Effectiveness of B14A probiotic candidates as enhancers on the physical characteristics and organoleptic properties of bekamal

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Abstract. Bekamal is a fermented meat typical of Banyuwangi Regency which is produced traditionally with the addition of sugar and salt as a preservative and flavor enhancer. The weakness of commercial bekamal takes a long time because the meat must be fermented for 3 to 6 months to get some unique taste, which has a juiciness, slightly sour and salty that consumers like. The B14A probiotic candidate is added to the bekamal ingredients to help speed up the fermentation process with similar characteristics to commercial bekamal. Bekamal with B14A isolate is begun by regenerating the isolate to become a starter culture. The 10% starter culture is inoculated into the pieces of meat and added by 7.5% palm sugar and 7% salt then fermented in some plastic jars with three variables of fermentation time, there are 7, 14, and 21 days. Bekamal in each fermentation time treatment was tested on physical characteristics that consist of yield, cooking loss, water holding capacity (WHC), and organoleptic properties like taste, color, aroma, and texture. The results showed that the B14A probiotic candidate can speed up the fermentation in less than 1 month (21 days) with physical characteristics and organoleptic properties as well as commercial bekamal.

1 Introduction

1.1 Background

The requirement of protein intake to fulfill a balanced amount of nutrition in the body is a top priority for society today, especially after the coronavirus pandemic that hit several countries in the world, namely COVID-19. Protein consists of nutritional content that is very important for body health in the form of amino acids which consist of essential amino acids and non-essential amino acids, essential amino acids are very important because our bodies

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are not able to produce them themselves, so they require additional supplements from outside the body, namely through food. Protein can be obtained from food sources, including types of animal food, one of which is meat.

However, meat generally experiences a decrease in quality, especially changes in the amount and structure of protein which is the main constituent of meat, these changes can be caused by processing, especially processing using high temperatures or through heating processes, whether by frying, drying, smoking, or grilling, which is generally done to make delicious processed meat products. One meat processing technology that can help minimize the decline in meat quality is fermentation technology. Fermentation technology is a food processing technology that is carried out without using mechanical heating, but by utilizing the metabolic capabilities of microorganisms and producing metabolite compounds that can increase the nutritional value and improve the physical quality of meat because the fermentation process can also prevent damage to meat proteins which in turn will affect the texture and tenderness of the processed meat produced.

Meat fermentation is one method used to extend shelf life, change and diversify meat products. Fermented meat products are the result of complex microbial activity, especially lactic acid bacteria (LAB) and coagulase-negative cocci bacteria which can cause biochemical changes in meat [1]. The LAB metabolic process during fermentation produces various enzymes, one of which is protease which functions to hydrolyze polypeptides into peptides and amino acids [2]. Amino acids produced in the protein hydrolysis process will be used by bacteria as growth nutrients, especially nitrogen compounds which will enter bacterial cells [3]. Apart from that, the principle in the formation of lactic acid in the fermentation process is the change in glucose by the glycolysis process. Carbohydrates will undergo a breakdown into glucose, then the glucose will be converted into lactic acid with the help of enzymes produced by lactic acid bacteria [4]. The enzyme used to convert pyruvic acid into lactic acid comes from BAL, namely the enzyme lactate dehydrogenase [5], the presence of the LAB metabolic process will trigger the growth of more LAB and will speed up the fermentation process. The addition of LAB starter to the fermentation process is usually done to produce standardized and safe products. Research on the addition of *Lactobacillus casei* starter culture to fermented sausages with a curing time of 28 days significantly increased LAB content and inhibited the growth of Enterobacteriaceae bacteria groups such as *Escherichia coli* and *Salmonella* sp. [6].

Bekamal is a fermented product made from beef and uses a lactic acid bacteria starter in the process. Lactic acid bacteria are gram-positive bacteria, do not produce spores, are round or rod-shaped, and can produce lactic acid as an important metabolic end product in the fermentation process [7]. Bekamal contains lactic acid bacteria which belong to the genus *Lactobacillus* sp. [8]. *Lactobacillus* sp. are lactic acid bacteria that have the ability to produce antibacterial properties so they can extend the shelf life of a product because they are able to inhibit food-destroying bacteria [9]. Research on the isolation and identification of indigenous lactic acid bacteria was carried out by [10] reporting that the isolation of indigenous lactic acid bacteria in bekamal produced 44 isolates which were suspected to be a group of lactic acid bacteria, including B14A isolate. B14A isolate added to the bekamal fermentation process was able to improve the chemical quality of bekamal fermented for 7, 14, and 21 days, namely in the form of protein content, pH, TAT, and water content [11]. Apart from that, the B14A isolate used was also proven to inhibit the growth of pathogenic bacteria, namely *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* sp. [12] hence, this B14A isolate is thought to have the potential to be a probiotic candidate isolate. Therefore, through this research, it is hypothesized that the addition of B14A isolate can influence the physical characteristics of bekamal in the form of water-holding capacity (WHC), cooking loss, yields, and organoleptic properties.
1.2 Objectives

This research aims to determine the effect of the B14A probiotic candidate addition in the fermentation process of bekamal on water holding capacity, cooking loss, yield, and organoleptic quality compared to commercial bekamal.

2 Research methods

2.1 Preparation of bekamal

Bekamal is prepared using 1 kg of topside, which is cut into pieces measuring 10 x 5 x 0.5 cm. The meat is then mixed with 7% salt and 7.5% brown sugar. Isolates are added to the entire mixture at a rate of 10%. The fermentation process is carried out for 0, 7, 14, and 21 days at room temperature, approximately 28-30 °C. The stages of bekamal fermentation can be seen in Fig 1.

![Flowchart of bekamal preparation](image)

Fig. 1 Flowchart of bekamal preparation [11].

2.2 Yield

Samples are cut and weighed at 20g, mixed with the prepared isolates, and fermented for the specified time. The bekamal, after fermentation, is weighed as the final weight, and the yield percentage is calculated using the formula [13] below.

\[
\text{% Yield} = \left( \frac{\text{initial weight}}{\text{final weight}} \right) \times 100
\]  

(1)

2.3 Cooking loss

Samples are cut and weighed at 20g, and boiled until they reach a temperature of 81°C. The meat samples are then removed and cooled until their weight stabilizes, and the cooking loss percentage is calculated using the formula [13] below.

\[
\text{% Cooking Loss} = \left( \frac{\text{initial weight} - \text{weight after cooking}}{\text{initial weight}} \right) \times 100
\]  

(2)

2.4 Water holding capacity

Samples are cut and weighed at 0.3g placed into double Whatman filter paper then pressed using a Carper press at a pressure of 35 kg/cm2 for 5 minutes. The wet area visible on the filter paper is drawn and measured using a planimeter. The water holding capacity is calculated using the Hamm formula [13] as follows:

\[
\text{mg H}_2\text{O} = \left( \frac{\text{wet area in cm}^2}{0.0948 - 8.0} \right)
\]  

(3)
Subsequently, mg H2O is converted into a percentage using this formula:

\[
\% \ H_2O = \left( \frac{\text{mg} \ H_2O}{\text{sample weight} \ (\text{mg})} \right) \times 100
\]

(4)

2.5 Sensory evaluation

This test is conducted to assess the acceptability of a product by consumers, including taste, color, flavor, and texture. In this study, 30 untrained panelists are used. This aligns with an organoleptic test in [14].

3 Results and discussions

3.1 Yield

Yield represents the final product obtained by calculating the ratio of the final product’s weight to the initial weight of the raw materials, expressed as a percentage [15]. The ANOVA results show that the fermentation duration significantly influences (P<0.05) the yield values in both bekamal B14A and commercial bekamal. Table 1 indicates that the highest average yield is observed in bekamal supplemented with B14A probiotic candidate at the 0-day fermentation, which also has a significant impact on bekamal fermentation for 7 days. When compared to commercial bekamal (Table 2), the yield value for commercial bekamal fermented for one month is nearly equal to that of bekamal fermented with a B14A probiotic candidate. This suggests that the fermentation time for bekamal can be shortened to 14 or 7 days to achieve a high yield. Yield is closely related to the water retained by proteins in the product, where the stronger the protein’s ability to retain water, the higher the percentage yield of a product.

Additionally, yield is greatly influenced by the loss of water during processing. Fermentation is a food processing process that generates water. This is supported by [16], stating that the increase in moisture content after fermentation is due to the metabolism of microorganisms that produce CO2 and H2O. A high percentage yield is preferred by both producers and consumers because the weight loss after cooking is reduced, leading to increased economic value of the product.

Table 1. Average percentage yield of bekamal with B14A isolate.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-day</td>
<td>96.97±2.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7-days</td>
<td>93.57±2.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>14-days</td>
<td>81.46±1.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>21-days</td>
<td>72.13±2.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 2. Average percentage yield of commercial bekamal.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-month</td>
<td>80.28±4.94&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2-months</td>
<td>72.21±2.62&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3-months</td>
<td>71.22±1.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

3.2 Cooking loss

Cooking loss represents the weight lost during processing, especially processes that utilize
heat energy. In essence, the higher the temperature and the longer the processing time, the greater the percentage of cooking loss until it reaches a constant value. Cooking loss also serves as an indicator for assessing the nutritional value of meat, particularly related to meat juiciness. The tenderness of meat is influenced by the availability of bound water within and between muscle fibers. Unlike yield, lower cooking loss values indicate relatively better meat quality compared to meat with higher cooking loss. This results in lower cooking loss values for meat [17]. Cooking loss is influenced by pH, temperature, cooking duration, and muscle type. It is also affected by the breed, age of the animal, and its diet.

ANOVA results show that the fermentation duration significantly influences (P<0.05) the cooking loss percentage in bekamal B14A (Table 3) but does not significantly affect (P>0.05) the cooking loss percentage in commercial bekamal. Table 3 indicates that the lowest average cooking loss is observed in bekamal supplemented with a B14A probiotic candidate during the 14-day fermentation. When compared to commercial bekamal (Table 4), the cooking loss value of commercial bekamal fermented for one month is nearly equal to that of bekamal fermented for 14 and 21 days with B14A probiotic candidate. This suggests that the fermentation time for bekamal can be shortened to 14 or 21 days to achieve low cooking loss.

Meat with low cooking loss is considered to have better quality compared to meat with high cooking loss because the risk of nutrient loss during cooking is reduced [17]. The low cooking loss is presumed to be due to the acidic pH of bekamal resulting from the fermentation process, which causes shrinkage in the meat's myofibrils. Additionally, the contraction of muscle tissue in bekamal is attributed to the heating process at 100°C during testing, causing the meat to shrink and protein oxidation, leading to the release of water from the meat. The mechanism of protein oxidation due to heating is supported by [18], which states that protein oxidation occurs due to the formation of protein cross-links through disulfide bonds, intended to maintain the protein's moisture content. However, when proteins are exposed to excessive hydrophobic residues continuously, it affects the protein's ability to retain water and results in evaporation during heating, leading to high cooking loss values.

### Table 3. Average percentage cooking loss of bekamal with B14A isolate.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cooking loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-day</td>
<td>41.48±3.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7-days</td>
<td>34.43±2.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>14-days</td>
<td>22.43±5.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>21-days</td>
<td>27.77±1.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### Table 4. Average percentage cooking loss of commercial bekamal.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cooking loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-month</td>
<td>28.3±1.55</td>
</tr>
<tr>
<td>2-months</td>
<td>40.72±5.16</td>
</tr>
<tr>
<td>3-months</td>
<td>37.85±5.96</td>
</tr>
</tbody>
</table>

#### 3.3 Water holding capacity

Water Holding Capacity (WHC) is the ability of meat to retain its water content during external treatments such as cutting, heating, grinding, and processing. The extent of WHC affects the color, tenderness, juiciness, elasticity, and texture of meat [19]. Factors influencing WHC include the water content within the muscles, fat content, and pH [20]. The data analysis results (Table 5 and 6) show that the fermentation duration significantly influences (P<0.05) the water-holding capacity of bekamal with the addition of B14A.
probiotic candidate, but it does not significantly affect (P>0.05) the water holding capacity of commercial bekamal. The addition of a B14A probiotic candidate can increase the WHC percentage in only 7 days of fermentation, while the highest WHC is observed in commercial bekamal, which is fermented for 2 months.

The differences in WHC percentages are likely because during the fermentation process, bekamal undergoes protein degradation, which results in structural changes in the meat's muscle. According to [21], the reduction in water-holding capacity occurs because of protein degradation, depolymerization, and increased protein solubility due to pressure, leading to structural changes in muscle protein, especially in actin and myosin. The physical properties of fermented meat that have changed can be analyzed by examining the meat's texture. Juicy textured of fermented meat has a high water-holding capacity, indicating that the protein in the meat is still capable of retaining moisture. Changes in meat structure can promote protein degradation, increasing meat tenderness and its ability to retain more water.

The fluctuation WHC in meat is also influenced by the meat’s pH. If the pH of the meat is below the isoelectric pH, then its water-holding capacity will be high. This aligns with the statement [20] that water holding capacity in meat decreases if the meat's pH is lower than the isoelectric pH (5.0-5.1) of meat proteins. At a lower pH, there are positive charges on the meat proteins, allowing water molecules to bind with the meat proteins. Otherwise, meat with a pH above the isoelectric pH has a lower water-holding capacity.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>WHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-day</td>
<td>48.53 ±3.74ab</td>
</tr>
<tr>
<td>7-days</td>
<td>58.73 ±4.86a</td>
</tr>
<tr>
<td>14-days</td>
<td>37.14 ±5.79c</td>
</tr>
<tr>
<td>21-days</td>
<td>33.74 ±6.43c</td>
</tr>
</tbody>
</table>

Table 5. Average percentage WHC of bekamal with B14A isolate.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>WHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-month</td>
<td>23.49±4.28</td>
</tr>
<tr>
<td>2-months</td>
<td>39.45±4.30</td>
</tr>
<tr>
<td>3-months</td>
<td>29.62±8.22</td>
</tr>
</tbody>
</table>

Table 6. Average percentage WHC of commercial bekamal.

3.4 Sensory evaluation

Sensory evaluation plays a significant role in determining the quality of a product. The organoleptic test in this study involved 25 untrained panelists using bekamal as the test sample. The results of the organoleptic evaluation of bekamal can be seen in Table 7.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste</td>
<td>3.56±0.71c</td>
<td>3.48±0.87c</td>
<td>3.96±0.79c</td>
</tr>
<tr>
<td>Color</td>
<td>2.72±0.61c</td>
<td>3.2±1.04f</td>
<td>4.04±0.73c</td>
</tr>
<tr>
<td>Flavor</td>
<td>3.80±1.12b</td>
<td>372±0.98b</td>
<td>4.88±0.43c</td>
</tr>
<tr>
<td>Texture</td>
<td>3.48±0.77bc</td>
<td>3.48±0.77bc</td>
<td>3.92±1.22c</td>
</tr>
</tbody>
</table>

Table 8. Average sensory evaluation of bekamal (continued).
Taste | 1.96±0.98<sup>a</sup> | 2.96±0.88<sup>b</sup> | 2.76±1.16<sup>b</sup> | 3.56±0.87<sup>c</sup>  
---|---|---|---|---  
Color | 2.92±0.91<sup>cd</sup> | 1.24±0.60<sup>a</sup> | 1.16±0.37<sup>a</sup> | 1.88±0.83<sup>b</sup>  
Flavor | 1.12±0.44<sup>a</sup> | 3.72±0.84<sup>b</sup> | 3.80±0.65<sup>b</sup> | 3.80±0.50<sup>b</sup>  
Texture | 2.80±1.12<sup>a</sup> | 2.84±0.80<sup>a</sup> | 2.96±0.61<sup>ab</sup> | 3.80±0.41<sup>c</sup>  

**Note:** Different notations (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup> and <sup>e</sup>) in the same column show significant differences (P<0.05), P1: Commercial bekamal fermented 1-month, P2: Commercial bekamal fermented 2-months, P3: Commercial bekamal fermented for 3-months, P0: Bekamal with B14A fermented 0-days, P7: Bekamal with B14A fermented 7-days, P14: Bekamal with B14A fermented 14-days and P21: Bekamal with B14A fermented 21-days.

3.4.1 Taste

The research results indicate that the addition of B14A isolate to beef significantly affects the taste of bekamal (P<0.01). Based on the data in Table 7, the highest taste score was obtained from commercial bekamal and bekamal fermented for 21 days, scoring 3.56. The lowest taste score was obtained from bekamal fermented for 0 days, with a score of 1.96. Fermentation for 21 days and commercial bekamal have a salty and acidic taste compared to the other treatments, while fermentation for 7 and 14 days has a salty and slightly acidic taste, and the 0-day fermentation has a slightly salty and acidic taste. The addition of salt in the bekamal-making process imparts a strong salty flavor. Salt added serves as a natural preservative for bekamal. Salt can also inhibit the growth of pathogenic bacteria in the meat and stimulate the growth of lactic acid bacteria in bekamal made from beef [21].

The sour taste in bekamal increases every week. The sour taste in bekamal results from the metabolism of lactic acid bacteria, which produce lactic acid from the breakdown of carbohydrates (brown sugar) and create a distinctive flavor in bekamal. The sour taste in bekamal is due to a decrease in pH value during the fermentation process, increasing total acidity [22]. During the fermentation process of bekamal, organic acids are formed. The compound of organic acids increases during the fermentation process, resulting in a stronger sour taste.

3.4.2 Color

The research results indicate that the addition of B14A isolates to beef significantly affects the color of bekamal (P<0.01). Based on the data in Table 7, the highest color score was obtained from bekamal fermented for 0 days, scoring 2.92. The lowest color score was obtained from bekamal fermented for 14 days, scoring 1.16. The 0-day fermentation and commercial bekamal have a brown color compared to the other treatments, while the 7 and 14-day fermentations have a pale brown color, and the 21-day fermentation has a light brown color. The color of meat is determined by several main factors, including the concentration of myoglobin pigment in meat, the type of molecules, and the chemical status of myoglobin [23]. Table 7 shows that naturally fermented bekamal (commercial bekamal) has a darker color compared to bekamal fermented with the addition of B14A isolate. The difference in color between commercial bekamal and bekamal with the addition of B14A isolate is due to the presence of H₂O₂ and enzymes produced by microorganisms. H₂O₂ compounds cause the oxidation of oxymyoglobin into metmyoglobin, which is brown [17]. The amount of H₂O₂ produced by naturally fermenting bacteria is likely greater than the amount produced by *L. plantarum* during the fermentation of beef. This causes naturally fermented meat to be darker than meat fermented with *L. plantarum* [24].
3.4.3 Flavor

The research results indicate that the addition of B14A isolates to beef significantly affects the aroma of bekamal (P<0.01). Based on the data in the table, the highest flavor score was obtained from commercial bekamal and bekamal fermented for 14 and 21 days, scoring 3.80. The lowest aroma score was obtained from bekamal fermented for 0 days, scoring 1.12. The 0-day fermentation has a non-acidic aroma compared to the 7, 14, and 21-day fermentations, and commercial bekamal. Lactic acid bacteria that produce lactic acid can also create a distinct flavor [25]. The sour aroma in fermented products is caused by the content of volatile compounds and lactic acid in the fermentation product, creating a distinctive sour aroma in bekamal [26].

The longer the fermentation time of bekamal, the stronger the sour flavor becomes. The emergence of a strong sour aroma during the fermentation process is influenced by the activity of lactic acid bacteria, which increases the acidity of bekamal. An increase in the amount of acid produced by Lactic Acid Bacteria (LAB) due to the accumulation of acid in bekamal, can increase the acidity of bekamal. The increase in acid in bekamal can be determined by the decrease in the pH of bekamal. The pH value of beef decreases, increasing the acid content in beef, which can give flavor to the product [27].

3.4.4 Texture

The research results indicate that the addition of B14A isolates to beef significantly affects the texture of bekamal (P<0.01). Based on the data in the table, the highest texture score was obtained from bekamal fermented for 21 days, scoring 3.80. The lowest texture score was obtained from bekamal fermented for 0 days (P0), scoring 2.80. The 21-day fermentation and commercial bekamal have a tender texture compared to the 0, 7, and 14-day fermentations. The longer the fermentation process, the softer the texture of bekamal becomes. The presence of protease enzymes produced from the metabolism of lactic acid bacteria also affects the texture of bekamal. According to [28], proteolytic enzymes will break down mucopolysaccharides from the basic matrix substance, thereby reducing the binding fibers, during which collagen and myofibrils are hydrolyzed, causing the loss of binding between meat fibers and making the meat more tender. [29] states that the longer the meat is soaked in proteolytic enzymes, the longer the time available for proteolytic enzymes to work in hydrolyzing proteins.

Higher water content also affects the texture of bekamal. High water content causes fermented bekamal to become softer and more watery. The tenderness of food is influenced by water content and aw. The higher aw causes the food become softer [30]. Meat tenderness is influenced by two factors, namely antemortem factors consisting of genetics, species,physiology, age, and gender, and postmortem factors consisting of salting methods, cooling, freezing, storage, and processing methods [31].

4 Conclusion

This research concludes that the process of making bekamal by adding the B14A probiotic candidate as a starter can speed up the fermentation of bekamal up to 7 days. In addition, the highest yield of bekamal was obtained while adding B14A in the 0th day, which was 96,97%. Moreover, the best percentage of cooking loss and WHC of bekamal with B14A isolate were 22,43 and 58,73%, respectively. These results are affected the the sensory attributes of bekamal, namely taste, color, flavor, and texture. In general, the sensory evaluation of commercial bekamal has higher acceptability than bekamal with B14A addition. Hence, the optimization of bekamal fermentation with B14A isolate is needed to
obtain the higher acceptable sensory attributes.

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