Tempoyak from Agam district of West Sumatera, Indonesia as a local probiotic super food candidate

N Hendry¹, Y Aldi², S Syukur³, I Juliarsi⁴, E Purwati⁴*  
¹ Post-graduate of Biotechnology, Andalas University, Padang, Indonesia, 25163  
² Lecture at Faculty of Pharmacy, Andalas University, Padang, Indonesia, 25163  
³ Lecture at Faculty of Natural Science, Andalas University, Padang, Indonesia, 25163  
⁴ Lecture at Faculty of Animal Science, Andalas University, Padang, Indonesia, 25163  
*Corresponding author e-mail: purwati17@yahoo.co.id / purwati17@ansci.unand.ac.id

Abstract. Tempoyak is a traditional food made from durian which has the potential to be a probiotic superfood. Tempoyak is made by fermenting durian fresh in anaerobic conditions. Tempoyak naturally contains probiotic microorganisms called Lactic Acid Bacteria (LAB). LAB is one of the most significant organisms and has benefits for help the healing process of various diseases such as diarrhea, constipation, irritable bowel syndrome, and infections. This study aims to determine the total colonies of LAB and chemical properties, namely protein value, fat content, water content, pH, and TTA (Total Titrable Acid) from tempoyak from Agam district. The method used is descriptive method and laboratory analysis. The sample used in this study was durian fresh (Durio zibethinus L.) with three treatments. The first treatment uses durian meat only (TK1), the second treatment uses durian meat with chili (TK2), the third treatment uses durian meat with salt (TK3). The results showed the total colonies of LAB 39x10⁷ - 98x10⁷, the highest was in tempoyak TK3. Protein content 2.84% - 3.90%, the highest was in tempoyak TK1. Fat content 3.37% - 3.74% and the highest was in tempoyak TK1. Water content 75.53% - 85.38% and the highest was in tempoyak TK3. pH 4.1 - 4.3, the highest was in tempoyak TK1. Total Titrable Acid 0.28% - 0.36% and the highest was in tempoyak TK1.

Keywords: Lactic Acid Bacteria, Superfood, Tempoyak, Traditional food

1. Introduction
Tempoyak is a traditional food from various regions in Indonesia, such as Sumatra and Kalimantan which is made by fermenting fresh durian under anaerobic conditions. Tempoyak is generally yellowish white and has a distinctive and sharp aroma. Tempoyak fermentation occurs spontaneously, and generally occurs around 4-7 days [1]. Tempoyak fermentation can be done with or without additional ingredients such as chili or salt. The addition of salt to tempoyak aims to draw water and nutrients from the fermented material network, which will be used as a substrate for the growth of bacteria involved in fermentation [2].

After completion of fermentation, tempoyak can be stored for 2 months to 1 year. This long shelf life is caused by the acid produced by lactic acid bacteria during the fermentation process, suppressing the growth of pathogenic microbes [3].

Tempoyak might be one of the unknown superfoods. One of the reasons tempoyak is considered a superfood is because fermentation makes all food nutrients more bioavailable than raw, so all the
vitamins, minerals and phytonutrients that durian fruit has to offer can become more bioavailable after fermentation. In previous studies, it was found that the nutritional content of tempoyak was 89.97% water content, 6.49% protein content, and 3.04% fat content [4]. In addition to increasing bioavailability, tempoyak also contains lactic acid bacteria. The presence of Lactic Acid Bacteria is what makes tempoyak as a local probiotic Superfood candidate.

Probiotics are living organisms that will provide health benefits to their host when consumed in certain amounts [5]. One of the groups of probiotic organisms that provide significant benefits is lactic acid bacteria (LAB). LAB can help the healing process of various diseases such as diarrhea, constipation, irritable bowel syndrome, and infections [6]. LAB also has various healing properties such as antioxidant, anti-allergic, and anti-anxiety properties. In addition, LAB can also increase the bioavailability of vitamins/minerals [7, 16].

While most studies limit LAB to dairy products such as yogurt and kefir or limit superfood research to non-fermented products such as moringa plant powder, this durian fermentation also allows producing superfoods as well as probiotics.

1.1. The purpose of the research
The purpose of this study was to determine the total colonies of Lactic Acid Bacteria and chemical properties including the nutritional value of Tempoyak, Agam Regency, West Sumatra Indonesia as a candidate for local super food probiotics.

2. Materials and methods

2.1. The material of the research
The materials used in making tempoyak are durian fresh, chilies, and salt. The materials used to calculate the total colonies of Lactic Acid Bacteria were de Mann Rogosa Sharpe (MRS) broth (Merck), MRS Agar (Merck), distilled water, and alcohol. While the materials used to see the nutritional value of tempoyak are distilled water, methyl red indicator, H2SO4, 30% NaOH, 0.1 N NaOH, spirits, benzene, phenolphthalein (pp). The equipment used in this study were label paper, porcelain plates, analytical scales, Kjeldahl flasks, pH meters, electric ovens, funnels, beaker glasses, Erlenmeyer, Bunsen, Eppendorf tubes, micropipette tips, goiter pipettes, hockey stick, magnetic stirrer & hot plate, test tube, distillation flasks, autoclave, volumetric flask, soxhlet, bunsen, grease paper, aluminum foil, anaerobic jar, Quebec colony counter, and laminar airflow.

2.2. Methods of the research.
The method used in this research is a descriptive method and laboratory analysis.

2.2.1. Total colonies of Lactic Acid Bacteria. Method [8], with the following work steps: All equipment to be used is sterilized first using an autoclave at a temperature of 121°C for 15 minutes with a pressure of 15 lb. 68.2 grams of de Mann Rogosa Sharpe (MRS) broth (Merck), MRS Agar (Merck), distilled water, and alcohol. While the materials used to see the nutritional value of tempoyak are distilled water, methyl red indicator, H2SO4, 30% NaOH, 0.1 N NaOH, spirits, benzene, phenolphthalein (pp). The equipment used in this study were label paper, porcelain plates, analytical scales, Kjeldahl flasks, pH meters, electric ovens, funnels, beaker glasses, Erlenmeyer, Bunsen, Eppendorf tubes, micropipette tips, goiter pipettes, hockey stick, magnetic stirrer & hot plate, test tube, distillation flasks, autoclave, volumetric flask, soxhlet, bunsen, grease paper, aluminum foil, anaerobic jar, Quebec colony counter, and laminar airflow.
Protein content of tempoyak. Protein content was determined by the Kjeldahl Micro Method based on guidelines [18], with the following working procedures: Destruction stage. A total of 1 gram of dry sample was put into a different Kjeldahl flask and then added 1 gram of selenium catalyst and 25 ml of H2SO4. Then digested over low heat in a fume hood and shaken from time to time to make it homogeneous. Heating is done until the solution is clear yellow.

Distillation Stage. The solution in the Kjeldahl flask is diluted into a 250 ml volumetric flask with distilled water to mark the line. A total of 25 ml of sample solution was put into a distillation flask given a boiling stone and then added with 25 ml of 30% NaOH which had been mixed with 75 ml of distilled water through a tighter. The solution is then distilled until 2/3 of the liquid has been distilled and captured by 25 ml of 0.05 N H2SO4 which is first mixed with 3 drops of methyl red indicator. Rinsing is carried out in the distillery into the collection flask.

Titration stage. The distillation result is titrated with 0.1 N NaOH using a micro buret until the color changes (X ml). Then made a blank titration, added 3 drops of MM indicator into H2SO425 ml 0.05 N, and titrated with 0.1 N (Y ml) NaOH.

2.2.2. Fat content of tempoyak. Method [9]. A total of 1 gram of the sample was put into a paper sleeve covered with cotton, then dried in an electric oven at 105°C for 12 hours. Then it is weighed hot and extracted with benzene for 4-6 hours until the benzene in Soxhlet becomes clear, then the sample is cooled to dryness, where the benzene will evaporate, then dried in an electric oven at a temperature of 105°-110°C for 4 hours to obtain constant weight. Samples were weighed one by one while still hot. Where the difference in weight before and after extraction is the weight of fat.

2.2.3. Water content of tempoyak. Method [9]. The aluminum plates were oven-dried at 110 ° C for 1 hour and then cooled in a desiccator. The dishes are weighed and filled with a 5-gram sample. Then dry in an oven at 105 ° C for 8 hours. Cool it in a desiccator and weigh it, then do it repeatedly until the weight is constant.

2.2.4. The pH of tempoyak. Tempoyak pH measurements were carried out with a pH meter. First, the pH meter is calibrated using a standard buffer solution of pH 7, to show the pH number mentioned above. Then the electrodes are washed with distilled water, then dried with tissue paper. PH measurements were carried out by diluting 1 g of tempoyak with 10 ml of distilled water in a container. Next, the electrode is immersed in the solution and let it move until it is a constant position and the number shown by the pH meter is the tempoyak pH.

2.2.5. TTA (Total Titratable Acidity). Method [9]. The sample was weighed using an analytical scale of as much as 5 g and dissolved with 10 ml of distilled water, stirred using a stir bar until the sample was homogeneous. The prepared biuret was filled with 0.1 N NaOH, then 2 ml of phenolphthalein (pp) indicator was added. Titrate with 0.1 N NaOH until a color change (equivalence point) occurs and the volume used for the titration is recorded and the last calculation is carried out. The description of the sample used can be seen in Table 1.

| Table 1. Sample description. |
|-----------------------------|
| Sample Code | Sample Type | Additional Material | Source             |
| TK1          | Durian (Durio zibethinus L.) | -                   | Kamang, Agam District |
| TK2          | Durian (Durio zibethinus L.) | Chili               | Kamang, Agam District |
| TK3          | Durian (Durio zibethinus L.) | Salt                | Kamang, Agam District |
3. Results and discussion

3.1. Total colonies of Lactic Acid Bacteria
The results of the total colonies of lactic acid bacteria from tempoyak can be seen in Table 2.

| Sample | Total LAB (CFU/g) |
|--------|------------------|
| TK1    | 75 x 10^7        |
| TK2    | 39 x 10^7        |
| TK3    | 98 x 10^7        |

In Table 2, this is the total colony of lactic acid bacteria. Plating onto MRS agar media, and anaerobic incubation for 24 hours at 37°C. Total bacterial colonies from tempoyak without addition or TK1 obtained total LAB colony 75 x 10^7 CFU/g, tempoyak addition of chili or TK2 with total LAB colony 39 x 10^7 CFU/g, and tempoyak addition of salt with total LAB colony 98 x 10^7 CFU/g.

When compared with the previous study, which was as much as 6.0 x 10^6 - 3.8 x 10^7 [10], the results of this study were much higher. This is in accordance with the criteria FAO/WHO (2002) because the LAB probiotic food produced must be in the amount of 10^6 - 10^8 CFU/Gram. The varying number of LAB colonies from this tempoyak isolate was due to the different nutritional conditions where the bacteria grew and developed. This difference in the growing environment of LAB will produce very varied LAB isolates [11].

3.2. Protein content of Tempoyak
The protein content of tempoyak can be seen in Table 3 below. The protein content of TK1 tempoyak was 3.90%, TK2 tempoyak was 3.39%, and TK3 tempoyak was 2.84%. The highest tempoyak protein content was found in TK1 without the addition of additional ingredients, which was 3.90% and the lowest tempoyak protein content was at TK3 tempoyak with the addition of salt, which was 2.84%.

| Sample | Protein Content (%) |
|--------|---------------------|
| TK1    | 3.90                |
| TK2    | 3.39                |
| TK3    | 2.84                |

The result of the tempoyak TK1 protein content was lower than the previous study, which was 6.49% [4]. While the lowest protein content was obtained tempoyak TK3 with the addition of salt, which was 1.42%. In general, salt will reduce the protein content of food [12]. This happens because salt is a strong electrolyte that can dissolve protein, so salt is able to break the bonds of water molecules in water and can change the nature of the protein.

3.3. Fat content of Tempoyak
The fat content of tempoyak can be seen in Table 4. The fat content of tempoyak in TK1 is the largest at 3.74%, followed by TK3 at 3.41%, and finally TK2 at 3.37%. The results of the fat content in this study were higher when compared to previous studies, where the fat content in tempoyak ranged from 1.03 - 3.04% [4]. This fat content can be influenced by LAB activity to reduce fat. During the fermentation process, lipase enzymes that are naturally present in food or produced by microbes that grow on fermented foods will degrade fat. The fat is then broken down into volatile and non-volatile fatty acids which will form the aroma and taste in tempoyak [4].
Table 4. Fat content.

| Sample | Fat Content (%) |
|--------|-----------------|
| TK1    | 3.74            |
| TK2    | 3.37            |
| TK3    | 3.41            |

3.4. Water content of Tempoyak
The water content of tempoyak, can be seen in table 5. Water content of tempoyak is TK1 was 75.53%, TK2 was 81.69%, and TK3 was 85.38%. The results of the moisture content in this study were not much different from the results of previous studies, ranging from 75.60% - 89.97% [4]. The difference in water content between one sample and another is due to differences in treatment between each sample.

Table 5. Water content.

| Sample | Water Content (%) |
|--------|-------------------|
| TK1    | 75.53             |
| TK2    | 81.69             |
| TK3    | 85.38             |

3.5. pH of Tempoyak
The pH of tempoyak, can be seen in table 6 below. The tempoyak pH range in this study was 4.1 – 4.3. With tempoyak TK1 has the highest pH of 4.3, followed by TK2 of 4.2, and finally TK3 of 4.1. The pH value produced in this study is acidic. The duration of fermentation also affects the pH value of tempoyak. Following previous research, the acid pH range was obtained with a value of 3.8 - 4.1 [4].

Table 6. pH.

| Sample | pH |
|--------|----|
| TK1    | 4.3|
| TK2    | 4.2|
| TK3    | 4.1|

3.6. TTA (Total Titratable Acidity) of Tempoyak
TTA (Total Titratable Acidity) of tempoyak, can be seen in table 7. TTA of tempoyak samples TK1 was 0.38%, TK2 was 0.31%, and TK3 was 0.28. The results of this study indicate a high acid content, this is due to the fermentation process. During fermentation LAB breaks down carbohydrates (glucose) into acids, which lowers the pH and causes a sour taste [13].

Table 7. TTA (Total Titratable Acidity).

| Sample | TTA (%) |
|--------|---------|
| TK1    | 0.38    |
| TK2    | 0.31    |
| TK3    | 0.28    |
4. Conclusion
Tempoyak is one of the typical foods of West Sumatra which has good nutritional content that is good for health and has the potential to become a local probiotic superfood. Tempoyak is a traditional food made from durian which is made by fermentation under anaerobic conditions or stored in closed conditions. The results showed that the highest number of LAB colonies $39 \times 10^7 - 98 \times 10^7$ was found in tempoyak TK3. Protein content $2.84\% - 3.90\%$, the highest in tempoyak TK1. Fat content $3.37\% - 3.74\%$ and the highest is in tempoyak TK1. The water content is $75.53\% - 85.38\%$ and the highest is at tempoyak TK3. pH $4.1 - 4.3$ highest at tempoyak TK1. Total Titrable Acid $0.28\% - 0.36\%$ and the highest at tempoyak TK1. In conclusion, tempoyak has good nutritional quality and can be used as a local superfood probiotic.

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