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Non-volatile taste components and amino acid profile of *jengkol* (*Pithecellobium jiringa*) seed flour after steam blanching

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Abstract

Jengkol (*Pithecellobium jiringa*) is a typical plant in the tropical region of Southeast Asia that is mostly liked by indonesian people because of savory taste. *Jengkol* seeds having high protein and healty effects, need a processing for keeping their nutrient quality. This study aims to determine the effect of steam blanching to non-volatile taste components of *jengkol* seeds. The results showed that steam blanching during 5 minutes decreased the total content of soluble sugars from 998.10 to 903.59 ppm, organic acid from 442.80 to 362.89 ppm and 5′-nucleotides from 2.92 to 2.57 mg/g dry weight. However, steam blanching could be able to increase the total content of free amino acids from 75.47 to 85.90 mg/g dry weight. The equivalent umami concentration (EUC) value in *jengkol* seeds also increased from 38.27 to 42.22 g MSG/100 g dry weight. The steam blanching treatment increased the MSG-like components in *jengkol* seed flour and also give the sweet taste and inhibit the formation of bitter taste. Hence, *jengkol* seed flour has high potential to serve as a functional food with a palatable umami taste.

Keywords: *Jengkol*, Steam blanching, Non-volatile taste components, Equivalent umami concentration (EUC)

INTRODUCTION

Pithecellobium jiringa or *jengkol* is belong to family of Fabaceae and classified as subfamily of Mimosaceae that is consumed raw or cooked in many tropical countries. *Jengkol* is traditionally used for treating diabetes, high blood pressures, overcoming dysentery, stomach disorders, and bladder stones (Shukri et al, 2011). *Pithecellobium jiringa* are popular as *jering* seeds in Malaysia, *jengkol* in Indonesia, *krakos* in Cambodia and *niang-yai* in Thailand. *jengkol* seeds, had a high potential for further use, processed and modified for the development of new food products. (Sridaran et al, 2012).

Jengkol was a seed that had flavor and could grow well in Indonesia (Handayani, 2017). *Jengkol* also had high protein and amino acids (Lim, 2012). The flavor was influenced by the presence of non-volatile flavor components such as organic acids, free amino acids, 5-nucleotides and dissolved sugars that provide a sensation called umami (Pei et al, 2014).

Umami was the fifth taste sensation received by the senses of human taste, besides four other traditional taste sensations (sweet, sour, salty, bitter) (Jinap and Hajeb, 2010). The umami component is found mostly in the form of amino acids L-glutamate and L-aspartate (Ney, 1971). It is also known that several compounds have contributed to the appearing of umami taste, namely monosodium glutamate (MSG), inosin monophosphate (IMP) and guanosine monophosphate (GMP) (Kuninaka, 1967). The mixture of the three compounds produces synergistic properties at certain comparisons (Yamaguci et al, 1971).

Jengkol seeds that are high in nutrients and have health effects require a processing because of due to perishable with high nutrient content. This processing aims to maintain the quality of *jengkol* seeds and extend shelf life. In the processing stage, the pre-process treatment needs to be carried out to determine the chemical characteristics of the *jengkol* before further processing such as packaging, marketing and storage. One of the pre-process treatments that is often used is blanching. Blanching is a preheating treatment carried out on fresh vegetables or fruit before the process of freezing, drying, or canning. In addition, blanching is an effective way to simplify the process of peeling the skin on green legumes and can maintain nutrition in beans, carrots and broccoli (Patras et al, 2011).

Blanching method that is often used is steam blanching. Steam blanching is the best method to maintain the nutritional quality of broccoli (Bongoni, 2014; Handayani, 2017). Steam blanching is the best method for maintaining the quality of nutrients such as carotenoids, glucosinolates, sulphorane, folate, and phytochemicals (Poelman et al, 2013). Though literature reported the beneficial effect of steam and other processing methods like fermentation in reducing the anti-nutritional factors and improving the digestibility of food grains (Theurer, Huber, Delgado-Elorduy, & Wanderley, 1999; Mohapatra et al, 2019). The blanching method also affects the sensory properties of food. One sensory attribute that is significantly affected by the blanching method is the flavor attribute. Blanching steam of vegetables was reported to be more flavorsome than vegetables using the boiling method. Steam blanching as a method for cooking vegetables was believed to be very suitable for vegetables such as broccoli. Steam blanching also reduced the washing of sugar into boiled water (Rennie, 2010).

So far, the research on *jengkol* seeds is still limited and the lack of information about the components of non-volatile taste compounds is the basis of this research. However, The aims of this research were to know the effect of steam blanching to the changes of non-volatile taste components of jengkol seeds. The non-volatile taste components consisting of soluble sugars, organic acids, 5′-nucleotides, and free amino acids were also evaluated and compared.

The data obtained from this study is considered useful to provide information on the potential of local plants in Indonesia.

MATERIALS AND METHODS

Material

Jengkol seeds were obtained from Kranggan Traditional Market, Yogyakarta. The chemicals used were analytical grade purchased from Sigma-Aldrich and Merck for analytical purposes. Sugar standards, and organic acid standards were HPLC grade from Sigma Aldrich. Nucleotide (IMP, GMP and AMP) standars and free amino acid standars were HPLC grade obtained from Sigma Aldrich. Mobile phases for HPLC analysis were HPLC grade quality and purchased from Merck.

Steam blanching methods

Jengkol seeds were first blanched for 5 minutes with steam blanching which refers to Opikasari, (2017) method. Fresh jegkol seeds were washed and then drained. As much as ± 1500 g of drained jengkol seeds were placed on the steaming stimulator below which contains boiling water (6 liters) for 5 minutes. The steamed jengkol was then immediately cooled with running tap water before slicing and drying. Fresh jengkol and steamed jengkol seeds were sliced to be smaller parts (reducing size) for helping in the proccess of drying and grinding. The small parts of fresh jengkol and steamed jengkol seeds were dried using a cabinet dryer at 50 ^{o}C for 18-20 hours. The dried seeds were then milled and sieved with a 50 mesh sieve for obtaining the jengkol seed powder.

Assay of soluble sugars

Soluble sugar in jengkol seed flour was extracted and analyzed according to W. Li et al (2014). Suspensions of powdered jengkol (0.25 g) in 50 ml 80% ethanol were shaken at 80 rpm for 45 min at room temperature. After filtration through Whatman No. 3 filter paper, the filtrate was evaporated to dry at 40 ^{o}C on a rotary evaporator. The extract was redissolved in deionized water to a final volume of 10 ml and the solution was filtered through (0.45 millex filter, Millipore) prior to analysis.

Soluble sugar was determined by an Knauer HPLC system (auto sampler, Knauer smartline 3950, German) equipped with a Refractive Index Detector (RID). The assay was performed on a Metacharb 87C column (300 x 7.8 mm) and the mobile phase used was H_2O

(LC grade) with injection volume of 20 µL at a flow rate of 0.5 mL/min. The oven temperature was maintained at 85 °C, and isocratic elution. The identification and quantification of sugar compounds was compared to calivartion curve of authentic standards.

Assay of organic acids

Organic acids were extracted as described by W. Li et al (2014). Powdered jengkol samples (5.0 mg) were suspended in 20 ml deionized water and subjected to ultrasound (400 W, 30 min, ambient temperature) using a 1500W High Intensity Ultrasonic Processor. The suspension was centrifuged at 4000 rpm for 30 min and the supernatant was filtered through a 0.45 μM cellulose membrane (Millipore) prior to analysis by HPLC.

The assay was performed on a MethaCarb H Plus column (7,8 x 300 mm) with the Knauer HPLC system (auto sampler, Knauer smartline 3950, lGerman). The injection volume was 20 µL and mobile phase was H_2SO_4 0,005 M at a flow rate of 0.5 mL/min. The oven temperature was maintained at 70 °C. In addition, the detection wavelength of organic acid was 215 nm by UV and each organic acid was identified by an authentic standard. Lastly, all the organic acids were quantified use of a calibration curve prepared from the external standards

Free amino acid assay

Free amino acids were extracted as the method that was described by Wang et al (2010). Sample (0.25 gram) is ground and dissolved in 10 ml of distilled water. The suspension is then heated at 90° for 20 minutes, cooled till ambient temperature. The extract was centrifuged at 4500 g and the supernatant was made up to 10 mL with distilled water and filtered with 0.45 μm nylon membrane filter. The SPE-PAKC18 cartridges were conditioned with 30 mL of methanol and 10 mL of distilled water. Sample infusion (1 mL) was loaded into the conditioned cartridges, and the retained compounds were eluted with 5.0 mL of 10% ethanol. The resulted elute was collected and filtered through a 0.45-μm nylon filter membrane before its pre-column derivatisation with o-phthalaldehyde (OPA).

The OPA derivatisation solution was freshly prepared as follows: 0.01 g of OPA was dissolved in 1 mL of methanol, and then added with 4 mL of 0.4 M borate buffer (pH = 9) and 25 μL of mercaptoethanol. The 10 μL of sample infusion or standard amino acid was mixed with 300 μL OPA solution, incubated at ambient temperature (27 °C) for 2 min, and used directly for HPLC analysis.

Analysis of free amino acids was carried out with High Performance Liquid Chromatography (HPLC-10AD Shimazu, Japan) that was equipped with a shimadzu

flourescence RF-10A detector. The column used was a lichoCART 125-4 reverse phase C-18 column 5 μm at a flow rate of 1.5 ml/min. The mobile phases used were 50 mM sodium acetate : tetrahydofuran (THF) : methanol (96 : 2 : 2) as solvent A and methanol 65% as solvent B. The gradient elution was performed as follows: 0–2 min, 100% solvent A; 2–35 min, 100% solvent B. Each amino acid was identified and quantifed by the external amino acid standars.

5′-Nucleotides assay

5′-Nucleotides were extracted as the method explained by Pei et al (2014). 1 gram of sample is mashed and dissolved in 10 ml of distilled water. The suspension is then heated for 1 minute, cooled and centrifuged 4500 g for 15 minutes. The residue from the previous process was washed 3 times with 10 ml of distilled water and then mixed with the filtrate to rotate the evaporator and diluted again with distilled water to a volume of 10 ml. The solution was filtered with 0.45 μm micropore membrane filter before analysing.

Analysis was carried out with High Performance Liquid Chromatography (HPLC-10AD Shimazu, Japan). The HPLC column is Zorbax Eclipse XDB C18 (250 x 4.6 mm, 5μm) with mobile phase aquades / methanol / acetic acid / tetrabutylammonium hydroxide (894.5 / 100/5 / 0.50). Injection volume was 20 μL with flow rate of 0.7 mL / minute and 5' nucleotides were detected by UV at 254 nm. External standar was used for quantification of each 5' nucleotide.

Equivalent Umami Concentration (EUC)

Calculation of the equivalent umami concentration (EUC) in fresh and steam *Jengkol* was based on the equation: $Y = \sum aibi + 1218(\sum aibi)(\sum ajbj)$ as described by Yamaguchi et al, (1971) where Y is the EUC of the sample (g MSG/100 g); ai is the concentration (g/100 g) of each umami amino acid (Asp or Glu); aj is the concentration (g/100 g) of each umami 5′-nucleotide (5′-IMP, 5′-GMP, 5′-AMP); bi is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1 and Asp, 0.077); bj is the RUC for each umami 5′-nucleotide (5′-IMP, 1; 5′-GMP, 2.3; 5′-AMP, 0.18) and 1218 is a synergistic constant based on the concentration (g/100g) used.

RESULT AND DISCUSSION

Soluble Sugars

The changes of soluble sugar in *jengkol* after steam blanching were presented in Table 1. Fructose + Galactose and Glucose were the most major soluble sugars in fresh *jengkol*. The content of Fructose + Galactose was around 342.70 ppm in fresh *jengkol* and it decreased in steam *jengkol* (224.53 ppm). The content of Glucose was around 271.80 ppm in fresh *jengkol* and it was higher than in steam *jengkol* (161.80 ppm) which showed a decrease caused by effecting from steam blanching treatment. There was a possibility that the short time hot water and steam blanching resulted in its decrease (Biao Li et al, 2017). In addition, soluble sugars also were considered as taste-active components contributed to the sweet perception so, It can be potential for flavour enhancer. Beside of that, the contents of fructose, Galactose and Glucose in steam *jengkol* decreased compared with fresh *jengkol*, which could be due to th decomposition of sugar and the Maillard reaction occurred during the heat treatment (Li, 2011; Biao Li et al, 2017).

Table 1. The changes of soluble sugar in *jengkol* seeds after steam blanching

	Content (ppm)	
Soluble Sugars	Fresh	Steam Blanching
Galacturonic Acid	227.10	325.63
Maltosa + Rafinosa	156.50	191.63
Glucose	271.80	161.80
Fructose + Galactose	342.70	224.53
Total	998.10	903.59

The total content of soluble sugars (Table 1) in fresh *jengkol* and steam *jengkol* ranged from 903.59 to 998 ppm, where the total content of soluble sugars in fresh *jengkol* was higher than the steam *jengkol*. According to Pei et al (2014) stated that during the thermal process, due to the higher temperature of samples, thermal decomposition could have led to suggar content decrease. The research reported by Saldivar et al (2010) stated that steam blanching effectively retained soluble sugars than another thermal treatment (hot water blanching). Hot water blanching decreased soluble sugars by leaching during the water blanching.

Organic Acids

The content of organic acids on fresh and steam *jengkol* was presented on Table 2. Organic acids are strongly related to the synthesis and metabolism of amino acids, aromatic compounds, esters, and phenols (Li, 2014; Biao Li et al, 2017). It could be seen that Succinic Acid was the major organic acid (1.145.87 ppm) in fresh *jengkol*, followed by Lactic Acid (259.61 ppm), Citric Acid (182.47 ppm), malic acid (0.72 ppm) and Oxalic Acid (< 0.158 ppm). The total content of organic acid in *jengkol* decreased from 442.80 ppm (fresh *jengkol*) to

362.89 ppm (steam *jengkol*) after blanching. The organic acids loss could be ascribed to the occurrence of decarboxylation due to the higher temperature of heat treatment during steam blanching (Handschumacher, 1960; Pei et al, 2014). In addition, the contents of Succinic Acid, malic acid, Lactic Acid, citric acid and Oxalic Acid also declined in different degrees, which may result in the decrease of astringency value (Sowalsky and Noble, 1998; Biao Li et al, 2017).

Table 2. The changes of organic acid in *jengkol* seeds after steam blanching

	Content (ppm)	
Organic Acids	Fresh	Steam Blanching
Oxalic Acid	< 0.158	<0.158
Malic Acid	0.72	0.42
Citric Acid	182.47	65.62
Succinic Acid	1.145.87	82.28
Lactic Acid	259.61	214.57
Total	442.80	362.89

Free amino acids

Free amino acid profile of fresh and steam *jengkol* could be seen in Table 3. free amino acid total of fresh *jengkol* was 76.47 mg/g dry weight and free amino acid total of steam *jengkol* was 85.90 mg/g dry weight. The total of free amino acid on steam *jengkol* was higher 10.78 % than raw or fresh *jengkol*. There is a possibility that increasing free amino acids that released from the proteolysis on steam *jengkol* is caused by heat treatment during the steam blanching. Yoneda et al (2005) reported that some free amino acids could be released from the proteolysis that occurred during heating treatment. Free amino acid total of fresh and steam *jengkol* was higher than reported by Li et al (2014) in five edible mushrooms (4.09 - 22.73 mg/g dry weight) and reported by Pei et al (2014) in *Agaricus bisporus* (44.2 mg/g dry weight).

According to Mau et al (2001) Amino acids differed into several clusters on the basis of their taste characteristics that could be seen on Table 4. Aspartic and glutamic acids were MSG-like components among all the free amino acids, that contributed to the characteristic umami taste on jengkol seeds (Tsai et al 2007). MSG-like components could also contribute to the level of EUC in jengkol seeds and EUC values tended high if the concentration of MSG-like components was also high (Biao Li et al 2017).

The steam jengkol contained MSG-like components (3.87 mg/g dry weight) that was higher than fresh jengkol (2.68 mg/g dry weight). As reported by Yang Joan-Hwa (2001) and Biao Li et al (2017), MSG-like components divided in three groups: high (>20 mg/g), middle (5–20 mg/g), and low (<5 mg/g). The level of MSG-like components on fresh and steam

jengkol was still under 5 mg/g. The content of sweet components on steam jengkol was 38.42 (mg/g dry weight) that was higher than fresh jengkol (30.58 mg/g dry weight). The content of bitter components on steam jengkol was 36.66 (mg/g dry weight) that was slightly higher than fresh jengkol (34.35 mg/g dry weight). Any bitterness in jengkol seeds would possibly be covered by the high amounts of soluble sugars, other sweet components and MSG-like amino acids, which together appear to be responsible for their natural taste (Li et al, 2014).

Table 3. Free amino acid levels on fresh and steam *jengkol*

Free amino acids	Content (mg/g dry weight)	
	Fresh *jengkol*	Steam *jengkol*
L-Threonine [a]	9.92	14.13
L-Valine [a]	17.62	20.64
L-Methionine [a]	6.07	4.36
L-Ileucine [a]	1.12	0.60
L-Leucine [a]	0.55	0.39
L-Phenylalanine [a]	4.21	3.25
L-Lysine [a]	1.71	0.81
Total	41.20	44.18
L-Aspartic acid [b]	1.28	2.15
L-Glutamic acid [b]	1.39	1.73
L-Alanine [b]	18.19	21.33
L-Serine [b]	1.48	1.86
L-Glutamine [b]	0.75	0.67
L-Glycine [b]	0.99	1.11
L-Tyrosine [b]	0.12	0.11
L-Asparagine [b]	6.28	5.35
Total	30.49	34.29
L-Histidine [c]	1.52	1.62
L-Arginine [c]	3.26	5.81
Total	4.78	7.42
Total content	76.47	85.90

[a] Essential amino acid
[b] Non-essential amino acid
[c] Non-essential amino acid

Table 4. Levels of amino acid taste components on fresh and steam *jengkol*

Taste components [a]	Content (mg/g dry weight)	
	Fresh *Jengkol*	Steam *Jengkol*
MSG-like	2.68	3.87
Sweet	30.58	38.42
Bitter	34.35	36.66
tasteless	1.83	0.92
Total	69.44	79.88

[a] MSG-like (Asp + Glu); sweet (Thr + Ser + Gly + Ala + Pro); bitter (Val + Met + Ile + Leu + Phe + His + Arg + Trp); tasteless (Cys + Tyr + Lys).

5′-Nucleotide Components

Changes of 5′-Nucleotide in jengkol seeds after steam blanching could be seen in Table 5. The total content of 5′-Nucleotide on fresh jengkol was 2.92 mg/g dry weight that was slightly higher 11.98% than steam jengkol (2.57 mg/g dry weight).

Table 5. Levels of 5′-nucleotide components on fresh and steam *jengkol*

5′-Nucleotide [a]	Content (mg/g dry weight)	
	Fresh *Jengkol*	Steam *Jengkol*
5′-AMP	1.98	1.77
5′-GMP	0.62	0.54
5′-IMP	0.32	0.26
Total	2.92	2.57

5′-AMP, 5′-adenosine monophosphate; 5′-GMP, 5′-guanosine monophosphate; 5′-IMP, 5′-inosine monophosphate

Decreasing the total content of 5′- Nucleotide components on steam jengkol seeds could be possible to the effect of thermal decomposition during the steam blanching treatment (Boekel, 2006). 5′-guanosine monophosphate (5′-GMP) and 5′-inosine monophosphate (5′-IMP) are considered as flavour 5′-Nucleotide responsible for the umami or palatable taste and 5′-GMP contributed for a meaty flavour, that is a flavour enhancer much stronger than MSG (Litchfield, 1967 and Yang et al, 2001). Based on Yang et al (2001), Flavor 5′-Nucleotide was ranged from low (<1 mg/g), medium (1–5 mg/g) and high (>5 mg/g) where Flavor 5′-Nucleotide of these jengkol seeds was at the first level (low). The main content of 5′-Nucleotide from these jengkol seeds was 5′-AMP, 5′-GMP and 5′-AMP respectively. 5′-AMP could also give the sweet taste and effectively inhibit the formation of bitter taste.

Equivalent Umami Concentration (EUC)

Changes of Equivalent Umami Concentration (EUC) in jengkol seeds after steam blanching could be seen in Figure 1. The EUC value of steam jengkol was 42.22 (g/100 g dry weight) and The EUC value of fresh jengkol was 38.27 (g/100 g dry weight). The The EUC value of steam jengkol was 9.36 % higher than fresh jengkol. According to Mau (2005), The EUC values of flavour components could be ranged into four levels as: (1) >1000 g MSG/100g dry weight, (2) 100–1000 g MSG/100g dry weight, (3) 10–100 g MSG/100g, and (4). It was visible that the EUC value of both fresh jengkol (38.27 g MSG/100 g dry weight) was at the first level and steam jengkol (42.22 g MSG/100 g dry weight) was also at the first level.

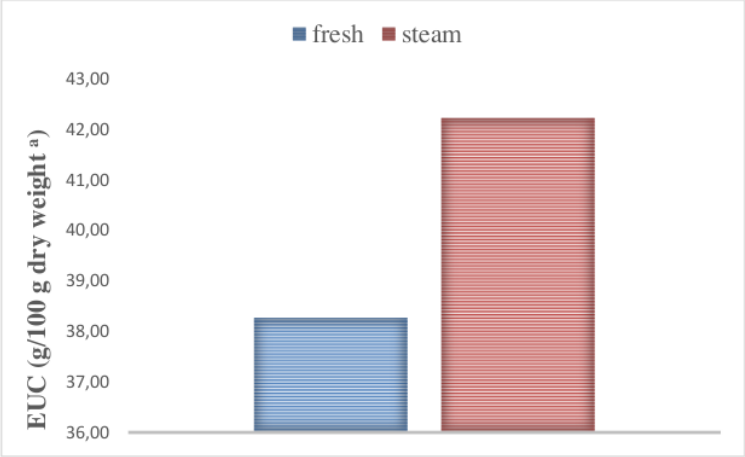

Figure 1 Changes of the equivalent umami concentration (EUC) in *Jengkol* after steam blanching treatment. [a] Calculation based on the equation: $Y = \sum aibi + 1218(\sum aibi)(\sum ajbj)$ (Yamaguchi et al., 1971) where Y is the EUC of the sample (g MSG/100 g); ai is the concentration (g/100 g) of each umami amino acid (Asp or Glu); aj is the concentration (g/100 g) of each umami 5′-nucleotide (5′-IMP, 5′-GMP, 5′-AMP); bi is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1 and Asp, 0.077); bj is the RUC for each umami 5′-nucleotide (5′-IMP, 1; 5′-GMP, 2.3; 5′-AMP, 0.18) and 1218 is a synergistic constant based on the concentration (g/100g) used.

Based on Yamaguchi et al. (1971) stated that the umami taste could be improved by synergistic effects between flavour 5′-Nucleotides (5′-AMP, 5′-GMP and 5′–IMP) and MSG-like components (L-glutaminc acid, L-aspartic acid) on jengkol seeds. The EUC value of jengkol seeds was higher than reported by Li et al (2014) at those mushroom of *P. cystidiosus* (13.32 g MSG/100 g dry weight) and *P. eryngii* (11.19 g MSG/100 g dry weight). The results have shown that jengkol seeds have a reasonably strong umami taste indicating with their equivalent umami concentration (EUC), as representing well-flavored foods and could be possible to serve as food flavoring materials and functional foods with a palatable umami taste.

CONCLUSION

Based on the results of this study, the contents of non-volatile taste components in *jengkol* seeds after the steam treatment decrased in soluble sugar from 998.10 to 903.59 ppm, in organic acid from 442.80 to 362.89 ppm and in 5′-nucleotides from 2.92 to 2.57 mg/g dry weight but increased in free amino acids from 75.47 to 85.90 mg/g dry weight. The EUC value in *jengkol* seeds after blanching treatment also increased from 38.27 to 42.22 g/100 g dry weight. It means that the steam blanching can increase the umami taste of *jengkol* seeds. The

steam blanching treatment was able to maintain the taste-active compounds of jengkol seed powder and also give the sweet taste and effectively inhibit the formation of bitter taste.

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Conflict of interest

All authors declare that there is no conflict of interest related to this paper.

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