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Effect of temperature and time on free amino acid profile in Thai chicken bone soup stock preparation

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ABSTRACT

Introduction: Soup stock is the basic material for cooking various types of food, including Thai food. The general practice to prepare good quality Thai soup stock is to gently boil chicken bone at 85 -95°C for 1 -3 h. Free amino acids (AAs) including essential AA (EAA) present in the soup stock play an important role for the supply of nutrients and the modulation of taste and palatability. Chicken bone contains key taste-active compounds such as glutamate (Glu) which is responsible for the umami taste. However, no data have been reported on the content of free AA and the changes in its concentration in chicken bone soup stock as a function of boiling time. **Method:** Therefore, this study aimed to investigate the effect of temperature and boiling time on the AA profile and their concentrations during soup stock production. The concentrations of free AA during the heating of the chicken bone soup stocks at three different temperatures (85°C, 90°C, and 95°C) for 30, 60, 120, and 180 min were analyzed by high-performance liquid chromatography. **Results and Conclusion:** The results showed the compositions of AA (19 AA) in Thai chicken bone stock prepared in each condition were similar, and major free AAs in the stock were Glu and alanine. The total free AA concentration slowly rose with increasing temperature and heating time, except for glutamine. The total AA concentration was the highest in soup stock boiled at 90°C and 95°C for 180 min (143.14 ± 3.30 and 145.99 ± 9.20 mg/100 ml, respectively) and was significant higher than those at 85°C, 180 min. The concentration of Glu was also found to be highest when the stock was boiled at 90 and 95°C, 180 min (28.61±1.0 and 28.80±2.21 mg/100 ml, respectively), whereas the concentration of EAA (52.00 ± 2.96 mg/100 ml) was significantly highest in the soup stock cooked at 95°C for 180 min. Sensory evaluation demonstrated that boiling time, but not temperature, improved sensory attributes of the soup stocks. Those boiled for 120 and 180 min obtained significantly higher scores for taste, color, thickness, and overall liking than those boiled for 30 and 60 min. This result corresponded to a significant increase of Glu concentration from 120 min of heating. This revealed that the palatability of Thai chicken bone soup stock might be affected by Glu, an umami taste substance. This finding might suggest that the condition to obtain good quality chicken bone stock in terms of taste and nutritional values, especially EAA content, is to gently boil at 90 -95°C for 180 min.

Keywords: Amino acid profile, free amino acid, soup stock, umami taste

INTRODUCTION

A soup stock is the flavorful extract that is made by gently boiling meat, bones, and/or vegetables in a thin clear liquid.[1] The soup stock is not usually served by itself but is used as a flavoring agent in many dishes, especially soups and sauces. The use of soup stock in food preparation has been found in many countries such as France, Spain, Japan, China, and Thailand. Recipes and techniques for making soup stock vary depending on culture and country.[2-4] Beef and chicken stocks are the most widely used in the world.[2]

In Thailand, chicken and pork bone soup stocks are the most popular ones for Thai cuisine. Stock is usually used as a base for soup, noodles, and stir-fried dish preparations, with the stock's sensory qualities underlying the palatability

of these foods. In general, practice to prepare Thai stock, chicken bone, or pork bone is gently boiled (85–95°C) for several hours (1–3 h) to obtain rich flavor substances.[5] The gentle boil method is recommended for improving extraction and preparing clear soup because this gradual heating process allows soluble proteins to coagulate and form large aggregates which will float to the surface and can easily be skimmed off.[6]

Free amino acid (AA) profile and its content in the stock play an important role for providing nutritional value and modulating taste and palatability.[1,7,8] Each free AA has its own taste; for example, alanine (Ala), glycine (Gly), glutamine (Gln), and serine (Ser) have sweet taste, valine (Val) and leucine (Leu) have bitter taste, whereas glutamate (Glu) and aspartate (Asp) have umami taste.[9,10] The different combinations of free AAs are key to the unique taste of each stock. It has been reported that ingredients, temperature and duration of heating can affect types of free AA and their concentration released in the stock.[7,8,11,12] Types of free AA in the stock are different depending on raw materials, while the concentrations are different depending on both boiling temperature and boiling time.[8] For example, in beef, the main free AAs are Ala, Gln, and Gly, while in chicken, predominant free AAs are Ala, Gln, Gly, and Ser.[13,14] Therefore, soup stock from beef and chicken should have the taste from their predominant AA. The higher temperature and boiling time show higher AA concentration.[8,15]

The stock, which is prepared by mixing beef, chicken bone, whole chicken, carrot, and onion, then boiled for 5 h, provided the highest free AA concentration compared to those cooked for 1, 2, 3, and 4 h. Moreover, the soup stock cooked at 95°C had higher free AA concentration than those boiled at 85°C for the same boiling time. Glu and Ala were two major free AAs in this soup stock; therefore, umami was the major characteristic taste along with thickness. The taste threshold of each AA is different and thus AA concentrations which are higher than their thresholds could influence taste quality in soup stock.[8]

Although several taste components, including AAs, in stock prepared from whole chicken with/without beef and vegetables, were previously investigated, there are no data on free AA profiles and concentrations in common Thai stock that is prepared from only chicken bone. The aim of this study was to investigate the effect of temperature and boiling time commonly used for making Thai chicken bone soup stock on released free AA profiles and concentrations as well as the impact on sensory attributes. A cooking condition would be identified to maximize chicken bone stock quality, for both nutritional and sensory aspects. This information could be used to set a new standard for quality control in Thai cooking which could ultimately increase the competitiveness of the Thai food industry against other countries.

MATERIALS AND METHODS

Raw Materials and Preparation

Chicken bones were bought from a supermarket in Bangkok and were prepared by taking the skin off and were cut into pieces (size approximately 10 cm × 10 cm). The bone pieces were cleaned with tap water and blanched in boiling water for 30 s to remove blood residue.

Chicken Bone Soup Stock Preparation

Chicken bone soup stock was prepared according to the method described in Culinary Technology and Service Program, Suan Dusit School of Culinary Arts.[5] Each boiling pot was prepared to contain the same amount of chicken bone pieces and tap water, a ratio of 1:2 (w/w). The chicken bone stock samples were cooked at three different boiling temperatures (85°C, 90°C, and 95°C). For each boiling temperature group, the samples were tested over four different boiling times (30, 60, 120, and 180 min). After that, the chicken bone stocks were filtered through cotton cloth sheets and collected. The chicken bone stock samples were taken for sensory analysis and kept at −20°C for free AA determination.

Determination of Free AAs And Total Free AAs by High-Performance Liquid Chromatography (HPLC)

All chicken bone soup stock samples were filtered through 0.45 μm nylon syringe filters before derivatization with o-phthalaldehyde using an autosampler (Agilent 1100, USA) with injector program as described by Henderson *et al.* (2010) before HPLC analysis.[16] Analysis of free AAs was performing using an HPLC equipped with a UV-VIS detector (Agilent 1100, USA) set at 338 nm. Free AAs were separated on a C18 (ZORBAX Eclipse-AAA, 4.6 × 150 mm, 5 μm) with a guard column (ZORBAX Eclipse-AAA, 4.6 mm × 12.5 mm, 5 μm). The temperature of the column was set at 40°C. The gradient elution system consisted of a mixture of 40 mM sodium phosphate dibasic, pH 7.8 as solvent A, and a mixture of acetonitrile:methanol:water (45:45:10 v/v/v) as solvent B. The gradient program was as follows: 0–1.9 min, 0%B; 18.1 min, 57%B; 18.6 min, 100%B; 22.3 min, 100%B; and 23.2 min, 0%B and then held for 2.8 min to reequilibrate to initial conditions before the next injection. The total running time was 26 min with the flow rate of 2.0 mL/min. Types of AA in the stock samples were identified by comparing the retention time with each standard AA. Quantity of AA was determined based on the external standard method using calibration curves fitted by linear regression analysis. Total free AAs were calculated from sum of all free AA concentration.

Sensory Evaluation

The chicken bone soup stock samples were analyzed for taste, odor, color, clearness, thickness, and overall liking using hedonic scales.[17] 32 untrained panelists were recruited to evaluate the samples. They were asked to rate the samples on the basis of a 7-point hedonic scale anchored by: 1 = "Strongly disliked;" 2 = "Moderately disliked;" 3 = "Slightly disliked;" 4 = "Neutral;" 5 = "Slightly liked;" 6 = "Moderately liked;" and 7 = "Strongly liked." The sensory evaluation was performed in a sensory laboratory under conditions of standard light and temperature (25°C). The samples were served at 60°C and presented monadically following a completely randomized design. To avoid carryover effects, mineral water was available as neutralizer between samples.

Statistical Analysis

Experiments were carried out in triplicate (n = 3). The measurements were performed in triplicate for each sample.

Data were expressed as mean ± standard deviation and analyzed using SPSS software version 22.0 (IBM Crop., Armonk, NY, USA, 2013). One-way analysis of variance (ANOVA) was performed and *post hoc* comparison with Duncan's multiple range test was applied to examine the difference between groups with the statistical significance level at $p < 0.05$. Principal component analysis (PCA) was performed to investigate the correlation among variables and categorization of AA components in chicken bone soup stocks using MultiExperiment Viewer (MeV) version 4.8 (www.tm4.org/mev/).

RESULTS AND DISCUSSION

Total Free AAs In Chicken Bone Soup Stocks

The concentration of total free AAs in chicken bone stocks boiled at 85, 90, and 95°C over 180 min is shown in Figure 1a. By increasing temperature of stock boiling, the results showed higher total free AA concentration with higher boiling temperature. By heating time effect, total free AA concentration also gradually increased with the longer boiling time. Over a 180 min boiling time, total free AA concentration changed from 97.82 ± 4.52 to 135.46 ± 6.68 mg/100 ml, 114.82 ± 4.19 to 143.14 ± 3.10 mg/100 ml, and 101.87 ± 4.25 to 145.99 ± 9.20 mg/100 ml, at 85, 90, and 95°C, respectively. Comparing within the same boiling temperature, total free AA concentration was highest at 180 min. Among all samples, the highest total free AA concentration was at both 90°C and 95°C, 180 min boiling time (statistically significant). This result suggests that the augmentation of temperature and boiling time could extract more AA content from raw food materials, which is in line with several other studies that showed the effect of temperature and time on improving AA extraction.[8,15] However, for 30 min long heating times, we found that soup stock boiled at 90°C could obtain a significantly higher total AA concentration than that cooked at 95°C. This result was similar to that of the study of Pereira-Lima *et al.* (2000), which showed that at high temperatures of cooking, soup stock could obtain lower free AA concentration. These decreases might be explained by Maillard's reaction and Strecker degradation reactions.[18] Interestingly, at 95°C, constant AA concentration was observed over 60–120 min boiling time. This could be partly contributed to by a dramatic loss of Gln content at 95°C, for 120 min.

Codex has set the standard for amino nitrogen in the stock to be 100 mg/L.[1] Research on Japanese and Western cuisine has also shown that AA concentration varied from 40 to 400 mg/100 ml in their stocks, which are usually prepared from a combination of various ingredients such as beef and/or whole chicken and/or chicken bone and/or vegetables.[19,20] In this study, it was shown that in the general practice of making Thai stock, gentle boiling of chicken bone alone for 2–3 h could provide AA concentrations at a level that aligns with the Codex standard and comparable to those found in the stock of other cuisines worldwide.

Free AAs Profiles in Chicken Bone Soup Stocks

A total of 19 free AAs including Asp, Glu, asparagine, Ser, Gln, histidine (His), Gly, threonine (Thr), arginine (Arg), Ala, tyrosine, cysteine (Cys), Val, methionine (Met), tryptophane (Trp), phenylalanine (Phe), isoleucine (Ile), Leu, and lysine (Lys) have been found in chicken bone stock in all tested conditions as shown in Table 1. These AAs could be categorized into an EAA group including Phe, Val, Thr, Trp, Met, Leu, Ile, and His and non-essential AA group (NEAA). This demonstrated that the temperature and boiling time had no effect on AA composition. However, they had an effect on concentration of each AA. Increasing the temperature and heating time increased the free AA concentration which could improve EAA and NEAA concentrations during heating at 85, 90, and 95°C. That of EAA significantly increased in the stock boiled for 180 min at both 90°C and 95°C compared to at 85°C. Especially, the stock boiled at 95°C for 180 min showed the highest concentration with statistical significance as shown in Figure 2. On the other hand, there was no significant difference observed between 90°C and 95°C at 180 min boiling time for NEAA. This suggests that gentle boiling of chicken bone stock at 95°C for 180 min was a good condition to extract EAA contents which could help meet the WHO recommended level of EAA.[21]

Concentration of most NEAAs rose with prolonged boiling time, except for Gln. As shown in Figure 1b, Gln was significantly degraded at longer boiling time (30 vs.

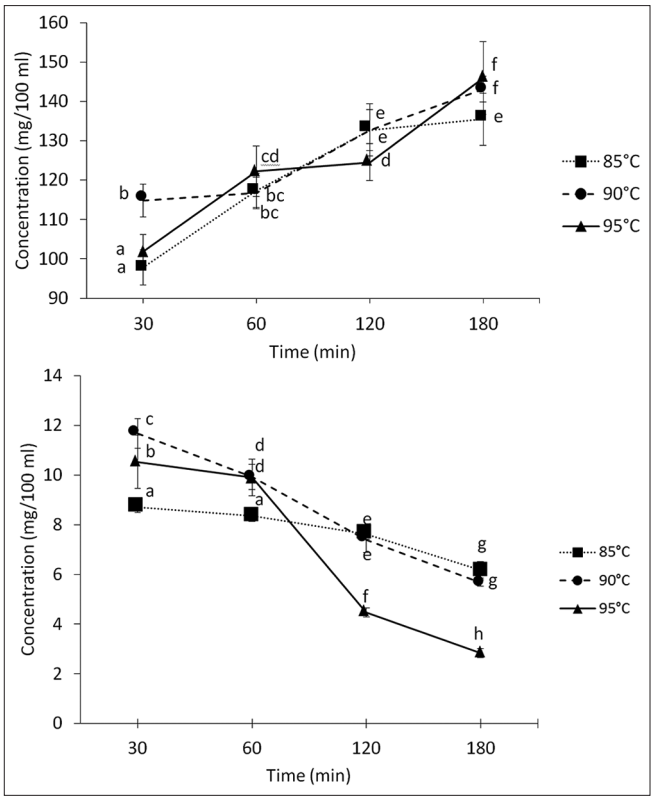

Figure 1: (a) Total free amino acids in chicken bone soup stock boiled at different temperatures. (b) Glutamine concentration in chicken bone soup stock boiled at different temperatures. All results are expressed as mean ± standard deviation ($n = 3$). The significant differences were found by a one-way ANOVA followed by *post hoc* multiple comparisons test (Duncan's test). $P < 0.05$ was considered statistically significant. Values with different alphabet are significantly different within same time

Table 1: Free amino acids in chicken bone soup stock boiled at different temperatures

Temperature (°C)	85				90				95			
Heating time (min)	**30**	**60**	**120**	**180**	**30**	**60**	**120**	**180**	**30**	**60**	**120**	**180**
Amino acid (mg/100 ml)												
Asp	5.89±0.69^{a}	6.70±0.34^{b}	7.78±0.43^{c}	7.95±0.27^{c}	6.72±0.29^{b}	7.07±0.20^{b}	8.61±0.39^{d}	9.33±0.26^{e}	6.21±0.26^{a}	8.24±0.44^{cd}	8.15±0.30^{cd}	9.42±0.62^{e}
Glu	15.91±0.55^{a}	19.58±0.66^{b}	23.44±0.21^{e}	24.28±0.76^{e}	18.36±1.31^{ab}	20.98±0.50^{cd}	23.93±1.80^{e}	28.61±1.00^{g}	17.25±0.97^{a}	21.71±0.73^{d}	23.12±0.90^{e}	28.80±2.21^{g}
Asn	0.87±0.10^{a}	1.00±0.03^{a}	1.20±0.03^{bc}	0.97±0.03^{a}	0.95±0.02^{a}	1.04±0.10^{ab}	1.01±0.06^{a}	1.20±0.10^{bc}	0.98±0.12^{a}	1.29±0.30^{cd}	1.45±0.27^{d}	1.76±0.17^{e}
Ser	5.77±0.23^{a}	7.10±0.26^{bc}	8.33±0.22^{d}	8.42±0.36^{d}	7.00±0.22^{bc}	7.30±0.22^{c}	8.31±0.58^{d}	9.57±0.23^{e}	6.82±0.19^{b}	8.41±0.28^{d}	8.46±0.28^{d}	10.04±0.58^{f}
Gln	8.71±0.20^{e}	8.36±0.23^{e}	7.62±0.21^{d}	6.18±0.33^{c}	11.67±0.60^{h}	9.92±0.50^{f}	7.39±0.51^{d}	5.66±0.13^{c}	10.53±1.06^{g}	9.90±0.74^{f}	4.47±0.19^{b}	2.84±0.19^{a}
His	2.63±0.11^{a}	2.89±0.07^{b}	3.38±0.11^{d}	3.45±0.21^{d}	2.91±0.10^{b}	3.10±0.16^{c}	3.80±0.21^{e}	4.01±0.04^{f}	2.46±0.05^{a}	2.89±0.29^{b}	4.24±0.14^{g}	4.28±0.16^{g}
Gly	5.70±0.17^{a}	6.93±0.28^{c}	7.74±0.15^{d}	8.40±0.31^{ef}	6.98±0.23^{c}	7.21±0.26^{c}	8.59±0.34^{f}	9.33±0.23^{g}	6.46±0.23^{b}	7.72±0.37^{d}	8.10±0.19^{de}	9.82±0.76^{h}
Thr	4.79±0.10^{a}	6.03±0.05^{cd}	6.82±0.23^{f}	6.64±0.19^{ef}	5.58±0.14^{b}	5.87±0.15^{c}	6.73±0.29^{f}	7.48±0.21^{g}	5.48±0.09^{b}	6.21±0.26^{d}	6.48±0.17^{e}	7.30±0.37^{g}
Arg	7.98±0.33^{c}	10.54±0.26^{g}	11.97±0.24^{h}	12.24±0.32^{h}	8.66±0.27^{d}	8.92±0.28^{d}	9.81±0.45^{f}	10.69±0.14^{g}	5.99±0.18^{a}	7.13±0.33^{b}	7.95±0.24^{cd}	9.42±0.60^{e}
Ala	15.30±0.75^{cd}	15.95±2.36^{d}	17.83±0.93^{ef}	18.31±2.15^{e}	14.35±0.92^{bc}	14.26±0.97^{bc}	15.86±1.00^{d}	16.79±0.25^{de}	10.53±0.23^{a}	13.13±0.53^{b}	13.89±0.41^{bc}	15.84±1.26^{d}
Tyr	3.08±0.15^{a}	3.89±0.10^{c}	4.86±0.16^{fg}	5.03±0.21^{g}	3.71±0.09^{c}	3.88±0.09^{c}	4.53±0.22^{e}	4.98±0.07^{g}	3.39±0.16^{b}	4.28±0.19^{d}	4.71±0.22^{ef}	5.31±0.26^{h}
Cys	0.60±0.29^{c}	0.33±0.03^{b}	0.59±0.04^{c}	0.56±0.09^{c}	0.24±0.00^{ab}	0.23±0.02^{ab}	0.34±0.15^{b}	0.19±0.01^{a}	0.27±0.02^{ab}	0.26±0.03^{ab}	0.73±0.08^{d}	0.73±0.06^{d}
Val	3.46±0.16^{a}	4.64±0.09^{bc}	5.74±0.12^{e}	5.71±0.26^{de}	4.38±0.11^{b}	4.58±0.12^{bc}	5.45±0.31^{d}	6.23±0.11^{f}	4.82±0.28^{c}	5.98±0.38^{ef}	6.21±0.24^{f}	7.31±0.35^{g}
Met	1.72±0.05^{a}	2.35±0.08^{c}	3.07±0.06^{f}	3.18±0.16^{fg}	2.29±0.07^{c}	2.39±0.06^{c}	2.83±0.15^{e}	3.12±0.06^{f}	2.03±0.08^{b}	2.55±0.12^{d}	2.63±0.11^{d}	3.27±0.17^{g}
Trp	0.44±0.03^{a}	0.95±0.12 ^{b}	1.51±0.13^{f}	1.53±0.15^{f}	1.21±0.04^{c}	1.35±0.07^{de}	1.49±0.03^{f}	1.45±0.02^{ef}	0.87±0.05^{b}	1.17±0.10^{c}	1.25±0.14^{cd}	1.52±0.08^{f}
Phe	2.43±0.12^{a}	2.85±0.21^{b}	3.64±0.23^{d}	4.11±0.25^{fg}	3.12±0.07^{c}	3.32±0.07^{c}	3.93±0.20^{ef}	4.30±0.10^{g}	3.13±0.06^{c}	3.90±0.22^{e}	4.21±0.14^{g}	4.94±0.18^{h}
Ile	2.36±0.10^{a}	2.99±0.21^{b}	3.81±0.09^{d}	3.64±0.26^{cd}	3.08±0.07^{b}	3.17±0.07^{b}	3.76±0.19^{cd}	4.10±0.10^{e}	3.00±0.09^{b}	3.58±0.22^{c}	3.76±0.12^{cd}	4.61±0.23^{f}
Leu	4.01±0.20^{a}	4.94±0.17^{b}	5.78±0.22^{c}	6.06±0.33^{cd}	5.02±0.13^{b}	5.18±0.07^{b}	6.27±0.26^{d}	6.90±0.06^{e}	4.90±0.14^{b}	5.85±0.30^{c}	6.15±0.22^{d}	7.55±0.45^{f}
Lys	6.16±0.39^{a}	7.40±0.43^{b}	9.48±0.25^{f}	8.80±0.35^{de}	6.78±0.23^{b}	7.01±0.34^{b}	8.39±0.42^{cd}	9.20±0.33^{ef}	7.12±0.41^{b}	8.07±0.74^{c}	8.58±0.56^{cd}	11.21±1.05^{g}
Total free AAs	97.82±4.52^{a}	114.64±4.32^{bc}	132.72±5.24^{e}	135.46±6.68^{e}	113.01±4.19^{b}	116.78±4.01^{bc}	131.03±6.59^{e}	143.14±3.30^{f}	101.87±4.25^{a}	122.26±6.41^{cd}	124.53±4.69^{d}	145.99±9.20^{f}
EAA	28.01±1.21^{a}	35.04±1.32^{b}	43.25±1.36^{d}	43.13±2.03^{d}	34.36±0.92^{b}	35.96±1.08^{b}	42.64±1.97^{d}	46.79±0.96^{e}	33.82±1.22^{b}	40.20±2.57^{c}	43.49±1.72^{d}	52.00±2.96^{f}
NEAA	69.81±3.33^{a}	79.60±3.01^{b}	89.47±3.92^{c}	92.33±4.65^{cd}	78.65±3.28^{b}	80.82±2.96^{b}	88.39±4.62^{c}	96.35±2.35^{d}	68.05±3.04^{a}	82.06±3.84^{b}	81.04±2.98^{b}	93.99±6.26^{cd}

All results were expressed as mean±standard deviation (n=3). The significant differences were found by a one-way ANOVA followed by *post hoc* multiple comparison test (Duncan's test). P<0.05 was considered statistically significant. Values with different alphabet in the same row are significantly different. AA: Amino acid, EAAs: Essential amino acids, NEAAs: Non-essential amino acids, Asp: Aspartate, Glu: Glutamate, Asn: Asparagine, Ser: Serine, Gln: Glutamine, His: Histidine, Gly: Glycine, Thr: Threonine, Arg: Arginine, Ala: Alanine, Tyr: Tyrosine, Cys: Cysteine, Val: Valine, Met: Methionine, Trp: Tryptophane, Phe: Phenylalanine, Ile: Isoleucine, Leu: Leucine, Lys: Lysine

180 min) and at higher temperature (8.71 ± 0.20–6.18 ± 0.33 mg/100 ml, 11.67 ± 0.60–5.16 ± 0.63 mg/100 ml, and 10.53 ± 1.06–2.84 ± 0.19 mg/100 ml for boiling at 85, 90, and 95°C, respectively). Gln in the stock boiled at 95°C for 180 min was most significantly decreased [Figure 1b]. This could be explained by degradation of Gln at temperatures higher than 70°C and being transformed to 2-pyrrolidone-5-carboxylic acid.[8] At boiling temperatures of both 85°C and 90°C, Glu, Ala, and Gln were the most abundant free AAs in the stock when boiled for 30 min. However, when boiling for 180 min, Glu, Ala, and Arg became predominant AAs. At 95 °C, 30 min, Glu, Ala, and Gln were also the major free AAs in the stock. This was changed at 180 min boiling, where Glu, Ala, Lys, and Ser became predominant free AAs instead [Table 1]. Similar to the other two temperatures, Gln level was high when boiled for 30 min, but dramatically decreased after 60 min boiling.

The most abundant free AAs in all chicken bone stocks in this study were Glu and Ala, whereas Cys and Trp were the least abundant as shown in Figure 3 and Table 1. This is due to their low content in chicken which agreed with findings by Ninomiya *et al*. (2010).[8] However, Dunkel *et al.* (2009) found that the major and minor AAs in chicken soup stock prepared from chicken meat and bone were Glu, Lys, Cys, and Trp, respectively,[22] but Pérez-Palacios *et al.* (2017) reported that Ala, Gly, and Asp were the predominant ones, and Cys and

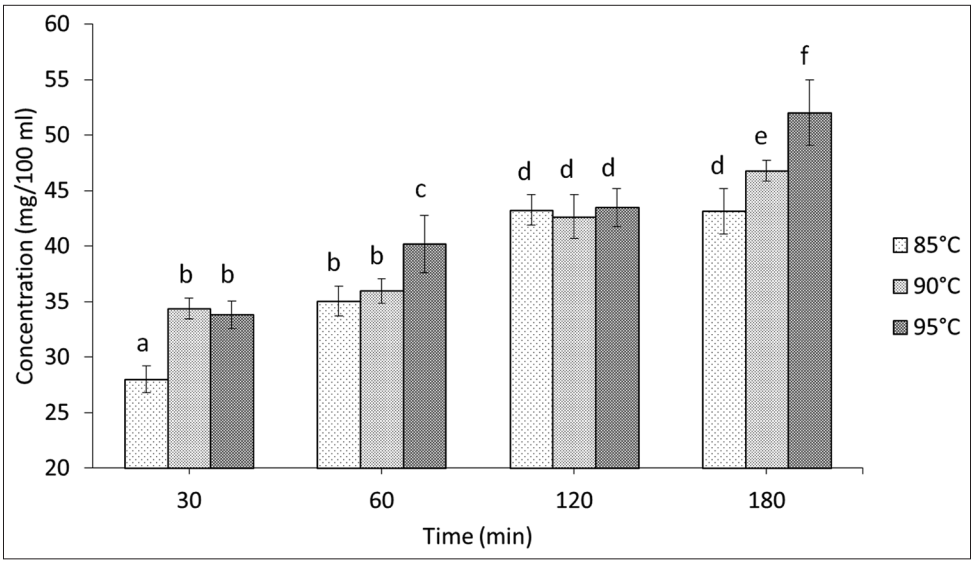

Figure 2: Concentration of total essential amino acid in chicken bone soup stock boiled at different temperatures. All results were expressed as mean ± standard deviation (n = 3). The significant differences were found by a one-way ANOVA followed by *post hoc* multiple comparison test (Duncan's test). $P < 0.05$ was considered statistically significant. Values with different alphabet are significantly different

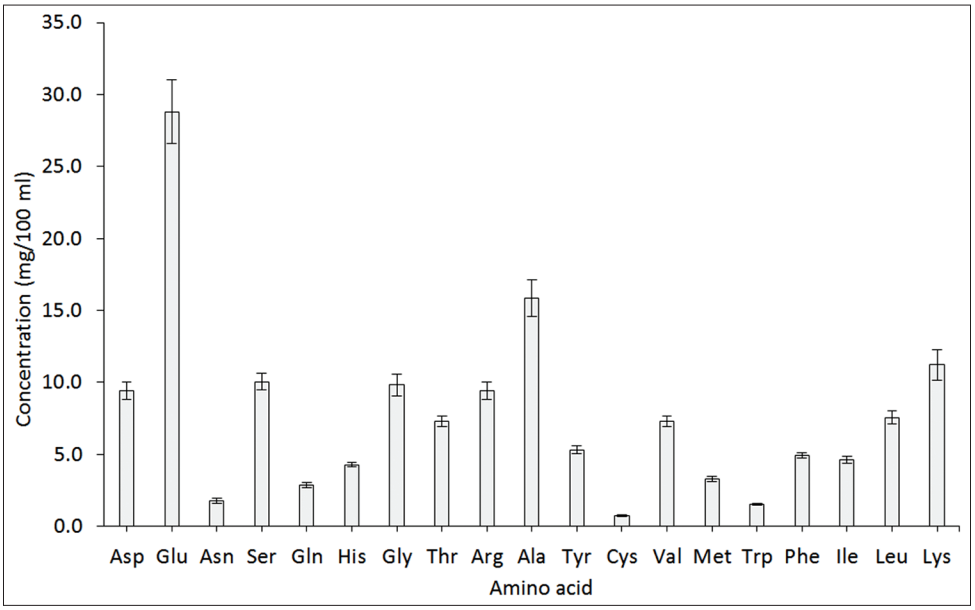

Figure 3: Free amino acids profile in chicken bone soup stock boiled at 95°C for 180 min. All results were expressed as mean ± standard deviation (n = 3)

Met were the minor free AAs in the soup stock prepared from chicken thigh, chicken carcass, onion, garlic, and clove.[15] The differences of major and minor AAs could be due to types of ingredient used such as meat, bone, and various vegetables in each stock recipe as different raw ingredients have different free AA profiles.[14]

Each AA has a different taste threshold.[22,23] Glu concentration was significantly higher in the soup stock boiled at 90 and 95°C than its threshold (16.7 mg/100 ml). This result agreed with Dunkel *et al.* (2009) who showed that among free AAs in chicken soup stocks, only Glu was higher than its threshold.[22] It has been reported that chicken soup stock also contained 5'-ribonucleotides which has a synergistic effect with Glu to enhance umami intensity.[24] Therefore, Glu in chicken bone soup stock in this study could be a key taste active substance and could have the synergistic effect with 5'-ribonucleotides usually found in chicken[14] which underlined the taste qualities, especially the umami taste of the soup stocks.

Sensory Evaluation of Chicken Bone Soup Stocks

The results on the sensory evaluation of the chicken bone soup stocks are shown in Figure 4a. Increasing the boiling temperature had no effect on sensory attributes, whereas longer boiling time could improve the sensory attributes. The chicken bone soup stocks boiled for 120 min and 180 min obtained significantly higher scores for taste, color, thickness, and overall liking than those boiled for 30 and 60 min. This result corresponded to an increase of free Glu concentration that was significantly higher with longer boiling time as shown in Figure 4b. This finding explains that an increase

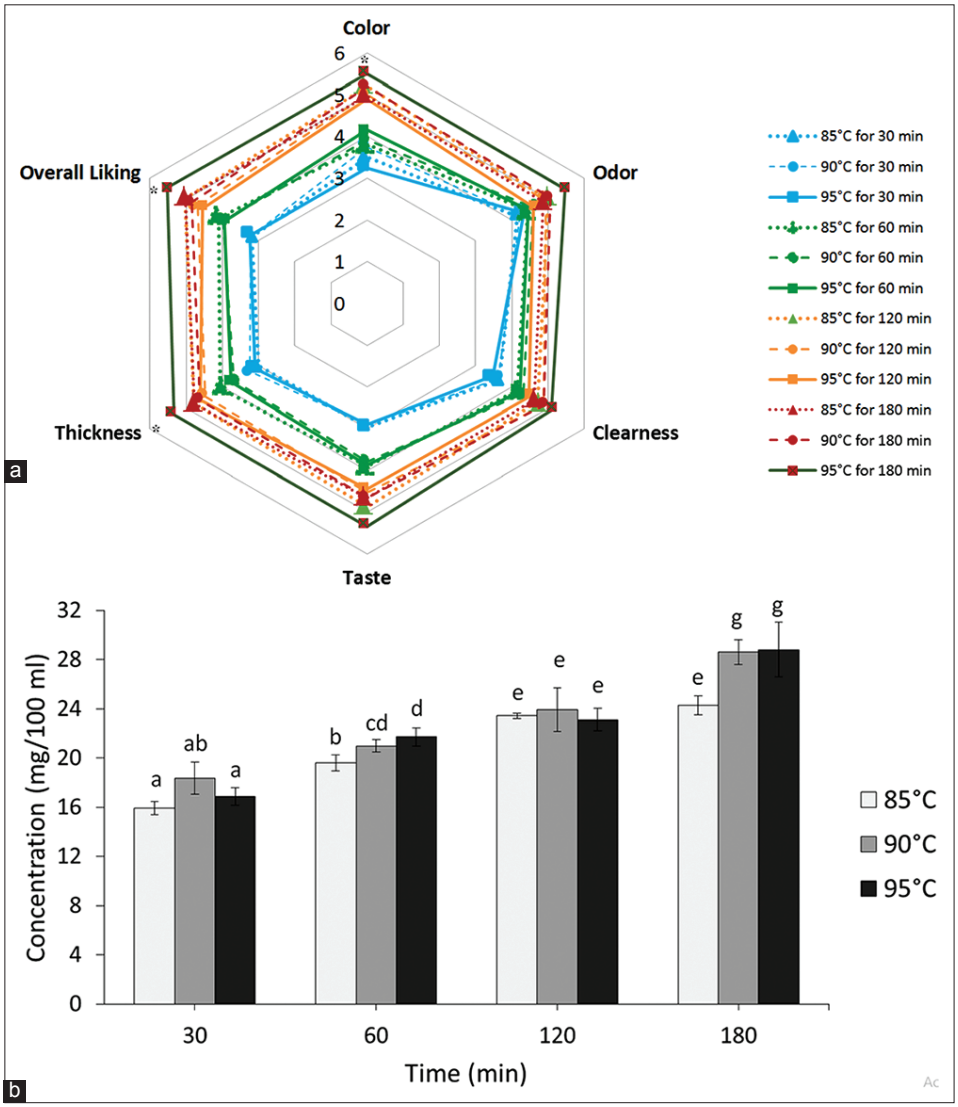

Figure 4: (a) Sensory evaluation of chicken bone soup stock boiled at 85, 90, and 95°C for 30, 60, 120, and 180 min by hedonic test (7 scales). All results were expressed as mean ± standard deviation (n = 32). The significant differences were found by a one-way ANOVA followed by *post hoc* multiple comparison test (Duncan's test). $P < 0.05$ was considered statistically significant. Asterisk indicates significant differences between 30–60 min and 120–180 min. (b) Concentration of glutamate in chicken bone soup stock boiled at different temperatures. All results were expressed as mean ± standard deviation (n = 3). The significant differences were found by a one-way ANOVA followed by post hoc multiple comparison test (Duncan's test). $P < 0.05$ was considered statistically significant. Values with different alphabet are significantly different

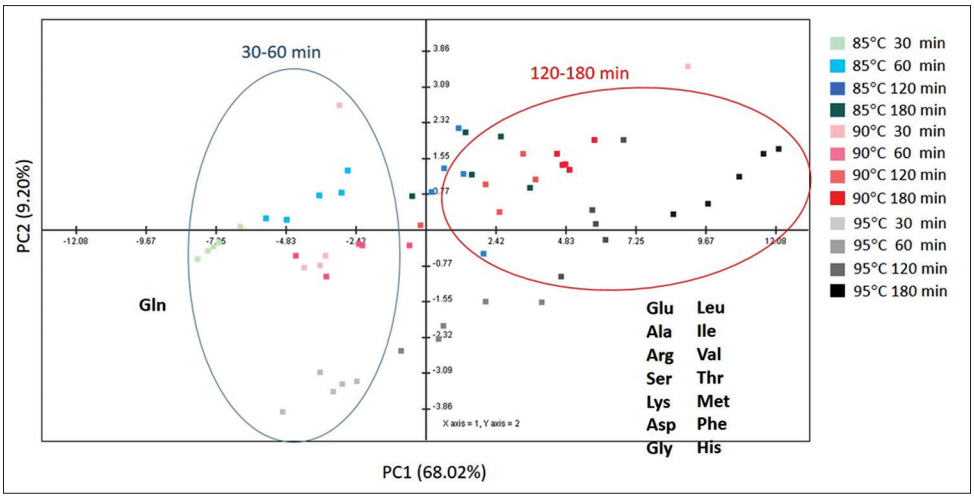

Figure 5: Score plot of principal component analysis for overall comparison of chicken soups cooked at different temperature 85, 90, and 95°C and time (30, 60, 120, and 180 min)

of free Glu, a key taste active substance, plays an important role in improving sensory attributes of the chicken bone stock. Moreover, it was reported that the Maillard reaction product, (S)-alapyridaine from hexoses and Ala, in beef broth cooked at 95°C for 180 min, significantly enhanced sweet and umami taste which affected the overall taste quality in beef broth.[25] Furthermore, Ogasawara *et al.* (2006) also showed that the Maillard peptide products, prepared from peptides of 1–5 kDa and xylose by boiling at 95°C for 3.5 h, could significantly improve mouthfulness and continuity of chicken consumed. [26] The Maillard reaction products of 180 min - long cooking might be one of the explanations of why increasing boiling time could improve the sensory attributes in the soup stocks. Further study would be required to investigate changes of Maillard reaction products in a time-dependent manner, which is involved in taste quality as well as palatability in Thai chicken bone stock production.

PCA of AAs Profile in Chicken Bone Soup Stocks

PCA was performed on AA profile using two first principle components (PC1 and PC2) which accounted for 68.02% and 9.20% of the total variances, respectively, as shown in Figure 5. The projection of the samples onto the PC space showed that the stocks boiled for 30–60 min at all three temperatures were on the left, this area defined by Gln which is one of the predominant AAs at the beginning of boiling. The stocks boiled for longer (120–180 min) were on the right side, defined by Glu, Ala, Arg, Ser, Lys, Asp, Gly, Leu, Ile, Val, Thr, Met, and Phe. Some of these were the predominant AAs which played the role of key taste-active components (Glu) and some of them were EAAs. The result demonstrated the difference in taste quality and nutritional value of the soup stock between the shorter and the longer boiling times.

CONCLUSION

This study revealed that the concentration of total free AAs and EAAs in chicken bone stock gradually rose with an increase of boiling time and temperature. The general practice of making the chicken bone stock for Thai cuisine by gentle boiling (85–95°C) for 1–3 h provided the same AA profile, of which Glu and Ala were the predominant free AAs. Boiling the stock at 90°C or 95°C for 180 min gave the highest concentration of total AA (143.14 ± 3.30 and 145.99 ± 9.20 mg/100 ml, respectively), significant higher than boiling at 85°C for 180 min. Stock boiled at 90°C or 95°C for 180 min also had the significantly highest concentration of Glu (28.61 ± 1.0 and 28.80 ± 2.21 mg/100 ml, respectively). The concentration of EAA (52.00 ± 2.96 mg/100 ml) was the significantly highest only in the stock cooked at 95°C for 180 min. Sensory evaluation demonstrated that sensory attributes improved with boiling time but not with an increased temperature. Stock boiled for 120 and 180 min obtained significantly higher scores for taste, color, thickness, and overall liking than those boiled for 30 and 60 min. This result corresponded to the significantly higher Glu concentration in the stocks boiled for 120 and 180 min. Therefore, Glu is a key taste active substance for umami taste and the palatability of the chicken bone stock. From this study, it can be concluded that gentle boiling at 90–95°C for 180 min was the best condition to obtain good chicken bone stock quality, for both taste and nutritional values and especially EAA content.

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