Growth dynamics of mold-yeast and bacteria during the production process of saga tauco [*Adenanthera pavonina*]

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Growth dynamics of mold-yeast and bacteria during the production process of saga tauco [*Adenanthera pavonina*]

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Abstract. Sagabean as a plant protein source, Sagabean has not been used by society. Several studies regarding Sagabean being a food product have been initiated. Sagabean can be used as tauco. The study was to observe the growth dynamics of microbe in Sagabean tauco during the production process. The manufacturing of Sagabean tauco was the same as making of soybean tauco. It was soaking, boiling pealing, soaking overnight, followed by steaming, and inoculating with tempe starter. Saga tempe was crushed then mixed with glutinous rice flour that had been roasted. After drying, it was put in the brine solution for fermentation. The observation were total microbes, total yeast-mold, total lactic acids bacteria, total protein, total dissolved solids, total acid, pH value, and ash content. The aim of this research was to see the relationship between the presence of microorganisms and their metabolic processes during tauco production. The result showed that the total number of microbes had increased during the fermentation process until a certain period, then it was constant and decreased according to the growth curve of microorganisms in general. For yeast and molds being relatively fluctuating and tending to increase. This seemed to relate to the presence of halophilic microorganisms in the product. Regarding the pH of the product during fermentation, it had a relevant value, while the ash content experienced a fluctuating value. If it had been related to the presence of microorganisms, the metabolism of the existing microorganisms would have affected the chemical properties of the saga tauco.

Keywords: Lactic acids bacteria, saga tauco [*Adenanthera pavonina*] yeast mold

1.Introduction

The import of soybeans in each period in Indonesia has increased, as an example from 2013 to 2018 with the total amount of imports in $ fluctuating but with an upward trend [1]. The use of soybeans in Indonesia is primarily for tempe, tofu, soy sauce, soy milk, and tauco. Tauco was originally produced in Cianjur, West Java [2]. Tauco can be used as a seasoning or food flavoring with a distinctive taste and is relatively durable because of its relatively high salt content [3]. Soybean as a raw material for Tauco has similar biological and chemical properties as well as functionally with Sagabean [*Adenanthera pavonina*]. The economic feasibility of the tauco production process must use at least 60 kg of soybeans once a process [4]. Thus, if Sagabeans are to be used as a substitute for soybeans, a lot of Sagabean production is needed. This is what is able to move the economy of the farming community to produce Sagabean. Sagabean are not only used as raw material for tempe but they can also be used for fresh cheese. [5] In making tauco, mold and bacteria greatly affect the quality of tauco. Several types of yeast and lactic acid bacteria had been identified in the processing of soybean tauco. In the first and second weeks, two types of yeast, namely *Sacharomyces* and *Phicia*, dominated, until the third and fourth weeks only *Sacharomces* appeared, while the fifth week onwards, lactic acid bacteria, namely *Streptococcus*. dominated [6]. Previous study reported that the dynamics of microbial

growth in tempe that were processed in different ways would provide different microbial profiles [7]. In our study, the microbiological and chemical reviews of tauco saga were examined during the production process.

2. Material and Methods

The materials used in this study were ripe Sagabean [*Adenanthera pavonina*, L] from the ITI Serpong campus, South Tangerang. Tempe starter culture was obtained from Bandung [Raprima], banana leaves which were used as a cover during first stage fermentation. The second stage fermentation used 10% salt solution. The chemicals and media used were NaOH, HCl, TCAA, H2SO4, [Merck], PDA, NA, and MRS Agar [All media from Difco].

The method in this research was descriptive quantitative. Sagabean tauco based was produced the same as manufacturing of soybean Tauco [Figure.1]. This research was repeated twice. The parameters observed were microbiological analysis of the product during the process [8] which included the total yeast mold [using PDA media], the total bacteria [using Nutrient agar], and the total lactic acid bacteria [using MRSA media]. The measurement of Total N [Kjeldahl], total dissolved solids [9] pH, and ash content was measured [10] to determine the role of the various types of microorganisms mentioned above. The experimental design for data analysis results of total microbe, total protein. pH, total acids, ash content, and total suspended solids were in Random Block Design with seven treatments [soaking process, mold fermentation, 0 weeks in the brine solution, one week in the brine solution, two weeks in the brine solution, three weeks in brine solution and four weeks in brine solutions] and the experiment was repeated twice.

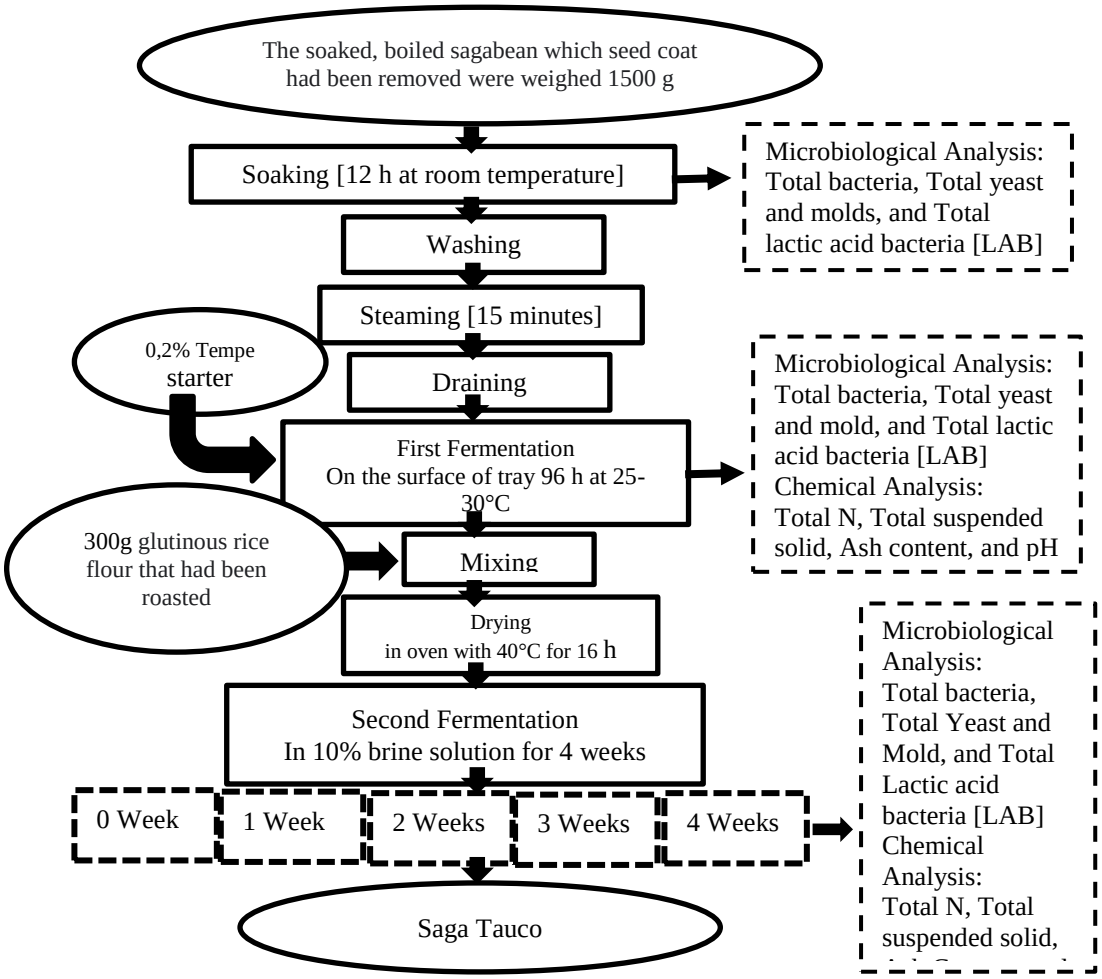

Figure 1. Research Flowchart of Saga Tauco Production Process

3.Results and Discussion

Based on the diagram in Figure 1, to carry out the saga tauco production process, two of good Sagabean were prepared then soaked insufficient clean water for 24 hours, after an imbibition event occurred, the Sagabeans were washed with water until they were clean then were boiled with boiling water for 1 hour. After 1 hour of boiling was stopped and cold water was added to make it easier to remove, separating the endosperm from the seed coat. This was to facilitate the process of stripping the Sagabean, the endosperm was then washed and soaked for 24 hours to provide an opportunity for microbes to carry out their activities. As shown in Figure 1, the microbiological analysis was carried out during the immersion including the total bacteria, the total yeast molds, and the total LAB. Then they have washed again and steamed for 15 minutes and continued with cooling to room temperature. Currently, they were ready to inoculate with tempe starter [2a]. The tempe was 96 hours old [2b] Up to this stage, it called the first fermentation process, then they were cut into small pieces and added the glutinous rice flour that had been roasted and finely ground to provide nutrients to microbes later when soaking in a salt solution [2c]. up to dry in an oven at 40 ° C for 16 hours [2d].

2a 2b 2c] 2d

Figure 2. Visualization of the endosperm of Sagabean up to first fermentation and it had been reduced in size and added with roasted glutinous rice flour ready to be continued in the second fermentation. Sagaseed endosperm that is ready to be inoculated with the tempe starter [2a], saga tempe 96 hours after fermentation [2b], saga tempe pieces mixed with roasted glutinous rice flour [2c], saga tempe pieces that have been mixed with roasted glutinous rice flour and dried in oven 40°C for 16 hours ready to process to the second fermentation with saline solution [2d]

The next step was preparing for the second fermentation in a previously prepared 10% salt solution. The tempe that had been cut into small pieces and was dried and had been added with roasted glutinous rice flour was then put in a 10% salt solution with a volume of 1000 ml [3a] while it was mixed in a jar so as the starting point of second fermentation, namely 0 weeks [Figure 3b] and incubated at 25-30 ° C for 4 weeks. Periodically, from 0 to 4 weeks of age, microbiological and chemical analyzes were carried out.

3a 3b 3c 3d

Figure 3. Fermentation II started from entering the fermentation product I into a 10% salt solution until Saga tauco was formed. Saga tempe pieces that have been mixed with roasted glutinous rice flour and dried in oven 40°C for 16 hours ready to process to the second fermentation with saline solution. Glass vessel filled with brine solution and saga tempe pieces that have been mixed with glutinous rice

flour, saga tempe pieces that have been mixed roasted glutinous rice flour and dried in oven 40°C for 16 hours ready to process to the second fermentation with saline solution [3a], the second fermentation was incubated for 0 weeks in a glass vessel containing brine solution [3b], second fermentation was incubated for 4 weeks [3c], tauco saga incubated for 4 weeks is ready for consumption [3d]

After 2 weeks of age, it could be seen that saga tauco had begun to form with a slightly brownish color change. The oil appeared at the top of the product [Figure 3c], then the mass of the tauco started to concentrate and coalesced into a compact mass, but the saga bean granules were still visible. In line with the fermentation time, the mass of the tauco became more massive and if taken with a spoon, the texture became softer, this showed that the metabolic process of microorganisms in the tauco production process carry out its function. It was due to the enzymes produced by the microorganisms that existed during the process, both from the first fermentation stage which was dominated by the molds from tempe starter, and the second fermentation stage which came from microorganisms that were resistant to salt solutions.

3.1 Profile of microorganisms during the Tauco saga production process

The role of microorganisms during the soaking process certainly affected the saga bean endosperm which would be processed into saga tauco. At least the microorganisms during soaking initiated the implementation of metabolic processes in the saga bean. This was due to the decrease in the pH of the saga bean endosperm from the initial 7.13 at the beginning of soaking and after 12 hours of soaking the pH of the saga bean endosperm fell to 6.42. This indicated the presence of organic acids produced by microorganisms during the soaking process. The number of microorganisms starting from soaking and first fermentation can be seen in Table 1.

Table 1. Profile of microorganisms during soaking and during fermentation with tempe starter or first fermentation stage in the age of 96 hours [log CFU / g product]

Batch	Number of the colony in Log CFU/g product					
	After soaking			After first fermentation with tempe starter 96 h		
	NA media	PDA media	MRSA media	NA Media	PDA media	MRSA media
1	7.477	6.623	-	7.362	8.204	6.491
2	7.146	5.623	-	7.255	8.176	6.380
Mean	7.312	6.123		7.308	8.190	6.436

The number of bacteria after soaking was much higher than that of yeast mold colonies. It could be understood that in the soaking water, bacteria and mold competition occurs. However, the LAB was not detected. This was a different result compared to the previous study. The previous study reported that the soaking water of soybean which would be processed into tempe had enough LAB colonies/g product [7]. The mold and yeast population [PDA media] in Saga tempe dominated reaching 8.19 log CFU /g of product compared to the total bacteria [NA media] which only reached 7.308 and even for the total LAB [MRSA media] only reached 6.436 log CFU/g product. This made a lot of sense because the tempe starter contains mainly the mold spores of *Rhizopus oligosporus* and *Rhizopus oryzae*. The mold and yeast present in tempe starters, hydrolyzed saga bean with the microbial enzymes they produced during fermentation into simple compounds and as a result produced organic acid which could lower the pH. This decrease in pH provided comfort for LAB as evidenced by the growth of LAB in MRSA reaching 6.436 log CFU / g of product.

The bacteria growth curve of on NA media during the incubation process from 0 weeks to 4 weeks shown in Fig 4a. Thus, it could be said that the total growth of bacteria in tauco fermentation followed the usual bacterial growth curve, namely, there was a lag phase or an early phase then an exponential

phase in the first and second weeks, and until the peak, then in the third and fourth week, it had decreased. Some of the factors determining this occurrence were the large variety of other microorganisms that contributed to the fermentation process of tauco. The variety of microbes in tauco greatly accelerated the substrate hydrolysis process, this was indicated by the increase in the total dissolved solids during fermentation [Figure. 5a]. The fermented soybean food is known for its attractive flavor, texture and superior digestibility [11] Therefore, the saga tauco also had the opportunity to be a product that was easily digested by the human body.

The dynamics of yeast and mold growth were very volatile [Figure 4b]. It was assumed that the growth of mold and yeast species alternately at the beginning was dominated by aerobic fungi, then if oxygen availability ran out, yeast was dominated by the anaerobic tolerant and relatively halophilic yeast. And near the end of the fermentation process, the number of halophilic microorganisms predominated. Usually, at the end of fermentation, salt-resistant yeast would dominate compared to other microbes.

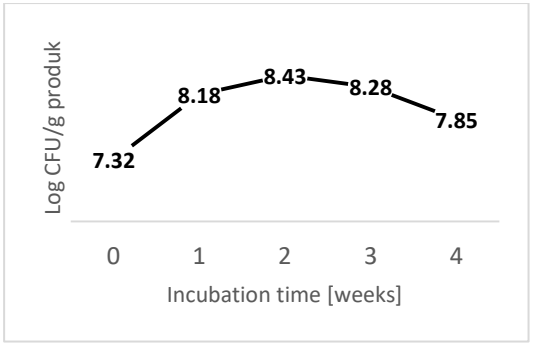

Figure 4a. Total growth of bacteria [NA media] in the Saga Tauco during the production process

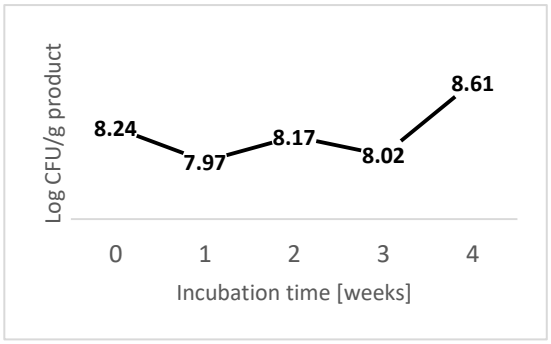

Figure 4b. Total growth of yeast and mold [PDA media] in the Saga Tauco during the production process

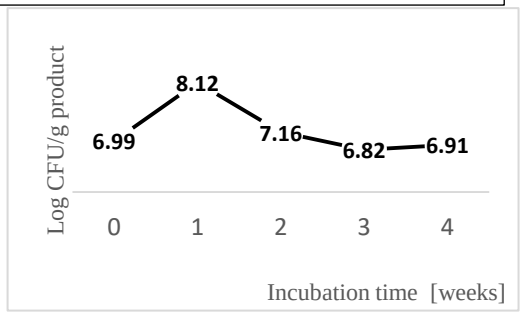

Figure 4c. Total growth of LAB [MRSA media] in the Saga Tauco during the production process

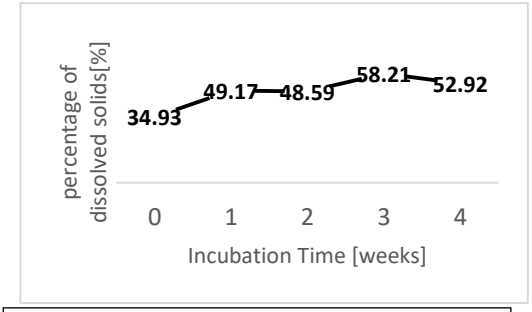

Figure 5a. The percentage of total dissolved solid in the Saga Tauco during the production process

During the fermentation process in a salt solution, all salt-resistant microbes, be they lactic acid bacteria [LAB], Yeast or even molds, synergized or might compete to hydrolyze the existing substrate so that the total dissolved solids increased as well as the pH value tended to rise slowly. When compared to Figures 4b and 4c there was a clear correlation in the first week of the maximum amount of LAB in contrast to minimal mold and yeast. It might be possible that LAB produces antimicrobials substance so that mold and yeast life was suppressed. This result was in line with previous research which proved that LAB was able to suppress the growth of mold and yeast at the beginning of tempe fermentation [7]. In contrast to the research conducted by Feng et al 2005 [12], who reported that the growth of *Rhizopus oligosporus* on grain fermentation was not affected by the presence of LAB. The presence of LAB in saga tauco is very beneficial because it had the potential as a probiotic

microorganism. Other researchers reported that traditional fermented food in Indonesia was a potential source of probiotics [13].

3.2 Chemical Analysis in Saga Tauco during the production process

The total dissolved solids in tauco during the production process had increased, [Figure 5a] indicating that tauco was a product rich in nutrients, at least a lot of dissolved solids that facilitated the absorption system of the human body. This was due to the many microbial enzymes in tauco that came from yeast, mold, and LAB that were present in tauco during the production process. The total nitrogen in tauco during its production process fluctuated which was not too different, while the pH value tended to increase slightly. The pH value of the saga tauco ranged from 4.5 to. 5.03 was due to the action of LAB. This was caused by protein hydrolysis by the protease enzyme from microorganisms, leading to the breakdown of amino acids into volatile compounds such as ammonia. Likewise, the ash content in the saga tauco during the production process slightly increased. [Table 2]. A previous study reported that LAB produced organic acids which contributed to the sensory value of the fermented product [14]. In addition to the sufficient total dissolved solids, tauco made from soybean also contained sufficient antioxidants and total phenol which have a positive effect on body health. [15].

Table 2. Total Nitrogen content, pH value and Ash content of saga tauco during production process*

Incubation time [week]	Total N content [%]	Total ash content [%]	pH value
0	13.80^{a}	6.85^{c}	4.50^{a}
1	13.68^{a}	5.84^{a}	4.80^{b}
2	13.53^{a}	5.94^{a}	4.83^{b}
3	14.05^{a}	6.34^{b}	4.82^{b}
4	14.08^{a}	6.14^{ab}	5.03^{c}

*average of two replicates. the same letter in the same column shows no significant difference

4.Conclusion

In saga tauco production process, this existing microbe's growth was very dynamic and contributed to the nutritional value of tauco proven by the increasing dissolved solids.

Acknowledgments

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