



Physicochemical Properties and Freshness of Spent Hen's Meat under Frozen or Refrigeration Conditions after Thawing

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Abstract

This study was conducted in order to investigate the effect of storage temperature abuse on the freshness of refrigerated and frozen spent hen's meat. After a room temperature storage condition, two different storage temperature were followed: refrigeration and frozen storage. All parts of the spent hen's meats were thawed at 4 d intervals up to 3 times (2, 6, and 10 d) for 24 h. The level of bacteria on the different parts of the refrigerated and frozen meats was higher than 6 Log CFU/g under the following storage conditions: refrigerated - breast, 12 h; leg, 6 h; wing, 12 h at the 1st analysis, frozen - breast, 12 h at the 2nd analysis; leg, 24 h at the 1st analysis; wing, 12 h at the 1st analysis. The pH value for the leg meat was higher than breast and wing meats. In the color measurements, under the room temperature storage condition, lightness and redness values decreased but the yellowness increased in refrigerated meats ($p < 0.05$). The K-value regarding refrigerated leg meats exceeded 60%, which is the threshold value to evaluate the degree of freshness, during the 1st analysis ($p < 0.05$). The VBN value of refrigerated leg meat was the highest and reached up to 96.93 mg%. Thus, studies regarding the possible decline in quality according to the change of storage temperature could be used in order to establish a basic database for the quality control of spent hen meat being traded with other countries.

Key words: spent hen, shelf-life, freshness, temperature abuse, thawing

Introduction

In recent years, food safety has become highly important due to the globalization and free trade in the food industry (Likar and Jevšnik, 2006). Consumer demand for highly qualified and safer food has increased. However, incidence of *Salmonella*, which was the most common infection associated with food, were reported to occur 1.2 millions in the United States annually (CDCP, 2010). Especially, raw poultry products are perceived to be related with a significant amount of food poisoning due to the frequent cases of poultry contaminated with pathogens (Kessel *et al.*, 2001; Zhao *et al.*, 2001). Also, it was reported that these sorts of disease can be caused by temperature abuse during storage and distribution (Juneja *et al.*, 2007). For this reason, the cold chain system is rec-

ommended as the most effective way to ensure freshness and safety of food products. Easily, perishable food products such as dairy products and fresh poultry meat, have a short durability and sell time so that distributors must ensure that appropriate storage temperatures are used.

Tenderness is the most important sensory characteristic of meat (Lawrie, 1991). However, the meat obtained from spent hens has poor quality attributes, such as toughness, which has decreased its remunerative price (Vaithyanathan *et al.*, 2008). On the other hands consumers in certain regions of East-South Asian countries, especially Vietnam, have a preference for spent hen meat. According to the Korean Agro-Fisheries Trade Corp. and Animal Plant & Fisheries Quarantine & Inspection Agency, spent hen exports have increased annually, most of which is sold to Vietnam (REPP, 2010). However, those countries has not equipped with efficient cold chain systems, so unfavorable changes related with freshness and quality attributes occur frequently in East-South Asia. Thus, there is a need to apply the cold chain system to ensure food safety during distribution, which would be very important

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not only to foreign consumers but also to Korean spent hen exporters.

Considering retail environment in certain East-South Asian Countries, study for possible decline in quality according to the change of storage temperature is needed. Therefore, the aim of this study was to examine the effect of storage temperature abuse on spent hen meats in regards to the shelf-life, using the K-value and volatile basic nitrogen (VBN), which have been used as physicochemical indicators of poultry. The results obtained in this study could be used to establish a basic database for the quality control of spent hen meat that is traded with East-South countries.

Materials and Methods

Samples preparation and condition of storage temperature

Frozen and refrigerated cuts of spent hen's meat (breast, leg, and wing) were obtained from a commercial slaughter house (JUNG WOO FOOD CO., LTD, Korea). Breast and leg meats deboned manually in commercial poultry processing unit and subcutaneous fat and visible connective tissue were removed. A total of samples (2 treatment \times 15 times \times 3 replication) were prepared for triplicate a parts and each cuts of spent hen's meat (30 g) samples vacuum-packed using a vacuum packer (FJ-500XL, Fujee Tech, Korea) and stored at 4°C and -18°C. In this study, the method of storage was divided into two groups: (a) refrigerated condition (4°C) followed by storage at room temperature (20°C), and (b) frozen condition (-18°C) followed by storage at room temperature (20°C), which is 1 cycle, repeated up to 3 times. While storage periods, at 4 d intervals (2, 6, and 10 d), all parts of the refrigerated and frozen meats were exposed at room temperature for 24 h. On room temperature condition, random carcasses were analyzed for 5 times (0, 2, 6, 12, and 24 h) and then remaining samples were restored at refrigerated and frozen conditions, respectively for 2nd and 3rd analysis. In the case of wing meats, skin was removed for analysis with the exception of color analysis.

Microbiological analysis

Samples were removed from the vacuum packaging using a sterile scalpel at 0, 2, 6, 12, and 24 h under room temperature. Sample (5 g) was placed in 45 mL of 0.1% peptone water in a sterile stomacher bag and homogenized using a Stomacher (Stomacher 400 Circulator, UK) for 2 min. The samples were then serially diluted with peptone

water for microbial counts. Plate count agar (PCA, Difco, USA) was used to obtain the total viable cell counts and experiments were performed in triplicate. The plates were incubated at 37°C for 48 h. Total viable cell counts were calculated as the mean of three determinations and expressed as Log CFU (colony forming unit)/g.

pH evaluation

The pH value of the samples were measured in homogenate prepared with 5 g samples with 20 mL distilled water at 2,000 rpm for 2 min in a homogenizer (Model AM-7, Nissei, Japan). The pH values were measured using a digital pH meter (F-51, Horiba, Japan) calibrated at pH 4.0 and 7.0.

Color evaluation

Color measurements were performed using a color meter (Chromameter, CR210, Minolta, Japan; illuminate C, calibrated with white standard plate $L^* = +97.83$, $a^* = -0.43$, $b^* = +1.98$). The measured region was 8 mm in diameter and the measured area and illumination area was 50 mm in diameter. Color values (CIE L^* , CIE a^* , and CIE b^*) were measured on the surface of the samples and measurements were acquired in triplicate for each sample.

K-value evaluation

To calculate the K-value, 200 mg of samples and 600 μ L of perchloric acid were placed in an eppendorf tube in order to precipitate the protein. The solution was then neutralized with 40-50 μ L of KOH. The K-value was calculated with a freshness checker system (Freshness checker system HF-1000, Huetech, Korea), which can divide ATP and ATP-related compounds into two spots : (a) ATP, ADP, AMP and IMP with negative charge because of phosphate groups, (b) H_xR and H_x with charge neutrality. It was detected by electrophoresis and visualized under UV illumination at 250 nm. The amounts of ATP and ATP-related compounds were estimated using the following formula, as described by Saito *et al.* (1959).

$$\text{K-value (\%)} = \frac{{}^1H_xR + {}^2H_x}{({}^3ATP + {}^4ADP + {}^5AMP + {}^6IMP + H_xR + H_x)} \times 100$$

¹H_xR, hypoxanthine; ²H_x, inosine; ³ATP, adenosine triphosphate; ⁴ADP, adenosine diphosphate; ⁵AMP, adenosine monophosphate; ⁶IMP, inosine monophosphate.

*The lower the K-value, the fresher the meat.

Volatile Basic Nitrogen (VBN) evaluation

Volatile basic nitrogen (mg%) tests were conducted to

assess the extent of protein deterioration by proteolysis during storage. VBN was measured using the modified micro-diffusion assay according to the method described by Pearson (1968).

$$\text{VBN (mg\%)} \\ = (a - b) \times (f \times 0.02 \times N \times 14.007 \times 100 \times 100) / S$$

Where, a=titer for sample, b=titer for blank, f=factor of reagent, N=normality, S=sample weight (g)

Statistical analysis

An analysis of variance were performed on all the variables measure using the General Linear Model (GLM) procedure in the SAS software (SAS, 2002). Duncan's multiple range test ($p < 0.05$) was used to determine difference between treatment mean applied. All data were expressed as mean \pm standard deviation.

Results and Discussion

Microbiological analysis

The microflora present on poultry is carried into the slaughter house and processing facility on the body and in the alimentary tract of the birds. Under proper conditions, the technological counts of bacteria range from 1 to 3 Log CFU/g (Sofos and Smith, 1998). The number of

microflora on carcasses after washing and cooling were reported to be approximately 3.58 Log CFU/cm² (Gill *et al.*, 2006).

In this study, the microflora of refrigerated spent hen meats increased from approximately 4.00 to 7.85 Log CFU/g and the bacterial counts on the frozen spent hen's meats ranged from 3.60 to 7.66 Log CFU/g during the thawing periods (Table 1). Barnes (1976) indicated that the phase of initial spoilage occurs when the number of microflora in poultry meat is more than 6 Log CFU/g. In this study, the bacterial counts on the refrigerated and frozen cuts of spent hen's meat exceeded this value when stored under the following conditions: refrigerated - breast, 12 h; leg, 6 h; wing, 12 h at the 1st analysis, frozen - breast, 12 h at the 2nd analysis; leg, 24 h at the 1st analysis; wing, 12 h at the 1st analysis. There were substantial differences in the growth rate among cut of refrigerated and frozen spent meat. An unpleasant odor was detected when the total number of bacteria reached 7 Log CFU/g bacteria for both refrigerated and frozen spent hen meat. Especially, during the 1st analysis, all parts of the refrigerated meats were incrustated with slime. In previous study, Sofos *et al.* (2000) reported that the number of microflora was more than 7 Log CFU/g if spoilage could be visibly detected.

Thomas and Mathews (2005) reported that temperature plays an important role in microbial growth and spoilage

Table 1. Total viable cell counts of spent hen's meat at different storage temperatures under room temperature storage condition

Analysis procedures & Storage periods (h)	Parts						
	Refrigeration ¹⁾			Frozen ²⁾			
	Breast	Leg	Wing	Breast	Leg	Wing	
1 st analysis on room temperature	2d+0	4.72±0.02 ^{J3)}	4.66±0.12 ^M	4.00±0.13 ^M	3.77±0.10 ^M	4.01±0.08 ^M	3.60±0.11 ^N
	2d+2	5.52±0.03 ^J	5.85±0.04 ^L	4.56±0.15 ^L	4.04±0.08 ^L	4.58±0.06 ^L	3.85±0.09 ^L
	2d+6	5.82±0.01 ^H	6.14±0.26 ^K	5.57±0.02 ^K	4.88±0.04 ^J	5.11±0.1 ^K	3.78±0.04 ^M
	2d+12	6.34±0.33 ^F	6.48±0.08 ^J	6.58±0.22 ^J	4.49±0.05 ^K	5.92±0.07 ^J	6.34±0.17 ^J
	2d+24	7.23±0.44 ^C	7.29±0.39 ^B	7.17±0.41 ^D	5.79±0.04 ^G	6.70±0.32 ^F	6.69±0.66 ^D
2 nd analysis on room temperature	6d+0	6.51±0.13 ^E	6.87±0.19 ^H	6.81±0.13 ^J	5.63±0.36 ^H	6.56±0.07 ^G	6.59±0.24 ^F
	6d+2	6.37±0.04 ^F	6.86±0.20 ^H	7.29±0.32 ^C	5.59±0.52 ^I	6.27±0.29 ^J	6.37±0.18 ^I
	6d+6	6.14±0.08 ^G	6.47±0.15 ^J	7.15±0.15 ^E	5.78±0.24 ^G	6.44±0.24 ^H	6.46±0.11 ^H
	6d+12	6.35±0.03 ^F	6.70±0.05 ^I	7.01±0.02 ^F	6.04±0.31 ^F	6.57±0.04 ^G	6.66±0.11 ^E
	6d+24	6.63±0.07 ^E	7.12±0.02 ^E	7.08±0.07 ^G	6.42±0.04 ^E	6.71±0.04 ^{EF}	6.93±0.02 ^A
3 rd analysis on room temperature	10d+0	6.54±0.05 ^E	7.08±0.04 ^F	7.10±0.05 ^H	6.53±0.08 ^D	6.75±0.11 ^D	6.87±0.07 ^B
	10d+2	6.87±0.11 ^D	7.18±0.26 ^D	7.47±0.15 ^B	6.77±0.30 ^C	6.72±0.19 ^E	6.77±0.16 ^C
	10d+6	7.85±0.13 ^A	7.36±0.42 ^A	7.64±0.29 ^A	7.30±0.47 ^B	7.66±0.28 ^A	6.28±0.13 ^K
	10d+12	7.68±0.15 ^B	7.24±0.15 ^C	7.46±0.24 ^B	7.45±0.07 ^A	7.35±0.13 ^B	6.37±0.43 ^I
	10d+24	7.32±0.22 ^C	7.06±0.11 ^G	7.08±0.28 ^G	7.30±0.32 ^B	6.96±0.17 ^C	6.56±0.35 ^G

Unit : Log CFU (colony forming unit)/g.

¹⁾Samples (stored at refrigerated condition) to room temperature

²⁾Samples (stored at frozen condition) to room temperature

³⁾Means±SD

^{A-N}Means with the different superscript in the same column are significantly different ($p < 0.05$).

of meat or meat products. Since microbial spoilage results in physicochemical changes, the initial microbial count is one of the most important parameters in determining the shelf-life of poultry meats (Cunningham, 1982).

pH

The changes in pH values of refrigerated and frozen spent hen meats were affected by storage temperature under room temperature storage condition (Table 2).

Differences in postmortem pH values were observed among the three parts (breast, leg and wing) of spent hens. Under room temperature storage condition, changes in pH values of three parts of refrigerated meats were as follows: breast; 5.87-6.72, leg; 6.56-7.36, and wing; 6.24-7.20 ($p<0.05$). In the case of frozen meats, the changes in pH values were as follows: breast; 5.91-6.19, leg; 6.43-7.27, and wing; 6.41-6.84 ($p<0.05$). Based on these findings, the pH value of the leg was shown to be higher than breast and wing. These results were consistent with those of Lesiak *et al.* (1996) and Northcutt *et al.* (2001), who reported that the pH value of leg meats was approximately 0.2-0.3 higher than that of breast meats of poultry. Geesink *et al.* (1995) also reported that the rate of the temperature decrease and type of muscle were also important determinants of the decrease in pH post mortem.

In generally, the pH fall will result from (a) denaturation of sarcoplasmic proteins and myofibrillar proteins,

(b) increase of actomyosin shortening and (c) internal structural changes (Offer and Trinick, 1983; Offer and Knight, 1988).

Color

The lightness, redness, and yellowness of refrigerated and frozen spent hen's meats during storage were significantly affected by storage temperature abuse (Table 3 and 4).

In the case of refrigerated wing meats, the yellowness were not influenced by storage temperature by elapsing storage periods until 2nd analysis ($p<0.05$). After 2nd analysis no significant difference were observed in the lightness. The yellowness of spent hen breast meats increased, while the redness values significantly decreased during refrigerated storage ($p<0.05$). Akamittath *et al.* (1990) reported that redness decreased with an increase in storage time because of metmyoglobin. When refrigerated meats were compared with frozen meats, the yellowness of the refrigerated wing meat was much higher than that of frozen wing meat, but the lightness value of frozen leg meat was higher than that of refrigerated leg meat.

Green color in meats results from sulphmyoglobin, when myoglobin reacts with (a) hydrogen sulphide (H_2S) and (b) hydrogen peroxide (H_2O_2). These compounds H_2S and H_2O_2 are produced by certain microorganisms (CFNS, 2006). In this study, refrigerated and frozen spent

Table 2. Changes in pH values of spent hen's meat at different storage temperatures under room temperature storage condition

Analysis procedures & Storage periods (h)	Parts						
	Refrigeration ¹⁾			Frozen ²⁾			
	Breast	Leg	Wing	Breast	Leg	Wing	
1 st analysis on room temperature	2d+0	6.72±0.03 ^{A3)}	6.71±0.05 ^{CD}	6.91±0.02 ^E	6.12±0.04 ^{ABC}	7.27±0.01 ^A	6.84±0.02 ^A
	2d+2	6.37±0.11 ^D	7.11±0.05 ^{EF}	6.66±0.01 ^C	6.00±0.11 ^{DEFG}	6.91±0.03 ^{CD}	6.72±0.03 ^C
	2d+6	6.28±0.02 ^E	6.85±0.05 ^{DE}	6.78±0.01 ^F	6.06±0.10 ^{CDE}	6.81±0.11 ^{DE}	6.62±0.03 ^{DEF}
	2d+12	6.27±0.10 ^E	6.56±0.02 ^{FG}	6.52±0.04 ^F	5.97±0.03 ^{FGH}	6.82±0.02 ^{DE}	6.80±0.05 ^{AB}
	2d+24	6.05±0.01 ^G	6.93±0.02 ^G	6.45±0.04 ^D	6.04±0.03 ^{CDEF}	6.68±0.03 ^{FG}	6.41±0.04 ^H
2 nd analysis on room temperature	6d+0	5.99±0.06 ^G	6.86±0.07 ^{GH}	6.42±0.03 ^D	5.99±0.06 ^{EF}	6.76±0.10 ^{EF}	6.74±0.03 ^C
	6d+2	6.02±0.06 ^G	6.87±0.02 ^{FG}	6.29±0.02 ^D	5.93±0.03 ^{HG}	6.70±0.01 ^{FG}	6.47±0.03 ^G
	6d+6	5.97±0.07 ^G	6.95±0.04 ^H	6.24±0.02 ^D	6.04±0.01 ^{CDEF}	6.68±0.07 ^{FG}	6.77±0.02 ^{BC}
	6d+12	5.87±0.02 ^H	7.23±0.11 ^{GH}	6.41±0.02 ^{ABC}	6.11±0.01 ^{ABC}	6.43±0.01 ^I	6.75±0.01 ^{BC}
	6d+24	6.04±0.06 ^G	7.22±0.01 ^{GH}	6.41±0.01 ^{ABC}	5.91±0.02 ^H	6.66±0.01 ^{FG}	6.60±0.03 ^F
3 rd analysis on room temperature	10d+0	6.16±0.02 ^F	7.18±0.04 ^{BCD}	6.96±0.06 ^{BC}	6.19±0.13 ^A	6.60±0.03 ^{HG}	6.60±0.02 ^{EF}
	10d+2	6.22±0.06 ^{EF}	7.16±0.04 ^{AB}	7.11±0.06 ^{BC}	6.16±0.02 ^{AB}	6.54±0.03 ^H	6.60±0.02 ^F
	10d+6	6.52±0.06 ^B	7.23±0.07 ^{DE}	7.01±0.01 ^{ABC}	6.08±0.06 ^{BCD}	7.00±0.02 ^C	6.66±0.02 ^D
	10d+12	6.50±0.01 ^C	7.29±0.03 ^{ABC}	7.05±0.01 ^{AB}	6.07±0.05 ^{CDE}	7.15±0.01 ^{CD}	6.66±0.03 ^{DE}
	10d+24	6.41±0.03 ^D	7.36±0.05 ^A	7.20±0.05 ^A	6.08±0.05 ^{BCDE}	7.15±0.02 ^B	6.50±0.05 ^G

¹⁾Samples (stored at refrigerated condition) to room temperature

²⁾Samples (stored at frozen condition) to room temperature

³⁾Means±SD

^{A-I}Means with the different superscript in the same column are significantly different ($p<0.05$).

Table 3. Changes in color values of refrigerated spent hen's meat at refrigeration storage temperature during the thawing periods¹⁾

Analysis procedures & Storage periods (h)	Breast			Leg			Wing		
	L ⁽²⁾	a ⁽³⁾	b ⁽⁴⁾	L [*]	a [*]	b [*]	L [*]	a [*]	b [*]
1 st analysis on room temperature	2d+0 56.35±1.41 ^{AS} 2d+2 53.68±1.90 ^{AB} 2d+6 53.76±4.09 ^{AB} 2d+12 53.73±5.73 ^{AB} 2d+24 52.03±4.74 ^{AB}	0.37±1.30 ^A 0.34±1.29 ^A -0.07±1.47 ^{AB} -0.40±1.83 ^{ABC} -0.67±1.80 ^{ABC}	1.83±1.50 ^F 1.89±1.63 ^F 2.88±0.62 ^F 6.09±0.30 ^E 5.90±0.67 ^E	47.59±2.75 ^A 44.39±2.92 ^{AB} 44.74±2.32 ^{AB} 43.85±2.86 ^B 46.35±3.62 ^{AB}	11.18±2.75 ^A 12.80±2.92 ^A 12.80±1.86 ^A 10.74±2.86 ^{AB} 7.39±3.62 ^{CD}	8.03±1.75 ^F 7.70±1.34 ^F 7.97±1.31 ^F 8.37±1.56 ^F 9.61±1.25 ^{EF}	73.03±1.43 ^A 70.34±2.87 ^{AB} 70.60±2.62 ^{AB} 70.44±3.93 ^{AB} 71.40±0.80 ^{AB}	0.46±0.57 ^C 1.39±0.31 ^{BC} 1.36±0.51 ^{BC} 1.58±0.71 ^{BC} 1.56±0.55 ^{BC}	13.10±1.22 ^C 12.08±1.22 ^C 12.32±0.86 ^C 11.80±2.46 ^C 11.73±0.95 ^C
2 nd analysis on room temperature	6d+0 45.58±1.90 ^{CD} 6d+2 49.33±4.23 ^{BC} 6d+6 43.37±4.23 ^D 6d+12 50.47±3.71 ^B 6d+24 51.36±3.62 ^{AB}	-1.77±1.41 ^{BCDE} -1.06±1.97 ^{ABC} -1.44±2.17 ^{ABCD} -2.10±1.87 ^{CDEF} -3.10±1.46 ^{DEFG}	8.19±0.72 ^{CD} 6.31±1.07 ^E 6.35±0.65 ^E 7.17±0.47 ^{DE} 8.82±1.41 ^C	44.68±1.22 ^{AB} 46.91±1.24 ^{AB} 45.64±0.82 ^{AB} 44.53±2.67 ^{AB} 44.47±1.07 ^{AB}	8.72±1.22 ^{BC} 6.37±1.24 ^{CDE} 6.70±0.82 ^{CDE} 5.20±2.67 ^{DE} 4.35±1.07 ^E	11.22±1.31 ^{CDE} 10.97±1.54 ^{CDE} 9.41±1.64 ^{EF} 11.73±1.57 ^{BCD} 10.67±1.57 ^{DE}	71.23±0.78 ^{AB} 69.73±2.20 ^B 70.33±1.72 ^{AB} 69.86±1.12 ^{AB} 68.46±1.55 ^B	1.27±0.46 ^{BC} 0.79±0.52 ^{BC} 0.39±0.94 ^C 1.00±0.87 ^{BC} 0.68±0.68 ^{BC}	12.28±1.75 ^C 12.50±1.40 ^C 11.91±0.99 ^C 12.28±0.97 ^C 12.40±1.63 ^C
3 rd analysis on room temperature	10d+0 51.86±2.39 ^{AB} 10d+2 52.84±2.19 ^{AB} 10d+6 52.46±1.27 ^{AB} 10d+12 52.98±1.94 ^{AB} 10d+24 52.90±1.31 ^{AB}	-3.71±0.71 ^{FG} -4.02±0.83 ^G -3.63±0.75 ^{EF} -3.60±1.08 ^{EF} -3.28±0.66 ^{DEFG}	12.61±1.35 ^B 11.97±0.90 ^B 12.50±0.52 ^B 13.11±1.71 ^B 14.52±1.11 ^A	47.22±1.90 ^{AB} 44.44±1.52 ^{AB} 44.62±1.33 ^{AB} 44.61±2.45 ^{AB} 44.36±1.32 ^{AB}	5.04±1.90 ^{DE} 4.79±1.52 ^{DE} 4.78±0.74 ^{DE} 4.51±0.78 ^E 4.42±1.32 ^E	15.89±1.42 ^A 12.79±2.31 ^{BC} 12.85±1.94 ^{BC} 13.55±0.67 ^B 13.67±1.08 ^B	60.93±3.85 ^C 63.94±3.84 ^C 62.17±1.81 ^C 62.67±1.29 ^C 57.87±3.18 ^D	2.85±1.47 ^A 1.07±2.31 ^{BC} 1.63±0.69 ^{ABC} 1.99±1.16 ^{AB} 1.96±1.04 ^{AB}	27.99±1.00 ^A 25.93±3.82 ^{AB} 26.56±1.33 ^{AB} 24.41±2.07 ^B 24.34±0.15 ^B

¹⁾Samples (stored at refrigerated condition) to room temperature, ²⁾L^{*}=Lightness, ³⁾a^{*}=Redness, ⁴⁾b^{*}=Yellowness, ⁵⁾Means±SD.
A-G/Means with the different superscript in the same column are significantly different (*p*<0.05).

Table 4. Changes in color values of frozen spent hen's meat at frozen storage temperatures during the thawing periods¹⁾

Analysis procedures & Storage periods (h)	Breast			Leg			Wing		
	L ⁽²⁾	a ⁽³⁾	b ⁽⁴⁾	L [*]	a [*]	b [*]	L [*]	a [*]	b [*]
1 st analysis on room temperature	2d+0 55.89±1.85 ^{BCS} 2d+2 50.97±1.97 ^{DEF} 2d+6 53.08±2.27 ^{CDE} 2d+12 54.48±4.08 ^{BCDE} 2d+24 50.32±5.57 ^{EF}	2.95±0.50 ^A 0.99±0.55 ^{BC} -0.02±1.01 ^{DE} -1.04±0.68 ^F -0.81±1.12 ^{EF}	2.48±0.95 ^{FG} -0.42±0.55 ^H 0.67±1.44 ^{GH} 1.66±1.47 ^G 4.22±1.16 ^F	44.90±5.12 ^{EF} 47.83±2.88 ^{BCDE} 51.15±2.23 ^{ABC} 51.15±1.15 ^{ABC} 49.36±1.82 ^{ABCD}	9.85±2.97 ^A 7.63±2.37 ^{AB} 8.30±2.89 ^{AB} 8.45±1.39 ^{AB} 6.79±1.35 ^{BC}	9.51±1.44 ^{GHI} 8.52±1.73 ^I 9.26±1.44 ^{HI} 11.57±2.37 ^{FGH} 13.46±3.03 ^{DEF}	59.52±2.16 ^{DEF} 70.02±3.92 ^{BC} 76.52±2.62 ^A 73.95±0.57 ^{AB} 73.86±3.25 ^{AB}	3.16±0.44 ^A 1.29±0.61 ^B 0.66±0.45 ^B 0.51±1.31 ^B 0.35±2.19 ^B	13.69±2.87 ^C 12.85±1.25 ^C 11.64±2.14 ^{CD} 8.62±1.92 ^E 8.62±1.92 ^E
2 nd analysis on room temperature	6d+0 58.93±0.98 ^A 6d+2 51.99±1.08 ^{CDE} 6d+6 50.53±5.08 ^{EF} 6d+12 47.61±4.66 ^F 6d+24 50.44±3.29 ^{EF}	1.57±0.26 ^B 0.84±0.49 ^{BCD} -1.61±0.93 ^F -2.85±1.10 ^G -3.10±0.38 ^{GH}	16.98±0.78 ^B 12.14±1.29 ^{CD} 4.21±0.61 ^F 9.50±1.96 ^E 14.08±4.11 ^C	47.80±5.63 ^{BCDE} 47.28±1.92 ^{CDE} 51.96±4.45 ^{AB} 48.61±4.68 ^{BCDE} 49.74±2.42 ^{ABCD}	7.12±1.39 ^{BC} 3.96±1.09 ^{DEF} 3.52±1.33 ^{EF} 6.29±2.37 ^{BCD} 6.23±3.30 ^{BCD}	12.27±1.80 ^{EF} 11.67±1.93 ^{FGH} 14.84±2.69 ^{CDE} 14.07±4.10 ^{CDEF} 16.12±3.49 ^{BCD}	64.52±0.40 ^{EF} 66.88±3.66 ^{CD} 69.20±1.28 ^C 69.03±2.33 ^C 65.21±4.15 ^{CD}	4.37±0.36 ^A 1.07±1.31 ^B 0.84±1.82 ^B 0.26±1.80 ^B 0.58±1.54 ^B	16.61±0.93 ^B 13.70±1.57 ^C 10.12±1.10 ^{DE} 9.96±1.16 ^{DE} 13.18±2.04 ^C
3 rd analysis on room temperature	10d+0 57.70±2.20 ^{AB} 10d+2 54.14±2.25 ^{BCDE} 10d+6 55.03±2.01 ^{BCD} 10d+12 54.32±1.35 ^{BCDE} 10d+24 53.90±2.43 ^{BCDE}	0.39±0.51 ^{CD} -2.67±1.22 ^G -2.80±0.68 ^G -2.65±0.75 ^G -3.97±0.61 ^H	21.51±1.37 ^A 9.86±2.22 ^E 11.86±1.01 ^F 12.16±1.78 ^{CD} 13.82±2.00 ^{CD}	43.16±2.58 ^F 46.76±1.78 ^{DEF} 47.83±1.30 ^{BCDE} 51.91±1.16 ^{AB} 54.35±1.70 ^A	8.16±0.80 ^{AB} 5.03±1.36 ^{BCD} 4.70±0.89 ^{CDE} 3.82±1.50 ^{DEF} 1.49±2.16 ^F	13.94±1.49 ^{CDEF} 14.81±1.43 ^{CDE} 16.68±0.71 ^{BC} 18.85±1.01 ^{AB} 20.49±2.57 ^A	58.37±5.80 ^F 60.13±5.93 ^{EF} 63.11±2.48 ^{DEF} 63.48±0.80 ^{DE} 68.39±5.14 ^C	3.34±0.55 ^A 0.02±1.27 ^B 0.39±1.09 ^B 0.15±0.46 ^B -0.01±0.31 ^B	20.40±1.47 ^A 18.91±2.29 ^{AB} 17.12±1.26 ^B 18.92±1.22 ^{AB} 16.50±3.13 ^B

¹⁾Samples (stored at frozen condition) to room temperature, ²⁾L^{*}=Lightness, ³⁾a^{*}=Redness, ⁴⁾b^{*}=Yellowness, ⁵⁾Means±SD.
A-H/Means with the different superscript in the same column are significantly different (*p*<0.05).

hen breast meats were observed under room temperature storage conditions.

K- value

The K-values of refrigerated and frozen spent hen meats were affected by storage temperature under room temperature storage condition (Table 5).

Terasaki *et al.* (1965) suggested that the K-value was an effective index of meat quality, especially for poultry. Many studies have found correlations between some of these metabolites or their ratios and freshness in some fish species (Hattula and Kiesvaara, 1996).

Adenosine triphosphate (ATP) is the main source of energy in muscle for biochemical reactions. After death, ATP is rapidly converted into adenosine diphosphate (ADP) and adenosine monophosphate (AMP) with subsequent accumulation of inosine 5'-monophosphate (IMP), which is further degraded into inosine (H_xR) and hypoxanthin (Hx) (Hernández-Cázares *et al.*, 2011).

Jolley *et al.* (1981) reported that the ATP concentration at any time post-slaughter was dependent on two factors: (a) the length of time during which the delay phase was operative and (b) the subsequent rate of ATP depletion. The quality of poultry products is significantly affected by its freshness and generally, fresh meats have a small

K-value.

During the 1st analysis, the K-value index of refrigerated breast, leg and wing meats was 41.6%, 65.7%, and 59.0%, respectively, whereas the K-value index of each part of the frozen meats was 35.4%, 56.8%, and 54.8%, respectively. K-value index 60% is defined as phase of initial spoilage to evaluate the degree of freshness and refrigerated spent hen leg meats exceeded at 1st analysis (Hashiguchi *et al.*, 1984; Usui, 1979).

The smallest change in the K-value was observed on frozen breast meats, which resulted in a longer shelf life on the storage. K-values of refrigerated leg meats significantly increased from 28.8 to 96.3 ($p < 0.05$), which showed higher increase than other meat components.

VBN

The changes in VBN values of refrigerated and frozen spent hen meats were affected by storage temperature under room temperature storage condition (Table 6).

The VBN value is a good indicator of protein deterioration and decomposition. Proteins in meat are decomposed into peptides and amino acids by enzymes and microorganisms when stored under cold conditions (Field and Chang, 1969).

During the 1st analysis, the VBN value of refrigerated

Table 5. Changes in K-values of spent hen's breast meat at different storage temperatures under room temperature storage condition
Unit : %

Analysis procedures & Storage periods (h)	Parts						
	Refrigeration ¹⁾			Frozen ²⁾			
	Breast	Leg	Wing	Breast	Leg	Wing	
1 st analysis on room temperature	2d+0	30.0±1.41 ^{F3)}	28.8±1.13 ^F	26.7±0.99 ^L	30.1±1.78 ^F	29.4±1.91 ^H	28.6±0.12 ^H
	2d+2	34.9±1.20 ^F	29.4±1.34 ^F	30.0±0.92 ^L	29.7±0.47 ^F	35.6±2.05 ^G	31.6±1.88 ^H
	2d+6	46.1±0.99 ^{CDE}	35.1±2.97 ^F	42.4±0.92 ^K	30.0±2.63 ^F	33.6±1.37 ^{GH}	30.1±0.67 ^H
	2d+12	42.6±2.30 ^{DE}	57.6±7.50 ^E	55.2±2.85 ^J	34.9±2.29 ^{CDE}	46.6±1.95 ^F	47.7±3.82 ^G
	2d+24	41.6±4.74 ^{DEF}	65.7±0.14 ^D	59.0±2.47 ^{IJ}	35.4±3.12 ^{BCDE}	56.8±2.75 ^E	54.8±0.75 ^F
2 nd analysis on room temperature	6d+0	39.6±0.64 ^{DEF}	71.7±6.36 ^{CD}	60.6±1.77 ^{HI}	32.7±1.28 ^{EF}	65.4±2.90 ^D	56.2±1.95 ^{EF}
	6d+2	44.7±0.78 ^{DE}	76.3±2.69 ^C	65.7±3.68 ^{GH}	34.1±0.64 ^{DE}	65.7±2.79 ^D	58.5±0.74 ^E
	6d+6	44.5±2.90 ^{DE}	85.3±0.21 ^B	69.1±4.31 ^{FG}	34.5±1.18 ^{DE}	65.6±4.47 ^D	63.3±2.08 ^D
	6d+12	48.3±2.55 ^{CD}	94.8±0.42 ^A	74.4±7.57 ^{EF}	34.6±1.34 ^{DE}	67.2±1.97 ^{CD}	65.8±2.20 ^D
	6d+24	49.4±4.38 ^{CD}	96.0±1.41 ^A	77.2±1.41 ^{DE}	36.7±0.91 ^{ABCD}	67.5±4.33 ^{CD}	70.4±1.80 ^C
3 rd analysis on room temperature	10d+0	48.8±1.84 ^{CD}	96.3±1.77 ^A	81.2±1.48 ^{CD}	38.2±0.64 ^{ABC}	71.6±1.31 ^{BC}	73.4±2.04 ^{BC}
	10d+2	48.8±3.89 ^{CD}	- ⁴⁾	85.4±1.41 ^{BC}	39.5±3.56 ^A	74.3±1.86 ^B	74.2±2.90 ^B
	10d+6	57.5±6.22 ^{BC}	-	87.9±2.33 ^{AB}	38.5±1.08 ^{AB}	86.6±1.35 ^A	75.8±1.41 ^{AB}
	10d+12	63.4±1.20 ^{AB}	-	89.5±1.34 ^{AB}	37.5±0.45 ^{ABCD}	86.0±1.97 ^A	76.9±1.47 ^{AB}
	10d+24	72.9±5.44 ^A	-	92.8±2.47 ^A	38.3±2.65 ^{ABC}	82.4±3.69 ^A	78.0±2.43 ^A

¹⁾Samples (stored at refrigerated condition) to room temperature

²⁾Samples (stored at frozen condition) to room temperature

³⁾Means±SD

⁴⁾-, means more than 100%.

^{A-L}Means with the different superscript in the same column are significantly different ($p < 0.05$).

Table 6. Changes in VBN (volatile basic nitrogen) of spent hen's meat at different storage temperatures under room temperature storage condition

Unit : mg%

Analysis procedures & Storage periods (h)	Parts						
	Refrigeration ¹⁾			Frozen ²⁾			
	Breast	Leg	Wing	Breast	Leg	Wing	
1 st analysis on room temperature	2d+0	13.17±0.40 ^{F3)}	8.68±0.32 ^J	8.40±0.79 ^{GH}	10.37±0.32 ^F	6.72±0.46 ^G	5.60±0.79 ^F
	2d+2	12.61±0.32 ^F	13.17±1.19 ^I	7.56±0.32 ^{GH}	12.61±0.32 ^{DE}	7.84±0.79 ^G	5.32±1.19 ^F
	2d+6	12.89±0.79 ^F	14.29±0.40 ^I	5.88±0.40 ^H	12.05±0.40 ^E	8.12±1.19 ^G	5.32±1.19 ^F
	2d+12	12.61±0.40 ^F	24.93±0.40 ^H	5.32±0.40 ^H	10.37±1.99 ^F	8.12±1.98 ^G	4.76±0.32 ^F
	2d+24	13.17±0.40 ^F	24.20±0.31 ^H	7.84±0.45 ^{GH}	9.53±0.80 ^F	12.61±0.32 ^F	5.60±0.79 ^F
2 nd analysis on room temperature	6d+0	14.01±0.79 ^F	30.82±2.38 ^G	10.65±0.79 ^{FG}	14.01±0.00 ^C	12.61±0.40 ^F	8.68±0.40 ^E
	6d+2	23.81±0.40 ^D	28.85±1.19 ^G	16.25±3.96 ^E	13.73±0.32 ^{CD}	14.85±0.40 ^E	8.68±0.40 ^E
	6d+6	23.81±0.40 ^D	48.47±5.15 ^F	14.29±0.40 ^{EF}	13.45±0.79 ^{CD}	22.13±0.40 ^D	9.53±0.80 ^E
	6d+12	22.41±1.58 ^E	58.27±3.17 ^E	14.01±0.79 ^{EF}	12.61±0.40 ^{DE}	21.94±0.71 ^D	9.19±1.98 ^E
	6d+24	21.01±0.40 ^{DE}	60.51±0.46 ^E	38.10±1.58 ^D	13.73±0.40 ^{CD}	22.41±3.17 ^D	9.81±0.40 ^{DE}
3 rd analysis on room temperature	10d+0	22.41±1.58 ^D	60.79±0.32 ^E	38.10±1.58 ^D	14.57±0.79 ^{BC}	23.81±0.32 ^D	10.93±1.19 ^{DE}
	10d+2	24.37±0.32 ^D	66.40±0.32 ^D	38.66±6.34 ^D	15.41±0.40 ^B	23.78±0.35 ^D	12.05±0.40 ^D
	10d+6	35.02±1.19 ^C	74.52±0.40 ^C	47.34±1.98 ^C	13.73±0.40 ^{CD}	30.41±1.80 ^C	17.37±2.38 ^C
	10d+12	39.50±0.32 ^B	87.68±0.32 ^B	58.27±3.17 ^B	15.41±0.32 ^B	36.70±0.32 ^B	22.13±0.40 ^B
	10d+24	44.54±0.32 ^A	96.93±2.38 ^A	69.47±0.00 ^A	19.05±0.79 ^A	46.50±2.38 ^A	30.54±1.20 ^A

¹⁾Samples (stored at refrigerated condition) to room temperature²⁾Samples (stored at frozen condition) to room temperature³⁾Means±SD^{A-J}Means with the different superscript in the same column are significantly different ($p<0.05$).

breast meat and frozen wing meat were not influenced by storage temperature by elapsing storage periods ($p<0.05$). The smallest change in VBN value was observed for frozen breast meats. VBN value significantly increased from 60.79 mg% to 96.93 mg% in refrigerated leg meat at 3rd analysis ($p<0.05$) and the difference was about 36 mg%. The VBN value of refrigerated leg meats reached up to 96.93 mg%.

Davies and Board (1998) suggested that a VBN value of 20 mg% can be used as a threshold value to evaluate the degree of freshness of raw and packed meat. The frozen breast meats did not exceed this range during the storage periods ($p<0.05$).

During the 1st, 2nd and 3rd analysis, the VBN values of both refrigerated and frozen leg meats were the highest. In this study, the VBN values as well as total viable cell counts were the highest in leg meat. This result was consistent with a previous report, where an increase in the VBN value was shown to be associated with the growth of bacteria and protein deterioration (Kang *et al.*, 2002).

Conclusions

Overall, inappropriate control of temperature accelerated the changes in freshness and physicochemical properties of spent hen meats even when the samples were

exposed to room temperature over a short period of time. Thus, it is highly important to maintain the appropriate storage temperature during distribution in order to ensure food safety and freshness of poultry meat products, which will require implementation of the cold chain system.

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