

Impact of Concentration of Glucono Delta Lactone (GDL), Tempe Starter and Soaking Time to Physical Characteristics of Tempe and Overripe Tempe

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Abstract

Tempe production requires 48 h of fermentation and another 72 h for overripe tempe. Adding Glucono Delta-Lactone (GDL) in soaking process could reduce the time, however appropriate concentration of GDL, tempe starter and length of soaking time are still unknown. In this study, tempe and overripe tempe were produced with variations of GDL concentration (4 g/l, 12 g/l, 20 g/l, 28 g/l and 36 g/l), tempe starter (2 g/kg, 3 g/kg, 4 g/kg) and soaking time (60 min, 90 min, 120 min, 150 min, 180 min). The best applications were selected based on the time required for fermentation from fresh tempe to overripe tempe and from visual, colorimeter evaluation for color and texture characteristics using penetrometer. As result, soaking with 36 g/l GDL concentration for 180 min with 4 g/kg tempe starter produced firm, full coverage of white mycelium and requires 54 h – 72 h to be overripe. Tempe firmness texture was observed having value of 25.53 ± 2.31 N, tempe color index value was determined as: $L^* 75.76 \pm 4.85$; $a^* 7.59 \pm 0.93$; $b^* 19.08 \pm 1.62$. Whereas, soaking with 36 g/l GDL concentration for 120 min with 4 g/kg tempe starter produced firm but visible grains and required 30 – 36 h to be overripe. Texture observed was 8.96 ± 1.78 N, color $L^* 50.39 \pm 5.61$; $a^* 10.75 \pm 0.69$; $b^* 25.90 \pm 1.73$. Best application for fresh tempe is 36 g/l GDL for 180 min with 4 g/kg starter; while for overripe tempe is 36 g/l GDL for 120 min with 4 g/kg starter. The protein profile compared shown significant different of the protein content, soluble amino acid and protein digestibility.

Keywords: Tempe, Overripe Tempe, Glucono Delta-Lactone, Soaking Time, Protein

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 grayish white color and has distinctive tempe aroma [1]. After inoculation, the fermentation process for tempe requires up to 48 hours (two days) and 120 hours (five days) for overripe tempe [2]. The mold fermentation which occur on tempe production is stop and taken over by the bacteria for further fermentation causing white mycelium on the fresh tempe into yellowish-brown velvety surface with softer consistency [3]–[5]. During the bacteria fermentation, proteins are hydrolysed which increases the amount of nitrogen, while fat are also hydrolysed causing pungent odor and softer texture [6]. There are 17 amino acids occurred in overripe tempe, with glutamic acid is the most abundant amino acid [7]. Overripe tempe has been found that it could be used as food ingredients and flavour enhancer that has natural ‘umami’ flavour due to the glutamic acid [8]. Glutamic acid was found to be the important substance for umami taste and has been used for savory seasonings around the world, although its taste is masked by flavors from fat or herbs [3].

The problem of long production time, especially in soybean soaking steps before fermentation could take place, has been of interest in many studies. Addition of Glucono Delta-Lactone (GDL) as chemically acidifying agent in the soaking process could reduce total production time up to 12 hours, which took 36 hours of fermentation with no changes of taste and aroma of tempe due to the odorless and tasteless character of the GDL solutions [5], [9], [10]. GDL itself had been recognized as safe (GRAS/Generally Recognized as Safe) and had usually been used in silken tofu production [11]. GDL could also save water usage during production, decrease water waste, and produce more environmentally friendly waste [9].

The exact formula for implementation of GDL in tempe production, however, is varied between studies with broad range. It is important to determine an appropriate range of GDL concentration used for soaking the soybean, especially due to the price of GDL that might have significant impact to production cost of tempe. It is also important to

understand how the GDL solution is affecting the tempe itself in relation to rate of fermentation to produce fresh tempe and further fermentation to overripe tempe. This study objectives were to obtain the appropriate concentration of GDL in tempe and overripe tempe production, the length of soaking time, and the amount of tempe starter needed in such production. Characteristics of tempe produced were observed as texture, color, and the protein profile. By understanding this basic production parameters, further studies related to implementation of GDL soaking process in tempe making could be explored.

Materials and methods

Raw material of soybean was obtained from local market in Tangerang (Indonesia), while tempe starter used was Raprima (RTI, Bogor, Indonesia). Soaking of soybean was done by diluting variable amount of GDL (Tristar Chem, Surabaya, Indonesia), from 4 to 36 g in one liter of water. Standard tempe making was used based on procedure set by Rumah Tempe Indonesia (RTI, Bogor, Indonesia).

The determination of GDL concentration was done by diluting 4, 12, 20, 28, and 36 g of GDL in one liter of water and used in soaking of soybean for 120 minutes, followed by tempe fermentation using 2 g of tempe starter per kg of dry soybean. Observations were made by measuring the texture using GY-4 Fruit Penetrometer Total (NK HP Series, China) for firmness, and determining the color using colorimeter colorimeter PCE-SCM7 (England).

Further step was determining the appropriate amount of tempe starter, by implementing the result from GDL concentration determination experiment. The amount of tempe starter was increased from 2 g/kg dry soybean to 3 g/kg and 4 g/kg dry soybean. The resulted tempe was observed of its texture, color, and time to reach the fresh tempe and time needed to reach overripe tempe state. Short time of tempe fermentation indicates that the process is suitable for fresh tempe production, while the time required to reach overripe tempe indicates its suitability of overripe tempe production.

After the amount of GDL concentration and amount of starter were determined, the process was repeated to determine the appropriate soaking time to produce fresh tempe and overripe tempe. The soaking time variables were 60, 90, 120, 150, and 180 minutes in predetermined GDL concentration, followed by fermentation using predetermined amount of tempe starter. The tempe firmness texture, color, and protein characteristics were measured. Protein was determined using Lowry method [10] by Biuret and Folin-Ciocalteu reagent (Merck, Germany). A 0.3 ml of sample extract was mixed homogeneously with 1.5 ml biuret reagent and was left for 10 minutes in room temperature. Then, 75 μ l Folin-ciocalteu (1:2) reagent was added, homogenized and incubated in room temperature for 30 minutes. The samples were analyzed using spectrophotometer (GENESYS 10S UV-Vis, Thermo Scientific, USA) at wavelength of 650 nm. The result was recorded and interpolated with the standard curve using BSA (Sigma-Aldrich, Germany) as the substrate. Soluble amino acid was determined by preparing sample added with 500 μ l distilled water and with phosphate buffer pH 8. The solutions were incubated for five min at 37°C. Then 750 μ l 10% of TCA (Merck, Germany) was added and centrifuged at 10.000 rpm for 10 minutes (Rotina 35R Hettlich, Germany). 300 μ l supernatant was collected, followed with the addition of 1000 μ l 0.5M Na₂CO₃ (Merck, Germany) and 200 μ l Folin-Ciocalteu reagent. The mixtures were analysed using UV-Vis spectrophotometer at 578nm against tyrosine standard curve. Protein digestibility was measured by digesting the protein using porcine pancreatin solution (Sigma-Aldrich, Germany) for 5 minutes in 37 °C. The protein after digestion was measured in similar method using spectrophotometer at 578nm against tyrosine standard curve.

Statistical analysis using t-Test (Paired Two Sample for Means, $p < 0.05$) were conducted to observed the differences in protein content, amino acid, and digestibility of fresh, overripe, and commercially available tempe.

Results and discussion

Standard tempe was produced using natural acidification method as reference point both for fresh tempe and overripe tempe (Fig. 1), and having the characteristics as presented

in Table 1. This definition is important because the Indonesian National Standard for tempe only shows qualitative reference of color and texture [1].



(A)

(B)

Fig.1 Standard reference tempe produced by natural acidification;

(A) fresh tempe; (B) overripe tempe

Table 1. Texture and color of standard reference tempe

| Standard | Firmness Texture (N) | Color | | |
|---------------------|----------------------|---------------|--------------|---------------|
| | | L* | a* | b* |
| Fresh Tempe (FT) | 14.79 – 21.39 | 55.53 – 84.11 | 4.18 – 9.55 | 11.03 – 26.74 |
| Overripe Tempe (OT) | 10.11 – 24.09 | 31.76 – 61.92 | 8.18 – 12.05 | 17.87 – 33.11 |

GDL Concentration Determination

Tempe was processed in five different variations of GDL concentration, ranging between 4 g/l to 40 g/l, which was based on previous researches. GDL was once used with range concentration 0.4% - 1.4% or 4 g/l – 14 g/l [12], however tempe made with concentration 4 g/l had low reproducibility. Another study showed successful tempe and overripe tempe production with GDL concentration 40 g/l [10]. Therefore, in this study five points were taken, taking 4 g/l as the minimal and 40 g/l as the maximal, resulting 4 g/l, 12 g/l, 20 g/l, 28 g/l and 36g/l as the points. Upon observation, soybean soaked by GDL concentration less than 28 g/l have a poor reproducibility in tempe fermentation. In several repetition, the fungal mycelium was not fully covered the soybean or unpleasant smell was detected, indicating that the fermentation condition was not reached through the acidification. There are several purposes of soaking process, namely to penetrate a desirable pH onto the soybean for the growth of mold later in the mold fermentation process, which is between pH range 3.5-5.2, and to prevent the growth of undesired microorganism such as pathogenic bacteria [13], [14]. Variations of GDL concentrations resulting in different of pH value. As the GDL concentration increases, the pH decreases. The pH of the GDL

soaking solutions were more acidic than the soaking water for natural acidification. However, the chemical acidification was only held for 120 minutes or two hours whereas the natural acidification is usually conducted for 24 hours. It is suspected that the cotyledons did not have pH within the range yet, and it had been stated that acidified beans allowed profuse growth of pathogenic contaminants [15]. The characteristics of tempe produced by 28 g/l and 36 g/l of GDL solution is presented in Table 2.

Table 2. Characteristics of tempe produced in various GDL concentration

| Sample Code | pH | Δt | Firmness Texture (N) | Color | | |
|-------------|-----|-----|----------------------|------------|------------|------------|
| | | | | L* | a* | b* |
| FT-28 | 3.6 | 48h | 18.99±3.21 | 69.51±1.16 | 8.19±0.65 | 20.24±1.32 |
| OT-28 | 3.6 | | 11.44±0.76 | 61.46±1.22 | 9.03±0.81 | 24.15±3.10 |
| FT-36 | 3.4 | 51h | 20.74±2.07 | 70.36±0.50 | 7.55±0.45 | 19.02±0.88 |
| OT-36 | 3.4 | | 6.53 ± 1.37 | 47.29±6.00 | 10.31±1.40 | 23.93±1.17 |

Sample Code: FT (fresh tempe); OT (overripe tempe) – XX (concentration of GDL in g/l)

Tempe Starter Determination

GDL concentration of 28 g/l and 36 g/l were used in further processing and the amount of 3 g/kg and 4g/kg of tempe starter were used. The usual concentration of tempe starter used in natural tempe is 2 g of inoculum, however the chemically acidified tempe production with GDL reduced the soaking time, whereas the aim of soaking process is for acidification or acid fermentation. It allows the growth of lactic acid bacteria which will lowers the pH of soybean, provide favorable environment and eliminate undesirable contaminant bacteria. Reducing soaking time might cause a not completed process of elimination of contaminant bacteria and successful tempe might not be obtained, thus this stage is expected to overcome that problem by increasing the amount of inoculum or tempe starter. The result of tempe produced using various tempe starter is presented in Table 3.

As shown in Table 3, from the visual character that all of the tempe surface were covered by mycelium and the cotyledons were visible very slightly, which all of the samples were considered as successful tempe. All the color index value were within the standard range.

Whereas, from the texture character, all of the sample were firm and compact and the cotyledons did not move when pressure were given. However, analysis using penetrometer, the FT-28-4, FT-36-3, FT-36-4, were harder than the standard. The tempe starter concentration then selected from the greater time differences needed from fresh tempe to overripe tempe. Thus, tempe starter concentration of 4 g/kg dried soybean that gives more than 54 hours of time differences was selected.

Table 3. Characteristics of tempe produced in various amount of tempe starter

| Sample Code | Firmness Texture (N) | Color | | |
|-------------|----------------------|--------------|--------------|--------------|
| | | L* | a* | b* |
| FT-28-3 | 20.20 ± 2.12 | 70.41 ± 1.57 | 7.29 ± 0.59 | 18.50 ± 1.51 |
| FT-28-4 | 24.28 ± 1.05 | 73.60 ± 0.48 | 7.15 ± 0.72 | 18.75 ± 1.50 |
| FT-36-3 | 21.57 ± 1.88 | 72.34 ± 4.93 | 7.29 ± 0.58 | 19.85 ± 1.25 |
| FT-36-4 | 26.21 ± 1.49 | 75.27 ± 1.30 | 7.11 ± 0.12 | 18.15 ± 0.10 |
| OT-28-3 | 11.75 ± 0.50 | 52.86 ± 4.35 | 10.27 ± 1.22 | 26.10 ± 0.40 |
| OT-28-4 | 18.42 ± 0.85 | 55.36 ± 2.61 | 10.94 ± 2.84 | 26.17 ± 0.43 |
| OT-36-3 | 15.98 ± 1.36 | 48.77 ± 4.48 | 10.54 ± 0.02 | 26.50 ± 2.61 |
| OT-36-4 | 21.65 ± 6.66 | 56.20 ± 6.73 | 12.19 ± 2.08 | 29.53 ± 2.32 |

Sample Code: FT (fresh tempe); OT (overripe tempe) – XX (concentration of GDL in g/l) – Y (amount of starter in g/kg)

Soaking Time Determination

Soybean was processed with the same method as before to produce tempe with the selected GDL concentration of 28 g/l and 36 g/l and selected tempe starter concentration 4 g/kg, with varied of soaking time, from 60, 90, 120, 150 and 180 minutes. The soaking time written on the patent is the range of 90-240 minutes [12], but the best length was not determined.

The soybean soaked in 60 minutes was found to be difficult to be dehulled thus further process could not be conducted. This soaking time is then eliminated. The 90 minutes soaking time resulting in hard tempe, that is difficult to be cut using knives. Based on this observation, the 90 minutes soaking time is also deemed not suitable. Among 120, 150

and 150 minutes of soaking time that resulting in acceptable tempe, the best length for fresh tempe production was the 180 minutes in 36 g/l GDL concentration, which is selected based on the ripeness time requirement. It shows the longest time to stand as fresh tempe (60 hours). This might happen due to the long soaking process with collaboration of more concentrated solution of of GDL resulted a bigger chance to lower the pH of soaking water due, make an optimum condition for mold growth development yet also prevent pathogenic contaminants. The long soaking process also contributes to an easier de-hulling process, which is important due to the hull is considered as barrier because it blocks mold enzymes to digest the cotyledons and resulted into a tender tempe, which in fact, the mycelium will penetrate into several layers of soybean cells up to 25% of the width of cotyledon [16]. In addition, soaking also increase the moisture content of the beans up to 62-65% and render the beans edible [17]. Whereas for the best length for overripe tempe was 120 minutes of soaking time with also the 36 g/l of GDL concentration. Inversely from the long soaking time, shorter soaking time could gave insufficient time of pH penetration onto the cotyledons which further have not prevent the bacterial growth. The time required to reach overripe tempe from fresh tempe was about 51 hours. The characteristics of tempe produced in different soaking time is presented in Table 4. The appearance of tempe produced is presented in Figure 2.

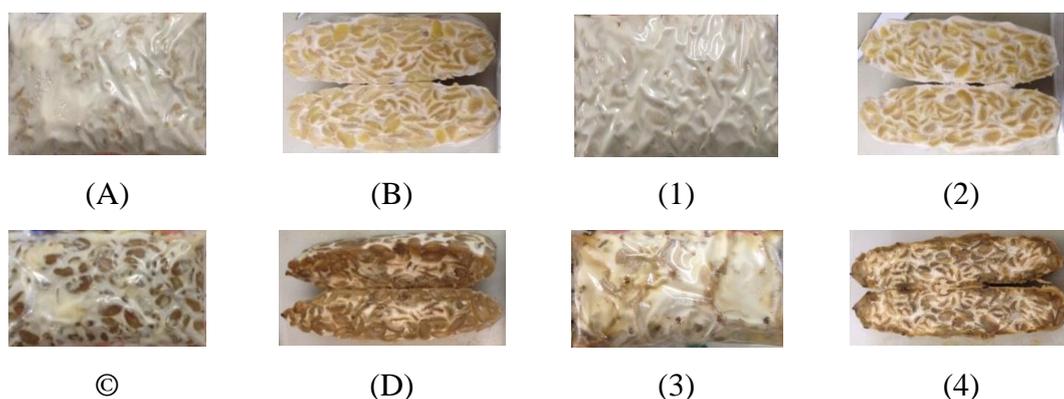


Fig. 2. Tempe produced in various soaking time; (A&B) fresh tempe, soaking time 120 min; (C&D) overripe tempe, 120 min; (1&2) fresh tempe, 180 min; (3&4) overripe tempe, 180 min

Protein Analysis

Protein analysis were conducted on fresh tempe produced using 36 g/l GDL concentration, 4 g/kg starter and 120 min soaking time and overripe tempe produced using 180 min soaking time to understand the profile and potential as umami flavor source. Table 5 shows the protein profile, and confirming previous studies, the amount of soluble amino acid and digestibility is increased in overripe tempe on both methods.

The overripe tempe had bigger soluble amino acid due to the further fermentation process which occurred on overripe tempe. During the growth of mold, the protein is hydrolyzed to amino acids and peptides by proteolytic enzymes [18]. Overripe tempe had been identified that there are amino acids present, which there is 17 different of amino acids were found and glutamic acid is the most abundant amino acid in overripe tempeh [7].

Table 4. Characteristics of tempe produced in various soaking time (GDL 36 g/l, starter 4 g/kg)

| Sample Code | Firmness Texture (N) | Color | | |
|-------------|-------------------------|--------------|--------------|--------------|
| | | L* | a* | b* |
| FT-28-4-120 | 25.39 ± 1.40 | 75.56 ± 1.57 | 7.73 ± 0.48 | 20.28 ± 1.72 |
| FT-28-4-150 | 25.05 ± 2.45 | 74.81 ± 3.66 | 7.03 ± 0.13 | 18.00 ± 0.11 |
| FT-28-4-180 | 26.99 ± 1.43 | 75.87 ± 4.98 | 7.40 ± 0.25 | 19.12 ± 0.55 |
| FT-36-4-120 | 21.36 ± 2.97 | 72.82 ± 6.29 | 8.13 ± 1.19 | 20.41 ± 2.46 |
| FT-36-4-150 | 25.85 ± 1.03 | 75.99 ± 4.36 | 8.54 ± 1.72 | 22.23 ± 4.15 |
| FT-36-4-180 | 25.53 ± 2.31 | 75.76 ± 4.85 | 7.59 ± 0.93 | 19.08 ± 1.62 |
| OT-28-4-120 | 15.79 ± 3.69 | 54.13 ± 0.02 | 11.16 ± 0.13 | 28.55 ± 0.16 |
| OT-28-4-150 | 12.82 ± 5.26 | 47.85 ± 0.59 | 12.00 ± 1.40 | 28.23 ± 1.17 |
| OT-28-4-180 | 12.5 ± 1.95 | 53.74 ± 1.49 | 10.34 ± 1.67 | 27.52 ± 2.09 |
| OT-36-4-120 | 8.96 ± 1.78 | 50.39 ± 5.61 | 10.75 ± 0.69 | 25.90 ± 1.73 |
| OT-36-4-150 | 15.68 ± 5.80 | 51.07 ± 4.86 | 11.48 ± 2.14 | 29.23 ± 0.24 |
| OT-36-4-180 | 17.45 ± 0.27 | 55.63 ± 0.70 | 10.70 ± 0.09 | 31.07 ± 2.82 |

Sample Code: FT (fresh tempe); OT (overripe tempe) – XX (concentration of GDL in g/l) – Y (amount of starter in g/kg) – ZZZ (soaking time in minutes)

Table 5. The protein profile, and confirming previous studies.

| Test Criteria | Unit | FT-36-4-180 | OT-36-4-120 | Natural acidification tempe [14] |
|-------------------------|---------------------------|-----------------------------|-----------------------------|----------------------------------|
| Protein Content (Lowry) | mg BSA eq/g dry base | 70.18±0.48 ^a | 76.79±2.29 ^b | 55.23±4.94 |
| Soluble Amino Acid | mg Tyrosine eq/g dry base | 387.06±23.83 ^{aa} | 459.66±29.13 ^{bb} | 391.51±35.67 ^{aa} |
| Protein Digestibility | mg Tyrosine eq/g dry base | 416.00±46.05 ^{aaa} | 515.97±69.85 ^{bbb} | |

Different superscript in the same row shows significant different in results (p<0.05)

Conclusions

From the study it is found that GDL acidification could be directed toward the production of fresh or overripe tempe, depending on the soaking time. Fresh tempe and overripe tempe produced by GDL acidification was having relative similarity in term of firmness texture and color with naturally acidified tempe. However, it is recommended to evaluate the difference in sensory characteristics and market acceptance of the product, in comparison with the naturally acidified tempe. To produce fresh tempe, the best concentration of GDL, tempe starter and length of soaking time was 36 g/l of GDL, 4 g/kg inoculum with 180 minutes. Whereas for overripe tempe production, is 36 g/l GDL, 4 g/kg inoculum with 120 minutes of soaking time was the method proposed. In addition, through protein analysis, the protein content, soluble amino acid and protein digestibility of fresh tempe compared to overripe tempe were significantly different.

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