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Antioxidant activity of meat and protein hydrolysate from various chicken meat

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ABSTRACT: This research aimed to compare the anserine and total phenolic contents of chicken breast meat and determine antioxidant activities of protein hydrolysates among broiler, Thai native chicken, and spent hen. The results showed breast muscle of Thai native chicken had a significantly higher anserine and total phenolic contents compared with the other chicken meat types ($P<0.05$). After that, the chicken breast meat was further hydrolyzed with commercial proteases Flavourzyme at 1% (w/w) of enzyme-substrate, pH value of 7.0, and incubation time of 50 °C for 4 hours. The chicken protein hydrolysate significantly exhibited antioxidant activities in the ferric reducing antioxidant power (FRAP) test which was high in Thai native chicken meat. However, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activities of the chicken protein hydrolysates were not significantly different. This study showed that breast meat of Thai indigenous chicken and its protein hydrolysate might potentially be classified as a functional food which is suitable for consumers who concerned health due to high in antioxidant activities. Moreover, protein hydrolysate obtained from Thai native chicken meat could be used in food supplements.
Keywords: Thai native chicken; protein hydrolysate; antioxidant activity

Introduction

A greater awareness of the association between diet and health has led to the growth of consumer demand for health-promoting and functional foods. Functional foods are defined as food products which provide health benefits together with the basic nutritional needs of the body (Abuajah et al., 2015). In recent years, protein has been recognized as a key ingredient for nutrition and health, leading to dietary protein supplementation to grow exponentially. However, protein consumption has also been associated with chronic diseases (Schaafsma, 2009). It has been reported that the molecular weight of an intact protein can be reduced via enzymatic hydrolysis to form protein hydrolysate, resulting in small peptides and amino acids, which can improve a protein's nutritive values (Daliri et al., 2017). Furthermore, several studies reported that protein hydrolysate could be enhanced recovery of physical function in older people (Landi et al., 2016; Nygård et al., 2018). Therefore, supplementing with protein hydrolysate represents an alternative to intact proteins by reducing the potential for adverse health outcomes.

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Recently, numerous studies have focused on bioactive peptides isolated from food sources, which contain diverse properties, for example, antioxidants, hypotensive agents, and antimicrobial activities (Bhat et al., 2015). Antioxidant peptides can be obtained from meat protein, such as duck, beef and fish, possessing an abundance of certain essential amino acids that are sparse in plant protein (Liu et al., 2016). Anserine, the predominant histidine dipeptide in chicken meat, which has an antioxidant function that is not found in pork or beef (Jung et al., 2013). Moreover, it has been known to play a role in quite a number of physiological functions in vertebrates such as inhibition of oxidative reaction in hydroxyl-radical and singlet oxygen-scavenging and lipid peroxidation (Intarapichet and Maikhunthod, 2005; Lee et al., 1998), inhibition of advanced glycated end-product (AGE) formation (Dukic-Stefanovic et al., 2001), and neurotransmitter in brain (Hipkiss and Brownson, 2000).

Chicken meat is an attractive source of food protein, which is used as a raw material for the production of physiological essential peptides (Liu et al., 2016; Yu, Field, & Wu, 2018). In Thailand, there are various chicken meat which are available in poultry market e.g., Thai native chicken, spent hen, and commercial broiler. The physicochemical properties and the growth performance of chicken have been exclusive studied in Thailand. However, there is limited data regarding the health benefit that can be obtained from the chicken meat in Thailand. Thus, the aim of this study was to compare the anserine and total phenolic contents of chicken breast meat and determine antioxidant activities of protein hydrolysates among broiler, Thai native chicken, and spent hen.

Materials and Methods

Sample preparation

Broiler (Ross 308), Thai native chicken (Pradu Hang Dam Chiang Mai), and spent hen (Hisex Brown) were selected in this study. All chickens were reared in one flock of a farm under identical conditions. The animal experimental designs were approved by the Animal Ethics Committee of the Faculty of Medicine, Chiang Mai University (No. 36/2562). The broilers were fed until they were six weeks old; whereas Thai native chicken and spent hen were fed until they were 16 weeks old and 72 weeks old. The chickens were slaughtered in a standard slaughterhouse at market weight (2.1 ± 0.2 kg for broiler, 1.3 ± 0.1 for Thai native, and 1.7 ± 0.1 kg for spent hen). The breast muscles were collected irrespective of their fat and connective tissue. The breast muscles were then packed in a polyethylene pouches and stored at -20 ℃ until further analysis.

Determination of anserine content

The samples were analyzed for anserine content following Bidlingmeyer et al. (1984) with slight modifications. The samples were homogenized with 10 mM HCL for 1 min and added to ACN. The mixture was centrifuged at 10,000 x g for 10 min at 4 ℃. The anserine standard was prepared using a concentration of 2.5 µmol/mL and was separated using a high performance liquid chromatography (HPLC) column with an L-column3 C18, 5 µm particle size (250 × 4 mm, Nacalai Tesque, Japan). A binary liner gradient was used with ACN as the mobile phase A, and 150 mM ammonium acetate (pH 6.2) containing 5% ACN was used as mobile phase B, at a flow rate of 0.6 mL/min. The gradient program

was performed as follows: 0-3 min 0-6% B; 3-20 min 6-22% B; 20-25 min 22-60% B; 26-37.1 min 100-0% B; 37.1-50 min 0% B. The separation was monitored using a diode array detector at a wavelength of 214 and 254 nm.

Determination of total phenolic content

The samples were determined the total phenolic content using Folin-Ciocalteu reagent as described by Jang et al. (2008) with some slight modifications. The meat was mixed in distilled water at a ratio of 1:3 (w/v) and homogenized at 3000 rpm for 5 min. Chloroform was added to the homogenates and mixed two to three times vigorously. A diluted sample was added to the Folin-Ciocalteu reagent, followed by addition of sodium carbonate solution. The reaction mixture was vortexed and the absorbance was evaluated with spectrophotometer at 700 nm after incubation 1 h. The gallic acid was used as standard. The total phenolic content was expressed as mg gallic acid equivalent per kg meat.

Preparation of the protein hydrolysates

The samples were defrosted overnight in a refrigerator at 4 °C. The meat was immersed in distilled water at a ratio of 1:3 (w/v) and homogenized using a homogenizer (Nissei AM-8, Japan). The mixture was heated to 50°C and the pH value was adjusted to 7.00 with 2 N NaOH. Subsequently, the protein slurry was heated to 50°C and then added with Flavourzyme (declared activity of ≥ 500 U/g) from *Aspergillus oryzae* (Sigma-Aldrich, USA) at a concentration of 1% (w/w) under a mild condition of stirring. Enzymatic hydrolysis of the meat proteins was performed for 4 h and immediately heated at 85 °C for 15 min to terminate the enzyme reaction. The hydrolysate obtained was cooled to room temperature and then centrifuged at 10,000 x g for 10 min. The supernatant was then dehydrated by freeze drying and stored at 4 °C for further use.

Determination of Antioxidant activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. DPPH radical scavenging activity was determined according to the modified method of Alam et al. (2013). Sample solution was added with 0.2 mM DPPH fresh in 95% ethanol and the mixture was incubated at room temperature for 30 min in the dark. The resulting solution was measured at 517 nm. The DPPH radical scavenging activity was determined according to the following equation.

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The antioxidant activity was expressed as EC_{50} (mg/mL), the concentration required to cause 50% DPPH inhibition.

2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activity. The ABTS radical scavenging activity was measured according to Alam et al. (2013) with a slight modification. The ABTS reagent was prepared by mixing 7.4 mM ABTS stock solution with 2.6 mM potassium persulphate at a ratio of 1:1 (v/v). To determine the scavenging activity, the sample was mixed with $ABTS^{\bullet+}$ solution. The mixture was incubated at room temperature for 10 min in dark and the absorbance was read at 734 nm. The ABTS radical scavenging activity was calculated similar to above equation. The antioxidant activity of the test sample was expressed as EC_{50}, the concentration necessary for a 50% reduction of ABTS.

Ferric reducing antioxidant power (FRAP). The ability of the samples to reduce ferric ion (Fe^{3+}) was evaluated following the method of Benzie and Strain (1996) with a slight modification. The working FRAP reagent was prepared by

mixing 300 mM acetate buffer with pH 3.6: 10 mM tripyridyltriazine (TPTZ) solution in 40 mM HCL: 20 mM ferric chloride solution, in a proportion of 10: 1: 1 at 37 °C. The reaction mixture was allowed to stand in the dark for 30 min at room temperature and the absorbance was read at 593 nm. The FRAP was calculated using Trolox as the standard curve.

Statistical Analysis

All the experiments were performed in triplicate. Data were performed using analysis of variance (ANOVA) and the comparison of the means was carried out using Duncan's multiple range tests. A significant difference was determined at the 95% confidence interval ($P<0.05$). Statistical analysis was performed using IBM SPSS Statistics 23 software.

Results and Discussion

Anserine content in chicken breast meat

The amount of anserine obtained from various chicken meat are presented in **Figure 1**. The anserine content of Thai native chicken (139.76) and spent hen (127.26) were significantly different compared with the broiler (72.77). Several factors, including chicken genotype, sex, and age, can affect the anserine content in chicken meat (Chan et al., 1994). In the present study, the Thai native chicken was found to possess the highest anserine content. In this experiment, all chickens were slaughtered at market weight, resulting in the difference in age. This factor did not influence on anserine content in native Korean chickens (Jayasena et al., 2014). However, our result was inconsistent with Kim et al. (2012) which reported that the amount of anserine in the spent hen was decreased with an increasing in age. Therefore, the significant differences in anserine content among white meat including broiler, Thai native chicken, and spent hen may be attributed to the genotype. Similarly, in comparison with the native and broiler chicken, the native chicken had a significantly higher anserine content in its meat than the broiler (Jayasena et al., 2015).

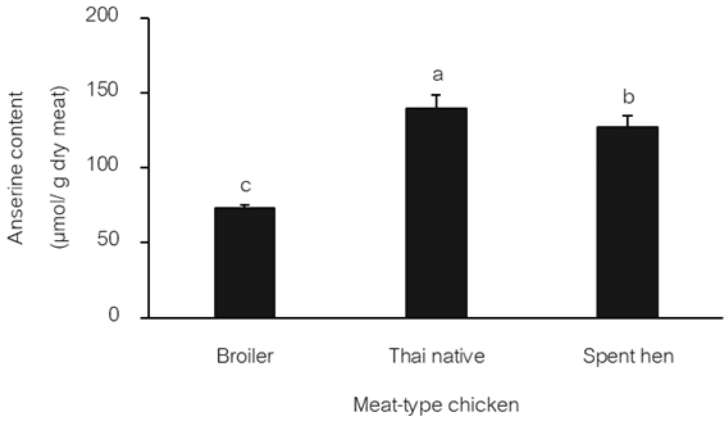

Figure 1 The difference of anserine content among chicken meat types (µmol per gram dry meat). Data are expressed as the mean ± standard deviation (SD). Bars with different alphabet are significantly different ($P<0.05$).

Total phenolic content in chicken breast meat

Polyphenolic compounds are distributed, retained, and remained functional in muscle (Sáyago-Ayerdi et al., 2009). Phenolic compounds generally presented in natural plant oils which added into chicken feed such as carotenoids, tocopherols, and gallagyl esters (Saleh et al., 2017). Total phenolic content of all chickens in this study were in the range of 42.44 to 49.41 mg of gallic acid equivalent/kg meat (**Figure 2**) and significantly difference ($P<0.05$). The result showed that the total phenolic content of Thai native chicken was higher than spent hen and broiler ($P<0.05$). Our finding was consistent with Okarini et al. (2013) who indicated that Bali indigenous chicken was high in total phenolic and antioxidant activity than spent laying hen and broiler due to eating behavior (scratch while eating), resulting in high consumption of phenolic antioxidants compounds. Furthermore, the antioxidant activity was associated with nonessential dietary antioxidants such as phenolic compound, anserine, and conjugated linoleic acid (Decker, 1995; Okarini et al., 2013). The phenolic compounds influence the quality, acceptability, and stability of foods by acting as flavors, colorants, and antioxidants (Decker, 1995). Anserine was known to contribute to endogenous antioxidant in meat. Our results showed that total phenolic content was similar found to be in anserine content.

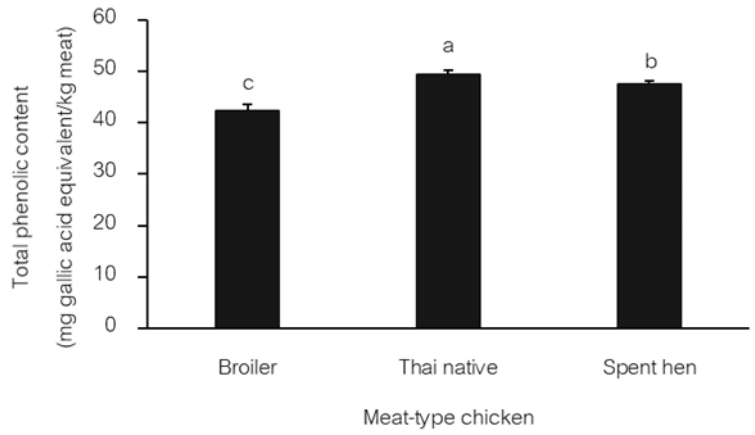

Figure 2 The difference of total phenolic content among chicken meat types (mg gallic acid equivalent/kg meat). Data are expressed as the mean ± standard deviation (SD). Bars with different alphabet are significantly different ($P<0.05$).

Antioxidant activities of chicken protein hydrolysates

The physiological proteins are commonly synthesized in forms of inactive polypeptides and are cleaved to the small functional peptides when they work at particular target molecules (Rizzello et al., 2016). Furthermore, bioactive peptides are released mainly by enzymatic processes (Daliri et al., 2017). In this study, the chicken meat was further hydrolyzed using Flavourzyme which was a mixture of endo- and exoprotease (Nchienzia et al., 2010). The antioxidant activities of their protein hydrolysates were critically analyzed and are presented in **Table 1**. Antioxidant activities of the protein hydrolysate were lower than L-glutathione (positive control) in all test assays. Our results showed that the

antioxidant activities of chicken hydrolysate were not significantly different in both the DPPH and ABTS tests. However, the protein hydrolysates were found to be significantly different in the FRAP test ($P<0.05$). This finding may have been related to the different antioxidant assays which vary in term of assay principle and experimental conditions (Rahman et al., 2015). It might be assumed that protein hydrolysate obtained from chicken meat has the ability to reduce the Fe^{3+}-ferricyanide complex to the ferrous form by donating an electron. Moreover, protein hydrolysate which obtained from Thai native chicken meat presented the highest ferric reducing antioxidant power. This result was consistent with the anserine and total phenolic content in chicken meat. The high antioxidant activities in protein hydrolysate might be due to anserine and total phenolic content. In addition, it has been reported that anserine has chelate prooxidative metals and its structure contained methyl group which could resist to enzymatic hydrolysis from both endogenous and exogenous sources (Decker, 2000; Pegova et al., 2000).

Table 1 The antioxidant activities of protein hydrolysates obtained from various chicken meat type[1/]

Treatments	EC_{50} (mg dry sample/mL)		FRAP test
	DPPH test	ABTS test	(μM Trolox equivalent/ g sample)
Broiler	4.71±0.22	24.27±3.36	14.82±1.08[c]
Thai native	4.41±0.14	24.13±1.15	18.33±1.61[a]
Spent hen	4.51±0.17	24.15±1.41	17.36±0.42[b]
GSH	0.09±0.02	0.28±0.05	757.57±73.37

[1/] Data are expressed as the mean ± SD. ND: not detected. GSH: L-glutathione at 0.1 mg/mL.

[a,b,c] Mean in the same column with different letters are significantly different ($P<0.05$).

Conclusions

Thai indigenous chicken meat provided a high anserine and total phenolic content which is suitable for consumers who concerned health. Moreover, protein hydrolysate obtained from Thai native chicken meat possessed a great antioxidant activity, which could be used in food supplements for people who face on health problem and ageing society. Other effects related to beneficial health effects of hydrolysates remain to be further determined.

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