

EFFECT OF YEAST AND LACTIC ACID BACTERIA IN CULLED LAYING HENS SALAMI AGAINST *ESCHERICHIA COLI*, *STAPHYLOCOCCUS AUREUS* AND *SALMONELLA SP*

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Abstract

The purpose of this study was to determine the effect of yeast and lactic acid bacteria in culled laying hens salami against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella sp.* The study was conducted using Completely Randomized Design (RAL) with 5 treatments and 4 replicates. The treatments were: P1 = Salami with 2% lactic acid and 1% yeast, P2 = Salami with 2% lactic acid and yeast 2 %, and P3 = Salami with 2% lactic acid and 3% yeast, and P4 = Salami with 2% lactic acid and 4% yeast. *Escherichia coli* and *Staphylococcus aureus* were analyzed with quantitative and qualitative method, and *Salmonella sp.* were analyzed with qualitative method. The test for inhibitory bacteria *Escherichia coli*, *Staphylococcus aureus*, *Salmonella sp.* used disc diffusion method, that the salami product of this research meets the requirements of SNI 01-3820-1995. It can be concluded that the use of yeast and lactic acid bacteria could inhibit the growth of pathogenic bacteria in culled laying hens salami.

Key words: lactic acid bacteria, pathogenic bacteria, salami, yeast.

INTRODUCTION

Nowadays the number of inventions by experts reported that microorganisms play a lot in fermentation processes through various kinds of biochemical reactions. Utilization of microorganisms is used both to produce food products and to preserve existing food products. Utilization of yeast in traditional food and fermented product in Indonesia is still relatively small, mainly utilizing only a few species such as *Saccharomyces cerevisiae*, *Kluyveromyces lactis* / *Kluyveromyces kefir* and *Zygosaccharomyces spp.*, especially in producing bread, tape, brem, wine, soy sauce, salt vegetables, etc. Whereas, in some other countries, yeast has been used in producing fermented milk and other products. Common cultures used in salami processing include classes of lactic acid bacteria such as *Lactobacillus*, *Pediococcus*, *Micrococcus* and *Streptococcus*.

Yeasts have antimicrobial properties with specific proteolytic activity, such as *Candida*, known to have proteolytic ability. In addition, there are extracellular proteolytic capabilities such as *Candida*, *Cryptococcus*, *Rhodotorula*,

Phicia, *Hansenulla* and *Metschnikowia*. Species *Saccharomycopsis fibuligera* R64 strains are known to have antimicrobial activity in some pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. *Kluyveromyces thermotolerans* and *Kloeckera apiculata* have long been known to inhibit the growth of *Lactobacillus plantarum*. A certain yeast culture belonging to *Debaryomyces* has been shown to help form and stabilize the color on the sausage surface. The presence of yeast in fresh meat is very different from that of processed meats: in fresh meat yeast growth is not expected because if the growth exceeds 10^5 - 10^6 cells / g it will be quickly damaged, therefore, in the sale of meat is expected to be at refrigerator temperature (4-5°C) or packed in a vacuum. In carcasses only 5 - 10% of the total number of microflora and not significant cause damage (Samelis and Sofos, 2003). Unlike the case in processed meat products, the presence of yeast can add flavor and aroma for example in fermented sausage. *Debaryomyces hansenii* on Italian salami, gives a good flavor to the product; and affect the quality of sausages (Samelis and Sofos, 2003). *Yarrowia lipolytica*

is also often isolated from fresh beef and sausage.

Meat is a food contains nutrients needed by the body, but it also has drawbacks such as perishable foodstuffs, considering the meat contains protein and fat as a source of nutrition for utilized by the development of microorganisms. Meat damage can occur due to early contamination of microorganisms as it enters the bloodstream during livestock slaughter. Fresh meat can be contaminated by a large number of bacteria including pathogenic bacteria, so the food is damaged. The type of bacteria such as *Bacillus cereus* concerned, *Clostridium perfringens*, *C. jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* sp. and *Staphylococcus aureus*. One of the requirements of quality poultry products are free of pathogens, such as *Salmonella* sp., *Staphylococcus aureus*, *Escherichia coli*, and *Campylobacter* sp. (Baumler et al., 2000). The microbial growth occurs in a short time and in appropriate conditions, such as the availability of nutrients, pH, temperature, and water content of food. The microbial group of spoil turns the fresh food into rotten and can even produce toxin (poison). Sometimes it shows no signs of change or physical damage (less really bad smell), so that food is still consumed (Djaafar and Siti, 2007). *Salmonella* and *Campylobacter* sp. is a major pathogen and is present in foods associated with poultry and often causes disease in humans (Mbata, 2005). However, other pathogens also exist such as *Clostridium perfringens*, *E. coli* O157 and *Listeria monocytogenes* and new pathogens, *Acetobacter* and *Helicobacter* sp. (Mbata, 2005). Therefore in this study will examine how the influence of yeast and lactic acid bacteria on quantitative analysis of *Escherichia coli*, *Staphylococcus aureus* and qualitative analysis of *Salmonella* and the inhibitory of each pathogenic bacterium against the culled laying hens salami.

MATERIALS AND METHODS

The material used was 30 culled laying hens strain Isa Brown 96 week old. Seasonings for the manufacture of salami such as garlic, ginger, pepper, nutmeg, sugar and salt, cornstarch, skim milk and fat. The starter used

is yeast *Trichosporon beigeli* yeast identification using RapiD™Yeast Plus System and lactic acid bacteria *Lactobacillus acidophilus* and *Lactobacillus plantarum* isolation from culled laying hens. The equipment used in this research is: Food Processor Philips HR 7620 brand used for grinding meat and mixing salami, basin for laying chicken, plastic to store spices, gloves, ohaus scales with capacity 600 g Model: Scout Pro SPS 6000F, knife and cutting board, thermometer to measure the temperature of the room, Harnir-Shuma brand brush as a filler into the sausage casings, Salami casings (casing) brand "NaloFaser" with the size Caliber 45, Lange 60.0 comes from Germany. Strap casing (mattress yarn), stove and oven for curing salami.

Salami production consists of culled laying hens and fat with a ratio of 80 : 20 (g/g). meat and fat are simultaneously ground, then frozen for 24 hours; the ground meat is frozen and then milled again using a food processor while added spices such as salt, sugar, garlic, ginger, pepper, nutmeg, cornstarch, skim milk and 2% lactic acid bacteria culture as well as yeast species (*Trichosporon beigeli* T0, T1, T1 (1%), T2 (2%), T3 (3%) and 4% (T4) until well-mixed. Into salami dough added cornstarch, milk powder and chicken fat. After mixing, the dough is inserted into the 30 mm diameter casing, then tied to a distance of 10 cm and then hung on a rack and held for 24 hours at room temperature (Arief et al., 2008), then fermentation process at room temperature for 6 days and interspersed with fumigation for one hour per day. Temperatures during fumigation are maintained 27 - 30°C, when heat exceeds that temperature, a temperature decrease by adding ice to the cubic chamber when the temperature exceeds 30°C. The fuel used is dry coconut shell.

The experiment was conducted using Randomized Completely Randomized Design with 5 treatments and 4 replications, so that were 20 treatment combinations (Gaspersz, 1995) were obtained: T0 = Salami with 2% lactic acid and 0% yeast without adding spices, T1 = Salami with 2% lactic acid bacteria and 1% yeast, T2 = Salami with 2% lactic acid and 2% yeast, and T3 = Salami with 2% lactic acid and 3% yeast, and T4 = Salami with the use of

2% lactic acid bacteria and 4% yeast. Quantitative analysis methods of *Escherichia coli* (APHA, 1992), quantitative analysis of *Staphylococcus aureus* (Fardiaz, 1992) and qualitative analysis of *Salmonella* sp. (Andriani et al., 2005) were then tested for *Escherichia coli* bacterial inhibition, *Staphylococcus aureus*, *Salmonella* sp. using disc diffusion method (Kirby-Bauer test).

RESULTS AND DISCUSSIONS

Quantitative analysis of *Escherichia coli* (MPN/g), *Staphylococcus aureus* (CFU/g) and qualitative analysis of *Salmonella* (per 25 g)

Table 1. Quantitative analysis of *Escherichia coli* (MPN/g), *Staphylococcus aureus* (CFU/g) and qualitative *Salmonella* sp. (per 25 g) against Culled Laying Hens Salami

Sample/ Salami	Quantitative Analysis		qualitative analysis	Infor- mation
	<i>Escherichia coli</i> (MPN/g)	<i>Staphylococcus aureus</i> (CFU/g)	<i>Salmonella</i> (per 25 g)	
T0	< 3	< 10	Negative	Qualify
T1	< 3	< 10	Negative	Qualify
T2	< 3	< 10	Negative	Qualify
T3	< 3	< 10	Negative	Qualify
T4	< 3	< 10	Negative	Qualify

Notes: Qualify SNI (Kesmawet Laboratory DKI Jakarta, 2015)

Based on the results of laboratory tests (Table 1) showed that salami culled laying hens using yeast starter and lactic acid bacteria, *Escherichia coli* salami bacteria of laying hens were <3 MPN/g for each sample. These data suggest that salami products are safe with negative results for *Escherichia coli* bacteria. In accordance with SNI 01-3820-1995 the *Escherichia coli* in sausage should be <3 MPN/g. The main habitat of this bacterium exists in the digestive tract (especially in the intestines) of humans and can be found in soil, water, and other places that are the native habitat of this bacterium (Jay, 2000). The bacteria are easy to contaminate the water, therefore the contamination of these bacteria in food usually comes from the contamination of the water used. Foodstuffs that are often contaminated by *E. coli* include, chicken, beef, pork during slaughter, fish and other seafood products, eggs and other dairy products, vegetables, fruits, juices, and beverages milk and others. This bacterium is very sensitive to heat and can be activated at food pasteurization

temperature or during cooking of food (Supardi and Sukanto, 1999).

Table 1 showed that the culled laying hens salami with starter yeast and lactic acid bacteria for all treatments against *Staphylococcus aureus* bacteria is <10 CFU/g; according to SNI 01-3820-1995 that the bacterium *Staphylococcus aureus* should be <10 CFU/g. The results of the study met the recommendations of National Standardization Agency (1995). The cells of *Staphylococcus aureus* are gram-shaped positive, generally arranged in groups like grapes. The bacteria are immobile, facultative anaerobic, growing on products containing up to 16% NaCl (Buckle et al., 2010). The presence of *S. aureus* in the diet comes from the skin, mouth, or nasal cavity of food processors, making it easy to contaminate food. Meat contaminated or containing enterotoxigenic *S. aureus* is very harmful to consumer health due to the absence of other competing microorganisms and can usually inhibit the growth and formation of *S. aureus* toxins (Djaafar and Siti, 2007).

In contrast to the qualitative test that laying chicken affection salami negative to *Salmonella*. The results are in accordance with the recommendation of the National Standardization Agency (1995), meaning that the resulting salami product is feasible and safe for consumption, because it is free from *Salmonella* bacteria.

Salmonella contamination in meat is most common, usually occurring during animal slaughtering processes (Hanes 2003; Goncagul et al., 2005; Stevens et al., 2006; Cortez et al., 2006). Cortez et al. (2006) and Nogrady et al. (2008) showed that the contamination of chicken meat in slaughterhouses occurs through feces, fur, soaking hot water before scalding water, evisceration water, chiller water, and carcass rinse water, as well as Humphrey (2006) stated that *Salmonella* contamination in carcass / poultry meat often occurs during the cutting process, especially during evisceration, and during dipping in soft scalding.

Salmonella is gram-negative pathogenic bacteria can be isolated from the soil, water, food, and digestive tracts of humans and animals (Anderson and Ziprin, 2001). Animals containing (infected) *Salmonella* often do not show clinical symptoms (subclinical), so the

bacteria tend to spread easily between flocks. In addition, animals become carriers of a persistent disease, so the prevalence of *Salmonella* incidence is not easily detected, except through routine sampling and examination (Namata et al., 2009). Another source of *Salmonella* infection in poultry is contaminated feed, rodents, worms, and other wild animals (Humphrey, 2006).

Some pathogenic microbes such as *Escherichia coli*, *Salmonella* and *Staphylococcus* sp. often contaminate meat. The microbial content of meat comes from farms and unhygienic animal slaughterhouses (Mukartini et al., 1995). Meat processing long enough, allowing the occurrence of microbial contamination in its processed products. Processed meat products such as sausages must meet the provisions of quality requirements. Based on SNI 01-3820-1995, *Salmonella* contamination in meat sausage must be negative, and *Clostridium perfringens* negative, and *S. aureus* up to 10^2 colonies /g.

Inhibition of *Escherichia coli* (MPN / g), *Staphylococcus aureus* (CFU / g), *Salmonella* (per 25 g)

The average inhibition of the *Escherichia coli* inhibition on the culled laying hens salami showed in Table 2.

Table 2. Mean of inhibition against *Escherichia coli* (MPN / g), *Staphylococcus aureus* (CFU / g) and *Salmonella* (per 25 g) in culled laying hens salami with Starter Yeast and Lactic Acid Bacteria

Sample / Salami	Bacteria inhibition		
	<i>Escherichia coli</i> (MPN/g)	<i>Staphylococcus aureus</i> (CFU/g)	<i>Salmonella</i> (per 25 g)
T0	0.27	0.23	0.45
T1	0.20	1.60	0.25
T2	0.27	1.98	0.80
T3	0.20	1.65	0.40
T4	0.27	2.33	0.40

Table 2 showed that the higher percentage of yeast used in laying chicken salami caused the resulting *Escherichia coli* inhibition to be relatively stable, that means all of treatments showed the same and not significantly different ($P>0.05$). The research on antimicrobial activity of laying hens salami was done by disc diffusion methods (Kirby-Bauer method). Figure 1 showed that the inhibition zone

activity of laying hens rejecting the growth of *Escherichia coli* bacteria in the presence of clear zones around each disc forming a drag zone with an average diameter of 0.275 mm; 0.2 mm; 0.275 mm; 0.2 mm and 0.275 mm, although not statistically significant ($P>0.05$). This means that lactic acid bacteria can produce lactic acid and other metabolites that are antibacterial, so the growth of pathogenic bacteria can be inhibited (Savadogo et al., 2004). In addition to producing organic compounds, several strains of lactic acid bacteria also produce bactericidal protein compounds against gram-positive and gram-negative bacteria called bacteriocin (Tahara et al., 1996), as well as yeasts capable of producing antimicrobial compounds in the form of organic acids hexanoate, octanoate and decanoate) and proteins.

Arief et al. (2008) reported that lactic acid bacteria of *Lactobacillus plantarum* sp. can produce antimicrobial compounds of hydrogen peroxide. Hydrogen peroxide serves to decrease the permeability of *E. coli* structure molecules through the mechanism of lactoperoxidase and thiocyanate, hydrogen peroxidase and can inhibit the growth of pathogenic bacteria *E. coli*, *Salmonella* and *Staphylococcus* (Jennie and Rini, 1995). The diameter of the growth zone of bacterial growth indicates bacterial sensitivity to anti-bacterial agents followed by the width of the diameter of the inhibition zone formed, so that the bacteria become more sensitive (Hastowo, 1992). *Escherichia coli* include harmless microorganisms, but also unfavorable under normal circumstances. These bacteria can be pathogenic with a moderate level of danger and rapid spread (Fardiaz, 1992).

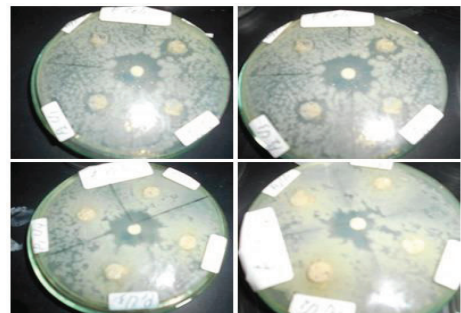


Figure 1. Inhibition of culled laying hens salami against *Escherichia coli* bacteria

Table 2 showed that the higher percentage of yeast with the addition of 2% lactic acid bacteria results in a significantly increased and significantly different inhibitory of *Staphylococcus aureus* ($P < 0.05$). This means that the starter yeast 4% is the best treatment in inhibiting the growth of *Staphylococcus aureus* bacteria in culled laying hens salami. Clarified again with the observations shown in Figure 2 that the higher percentage of yeast starter shows clear zone as the percentage of yeast is T0 (0.23 mm), T1 (1.60 mm), T2 (1.98 mm), and T3 (1.6 mm) and T4 (2.33 mm). Means yeast in inhibiting the growth of different bacteria *Staphylococcus aureus* for each treatment so it can be concluded that yeast can inhibit the growth of *Staphylococcus aureus* bacteria, especially at 4% treatment (T4) obtained the highest is 2.33 mm. Differences zone resistor for each treatment due to the difference of yeast starter; this is because the higher starter yeast the higher the content of active substances in it. Thus the formation of a strong inhibitory zone can be due to the work of active substances as antimicrobials contained in culled laying hens salami.

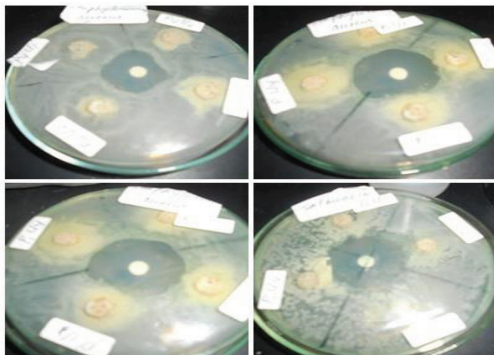


Figure 2. Inhibition of culled laying hens salami against *Staphylococcus aureus* bacteria

Based on the measurement of the inhibition zone diameter, the clear zone is visible. Means of salami with starter yeast has an inhibitory effect on the growth of *Staphylococcus aureus* bacteria and occurs in all treatments.

The properties of yeast antimicrobials such as organic acids (hexanoate, octanoate, and decanoate) and proteins are known to inhibit the growth of bacteria and molds (Roostita, 2004). Bilinski et al. (1985) reported several

types of yeast such as *Kluyveromyces thermotolerans* and *Kloeckera apiculata* showed activity in inhibiting the growth of *Lactobacillus plantarum*. The results of Arkoudelos et al. (1998) showed inhibition of pathogenic *S. aureus* bacteria in fermented meat products by starter *Lactobacillus plantarum*. Other antimicrobial compounds that can inhibit *S. aureus* growth are hydrogen peroxide as a result of the action of lactic acid bacteria (Leroy and Vuyst, 1999).

Similarly, with *Salmonella* spp bacteria (Table 2), the higher percentage of yeast in laying chicken salami was higher causing inhibitory effect on *Salmonella* bacteria growth and relatively stable although statistically not significantly different ($P > 0.05$). T2 showed that the largest *Salmonella* bacterial inhibition (0.80 mm) increased from 0.25 mm for T1 treatment, then decreased again in treatment of T3 and T4 to 0.40 mm. The high drag in T2 treatment is caused by 2% yeast starter able to inhibit *Salmonella* bacteria growth optimally so that it has strong resistor power; whereas in the treatment of T3 and T4 the inhibitory power to the growth of *Salmonella* bacteria decreased or weakened. Optimum point of *Salmonella* bacteria in culled laying chicken salami obtained at percentage of starter yeast 2% (P2). Means 2% yeast starter is an optimal point in inhibiting *Salmonella* bacteria as shown in Figure 3.

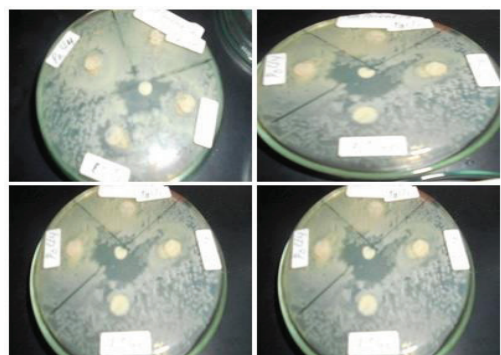


Figure 3. Inhibition of culled laying hens salami against *Salmonella* sp.

Figure 3 showed that the inhibition zone activity of laying hens salami on growth of *Salmonella* spp bacteria create a clear zone around the disc. Evidence that a test material

has antibacterial activity is indicated by the formation of a clear zone or zone of inhibition around the disc. The result of antibacterial activity of culled laying hens salami on growth of *Salmonella* sp. bacteria was obtained from each treatment of T0, T1, T2, T3 and T4 on the percentage of yeast 0%, 1%, 2%, 3% and 4%. Each treatment formed an inhibitory zone with an average diameter of 0.45 mm, 0.25 mm, 0.8 mm, 0.40 mm and 0.40 mm, although not statistically significant ($P > 0.05$).

It can be concluded that the yeast starter used in the study can produce bioactive components i.e antimicrobial compounds so useful as biopreservative agents. Lactic acid bacteria is a bacterium that produce lactic acid as a primary metabolite product and also produces other antibacterial substances such as hydrogen peroxide, diacetyl and antibacterial bacteriocin that can inhibit the growth of other bacteria (Tagg et al., 1976).

As it is known that some of the common pathogenic microbes contaminating meat are *Escherichia coli*, *Salmonella* and *Staphylococcus* sp. The microbial content of meat can from non hygienis animal farms and slaughter houses (Mukartini et al., 1995) as well as the long process of meat processing also allows the occurrence of microbial contamination in its processed products. Based on SNI 01-3820-1995, *Salmonella* contamination in meat sausage must be negative, and *S. aureus* up to 10^2 colonies /g.

CONCLUSIONS

Salami products (fermented sausages) are safe from *Escherichia coli* bacteria (<3 AMP / g), *Staphylococcus aureus* (<10 CFU / g), and *Salmonella* (negative). Means that salami products are feasible and safe for consumption because they are free from pathogenic bacteria and SNI 01-3820-1995 requirements for all treatments

The use of yeast and lactic acid bacteria may inhibit the growth of pathogenic bacteria such as *Escheriacia coli*, having inhibitory zone activity of each mean diameter T0 (0.275 mm); T1 (0.2 mm); T3 (0,275 mm); T3 (0.20) mm and T4 (0.275 mm); *Staphylococcus aureus* with clear zones grew in line with increasing percentage of yeast ie T0 (0.23 mm), T1 (1.60

mm), T2 (1.98 mm), and T3 (1.6 mm) and T4 (2, 33 mm) and *Salmonella* formed inhibition zone with mean diameter T0 (0.45 mm), T1 (0.25 mm), T2 (0.8 mm), T3 (0.40 mm) and T4 (0.40 mm).

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