Taste compounds from crude extract of *bekkai lan* (*Albertisia papuana* Becc.)

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Abstract: *Bekkai lan* leaves are commonly known as food flavoring in the culinary world of Dayak tribe, Borneo, Indonesia. No report has been made concerning the profile of the taste compounds in the extract of *bekkai lan* leaves. Thus, this study was aimed to investigate the profile of taste compounds in the leaves. The experiment was conducted on the crude extract of homemade leaves powder by using HPLC method. The results showed that the total content of taste compounds was 73.72 mg/g and there were 22 selected taste compounds from which the higher contribution came from some bitter compounds (59.48 mg/g) while the lower contribution came from soluble sugars (0.19 mg/g) and salty compounds (0.28 mg/g). An analysis using the computation of DoT (Dose-over-Threshold) factors showed that gallic acid served as the dominant tastant with DoT factors of 279. This study finds that the higher concentration of taste compounds are not the higher the DoT factors. The taste compounds that potentially had an impact on the extract taste including tyrosine, Ca, P, Guanosine monophosphate (GMP), malic acid, alanine, valine, aspartic acid, methionine and Adenosine monophosphate (AMP). This finding is important to food scientists to understand the profile of taste compounds contributing to flavor in the crude extract leaves.

Keywords: *Bekkai Lan*, Taste Compounds, Dose Over Threshold

1. Introduction

Bekkai lan leaves are well-known as food flavoring in the Dayak tribe's foods. According to (1), flavoring can change, modify, enhance, mask, form or neutralize aroma. These flavoring can be either natural or synthetic flavoring. Although today synthetic flavoring is already available in the markets, however the natural flavoring still becomes the main option to obtain palatability. Some of the natural flavoring contains nutrition or phytochemicals that are essential to health of humans and more favorable than the synthetic one.

Taste is one of the flavor components which is important to consumer to evaluate the quality of foods. Traditionally, taste sensations are classified into five basic qualities: sour, sweet, salty, bitter, and umami. Although most of researchers reported that aroma compounds influenced more to the food flavor, the taste compounds have been identified as a precursor to aroma. Most of researchers report about the contribution of taste compounds from animal-derived food material, while that from plant-derived compounds is rarely

reported. Some of the precursors were amino acids, sugars, nucleotides, organic acids and minerals. These compounds are well-known as taste active of food ingredients (2 & 3). Many studies of taste active have been reported: in oval squid (4), cheese (5 & 6), crab (7), mushrooms (8 & 9), fish (10), soy sauce (11), tomato juice (12), dried scallop (13), green tea (14). Although the perception of taste is so complicated due to the great number of involved taste and aroma compounds, the role/contribution of taste compounds of extract still needs to be investigated. Meanwhile, the taste active in *bekkai lan* leaves has never been reported. Therefore, the exploration of taste active is necessary.

2. Materials and Methods

2.1. Plant Materials

Bekkai lan leaf powder was obtained from the indigenous people of Dayak Kenyah tribe. The leaves were harvested in May, 2011 from the secondary forests surrounding the village of Long Lees (LU 04 43 '37"; LS 00 87' 02.4") and the village

of Long Pejeng (LU 04 53 '58.5"; LS 00 85' 75, 2"), Busang Sub-district, East Kutai District, East Kalimantan Province, Indonesia. To obtain the leaf powder, fresh leaves were sliced, sun dried for 2 days, and milled or grounded into powder of 60 Mesh. The powder samples were then packaged in plastic containers, covered with aluminum foil paper, and stored at freezing temperature (-20°C) until used.

2.2. Preparation of Water Soluble Extraction

Table 1. Profile of Taste Compounds in Crude extract of *Bekkai lan*, Treshold and The Value of DoT factors

Taste Compounds			Concentration (mmol/L)	Threshold (mmol/L)*	DoT
1	I Sweet	Sucrose	0.04	12.50	0.003
2		Glucose	0.03	48.00	0.001
3		Fructose	0.96	5.00	0.19
4		Alanine	32.97	8.00	4.12
5		serine	18.22	30.00	0.61
6	II Umami	Aspartic acid	20.59	7.50	2.74
7		AMP	2.55	2.00	1.27
8		GMP	2.61	0.30	8.70
9	III Acid	Malic acid	16.37	3.70	4.43
10		Oxalic acid	2.75	5.60	0.49
11		Glucuronic acid	10.03	-	-
12	IV Bitter	Tyrosine	186.81	5.00	37.36
13		Methionine	10.66	5.00	2.13
14		Valine	74.66	21.00	3.56
15		Isoleucine	9.64	11.00	0.88
16		Lysine	30.84	34.20	0.90
17		Gallic acid	55.95	0.20	279.77
18	V Salty	Na	0.31	7.50	0.04
19		K	3.53	15.00	0.24
20		P	3.14	0.16	19.46
21		Ca	0.09	3.12	29.11
22		Mg	1.30	4.00	0.32

Note: - = no data or no references

* Taste threshold value according to (7,9, 14, 22- 24)

2.3. Chemical Analysis

Chemical analysis were performed on water-soluble extracts such as free amino acid compounds (15) with a modification method as proposed by (16), soluble sugar compounds (17), sour compounds (18), 5'-nucleotides (19), phenolic acid compounds (20). The salt analysis (Na, K, Mg, Ca) was performed using an atomic absorption spectrophotometer (AAS) while the phosphate was analyzed photometrically (21).

2.4. DoT Analysis

The DoT factors were calculated as the ratio between the concentration of each compound in samples and its corresponding taste threshold (14).

2.5. Statistical Analysis

The data results were tabulated and analyzed descriptively

The process of making extracts from powder leaves was as follows: 5 g of sample was homogenized in 150 ml water (1/30; g/v). Vortex and mixed until dissolved leaves for approximately 5 minutes and boiled (100°C) for 3 minutes. The mixture was vacuum filtered and the residues were re-extracted with water in the previous stage. The collected filtrate referred to water soluble extract (WSE), freeze dried and stored at freezing temperatures (-20°C) until used.

3. Result and Discussion

3.1. Profile of Taste Compounds

All five basic quality of taste from *bekkai lan* leaves compounds have been detected. A complete component of the taste can be seen in Table 1 in which 22 taste compounds are detected. Some of the taste compounds are identified as 'the unknown', which can be seen in all Figures in this paper.

From the total compounds, it has been identified that most of the compounds are free amino acid as the main contributor. There were five amino acids (tyrosine, methionine, valine, isoleucine, lysine) contributed to bitter, two responsible for sweet taste (serine and alanine) and one indicated to umami (aspartic acid). Fig.1 and Fig.2 shows the chromatography of amino acid and nucleotides. The nucleotides were detected only GMP and AMP. A report by (15) shows that the nucleotides can be extracted directly from the crude extract as referred to the analysis conducted by (19). The soluble carbohydrates and acid compounds in crude extract were fructose, glucose, sucrose, malic acid, oxalic acid and glucuronic acid (Fig.3a & b). Not only can be extracted from

ethanol (25 & 26) or methanol (27), the soluble carbohydrate is also able to be extracted from hot water as reported by (28). Reports by (29 & 30) that organic acids can be extracted from the crude extract while the most efficient extraction is performed by alkaline medium (31).

Fig 1. Chromatogram of the amino acid: (1) aspartic acid; rt 2.255, (2) serine; rt 10.221, (3) alanine; rt 17.940, (4) tyrosine; rt 19.173, (5) methionine; rt 25.370, (6) valine; rt 27.083, (7) isoleucine; rt 29.743, (8) lysine; rt 35.037

Fig 2. Chromatogram of umami 'nucleotides' (1) GMP (rt 9.094) and (2) AMP (rt 18.030)

Fig 3(a). Chromatogram of the carbohydrate compounds: (1) glucuronic acid; rt 5.64, (2) sucrose; rt 7.602, (3) glucose; rt 9.605, (4) fructose; rt 11.908

Fig 3(b). Chromatogram of the organic acid compounds: (1) oxalic acid; rt 2.973 and (2) malic acid; rt 3.43

Interestingly, this study finds that gallic acid is the only of phenolic acids highly extracted from hot water (Fig.4). Our results consistent in reported by (15, 28-30, 32, 33), there for we suggested that the taste compounds can extracted directly from crude extract.

Fig 4. Chromatogram of gallic acid at rt 1.285

Table 2 showed that the total content of taste compounds in the extract was 73.72 mg/g, from which the higher contribution comes from bitter compounds (59.48 mg/gr) especially from tyrosine which has the higher concentration 186.81 mmol/l (Table 1) $\approx$ 33.85 mg/g. The compounds very low extracted are minerals (0.28 mg/g) and soluble sugars 0.19 mg/g.

Table 2. Total Content of Taste Compounds (mg/g)

Taste Compounds	Content
Soluble sugars	0.19
Sweet amino acid	4.85
Umami	4.53
Acid	4.39
Bitter	59.48
Salty	0.28
Total of taste compounds	73.72

Note: The value of content from conversion mmol/l to mg/g in Tabel 1

3.2. Calculation of DoT Factors

The value of DoT factors (9 & 14) is the same as the value of taste activity (TAV) as claimed by (7 & 34). Although they have different term, still they have the same meaning. The TAV value is obtained from the ratio between the concentration of sample and its threshold value, and generally measured in water or in a simple matrix (8). The compounds whose TAV values are > 1 are considered as taste active compounds in food (8) while those having DoT factors of > 1 can contribute to the taste (4, 18).

The evaluation of the contribution of each taste including 8 amino acids, 3 organic acids, 2 nucleotides, 5 inorganic salts, 3 soluble sugars and 1 phenolic acid is quantitatively conducted in the extract. Since the objective of this study is to elucidate the key contributors for each taste quality, one taste compound is grouped into five classes with different taste qualities (Table 1).

Tastant Group I, the compounds producing sweet taste containing sucrose, glucose, fructose and serine, shows a taste threshold concentration (DoT factors) of < 1 while only alanine shows DoT factors of 4. Meanwhile, Tastant group II are the compounds producing an umami-like taste sensation containing aspartic acid, AMP and GMP. All of the umami have DoT factors of 1-8. In contrast, the standard of umami taste (glutamic acid) can be not detected; it might be caused by the less concentration.

Tastant Group III are the compounds producing sour taste containing malic acid, oxalic acid, and glucuronic acid. Nevertheless, the taste threshold of glucuronic acid has not been reported yet so the analysis of DoT factors of glucuronic acid cannot be performed. In this group, only malic acid shows DoT factors of 4. From the bitter tastant group (Table 1), it can be seen that gallic acid is the first bitter tastant with DoT factors of > 1 and exceeds its own threshold concentration in extract by a value of 279. The second bitter tastant which also has high DoT factors is tyrosine. It is assumed that the tyrosine also significantly affects the taste of extract due to its Dot factors of 37.

The last tastant group (mineral), only cation Ca and P have DoT factors of > 1 respectively. The DoT factors of P and C was 19 and 29, higher than other minerals which have DoT factors < 1 (Na, K, Mg). This concentration implies that Ca and P were great potential to have an impact on the taste of extract. Since the taste compounds which have DoT factors of

1-10 may have less impact on the extract taste.

In regard with the data above, the sweet compounds from soluble sugar are not important in the responsible on taste of *bekkai lan* leaves.

4. Conclusion

The profile of taste compounds in the extract of *bekkai lan* leaves has been identified in this study. Gallic acid and tyrosine shows higher DoT factors. We concluded that 11 of taste compounds (gallic acid, tyrosine, Ca, P, GMP, malic acid, alanine, valine, aspartic acid, methionine and AMP) has been responsible for taste of crude extract *bekkai lan* leaves based on DoT factors.

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