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THE LACTIC ACID BACTERIA AND YEAST EFFECT ON TOTAL BACTERIA, ACIDITY DEGREE AND WATER ACTIVITY OF CULLED LAYING HENS SALAMI

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Abstract

The research has been done in the Laboratory of Products Processing Technology - Faculty of Animal Husbandry - Universitas Padjadjaran, Bandung. The research was conducted using culled Isa Brown laying hens, obtained from Private laying hens farm, Sumedang, West Java- Indonesia. The experiment method using Completely Randomized Design (CRD) with 5 treatments and 4 replicates i.e. P0 = 2% Lactic Acid Bacteria (LAB) + 0% yeast, and without spices; P1 = 2% LAB and 1% yeast; P2 = 2% LAB and yeast 2%, P3 = 2% LAB and 3% yeast; P4 = 2 % LAB and 4% yeast, followed by Duncan's Multiple Range Test. Results indicated that salami bacteria total, were significant difference decreased when the yeast percentage as starter increased : P0 (5.08 x 10^4cfu/g); P1 (5.06 x 10^4cfu/g); P2 (4,97 x 10^4cfu/g) ; P3 (4.93 x 10^4cfu/g) and P4 (4.91 x 10^4cfu/g). The salami average pH, on P0 = 5.39; P1 = 4.54; P2 = 4.37; P3 = 4,3 and P4 = 4.24, also decreased as the yeast increase. At last, the mean of salami water activity, on P0 = 0.986; P1 = 0.966; P2 = 0.959; R3 = 0.946 ; and P4 = 0.953. In general, the P4 treatment give the best culled laying hens salami.

Key words**:** *lactic acid bacteria, pH, total bacteria, salami, water activity, yeast*

INTRODUCTION

Today, the needs for food around the world was raised. Chicken meat is one of food source of animal protein for humans. Every year was reported that the needs of chicken meat in Indonesia also continues to increase, as the most widely traded commodity, so the demand of food safety of its products need to be improved. At the other side, the chicken is a perishable food, easily damaged due to the high nutritional content (Simmons, 2014) [13].

In Indonesia, producing traditional foods and fermented by yeast not yet entrenched in using bacteria and fungi such as *Rhizopus, Aspergillus spp, Penicillium spp, Mucor spp,* and others, compared to lactic acid bacteria (LAB) as starter, which is usually used as a probiotic. Utilization of yeast is rarely small, especially on a few types such as *Saccharomyces cerevisiae, Kluyveromyces lactis / Kluyveromyces kefyr* and *Zygosaccharomyces spp*., for making bread, wine, soy sauce, salted vegetable, and some traditional food e.g. tape and brem. In some European countries the use of yeast in fermented products, especially milk, and other products are common. The existence of yeast in food is very difficult to avoid, but should be considered, given the yeast capable contributing positively to shelf-life of the fermentation products. Positive role in the fermentation products is very diverse, among others, the fermentation process in sausage (salami).

Salami or fermented sausage is a processed meat product, mixed meat with fat through a process of fermentation using lactic acid bacterial culture, that able to convert the carbohydrates into lactic acid. Salami is meat product that easily damaged (perishable food), so it need special handling to extend the keeping of the product, e.g. preservation. Preservation usually used chemicals, but it was toxic and carcinogenic, which harmful to human health. Now, using biological material as preservative material (bio-preservative),

using the microorganisms activity or their metabolites result as an antimicrobial agent. Culture of microorganisms could be lengthen the shelf life of meat products as a result of acid formation Heinz and Hautzinger (2007) [5].

Fresh meat, generally contaminated with large amounts of bacteria including pathogenic bacteria, such as *Salmonella sp, Escherichia coli sp, Staphylococcus aureus* and other bacterial pathogens. Fresh meat can also be contaminated by the extent of yeast population and also the fermented meat. Soon after slaughtered, the pH of meat, ranges from 6.8 to 7.2, and then decline because of lactic acid accumulation in muscle tissue as a result of anaerobic glycolysis process. Then the pH increase, because of microorganisms growth. The pH of chicken meat will reach 5.8 to 5.9 after post-mortem, between 2 - 4.5 hours, but in ambient temperature, or at high temperatures, the pH will drop quickly, or by the physical condition of muscle tissue (Muchtadi and Sugiyono, 1992) [10].

Culled hens meat generally have a tough and hard, and smells difference than the smell of broilers or fresh chicken meat. In addition, the lack of consumer interest in culled hens, because the process has not been accustomed in a variety of variations to be preferred by consumers. Therefore, culled hens meat, must processed in other style, into salami or sausages. However it is necessary to study the changes that should appeared are safe for consumption, by total bacteria counts, acidity (pH) and water activity (a_w) of the product. From the processing point of view, meat with pH 5.6-6.0 is better for products where good water binding is required, looks like sausages, as meat5 with higher pH has a higher binding capacity (Heinz, and Hautzinger, 2007) [5].

Science and technology must spearhead agricultural production in the next 30 years, as a pace faster than the green revolution did during the past three decades. This is an unacceptable situation today and will require a new approach to food production to avert an even worse scenario in the coming decades (Simmons, 2014) [14].

MATERIALS AND METHODS

Raw Materials and Sausage Preparation

The Isa Brown culled layer hens, were obtained from the Private laying hens farm, Tanjung Sari Sumedang, West Java-Indonesia. The meat and chicken fat was kept overnight in refrigerator and the meat was manually deboned. The other materials, spices, salt, sugar, garlic, ginger, pepper, nutmeg, cornstarch, powdered milk were purchased from a local wholesaler also in Tanjung Sari Sumedang, West Java-Indonesia. *Trichosporon beigilii* (yeast) used as starter, and lactic acid bacteria, was isolated from culled laying hens meat.

Formulations Methods

Five formulations of culled layer hens were prepared, were repeated five times in a completely randomized design. The sausage dough, the meat : fat is 80: 20 was mixed after frozen for 24 hours. After ground and mixed carefully with food processor, then added with spices, salt, sugar, garlic, ginger, pepper, nutmeg. The sausage dough was added with 2% lactic acid bacteria, then separate into five parts according the treatments (P0, P1, P2, P3 and P4). Each treatment was also added with yeast (*Trichosporon belgilii*, that was isolated from meat of culled hens) in accordance with the treatment that is 0% (P1), 1% (P2), 2% (P3), 3% (P4) and 4% (P5). The dough was added with cornstarch, powdered milk and chicken fat then mixed carefully and stuffed into the casing and tied up. The pH, a_w, moisture content (physico-chemical), and total bacteria (microbiological). The resultant mixture was filled in casings, and then hung on a rack and allowed for 24 hours at room temperature and keep for six days so the fermentation allowed and interspersed with the process of curing for one hour per day. The temperature during curing is maintained at 27 - 30°C in the fumigation chamber using coconut shells, when the temperature exceeds more than 30°C, the room was spray with ice water.

The tools, are: Philips HR 7620 Food Processor, for grind, and mixed the sausage dough, minimum and maximum thermometer to measure the room temperature, stuffer

filler, Nalo Faser casing size of Lange Calibre 45 60.0 Menge 25 Germany and O'haus storage scale. Research has been conducted in the Laboratory of Animal Products and Processing Technology Research and Testing Laboratory at Faculty of Animal Husbandry Universitas Padjadjaran, Bandung between September 2015 to December 2015.

Physico-chemical analysis

The physico-chemical analysis (pH, a_w), were determined according to AOAC standard procedures (1990), was performed in duplicate. For the determination of pH, 10 g of each sample was homogenized with distilled water in the ratio of 1:10. The homogenate was subjected to a pH test using pH-meter. The pH value was determined on production days by taking the average of two readings. Water Activity System apparatus was used to measure the water activity (a_w). The a_w values were determined in duplicate in order to optimize the weights of samples at 25^0C until equilibrium was reached.

Microbiological analysis

To evaluate the microbiological characteristics, aliquots of 25g were collected, homogenized with 225 mL of 0.1% peptone water, and serially diluted on a decimal scale. The microbiological analyses were performed in duplicate. The data was transformed into alogarithm of the number of colony-forming units ($cfug^{-1}$). Total Plate Count (TPC) for determination of the number of viable microorganisms in the sample (Marturin and Peeler, 2001) [9] .

Statistical analysis

The experiment, used a completely randomized design and was repeated five times. All analyses were performed in duplicate, and the data was evaluated through an analysis of variance (ANOVA). The means were compared by Tukey's test at a confidence level of 5% ($p \leq 0,05$) .

RESULTS AND DISCUSSIONS

Effect of Treatment on Sausage Total Bacteria. The results of the analysis of the treatment effect using starter yeast and lactic acid bacteria on sausage bacteria total are presented in Table 1.

Table 1. Culled laying hens Salami total bacteria with various treatments

Replication	Treatments				
	P0	P1	P2	P3	P4
	….…… X 10^4 colonies/gram …….				
1	12.97	11.12	9.13	6.73	7.47
2	12.62	11.23	9.12	8.67	7.72
3	11.23	10.87	9.57	9.51	8.78
4	11.92	12.27	9.35	8.92	8.54
Total	48.74	44.49	37.17	33.83	32.51
Average	12.19	11.12	9.29	8.46	8.13

Table 1 showed that the total bacteria were decrease as the percentage of yeast as starter in culled laying hens salami.

The total bacteria between 8.13 x 10^4 cfu/g to 12.19 x 10^4 cfu/g.

The lowest total bacteria was obtained in treatment using 4% yeast (P4) 8.13 x 10^4 cfu/g; and the highest was obtained in R0 treatment (0% yeast) is 12.19 x 10^4cfu/g.

The data from Table 2 showed that the total bacteria of salami decreased, as the percentage of starter yeast increased.

Analysis of variance showed that the starter yeast used in chicken salami has significant effect ($P \leq 0.05$) to total bacteria.

Duncan's Multiple Range Test was conducted to determine the influence of different yeast percentage, in salami total bacteria, as seen in Table 3.

Based on the data in Table 3, it appears that the *lactic acid bacteria* and *yeast* as salami starter in treatment (P4), has the same effect with the treatment (P3) starter yeast 3%, while treatment (P3), 3% starter yeast was not significantly different with the treatment (P2), 2% yeast starter.

Table 2. Total bacteria transformation logarithm of culled laying hens salami with various treatments

Replication	Treatment				
	P0	P1	P2	P3	P4
	……X 10^4 colonies/gram ……..				
1	5.11	5.05	4.96	4.83	4.87
2	5.10	5.05	4.96	4.94	4.89
3	5.04	5.04	4.98	4.98	4.94
4	5.08	5.09	4.97	4.95	4.93
Total	20.34	20.23	19.87	19.70	19.63
Average	5.08	5.06	4.97	4.93	4.91

Table 3. Duncan's Multiple Range Test Results Effect of Treatment on Total Bacteria Salami

Treatments	Total Bacteria	Significancy $\alpha_{0,05}$
	(x 10^4 colonies / gram)	
P0	5.08	a
P1	5.06	a
P2	4.97	b
P3	4.93	bc
P4	4.91	c

Treatment P0, with 0% starter yeast, and the treatment P1, with 1% yeast starter did not significant differences ($P \geq 0.05$). It shows, that the increase of yeast starter since 3% gave significant effect on the total bacteria of culled laying hens salami, but the salami under 2% yeast as starter, has no significant differences. Total plate count in native chicken sausages using some oil and fats, between 5.25 $\pm$ 0.01 with corn oil to 5.37 $\pm$ 0.02 with beef fat, (Lengkey, et al., 2016) [6] means that this results (using culling hens) is good choice as meat sources.

The use of lactic acid bacteria and yeast as a starter to culled hens salami fermentation is very advantageous, because it is an anti-synergic to pathogenic microbes, in line with Lindren and Dobrogosz, (1990) [7]. Ray (2004) [11] reported that the anti-synergic growth with two or more microorganisms in food, could have effects on the growth of microorganism, also will intervene the growth of one or more types of microorganisms; sometimes will kill the microorganism. Further said, that the growth of anti-synergic could be found on some strain of microbes, including the bacteria with yeasts, molds and yeasts or fungi and bacteria.

Roostita *et al*., (2013) [13] reported that the addition of 2% crude yeast extract as bio-preservative in sausage products; is proven to produce the lowest of total bacterial population (1.7 x 10^3) that affect the storability at room temperature, which reached six days and also at refrigeration temperatures up to 60 days. Arief, *et al*., (2008) [2] said that the average of total lactic acid bacteria in beef and mutton fermented sausage are 1.93 x 10^{12}cfu/g and 5.73 x 10^{10}cfu/g respectively, by using dried culture of *Lactobacillus plantarum* 1B1.

Effect of the Treatment on Salami pH

The analysis result of the treatment, using yeast and lactic acid bacteria as starter on the pH of salami sausage, is presented in Table 4.

Table 4. The pH of culled hens salami with various treatments

Replication	Treatment				
	P0	P1	P2	P3	P4
	…… X 10^4 colonies/gram ……..				
1	5.59	4.30	4.29	4.29	4.21
2	5.33	4.26	4.37	4.32	4.24
3	5.15	5.26	4.39	4.35	4.13
4	5.49	4.35	4.42	4.22	4.38
Total	21.56	18.17	17.47	17.18	16.96
Average	5.39	4.54	4.37	4.30	4.24

Based on Table 4, when the yeast as the culled layer hens starter, increase than the pH will decrease. The average of pH of culled layer hens salami that using yeast and lactic acid bacteria as starter, ranged from 5.39 to 4.24.

The results of variance analysis showed that using yeast and lactic acid bacteria as starter, will provide significantly different effect ($P \leq 0.05$) on the salami pH. The results of analysis of variance showed that the starter yeast used in culled layer hens salami, has significant effect ($P \leq 0.05$) on the sausage pH. By Duncan's Multiple Range Test, to determine the effect between different percentages of salami yeast on the pH of culled layer hens, was gave in Table 5.

Table 5. Duncan's Multiple Range Test, the Effect of treatment on the Salami culled layer hens pH

Treatment	PH	Significant $\alpha_{0,05}$
P0	5.39	a
P1	4.54	ab
P2	4.37	ab
P3	4.30	ab
P4	4.18	b

The final pH value of culled layer hens salami, in this study is more acidity (4.18 to 5.39) than the final pH of usual salami, mostly European – style (4.8 to 5.0) (Lucke, 1997) [8], but for some types of sausages such as summer sausages, German sausages, Bologna and cervelat the pH between 4.4 to 5, still acceptable (Rose, 1982) [12]. A decrease in pH, resulting from the addition of acid in foodstuffs or meat, will give a distinct advantage. Foods with low pH is more likely to be stable against microbial damage compared with a neutral pH (Frazier and Westhoff, 1998) [4]. Meat that has high pH, generally is very good for bacteria growth (Aberle, *et al.*, 2001) [1].

Effect of treatment on water activity (a_w)

The effect of treatment on salami water activity, using lactic acid bacteria and yeast as starter is presented in Table 6.

Table 6. The Effect of Treatment on Culled Layer Hens Salami Water activity (a_w)

Replication	**Treatment**				
	P0	P1	P2	P3	P4
1	0.995	0.955	0.954	0.882	0.948
2	0.977	0.962	0.946	0.988	0.966
3	0.971	0.978	0.979	0.956	0.952
4	0.989	0.968	0.958	0.959	0.947
Total	3.942	3.863	3.837	3.785	3.813
Average	0.986	0.966	0.959	0.946	0.953

The data on Table 6 showed as the percentage yeast in the treatment of culled layer hens salami increased, the water activity (a_w) tends to decrease. Analysis of variance showed that the treatment using of lactic acid bacteria and yeast as starters on culled laying hens salami showed no significant differences ($P \geq 0.05$) on water activity.

Water activity (a_w) is the amount of free water that can be used by microbes for growth (Winarno, 1991; Syarief and Hamid, 1993) [17, 16]. Water activity is the amount of water in the material available for microbial growth. High water activities have an impact on the increasing of microorganisms number in foodstuffs (Syarief and Hamid, 1993) [16]. In this study, there is a tendency to decrease water activity as the yeast increased, but in R4 treatment (4% yeast), the water activity increased again to 0.953, although has no statistically significant differences ($P \geq 0.05$). The a_w value of fermented sausages ranged from 0.85 to 0.93 (Sopandi, 2014) [15].

CONCLUSIONS

The results indicated that the total bacteria of culled laying hens salami was significant difference decreased as the percentage of yeast as starter increase, P0 (5.08 x 10^4cfu / g), P1 (5.06 x 10^4cfu/g), P2 (4, 97 x 10^4cfu/g) P3 (4.93 x 10^4cfu/g) and P4 (4.91 x 10^4cfu/g).
The average pH of salami decreased as the percentage of yeast as starter increase (P0 = 5.39; P1 = 4.54; P2 = 4.37; P3 = 4.30 and P4 = 4.24). The decreasing of pH gave significantly different with culled layer hens salami using yeast and lactic acid bacteria as starter, for each treatment.
The mean of water activities (a_w) in each treatment decreased as the yeast and lactic acid bacteria as starter, increased; {P0 (0.986); P1 (0.966); P2 (0.959); R3 (0.946) and P4 (0.953)}, even from P3 treatment and also P4, the water activity was decreased, although has no significant differences statistically.

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