Short Communication:

Microstructure of Fermented Catfish (*Clarias* sp.) Sausages infected by *Listeria monocytogenes*

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Abstract

The effect of several lactic acid bacteria was studied on the fermented African Catfish (Clarias sp) sausages infected by Listeria monocytogenes. Pediococcus acidilactici and Lactobacillus casei were used as starter of fermented sausage. The aim of this study was to investigate the microstructure of the infected fermented sausage. It was noted that myosin filaments which existed in all sausage were affected by the breakdown of tissue cells as a result of chopping the fish flesh. The formation of elongated fibrils indigenous sausage was presumably caused by lactic acid bacteria which was lower than fermented sausage using lactic acid bacteria starter.

The structure of Pediococcus acidilactici sausage starter has cavities looks and there are granules around it. Meanwhile, Lactobacillus casei starter was dominated by wider and compact swollen myofibril. Pediococcus acidilactici-fermented sausage had rougher swollen myofibril than that of combination of Pediococcus acidilactici and Lactobacillus casei starter-fermented sausage.

In conclusion, the combination of Pediococcus acidilactici and Lactobacillus casei gave a compact microstructure in fermented catfish sausage.

Keywords: Catfish, *Listeria monocytogenes*, Sausages, Fermentation.

Introduction

The basic structures of matrix composition, fibers, fat globules and matrix proteins changes in the sausages are stored at particular interval formed in fermented sausage emulsion product and can be seen by using Scanning electron microscopy (SEM)[1].

Furthermore, it can display microstructure of sausage with pre-blending treatment without salt; phosphate addition and/or mechanical treatment indicates that the tissue looks very sharp, its edges are illuminated by water without any physsical irregularities.

Long period of pre-blending time causes the fiber tissue becomes rougher. Fiber that appears to be broken and out of the unity indicates the maximization of protein solubility due to salt and water infusion during pre-blending. More dissolved proteins make it difficult to identify the fiber tissue; the fibers are unclear, fat globules cover the protein and there is thick matrix protein[2].

In myofibril cell, there are protein aggregates that look like stems. Myofibrils are long elements which are able to contract inside sarcoplasm and unstable in frozen storage[3].

The accumulation of lactic acid causes acidification rise in myofibril tissue, thus reducing the water-holding capacity. Cross-link is formed by actin and myosin and glycogen which is stored out of the tissue[3]. In this study we investigate the microstructure of fermented fish sausage with and without *Listeria monocytogenes* infection.

Material and Methods

Materials: Catfish *(Clarias sp.)* was purchased from local market near Brawijaya University. *Pediococcus acidilactici* 0110<TAT-1(PA), *Lactobacillus casei* and *Listeria monocytogenes* ATCC-1194 (LM) were obtained from Center for Universities, Gadjah Mada university. All materials for this study were of analytic grade.

Sausage formulation: The formulation of sausage in detail is explained elsewhere[5].

SEM analysis: The microstructure condition was analyzed by using Scanning electron microscopy[6], which was described as follows: sausage (0.5 cm in diameter and 0.2-0.3 cm thick) was placed into 2 % Glutaraldehyde fixative soltion for 2 – 3 hours at a temperature of 4 ºC. It was then triplicate rinsed with phosphate buffer saline (PBS), pH 7.4 for 5 minutes at 4 °C. The solution was substituted by 1% osmic acid as post fixation solution for 1-2 hours at 4 °C.

It was further rinsed with PBS, pH 7.4, three times for 5 minutes each at 4°C. Dehydration was performed by using increased alcohol level: 30 %, 50 %, 70 %, 80 %, 90 % with two times absolute value for 15 -20 minutes each. 30 % and 70 % dehydration were performed at a temperature of 4°C while 80 % absolute dehydration was at a room temperature.

It was dried by using Critical Point Drying (CPD) and attached to stub (holder) by using special glue. It was gold coated and observed using FEI, Type Inspect-S50 with 20 kV. The photograph data were analyzed descriptively using 30.000X magnification.

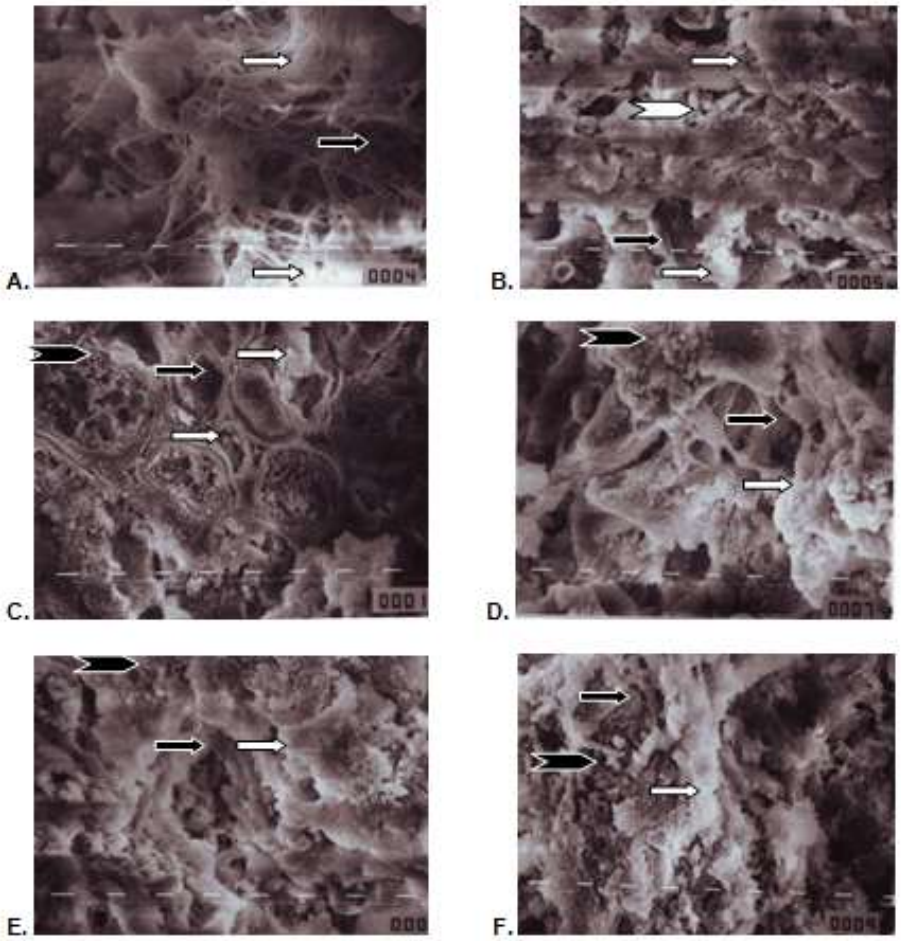

Figure 1: Microstructure of fermented sausages microstructure of indigeneous fermented sausages (A and B); *Pediococcus acidilactici*-fermented sausage (C and D); Combination of *Pediococcus acidilactici* and *Lactobacillus casei*-fermented sausage (E and F); Sausages without *Listeria monocytogenes* infection (A,C,E); Sausages with *Listeria monocytogenes* infection (B,D,F).

Results and Discussion

Microstructural fermented sausages were fermented by using indigenous and lactic acid bacteria (*Pediococcus acidilactici* and *Lactobacillus casei*) with or without infection of *Listeria monocytogenes* depicted in figure 1.

Microstructure of fermented sausages showed different myosin filaments among treatments. Myosin filaments that existed in all sausage shapes were caused by the breakdown of tissue cells as a result of chopping the fish flesh so that its integrity was damaged due to the opening of sarcolemma of myofibrils.

Figure 1A showed that sausage with indigenous starter was dominated by elongated fibers. Those long fibers were presumed as collagen in triple helix structure as tropocollagen molecules that formed fibrils. It was likely caused by the growth rate of lactic acid bacteria in indigenous sausage. As a consequent the heat energy formed during fermentation was low. Hence, it failed to break hydroxyproline from the triple helix structure. Hydroxyproline stability depends on the heat stability and it affects the collagen stability. Rose and Mandal[7] stated that the enthalpy of collagen depends on the content transition of hydroxyproline and hydroxyxylene.

Figure 1C showed that the structure of *Pediococcus acidilactici* fermented sausage had cavities looks and there are granules around it. It is related to the pH value and the production of acetic acid. The higher is the content of acetic acid, salt penetration into the myofibril fibers decreases. The formation of cavities and rough structures indicated that there was remaining actomyosin tissue from the unity of myofibril tissue that was not extracted. As a result, fat emulsion occupied the space between the protein film surfaces. It was caused by the effect of shrinkage during incubation.

The existence of cavities in sausage products causes unstable fat emulsification and the size of the cavity formed depends on the melted fat from the unity of matrix proteins emulsion[8]. Woloszyn[9] stated that myofibril proteins and stromal proteins contribute to gel formation of fermented sausage. Hence, the ability of gel forming from proteins tissues is directly related to myosin and a number of actin sarcoplasm protein as well as pH and ionic strength[10].

Combination of *Pediococcus acidilactici* and *Lactobacillus casei* starter was dominated by wide and compact swollen myofibril compared to sausage with indigenous and *Pediococcus acidilactici* starter (Figure 1E). This situation was plausible related to dibasic amino acids and diacid collagen which were also high, but it did not contain tryptophan and cysteine.Several produced enzyme will affect gel forming ability through sarcoplasm decomposition. Sanz et al[11] suggested that the proteolysis activity of Lactobacillus casei is very responsive in decomposing the sarcoplasm proteins. Indigenous sausages infected by *Listeria monocytogenes* displayed a number of myosin filaments pieces (Figure 1B). Fiber pieces of myosin showed that actin interacted with myosin as actinomyosin apart from the gel. Visessanguan et al[12] reported that myosin and actomyosin play an important role in the formation of gel unity. Myosin is a protein that has the ability to form gel while actin is related to skeletal protein arrangement and it does not form a gel but it affects viscoelasticity[2].

Lactic acid bacteria-fermented sausages which were infected with *Listeria monocytogenes* had a consistent microstructure (Figure 1 D and F). Sausages which were fermented using Pediococcus acidilactici starter (Figure 1D) had rougher swollen myofibril than sausages combination of *Pediococcus acidilactici* and *Lactobacillus casei* starter (Figure 1F). It was presumably caused by the hemolysin produced by *Listeria monocytogenes* which was less than the indigenous-fermented sausage.

Purchas and Aungsupakorn[4] stated that isoelectric point of fermented sausage was achieved at pH of 5.2 to 5.3. Further report by Woloszyn[9] indicated that protein gel will turn to rough if the matrix protein was bound between myosin fibers. Increase in temperature will cause the protein coat between fat globules becomes thin which will lead to the decline in the stability of sausage emulsions.

Conclusion

From the study it was concluded that *Pediococcus acidilactici* starter served to open the myofibril fibers and formed cross-link between protein molecules through the coagulation process by acetic acid. Swollen myofibrils due to water molecules absorption freely flown into fibrils were controlled by the lactic acid produced by *Lactobacillus casei*. The combination of *Pediococcus acidilactici* and *Lactobacillus casei* starter produced compact microstructure in fermented catfish sausage.

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