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Research Article

Determination of taste enhancing compounds from alkaline extraction of Bekkai lan (*Albertisia papuana* Becc.) leaves by nanofiltration technique

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Abstract

Taste enhancer compounds from specific ethnic culinary herbs have very little information in the literature but this is important to food scientiests because these compounds can have a positive effect on food flavour. For many years, *bekkai lan* leaves have been used as flavour enhancer by the Dayak people in Borneo. Alkaline extraction of crude *bekkai lan* leaves was done at pH 8 and the isolation of taste compounds was done using the nanofiltration membrane method. Analysis of the water-soluble leaf extract compounds, especially taste enhancers with molecular weight less than 500 Da, were studied. It was found that total sugars were higher than total salts during the experiment, especially with total salts decreasing until 50% with decline of molecule weight cut off (MWCO) membrane. Sweet compounds were found as soluble sugar (glucose, fructose, sucrose); sugar alcohols (sorbitol, mannitol), including alanine. The major minerals contributing to salty taste were found as K and P. These taste enhancer compounds that were investigated are potential enhancers to umami taste in cooking.

Keywords: Dayak, Borneo, traditional food, sugar alcohol, flavor, nanofiltration.

Introduction

Palatability is one of the most important factors in food ingestion because it promotes appetite, digestion and the feeling of satisfaction [1]. Beside MSG and 5'-nucleotides, other chemical compounds which have a positive role for food palatability are sweet and salty compounds. Generally, table salt and 5'-nucleotides are known as potentiators (enhancers) of food flavour [2]. The sweet compounds are flavour enhancers too, because it could increase vegetables, meat and condiment flavours and also other product non sweet [3]. The sweet compound was important to form non-savoury flavour and as well as for salty compound in the formation savoury food flavour, so that salt and sugar as a flavour enhancers [4]. The terms taste enhancer is used by Schiffman [5], so that in the research we used the same terms of the taste enhancer for sweet and salty compounds.

We did not discuss the issue of MSG and 5'-nucleotides, because umami flavour components from the same leaf extract have been reported by Purwayanti, *et al* [6].

Sweet compound was not just the soluble sugars but other chemical compounds have a sweet contribution such as glycine, alanine, threonin and serin [7, 8], protein [9], low molecule weight (MW) of carbohydrate, aminoacyl sugars, peptida, terpenoid, chlorinated hydrocarbon, halogenated sugar, N-sulfonyl amides, sulfamat, polyketida, aniline and urea [10]. Other sweet compound was sugar alcohols, higher and lower MW non-caloric sweeteners (sucralose, siclamat, aspartame) [11]. Sugar and sugar alcohols often used to provide texture and effected to the release of flavour compounds [12, 13], especially for sucrose [14].

Generally, prototype of salty compounds was salt of NaCl. The mayor compounds which activating the salty receptor 'ENaCs' (*Epithelias Na Channels*) was Na and Li [15]. Other receptor when some salty from cation was not ligand with ENaC was K, Ca and ammonium [16]. For some products, salty taste was important because could improve the thickness, increasing the sweeteners, masking metallic taste or chemical off-notes and round out overall flavour when fitting the flavour intensity [17]**.** NaCl also is known impression strengthener to aroma [4]. The presence of salt can lowering the activity of water, so effective in increasing concentrations of volatile flavour components and improve the flavour [18]. Moreover, cation K, P and Ca are imployed to induce flavour release as controlling transition flavor release from a medium [19]. So that minerals effected to flavour.

This study focuses on extraction under alkaline condition, while the effect of acid medium is still running to investigate in the same material. We only discussed the sweet and salty component which resulted during cooking, because the mix of sweet and salty compounds has been significant potential as a taste enhancer in food. Taste enhancer compound has range MW <500 Da, we chose the isolation method with filtration membrane (nanofiltration/NF) for molecule weight cut off (MWCO) 400Da and 1000Da. This method very applicable for industry and has now become the standard for numerous manufacturing applications [20]. Besides that, compound that can elicit the umami or savoury taste belong to a broad class and were found to have MW less than 1000Da [21]. So that, the aim of this research, is to determine the taste enhancer that forms during the extraction process by boiling method under alkaline conditions. This research was important because the presence of taste enhancer can predict how far the food flavour improvement desired.

Materials and Methods

Materials

Bekkai lan leaf powder was obtained from local indigenous people of Dayak-Kenyah ethnic group. The leaves were harvested in May 2011 from the secondary forests that sorround Long Lees village (LU 04 43 '37"; LS 00 87' 02.4") and Long Pejeng village (LU 04 53 '58.5"; LS 00 85' 75, 2"), Busang Subdistrict, East Kutai District, East Kalimantan Province, Indonesia. To obtain leaf powder, fresh leaves were sliced, sun dried for 2 days and ground into powder of 60 mesh in size. The powder samples were packaged in plastic sachets, covered with aluminum foil paper and stored at freezing temperature (-20^{o}C) until used.

Preparation of water soluble extraction

Extract were prepared from powder leaves as follows: 6 grams of sample was homogenized in 180 ml solution of 0.2 M tris-HCl buffer pH 8. Vortex and mixed until dissolved leaves approximately 3 minutes and boiled (100^{o}C) for 3 min. The mixture was vacuum filtered and the residues were re-extracted with the same buffer as in the previous stage. The collected filtrate referred to as water soluble extract (WSE), freez dried and stored at freezing temperature (-20^{o}C) until used.

Multistep membrane

Water-soluble extracts fractionated by the method of nanofiltration (NF) membrane using a 'dead end' filtration system with polyethersulphon (PES) at Membrane Research Centre, Bioseparation Laboratory, Faculty of Chemical Engineering, Diponegoro University, Semarang. The tools consist of modules such as plate and frame, 3 liter capacity, 47mm diameter membrane, the active surface area of 4 cm^2 with separation mechanism sieving. Worked system: multistep filtration start from microfiltration/MF (PES; the compacting pressure and filtration pressure (<1 and 3 bar), NF 30kDa (PES; 4 and 5 bar), NF 10kDa (PES, 5 and 7.5 bar), NF 1kDa (PES, 8 and 12 bar) and 400Da (PES, 12 and 15 bar). PES membrane obtained from Membrane Research Centre and NADIR, Germany. Permeate has been freeze dried and stored at -20°C. NF permeate results 1000Da and NF 400Da respectively referred to as fractions of 1000 Da and fractions of 400 Da.

Chemical analysis

Chemical analysis was performed on water-soluble extracts such as total sugar [22], total salt, modified from [23] whereas sugars with HPLC Aminex-87H [24], modification method from [25]; free amino acid with HPLC Shimadzu LC10 [6], modification method from [26] and salty compounds (minerals) were analyzed by AAS.

Results and Discussion

Contributing compounds

In the early stages of determining whether or not a taste compound can be done with the general calculation approach such as total sugar for sweetness and minerals for salty. Total sugars explain all kind of sugar regardless of the source, the natural or the product results during the extraction process. As well as, NaCl which describes to total salts. Total sugars and total salts contained in the leaf extract can be seen at the Table 1. Table 1 showed that the total sugar was higher than the salty, and decreasing during NF membrane filtration. Decreasing total sugar by ≈ 5%, while the total salt > 50%. This may be due to the system filtration of 'dead end', the weakness in this system was easily formed fouling.

Table 1. Total levels of sugars and salts in extract *Bekkai lan* leaf (%; w/v)

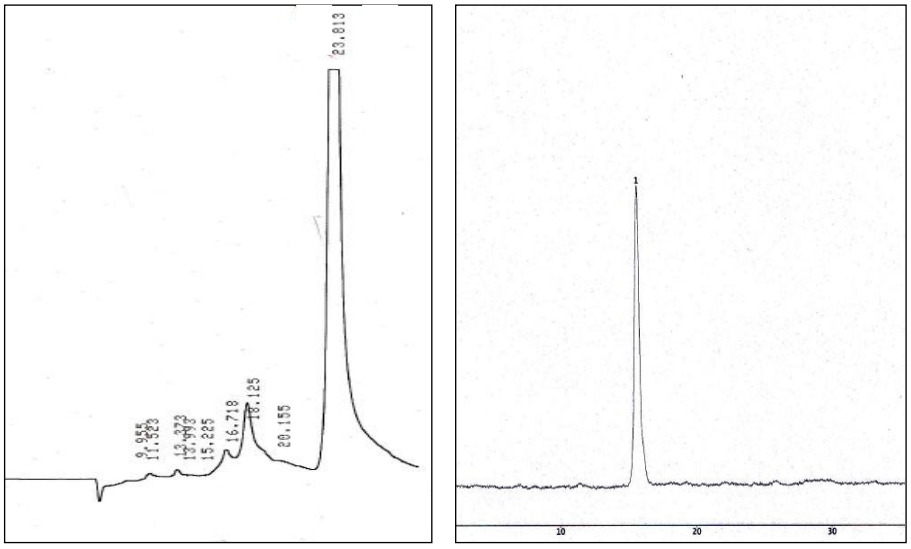
Parameter/Type of samples	Total sugar	Total Salt
Water soluble extract	2.01	0.54
Fraction of 1000Da	1.99	0.29
Fraction of 400Da	1.91	0.23
Decreasing Filtration (%)	5	57

Note : Mean values from the experiments were done in triplicates report.

Fouling was the accumulation of permanent component due to filtration itself. The nature of fouling was inevitable in membrane technology [27], but the fouling on the system 'dead end' higher than the system 'cross flow' [28].

Sweet compound analysis

The total sugar was reflecting to sweet compound in the extract leaves of Bekkai lan. Detection by HPLC Aminex-87H has shown the presence of glucose, sucrose, mannitol, sorbitol whether alanine from HPLC Shimadzu LC10 (Figure 1).



(a) (b)

Figure 1. Chromatogram of sweet compounds from fraction of 400 Da (etanol: water /80:20 w/v) extract leaves *Bekkai lan*. Sugars and sugar alcohols (a) and alanine (b)

The sugar compounds more detectable from dissolved materials of freezing with 96% ethanol: water (80:20, v/v) compared to the original crude extract. Samples were tested in both crude extract, ethanol: water and the fraction of 1000Da, none of which detected all of sugars, but it was generated on the fraction of 400Da (ethanol: water). Just one sample detected of fructose from crude extract (data not shown). Not all sugar compounds was detected, some unknown at retention time of 18.125 and 20.155. A complete sugar compounds were detected in fraction 400Da can be seen in Table 2.

Table 2. Content of sweet compound (mg/gr).

Sweet compound	Retention time	Fraction of 400Da
Sucrose	9.955	0.76
Glucose	11.523	1.42
Fructose*	12.5	Not detected
manittol	13.373	2.72
sorbitol	13.993	5.62
alanine	23.6	0.06

Note: *fructose just detected in water soluble extract

Data of sugars which shown comes from HPLC type as the same as that used [24]. More over [24], it should be noted that the elution between xylitol and arabitol have the same retention time, as well as glycerol and formic acid. We suspected that all of the sugar compounds were detected from the boiling. The vary result in difference alkaline condition from some research has been studied [29] and [30]. The reports said in the alkaline condition, glucose and fructose free would be degraded, whereas sucrose stable, relatively unreactive but some report said that sucrose was degradated too. While, generally the aldo and keto groups of carbohydrate can be readly reduced in aqueous solutions to give sugar alcohols [31]. That was explined that carbohydrate can be reduced easly in sodium borohydrate at pH 6-10. The reduction glucose yields sorbitol, whereas fructose results in two epimer sorbitol and mannitol [31].

In addition, we assumed that glucose or sorbitol could be derived from the degradation products of hydrolysable tannins too. To get a free phenolic acid commonly used extraction under alkaline conditions [32]. Hydrolisis or saponification was still a method to get a free phenolic acid although it remains unclear how decomposition of phenolic acids occur in these conditions [33]. The hydrolysable tannin components commonly found in leaves was gallotanin. The main composition of hydrolysable tannins was gallic acid (non-flavonoid phenolic acids) and glucose or sorbitol [34]. More over, gallic acid was rarely found in the free form, but generally in the form of hydrolysable tannins [35]. We have proved in another study (data not shown) in the same condition, the extract has been contained gallic acid. In our opinion, alkaline hydrolisis at pH 8 may cause hydrolysable tannins were decomposed into monomers such as gallic acid and glucose or sorbitol. Thus, we concluded that application of 0.2M Tris-HCl to result in alkaline medium (pH 8) as a buffer, when boiled at 100^{o}C it could be as a catalytics which promotes some reaction (reduction, hydrolisis or decomposition). However, especially for sucrose, the hydrolisis was still not completely because the time of boiling was shortly (3 min).

Other sweet compounds was detected is the amino acid alanine at retention time 15.575. It was surprising because alanine was the only free amino acids were detected in *Bekkai lan* leaf extracts cooked in alkaline conditions. Alanine has slightly sweet and umami taste [36]. Moreover, the higher concentrations of MSG can be reduced by the presence amino acids glucogenic (glycine, proline, alanine) and sugar (glucose) [37]. MSG and L-alanine resemblance taste quality because both amino acids activated the same taste receptors in mice [38]. The report mentions that the similarity occurs when the levels Na of MSG eliminated, resulted in two amino acids which have a similar taste. Different things, at high concentrations taste quality between the MSG (without omitted Na) with alanine, can be distinguished by the mice. Thus, between the glutamate and alanine was a component that was similar although not identical taste quality. Na ions may be masking of perceived similarities between MSG with other amino acids. The same mentioned that alanine compound belonged to the dominant taste sweet, whereas the L-alanine and L-serine at high concentrations could enhance the umami taste [39].

Salty compounds analysis

Minerals (cations and anions) had been known to be associated with salt-forming compounds. Until now, has never been tested on mineral composition of *Bekkai lan* leaves extract. Minerals were tested only Na, K, Ca, P and Mg, not for Li because Li was toxic and was rarely found in plants. Table 3 shown the minerals that contributed to the salty taste were higher from P and K than others, but all minerals decreased with the decline of the MWCO membrane. Interestingly, at fraction of 400Da, mineral K was higher than others. Sharply decline was happens to P and Ca almost decreasing at 86%, probably due to the nature of P and Ca to form the salt. Especially for the P, we have proved that in other research (unpublished data) which used of phosphate buffer greatly affect the efficiency of the filtration membrane process.

Table 3. Minerals Composition of *Bekkai lan* Extract (ppm)

Type of samples/ minerals	Water soluble extract	Fraction of 1000Da	Fraction of 400Da	Decreasing filtration (%)
Phosphor (P)	112,13	19,42	15,55	86
Potassium (K)	109,76	63,60	41,8	62
Magnesium (Mg)	15,61	5,28	4,76	70
Sodium (Na)	6,81	6,16	3,71	46
Calcium (Ca)	3,72	0,89	0,52	86

Note : Mean values from the experiments were done in triplicates report.

It seems has phenomenon of fouling, and occured start from microfiltration and nanofiltration especially with PES membrane. We sugested, at during filtration occured membrane pore blocking because fouling phenomenon salt of P and Ca.

Although the really salty taste is NaCl [40], K has been reported as an elements contributed to salty than Na in seaweed [41], in soy paste Doenjang [42]. Thus, the high element of K in *Bekkai lan* leaf extracts may be related in salty taste. There was a relation between K with the introduction of both the sweetness and the taste cells in the pancreatic ß-cells and was mediated by the cytosolic Ca [43]. This means that without K and Ca can reduce or inhibit the formation of sweetness. Calcium also affects the taste of salt and umami in the cheese [44].

Conclusion

This research reported about taste enhancer from *Bekkai lan* leaves extract obtained from alkaline extraction was first study, especially determination of compounds with nanofiltration. By the condition in the 0.2 M Tris-HCl buffer at pH 8 for 3 minutes, we got the sweety and salty compounds. The sweet compounds of *Bekkai lan* leaf extract were glucose, sucrose, fructose, sorbitol, mannitol and alanine, sweet amino acid. The minerals which contributed to salty compounds of *Bekkai lan* leaf extract were K, P, Mg, Na and Ca. Finally, these results indicated that there were some potential to enhanced umami flavour from the sweet and salty compounds of *Bekkai lan* leaf extract.

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