大韓獸醫學會誌(2004) 第44卷 第2號
Korea J Vet Res(2004) 44(2) : 225~231

Bacteriological profiles of dressed broilers at different conditions and frozen storage periods

M.A. Ehsan, M.S. Rahman, Joon-Seok Chae[1], Seong-Kug Eo[1], Ki-Won Lee[1], In-Shik Kim[1], Hyun-A Yoon[1], and John-Hwa Lee[1]*

Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[1]College of Veterinary Medicine and Bio-Safety Research Institute, Chonbuk National University, Jeonju 561-756, Korea
(Accepted: May 10, 2004)

Abstract : This study was conducted to determine the incidence of microorganisms associated with dressed broiler with intact skin and without skin at different frozen storage periods such as 0, 10, 20, 30 days and to demonstrate the role of packaging and pretreatment chilling on the changes of carcass quality. The values of total viable count (TVC), total coliform count (TCC), total streptococcal count (TStC) and total staphylococcal count (TSC) were determined for meat samples of thigh and breast and swab samples of visceral surfaces of the broilers with intact skin and without skin. It was observed that the values of TVC, TCC, TStC and TSC in both cases of dressed broiler with intact skin and without skin exceeded the International Commission on Microbiological Specification for Foods. However, numbers of microorganisms were considerably decreased during the frozen storage. Packing and prechilled conditions were generally better effective in decrease of the loads of microorganisms than without packing and prechilled conditions, and lower bacterial numbers were also found in dressed broiler with intact skin than that without skin. The highest sensory panel score was obtained at 10 days of frozen storage. These results, thus, indicate that usages of appropriate periods and conditions of frozen storage and packaging systems can minimize the potential health hazards associated with contaminants gaining access to the dressed or processed broilers and improve the quality and shelf life of dressed broilers.

Key words : broiler, microorganism, frozen storage, packing, public health

Introduction

Microbial food safety for poultry products is an increasing public health concern worldwide. The last decade has seen an exponential increase in consumer demands for poultry and poultry products, fueled in part by dietary health considerations. Fears over microbiological food safety issues, in conjunction with consumer demand for a product of consistently high quality, have focused attention on the food production industry for microbiologically spoiled or contaminated meat [2].

Muscle food, such as poultry, is considered as spoiled if organoleptic changes make them unacceptable to the consumer. These organoleptic characteristics may include changes in appearance (discoloration), the development of off odors, slime formation, changes in taste, or any other characteristic which makes the food inappropriate for consumption [5, 6]. While endogenous enzymatic activity within muscle tissue postmortem can contribute to changes during storage [5, 12], it is generally accepted that detectable organoleptic spoilage is a result of decomposition and formation of metabolites caused by the growth of microorganisms [7, 10]. The organoleptic changes which take place also vary according to the species of microflora present, the characteristics

*Corresponding author: John-Hwa Lee
College of Veterinary Medicine and Bio-Safety Research Institute, Chonbuk National University, Jeonju 561-756, Korea
[Tel: +82-63-270-2553, Fax: +82-63-270-3780, E-mail: johnhlee@chonbuk.ac.kr]

of the meat, processing methods, product composition, and the environment in which the food is stored [5]. The poultry during processes of slaughter and dress carry initial contamination and there occurs biomagnification at all levels of handling, poor transport and retailing conditions [13].

Little is known about the microbiological aspects, shelf life, maintenance of quality and food safety of processed chicken meat. There is also little information available concerning with the interrelatedness among the status of microbial growth, sanitary quality and the quality maintenance of broilers stored at different conditions. The objective of this study is to determine the incidence of microorganisms associated with dressed broiler, to investigate distribution of selected microbial attributes related to the sanitary quality of dressed carcass, and to demonstrate the roles of packaging and pretreatment-chilling, which concerns with the changes in carcass quality.

Materials and Methods

A total number of 52 broilers were collected from the poultry farm of Bangladesh Agricultural University campus from May 2002 to September 2002. Poultry at similar ages were brought to convenient slaughter points and then killed. The participants immersed the slaughtered broilers in a special tank containing hot water for 5 minutes. The immersed birds were defeathered by plucking and subsequently were eviscerated. All these eviscerated birds were prepared for microbiological analysis. Twenty-six broilers remained with skin intact and the other 26 were kept without skin. The dressed birds with or without skin were then grouped into two classes. Class 1 dressed birds were prechilled at 2-3^{o}C for a period of 6 hours and then stored in frozen conditions at -20^{o}C while class 2 was directly frozen. At the time of storage all birds were divided again into two groups of packed and unpacked. The samples of this study included raw meat tissues and swab samples from the external surfaces of the skin and internal surface of abdominal cavities. Twenty-five grams of surface meat samples were aseptically excised from the regions of thigh and breast of carcasses and were macerated in a mechanical blender. As per recommendation of ICMSF [4] cotton wool swabs were used for this study. The swabs were prepared using non-absorbent cotton wool, which were wound roundly to a length of four centimeters and a thickness of 1 to1.5 centimeters on wooden sticks. They were placed in glass tubes, and were plugged and sterilized. Before use, the swabs were moistened with sterile 0.1% peptone water. These were rubbed firmly over the skin surface using parallel strokes with slow rotation and rounded the surface of the abdominal cavities using circular strokes. The surface to be examined was swabbed twice using parallel strokes at right angles to the first strokes and circular strokes. In case of abdominal cavity the whole area was swabbed. After swabbing, the swabs were transferred to the respective tubes containing ten milliliter of sterile 0.1% peptone water. Before examination, the swabs were agitated up and down in the tubes to assist the rinsing of bacteria from the surface.

Samples were collected aseptically in sterile containers and were brought to the laboratory within 30 minutes. During transportation the sterile containers were kept cool.

After collection, bacteriological analysis of the samples was performed under two major principal assessments [4, 9]: firstly, to evaluate the sanitary quality of meat, total viable count (TVC), total coliform count (TCC), total streptococcal count (TStC), total staphylococcal count (TSC) were determined by using nutrient agar, plate count agar, MacConkey agar, blood agar, Eosin-Methylene-blue agar, Salmonella-Shigella agar, crystal violate Blood agar, triple sugar iron agar, Streptococcus selective broth, and Staphylococcal Media No. 110 (SM-110) (Difco, Detroit, Mich., USA). Secondly, bacteria contaminating the meat samples were isolated and identified by using different biochemical tests such as the catalase test, coagulase test, haemolytic activity, oxidation-fermentation test, oxidase test, IMVIC Test, and API Identification system (Biomerieux, Lyon, France).

The degrees of correlation between the bacterial counts and the quality of stored birds were meassured by sensory tests [4]. The birds were examined organoleptically for defects of pigmentation in skin and muscles, firmness of meat, colour and odour. They were scored in a 0 to 10 point hedonic scale for quality: excellent, 10; good, 8; fair, 6; poor, 4; half-spoiled, 2; spoiled, 0. Grade A corresponded to 8 to 10 scores; Grade B , 4 to 7; and Grade C, 0 to 3. Birds with scores of 4 or less were considered unacceptable for human consumption.

Results and Discussion

Total viable count (TVC)

The data presented in Table 1 represent the mean values of TVC per gram for meat samples from breast and thigh regions of dressed broiler with/without skin for 0 day, 10 days, 20 days and 30 days of storage. The bacterial counts for the swab samples obtained from per square centimeter of visceral cavities are shown in Table 2. At storage conditions the counts of total viable bacteria were considerably decreased. The study of TVC per gram for meat samples from breast and thigh regions of dressed broiler without skin for 0 day, 10 days, 20 days and 30 days of storage revealed the similar results with slightly higher bacterial counts than those with intact skin. The slightly higher bacterial count was seen in meat tissues of thigh regions than in breast regions. Variations that occurred in TVC of dressed broilers kept at different prolonged storage periods were remarkable. The general reduction in numbers of organisms during storage might have occurred due to unfavorable low temperature, whereas the variation in counts may be due to difference in sanitary conditions practiced and handling of birds during dressing operations. The prechilling process was also found to be very effective in reducing bacterial load of dressed carcasses and this has been emphasized by many researchers [1, 8, 14, 15]. The bacterial counts were generally lower in packed condition than in unpacked condition, which implicated that packing broilers are more efficient in preventing microbial contamination during handling and storage and inhibiting growth of bacteria. This was also suggested by a previous report that packing had influence on shelf life of chicken carcasses during storage [11].

Total coliform count (TCC)

TCCs per gram for meat of dressed broiler from breast and thigh regions with/without skin for 0 day, 10 days, 20 days and 30 days of storage are shown in Table 3. The swab samples per square centimeter from visceral cavities are revealed in Table 4. For 30 days of storage, numbers of coliforms were detected from samples of muscles, skin or abdominal cavities at the lowest level in any condition. This indicated that frozen storage for this period significantly inhibit the growth of coliforms.

Table 1. Total viable plate counts (numbers in log) per gram for meat samples obtained from different regions of fresh and stored dressed broilers

Dressed broiler with	Sample region	For 0 day storage	For 10 day storage				For 20 day storage				For 30 day storage			
			Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen	
			P	UP	P	UP	P	UP	P	UP	P	UP	P	UP
Intact skin	Breast	5.95	2.21	2.38	2.34	2.56	1.89	1.95	1.92	2.01	1.54	1.81	1.69	1.91
	Thigh	5.47	2.52	2.64	2.60	2.69	2.04	2.09	1.95	1.97	1.78	1.90	1.81	1.92
No skin	Breast	6.50	2.35	2.63	2.61	2.68	2.02	2.13	2.09	2.24	1.83	1.92	1.95	2.04
	Thigh	6.65	2.42	2.69	2.69	2.78	2.25	2.39	2.50	2.59	1.87	2.01	2.09	2.26

P, Packed; UP, Unpacked

Table 2. Total viable plate counts (numbers in log) per cm^2 for swab samples obtained from external and internal surfaces of dressed broilers

Dressed broiler with	Sample region	For 0 day storage	For 10 day storage				For 20 day storage				For 30 day storage			
			Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen	
			P	UP	P	UP	P	UP	P	UP	P	UP	P	UP
Intact skin	Visceral	5.47	3.62	3.74	3.51	3.07	2.47	2.69	2.54	2.77	1.88	1.94	1.95	1.99
No skin	cavity	5.69	3.71	3.76	3.62	3.97	2.54	2.81	2.60	2.84	1.95	2.07	2.15	2.20

P, Packed; UP, Unpacked

Table 3. Total coliform counts (numbers in log) per gram for meat samples obtained from different regions of fresh and stored dressed broilers

Dressed broiler with	Sample region	For 0 day storage	For 10 day storage				For 20 day storage				For 30 day storage			
			Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen	
			P	UP	P	UP	P	UP	P	UP	P	UP	P	UP
Intact skin	Breast	2.31	1.69	1.81	1.80	1.83	0.69	0.90	0.95	1.04	0.42	0.55	0.74	0.90
	Thigh	3.17	1.79	1.85	1.89	1.94	0.91	1.08	1.08	1.18	0.66	0.77	0.69	0.84
No skin	Breast	2.77	1.96	2.04	2.02	2.27	1.47	1.26	1.48	1.85	0.93	0.85	0.88	0.96
	Thigh	3.76	2.01	2.11	2.04	2.31	1.61	1.34	1.65	1.78	1.02	0.93	1.12	1.11

P, Packed; UP, Unpacked

Table 4. Total coliform counts (numbers in log) per cm^2 for swab samples obtained from visceral cavity of dressed broilers

Dressed broiler with	Sample region	For 0 day storage	For 10 day storage				For 20 day storage				For 30 day storage			
			Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen	
			P	UP	P	UP	P	UP	P	UP	P	UP	P	UP
Intact skin	Visceral	4.01	2.73	2.79	2.75	2.81	1.56	1.64	1.61	1.69	1.02	1.01	1.13	1.15
No skin	cavity	3.97	2.91	2.94	3.03	3.17	1.62	1.77	1.67	1.82	1.05	1.15	1.09	1.11

P, Packed; UP, Unpacked

Total streptococcal count (TStC) and total staphylococcal count (TSC)

The mean values of TStC per gram for meat of dressed broiler from breast and thigh regions with/without skin for 0 day, 10 days, 20 days and 30 days of storage are shown in Table 5 and the TStc in the swab samples per square centimeter from visceral cavities are shown in Table 6. The mean values of the total staphylococcal count (TSC) per gram for meat of dressed broiler from breast and thigh regions with/without intact skin for 0 day, 10 days, 20 days and 30 days of storage are in Table 7 and the TSC in the swab samples per square centimeter from visceral cavities are shown in Table 8. In frozen dressed broilers streptococci and staphylococci occurred more persistently than coliforms. A previous report also suggested that there was no appreciable decrease in the numbers of these bacterial groups in comparison to coliforms during the storage period [3]. These data indicated that the groups of streptococci and staphylococci were more resistant to frozen condition than the groups of coliforms.

Table 5. Total streptococcal counts (numbers in log) per gram for meat samples obtained from different regions of fresh and stored dressed broilers

Dressed broiler with	Sample region	For 0 day storage	For 10 day storage				For 20 day storage				For 30 day storage			
			Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen	
			P	UP	P	UP	P	UP	P	UP	P	UP	P	UP
Intact skin	Breast	2.47	1.34	1.49	1.56	1.99	1.23	1.36	1.44	1.81	1.07	1.27	1.14	1.32
	Thigh	2.77	2.07	2.58	2.49	2.61	1.63	1.69	1.72	1.85	1.23	1.39	1.25	1.46
No skin	Breast	3.43	1.56	1.82	2.05	2.08	1.41	1.79	1.57	1.92	1.52	1.53	1.46	1.56
	Thigh	3.70	2.15	2.62	2.57	2.71	1.77	1.89	1.85	2.04	1.36	1.61	1.44	1.66

P, Packed; UP, Unpacked

Table 6. Total streptococcal counts (numbers in log) per cm^2 for swab samples obtained from visceral cavity of dressed broilers

Dressed broiler with	Sample region	For 0 day storage	For 10 day storage				For 20 day storage				For 30 day storage			
			Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen	
			P	UP	P	UP	P	UP	P	UP	P	UP	P	UP
Intact skin	Visceral	2.69	2.25	2.29	2.32	2.36	1.93	2.15	1.87	2.17	1.70	1.82	1.74	1.84
No skin	cavity	2.67	2.24	2.35	2.35	2.45	2.18	2.25	2.15	2.25	1.95	2.07	2.04	2.19

P, Packed; UP, Unpacked

Table 7. Total staphylococcal counts (numbers in log) per gram for meat samples obtained from different regions of fresh and stored dressed broilers

Dressed broiler with	Sample region	For 0 day storage	For 10 day storage				For 20 day storage				For 30 day storage			
			Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen	
			P	UP	P	UP	P	UP	P	UP	P	UP	P	UP
Intact	Breast	2.30	1.25	1.41	1.46	1.93	1.07	1.27	1.34	1.75	0.84	1.11	0.95	1.21
skin	Thigh	2.69	2.06	2.50	2.44	2.57	1.57	1.65	1.65	1.81	1.04	1.23	1.14	1.30
No	Breast	3.32	1.49	1.69	1.95	2.06	1.30	1.74	1.54	1.87	1.20	1.46	1.25	1.49
skin	Thigh	3.66	2.13	2.59	2.51	2.69	1.79	1.84	1.81	1.95	1.27	1.50	1.34	1.59

P, Packed; UP, Unpacked

Table 8. Total staphylococcal counts (numbers in log) per cm^2 for swab samples obtained from visceral cavity of dressed broilers

Dressed broiler with	Sample region	For 0 day storage	For 10 day storage				For 20 day storage				For 30 day storage			
			Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen		Prechilled & frozen		Direct frozen	
			P	UP	P	UP	P	UP	P	UP	P	UP	P	UP
Intact skin	Visceral	2.61	2.11	2.23	2.20	2.40	1.90	2.04	1.05	1.14	1.63	1.76	1.69	1.79
No skin	cavities	2.65	2.34	2.41	2.41	2.45	2.20	2.31	2.35	2.40	1.90	1.96	1.99	2.01

P=Packed; UP=Unpacked

Correlation between TVC, TCC, TStC and TSC

Results of correlation between TVC, TCC, TStC and TSC depicted some important facts. The levels of microbial contamination of dressed broiler with intact skin for 10 days storage showed highly significant correlation between TVC, TCC, TStC and TSC ($P<0.001$). On subsequent storage of 20 days, it showed highly significant relationship between TVC, TStC and TSC ($P<0.001$), but not between TVC and TCC ($P>0.05$). For 30 days storage the result showed highly significant correlation between TVC and TStC ($P<0.001$) but not between TVC, TSC and TCC ($P>0.05$).

In this study the values of TSC obtained were highly significant ($P<0.001$) in meat samples with intact skin kept at 10 days storage. But in case of birds without skin there were no significant values ($P>0.05$), whereas TCC and TStC were highly significant ($P<0.001$) in both cases. These results indicated that no statistical interactions occurred between temperatures of incubation and sampling site of frozen chicken carcasses.

Frequency in distribution of microorganisms

Rate in distribution of different types of microorganisms of dressed broiler with skin and without skin are presented in Table 9. Presence of high rate of *E. coli* in both dressed broiler with and without skin is alarming

Table 9. Contamination of different bacteria isolated from freshly dressed broilers

Dressed broiler with	Bacteria isolated	No. of sample		%
		Examined	Isolated	
Intact skin	*Staphylococcus aureus*	80	18	23
	Streptococcus pyogenes	80	22	27
	Escherichia coli	80	43	54
	Pseudomonas aerogenosa	46	14	31
	Micrococcus spp	45	14	31
	Salmonella spp	54	8	14
No skin	*Staphylococcus aureus*	80	17	21
	Streptococcus pyogenes	80	26	32
	Escherichia coli	80	54	67
	Pseudomonas aerogenosa	46	22	48
	Micrococcus spp	45	16	36
	Salmonella spp	54	11	17

for meat consumption. The organisms gaining access to meat were not only the cause of deterioration and spoilage but were also responsible for many outbreaks of foods borne diseases [5, 6]. Presence of *Staphylococcus, Streptococcus, Pseudomonas and Salmonella* in meats are also often responsible for causing of public health problems [5].

Correlation between sensory panel score (SPS) and different storage days

The highest sensory panel score was 288 of 400 at frozen storage of 10 days. For 10 days storage, panel score was higher than 20 days and 30 days storage. For 30 days storage, the score was only 43. The growth of microorganisms during frozen storages was ascribed to the development of undesirable organoleptic changes. These facts indicate that longer period of frozen storage can decrease the quality of broilers.

In conclusion, numbers of microorganisms were considerably decreased during the frozen storages and the highest sensory panel score was measured at storage of 10 days. Lower numbers of the growth of microorganisms were generally detected in packed than in unpacked conditions. Lower bacterial load was also found in dressed broiler with intact skin than that without skin, indicating that the skin acted as a barrier, while dressed broilers without skin were more prone to contamination. The prechilling process was also found to be very effective in reducing bacterial load. Longer periods (ie, 30 days) of frozen storage completely inhibited the growth of certain bacterial group (ie, coliform bacteria). Collectively, all these results indicate that usages of appropriate periods and conditions of frozen storage and packaging systems can minimize the potential health hazards associated with contaminants gaining access to the dressed or processed broilers and improve the quality and shelf life of dressed broilers.

Acknowledgements

This study was supported by the Brain Korea 21 Project in 2003, Republic of Korea and a research grant from Bio-Safety Research Institute, Chonbuk National University, Korea in 2004.

References

1. **Anand, S. K., Mahapatro, C. M., Pandey, N. K. and Verma, S. S.** Microbial changes on chicken's carcasses during processing. Indian J. Poultry Sci. 1989, **24**, 203-209.
2. **Archer, D. L.** The validation of rapid methods in food microbiology. Food Control. 1996, **7**, 3-4.
3. **Comi, G., Valenti, M., Aubert, D. S. and Giorgetti, D.** Valuation of a rapid conductance method for the quantitative estimation of coliforms in H. A. C. C. P. [Hazard analysis critical control point] system during poultry meats production. *Ingegneria Alimentare*, Le conserve Animal, 1990, **6**, 16-26.
4. **International Commission on Microbiological Specification for Foods** (ICMSF). Microorganism in

foods; samples for Microbiological Analysis: Principles and specific applications. Recommendation of the International Commission on Microbiological Specification for Foods. Association of Microbiological Societies. Toronto, University of Toronto Press, 1986.

5. **Jackson, T. C., Acuff, G. R. and Dickson, J. S**. Meat, poultry, and seafood, p 83-100. In M. P. Doyle, L. R. Beuchat, and T. J. Montville (ed.), Food microbiology: fundamentals and frontiers. ASM Press, Washington, D.C., 1997.
6. **Jay, J. M.** Modern food microbiology, 5th ed. Chapman & Hall, London, United Kingdom, 1996.
7. **Kakouri, A. and Nychas, G. J. E.** Storage of poultry meat under modified atmospheres or vacuum packs: possible role of microbial metabolites as indicators of spoilage. J. Appl. Bacteriol. 1994, **76**, 163-172.
8. **May, G. C. and Pooni, G. S.** Shelf-life prediction for chilled poultry products. Biennial Report AFRC Food Research Institute, Nowich, No. 67, 1974.
9. **Notermans, S., Kampelmacher, E. H. and Schothorst, M. V.** Studies of different sampling methods for the determination of bacterial counts from frozen broilers. J. Appl. Bacteriol. 1975, **39**, 125-131.
10. **Nychas, G. J. E. and Tassou, C. C.** Spoilage processes and proteolysis in chicken as detected by HPLC. J. Sci. Food Agric. 1997, **74**, 199-208.
11. **Sawaya, W. N., Elnawawy, A. S., Abu-Ruwiada, A. S. and Klralafai, S.** The influence of modified atmospheric packaging on shelf life of chicken carcasses under refrigerated storage conditions. J. Food Safety, 1995, **15**, 35-51.
12. **Schreurs, F. J. G.** Post-mortem changes in chicken muscle. Worlds Poult. Sci. J. 2000, **56**, 319-346.
13. **Sklan, N. J. and Tenne, C. E.** Bacterial quality of broilers and alternative processing procedures. J. Appl. Poultry Res. 1984, **4**, 164-169.
14. **Thompson, J. K. and Patterson, J. T.** *Staphylococcus aureus* from astir of contamination in a broiler processing plants. Rec. Agricultural Res. 1974, **31**, 53-23.
15. **Verma, A. L., Rathgeber, B. M., Hierholzer, R. E., Smoot, L., Martin, L. M., Bilgili, S. F., Fletcher, D. L., Chen, T. C. and Wabeck, C. J.** Effects of reprocessing on microbiological quality of commercial prechill broiler carcasses. J. Appl. Poultry Res. 1993, **2**, 111-116.