The effect of fermentation with lactic acid bacteria to chemical and sensory characteristics of Sumbawa’s Buffalo Jerky

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Abstract. Jerky is a traditional food made from sliced or minced meat that were marinated in spices and dried to extend its shelf life. Most of the marketed jerky available in Sumbawa were half-cooked. The aim of this study was to determine the concentrations of Lactic Acid Bacteria to chemical and sensory characteristics of buffalo jerky. A laboratory experiment was conducted using Completely Randomized Design with a single factor namely LAB concentrations (P1=0%, P2=0.5%, P3=1% and P4=1.5%). Data was analyzed using Co-Stat software at 5% significance level. The results showed that the LAB concentrations gave significant differences on water content, protein, fat, color, flavor and texture of fermented buffalo jerky, but gave non-significant difference on the ash content. The best LAB concentrations were 0.5% that resulted on jerky with protein content of 74.32%, fat content of 6.37%, water content of 12.51%, ash content of 1.14%, also color, flavor and texture that were acceptable to panelists.

1. Introduction
Buffalo (Bubalus bubalis) is commonly used as livestock to help cultivate agricultural lands in Sumbawa, an Island in West Nusa Tenggara Indonesia. Buffalo meat is a potential source of nutrients, since it is high in protein content, low in water content and contains higher myoglobin than beef [1]. Apart from its high nutritional content, many consumers prefer beef compared to buffalo meat, this is because buffalo meat is tougher, since it is usually slaughtered from older animals, has longer muscle fibres, less juicy and has certain natural odour. Fresh meat will go through biochemical and physicochemical changes that can affect its quality and quantity, therefore a correct processing method is needed in order to have products that are favourable to consumers, such as jerky. Jerky is a traditional food made from sliced or minced beef and marinated in spices and dried to extend its shelf life. Most of the marketed jerky available in Sumbawa is half-cooked; therefore, cooking is needed prior to consumption. The incorrect cooking method and temperature will result on having low quality jerky with a very tough texture, therefore ready-to-eat jerky is an innovation to make the jerky is easier to prepare and maintain its nutritional content [2].

There are many processing techniques available to increase the tenderness of buffalo’s meat; one well known technique is fermentation with lactic acid bacteria (LAB). Ready-to-eat jerky flavoured with traditional Sumbawa spices could be made by fermentation. The high protein content causes the texture of the buffalo meat to be tougher, so it is necessary to break down protein molecules...
into simpler amino acids to make the meat texture more tender [3]. This can be achieved in various ways, one of which is the addition of proteolysis enzymes. LAB isolates contain proteolysis enzymes that can break down proteins and inhibit the growth of spoilage microorganisms [4]. Fermentation is a method of processing or preservation commonly used to increase the nutritional value of meat. The advantage obtained is that the product will have longer shelf life and improved flavour and aroma [5]. There are various factors that influence the rate of fermentation, including the type of microorganism used, temperature, substrate availability, fermentation time and microorganism concentrations [6]. The right concentration of LAB used will contribute to the quality of jerky. If the concentration is low, the fermentation process of converting complex compounds into simpler compounds will not run optimally, and vice versa. Therefore, a study was conducted to find the best LAB concentration to produce the best quality jerky based on its chemical and sensory characteristics.

2. Materials and Methods

2.1. Time, Place and Experimental Design

This experiment was conducted in Food and Agroindustry Laboratory in Universitas Teknologi Sumbawa from February to April 2021. The materials used in this study were buffalo meat obtained from KeratoBerangBiji Market in Sumbawa, garlic, coriander, cinnamon, cloves, star anise, cumin, salt, pepper, galangal, saparwantu, brown sugar and Lactic Acid Bacteria starter (a combination of Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei and Streptococcus thermophillus). The tools used in the research were digital scales (Mettler Toledo AL-204 analytical balance), meat slicer (Fomac MSC-HS10), stainless steel knife, baking sheet, frying pan, spatula, blender (Phillips), tissue, spoon, plate, porcelain cup, Kjeldhal flask (Pyrex), Soxhlet extraction tool (IWAKI), desiccator (18.5 L Duran), food chopper (Oxone OX-272 Jumbo) and oven (Memmert oven UNB 400, Germany). This study used a completely randomized design with 4 treatments (LAB concentrations); P1 (0%), P2 (0.5%), P3 (1%), and P4 (1.5%). Each treatment was repeated 3 times. The variables measured were water content, protein content, fat content, ash content and sensory analysis on color, texture, aroma, flavor and overall acceptance. Data was then analyzed using CoStat software (CoHort 6.451) at 5% significance level. Any significant differences was then tested with the Duncan Multiple Range test [7].

2.1.1. Jerky Production

The production of this jerky is done as follow [8]: external fat is removed from fresh buffalo meat and the meat was sliced until the thickness is 2-2 mm using meat slicer. Marination sauce is made by mixing all the spices and LAB and sliced meat is added to the mixture. The marination time is 12 hours. Meat was then heated in the oven at 120˚C for 15 minutes [9]. Chemical and sensory analysis was then carried out to measure the quality of the jerky.

2.1.2. Water Content

Measurement of moisture content was carried out using the oven method [9]. The working procedure for determining water content is as follows: a clean porcelain cup is dried in a drying oven at 105 °C for 1 hour. Furthermore, the porcelain plate was cooled in a desiccator for 1 hour (equivalent to room temperature), then weighed in a closed state (A gram). A sample of 1.5 - 2.0 grams was put into a porcelain dish (B grams). Then dried in an oven 105 °C for 8-12 hours. After that the plate containing the sample was cooled in a desiccator for 1 hour, then weighed (C gram) [10]. The calculation of water content is carried out as follows:

\[ \text{Water content (\%)} = \frac{B - C}{B - A} \times 100\% \]

2.1.3. Protein Content

Determination of protein content was carried out using the Kjeldahl method[10] which consists of 3 steps: extraction, destillation and titration. 1 gram of the buffalo jerky sample that has been blended was placed into a 100 mL Kjeldahl flask, then added 10 mL concentrated sulfuric acid. Then the
Kjeldahl flask is heated over low heat until the solution becomes clear greenish color. The solution is then cooled and aquadest to 100 ml was added. 10 mL of 30% sodium hydroxide solution was added through the walls of the distillation flask until a layer is formed below the acid solution. The distillate flask is installed and connected to the condenser, then the end of the condenser is immersed in the collecting liquid. The steam from the boiling liquid will flow through the condenser to the reservoir Erlenmeyer. Erlenmeyer reservoir is filled with 10 mL of 0.1 N hydrochloric acid solution which has been dropped by methyl red indicator. The solutions were then titrated using 0.1 N sodium hydroxide. The end point of the titration is reached after the solutions becomes pink to yellow color. This treatment was carried out 3 times for each sample. Calculation of protein content is carried out as follows:

\[
\text{Protein content (\% ) } = \frac{\text{ml HCL (sample standards)}}{\text{sample weight (grams)}} \times N \text{ HCl 100\% x 14,000 x 100}\% 
\]

2.1.4. Fat Content
Fat content was determined using the Soxhlet method [11]. The working procedure for determining fat content is as follows: Fat flask is dried in an oven at 105 °C for 30 minutes, then cooled in a desiccator (15 minutes) and weighed (A). The jerky sample was weighed as much as 5 g (S) then wrapped in filter paper and put in a fat sleeve. The fat sleeve was covered with fat-free cotton and put into the Soxhlet tube extractor chamber, then doused with fat solvent (hexane), then the tube was attached to the Soxhlet distillation device. The prepared fat flask is then attached to a distillation device over an electric heater with a temperature of about 80 T. Reflux is carried out for a minimum of 5 hours until the solvent that drops back to the fat flask is clear in color. The solvent in the fat flask is distilled, then the flask containing the extract is heated in an oven at 105 °C for 60 minutes or until its weight remains. Then the fat flask is cooled in a desiccator for 20-30 minutes and weighed (B). The calculation of fat content is carried out as follows:

\[
\text{Fat content (\% ) } = \frac{(A-B)}{\text{sample weight}} \times 100\%
\]

2.1.5. Ash Content
Testing the ash content by means of a porcelain plate, oven for 1 hour, then removed and cooled for 30 minutes in a desiccator. The empty plates are weighed as a gram. After that, 5 grams of the test material are put into a cup, weighed and recorded as b grams. This process is carried out at a temperature of 600°C until perfect ash is formed. Then weighed and calculated the percentage of ash content in the sample [12]. The calculation of the ash content is carried out as follows:

\[
\text{Ash content (\% ) } = \frac{\text{ml HCL (sempel blanko)}}{\text{berat sampel (grams)}} \times 100\%
\]

2.1.6. Sensory Analysis
Sensory analysis was carried out to determine the acceptance level of panelist on the jerky products. In this test, there were 30 panelists who gave an assessment of the jerky which included color, texture, and taste. Tests conducted using the scoring method with a rating scale of 1-3, namely for the color (1) blackish brown, (2) dark brown and (3) reddish brown, for textures (1) tough, (2) tender and (3) very tender. and for the taste (1) very sour-sweet, (2) quite sour-sweet and (3) normal-typical jerky taste [13].
3. Results and Discussion

3.1. Water Content
Water content plays an important role in the quality and shelf life of buffalo jerky. Figure 1 shows the water content for each treatment. Results obtained that different LAB concentrations give significant differences on the water content of buffalo jerky.

![Water Content of Buffalo Jerky](image)

Figure 1. Water content of buffalo jerky.

In this study, the moisture content of buffalo jerky ranges from 10.17% to 15.27% (p<0.05). The highest moisture content is in P4, with the highest concentration of LAB added. Moisture content has a decisive effect on the stability of intermediate-moisture (IM) foods, according to the Indonesian Standard (SNI 2908-2013) for jerky the maximum moisture content is 12%, whilst in general commercial IM foods have moisture content of 20% to 40%. The increase of water content is due to LAB began to utilize easily fermented carbohydrates as substrates to grow and develop. These complex carbohydrates are then broken down into simple sugars, and then converted into energy with by-products in the form of metabolites, alcohol, acids, carbon dioxides and water [14]. Water is one of the by-products of the fermentation process that will affect the moisture content of fermented products. Therefore, the higher the concentration of LAB added will increase the fermentation rate and eventually increase the water content of jerky products.

3.2. Protein Content
Figure 2 shows the protein content of fermented buffalo jerky, with the protein content ranges from 62.36% to 82.21%, and this is corresponding to the minimum protein content requirements from the Indonesian Standards of jerky, which is minimum of 18%. Results obtained that different LAB concentrations give significant differences on the protein content of buffalo jerky.
Solid state fermentation increases crude protein content due to the secretion of microbial proteins like enzymes, hydrolyzed peptides and other nitrogenous microbial components like chitin [16]. In general, microorganisms utilize carbohydrates as a source of energy and bio-converted them into microbial proteins through intermediary metabolism. Microorganisms utilize carbohydrates as an energy source and produce carbon dioxide as a by-product. This causes the nitrogen in the fermented buffalo marination to be concentrated, and thus, the proportion of protein in the total mass increases. During fermentation, LAB releases proteolytic enzymes that breaks down complex protein to simpler amino acids. Determination of protein content with Kjeldahl method will result on number of crude protein or total Nitrogen in the sample, therefore the higher LAB concentrations added, more protein will be converted into amino acids, overall crude protein content will increase.

3.3. Fat Content
Figure 3 shows the fat content of fermented buffalo jerky, with the fat content ranges from 5.11% to 6.61%, and this is corresponding to the minimum protein content requirements from the Indonesian Standards of jerky, which is maximum of 3%. Results obtained that different LAB concentrations give significant differences on the fat content of buffalo jerky.

The higher the concentrations of LAB added, the higher the rate of fermentation will occur. LAB has lipolysis enzymes that can breaks down complex fat into simpler fat molecules; this will result on the decrease of jerky’s fat content. The increasing fat content is caused by decreased water content in jerky. Decreased water content on foodstuffs will increase compounds such as carbohydrates and fat.
3.4. Ash Content
Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600 °C. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to CO$_2$, H$_2$O and N$_2$ [15]. Figure 2 shows the ash content of jerky for all treatments. Results obtained that different LAB concentrations give non-significant differences on the ash content of buffalo jerky.

![Ash Content of Buffalo Jerky](image1)

Figure 4. Ash content of buffalo jerky.

The ash content of buffalo jerky ranges from 0.97% to 1.21%. According to the Indonesian National Standards for Jerky (SNI 2908-2013) the ash content for jerky is maximum 0.5%, therefor the ash content for all treatments beyond the standards. Changes in the ash content of substrates during the fermentation process caused by changes in organic matter that occur during the bioconversion process. Increased ash content during fermentation is caused by the increasing period of the body's cell time of anytime and the occurrence of increased concentration in the product due to changes in organic matter due to bioconversion processes that produce H$_2$O and CO$_2$.

3.5. Sensory Analysis
3.5.1. Color
Figure 5 shows the score for jerky’s color that ranges from 1.45 until 2.21, and were stated deep brown to blackish brown in color. The addition of LAB with different concentrations give significant differences in color appearance of fermented buffalo jerky.

![Jerky's Color](image2)

Figure 5. Color appearance of buffalo jerky
The addition of LAB affected the color appearance of the jerky, since the more LAB added the color will turn from blackish brown into brighter deep brown color. This is due to the ability of LAB to do the color degradation. This color degradation occurs because the LAB can utilize pigment conversion. Buffalo’s meat is usually deep red in color, red / dark meat contains myoglobin and hemoglobin which are prooxidants as well as rich in fat caused high hemoprotein content composed of protein moiety, globin and structure heme [17]. The higher the LAB concentrations added, the more LAB that can breaks down red pigments. The difference in meat color is caused by the presence of H$_2$O$_2$ and the enzymes produced by LAB. H$_2$O$_2$ compound cause oxidation of oxymyoglobin to metmyoglobin which is brown in color [18] this causes the color of the meat without added LAB is darker compared to fermented meat that was added LAB.

3.5.2. Flavor

Figure 6 shows the score for jerky’s flavor that ranges from 1.43 until 2.31, and were described as mild acidic flavor to strong acidic flavor. The addition of LAB with different concentrations give significant differences in flavor of fermented buffalo jerky.

![Bar chart showing Jerky's Flavor](image)

**Figure 6.** Flavor of buffalo jerky

The addition of LAB affected the flavour of the jerky, since the more LAB added the panellist were able to taste more acidic flavour in the jerky. Lactic acid fermentation continues to be highly desirable methods of processing and preserving foods because they are of low cost, have low energy requirements, and yield highly acceptable and diversified flavours. The production of lactic acid has also a direct impact on sensory product quality by providing a mild acid taste, and by supporting the drying process which requires a sufficient decline in pH. Furthermore, LAB influence the sensory characteristics of the fermented jerky by the production of small amounts of acetic acid, ethanol, acetoin, pyruvic acid, carbon dioxide, and their ability to initiate the production of aromatic substances from proteinaceous precursors [19].

3.5.3. Texture

Fermentation with different LAB concentrations added give significant differences in buffalo’s jerky texture, this result is shown in Figure 7.
Jerky’s texture score ranges from 1.14 until 2.09, and were described as tough to tender. The addition of LAB with different concentrations gave significant differences in the texture of fermented buffalo jerky. As the concentrations of LAB increased, the toughness of the jerky decreases. This significant changes in texture is caused by the increased water content during fermentation and also the ability of proteolytic enzymes from LAB that breakdown complex muscle fibers and proteins into smaller molecules, that contributes to the tenderness of the jerky.

Juiciness and tenderness are two very important factors when it comes to meat quality. Meat consists of muscle and connective tissues that are made up of proteins. Collagen is a long, stiff protein that is the most prevalent protein in mammals. It’s made up of three separate molecules composed of amino acid chains, twisted around each other [20]. This structure is what makes the collagen so strong; this strength is also what makes it more difficult to break down. The more collagen there is in a piece of meat, the tougher it is to cut and to chew. LAB has protease enzymes that can breakdown large protein molecules into smaller ones [21], this will result on softer and more tender meat. Protease treatment is an efficient method used for meat tenderization. Proteases play an important role in degrading the structural proteins in the connective tissues, thus reducing toughness of meat.

Conclusions

The best LAB concentrations to produce the best buffalo jerky was 0.5% that gave protein content of 74.32%, fat content of 6.37%, water content of 12.51%, ash content of 1.14% that are in accordance to the Indonesian National Food Standards for Jerky (SNI 2908-2013), and with color (bright deep brown color), flavor (mild acidic flavor) and texture (tender) that were acceptable to panelists.

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