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THE EFFECT OF THE STORAGE TO THE MICROSTRUCTURE OF ANGKAK SAUSAGES OF LAYING CHICKEN MEAT WITH THE SCANNING ELECTRON MICROSCOPE (SEM) METHOD

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Abstract

The purpose of this study was to evaluate the microstructure of the sausage meat of laying hens without giving Angkak (0%), with 0.5% Angkak, 1% Angkak and 1.5% Angkak and storage at 0 days, 10 days, 20 days and 30 days at 5^0C with the method Scanning Electro Microscope (SEM). The method used is descriptive observation using a Scanning electron microscope. The results showed that the higher the addition of Angkak concentrations in sausages the greater the cavity on the surface of the sausage structure. At 0 day storage, the surface of the structure was still compact and there was little bacterial contamination. After 10 days of storage, the surface texture began to loosen and the cavities began to spread. Storage for 20 days the surface texture of the sausage is more cavity and begins to grow bacteria. 30 days of storage, there was a visible growth of fungus on the surface of the meat sausage. The conclusion is that the addition of Angkak affects the formation of starch granules and texture on the sausage surface. At 20 days of storage there was no effect of preservation on the addition of Angkak, it was shown the growth of bacteria and fungi. Furthermore, the 30 day shelf life creates a new ecosystem that allows mold to appear on the surface of the sausages.

Key words: *Angkak sausage, microstructure, scanning electron microscope, storage.*

INTRODUCTION

Laying hens chicken meat has low quality because the cutting is done at old age and does not produce so that the meat tenderness is lower and is less liked by the community. To increase the preference for rejected layer chicken meat, it is is necessary to innovate and diversify the rejected layer chicken meat, one of which is by making sausages (Purnamasari, 2012).

Sausage or sausage comes from the Latin salsus which means to be salted is meat that is prepared through salting. Sausage is a food made from mashed, ground, seasoned meat and then wrapped in a casing that is symmetrical and has a distinctive taste (Ernawati, 2015). Sausage is a source of protein (Elvira, 2009). The characteristics of a good sausage are chewy texture, do not contain preservatives, are free from harmful chemicals and do not contain harmful synthetic dyes (Palandeng et al., 2016). The composition of sausage processing consists of animal tissue, water, ingredients for curing, spices, fillers and binders. The basic principles of making sausages include the stages of grinding and mixing the meat with spices, filling in the shell, smoking, drying and storing. The ingredients for curing (cured meat) in the manufacture of sausages are salt (NaCl), sugar, and Angkak.

The purpose of curing is to get a stable color, aroma, texture, good taste and to extend the shelf life of the product (Soeparno, 2011). Nitrite salt functions as a curing agent but can be a precursor to carcinogens because it reacts with amines from protein components to form nitrosamines (Zahran and Kassem, 2011). One of the ingredients that can replace the function of nitrite as a preservative, flavor giver, texture improvement as well as natural dyes and colors that are not harmful is Angkak.

Angkak is fermented rice using the fungus Monascus purpureus so that its appearance is red. Angkak has been used widely in Asia as a natural food coloring in fish, Chinese cheese, red wine, and sausages (Blanc, Lorel and Goma, 1997). Apart from being a natural dye, Angkak is also used as a flavoring, preservative, texture improvement and medicine because it contains nutritious bioactive ingredients. *Monascus purpureus* fungus produces pigments that are not toxic and do not interfere with the immune system (Fardiaz and Zakaria, 1996). The red color of angkak is very potential as a substitute for synthetic red which is currently very widely used in various food products. As a natural dye, Angkak has fairly stable properties, can mix with other color pigments and is non-toxic. Monascus mushrooms which produce Angkak by converting the substrate of starch into several metabolites, such as alcohol, antibiotic agents, antihypertensives, enzymes, fatty acids, aromatic compounds, ketones, organic acids, pigments and vitamins (Yongsmith, Tabloka, Yongmantichal and Bavavoda, 1993). In addition to the curling material, Angkak can also be used as a binder (Rumondor et al., 2019).

The purpose of this study was to evaluate the structure of the sausage meat of laying hens without giving Angkak (0%), 0.5% Angkak, 1% Angkak and 1.5% Angkak and storage at 0 days, 10 days, 20 days and 30 days at 5 0C with the method Scanning Electro Microscope (SEM). The method used is descriptive observation using a Scanning electron microscope.

MATERIALS AND METHODS

This research is an experimental laboratory conducted in the Microbiology Laboratory of Gajah Mada University. Microstructure testing of chicken meat Angkak sausage was carried out to determine the microstructure of sausages without giving Angkak, giving Angkak 0.5 %, 1 % and 1.5% and Angkak sausages stored at 0 days, 10 days, 20 days and 30 days.

Angkak Sausage Microstructure Research Procedure Using SEM Method

The method used is microscope observation using a Scanning electron microscope. The sausage sample to be observed is fixed first. Substance or 3% gluterldehyde substance which is diluted at 0.1 M phosphate buffer for 2 - 4 hours. Then washed with 0.1 M phosphate buffer with a pH of 7.3, carried out 3 times for 10 minutes. After fixation 2% osmium was added in 0.1 M phosphate buffer for 2 - 4 hours at room temperature. Then do the dehydration with 100% ethanol for 2 times for 15 minutes. Then it was dried and coated with gold, after which it was observed on the Scanning Electron Microscope. SEM equipment preparation: turn on the switch beside the tool (SEM) and leave it for 30 minutes to warm up the tool, set the specimen on the specimen holder. Then press the EVAC / AIR button to enter air into the specimen chamber. LED light (which is blinking and yellow). To indicate that air has entered the specimen camber, the AIR LED light will light up constantly and not blink again. Insert the sample slowly by adjusting the sample support to the main unit. Then adjust the position of the sample surface you want to see by turning the XY button, its position can be seen on the display screen. Furthermore, an enlargement is carried out on each sample observation.

RESULTS AND DISCUSSIONS

Angkak Sausage Microstructure

Analysis of differences in the treatment of Angkak on sausages using a Scanning Electron Microscope at a magnification of 100x. Scanning Electron Microscope results (Figure 1) show a difference in the structure of sausages without the addition of Angkak (Figure 1a) and the addition of Angkak (Figures 1a, b, c and d).

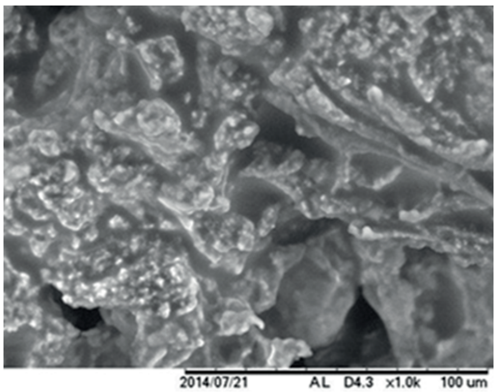

Figure 1a. Sausage Without Giving Angkak (0%)

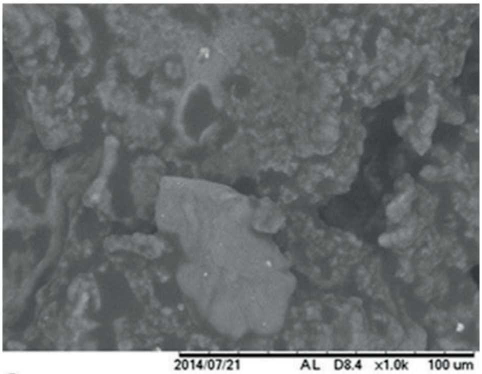

Figure 1b. Sausage with the addition of Angkak 0.5%

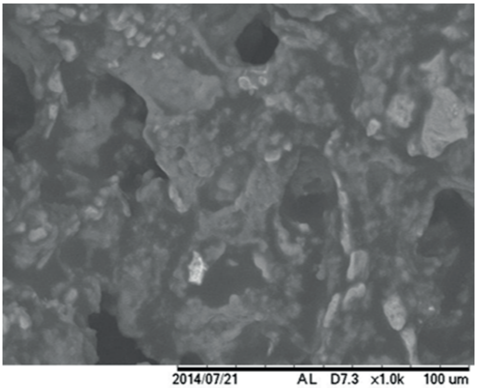

Figure 1c. Sausage with the addition of Angkak 1%

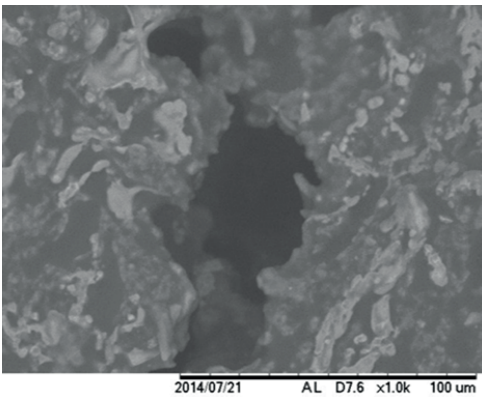

Figure 1d. Sausage with the addition of Angkak 1.5%

The presence of starch granules from tapioca which are irregularly rounded and the surface of a slightly rough texture, seen in sausages without the addition of Angkak (sausages with the addition of 0.5% Angkak), visible starch granules from Angkak rice in the form of small spheres and starch from tapioca flour. Sausage with the addition of 1.0% and 1.5% Angkak showed the presence of starch granules in the form of small spheres and irregular spheres originating from Angkak rice flour and tapioca flour. Qualitative SEM analysis results inform that the addition of Angkak affects the formation of starch granules and texture on the sausage surface.

The difference in surface texture and presence of pore cavities both qualitatively (the size of the cavity) and quantitatively (the number of cavities) formed in Figure 1 is due to differences in the concentration of Angkak given to each treatment. The higher the increase in the concentration of Angkak in sausages the more and the greater the cavity on the surface of the sausage structure. This shows a decrease in water content in the cells due to the osmosis and dehydration processes as a result of the release of water through the evaporation of water substance (H2). The cooking temperature also affects the structure of the sausages, the process of increasing the temperature results in the sausage cavity being wider and easier to absorb water (Ayu and Yuwono, 2014). Carbon dioxide (CO_2) and water (H2) easily evaporate, this is due to the gas produced through the fermentation process of this Angkak will try to make a way out that shows the presence of air cavities on the surface of the sausage. Gas production in the fermentation process of Angkak is preceded by the release of energy from carbohydrates in the form of glycogen in meat and starch from rice in the form of one part of the phosphate. The amylase enzyme from Angkak converts starch or starch into a simple monosaccharide. This concurs with Pattanagu et al. (2007) that Angkak produces α-amylase, β-amylase enzymes which can improve the texture of meat sausages. The release of this energy changes ATP to ADP + Pi, then there is the release of water (HOH) which reacts with carbohydrates to form vinegar (antibiotics) and its aromatic compounds as well as carbon dioxide (CO_2) and water (H_2).

Angkak Sausage Microstructure during Storage

Angkak comes from fermented rice using the fungus *Monascus purpureus* which is a natural coloring agent and also as a preservative that can suppress the moisture content of sausages, inhibit the growth of decomposing bacteria and contain bioactive ingredients. The fungus *Monascus purpureus* produces Angkak by converting a starch substrate which is alcohol, antibiotic and aromatic compounds (Yongsmith, 1993). The results of observations through the Scanning Electron Microscope in Figure 2, the sausage shelf life of 0 days, 10 days, 20 days and 30 days at a temperature of 5 0C with a magnification of 100x, which is indicated by a circle sign, namely bacterial growth.

At 0 day storage, the surface of the structure was still compact and there was little bacterial contamination. After 10 days of storage, the surface texture began to loosen and the cavities began to spread. Storage for 20 days the surface texture of the sausage is more cavity and begins to grow bacteria. Storage of 30 days has seen a growth of fungus on the surface of the meat sausage.

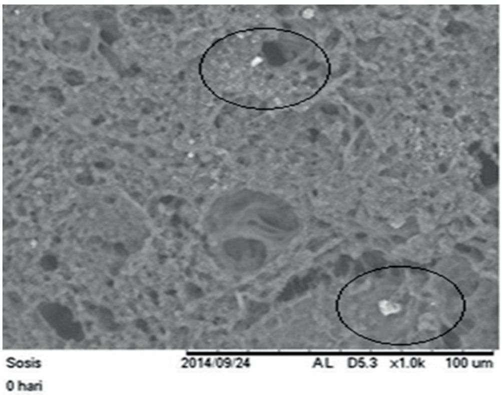

Figure 2A. Sausages shelf life of 0 days

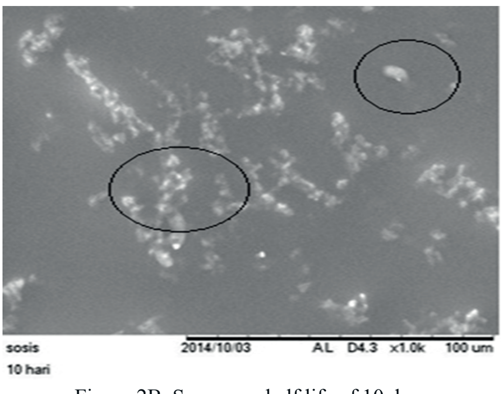

Figure 2B. Sausages shelf life of 10 days

The results of the analysis showed that at 0 days of storage at first there were no visible physical, biological and chemical changes. 10 days of storage, there is an effect of giving Angkak, while in 20 days of storage there is no effect of preservation on the addition of Angkak, it is shown the growth of bacteria and fungi. Furthermore, the shelf life of more than 20 days to 30 days creates a new ecosystem that allows the appearance of mold on the surface of the sausage. Angkak compounds are able to control water content, dehydration and cell osmotic pressure so that it can suppress the decomposing microbial population in sausages.

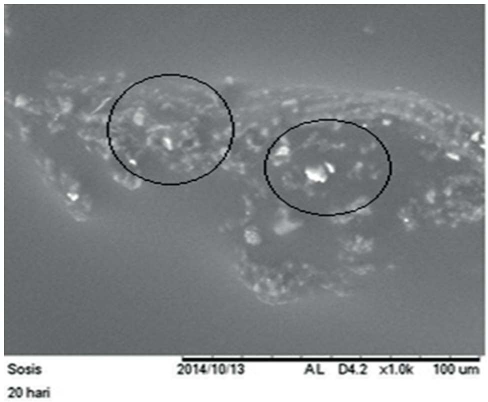

Figure 2C. Sausages shelf life of 20 days

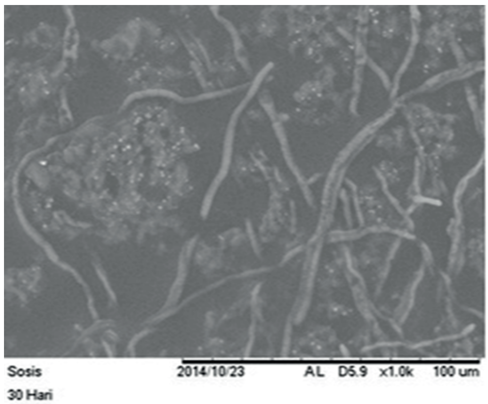

Figure 2D. Sausages shelf life of 30 days

The change in sausage in Figure 2 occurs due to the re-fermentation process by fungi. The end result of fermentation is in the form of gas and water which is released resulting in structural changes in the sausages in the form of physical changes in the presence of a space or cavity which is the way out of the gas.

The carbon dioxide produced from Angkak evaporates when the space or cavity is still small and the cavity space extends or increases. The enlarged space or cavity for the production of carbon dioxide gas evaporates and decreases along with the decreasing power of carbon dioxide production. As an antibiotic agent that kills thermophilic bacteria, the compounds that play a role are Ankalactone compounds from Angkak which are more acidic (Mostafa and Abbady, 2014). According to Barbara (2001) bacteria that can damage sausages are *Staphylococcus aureus* which grows at a pH of 5.0 - 6.5 which is not too acidic and a material with a high protein content.

The lovastatin compound in Angkak together with water will react to become acid, carbon dioxide and water. This carbon dioxide will evaporate, thus inhibiting the growth of the bacteria *Pseudomonas* sp, *Bacillus cereus* and *Bacillus stearothermophyllus*. The results of the reaction of the lovastatin compound and water have the ability to bind and release water. The production of carbon dioxide gas also comes from the garlic seasoning as an anti-bacterial which can bind water and change the osmotic pressure in cells.

Inhibition of the decay process due to reduced water content which inhibits the growth of decomposing bacteria from the sausages. Evaporation of light gaseous carbon dioxide and oxygen will come out of the sausage and leave a pore in the form of a cavity as a way out of gas in the sausage. This is supported by Adams & Moss (2002) that the growth of microorganism contaminants on the surface of sausages can result in changes in color, taste and smell.

CONCLUSIONS

The addition of Angkak affects the formation of starch granules and texture on the sausage surface. At 20 days of storage there was no effect of preservation on the addition of Angkak, it was shown the growth of bacteria and fungi. Furthermore, the 30 day shelf life creates a new ecosystem that allows mould to appear on the surface of the sausages.

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