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Effectiveness of gamma and electron beam irradiation techniques in extending the shelf-life of pasteurized sausage in natural conditions

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Abstract: Co-research group in cooperation between Nong Lam University and VINAGAMMA Center in Ho Chi Minh City carried out this irradiation experiments on instant food by using two different irradiation techniques. The stationary irradiation sourced from Co^{60} was applied at different doses (1.0 kGy, 2.5 kGy, 5.0 kGy) to ready to eat pasteurized sausage and mobile irradiation sourced from Electron –Beam (EB) was applied at same doses to pasteurized sausage in order to prolong the shelf-life of the products. After irradiation, the microbial and sensory quality of sausage was analyzed and evaluated monthly upto six months of storage in natural conditions. The microbial parameter included total aerobic bacteria, yeast, mold, *E. coli*, *clostridium* spp. and sensory parameters of color, structure, smell, and taste was evaluated for each dose applied upto six months of storage at natural condition. The study results showed that despite the irradiation sources, application of 5.0kGy dose of irradiation was able to extend the shelf life of ready to eat sausages up to six months at natural condition without compromizing the microbial and sensory parameters. Thus, this irradiation technology could be applicable in extending the shelf life of meat products.

Keywords: Stationary Irradiation, Mobile Irradiation, Co^{60}, EB (Electron Beam), Pasteurized Sausage, Yeast, *E. coli*, Mold, Total Aerobic Bacteria (TAB), *Clostridium* spp, and Sensory parameter

1. Introduction

Ready-to-eat pasteurized sausage is getting more and more popular because of its convenience, high nutrition content and safety; therefore, the requests of food hygiene safety and shelf-life are very important for consumers and producers (QCVN 8-3: 2012/BYT Vietnam). The current practices in Vietnam could able to store ready-to-eat package sausages only 3 months in natural condition. This three months storage in natural condition is not enough to export these products to the international market. Therefore, the producer wanted at least 6 months to one year shelf-life for their products to export in international market. To overcome this challenges use of irradiation is thought to be the appropriate techniques in extending the shelf life of ready to eat food products. In addition, The U.S. Food and Drug Administration has approved the use of irradiation up to 4.5 kGy and 7 kGy to control bacterial pathogens on refrigerated meat and frozen meat, respectively (FDA 1997). Furthermore, many study result reported that food irradiation using electron beam – EB, gamma rays or X rays to inhibit and reduce the growth of harmful microorganisms in ready-to-eat food products and many countries including USA already permitted irradiated food to be sold in the market with proper labeling. (Jo et al., 2000, 2002; Bari et al., 2006). On the other hand, Bari et al (2006) reported that higher dose (>5.0kGy) of irradiation has a negative effect on the organoleptic qualities of the meat and therefore, in this study we used maximum 5.0 kGy of irradiation.

Based on the above mentioned fact we carried out this

study using gamma and electron beam irradiation to prolong shelf-life of pasteurized sausage stored in natural condition.

2. Materials and Methods

2.1. Materials

2.1.1. Sample Preparation

Pasteurized sausage: 25 g and 35 g (collected from supermarkets in Ho Chi Minh City)

2.1.2. Equipment and Tools

Equipment: drying cabinet, incubator, autoclave, refrigerators, micropipette…

Tools: test tubes, petridishes, stirring rod, flask, alcohol lamps, absorbent cotton…

2.1.3. Chemicals

Alcohol 96^o, distilled water, agar, n-butanol, TBA-reagent (TBA: Thiobartiburic acid).

2.1.4. Culture Medium

Plate Count Agar, Sabouraud Dextrose Agar, Brilliant Green Bile Agar, Iron Sulphite Agar, Peptone dilute solution. (Tran Linh Thuoc. 2006; TCVN 5165 – 90)

2.2. Methods

2.2.1. Irradiation Techniques

The sausages samples were irradiated from Co-60 source (SVST-Co60/B; made in Hungary) and 1.0 kGy; 2.5 kGy and 5.0 kGy doses were applied. Same doses were applied to the sausages samples using EB accelerator (UERL-10-15S2; made in Russia). All the samples were irradiated at VINAGAMMA Center of Ho Chi Minh City.

2.2.2. Experimental and Anlytical Methods

Sausage samples were irradiated and the changes of microbiological and sensory quality of irradiated sausage was analyzed and evaluated monthly up to 6 months of storage at room temperature: 25-27^{o}C (TCVN 7247: 2003: CODEX STAN 106-1983).

2.2.3. Determination of the Optimal Irradiation dose for the Extended Shelf-Life with the Better Quality

The optimal irradiated dose has been determined by analyze monthly during six months to evaluate quality changes of irradiated sausage stored in natural condition in Ho Chi Minh city consisting of sensory evaluation, aerobic microorganism total yeast, mold, *E. coli,* and *clostridium spp* as the followings:

2.2.3.1. Total Aerobic Bacteria (TCVN 5165-90 Vietnamese Standards)

Twenty-five grams of irradiated, non-irradiated, or post-irradiated sausage sample was aseptically ground and mixed with 225ml peptone water into a sterile flask. The content was mixed thoroughly using a sterile glass rod and then serial decimal dilutions were prepared with 0.1% peptone water. The diluted and undiluted samples were pour plated (1.0 ml, in duplicate) on Plate Count Agar (PCA). The plates were incubated at 37^{0}C for 24 to 48 h before colonies were counted. Number of colonies was calculated using the following formula:

$$A = \frac{N}{n1 \times V1 \times f1 + \ldots + ni \times Vi \times fi} \quad (CFU/g)$$

Where: N: total number of colonies

n_i: number of colonies existing in the test dish

v: volume of solution cultured in each dish

f_i: dilution concentration

2.2.3.2. Yeast - Mold

The previously prepared diluted and undiluted samples were pour plated (1.0 ml, in duplicate) on Sabouraud Dextrose Agar. The plates were incubated at 25^{0}C for 5 to 7 days before colonies were counted.

2.2.3.3. E. coli

The previously prepared diluted and undiluted samples were pour plated (1.0 ml, in duplicate) on Brilliant Green Bile Agar. The plates were incubated at 37^{0}C for 24 to 48 h before colonies were counted.

2.2.3.4. Clostridium

The previously prepared diluted and undiluted samples were pour plated (1.0 ml, in duplicate) on Iron Sulfite Agar. The plates were incubated at 37^{0}C for 24 to 48 h before colonies were counted.

Table 1. *Scored method under TCVN 3215-79*

Parameters Score interval	Important coefficient
Color	0.25
Structure	0.35
Smell	0.20
Taste	0.20
Total	1.00

2.2.3.5. Sensory Evaluation (TCVN 3215-79)

The irradiated, or non-irradiated, sausage sample were stored at room temperature (25-27^{0}C) up to six months and evaluated periodically for its sensory characteristics of color, structure, smell, and taste using Vietnam national standard method (TCVN 3215-79). The evaluation was based on score scale rated in the interval from 1.0 to 5.0 for 4 parameters: color, structure, smell, and taste (*Table 1*), important coefficient was rated from 0.25 to 1.00 to express the important level of each parameter

The total scores of each parameter were to multiply the score of each parameter and its important coefficient. The last sensory score of product was the total scores of the 4 parameters. If the last sensory score >= 4, the sample meets the highest quality.

3. Results and Discussion

3.1. Sensory Evaluation

The results presented in Table 2 and Figure 1, showed that

the sensory score of the non-irradiated samples gradually decreases on storage time. The highest sensory score of the non-irradiated sample was 3.7 and the lowest score was 2.9; and the score disparity was 0.8. On the other hand, the highest sensory score of 5.0 kGy Co^{60} irradiate sample was 4.0 and the lowest score was 3.0, and the score disparity was 1.0. For 1.0 kGy EB irradiate sample, the highest score was 4.0, and the lowest score was 3.0, the score disparity was also 1.0. The other Co^{60}irradiated and EB irradiated samples exhibited similar scores. Overall, sensory score difference among samples was not found significant. Therefore, sensory quality of sausages samples at different irradiation dose was found equally good up to six months of storage in natural condition.

Table 2. *The last sensory score of irradiated sausage stored in natural condition during 6 months.*

Samples	Parameters	1 month	2 months	3 months	4 months	5 months	6 months
Control	Color	0.96	0.93	0.87	0.87	0.75	0.88
	Structure	1.20	1.23	1.17	1.19	0.98	1.28
	Smell	0.81	0.64	0.62	0.63	0.51	0.64
	Taste	0.71	0.70	0.71	0.60	0.61	0.68
	Last score	*3.70*	*3.5*	*3.4*	*3.3*	*2.90*	*3.50*
Gamma 5.0 (G5.0)	Color	1.04	0.91	0.91	0.82	0.83	0.93
	Structure	1.38	1.23	1.17	1.10	1.05	1.21
	Smell	0.81	0.68	0.61	0.53	0.57	0.60
	Taste	0.80	0.69	0.63	0.60	0.57	0.53
	Last score	***4.00***	***3.50***	***3.30***	***3.00***	***3.00***	***3.30***
Gamma 2.5 (G2.5)	Color	0.96	0.94	0.84	0.85	0.77	0.90
	Structure	1.28	1.26	1.13	1.10	1.12	1.28
	Smell	0.83	0.69	0.60	0.53	0.52	0.67
	Taste	0.71	0.76	0.70	0.55	0.60	0.61
	Last score	*3.80*	*3.60*	*3.30*	*3.00*	*3.00*	*3.50*
Gamma 1.0 (G1.0)	Color	1.09	0.88	0.88	0.85	0.80	0.88
	Structure	1.20	1.21	1.20	1.05	1.05	1.24
	Smell	0.77	0.65	0.57	0.60	0.61	0.56
	Taste	0.79	0.66	0.65	0.53	0.65	0.59
	Last score	*3.80*	*3.40*	*3.30*	*3.00*	*3.10*	*3.30*
EB 5.0	Color	0.96	0.88	0.81	0.90	0.82	0.80
	Structure	1.23	1.23	1.14	1.19	1.00	1.14
	Smell	0.87	0.71	0.63	0.61	0.57	0.60
	Taste	0.78	0.70	0.69	0.69	0.63	0.60
	Last score	***3.80***	***3.50***	***3.30***	***3.40***	***3.00***	***3.10***
EB 2.5	Color	1.05	0.90	0.89	0.87	0.75	0.88
	Structure	1.33	1.20	1.20	1.05	1.03	1.24
	Smell	0.81	0.63	0.63	0.59	0.49	0.65
	Taste	0.84	0.66	0.71	0.61	0.60	0.65
	Last score	***4.00***	***3.40***	***3.40***	***3.10***	***2.90***	***3.40***
EB 1.0	Color	1.02	0.88	0.87	0.80	0.78	0.88
	Structure	1.38	1.13	1.16	1.17	1.14	1.28
	Smell	0.84	0.65	0.63	0.52	0.68	0.64
	Taste	0.79	0.61	0.66	0.48	0.64	0.68
	Last score	***4.0***	***3.3***	***3.3***	***3.0***	***3.2***	***3.50***

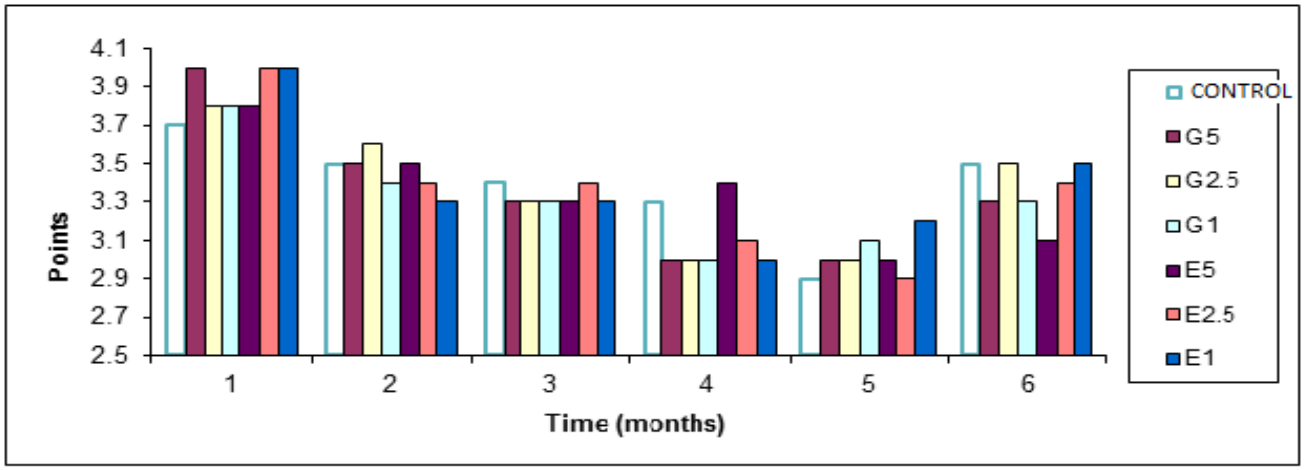

Fig. 1. *Sensory total score evaluated by TCVN 3215-79 of the sausages irradiated by gamma and EB with the different doses during 6 months stored in natural conditions in Ho Chi Minh City, Vietnam*

3.2. Total Aerobic Bacteria (TAB)

Table 3. *TAB of the sausages irradiated stored in natural conditions in HoChiMinh City during 6 months*

Treatments	CFU/g 1month	CFU/g 2months	CFU/g 3months	CFU/g 4months	CFU/g 4.5months	CFU/g 5months	CFU/g 5.5 months	CFU/g 6 months
Control	1.1×10^2	< 10	<10	<10	7.3×10^2	1.4×10^3	9.4×10^2	3.6×10^2
G 5.0	0	0	0	<10	<10	<10	<10	1.9×10^2
Gamma 2.5	5×10^2	< 10	<10	<10	3.4×10^2	1×10^3	2.9×10^2	<10
Gamma 1.0	1.5×10^2	0	0	<10	<10	7.9×10^2	<10	<10
EB 5.0	< 10	< 10	<10	<10	<10	5.7×10^2	<10	<10
EB 2.5	0	< 10	< 10	<10	<10	<10	<10	<10
EB 1.0	< 10	< 10	< 10	<10	2.3×10^2	2.4×10^2	<10	<10

Data presented in Table 3 showed that number of aerobic bacterial count gradually increase on storage time. The initial TAB was recorded as 1.1 x 10^2 CFU/g in non irradiated control sample at 1st month of storage and did not increased significantly at 6th month of storage. For irradiated samples there was a decreased trend observed up to 5 months and at 6th month of storage with 5.0 kGy Co-60 irradiated samples showed 1.9 x 10^2 CFU/g population of total aerobic bacteria in the sample. In contrast, other low irradiation dose from the same source did not show any TAB. This finding suggested that the applied dose did not cover every side of the products. On the other hand, EB irradiated sample showed no bacteria from the first months of storage to the 6th months of storage. From the results, 2.5 kGy and 5.0 EB irradiated samples showed better results compared to other doses of EB or Co^{60} sources of irradiated samples.

3.3. Yeast – Mold

Table 4. *Yeast - mold analysis of the irradiated sausages during 6 months stored at room temperature*

Samples	(CFU/g) 1 month	(CFU/g) 2months	(CFU/g) 3months	(CFU/g) 4months	(CFU/g) 4.5months	(CFU/g) 5months	(CFU/g) 5.5months	(CFU/g) 6months
Control	1.1×10^2	< 10	<10	<10	<10	<10	4.5×10^2	2.2×10^2
Gamma 5.0	0	0	0	<10	<10	<10	<10	<10
Gamma 2.5	8.2×10^2	< 10	<10	<10	<10	<10	<10	<10
Gamma 1.0	0	0	0	<10	3.2×10^2	3.5×10^2	<10	<10
EB 5.0	< 10	0	0	<10	<10	<10	<10	<10
EB 2.5	0	< 10	< 10	<10	2×10^2	2.3×10^2	<10	1.7×10^3
EB 1.0	0	< 10	< 10	<10	<10	<10	<10	<10

The non irradiated control samples showed yeast & mold growth at the first months analysis results (Table 4). However, irradiated samples did not show any yeast & mold at the first months of analysis. This finding suggested that the number of yeasts and mold colonies in the control samples was much more than that of the irradiated samples. Data presented in table 4 also demonstrated that irrespective of irradiation sources, 5.0kGy dose of irradiation was able to inactivate the yeast and mold successfully and thus, did not appear in the medium.

3.4. E. coli and clostridium spp.

Table 5. *Quantification analysis of E. coli and Clostridium in the irradiated sausages during six months stored in natural conditions in HCM City.*

Samples	Parameters	(CFU/g) 1 month	(CFU/g) 2 months	(CFU/g) 3 months	(CFU/g) 4 months	(CFU/g) 5 months	(CFU/g) 6 months
Control	*E. coli*	0	0	0	0	0	<10
	Clostridium	0	0	0	0	0	0
Gamma 5.0	*E. coli*	0	0	0	0	0	<10
	Clostridium	0	0	0	0	0	0
Gamma 2.5	*E. coli*	0	0	0	0	0	<10
	Clostridium	0	0	0	0	0	0
Gamma 1.0	*E. coli*	0	0	0	0	0	0
	Clostridium	0	0	0	0	0	0
EB 5.0	*E. coli*	0	0	0	0	0	<10
	Clostridium	0	0	0	0	0	0
EB 2.5	*E. coli*	0	0	0	0	0	<10
	Clostridium	0	0	0	0	0	0
EB 1.0	*E. coli*	0	0	0	0	0	<10
	Clostridium	0	0	0	0	0	0

The *E. coli* and *Clostridium spp*, was not found in any samples stored in natural conditions for 6 months. However, in case of their presence, they might survive or grow during the long storage; therefore application of irradiation could maintain the safety of the sausages. By comparing the other parameters, the 5 kGy dose irrespective of irradiation sources could inactivate pathogen by extending the shelf-life at room temperature.

4. Conclusion

The study results concluded that irrespective of the irradiation sources, application of 5.0kGy dose of irradiation was able to extend the shelf life of ready to eat sausages up to six months at natural condition without compromizing the microbial and sensory qualities of the products. Thus, this irradiation technology could be applicable in extending the shelf life of meat products. However further research is needed to evaluate the influences of 5.0 kGy irradiation dose on bio-chemical composition, including oxidation of fat, protein and other nutrient.

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