Comparison of the quality characteristics of chicken breast meat from conventional and animal welfare farms under refrigerated storage

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ABSTRACT In this study, we aimed to investigate the meat quality characteristics, bioactive compound content, and antioxidant activity during refrigerated storage of breast meat of Arbor Acres broilers (carcass weight: 1.1 kg, raised for 35 D) obtained from a conventional farm (**BCF**, n = 30) and an animal welfare farm (**BAF**, n = 30) in Korea. The BCF and BAF did not differ in their proximate composition, color, water-holding capacity, creatine, creatinine, and carnosine contents. However, the shear force value was significantly higher in BAF than in BCF ($P < 0.05$). The 2-thiobarbituric acid reactive substance (**TBARS**) levels in BCF on days 7 and 9 were significantly higher than those in BAF ($P < 0.001$). During storage, the total volatile basic nitrogen (**VBN**) content of BAF was significantly lower, except on day 1. The fatty acid composition of samples was not affected by the storage period, however, saturated fatty acid and unsaturated fatty acid contents did differ among the types of farm systems ($P < 0.05$). Although the creatine, creatinine, and carnosine contents in BAF and BCF did not differ significantly, the carnosine and creatinine contents decreased with the increase in storage period ($P < 0.05$). The anserine content of BAF was significantly higher than that of BCF throughout storage. Superoxide dismutase activity was not affected by the type of farm system but was affected by storage period. Overall, BAF showed lower pH, microorganism, TBARS, and VBN values, and higher anserine contents than BCF. These findings can serve as reference data for the evaluation of chicken meat quality of broilers raised in animal welfare farm and conventional farm.

Key words: chicken, breast meat, animal welfare, meat quality, anserine

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INTRODUCTION

Chicken meat is recognized for its several health benefits due to its high nutritional value and high protein content, and low cholesterol, calorie, and fat contents. Moreover, chicken meat is less expensive than other meats, such as pork, beef, and lamb (Sujiwo et al., 2018). In 2017, chicken consumption, as calculated by the Organization for Economic Cooperation and Development in countries with GDP per capita of $30,000 or more, was 30.2 kg and higher than that of other meat types, such as pork (23.6 kg) and beef (14.5 kg) (OECD, 2019). An increase in chicken meat (especially breast meat) consumption is expected due to the increase in health awareness and demand for low-cost protein sources.

Previously, research on broilers focused on an intensive system of mass production, feeding, and management to increase productivity. Production systems involving battery cages and the use of growth accelerators have effectively increased productivity; however, the incidence of diseases such as avian influenza, as well as mortality rates resulting from stress have increased (Dawkins, 2017). Stress deteriorates broiler carcass quality and meat characteristics, resulting in

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heavy economic losses. Stressors are related to rearing conditions, such as stocking density, temperature, and ventilation system, as well as health status of broilers, duration of fattening, transport conditions, and distance covered to the abattoir (Feddes et al., 2002). Under a high rearing density, high levels of heat and ammonia are produced that are associated with excessive production of reactive oxygen species, which reduce the immune function and antioxidant activities (An et al., 2012). As a result, there has been a rise in consumer concerns regarding chicken meat safety and an interest in animal welfare for safe, healthy, and sustainable broiler production.

According to the World Organization for Animal Health (OIE, 2019), an animal is considered to be in a good state of welfare "if (as indicated by scientific evidence) the animal is healthy, comfortable, well nourished, safe, able to express innate behavior, and if it is not suffering from unpleasant states such as pain, fear, and distress." The physical needs of broilers have been described and evaluated to some extent; however, it is more difficult to characterize and describe their mental states and needs. Numerous studies have aimed to improve animal welfare and breeding systems and have focused on animal welfare regulations, breeding protocols, livestock diseases, environment of livestock facilities, and consumer preference (Yoon et al., 2018). A certification system for animal welfare farms was first initiated for laying hens in 2012 in Korea; this system then expanded to pigs in 2013, broilers in 2014, Korean native cattle (Hanwoo), beef cattle, cows, and goats in 2015 (Yoon et al., 2018).

For broilers, the most important differences between animal welfare farms (**AF**) and conventional farms (**CF**) are stocking density, appropriate facilities for roosting, and the provision of vegetables. In Korea, AF should be stocked at the minimum stocking density (less than 19 broilers or less than 30 kg/m^2), provide 2 m length roosts per 1,000 broilers, and provide cabbage and other vegetables as materials for pecking without supplementation of animal source protein. Since 1994, the Royal Society for the Prevention of Cruelty to Animals (RSPCA, 2013) has been certifying meat from AF, labeling the products as "Freedom Food." This meat is sold at a higher price than meat from CF. Furthermore, Germany, the United States, and Japan have continuously managed to supply meat products via AF systems. In Korea, currently 61 broiler farms were designated as AF, and chicken products from these certified farms are mainly distributed in the market by 2 major chicken meat companies namely Harim Co., Ltd and Charmfre Co., Ltd.

However, the differences in chicken breast meat quality and endogenous bioactive compounds between CF and AF have not been investigated in detail. Therefore, in this study, we aimed to compare the quality, bioactive compound content (creatine, creatinine, carnosine, and anserine), and antioxidant activity of chicken breast meat from CF and AF during refrigerated storage.

MATERIALS AND METHODS

Birds and Meat Sampling

We used one-day-old Arbor Acres chicks reared under CF and AF conditions for 35 D in an indoor system. Rice husk was provided as litter. The CF conditions were as follows, floor size: 929 m^2, stocking density: 25 chickens/m^2, ammonia levels in the air: 50 to 100 ppm, and 1 water nipple was shared by 13 to 15 chickens. The AF conditions were as follows, floor size: 1,027 m^2, stocking density: 17 chickens/m^2, regulated ammonia levels in the air: <25 ppm and 1 water nipple was used by 10 chickens. In addition, broilers in the AF were provided rice straw, saw dust, and plant sources to allow for pecking according to the guidelines for AF authorized from the Ministry of Agriculture, Food and Rural Affairs (**MAFRA**) in Korea.

The chicks in the CF were fed a diet formulated with 22.5% crude protein (CP) with 3,040 kcal/kg of apparent metabolizable energy (**AME**) for starter diets (0 to 7 D), 21.0% CP with 3,150 kcal/kg of AME for grower diets (8 to 21 D), and 20.0% CP with 3,200 kcal/kg of AME for finisher diets (22 to 35 D). The chicks in AF were fed diet formulated with 22.5% CP with 3,040 kcal/kg of AME for starter diets (0 to 7 D), 21.5% CP with 3,150 kcal/kg of AME for grower diets (8 to 21 D), and 20.5% CP with 3,200 kcal/kg of AME for finisher diets (22 to 35 D). The protein component of the AF diet was derived from non-animal sources, according to guidelines of MAFRA in Korea.

Chicken carcasses (mean ± SE, 1.1 ± 0.2 kg) from the CF (n = 30) and AF (n = 30) were collected randomly from the abattoir (Harim Co., Iksan, Korea) after slaughter and transferred at a temperature of 2 ± 2°C to the laboratory. Chicken breast meat was then dissected (BCF and BAF for chicken breast meat obtained from CF or AF, respectively) and directly arranged on polystyrene trays and wrapped with low-density polyethylene. Breast meat samples were then immediately stored in the refrigerator at 4°C in the dark for 9 D. Analyses were conducted on experimental days 1, 3, 5, 7, and 9 of storage.

Proximate Analysis and Muscle pH

The proximate composition of meat was measured according to the AOAC methods (1998). Meat pH was measured as follows: 10 g of meat was homogenized with distilled water (90 mL) for 15 s using a homogenizer (Polytron PT-2500E; Kinematica, Lucerne, Switzerland), according to Kim et al. (2019a), and the pH of the homogenate was determined using an Orion 230A pH meter (Thermo Fisher Scientific, Waltham, MA).

Color

Chicken breast meat color was measured with a Chroma Meter CR-400 instrument (Minolta Co., Osaka, Japan) using CIE L* (lightness), CIE a* (redness), and

CIE b* (yellowness), according to Shim et al. (2018). The Chroma Meter was calibrated using white plate references (Y value: 93.60, x value: 0.3134, y value: 0.3194).

Water-holding Capacity

The water-holding capacity (**WHC**) was evaluated according to Jang et al. (2011). Briefly, chicken breast meat (0.5 g) was placed on a round plastic plate in a tube (Millipore Ultrafree-MC; Millipore, Bedford, MA), heated in a water bath (20 min, 80°C), cooled to 23 ± 1°C, and then centrifuged for 10 min at 4°C (2,000 × *g*) to measure the water loss.

$$\text{WHC} = (\text{moisture content} - \text{water loss}) / \text{moisture content} \times 100$$

$$\text{Water loss} = (\text{weight before centrifugation} - \text{weight after centrifugation}) / (\text{sample weight} \times \text{fat factor}) \times 100$$

$$\text{Fat factor} = 1 - (\text{crude fat} / 100).$$

Shear Force

Chicken breast meat was placed in a polyethylene bag and heated in a water bath (75°C) for 45 min. The samples were cut into 1 × 2 × 2 cm pieces, and their shear force values were measured using a TA1 texture analyzer (Lloyd Instruments, Berwyn, IL) with a V blade. The analyzer settings were as follows: 500 N load cell and a cross-head speed of 50 mm/min.

Microorganisms

Chicken breast meat (10 g) was homogenized with sterile saline solution (90 mL) for 40 s using a Bag Mixer 400 stomacher (Interscience, Saint-Nom-la-Bretèche, France). After serial dilution of the homogenate, the total counts of aerobic bacteria and coliforms were determined using 3 M Petrifilm (3 M Company, Saint Paul, MN) after incubation for 48 h at 37°C, per the manufacturer's protocol. A total of 3 replicates were performed, and the results were expressed as log CFU/g.

2-Thiobarbituric Acid Reactive Substances

The content of 2-thiobarbituric acid reactive substance (**TBARS**) was determined using the methods described by Buege and Aust (1978). Briefly, 5 g chicken breast meat was added to 15 mL distilled water with 50 μL of 7.2% *tert*-butyl-4-hydroxyanisole (**BHA**) and homogenized for 30 s using a Polytron PT-2500E homogenizer. A 1 mL sample of the meat homogenate was then transferred to a tube, and 2 mL of 20 mM thiobarbituric acid in 15% trichloroacetic acid was added. The sample was heated in a water bath at 90°C for 15 min, cooled for 10 min, and centrifuged at 2,000 × *g* (4°C, 10 min). The absorbance of the supernatant solution was evaluated at 531 nm using a spectrophotometer (M2e; Molecular Devices, Sunnyvale, CA). The TBARS content was expressed as milligrams of malondialdehyde (MDA) per kilogram of meat as follows:

$$\text{TBARS}(\text{mg MDA / kg of meat}) = (\text{absorbance of sample} - \text{absorbance of blank sample}) \times 5.88.$$

Total Volatile Basic Nitrogen

The volatile basic nitrogen (**VBN**) content was determined using the micro-diffusion method (Kim et al., 2018). Chicken breast meat (10 g) and 50 mL distilled water were homogenized using a magnetic stirrer for 30 min. The homogenate was filtered using a filter paper (Whatman No. 1, Whatman, Maidstone, UK), and 1 mL of the filtrate was added to the outer chamber of a Conway micro-diffusion cell. Then, 0.01 N H_2SO_4 (1 mL) was added to the inner cell, and 1 mL of saturated K_2CO_3 was added to the other outer cell. The cell was covered immediately and incubated for 1 h at 25°C. After incubation, 10 μL Brunswick reagent was added to the inner cell and titrated with 0.01 N NaOH.

$$\text{VBN}(\text{mg / 100 g}) = 0.14 \times (\text{b} - \text{a}) \times \text{F / W} \times \text{d} \times 100,$$

where a is the volume of 0.01 N NaOH (mL) added in the sample, b is the volume of 0.01 N NaOH (mL) added in the blank, F is the standard factor of 0.01 N NaOH, W is the sample weight (g), and d is the dilution factor.

Fatty Acid Composition

Lipids were extracted from chicken breast meat (1 g) with the addition of 20 μL of BHA and 15 mL of Folch's solution (2:1 mixture of chloroform and methyl alcohol, v/v). The homogenates were filtered through filter paper (Whatman No. 1). The filtrate was vortexed with 3 mL KCl (0.88%) and incubated overnight in the dark to separate the 2 layers. The lower lipid-containing layer was condensed with N_2. A 25 mg lipid sample was mixed with 1.5 mL of 0.5 N NaOH (in methyl alcohol) in glass tubes and heated to 100°C for 5 min. The mixture was mixed with 1 mL 10% boron trifluoride and heated to 100°C for 2 min. After the addition of 2 mL iso-octane and 1 mL saturated NaCl (40 g NaCl/100 mL distilled water), samples were centrifuged at 783 X g for 3 min. Iso-octane extract aliquots were injected into an Agilent 6890N gas chromatograph (Agilent Technologies, Wilmington) equipped with an Omegawax 250 capillary column (30 m × 0.25 mm × 0.25 μm, Supelco, Bellefonte, PA). The carrier gas, flow rate, and split ratio were helium (99.99%), 1.2 mL/min, and 1:100, respectively. The analytical temperatures of the injector and flame

ionization detector were 250°C and 260°C, respectively. The optimized column temperature program was as follows: initial temperature of 150°C, held for 2 min; gradual increase in temperature to 220°C at a rate of 4°C/min, held at 220°C for 30 min.

Creatine, Creatinine, and Di-Peptide (Anserine and Carnosine)

The creatine, creatinine, and di-peptide (anserine and carnosine) contents were determined using the methods described by Mora et al. (2007). Briefly, 2.5 g chicken breast meat was homogenized with 0.01 N HCl (7.5 mL) for 1 min. The homogenate was centrifuged for 30 min (3,000 × *g*, 4°C). The supernatant was filtered through a glass microfiber filter (Whatman GF/C), and 250 µL of the filtrate was mixed with 750 µL acetonitrile. The solution was left undisturbed for 20 min, followed by centrifugation for 10 min (10,000 × *g*, 4°C). The supernatant obtained was filtered using a 0.22 µm membrane filter, and 20 µL of the filtrate was injected into an Atlantis HILIC silica column (150 × 4.6 mm, 3.0 µm; Waters, Milford, CT) equipped with an HPLC system (Agilent Infinity 1260 series, Agilent Technologies, Palo Alto, CA). The creatinine content was determined at 236 nm, and the creatine and di-peptide (anserine and carnosine) contents were assayed at 214 nm. The mobile phases consisted of solvent A (pH 5.5, 0.65 mM ammonium acetate in water: acetonitrile, 25:75, v/v) and solvent B (pH 5.5, 4.55 mM ammonium acetate in water:acetonitrile, 70:30, v/v). Solvent B was supplied at a linear gradient (0 to 100%) at 1.4 mL/min for 13 min. Creatine, creatinine, anserine, and carnosine contents were determined from standard curves generated using the respective standard reagents purchased from Sigma Co. (Sigma-Aldrich, St. Louis, MO).

Superoxide Dismutase Assay

Superoxide dismutase (**SOD**) activity was assayed using the SOD assay Kit-WST (Dojindo, Tokyo, Japan). Absorbance at 450 nm was recorded using a microplate reader (SpectraMax M2e; Molecular Devices), and the superoxide inhibition rate was calculated per the manufacturer's formula.

Statistical Analysis

The effects of different farm treatments, different storage periods, and the interaction between them on the variables were evaluated with a two-way ANOVA using the general linear model of SAS program (ver. 9.4, 2018, SAS, 2018). A Duncan's multiple range test was performed when differences among storage days were detected ($P < 0.05$).

RESULTS AND DISCUSSION

Proximate Composition and Physicochemical Properties

No significant differences were found in the moisture (75.03 to 76.22%), crude protein (21.80 to 22.50%), crude fat (1.10 to 1.44%), or crude ash (1.67 to 1.76%) contents between BCF and BAF ($P > 0.05$) (Table 1). These findings are consistent with those reported by Alvarado et al. (2005) who showed that the crude protein, fat, and ash contents of breast fillets obtained from commercial and organic free-range chickens were similar, within ranges of 19.54 to 20.84%, 1.80 to 2.14%, and 1.65 to 1.83%, respectively. Wang et al. (2009) also found no difference in the moisture, crude protein, and crude fat content of chicken muscle obtained from different raising systems (free-range vs. conventional systems).

The type of farm and storage period significantly affected the pH values of chicken breast meat (Table 2). Although, the pH values of BCF and BAF were not significantly different on days 1, 3, 5, and 7, the pH of BCF was significantly higher (6.34) than that of BAF (6.15) on day 9. Husak et al. (2008) also reported that the pH of breast meat from free-range and conventional broiler farms did not differ on day 1. The increase in meat pH during storage may be attributed to the accumulation and proteolytic degradation of metabolites due to bacterial action on meat (Kim et al., 2019a). However, no significant interaction was found between farm types and storage period.

Meat color is an important criterion that affects consumer choice and is a crucial trait of meat quality. Meat myoglobin content is the main factor that contributes to meat color, and its value depends on the type of muscle, species, and age of the bird. The type of farm (AF and CF) did not affect the CIE L* (lightness), CIE a* (redness), and CIE b* (yellowness) values. However, storage period did significantly affect the CIE L* and b* values (Table 2). Castellini et al. (2002) also reported that the CIE a* values of breast meat from caged and organic broilers did not significantly differ.

Table 1. Proximate composition of chicken breast meat from conventional and animal welfare farms during cold storage.

	BCF					BAF						Significance		
Items (%)	1	3	5	7	9	1	3	5	7	9	SEM	T	S	T*S
Moisture	76.10	76.09	75.86	75.93	76.22	75.03	75.95	76.10	76.02	75.53	0.359	n.s.	n.s.	n.s.
Crude protein	21.95	22.00	22.14	22.04	21.80	22.50	22.07	22.07	21.89	21.99	0.360	n.s.	n.s.	n.s.
Crude fat	1.29	1.28	1.31	1.35	1.34	1.10	1.44	1.19	1.22	1.33	0.091	n.s.	n.s.	n.s.
Crude ash	1.67	1.71	1.70	1.73	1.73	1.73	1.73	1.75	1.73	1.76	0.036	n.s.	n.s.	n.s.

Abbreviations: BCF, chicken breast meat obtained from a conventional farm; BAF, chicken breast meat obtained from an animal welfare farm; S, storage; T, treatment; n.s., not significant.

Table 2. Meat pH, instrumental color, water holding capacity (WHC), and shear force of chicken breast meat from conventional and animal welfare farms during cold storage.

	BCF					BAF						Significance		
Items	1	3	5	7	9	1	3	5	7	9	SEM	T	S	T*S
pH	5.95^{d,e}	6.05^{b-d}	6.07^{b,c}	6.08^{b,c}	6.34^{a}	5.92^{e}	6.00^{c-e}	6.03^{c,d}	6.07^{b,c}	6.15^{b}	0.035	**[1]	***	n.s.
Color														
L*	54.68^{a-c}	54.19^{a-c}	54.67^{a-c}	53.74^{b,c}	54.03^{a-c}	55.33^{a}	54.90^{a,b}	54.79^{a,b}	54.49^{a-c}	53.28^{c}	0.444	n.s.	*	n.s.
a*	1.89	1.78	1.86	1.52	1.69	1.74	1.77	1.79	1.74	1.70	0.093	n.s.	n.s.	n.s.
b*	2.25^{c}	2.79^{b}	3.16^{a,b}	3.28^{a,b}	3.56^{a}	2.26^{c}	3.34^{a}	3.53^{a}	3.46^{a}	3.44^{a}	0.175	n.s.	***	n.s.
WHC (%)	47.41^{a,b}	48.70^{a}	48.70^{a}	49.11^{a}	49.39^{a}	44.50^{b}	48.46^{a}	48.34^{a}	48.43^{a}	48.82^{a}	1.074	n.s.	*	n.s.
Shear force (N)	25.27^{b}	22.52^{c,d}	21.59^{c,d}	20.40^{d,e}	18.55^{e}	27.44^{a}	25.39^{b}	23.63^{b,c}	21.97^{c,d}	20.92^{d}	0.671	***[1]	***	n.s.

Abbreviations: BCF, chicken breast meat obtained from a conventional farm; BAF, chicken breast meat obtained from an animal welfare farm; S, storage; T, treatment; n.s., not significant.

[a–e]Means within the same row with different letters are significantly different at $P < 0.05$ (*) and $P < 0.001$ (***).

[1]Means between BCF and BAF are significantly differ at $P < 0.01$ (**) and $P < 0.001$ (***).

Consistent with these findings, Fanático et al. (2007) suggested that access to free-range conditions in fast-growing chickens does not affect chicken meat color.

The WHC was similar between BCF and BAF, but was significantly affected by storage period (Table 2). Similarly, Wang et al. (2009) also found that the WHC of free-range chicken breast meat was not significantly different from that of indoor chicken breast meat.

Shear force is associated with connective tissue, which contributes to meat preference, flavor, and cooked meat toughness (Beilken et al., 1986). Shear force measurement is an effective method for evaluating meat tenderness (Kim et al., 2019b), and depends on the level of proteolysis of myofibrillar proteins (Marcinkowska-Lesiak et al., 2016). The type of farm and storage period significantly affected the shear force value of chicken breasts, whereby the shear force of BAF was significantly higher than that of BCF on days 1, 3, and 9 ($P < 0.05$), and ranged from 18.55 to 25.27 N for BCF and 20.92 to 27.44 N for BAF. The shear force of BAF was significantly higher than that of BCF; however, in terms of sensory characteristics, chicken breast meat with a shear force of less than 45 N is still regarded as tender by the majority of consumers (Schilling et al., 2008). Consistent with our findings, Husak et al. (2008) reported that the shear force of breast meat was significantly higher in broilers from free-range farms than those from conventional farms. Also, Castellini et al. (2002) reported that different farming systems affect the shear force, as a consequence of the higher amount of exercise in free-ranging systems. In contrast, Wang et al. (2009) reported that a free-range raising system did not affect chicken breast meat tenderness. In the present study, the shear force of BCF and BAF decreased significantly during storage from 25.27 and 27.44 N on day 1 to 18.55 and 20.92 N on day 9, respectively ($P < 0.05$). This decrease may be attributed to the degradation of meat protein during storage, which be caused by either enzymatic or bacterial processes (Kruk et al., 2011).

Microorganisms

The initial total aerobic bacterial counts (day 1) for BCF and BAF were similar and ranged from 2.60 to 2.82 log CFU/g; but increased significantly throughout storage (Table 3). No significant difference in bacterial counts were detected between the treatments on days 1, 3, 5 and 7 of storage, but the total aerobic bacterial count for BCF was higher than that for BAF on day 9. This result is consistent with the higher pH values of BCF (as shown in Table 2) due to a higher bacterial count compared to that of BAF. On day 9, the total aerobic bacterial count exceeded 7 log CFU/g in BCF and was 6.58 log CFU/g in BAF. Meats with bacterial counts higher than 7 log CFU are considered spoilt by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986). Wang et al. (2019) reported that microorganisms can spread easily within chicken flocks stocked at high densities and that bacteria found in chicken carcasses originate from feces or the environment, which contaminate chicken meat and skin during processing in slaughter houses. Moreover, Patria et al. (2016) reported that the total aerobic bacterial count of breast meat of Kampong-broiler chickens was higher in those raised under high density (12 birds/m^2) conditions than those raised under low density (8 and 10 birds/m^2) conditions. During storage, *E. coli* was not detected in chicken breast meat (data not shown). Coliforms in chicken breast meat were not detected on day 1, but were significantly affected by the type of farm and storage period.

TBARS

The TBARS assay measures MDA, ketones, and oxidation products, and the TBARS value obtained represents the lipid oxidation level. TBARS values ≥ 0.8 mg MDA/kg are indicative of perceptible rancidity (O'Neill et al., 1998). The TBARS values in BAF were lower than those in BCF on days 7 and 9 ($P < 0.001$) and the TBARS values of both BCF and BAF increased significantly ($P < 0.001$) throughout the storage period (Table 3). The TBARS values also showed a significant interaction between the type of farm and storage period. Alvarado et al. (2005) reported that lipid oxidation (TBARS) levels in breast meat from commercial chicken farms were higher than those in breast meat from free-range chicken farms. Furthermore, Husak et al. (2008) reported that the TBARS values of raw breasts were

Table 3. Microorganisms, 2-thiobarbituric acid reactive substance (TBARS), and volatile basic nitrogen (VBN) value of chicken breast meat from conventional and animal welfare farms during cold storage.

	BCF					BAF						Significance		
Items	1	3	5	7	9	1	3	5	7	9	SEM	T	S	T*S
Microorganisms (log CFU/g)														
Total aerobic bacteria	$2.82^{e,f}$	$2.82^{e,f}$	3.48^{d}	5.55^{c}	7.01^{a}	2.60^{f}	2.91^{e}	3.38^{d}	5.49^{c}	6.58^{b}	0.099	*[1]	***	n.s.
Coliforms	ND	$1.12^{a,b}$	$1.17^{a,b}$	$1.27^{a,b}$	1.40^{a}	ND	0.43^{b}	0.40^{b}	$0.96^{a,b}$	$1.14^{a,b}$	0.276	*[1]	***	n.s.
TBARS (mg MDA/kg)	0.13^{f}	0.15^{e}	0.18^{d}	0.30^{b}	0.33^{a}	0.11^{f}	0.14^{e}	0.18^{d}	0.19^{c}	0.27^{b}	0.005	***[1]	***	***
VBN (mg/100 g)	$9.69^{g,h}$	10.61^{f}	11.69^{e}	16.35^{c}	25.07^{a}	9.17^{h}	10.01^{g}	10.92^{f}	14.32^{d}	22.88^{b}	0.196	***[1]	***	***

Abbreviations: BCF, chicken breast meat obtained from a conventional farm; BAF, chicken breast meat obtained from an animal welfare farm; S, storage; T, treatment; n.s., not significant; ND, not detected.

[a–h]Means within the same row with different letters are significantly different at $P < 0.05$ (*) and $P < 0.001$ (***).

[1]Means between BCF and BAF are significantly differ at $P < 0.05$ (*) and $P < 0.001$ (***).

significantly lower from free-range broilers than from conventional broilers.

Total VBN

The VBN value of BCF was significantly higher ($P < 0.001$) than that of BAF throughout the storage period, except on day 1 (Table 3). The VBN values in meat samples of both types of farms increased with storage ($P < 0.001$). Moreover, the VBN values showed a significant interaction between the type of farm and storage period. VBN values are used as an indicator of meat freshness; and meats with low VBN values are considered to be fresh. Meats with VBN values higher than 20 mg/100 g are considered spoilt meat according to the Food Code in Korea (MFDS, 2018). Therefore, both BCF and BAF were considered to be spoilt on day 9. This trend is similar to the increase in total aerobic bacterial counts in chicken breast meat. Jung et al. (2010) showed that microorganisms and enzymes in meat increase proteolysis and lead to increases in the VBN value. These authors also reported that the VBN of chicken significantly increased over 12 D of storage (from 11 to 20 mg/100 g) at 4°C. However, in the present study, we found that the total aerobic bacterial counts of BAF and BCF were similar until day 7. Further studies are needed to understand these differences in study findings.

Fatty Acid Composition

The 3 major fatty acids in BCF and BAF were oleic acid (33.85 to 38.04%), palmitic acid (22.43 to 23.27%), and linoleic acid (16.01 to 17.52%). The fatty acid profiles of BAF and BCF did not show any significant differences throughout the entire storage period (Table 4). Our results are consistent with those of Nkukwana et al. (2014) who reported that the 3 most abundant fatty acids in chicken breast meat were oleic acid (28.04 to 33.55%), palmitic acid (21.64 to 25.31%), and linoleic acid (16.27 to 21.26%). Soares et al. (2009) reported that saturated fatty acid, monounsaturated fatty acid, and PUFA contents of chicken breast meat were 31.44 to 31.48%, 21.77 to 24.15%,

Table 4. Fatty acid composition of chicken breast meat from conventional and animal welfare farms in Korea.

	BCF					BAF						Significance		
Fatty acid (%)	1	3	5	7	9	1	3	5	7	9	SEM	T	S	T*S
C14:0 (Myristic acid)	1.07	1.04	0.89	0.98	1.02	1.02	1.03	1.02	1.05	1.02	0.070	n.s.	n.s.	n.s.
C16:0 (Palmitic acid)	23.20	23.33	23.50	23.41	23.71	23.01	23.37	22.43	22.70	23.24	0.385	n.s.	n.s.	n.s.
C16:1n7 (Palmitoleic acid)	3.86	3.94	4.50	4.11	4.34	4.62	4.01	4.12	4.14	4.23	0.192	n.s.	n.s.	n.s.
C18:0 (Stearic acid)	9.31	9.43	8.73	9.62	9.97	8.39	9.35	9.14	9.22	9.22	0.249	n.s.	n.s.	n.s.
C18:1n9 (Oleic acid)	36.93	34.91	35.63	33.85	35.07	38.04	35.51	35.35	36.15	35.61	0.951	n.s.	n.s.	n.s.
C18:1n7 (Vaccenic acid)	3.27	3.20	3.35	3.46	3.55	3.35	3.82	3.36	3.22	3.37	0.201	n.s.	n.s.	n.s.
C18:2n6 (Linoleic acid)	17.14	17.52	16.91	17.46	16.01	16.69	16.32	17.44	17.08	17.01	0.524	n.s.	n.s.	n.s.
C18:3n6 (γ-Linolenic acid)	0.46	0.57	0.69	0.66	0.56	0.41	0.61	0.60	0.57	0.54	0.084	n.s.	n.s.	n.s.
C18:3n3 (Linolenic acid)	0.84	0.81	0.78	0.75	0.70	0.83	0.75	0.77	0.81	0.77	0.035	n.s.	n.s.	n.s.
C20:1n9 (Eicosenoic acid)	0.33	0.40	0.40	0.53	0.24	0.34	0.37	0.41	0.53	0.43	0.086	n.s.	n.s.	n.s.
C20:4n6 (Arachidonic acid)	2.37	3.34	3.21	3.53	4.03	2.25	3.22	3.72	3.14	3.14	0.416	n.s.	n.s.	n.s.
C20:5n3 (Eicosapentaenoic acid)	0.17	0.20	0.19	0.26	0.25	0.19	0.22	0.19	0.17	0.22	0.034	n.s.	n.s.	n.s.
C22:4n6 (Adrenic acid)	0.63	0.84	0.80	0.93	0.81	0.55	0.92	0.96	0.84	0.78	0.099	n.s.	n.s.	n.s.
C22:6n3 (Docosahexaenoic acid)	0.31	0.49	0.41	0.45	0.43	0.31	0.48	0.50	0.39	0.43	0.056	n.s.	n.s.	n.s.
SFA	33.58	33.79	33.13	34.01	34.70	32.42	33.76	32.59	32.97	33.47	0.476	*[1]	n.s.	n.s.
USFA	66.42	66.21	66.87	65.99	65.30	67.58	66.25	67.41	67.03	66.53	0.476	*[1]	n.s.	n.s.
MUFA	44.40	42.45	43.88	41.95	43.20	46.36	43.72	43.24	44.04	43.64	0.764	n.s.	n.s.	n.s.
PUFA	22.02	23.76	22.99	24.04	22.10	21.22	22.53	24.17	22.99	22.89	0.654	n.s.	n.s.	n.s.
n6/n3	15.70	14.88	15.71	15.50	15.08	15.07	14.63	15.64	15.86	15.10	0.648	n.s.	n.s.	n.s.

Abbreviations: BCF, chicken breast meat obtained from a conventional farm; BAF, chicken breast meat obtained from an animal welfare farm; S, storage; T, treatment; n.s., not significant; SFA, saturated fatty acid; USFA, unsaturated fatty acid: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; n6/n3, ratio of n6 fatty acid and n3 fatty acid.

[1]Means between BCF and BAF are significantly differ at $P < 0.05$ (*).

and 42.08 to 45.94%, respectively; which are similar to the findings of the present study. Stadig et al. (2016) reported that the PUFA composition was higher in the breast meat of free-range chickens than that of chickens raised indoors. These authors suggested that this may be due to the higher plant intake of free-range chickens compared to that of conventional farm-raised chickens. In the present study, the broilers in AF were raised in more spacious area with access to some plant resources for pecking. Therefore, the access to such plant resources may account for the significantly higher unsaturated fatty acid composition of BAF than BCF. The fatty acid composition of chicken breasts did not show a significant interaction between the type of farm and storage period.

Creatine, Creatinine, and Di-Peptide (Anserine and Carnosine) Content

Several bioactive compounds such as di-peptides, free amino acids, creatine, and creatinine are present in the skeletal muscle tissue of vertebrate animals. Creatine, creatinine, and di-peptide (anserine and carnosine) can only be obtained by meat consumption and are absent in vegetarian foods (Schmid, 2009). Creatine and creatinine have neuroprotective effects (Schmid, 2009); in the present study, we found no significant differences between the creatine and creatinine contents of BCF and BAF (Table 5). However, the creatine content of BCF decreased ($P < 0.01$), but the creatinine content of BCF increased significantly during storage ($P < 0.001$). These findings may be attributed to non-enzymatic transformation whereby creatine is transformed into creatinine in the muscle via the removal of water and the formation of a ring structure (Mora et al., 2007). Moreover, creatinine did show a significant interaction between the farm type and storage period ($P < 0.05$).

As a di-peptide, carnosine (β-alanylhistidine) plays a key role in physiological functions such as oxidation inhibition and neurotransmission, being a potent intracellular buffering agent for pH maintenance (Wu and Shiau, 2002). Carnosine content varies with muscle type, age, breed, and sex of the animal (Intarapichet and Maikhunthod, 2005). Manhiani et al. (2011) reported that carnosine might facilitate the expression of heat stress genes and result in the production of stress proteins, therefore, carnosine can be used as an indicator of muscle stress. However, Dunnett et al. (2002) reported that plasma carnosine concentrations in horses increased after exercising for 5 to 30 min and then decreased at 120 min. They also suggested that the carnosine concentration returned to normal level after 1 D. In the present study, no significant differences were observed between the carnosine contents of BCF and BAF. It can be assumed that the carnosine content of chicken breast meats from both farms may decrease over time after slaughtering and chilling even if it was increased during transportation, resting in lairage, and slaughtering under stress. However, the carnosine contents of BCF and BAF decreased during storage ($P < 0.001$) and the value of BAF and BCF on day 9 were significantly lower than those on day 1. Nishimura et al. (1988) reported that the carnosine contents of chicken breast meat stored at 4°C for 2 D were significantly lower than those of meat obtained on the day of slaughter. These authors also reported that carnosinase in breast meat seemed to degrade carnosine during storage. In contrast, Moya et al. (2001) reported that carnosine contents were maintained during ageing of pork meat due to a shortage of proteases capable of hydrolyzing them. In the present study, we did not determine the carnosinase content in the breast meat; and further studies are needed to identify changes in carnosinase activity in chicken muscle during storage or ageing.

Anserine is an N-methylated derivative of carnosine and is abundant in nonmammalian species (such as poultry). This compound shows biological activity similar to that of carnosine (Abe and Okuma, 1995). The anserine contents of BCF and BAF significantly decreased throughout storage; however, the anserine content of BAF was higher than that of BCF ($P < 0.001$) on all days. Juniper and Rymer (2018) reported that the anserine contents were significantly higher in free-range chicken breast meat than in conventional chicken breast meat. These authors reported that the increase in anserine contents may be due to a higher amount of exercise in the free-range chickens. We assumed that the broilers in the AF were able to exercise more than those in the CF, which thus may account for the higher anserine content of the BAF. Further studies are needed to evaluate if the anserine value can be used as a bio marker for chicken meat from AF.

Table 5. Creatine, creatinine, anserine, and carnosine contents of chicken breast meat from conventional and animal welfare farms during cold storage.

	BCF					BAF						Significance		
Items (mg/100 g)	1	3	5	7	9	1	3	5	7	9	SEM	T	S	T*S
Creatine	183.18^{a}	182.67^{a}	171.96^{a-c}	167.51^{b,c}	161.62^{c}	178.73^{a,b}	182.82^{a}	174.85^{a,b}	176.00^{a,b}	172.91^{a-c}	3.852	n.s.	**	n.s.
Creatinine	1.04^{c}	1.50^{a}	1.52^{a}	1.60^{a}	1.54^{a}	1.22^{b}	1.48^{a}	1.44^{a,b}	1.52^{a}	1.54^{a}	0.052	n.s.	***	*
Anserine	92.60^{d-f}	94.99^{d-f}	104.57^{c-e}	88.36^{e,f}	81.85^{f}	130.10^{a}	117.54^{a-c}	123.55^{a,b}	129.54^{a}	110.19^{b-d}	5.791	***[1]	*	n.s.
Carnosine	63.16^{a,b}	58.01^{a-d}	54.69^{b-d}	55.88^{b-d}	46.38^{d}	68.52^{a}	65.30^{ab}	59.29^{a-c}	49.83^{c,d}	48.55^{c,d}	3.768	n.s.	***	n.s.

Abbreviations: BCF, chicken breast meat obtained from a conventional farm; BAF, chicken breast meat obtained from an animal welfare farm; S, storage; T, treatment: n.s., not significant.

$^{a-f}$Means within the same row with different letters are significantly different at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

[1]Means between BCF and BAF are significantly differ at $P < 0.001$ (***).

Table 6. Superoxide dismutase (SOD) activity of chicken breast meat from conventional and animal welfare farms during cold storage.

Items (U/g wet tissue)	BCF					BAF					SEM	Significance		
	1	3	5	7	9	1	3	5	7	9		T	S	T*S
SOD activity	3068.74^{a,b}	2740.19^{a-c}	2919.20^{a-c}	2926.62^{a-c}	2500.23^{b,c}	3010.72^{a-c}	3119.43^{a}	3025.07^{a,b}	2793.79^{a-c}	2555.15^{c}	157.25	n.s.	*	n.s.

Abbreviations: BCF, chicken breast meat obtained from a conventional farm; BAF, chicken breast meat obtained from an animal welfare farm; S, storage; T, treatment; n.s., not significant.
$^{a-c}$Means within the same row with different letters are significantly different at $P < 0.05$ (*).

SOD Activity

SOD is an antioxidant enzyme that inhibits the accumulation of excess reactive oxygen species in tissues and helps prevent subsequent oxidative damage (Bai et al., 2016). SOD immediately reacts with free radicals and accelerates the production of H_2O_2 and O_2 from O_2^-. No significant changes in SOD activity were observed between BCF (2500.23 to 3068.74 U/g wet tissue) and BAF (2555.15 to 3119.43 U/g wet tissue) throughout the entire storage period (Table 6). However, storage period did significantly affect the reduction of SOD activity ($P < 0.05$).

CONCLUSION

The present study demonstrates that the proximate composition, color, WHC, creatine, creatinine, and carnosine contents, and SOD activity did not differ between BCF and BAF during storage. However, some traits, such as the shear force value, unsaturated fatty acids, and anserine contents were higher in BAF than BCF in Korea. Furthermore, the pH, microorganism content, TBARS and VBN values, and saturated fatty acids were lower in BAF than in BCF. This work is a preliminary study, and further research is needed to check whether the advantage of AF is sustainable and if it is reasonable to pay a higher price for chicken meat from AF than from CF in Korea.

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