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Comparison of the Quality of Fermented Sausages with the Use of Yogurt Starter and *Lactobacillus plantarum* (pH, a_w, and Proximate values)

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Abstract: This research is related to the application of probiotic yogurt starter and *L. plantarum*. The purpose of this study is related to know and comparing the quality of fermented sausages in terms of pH, aw, water content, protein content, fat and ash content so that they become healthy food and practical and easy to manufacture. Treatment with differences in the use of yogurt starter and *L. plantarum* respectively 0%, 1%, 2%, and 3%. The results showed that the use of yogurt starter and *L. plantarum* each did not provide a significant difference (P>0.05) for the value of pH, aw, moisture content, protein content, fat content, and ash content. it can be concluded that by comparison of the use of yogurt starter (which contains several types of bacteria) with *L. plantarum* each has differences in pH, aw, moisture content, protein content, fat content, and ash content but all are still in accordance with SNI.

Keywords: Fermented sausage; *L. plantarum;* Yogurt starter

Introduction

Probiotics are widely used in dairy products, but their application in meat products is still being explored. Meat is generally heated before consumption, which kills probiotic bacteria, but dry sausages are processed by fermentation, without heating, and can be a good medium for application of probiotics to meat products. In addition, the sausage matrix protects the viability of probiotic lactobacilli through the digestive tract. *Lactobacillus plantarum, Lactobacillus bulgaricus* and/or mixtures are probiotic lactic acid bacteria which have beneficial effects on consumer health when ingested in certain amounts. Unheated fermented meat products can serve as a suitable matrix for assessing probiotic LAB as a starter culture (Rubio et al., 2014). In addition, it is believed that the sausage matrix supports the survival of probiotic bacteria during their passage in the digestive tract (Klingberg & Budde, 2006). It is also generally accepted that different starter cultures affect differently the sensory characteristics of dry fermented sausages (Cavalheiro et al., 2015). The increase in nutritional value in fermented foods is caused by the fermentation of microorganisms present in them, and the three different ways of fermentation by microorganisms include microorganisms that are catabolic and anabolic, break down complex compounds, and synthesize complex vitamins and other growth factors (Kennedy, 2016). Fermentation with bacteria, fungi, and yeast that decomposes or damages the cell wall and this non-digestible layer both physically and chemically (Hasan et al., 2015). The long-lived fermented meat product group with the addition of a special starter (lactic acid bacteria) has the ability to lower the product's pH and water activity (a_w) thus contributing to a longer shelf life even without heat treatment. With the different types of starter used in sausage fermentation, it will certainly affect protein degradation activity, so it is necessary to analyze the quality of the sausage from the physico-chemical and proximate aspects of the fermented sausage that has been made. The purpose of this research is as an effort to apply probiotic starter from yogurt and *L. plantarum* on the market in making healthy fermented sausages so that it can be applied practically

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and easily so it is necessary to know the quality of fermented sausages in terms of pH value, a_w value, water content, protein content, fat content, and ash content.

Method

The materials and tools used in this study were chicken meat (breast and upper thighs that had been ground), *Lactobacillus plantarum* starter (Agroteknoshop) and yogurt starter (Yogourmet), spices (salt, sugar, garlic, tapioca flour, ice cubes, white pepper, ginger, and sausage casings.The tools used in this study included a meat grinding machine, digital scales, stove, pH meter, Aw meter, oven, soxhlet, and desiccator.

Procedure for Making Fermented Sausages

1) Fermented Sausage Manufacturing

The ground meat is mixed with tapioca flour, ice water, STPP, spices (salt, sugar, ginger, white pepper) and bacterial starter according to each treatment. The dough that has been formed is put into the stuffer, then put into the sleeve and the sausages are pricked with a sterile needle to taste. The sausages were then broiled for 24 hours at room temperature (± 27^{o}C). Treatment with differences in the use of yogurt starter and L. plantarum respectively 0%, 1%, 2%, and 3%. Variable measurements include pH, aw, water content, protein content, fat content, and ash content.

2) Fermented Sausage Analysis pH Value (AOAC 2005)

The pH value was measured using a pH meter. The pH meter tool is calibrated at pH 4 and 7. The sausage is chopped first then pierced with an electrode and left until the number printed on the digital measurement does not change.

3) a_w Value (AOAC 2005)

Sausage aw measurements were measured using a calibrated aw meter. Four grams Sausages are mashed and then placed in a measuring cup. The tool is run until it shows the completed sign.

4) Modified AOAC (2005) Proximate Analysis

Analysis of water content, ash content, protein content, and fat content were calculated using a manual formula.

Data Analysis

The study used analysis of variance arranged in a Completely Randomized Design (CRD). If the results of the treatment in this study had a significant effect ($P<0.05$) or very significant ($P<0.01$), then further testing would be continued using Duncan's Multiple Range Test (DMRT).

Result and Discussion

Comparison of pH and a_w Values

The use of yogurt starter and *L. plantarum* did not give a significant difference ($P>0.05$) for the pH and a_w values of fermented sausages. The decrease in pH was confirmed by the number of lactic acid bacteria at the same time. Figure 1 and Figure 2. shows the addition of the percentage of yogurt starter, the pH of sausages ranges from 4.41 to 4.47, in contrast to the use of *L. plantarum* starter, the higher the percentage of *L. plantarum* starter, the pH value tends to increase from 4.63 to 4.9. The final pH of semi-dry sausage is acidic, reaching a pH of 4.7 to 5.2 – 5.4, with a lactic acid content of 0.5% to 1.3%; and humidity of 35% or higher (Vignolo et al., 2010). According to Mitrović (2016), the decrease in the pH of the fermented (pH 5.6 to 5.8 down to 4.6 to 4.9) is more important and significant for the preservation of sausages in Mediterranean countries, the reduction in aw is more important too. According to Zhou et al. (2018), Lactobacillus produces lactic acid and sour acids, and reduces the pH of fermented products. Lowering the pH inhibits the growth and reproduction of harmful microorganisms, especially spoilage bacteria, thus potentially extending the shelf life of fermented sausages and increasing preservation. The difference in pH value and aw level in the final fermented sausage product after 24 hours of fermentation is in accordance with the opinion of Lopez et al. (2006) and Ivanović et al. (2017) so that fermented sausages have a pH value of 4 to 7. Lactic Acid Bacteria (LAB) can produce organic acids such as lactic acid through the metabolism of carbohydrates in fermented meat, resulting in a decrease in the pH environment (Hu et al., 2021). According to Virgili et al. (2007), in dry cured ham production found higher pH values after aging due to the large release of low-weight nitrogen molecules and the formation of ammonia ascribed to endogenous and exogenous proteolytic, deaminase and deaminase activities. The a_w value of fermented sausages with differences in yogurt starter increased the a_w value with the increasing number of percentages of yogurt starter, in contrast to the use of *L. plantarum* starter yogurt, the a_w value tended to be stable at 0.88. Regarding aw, this parameter is very important for the safety of products such as fermented sausages, because it ensures the value is below 0.9 and the product is stable at room temperature, to limit the growth of spoilage and bacterial pathogens (Taormina and Sofos, 2014). The reduction in a_w value that occurs in fermented sausages is a consequence of the loss of moisture during ripening of fermented sausages similar to that which occurs in Italian-type salami with the addition of additional *L. acidophilus* and *B. lactis* as free cells (Ruiz et al., 2014).

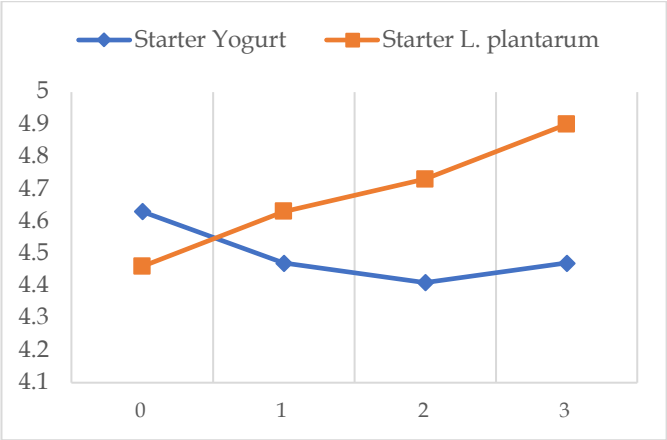

Figure 1. pH value of fermented sausages using different types of starter yogurt and *L. plantarum*

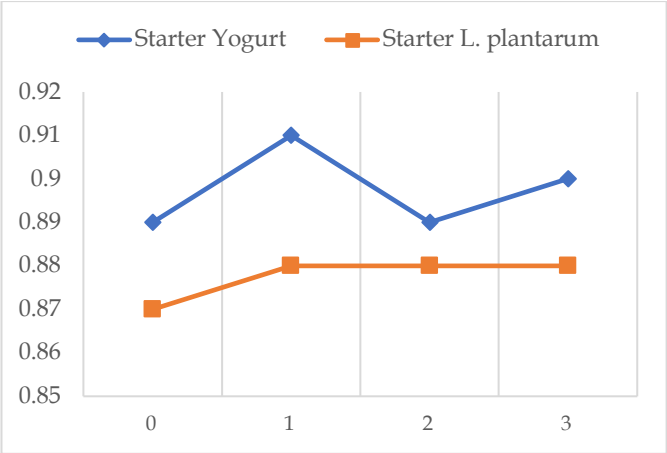

Figure 2. A_w value of fermented sausages using different types of starter yogurt and *L. plantarum*

Comparison of Moisture Content

The use of yogurt starter and *L. plantarum* did not provide a significant difference (P>0.05) for the water content of fermented sausages. In Figure 3. The addition of the percentage of yogurt starter and L. plantarum resulted in a percentage of water content of 63.35-65.17%. The water content in food ingredients also determines the acceptability, freshness and resistance of ingredients to microbial attack (Winarno 2002). The water content of beef sausages in this study met the quality standards of meat sausages based on SNI requirements, namely a maximum of 67% (BSN 2015). Based on this, the results of the water content test obtained in this study are in accordance with the standards of good sausage according to SNI. Microbial activity in a food ingredient is influenced by the water content and aw of the product so that all factors will affect microbial activity. The higher the water content of the product, the fermentation rate will increase.

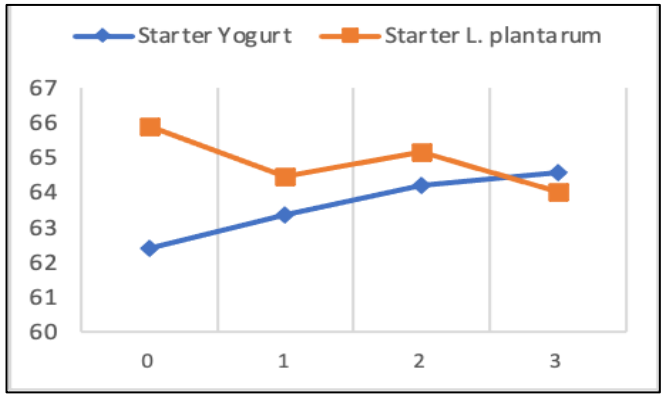

Figure 3. Moisture Content of fermented sausages using different types of starter yogurt and *L. plantarum*

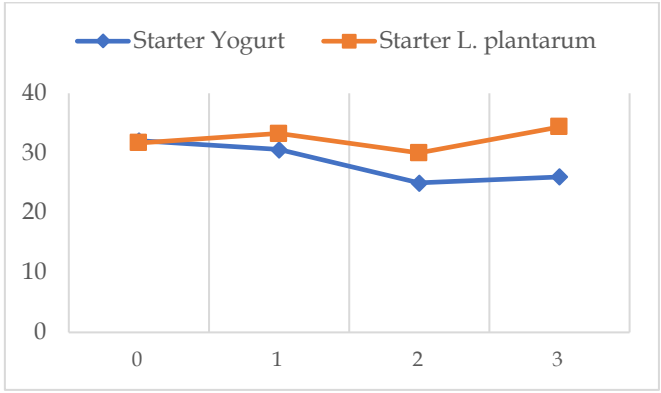

Figure 4. Protein Content of fermented sausages using different types of starter yogurt and *L. plantarum*

Comparison of Protein Content

The use of yogurt and *L. plantarum* starter types did not provide a significant difference (P>0.05) for the protein content of fermented sausages. In Figure 4. shows a graph of the differences in the protein content of fermented sausages, during the fermentation process, the yogurt starter hydrolyzes some of the chicken meat protein thereby reducing the value of the protein content, so that the fermented product becomes easier to digest and absorb. According to Dalmis and Soyer (2008), polypeptides are further broken down into small peptides, free amino acids, aldehydes, organic acids, and amines by endogenous aminopeptidases, microbial proteases, and microbial metabolites, which causes changes in physical and chemical criteria. Sausages that are fermented either with yogurt starter or *L. plantarum* in the early stages of sausage fermentation, will produce lactic acid as a microbial metabolism such as LAB. Ruiz et al. (2011) and Ikoni´c et al. (2013) explained that LAB lowers the pH of the product, alkaline metabolites accumulated by protein degradation at a later stage can act as a buffer against organic acids and cause a rebound in the product's pH. Based on Wang et al. (2021), protein hydrolysis index, total volatile base nitrogen, and meat free amino acid content significantly increased during fermentation.

Comparison of Fat Content

The use of yogurt starter and *L. plantarum* did not provide a significant difference (P > 0.05) for the fat content of fermented sausages. In Figure 5. shows a graph of the differences in the fat content of fermented sausages. The fat content of fermented sausages with stable yogurt starter ranged from 15.01-15.26%, whereas with *L. plantarum* starter there was a decrease of 9.81-13.11%. The amount of fat between types of sausage is different. The value of this study is lower than the 18.4% presented by Ferreira et al. (2007), 19.5% reported by Patarata et al. (2008), or 18.1% Marcos et al. (2016). The fat content affects the volatile compounds so that the total amount of volatile compounds in the sausage (Gómez and Lorenzo 2013). Esters were the most abundant volatile compounds detected in dry sausages which showed higher values in low-fat sausages (Olivares et al. 2011). Esters are formed through the enzymatic esterification of fatty acids and alcohols during the fermentation process, mostly by the action of microorganisms such as lactic acid bacteria and Micrococcaceae (Purriños et al. 2011). Sausage fat content in this study met the SNI requirements, namely maximum 25% (BSN, 2015).

Ash Content Comparison

The use of yogurt and *L. plantarum starter* types did not provide a significant difference (P > 0.05) for the ash content of fermented sausages. In Figure 6. shows a graph of the differences in the ashcontent of fermented sausages. The maximum limit for the ash content of beef sausages according to SNI is 3% (BSN 2015), thus the ash content of sausages in this study exceeds the SNI requirements. Ash content is a mineral content in a material or product. These minerals can come from natural minerals contained in an ingredient or the addition of mineral salts that occur during the process of making sausages Vuyst and Vandamme (1994). The decrease in ash content is influenced by the use of minerals to maintain the life of microorganisms. Because microorganisms need minerals to survive even in small amounts (Fardiaz, 1992).

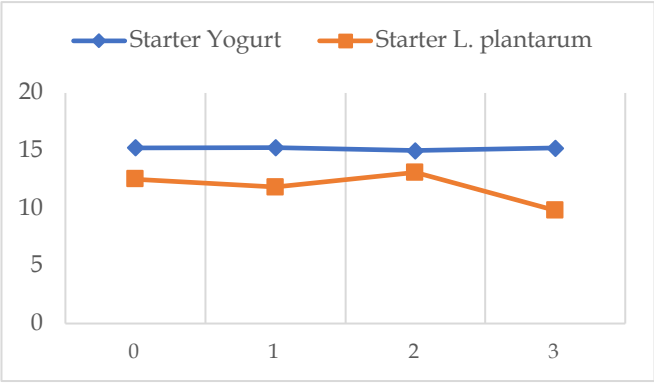

Figure 5. Fat Content of fermented sausages using different types of starter yogurt and *L. plantarum*

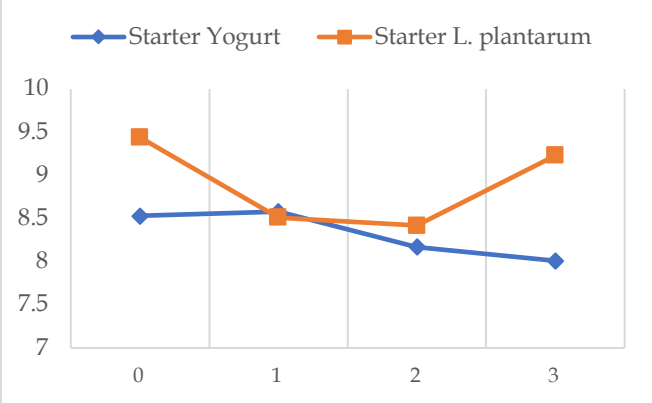

Figure 6. Fat Content of fermented sausages using different types of starter yogurt and *L. plantarum*

Conclusion

The use of different types of starters will produce different characteristics of fermented sausages. The pH of the fermented sausage that is close to the standard is the use of *L. plantarum* starter. Likewise, fermented sausages with *L. plantarum* produced lower water activity (a_w). The water content with the difference between yogurt starter and *L. plantarum* produces a percentage that is still according to the standard. The protein content of the fermented sausage with yogurt starter hydrolyzed some of the chicken meat protein thereby reducing the value of the protein content, so that the fermented product became easier to digest and absorb, while the fat content of the fermented sausage with *L. plantarum* starter decreased. Suggestions for the use of the percentage of starter yogurt and starter *L. plantarum* as much as 2%.

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Author Contributions

Conceptualization, A.S. and M.W.A.; methodology, E.S.W.; validation, H.E. and A.S.; formal analysis, M.W.A.; investigation, E.S.W.; data curation, H.E.; writing—original draft preparation, A.S. and M.W.A; writing—review and editing, M.W.A. and H.E.; supervision, A.S.; project administration, E.S.W.

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Conflicts of Interest

The authors declare no conflict of interest.

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