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Umami potential from crude extract of *Bekkai lan* (*Albertisia papuana* Becc.) leaves, an indegenous plant in East Kalimantan-Indonesia

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Abstract

Umami-taste active compounds of *Bekkai lan* leaves crude extract (*Albertisia papuana* Becc.) were analyzed and their potential were evaluated by equivalent umami concentration (EUC) method. The EUC was found to be 48.31 g MSG/100 g in the crude extract, obtained at pH 8 for 3 minutes. The umami potential of the crude extract was moderate. The yield of the umami compound depend on pH and extraction time, therefore the lower the pH, the longer the time required for the extraction of umami compound.

Introduction

Recently, umami taste has become widely accepted in Western countries, and umami is considered as the fifth basic taste sensation (Conn, 1992) along with sweet, salty, bitter and sour. Umami was first defined as the characteristic taste elicited by glutamates, and has since also been associated with monosodium glutamate (MSG), Yamaguchi (1991). Umami taste, also called the palatable taste or the perception of satisfaction, is a good taste commonly provided by an overall food flavor induced or enhanced by MSG (Yamaguchi, 1979). Umami is also provided by disodium salts of 5'-nucleotides: IMP (disodium 5'-inosine monophosphate), GMP (disodium 5'-guanosine monophosphate) and AMP (disodium 5'-adenosine monophosphate). There are synergistic effects between MSG, IMP, GMP, AMP which together in certain ratios produce a strong umami taste (Yamaguchi *et al*., 1971).

Among the Dayaks tribe in East Kalimantan, Indonesia, there are herbs that are used as food flavouring. Traditionally, bekkai lan (*Albertisia papuana* Becc.) is used for the treatment of cancer or tumor from root and bark. Extensive research have been carried out for several therapeutic effects including potential anti plasmodium (Lusiana, 2009) and antitoxin (Wet, 2005). The same plant was also used for the same purpose by the Dayak's from West Kalimantan (Purwayanti, 2009) and Central Kalimantan (Lusiana, 2009). However, the perception of the Dayaks in East Kalimantan about these leaves known as 'vetsin of Dayaks Kenyah' (Susiarti and Setyowati, 2005). In the Indonesian food culinary, vetsin means MSG, while MSG was reference of umami taste. So, we think bekkai lan leaves extract have a contribution for umami taste.

Naturally, umami compound are present in many protein rich foods, such as meat, fish and fungi, however these compound had not been properly investigated from indigenous plant like *A. papuana* Becc. There is a need for an extraction method that can produce higher yields of umami compounds from *A. papuana* Becc. Soldo *et al*. (2003) reported that pH values can greatly affects recovery of umami compounds, especially at pH 5-7. Sommer (1996) explained that glutamate as a flavor enhancher can be achieved within a pH range of 5-8 (slightly acid to neutral). Besides that, the ionic environment of taste cell affected the interaction of taste eliciting molecules and receptor cells (Spielman, 1999). Hence umami taste perceptions are pH-dependent.

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The EUC is the concentration of MSG equivalent to the umami intensity given by the mixture of MSG-like amino acids and the flavor 5'-nucleotides. Composition of umami from bekkai lan crude extract leaves had not been investigated until now, while the composition could be explained the potential of umami by the EUC.

To evaluate the umami potential of bekkai lan crude extract leaves can be calculated by EUC, therefore the objective of this study was to examine the effects of pH and extracting time on the umami potential from bekkai lan leaves.

Materials and Methods

Materials

Dried leaves and leaves powder of *Albertisia papuana* Becc. were obtained from local indigenous people of Dayak Kenyah ethnic group. The leaves were harvested in Mei 2011 from the secondary forests that surround Long Le-es village in the Busang Subdistrict, East Kutai District, East Kalimantan Province, Indonesia.

The dried leaves were made from fresh leaves dried in a room to prevent them from being exposed to direct sunlight for more than 3 months at room temperature. To obtain leave powder, fresh leaves were sliced, dried under sunlight for 2 days and ground into powder of 60 mesh in sizes. The dried leaves and powder samples were stored in the dark at low temperature (-20^{o}C) until used.

Proximate analysis

The proximate composition of bekkai lan leaves, i.e: moisture, crude ash, crude fat, crude fiber and crude protein, were determined according to the methods of AOAC (2006). The carbohydrate content (%) was calculated by subtracting the contents of crude ash, fat and protein from 1000 mg/g of dry matter and expressed as % of dry mass.

Preparation of water-soluble extract

Extracts were prepared from bekkai lan powder as follows: 1 g powder was homogenized with 30 ml buffer phosphate pH 5, 6, 7 and 8. The homogenates were heated and boiled for 3, 6, 9 and 12 min and then left to cool. As a comparison, powder sample left to infused in the buffer solutions for 30 min without heating were used. The mixture was vacuum filtered and the residues were re-extracted with the same buffer phosphate as in the previous stage. The collected filtrate referred to as crude leaves extract or water soluble extract (WSE) were frozen at -20^{o}C until use.

Free amino acid (FAA) analysis

FAA were analysed as described modification from Jork *et al*. (1990), WSE (4 ml) was centrifuged for 2 min and 25 µl of supernatant was mixed with 300 µl of OPA (o-Phthaldialdehyde) solution. The mixture was vortexed for 1 min and 20 µl aliquot was injected and analyzed using HPLC (SHIMADZU LC 10) with a Licrospher 100 RP 18 (5 µm) and a 125 x 4 mm coloumn.

The separation of OPA-derivatives was performed with a mobile phase, consisting of methanol, 50mM Na-asetat, THF (2:9:2) at pH 6,8 as solvent A and 65% methanol as solvent B. The gradient elution programme was held at 100% of A for 0.1 min, ramped at 100% of B for 45 min and stop at 50 min with a flow rate of 1 ml/min. Detection was performed with a flourecence Shimadzu RF-138 set at 360nm (Ex) and 460nm (Em). Each FAA was identified by using the authentic standard (Sigma-Aldrich) and quantified by the calibration curve of the authentic compound (external standard method).

5'-Nucleotides analysis

5'-Nucleotides were analysed as described by Huang *et al*. (2006) from WSE. Samples (10 ml) was filtered through 0.45µm filters and 20 µl aliquot was analyzed using the HPLC system which consisted of a HPLC Waters E2695 automatic sampler, Waters SM7 injector, a 20 µl sample loop, a sunfire C18 column (4.6 x 125 mm, 5µm), Waters 2489 uv/vis detector. The mobile phase was 500 mmol/L KH_2PO_4/H_3PO_4, pH 4.3 at a flow rate of 1 ml/min and UV detection at 254 nm. Each 5'-nucleotide was identified and quantified by using the authentic standart of 5'-nucleotide: IMP and AMP (Sigma-Aldrich), GMP (Ajinomoto Int, Japan) by the calibration curve of the authentic compound (external standard method).

Equivalent umami concentration (EUC) Calculation

EUC (g MSG/100 g) is the concentration of MSG equivalent to the umami intensity given by a mixture of MSG and 5'-nucleotides and is represented by the following equation (Yamaguchi *et al*., 1971):

$$Y = \Sigma aibi + 1218[\Sigma aibi]\,[\Sigma ajbj]$$

where Y is the EUC of the mixture in terms of g MSG/100 g; ai is the concentration (g/100 g) of each umami amino acid (Asp or Glu); bi is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1 and Asp, 0.077); aj is the concentration (g/100 g) of each umami 5'-nucleotide [5'-IMP, 5'-GMP, 5'-XMP, 5'-AMP]; bj is the RUC for each umami 5'-nucleotide to 5'-IMP (5'-IMP, 1; 5'-GMP, 2.3; 5'-XMP, 0.61 and 5'-AMP, 0.18);

and 1218 is a synergistic constant based on the concentration (g/100 g) used.

Statistical analysis

The resulting data were tabulated and analyzed descriptively.

Results and Discussion

To our knowledge this is the first report on the chemical composition of *A. papuana* Becc leaves from Indonesia. The data presented here are on the comparisons of the proximate analysis of the whole dried leaves and the powder forms of the leaves.

Table 1 shows the proximate component of leaves powder and dried leaves of bekkai lan are different process used. Hence, based on the data obtained, leaves powder was chosen as it has a much higher protein content. Nijima (2000) suggested that umami taste could be a marker for protein-rich foods, whereas Yamaguchi and Ninomiya (2000) reported that glutamic is a major constituent of food plant/ animal protein. Taken together, the data obtained in this study indicated that the Albertisia papuana Becc leaves powder have component associated with umami character.

Free amino acid assay

Free amino acid were analyzed by using HPLC and quantified by comparison to authentic reference standards. Determination of the FAA content from plant based foods are still rarely performed but amino acids from protein hydrolysis has been extensively published (Loliger, 2000; Yamaguchi and Ninomiya, 2000).

In Fig. 1, the chromatogram of the FAA profile for all the treatments shows a similar phenomenon, whereby the detection of a group of uncharged amino acids is higher than a group of highly polar and charged amino acids 'umami'. Tyrosine represented the highest amount of uncharged amino acids extracted with phosphate buffer at pH 5 to pH 8, followed by a group uncharged amino acids comprising of methionine, alanine, isoleucine, valine and phenylalanine. The other group of charged amino acids comprised of the aspartic acid, histidine, glutamic acid and arginine.

Table 2 showed that a much higher amount of the total FAA compound of 'umami' (glutamate and aspartate) can be obtained when the extraction was carried out at pH 5 for 6 minutes (16.82 mg/g). The solubility characteristic of amino acid or protein is the same in water. This can be explained by the titration curve of component as reported by Nelson (2004). Since glutamate and aspartate have a similar

Table 1. Proximate composition of *bekkai lan* leaves

	Content[a] (%)				
Component	Moisture content	Crude ash	Crude protein	Crude fat	Crude carbohydrate
Leaves powder	9.73	4.27	20.65	5.24	60.10
Dried leaves	7.33	3.20	11.35	2.80	75.31

[a]Moisture were presented based on wet mass, others were presented based on dry mass

Table 2. Content of free glutamic acid and free aspartic acid from WSE of *bekkai lan* leaves extract (mg/g)

Time (minute)	pH 5		total	pH 6		total	pH 7		total	pH 8		total
	asp	glu		asp	glu		asp	glu		asp	glu	
0	1.47	0.81	2.27	2.82	3.76	6.57	0.95	0.87	1.82	1.82	3.39	5.21
3	8.83	5.52	14.05	1.80	0.16	1.96	0.16	0.55	0.71	10.48	4.88	15.22
6	11.50	5.32	**16.82**	1.07	0.96	2.03	5.39	3.85	9.24	7.29	4.37	11.66
9	8.27	3.87	12.15	2.16	0.10	2.26	6.92	3.53	10.45	6.30	3.43	9.72

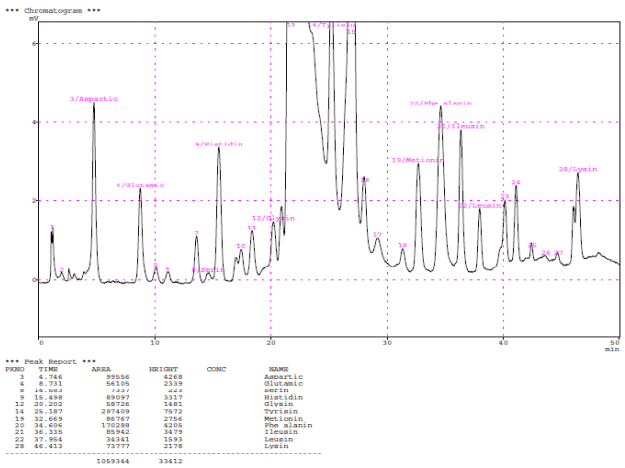

Figure 1. FAA chromatogram profile of crude extract *bekkai lan* leaves

chemical structure, their titration curves are also very similar. If the solubility is close to the isoelecrtic point, the dissociation will not increase but instead this may lead to deposition. Conversely, when the solubility is close to the pKR or the pKa, the dissociation is increased. This could explained for the observation made in this study in which the glutamate and aspartate were extract in higher amount at pH 5 compared to at pH 8.

The amount of aspartic acid was found to be higher than glutamic acid as the umami compound from FAA in bekkai lan leaves extract Elsewhere, unpublished reports from Purwayanti (2009) and Eva (2012) showed that the contents of aspartic acid was higher than glutamic acid from Albertisia papuana Becc. leaves, indication that Albertisia papuana from East Kalimantan, West Kalimantan and Central of Kalimantan have the same profile of MSG-like components.

Contents of MSG-like components from bekkai lan leaves extract were higher compared with crab meat (Chen and Zhang, 2007) but lower than mushrooms (Huang *et al*., 2006) and potatoes tuber (Morris *et al*., 2007). Yang *et al*. (2001) reported that the content of MSG-like components (glutamate and aspartate) were in middle range of 5-20 mg/g.

Table 3. Content of Inosine mono phosphate (IMP) and Adenosine mono phosphate (AMP) from WSE of bekkai lan leaves extract (mg/g)

Time (minute)	pH 5		total	pH 6		total	pH 7		total	pH 8		total
	IMP	AMP		IMP	AMP		IMP	AMP		IMP	AMP	
0	0.00	0.00	0.00	0.00	0.05	0.05	0.04	0.05	0.09	0.09	0.07	0.16
3	0.00	0.00	0.00	0.57	0.00	0.57	0.53	0.00	0.53	0.68	0.00	0.68
6	0.00	0.00	0.00	0.54	0.00	0.54	0.64	0.00	0.64	0.60	0.00	0.60
9	0.52	0.08	0.06	0.0002	0.00	0.00	0.60	0.00	0.60	0.54	0.00	0.54

Table 4. The EUC in soluble water extract leaves

Condition (pH; mnt)	KEU (%)
5;0	0.09
5;3	0.59
5;6	0.62
5;9	28.89
6;0	0.80
6;3	2.51
6;6	6.71
6;9	0.03
7;0	0.68
7;3	3.81
7;6	31.97
7;9	29.92
8;0	5.21
8;3	48.31
8;6	36.76
8;9	24.05

5'-nucleotide assay

5'-Nucleotides 'umami' were analyzed using HPLC and quantified by comparison to authentic reference standards. In Fig 2, the chromatograph of 5'-nucleotides 'umami' profile for all the treatments, showing the same phenomenon, producing IMP and AMP, but not GMP.

Table 3 shows that more of the total 5'-nucleotide compound of 'umami' (IMP and AMP) were extracted at pH 8 for 3 minutes (0.68 mg/g). This can be explained by the titration curve of the component which was similar to the FAA, as both the FAA and 5-nucleotides has a charge. The net charge depends on the pH of solvent. Amino acids with side ring has a net charge at amine and carboxyl group and the carboxyl at R group (Nelson, 2004), while 5'-nucleotides compound has a net charge at the phosphate group and the N-base group (Motley, 2008).

The level of nucleotides 'umami' that have been reported from the vegetables foods were from fungi (Cho *et al*., 2010; Huang *et al*., 2006) and potatoes tuber (Morris *et al*., 2007), whereas in animal foods were from crab (Chen and Zhang, 2007), the cube of chicken broth, pork and seafood (Chiang *et al*., 2007). The value of flavor 5'-nucleotides of bekkai lan leaves at pH 8 for 3 minutes is still low (< 1mg/100g) compared with mushrooms, broth cubes and crab mitten. According to the classification reported by Yang *et a*l. (2001), contents of flavor 5'-nucleotides lower than 1 mg/g are classified in the low ranges.

Loliger (2000) reported that AMP are abundant and plentiful in crustacean, mollusks and some vegetable, which eventually serves as the precursor for the formation of IMP. Thus, the discovery of IMP clearly suggested that AMP is associated with the presence of IMP in bekkai lan leaves.

The EUC

The intensity of umami flavor depends on the synergistic interaction between the 5'-nucleotides and 'umami amino acid. EUC value were calculated as describes above using the levels of glutamic acid, aspartic acid, IMP and AMP from the equation $Y = \Sigma aibi + 1218[\Sigma aibi]\ [\Sigma ajbj]$. Result shows that the EUC value was higher when the umami compound were extracted at pH 8 for 3 minute with phosphate buffer solution (Table 4).

According to the equation, the value of EUC was calculated as 48.31 g MSG/100 g (wet base). In other words, the umami concentration of one gram of leaves powder were equivalent to 0.48 g MSG. Compared with other foods, this value of EUC is higher than in mitten crab meat (4.2g MSG/100g (Chen and Zhang, 2007) but very low when compared to fruit bodies (622 g MSG/100 g) and mycelia of mushrooms (608g MSG/100 g) (Huang *et al*., 2006). Mau (2005) grouped EUC values into four levels: first level are > 1000g/100g (> 10 g MSG g^{-1} dry weight), second level are 100–1000g/100g (1–10 g MSG g^{-1}), third level are 10–100g/100g (0.1–1 g MSG g^{-1}), and fourth level are 10-100g/100gr (<0.1 g MSG g^{-1}). Based on the category above, the EUC values obtained in this study are group into the third level.

Conclusion

This is the first time reported test for umami compound of Albertisia papuana Becc. Result of this research showed that the major content of umami compound was relatively low and the EUC value from the major of umami compound of bekkai lan crude extract leaves were moderate. Effects of pH and time extraction has pattern based on the EUC obtained. The lower the pH extraction, the longer time needs to get umami compound.

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