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Potential of *Leucaena leucocephala* (white leadtree) seed flour as a functional food with umami taste

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Abstract

Leucaena leucocephala (White leadtree) legume seed is promoted in several countries of Southeast Asia. The young pods are edible and occasionally eaten by Indonesians because of their unique combination taste of savory, sweet and slightly bitter. This study investigated the processing effects of steam blanching on the protein profile (total amino acids) and the taste components of *L. leucocephala* seed flour. A 5-minute steam blanching on seeds increased the soluble sugar components from 10.59±1.091 to 14.78±1.590 mg/g (dry basis) and free amino acids content from 39.25±2.260 to 49.66±2.070 mg/g on the seed flour. On the other hand, steam blanching could decrease the content of the organic acid (167.78±4.800 mg/g to 149.29±10.420 mg/g) and the value of total amino acids from 23.86±1.293 (%w/w) to 24.43±1.411 (%w/w). *Leucaena leucocephala* seed flour had an essential amino acids ratio (E/T) value of 37.09±2.340–37.94± 1.130, predicted protein efficiency ratio (P-PER) of 0.27±0.002–0.30±1.668. In addition to the umami taste contributor component, a nucleotide compound was detected in fresh and steamed seed flour namely inosine-monophosphate (IMP). Equivalent umami concentration (EUC) showed that *L. leucocephala* seed flour contains high MSG-like components (425.92±4.76 to 540.12±13.05 mg MSG/100 g). Glutamic acid and aspartic acid, in line with Inosine Monophosphate (IMP), are MSG-like components providing a savory taste, known as 'umami' by receptors in humans. Steam blanching for five minutes prevented the degradation of sweet and savory compounds. Thus, the seed flour of *L. leucocephala* is notably marked to be utilized as an alternative to functional food with an umami taste.

1. Introduction

White leadtree legume (*Leucaena leucocephala*), also commonly known as '*jumbay*', '*ipil-ipil*', '*lamtoro*', '*petai Jawa*', '*guaje*' is promoted in several countries of Southeast Asia. The young pods are edible and occasionally eaten by Indonesian because of their unique combination taste of savory, sweet, and slightly bitter. *Leucaena leucocephala* is part of *Fabaceae* (*Leguminoceae*) family. In some areas of Indonesia, the seeds are eaten as sprouts or beans, and even made as traditional fermented food "tempeh" (Sayudi *et al.,* 2015; Nursiwi *et al.,* 2018). In Thailand, Cambodia, and Philippines, young seeds are consumed as a common vegetable. With a protein content of 31.1% (Ahmad and Abdelati, 2009), those seeds are considered an alternative protein source. The protein content is higher than in lentils (31.12%), followed by chickpeas (22.83%), broad beans (22.61%) and kidney beans

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(20.09%) (Qayyum *et al.,* 2012).

Proteins in food are various in composition, structure and functionality (Loveday, 2019). The interesting finding is that proteins either from plants or animals with their primary structure and specific amino acid sequence when broken down by a proteolytic enzyme, have specific bioactivities (Sgarbieri, 2017) that have a positive impact on the human body. Any food that has a positive impact on an individual's health, physical performance, or state of mind, in addition to its nutritious value, is a definition of 'functional food', with the provision that it is a food (not as a drug) from natural ingredients; being consumed as part daily diet; has a particular function when eaten; and serving to regulate a particular body process (Rincón-León, 2003).

Due to the perishable properties of common vegetables, legume seeds are very susceptible to microbial contamination regarding the high moisture content. A way of processing is needed to extend the shelf-life of *L. leucocephala* seeds. Among the ancient to the advanced preservation methods, drying is considered the best way to preserve and maintain the nutrition content and quality attributes, since Nobosse *et al.* (2017) reported that drying of the blanched *Moringa* leaves could retain the carotenoid content, compared to the fresh samples. It could also minimize microbial deterioration and give a practical way for storage regarding the low moisture content.

To prevent the development of undesired flavor in subsequent storage, most of the vegetables must be blanched before. Blanching is effective for reducing anti-nutritional compounds (Nkafamiya *et al.,* 2010), eliminating microbes (Tavares *et al.,* 2022), inactivating enzymes (Pervin *et al.,* 2017), and maintaining the chemical quality of food (Lin and Brewer, 2005). 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 have better retention of antioxidant activity and ascorbic acid in broccoli carrots, and green beans (Patras *et al.,* 2011).

Regarding the protein content, only a little is known about the composition of total amino acids within. The purpose of this study was to investigate the total amino acids regarding the protein content in *L. leucocephala* seed flour and the functional properties, with the changes in the taste components after steam blanching of the seeds.

2. Materials and methods

2.1 Seed flour preparation

The seeds were obtained from Yogyakarta, Indonesia. It was steamed for 5 mins, then immediately cooled down by using tap water and then drained. Drying was carried out in cabinet drying with the temperature of 50°C to reach a moisture content level of 5%. Dried seeds were grounded with the milling machine XiangRong 6NF-4 and then sieved for 60 mesh. The final product was stored in tight polyethylene packaging and kept in the freezer chamber at -18°C before being analyzed for quality attributes.

2.2 Quality analysis

Protein content was analyzed according to the method by Association of Official Analytical Collaboration (AOAC) International (2005) and the total amino acid profile analysis based on Fisher *et al.* (2001). Taste in food is primarily ascribed by free amino acid composition, 5'nucleotides, organic acids, and soluble sugars – polyol. The taste components assay of soluble sugars and free amino acids assays were performed based on Tsai *et al.* (2008). Organic acids assay was performed based on Li *et al.* (2015). Analysis of 5'-nucleotides was conducted according to Pei *et al.* (2014). Calculation of umami equivalent concentration is used to equalize the food umami *values* in units of mg MSG/100 g of dried matter (Tsai *et al.,* 2008).

2.3 Statistical analysis

All samples were run in triplicates. The data were expressed as mean ± standard of deviation (SD). Data was analyzed for variation using one-way analysis of variance (ANOVA) and the means separated by Duncan's multiple-range test. The significance between related samples was analyzed at the level of 0.05 ($p < 0.05$).

3. Results and discussion

3.1 Protein and total amino acids

Steamed *L. leucocephala* seed flour had lower protein content (27.10±0.374%) than in the fresh seed flour (30.79±1.314%) (Table 1). The protein content is higher than in lentils (31.12%), followed by chickpeas (22.83%), broad beans (22.61%), and kidney beans (20.09%) (Qayyum *et al.,* 2012). With a relatively high value of protein content, this seed has very potential to be used as an alternative source of protein which has been implemented by Sayudi *et al.* (2015) and Nursiwi *et al.* (2018) as a substitute ingredient for making tempeh (traditional fermented food based from soybean).

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Table 1. Total amino acids in *L. leucocephala* seed flour.

Groups	Amino acids	Value (%w/w)	
		Fresh seed flour	Steamed seed flour
Essential Amino Acids (EAA)	Methionine	0.22±0.008[a]	0.19±0.002[b]
	Threonine	0.85±0.056[a]	0.82±0.004[a]
	Lycine	2.21±0.033[a]	2.15±0.002[b]
	Valine	1.05±0.065[a]	1.03±0.002[a]
	Leucine	1.83±0.069[a]	1.84±0.004[a]
	Isoleucine	0.77±0.030[a]	0.77±0.002[a]
	Phenylalanine	1.08±0.048[a]	1.10±0.002[a]
	Total EAA	8.00±0.502[a]	7.89±0.183[a]
Semi essential	Histidine	1.26±0.088[a]	0.96±0.002[b]
	Arginine	2.30±0.275[a]	2.24±0.002[b]
	Total Semi Essential AA	3.56±0.203[a]	3.20±0.084[a]
Non-esential	Glutamic acid	3.86±0.188[a]	3.97±0.004[a]
	Aspartic acid	2.93±0.066[b]	3.03±0.002[a]
	Proline	1.23±0.038[a]	1.22±0.004[a]
	Serine	1.24±0.063[a]	1.23±0.002[a]
	Glycine	1.35±0.103[a]	1.16±0.002[a]
	Cysteine	0.04±0.002[b]	0.07±0.002[a]
	Alanine	1.46±0.048[a]	1.41±0.002[a]
	Tyrosine	0.75±0.013[a]	0.69±0.004[b]
	Total Non-Essential AA	12.85±0.889[a]	12.77±1.360[a]
	Total Amino Acids (TAA)	24.43±1.411[a]	23.86±1.293[a]
E/T ratio		37.94±2.340[a]	37.09±1.130[a]
P-PER		0.27±0.002[b]	0.30±1.668[a]
Hydrophobic AA		30.36±1.760[a]	32.00±2.540[a]
Protein content		30.79±1.314[a]	27.10±0.374[b]

Values are presented as mean±SD, n = 3. Values with different superscripts within the same row are statistically significantly different ($p<0.05$) compared to fresh samples.

Protein structure is comprised of amino acids. The total amino acids (TAA) exposed all of the amino acids in the food sample. Total amino acids in fresh *L. leucocephala* seed flour and steamed seed flour were 24.43±1.411 (%w/w) and 23.86±1.293 (%w/w) respectively (Table 1). Amino acids are grouped into "essential" and "non-essential". The essential amino acids are indispensable and required for growth, or nitrogen balance; even when there is an adequate amount of alternative amino acids. Ingesting protein-containing supplements and foods provides essential amino acids (EAA) necessary to increase muscle and whole-body protein synthesis (WBPS) (Church *et al.*, 2020).

The total essential amino acid of steamed flour is lower than that of fresh one. This finding is in accordance with Kabau flour (Fitriani, 2021). Though methionine, threonine, lysine, valine, leucine, isoleucine, histidine, and phenylalanine had no significant difference sum value of 8.00±0.502 (%w/w) for fresh seed flour and 7.89±0.183 (%w/w) for steamed seed flour (Table 1). The essential amino acids are obtainable by a single complete protein. A complete protein, by definition, contains all the essential amino acids. Complete proteins are usually derived from animal-based sources of nutrition, except for soy. The essential amino acids are also available from incomplete proteins, which are usually plant-based foods.

Fresh seed flour had a ratio of essential amino acid to total amino acids (E/T value) of 37.94±2.340%, meanwhile, the steamed seed flour was 37.09±1.130% (Table 1). Both values were not significantly different. The E/T values are considered to be adequate as per FAO and WHO guidelines (FAO, 1991) where values above 35.9% are acceptable for infants, 32.6% for children, and 15.2% for adults. This means that essential amino acids in *L. leucocephala* seeds have a high potential as alternative protein sources for all ages. The ratio of essential amino acids to total amino acids could be a representation of the effectiveness of a product and for enhancing the value of a commodity (Khanifar *et al.*, 2011). These amino acids must be supplied from daily intake of food because the human body lacks the metabolic pathways required to synthesize these amino acids (Lopez *et al.,* 2022).

Some amino acids are called semi-essential or conditionally essential amino acids because the body's necessity amount fluctuates, especially in the period of infancy or in certain health conditions (sick, injured or after surgery). Nonessential amino acids are synthesized by most of the cells. Nonessential amino acids are mainly synthesized from glucose (alanine, arginine [from the urea cycle in hepatic cells], asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline and serine), except for tyrosine, which is synthesized from phenylalanine.

The value of the predicted protein efficiency ratio (P -PER) was 0.27±0.002 for fresh seed flour and 0.30±1.668 for steamed seed flour (Table 1). Protein efficiency ratio measures the nutritive value of protein sources. The higher the PER value, the more beneficial it is to humans and animals. These predicted PER values were lower than *Moringa oleifera seeds* reported by Mune *et al.* (2016) ranging from 2.30 to 2.61. The higher P-PER score, the better the biologically available protein of a product.

Among the total amino acids, some of them belong to the hydrophobic amino acids. The hydrophobic amino acids are Ala, Val, Leu, Ile, Phe, Pro, Trp and Met. They might act as bioactive peptides and have the potential capability as Angiotensin-converting enzyme (ACE) inhibitors. Angiotensin-converting enzyme (ACE) increases blood pressure by producing the hormone angiotensin II, which constricts blood vessels. The ACE inhibitors work by helping to relax the veins and arteries to lower blood pressure. Bioactive peptides are natural ACE inhibitors derived from food protein, mostly have low molecular weight and consist of hydrophobic as well as negatively charged amino acids (Pebrianti *et al.,* 2019).

ACE-inhibitory peptides from food origins could be a good alternative to synthetic drugs. There has been much research conducted to investigate the beneficial properties of functional foods to reduce high blood pressure. Some plant-based peptides have been investigated for their potential ACE inhibitor (Daskaya-Dikmen *et al.,* 2017). The percentage of hydrophobic amino acids in *L. leucocephala* fresh seed flour was around 30.36±1.760% and 32.01±2.540% in steamed seed flour (Table 1). This result was lower than the amount of hydrophobic AA reported by Rahmadian *et al.* (2019) from jengkol (*Pithecellobium jiringa*) seed flour (36%-36.41%).

3.2 Taste components

3.2.1 Soluble sugars

Total soluble sugars in steamed seed flour tended to be significantly higher (14.78±1.590 mg/g) than in the fresh seed flour (10.59±1.091 mg/g) dry basis (Table 2). Compared to the fresh seed flour without blanching, the glucose content in steamed seed flour decreased (Table 2). This phenomenon could be caused by the leaching of the sugar components along with thermal processing. Saldivar *et al.* (2010) revealed that soluble sugars decreased in soybean seeds during hot water blanching for 10 mins as a result of leaching. The glucose content in both powdered seeds was lower than in *A. blazei* (27.59 mg/g) and *A. cylindracea* (17.28 mg/g) studied by Tsai *et al.* (2008). On the other hand, maltose and raffinose in steamed seed flour tended to have increased amounts.

3.2.2 Organic acids

Organic acids determine sour taste intensity (Neta *et al.,* 2007). The total organic acid content (oxalic acid, malic acid, citric acid and succinic acid) in fresh seed flour tended to be higher (167.20±4.800 mg/g) than in the steamed one (149.29±10.420 mg/g) dry basis (Table 2). These results showed that steam blanching continued with drying had a negative effect on the reduced amount of organic acids. This was supported by the research by Li *et al.* (2015), who found that the content of malic acid and ascorbic decreased by 51.92% to 90% after various methods of drying (hot air, microwave, vacuum, and natural air drying). Compared to the total organic acids content in *L. edodes* by hot water blanching (117.40 mg/g dry basis) and microwave blanching (44.76 mg/g db), both the fresh and steamed *L. leucocephala* seeds had a higher total amount. Not only act as a flavor enhancer, citric acid and malic acid are also able to inhibit the growth of food spoilage and pathogenic microorganism in food. It is well known that the main effect of organic acid is accounted for due to pH, different organic acid has a different inhibitory effect (Saha *et al.,* 2013).

3.3.3 Free amino acids

Free amino acids are different from the total amino acids. Total amino acids are all amino acids that are bound in proteins as well as those amino acids that may be present as individual unbound units. Free amino acids are unbound individual amino acids in a protein. Free amino acids and peptides play important roles in eliciting characteristic tastes of foods (Lioe *et al*., 2010). In general, steamed *L. leucocephala* seed flour had a significantly higher free amino acid (49.66±2.070 mg/g) than the fresh one (39.25±2.260 mg/g) (Table 2), due to the release of amino acids during the steam blanching process. It happened as a result of protein hydrolysis by thermal treatment where the hydrogen bond was disrupted and the structure became unfolded.

The study of free amino acids as the taste component

Table 2. Taste components in *L. leucocephala* seed flour.

Taste Components		Content (mg/g)	
		Fresh seeds	Steamed seeds
Soluble sugars	Glucose	6.86±0.518[a]	5.83±0.004[a]
	Maltose + Raffinose	3.73±0.051[b]	8.95±0.065[a]
	Maltose	ND	ND
	Galactose + fructose	ND	ND
	Total Soluble Sugars	10.59±1.091[b]	14.78±1.590[a]
Organic Acids	Oxalic acid	1.42±0.128	ND
	Malic acid	0.05±0.003[a]	0.03±0.001[b]
	Citric acid	19.93±1.257[a]	10.40±0.121[a]
	Succinic acid	145.80±7.190[a]	129.87±2.970[a]
	Lactic acid	ND	ND
	Total Organic Acids	167.20±4.800[a]	149.29±10.420[a]
Free Amino Acids (FAA)	L-Methionine	3.43±0.164[a]	4.32±0.526[a]
	L-Threonine	6.28±0.374[a]	6.13±0.389[a]
	L-Lycine	0.54±0.021[a]	0.21±0.015[b]
	L-Leucine	0.88±0.044[a]	1.16±0.171[a]
	L-Isoleucine	0.48±0.007[b]	0.66±0.027[a]
	L-Phenylalanine	0.23±0.007[b]	0.39±0.002[a]
	L-Histidine	2.77±0.296[a]	3.80±0.338[a]
	L-Arginine	2.22±0.127[a]	3.81±0.609[a]
	L-Glutamic acid	2.94±0.117[b]	4.46±0.134[a]
	L-Aspartic acid	0.79±0.048[a]	0.80±0.028[a]
	L-Serine	9.31±0.382[b]	13.82±0.118[b]
	L-Glycine	0.59±0.060[b]	0.82±0.066[a]
	L-Alanine	8.10±0.062[a]	8.84±0.122[a]
	L-Tyrosine	0.67±0.032[a]	0.44±0.005[b]
	Total FAA	39.25±2.260[b]	49.66±2.070[a]
5'-Nucleotide	Inosine Monophosphate (IMP)	1.22±0.130[a]	1.02±0.001[a]
EUC value		425.92±4.76[b]	540.12±13.05[a]

Values are presented as mean±SD, n = 3. Values with different superscripts within the same row are statistically significantly different ($p<0.05$) compared to fresh samples. ND: not detected.

provides significant information in identifying non-volatile food flavor contributors. The importance of amino acids and their contribution to the taste character was explained by Ikeda in 1908. He found out that monosodium L-glutamate (MSG), a form of salt from the glutamic acid, was an essential compound in food that gives an impression that nowadays well known as 'umami'. The word 'umami' derives from the Japanese for 'delicious' and the umami taste can be described as savory, meaty, or broth-like (Kurihara, 2015).

The results of this study proved that the content of glutamic acid and aspartic acid as the main umami taste contributors in steamed seeds powder flour was higher than the fresh seed flour (Table 2). Hydrolysis of protein into amino acids is a result of fermentation, aging, maturation, and heating during cooking. Glutamic acid level in those seed flour was higher than in Chinese mitten crab (*Eriocheir sinensis*) amounted to 0.62 mg/g (Chen *et al.,* 2007) and *A. blazei* mushroom at 1.09 mg/g (Tsai *et al.*, 2008), but lower than in button mushroom amounted to 5.63–7.67 mg/g dry weight (Pei *et al.*, 2014). Daly *et al.* (2013) explained that a salient taste from amino acids in animals and umami taste in humans is influenced by the effect of synergistic enhancement of Glutamic acid or other amino acids that combined with the 5'-nucleotides compounds.

The highest free amino acid in both seed flour samples was L-Serine. It has a significant difference value in steamed seed flour (13.82 mg/g), compared to fresh seed flour (9.31 mg/g). Serine has a constant, clear, sweet taste. It is also judged to be good, soft, smooth, and food-like (Schiffman and Dackis, 1975). The lighter-weight amino acids (serine, alanine and glycine) build the taste sweet impression regarding their ability to bind

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to the sweet receptors, while the heavier ones tend to be bitter. Methionine is one of the amino acids forming a bitter taste, and had a value of 3.43 mg/g in fresh seed flour, then 4.32 mg/g in steamed seed flour (Table 2).

3.3 5'-Nucleotides

Nucleotide compounds that are mostly known to contribute to the formation of umami taste are adenosine monophosphate (AMP), inosine monophosphate (IMP) and guanosine monophosphate (GMP) (Kurihara, 2015). A mixture of the three compounds produces synergistic properties at certain comparisons (Hwang *et al*., 2020). An addition to the umami taste, the only nucleotide compound detected in fresh and steamed *L. leucocephala* seed flour was inosine-monophosphate (IMP).

The IMP value was 1.02±0.001 mg/g db and 1.22±0.130 mg/g db in the fresh seed flour (Table 2). The decreasing value could be attributed to the nature of the IMP which is easily degraded by high temperatures and broken down into ribose (Van Boekel, 2006). The IMP values in both seed flour samples were higher when compared with the IMP values in three types of fungi (*A. blazei, A. cylindracea*, and *B. edulis*) studied by Tsai *et al.* (2008) which ranged from 0.04–0.07 mg/g dry weight

The synergistic enhancement of umami taste by IMP or GMP is detected by the common sensing receptor in humans that has been hypothesized to function in combination with sweet taste receptors (Li *et al.,* 2002). These receptors also are a determinant to differentiate between sensitivity and insensitivity in animals (mice) (Bachmanov *et al.,* 2001).

3.4 Equivalent umami concentration

EUC obtained from fresh *L. leucocephala* seed flour was 425.92±4.76 g MSG/100 g and 540.12±13.05 g MSG/100 g for steamed seed flour. EUC values of the two samples were higher than *A. blazei* (135.90 g MSG/100 g), *A. cylindracea* (46.73 g MSG/100 g), and *B. edulis* (10.46 g MSG/100 g) in dry weight. According to Mau (2005), EUC value categorized into four levels that are: low (<10 g MSG/100 g db), medium (10–100 g MSG/100 g db), high (100–1000 g MSG/100 g db), and very high (>1000 g MSG/100 g db). This *L. leucocephala* seed flour had a "high" category.

4. Conclusion

Five minutes of steam blanching had a negative effect on the crude protein content of steamed *L. leucocephala* seeds powder, the content of organic acids, total amino acids, and 5'-nucleotide (IMP) were decreased. On the contrary, free amino acids and sugar compounds tended to increase in steamed *L. leucocephala* seed powder. Equivalent umami concentration (EUC) showed that white leadtree seed flour contained high MSG-like components. Steam blanching for five minutes prevented the degradation of sweet and savory (umami) taste components. Thus, the seed flour of *L. leucocephala* is notably marked to be utilized as an alternative to functional food regarding its essential amino acids and the hydrophobic acids content that potentially act as an ACE inhibitor.

Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript.

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