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Effect of extraction time on the amounts of neurotransmitters and amino acids in chicken essence

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ABSTRACT

This study aimed to quantify yields of chicken essence, neurotransmitters, and amino acid concentrations in chicken essence. Kasetsart University, Thailand, bred three chicken lines to improve carcass percentages, and we used the chicken lines as the sources for chicken essence preparation. The selected chicken lines were Betong chicken (KU line), Tapaotong Kasetsart, and KU-Phuparn. Treatments were different extraction times for 1.0, 1.5, and 2.0 hours at 100 degrees Celsius. The results showed that Betong chicken (KU line) yielded higher amounts of chicken essence when employing 1.5 and 2.0 hours of extraction time compared to others from the Tapaotong Kasetsart and KU-Phuparn lines. Also, the concentration of each neurotransmitter in chicken essence with different extraction times was significantly different ($P < 0.05$). In addition to extraction time, Betong chicken (KU line) carcasses as the source of chicken essence preparation generated the highest amounts of anserine, carnosine, and creatine, followed by KU-Phuparn and Tapaotong, Kasetsart, respectively. Like neurotransmitters, extending extraction time improved the contents of amino acids. Compared to chicken essence extracted from Betong chicken (KU line) and Tapaotong Kasetsart, chicken essence originating from the KU-Phuparn line tended to generate a higher concentration of amino acids. The findings suggest that chicken lines and extraction affected chicken essence yields and amounts of neurotransmitters and amino acids in chicken essence.

Keywords: chicken essence (golden soup), endogenous compound, Betong chicken (KU line), Tapaotong Kasetsart line, KU-Phuparn line

INTRODUCTION

Nowadays, everybody emphasizes good health without any diseases, and the changed lifestyle, especially among people living in urban areas, causes physical fatigue. Good health begins with eating nutritious food and exercising regularly. Chicken meat is popular among consumers since it is considered an inexpensive meat compared to others (Sujiwo et al., 2018). Also, it is nutritious and contains high protein but low cholesterol. In 2022, the global consumption of chicken meat had grown at a rate of 2.09 percent per year, while domestic consumption accounted for 66.26% of total production (OAE, 2023). Thai native chickens have been raised either free- or semi-range. The advantages of Thai native chicken lines are tolerance to various environmental conditions and diseases, easy nurture, and low cost. Moreover, their meat textures are tough, soft, tender, and good taste, suitable for both cooking and human consumption. Therefore, the demand for poultry meat is high in markets. However, there are a few disadvantages due to its low carcass percentages (Jaturasitha et al., 2008).

Three Thai native chicken lines, namely Betong chicken (KU line), Tapaotong Kasetsart, and KU-Phuparn, were bred by Kasetsart University, with many advantages. Betong is a famous broiler chicken line in lower southern Thailand; it was bred in Betong District, Yala, Thailand. The breed originates in the Laeng Chan line in Guangdong Province, China. Betong is usually raised in rubber plantations for 7 to 8 months before slaughtering. The particular characteristics of their carcass meat are that it is delicious, tender, soft, low fat, and has a good odor, which makes Betong chicken meat popular among Thai people. However, the Betong chicken line has a low breeding rate and lacks scientific information support, so the number of raising the Betong chicken line has declined. For this reason, the Betong chicken

(KU line) was bred at Luang Suwan Wachakasikit Poultry Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bang Khen campus, Bangkok, Thailand, with the new character of fast-growing, with light brown to dark brown hair appearance (Sopannarath et al., 2015), raised for only 16 weeks (Sopannarath et al., 2015; Putsakul et al., 2010), and weights range from 962 to 2,949 grams. Males usually are heavier than females, and the mean live weights of males and females are 2,282.63 and 1,625.86 grams, respectively (Bungsrisawat et al., 2018). For the Tapaotong chicken line, Thai people with Chinese ethics call it Tapaotong due to its golden hair. The Tapaotong chicken line is significant in body size, with big breasts and much meat. Later, researchers of the Department of Animal Science, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand, bred the Tapaotong Kasetsart chicken line to increase their tolerance to environments, and more resistant to diseases with good shape appearance, including hybrids of the stone crest and chakra crest with golden hair, yellow beak, and yellow skin. Its meat is soft, tender, and good taste. Black bone chicken is famous in China; the Chinese believe that black bone chicken can remedy many diseases, such as diabetes and anemia. Based on medical studies, black bone chicken has antioxidant capacities, strengthening muscle enhancers, wrinkle reducers, and carnosine, an abundant non-protein nitrogen-containing meat compound, resulting in high domestic demand. For these reasons, the KU-Phuparn chicken line, a black bone chicken, was bred by Kasetsart University, Chaloem Phrakiat Campus, Sakon Nakhon, Thailand. The unique characteristics are white hair, black meat and skin, chakra crest, black bone, and fast growth. According to the study, results show that males usually are heavier than females due to hormones affecting growth and better feed conversion ratio in males. Markets prefer chickens that average 1.2 kilograms in weight and are raised only ten weeks (Khumpeerawat, 2016).

Chicken essence is a dark brown drink extracted from chicken meat. Therefore, it is categorized as a functional food popular in Asia, especially South East Asia. Generally, chicken essence is a supplement that improves exercise performance, ameliorates physical fatigue, reduces stress, vanishes worry, enhances memory, and improves student learning (Jiang and Groen, 2000). Vertebrate animal meat is composed of anserine (a methylated product of carnosine; β-alanyl-1-methyl-l-histidine), carnosine (a dipeptide; β-alanyl-l-histidine), and creatine (a metabolite of arginine, glycine, and methionine). They are known as bioactive compounds (Li et al., 2012). Most animals, except humans and plants, possess anserine, carnosine, and creatine. These dietary nutrients are beneficial for preventing and treating obesity, cardiovascular dysfunction, and aging-related disorders, as well as inhibiting tumorigenesis, improving skin and bone health, and ameliorating neurological abnormalities. Furthermore, by improving the metabolism and operations of immune system cells, such as monocytes, macrophages, and others, they may enhance people's immunological defense against infections by microorganisms (Jung et al., 2013).

The chicken carcasses of Betong chicken (KU line), Tapaotong Kasetsart, and KU-Phuparn lines were the benefit source for chicken essence production since Kasetsart University bred these lines for increasing carcass percentages. The objectives of this study were to observe the effect of extraction time on yields of chicken essence, neurotransmitters, and amino acids.

MATERIALS AND METHODS

Sources of samples

The selected chicken lines in this study were as follows: Betong chicken (KU line), Tapaotong, Kasetsart, and KU-Phuparn lines. Betong chicken (KU line) carcasses were obtained from Luang Suwan Wachakasikit Poultry Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bang Khen campus, Bangkok, Thailand, while Tapaotong Chicken Farm, Song Phi Nong District, Suphan Buri Province, Thailand, supported Tapaotong Kasetsart chicken carcasses. Khun Tam Farm, Bangkok, Thailand, provided KU-Phuparn chicken carcasses. The weight of all chicken carcasses was between 1.0 and 1.5 kilograms. The prices of Betong chicken (KU line), Tapaotong Kasetsart, and KU-Phuparn chicken carcasses are 190, 130, and 210 Baht per kilogram, respectively.

Chicken essence preparation and experimental design

The method was modified by Wu (2020). The whole carcasses were thawed at 4 degrees Celsius in a refrigerator for 24 hours. The carcasses were washed with water and cut into pieces, and bone parts were smashed. Then, carcasses were weighed and steamed. The outer pot contained approximately 33% water (v/v), while the inter pot had pores to let chicken essence drip into the bottom. The experiment was assigned using the randomized complete block design (RCBD). Treatments were three times extraction conditions: 1.0, 1.5, and 2.0 hours at 100 degrees Celsius, respectively. Each treatment consisted of three replications. The chicken essences were filtered by using a cotton sheet and weighed. Yields were calculated using Equation 1.

$$\text{Yield}(\%) = \frac{\text{weight chicken essence}}{\text{weight of chicken carcass}} \times 100 \quad (1)$$

Quantitative analysis of neurotransmitters

The methods of anserine, carnosine, and creatine quantification were modified by Li et al. (2012). One gram of chicken essence was homogenized with 7.5 mL of 0.01 N HCl at 1,130 × g for one minute, and then the homogenate was centrifugated at 17,030 × g for 15 min at 4 degrees Celsius. The 2.5 ml supernatant was mixed with 7.5 ml of acetonitrile and centrifugated at 17,030 × g for 15 min at 4 degrees Celsius. The clear supernatant was filtered through a 0.22 µm syringe filter, and 10 µl of samples were injected into the HPLC instrument (Waters 600E). The HPLC instrument consisted of a HILIC silica column (4.6 mm × 150 mm, 3 µm; Millipore) and a diode array detector (Waters 2487 HPLC UV/VIS Detector) at 214 nm to measure carnosine, anserine, and creatine contents. The mobile phase A was 0.65 mM ammonium acetate in water- acetonitrile mix (25:75 vol/vol, pH 5.5), while the mobile phase B was 0.65 mM ammonium acetate in water- acetonitrile mix (70:30 vol/vol, pH 5.5). The mobile phase B was supplied at 1.2 mL/min for 16 min with a linear gradient from 0 to 100%. The contents of carnosine, anserine, and creatine were calculated using standard linear regression, and the standards were purchased from Sigma Company (St. Louis, MO, USA). Neurotransmitter quantification was replicated at least three times on three independent occasions. The means and standard deviation of neurotransmitter amounts were reported.

Quantitative analysis of amino acids

The amino acid measurement was performed at the Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand. Based on Journal of Chromatography B (2014) 116–127, The in-house Method WI-TMC-06 Dong et al. (2014) was used to quantify amino acids. Eighteen amino acids were measured as follows: alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

Data analysis

All data were analyzed for variance (Analysis of Variance: ANOVA) according to the randomized complete block design, and the mean differences were compared by Duncan's New Multiple Range Test (DMRT) using the statistical package SPSS version 26.0 (IBM Corp, 2019).

RESULTS AND DISCUSSION

Yields of chicken essence

In the current study, we set 100 degrees Celsius as extraction temperature and varied extraction times of 1.0, 1.5, and 2.0 hours. When we increased the processes of chicken essence preparation from 1.0 to 1.5 hours, it enhanced the yield of chicken essence, as shown in Figure 1. However, increasing extraction time did not cause higher yields after 1.5-hour extraction time. In addition, chicken meat from Tapaotong Kasetsart and KU-Phuparn lines generated equal amounts of chicken essence at every extraction time. In contrast to the chicken essence originating from Tapaotong Kasetsart and KU-Phuparn meat, chicken carcasses of Betong chicken (KU line) gave higher percentages when extracting for 1.5 and 2.0 hours. Extraction time affects the contents of chicken essence because heat denatures myosin and actin (Lin et al., 2016). Over a longer time, the heating process denatures myosin, and actin expels sarcoplasmic fluid from muscle fibers (Dai et al., 2014). Finally, meat tissue loses water.

Quantitation of neurotransmitters in chicken essence

The current study measured amounts of anserine, carnosine, and creatine, as shown in Tables 1 to 3. The approximate concentrations of anserine, carnosine, and creatine ranged from 669–1,150, 104–336, and 316–545 mg/100g, respectively. Different extraction times yielded significant contents of anserine, carnosine, and creatine ($P < 0.05$). When a 2-hour extraction time was employed, we measured the highest concentration of anserine, carnosine, and creatine. According to chicken lines, the highest concentration of anserine, carnosine, and creatine was observed in chicken essence extracted from Betong chicken (KU line) meat, followed by chicken essence originating from Tapaotong Kasetsart and KU-Phuparn meat, respectively.

Table 1. Anserine concentrations in chicken essence (mg/100g) extracted from three Thai native chicken lines

Items	Extraction time		
	1.0 hour	1.5 hours	2.0 hours
Betong chicken (KU line)	691.00 ± 51.21^{c}	924.20 ± 27.82^{bA}	$1,150 \pm 48.21^{aA}$
Tapaotong Kasetsart	669.33 ± 61.64^{b}	751.93 ± 22.65^{abC}	839.94 ± 39.09^{aB}
KU-Phuparn	676.01 ± 60.65^{c}	830.40 ± 51.53^{bB}	937.10 ± 35.92^{aB}

^{abc}Means in the same row with different superscripts are significantly different (P < 0.05).
^{ABC}Means in the same column with different superscripts are significantly different (P < 0.05).

Table 2. Carnosine concentrations in chicken essence (mg/100g) extracted from three Thai native chicken lines

Items	Extraction time		
	1.0 hour	1.5 hours	2.0 hours
Betong chicken (KU line)	198.86 ± 18.64^{cA}	265.86 ± 9.50^{bA}	336.42 ± 11.06^{aA}
Tapaotong Kasetsart	103.86 ± 19.78^{cB}	169.89 ± 8.25^{bB}	205.18 ± 16.89^{aC}
KU-Phuparn	198.69 ± 14.34^{cA}	251.16 ± 18.02^{bA}	273.56 ± 11.93^{aB}

^{abc}Means in the same row with different superscripts are significantly different (P < 0.05).
^{ABC}Means in the same column with different superscripts are significantly different (P < 0.05).

Table 3. Creatine concentrations in chicken essence (mg/100g) extracted from three Thai native chicken lines

Items	Extraction time		
	1.0 hour	1.5 hours	2.0 hours
Betong chicken (KU line)	346.67 ± 20.08^{c}	450.97 ± 9.87^{bA}	545.09 ± 13.99^{aA}
Tapaotong Kasetsart	319.89 ± 32.89^{b}	367.91 ± 10.29^{aB}	399.67 ± 21.76^{aB}
KU-Phuparn	316.09 ± 16.13^{c}	366.28 ± 20.81^{bB}	413.87 ± 12.64^{aB}

^{abc}Means in the same row with different superscripts are significantly different (P < 0.05).
^{ABC}Means in the same column with different superscripts are significantly different (P < 0.05).

Our study found different amounts of anserine and carnosine compared to previous studies, as shown in Tables 5 and 6. Many studies show that various factors, particularly chicken lines (Dai et al., 2014), cause different amounts of neurotransmitters, such as diverse types of meat, chicken lines, genders, and cooking conditions (Qi et al., 2018). For example, commercial chicken's edible meat delivers more anserine and carnosine contents than bones (Maikhunthod, 2003). In general, fresh breast meat of five lines of Korean native chickens consists of more anserine and carnosine than fresh thigh meat of the same Korean native chicken lines (Li et al., 2012). In addition to depending on the type of meat, most fresh meat of different Korean native chicken contains significant variations of anserine, carnosine, and creatine (Li et al., 2012). Like different kinds of meat and chicken lines, most fresh breast and thigh meat of the same Korean native chicken lines from females is composed of significantly higher amounts of anserine and carnosine (Li et al., 2012). The results disagreed with a report that prolonging extraction time at 105 and 115 degrees Celsius adversely influences anserine and carnosine concentration (Dai et al., 2014). Due to divergent chicken lines in these studies and the lack of scientific information on creatine, we could not compare these neurotransmitter amounts with other chicken essence (Dai et al., 2014). The chicken essence originating from the breast meat of Thai native chicken generates the highest concentration of carnosine, followed by the others extracted from hybrid Thai native chicken lines and broilers, respectively (Qi et al., 2018).

Amino acid profiles of chicken essence extracted from three chicken lines

We determined amino acid quantification in chicken essence, as shown in Table 4. For chicken essence generated from chicken carcasses of Betong chicken (KU line) and Tapaotong Kasetsart lines, shifting extraction time from 1.5 to 2.0 hours resulted in increasing amounts of all amino acids, except proline and tryptophan. However, most amino acids in chicken essence derived from Betong chicken (KU line) and Tapoundthong Kasetsart line were equal between 1.5 and 2.0 hours of extraction time. Commonly, when we extended extraction time from 1.0 to 1.5 hours, all amino acids in chicken essence originating from chicken carcasses of the KU-Phuparn line increased. In contrast to others, chicken essence extracted from KU-Phuparn carcasses contained higher amounts of most amino acids, except valine, cysteine, and leucine, when we employed a 2.0-hour extraction time.

When we compared amino acids in our chicken essence with others reported in previous studies, as shown in Table 7. The concentration of most amino acids is similar to the others extracted from the Taiwanese native chicken line, except aspartic acid, arginine, methionine, cystine, isoleucine, and phenylalanine (Dai et al., 2014). In our study, extending extraction time enhanced the increasing amount of amino acid in chicken essence. In agreement with our results, shifting from 4 to 6 hours with 115 degrees Celsius incubation improved most amino acids in chicken essence (Dai et al., 2014). Like the amounts of neurotransmitters, amino acid concentration in chicken essence possibly depends on the chicken line. For instance, Wu and Shiau (2002) demonstrated that chicken essence extracted from divergent chicken lines contains significantly different amounts of amino acids.

The requirements of carnosine and creatine for a 70-kg healthy adult are 606 and 1,700 mg per day, respectively (Jung et al., 2013). According to Jung et al. (2013), arginine, glycine, and methionine are the precursors of creatine synthesis via the cooperation of multiple organs, and a 70-kg healthy adult releases 1.7 g creatine per day as the excretion of urine; on the other hand, the production of 1.7 g creatine needs 2.3 g arginine, 1.0 g glycine, and 2.0 g methionine (Jung et al., 2013). Possibly, the amounts of neurotransmitters in chicken essence may not meet human physiological requests. Therefore, we recommend that people should eat meat as an alternative source of these dietary nutrients.

Table 4. Amino acid profiles of chicken essence extracted from KU Betong, Tapaotong Kasetsart, and KU-Phuparn meat (mg/100 g) with 1.0-, 1.5-, and 2.0-hour extraction time

Items	Chicken line	Betong chicken (KU line)			Tapaotong Kasetsart			KU-Phuparn		
	Extraction time (hour)	1.0	1.5	2.0	1.0	1.5	2.0	1.0	1.5	2.0
Amino acid	Taste									
Aspartic acid	Umami	167.78	168.68	205.52	156.00	156.59	185.97	175.37	201.93	204.62
Glutamic acid	Sour	465.26	468.98	546.86	461.71	451.28	520.55	526.9	589.92	583.03
Total		**633.04**	**637.66**	**752.38**	**617.71**	**607.87**	**706.52**	**702.27**	**791.85**	**787.65**
Serine	Sweet	100.07	99.35	121.04	97.60	99.49	115.65	113.29	128.32	126.74
Threonine		77.22	77.80	92.09	77.66	75.04	88.32	83.93	96.09	94.68
Glycine		501.99	522.8	642.39	505.74	460.00	520.67	489.47	583.56	608.62
Alanine		240.18	247.55	298.85	246.59	226.7	261.05	251.41	289.63	295.27
Tyrosine		34.59	33.49	38.11	21.88	31.54	36.67	36.47	39.25	39.50
Proline		193.95	232.77	188.95	204.80	154.84	203.95	135.03	177.12	238.66
Total		**1,148**	**1,213.76**	**1,381.43**	**1,154.27**	**1,047.61**	**1,226.31**	**1,109.6**	**1,313.97**	**1,403.47**
Histidine	Bitter	144.03	140.54	151.31	91.38	140.50	148.23	132.95	136.81	154.75
Arginine		185.34	181.81	236.12	185.67	172.93	200.57	195.47	226.40	236.25
Valine		68.35	70.33	85.75	71.47	63.47	81.81	72.84	86.72	50.00
Methionine		56.26	60.53	71.74	60.11	56.91	70.01	70.14	76.97	75.70
Cystine		317.79	338.07	414.6	327.72	301.89	341.51	313.89	382.83	384.95
Isoleucine		47.65	48.86	59.74	49.93	43.61	55.35	49.73	59.39	56.81
Phenylalanine		67.00	68.27	83.79	67.20	64.88	74.76	68.60	80.85	82.19
Tryptophan		4.44	4.59	2.94	3.91	3.78	3.66	3.85	3.73	4.50
Leucine		105.41	106.99	127.8	108.63	103.88	123.90	114.58	183.35	129.67
Lysine		150.26	152.12	179.16	129.77	136.43	165.09	163.57	183.35	180.33
Total		**1,146.53**	**1,172.11**	**1,412.95**	**1,095.79**	**1,088.28**	**1,264.89**	**1,185.62**	**1,420.40**	**1,355.15**
Total amino acid		**2,927.57**	**3,023.53**	**3,546.76**	**2,867.77**	**2,743.76**	**3,197.72**	**2,997.49**	**3,526.22**	**3,546.27**

Table 5. Anserine concentrations in chicken essence (mg/100g) detected in this study and reported in previous studies

Source for chicken essence preparation	Anserine concentration (mg/100g)	Reference
Hybrid Thai native chicken	669–1,150	This study
Commercial chicken	36–437	Wu and Shiau, 2002
Taiwanese native chicken	377–518	Lin et al., 2016
Taiwanese native chicken	3,419–6,304	Lin et al., 2017

Table 6. Carnosine concentrations in chicken essence (mg/100g) detected in this study and reported in previous studies

Source for chicken essence preparation	Carnosine concentration (mg/100g)	Reference
Hybrid Thai native chicken	104–336	This study
Thigh meat from many chicken lines[1]	6–11	Maikhunthod, 2003
Breast meat of many chicken lines [1]	18–33	Maikhunthod, 2003
Commercial chicken	8–162	Wu and Shiau, 2002
Taiwanese native chicken	139–172	Lin et al., 2016
Taiwanese native chicken	2,525–3,263	Lin et al., 2017

[1] In this study, they prepared chicken essence from Thai native chicken, hybrid Thai native chicken lines, and broiler.

Table 7. Amino acid profiles in chicken essence (mg/100g) detected in this study and reported in previous studies

Items	Source for chicken essence preparation			
Amino acid	Hybrid Thai native chicken	Taiwanese native chicken	Taiwanese native chicken	Commercial chicken
Aspartic acid	168–205	212–307	172–214	4.4–34.1
Glutamic acid	451–589	484–665	536–710	13–107
Serine	97–128	100–136	249–333	3.4–43
Threonine	75–96	73–106	155–175	3–28
Glycine	460–642	101–671	219–249	8–383
Alanine	226–298	296–426	382–413	10–59
Tyrosine	21–39	26–38	53–75	4–18
Proline	135–239	217–358	118–151	4–23
Histidine	91–155	127–156	41–63	1–10
Arginine	172–236	593–758	128–176	3–28
Valine	50–86	96–127	111–152	8–23
Methionine	56–76	10–30	45–63	4–11
Cystine	301–414	0–16	1–2	-
Isoleucine	43–59	19–78	65–89	2–12
Phenylalanine	64–83	74–107	66–88	4–14
Tryptophan	3–4	14–54	-	-
Leucine	103–183	111–170	136–178	4–23
Lysine	129–183	179–256	170–250	5–36
Reference	This study	Lin et al., 2016	Lin et al., 2017	Wu and Shiau, 2002

CONCLUSIONS

According to Betong chicken (KU line), Tapaotong Kasetsart, and KU-Phuparn chicken lines as the sources of chicken essence preparation in the present study, the information of prices, yields of chicken essence, and amounts of neurotransmitters and amino acids could be the guideline for producing chicken essence. The clear results showed that Betong chicken (KU line) carcasses gave the highest chicken essence yield when we applied with either 1.5 or 2.0-hour extraction time at 100 degrees Celsius. Many factors, especially chicken lines, affected neurotransmitter amounts. Chicken essence generated from the Betong chicken (KU line) line provided the highest amounts of anserine, carnosine, and creatine, followed by others extracted from KU-Phuparn and Tapaotong Kasetsart, respectively. Amounts of each neurotransmitter in chicken essence extracted from three chicken lines significantly depended on extraction time ($P < 0.05$). To maintain physiological regulations, people should eat animal meat since chicken essence may contain insufficient amounts of neurotransmitters. Extraction time influenced amounts of amino acids in chicken essence. When we increased extraction time, a higher concentration of most amino acids in chicken essence was observed.

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