Fermentation Time in The Tape Making Process Affects the Chemical Quality of Corn Tape

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Abstract. Consuming fermented foods to increase microbial diversity and high-fiber foods will provide more significant synergistic benefits. The purpose of this study was to analyze the chemical quality of corn tape with different fermentation times. This research was an experimental study using a completely randomized design. The treatment in this study was the length of fermentation in making corn tape, including five days, six days, and seven days. The analysis results showed significant differences in the protein content, lactic acid bacteria, pH, antioxidant capacity, and flavonoid content of fermented corn tape at different times. The longer the fermentation, the higher the protein content, lactic acid bacteria content, and flavonoid content, the stronger the antioxidant capacity and the more acidic the pH of corn tape. The conclusion is that the fermentation duration can affect corn tape's chemical quality.

1 Introduction

The consumption of healthy foods continues to increase human efforts to maintain the body's immune system. One of the health foods is food rich in probiotics which play a role in maintaining the body's metabolism in the body remains normal. Fermented foods are the staple food of humans and have been produced and consumed since the development of human civilization [1]. Food fermentation processes can be categorized based on the primary metabolites and microorganisms involved: Lactic Acid Bacteria (LAB) belong to genera such as Leuconostoc, Lactobacillus, and Streptococcus.

In some cases, materials containing high monosaccharides and disaccharides, or starch, are fermented by Lactic Acid Bacteria (LAB) [2]. Regularly, LAB-fermented foods boost the immune system and strengthen the body in fighting pathogenic bacteria [3]. Bacteriocins produced by LAB can be potential drug candidates to replace antibiotics to treat several drug-resistant pathogens [4].

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Tape or tapai is a traditional food or snack that goes through a fermentation process in manufacturing. There are two types of tape based on the raw material: cassava tape, which is the primary ingredient of cassava, and sticky tape made from sticky rice, both white sticky rice, green sticky rice, and black sticky rice. Some researchers say that probiotics are most widely applied to fermented foods, making tape one of the processed probiotic foods. The positive thing, the effect of probiotics on human health has been proven by increasing the body's immunity (immunomodulation). Many diseases start with an initial imbalance of human resident microflora and associated immunobiological reactivity [5]. Past research has shown that consuming probiotics that thrive in foods such as yogurt, sauerkraut, and kimchi can improve overall gut and respiratory health and even cut the risk of chronic conditions such as type 2 diabetes and heart disease [6].

Several studies have shown that consuming fermented foods increases microbial diversity, and also consuming high-fiber foods will provide more significant synergistic benefits. Corn is a food that has a high dietary fiber content with a relatively low glycemic index (GI) compared to rice. Recommend corn rice for people with diabetes. The purpose of this study was to analyze the chemical quality of corn tape with different fermentation times, which are five, six, and seven days.

2 Methodology

2.1 Types of research

This research is experimental research using a completely randomized design. The treatment in this study was the length of fermentation in the process of making corn tape and the percentage of bacteria used. The research determined fermentation times of two, three, and four days with yeast percentages of 0.3%, 0.4%, and 0.5%. Table 1 shows the research design.

Table 1. Research design

Repetition	**Treatment**		
P	A_1	A_2	A_3
P_1	A_1P_1	A_2P_1	A_3P_1
P_2	A_1P_2	A_2P_2	A_3P_2

Note:
Treatment : Fermentation Time (A)
A_1 = Corn tape with two days of fermentation
A_2 = Corn tape with three days of fermentation
A_3 = Corn tape with four days of fermentation
Repetition:Repe tition Factor (P)
P_1 = Repetition 1
P_2 = Repetition 2

2.2 Research procedure

Prepare the tools and materials for making corn tape, and weigh the shelled corn according to the treatment. Furthermore, the shelled corn was washed twice and soaked for 30 minutes. After the soaking process, the corn is steamed until cooked, then cooled. Then, following the given treatment, sprinkle the yeast onto the tape and gently stir until evenly distributed. Then put it in a closed container covered with banana leaves. Finally, store it in a place not exposed to direct sunlight.

The stages in making sticky rice tape have their respective functions, including the washing process twice serves to clean dirt and remove foreign object contamination in glutinous rice—the soaking process functions in the gelatinization process at a later stage—the steaming process has a function in helping the ripening of glutinous rice, killing pathogenic microbes, and obtaining a soft texture on glutinous rice—the process of giving yeast has the function of fermenting sticky rice into the tape—the tape packaging process obtains an anaerobic atmosphere to support the fermentation process by amylolytic microbes and keep it sterile.

2.3 Materials and tools

2.3.1 Corn tape materials

The material used in making corn tape is ground corn which has been cleaned and then soaked in water. The materials used for chemical analysis use chemicals. Table 2 shows the materials used in making corn tape.

Table 2. Corn tape materials

Number	Ingredient's Name	Percent	Amount
1.	Ground corn	100%	100 g
2.	Tape yeast	1%	g

2.3.2 Tools

The tools used in this study are shown in Table 3.

Table 3. List of tools used in manufacturing of corn tape

Number	Tool's Name	Amount
Preparation Tools		
1.	Scale	1 piece
2.	Bowl	1 piece
3.	Spoon	1 piece
Processing Tools		
1.	Stove	1 piece
2.	Steamer pot	1 piece
3.	Tray	1 piece
4.	Spoon	1 piece
5.	Plastic cups	12 piece

2.4 Time and place of research

The research was conducted from April to August 2022. The first stage began with testing shelled corn's soaking and steaming time at the Industrial Laboratory, Department of Industrial Technology, Faculty of Engineering, Universitas Negeri Malang. The next stage is corn tape's phytochemical and physical analysis with different fermentation treatments and yeast percentages at the Chemical Analysis Laboratory, Universitas Muhammadiyah Malang.

3 Results and Discussion

The chemical analysis of corn tape is shown at Table 4 below.

Table 4. Chemical analysis of corn tape processed with different fermentation time

Fermentation time	Content of Protein	Content of Lactic Acid Bacteria	pH	Antioxidant Capacity	Flavonoid
Five days	5.555 ± 0.0636[a]	7.97×10^6 ± 261629.509[a]	5.235 ±0.00707[a]	87.319 ±0.620133[a]	0.066[a]
Six days	5.810 ± 0.0636[b]	9.37×10^6 ± 176776.695[b]	4.885 ±0.00707[b]	75.604 ±0.659024[b]	0.074[b]
Seven days	5.991 ± 0.0636[c]	1.03×10^7 ± 141421.356[c]	4.595 ±0.02121[c]	69.022 ±0.345775[c]	0.078[c]

3.1 Protein content in corn tape

According to [7], protein content is an essential food substance for the body because it functions as a builder and regulatory substance. Protein plays a role in forming layers and resists some of the expansion of water vapor. Meanwhile, according to [8], protein is a polymer of about 21 amino acids connected by peptide bonds. Amino acids create a variety of side chains, forming connections with different proteins that can exhibit diverse properties and distinct secondary and tertiary structures. The side chains can be polar and nonpolar. The high content of polar amino acid moieties in proteins increases their solubility in water.

Based on the results (Table 4) and ANOVA test analysis, the protein content of corn tape at five days, six days, and seven days of fermentation showed a significant difference at the 5% significance level. The results of the DMRT test analysis showed that corn tape at seven days of fermentation had the highest protein content, with an average of 5.991%. According to [9], corn protein (8-11%) consists of five fractions: albumin, globulin, prolamin, glutelin, and non-protein nitrogen. Meanwhile, according to data from [10], the protein content in corn is 3.22g. Furthermore, [11] found that corn flour, fermented using tape yeast for 80 hours, yields a total protein content of 8.4[a], with total protein levels ranging from 7.19% to 8.46%.

Various reasons can cause the high protein content observed in corn tape with a 7-day fermentation time. As [11] report, fermentation can elevate dissolved protein levels. Lactobacillus casei caused the increase in dissolved protein levels during the fermentation process by reshuffling the substrate in the form of corn starch. Under these conditions, the dissolved protein was hydrolyzed into amino acids so that the dissolved protein content increased during the 7-day fermentation with a protein content of 5.991%. This protein content is higher than the results of a study by [12], namely the results of a protein content of 5.07%, and the results of research by [13], namely 5.4%. However, it is similar to the research conducted by [4], namely, with a protein content of 8.7% to 10%. Therefore, the longer the time used for fermentation, the higher the dissolved protein content produced.

Protein hydrolysis is one of the leading causes of decreased protein levels observed in corn tape with a 5% fermentation time, although various factors can contribute to this reduction. Research by [14] in [15] reported that protein hydrolysis into simpler compounds caused the microbes present in tape yeast starter, especially Rhizopus sp., to produce proteases. Therefore corn tape with five days of fermentation produced a lower protein content than corn tape with six and seven days of fermentation.

3.2 Content of lactic acid bacteria in corn tape

Based on the results (Table 4) and the ANOVA test analysis, the content of lactic acid bacteria in corn tape at the fermentation time of five days, six days, and seven days showed that there was a significant difference at the 5% significance level. The results of the DMRT test analysis showed that corn tape at seven days of fermentation had the highest lactic acid bacteria content, with an average of 1.03×10^7 cfu/g.

Lactic acid bacteria are commonly used as probiotics because they are non-pathogenic and non-toxigenic. Lactic acid bacteria usually produce bacteriocins, peptides with antibacterial properties [16]. Lactic acid bacteria are a group of bacteria that produce lactic acid as a metabolic product. Lactic acid bacteria are gram-positive bacteria in the form of cocci, non-sporing rods and are microaerophilic. These organisms are heterotrophic and generally require complex nutrition during their growth and development [17]. Lactic acid bacteria are commonly used as probiotics because they are non-pathogenic and non-toxigenic. Lactic acid bacteria usually produce bacteriocins, peptides with antibacterial properties [16].

Furthermore, [18] asserts that lactic acid bacteria microorganisms strongly influence fermentation in food. Lactic acid bacteria are normal human flora in the mouth and digestive tract. Furthermore, lactic acid bacteria inhabit nutrient-rich environments like dairy products, meat, and vegetables. Lactic acid bacteria intended for fermentation include L.bulgaricus, L.casei, L.plantarum, and L. acidophilus. L.bulgaricus bacteria is one of several bacteria used to produce yogurt. Other fermented products also contain it. This bacterium is in the form of a long, filamentous rod, does not form spores, and can ferment carbohydrates to produce lactic acid. L.bulgaricus bacteria inhibit Helicobacter pylori bacteria that cause digestive tract infections [19].

Based on research conducted by [20], the highest average lactic acid bacteria (LAB) was 19.00 x $10^8 \pm 9.05$, and the lowest was $16.15 \times 10^8 \pm 9.6$. The high content of lactic acid bacteria in corn tape is safe for our digestion. Meanwhile, [21], apart from its ability to inhibit pathogenic bacteria, bacteriocins do not harm the normal intestinal flora because digestive enzymes quickly digest them. L. casei bacteria are homofermentative lactic acid bacteria. These namely bacteria can ferment glucose into large amounts of lactic acid and produce citric acid in small amounts, which affects the taste of lactic fermentation [6]. Several factors, including nutrient loss, can cause the low content of lactic acid bacteria in corn tape during five days of fermentation, as observed in the analysis. The decrease in the number of lactic acid bacteria and yeast after the second day of fermentation was due to the reduced amount of nutrients available in the fermentation medium, as well as the presence of antimicrobial components produced by LAB [22]. Besides that, the death of microbes is because the nutrients in the medium have run out, and the reserve energy in the cells has run out [23].

The high content of lactic acid bacteria in corn tape with seven days of fermentation with an average of 1.03×10^7 cfu/g is influenced by several things, according to [20], changes in total lactic acid bacteria occur during fermentation. Lactic acid bacteria convert lactose into lactic acid during the fermentation process. The greater the sugar used to produce lactic acid, the greater the activity of lactic acid bacteria. Several factors, such as extended curing time and the proliferation of microorganisms, mainly lactic acid bacteria, contribute to the high levels of lactic acid bacteria. This extended growth period produces more lactic acid, leading to more significant denaturation of proteins. In addition, [2] stated that the longer the curing time, the lactic acid bacteria became more active and produced more and more lactic acid, which could increase viscosity. The presence of lactic acid bacteria (including L. bulgaricus and L. casei) could degrade the corn cell walls so that the starch granules came out of the cells, facilitating the milling process. L. bulgaricus and L. casei can also degrade starch in their growth media into simple sugars and degrade proteins and peptides into amino acids

[24]. Therefore corn tape with seven days of fermentation produced a higher protein content than corn tape with six days and five days of fermentation.

3.3 pH content in corn tape

Based on the results (Table 4) and the ANOVA test analysis, the pH content of corn tape at five days, six days, and seven days of fermentation showed a significant difference at the 5% significance level. The results of the DMRT test analysis showed that the highest pH was corn tape with a 7-day fermentation time with an average of 4.595.

According to [25], pH is the degree of acidity used to express a solution's level of acidity or alkalinity. pH test aims to determine the level of acidity contained in corn flour. According to [26], pH measures the hydrogen ion concentration of the solution. The pH (hydrogen potential) measurement will measure if the solution is acidic or basic. Regarding pH, a solution considers itself neutral when it possesses an equal number of acid and base molecules. Measure pH value and total acid needs to be done to determine the level of acidity or alkalinity of a product. It is related to the safety and shelf life of the product. According to [27], a normal pH has a value of 7, while a pH value > 7 indicates that the substance has alkaline properties, while a pH value < 7 indicates acidity. pH 0 indicates the highest degree of acidity, and pH 14 indicates the highest degree of alkalinity. Generally, a simple indicator used is litmus paper which turns red when the acidity is high and blue when the acidity is low.

Fermentation time also affects the pH value. Both [28] and [5] stated, the longer the fermentation time, the greater the number of microorganisms that will grow to produce acid, so that the pH value will decrease as the concentration of dissolved acid increases. Whereas [29] stated that changes in pH in fermentation are due to the activity of yeast cells; in addition to producing ethanol as a primary metabolite, they also produce acids such as malic acid, tartaric acid, citric acid, lactic acid, acetic acid, and butyrate as a by-product. This decrease in pH value is due to a fermentation process by microbes that degrades starch into organic acids. According to [30], the more microorganisms that are active and multiply in fermentation, the better the ability to break down the substrate, resulting in large amounts of lactic acid. The lactic acid produced during fermentation can increase the taste and acidity or lower the pH. As a result, the formation of lactic acid will affect the physical properties of flour [20].

The results of a similar study conducted by [26] stated that the pH content found in the best treatment of corn tape products with a yeast concentration of 0.5% with a fermentation time of 3 days was 3.56 ± 0.03. The higher the yeast concentration and the longer the corn tape fermented, the lower the average pH value. The decrease in pH is related to the presence of microorganisms in tape yeast which can form various acids such as lactic acid, acetic acid, formic acid, and others [31]. Meanwhile, according to [3] that the longer the fermentation time, the higher the acidity level, so the resulting pH value decreases. According to [32], the longer the fermentation, the more Saccharomyces cerevisiae. This is related to the growth phases of Saccharomyces cerevisiae, which start from the adaptation phase (Lag phase), the exponential phase (Log phase), the stationary phase (Stationary phase), and the death phase (Death phase). The longer the fermentation, the more microorganisms will grow so that the acid formed from the results of alcoholic fermentation also increases [1]. Hence, the pH content decreased in correlation with the fermentation time of the corn tape, resulting in a lower pH in the corn tape fermented for seven days. Therefore, the resulting taste was more sour than the corn tape with six and five days of fermentation.

The decrease in pH that occurs indicates the presence of microbial activity in breaking down carbohydrates [33]. When fermented under anaerobic conditions, sugars such as glucose, fructose, and sucrose as essential ingredients will produce ethanol, lactic acid, and

hydrogen. This fermentation process will result in a change in acidic conditions or a decrease in pH [34]. According to [35], the lactic acid produced during fermentation can enhance taste and increase acidity or lower pH. According to [36], a decrease in pH can inhibit other microorganisms. According to [37], lactic acid bacteria will ferment food and cause the formation of lactic acid, which will lower the pH value of the growth environment. This results in inhibiting the growth of several types of pathogenic microorganisms. The pH of foodstuffs can drop below 4 to inhibit other microorganisms, including pathogenic microbes, so that products can last longer [38]. So corn tape with a seven-day fermentation time will last longer.

The research results by [39] statet that cassava tape has a pH of 4.93 to 5.1. [40] reported that the average pH of glutinous tape is 4.0-4.2. Meanwhile, according to [41], cassava tape has a pH of 4.38-4.75. From the studies above, the average pH of tape is 4.0 to 5.1, which corresponds to the average pH of corn tape. According to [42], the activity of enzymes in yeast can form by-products such as lactic acid, acetic acid, glycerol, and more, which cause a decrease in pH. When oxidation or the presence of bacteria occurs, they form acetic acid, which also causes a decrease in pH.

3.4 Content of antioxidant capacity in corn tape

The ANOVA test analysis revealed a significant difference in the antioxidant capacity of corn tape's content at the 5% significance level, prompting the continuation of the DMRT test. Corn tape at seven days of fermentation had the most vital antioxidant capacity compared to the increase in antioxidant capacity of six days and five days of corn tape. The antioxidant capacity of fermented corn tape for seven days had the lowest average, namely 69.022 ppm.

According to [43], antioxidants are electron donors or reductants. Biologically, antioxidants are compounds that can counteract or reduce the adverse effects of oxidants in the body. Antioxidant status is an important parameter to monitor one's health. This compound has a small molecular weight but can inactivate the development of oxidation reactions by preventing the formation of radicals. Meanwhile, according to [44], antioxidants in food or beverages can be natural antioxidants, such as those contained in vegetables, fruits, and beverages, as well as synthetic antioxidants that are intentionally added (additives) to the food and beverages consumed. A compound is said to have potent antioxidant activity if the IC50 value is less than 50 ppm and strong for IC50 values of 50-100 ppm [45].

According to research by [46], yellow corn contains an antioxidant capacity of 01.8 ± 22.3 µmol. In this study, corn tape has a higher antioxidant capacity than research by [47], which stated that the highest antioxidant activity content in black sticky rice tape has an IC50 value of 81.9834 ppm. Based on previous references, the antioxidant capacity of corn tape, with a value of 69.022 ppm, is classified as a potent antioxidant.

3.5 Flavonoid content in corn tape

Based on the ANOVA test analysis results, the flavonoid content of corn tape at five days, six days, and seven days of fermentation showed a significant difference at the 5% significance level. The results of the DMRT test analysis showed that corn tape at seven days of fermentation had the highest flavonoid content, namely 0.078%.

Flavonoids are one of the largest groups of natural phenolic compounds in plants. Furthermore, it comprises 15 carbon atoms as its fundamental core and a C6-C3 - C6 configuration, namely two aromatic rings connected by three carbon atoms which may or may not form a third ring [48]. Flavonoids are secondary metabolites of polyphenols, found widely in plants and foods and have various bioactive effects, including anti-viral, anti-

inflammatory [49], anti-aging, and contain antioxidants [50]. Flavonoids inhibit microbial growth, lower blood cholesterol, lower blood glucose levels, are antibiotics, and increase the body's immunity [51]. [52] stated that the content of flavonoids relies on the molecular structure of several phenolic hydroxyl components from the aromatic ring structure found in garlic, plant oils, and ginger, which constitute potential antioxidant components.

Corn tape has a higher flavonoid content than previous studies. [53] reported that the flavonoid content in yellow corn measured 0.056. In a previous study by [54], they reported that the total flavonoid levels of jackfruit seed tape extract changed during fermentation. Total flavonoid content increased from the 0th day of fermentation (2.9146 mgQE/gram extract) to the 3rd day (4.0488 mgQE/gram extract). In addition, according to [55], the longer the fermentation time, the higher the total levels of kombucha flavonoids in butterfly pea flower (*Clitoria terna tea* L.). Based on research on corn tape, the flavonoid content increases with the length of fermentation time, namely from days five, six, and seven. After reviewing several previous studies, it is evident that several factors can undeniably influence the high flavonoid content, one of which is fermentation time. [55] stated that lactic acid bacteria's activity can increase total flavonoid levels during fermentation. During this process, lactic acid bacteria produce enzymes that break down sugar, degrade complex phenolic compounds, release phenolic compounds from the substrate, and add phenol groups to form flavonoid compounds. Therefore, the highest content of flavonoids found in corn tape is the most extended fermentation, which is seven days with a value of 0.078% compared to fermentation on days six and five.

4 Conclusion

The length of fermentation showed significant differences in the protein content, lactic acid bacteria, pH, antioxidant capacity, and flavonoid content of maize tape fermented at different times. The longer the fermentation, the higher the protein content, lactic acid bacteria content, and flavonoid content, the stronger the antioxidant capacity and the more acidic the pH of the corn tape. The conclusion is that the fermentation duration can affect corn tape's chemical quality.

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