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Int. J. Trop. Vet. Biomed. Res.
Vol. 2 (1) : 1-7; May 2017

www.jurnal.unsyiah.ac.id/IJTVBR
E-ISSN : 2503-4715

The Presence of *Salmonella* in *Sie Balu*, Acehnese Dried Meat After Gamma Irradiation

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Abstract

Sie balu is an Acehnese dried meat preserved by the addition of salt, acid and dried. However, long processing and drying it under the sun can cause meat products contaminated by *Salmonella*. Irradiation can eliminate *Salmonella* and other pathogenic bacteria in food. This study aims to determine the presence of *Salmonella* in *sie balu* after irradiated with increasing doses and 2 to 4 months shelf life. *Sie balu* was made of 5 kg fresh beef, dried under the sun to dry, vacuum and irradiated at doses of 5, 7 and 9 kGy. *Salmonella* was detected using bacteriological and biochemical tests. Results showed *sie balu* contaminated by *Salmonella* paratyphi B and *Salmonella cholerasuis*. The irradiation and shelf life significantly affect ($P<0.05$) the count of *Salmonella* in *sie balu* compared with unirradiated one. Irradiation doses of 5, 7 and 9 kGy do not affect the count of *Salmonella* in *sie balu*. Extending the shelf life up to 4 months can increas the count of *Salmonella*. This study concluded that irradiated *sie balu* at doses of 5, 7 and 9 kGy can be stored for up to two months

Key words : Meat Preservation, Salmonella, Sie Balu, Irradiation, Shelf Life.

Background

Meat is a food of animal origin, nutritious so that it becomes an excellent medium for the growth and proliferation of microorganism. Microorganism cause damage and degrade the quality of the meat and cause disease in humans (foodborne diseases). Raw meat and poultry is a major source of transmission of *Salmonella* in humans in which 40% of cases as a result of consuming meat products, eggs and poultry meat that not properly cooked or handled (Jamshidi *et al.*, 2009). Typhoid fever cases in the world occurred 16 million a year, 1.3 billion gastroenteritis cases and 3 million deaths are caused by *Salmonella.* The economic losses are also a consideration for the investigation, treatment and prevention of salmonellosis in humans (Kemal, 2014).

Many efforts to improve the meat quality is done through the better processing or handling to reduce the damage during storage. Meat preservation is an attempt to control the activities of microorganisms that caused enzymatic activity and chemical reactions in meat (Nurliana *et al.*, 2003). This process is also aimed to maintaining and improving the quality of food products or for food safety for consumers (Andini and Harsojo, 2010). One of the traditional food is *sie balu*, the Acehnese dried meat preserved by the addition of salt and acid, which provide the flavor as well. Further pressed with ballast and dried. The Acehnese people have been doing this since decades ago. *Sie balu* with the addition of vinegar, garlic and ginger can reduce the count of bacteria after preservation for 216 hours (Nurliana *et al.*, 2003). However, long processing and drying under direct sun caused the meat contamination, especially by *Salmonella*. Sources of contaminants can originate from the water, equipment, dust, wind, rain, insects and birds. Naidoo and Lindsay (2010) describes that had happened salmonellosis case caused of eating *biltong* (traditional dried meat of South Africa). Therefore we need the technology that can eliminate the microorganisms so that *sie balu* product will be safe to eat and can be stored longer.

Irradiation technology can eliminate or reduce *Salmonella* and other pathogenic bacteria in meat and other foodstuffs (Sarjeant *et al.*, 2005; EFSA, 2011). Irradiation at a dose of 2-7 kGy, can

eliminate pathogenic bacteria from food products, especially *Salmonella, Staphylococcus aureus, Campylobacter* and *Listeria monocytogenes* depending on irradiation conditions, the initial contamination and the type of food (Spoto *et al.*, 2000). Sarjeant *et al.* (2005) reported that low-dose irradiation (1-3 kGy) effectively reduced *Salmonella enterica* serovar Typhimurium and spoilage organisms from raw chicken meat. Therefore to know the effects of increasing doses of gamma irradiation and shelf life to the presence of *Salmonella* in *sie balu,* some researches need to be done.

Materials and Methods

Research design

Each experiment was performed at three separate times with four samples analyzed in each triplicate. Experimentally in this study was to calculate the count of *Salmonella* in *sie balu* before and after irradiation with a shelf life of 2 to 4 months.

The process of making *sie balu*

Sie balu was made of 5 kg fresh beef cut into pieces with a thickness of 2.5 cm. Furthermore, the meat was washed and covered with 250 g salt, 200 ml vinegar, garlic 200 g and 100 g ginger that had been smoothed. The meat left on for 30 minutes, then put it into *umpang* and pressed with a weight of 10 kg for one night (± 12 hours). The meat dried under the sun to dry (Nurliana *et al*., 2003).

Irradiation of *sie balu*

Sie balu samples packaged in vacuum packs subsequently irradiated gamma rays at the Center for Isotope and Radiation Technology Applications at National Nuclear Energy Agency, Jakarta in irradiators with ^{60}Co source at doses of 5, 7 and 9 kGy.

Detection and Identification of *Salmonella*

A total of 25 gram of *sie balu* was sliced and introduced into 225 ml of Selenite Cysteine broth then stomachered and incubated at 37°C for 2 hours. Further dilution of up to 10^4 and cultured in Deoxycholate CM 0163 Agar then incubated at 37°C for 24 hours. The count of colonies of *Salmonella* (gray-black) was calculated using the Total Plate Count. The separated colony continued with biochemical test for identification of the species consists of Indol, Methil Red, SIM, Simmons Citrate and sugar: Triple Sugar Iron Agar (TSIA), mannitol, lactose, sucrose, glucose and maltose (SNI 2897-2008).

Data analysis

Analisys of variance was performed using SPSS for Windows version 17.0. A post-hoc Duncan test was used to obtain comparisons among treatments. *Salmonella* counts were converted to log cfu/gr.

Results and Discussion

Irradiation

Effect of irradiation and shelf life on the counts of *Salmonella* in *sie balu* before and after irradiation shown in Table. *Sie balu* was contaminated by *Salmonella* Paratyphi B and *Salmonella cholerasuis* during processing. The sample of fresh meat for the *sie balu* processing had been tested and did not found *Salmonella*. *Sie balu* contaminated by *Salmonella* in large counts (6.4 log cfu / g) during processing. This is consistent with research Ingham *et al*., (2006) and Naidoo and Lindsay, (2010) that the meat products may be contaminated with *Salmonella* during processing. The count of *Salmonella* had very significant reduction in newly irradiated *sie balu* (negative).

Table. Means (±SD) Total Plate Count of *Salmonella* in *sie balu* (log cfu/g).

Shelf life (month)	Irradiation Dose (kGy)				Means
	0	5	7	9	
0	6,40± 0,00^{a,x}	0^{a,y}	0^{a,y}	0^{a,y}	1,60 ± 3,20
2	2,65± 0,64^{ab,x}	1,98 ±0,28^{ab,y}	2,01 ±0, 87^{ab,y}	1.97 ±0,81^{ab,y}	2,15 ± 0,33
4	2,73 ± 1,24^{b,x}	2,17 ± 0,77^{b,y}	2,13 ±0, 71^{b,y}	2,27 ± 0,86^{b,y}	2,33 ± 0,28
Rata-rata	3,93 ± 2,14	1,38 ± 1,20	1,38 ± 1,20	1,41 ± 1,23	

Note: lowercase different superscripts in the same column (a,b) and the same row (x,y) showed significantly difference (P<0.05).

Anova test results showed that irradiation significantly affects (P<0,05) the count of *Salmonella* in *sie balu* compared with unirradiated one. Increased irradiation dose did not show a significant effect. The Duncan test results the values of all irradiation doses were in the same subset. This could be concluded that irradiation at doses of 5, 7 and 9 kGy could eliminate *Salmonella* in *sie balu*. It was caused by irradiating dried meat. Low water activity in irradiated foodstuffs caused the effect of irradiation on bacteria is reduced. This is in agreement with Dickson, (2001) that microorganisms are more resistant when irradiated in dry conditions due to the formation of free radicals from water that occurred during the irradiation process is quite low or even non-existent. Therefore, the indirect effects of the low microbial cell DNA did not even exist. The effectiveness of irradiation to decrease the number of microbes is affected by the condition of foodstuffs and early contamination (EFSA, 2011).

The varying count of *Salmonella* is influenced by the condition of the sample during processing strongly. Meat was divided into several sections that were thinner and after dried cut to a small size. This process makes the surface of the sample more widely. According to Naidoo and Lindsay (2010) contamination of the surface of the dried meat is higher than the inside because it is exposed to the environment faster. *Sie balu* traditionally made so it can not be ascertained high and low contamination early. Gamma irradiation has a high penetration power in a solid material (Dwiloka, 2002). *Sie balu* with an average thickness of 1-2 cm is able to be penetrated by this ray and therefore contributes to contaminants inside. Meat extensive surface and irregular requires high energy radiation and high penetration to ensure uniformity of treatment (Arthur *et al*., 2005).

The long processing in long time lead *sie balu* easily contaminated by *Salmonella*. Efforts to reduce microbes during processing *sie balu* conducted by additioning salt, acid and addition of spices such as garlic and ginger that containing anti-microbial compounds. The salt can reduce the moisture content in meat with the osmolarity of the cell so that water comes out of the meat and the spices to infuse into it. Reduction of water content in food products can reduce the count of microbes in addition of the salt gives bacteriostatic effect by means of autolysis and plasmolysis (Nurwantoro, 2012). Meat that has been seasoned pressed with ballast to accelerate the process of water discharge from meat so that it will speed up the drying process. Drying under direct sun causes *sie balu* exposed to contaminants from a variety of sources such as dust, wind, insects, rain and birds (Naidoo and Lindsay, 2010).

Sarjeant *et al.,* (2005) reported that irradiation 1-3 kGy effectively reduce *Salmonella enteritica* serovar Typhimurium and spoilage organisms from raw chicken meat. Irradiation 2-7 kGy was able to eliminate a variety of microbial pathogens such as *Salmonella, Staphylococcus aureus, Campylobacter* and *Listeria monocytogenes* in foodstuffs (Spoto *et al*., 2000; Natalia *et al*., 2009). The accurate irradiation dose could not be determined because of many factors influencing such as kinds of foodstuffs and initial contamination. This proved *Salmonella* in unirradiated *sie balu* as much as 6,4 log cfu/g. *Salmonella* even less negative in irradiated *sie balu* (after irradiation without storage). Jouki (2013) proved that *Salmonella* was not detected in turkey meat that irradiated at dose 4 kGy, while the count of mesophilic and coliform bacteria decreased by 5 log. Dickson (2001) describes the process of irradiation can damage DNA so that *Salmonella* can not adapt and breed in a short time. Irradiation 3-7 kGy is used to prevent foodborne diseseases by destroying pathogenic bacteria such as *Salmonella* in fresh and frozen food (EFSA, 2011). According to Sedeh *et al*., (2007) in Henriques *et al*. (2013) irradiation 3 kGy can eliminate *Salmonella* in beef.

A study conducted by Kundu *et al*., (2014) showed irradiation causes the bacterial cells killed or injured by the destruction of vital macromolecules such as DNA, RNA and protein. The effects of irradiation showed that *Salmonella* more resistant than *E. coli*. D_{10} value can be used

to see the radiation resistance. D_{10} value is the value of a decimal reduction of mikroorganisne irradiated. D_{10} value for *E. coli* O157: H7 is 0.241 - 0.307 kGy and 0.618 - 0.800 kGy for *Salmonella* in raw minced beef patties (Clavero *et al.,* 1994 cited by Kundu *et al*., 2014). Differences in the sensitivity of irradiation not only between species but between strain. The size of cell and structural arrangement of DNA within the cell affect the sensitivity of irradiation. Irradiation resistance of nonpigmentasi bacteria species strongly influenced by the ability of the organism in repairing the damage due to irradiation and physiological activity such as the metabolic phase of the organism when separately irradiated (Kundu *et al*., 2014). Irradiation directly lead to the breakup of chemical bonds in DNA and indirect effects of reactive oxygen due to water radiolysis to cell membranes and chromosomes of microorganisms (Min *et al*., 2003; Huq *et al.,* 2015).

Combination of vacuum prossesing and irradiation can inhibit microbial growth and extend shelf life. The combination of Modified Athmosphere Packaging (MAP) and low dose irradiation has been used to extend the shelf life of various meat products (Chouliara *et al*., 2008). Irradiation of the food that is processed based on the Good Manufacturing Practices (GMP) can increase food security and decrease foodborne diseases, extending the shelf life for perishable product, and lowers the risk of contamination. The food is sterilized with high-dose irradiation is able to kill all microorganisms (Zhu *et al*., 2012).

Shelf life

Anova test results indicated the shelf life of *sie balu* significantly effect on the count of *Salmonella* ($P<0.05$) (Table). Count of *Salmonella* in unirradiated *sie balu* decreased after 2 months of shelf life in vacuum packaging at room temperature. *Salmonella* growth was inhibited due to low levels of water and nutrients in *sie balu*. However, the increase in the count of *Salmonella* occur after the shelf life of 4 months becouse of the ability to adapt even under anaerobic conditions. This adaptation process because there are nutrients and suitable conditions for growth such as temperature, moisture, oxygen and pH (Cox, 2000; Levin, 2010). Increasing the count of *Salmonella* in irradiated *sie balu* lower than those not irradiated.

Mackey and Derrick (2008) discribed cells damaged by the process of heating and freezing takes longer to repair itself than the cells damaged by the drying process or gamma radiation. Irradiation inactivate microbes by damaging the genetic material of cells which can result in lethal or preventing cell multiplication and within a certain time can be repaired by the cell itself (Zahradka *et al*., 2006 in EFSA, 2011). Radiation also can cause lethal and sublethal effects on other cell structures such as membranes, enzymes and plasmids. Dickson (2001) discribes that was difficult to distinguish the influence of genetic and nongenetik damage due to irradiation. Damage is not specific to the genetic component alone but also other cellular components.

Results of microbiological tests of the shelf life can be concluded that the combination of irradiation and vacuum in *sie balu* can extend the shelf life. The study also showed a correlation between irradiation and shelf life of *sie balu*. Irradiation and shelf life showed significant effect ($P<0.05$) on the count of *Salmonella* in *sie balu.*

Salmonella colonies growing on Desoxychocolate agar to be gray-black because it absorbs ferric acid contained in the media. Results of some biochemical tests consisting of Indol test and fermentation of lactose were negative while the MR test, SIM, Simon citrate, TSIA, mannitol, sucrose, glucose and maltose were positive showed the characteristic of *Salmonella* Paratyphi B. The other colonies identified as *Salmonella cholerae suis* with negative for Indol and TSIA, while positive for MR test, SIM, Simon citrate, mannitol, sucrose, glucose and maltose. This finding concluded that there were some other species of *Salmonella* in *sie balu.*

Salmonella infections in humans and animals caused asymptomatic disease to severe systemic infection that ended with

high mortality. Infections in animals are economically important because the effect on morbidity and mortality. Even more important to human health, salmonellosis can be contracted as a result of direct or indirect contact with the reservoir animals (Libby *et al*., 2004). *Sie balu* is one of dried meat product that can not be consumed directly. *Sie balu* consumed after frying, so *Salmonella* would be die and *sie balu* products are safe for consumption.

Chart (2002) describes strains of *Salmonella* Paratyphi B is not the major cause enteric fever but usually cause gastroenteritis in humans. *S.* Paratyphi B can survive in the digestive tract and tolerant of low pH, further penetration through the mucosa and colonizes the intestinal epithelium. According to Prager *et al*., (2003) among Salmonella enteritica, the two major pathogens that infect humans is *S. enteritica* serotype Typhi and *S. enterica* serotype Paratyphi A, B and C lead to septicemia and fever tiphoid. Infections caused by *S. enteritica* serotype Paratyphi B is not only limited on the systemic infection but associated with gastroenteritis and foodborne infections.

The ability of *Salmonella typhi* passes through the transition period of the dynamic response of the host while enter the human body such as hiperosmolaritas, low pH (acidic stress), bile salts, and other immune responses, is a form of bacterial strategy to survive in the host environment. An increase in the virulence of *Salmonella typhi* happens when positioned on the environmental conditions of low oxygen, high osmolarity and low pH (Kundera *et al*., 2012). EFSA (2011) describes the irradiation can not cause non-pathogenic microbes to be pathogens. Irradiation causes reduced levels of microbial virulence.

CONCLUSIONS

Irradiation can reduce microbial pathogens that contaminate food, especially *Salmonella* and extend the shelf life of *sie balu*. Irradiation doses of 5, 7 and 9 kGy can eliminate *Salmonella* in *sie balu*. The shelf life can increase the count of *Salmonella* at room temperature although the foodstuffs have been dried and irradiated.

SUGGESTION

Based on the research conducted, it is suggested the processing of *sie balu* should to be hygiene and in high sanitation condition of all stages of processing. Further observation is needed about the irradiation effect on the growth of microbes in dried food.

ACKNOWLEDGMENT

Great thanksfulness for UNSYIAH that has funded this research through Unsyiah Postgraduate Grant Incentives Fiscal Year 2014, Number: 189/UN11.2/LT/SP3/2014, June 5, 2014.

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