

Bioactive and Taste-related Compounds in Defatted Freeze-dried Chicken Soup Made from Two Different Chicken Breeds Obtained at Retail

Dinesh D. Jayasena^{1,2}, Samooel Jung¹, Amali U. Alahakoon¹, Ki Chang Nam³,
Jun Heon Lee¹ and Cheorun Jo⁴

¹Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea

²Department of Animal Science, Uva Wellassa University, Badulla 90000, Sri Lanka

³Department of Animal Science and Technology, Suncheon National University, Suncheon 540-742, Republic of Korea

⁴Department of Agricultural Biotechnology, Center for Food and Bioconvergence,

Research Institute for Agriculture and Life Science, Seoul National University, Seoul 151-921, Republic of Korea

The aim of this study was to compare the bioactive and taste-related compounds in defatted freeze-dried chicken soup (DFDS) made from Korean native chickens (*Woorimatdag*TM, KNC) and commercial broilers (CB) available at retail. The betaine, carnitine, histidine dipeptide, creatine, nucleotide, free amino acid content, and fatty acid composition were analyzed in six DFDS samples from each breed. Histidine dipeptides were not detected in any DFDS samples. DFDS made with KNC had significantly higher betaine, carnitine, inosine-5'-monophosphate, inosine, and cysteine content compared to that prepared from CB. Furthermore, lipid layer separated from soup made with KNC showed a significantly higher linoleic, α -linolenic, arachidonic, and docosahexaenoic acid content, and lower saturated fatty acid content. In addition, DFDS from CB possessed a higher valine, isoleucine, leucine, phenylalanine, methionine, creatine, and hypoxanthine content than that from KNC. Our findings suggest that DFDS from KNC was qualitatively superior due to enhanced nutritional and taste-related factors compared to that made with CB.

Key words: arachidonic acid, betaine, broiler, carnitine, inosine-5'-monophosphate, Korean native chicken

J. Poult. Sci., 52: 156-165, 2015

Introduction

The unique culture of diverse ethnic groups or nations is generally linked with the food and consumption habits. The meat consumption culture in Korea developed because of the influence of ancient nomadic lifestyle (Nam *et al.*, 2010). Different cooking methods including grilling, steaming, and stewing have been well developed since the 13th century when the Mongolians invaded Korea (Nam *et al.*, 2010). Meats from cattle, pig, and chicken dominate the Korean meat cuisines, in which they are consumed either roasted or as soups or stews (Nam *et al.*, 2010). In Korean culture, chicken is frequently served roasted or braised with vegetables or in soups of different types.

Soup is generally considered as a heterogeneous food category with high satiety value, and reduces hunger (food

source) as well as quenches thirst as a beverage (Mattes, 2005). Of many meat-based soups found in Korea, *samgyetang* and *baeksuk* are well-known traditional soups prepared using young and mature Korean native chickens (KNC), respectively (Nam *et al.*, 2010). Korean consumers prefer KNC meat to that from commercial broilers (CB) because of the characteristic flavor and texture of the KNC meat. Nevertheless, the traditional dishes such as *samgyetang* and *baeksuk* are now being prepared using CB because KNC production is inadequate to meet the consumer demand compared to the production of CB (Jeon *et al.*, 2010; Jayasena *et al.*, 2013c). As a result, the original characteristics contributed by KNC to enhance the quality of meat-based soups have been affected. Hence, it is vital to elucidate whether the breed of chicken significantly influences the quality of meat-based soups. Scientific information regarding the quality traits and the molecules responsible for the flavor of meat-based soups, particularly in those prepared with native chickens such as KNC is limited. Recently, similar color, taste, and flavor characteristics were found in soups made with KNC and CB (Jayasena *et al.*, 2013c).

Received: June 24, 2014, Accepted: October 7, 2014

Released Online Advance Publication: November 25, 2014

Correspondence: Dr. Cheorun Jo, Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute for Agricultural and Life Science, Seoul National University, Seoul 151-921, Republic of Korea. (E-mail: cheorun@snu.ac.kr)

Chiang *et al.* (2007) reported that foods have four functionalities; the nutritional value, taste properties, physiological effects, and cultural characteristics. Similarly, soups have been shown to possess similar characteristics. For instance, soups can boost the appetite by stimulating the secretion of saliva. Furthermore, they support peristalsis of the stomach, which assists food intake (Chiang *et al.*, 2007) and promotes easy nutrient delivery (Ke *et al.*, 2011). Soups are also an excellent food for convalescents primarily due to their high digestibility and nutritive value including low fat content (Gadekar *et al.*, 2009). In addition, chicken soup is usually popular as the most ubiquitous medicinal soup in the world (Ke *et al.*, 2011). *Samgyetang* is consumed by Korean consumers to reduce heat during summer times (Nam *et al.*, 2010). Ke *et al.* (2011) reported that the amount of nutrients, including carbohydrates, proteins, lipids, and micronutrients, which is extracted from meat into soups during boiling, was in relatively small. Nevertheless, the availability of distinct bioactive compounds such as betaine, carnitine, creatine, carnosine, and anserine in meat-based soups are yet to be well documented.

One of the most important quality traits that promote the extensive consumption of soup is the flavor. The distinctive flavor of soup is facilitated by the presence of non-volatile taste components including nucleotides, free amino acids, and soluble sugars other than the volatile compounds (Chiang *et al.*, 2007). Previous findings have shown that the content of nucleotides and free amino acids, and the fatty acid composition in meat are significantly different between KNC and CB (Choe *et al.*, 2010; Jayasena *et al.*, 2013c), and that this difference might influence the availability and the content of similar compounds in the soups made with these meats. Therefore, this study was designed to compare the availability and quantity of bioactive and taste-related compounds in the defatted freeze-dried chicken soups (DFDS) made with KNC and CB available at retail.

Materials and Methods

Chemicals and Reagents

Sodium phosphate dibasic (Na_2HPO_4), silver oxide (Ag_2O), 2,4'-dibromoacetophenone, 18-crown-6, ammonium acetate, and all the standards [carnosine, anserine, creatine, betaine, l-carnitine hydrochloride, hypoxanthine, inosine, inosine-5'-monophosphate (IMP), adenosine-5'-monophosphate (AMP), fatty acid esters, and free amino acids] were purchased from Sigma Corp. (St. Louis, MO, USA). Hexane, ethanol, chloroform, hydrochloric acid (HCl), perchloric acid, potassium hydroxide, and sodium chloride (NaCl) were obtained from Samchun Chemicals Co. Ltd. (Gyeonggi-do, Korea). Trichloroacetic acid was acquired from Alfa Aesar (Ward Hill, MA, USA). Acetonitrile, triethylamine and methanol were purchased from Avantor Performance Materials Inc. (Central Valley, PA, USA).

Instrumentation

The soup samples were lyophilized using a freeze dryer (TFD 5505, IL Shin Lab Co. Ltd., Gyeonggi-do, Korea). The samples were homogenized using a T25b disperser [Ika

Works (Asia), Rawang, Malaysia]. Centrifugation of the samples was done using a centrifuge machine (HM-150IV) supplied by Hanil Co. Ltd. (Incheon, Korea). The carnosine, anserine, creatine, betaine, and carnitine content were quantified by high-performance liquid chromatography (HPLC) using a Ultimate-3000 chromatography instrument (Thermo scientific, Germering, Germany) equipped with a 2487-diode array detector, a 1525 pump, a 717 plus autosampler, and a Atlantis HILIC silica column (Waters Corp., Milford, MA, USA) of 4.6×150 mm ($3 \mu\text{m}$ particle size). The quantification of free amino acids was done using AccQ-TagTM reverse-phase HPLC column (3.9×150 mm; Waters Corp.). The nucleotide content was quantified using ACME 9000 HPLC system (Younglin Instruments Inc., Seoul, Korea) and a Waters-Atlantis dC18 reverse-phase column (4.6×250 mm, $5 \mu\text{m}$ particle size; Waters Corp.). The fatty acid composition was analyzed using a gas chromatography instrument (HP 7890, Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary column ($30 \text{ m} \times 0.32$ mm; $0.25 \mu\text{m}$; Omegawax 320, Supelco, Bellefonte, PA, USA).

Preparation of DFDS Samples

Meat from six commercial KNC (*Woorimatdag*TM) aged approximately 80 d and six CB aged approximately 32 d available at retail were used to prepare the DFDS samples in this study. The meat from both breeds was purchased from a local market (Homeplus, Daejeon, Korea) and stored in a freezer at -20°C until further analysis. For the preparation of the DFDS samples, each frozen carcass of KNC and CB was first thawed in a refrigerator (4°C) for 24 h and portioned in to two halves. After trimming the visible skin and fat, the thawed meat was boiled separately in water (1:1.5 wt/vol) for 40 min. After removing the cooked meat from the boiling water, the remaining soup samples were further boiled for 2 h and concentrated. The concentrated soup samples were subsequently filtered through a testing sieve (wire diameter of $100 \mu\text{m}$, aperture of $150 \mu\text{m}$; Chunggye Sanggongsa, Seoul, Korea) in order to remove the solid particles. The filtrates were mixed with hexane (1:1 vol/vol), stirred using a magnetic stirrer (C-MAG HS 7, Ika Korea Co. Ltd., Seoul, Korea) for 1 h, and allowed to settle. The resulting supernatant layers that mainly consisted of fat were carefully removed to separate containers and used for the analysis of fatty acid composition. The remaining solutions were finally lyophilized at -53°C under pressurized vacuum at 7 mTorr. The DFDS samples were then stored in a freezer (-80°C) until further analysis.

Determination of Carnosine, Anserine, and Creatine Content in DFDS Samples

The content of carnosine, anserine, and creatine were determined using the method of Mora *et al.* (2007) with some modifications. Each DFDS sample (2 g) was homogenized with 7.5 mL of 0.01 N HCl at 13,500 rpm for 1 min, and then centrifuged at $17,030 \times g$ for 15 min. The supernatant ($250 \mu\text{L}$) was mixed with $750 \mu\text{L}$ acetonitrile. Following a storage period of 20 min at 4°C and centrifugation at $10,000 \times g$ for 10 min, the supernatant was filtered through a 0.2

μ m-polyvinylidene difluoride (PVDF) syringe filter (Whatman International Ltd., Pittsburgh, PA, USA) and injected into the HPLC column. The partitioned fractions were detected at 214 nm to determine the creatine, carnosine, and anserine contents. A gradient elution was performed using a linear gradient (0 to 100%) mobile phase B at a flow rate of 1.2 mL/min for 16 min with mobile phase A as a background. Mobile phase A consisted of 0.65 mM ammonium acetate in water:acetonitrile (25:75, vol/vol, pH 5.5), and phase B consisted of 4.55 mM ammonium acetate in water:acetonitrile (70:30, vol/vol, pH 5.5). The content of each compound in the samples was determined using the standard curve derived from the respective standards.

Determination of Betaine and Carnitine Content in DFDS Samples

Betaine and carnitine content in each DFDS sample were determined using the method described by Li *et al.* (2007) with some modifications. First, the DFDS samples (1.5 g) were homogenized separately in 10 mL precipitating reagent (acetonitrile: methanol solution, 9:1 vol/vol) at 13,500 rpm for 30 s and centrifuged at $2,090\times g$ for 5 min. The supernatant was then filtered into a 20-mL volumetric flask through a funnel plugged with glass wool. This procedure was repeated after adding 10 mL precipitating reagent and the supernatant was again collected into the same volumetric flask. This filtrate was made up to 20 mL with the precipitating reagent and 2 mL of the resulting solution was thoroughly mixed with 0.810 g Na_2HPO_4 and 0.090 g Ag_2O (9:1 wt/wt) in a 15-mL tube. The sample tubes were air-dried in a shaker without their caps for 30 min, and then centrifuged at $2,090\times g$ for 5 min. The supernatant (0.5 mL) was added to 0.5 mL derivatizing reagent (1.39 g 2,4'-dibromoacetophenone and 0.066 g 18-crown-6 in 100 mL acetonitrile) in a 15-mL tube and vortex-mixed. The mixture was heated at 80°C for 60 min in a water bath and then cooled for 5 min under running water. This mixture was filtered through a 0.2- μ m PVDF syringe filter (Whatman International Ltd.) and analyzed in the HPLC system at 254 nm. An isocratic elution was performed where the mobile phase was a 9:1 mixture of 25 mM ammonium acetate in formic acid (pH 3.0) and acetonitrile. The mobile phase was supplied at 1.4 mL/min for 20 min. The betaine and carnitine content were calculated using a standard curve for each compound.

Determination of Nucleotide Content in DFDS Samples

Nucleotide content of each DFDS sample was measured according to the procedure of Jung *et al.* (2011). The nucleic acids were extracted from the DFDS samples (0.5 g each) using 25 mL of 0.7 M perchloric acid. The pH of the extract was then adjusted to 7 with 5 N KOH. Subsequently, it was placed into a volumetric flask, and made up to a volume of 100 mL with 0.7 M perchloric acid (pH 7). After 30 min of cooling, the mixture was centrifuged at $1,130\times g$ (0°C), and the supernatant was filtered through a 0.2- μ m-PVDF syringe filter (Whatman International Ltd.). The filtrate (5 mL) was analyzed using the HPLC system. A mobile phase of 0.1 M of triethylamine in 0.15 M of acetonitrile (pH 7.0) with a flow rate at 1.0 mL/min was used. The injection volume was

10 μ L and elution time was 25 min. The column temperature was maintained at 35°C and detection was monitored at a wavelength of 260 nm. The peaks of individual nucleotides were identified using the retention times for standards and the concentrations were calculated using the area under each peak.

Determination of Fatty Acid Composition of Lipid Layers Separated from Soups

Total lipid content of the supernatant layer separated from each soup sample was extracted according to the method of Folch *et al.* (1957) with some modifications. Each sample was mixed (1:2, vol/vol) with chloroform-methanol solution (2:1, vol/vol) and stirred using a magnetic stirrer (Ika Korea Co. Ltd.) for 1 h. Subsequently, each mixture was added with 40 mL of 0.88% NaCl, stirred well and allowed to settle overnight. The resulting supernatant layer containing methanol was removed and the mixture was filtered (filter paper No.4, Whatman International Ltd.). The remaining chloroform in the filtrate was evaporated by an Eyela N-1000 rotary evaporator (Tokyo Rikakikai Co. Ltd, Tokyo, Japan). The extracted oily solution was further evaporated by N_2 gas (99.999%) and used for the analysis of fatty acid composition.

The extracted oily samples (0.5 g) were first mixed with 1 N KOH in ethanol (50 mL) and heated in a water bath (70°C) for 1 h. After cooling, 10 mL of each sample was transferred to a 50-mL tube, vortex-mixed with distilled water and hexane (10 mL each), and centrifuged at $1,130\times g$ for 3 min. The supernatant was removed and the remaining mixture was then mixed with 6 mL of HCl-distilled water solution (1:1, vol/vol) and 5 mL of hexane, vortex-mixed and centrifuged at $1,130\times g$ for 3 min. The resulting supernatant hexane layer was collected in a 15-mL tube. A similar extraction was repeated after adding 5 mL of hexane to the remaining original mixture. The resulting supernatant hexane layer was again collected in the same tube, which was then completely evaporated using N_2 gas (99.99%). Next, 1 N H_2SO_4 in methanol (5 mL) was added to each tube and heated in a water bath (50°C) for 1 h. After cooling, 2 mL of distilled water and 3 mL of hexane were added to the same tubes, which were then centrifuged at $1,130\times g$ for 3 min. The top hexane layer containing FAME (fatty acid methyl esters) was transferred to another 15-mL tube, concentrated up to 1.5 mL using N_2 gas (99.999%), and dehydrated through anhydrous Na_2SO_4 in to a vial. Fatty acid composition was then analyzed using gas chromatography. A split inlet (split ratio, 100:1) was used to inject the samples into the capillary column and ramped oven temperature was used for the analysis (150°C for 5 min, increased to 170°C at $5^\circ\text{C}/\text{min}$ and maintained for 8 min, then increased to 190°C at $5^\circ\text{C}/\text{min}$ and maintained for 15 min and finally increased to 220°C at $5^\circ\text{C}/\text{min}$ and maintained for 30 min). The inlet temperature was 210°C . N_2 gas was used as the carrier gas at a constant flow rate of 0.7 mL/min.

Determination of Free Amino Acid Content in DFDS Samples

Free amino acid content was analyzed using the method

described by Hughes *et al.* (2002) with some modifications. Each DFDS sample (1.5 g) was mixed with 20 mL of 2% trichloroacetic acid solution and homogenized at $1,130 \times g$ for 30 s. The homogenate was centrifuged at $17,000 \times g$ for 30 min and filtered through a 0.45- μ m PVDF syringe filter (Whatman International Ltd.). The filtrate was derivatized using AccQ-TagTM (Waters Corp.) according to the manufacturer's protocol, and 5 μ L was injected into the reverse-phase HPLC column. The column temperature was 37°C and a fluorescent detector (WatersTM 2475, Millipore, Billerica, MA, USA) was used with 250 nm and 395 nm of excitation and emission wavelengths, respectively. The separation was done by using buffers: A (Waters AccQ-Tag eluent) and B (60%, vol/vol, acetonitrile). Individual amino acids were identified by comparison of their retention times with those of calibration standards. Peak areas were processed using Millennium 32 software and the concentrations of individual amino acids were expressed as mg/100 g.

Statistical Analysis

The concentration of bioactive and taste-related compounds in the DFDS made with each breed is expressed as mean and standard error from six independent DFDS samples. The experimental data were subjected to an analysis of variance for a completely randomized design using the procedure of General Linear Model using SAS software system (Release 9.3, SAS Institute Inc., Cary, NC, USA). Comparisons of means were performed by Duncan's multiple range tests at $P < 0.05$.

Results and Discussion

The effect of different breeds of chicken on bioactive and taste-related compounds in DFDS was examined in this study. Scientific literatures regarding the availability of such compounds in meat-based soups made with poultry or other species are scarce.

Carnosine, Anserine and Creatine Content in DFDS Samples

Carnosine and anserine, two well-known histidine-derived dipeptides, have strong buffering and antioxidant properties (Peiretti *et al.*, 2012). In addition, carnosine possesses anti-ageing properties (Purchas *et al.*, 2004) and a strong defense mechanism against glycation and oxidation (Peiretti *et al.*, 2011). Anserine is found abundantly in most types of poultry meat including KNC (Jung *et al.*, 2013). The histidine dipeptide content of DFDS prepared from KNC and CB obtained at retail are given in Table 1. A clear-cut effect of heat treatment on the retention of histidine dipeptides in the cooked meat has been previously reported. The content of carnosine in lamb (Purchas *et al.*, 2004), and the amount of carnosine and anserine in beef, and turkey (Peiretti *et al.*, 2012) were depleted during cooking, particularly during boiling in water, and the cooking liquor was primarily attributed to be responsible for the high water solubility of carnosine (Purchas *et al.*, 2004; Peiretti *et al.*, 2012). However, both histidine dipeptides were not detected in any of the DFDS samples in this study (Table 1). Recently, Jayasena *et al.* (2015) revealed that the carnosine content in raw and

Table 1. **Carnosine, anserine, and creatine content (mg/100 g) of defatted freeze-dried chicken soups made from two different chicken breeds obtained at retail**

Breed	Carnosine	Anserine	Creatine
Korean native chicken	— ¹	—	96.31 ^b
Commercial broiler	—	—	146.41 ^a
SEM ²	—	—	12.64
p-value	—	—	0.04

¹ Not detected.

² Standard error of the means ($n=12$).

^{a,b} Means in the same column with different letters differ significantly ($P < 0.05$).

cooked meat from KNC and CB differed significantly. Carnosine and anserine contents significantly decreased after cooking, with average values of 94.771 and 319.850 mg/100 g in cooked meat, respectively compared to average values of 115.740 and 383.793 mg/100 g in raw meat, respectively ($p < 0.05$; Jayasena *et al.*, 2015). Hence, it can be suggested that a depletion of carnosine and anserine into liquor (soup) has been taken place. However, in the same study, the retention values for carnosine and anserine were much higher (86 to 88% for carnosine and 78 to 91% for anserine) in KNC and CB meats compared to those observed in lamb meat (carnosine, 76%; Purchas *et al.*, 2004), beef (carnosine, 50% and anserine, 30%), and turkey meat (carnosine, 60% and anserine, 65%; Peiretti *et al.*, 2012). Therefore, the higher retention value of these dipeptides in KNC and CB meats during cooking might be the reason for their not being detected in the DFDS samples during this study. Similarly, these two histidine dipeptides were not found in freshwater clam and hard clam essences (Wu and Shiau, 2002). In contrast, minute amounts of carnosine were detected in Conger eel extract and lobster extracts (Sri Kantha *et al.*, 2000). Although the eel meat was rich in carnosine, the eel essence contained only very little carnosine with no anserine. However, several other authors have stated that the meat extracts of chicken, turkey, and beef and six chicken essences had considerable amount of anserine and carnosine (Crush, 1970; Sri Kantha *et al.*, 2000; Wu and Shiau, 2002). The carnosine content of a commercially available chicken muscle extract (70 to 90 mg/100 g) was lower than that of beef muscle extract (3,750 to 3,820 mg/100 g; Sri Kantha *et al.*, 2000). However, further investigations are needed to find out the reasons for the absence of these histidine dipeptides in DFDS made from KNC and CB.

Creatine and creatine phosphate are vital components in muscle energy metabolism and they provide the energy required for vigorous muscle contraction (Wyss and Kaddurah-Daouk, 2000). Generally, the glycolytic muscles have higher creatine content mainly owing to their anaerobic metabolism (Mora *et al.*, 2008), which demand higher levels of phosphocreatine for immediate regeneration of ATP. Furthermore, creatine can contribute certain sensory properties to food such as the overall flavor of meat extracts (Mora

et al., 2010). In the present study, the DFDS made from CB had higher content of creatine ($P < 0.05$) as opposed to that of KNC (Table 1). Cambero *et al.* (2000) showed that the creatine content of beef broth ranged between 6,112 and 6,600 $\mu\text{mol/L}$. Jayasena *et al.* (2015) found that CB meat contained a significantly higher level of creatine than did KNC meat, which is likely to be due to the distinctive muscle fiber composition of the former breed compared with that of the latter. Jurie *et al.* (1995) and Jaturasitha *et al.* (2008) explained that the CB mainly possess more type IIB glycolytic muscle fibers compared to the indigenous breeds such as KNC, which eventually facilitate a much higher creatine content in CB meat.

Betaine and Carnitine Content in DFDS Samples

Betaine acts as an osmolyte that protect cells, proteins, and enzymes from environmental stress. It is also involved in the methionine cycle as a methyl donor (Craig, 2004), and has the potential to improve the growth performance and fat distribution of livestock (de Zwart, 2003). Carnitine is responsible for transporting long-chain fatty acids across the inner mitochondrial membranes for β -oxidation and thereby performs its role in fatty acid metabolism in animals. In addition, it has a strong buffering potential against excess acetyl group formation during exercise (Constantin-Teodosiu *et al.*, 1996). Figure 1 shows the differences in betaine and carnitine contents of DFDS derived from different breeds of chicken obtained at retail. There were significant differences in the carnitine ($P = 0.0008$) and betaine contents ($P = 0.002$) between the DFDS made from KNC and CB. The betaine content of the DFDS prepared from KNC was approximately two times higher than that made from CB. Scientific literatures on the quantification of the amount of betaine in meat soups are rare. Zeisel *et al.* (2003) and Patterson *et al.* (2008) reported that chicken noodle soup contained 11.87 to 12.00 mg of betaine/100 g. These authors further found the same bioactive compound in New England clam chowder soup (23.93 to 24.00 mg/100 g). Jayasena *et al.* (2015) recently demonstrated that betaine content of raw KNC and CB meat was depleted significantly during cooking due to its high water solubility (de Zwart *et al.*, 2003). This observation is consistent with the considerable amount of betaine detected in the DFDS samples of KNC and CB in this study. In addition, Jayasena *et al.* (2015) observed a higher retention of betaine in the cooked meat of CB (82%) compared to that of KNC (63%). This higher depletion of betaine from KNC meat could be the reason for the greater content of this bioactive compound in the DFDS samples derived from KNC than that of CB.

KNC showed the highest carnitine content in the DFDS samples during this study (Fig. 1; $P < 0.05$), which was approximately 3.5 times greater than the carnitine content observed in the DFDS from CB. According to the recent findings of Jayasena *et al.* (2015), KNC possessed a higher concentration of carnitine in their meat compared to CB, which could be attributed to higher levels of type I fibers in the indigenous chickens (Jaturasitha *et al.*, 2008) such as KNC. As a result, KNC require higher carnitine content in

order to buffer the excessive acetyl groups produced due to higher mitochondrial contents in type I fibers (Constantin-Teodosiu *et al.*, 1996). Additionally, Jayasena *et al.* (2015) showed that the cooked meat of KNC and CB had significantly lower carnitine content than the corresponding levels in their raw meat. Therefore, the higher water solubility of carnitine (Arslan *et al.*, 2003) and the longer boiling period during the preparation of soup samples resulted in substantial amount of carnitine in the DFDS samples, irrespective of the chicken breed. However, similar to betaine, CB showed much higher retention of carnitine (91%) in the cooked meat as opposed to KNC (67%; Jayasena *et al.*, 2015). This higher retention of carnitine in CB meat might result in the lower carnitine content in its DFDS samples compared to that of KNC.

Nucleotide Content in DFDS Samples

IMP is the major nucleotide found in muscle and it is considered one of the major precursors responsible for flavor (Jayasena *et al.*, 2013b). Degradation of IMP to inosine takes place during aging and cooking and inosine can further be degraded to hypoxanthine and ribose (Tikk *et al.*, 2006). Ribose in meat ultimately contributes to flavor via the Maillard reaction. The umami taste is attributed to the synergistic effect of inosinic acid and glutamic acid (Jo *et al.*, 2012). Additionally, IMP possesses the characteristics of umami flavor (Tikk *et al.*, 2006; Jayasena *et al.*, 2013b). In contrast, hypoxanthine together with several free amino acids, anserine, carnosine, and other dipeptides might contribute to bitter flavor characteristics (Tikk *et al.*, 2006). The content of ATP breakdown products in the DFDS prepared from KNC and CB obtained at retail are shown in Table 2. The breed of chicken significantly influenced the content of all ATP breakdown products in the DFDS samples. It has been well documented that the nucleotides content in muscles varied with the species, breed, age, and sex (Bailey, 1983). KNC showed higher content of AMP, IMP, and inosine in the DFDS samples than CB ($P < 0.05$). In contrast, CB had a significantly higher content of hypoxanthine compared to KNC. Similar to the findings of the present study, Wei *et al.* (2012) stated that soups prepared with Jiangsu traditional chickens contained higher AMP and IMP contents together with the best overall flavor in comparison to that of a CB breed (817 broiler). Chiang *et al.* (2007) detected higher content of AMP and IMP in broth cubes of pork than those of chicken. The concentrations of AMP, IMP, inosine, and hypoxanthine in beef broth at 95°C were 156, 1081, 551, and 562 $\mu\text{mol/L}$, respectively (Cambero *et al.*, 2000). Jayasena *et al.* (2013a) reported that IMP content was significantly higher in the meat of indigenous chickens such as KNC (Jung *et al.*, 2011) and Hinai-jidori chicken (Rikimaru and Takahashi, 2010), and slow-growing Wenchang and Xianju genotypes in China (Tang *et al.*, 2009) compared to that of CB. On the other hand, Jung *et al.* (2011) and Jayasena *et al.* (2013c) observed higher inosine content ($P < 0.05$) in the CB meat compared to that in KNC meat, but similar hypoxanthine content ($P > 0.05$) in both chicken breeds.

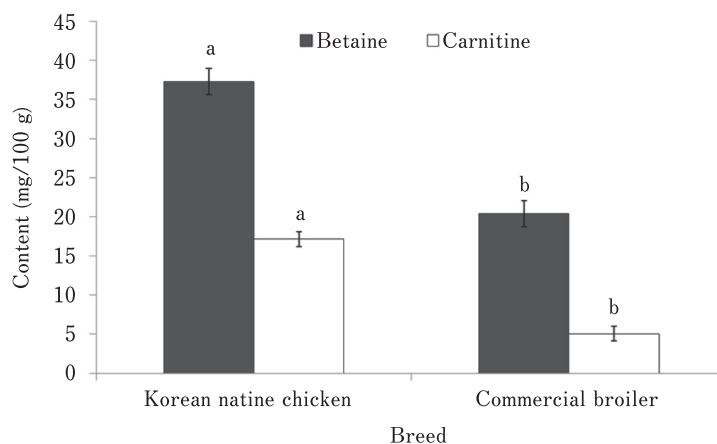


Fig. 1. **Betaine and carnitine content (mg/100 g) of defatted freeze-dried soups made from two different chicken breeds obtained at retail.** Results are expressed as means with error bar indicating standard error of the means ($n=12$). Means with different letters within each compound indicate significant differences ($P<0.05$).

Table 2. **Nucleotide content (g/100 g) of defatted freeze-dried chicken soups made from two different chicken breeds obtained at retail**

Breed	AMP ¹	IMP ²	Inosine	Hypoxanthine
Korean native chicken	0.28 ^a	1.50 ^a	1.32 ^a	0.48 ^b
Commercial broiler	0.20 ^b	0.63 ^b	1.23 ^b	0.78 ^a
SEM ³	0.002	0.007	0.012	0.007
p-value	<0.0001	<0.0001	0.0005	<0.0001

¹ Adenosine-5'-monophosphate.

² Inosine-5'-monophosphate.

³ Standard error of the means ($n=12$).

^{a,b} Means in the same column with different letters differ significantly ($P<0.05$).

Fatty Acid Composition of Lipid Layers Separated from Soups

Table 3 presents the fatty acid composition of the lipid layers separated from soup prepared with KNC and CB. No previous reports were found regarding the fatty acid composition of meat-based soups. The composition of all fatty acids found in the lipid layers was significantly affected by the breed of chicken. However, diet of the bird may also have affected the fatty acid composition of these lipid layers because the meat was obtained from a local market in this experiment. Oleic acid (C18:1), linoleic acid (C18:2), and palmitic acid (C16:0) were the main fatty acids found in both KNC and CB meats (Choe *et al.*, 2010; Jung *et al.*, 2011; Jayasena *et al.*, 2013c). The same fatty acids were detected as the predominant fatty acids in the lipid layers removed during DFDS preparation using the meat of these two strains during this study (Table 3). However, among all the fatty acids, oleic acid was the principal fatty acid detected in the DFDS samples, irrespective of the breed of chicken. Oleic acid is considered as one of the major fatty acids related to meat flavor (Choe *et al.*, 2010).

The content of myristic (C14:0) and palmitic (C16:0) acids in the lipid layer separated from the soup made with CB were significantly higher than the corresponding levels in the lipid layers removed from the soup made with KNC. Similarly, the lipid layer made with CB had greater content of monounsaturated fatty acids, palmitoleic acid (C16:1), and oleic acid, compared to that of KNC (Table 3). On the other hand, the lipid layer separated from the soup derived from KNC had the highest content of stearic acid (C18:0). The polyunsaturated fatty acid (PUFA) contents were also significantly higher in the lipid layer from KNC than that from CB. Among these PUFAs, the essential fatty acids such as linoleic (C18:2), α -linolenic (C18:3), and arachidonic acids (C20:4) are vital for the well-being of humans as they cannot be biosynthesized within the human body (Jung *et al.*, 2011). In addition, arachidonic and docosahexaenoic (C22:6; DHA) acids are well-known for their flavor enhancing properties (Koriyama *et al.*, 2002; Kiyohara *et al.*, 2011; Jayasena *et al.*, 2013a). Koriyama *et al.* (2002) showed that DHA suppressed sourness and bitterness but increased sweetness and umami characteristics. Additionally, Kiyohara *et al.* (2011)

Table 3. Fatty acid composition (%) of lipid layer separated from chicken soups made from two different breeds obtained at retail

Fatty Acid	Korean Native Chicken	Commercial Broiler	SEM ¹	p-value
Myristic acid (C14:0)	1.02 ^b	1.30 ^a	0.004	<0.0001
Palmitic acid (C16:0)	22.92 ^b	27.47 ^a	0.073	<0.0001
Palmitoleic acid (C16:1)	3.44 ^b	7.14 ^a	0.014	<0.0001
Stearic acid (C18:0)	6.89 ^a	6.59 ^b	0.039	0.0017
Oleic acid (C18:1)	36.39 ^b	43.04 ^a	0.074	<0.0001
Linoleic acid (C18:2)	26.74 ^a	13.25 ^b	0.034	<0.0001
?-linolenic acid (C18:3)	1.65 ^a	0.68 ^b	0.008	<0.0001
Eicosenoic acid (C20:1)	0.59 ^a	0.39 ^b	0.006	<0.0001
Arachidonic acid (C20:4)	0.29 ^a	0.07 ^b	0.002	<0.0001
Docosahexaenoic acid (C22:6)	0.06 ^a	0.01 ^b	0.000	<0.0001

¹ Standard error of the means ($n=12$).

^{a,b} Means in the same row with different letters differ significantly ($P<0.05$).

showed that total taste intensity and aftertaste of both chicken soup and steamed minced meat were significantly greater when Hinai-jidori chickens were fed an arachidonic acid-enriched diet. Choe *et al.* (2010) observed comparable linoleic acid contents between the KNC and CB meats. However, the lipid layer of soup made with CB had a significantly lower content of linoleic acid than that of KNC (Table 3). Furthermore, lipid layer from KNC showed approximately two and four times higher content of α -linolenic and arachidonic acids, respectively, compared to that from CB. Similarly, several other studies also indicated that the indigenous chicken meat such as KNC (Jeon *et al.*, 2010; Jung *et al.*, 2011; Jayasena *et al.*, 2013c) and Hinai-jidori chickens (Rikimaru and Takahashi 2010) contained significantly higher content of arachidonic acid in their meat than CB. Differences in diet (Cherian *et al.*, 2002) and feeding behavior between the breeds (Wattanachant *et al.*, 2004) may be responsible for the differences in the fatty acid composition of lipid layers separated during DFDS preparation in addition to the breed effect. The fat layer of soup made with KNC showed 6 times higher content of DHA as that made with CB (Table 3). Similar to the findings of this study, higher DHA levels were found in the meat of KNC (Jeon *et al.*, 2010; Jayasena *et al.*, 2013c) and Hinai-jidori chickens (Rikimaru and Takahashi, 2010) as opposed to that of CB. Thus, the relatively higher content of DHA, linoleic, α -linolenic, and arachidonic acids in the fat layer of soup made with KNC can be considered to enhance its nutritional value if it is also incorporated in the freeze-dried soup during preparation.

Free Amino Acid Content in DFDS Samples

The breed of chicken significantly influenced the free amino acid content of DFDS samples, except serine, glutamic acid, glycine, histidine, and alanine (Table 4). Scientific literatures regarding the free amino acid content of meat-based soups were scarce, which prevented a comparison of the data of the present study with previous reports. Free amino acids are vital for flavor development and

therefore for enhancing edible value of meat (Toldrá, 1998; Lim *et al.*, 2013). For instance, amino acids such as glycine, alanine, lysine, and serine have been shown to be closely associated with a sweet flavor whereas glutamic and aspartic acid contributed to the pleasantly fresh or umami taste of meat (Zhu and Hu, 1993; Lim *et al.*, 2013). On the other hand, valine, isoleucine, leucine, phenylalanine, methionine, arginine, and histidine are associated with a bitter taste (Zhu and Hu 1993; Lim *et al.*, 2013). According to Table 4, there was no difference ($P>0.05$) in the content of umami taste related free amino acid, glutamic acid, in the DFDS samples between KNC and CB, but the content of aspartic acid which contributes to the same sensory character was higher ($P<0.05$) in the DFDS from CB compared to that of KNC. Both these free amino acids were previously found in similar amount in the CB and KNC meat (Jung *et al.*, 2011). Additionally, DFDS derived from CB had a significantly higher level of lysine than that from KNC, but all the other free amino acids related to the sweet flavor were comparable between the DFDS samples made with KNC and CB. All the free amino acids were detected in higher amount in the DFDS samples made with KNC and CB compared to those found in commercial chicken essences (Wu and Shiau, 2002). However, the DFDS samples from KNC and CB had lower content of aspartic acid, valine, methionine, isoleucine, leucine, and lysine than beef essence in the same study. Alanine was the major free amino acid found in essences from hard clam and freshwater clam whereas eel essences contained leucine, lysine, taurine, phenylalanine, and arginine as the predominant free amino acids (Wu and Shiau, 2002). In addition, much lower levels of free amino acids were reported in chicken broth cubes (Chiang *et al.*, 2007) as compared to the levels found in this study. Furthermore, broth cubes made with pork only contained minimal amount of alanine, arginine, glutamic acid, histidine, serine, and tyrosine (Chiang *et al.*, 2007). These variations could be attributed to different processing methods and raw materials used during the manufacturing of these essences, broth

Table 4. Free amino acid content (mg/100 g) of defatted freeze-dried chicken soups made from two different chicken breeds obtained at retail

Free Amino Acid	Korean Native Chicken	Commercial Broiler	SEM ¹	p-value
Aspartic acid	22.02 ^b	29.68 ^a	0.63	<0.0001
Serine	116.71	103.12	14.80	0.53
Glutamic acid	76.60	107.57	19.79	0.30
Glycine	82.06	85.38	5.12	0.66
Histidine	52.13	80.14	9.42	0.06
Arginine	1363.79 ^a	519.59 ^b	14.61	<0.0001
Threonine	1100.84 ^a	500.42 ^b	11.24	<0.0001
Alanine	76.92	82.49	2.14	0.10
Proline	39.74 ^b	76.95 ^a	2.26	<0.0001
Cysteine	11.11 ^a	3.67 ^b	0.33	<0.0001
Tyrosine	70.21 ^b	92.47 ^a	2.10	<0.0001
Valine	38.10 ^b	56.04 ^a	1.18	<0.0001
Methionine	28.64 ^b	41.93 ^a	0.78	<0.0001
Lysine	15.36 ^b	57.69 ^a	3.41	<0.0001
Isoleucine	25.10 ^b	40.74 ^a	0.86	<0.0001
Leucine	51.52 ^b	68.65 ^a	1.55	<0.0001
Phenylalanine	57.43 ^b	73.68 ^a	1.91	0.0003

¹ Standard error of the means ($n=12$).

^{a,b} Means in the same row with different letters differ significantly ($P<0.05$).

cubes, and DFDS. Furthermore, in contrast to the findings of the present study, a previous literature showed that KNC meat had significantly higher content of glycine and alanine relative to that of CB meat (Choe *et al.*, 2010). The DFDS samples prepared from CB had greater contents of valine, isoleucine, leucine, phenylalanine, and methionine, which are responsible for the bitter taste. Similarly, these free amino acids were present at higher levels in CB meat compared to those in Hinai-jidori chicken meat (Rikimaru and Takahashi, 2010). Further, the content of phenylalanine was significantly higher in the CB meat compared to KNC meat (Choe *et al.*, 2010). The results of the current study revealed that the DFDS samples from KNC had significantly greater content of arginine, threonine, and cysteine than that derived from CB. Jayasena *et al.* (2013b) stated that cysteine is one of the principal precursors for the formation of 2-methyl-3-furanthiol, which is responsible for the meaty flavor of chicken broth.

Conclusions

The results of this study demonstrated that the breed of chicken significantly influences the content of creatine, betaine, carnitine, nucleotides, and free amino acids in the DFDS samples and the fatty acid composition of lipid layers separated during the preparation of DFDS. However, histidine dipeptides were not detected in any DFDS sample tested in this study. DFDS derived from KNC had significantly higher content of betaine, carnitine, IMP, inosine, and cysteine than that from CB. Additionally, a higher content of PUFAs including essential fatty acids and DHA were found in the lipid layer separated from soup samples made from

KNC compared to those from CB. These compounds are considered to enhance the nutritional value and taste of the DFDS from KNC as opposed to those prepared with CB.

Acknowledgment

This research was supported by Golden Seed Project, Ministry of Agriculture, Food and Rural Affairs, Ministry of Oceans and Fisheries, Rural Development Administration Korean Forestry Services.

References

- Arslan C, Citil M and Saatci M. Effects of L-carnitine administration on growth performance, carcass traits, blood serum parameters and abdominal fatty acid composition of ducks. *Archives of Animal Nutrition*, 57: 381–388. 2003.
- Bailey M, E. The Maillard reaction and meat flavour. American Chemicals Society, Washington, USA. 1983.
- Cambero MI, Pereira-Lima CI, Ordoñez JA and García de Fernando GD. Beef broth flavour: relation of components with the flavour developed at different cooking temperatures. *Journal of the Science of Food and Agriculture*, 80: 1519–1528. 2000.
- Cherian G, Selvaraj R, Goeger M and Stitt P. Muscle fatty acid composition and thiobarbituric acid-reactive substances of broilers fed different cultivars of sorghum. *Poultry Science*, 81: 1415–1420. 2002.
- Chiang P-D, Yen C-T and Mau J-L. Non-volatile taste components of various broth cubes. *Food Chemistry*, 101: 932–937. 2007.
- Choe JH, Nam KC, Jung S, Kim B, Yun HJ and Jo C. Differences in the quality characteristics between commercial Korean native chickens and broilers. *Korean Journal for Food Science of Animal Resources*, 30: 13–19. 2010.
- Constantin-Teodosiu D, Howell S and Greenhaff PL. Carnitine

- metabolism in human muscle fiber types during submaximal dynamic exercise. *Journal of Applied Physiology*, 80: 1061–1064. 1996.
- Craig SA. Betaine in human nutrition. *The American Journal of Clinical Nutrition*, 80: 539–549. 2004.
- Crush KG. Carnosine and related substances in animal tissues. *Comparative Biochemistry and Physiology*, 34: 3–30. 1970.
- de Zwart FJ, Slow S, Payne RJ, Lever M, George PM, Gerrard JA and Chambers ST. Glycine betaine and glycine betaine analogues in common foods. *Food Chemistry*, 83: 197–204. 2003.
- Folch J, Lees M and Stanley GHS. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, 226: 497–509. 1957.
- Gadekar YP, Anjaneyulu ASR, Thomas R, Mendiratta SK and Kondaiah N. Quality changes in soup from deboned chicken frames at refrigerated ($4\pm 1^{\circ}\text{C}$) and frozen ($-18\pm 1^{\circ}\text{C}$) storage. *International Journal of Food Science and Technology*, 44: 1763–1769. 2009.
- Hughes MC, Kerry JP, Arendt EK, Kenneally PM, McSweeney PLH and O'Neill EE. Characterization of proteolysis during the ripening of semi-dry fermented sausages. *Meat Science*, 62: 205–216. 2002.
- Jaturasitha S, Srikanchai T, Kreuzer M and Wicke M. Differences in carcass and meat characteristics between chicken indigenous to Northern Thailand (Black-Boned and Thai Native) and imported extensive breeds (Bresse and Rhode Island Red). *Poultry Science*, 87: 160–169. 2008.
- Jayasena DD, Ahn DU, Nam KC and Jo C. Flavour chemistry of chicken meat: a review. *Asian Australasian Journal of Animal Science*, 26: 732–742. 2013a.
- Jayasena DD, Ahn DU, Nam KC and Jo C. Factors affecting cooked chicken meat flavour: a review. *World's Poultry Science Journal*, 69: 515–526. 2013b.
- Jayasena DD, Jung S, Kim HJ, Bae YS, Yong HI, Lee JH, Kim JG and Jo C. Comparison of quality traits of meat from Korean native chickens and broilers used in two different traditional Korean cuisines. *Asian Australasian Journal of Animal Science*, 26: 1038–1046. 2013c.
- Jayasena, DD, Jung S, Bae YS, Park HB, Lee JH and Jo C. Comparison of the amounts of endogenous bioactive compounds in raw and cooked meats from broilers and indigenous chickens. *Journal of Food Composition and Analysis*, 37: 20–24. 2015.
- Jeon H-J, Choe J-H, Jung Y, Kruk ZA, Lim D-G and Jo C. Comparison of the chemical composition, textural characteristics, and sensory properties of North and South Korean native chickens and commercial broilers. *Korean Journal for Food Science of Animal Resources*, 30: 171–178. 2010.
- Jo C, Cho SH, Chang J and Nam KC. Keys to production and processing of Hanwoo beef: A perspective of tradition and science. *Animal Frontiers*, 2: 32–38. 2012.
- Jung S, Bae YS, Kim HJ, Jayasena DD, Lee JH, Park HB, Heo KN and Jo C. Carnosine, anserine, creatine, and inosine 5'-monophosphate contents in breast and thigh meats from five lines of Korean native chicken. *Poultry Science*, 92: 3275–3282. 2013.
- Jung Y, Jeon HJ, Jung S, Choe JH, Lee JH, Heo KN, Kang BS and Jo C. Comparison of quality traits of thigh meat from Korean native chickens and broilers. *Korean Journal for Food Science of Animal Resources*, 31: 684–692. 2011.
- Jurie C, Robelin J, Picard B and Geay Y. Postnatal Changes in the Biological Characteristics of Semitendinosus Muscle in Male Limousin Cattle. *Meat Science*, 41: 125–135. 1995.
- Ke L, Zhou J, Lu W, Gao G and Rao P. The power of soups: Superhero or team-work? *Trends in Food Science and Technology*, 22: 492–497. 2011.
- Kiyohara R, Yamaguchi S, Rikimaru K and Takahashi H. Supplemental arachidonic acid-enriched oil improves the taste of thigh meat of Hinai-jidori chickens. *Poultry Science*, 90: 1817–1822. 2011.
- Koriyama T, Wongso S, Watanabe K and Abe H. Fatty acid compositions of oil species affect the 5 basic taste perceptions. *Journal of Food Science*, 67: 868–873. 2002.
- Li K, Li W and Huang Y. Determination of free L-carnitine in human seminal plasma by high performance liquid chromatography with pre-column ultraviolet derivatization and its clinical application in male infertility. *Clinica Chimica Acta*, 378: 159–163. 2007.
- Lim D-G, Kim K-T, Lee K-H, Seo K-S and Nam K-C. Physicochemical traits, fatty acid and free amino acid compositions of two-way crossbred pork belly. *Korean Journal for Food Science of Animal Resources*, 33: 189–197. 2013.
- Mattes R. Soup and satiety. *Physiology and Behavior*, 83: 739–747. 2005.
- Mora L, Sentandreu MA and Toldrá F. Hydrophilic chromatographic determination of carnosine, anserine, balenine, creatine, and creatinine. *Journal of Agricultural and Food Chemistry*, 55: 4664–4669. 2007.
- Mora L, Sentandreu MA and Toldrá F. Contents of creatine, creatinine and carnosine in porcine muscles of different metabolic types. *Meat Science*, 79: 709–715. 2008.
- Mora L, Hernández-Cázares AS, Sentandreu MA and Toldrá F. Creatine and creatinine evolution during the processing of dry-cured ham. *Meat Science*, 84: 384–389. 2010.
- Nam K-C, Jo C and Lee M. Meat products and consumption culture in the East. *Meat Science*, 86: 95–102. 2010.
- Patterson KY, Bhagwat SA, Williams JR, Howe JC, Holden JM, Zeisel SH, Dacosta KA and Mar M-H. USDA database for the choline content of common foods. pp. 12–36. U.S. Department of Agriculture, Maryland, USA. 2008.
- Peiretti PG, Medana C, Visentin S, Dal Bello F and Meineri G. Effect of cooking method on carnosine and its homologues, pentosidine and thiobarbituric acid-reactive substance contents in beef and turkey meat. *Food Chemistry*, 132: 80–85. 2012.
- Peiretti PG, Medana C, Visentin S, Giancotti V, Zunino V and Meineri G. Determination of carnosine, anserine, homocarnosine, pentosidine and thiobarbituric acid reactive substances contents in meat from different animal species. *Food Chemistry*, 126: 1939–1947. 2011.
- Purchas RW, Rutherford SM, Pearce PD, Vather R and Wilkinson BHP. Concentrations in beef and lamb of taurine, carnosine, coenzyme Q₁₀, and creatine. *Meat Science*, 66: 629–637. 2004.
- Rikimaru K and Takahashi H. Evaluation of the meat from Hinai-jidori chickens and broilers: Analysis of general biochemical components, free amino acids, inosine 5'-monophosphate, and fatty acids. *Journal of Applied Poultry Research*, 19: 327–333. 2010.
- SAS. 2011. SAS/STAT software for PC. Release 9.3. SAS Inst. Inc., Cary, NC, USA.
- Sri Kantha S, Takeuchi M, Watabe S and Ochi H. HPLC Determination of carnosine in commercial canned soups and natural meat extracts. *LWT-Food Science and Technology*, 33: 60–62. 2000.
- Tang H, Gong YZ, Wu CX, Jiang J, Wang Y and Li K. Variation of meat quality traits among five genotypes of chicken. *Poultry*

- Science, 88: 2212–2218. 2009.
- Tikk M, Tikk K, Tørngren MA, Meinert L, Aaslyng MD, Karlsson AH and Andersen HJ. Development of inosine monophosphate and its degradation products during aging of pork of different qualities in relation to basic taste and retronasal flavor perception of the meat. *Journal of Agricultural and Food Chemistry*, 54: 7769–7777. 2006.
- Toldrá F. Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Science*, 49: 101–110. 1998.
- Wattanachant S, Benjakul S and Ledward D. Composition, color, and texture of Thai indigenous and broiler chicken muscles. *Poultry Science*, 83: 123–128. 2004.
- Wei W, YongZhi Z, Yu S and MuHan Z. Comparative study on flavor quality of chicken soup made of different chicken breeds. *Acta Agriculturae Jiangxi*, 24: 149–152. 2012.
- Wu H-C and Shiau C-Y. Proximate composition, free amino acids and peptides contents in commercial chicken and other meat essences. *Journal of Food and Drug Analysis*, 10: 170–177. 2002.
- Wyss M and Kaddurah-Daouk R. Creatine and creatinine metabolism. *Physiological Reviews*, 80: 1107–1213. 2000.
- Zeisel SH, Mar M-H, Howe JC and Holden JM. Concentrations of choline-containing compounds and betaine in common foods. *Journal of Nutrition*, 133: 1302–1307. 2003.
- Zhu SW and Hu JX. Studies on Jinhua ham tastes and taste substances. *Journal of Food Science*, 159: 8–11. 1993.