Lactic Acid Bacteria and Histamine Levels of Sie Balu After Gamma Irradiated.

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Abstract

The presence of lactic acid bacteria (LAB) and histamine in foodstuffs indicate the level of deterioration in the quality of food and cause poisoning. Sie Balu is the Acehnese dried meat preserved by the addition of salt, acid and dried, but the long processing and drying it under the sun can cause microbial contamination in meat products. Irradiation can eliminate bacteria in foodstuffs. This study aimed to determine the amount of LAB and histamine levels of Sie Balu after irradiation doses of 5, 7 and 9 kGy and stored 2 to 4 months. Sie Balu was made of fresh beef 5 kg, dried in the sun to dry, vacuumed and irradiated with gamma rays. The samples for LAB determination cultured in MRS agar and incubated at 37°C for 24 hours. The number of colonies was counted using Total Plate Count. The histamine level of Sie Balu conducted by ELISA. Irradiation did not significantly (P>0.05) affect the amount of LAB, but the shelf life significantly (P<0.05) affected the amount of LAB in Sie Balu. Extending the shelf life up to 4 months can increase the amount of LAB. Irradiation dose and shelf life had no effect on histamine levels of Sie Balu (P>0.05). This study concluded that irradiated Sie Balu cannot be stored for more than two months.

Keywords: Lactic acid bacteria, histamine, meat, Sie Balu, irradiation, shelf life.

Background

Sie Balu is one of the traditional food products that is processed and preserved in a conventional way. Sie Balu is dried meat from Aceh which is preserved with the addition of salt and acid as a flavour as well as to preserve. Then pressed with ballast and dried. Conventional meat preservation such as drying, salting, heating, freezing and fuming can be done to maintain or improve the quality and safety of food products. The method of preserving meat is used to control the activity of microorganisms that cause enzymatic activity and chemical reactions in meat (Nurliana et al., 2003). One of the compounds produced from enzymatic reactions and chemical changes due to the activity of microorganisms in meat is biogenic amines, including histamine (Keer et al., 2002; Tjay and Rahardja, 2007).

Histamine is formed because of an error during the handling and processing process. Histamine formation in meat depends on histidine concentration in tissues, number and type of bacteria containing histamine decarboxylase (HDC) enzymes, meat locations and environmental conditions (Lehanne and Olley, 2000; Barceloux, 2008). Histidine is an amino acid that can be decarboxylated by HDC produced by meat and bacteria, becoming histamine (Keer et al., 2002). High levels of histidine as histamine precursors in fish can increase the chances of high histamine formation.

Histamine levels in meat with various treatments need to be known, so that it is necessary to find a good and appropriate treatment method which makes histamine levels in processed meat products do not increase. One of the efforts includes applying non-thermal technology such as ionizing radiation to food, because it has several advantages including hygienic, safe, leaves no residue, is effective and efficient, and is able to maintain quality but the freshness of food products is maintained (Irawati, 2007).

The sterilization application by doing irradiation using high doses on several
ready-to-eat foods based on traditional Indonesian recipes has been good from microbiological aspects, physicochemical characteristics and sensory tests (Irawati, 2007). However, the effects of irradiation on traditional foods based on toxic compositions that may be irradiated are only slightly collected. According to Ham et al. (2009) yoghurt irradiated by Gamma rays at a dose of 10 kGy can reduce allergen substances that cannot be removed during the fermentation process so that the product becomes safer for consumption.

Meat processing requires an optimization effort to reduce and eliminate bacteria, especially groups of lactic acid bacteria and histamine levels to suit the desired goals. For this reason, knowledge about the effects of processing on food security is important. However, the more important thing is how to do food processing that produced food with a high nutritional value and safe.

Materials and Methods

This research was begin with the making of Sie Balu in one of the Sie Balu producers in Banda Aceh, irradiation at the Isotopes and Radiation Technology Application Center of National Nuclear Energy Agency Jakarta, LAB examination at the Veterinary Public Health Laboratory and Research Laboratory of Veterinary Medicine Faculty Unsyiah, Darussalam, Banda Aceh, which runs from February to June 2015.

The Making Process of Sie Balu

Sie Balu was made as follows: fresh beef 5 kg cut into pieces with a thickness of 2.5 cm. Next, the meat is washed and covered with 250 g of salt, 200 ml vinegar acid, 200 g of garlic, and 100 g of ginger which has been mashed. The meat was left for 30 minutes. Then put in a rice bag and pressed with 10 kg ballast for one night (± 12 hours). Then dried in the sun at 37 – 40 °C to dry for 8-10 days (Nurliana et al., 2003).

The Irradiation of Sie Balu

Samples of Sie Balu were packaged in vacuum packaging and then irradiated by gamma rays at the Isotopes and Radiation Technology Application Center of National Nuclear Energy Agency Jakarta in an irradiator with a source of 60Co at doses 5, 7 and 9 kGy.

The Examination of Lactic Acid Bacteria

Examination of lactic acid bacteria was carried out by entering a Sie Balu sample of 10 g into a solution of 90 ml of peptone with 0.1% sterile and dilution until 104 dilutions were then cultured on MRS agar media and incubated at 37 ° C for 24 hours. The lactic acid bacteria colonies that grew on the cup media were observed and calculated using the Total Plate Number (TPL) method.

The Examination of Sie Balu's Histamine Level

Examination of histamine levels was carried out by the ELISA method. The commercial ELISA kit used is ELISA His (histamine) kit. There were 50 samples added to each of the available ELISA plate 50 wells, 50 μL of biotinylation Ab detection in each well then incubated for 45 minutes at 37ºC. Then the samples were added with a buffer (350 μL) 3 times and 100 μL of the HRP conjugate (horseradish peroxidase) to each hole and then incubated at 37ºC for 30 minutes. Then wash 5 times and 90 μL of substrate reagent were added and incubated at 37 ºC for 15 minutes. Then 50 μL stop solution was added (the colour immediately turns yellow). Then the level of histamine was read using a microplate reader on 450 nm OD (Optical Density).

Data Analysis

The number of lactic acid bacteria and histamine levels were analyzed using ANAVA with the SPSS for Windows version 17.0 program and continued with the Duncan Multiple Range Test (DMRT) at a significance level of α 0.05.

Results and Discussion

The Amount of Lactic Acid Bacteria in Sie Balu

The total amount of LAB plates in Sie Balu before and after irradiation and also stored at 2 and 4 months is presented in
Table 1. The results show that LAB contaminated *Sie Balu* during and after the processing occurred. The amount of LAB in *Sie Balu* before irradiation is 5.01 log cfu / g. Meat is one medium that is very suitable for microbial growth including lactic acid bacteria. According to Pramono et al. (2008), Lactic acid bacteria is one of the natural microorganisms that can be found in meat. One of the LAB found in meat can be caused by poor handling during the slaughter, processing, distribution and marketing process.

Table 1. Average (± SD) number of total LAB (log cfu) plates in *Sie Balu*

| Irradiation Dose (kGy) | Storage Duration (Month) | 0         | 2             | 4             |
|------------------------|--------------------------|-----------|---------------|---------------|
| 0                      |                          | 5.01 ± 0.04a | 3.54 ± 0.51b | 4.85 ± 0.33b |
| 5                      |                          | 3.02 ± 1.05c | 4.67 ± 1.41b | 4.28 ± 0.85b |
| 7                      |                          | 2.56 ± 0.00c | 5.03 ± 0.73b | 4.07 ± 0.71b |
| 9                      |                          | 1.48 ± 0.67c | 4.01 ± 1.00b | 5.09 ± 0.62b |

The results of statistical analysis using Anava shows that irradiation did not affect (p > 0.05) the amount of LAB in *Sie Balu*. However, based on Table 1, irradiation can reduce the amount of LAB to 3.53 logs before being stored. The decrease in the amount of LAB without storage shows that irradiation is able to eliminate the LAB. This shows that the irradiation dose plays a role in reducing the amount of LAB. The results of this study are in line with the research of Yazdi and Jouki (2012) which showed that LAB decreased in doses 1 and 3 kGy with a shelf life of 21 days. Dickson (2001) explained that the irradiation process can damage DNA so that bacteria are unable to adapt and reproduce in a short time. The irradiation dose of 3-7 kGy is used to prevent foodborne diseases by damaging pathogenic bacteria in fresh and frozen food (EFSA, 2011).

The increase of irradiation doses can reduce the amount of LAB in food. This is caused by the ability of irradiation rays to penetrate the LAB cell wall. One of the roles of irradiation in food preservation processes is to permanently destroy and damage the organism's DNA. According to research by Kundu et al. (2014) Irradiation causes bacterial cells to be injured or die due to the destruction of important macromolecules such as DNA, RNA and protein.

The increase in irradiation dose did not show a difference (p > 0.05) in the amount of LAB in *Sie Balu*. Microorganisms will be more resistant when irradiated in dry conditions because the formation of free radicals from water that occurs during the irradiation process is quite low or even nonexistent. Therefore indirect effects on microbial cell DNA are low and even nonexistent (Dickson, 2001). The effectiveness of irradiation on decreasing the number of microbes is influenced by food conditions and initial contamination (EFSA, 2011).

The results of statistical analysis using Anava shows that the shelf life of *Sie Balu* had a significant effect on the amount of LAB (P < 0.05). The amount of LAB in *Sie Balu* in a vacuum that is not irradiated has decreased after a shelf life of 2 months at room temperature. LAB growth is inhibited due to low water and nutrient levels in *Sie Balu*. However, an increase in the amount of LAB occurs after a shelf life of 2-4 months due to adaptability even under anaerobic conditions. This adaptation process is caused by the existences of nutrients and the conditions that are suitable for the growth of LAB such as temperature, water content, oxygen and pH (Levin, 2010).

The total plate number of LABs in different varieties after storage of 2-4 months did not show a significant difference (p > 0.05). But ALT of LAB is significantly different between a shelf life of 0-2 months with a shelf life of 2-4 months. Based on Table 1, the shelf life period is below 2 months better than the shelf life of more than 2 months. The condition of 0-month shelf life with various irradiation doses indicates a decrease in the number of LABs. The growth of lactic acid bacteria in irradiated samples does not exceed 107 cfu / g and this is in accordance with the limits set by ICMSF (1986) as the total number of microbes in meat.
According to Corapci and Kaba (2011), the use of gamma irradiation is quite effective in reducing the number of pathogenic bacteria and extending the shelf life of the product, but some pathogenic bacteria have the ability to survive and multiply after post-radiation. Therefore, it is necessary to combine gamma irradiation with other preservation techniques as a technology to provide optimal results. One of the preservation techniques that can be used is frozen storage (Genc and Diler, 2013).

In addition to the frozen storage modification, it is also supported by modified atmosphere packaging (MAP). In the MAP application, a specific atmosphere is generated by injecting the desired initial gas mixture into the package. Changes in the atmosphere can affect the microflora of products that aim to extend the shelf life. The common gas used in MAP is carbon dioxide (to inhibit bacterial growth), oxygen (to suppress aerobic growth, to prevent anaerobic growth and to maintain flesh colour) and nitrogen (to avoid fat oxidation and packaging damage). These gases can be applied individually or in combination to achieve optimal effects (Chouliara et al., 2008).

Irradiation of food processed based on Good Manufacturing Practices (GMP) can improve food security and reduce foodborne diseases, extend the shelf life of perishable products, and reduce the risk of contamination after packaging product processing. Sterile foods with high-dose irradiation can kill all microorganisms (Zhu et al., 2012).

**Histamine Levels in Sie Balu**

The average histamine levels in *Sie Balu* irradiated with various doses can be seen in Table 2. The results show that there is a decrease in histamine levels after the irradiation process. The decrease in histamine levels in *Sie Balu* correlates with the reduction of histamine decarboxylase enzyme-forming microbes. Histamine is an amino biogenic compound which results from decarboxylation of histidine amino acids and histamine-forming microbial activity found in meat and can provide poisoning or allergic effects (Lehana and Olley, 2000; Mc Lauchlin et al., 2005). The presence of large amounts of histamine can cause decay, poisoning, and even death (Paleologos et al., 2004; Kung et al., 2005; Jiang et al., 2007).

| Irradiation Dosage (kGy) | Storage Duration (Month) | Average Histamine (± SD) (ppm) in *Sie Balu* |
|-------------------------|---------------------------|-----------------------------------------------|
| 0                      | 0                         | 12.36 ± 16.16 x 10^3 1.69 ± 1.28 x 10^3 9.29 ± 1.08 x 10^3 |
| 0.5                    | 0                         | 8.36 ± 4.71 ± 0.82 x 10^3 8.05 ± 1.11 x 10^3 |
| 1                      | 0                         | 12.18 ± 2.34 ± 4.40 x 10^3 8.05 ± 1.11 x 10^3 |
| 1.5                    | 0                         | 0.87 ± 0.93 ± 1.55 x 10^3 7.37 ± 3.69 x 10^3 |
| 2                      | 0                         | 0.67 ± 2.48 x 10^3 6.23 ± 3.69 x 10^3 |

Based on the results of statistical analysis using Anava shows that the dosage and shelf life did not have a significant effect (p>0.05) on histamine levels. The results of the analysis of histamine levels in *Sie Balu* with a shelf life of 0, 2, and 4 months are still below the maximum limit of histamine level of 100 ppm, so it is still safe and feasible for consumption (BSN 2006).

This study showed that irradiation was able to reduce histamine levels in food products and in line with the research of Kim et al. (2002) that irradiation was effective in reducing histamine in soybean paste. Kanatt et al. (2005) stated that irradiation 1, 2 and 3 kGy significantly reduced the number of bacteria. Decreasing the number of living bacteria will also affect the histamine levels produced by bacteria. Allegedly the formation of histamine in *Sie Balu* is related to the number of bacteria found in the *Sie Balu* and histamine-producing decarboxylase enzyme activity. In particular, Min et al. (2007) concluded that irradiation effectively reduces histamine in beef and pork. This is consistent with the statement of McLauchlin et al. (2005) the results of the decomposition of proteins will be used by bacteria to grow, including bacteria that produce histidine decarboxylase enzymes. Histidine decarboxylase producing bacteria can grow by using histidine amino acids and turning them into histamine.
The results of this study showed that histamine levels of Sie Balu without irradiation and with irradiation at 7 and 9 kGy varied with the length of storage. On the contrary, histamine levels of Sie Balu with irradiation doses of 5 kGy were lower with the length of storage (Table 2). Histamine is formed from histidine during decay by bacteria that have histidine decarboxylase enzymes (Taylor, 1983). It is certain that histamine levels are formed in the presence of histidine decarboxylase enzymes. According to Allen (2004), histamine production does not always correlate with the number of histamine-producing bacteria, because the response and ability of bacteria to produce histamine vary. Furthermore, Kim et al. (2004); Tsai et al. (2007) explained that bacterial activity and histamine formation were influenced by temperature and incubation time. Each species has a different optimum temperature and time. Histamine-forming bacteria can be grouped into species capable of producing large amounts of histamine (> 100 ppm) at temperatures above 15 °C with incubation times <24 hours and species that produce histamine in small amounts (<25 ppm) after incubation at 30 °C for > 48 hours.

Histamine formation is influenced by time, temperature, type of raw material and histamine forming bacteria in meat (McLauchlin et al., 2005). Alasalvar and Taylor (2002) state that histamine is generally formed at high temperatures (> 20 °C). Storage temperature is the most important factor contributing to the formation of biogenic amines. Reports of the optimum temperature and the lowest temperature limit for histamine formation is very various. The optimum temperature for histamine formation is 25 °C (Du et al., 2002; Keer et al., 2002; Kim et al., 2002; Rodtong et al., 2005). According to Fletcher et al. (1995), the formation of histamine at a temperature of 0-5 °C is very small and even negligible. Results of Price et al. (1991) also showed that histamine formation would be inhibited at 0 °C or lower. Therefore, the Food and Drug Administration (FDA) sets the critical temperature limit for histamine growth in fish bodies which is 4.4 °C (FDA 2001).

Histamine-producing bacteria isolated from fermented products are mostly from halophilic (high salt-resistant) lactic acid bacteria such as Staphylococcus epidermidis, S. captitis and Tetragenococcus muriaticus (Gildberg and Thongthai, 2001; Kimura et a. L, (2001). Tuna meat fermentation stored at room temperature can produce histamine compounds more dominated by lactic acid bacteria, especially Pediococcus acidilactid (Ishizuka et al., 1993; Leuschner at al., 1998).

The variation of histamine amount in Sie Balu is also influenced by the condition of the sample during the processing. The meat is divided into thinner parts. After being dried, it cut into small sizes. This process makes the surface of the sample wider. According to Naidoo and Lindsay (2010) contamination of the surface of dried meat is higher than the inside because it is more quickly exposed to the surrounding environment. Sie Balu is made traditionally so it cannot be ascertained that the initial contamination is high and low. Irradiation is able to eliminate bacteria in Sie Balu. Irradiation with gamma rays has a high penetration power in solid materials (Dwiloka, 2002). Sie Balu with an average thickness of 1-2 cm can be penetrated by this ray. So that it affects the inner contaminants. Extensive and irregular meat surfaces require high radiation energy and high penetration to ensure uniformity of treatment (Arthur et al., 2005).

**Conclusion**

The results of this study are various irradiation doses can reduce the amount of LAB which contaminates Sie Balu. While the long shelf life can increase the amount of LAB even in the food that has been dried and irradiated. LAB can adapt and improve itself at room temperature even in anaerobic conditions.

The results of the analysis of the histamine level of Sie Balu is it has no irradiation effect and shelf life. Irradiation doses 5, 7 and 9 kGy can reduce the histamine level of Sie Balu without storage. Overall histamine levels in Sie Balu do not exceed 100 ppm the prescribed limit and are still suitable for consumption.
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