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PROTEINS PROFIL OF SAUSAGE LAYING CHICKEN MEAT WITH ANGKAK (RED RICE) USED AS NATURAL FOOD MATERIAL

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Abstract

This study examines the potential of Angkak as a binding compound, color improvement in the process of making sausage chicken layered rejects as a curing material. Testing protein profiles related to dissolved protein, texture, water binding capacity to form a compact and soft texture. The design was carried out in a completely randomized design (CRD) 4 x 4, as a treatment using the Angkak level, ie without Angkak (0%), Angkak 0.5%, Angkak 1% and Angkak 1.5% with replications 4 times followed by a test BNJ. The data obtained were analyzed by analysis of variance (Analysis of Variance) which included chemical, color, total protein, and protein profile determination using the SDS-PAGE method (Sodium Dodecyl Sulphate Polyacrilmide Gel Electrophoresis). The results of profil proteins with SDS-PAGE (sodium dodecyl sulphate polyacrilmide gel electrophoresis), where R0 is a sausage without the addition of Angkak, R1: sausages that use 0.5% Angkak concentration, R2: sausages that use a concentration of Angkak 1.0% and R3: sausage who use the Angkak concentration of 1.5%. The conclusion of the study was that the addition of Angkak gave a chemical change and protein profile in the sausages of rejected laying hens and the range of molecular weights found in R0, R1, R2 and R3 were the same, namely 12.44 - 47.89 kDa.

Key words*: sausage meat, laying chicken, Angkak, curing.*

INTRODUCTION

Reed laying chicken meat as well as other livestock products is livestock commodities that need to be developed and improved. In general, the Indonesian people have known laying chicken meat rejects as a source of food that is mainly expected to be eggs, but the obstacles that have existed during this time rejects laying chicken meat is not much in demand by the public, because laying chicken meat apart from clay meat that is not favored by consumers also variations in processing chicken rejected laying into processed products is very limited, so that a processing technology is pursued to utilize and increase the added value of rejected laying chicken meat. One processing technique that can be develop.

According to Tisnadjaja (2006) that the use of nitrite in the process of curing in meat is 125 ppm, but this use has not been strictly monitored on food products, while the use of Angkak as a substitute for saltpeter or nitrite can be reduced by up to 60% without any apparent changes in organoleptic properties. Angkak pigments are used as a partial substitute for nitrite in the processing of meat cured like ham and sausage beef, both in improving the red color of meat products and in inhibiting the growth of spore-forming bacteria such as *Bacillus cereus* and *Bacillus stearothermophillus*.

According to Astawan (2012), the use of Angkak can reduce the use of nitrite in food. Nitrite is often used as a component of saltpeter, a substance used to maintain the red color of meat, especially in making sausages, smoked meat and cornet. Through its antimicrobial properties, the use of Angkak in making sausages is not only a red giver, but also as a safe preservative for health. Another advantage of using Angkak in making sausages is improving the texture and flavor. Furthermore, according to Sheu et al. (2000), states that the pigments produced by *Monascus purpureus* are very stable and do not change the taste of nata de coco. The dosage used for animal food coloring ranges from 2000-4000 ppm Monascus extract while for soft drinks, the concentration used can be lighter which is 0.002% - 0.005% (2-5 ppm).

The curing solution formulation can increase the red color stability of the product during storage, in ham and beef sausage products, the use of nitrite in the curing solution can be reduced from 125 ppm to 80 ppm by adding 2.5 g / kg meat of Angkak pigment. (Fardiaz et al., 2008). Farisandi and Pangesthi (2013) examined the combination of administration of Angkak with sodium nitrite to the organoleptic properties of corned beef, the result was that giving 1% Angkak combined with 50 ppm sodium nitrate affected the color of corned beef products but not flavor and aroma. According to Pattanagu et al. (2007), the optimum use of Angkak in meat products is 1.6% (w / w).

Proteins are high molecular weight complex organic compounds which are polymers of amino acid monomers that are connected to each other by peptide bonds. Proteins generally have a high molecular weight, because of the large weight of protein molecules, so proteins tend to form colloids. Protein solubility depends more on its structure and function and not on its molecular weight. Soluble proteins are good buffers and are very important in maintaining equilibrium reactions. Proteins are formed by units of amino acids that make up polymers so they are long compounds. Protein quality depends on the amino acids it contains. The principle of determining protein profiles by electrophoresis is to separate protein molecules with different charges.

MATERIALS AND METHODS

The ingredients used for the manufacture of sausages are 20 - 24 month old reject chicken meat which has been skinned, washed, 2x2 cm in size taken from the chest and thighs, then separated into 4 parts.

Then the meat is ground and then seasoning is added with a formula from a combination of Bhattacharyya, Mita and Biswas (2005) and Pearson and Dutson (1988), namely: 2% salt, 1.67% sugar, 1.5% garlic, 0.5% pepper, ginger 0.75%, nutmeg 0.5%, which is given in powder form, oil 15%, tapioca flour 5.7%, skim milk 3.5%, ice cubes 16.7% and STPP 0.3% by weight meat.

Each part is given Angkak 0%, 0.5%, 1% and 1.5% in the mixture put in a sleeve with a length of 10 cm and a diameter of 2.5 cm. Then cooked by steaming at 85^0C for 30 minutes. Then cooled and analyzed.

The research design carried out was descriptive research. The independent variable in this study was the addition of Angkak to the sausage meat of layered laying hens with concentrations of 0%, 0.5%, 1.0% and 1.5%.

Tools used for protein profile analysis are microtube, beaker glass, Erlenmeyer, electrophoresis chamber, power supply, rotator, mortal cup and spectrophotometer. The method used is SDS-PAGE (Rantam, 2003): Supernatant sample: 20 ml PB (Posphate Buffered Saline) solution added 0.5 M NaCl at pH 7.2. A 10 gram sausage sample was pounded with mortar then add a 0.01 ml PBS buffer of 3 ml. Then centrifuged at 6000 rpm for 15 minutes at 4^0C.

The work procedure of SDS-PAGE is as follows:

Prepare samples:

The protein sample is supplemented by 1:1 Reducing Sample Buffer (RSB) in the Eppendorf tube. Then the sample is heated at 100^oC for 5 minutes. After being cold, if the sample is not directly used, the sample can be stored at -20^0C

Prepare separating and stacking gel for 2 plates: Gelling plate is arranged as a guide. 15% separating gel is made by: 10% SDS - 60 ml, 10% APS – 60 ml, TEMED 10 ml

Enter the sample in the gel well

A plate that already contains gel is inserted into the electrophoresis chamber. Running buffer is poured until the top and bottom of the gel are submerged. If air bubbles form on the base of the gel or between sample wells, they must be removed. A standard 10 µl marker is inserted in one of the wells (can be gargled at the edge or in the middle well).

Samples of 10-20 µ (with a minimum protein content of 0.1 µ and a maximum of 20-40 µ) are carefully inserted into the bottom of the gel well, using Hamilton syringe. Syringes are rinsed to 3x using water or by running buffer before being used to insert different samples in the next gel well.

Running sampel

To start running the electrophoresis device is connected to the power supply. Running is carried out at a constant current 20 mA for approximately 40-50 minutes or until tracking

dye reaches a distance of 0.5 cm from the bottom of the gel. After completion, the running buffer is poured and the gel is taken from the plate

Coloring of Gel:

For this stage, a staining solution is needed for coloring gel proteins, the coloring used is Comasie Brilliant Blue or Silver Stain depending on usability. Staining is carried out for 30 minutes. Destaining solution to remove color in the gel and clarify the protein bands formed.

RESULTS AND DISCUSSIONS

Water Content

Decreasing the water content of duck sausage is caused by the increased concentration of Angkak used. Angkak can experience oxidation when heated which causes a decrease in the water in the meat is lost, so that the water content drops (Fardiaz and Zakaria, 1996).

According to Zanardi et al. (2002), the addition of the Angkak concentration produced from the *Monascus purpureus* mushroom resulted in a positively charged meat protein and binding to the H + charge, consequently there is no H + that is free or which binds to O which produces free water molecules.

The sausage water content according to the Indonesian National Standard (1995) is a maximum of 67.0%, so the sausage water content from the research which ranges from 63.70 - 64.16% still meets SNI standards.

Fat

This decrease in fat levels is caused by lovastin compounds in Angkak which act as inhibitors of HMG-CoA reductase (an enzyme that plays a role in cholesterol biosynthesis), where lovastin is hydrophilic and lipophilic but tends to be lipophilic (Dalimartha, 2001).

The fat content of this study ranged from 8.38 - 9.43%, while fat content according to the Indonesian National Standard (1995) was a maximum of 25%, so the fat content of the results of this study still met the standards.

Table 1. The average value of the chemical properties of sausage meat in laying hens is rejected

Parameter	Consentration Angkak			
	0%	0,5%	1%	1,5%
Water Content	$64.16 \pm 0,168^{a}$	$63.26 \pm 0,078^{a}$	$63.33 \pm 0,213^{b}$	$63.70 \pm 0,082^{c}$
Fat	$9.43 \pm 0,056^{a}$	$9.21 \pm 0,055^{b}$	$8.98 \pm 0,057^{c}$	8.38 ± 0.089^{d}
Carbohidrate	$7,75 \pm 0,090^{a}$	$8.14 \pm 0,110^{b}$	$9.31 \pm 0,029^{c}$	$10.89 \pm 0,028^{d}$
Protein	$15,48 \pm 0,369^{a}$	$15,81 \pm 0,088^{a}$	$16,09 \pm 0,118^{c}$	$16,83 \pm 0,131^{d}$

Remarks: different notations show significant differences between treatments ($P<0.05$)

Carbohidrate

The results of the analysis of carbohydrate values indicated that the higher the concentration of Angkak added, the higher the carbohydrate level is. While the carbohydrate carbohydrate levels in this study were 10.89% so that the higher the Angkak added, the higher the carbohydrate content of sausage chicken meat sausages.

Protein

The results of the study for protein levels showed that the higher the concentration of Angkak added, the higher the level of sausage protein in the rejected laying chicken meat.

The addition of Angkak in this experiment was immediately given together with seasonings in the process of making sausages. Monascus mushrooms that produce enzymes such as α-amylase, β-amylase, glucoamylase, lipase, protease, glucosidase and ribonuclease are able to grow in materials containing starch, protein or lipids (Pattanagu et al., 2007), this condition allows an increase in levels of chicken sausage protein with increasing levels of addition of Angkak to treatments R1, R2, and R3.

Angkak proteins undergo decomposition (oxidative degradation) through a transamination reaction (the enzymatic transfer of amino acid groups from one amino acid to another amino acid) that can bind meat proteins, this is supported by Kramlich (1971) which states that meat protein plays a role in increasing destruction meat during cooking to

form a compact product structure. The role of other proteins is the formation of meat emulsions, which are proteins that function as fat emulsifiers.

Protein content of chicken sausages with Angkak colorant added to the results of this study ranged from 15.48 - 18.83% so that the sausage protein levels still meet SNI standards.

Proteins generally have a high molecular weight, because of the large weight of protein molecules, so proteins tend to form colloids. Protein solubility depends more on its structure and function and not on its molecular weight. Soluble proteins are good buffers and are very important in maintaining equilibrium reactions.

Profile protein

The results of SDS-PAGE sausages from rejected laying hens have 7 protein bands that appeared in sausages without the addition of Angkak and the addition of Angkak (0.5%, 1%, 1.5%) with protein molecular weight ranging from 12.44 - and 47.89 kDa (Table 2). Calculation of molecular weight (BM) of protein bands contained in the gel by comparing the molecular weight of the marker and Retardation Factor (Rf), then proceed with making a standard curve with the value of Rf as the x axis and the molecular weight logarithm value as the y axis.

Table 2. Weight of sausage protein molecules

Pita	Rf	Ro	R1 BM	R2	R3
2.1	0.33	47.89	47.89	47.89	47.89
2.3	0.36	42.36	42.36	42.36	42.36
2.5	0.39	37.48	37.48	37.48	37.48
2.1	0.45	29.33	29.33	29.33	29.33
3.9	0.48	25.95	25.95	25.95	25.95
3.6	0.56	19.10	19.10	19.10	19.10
4.3	0.67	12.44	12.44	12.44	12.44

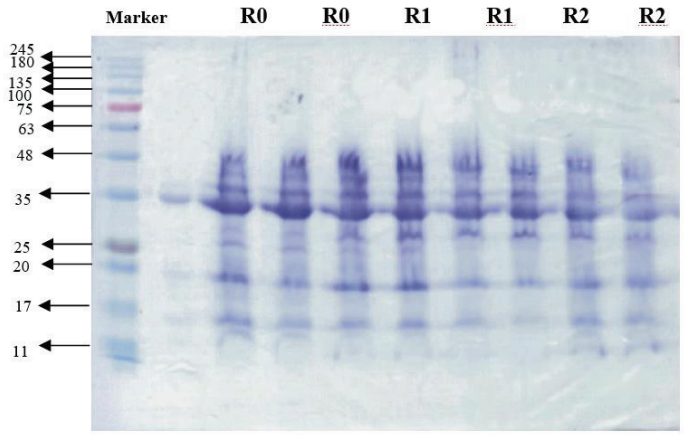

Figure 1. SDS-PAGE Electrophoresis Results
(R0: Sausage + Without Angkak,
R1: Sausage + Angkak 0.5%, R2: Sausage + Angkak 1%
and R3: Sausage + Angkak 1.5%, M: Marker)

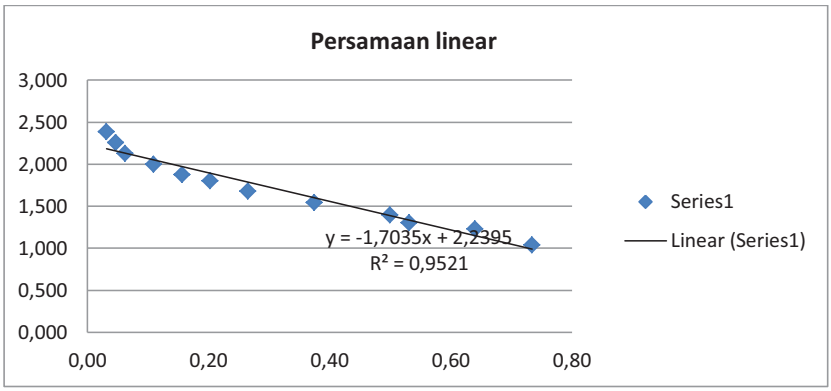

Figure 2. Molecular Weight Curve

According to Laemmli (1970), protein bands that are close together indicate that the protein has the same number of amino acids, whereas according to Soeparno (2011) that proteins are formed from amino acids which are bound together to form a series. A small difference in the formation of a series will produce a different type of protein. The types of protein bands detected in processed products are closely related to the functional level of protein damage. Amino acids are increasingly showing the low functional damage to proteins. This is evidenced by the type of protein bands found in meat sausages without the addition of Angkak and added ones which have molecular weights of 12.44 kDa, 19.10 kDa, 25.95 KDa, 29.33 kDa, 42.36 kDa, 37.48 kDa, and 47.89 kDa.

CONCLUSIONS

The addition of Angkak to laying hens sausages can provide changes in water, fat, carbohydrate and protein levels. The molecular weight of the treatment is 0%, 0.5%, 1% and 1.5% ranging from 12.44 kDa - 47. kDa.

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