# Quality of Black-Boned Chicken (*Gallus Domesticus*) Carcass and Development to Black-Boned Chicken Soup

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## Abstract

Black-boned chickens are one of the native fowl with distinctive characteristics. It was found that it has high protein and low-fat content. Additionally, it had melanin which is a natural antioxidant compound. Chicken soup is a popular functional food that contains carnosine and anserine. These substances are the most prevalent histidine-containing dipeptides not found in plants. Thus, this study aimed to examine the quality of black-boned chicken carcasses and evaluate the biological properties of melanin, carnosine, and anserine were evaluated in various part of black-boned chicken: breast meat, thigh meat, and femur bone as well as the soups derived from each part. The chicken breast meat and its soup contained more carnosine and anserine than the those found thigh meat and femur bone soups ($P<0.05$). Although, the melanin content showed different amount in raw samples. The chicken soups were not significantly different ($P\geq0.05$). The anserine content in chicken muscles were found to be higher compared to that of carnosine. The biological properties of carnosine and anserine extracts from chicken muscle increased proportionally with the amount of these peptides in the muscle. Meanwhile, the femur bone had fewer peptides than the muscle. The femur bone exhibited higher antioxidant activities than the chicken thigh soup, as measured by ABTS and FRAP assays. Hence, the antioxidant activities corresponding with the dipeptides except femur bone.

**Keywords :** Black-boned chicken, Soup, Functional foods, Carnosine, Anserine, Melanin

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## Introduction

The expansion of the chicken meat in Thailand can be attributed to the rising demand from both local and international markets [1]. The Thai poultry industry predominantly relies on the broiler genotype owing to its superior growth performance, which leads to reduced production costs. In addition, Thailand offers alternative genotypes for meat consumption, including Thai indigenous and the black-boned chicken, which are commercially available.

Thai indigenous chickens are a sought-after meat for culinary purposes due to their distinctive texture, favorable taste, and low-fat content, despite their high cost and slow growth rate [2]. The black-boned chicken, despite its distinct physical characteristics, is associated with a relatively high price point. However, it is widely believed that the breed's purported health benefits have resulted in increased consumer interest when compared to other chicken breeds.

Black-boned chicken also contains melanin that represents the black color of skin, flesh, and bone [3]. It acts as an antioxidant and is also associated with the immune system function in the body [4]. This chicken has higher amounts of carnosine and anserine than conventional chickens about two times [5].

Moreover, carnosine content was higher in breast meat than thigh meat. The breast meat contained higher anserine content compared with thigh meat. The sex effect on anserine was not consistent between breast and thigh meat [6].

The amount of melanin pigment in the tissues of different organs of black-boned silky chickens was significantly different ($P<0.05$). The melanin pigment in femur bone was the highest at 21.3 mg/g of tissue followed by ovarian and testicular tissue, trachea, muscle tissue. and skin respectively [7].

Our world has changed in various ways recently, including lifestyle adaptation [8]. Consumers are more conscious of their food choices due to time limitations, especially students and working age who do not have much time to cook [9]. Therefore, functional food is an alternative that can satisfy this consumer group.

Functional foods containing bioactive peptides have been found in plants and animals [10]. In particular, chicken is a good source of bioactive peptides. It is not only rich in protein but also has high bioactive peptide content [11]. Thus, making it highly desirable to consumers. The important bioactive peptides include carnosine and anserine [12].

The soup of chicken has been long one of the most popular functional foods. Because it contains carnosine and anserine, which are significant dipeptides [13]. Carnosine is one of the anti-aging nutrients and can also help restore tiredness during exercise by reducing the buildup of lactic acid in the muscles [14]. Anserine can help reduce stress and fatigue [15], stimulate brain activity, increase concentration, improve learning and memory capacity [16].

## Research Objectives

1. To examine the quality of black-boned chicken carcasses.

2. To evaluate the biological properties of melanin, carnosine, and anserine in a black-boned chicken parts and soups derived from each part.

## Research Methodology

### 1. Materials

Black-boned roosters (royal project breed) averaging 1.6 kg and four weeks of maturation were obtained from the royal project farm in Chiang Mai, Thailand. Samples were taken to reduce the residual blood by soaking in water at 25±0.5 °C for 10 min before further processing (adapted from [17]).

### 2. Methods

#### 2.1 Sample preparation

Raw chickens consisted of three parts: breast meat, thigh meat, and femur bone were studied separately. The breast and thigh meats were cut into 1x1x1 $cm^3$, and femur bones were reduced to small pieces about 1-2 cm.

#### 2.2 Stewing Process

Each treatment contained the chicken part-to-water ratio of 1:1. The contents were filled in a pressure-resistant glass bottle. Subsequently, autoclaved at 115 °C and 10 psi for 2 h (HVA-85, Hirayama, Saitama, Japan). Then, separated meat and soup by filter paper no.4 (Whatman). The extracts were kept under -20 °C with light protection for further analysis.

#### 2.3 Proximate analysis

Determined the amount of moisture, fat, and ash in raw samples and soups [18]. Kjeltec 2300 Analyzer (Foss Tecator, Hoganas, Sweden) was used to determine crude protein according to the Kjeldahl technique. The derived protein amount was multiplied by an overall nitrogen value of 6.25.

### 2.4 Physical and chemical properties

#### 2.4.1 pH

The pH of the raw materials and their soup samples were measured potentiometric (Lab284, Metrohm, Switzerland). The raw materials were mixed with water in a ratio of 1:5 at a temperature of 25 ºC. The pH of the soup samples was directly measured.

#### 2.4.2 Color

The color parameters of the raw chicken parts and their soup samples were monitored using a colorimeter (ColorQuest XE, HunterLab, Reston, VA). Reported in terms of the CIE system and displayed in the form of values $L^*$ (100 to 0 represents lightness to darkness), $a^*$ (where red has a positive value and green has a negative value), and $b^*$ (a positive value indicates yellow, whereas a negative value illustrates blue).

### 2.5 Carnosine and anserine

Each part of the raw chicken samples was homogenized with 0.01 N HCl, then centrifuged at 10,000 rpm for 20 min (3-30KS, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The collected clear upper solution was stirred with acetonitrile and preserved at the refrigerator temperature for 20 min. Subsequently, the solution was centrifuged at the same speed for 10 min. The HPLC system was made on an Agilent (Santa Clara, California, USA). The operation was performed according to [19]. A Restek C18 column (4.6 ×250 mm 5$\mu$m) was used. Twenty microliters of each sample were injected into the process. The mobile phase consisted of two parts: a 25:75 mixture of 0.65 mM ammonium acetate (pH 5.5) and acetonitrile. The other was a 70:30 mixture of 4.55 mM ammonium acetate (pH 5.5) and acetonitrile. A gradient system was employed in this operation (percentage ranging of the second solvent from 0% (v) to 100% (v)) at a flow rate of 0.8 mL/min for 16 min. A diode array detector was used to measure at 214 nm of absorbance. Standard carnosine and anserine were purchased from Sigma-Aldrich (Sigma Co. St. Louis, MO, USA).

### 2.6 Melanin content

For melanin isolation, approximately 20 g of muscle and bone samples were randomly collected from each sample using a slightly modified method described by [20]. The extracted

melanin samples were stored at -20 °C for further determination of melanin content compared to the synthetic melanin linear standard curve (Sigma No. M8631, USA). A suspension of synthetic melanin was prepared for the standard curve by preparing 0.01 g of synthetic melanin in 100 mL of 0.1 M NaOH at a concentration of 100 ppm. The melanin suspension was diluted to obtain a calibration curve by 0, 5, 10, 20, 25, and 30 mg/mL. The absorbance was read at 490 nm with a microplate reader (Spark, Tecan, Switzerland) illustrated in milligrams per milliliters. Then, the melanin content was converted to milligrams per gram.

**2.7 Antioxidant activities**

**2.7.1 DPPH (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay)**

DPPH radical scavenging capacity was tested according to [21]. The 0.06 mmol DPPH solution in 99.5% methanol was mixed with black-boned chicken soup at 1:39 %v/v and left for 30 min with light protection. The absorbance was detected at a wavelength of 517 nm, compared to the Trolox concentration curve standardized. Reported as micromole Trolox equivalent per gram of black-boned chicken parts and soups.

**2.7.2 FRAP (Ferric reducing antioxidant power assay)**

Measurement of the ability of the sample to reduce ferrous ions was based on the method of [22]. Freshly mixed acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM TPTZ in 40 mM of HCl), and $FeCl_3 \cdot 6H_2O$ solution (20 mM) in a 10:1:1 ratio were used to prepare the FRAP solution. The solution was incubated at 37 °C before using for 4 min. Then, 100 $\mu$L of the sample was mixed with 2800 $\mu$L of the FRAP solution and incubated at 37 °C for 30 min. The absorbance was read at 593 nm, the Trolox concentration curve was standard. The values were reported as micromole Trolox equivalent per gram of black-boned chicken parts and soups.

**2.7.3 ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity assay)**

A modified method of ABTS radical scavenging activity was described by [23]. The ABTS solution was made by incubating 7.4 mM ABTS and 2.6 mM potassium persulfate for 12 h. Before ABTS solution was used, it was diluted with distilled water until the absorbance value was achieved of 0.700±0.020. The sample was used at 100 $\mu$L and the diluted ABTS 2800 $\mu$L. The mixture was incubated for 6 min in the dark at 37 °C. The absorbance was detected at 734 nm, compared with the Trolox standard. Exhibited data in micromole Trolox equivalent per gram sample of black-boned chicken parts and soups.

Table 1: Macronutrients of black-boned chicken parts.

| Chicken parts | Moisture (%) | Protein (%) | Fat (%) | Ash (%) |
|---|---|---|---|---|
| Breast meat | $74.68\pm0.28^{a}$ | $23.44\pm0.19^{a}$ | $0.48\pm0.03^{c}$ | $0.90\pm0.04^{b}$ |
| Thigh meat | $73.36\pm0.10^{b}$ | $20.49\pm0.17^{b}$ | $2.11\pm0.06^{b}$ | $0.89\pm0.03^{b}$ |
| Femur bone | $46.18\pm0.13^{c}$ | $17.67\pm0.14^{c}$ | $13.15\pm0.11^{a}$ | $22.43\pm0.24^{a}$ |

Data are presented as mean ± SD, the alphabet in each column indicates a significant difference (P<0.05).

Table 2: Macronutrients of black-boned chicken soups.

| Chicken parts | Moisture (%)$^{ns}$ | Protein (%) | Fat (%) | Ash (%)$^{ns}$ |
|---|---|---|---|---|
| Breast meat | $94.61\pm0.78$ | $2.38\pm0.03^{c}$ | $0.10\pm0.02^{b}$ | $0.57\pm0.07$ |
| Thigh meat | $95.01\pm1.05$ | $3.42\pm0.25^{b}$ | $0.13\pm0.03^{b}$ | $0.56\pm0.14$ |
| Femur bone | $94.70\pm1.02$ | $3.89\pm0.61^{a}$ | $0.27\pm0.04^{a}$ | $0.62\pm0.09$ |

Data are presented as mean ± SD, the alphabet in each column indicates a significant difference (P<0.05), ns indicates no significant difference (P≥0.05)

### 3. Statistical analysis

The experiment was planned to have completely randomized design (CRD) with three repetitions. Data are shown as mean ± standard deviation. All investigations were carried out using the SPSS statistical program (17.0, SPSS Inc., California). Compared mean using analysis of variance (ANOVA) via Duncan's least significant test that significance was determined at P<0.05.

## Results

### 1. Proximate analysis

The chemical composition of the raw samples is shown in Table 1. Breast meat contained the highest protein content (23.44%), whereas the fat content was the lowest (0.48%). The moisture content of the meat parts was significantly different compared to the bone (P<0.05). The muscle parts showed significant differences in protein and moisture content compared to the bone (P<0.05). Meanwhile, the femur bone was found as the greatest fat and ash contents due to it rich in bone marrow and minerals, respectively [24]. This result showed that breast meat can be used as a raw

Table 3: Physical chemical properties of black-boned chicken parts.

| Chicken parts | pH | Color | | |
|---|---|---|---|---|
| | | $L^*$ | $a^*$ | $b^*$ |
| Breast meat | 5.94±0.03$^c$ | 38.87±0.96$^a$ | 0.80±0.26$^c$ | 1.97±0.66$^b$ |
| Thigh meat | 6.37±0.01$^b$ | 36.26±0.97$^b$ | 2.31±0.42$^b$ | 3.68±0.36$^a$ |
| Femur bone | 7.10±0.04$^a$ | 35.22±0.86$^c$ | 4.44±0.93$^a$ | 3.41±0.65$^a$ |

Data are presented as mean ± SD, the alphabet in each column indicates a significant difference (P<0.05).

material to produce high-protein, low-fat chicken soups.

The basic composition of the black-boned chicken soups is shown in Table 2. There were no statistically significant differences in moisture and ash contents among the different chicken parts (P≥0.05). The protein content of the femur bone soup was found to be the highest at 3.89%, while the thigh meat and breast meat soups showed protein contents of 3.42% and 2.38%, respectively. The chicken soup derived from the femur bone had the highest fat content compared to soup derived from breast meat and thigh meat.

**2. Physical and chemical properties**

The physical and chemical parameters of untreated chicken parts are shown in Table 3. The pH level is an important indicator of meat quality [25]. Each piece of fresh chicken had a varied pH value (P<0.05). The pH of the muscles was lower than that of bones. Furthermore, it was observed that the pH of the breast muscle was lower in comparison to that of the thigh muscle.

The distinguishing characteristic of black-bone chicken is the black color of the muscles and bones. Considering that the various parts of a black-boned chicken have distinct hues due to the accumulation of black pigments and the chemical composition of each part. The present investigation resulted in statistically significant differences (P<0.05) between the breast and thigh muscles. The breast muscle had the highest levels of brightness ($L^*$) and the lowest levels of redness ($a^*$) and yellowness ($b^*$). Compared to the bone, the muscles had a higher $L^*$ value but a lower $a^*$ value. The tissue of the thigh was more yellowness than that of the breast.

As shown in Table 4, this study observed the increase in pH after stewing compared to raw chicken. The pH of each chicken part was significantly different (P<0.05). The results indicated that the pH levels of the muscle soups were significantly lower than those of the bone soup (P<0.05).

Table 4: Physical chemical properties of black-bone chicken soups.

| Chicken parts | pH | Color | | |
|---|---|---|---|---|
| | | $L*$ | $a*$ | $b*$ |
| Breast meat | 6.37±0.01$^{c}$ | 25.58±0.20$^{c}$ | -1.50±0.35$^{b}$ | 0.50±0.04$^{a}$ |
| Thigh meat | 6.57±0.01$^{b}$ | 27.67±0.28$^{b}$ | -1.35±0.08$^{b}$ | -0.06±0.23$^{b}$ |
| Femur bone | 7.12±0.06$^{a}$ | 29.73±0.05$^{a}$ | -1.00±0.04$^{a}$ | -0.60±0.03$^{c}$ |

Data are presented as mean ± SD, the alphabet in each column indicates a significant difference (P<0.05).

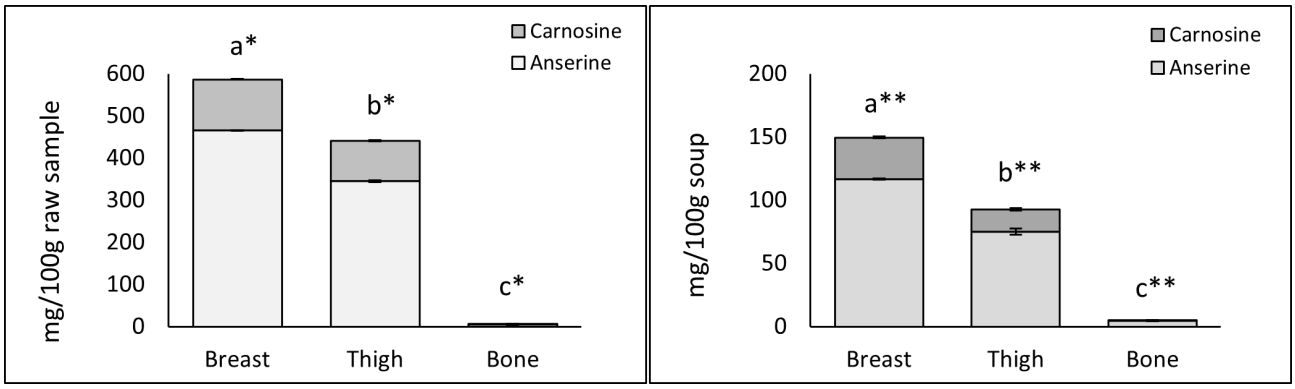


Figure 1: Carnosine and anserine contents in a) black-boned chicken parts and b) black-boned chicken soups, a small letter in each column indicates a significant difference in chicken type (P<0.05), and *, ** indicates a significant difference in each sample type (P<0.05).

Following stewing, a notable reduction in the brightness ($L^*$) of the broth derived from each chicken part was observed, with statistical significance (P<0.05). The femur bone soup exhibited the highest brightness, whereas the thigh and breast muscle samples displayed lower levels of brightness in descending order.

The study found that there was no significant difference in the redness of chicken soup made from breast and thigh muscles. The femur bone soup exhibited the highest level of redness. This study revealed that the $b^*$ values, which indicate yellowness, of chicken soup varied significantly (P<0.05) among different parts. Specifically, the breast muscles exhibited the highest degree of yellowing, followed by the thigh muscles and femur bone.

### 3. Carnosine and Anserine

Carnosine and anserine were found in the greatest numbers in raw breast tissue, followed

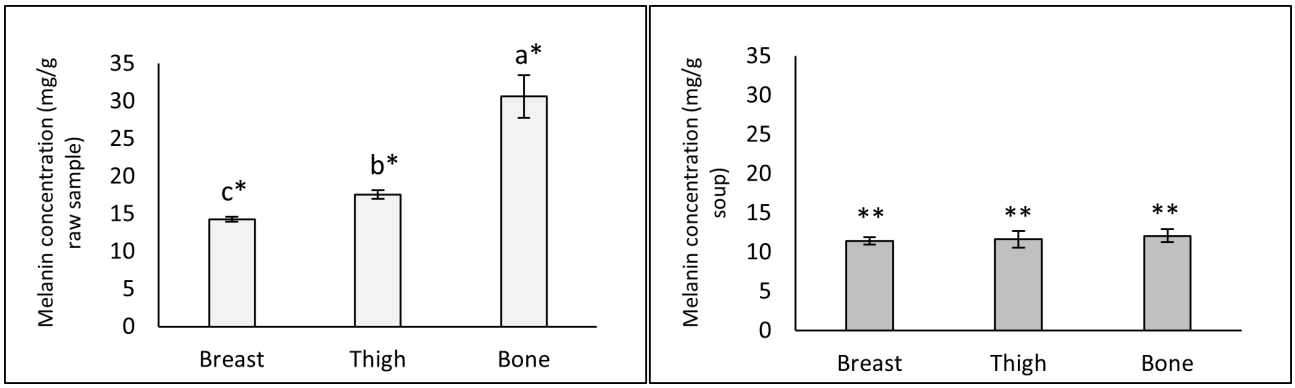


Figure 2: Melanin contents in a) black-boned chicken parts and b) black-boned chicken soups, small letter in each column indicates a significant difference in chicken type ($P<0.05$), and *, ** indicates a significant difference in each sample type ($P<0.05$).

by thigh tissue and femur bone, respectively (Figure 1a). This work found that these dipeptides were found at low levels in raw femur bone. After stewing chicken broth using breast muscle, it had the highest histidine-dipeptides compared to thigh and femur bone soup (Figure 1b). In addition, the anserine content in chicken breast broth was 1.35 and 58.1 times higher than that in thigh tissue and femur bone, respectively.

The ratios of anserine and carnosine contents in the black-boned chicken broth made from breast muscle, thigh muscle, and femur bone were 3.57, 4.24, and 24.15, respectively. Compared to similar raw meat samples, processing caused carnosine losses of 73%, 82%, and 88% in the cases of breast, thigh, and bone, respectively. At the same time, anserine was found to be lost by 75%, 78%, and 43% for the meats (breast and thigh), and bone, respectively.

**4. Melanin**

The findings revealed that the amount of melanin accumulated in various chicken parts varied (Figure 2a). After stewing, there were no significant differences ($P\geq0.05$) in the chicken broth from each part (Figure 2b). In contrast, there were significant decrease ($P\geq0.05$) from 14.29 to 11.4 mg/g for breast soup, 17.57 to 11.63 mg/g in thigh soup, and 30.63 to 12.08 mg/g in femur bone soup. Due to the absence of the black pigment from melanin, the color values obtained were consistent with the melanin content.

**5. Antioxidant activities**

The antioxidant activities of the raw black-boned chicken samples were determined using the DPPH, ABTS, and FRAP methods (Table 5). The breast and thigh meats exhibited significantly higher antioxidant activity in the DPPH assay compared to the femur bone ($P<0.05$).

Table 5: Antioxidant activities of black-boned chicken.

| Chicken parts | DPPH (μmol Trolox eq/g) | ABTS (μmol Trolox eq/g) | FRAP (μmol Trolox eq/g) |
|---|---|---|---|
| Breast | $0.390 \pm 0.014^{a}$ | $0.364 \pm 0.030^{b}$ | $0.230 \pm 0.024^{a}$ |
| Thigh | $0.374 \pm 0.018^{a}$ | $0.338 \pm 0.039^{b}$ | $0.174 \pm 0.021^{b}$ |
| Femur bone | $0.289 \pm 0.004^{b}$ | $0.440 \pm 0.029^{a}$ | $0.219 \pm 0.027^{a}$ |

Data are presented as mean ± SD, small letter in each column indicates a significant difference in chicken part ($P<0.05$).

Table 6: Antioxidant activities of black-bone chicken soup.

| Chicken parts | DPPH (μmol Trolox eq/g) | ABTS (μmol Trolox eq/g) | FRAP (μmol Trolox eq/g) |
|---|---|---|---|
| Breast | $0.524 \pm 0.030^{a}$ | $0.854 \pm 0.046$ | $0.254 \pm 0.023^{a}$ |
| Thigh | $0.501 \pm 0.054^{a}$ | $0.844 \pm 0.101$ | $0.217 \pm 0.021^{b}$ |
| Femur bone | $0.434 \pm 0.034^{b}$ | $0.910 \pm 0.025$ | $0.241 \pm 0.024^{ab}$ |

Data are presented as mean ± SD, small letter in each column indicates a significant difference in chicken part ($P<0.05$), ns indicates no significant difference in chicken part ($P \geq 0.05$).

The ABTS antioxidant activity test indicated that the femur bone had the highest antioxidant activity. This activity allowed it to scavenge $ABTS^{\cdot+}$ free radicals more effectively than the breast and thigh meats. Furthermore, the FRAP assay revealed the electron-donating capacity. This study revealed that the FRAP values of breast muscle and femur bone were comparatively higher than those of thigh muscle.

## Discussion

### 1. Proximate analysis

The chemical composition of chickens varied due to growth and fat accumulation [26]. Muscle components exhibited high protein content due to their prevalence in meat, and characteristics and respective distribution within the tissue contributed to the meat's leanness and overall quality [27].

Furthermore, the femur bone exhibits a high concentration of minerals. Calcium was a crucial constituent of bone structure. Also, iron content, which is a component within the bone [24].

Change in protein might be due to the possible degradation of myofibrils and connective tissue. This resulting in a decrease in protein content [28]. The femur bone soup contained the fattest. Comparable to chicken breast and thigh broth. Because the bone is composed of bone marrow, which includes fat and fatty acids [29]. These results are consistent with [30] and [2].

**2. Physical and chemical properties**

The physical properties have a significant impact on the visual quality of meat. In normal conditions, the pH of a freshly slaughtered chicken carcass typically fell within the range of 5.30-6.50 [31]. After dressing, chicken meats exhibited a normal pH range between 6.26 and 6.30. This study pH of the carcass could be related to lactic acid buildup induced by the anaerobic respiration process. The observed phenomenon may be attributed to the highest accumulation of lactic acid in the breast muscles, consequently leading to the lowest pH in the meat.

Black-boned chickens are characterized by the dark coloration of their bones and other anatomical structures. The presence of melanin pigment throughout the chicken's body is responsible for its relatively low levels of redness and yellowness in comparison to other chickens. Additionally, chicken meat is classified as white muscle, accumulating myoglobin in smaller quantities than red muscle [32]. Red muscle functions by storing and absorbing oxygen from the capillaries, delivering it to various cells that require oxygen for the oxidation process. The breast muscle exhibited brighter than the thigh muscle. A lower proportion of red and intermediate fibers in comparison to white fibers was observed in the thigh muscle. The results of this study are consistent with previous research by [33].

The color of chicken meat typically correlates with a pH range ranging from 5.70 to 6.30 [34]. Chicken meat that has not been contaminated will be vibrant yellowish-white in color and have a pH between 5.70 and 6.50. Several factors, such as pH value, influence the color of chicken meat: the lower the pH value, the paler the color of the meat.

After stewing, the change of protein occurs due to the degradation of the protein and the shortening of the amino acid chain. It might increase the peptide that has a lower molecular weight than fresh samples. Moreover, this phenomenon could be attributed to the hydrolysis of proteins, which may lead to the separation of globin from the heme group, thereby inhibiting the formation of the dark color. So, the chicken soups were lighter yellow color [35]. The results align with [36], which demonstrated that protein hydrolysis led to a lighter yellow color in chicken soup, particularly

when combined with mushroom flavoring.

Furthermore, it can be observed that after heat treatment, melanin was not extracted from the raw samples into the broths. Consequently, this phenomenon contributed to a reduction in the overall brightness of the soups when compared to the raw samples.

**3. Carnosine and Anserine**

Different chicken parts contained varying levels of carnosine and anserine. Anserine is mainly found in poultry meat, whereas carnosine is more common in beef and pork [37]. The anserine to carnosine ratio of poultry meat was higher than that of pork and cattle. This finding is corresponded to the research of [38].

These outcomes are consistent with the findings of [39], that reported carnosine loss in beef and turkey meat due to boiling processes. Furthermore, heat treatments, which remove proteins from untreated samples, can lead to protein denaturation through aggregation. Additionally, sterilization processes could cause the pyrolysis and decomposition of certain amino acids [40]. Under these circumstances, not only the essential amino acids but also the crucial dipeptides may be lost.

The quantities of carnosine and anserine were found to be influenced by factors such as muscle fiber type, genotype, sex, age, and mating [41, 42]. Those substances were generally found in muscles, which help regulate body pH. Therefore, play an essential role in maintaining the balance of the anaerobic glycolysis [39]. Notably, the breast muscle exhibited a higher accumulation of these dipeptides compared to the thigh muscle. Conversely, due to the relatively small amount of muscle in the femur bone, the presence of these crucial substances was the lowest.

**4. Melanin**

The femur bone exhibited the highest concentration of melanin due to the presence of genes that influence the production of dark pigments. Consequently, melanin from this region diffused throughout the chicken, resulting in lower melanin levels in the thigh and breast muscles.

This study's findings align with previous research on Silky Fowl (black chicken) tissues, which also reported consistent melanin content [3]. Notably, the periosteum of the femur displayed the highest melanin concentration compared to other organs. Furthermore, the melanin pigment levels in this chicken's muscle tissues exhibited statistically significant differences [7].

The stewing conditions might limit the ability of melanin extraction from the cell. A high basic solution is required for melanin to be removed from cells [43]. In this study, the sample preparation maintained a weakly acidic to weak base acidity, thus preventing further melanin extraction.

According to [44], melanin could break down because of the high temperature since 114.49 °C. This process is attributed to the complex structure of melanin, which comprises eight repeating indole-5,6-quinone units that can lose one unit at high temperatures. This reaction could form a complex web of hydrogen bonds with naturally occurring complementary peptide-type structures. Changes were made to the physiological environment. The complicated structure of melanolipoprotein had the potential to degenerate, resulting in depigmentation.

The antioxidant properties of melanin include reducing power and ferrous ion binding capacity ($Fe^{2+}$) [20]. Melanin was not the only factor in the system that prevents free radicals from melanin. However, it was the outcome of several mechanisms combined in chicken meat.

**5. Antioxidant activities**

The free radical scavenging properties using DPPH and ABTS methods. These methods are considered easy, convenient, and fast due to the relative stability of DPPH and ABTS as free radicals. It measures the ability of the test substance to scavenge free radicals based on the hydrogen atom principle.

The results obtained from the ABTS method contrasted with those obtained from the DPPH and FRAP methods when analyzing raw samples. It is noteworthy that raw femur bone may exhibit a high concentration of unsaturated fatty acids [2]. This observation suggests that the presence of unsaturated fatty acids led to the depletion of intra-bone antioxidants due to the inhibition of polyunsaturated fat oxidation. Consequently, this phenomenon may contribute to the reduced free radical scavenging capacity observed in the bone when compared to the breast and thigh muscles. These findings are consistent with prior research presented in [45].

The assessment of antioxidant activity in chicken broths from individual parts using the DPPH method indicated a proton donor ability that aligned with the results of the ABTS and FRAP methods. Particularly, the ABTS results of the soup samples exhibited higher activity than the DPPH results. This discrepancy can be attributed to the hydrophobic nature of the free radicals generated in the DPPH method, resulting in a higher specificity of radicals in the DPPH assay for proton donation reactions when compared to the ABTS assay.

When considering the antioxidant activity assessed by FRAP, it may imply that the primary constituents in chicken broth derived from breast meat had a notable electron transport potential. Prior research had indicated that the imidazole group on the histidine residue of peptides could effectively bind metal ions, inhibiting residual oxygen activity and scavenging hydroxyl free radicals [46, 47].

In addition, the DPPH and FRAP results exhibited correlations with the carnosine and anserine content in both breast and thigh soups. However, the femur bone presented an anomalous observation: it displayed a high antioxidant activity according to the FRAP assays, despite containing minimal amounts of carnosine and anserine. The femur bone might contain other proteins or peptides, such as collagen, which undergo hydrolysis, resulting in the production of peptides with significantly higher antioxidant activity than carnosine and anserine, as suggested in previous research [48].

Moreover, proteins may undergo conversion into low molecular mass peptides through stewing, accompanied by Maillard reactions. This process led to the formation of brown compounds with hydroxyl and pyrrole groups, capable of donating electrons to create stable molecules, effectively terminating the free radical chain reaction [49, 50].

## Conclusion

Differences in chemical composition, bioactive components, and biological activities were observed among various parts of black-boned chicken. Breast meat exhibited a high protein content and low-fat content. The breast muscle contained a high concentration of carnosine and anserine, while the femur bone showed the highest melanin concentration. Chicken soup derived from the breast muscle exhibited the highest carnosine and anserine content among all the soups. Melanin concentrations exhibited no significant variations among soups prepared from various chicken parts. Furthermore, the antioxidant activities of both the raw samples and their soup samples were associated with carnosine and anserine, except for the femur bone.

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