.00±0.00 ^a	8.52±0.08 ^b	7.96±0.12 ^c	7.32±0.08 ^d	7.05±0.15 ^e	6.42±0.10 ^f	5.93±0.15 ^g
Skin appearance	9.00±0.00 ^a	8.54±0.15 ^b	7.97±0.10 ^c	7.30±0.05 ^d	7.02±0.08 ^e	7.03±0.15 ^e	6.23±0.15 ^f
Odour	9.00±0.00 ^a	8.56±0.08 ^b	8.00±0.15 ^c	7.35±0.05 ^d	6.93±0.06 ^e	6.42±0.19 ^f	5.80±0.10 ^g
Taste	9.00±0.00 ^a	8.59±0.13 ^b	7.92±0.25 ^c	7.30±0.10 ^d	7.03±0.06 ^e	6.33±0.21 ^f	5.95±0.10 ^g

Means±standard deviation.

Data within the same row with different letters are significantly ($p<0.05$) different.

with the growth of lactic acid bacteria and the formation of lactic acid. It has been suggested by Gill (1988) that the solubility of CO₂ in meat should increase with increasing pH, therefore, since changes in pH during storage were not statistically significant, the CO₂ solubility in water-cooked salted duck is likely to be variable.

TVB-N is the traditional chemical means most widely used for evaluation of the degree of spoilage in meat. The initial TVB-N was 9.12 mg/100 g duck and showed significant differences ($p<0.05$) between days 0 and 4. From day 6 onwards, no significant differences ($p>0.05$) were recorded. During storage, slight decrease in the TVB-N value was first noticed, then significantly increased ($p<0.05$) by the day 4. The slight decrease in TVB-N during the first stage of storage may be initiated by autolytic degradation of nucleotides and free amino acids while the subsequent changes in TVB-N during the later stage of storage is most likely caused by a combination of microbiological and autolytic activities.

Sensory analysis

Table 1 shows the average scores given by the panelists to each of the attributes examined on each sampling day. All sensory parameters were correlated with time significantly. The initial quality characteristics of the water-cooked salted duck were very bright muscle and skin appearance, fresh odours and delicate taste. The acceptable shelf life was found to be 10 days for water-cooked salted duck (odour and taste score less than 6). According to the scale used, muscle appearance, odour and taste shown significant differences ($p<0.05$) among each sampling day. The scale of skin appearance became bigger from day 8 to 9 and then decreased sharply.

Microbiological changes

Microbial profiles in water-cooked salted duck during storage at 4±1°C are shown in Figure 2. The initial (day 0) total viable count (2.86 log cfu/g) indicates that the water-cooked salted duck was of a good quality. Then the count

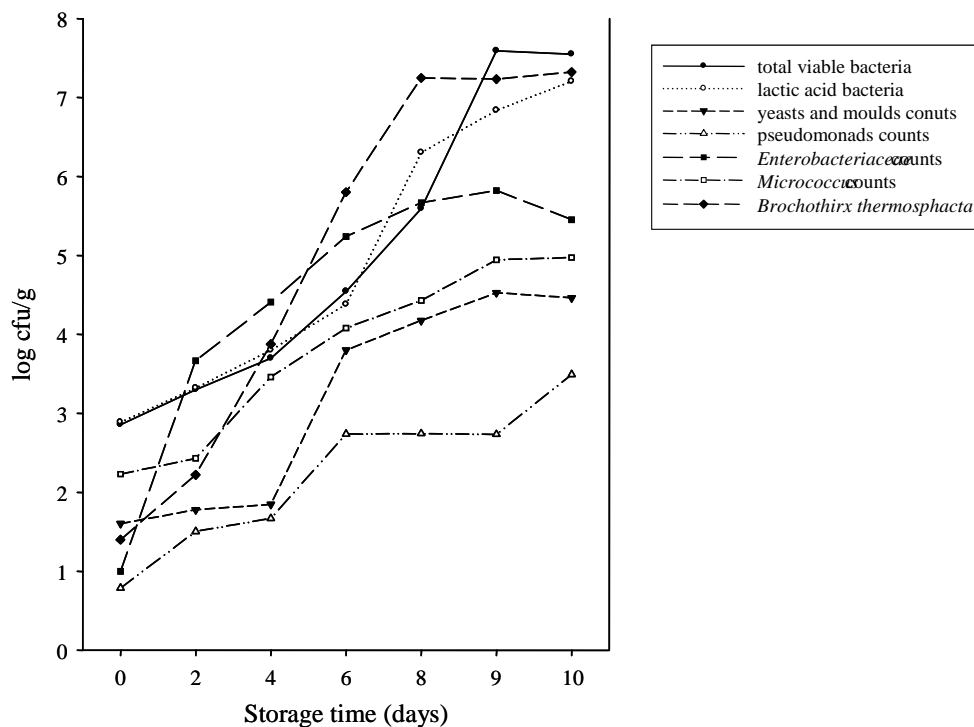


Figure 2. Microbial profile of water-cooked salted duck stored at 4±1°C for 10 days.

increased up to day 9 and then slightly decreased (Figure 2). The maximum level of bacteria during refrigerated storage of meat is 10^7 - 10^9 cfu/cm², and of meat products about 10^7 - 10^8 cfu/g (Elisabeth Borch et al., 1996). In our study, this count was not reached until day 9 (for total aerobic count and *Brochothrix thermosphacta*) and 10 (for LAB).

The count of *B. thermosphacta*, which was the predominant spoilage bacteria during last stage, increased sharply from day 0 to 8, and increased slightly to day 9 and 10. Available information indicates that *B. thermosphacta*, which is common to the meat habitat, originates in soil and faeces and is introduced into slaughterhouses from these sources. Its ability to grow at low temperatures and at low water activity favours the subsequent proliferation (Skovgaard, 1985).

It is well established that strains of LAB is the major group of spoilage bacteria developing on various types of meat and meat products (Gill and Newton, 1978; Schillinger and Lücke, 1987; Yang and Ray, 1994; Bjorkroth and Korkeala, 1996; Samelis et al., 2000). The present study indicated that LAB was also the predominant spoilage bacteria of water-cooked salted duck during $4\pm 1^\circ\text{C}$ storage, which reached to 7.21 log cfu/g at day 10.

Count of *Enterobacteriaceae* was increasing from day 0 to 9 and then decreased to 5.45 log cfu/g at day 10. *Pseudomonas* spp. are the most common spoilage organisms, particularly in aerobically stored foods with a high water content and natural pH, e.g., red meat (Dainty and Mackey, 1992; Dainty, 1996; Jos et al., 1996) and poultry (Lahellec and Colin, 1979; Gallo et al., 1988; Regez et al., 1988). *Pseudomonas* spp., like most of the other Gram-negative rod shaped bacteria; usually comprise only a small proportion of the initial microflora of fresh foods. They are, however, widely distributed in the environment and may contaminate foods from many sources and are able to utilize a wide range of materials as substrates for growth. In our present study, *Pseudomonas*, which was not the predominant spoilage bacterial, increased from the very beginning to the last sampling day (Figure 2).

Micrococcus spp. are able to grow in the presence of salt and may be responsible for the spoilage of meat products, such as bacon producing slime, souring or pigment formation. Many strains are thermophilic and may survive pasteurization causing subsequent spoilage, particularly if the other spoilage organisms are eliminated by heat treatment (Jos and Huis in't Veld, 1996). In our study, *Micrococcus* increased slightly from day 0 (2.23 log cfu/g) to 10 (4.97 log cfu/g) (Figure 2).

Yeasts and moulds increased from day 0 (<2 log cfu/g) to 9 (4.53 log cfu/g) and then decreased to day 10 (4.46 log cfu/g) (Figure 2). According to previous research, yeasts and moulds can be found in a wide variety of environments, such as in plants, animal products, soil, water and insects

(Jos et al., 1996). This broad occurrence can be explained by the fact that yeasts and moulds can utilize a variety of substrates such as carbohydrates, organic acids, proteins and lipids. Moreover, yeasts and moulds are relatively tolerant to low pH, low water activity and low temperature.

CONCLUSIONS

The shelf-life of water-cooked salted duck was 10 day. pH value and total volatile basic nitrogen (TVB-N, mg N/100 g) varied from 6.47 ± 0.16 to 6.69 ± 0.10 and from 5.90 ± 0.93 to 13.42 ± 2.46 , respectively. The predominant spoilage bacteria were found to be *B. thermosphacta* and lactic acid bacteria at the end of the shelf-life.

To our knowledge, this is the first study reporting physicochemical and microbiological changes of water-cooked salted duck during the shelf-time. Further studies are needed with regard to preservation of water-cooked salted duck, which will maximize shelf-time, while at the same time maintaining good sensorial characteristics.

REFERENCES

- BAM. 1998. Bacteriological analytical manual (8th ed.). Gaithersburgh, MD: US Food and Drug Administration.
- Bjorkroth, J. and H. Korkeala. 1996. rRNA gene restriction patterns as a characterization tool for *Lactobacillus sake* strains producing rosy slime. *Int. J. Food Microbiol.* 30:293-302.
- Dainty, R. H. 1996. Chemical/biochemical detection of spoilage. *Int. J. Food Microbiol.* 33:19-33.
- Dainty, R. H. and B. M. Mackey. 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *J. Appl. Bacteriol. Symp. Suppl.* 21, 103S-114S.
- Daoying Wang, Weimin Xu, Xinglian Xu, Guanghong Zhou, Yongzhi Zhu and Chunbao Li. 2009. Determination of intramuscular phospholipid classes and molecular species in Gaoyou duck. *Food Chem.* 112:150-155.
- Elisabeth Borch, Marie-Louise Kant-Muermans and Ylva Blixt. 1996. Bacterial spoilage of meat and cured meat products. *Int. J. Food Microbiol.* 33:103-120.
- Gallo, L., R. E. Schmitt and W. Schmidt-Lorcllz. 1988. Microbial spoilage of refrigerated fresh broilers. I. Bacterial flora and growth during storage. *Lebensm. Wiss. u.-Tcchnol.* 21:216-223.
- ISO 8586-1 1993. Sensory analysis. General guidance for the selection, training and monitoring of assessors. Part 1: Selected assessors.
- García-López, M. L., M. Prieto and A. Otero. 1998. The physiological attributes of Gram-negative bacteria associated with spoilage of meat and meat products. In: *The microbiology of meat and poultry* (Ed. A. Davies and R. G. Board) pp. 1-34. London: Blackie Academic and Professional.
- Gill, C. O. and K. G. Newton. 1978. The ecology of bacterial spoilage of fresh meat at chill temperatures. *Meat Sci.* 3:207-217.

- Jos, H. J. and Huis in't Veld. 1996. Microbial and biochemical spoilage of foods: an overview. *Int. J. Food Microbiol.* 33:1-18.
- Lahellec, C. and P. Colin. 1979. Bacterial flora of poultry. Changes due to variations in ecological conditions during processing and storage. *Arch. Lebensm. Hygiene*, 30:95-98.
- Samelis, J., A. Kakouri and J. Rementzia. 2000. Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4°C. *Food Microbiol.* 17:329-340.
- Schillinger, U. and F. K. Lücke. 1987. Identification of Lactobacilli from meat and meat products. *Food Microbiol.* 4: 199-208.
- Skovgaard, N. 1985. *Brochothrix thermosphacta*-comments on its taxonomy, ecology and isolation. *Int. J. Food Microbiol.* 2(1-2), 71-79.
- Ministry of Health of the P. R. China. 1996. Method for analysis of hygienic standard of meat products. GB/T 5009.44-1996 (in Chinese).
- Regez, P., L. Gallo, R. E. Schmitt and W. Schmidt-Lorenz. 1988. Microbial spoilage of refrigerated fresh broilers. III. Effect of storage temperature on the microbial association of poultry carcasses. *Lebensm. Wiss. u.-Technol.* 21:229-233.
- Weimin Xu, Xinglian Xu, Guanghong Zhou, Daoying Wang and Chunbao Li. 2008. Changes of intramuscular phospholipids and free fatty acids during the processing of Nanjing dry-cured duck. *Food Chem.* 110:279-284.
- Yang, R. and B. Ray. 1994. Factors influencing production of bacteriocins by lactic acid bacteria. *Food Microbiol.* 11:281-291.
- Yuan Liu, Xing-lian Xu and Guang-hong Zhou. 2007. Changes in taste compounds of duck during processing. *Food Chem.* 102(1):22-26.