Application of quick tempe technology for production of overripe tempe

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Abstract. Recent studies indicated overripe tempe potencies as source of umami flavor. However, further development of overripe tempe as food ingredient was hindered by its long production time compared to tempe and its unavailability in commercial market. In this study, quick tempe technology that replace natural lactic acid fermentation with Glucono Delta Lactone (GDL)-chemical acidification, was applied to shorten the production time of overripe tempe. In this study, three variations of GDL-chemical acidification were applied, whether it is applied in the first soaking (Tempe GDL I), second soaking (Tempe GDL II), or both soaking stages (Tempe GDL III). Visual documentations were done every 12 hours to observe mycelium growth rate and the fermentation time required to produce overripe tempe. Each variation show different mycelium growth rate. Tempe GDL I became overripe tempe after 72 hours starter inoculation, while GDL II and GDL III started to become overripe tempe after 96 hours of starter inoculation. The result suggest different method of GDL-chemical acidification for different target of production, whether it is targeting the production of fresh tempe, overripe tempe, or tempe that will be further processed as ingredient.

Keywords: tempe, technology, overripe tempe

1. Introduction
Tempe is a traditional fermented food product originated from Java, Indonesia, that made from soybean seeds by using Rhizopus spp. into a compact solid form with slightly gray-ish white color and has distinctive tempe aroma [1]. A further tempe fermentation could be developed into overripe tempe, which most of the protein content has been breakdown into free amino acids with the composition of glutamate and aspartate and causes overripe tempe to have a delightful ‘umami’ taste and pungent aroma, which are potential to be used as a raw material in flavour enhancing industries [2-3].

After inoculation, the fermentation process for tempe requires up to 48 hours (two days) and 120 hours (five days) for overripe tempe [4-5]. By the addition of Glucono Delta-Lactone (GDL) as chemically acidifying agent the fermentation time could be reduced up to 12 hours, which took 36 hours with no changes of taste and aroma of tempe due to the odourless and tasteless character [5-8]. GDL could also save water usage during production, decrease water waste, and produce more environmentally friendly waste [9].
By using GDL acidification, it is expected to produce tempe in shorter production time, higher consistency level, with the expected quality so that further could increase the tempe production and be produced not only in tropical countries [10]. In this study, three variations of GDL-chemical acidification were applied to find the best application of GDL in tempe production.

2. Materials and Methods

2.1. Materials
Soybean as the raw materials was obtained from Pasar Modern BSD, Tangerang Selatan, Indonesia while tempe starter Raprima® was obtained from Rumah Tempe Indonesia in Bogor and technical grade glucono delta-lactone (GDL) obtained from Tristar Chemical.

2.2. Overripe tempe production
Overripe tempe was produced using method described in previous study [6-8, 11] with modification. The soybean was washed, sorted, soaked twice, boiled for 30 minutes every after soaking, drained, dried, cooled down and then de-hulled. In natural acidification, soaking was done in water over night twice at room temperature (25°C, 2x 12 hours) resulting Tempe Control. Three variations of GDL-chemical acidification using 4% GDL for 2 hours were applied to replace water, whether it is applied in the first soaking (Tempe GDL I), second soaking (Tempe GDL II), or both soaking stages (Tempe GDL III). For each variation of soaking, pH evaluation was done to the soaking solution in the first and second soaking before and after boiling. Solid-state fermentation was started by the inoculation of Raprima® tempe starter with ratio 2 g inoculum for each kilogram of soybean and take place at room temperature for 96 hr further fermentation was done to obtain overripe tempe.

2.3. Sensory evaluation
Visual observation was done every 12 hours after tempe starter inoculation up to 96 hours fermentation period. Sensory evaluations by trained panellist were done to evaluate taste, aroma, and texture of raw and oven dried tempe resulted after 48 hours fermentation. Prior to the analysis, the panelist went through 50 hr training of sensory attributes and protocol, taste, aroma, and flavor recognition and ranking, vocabulary training, attribute generation, and descriptive evaluation. During focus group discussion, the panelists were requested to generate attributes related to tempe and to describe the samples according to the attributes defined.

3. Results and Discussion
Soaking of soybeans is one important steps in tempe processing aimed to hydrates the beans, allowing the increment of moisture content up to three times greater than the original soybean and the size [5]. This process also allows lactic acid bacteria to lowers the pH of soybeans, providing a favorable environment for the molds to grow and eliminate the undesirable contaminant bacteria [12]. It had been stated that acidified beans allow profuse growth of pathogenic contaminants [13]. The usage of chemically acidification using gluconodeltalactone (GDL) in production of tempe had been patented for its ability to speed up the soaking process from 20-30 hours of natural acidification [12] into 30-150 minutes without significant effect to the flavor profile of resulted tempe [7-8] and its ability to save up water usage and increase potential profit up to 50% due to the process efficiency [9-10].

Evaluation of the soaking solution after soaking and subsequent boiling showed large pH gap between the soaking solution after natural acidification and chemical acidification while boiling did not affect the pH significantly. The pH of the GDL soaking solutions were in the range of 1.8 - 2.7, more acidic than the soaking water for natural acidification that were in the range of 5.3 - 6.8 (Table 1). However, the chemical acidification only held for 2 hours whereas the natural occurs for 12 hours and the lower pH might be required to ensure the acidification of the soy cotiledon in GDL soaking treatments.
Tempe soaked with natural acidification supports lactic fermentation which lactic acid bacteria dominates during the process that contributes to low levels of pathogenic and spoilage microorganisms in tempe such as *Salmonella*, *Enterobacter aerogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas cocovenenans*, and *Clostridium botulinum* [13]. The lactic acid bacteria is still presence during solid state fermentation and reported it is increased until $10^6$ CFU/g and considered that it has a potential to improve quality and safety of tempe [14]. In this study, the mold growth in solid state fermentation of tempe processing was observed every 12 hours after the inoculation of tempe starter in soybean that went through GDL-chemical acidification in comparison to the natural acidification.

### Table 1. pH value of soaking water in soybean processing prior tempe starter inoculation

| Samples | Treatments                  | pH  |
|---------|-----------------------------|-----|
| Control | 1st soaking (water, 12 hours, 25°C) | 5.6 |
|         | 1st boiling (water, 30 min, 100°C) | 6.4 |
|         | 2nd soaking (water, 12 hours, 25°C) | 5.6 |
|         | 2nd boiling (water, 30 min, 100°C) | 6.8 |
| GDL I   | 1st soaking (GDL 4%, 2 hours, 25°C) | 2.4 |
|         | 1st boiling (GDL 4%, 30 min, 100°C) | 1.8 |
|         | 2nd soaking (water, 12 hours, 25°C) | 5.6 |
|         | 2nd boiling (water, 30 min, 100°C) | 5.5 |
| GDL II  | 1st soaking (water, 12 hours, 25°C) | 5.3 |
|         | 1st boiling (water, 30 min, 100°C) | 6.8 |
|         | 2nd soaking (GDL 4%, 2 hours, 25°C) | 2.7 |
|         | 2nd boiling (GDL 4%, 30 min, 100°C) | 2.3 |
| GDL III | 1st soaking (GDL 4%, 2 hours, 25°C) | 2.4 |
|         | 1st boiling (GDL 4%, 30 min, 100°C) | 1.8 |
|         | 2nd soaking (GDL 4%, 2 hours, 25°C) | 2.8 |
|         | 2nd boiling (GDL 4%, 30 min, 100°C) | 2.2 |

All variation of GDL chemical acidification enhance the mould growth as shown by shorter time required to form a compact cake (24 hours) and to cover all soybean by the white mycelium (36 hours) and to reach the overripe state in comparison to control. GDL III was shown to require lowest time to produce overripe tempe followed by GDL II, GDL I, and then control (Table 2). GDL acidification process provide desirable environment for tempe mold growth without domination of lactic acid bacteria, and therefore reduce the competition even more for the mold to grow and produce mycelium. Therefore in GDL III where natural acidification process was completely eliminated from the process, shorter time was required for the mold to reach lag, stationary, and death phase as shown in the speed of overripe tempe production process after starter inoculation. It is interesting that GDL I and GDL II have different speed to reach overripe state due to the different sequence of natural and chemical acidification. The later GDL-chemical acidification in the 2nd soaking treatment followed by boiling in GDL II might reduce the domination of lactic acid bacteria greater than those in GDL I and therefore support the mould growth better.

Tempe resulted from 48 hours solid state fermentation of soybean that went through GDL-chemical acidification was then evaluated for its sensory characteristic in comparison to those went through natural acidification (Tempe Control). The sensory evaluation showed that in tempe GDL II there was slight acidic taste and aroma detected while in tempe GDL III there was also bitter taste detected, whereas both taste and aroma were still acceptable as normal tempe sensory character, they were not detected in Tempe Control (Table 3).
| Hours | Control | GDL I | GDL II | GDL III |
|-------|---------|-------|--------|---------|
| 0     | ![Image](image1.jpg) | ![Image](image2.jpg) | ![Image](image3.jpg) | ![Image](image4.jpg) |
| 12    | ![Image](image5.jpg) | ![Image](image6.jpg) | ![Image](image7.jpg) | ![Image](image8.jpg) |
| 24    | ![Image](image9.jpg) | ![Image](image10.jpg) | ![Image](image11.jpg) | ![Image](image12.jpg) |
| 36    | ![Image](image13.jpg) | ![Image](image14.jpg) | ![Image](image15.jpg) | ![Image](image16.jpg) |
| 48    | ![Image](image17.jpg) | ![Image](image18.jpg) | ![Image](image19.jpg) | ![Image](image20.jpg) |
| 60    | ![Image](image21.jpg) | ![Image](image22.jpg) | ![Image](image23.jpg) | ![Image](image24.jpg) |
| 72    | ![Image](image25.jpg) | ![Image](image26.jpg) | ![Image](image27.jpg) | ![Image](image28.jpg) |
| 84    | ![Image](image29.jpg) | ![Image](image30.jpg) | ![Image](image31.jpg) | ![Image](image32.jpg) |
| 96    | ![Image](image33.jpg) | ![Image](image34.jpg) | ![Image](image35.jpg) | ![Image](image36.jpg) |
Glucono Delta Lactone is simply the dry form of gluconic acid which is obtained by the removal of water during the crystallization process. When it is dissolved in water, the reaction will reverse, resulting in a solution of gluconic acid. Gluconic acid is a molecule of glucose in which the aldehyde function has been oxidated to an organic acid which occurs naturally in fruits, plants, honey (up to 1%) or wine (up to 0.5%) [15]. It is very unique compared to other acidulants do to its structure; when it is mixed with aqueous solution, it initially tastes sweet then slowly hydrolyzes to acidic and further the final flavor is much less tart than other common food acids. The relative sourness of gluconic acid is only 1/3 of lactic and citric acids and ¼ of acetic, tartaric and malic acids [15].

| Table 3. Sensory evaluation of tempe resulted from GDL-chemical acidification process |
|---------------------------------|---------------------------------|---------------------------------|
| Raw Tempe                       | Tempe GDL I                     | Tempeh GDL II                   | Tempeh GDL III                  |
| • Similar taste to Tempe Control| • Similar taste to Tempe Control with slight acidic taste | • Similar taste to Tempe Control with slight bitter taste |
| • Compact texture but slightly harder than Tempe Control | • Compact texture but slightly harder than Tempe Control | • Compact texture but slightly harder than Tempe GDL I and II |
| • Fully covered with white mycelium | • Fully covered with white mycelium | • Fully covered with white-grey mycelium |
| Grilled tempe (Oven180° C, 30 minutes) |                                      |                                 |
| • There is no significant taste and no after taste, similar to Tempe Control | • Acidic after taste is detected, but in overall similar taste to Tempe Control | • Acidic and bitter after taste is detected, but in overall similar taste to Tempe Control |
| • Beany aroma, similar to Tempe Control | • Beany aroma, similar to tempe control but with additional acidic aroma | • Beany aroma, similar to Tempe Control |

4. Conclusion
Considering the production time, GDL-chemical acidification in both soaking or 2nd soaking step prior tempe starter inoculation seemed to be more suitable for the production of overripe tempe. Though such application of GDL-chemical acidification may result in the more detectable sourness and bitterness of resulted tempe, the overall taste and aroma was still accepted as normal tempe. Further processing of resulted tempe and overripe tempe into ingredient might be beneficial in masking the acidic and bitter taste and aroma. While GDL-chemical acidification in the first soaking seemed to be more suitable to produce fresh tempe as it stayed longer as tempe and had no taste and aroma difference compared to those tempe produced with natural chemical acidification process.
Further microbial observation to analyze the mould growth over bacterial competition might be beneficial to be conducted in the next study.

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