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Low Molecular Weight Compounds Responsible for Savory Taste of Indonesian Soy Sauce

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Indonesian soy sauce is made using only soybeans as the nitrogenous source. Moromi obtained from fermentation of yellow soybeans using *Aspergillus sojae* as the starter was investigated. The fraction with molecular weights of less than 500 Da obtained by stepwise ultrafiltration was then fractionated by several chromatographic procedures, including gel filtration chromatography and RP-HPLC. Several chemical analyses, CE profiles, and taste profiles were performed to obtain the most intense umami fraction. The main components eliciting or enhancing the umami taste present in the fraction were purified and identified by protein sequencing, ESI-MS, and ^{1}H NMR at 400 MHz. Besides free L-glutamic acid and aspartic acid, free aromatic amino acids such as L-phenylalanine and L-tyrosine may also play an important role in impressing savory or umami taste of Indonesian soy sauce at their subthreshold concentrations and in the presence of salt and free acidic amino acids. This is reported as a new phenomenon of the so-called bitter amino acids.

KEYWORDS: Soy sauce; moromi; ultrafiltration; gel filtration chromatography; reversed-phase HPLC; capillary zone electrophoresis; umami taste; savory taste

INTRODUCTION

Indonesian soy sauce is a Chinese type of soy sauce, produced using soybeans as the only ingredient and a two-step fermentation; the first step is mold fermentation, and the second step is brine fermentation at a high concentration of salt (*1*−*4*). Compounds responsible for the umami or savory taste of the soy sauce have not been studied extensively yet. However, Apriyantono et al. (*5*) investigated the sensory characteristics of its ultrafiltration fractions. They reported that fractions with a molecular weight of less than 500 Da had the most intense umami taste.

Compounds that can elicit the umami or savory taste belong to a broad class and were found to have molecular weights less than 1000 Da. Besides monosodium glutamate (MSG), a well-known umami taste-eliciting compound, an octapeptide, Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala, isolated from beef extract treated with papain (*6, 7*), *N*-(1-methyl-4-hydroxy-3-imidazolin-2,2-ylidene)alanine in cooked beef (*8*), and glycoconjugates of glutamate from wheat gluten hydrolysate (*9, 10*) were reported to have an umami, savory, or brothy taste.

The major components of soy sauce that directly impart the savory taste of this product, especially in Indonesian soy sauce, have not yet been isolated and identified. Therefore, several chromatographic procedures, as well as capillary zone electrophoresis (CE) profiles and taste profiles, were conducted to identify the major components that contribute to the intense umami taste of Indonesian soy sauce.

MATERIALS AND METHODS

Preparation of Moromi. Yellow soybeans from a local market in Bogor (Indonesia) were boiled in water (1/3, w/v) for 1 h, drained, and cooled. The cooked soybeans were inoculated with 0.5% (w/w) *Aspergillus sojae* starter (obtained from Konno Moyashi Co., Kobe/Osaka, Japan). After incubation for 3 days at room temperature, the koji produced was soaked in 20% NaCl solution (1/3, w/v) for 8 weeks at the same temperature to let brine fermentation occur naturally. The resulting product is called moromi. The moromi was then homogenized and filtered through cheesecloth as well as pressed by hand. To remove the fine precipitate and fat, the suspension was centrifuged at 14000 rpm for 10 min at 4 °C. The transparent-light brown supernatant was collected.

Ultrafiltration. The supernatant was submitted to an ultrafiltration cell using an Amicon model 8050 ultrafiltration unit (Amicon Inc., Beverly, MA) at 2−4 °C under 2−3 bar N_2 pressure. Stepwise ultrafiltration was carried out to obtain fractions with molecular weights of less than 500 Da (**F-500**) using a 0.45 μm membrane, followed by YM10 (MWCO 10 000), YM3 (MWCO 3 000), and YC05 (MWCO 500) membranes (Amicon Inc.). Fraction **F-500** was collected, lyophilized, and stored in the freezer until further use.

Gel Filtration Chromatography. One gram of the lyophilized **F-500** was dissolved in 5.0 mL of deionized water and then chromatographed

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Table 1. Standard Solutions Used for Taste Intensity Analysis

taste attribute	standard	score	% (w/v)
umami	MSG	50	0.05
		80	0.10
		150	0.20
		200	0.25
salty	NaCl	50	0.25
		80	0.50
		150	1.25

on a Sephadex G-25 SF column (2.5 × 57 cm) with deionized water as the eluant. Sixty tubes of 7.5 mL each of the eluate were collected using a TOYO SF-100 fraction collector (Toyo Kagaku Sangyo Co., Japan). The UV absorbance of each tube was measured at 214 and 280 nm using a Shimadzu UV-160 spectrophotometer (Shimadzu Corp., Japan). Since the other aim of this fractionation was to desalinate **F-500**, its NaCl content was also analyzed by the Mohr method (*11*). The absorbance and salt content were plotted against the tube number. The collected fractions were lyophilized. Analysis of soluble peptides by the Lowry method (*12*), amino acid composition determined after acid hydrolysis (*13*), CE profile using capillary zone electrophoresis coupled with a photodiode array detector (see below), taste profile (see below), and free L-glutamic acid content (*14*) were performed for each fraction. These analyses were done in duplicate, except for the CE profile and taste profile.

The most intense umami fraction was then chromatographed further by using Sephadex G-10 (1.2 × 82.5 cm). The lyophilized fraction was dissolved in 2.5 mL of deionized water and then eluted with deionized water through the column. Sixty tubes of 2.5 mL each eluate were collected. The same plots or analyses as above were conducted for each tube or subfraction collected.

CE Profile. The CE profile of each fraction was performed by capillary zone electrophoresis (CZE) using a Photal CAPI-3300 System (Otsuka Electronics Co., Japan) on an uncoated fused silica column (i.d. 50 μm, l 50 cm, l_{eff} 37.8 cm) with conditions as follows: voltage, 15 kV; injection volume, 15 nL; running time, 25 min; temperature, 25 °C; buffer, phosphate 50 mM pH 2.5; detection at 190−400 nm (photodiode array detector/PDAD). Sample solutions were filtered through a 0.45 μm membrane prior to analysis.

Taste Profile. Taste profiles were obtained by determining scores for both umami and salty tastes (*5*). A series of standards, with a pH range of 5.7−7.1, for judging taste intensity was used (**Table 1**). Deionized water was used to prepare each sample and standard solution tasted and also for oral rinsing. Fourteen trained panelists evaluated the taste of **F-500** and its fractions yielded in three sessions. In this evaluation, each lyophilized fraction other than **F-500** and **F2** was dissolved in deionized water so that the concentration of soluble peptides present in each fraction was 10.0 mg/10 mL, about 10 times the monosodium glutamate (MSG) threshold value (*15*). Salt was present in the solutions at their original concentrations. In the case of high salt contents, **F-500** was tasted at the concentration of soluble peptides of 5.0 mg/10 mL and **F2** tasted at that of 1.0 mg/10 mL. Both solutions contained sodium salt of 82.0 mg/10 mL and 76.0 mg/10 mL, respectively. To verify taste intensities of the fractions of **F-500**, the same sample solutions adjusted to the same salt concentration (76.0 mg/10 mL) were also evaluated. In the other taste evaluation, eight trained panelists performed taste intensity analyses of subfractions at a concentration level of 3.0 mg/10 mL of soluble peptides. All sample solutions tasted were in the pH range of 5.2−6.2. Their pH values were measured without any pH adjustment. The pH range of 5−7 is known to give an optimal umami taste intensity of MSG, as a basic umami tastant (*16*).

Separation and Purification by Reversed-Phase HPLC. The lyophilized subfractions yielded above were separated further by a reversed-phase HPLC (RP-HPLC) method using an LC-9A Shimadzu liquid chromatograph (Shimadzu Corp., Japan). This HPLC was equipped with a Cosmosil 5C18-AR-300 semipreparative column (5 μm particle size, 300 Å pore size, 15 cm × 4.6 mm i.d.) (Nacalai Tesque Inc., Japan). These fractions were filtered through 0.45 μm filters prior to injection. A linear gradient of acetonitrile (HPLC grade, Cica, Kanto Chemical Co., Japan) containing 0.05% trifluoroacetic acid (TFA) (Wako, Wako Pure Chemical Industries Ltd., Japan) from 0% to 50% for 60 min in Milli Q water (Milli-Q SP Reagent water system, Millipore Corp., Bedford, MA) containing 0.05% TFA was applied at a flow rate of 0.5 mL/min to each purification. UV absorbance at 214 nm was monitored to perform each separation profile. The major peaks were then purified by RP-HPLC using the same procedures, and the purity was checked by CZE under the same conditions as above.

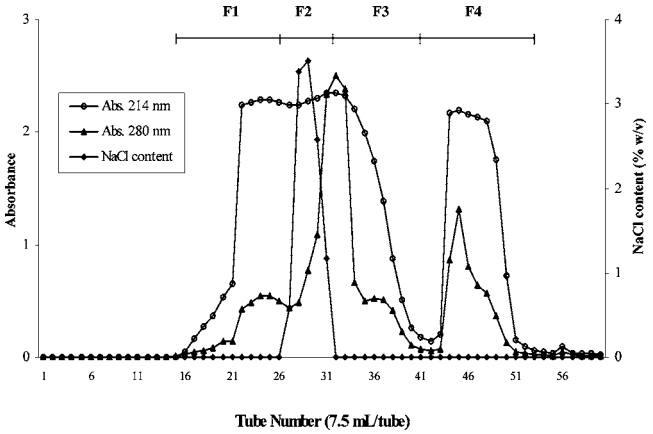

Figure 1. Gel filtration chromatogram of **F-500** (MW less than 500 Da) obtained from stepwise ultrafiltration of soy sauce moromi. Fractionation was performed on a Sephadex G-25 SF column (2.5 × 57 cm) using deionized water as the eluant.

Identification of Major Components. Identification of the compounds was carried out by automated Edman degradation using an Applied Biosystems model 473A sequencer (Applied Biosystems Japan Ltd., Japan). Molecular weight was determined on an Applied Biosystems API 2000 mass spectrometer with an electrospray ion source. Mass spectra were performed in the mass range m/z 80−500 in positive-ion mode. NMR spectroscopy was also performed to confirm the structure of the major compound. ^{1}H NMR spectra were recorded on a JEOL JNM-LA400 spectrometer (JEOL Ltd., Japan) at 400 MHz. The dried sample (1 mg) was dissolved in D_2O and applied at room temperature. Tetramethylsilane was used as the internal standard.

RESULTS AND DISCUSSION

Chromatographic Separation of F-500. The separation profile of the moromi fraction with a molecular weight of less than 500 Da (**F-500**) based on 1 g of lyophilized fraction (equal to about 5 mL of the liquid) is shown in **Figure 1**. Fraction **F-500** can be separated further into four fractions, **F1**−**F4**, by gel filtration chromatography using a Sephadex G-25 SF column. Because the aim of the fractionation was not only to separate the fractions but also to desalinate them, and in fact, salt was effectively separated into one peak, the eluates containing salt were collected as one fraction, i.e., **F2**. Thus, the peak eluted before the salt peak was collected as **F1**, whereas the other peaks were collected as **F3** or **F4**. In this case, the third peak was collected together with the second peak in **F3** because its yield was relatively low. As a result, **F2** and **F3** have relatively high absorption at 280 nm, which corresponds to an aromatic group (*17*). It is known that separation on Sephadex G-25 is based not only on molecular size, but also on interaction between aromatic groups of the components and the matrix gel (*18*).

The quantitative data of the fractionation results are described in **Table 2**. The results showed that the nonsalt dry matter of **F-500** was present mainly in **F1** and **F2**. Free L-glutamic acid was also found prominently in both fractions. Its concentration was relatively high in **F1** as compared to **F2**. This amino acid

Table 2. Chromatography of 1 g of Lyophilized F-500 Using Sephadex G-25 SF

	absolute amount, mg			
fraction	dry matter	NaCl content	soluble peptides	free L-glutamic acid
F1	94	0	9.9	10.09
F2	773	675	8.9	7.03
F3	18	0	6.2	0.01
F4	15	0	3.2	0.01

Table 3. Amino Acid Composition of 1 g of Lyophilized F-500 and Its Four Fractions

	absolute amount, mg				
amino acid	F-500	F1	F2	F3	F4
aspartic acid	18.4	6.8	6.6	0.1	0.2
glutamic acid	35.3	14.6	10.4	0.1	0.2
serine	5.4	1.1	2.6	tr[a]	0.1
histidine	3.6	0.7	1.5	tr	0.1
glycine	6.2	1.2	2.7	0.1	0.2
threonine	5.9	1.3	2.6	0.1	0.1
arginine	1.0	0.5	0.4	tr	0.1
alanine	7.9	1.7	3.5	0.1	0.2
tyrosine	1.9	0.1	0.3	0.4	0.1
methionine	1.8	0.1	1.2	tr	nd[b]
valine	9.3	2.6	3.7	0.1	0.2
phenylalanine	7.7	0.2	3.9	0.4	0.1
isoleucine	7.5	1.9	3.7	0.1	0.1
leucine	10.8	2.0	6.3	0.1	0.1
lysine	11.0	5.3	2.1	0.1	0.1

[a] tr = trace (less than 0.05 mg). [b] nd = not detected.

is well known for exhibiting a sour taste on its own or an umami taste as the sodium salt (*16*, *19*−*23*). On the basis of these results, it can be expected that the tastes of both fractions affected the taste of **F-500**.

The amino acid composition analysis also indicated that glutamic acid was the major amino acid of **F-500** (**Table 3**). After fractionation it was found to be the major one in **F1** and **F2**. According to the separation results above and considering the analysis results in the table, **F1** contained much higher acidic and basic amino acids than hydrophobic amino acids. On the other hand, **F2** contained acidic amino acids and hydrophobic amino acids in equal amounts, and **F3** contained much higher concentrations of aromatic amino acids than the other amino acids. Aromatic amino acids and most hydrophobic amino acids have an intense bitter taste (*19*, *21*). However, Salles et al. (*22*) found that hydrophobic amino acids could also exhibit an umami taste in the presence of salt. Thus, it is more likely that **F1** and **F2** are the umami or savory taste fractions of **F-500**.

The CE profile of **F-500** showed two main peaks, peaks 1 and 2, which correspond to at least two different major compounds contained in this fraction, as indicated by the different spectra (**Figure 2**). After fractionation, peak 1, which was eluted earlier, was also found in **F4** whereas peak 2 was found in **F2** and **F3**. No main peak could be detected in **F1**. It is obvious that **F1** and **F2** as the major fractions of **F-500** had different CE profiles. They, therefore, were expected to consist of different major components. Considering the spectra, both peaks 1 and 2 have maximum absorption at 200−215 and 240−280 nm, indicating aromatic compounds (*17*, *24*). In this case, the aromatic group present in peak 2 has a weak absorption.

Taste intensity analysis of **F-500** revealed that this ultrafiltration fraction had an intense umami taste rated with a score of 98 ± 9 and salty taste (rated score 103 ± 7) at a peptide

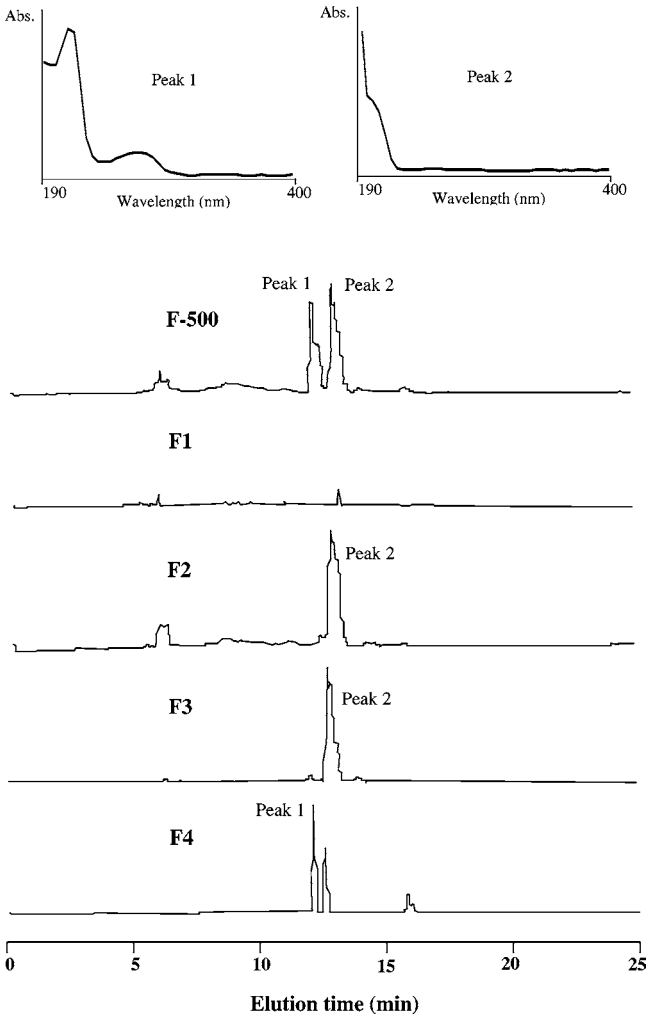

Figure 2. CZE electropherograms of **F-500** and its four fractions (**F1**–**F4**), detection at 214 nm. The two major peaks of **F-500** are named Peaks 1 and 2. (Inset) Spectra of the major peaks obtained from PDAD. CZE was performed on an uncoated fused silica column, l_{eff} 37.8 cm, using 50 mM phosphate buffer at pH 2.5 as an eluting buffer.

concentration of 5.0 mg/10 mL and NaCl concentration of 82.0 mg/10 mL. The results of its four fractions are shown in **Figure 3A**. This analysis was done at the original salt concentration but at the same soluble peptide concentration, i.e., 10.0 mg/10 mL, except for **F2**, which was 10 times more diluted than the others because of its high salt concentration. **Figure 3A** shows that **F1** had the highest score for umami taste, whereas **F2**, as predicted, had the highest score for salty taste. Although **F2** had been diluted 10 times compared to the other fractions, **F2** had a considerably intense umami taste, and its intensity may increase at the same soluble peptide concentration. These results were verified by evaluation at identical salt concentrations as presented in **Figure 3B**, because of the possible synergistic effect between salt and umami compounds. The same result was obtained, **F2** at a peptide level 10 times lower than the other fractions still had a relatively intense umami taste compare to the other fractions. This indicated that the major components of **F2** were the dominant contributors to the umami or savory taste of **F-500**. **F2** was then subjected to further analysis. Of these results, it is noted that after salt adjustment **F3** also had a considerably intense umami taste as compared to **F1**, although it contained more aromatic compounds, as shown by its amino acid composition (**Table 3**). Moreover, referring to the CE

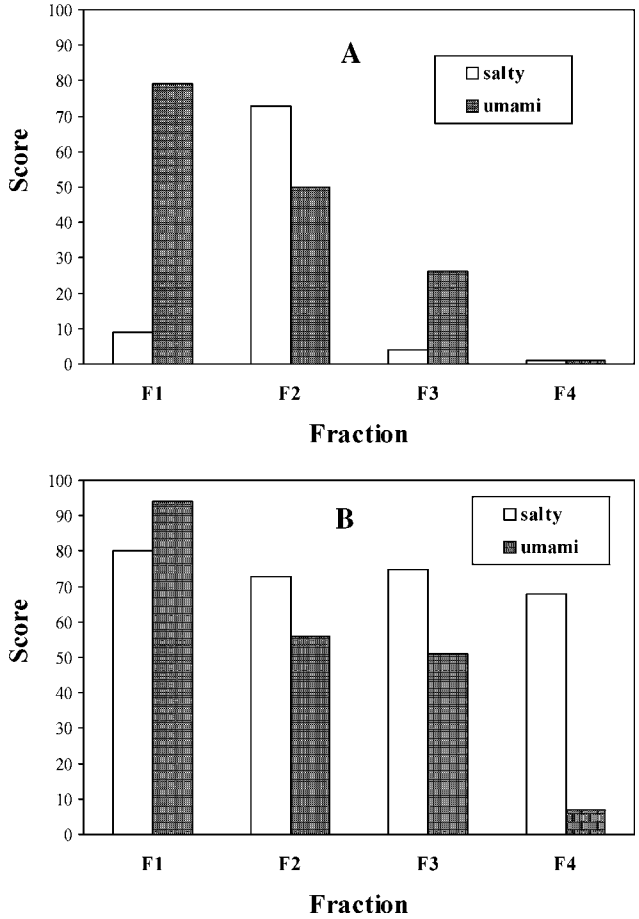

Figure 3. Umami and salty taste intensities of **F1**–**F4**. Evaluations were done (**A**) on the original salt concentration contained in each fraction and (**B**) on the same salt concentration (76.0 mg/mL). The score range was based on intensity of standard solutions presented in **Table 1**. The taste profiles were obtained from the average scores from 14 trained panelists in three sessions. Each fraction contained 10.0 mg of soluble peptides/10 mL of test solution, except **F2**, which contained 1.0 mg/10 mL.

profiles in **Figure 2**, **F2** and **F3** may have the same major components.

Isolation of Major Components. Further fractionation of **F2** by using Sephadex G-10 yielded five subfractions, **F2.I**−**F2.V**, as shown in **Figure 4 A**. High absorption at 280 nm of **F2.III** and **F2.IV** means that both were aromatic fractions. In this separation, salt was also effectively separated into one peak. Most of the salt was collected in **F2.III**. Therefore, to identify the major components in **F2.III**, rechromatography of this subfraction on a Sephadex G-10 column was done, and the result is shown in **Figure 4B**.

Table 4 shows the chemical composition of the fractionation results. Free L-glutamic acid was present only in **F2.I** and **F2.II**, and consequently these subfractions were expected to have an umami taste. On the other hand, RP-HPLC separation profiles combined with CE profiles (**Figure 5**) indicated that peak 2, the major peak of **F-500** and **F2,** was then found in **F2.III** (especially in sub-subfraction **F2.III-d**) and **F2.IV**. In this case, **F2.III-c** was predicted to have the same major peak in **F2.III-d**, but this sub-subfraction could not be separated further due to its high salt content. The overall result of HPLC separations revealed that there were actually three major components. They had retention times at ∼4 min or at the void volume of the column (found in **F2.I** and **F2.II**), ∼16 min (found in **F2.V**), and ∼21 min (found in **F2.III** and **F2.IV**).

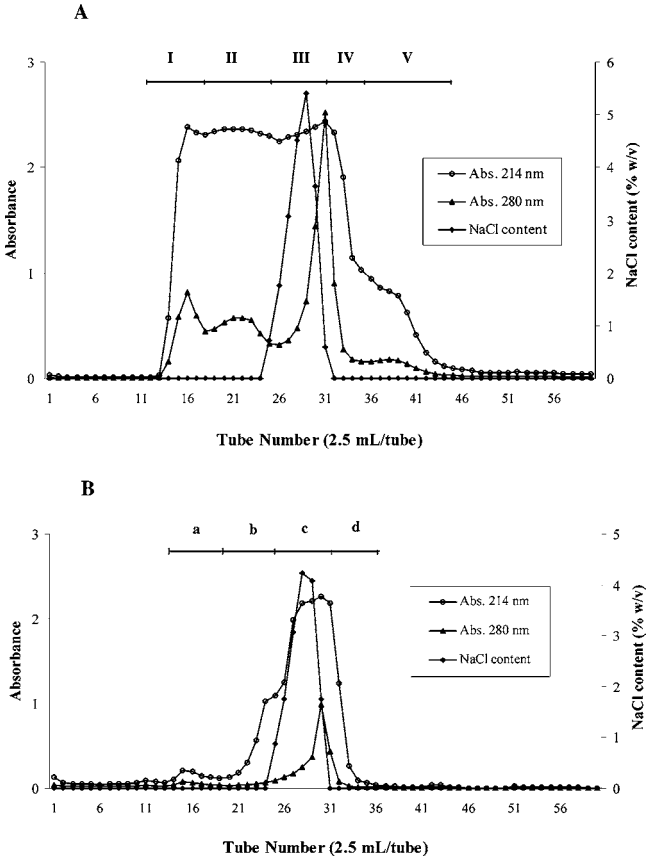

Figure 4. Gel filtration chromatograms obtained from (**A**) Sephadex G-10 chromatography (on a 1.2 × 82.5 cm column) of the most intense umami fraction, **F2**, and (**B**) rechromatography of the subfraction that had a high salt concentration, **F2.III**. Deionized water was used as the eluant.

Table 4. Chromatography of F2 (with amounts from the result in Table 2) and Rechromatography of Its Subfraction Using Sephadex G-10

	absolute amount, mg			
subfraction/ sub-subfraction	dry matter	NaCl content	soluble peptides	free L-glutamic acid
F2.I	8	0	1.3	0.97
F2.II	89	18	2.8	6.24
F2.III	425	394	1.3	nd
F2.IV	15	11	1.5	nd
F2.V	4	0	1.5	nd
F2.III-a	1	0	0.1	nd
F2.III-b	27	21	0.1	nd
F2.III-c	360	353	0.7	nd
F2.III-d	4	0	0.2	nd

The most abundant components are those with a retention time ∼4 min, followed by those with a retention time ∼21 min, and the least at ∼16 min.

Figure 6 shows taste profiles of subfractions of **F2** performed at the same soluble peptide concentration of 3.0 mg/10 mL, three times higher than that of **F2** in **Figure 3**. Most of the subfractions had an intense umami taste, as presented by the tastes of **F2.II**, **F2.III**, and **F2.IV**. The intense umami taste of **F2.II** can be easily explained by the high concentration of free L-glutamic acid in the presence of sodium salt (**Table 4**), which is known to result in the flavor potentiation effect (*20*, *22*). However, **F2.III** and **F2.IV**, recognized as aromatic fractions and had no free L-glutamic acid (T**able 4**), interestingly also exhibited an intense umami taste in the presence of salt. This phenomenon was also found in the other study that characterized

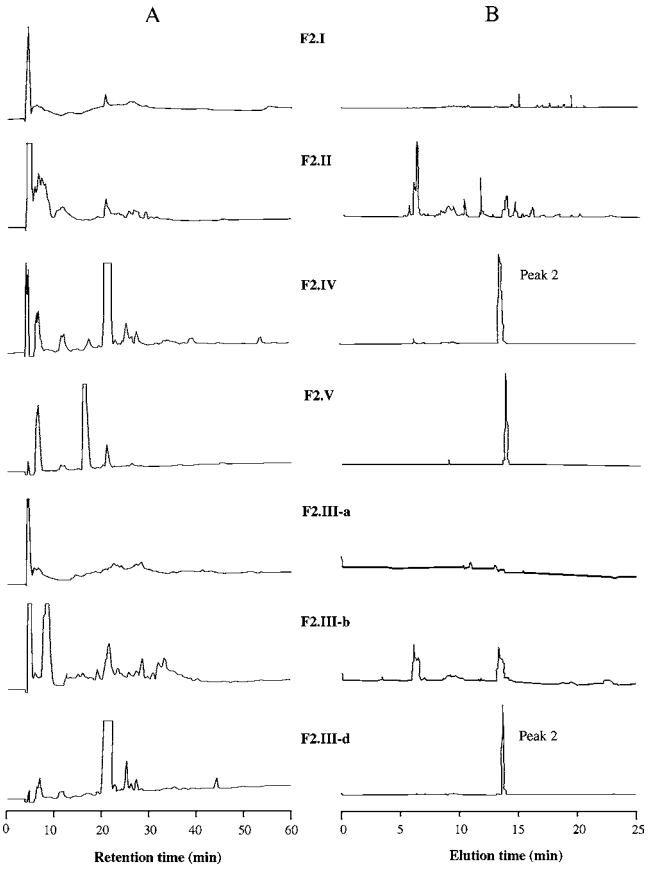

Figure 5. RP–HPLC chromatograms (**A**) and CZE electropherograms (**B**) of subfractions, **F2.I**–**V**, and sub-subfractions of **F2.III**. Peak 2 is the major peak of **F-500** shown in **Figure 2**. RP-HPLC was performed on an ODS column (15 cm × 4.6 mm i.d.) with a linear gradient of 0.05% TFA–acetonitrile (0–50% for 60 min). CZE was performed on an uncoated fused silica column, l_{eff} 37.8 cm, using 50 mM phosphate buffer at pH 2.5 as an eluting buffer.

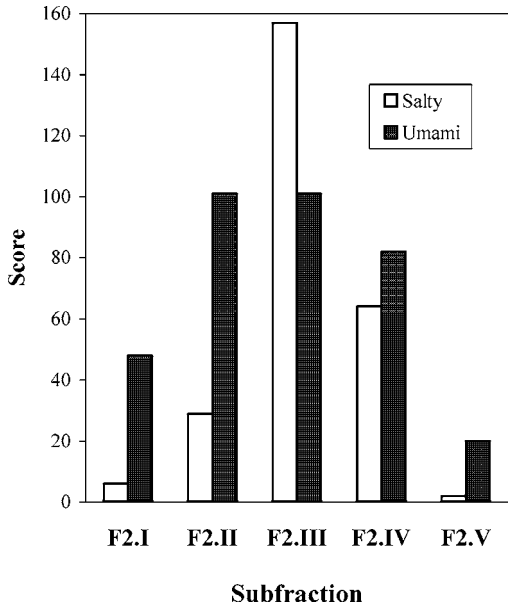

Figure 6. Umami and salty taste intensities of subfractions, **F2.I**–**V**. The score range was based on the intensity of standard solutions presented in **Table 1**. The taste profiles were obtained from the average scores from eight trained panelists. Each fraction contained 3.0 mg of soluble peptides/10 mL of test solution.

sensorially the fractions of water-soluble Comté cheese extract (*22*). This indicates that besides free L-glutamic acid, other compounds that have aromatic groups may contribute to or produce the effect of the intense umami taste of soy sauce in the presence of a high sodium salt concentration.

Identification of Major Components. Identification by protein sequencing of major components in **F2.I** and **F2.II**, which were eluted at ∼4 min or at the void volume of the reversed-phase column, revealed that they were the mixture of three major single amino acids, i.e., glutamic acid, aspartic acid, and alanine. Glutamic acid and aspartic acid are the dominant amino acids in soybeans (*25*) and commonly occur in fermented soybean products (*26*−*28*). Other savory food also contain glutamic acid as the principal free amino acid (*29*). As L-α-amino acids, glutamic acid, and aspartic acid have a sour taste, in the presence of sodium salt they can exhibit umami taste (*21*, *22*), whereas alanine has a strong sweetness (*21*). The possible interactions between alanine as a sweet stimuli and monosodium glutamate may also contribute to the intense umami taste of soy sauce (*30*).

Besides those amino acids, the major component present in **F2.III** and **F2.IV**, which eluted at ∼21 min in the reversed-phase HPLC column and indicated as peak 2 (**Figures 2** and **5**), was an interesting compound, since they were able to produce an umami taste in the presence of salt (**Figure 6**). Identification of the component by protein sequencing showed that it was single aromatic amino acid, i.e., phenylalanine. Since it was a single component and present in a relatively high amount (about 70% of the total peak area integration in both HPLC profiles of **F2.III** and **F2.IV**), determination of the molecular weight by ESI-MS and confirmation of the structure by ^{1}H NMR 400 MHz were possible. The ESI-MS spectrum showed the ion MH^+ at *m*/*z* 166 and its NMR spectra fit the database of L-phenylalanine spectra and therefore confirmed the identification. This result has also been proved using the authentic sample, pure L-phenylalanine. UV spectra of this authentic sample also confirmed with that of peak 2 (**Figure 2**). Belitz and Grosch (*24*) described that the aromatic group of L-phenylalanine has maximal absorption at 250−260 nm with a molar absorption coefficient of ca. 200 M^{-1} cm^{-1}, which is very weak to be observed. It can be inferred that free L-phenylalanine is the principal component of **F2.III** and **F2.IV.** Since it was present in the umami fractions, consequently that compound is an important component which contributes to the intense umami taste of **F2** and, certainly, **F-500** as well as free L-glutamic acid.

The other major compound found in **F2.V**, which eluted at ∼16 min in the reversed-phase HPLC column, was identified as the other single aromatic amino acid, tyrosine, based on the sequencing result and ESI-MS spectrum that showed the ion MH^+ at *m*/*z* 182. This compound was also predicted to have the same effects as phenylalanine in soy sauce, because this also occurs dominantly in **F3** (**Table 3**), which had a considerably intense umami taste (**Figure 3**) in the very low−almost not detected free L-glutamic acid content (**Table 2**). Thus far, there has been no report on the enhancing effect of aromatic amino acids on the umami taste of mixtures consisting of L-glutamic acid and NaCl. In this case, we just found reports of taste enhancement between various free amino acids and IMP (*31*) and among free amino acids, MSG and IMP (*20*). However, the hypothesis of the synergistic effects between several amino acids, including aromatic and acidic amino acids, and salts has been postulated in a similar study of the other food product (*22*).

The concentrations of free L-phenylalanine and tyrosine contained in the subfractions tasted (**Figure 6**) were roughly

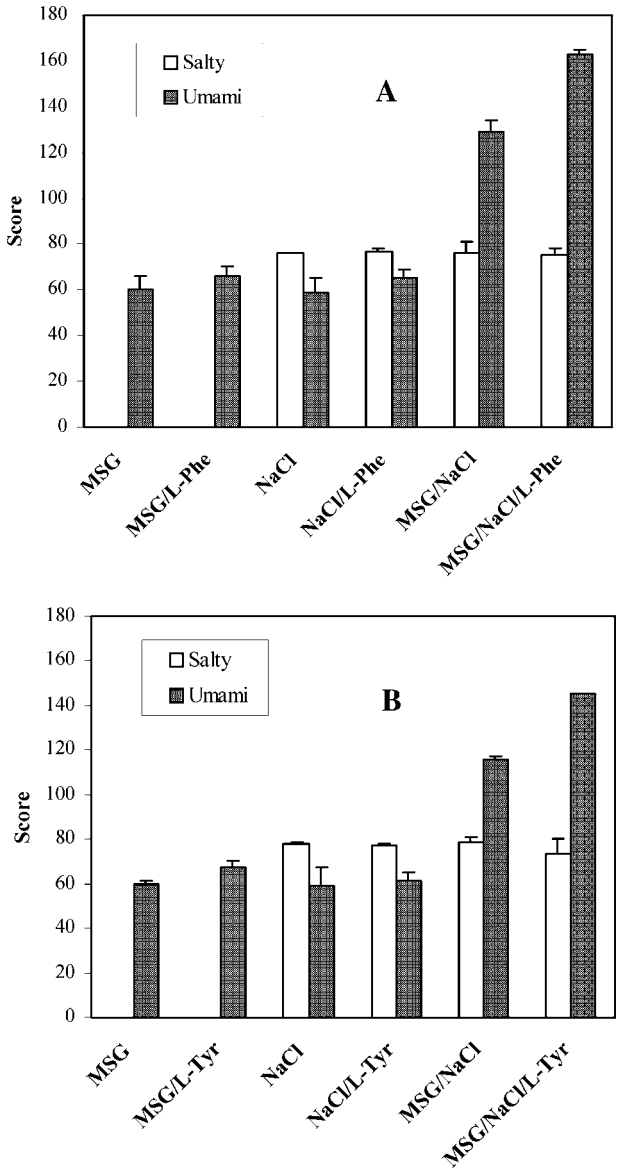

Figure 7. Effect of aromatic amino acids, (**A**) L-phenylalanine (L-Phe) and (**B**) L-tyrosine (L-Tyr), on the tastes of MSG (4.0 mM) and NaCl (80 mM), either alone or mixed, at a subthreshold concentration of 1.5 mM. Scores were geometric means of two sessions. The error bars show the standard deviation of the mean.

calculated as being as much as 1.7 and 1.0 mM, respectively. These concentrations are lower than their threshold values (*16*, *19*). In the further study, umami-enhancing effects of the subthreshold L-phenylalanine or L-tyrosine on the mixture of suprathreshold L-glutamic acid (as sodium salt form, MSG) and NaCl were studied and evaluated statistically for their significances. Nine to ten trained panelists were involved in this study. Pure chemicals (Wako, Japan) and Milli Q water were used for preparing the sample and standard solutions. All solutions tasted were in the pH range of 5.7−7.1. Data are shown in **Figure 7**. The results proved that there were significant enhancing effects of the aromatic amino acids at a certain subthreshold concentration (1.5 mM) on the umami taste intensity of the MSG/NaCl mixture. In an in-depth study, both L-phenylalanine and L-tyrosine significantly enhanced the umami taste of the mixture when they were added at subthreshold concentrations of 0.5 and 1.5 mM whereas neither the umami taste of MSG alone nor the salty taste of NaCl alone was intensified. In other words, this sensory phenomenon confirmed the results obtained in this work and was newly found. Furthermore, model solutions for **F2.I**−**F2.V** using the authentic compounds have also been evaluated sensorially and gave the same profiles as those described in **Figure 6**.

Moreover, small peptides which may be contained in soy sauce (*32*, *33*) were not detected as the major taste-active compounds in Indonesian soy sauce. Therefore, the contribution of peptides in producing the umami or savory taste of soy sauce cannot be found in our work. This confirmed the same result in a similar study of the other fermented food product (*22*) that the presence of amino acids and salts were more responsible for the sensory characteristics than the presence of peptides.

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