

## Study on *Leucaena leucocephala* seed during fermentation : sensory characteristic and changes on anti nutritional compounds and mimosine level

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**Abstract.** Lamtoro (*Leucaena leucocephala*) seed is one of the leguminosae which have high level of protein but it contains toxic compound such as mimosine and some anti nutritional compounds such as phytic acid and tannin. The objectives of the research was to investigate the sensory characteristic and the changes on anti nutritional compounds and mimosine level in *Leucaena leucocephala* seed during fermentation. Lamtoro tempeh processing was carried out by boiling the seed, crushing to separate the hull, soaking, boiling, and fermentation. The best concentration inoculum in lamtoro tempeh processing was determined by hedonic test. Fermentation was carried out in 36 hours and every 6 hours mimosine, tannin, and phytic acid content was analyzed. From hedonic test, inoculum concentration of 1% was used in lamtoro tempeh processing. During 36 hours fermentation, phytic acid content and mimosine content was decreased significantly, from 0.0558 % to 0.0453 % and from 0.00393 % to 0.00173 % respectively. Whereas tannin content was increased signifacntly, from 0.0822 % to 0.00173 %.

**Keywords :** *Leucaena leucocephala*, mimosine, phytic acid, tannin

### 1. Introduction

Lamtoro (*Leucaena leucocephala*) plant belongs to the leguminous class which grows in several areas in Indonesia, such as the area around Gunungkidul and Trenggalek. The parts of this plant are often used, wood and twigs are used to make paper, leaves, seed, young stems and flowers are used as food sources for humans and livestock. Several areas in Indonesia use ripe seed as material for making tempeh [1].

Lamtoro seed have high level of protein and minerals. Ripe lamtoro seeds contain 30.81% protein (db), 20.45% (db) crude fiber, 8.80% (db) ash content, 1.09% (db) calcium, 0.69% (db) phosphorus, 1.50% (db) potassium, 1.11% (db) magnesium, and 0.05% sodium (db) [2]. However, lamtoro seed contain toxic compounds (mimosine) and anti-nutritional compouds such as phytic acid and tannin.

Mimosine is a non-protein amino acid structurrally having toxic properties by blocking the metabolic pathways of aromatic amino acids and tryptophan. It probably acts as tyrosine analogue because its structure resemblance to L-tyrosin that inhibits protein biosynthesis in the living body [3]. Negative impacts caused by mimosine if consumed by women and children, causes hair loss and eyebrows, causing pain in the scalp and edema occurs part of the body at a certain point [4]. Feeding lamtoro tempeh in rats causing impaired thyroid function [1]. Whereas feeding lamtoro leaf with mimosine content of 1% causes hair loss, liver tissue damage, edema, and increase death percentage



[3]. Mimosine content in ripe lamtoro seed was 2-9%. It can be reduced by washing, soaking, boiling, steaming, and fermentation during tempeh processing [5].

Several groups of tannins lead to the formation of complexes of protein, metallic ions, and other macromolecules such as polysaccharides [6]. It causes inhibition of iron absorption in the body and inhibition of enzymes due to the formation of the protein-tannin complex. Tannin content in 100 g lamtoro seed is 1.194 grams [7]. Phytic acid form complex bonding with Fe or other minerals such as Zn, Mg and Ca. The formation make it insoluble so that it is difficult to be absorbed by the body. Each 100 gram of lamtoro seed contains 2.325 gram of phytic acid [7].

Stages of traditional tempeh processing include boiling, cooling, removing the hull, washing, soaking, boiling, draining, cooling, inoculation, packaging and incubation [8]. Boiling, washing, and steaming reduce the antioxidant compounds in lamtoro seeds such as phytic acid and tannins. Phytic acid content in lamtoro seed reduced 37.9% by boiling and soaking whereas tannin content reduced 92% [7]. During fermentation, complex compounds were degraded into simpler compounds through enzymes produced by inoculum (protease, amylase, lipase, and phytase) [9].

Lamtoro tempeh processing in Wonogiri use *usar* as inoculum. *Usar* contains many types of molds such as *Rhizopus sp.* and other microorganism [10]. It makes sensory quality of tempeh is not consistent [11]. Beside *usar*, there is a commercial tempeh inoculum 'Raprima' which can be used as an alternative in lamtoro tempeh processing. It contains *Rhizopus oligosporus*, the dominant microorganism that play a role in tempeh fermentation. The objectives of the research was to investigate the sensory characteristic and the changes on anti nutritional compounds and mimosine level in *Leucaena leucocephala* seed during fermentation.

## 2. Material and Methods

### 2.1 Materials

Lamtoro seed was obtained from local market in Wonogiri. Commercial tempeh inoculum 'Raprima' was obtained from local market in Surakarta.

### 2.2 Lamtoro Tempeh Processing

Lamtoro seed was boiled with the addition of ash for about 2 hours (first boiling). Having been boiled, the lamtoro seed was separated with the hull. After that, the lamtoro seed was soaked for 9 hours then boiled (second boiling) for 2 hours. The boiled lamtoro seed was drained and cooled down. Afterwards, it was inoculated with commercial tempeh inoculum 'Raprima', packaged, and incubated on 27°C-29°C for 36 hours.

The inoculum concentrations used were 0.2%, 0.5% and 1%. Twenty five panelists were selected for sensory evaluation using 7-points hedonic scale.

### 2.3 Analysis of Phytic Acid Content

The analysis of phytic acid content was determined by Spectrophotometric method [12]

### 2.4 Analysis of Tannin Content

The analysis of tannin content was determined by spectrophotometric method [13].

### 2.5 Analysis of Mimosine Content

The analysis of mimosine content was determined by using colorimetric method [14].

## 3. Results and Discussion

### 3.1 Sensory Characteristics of Lamtoro Tempeh

3.1.1 Color is one of important parameter affecting panelists acceptance. Color parameter of lamtoro tempeh was evaluation on raw tempeh and fried tempeh. The result of color parameter evaluation was shown in Table 1.

**Table 1.** Sensory characteristic of lamtoro tempeh : color

Inoculum concentration	Raw tempeh	Fried tempeh
0.2 %	4.90±1.39 <sup>a</sup>	4.50±1.59 <sup>a</sup>
0.5 %	5.07±1.14 <sup>a</sup>	4.33±1.37 <sup>a</sup>
1 %	5.70±0.91 <sup>b</sup>	4.17±1.36 <sup>a</sup>

Notes : Scoring scale 1: dislike very much, 2: dislike, 3 : dislike slightly 4: Neither like nor dislike, 5:like slightly,6: like, 7: like very much

Sensory evaluation for color parameter showed that only at inoculum concentration of 1% significantly difference with other raw tempeh, but there were no significantly difference in all fried tempeh. Raw tempeh has white color, formed as the result of the growth of mycelium on the surface of tempeh. The less inoculum concentration decrease the ability of the microorganisms to form the hypha so the mycelium less of dense and the surface of tempeh is not throughout covered by hypha. The addition of inoculum concentration properly, produce mycelium more evenly so that the color of tempe is preferably by panelist [10]. Fried tempeh has golden brown color. The color is formed due to Maillard reaction during frying process. During frying tempe occurs reaction between active side of amino acid and reducing sugar due to heating to form melanoidin which produce brown color [15].

**3.1.2. Appearance** Good appearance of tempeh can be seen by the mycelium covering the tempeh surface. The dense of the mycelium determine the compactness of tempeh. Appearance parameter of lamtoro tempeh was evaluation on raw tempeh and fried tempeh. The result of appearance parameter evaluation was shown in Table 2.

**Table 2.** Sensory characteristic of lamtoro tempeh : appearance

Inoculum concentrations	Raw tempeh	Fried tempeh
0.2 %	3.97±1.32 <sup>a</sup>	4.63±1.35 <sup>a</sup>
0.5 %	5.10±0.88 <sup>b</sup>	4.60±1.16 <sup>a</sup>
1 %	5.93±0.58 <sup>c</sup>	4.73±1.14 <sup>a</sup>

Notes : Scoring scale 1: dislike very much, 2: dislike, 3 : dislike slightly 4: neither like nor dislike, 5:like slightly, 6: like, 7: like very much

Three different inoculum concentrations in raw tempeh were significantly different. Higher inoculum concentration, higher panelists acceptance. Inoculum concentration at 1% has dense and compact texture, and the appearance is similar to soybean tempeh. The addition of inoculum concentration properly, produce good mold micelium so that the arrangement of seed appears more compact [10]. There were no significantly difference in all fried tempeh.

**3.1.3 Aroma** The aroma of lamtoro tempeh resulted by the microorganism on lamtoro seed during fermentation. Aromaparameter of lamtoro tempeh was evaluation on raw tempeh and fried tempeh. The result of aroma parameter evaluation was shown in Table 3.

**Table 3.** Sensory characteristic of lamtoro tempeh : aroma

Inoculum concentrations	Raw tempeh	Fried tempeh
0.2 %	5.03±1.37 <sup>a</sup>	5.70±0.87 <sup>a</sup>
0.5 %	4.97±1.15 <sup>a</sup>	5.47±0.77 <sup>a</sup>
1 %	5.23±1.25 <sup>a</sup>	5.73±1.14 <sup>a</sup>

Notes : Scoring scale 1: dislike very much, 2: dislike, 3 : dislike slightly 4: neither like nor dislike, 5:like slightly, 6: like, 7: like very much

The addition of inoculum concentration properly causes mold grow better with a lot of mycelium resulting in a distinctive tempeh aroma [10]. But in the research using different inoculum concentrations (0.2%, 0.5% and 1%) resulted no significantly difference on aroma parameter in all raw tempeh and in all fried tempeh. The aroma of lamtoro tempeh is similar to soybean tempeh, and it does not have unpleasant aroma.

**3.1.4 Taste** Taste parameter of lamtoro tempeh was evaluation on fried tempeh. The result of taste parameter evaluation was shown in Table 4.

**Tabel 4.** Sensory characteristic of lamtoro tempeh : taste

Inoculum concentrations	Fried tempeh
0.2 %	5.13±0.90 <sup>a</sup>
0.5 %	5.27±0.82 <sup>a</sup>
1 %	5.33±1.24 <sup>a</sup>

Notes : Scoring scale 1: dislike very much, 2: dislike, 3 : dislike slightly 4: neither like nor dislike, 5: like slightly, 6: like, 7: like very much

Different inoculum concentrations resulted no significantly difference on taste parameter in all fried tempeh. It has pleasant taste, formed during fermentation by *Rhizopus oligosporus* [16]. The frying process also increase the taste of lamtoro tempeh, because the frying oil is absorbed into the lamtoro tempeh. The components of frying oil and tempeh affect the characterization of tempeh [17]. The frying oil increase the tasty taste of tempeh [15].

Based on all parameter evaluated by hedonic test, the best inoculum concentration for tempeh processing was 1 %.

### 3.2 Anti nutritional and Mimosine Level

**3.2.1** Phytic acid is one of the anti nutritional compounds found in lamtoro seeds. It is capable to bind mineral substances, so the mineral is absorbed difficulty by the body. By fermentation, phytic acid level can be lowered. The decrease of phytic acid level by fermentation is greater than boiling, steaming and soaking process [18]. Phytic acid level in lamtoro seed during fermentation shown in Table 5.

**Table 5.** Phytic acid contents in lamtoro seed during fermentation

Fermentation time (hours)	Phytic Acid Content (%)
0	0.0558±0.0022 <sup>b</sup>
6	0.0525±0.0029 <sup>ab</sup>
12	0.0504±0.0035 <sup>ab</sup>
18	0.0499±0.0019 <sup>ab</sup>
24	0.0488±0.0058 <sup>ab</sup>
30	0.0455±0.0070 <sup>ab</sup>
36	0.0453±0.0101 <sup>a</sup>

The result of the research showed that phytic acid content in lamtoro seed during fermentation was significantly decreased from 0.0558 % before fermentation to 0.0453 % after 36 hours fermentation. The decrease in phytic acid content occurs due to the activity of the phytase enzyme produced by the *Rhizopus oligosporus* during fermentation. By phytase enzymes, phytic acid is hydrolyzed into inositol and organic phosphate compounds [19, 20]. Other study reported the similar result, phytic acid content was decreased from 1.442g/100g to 1.116g/100g after 36 hours fermentation [7].

**3.2.2 Tannin** Tannin are polyphenolic compounds with different molecular weight and complexity. Tannins are found in many vegetables which are used as comestibles or animal feeds. Several groups of tannins lead to the formation of complexes of protein, metallic ions, and other macromolecules such as polysaccharides [6]. Tannin level in lamtoro seed during fermentation shown in Table 6.

**Table 6.** Tannin contents in lamtoro seed during fermentation

Fermentation time (hours)	Tannin content (%)
0	0.0822±0.0021 <sup>a</sup>
6	0.0650±0.0114 <sup>a</sup>
12	0.0681±0.0238 <sup>a</sup>
18	0.0784±0.0224 <sup>a</sup>
24	0.0904±0.0213 <sup>a</sup>
30	0.1206±0.0321 <sup>b</sup>
36	0.1522±0.0316 <sup>c</sup>

The result of the research showed that tannin content on lamtoro seed during fermentation was significantly increased, from 0.0822% before fermentation to 0.1522% after 36 hours fermentation. This was similar with studies that has been done before. From the studies, reported that after tannins are hydrolysed by enzymes into simpler compounds, the pH increase, it weakens the bond of tannin with protein and carbohydrate, in the result, tannin will be detected. During fermentation for 48 hours tannin level was increased from 0.095 g/100 g to 0.136g/100 g [7]. Other study also had similar result, tannin content was increased after fermentation from 60 mg/100g to 122mg/100g [21].

**3.2.3 Mimosine** Mimosine is a group of aromatic amino acids with the chemical formula of (β-N-(3-hydroxy-4-pyridone)-α-amino-propenoic acid). It is a non-nutritional amino acid whose structure is nearly similar to that of tyrosine and is found in several mimosa species within the *Leucaena* genus. The mimosine level of lamtoro gung leaves ranged from 2% to 6% and varies according to their ripening level [22]. Mimosine level in lamtoro seed during fermentation shown in Table 7.

**Table 7.** Mimosine contents in lamtoro seed during fermentation

Fermentation time (hours)	Mimosine Content (%)
0	0.00393±0.00011 <sup>e</sup>
6	0.00382±0.00022 <sup>e</sup>
12	0.00309±0.00010 <sup>d</sup>
18	0.00210±0.00015 <sup>b</sup>
24	0.00230±0.00011 <sup>c</sup>
30	0.00209±0.00010 <sup>b</sup>
36	0.00173±0.00015 <sup>a</sup>

The result of the research showed that mimosine content on lamtoro seed during fermentation was significantly decreased, from 0.00393% before fermentation to 0.00173% after 36 hours fermentation. During fermentation, mimosine in lamtoro seed was metabolized by microorganism from inoculum. It caused by the mimosine structurally resemblance to L-tyrosin [23].

#### 4. Conclusion

From hedonic test, inoculum concentration of 1% was used in lamtoro tempeh processing. During 36 hours fermentation phytic acid content and mimosine content was decreased significantly, from 0.0558 % to 0.0453 % and from 0.00393 % to 0.00173 % respectively. Whereas tannin content was increased significantly from 0.0822 % to 0.00173%.

## Acknowledgement

The research was financially supported by research project of PNBP UNS No : 623/UN27.21/PP/2017 from Universitas Sebelas Maret, Surakarta, Indonesia.

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