Effect of Hurdle Technology in Angkak Sausage Preservation

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Abstract. Angkak (red rice fermented) sausage is a product of research from the application of a combination of potassium nitrate and angkak powder as an ingredient in the process of dry curing on beef. Thus the food product is relatively healthier than the sausages in the market that rely solely on nitrite ability in the curing process. However, this product has a shelf life of up to 5 days on chill room storage temperature (2°C). In order to improve the quality of the shelf life is pursued the use of natural preservatives chitosan type. Chitosan is selected, because it is a natural preservative containing polycation capable of binding the negative charge of other compounds and easily degrades biologically and non-toxic, thus able to inhibit the growth of bacteria and moulds. The results of research before that the application of chitosan use on the best sausage product is 0.4%. This study aims to know the effect of shelf life of the best angkak sausage application of 0.4% chitosan with the treatment of application with or without packaging materials with vacuum system on chill room storage at 2-4°C. This research includes descriptive research type, because it is intended to know the ability of chitosan as a preservative in sausage. Thus the variables in this research are the application with or without packaging materials with a vacuum system on chill room storage temperature of 2-4°C. The data of angkak sausage shelf life were analysed by T test. The results showed that: 1) the best shelf life of angkak sausage from application of 0.4% chitosan without vacuum system packaging materials has 7 and 10 days shelf life for angkak sausage using vacuum system packaging, 2) The angkak sausage nutrition contains 16.44% of protein, 20.94 of fat, 2.93% of carbohydrate and 36.52% of water.

1. Introduction

Angkak (red rice fermented) sausage is a product of angkak utilization as saltpeter substitution [1]. The results showed that the application of alternative saltpeter from a combination of potassium nitrate 300 ppm and 1% angkak powders on the manufacture of sausage nitrate with dry curing method able to provide organoleptic quality in terms of color, flavor and taste resemble sausage products with a shelf life of 5 days at chill room storage (2°C). Thus angkak sausage as a food of meat type is a good medium for the growth of microorganisms with the ability to decompose proteins, ferment carbohydrates and make fat smell rancid so that the sausage can be spoil. The spoilage in sausage angkak can be known from the assessment of organoleptic quality characterized by the smell becomes off odor, the color becomes faded, the surface texture becomes sticky and even slimy [2]. This spoilage caused by microorganisms of bacteria and fungi. Some types of bacteria will do the decomposition process at different temperatures depends on the type of bacteria, at temperature 45-55°C (thermophilic bacteria), such as Bacillus stearothermophilus; at temperature 20-45°C (mesophilic bacteria); and below 20°C (psycho-physe bacteria), such as Pseudomonas, Flavobacterium, Alcaligenes [3].

In general, the spoilage of animal protein caused by slime mold [4]. The growth of slime mould can occur under any conditions and at various temperatures. Its growth looks like it contains powder cotton and woven fine yarn that often show brilliant colours. The growth of these yarns will be followed
by the presence of slime along with the growth of more and more moulds accompanied by the typical smell of slime mould. Appearance change is the indicator of food spoilage. According to Ref.[5], that the number of microbes in the range 103-104 has not shown signs of spoilage. Thus, the amount of microbial sausage still eligible for human consumption during the storage period does not exceed the maximum threshold of 5.5 x 105 cells / gram [6] or 1 x 105 (CFU / g) according to SNI 7388: 2009. Rottenness in processed meat products usually occurs in the range of > 106 CFU / g characterized by changes in aroma, changes in the texture of perishable products and the formation of slime.

One of the natural preservatives that have been developed and can be applied to animal protein is chitosan. Chitosan (poly-β-(1,4)-D-glucosamine) is a cationic polyelectrolyte with free amino groups distributed regularly in its molecular chain [6]. Chitosan is a derivative product of chitin polymers derived from crustacean skin processing in the fishing industry especially crabs, shrimp, lobsters and crabs [7] by removing acetyl groups (-COCH3) from chitin with an alkaline solution [6]. Chitin has a long crystal structure with a strong bond between the nitrogen and the acetyl groups. Therefore, in deacetylation process used high concentration sodium hydroxide solution (40-50%) and high temperature (100-150°C) to obtain chitosan from chitin. The process begins with the removal of proteins (deproteination) using alkaline solutions and mineral removal (demineralization) with an acid solution to produce chitin. Chitin will further be deacetylated by heating in an alkaline solution. The result is a chitosan mush that is still to be washed and dried so as to produce a white crystal [8].

Chitosan is water-insoluble but soluble in weak organic acid solutions [9]. Chitosan contains polycation (positively charged) that can bind the negative charge of other compounds and is susceptible to biological and non-toxic degradation. With such properties then chitosan can inhibit the growth of bacteria and mold or act as a bacteriostatic activity. Even according to [10], chitosan acts as a bactericidal activity, because the ability to change the permeability of cell walls or active transport of bacterial cell walls to lysis. Chitosan products are marketed in powder and liquid form. Its use is dissolved in water of different concentrations by means of direct addition or by immersion of foodstuffs to be preserved into chitosan solution. Addition of chitosan reduced lipid oxidation, inhibited the growth of spoilage bacteria, resulted in better sensory attributes and had a good effect on the development of the red colour of minced beef during storage [7]. The ability of preservation has been tested on food products through trials that have been conducted by the Research Team of the Faculty of Fisheries and Marine Sciences Bogor Agricultural University (IPB). Trials were performed on salted fish, dried anchovies and salted squid. The result turned out that food products can be stored until three months. This shelf period is much more durable than those without chitosan, that is two months. In addition to chitosan effect on the shelf life of salted fish also on the taste of a better and better appearance. A similar study was conducted on anchovies [11]. The results showed that on the use of 0.5% liquid chitosan can reduce the total bacteria of anchovy. The ability of preservation of chitosan is selected, because it is a natural preservative that can improve the quality of angkak sausage relatively healthy, competitive and safe compared to the sausages in the market.

Efforts to inhibit the acceleration of food spoilage in addition to the use of preservatives are also done by providing storage treatment at low temperatures, i.e., 0-2°C. Similar explanation by [9] that at low temperatures ranging from 2 to 4°C can slow or limit the rate of decay. According to [6] that low temperature storage can create an environment uncomfortable for bacterial growth, but bacteria can still develop even at low speeds. It further explained that cold temperatures slow down the chemical and biological processes in food and the spoilage that causes deterioration of nutritional quality. In this way the spoilage to sausage can be inhibited so that the shelf life can be extended.

The inhibition of food spoilage can also be attempted through the application of packaging material of polyethylene LD (04) vacuum system. According to Ref. [12] a vacuum packaged product creates an unfavourable environment for microbial growth in processed products. Ref. [13] also stated that the packaging aims to prevent re-entry of microorganisms after the process of elimination (cooking) again, minimize the probability of disease, infection of food and reduce the decay that can cause toxin. According to Ref. [12] vacuum packaging will result in low oxygen levels in enclosed packaging. The anaerobic environment of vacuum packaging prevents the growth of anaerobic, especially spongy microorganisms responsible for the occurrence of odour’s, slime, and changing textures. By using gas-impermeable packaging materials, the growth of aerobic spoilage microorganism is inhibited, so that
shelf-life sell-by dates of products can be extended [14]. In addition, vacuum packaging keeps the product from water, so it does not affect the water activity. Thus, indirectly the packaging is intended to control the water activity. Most hygroscopic dry products can absorb water, otherwise the wet product is susceptible to water loss

2. Materials and Method

2.1. Materials and Methods
The ingredients used in the manufacture of sausages are: beef, angkak powder, Koepoe-koepoe potassium nitrate powder, chitosan powder, Indomilk skimmed milk powder, Petani tapioca, Happy Salad Oil, Garlic, Kapal salt, Gulaku sugar, Indomaret pepper and nutmeg. The equipment used in the manufacture of sausages are: Tanita brand digital scales, knives of steel, spoon of material steel, meat crusher with brand Blixter, plastic box for cradle, Nayati brand refrigerator with temperature control indicator, pan of st.steel, Sango brand ceramic bowls, Rinai brand LPG stoves, temperature gauge thermometers, sausage casing type of High Density Poly Ethylene (HDPE) plastic, Akebono brand sausage stuffers, polyaniline LD (04) vacuum packaging materials and brand vacuum packing tools Panasonic.

2.2. Dilution of Chitosan
Dilution of chitosan was done with vinegar acid concentration 25% as much as 5% (15 g), 100% aquades (300 g), chitosan 3.3% (10 g). The percentage of vinegar and chitosan is based on the total amount of aquades. The dilution procedure is carried out as follows: chitosan powder is placed on a ceramic bowl. Vinegar and aquades are poured into a ceramic bowl. Stir the mixture using a stirrer until the chitosan is submerged with the solution. Cover the surface of the bowl with plastic wrap and leave until the chitosan dissolves entirely.

2.3. Sausage Production Methods
Sausage is made with dry curing technology which is done with the following procedure: chitosan diluted. Beef trimmed from connective tissue and fat, then washed under running water, drained and cut into 1 cm dice. Angkak powder, potassium nitrate, salt, and sugar are mixed and evenly distributed throughout the beef surface. Cured beef is placed in a closed plastic container and incubated at 2-4°C for 48 hours. Meat curing milled with 0.4% chitosan solution added, ice cubes, skim milk powder, oil, tapioca, garlic, pepper, and nutmeg. Sausage dough is loaded into sausage casing and cooked simmering at 85°C for 30 minutes. Sausage is cooled by soaking in cold water. Sausages are cut into pieces and packed in a vacuum.

2.4. Shelf life Test
Microbiological testing was performed by total plate counting method against the angkak sausage from the application of treatment with and without packing type polyethylene LD (04) vacuum system and storage treatment at cold temperature ranged 2-4°C. The application of this temperature is based on the guidance that fresh meat generally has a shelf life of 48 hours or two days at a temperature of 2-4°C. Implementation of storage carried out for 11 days. The testing process starts from the first day with a span of 1 day in a row until the 11th day. The shelf life quality is based on microbial growth at a maximum threshold of 5.5 x 10^5 cells/gram [15] or 1 x 10^5 (CFU / g) according to SNI 7388: 2009.
3. Results and Discussion

The results of microbiological testing by total plate counting method against the best sausage product from the application of 0.4% chitosan on treatment with and without the application of polyethylene LD (04) packaging materials vacuum system and storage at cold temperature ranging from 2-4°C as a whole presented at Table 1.

| Day | Total Bacteria of Angkak Sausage |
|-----|---------------------------------|
|     | Without Packaging | With Polyethylene |
|     | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 | Rep 1 | Rep 2 | Rep 3 |
| 1   | 62   | 54   | 58   | 58   | 31   | 28   | 34   | 31   |
| 4   | 95   | 88   | 110  | 97.6 | 61   | 70   | 64   | 65   |
| 6   | 280  | 310  | 360  | 316  | 85   | 81   | 88   | 84.6 |
| 7   | 410  | 390  | 430  | 410  | 280  | 210  | 250  | 246  |
| 8   | 830  | 720  | 910  | 820  | 230  | 310  | 250  | 263  |
| 11  | 4100 | 5200 | 3900 | 4400 | 860  | 920  | 780  | 853  |

The result of microbiological testing by total plate counting method against the angkak sausage showed that the number of microbes grown in the angkak sausage with treatment without packaging materials at chill room storage temperature (2°C) 7th day reached an increase that was at the maximum threshold of the required microbial provisions according to SNI 7388: 2009 is 4.1 x 102. Thus the shelf life of angkak sausage without packaging only reached day 7. In the microbiological test results by angkak sausage showed that the number of microbial sausages with packing polyethylene LD (04) vacuum system on chill room storage temperature (2°C) day 10 reached an increase that almost reached the maximum threshold of the required amount of microbial requirements based on SNI 7388: 2009. Thus the age of sausage shelf life only reaches approximately 10 days.

The result of T test analysis on the product of angkak sausage with and without packaging of polyethylene LD (04) vacuum system per day presented in Table 3. Table 3. shows the angkak sausage by adding 0.4% chitosan with and without polyethylene packing material with vacuum packaging system each day significantly different on days 1, 6, 7, 8, and 11 based on t value of 12.970 respectively; 10.414; 9.800; 7.382 and 6.630 are greater than t table value 0.05 (2) = 4.303 with probability (p) days 1, 6, 7, 8, and 11 respectively 0.06; 0.009; 0.10; 0.018 and 0.11 is smaller than the real level of 0.05 (> 5%). However, the result of T test from angkak sausage from adding 0.4% chitosan with and without polyethylene packaging material of vacuum packing system on the 4th day showed no difference based on t value 4.028 smaller than t table value 0.05 (2) = 4.303 with probability (p) day 4. The result of T test analysis on angkak sausage product with and without of polyethylene LD (04) vacuum packaging system on the day 1-11 presented in Table 4.

Based on Table 4, angkak sausage with and without polyethylene packaging materials with day to day 1-11 vacuum system shows a significant difference based on t value = 2.452 greater than t table value 0.05 (17) = 2.110 with probability (p) = 0.000 greater than the real level of 0.05 (> 5%). So it can be concluded that there is a difference of shelf life of angkak sausage with and without polyethylene packaging material with day to day 1-11 vacuum system.
Table 2. Maximum Limit of Microbial Contamination in Food (SNI 01-7388:2009)

| Number of Food Category | Food Category | Type of Microbes | Maximum |
|-------------------------|---------------|------------------|---------|
| Processed meat, poultry, and game product | Processed meat and chicken (meatball, sausage, nugget, patty) | ALT (30°C, 72 hours) | $1 \times 10^3$ colony/g |
| | | APM Coliform | 10/g |
| | | APM Escherichia coli | $< 3/g$ |
| | | Salmonella sp | Negative/25g |
| | | S. aureus | $x 10^2$ colony/g |
| | | C. Perfringens | $x 10^2$ colony/g |
| Processed meat, poultry, and game product | Cooked sausage (without canning, ready to eat) | ALT (30°C, 72 hours) | $1 \times 10^4$ colony/g |
| | | APM Coliform | 3/g |
| | | APM Escherichia coli | $< 3/g$ |
| | | Salmonella sp | Negative/25g |
| | | S. aureus | $x 10^2$ colony/g |
| | | C. Perfringens | colony/g |
| | | Listeria Monocytogenes | Negative/25g |

Table 3. Angkak Sausage T Test Results with and without Polyethilane Vacuum Packaging System

| Day | t | df | Sig. (2-tailed) |
|-----|---|----|----------------|
| 1   | 12.970 | 2 | 0.06 |
| 4   | 4.028  | 2 | 0.56 |
| 6   | 10.414 | 2 | 0.009 |
| 7   | 9.800  | 2 | 0.10 |
| 8   | 7.388  | 2 | 0.018 |
| 11  | 9.630  | 2 | 0.11 |

Table 4. T Test Results of Angkak Sausage Product with and without Packaging of Polyethilane Ld (04) Vacuum Packaging System on The Day 1-11

| Angkak Sausage | Paired Sample test |
|----------------|--------------------|
| Pair 1 Non Vacuum-Vacuum | T | df | Sign |
| | 2.452 | 17 | .025 |

Chitosan has antimicrobial properties. Ref. [11] revealed that chitosan binds the outer membrane and removes barrier function of the cell membrane, so that the cell undergoes cell surface changes and loss of protective function in the bacterial cell. The mechanism occurs through the interaction between the positive charge (cluster NH$_3$) on the chitosan molecule with the negatively charged molecule present in the bacterial cell membrane. These interactions result in the release of protein elements and other cell constituents resulting in changes in cell membrane permeability (osmotic internal imbalance of cells) resulting in the growth of microorganisms inhibited. Coating sausages with chitosan layer slowed down the moisture loss, but moisture loss was still significant after 150 days of storage [16]. The spoilage of chitosan angkak sausage without packaging can be stored only approximately 7 days, while the use of vacuum packaging can be stored up to 10 days. The shelf life of sausage with or without the vacuum process is still appropriate for the type of cooked sausage. According to Ref. [8] the type of cooked sausages can be stored in the refrigerator up to 7 days after packaging opened.
The decrease sausages’ shelf life; according to Ref. [17] due to the increase in the number of bacteria during storage due to increased moisture content and the availability of adequate nutrients for bacterial growth and environmental temperature. The spoilage of angkak sausage product can occur faster because in the processing stage the sausage is experiencing indirect contact or contamination of pathogenic bacteria in the form of impurities through water or equipment or work table used during processing. On that basis, according to the observer the cause of the acceleration of angkak sausage because at the stage of the process of cooling the sausage is done by using PDAM water. Thus, microbial contamination of the sausage has occurred before the storage process. Another cause of spoilage to sausage products chitosan-angkak is the existence of pathogen bacteria that are resistant to heat. while the process of boiling sausage chitosan angkak only done at a temperature of 85-90 °C for 30 minutes. The bacteria in question is S. aureus. According to Ref. [18] that S. aureus cannot be destroyed even able to produce enterotoxin. This toxin is resistant to high temperatures. although the bacteria die by heating but the resulting toxin able to survive even with cooling or heating 100-120°C. However all bacterial spores will die at 121°C for 15 minutes [18].

Proximate test results from the best angkak sausage presented in Table 5.

| Chitosan (%) | Protein (%) | Fat (%) | Carbohydrate (%) | Water (%) |
|--------------|-------------|---------|------------------|-----------|
| S3 (0.4%)    | 16.44       | 20.94   | 2.93             | 36.52     |

Based on Table 5, the best proximate test results of the best sausage from the application of 0.4% chitosan has a protein content of 16.44%, fat 20.94%, 2.93% carbohydrate and 36.52% water. The value of the sausage especially on the nutrition of protein and carbohydrate still in accordance with the standard of sausage quality according to SNI 01-3820-1995. At the level of protein sausage angkak higher 1.26% than the standard quality of protein set by SNI. The addition of protein content comes from the use of skim milk. Test results on fat, carbohydrate and water levels in the sausage angkak still in accordance with the standard of quality of fat set by SNI.

| Nutritional Value | Total |
|-------------------|-------|
| Water (max b/b)   | 67.0  |
| Protein (min b/b) | 13.0  |
| Ash (max b/b)     | 3.0   |
| Fat (max b/b)     | 25.0  |
| Carbohydrate (max b/b) | 8.0 |
| Food Additive     | According to SNI 01-0222-1995 |

4. Conclusions
Best angkak sausage application of 0.4% chitosan without packaging materials have 7 days storage and reach 10 days in angkak sausage with polyethylene packaging LD (04) vacuum system. The best value of angkak sausage nutrition result of application of chitosan 0.4% have protein content 16.44%, fat 20.94% carbohydrate 2.93% and water 36.52%. The ability of adding 0.4% chitosan to improve the shelf life of the angkak sausage at chill room storage (2-4°C) can achieve twice as much storage time than without chitosan. Thus it is suggested that the control of microbial quantities can occur more effectively in order to improve the shelf life of angkak sausage then in addition to the use of vacuum system packaging is also given the storage treatment at freezing temperatures (0°C). The ability to save the angkak sausage at chill room storage (2-4°C) with preservative chitosan 0.4% to reach 10 days need to be followed by testing of organoleptic angkak sausage to determine the level of acceptance of panellists.
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Acknowledgment

The authors would like to thank the Indonesian Ministry of Research and Higher Education who has provided on Tinggi funding through the research Scheme Penelitian Kerja Sama Perguruan Tinggi (Pekerti) in 2014 and the Universitas Negeri Surabaya which has given the author the opportunity to carry out research in the Food Technology Laboratory Department of Home Economics. Faculty of Engineering and supported by alumni of the Department of Home Economics who have assisted this research