

Changes in chemical characteristics and dynamics population of microorganisms in pre-fermentation treatment of lamtoro seed (*Leucaena leucocephala*) during mlanding tempeh's production

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Abstract

Mlanding tempeh is an indigenous fermented food from lamtoro (*Leucaena leucocephala*) seed that uses *usar* as the inoculum in its production. Lamtoro seed contains antinutritional compounds namely phytic acid and tannin, and toxin compounds namely mimosine. Antinutritional compounds decrease the quality of nutrients in mlanding tempeh. The purpose of the research was to investigate the changes in nutritional compounds, antinutritional and toxin compounds, total phenols, antioxidants activity, dietary fiber and dynamics population of microorganisms in pre-fermentation treatment in lamtoro seed during mlanding tempeh's production. The analysis was carried out at the pre-fermentation stage, namely before the inoculation process. The result showed the change of nutrient compounds, antinutritional and toxin compounds, total phenols, antioxidants activity, dietary fiber, and dynamics population of microorganisms in pre-fermentation treatment in lamtoro seed during mlanding tempeh's production. Moisture, protein, and fat content increased, whereas ash, carbohydrate, phytic acid, tannin, and mimosine decreased. The total phenols content, antioxidant activity, and dietary fiber content of lamtoro seed also decreased. Meanwhile, the population of microorganisms (yeast and lactic acid bacteria) increased in pre-fermentation treatment in lamtoro seed during mlanding tempeh's production.

1. Introduction

Lamtoro seeds (*Leucaena leucocephala*) are legumes used as raw materials to produce non-soybean tempeh. This kind of tempeh is often referred to as mlanding tempeh or lamtoro tempeh commonly found in traditional markets around Gunungkidul and southern Java (Shurtleff and Aoyagi 2007; Prameswari *et al.*, 2021), including Wonogiri (Nursiwi *et al.*, 2019) and Pacitan (Ishartani *et al.*, 2021).

Lamtoro seeds contain nutrient compounds such as protein and fat. Moreover, lamtoro seeds also contain antinutritional compounds and toxins such as phytic acid, tannins, and mimosine, but also functional compounds such as phenolic compounds, antioxidants, and dietary fiber (Almasyuri *et al.*, 1990; Soedarjo *et al.*, 1994; Boateng *et al.*, 2008; Benjakul *et al.*, 2013). These antinutritional compounds can inhibit the absorption of several types of minerals and proteins. Mimosine, a toxin compound in lamtoro seeds, inhibits thyroid hormone synthesis (Listyawati, 2003).

The production process of non-soy tempeh such as tempe mlanding is essentially the same as the production of soy tempeh (Radiati and Sumarto, 2016), consisting of a pre-fermentation stage and a fermentation stage. The pre-fermentation stage is a pre-treatment given to the beans before the fermentation process. It consists of boiling, peeling, washing, and soaking. Meanwhile, the fermentation stage is the stage after the inoculum is given.

Fermentation of legume seeds has several advantages, namely decreasing antinutritional compounds, increasing nutrient digestibility, increasing antioxidant activity, and increasing phenolic levels compounds in relation to a reduced risk of chronic disease (Vital *et al.*, 2018). An increase in the solubility of protein and carbohydrates occurs during the fermentation process of lamtoro tempeh (Komari, 1999). Moreover, there is also an increase in dissolved protein levels and total phenol levels (Nursiwi *et al.*, 2018), while phytic acid and mimosine levels decrease during 36 hrs of lamtoro seed fermentation (Nursiwi *et al.*,

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2018). The studies that have been conducted focused on changes after the fermentation process. On the other hand, a study on the pre-fermentation stage of mlanding tempeh has not yet been widely carried out. Whereas, the pre-fermentation stage contributes to changes in levels of nutritional, antinutritional, and functional compounds.

Mayasari (2012) with corn tempeh samples and Karisma (2014) with red bean samples reported the effect of pre-fermentation treatments such as boiling and soaking on the content of nutritional compounds. The content of anti-nutritional compounds such as phytic acid and tannins in several types of beans decreased after boiling, soaking, and seed coat removal (Almasyuri, 1990; Egounlety and Aworh, 2003). Meanwhile, the research conducted by Soedarjo and Borthakur (1996) and Laconi and Widiyastuti (2010) found that there was an effect of boiling and soaking on the changes in mimosine levels in lamtoro seeds and leaves. Phenol levels, which were in the study by Srivastava and Khokhar (1996) on peas, Sinha and Kawatra (2003) on cowpeas, Siah *et al.* (2014) on faba beans, and Pal *et al.* (2016) on horsegram, decreased after pre-fermentation treatments such as boiling, soaking, and seed coat removal. The antioxidant activity of the grains also decreased after pre-fermentation treatments such as boiling, soaking and seed coat removal. This is mentioned in the study of Segev *et al.* (2011) on green beans and Salem *et al.* (2014) on faba beans, white beans, lentils, and fenugreek seeds, as well as on the research of Pal *et al.* (2016) with a horsegram sample.

The study conducted by Mulyowidarso *et al.* (1989) and Sutardi and Buckle (2004), found the growth of yeast and lactic acid during the soaking process in the production of soybean tempeh. Meanwhile, Ashenafi and Busse (1991) and Efriwati *et al.* (2013) reported the dynamics of the population of microorganisms, especially yeast and lactic acid during the soaking process in the production of soybean tempeh.

Thus, this study aims to determine changes in chemical characteristics which include nutritional and antinutritional compounds (phytic acid and tannins), toxin compounds (mimosine) functional compounds (total phenol, antioxidant activity, dietary fiber) and population dynamics of microorganisms during pre-fermentation treatment, which was before the inoculation of *usar* in the production of mlanding tempeh.

2. Materials and methods

2.1 Materials

The materials used in the production of mlanding tempeh are ripe lamtoro seeds, water, ash, and *usar* as a starter obtained from mlanding tempeh producers in

Wonogiri. Materials for chemical analysis include concentrated sulfuric acid (H_2SO_4), mercuric oxide (HgO), potassium sulfate (K_2SO_4), hydroxide-sodium thiosulfate solution ($\text{Na}_2\text{S}_2\text{O}_3$), boric acid solution (H_3BO_3), HCl solution, petroleum ether, nitric acid solution (HNO_3), FeCl_3 solution, amyl alcohol, ammonium thiocyanate, sodium phytate, Na_2CO_3 solution, tannic acid, Folin-Ciocalteu reagent, L-mimosine, activated charcoal, pure phenol, methanol, DPPH crystals, buffer solution, PP indicator, ethanol, aquadest, termamyl solution, protease, sodium hydroxide (NaOH) solution, amyloglucosidase enzyme, crucible, acetone, celite. Meanwhile, the microbiological analysis used physiological saline, MRSA media (de Man Rogosa Sharpe Agar) to calculate the total number of lactic acid bacteria (LAB), and MEA (Malt Extract Agar) media to calculate the total number of yeasts.

2.2 Preliminary analysis of lamtoro seeds

Lamtoro seeds were sorted from dirt, stones, and damaged seeds, then it was analyzed for moisture, ash, protein, mimosine, phytic acid, tannin, and phenol content and antioxidant activities.

2.3 Production of mlanding tempeh

The production of mlanding tempeh was the same as the production of the home industry in Wonogiri, Central Java. A total of five kg of ripe lamtoro seeds and 600 grams of ash were boiled for 2 hrs (Boiling 1), and then drained for 18 hrs. Then, the processes were rolling (Rolling 1), washing, peeling off the seed coat, and plunging into the water to separate the seeds from the coats. Afterwards, the seeds without the coats were soaked for 15 hrs (Soaking 1). Then, a second rolling and seed coat removal (Rolling 2) were carried out. The process was followed by a second soaking process for 9 hrs (Soaking 2). Then, lamtoro seeds were reboiled for 2 hrs (Boiling 2) and drained for 4 hrs or until the seeds were not too wet. Then they were inoculated with *usar* and then wrapped using oil paper. Next, fermentation was carried out at a temperature of 27-29°C for 36 hrs. Chemical analysis and microbiological analysis of lamtoro seeds were carried out at all pre-fermentation stages (Boiling 1; Rolling 1; Soaking 1; Rolling 2; Soaking 2; and Boiling 2). Meanwhile, the analysis of pH and total titratable acidity was carried out on boiling water 1, soaking water 1, soaking water 2, and boiling water 2.

2.4 Chemical analysis

Nutritional compound analysis which was carried out was moisture content, ash content, fat content, protein content, and carbohydrate content using Association of Official Analytical Chemists (AOAC)

(2005). Analysis of phytic acid content used the spectrophotometric method as described by Yunarti *et al.* (2013), tannin content used the spectrophotometric method as described by Fathurrohman (2012), mimosine content used the spectrophotometric method (Ilham *et al.*, 2015), total phenol content used the Folin-Ciocalteu method as described by Parnanto *et al.* (2012), antioxidant activity used the DPPH method (Pal *et al.*, 2016), dietary fiber content used the method described by Jelita (2011), total acid content used the titration method (Aristya *et al.*, 2013), and pH values used a pH meter (Silva *et al.*, 2013).

2.5 Microbiological analysis

Calculation of the number of yeast and total lactic acid bacteria used the total plate count method (Silva *et al.*, 2013).

2.6 Statistical analysis

The data obtained were then processed statistically using One-Way Analysis of Variance at a significance level of 5% ($\alpha = 0.05$) to determine whether there was a difference. If the difference was found, it is continued with a significant difference test using Duncan's Multiple Range Test (DMRT).

3. Results and discussion

3.1 Changes in nutrient compounds in lamtoro seeds during pre-fermentation treatment of tempeh

3.1.1 Moisture content

Based on the results of the study presented in Table 1, there was a change in the moisture content of the lamtoro seeds during the pre-fermentation treatment of tempeh. Raw lamtoro seeds had a moisture content of 13.17%. Lamtoro seeds had a significant increase in moisture content to 71.35% after the process of boiling 1. The increase in moisture content occurred because boiling softens the seed coat, so a lot of boiled water was absorbed into the seeds. The moisture content of lamtoro seeds increased to 79.44% after the process of rolling 1.

After soaking 1, the moisture content of lamtoro seeds increased to 81.58% due to the fact that the grain structure became soft during the soaking process, so water entered the cell structure with ease. In the next process, which was rolling 2, the moisture content of lamtoro seeds decreased to 78.00% and the moisture content of lamtoro seeds increased to 82.73% after the process of soaking 2.

3.1.2 Ash content

The results of the analysis of ash content are displayed in Table 1. The ash content of raw lamtoro seeds is 3.53%. The ash content of lamtoro seeds decreased to 1.69% after the process of boiling 1. This decrease occurred because the minerals contained in the seeds were soluble in water (Karisma, 2014). After the process of rolling 1, the ash content of lamtoro seeds decreased to 0.88%. It is predicted that this decrease in ash content occurred because some of the minerals in the lamtoro seeds were found in the seed coat. Minerals in legumes and seeds concentrated in the seed coat and epidermis. Afterwards, the ash content of lamtoro seeds decreased to 0.53% after the process of soaking 1. The process of rolling 2 slightly increased the ash content to 0.54%. Then, after soaking 2 there was a decrease back to 0.47% and did not change after boiling 2.

3.1.3 Protein content

Based on the results of the study presented in Table 1, there was a change in the protein content of the lamtoro seeds during the pre-fermentation treatment of tempeh. Raw lamtoro seeds had a protein content of 23.88%. Boiling 1 caused the protein content of lamtoro seeds to decrease to 19.48%. Meanwhile, based on the research conducted by Haron and Raob (2014) with soybean samples, the protein content decreased to 16.48% after the boiling process. In addition, research by Sinha *et al.* (2007) on the cowpea sample also reported a decrease in protein content after the boiling process. The protein contained in Lamtoro seed is a globular protein easily soluble in water and more easily changed under

Table 1. Nutritional content of lamtoro seeds in the pre-fermentation.

Sample	Moisture content (%)	Ash Content (% db)	Protein Content (% db)	Fat Content (% db)	Carbohydrate Content (% db)
Raw lamtoro seed	13.17±0.34 ^a	3.53±0.03 ^f	23.88±2.34 ^b	6.12±0.45 ^b	54.51±4.23 ^g
Boiling 1	71.35±0.47 ^b	1.69±0.01 ^e	19.48±1.77 ^a	3.97±0.39 ^a	43.77±1.39 ^f
Rolling 1	79.44±0.50 ^d	0.88±0.01 ^d	40.13±2.62 ^c	12.95±1.21 ^c	34.47±2.65 ^e
Soaking 1	81.58±0.37 ^c	0.54±0.01 ^b	51.21±3.00 ^e	18.47±1.29 ^e	16.88±2.62 ^d
Rolling 2	78.00±0.42 ^c	0.56±0.01 ^c	45.29±2.21 ^d	15.91±1.55 ^d	13.68±1.93 ^c
Soaking 2	82.73±0.24 ^f	0.47±0.01 ^a	56.44±2.44 ^f	19.06±0.71 ^e	9.91±3.12 ^b
Boiling 2	81.32±0.18 ^c	0.48±0.01 ^a	55.89±2.19 ^f	19.66±0.93 ^e	5.88±2.04 ^a

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($\alpha = 0.05$).

the influence of temperature, so it causes denaturation (Sayudi, 2015).

The protein content of lamtoro seeds increased significantly to 40.13% or increased by 2 times after the process of rolling 1 which aims to remove the seed coats. Likewise, similar results were found in the study of Sudha *et al.* (1995) and Pal *et al.* (2016) with horsegram samples, as well as in the research of Ghavidel *et al.* (2007) with samples of cowpeas, green beans, green grams, and lentils. An increase in protein content occurred due to the seed coat removal during rolling process. The protein found in the seed after the seed coat removal process is the protein contained in the endosperm of the grains (Ghavidel *et al.*, 2007; Pal *et al.*, 2016).

The process of soaking 1 caused the protein contained in lamtoro seeds to increase to 51.21%. An increase in protein contents in gude beans after soaking was found in the study of Ragab *et al.* (2010). This is predicted to the growth of yeast and lactic acid bacteria during the soaking process (Figure 1). It was supposed that the increase in protein during the soaking process was caused by the analysis of nucleic acids, purines, and pyrimidines from yeast and lactic acid bacteria growing during the soaking process. When the amount of yeast and lactic acid bacteria in lamtoro seeds increases, the amount of nucleic acids, purines, and pyrimidines or nitrogen elements from non-protein substances in lamtoro seeds increases.

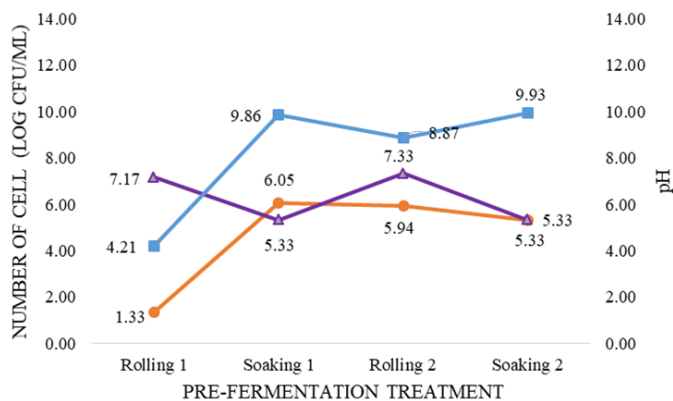


Figure 1. Dynamics population of microorganisms in lamtoro seeds during pre-fermentation. Legend: treatment of Tempeh (▲) pH of water (■) LAB (●) yeast

In rolling 2, there was a decrease in protein content in lamtoro seeds to 45.29%. The process of soaking 2 increased the protein content of lamtoro seeds to 56.43%. Ragab *et al.* (2010) reported an increase in the protein content of gude beans after soaking. Like soaking 1, this was due to the growth of LAB during soaking process (Buckle, 2004). Meanwhile, LAB has the ability to produce proteolytic enzymes (Mardiani *et al.*, 2012). Thus, it is suspected that the protein analyzed in lamtoro

seeds after soaking 2 is the protein in the lactic acid bacteria and small peptides due to the breakdown of lamtoro seed protein by proteolytic enzymes produced by lactic acid bacteria. Based on Table 1, it is known that the boiling process has no effect on the protein content of lamtoro seeds during the production of mlanding tempeh because the protein content of lamtoro seeds increased insignificantly to 55.89% after this process.

3.1.4 Fat content

Based on the results of the study shown in Table 1, there was a change in fat content during the pre-fermentation treatment of lamtoro seeds in the production of tempeh. The fat content of raw lamtoro seeds is 6.12%. Moreover, there is a decrease in fat content to 3.97% in lamtoro seeds after boiling 1. The finding is supported by the research of Haron and Raob (2014) showing that the boiling process reduced fat content from 10.60% to 4.66%. It is caused by the characteristics of the fat which is not heat-resistant, so that the fat evaporates into other components such as flavor. In the boiling process, fat is hydrolyzed into fatty acids and glycerol (Sundari *et al.*, 2015).

After rolling 1, the fat content of lamtoro seeds increased to 12.95%. The research conducted by Ghavidel *et al.* (2007) with samples of cowpeas, green beans, green gram, and lentils, as well as in the research by Pal *et al.* (2016) with horsegram samples found an increase in fat content after seed coat removal. The fat found in grains after the seed coat removal process is the fat found in the endosperm of the seed (Ghavidel *et al.*, 2007; Pal *et al.*, 2016).

The process of soaking 1 caused the fat content of lamtoro seeds to increase to 18.47%. Based on the study by Obizoba and Atii (1991), the fat content in soaked grains increased by 4 times. In addition, the fat content of gude beans increased from 1.25% to 1.44% after soaking according to the study by Ragab *et al.* (2010). During the soaking process, LAB growth occurs (Sutardi and Buckle, 2004). Lactic acid bacteria have the ability to produce lipase enzymes that function to break down fats in foodstuffs into glycerol and fatty acids then used by LAB to produce fat and other cell components (Suciati *et al.*, 2016).

Moreover, in the rolling process 2, the fat content decreased to 15.91%. In the process of soaking 2, the fat content of lamtoro seeds increased to 19.06%. Ragab *et al.* (2010) also found an increase in fat content in gude beans after soaking process. The fat analyzed in lamtoro seeds after soaking is considered to be fat within the lamtoro seeds and fat from the body of lactic acid bacteria because cell components such as cytoplasm in

lactic acid bacteria contain a lot of fat (Pelczar and Chan, 2008). The fat content of lamtoro seeds increased insignificantly to 19.66% after the process of boiling 2.

3.1.5 Carbohydrate content

Based on the results of the study presented in Table 1, there was a change in the carbohydrate content of lamtoro seeds during the pre-fermentation stage. The carbohydrate content in raw lamtoro seeds was 54.51% and then decreased to 43.77% after boiling 1. Afterwards, the carbohydrate content decreased to 34.47% after rolling 1. Following soaking 1, the carbohydrate content decreased to 16.88%. This occurred because there was a growth of lactic acid bacteria during the soaking process (Sutardi and Buckle, 2004) which utilized sucrose and glucose to be metabolized into lactic acid. The process of rolling 2 reduces the carbohydrate content to 13.68%. After the soaking 2, the carbohydrate content decreased to 9.91%. After the process of boiling 2, the carbohydrate content of lamtoro seeds decreased to 5.88%.

3.2 Changes in antinutrient compounds and toxic compounds in the pre-fermentation treatment of lamtoro seeds during the production of tempeh

3.2.1 Phytic acid content

The levels of phytic acid in lamtoro seeds changed during the pre-fermentation treatment of tempeh. This can be seen in Table 2. Raw lamtoro seeds contain 1.23% phytic acid, while the seed coat of lamtoro contains 1.21% phytic acid. Phytic acid content in raw lamtoro seeds of 2.325% was reported in Almasyhuri *et al.* (1990). The levels of phytic acid in lamtoro seeds after boiling 1, rolling 1, and soaking 1 decreased more insignificantly than the levels of phytic acid in raw lamtoro seeds.

The decrease of phytic acid levels significantly occurred after the process of rolling 2 to 0.90% or 20%. There are still a lot of seed coats in the process of rolling 1, while the seed coats have been peeled off a lot in the process of rolling 2. This significant decrease in phytic

acid levels is predicted to occur because a lot of phytic acid in lamtoro seeds was removed along with the peeled lamtoro seed coats. It is supported by the statement of Yanuartono *et al.* (2017) highlighting that most of the phytic acid in legume grains is found in the aleurone layer or the outermost layer of the endosperm and outer bran. Moreover, the research conducted Egouley and Aworh (2003) shows a decrease in phytic acid levels in soybeans, cowpeas, and peanuts by 9.9%, 10.1%, and 4.8%, respectively, after the peeling process of seed coats.

After the process of soaking 2, the phytic acid content in lamtoro seeds decreased significantly to 0.43% or 52.2%. A significant decrease of 50.3% also occurred in green beans after the soaking process for 8 hrs (Ertas and Turker, 2012). The decrease in phytic acid levels after the soaking process also occurred in the study conducted by Vidal-Vaverde *et al.* (1998) with a sample of faba bean and Huma *et al.* (2008) with samples of the white gram, green beans, kidney beans, white kidney beans, and lentils. This occurs because of an increase in phytase activity from the growth of the yeast and lactic acid bacteria during the soaking. Therefore, the breakdown of phytic acid into myo-inositol and inorganic phosphate occurs (Sutardi and Buckle, 2004). In addition, phytic acid is also soluble in water and sodium-potassium salt solution (Lolas and Markakis, 1975), so its levels decrease after the soaking process.

3.2.2 Tannin content

Based on the results of the study shown in Table 2, there was a change in the tannin content of lamtoro seeds during tempeh pre-fermentation treatment of lamtoro seed. The tannin contained in raw lamtoro seeds is 1.08%. The tannin content of lamtoro seeds decreased significantly to 0.19% after the process of boiling 1. This was also reported by Almasyhuri *et al.* (1990) on boiling several types of legumes and Adewusi and Falade (1996) on boiling several types of Nigerian beans. The decrease in tannin levels in lamtoro seeds after boiling is thought

Table 2. Anti-nutritional and toxin compound content of lamtoro seeds in the pre-fermentation.

Sample	Phytic Acid Content (%db)	Tannin Content (%db)	Mimosine Content (%db)
Raw lamtoro seed	1.23±0.07 ^d	1.08±0.04 ^c	5.32±0.24 ^g
Boiling 1	1.16±0.09 ^{cd}	0.19±0.01 ^d	1.84±0.09 ^f
Rolling 1	1.16±0.06 ^{cd}	0.19±0.02 ^d	1.73±0.08 ^c
Soaking 1	1.08±0.07 ^{cd}	0.18±0.02 ^{cd}	1.52±0.07 ^d
Rolling 2	0.90±0.04 ^b	0.17±0.01 ^c	1.27±0.02 ^c
Soaking 2	0.43±0.03 ^a	0.14±0.01 ^b	1.18±0.04 ^b
Boiling 2	0.37±0.03 ^a	0.11±0.01 ^a	1.11±0.04 ^a

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($\alpha = 0.05$).

to occur because the tannins are water-soluble. Tannins are also not resistant to heating above 80°C (Oematan, 2015), resulting in a diffusion process from the seeds to the boiling water (Almasyhuri *et al.*, 1990). In addition, it is also caused by the heating process that is too high which results in the tannins being hydrolyzed into glucose and tannic acid (Oematan, 2015).

The tannin content of lamtoro seeds did not change significantly after the process of rolling 1 and soaking 1. The tannin level decreased after the rolling process to 0.17%. These results are supported by the research of Deshpande *et al.* (1982), who mentioned that seed coat removal during the rolling process led to a decrease in tannin levels in green beans. Since tannins are found in the pericarp and seed coat of grains (Deshpande *et al.*, 1982). This is associated with the results of this study which showed that the seed coat of lamtoro contains tannin of 0.10%.

After the process of soaking 2, the tannin content of lamtoro seeds decreased to 0.14%. Huma *et al.* (2008) with samples of white gram, green beans, kidney beans, white kidney beans, and lentils reported a decrease in tannin levels after the soaking process since tannins dissolve into water during the soaking process. After the process of boiling 2, the tannin content of lamtoro seeds decreased to 0.11%.

3.2.3 Mimosine content

Mimosine is a toxin compound contained in lamtoro seeds. As displayed in Table 2, there was a change in mimosine content in lamtoro seeds during the tempe pre-fermentation treatment. Raw lamtoro seeds have mimosine content of 5.32%. Mimosine content in lamtoro seeds significantly decreased from 5.32% to 1.84% or decreased by 65.4% after the process of boiling 1. In the research conducted by Soedarjo and Borthakur (1996), boiling caused a decrease in mimosin levels in lamtoro seeds by 55.3%. The decrease in mimosine levels in lamtoro seeds occurred since mimosin is sensitive to heating above 70°C and is soluble in water

(Astuti, 1989). The heating treatment degraded mimosine to 3,4 DHP (3-hydroxy-4(IH)-pyridone) and a lower level of toxicity (Kurnia, 2017).

The rolling process 1 caused the mimosine content of lamtoro seeds to decrease to 1.73%. This happened because the lost mimosine was in the lamtoro seed coat wasted during the rolling process. In this study, there was a mimosine content of 1.16% in the seed coat of lamtoro. Mimosine content in lamtoro seeds decreased to 1.52% or decreased by 71.3% after the process of soaking 1 when compared to mimosine levels in raw lamtoro seeds. According to Laconi and Widiyastuti (2010), the soaking process can reduce mimosine levels in grains by 51.24%.

The process of rolling 2 reduced mimosine levels in lamtoro seeds to 1.27%. Like rolling 1, the decrease in mimosine levels occurs because the lost mimosine was in the lamtoro seed coat wasted during the rolling process. After following the process of soaking 2, the mimosine content of lamtoro seeds decreased to 1.18% and to 1.11% after boiling 2.

3.3 Changes in functional compounds in pre-fermentation treatment of lamtoro seeds during tempe production

3.3.1 Total phenol content

Based on the research results presented in Table 3, there was a change in the total phenol content of lamtoro seeds during the pre-fermentation treatment of tempeh. The total phenol content in raw lamtoro seeds was 0.0100%. The total phenol content of lamtoro seeds decreased to 0.0079% and to 21% after the process of boiling. According to the study by Srivastava and Khokhar (1996) with a sample of peas, boiling process caused the total phenol to decrease by 30-37%. The decrease in phenol after boiling process was also presented in the study conducted by Sinha and Kawatra (2004) on cowpeas and in the study conducted by Siah *et al.* (2014) on faba beans. This decrease occurs because the heating process can increase the degradation between

Table 3. Functional compound content of lamtoro seeds in the pre-fermentation.

Sample	Total Phenol Content (%)	Antioxidant Activity (%)	Dietary Fiber Content (%db)
Raw lamtoro seed	0.0100±0.0006 ^a	45.01±3.11 ^c	61.26±0.41 ^f
Boiling 1	0.0079±0.0006 ^d	27.51±2.49 ^d	56.19±1.68 ^c
Rolling 1	0.0073±0.0005 ^e	26.48±1.48 ^d	39.27±2.70 ^c
Soaking 1	0.0065±0.0004 ^e	16.18±0.94 ^c	50.26±2.19 ^d
Rolling 2	0.0052±0.0002 ^c	7.96±0.69 ^b	32.07±1.53 ^b
Soaking 2	0.0025±0.0001 ^b	4.28±0.30 ^a	38.02±1.21 ^c
Boiling 2	0.0021±0.0001 ^a	2.10±0.18 ^a	26.89±1.79 ^a

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($\alpha = 0.05$).

the aromatic rings of phenolic compounds which leads to polymerization reactions or structural cleavage (Nergiz *et al.*, 2007). Moreover, high temperatures cause the release of phenolic compounds from the cell wall due to damaged cell components, so soluble phenolic compounds dissolve in boiling water (Soehendro *et al.*, 2015)

After the process of rolling 1 and soaking 1, the total phenol content did not change significantly. After soaking 1, the total phenol of lamtoro seeds decreased to 0.0065%. It occurs because the soaking process softens the grain cell wall tissue, causes polyphenols to be dissolved into water, and causes polyphenols to bind with carbohydrates or proteins (Boateng *et al.*, 2008).

The process of rolling 2 reduced the total phenol content to 0.0052%. Likewise, it occurred in the research conducted by Sinha and Kawatra (2003) with cowpeas, Han and Baik (2008) with beans, peas, and soybean, and Pal *et al.* (2016) with horsegram. Peeling off the seed coat causes the loss of a number of phenols in the grain. This happens because the seed coat contains phytochemical bonds making phenolic compounds difficult to dissolve in water (Han and Baik, 2008). It is supposed that when the seed coat is removed due to the rolling process, the phenolic compounds in the seed coat are also removed. Thus, the phenol content in the lamtoro seeds decreases.

In Soaking 2, the total phenol in lamtoro seeds decreased significantly to 0.0025% or 51.9%. Sinha and Kawatra (2004) and Siah *et al.* (2014) reveal a decrease in phenol levels in cowpeas and faba beans after soaking process. After the process of Boiling 2, the total phenol content of lamtoro seeds decreased to 0.0021%.

3.3.2 Antioxidant activity

Based on the results of the study displayed in Table 3, there was a change in antioxidant activity during the pre-fermentation treatment of lamtoro seeds in the production of tempeh. The antioxidant activities of raw lamtoro seeds were 45.01%. Lamtoro seeds decreased their antioxidant activities to 27.51% after boiling 1. In the study conducted by Silva *et al.* (2017) with a sample of green beans, boiling caused its antioxidant activity to decrease to 29.25%. Segev *et al.* (2011) on green beans and Salem *et al.* (2014) on faba beans, white beans, lentils, and fenugreek seeds also revealed a decrease in antioxidant activity due to the boiling process. This decrease occurred due to the dissolution of antioxidant compounds into the boiling water. Moreover, there is also a change in the structure of the phenolic compounds during the boiling process, so that the level of free radical scavenging also decreases (Silva *et al.*, 2017).

The antioxidant activity of the grain is also affected by the polyphenol content in the grain. In this study, there was a decrease in tannin levels to 0.19% (Table 3) and a decrease in total phenol to 0.0079% (Table 3) in lamtoro seeds after the boiling process. Tannins are one of the polyphenolic compounds in lamtoro seeds.

Rolling 1 did not affect the antioxidant activity of lamtoro seeds during the production of mlanding tempeh. This is indicated by the insignificant decrease in antioxidant activity to 26.48% in lamtoro seeds. This result is associated with the results of tannin levels and total phenol in this study which found a significant decrease in lamtoro seed samples after the process of rolling 1 (Table 3). The antioxidant activity of lamtoro seeds decreased to 16.18% after the process of soaking 1. The decrease in antioxidant activity of faba beans, white beans, lentils, fenugreek seeds, and green beans after soaking was also displayed by Salem *et al.* (2014) and Segev *et al.* (2011). Moreover, according to Cabrejas *et al.* (2009), the phenol at the periphery of the grain dissolves into the water through the seed coat during the soaking process.

The process of rolling 2 reduced the antioxidant activity of lamtoro seeds to 7.96%. This is in accordance with the research conducted by Han and Baik (2008) on green beans, green peas, yellow peas, and lentils, as well as the research of Pal *et al.* (2016) on horsegram showing decreased antioxidant activity after peeling off seed coat. This decrease in antioxidant activity occurred due to a decrease in phenolic compounds as a result of the seed coat removal after the second stripping (Han and Baik, 2008).

After the process of soaking 2, the antioxidant activity of lamtoro seeds decreased back to 4.28%. The results of antioxidant activity in lamtoro seeds after Soaking 2 were in accordance with the results of tannin levels (Table 3) and total phenol yields in lamtoro seeds after Soaking 2 (Table 3) in this study. Following the process of boiling 2, the antioxidant activity of lamtoro seeds did not change significantly.

3.3.3 Dietary fiber levels

The results of the analysis of dietary fiber content are shown in Table 3. The content of dietary fiber in raw lamtoro seeds is 61.26%. After boiling 1, dietary fiber content of lamtoro seeds decreased to 56.19%. It is because the chemical degradation of cellulose from seed cell walls into glucose and hemicellulose into arabinose, xylose, and galactose occurred during the heating process (Vidal-Vaverde and Frias, 1991; Wang *et al.*, 2009). Hemicellulose is included in Insoluble Dietary Fiber (IDF). As a result, when the hemicellulose in

grains decreased, the total dietary fiber (TDF) in these grains decreased as TDF is the sum of ISD and Soluble Dietary Fiber (SDF) (Jelita, 2011).

The dietary fiber contained in lamtoro seeds decreased to 39.27% after rolling 1. The decrease occurred due to a decrease in the content of IDF in the grains after the seed coat removal process (Wang *et al.*, 2009). The process of soaking 1 causes the dietary fiber content of lamtoro seeds to increase to 50.26%. Meanwhile, the rolling 2 reduces the dietary fiber content of lamtoro seeds to 32.07%. In the soaking 2 process, the dietary fiber content of lamtoro seeds increased again to 44.83%. After Boiling 2, the fiber content of lamtoro seeds decreased to 26.89%.

3.4 Dynamics population of microorganisms in lamtoro seeds during pre-fermentation treatment of tempeh

Based on the research results displayed in Figure 1, there are the population dynamics of microorganisms in lamtoro seeds during the pre-fermentation treatment of tempeh. After the process of rolling 1, in lamtoro seeds, yeast growth was found in lamtoro seeds at 1.33 log CFU/g. Then, after the process of soaking 1 for 24 hrs, the amount of yeast increased significantly to 6.05 log CFU/g. Like the process of producing soybean tempeh, the amount of yeast after soaking increased by 3-4 log CFU/g (Mulyowidarso *et al.*, 1989). Yeasts such as *Endomycopsis burtonii*, *Candida diddensiae*, and *Candida tropicalis* grow during the soaking process (Sutardi, 2003). Furthermore, there was a relatively small decrease in the amount of yeast to 5.94 log CFU/g in lamtoro seeds after rolling 2. The amount of yeast growing on lamtoro seeds increased slightly to 6.07 log CFU/g yeast after Soaking 2.

Meanwhile, there was a growth of lactic acid bacteria (LAB) of 4.21 log CFU/g after rolling 1. There was a significant increase in LAB amount to 9.86 logs CFU/g in lamtoro seeds after soaking 1 for 24 hrs. Mulyowidarso *et al.* (1989) reported an increase in the amount of lactic acid by 5-7 log CFU/g after soaking process. Efriwati (2013) also found an increase in the amount of LAB by 2 log CFU/g of sample after soaking process. The decrease in the pH of the soaking water from 7.17 to 5.33 also supports the growth of LAB after soaking 1 (Figure 1).

According to Mulyowidarso *et al.* (1989), there were 10 species of LAB and 4 species of yeast after the soaking process in the production of soybean tempeh. LAB growing during the soaking process were *Streptococcus faecium* and *Streptococcus dysgalactie* (Sutardi and Buckle, 2004). Bacterial growth during the soaking process is characterized by the release of an

acidic odor during soaking process and the presence of foam on the surface of the soaking water (Radiati and Sumarto, 2016). LAB growing during the soaking process increases the content of organic acids in lamtoro seeds, inhibits the growth of natural microflora such as coliforms, *Klebsiella pneumoniae*, and yeast, and extends the shelf life of tempeh (Nout and Kiers, 2004). The next process, particularly rolling 2, found a decrease in the amount of LAB to 8.87 log CFU/g in lamtoro seeds. Meanwhile, the amount of LAB growing on lamtoro seeds increased to 9.93 log CFU/g in Soaking 2.

3.5 Changes in pH value and total titrated acid in lamtoro seeds during pre-fermentation treatment of tempeh

Based on the research results presented in Figure 2, it is known that the pre-fermentation treatment affects the pH of lamtoro seeds during the production of tempeh. The lamtoro seeds after boiling 1 have a pH on seed of 7.60 and the pH of boiling water is 7.50. Furthermore, the pH of the seeds and the pH of the water in the lamtoro seeds decreased to 7.06 and 7.17, respectively, after Rolling 1 process.

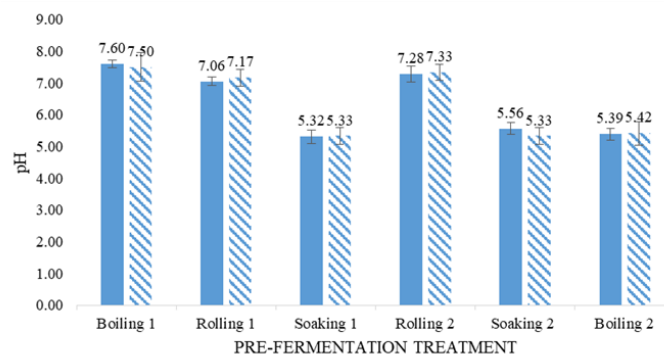


Figure 2. Changes in pH in Lamtoro seeds during pre-fermentation treatment of tempeh in the production of tempeh (■) pH of seeds (▨) pH of water.

The process of soaking 1 caused the pH of lamtoro seeds to decrease to 5.32 and the pH of the soaking water to decrease to 5.33. Buckle and Sambudi (1990) reported a decrease in the pH of Winged beans after soaking. This occurred due to the growth of LAB-producing acid during the soaking process (Mulyowidarso *et al.*, 1989; Sutardi and Buckle, 2004; Efriwati, 2013). This study also showed an increase in the amount of LAB by 9.86 log CFU/g in lamtoro seed sample after the process of soaking 1 (Figure 1). The acid content of lamtoro seeds after the process of soaking 1 increased to 3.60% from 0.82% after rolling 1 (data not presented).

The process of rolling 2 increased the pH of the lamtoro seeds to 7.28. The process of soaking 2 caused the pH of the seeds and water to decrease to 5.56 and 5.33. Like Soaking 1, the decrease in pH was due to the growth of LAB during the soaking process. In this study,

there was an increase in the amount of LAB by 8.87 log CFU/g in lamtoro seed sample after the process of Soaking 2 (Figure 1) and an increase in the titratable acidity content from 0.98% after Rolling 2 to 1.92% after Soaking 2 (data not presented). After the process of boiling 2, the pH of the seeds and water decreased insignificantly to 5.39 and 5.42.

4. Conclusion

Pre-fermentation treatment in the process of mlanding tempeh production affects the population dynamics of yeast and LAB, changes in nutritional compound levels, changes in anti-nutritional compounds and toxins levels, and changes in functional compounds in lamtoro seeds. The number of yeast and lactic acid bacteria increased during Soaking 1 or 2. This was in line with the results of pH measurements and total titrated acid in lamtoro seeds after the soaking process, in which the pH value decreased and the total titratable acidity content increased. The moisture, protein and fat content of lamtoro seeds during the pre-fermentation treatment tended to increase. Meanwhile, the levels of ash, carbohydrates, phytic acid, tannins, and mimosine as well as the levels of total phenol, antioxidant activity, and dietary fiber of lamtoro seeds during the pre-fermentation treatment decreased. Based on the results of this study, it can be concluded that the pre-fermentation in the process of producing mlanding tempeh also plays a role in increasing nutritional compounds and reducing anti-nutritional compounds and toxins.

Conflict of interest

The authors declare no conflict of interest.

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