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Characteristic of black soy miso with crude bromelain and *Lactobacillus plantarum*

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ABSTRACT

This research aims to improve the diversity of functional foods rich in bioactive peptide components and have a unique flavor for black soybean miso through the addition of crude bromelain and *Lactobacillus plantarum*. This research uses a completely randomized design with 5 treatments and 4 repetitions to obtain a total of 20 experimental units. The treatments are the different concentrations of crude bromelain addition, namely the control treatment or 0% (T0), 3% (T1), 6% (T2), 9% (T3), and 12 % (T4). The parameters observed are water content, water activity, pH value, and lactic acid bacteria (LAB) viability. The result showed that an increase in the crude bromelain concentration may increase the water content and water activity of the black soybean miso, while the pH value decreases. Based on the viability of the LAB showed a wavering result. Miso with the 9% concentration of crude bromelain is the best result among the others.

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1. INTRODUCTION

Miso is a traditional Japanese condiment in the form of soybean paste which comes from the process of mixing boiled soybeans, salt, and koji culture which is fermented in three to twenty-four months [1]. Miso is one of the soybeans paste that is produced by fermenting soybeans with brine and koji mold under specific humidity and temperatures [2]. The process of making miso takes months because it goes through a fermentation process to obtain optimal nutritional value, amount of lactic acid bacteria (LAB), flavor, and other supporting factors [3].

Miso has many benefits for the human body because it contains isoflavones which are antioxidant compounds found in soybeans [4]. Apart from being rich in antioxidants, miso also has many nutritional contents. The nutritional content of miso can be increased further by changing the basic ingredient, namely yellow soybeans to black soybeans. Black soybeans have their own advantages because they have quite high nutritional content, especially protein and carbohydrates [5]. The glutamic acid content in black soybeans is also higher when compared to yellow soybeans. These amino acids are components that form the savory taste of miso [6]. The process of making miso will involve a fermentation process, where the process can be accelerated by adding crude bromelain enzyme because microbes do not need to produce protease enzymes to break down proteins.

The protease enzyme contained in the bromelain enzyme is found in pineapple and can hydrolyze peptide bonds in proteins into simpler molecules, namely amino acids, making them easier for the body to digest [7]. Protease enzymes can also be found in papaya fruit, namely the papain enzyme, but the papain enzyme will give a bitter taste so it is not suitable if added to miso products [8]. Another advantage of using the bromelain enzyme is that it can speed up the fermentation process. The miso fermentation process can be further accelerated by adding *Lactobacillus plantarum* [9].

Lactobacillus plantarum is one of the LAB species that is often used for fermentation because of its amylolytic properties [10]. The presence of amylolytic activity of *Lactobacillus plantarum* will be able to produce more glucose, then the glucose will be converted into pyruvate by freeing water molecules [11]. The microbiological amylolytic activity of LAB which can hydrolyze starch into lactic acid by freeing water molecules can cause the water content to increase [12]. *Lactobacillus plantarum* has a higher inhibitory power compared to other types of LAB in inhibiting the growth of pathogenic bacteria. Lactic acid metabolite compounds will be produced from optimal LAB growth which can lower the pH thereby increasing acidity because lactic acid dissociates in the form of H^+ [13].

In the process of making miso, salt is added to keep another microbe besides *Lactobacillus plantarum* from growing [14]. Apart from that, in the fermentation process salt also plays a role in binding water and forming the taste of miso. The water in the product will be bound by salt so that it has value a_w decreased because salt is hygroscopic [15]. In general, optimal environmental conditions for bacteria to grow and develop have a value a_w ranges from 0.6 to 0.9. The existence of this attachment makes researchers want to know the effect of the addition of crude bromelain enzyme on water content, water activity, pH value, and LAB viability.

2. RESEARCH METHOD

2.1. Research design

Samples were prepared in a completely randomized design which was carried out in stages including: i) preparation of crude bromelain, ii) manufacturing of black soy miso, and iii) comparative analysis of chemical and microbial parameters of black soy miso. The treatment given to black soy miso is the crude bromelain concentration of 0% as control, 3, 6, 9, and 12%. This research was carried out at the Food Chemistry and Nutrition Laboratory and the Food Engineering and Agricultural Products, Faculty of Animal and Agriculture, Universitas Diponegoro from June to September 2023. The main ingredients in this study are black soybeans (500 g) ordered from Gang Baru Market, Kranggan, Central Semarang, Semarang, with the variety used being Mallika and pineapples obtained from Damar Market, Padangsari, Banyumanik, Semarang.

2.2. Research procedure

2.2.1. Preparation of crude bromelain

The preparation method of crude bromelain has been adopted from [16], with modifications. First, pineapples were peeled off and separated from the bracts then cut into small pieces and mashed using a blender. The blender speed must be at the lowest or on a scale of 1, so the pineapples mashed carefully. 0.1 M phosphate buffer pH 7 was added to mashed pineapples in a 1:1 ratio gradually. The pineapple juice was filtered using a filter cloth and followed by centrifugation at 4500 rpm for 15 minutes to separate the supernatant and the pellet so that got the crude bromelain.

2.2.2. Manufacturing of black soy miso

The manufacturing process of miso refers to [17] with modification. Black soybeans were washed first before being mashed and soaked with water for 12 hours. To make a sterilized black soybean, boil it for 20 minutes at ±100 ºC. The boiled black soybean was then filtered to separate the water and its solids. The black soybeans were divided into five parts, with each part weighing 150 g of wet basis. Each sample of miso was mixed with crude bromelain according to the treatment (w/w) namely 0% (T0), 3% (T1), 6% (T2), 9% (T3), and 12% (T4) and rest for 3 hours then added with 25% (w/w) black soybean koji, 13% (w/w) salt, 4% (w/w) sugar, and 4% (w/w) *Lactobacillus plantarum* suspension. The mixture was stirred until distributed, compacted in a plastic container, and fermented at room temperature for 7 days.

2.2.3. Parameters testing

The parameter assessment was carried out on black soybean miso treated with crude bromelain (w/w) 0% (T0) as a control, 3% (T1), 6% (T2), 9% (T3), and 12% (T4). The water content testing uses the oven drying method by weighing the miso and putting it in the oven for 8 hours [18]. The water activity test was carried out using a calibrated a_w meter. The pH testing was carried out [19] using a pH meter. LAB viability was carried out to [20] using the pour plate method with 10^{-7} dilution.

2.3. Data analysis

The data were analyzed using an application named SPSS 26.0 for Windows. The data was obtained by testing the water content, water activity, and LAB viability. It was analyzed using the analysis of variance (ANOVA) parametric test with significance level ($p<0.05$) and when there is a significant difference in treatment will be continued by Duncan's multiple range test (DMRT) while the LAB viability was statistically analyzed using Excel application.

3. RESULTS AND DISCUSSION

3.1. Black soy miso's chemical characteristics

The chemical characteristics of black soy miso were water content, water activity, and pH value as described in Figure 1. Chemical characteristics of black soy miso were water content, water activity, and pH value as described in Figure 1 can be seen that the addition of crude bromelain with concentrations of 0, 3, 6, 9, and 12% gives a significant effect ($p<0.05$). The average water content of black soybean miso in this study ranged from 56.92-61.00%. Miso treatment with the addition of 0% crude bromelain or acting as a control has the smallest a_w value which is 0.653 while the highest a_w value of the treatment is miso with the addition of 9% crude bromelain which is 0.671. The highest pH value is owned by miso with the addition of 0% crude bromelain which the value is 5.10 and the lowest pH value is owned by miso with the addition of 12% crude bromelain which the value is 4.70.

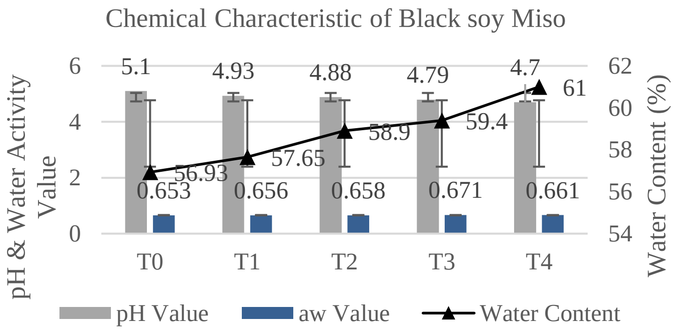

Figure 1. Chemical characteristics of black soy miso

Figure 1 shows that the addition of crude bromelain can increase the water content of black soybean miso. The high-water content is thought to be caused by the amylolytic activity of *Lactobacillus plantarum*. Amylolytic activity by *Lactobacillus plantarum* can hydrolyze starch and simple proteins to produce glucose and other sugars which are then converted into pyruvate by freeing water molecules [10] so that the amount of water contained will also be more.

Another factor that influences water content is salt. According to Tuhumury [21], the reduced water content of a product can be caused by the presence of salt which can reduce the water activity. Salt is hydrophilic which has the ability to absorb water. Salt has a higher osmotic pressure because of this difference in pressure so the salt will absorb the water in the material until there is a balance between the two, the water that continues to be absorbed will affect the decrease in the water content value of the material [22]. However, in this study, salt had no effect on the water content of black soybean miso because the concentration used was the same.

The higher adding crude bromelain concentration given to the miso product, the higher the water activity value contained. The a_w value of miso treatment had a mean range between 0.653-0.671. When it is compared, the water activity value of treated miso is lower than miso with low salt content. According to Allwood *et al.* [23], miso with a low salt content of 2.36-5% has a water activity value of 0.835-0.875. The low of a_w value on the miso sample is influenced by the added salt content. Salt itself has hygroscopic properties which can bind water. This is in line with Wang *et al.* [24] which states that salt has the ability to attract water molecules so that water activity levels decrease and can inhibit the growth of pathogenic microbes in the product.

The addition of crude bromelain and *Lactobacillus plantarum* can speed up the fermentation process by breaking down proteins into simpler compounds, namely amino acids. The fermentation process is closely related to LAB. *Lactobacillus plantarum* is a type of LAB that can ferment sugar into lactic acid and is often found in fermented food products such as miso. Sugar is used by LAB as a substrate or source of nutrients to develop. This is in line with Liu *et al.* [25] which states that glucose and sucrose function as carbon for LAB fermentation and will decrease along with LAB growth and metabolism. The fermentation process will cause the metabolism of LAB. The increase in water activity in miso can also be due to the amylolytic activity of *Lactobacillus plantarum* because it uses starch as a substrate. Amylolytic activity in *Lactobacillus plantarum* can hydrolyze starch and protein into simple sugars and will be converted into pyruvate by freeing water molecules so that the hydroxyl groups in the starch granules disappear and the water content in the product is greater.

The pH value is an indicator to determine the acidity level of a product. The amount of H^+ ion concentration contained in the solution becomes a reference in determining the pH value. The pH value will decrease or become more acidic as the crude bromelain concentration increases. Miso with the addition crude bromelain concentration of 12% showed a lower pH value compared to other treatments. Miso with the addition of different concentrations of crude bromelain will produce different pH values. The higher of crude bromelain concentration added, the acidity level tends to increase.

Crude bromelain is classified as a protease enzyme that is able to speed up the fermentation process by breaking down proteins into simpler compounds, namely amino acids. The types of amino acids that are quite often found in fermented products are non-essential amino acids such as glutamic acid and aspartic acid due to the abundant amount of protein [25]. These two types of amino acids are what make it possible to reduce the pH value of the product because they are classified as acidic amino acids. This is in line with the opinion of Agius *et al.* [26] which states that glutamic acid and aspartic acid are the only proteinogenic amino acids with acidic side chains because these side chains are charged and hydrophilic and are often found on the surface of proteins.

The high LAB content in black soybean miso koji is due to the fermentation process itself and the additions of *Lactobacillus plantarum* This also aims to speed up the fermentation process. Hakim *et al*. [27] stated that the LAB metabolite process during the fermentation process will produce lactic acid and other organic acids so that it can reduce the pH value of fermented soybean paste products. In the fermentation process, there will be a breakdown of glucose into lactic acid which is followed by an increase in the concentration of H^+ which can lower the pH.

3.2. Black soy miso's microbial characteristic

The microbial characteristic of black soy miso is the LAB viability as described in Figure 2. The microbial characteristic of black soy miso shows a waving LAB growth as described in Figure 2 because of the addition of crude bromelain with different concentrations. LAB growth experienced its optimal phase on the 3rd day of fermentation, it reached 9.11 log CFU/mL in miso with the addition of 3% crude bromelain. On the 7th day of fermentation, LAB growth decreases until 8.35 log CFU/mL in miso with the addition of crude bromelain of 3% as well. On the 10th day of fermentation actually was an increase in LAB growth in several treatments and some growth was stable or even decreased.

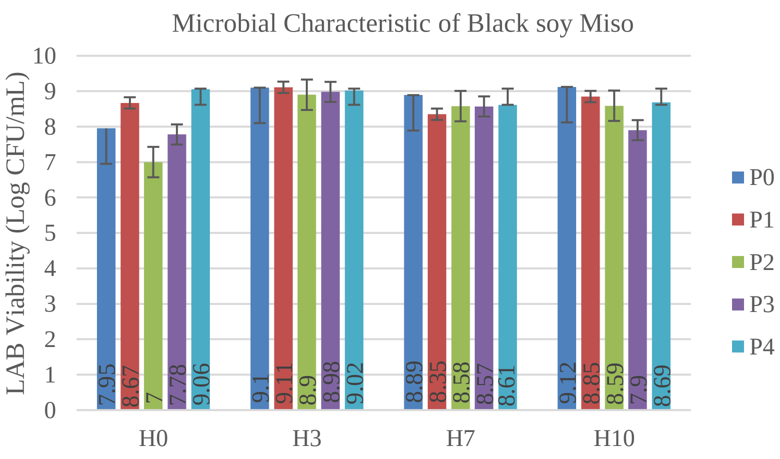

Figure 2. Microbial characteristics of black soy miso

The number of LAB growth on fermentation day 0 is still small because the bacteria need to adapt to their new environment. This is in accordance with Nicola and Baath [28] which states that on day 0 of fermentation, *Lactobacillus* sp. is in the lag phase so their growth is not optimal because the bacteria are carrying out an acclimatization process to environmental conditions, in this case, are pH value, temperature, and nutrition. Entering the 3rd day of fermentation, LAB growth increased in all treatments because LAB had begun to grow and entered the exponential phase where bacterial growth occurred very quickly. According to Sha *et al.* [29] stated that in this exponential phase, microbes are carrying out cell division or multiplication. The greater the crude bromelain concentration added, the faster the growth of LAB. This is in accordance with Utami *et al.* [30] who stated that the bromelain enzyme is a protease enzyme that can accelerate the conversion of protein bonds into amino acids whose nitrogen content plays a role in the formation of biomass cells in LAB growth. The faster the fermentation process, the more LAB growth will increase.

LAB growth began to decline on the 7th day of fermentation because it was approaching its death phase after entering the stationary phase between the 3rd and 7th days. Bacterial growth in the stationary phase is relatively constant because the number of cells that grow is the same as the number of cells that die, and even relatively decreases because the nutrients in the media begin to decrease [31]. This is consistent with the 10th day of fermentation showing that LAB growth has increased again in several treatments, is stable, or even decreased. The increase in LAB growth means that the bacteria are still in the exponential phase. A stable number of LAB colonies indicates that the bacteria are in the stationary phase.

4. CONCLUSION

The addition of crude bromelain in miso made from black soybeans gives different results in each parameter. The addition of crude bromelain in miso can reduce the pH value, and increase the water content and a_w value, but has not provided optimal results from a microbiological aspect. The best treatment from the research conducted is miso with additions of crude bromelain enzyme 9% because it is very close to the desired criteria which are having a high-water content, a_w value high, and low pH values.

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