The Utilization of Lamtoro leaves *(Leucaena leucocephala L.)* Extract as an Alternative Nitrogen Source on The Formation of Nata de Soya Cellulose from Tofu Whey Waste

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ARTICLE INFO	ABSTRACT

Nata is not only made from coconut water; it is also possible to produce it from other raw materials. In our study, nata de soya has successfully made using tofu whey as raw material. Research focused is on the utilization of lamtoro (*Leucaena leucocephala L.*) leaves as an alternative source of nitrogen in addition to the use of ammonium sulphate and urea which have generally been used in nata production. The selected research method was experimental. First, the Lamtoro leaves were mashed and then inserted into the medium and cooked until it boils. The medium was incubated at room temperature for 12-14 days and the thickness of the cellulose was then measured. The purpose of this study was to determine the effect of the using of lamtoro leaf extract (*Leucaena leucocephala L.*) as an alternative source of nitrogen for nata de soya cellulose formation. The optimization of pH and nitrogen contentration have been obyained. Three types of formula have been arranged, i.e. 1/2 of the control nitrogen concentration, equivalent, and 2 times of the control nitrogen concentration, to provide the variation concentration. Results found that the optimum pH was 3 and the optimum nitrogen concentration was ½ of the control nitrogen level. Nata de soya has then prepared using optimum condition of pH and nitrogen concentration. The maximum average of nata de soya thickness was 7.6 mm. In addition to leveraging the ability of medicinal plants and reducing the use of chemicals in the food processing, the use of alternate sources of nitrogen may also be adopted in the wider cellulose nata application.

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BACKGROUND

Food is very important for human life. the development of science and technology in the field of food will produce several new products. In general, people only consume food products without knowing the content and process of making the food product. According to Law of the Republic of Indonesia Number 7 of 1996 Article 1 Paragraph 1 Concerning Food, "Food is everything that comes from biological sources and water, whether processed or not processed, which is intended for human consumption, including food additives, food raw materials, and other materials used in the process of preparing, processing and or making food or beverages. The lack of public knowledge about the nutritional content in food has resulted health problems. So that people need food with a high enough nutritional content. One of the foods that have good nutritional content is soybeans.

Soybeans are a producer of vegetable protein that is very healthy for the body. Isoflavones are among the ingredients found in soybeans. In the term for reducing the risk of degenerative diseases, the isoflavone content is very useful. The concentration of isoflavones ranging from 130-380 mg / 100 g are presented in soy foods, such as tofu, milk, soybeans, soy flour and whole soybeans (Sutrisno, 2006).

One of the processed foods produced from soybeans is tofu. Besides that, tofu, especially for Indonesians, is a favorite food. The explanation is because tofu also has good nutritional value in addition to its inexpensive price and is very simple to obtain. Tofu is provided by the white soybean (Glycine max) fermentation process that is obtained from its extract. In many methods, tofu processing is carried out. Starting from the soybean washing part, boiling up to tofu printing. It produces two wastes from the production process, i.e. solid waste and liquid waste. Solid waste in the form of dregs of tofu is commonly used as feed for livestock. In the meantime, liquid tofu is often immediately disposed of to the environment without providing treatment and still a handful who profit from it. If this condition is not carefully presented, an environmental problem may be caused. In fact, tofu liquid waste can be further processed to produce a product that can be consumed (Muhammad, 2015; Indrawati *et al.*, 2019).

The modified product of Nata has also grown very quickly, in line with the advancement of science and technology. Nata does not only produce from coconut water; it can be made from other raw materials as well. Basic products, such as coconut water, tofu liquid waste, and pineapple waste, can be used. Nata de coco is created as a basic ingredient using coconut water, while nata de pina is made from pineapple waste water, and nata de soya is produced using tofu liquid waste. The aspect of nutritional value viewed that nata is a food product and good for consumers who are implementing a low calorie diet program. Nata de soya has a very high fiber content, so it can improve the digestion.

There are many factors in the fermentation process that can influence the quality of nata, such as microorganism as starter, sources of carbon and nitrogen. Nutrients found in the medium are converted into nata by the starters activities. The formation of nata de soya is attributable to *Acetobacter xylinum's* using glucose from carbohydrates from tofu whey waste. Then with fatty acids, glucose will be combined to form precursors on the cell membrane and enzymes will polymerize glucose into cellulose (Wisnu, 2006; Sri and Jumiati, 2019). In her research, Retni (2008) concluded that the concentration of *Acetobacter xylinum* can influence the thickness and yield of nata de Soya cellulose. The optimum concentration was obtained at 15% of starter concentration, resulting in an average thickness and cellulose yield of 1.32 percent and 22.2 g / liter, respectively. In addition to the formation of nata follicles, sugar as a carbon source has been used by Acetobacter xylinum bacterial cells for energy. Nata bacteria are able to synthesize galactose, maltose, lactose and glycerol (Yanti *et al.*, 2017)

On the other hand, one of the factors that

also influence the formation of nata is a source of nitrogen. For the growth of Acetobacter xylinum bacteria and source of nutrition, normally nitrogen was added to the fermentation medium (Ismawati, Maswati and Wahyu, 2013). Ammonium sulphate and urea are the nitrogen sources that are widely used, due to the price of this commodity is reasonably inexpensive and simple to be obtained. However, the source of nitrogen from this material is not a natural food ingredient.

Some studies to improve the consistency of nata by adding inorganic nitrogen have been carried out. Ismawanti (2013) reported the effect of adding ammonium sulphate to the thickness of nata de soya generated from tofu liquid waste. Meanwhile the consumption of ammonium sulphate in nata de banana production has been optimized by Evy (2008). However, the use of nitrogen from natural resources needs to be investigated for further study. In our study, lamtoro leaves have been selected as nitrogen source for nata the soya formation. Lamtoro is a multipurpose plant, with all parts of the plant being useful for both humans and animals. Lamtoro can grow in arid climates, villages, gardens, forests, and mountains. This plant is not only native to Indonesia, but it can also be found in almost all continent of the world. The advantages of using Lamtoro leaves are they can be functioned for nata production because has a lot of nitrogen, easily available, and has a good economic value. The leaves of lamtoro as a legume plant contain relatively high nitrogen elements compared to other plants. The nutrient content of lamtoro leaves consists of 3.84% N; 0.2% P; 2.06% K; 1.31% Ca; 0.33% Mg (Palimbungan, Robert and Faisal, 2006). Lamtoro plants are widely used as soil fertilizers, not only because of their leaves, but also because their roots contain a significant amount of nitrogen, which has aided the fertility of the surrounding soil. The presence of nitrogen in lamtoro leaves, on the other hand, can be used as one of the essential ingredients in the preparation of nata and minimize dependence on the use of synthetic material as nitrogen sources (Ammonium sulphate and urea). Based on the description above, research on the utilization of the use of lamtoro (*Leucaena leucocephala* L.) leaf extract as an alternative source of nitrogen in the formation of cellulose nata de soya from tofu wastewater has attracted researchers to be investigated more. The amount of lamtoro leaf extract needed has been correlated to the use of ammonium sulfate, which is widely applied to achieve nitrogen requirements in the nata formation process.

MATERIALS AND METHODS

The principle of research

The methodology approach in our study is an experimental method. The principle is that the bacteria will grow and reproduction during them perform to produce a cellulose layer and facilitated with glucokinase enzyme. Meanwhile, glucose in the medium will be converted by Acetobacter xylinum into glucose-6-phosphate (G6P). With the aid of the enzyme phosphoglucomutase, G6P will then be converted into glucose-1-phosphate (G1P). After that, G1P with the UDP Glucose phospholyrase enzyme will be converted back to Uridine Diphospate (UDP). With the assistance of the enzyme cellulose synthase, UDP is then converted into cellulose (Miguel, 2013). Acetobacter xylinum can replicate with denatured proteins and a cellulose layer begins to form in the next step.

Materials and Equipment

The equipment used in this study were laboratory glassware such as measuring glass, stirring rods, dropper pipettes. Besides that, calipers, baking pans, analytical balances, stoves, universal pH, rubber bands, tofu liquid waste containers, filters, paper and medium pots have been functioned as tools of work. Meanwhile, food grade ammonium sulphate, acetic acid, *Acetobacter xylinum*, sugar, lamtoro leaves, and tofu liquid waste have been chosen as materials of

research.

Procedure

In our work, a positive control of nata de soya means that ammonium sulphate has been functioned as source of nitrogen. Generally, urea was also used as a nitrogen source, but in this study ammonium sulphate has been chosen. As preliminary step, the formation of nata de soya using ammonium sulphate has been conducted. 500 mL of tofu liquid waste has been prepared and collected in the clean containers, then the waste has been stirred to homogenization process. Meanwhile, the funnel and filter have been chosen to filter, and the solution has been collected into the pan. While stirring, the filtered tofu liquid waste has been heated to a boil over heat. Glacial acetic acid was then supplemented until the medium's pH reached 3-4. Sugar has been added to medium with a composition of 80 g of sugar per 1 liter of whey, then stirred until the sugar dissolves and this solution was known as sugary acid whey. Food Quality Ammonium Sulphate (with a composition of 15.75 g for 1 liter of acid whey) has been added into solution. 1.67 g of nitrogen was set as a concentration of control nitrogen for the formulation of nata as details. In the meantime, 10.60% of the amount of nitrogen was found in the molecule of ammonium sulphate, so 15.75 g of ammonium sulphate was calculated to be modified in the medium solution.

The next step has been performed, the solution has then boiled again and resulted a nata media. Furthermore, the pan was used to collect the nata media, then immediately covered with paper and allowed to cool. The *Acetobacter xylinum* composition of 10:1 was applied to the nata medium volume and then fermented for 12-14 days. The thickness of the nata was measured using a caliper and it was observed from the third to the eighth day.

Determination of the optimum pH

In this segment, as a nitrogen sources,

lamtoro leaves have been presented to the nata de soya formation. A 500 mL of tofu liquid waste has been prepared as a medium and 27.385 g of lamtoro leaves has been added into solution, then poured into the pan. The tofu liquid waste was combined with lamtoro leaves. While stirring, the solution was heated to a boil over heat. Glacial acetic acid has been adjusted after boiling to maintenance the pH solution. The pH variation of the nata medium was identified at pH 3 and 4, respectively. While, sugar has been added with the formulation of 80 g for every 1 liter of whey. This mixture then stirred until the sugar dissolves. To separate the dregs of Lamtoro leaves with nata medium, the next stage is filtered, poured into a baking sheet and then covered with paper and allowed to cool. After that, *Acetobacter xylinum* has been added into medium with a 10: 1 of formulation volume. Nata de soya will be formed after the fermentation process for 12-14 days. The measurement of nata de soya thickness has been carried out and started on the 8th day untul 14th day of the observation.

Determination of the optimum nitrogen concentration

In general, all procedures are nearly identical to the previous step, but the concentration of lamtoro leaves has been varied in this segment. Ammonium sulphate as a nitrogen control has been fixed. Three formulas were made, i.e. the nitrogen content in lamtoro leaves was 1/2 of the nitrogen control; equivalent; and 2 times of the nitrogen control, respectively. Lamtoro leaf contain 3% of nitrogen so 55.67 g of lamtoro leaves have been prepared and equivalent with nitrogen quantity in 15.57 g of ammonium sulphate as nitrogen control. In order to provide the different concentrations of nitrogen, some lamtoro weights have been formulated. 27.83 g; 55.67 g; and 111.34 g of lamtoro leaves were weighted at 1/2 of the control nitrogen concentration, equivalent, and 2 times the control nitrogen concentration, respectively. The next process was in accordance with the previous stage, and the identification of the

thickness of nata de soya was performed in a fermentation period of 14 days.

Data analysis

The last experiment of nata de soya formation has been performed using the optimum pH and optimum of nitrogen concentration. Data analysis has been investigated to observe each experiment part and displayed in some figure. Nata de soya thickness has been fixed as a reference for the effects of the various pH and concentrations tested.

RESULTS AND DISCUSSION

First, liquid tofu waste was prepared and applied as a medium by the *Acetobacter xylinum* bacteria for the formation of nata de soya cellulose. Fresh tofu liquid waste has been selected and needed for our work. Siti (2008) has asserted this situation in her study. After studying variations in the storage time of tofu liquid waste, she confirmed that there was a significant impact on the thickness of the nata de soya.

The fresh tofu liquid waste has cloudy white color and did not have a bad smell. First, tofu liquid waste was homogenized in order to have the same composition of all ingredients. After that, to extract the sediment that was still present, the waste was then purified and put into the pan for heating. In our study, the optimization of the nata de soya formation has been conducted in two stages: the identification of the optimum pH and the optimum concentration of nitrogen formulated by the addition of lamtoro extract.

Identification of optimum pH on the formation of nata de soya has been explored in our work. In the nata medium, glacial acetic acid was applied before pH 3 and 4 were obtained. Lamtoro leaves have been chosen as a source of nitrogen for sample, while ammonium sulphate has been used as nitrogen controls. Using a caliper, the thickness of the cellulose nata de soya was calculated. Research can be displayed in Table 1 and Figure 1.

Table 1 and Figure 1. demonstrate the thickness of cellulose nata de soya on varied pH at 14th day of incubation period. The nitrogen content in lamtoro leaves has been applied in the sample and was equivalent to 15.57 g of ammonium sulphate. In this study, the optimum pH on sample was obtained at pH 3 and the average of thickness was 4.2 mm. Meanwhile at pH 4 was achieved 2.8 mm and it was lower than generated on pH 3. In the meantime, at the control medium, the optimum average of nata de soya thickness at pH 3 and 4 were attained 6.6 and 4.6 mm, respectively. The results confirmed that the optimum pH of nata formation was produced at pH 3, either by adding of ammonium sulphate or lamtoro leaves. However, there was a significant difference produced when the formation of nata de soya was carried out at pH 3 and 4. It was assumed by several factors and one of factor is due to optimum pH for *Acetobacter xylinum* bacteria during the formation of nata de soya cellulose, besides that the texture and degree of whiteness of nata color is influenced by pH. At pH 3, the color of the nata was lighter than at pH 4, the texture was suppler and the layer was thicker than at pH 4. However, when nata produced, it's important to provide a conducive condition for bacteria growth. In addition, the organoleptic form of the nata formed must be taken into considered.

Determination of optimum pH

Table 1. The thickness of nata de soya cellulose from identification of optimum pH

pH of medium	The thickness of nata de soya cellulose (mm)	
	mean	SD
control medium i.e ammonium sulphate was used as nitroge source		
pH 3	6.6	± 0.176
pH 4	4.6	± 0.193
Sample i.e lamtoro leaves were used as nitrogen source		
pH 3	4.2	± 0.172
pH 4	2.8	± 0.158
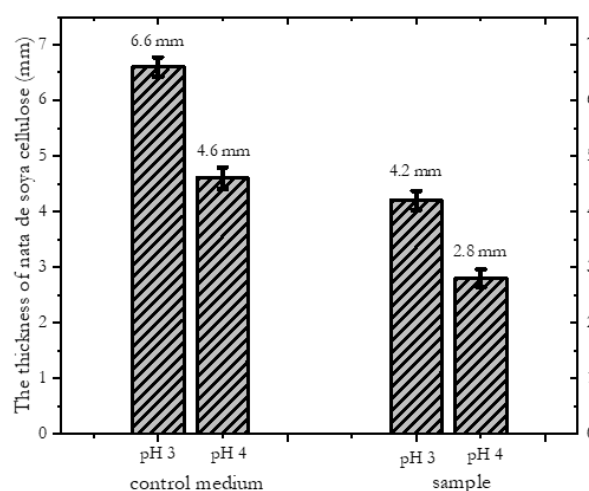
Figure 1 The thickness of nata de soya formation from varied of pH medium on sample and control medium

Meanwhile, compared with control medium, the quantity of nitrogen found in lamtoro leaves were lower. This can be seen from the results of observations made at the same pH, the thickness of the nata from the control is greater than that of the sample. However, the use of nitrogen from lamtoro leaves can be an alternative source besides the use of nitrogen from synthetic material which have been supported by ammonium sulphate or urea. By heating, this protein will be denatured, as well as acids to form amino acids that are zwitter ions and have groups of nitrogen in them. The more acidic a medium's pH is; the more amino acids will produce denatured protein. In this way, the nitrogen that *Acetobacter xylinum* can use for cell growth and enzyme formation will be optimal. As a preliminary study, the observation of pH variations from the range of 1 to 6 have been

investigated, and the optimum pH was generated between the pH range of 3 to 4. Result showed that on the experiment carried out under pH 3, the nata layer formed was extremely thin, the texture of the nata was irregular, and the acid smell was very intense. Similarly, according to the result from pH above the optimum level, the nata produced has brownish in color, an irregular texture, and brittle fiber. However, the use of a medium pH that is too acidic (pH 1-2) can disrupt the bacterial activity and this result is in accordance with the statement of Iryandi (2014) who found that *Acetobacter xylinum* can grow and evolve in acidic media with an optimum pH of 3-4. Saptarina (2017) also claims that lowering the pH of the fermentation medium causes oxidation of sucrose (a transition in sugar to acid) in *Acetobacter xylinum* cells, resulting in plasmolysis, which prevents cellulose formation and produce

a thinner layer. Meanwhile at pH condition upper than 3, it was assumed that this condition is not appropriate with the optimum condition for the *Acetobacter xylinum* growth (Iryandi, Hendrawan, and Komar, 2014)

Denatured protein also offers a benefit since the proteins that are still in the form of complex molecules cannot be extracted by microorganisms. The production of the enzyme *cellulose synthetase*, which acts as a biocatalyst for the cellulose formation response, will increase the optimal growth and development of *Acetobacter xylinum* (Evy, Usman and Damanik, 2008; Syarifah and Fatkhun, 2019)). In order to use medium glucose in combination with fatty acids, *Acetobacter xylinum* cells can form precursors on cell membranes and polymerize glucose into cellulose outside the cells along with enzymes

(Wisnu, 2006). Nitrogen deficiency causes cells to develop less well and can prevent the production of the necessary enzymes to make the fermentation process imperfect.

Determination of nitrogen concentration optimum

The next method is the determination of the optimum nitrogen concentration and was performed at pH 3 as optimum pH. The varied concentrations were arranged and this variation was based on the amount of nitrogen required by *Acetobacter xylinum*, usually adding 15.57 g of ammonium sulphate as a normal procedure. From the 3rd to the 14th day of the fermentation period, the thickness of nata de soya cellulose was observed. Figure 2 displays the results.
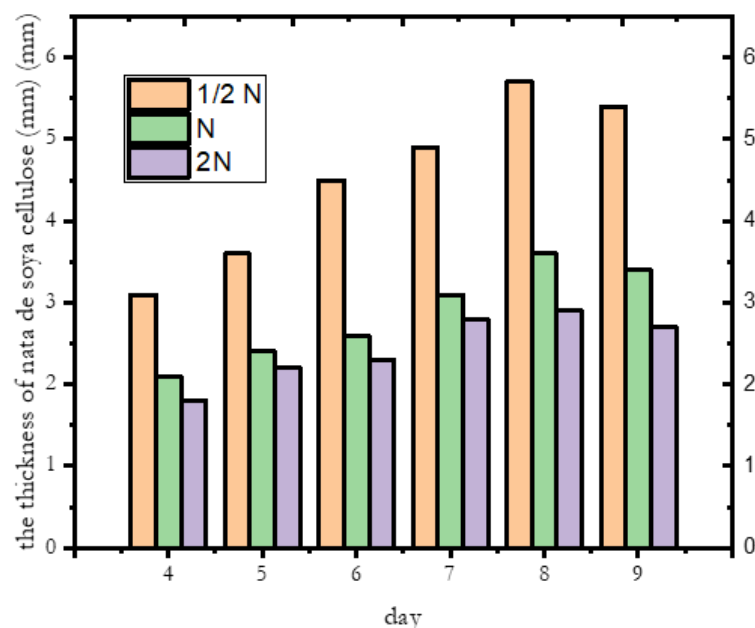

Figure 2 The evolution of nata de soya thickness to varied nitrogen concentration over 14 days of fermentation period.

Results confirmed that there was a substantial difference in the quality of nata de soya produced after the addition of nitrogen concentrations (Figure 2). It has been assumed that the existence of nitrogen has been believed

to be one of the determinants of this situation. The denaturation of protein that occurs due to heating and acid addition will have an effect on the thickness of the processed cellulose nata de soya. At the concentration of 1⁄2 control

nitrogen, the best thickness of nata de soya with an average of 5.75 mm was obtained. The thickness of the nata de soya decreases as the nitrogen content rises as well. As Ismawanti (2013) and Sulik (2007) have reported in their research, that the reduction in the thickness of nata may be influenced by the addition of excess nitrogen material. It was assumed that the pH shifts towards to an alkaline due to excessive levels of nitrogen, so the pH of the medium is not suitable for bacterial growth. Therefore, that the bacteria are denatured and that the activity of the bacteria *Acetobacter xylinum* declines. The growth of *Acetobacter xylinum* cells in the synthesis of cellulose from glucose in the medium will be stimulated by the sufficient amount of nitrogen in the medium. Thus, as a commodity, nata has strong cellulose bonds and is not easily destroyed. On the other hand, if the concentration of nitrogen in the medium is extreme, the activity of *Acetobacter xylinum* bacteria will be eliminated, thereby inhibiting the cellulose formation process (Yanti et al., 2017).

Nata de soya production with pH and and nitrogen concentration optimum

In our study, both optimal conditions of nata production has been applied for next step with an incubation period of 14 days. Result displays in Table 2. Figure 3 a shows that the average thickness of the nata de soya cellulose obtained from optimum condition was 7.6 mm. Meanwhile, 14 days of incubation time have been set and it is not recommended to do any more than that and product can be seen in Figure 3b. This is because other microorganisms, such as fungi or other bacteria, are proliferating. Besides that, the life time of *Acetobacter xylinum* was terminated due to a decrease of nutrient source in the medium. In addition, the source of carbohydrates is another aspect that can affect the formation of nata de soya. *Acetobacter xylinum* was mixed glucose in the medium with fatty acids to form a precursor to the cell membrane and glucose becomes cellulose outside the cell along with the enzyme (Wisnu, 2006).

Table 2. The thickness of nata de soya cellulose generated from pH 3 and nitrogen concentration of ½ control nitrogen

Description	The thickness of nata de soya cellulose (mm)			
	I	II	II	mean
Samples were treated at pH 3 and nitrogen contentration of ½ control nitrogen	7.5	7.8	7.6	7.6

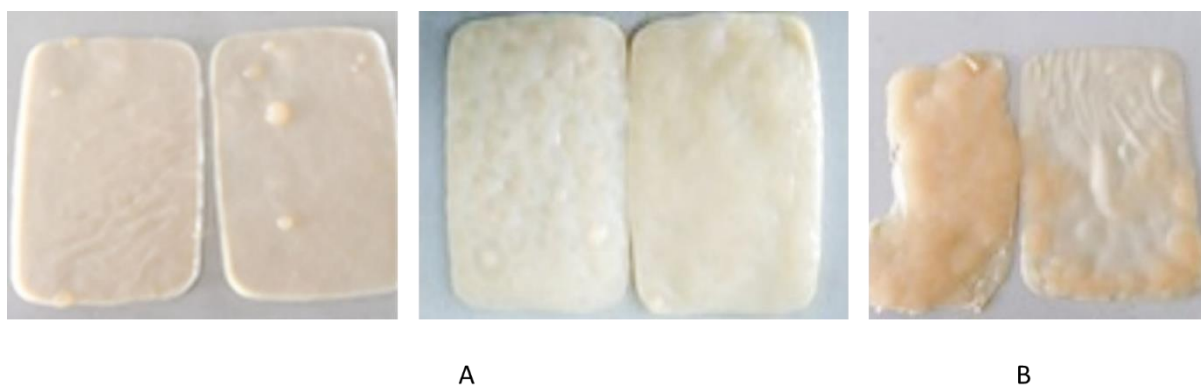A   B

Figure 3. Nata de soya cellulose is processed under the following treatments: (A) optimum pH and optimum nitrogen concentration; (B) more than 14 days of fermentation.

The source of nitrogen is the next element that is also very significant in the formation of nata de soya. Lamtoro leaves were used in this study as a nitrogen source to substitute ammonium sulphate. The quantity of nitrogen present in lamtoro leaves is 3% (Imam, 2007). However, lamtoro leaf has been active in the development of nata de soya as an alternative source of nitrogen and this can diminish the use of unsuitable nitrogen sources as ingredients for nata as a food product. Even though only ammonium sulphate in the food grade group is permitted to be added to food production. If this is allowed the use of ammonium sulphate outside the specified category, it may cause health issues such as sore throat, vomiting and stomach pain. Nonetheless, a toxicity test is required to ensure the safety of the food produced. In this way, nata de soya, which is formed from the leaves of lamtoro with a nitrogen source, can be determined to be fully safe for consumption. This needs to be further studied because it is feared that if toxic compounds are present in lamtoro leaves, they can interfere with the body's health.

CONCLUSION

Results confirmed that lamtoro leaves can be used as an alternative source of nitrogen in nata de soya formation. The optimum concentration was identified at ½ of the control nitrogen level (27.83 g of lamtoro leaves have been prepared for each one liter of tofu liquid waste), meanwhile the optimum pH was resulted at 3. The optimum average thickness of nata de soya under pH 3 and the optimum nitrogen concentration is 7.6 mm after 14 days of incubation. In our present work, the thickness of nata de soya layer produced on pH 3 was better than pH 4. On the nata formation, the pH selection has a significant impact not only for the bacterial activity which has correlated with the thickness of the nata de soya layer but also the degree of whiteness of nata de soya produced. Results have been verified that lamtoro leaves can

be used to provide the nitrogen source as an alternative material for bacteria to arrangement the cellulose of nata de soya. Furthermore, it can also be chosen as a substitute for nitrogen source in a wider application specifically for the nata production.

CONFLICT OF INTEREST

We have no conflict of interest related to this work

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