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Yam Starch and Chitosan-Based Bioplastics to Improve Shelf Life of Pempek

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Abstract: This study aimed to determine the effect of yam starch and chitosan based-bioplastics packaging on the microbiological and sensory qualities of pempek during storage. This research was carried out by packing pempek using 2 different packaging: bioplastics and polyethylene plastic. Pempek in each group was stored for 0, 8, 16, 24, 48 hours at room temperature. The total of *Staphylococcus aureus* bacterial was determined using colony forming unit. Sensory quality was obtained by determination of color, texture and aroma of pempek using 5 skilled panelists. The result showed that the use of bioplastics packaging from yam starch and chitosan decreased the growth of *Staphylococcus aureus* bacteria in pempek compared to the use of polyethylene plastic packaging. The decrease in pH was in line with the growth of *S.aureus* that is higher in pempek packaged with polyethylene plastics. The use of bioplastics also maintained sensory qualities of pempek during storage better than polyethylene plastics.

Keywords: Bioplastics; Chitosan; Pempek; *S.aureus*

Introduction

Bioplastics is plastics derived from biomaterial that can be destroyed naturally (Ashter, 2016). The main ingredients for bioplastics production are polysaccharides, proteins and lipids. In general, bioplastics are divided into two types, namely edible bioplastics (edible film) and non-edible bioplastics. Unlike conventional plastics which are difficult to degrade in the environment, both types of bioplastics can be degraded easily in a suitable environment. Bioplastics can be used as a packaging material that functions as a barrier to water and gas transfer, preventing the interaction of the packaged material with conditions outside the package. Bioplastics can also be used as a carrier for food components such as vitamins and minerals (Yamada et al., 2020; Perotto et al., 2020). Bioplastics may act as a barrier, either gas, oil or more importantly water. The moisture content of food is important for maintaining freshness and controlling microbial growth. Bioplastics can control Aw (water activity) through the release and acceptance of water.

One of a promising biomaterial for bioplastics is starch. Several starches have been used for bioplastics such as cassava, corn and yam. One of the advantages of using yam starch for bioplastics is a better film transparency compared to cassava starch based bioplastics (Ulyarti et al., 2018; Ulyarti et al., 2020). In Jambi, yam currently is not consumed for staple food therefore its utilization for industrial purposed does not interfere with the availability of staple food.

Bioplastics used as packaging for food products is expected to inhibit the quality degradation of the food products. However, the bioplasics itself perform limitations such as low mechanical strength and low barrier properties (Abang et al 2023). The bioplastics characteristics can be improved by adding another material such as chitosan (Jabbar et al., 2017; Kumar et al., 2020; Ulyarti et al., 2021). Chitosan is a derivative of chitin, the main component in shrimp and crab shell (Pakizeh et al. 2021; Dominic et al. 2020). It is produced

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by deacetylation of chitin (Pakizeh et al., 2021) and performs an antimicrobial properties (Abdallah et al. 2017; Chao et al., 2019). The combination of antimicrobials with bioplastics packaging can control microbial growth in food and inhibit food quality degradation during storage (Warsiki, Sunarti, & Martua, 2009). Chitosan is not toxic and is a biodegradable polymer. Chitin from shrimp and crab shells are usually thrown away by the community and become waste. The price of chitosan in the market is cheaper when compared to other antimicrobial compounds such as essential oils. Kaffir lime oil, which is an example of essential oil, has a selling price of USD 65.00-75.00 per kilogram (Warsito et al., 2017).

Pempek is a traditional food made from ground fish and tapioca. Pempek contains 58.59% water content and 18.26% protein content which makes it easily damaged. According to Karneta et al. (2015), pempek stored at room temperature can only last for one day. The decline in the quality of pempek is marked by the changes in the texture of the pempek, the formation of mucus on the surface, the appearance of a foul odor and a decrease in pH. These occur partly due to microbial activity which degrades protein into amino acids and is further degraded into gaseous ammonia (NH_3), hydrogen sulfide (H_2S), nitrogen oxides (NO) and sulfur dioxide (Karneta et al., 2015).

The dominant bacteria that contaminate fish products is *Staphylococcus aureus* (Tavakoli., Soltani, & Bahonar, 2012). Contamination may occur during the processing or packaging, at the time of the product contact with air, dust, utensils or infected hands (Al-Bahry, Mahmoud, Al-Musharafi, & Sivakumar, 2014). The dominance of *S. aureus* occurs because this bacterium has physiological properties that are more suitable than other bacteria. *S. aureus* is a facultative anaerobic group of bacteria, resistant to high salt up to 20%, survive in the range of pH 4-10 and 6-48.5^{o}C which allow it to grow in various conditions. As a major putrefactive bacterium, *S. aureus* in pempek may be inhibited by using an antibacterial packaging.

Bioplastics with the addition of chitosan and its applications have been previously developed. The addition of chitosan to bioplastics produced antimicrobial activity against *Staphylococcus aureus* with an inhibition zone diameter of 41 mm (Ulyarti et al., 2021). Chitosan films have the ability to inhibit microbial growth in peeled melons for up to 2 days with a microbial count of 21.6×10^5 CFU/g when compared to controls with a microbial count of 3.4×10^7 CFU/g (Cahyana et al., 2012). This study aims to compare the microbiological and sensory properties of pempek which is packaged with either bioplastics from yam starch-chitosan or conventional plastic during storage.

Method

Material and Chemical

The materials used in the pempek processing are tapioca flour, fresh mackerel fish, fine salt and garlic. The materials used for the manufacture of bioplastics are white yam starch, chitosan, plasticizer (glycerol), acetic acid, and distilled water. The materials used for analysis were BPA media (Baird Parker Agar) from mercks, *Staphylococcus aureus* culture obtained from the Jambi Provincial Health Laboratory and 70% ethanol.

Research Design

This research was carried out using 2 types of packaging as a treatment: polyethylene plastic and yam starch-chitosan based bioplastics. Each treatment was analysed for its microbiological and sensory quality during storage (0, 8, 16, 24, 48 hours). Storage was carried out at room temperature and each treatment was repeated twice to obtain a total of 20 experimental units.

Preparation of 1% Chitosan Solution (Ulyarti et al., 2021)

One gram of chitosan powder was weighed carefully and placed in a 100 ml beaker glass. The powder was dissolved using 1% acetic acid (CH_3COOH) and transferred into 100 ml volumetric flask. The volume was added up to 100 ml using 1% acetic acid.

Bioplastics Preparation (Ulyarti et al., 2021)

7 g of yam starch was placed in a beaker glass and dissolved in 188 g of distilled water. The starch suspension was heated on a hot plate for 10 minutes until it reached a temperature of 50-60^{o}C while continuing to stir, then 50 g of 1% chitosan solution and 2% of glycerol was added to the suspension. The starch solution was continued to be heated at 80^{o}C with stirring using a magnetic stirrer for 30 minutes. The homogeneous film solution (220 gram) was then poured into a square glass mould (25 cm x 25 cm). The film on the glass mould was dried in an oven at 50°C for 24 hours. The film is then removed from the mould and stored in a closed and airtight container.

Pempek Preparation (Pratama, Warsiki, & Haditjaroko, 2016)

Mackerel mince were weighed as much as 500 g, 333 g tapioca flour, 30 g salt and 11.01 g crushed garlic. These inggredients, except for tapioca, were mixed and tapioca flour was added gradually and kneaded until smooth. The dough was printed into elliptic weighing 20 g and 5-6 cm long. The dough was further boiled in the boiling water at 100^{o}C for 15 minutes or until it floats, then removed from water, drained, cooled to room temperature and stored in a sealed and airtight container until further use.

Pempek Packaging

Four pieces of pempek were packed using a piece of bioplastics. Pempek were arranged in a row on half side of bioplastics 25 cm x 12.5 cm size. The other half side was further taken up to cover the pempek. Each side of bioplastics was seal pressed at 100°C for 5 seconds. The packaged pempek was placed on mica plastic which is left open and stored at room temperature. Tests were carried out right away (no storage) and after 8, 16, 24 and 48 hours.

Determination Staphylococcus aureus

The number of *S. aureus* was determined by Total Plate Count (TPC) using Method described in SNI 2332.9:2011. The stages in this analysis include the preparation of BPA (Baird Parker Agar) media and sterilizing equipment, rejuvenating and identifying *Staphylococcus aureus* bacteria and the colony counting. BPA media was prepared by dissolving 58 g BPA powder using 950 ml distilled water and boiled on a hotplate. The media was further sterilized using an autoclave at 121°C for 15 minutes.

Cultivation and identification of Staphylococcus aureus bacteria was carried out by pouring 15-20 ml Baird Parker Agar (BPA) media into a petri dish and then allowing it to harden. A pure culture stock of *S. aureus* bacteria was taken with an ose needle and then streaked aseptically on BPA media in a petri dish. The petri dish was wrapped in coffee paper and incubated for 48 hours at 37°C in an inverted position. After 48 hours of incubation, macroscopic identification of *S. aureus* colonies was carried out. Colonies of *S. aureus* were macroscopically characterized as round, clustered like an arrangement of grapes and shiny gray to dark yellow colonies.

The determination for the number of *S. aureus* contained in pempek was carried out by taking a sample of 1 g of pempek, diluted in 9 ml of physiological salt solution (0.85% NaCl solution) and counted as a 10^{-1} dilution. These procedures were repeated to make up to 10^{-7} dilutions using 10^{-1-} microbial solution. Dilution levels 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were grown by taking 1 ml of each dilution and inoculating it in a petri dish. 15-20 ml BPA media was added to the petri dish. The inoculum was incubated for 48 hours at 37°C under inverted conditions. The number of *S.aureus* is expressed in units of cfu/g and calculated using the equation below.

$$\text{Number S. aureus (cfu/g)} = \text{number of colony} \times \text{dilution factor} \quad (1)$$

Changes in pH (SNI 06-6989 11-2004)
pH

Changes in pH were measured by suspending the refined pempek samples into distilled water with a volume ratio of 1:1 (10g:10ml). The mixture is then homogenized using a vortex. The homogeneous mixture is then measured for its pH using a previously calibrated pH meter.

Sensory Test (SNI 2346:2011)

The sensory test was carried out by giving a direct assessment of the aroma, texture and color of pempek by taking into account the reference provided. The sensory quality assessment involved 5 trained panelists. The sensory quality scale was changed to a numeric scale from 1 to 5.

Table 1. Sensory score

Score	Color*	Texture**	Aroma
1	Extremely dislike	Extremely not springy	Extremely stink
2	Dislike	Not springy	Stink
3	Rather like	Rather springy	Rather stink
4	Like	Springy	Not stink
5	Extremely like	Extremely springy	Extremely not stink

* Reference was available for each panelist during the analysis to compare the color and the texture of pempek. The reference was given score 4 for both parameters.

Result and Discussion

The Packaging

This yam-chitosan bioplastic was odorless and elastic, but has poor tensile strength when compared to polyethylene plastic. The yam-chitosan bioplastics tears more easily when it is stretched. The bioplastics surface was also rougher when compared to the polyethylene plastic surface but has a good transparency. The appearance of the yam-chitosan bioplastics used can be seen in Figure 1.

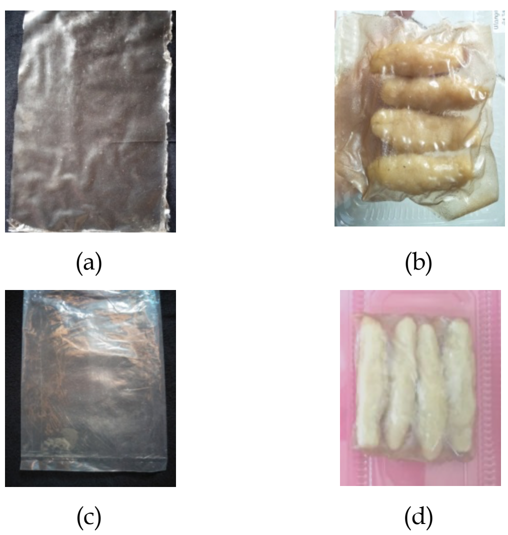

Figure 1. (a) yam-chitosan bioplastics (b) pempek wrapped in yam-chitosan bioplastics (c) *polyethylene* plastics (d) pempek wrapped in *polyethylene* plastics

The Growth of S aureus

The process of pempek decomposition is fast due to the growth of microorganism. Bioplastics from yam starch-chitosan in this study was applied to inhibit the growth of microorganism S.aureus during storage. For this purpose, the number of S.aureus that develops in the product was determined at interval time for 2 days. The growth curve of S.aureus in pempek which is packaged in yam starch-chitosan bioplastics and polyethylene plastic during storage can be seen in Figure 2.

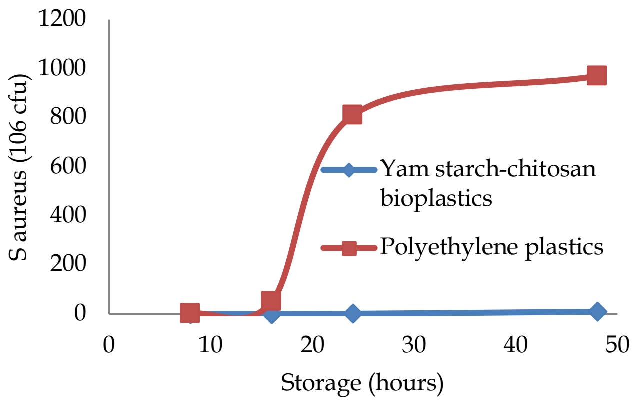

Figure 2. The number of *S aureus* of pempek during storage

The growth curve of *S.aureus* in pempek shows that the number of *S.aureus* in pempek packed with polyethylene plastic has increased significantly during storage. Storage conditions for pempek at room temperature (29-30°C) allow *S. aureus* bacteria to grow properly. *Staphylococcus aureus* can grow at an ambient temperature of 6.7°C-45.5°C with an optimum temperature of 37°C (Omotoyinbo and Omotoyinbo, 2016) and a pH of 4.0-9.8 with an optimum pH of around 7.0-7.5. *S. aureus* in pempek metabolizes H_2O_2 to water and oxygen. The resulting water was observed on the inner surface of both yam starch-chitosan bioplastics and polyethylene plastic.

Based on Figure 2, the growth of *S. aureus* in pempek packaged in both packaging follows the standard microbial growth curve, consisting of several growth phases. During first 8 hours to 16 hours storage *S. aureus* underwent initial growth. This stage was followed by an exponential growth stage or a logarithmic growth stage from 16 h to 24 h storage. The stationary phase occurred during 24 hours to 48 hours of storage. In this phase, the speed of microbial growth begins to decrease. This decline phase can be caused by several factors including the amount of nutrients and oxygen that has been greatly reduced, changing the pH of the substrate, the formation of the product of microbial metabolism that inhibit their growth.

The growth of *S. aureus* bacteria in pempek packaged with yam starch-chitosan bioplastics tended to remain constant. The growth curve of *S. aureus* as shown in Figure 2 indicates that *S. aureus* has not attained the logarithmic phase. The bioplastics packaging from yam starch-chitosan showed an inhibition for *S. aureus* in pempek. The antibacterial properties of chitosan come from the polymer structure which has a positively charged amine group. Chitosan as a cation has the potential to bind many components such as proteins. The positive charge of the NH_3^+ group in chitosan can interact with the negative charge on the surface of the bacterial cell causing the damage to the cell wall (Dazhong et al., 2021). The damage may change the shape of the cell wall and enlarge the pores of the cell. This condition results in the inability of cell wall to regulate the exchange of substances to and from the cell which further hamper metabolic activity and eventually death to the cells.

pH

pH is an indicator for the freshness of a product. The pH of pempek packed with polyethylene plastic decreased more sharply when compared to the pH of pempek packaged with yam starch-chitosan bioplastics during storage (Figure 3). The pH of pempek packaged with yam starch-chitosan bioplastics decreased slightly, from 6.75 to 6.55. These decreases in pH were in line with the increase in the number of *S. aureus* in pempek during storage. *S. aureus* degrades the protein in pempek to produce acidic gases including hydrogen sulfide (H_2S), sulfur dioxide (SO_2) and nitric oxide (NO). Pempek's protein consists 0.27% methionine, 0.66% arginine, 0.4% cysteine. The amino acids cysteine and methionine are degraded by *S. aureus* into sulfur dioxide and hydrogen sulfide.

The decrease in pH is also due to the fermentation process carried out by *S. aureus* on the substrate (pempek). The pempek used was made of 38.1% (w/w) tapioca flour which is a carbohydrate. The glucose contained in pempek under anaerobic conditions by *S.aureus* is degraded and produce acetoin which cause a decrease in the pH of the substrate to 5 (Karimela, Ijong, & Dien, 2017).

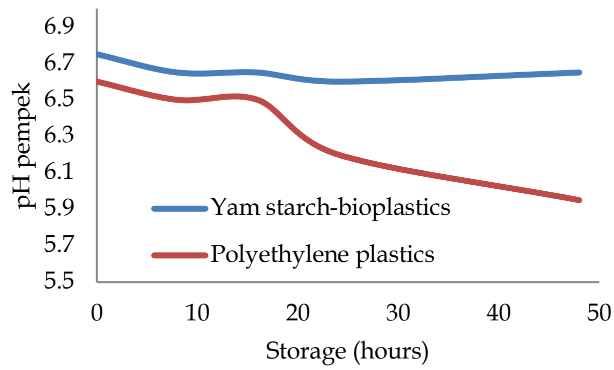

Figure 3. The changes of pH of pempek during storage

Sensory properties

Color can be used as signs of damage, indication of quality level and guidelines for processing of food products. Color is the most important quality attribute. Along with texture and taste, color plays a role in determining the acceptability of a food. When a product is nutritious, tastes good and has a good texture, but has dull color, then it is most likely be less attractive.

Pempek used in this study has a light gray color with the average values of hedonics for color of pempek packaged in yam starch-chitosan bioplastics were higher than pempek packaged in polyethylene plastics (Table 2). Pempek packaged in polyethylene plastic experienced a decrease in color during storage.

After 48 hours of storage, the color has changed to pale gray (2.4 or dislike) and the surface contained white mucus. The presence of this mucus is thought to affect panelist's evaluation for the color of pempek. The different changes in color of pempek packaged in both packaging, again, may be due to the activity of *S. aureus* bacteria. The decomposition of the protein content in fish meat by proteolytic bacteria causes a decrease in color to become paler (Fajri & Dasir, 2017).

The other important sensory property for pempek is the texture. A good quality of pempek should have a springy sense when bitten. This property comes from the gelling properties of fish meat and tapioca and the homogeneity of the dough. During storage, the texture changed (Table 2). Both packaging showed similar effect to the pempek but this does not relate to the activity of *S aureus* as the directions of the change were also different. Bioplastics packaging turned the product into harder and dryer surface due to the physical characteristics of the bioplastics. Yam starch-chitosan bioplastics is known to have high water vapor transmission rate (Ulyarti et al., 2021) allowing water vapor to easily moves in and out of the packaging depend on its humidity. In this experiment, the water vapor moves out of the packaging rendering pempek's surface becomes dry and hardened. The polyethylene plastics, on the other hands, turned the product into soggy pempek that might be due to proteolytic activity of *S aureus* or enzymatics activity. Within 48 hours of storage, *S aureus* bacteria were in the logarithmic phase, which began to decompose organic components, especially protein, which caused the texture of pempek to become soft. The low degree of meat acidity may activate several enzymes that are active in acidic conditions (low pH) and decompose protein.

Table 2. The average values of sensory evaluation of pempek

Storage (hours)	Color*		Texture*		Aroma*	
	Bioplastics	Polyethylene	Bioplastics	Polyethylene	Bioplastics	Polyethylene
0	4	4.4	4	4.2	4.6	4.4
8	3,8	3.8	3.8	3.8	4.2	3.6
16	3,6	4	3.6	3.8	4.2	3.6
24	3,8	3.4	3.4	3.2	4	3.2
48	3	2.4	2	2	3.8	1.2

*sensory scores as shown in Table 1

Aroma can be defined as the properties of products that are perceived by smell. Aroma is one of the supporting factors for taste that determines the quality of a product. Aroma also can be used as an indicator to determine the level of acceptance of a product by consumers. The average aroma of pempek packaged in yam starch-chitosan bioplastics and polyethylene plastic during storage can be seen in Table 2. Yam starch-chitosan showed the ability to preserve aroma of pempek when compared to polyethylene plastic. The rotten smell developed in line with the growth of *S. aureus* bacteria which degrade the protein into volatile bases that responsible for the stinky odor such as ammonia, histamine, hydrogen sulfide, and trimethylamine (Ali, Baehaki, & Lestari, 2017). The stinky smell of pempek can also be caused by the oxidation of fat. Fat is hydrolyzed so that short fatty acid chains are formed. The compounds such as amino acids, glucose and lipids can also be converted by bacteria into products that can be used as indicators of spoilage such as hydrogen sulfide (H_2S), carbonyl, histamine and ammonia.

Conclusion

Bioplastics packaging from yam starch and chitosan has shown to inhibit the growth of *Staphylococcus aureus* bacteria in pempek when compared to *polyethylene plastic* packaging. The pH of pempek during storage was in line with the growth of *S. aureus* in pempek and both are relate to the sensory properties of pempek.

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Author Contributions
Ulyarti contributes in the conceptualization and methodology of the research, Ninada contributes in the Laboratory work and manuscript preparation, Agus Salim contributes in the

microbiological analysis, Silvi Leila Rahmi and Nazarudin contribute in the supervision and review.

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Conflicts of Interest

Authors declare that no conflict of interest in this publication.

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