สำนักงานพลังงานปรมาณูเพื่อสันติ

พปส/OAEP-1-124

IMPROVEMENT OF BACTERIOLOGICAL QUALITY OF FROZEN CHICKEN BY GAMMA RADIATION

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ธันวาคม 2529

DECEMBER 1986

OFFICE OF ATOMIC ENERGY FOR PEACE

พปส.
OAEP -1-124

การปรับปรุงคุณภาพทางจุลินทรีย์ของเนื้อไก่แช่แข็งด้วยรังสีแกมมา

IMPROVEMENT OF BACTERIOLOGICAL QUALITY OF FROZEN CHICKEN BY GAMMA RADIATION

โกวิทย์ นุชประมูล ยุทธพงศ์ ประชาสิทธิศักดิ์ และ เสาวพงศ์ เจริญ
กองวิทยาศาสตร์ชีวภาพ สำนักงานพลังงานปรมาณูเพื่อสันติ

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DECEMBER 1986

สำนักงานพลังงานปรมาณูเพื่อสันติ
OFFICE OF ATOMIC ENERGY FOR PEACE

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เอกสารฉบับนี้ จัดทำขึ้นโดยสำนักงานพลังงานปรมาณูเพื่อสันติ (พปส.) สำนักงานฯ ไม่ประกันความรับผิดชอบทางกฎหมายในเรื่องความแน่นอน ความสมบูรณ์ หรือประโยชน์ของข้อมูล เครื่องมือ ผลิตผล หรือกระบวนการใด ๆ ที่เปิดเผยในเอกสารนี้"

ISBN 974-7399-34-2

<u>บทคัดย่อ</u>

ได้ทำการศึกษาการปรับปรุงคุณภาพทางจุลินทรีย์ของเนื้อไก่แช่แข็ง โดยการใช้รังสีแกมมา ปริมาณ 1.6 ถึง 4.0 กิโลเกรย์ และได้ทำการศึกษาผลของรังสีแกมมาที่มีต่อเชื้อซัลโมเนลลา ในเนื้อไก่แช่แข็ง และที่มีต่อคุณภาพทางประสาทสัมผัสของเนื้อไก่-แช่แข็ง ค่าความต้านทานต่อรังสีของเชื้อซัลโมเนลลาสายพันธุ์ต่าง ๆ ในเนื้อไก่แช่แข็งมีค่าระหว่าง 0.41 ถึง 0.57 กิโลเกรย์ การทำลายเชื้อซัลโมเนลลาในเนื้อไก่แช่แข็ง-ให้ลดลง 7 log cycles จะต้องใช้รังสีปริมาณ 4 กิโลเกรย์ จากการตรวจเนื้อไก่-แช่แข็ง พบว่า ร้อยละ 21 ของตัวอย่าง มีเชื้อซัลโมเนลลาปะปนอยู่ เชื้อที่ตรวจพบมากได้แก่ <u>Salmonella typhimurium</u>, <u>Salmonella virchow</u> และ <u>Salmonella java</u> การฉายรังสีเนื้อไก่แช่แข็งที่ปริมาณรังสีต่ำสุด 3.2 กิโลเกรย์ สามารถทำลายเชื้อซัลโม-เนลลา Coliform, <u>Escherichia coli</u> และ <u>Staphylococcus aureus</u> ที่มีอยู่ได้หมด อีกทั้งยังช่วยลดจำนวนบักเตรีทั้งหมดลงได้ 2 log cycles ส่วนเชื้อ Faecal streptococci นั้น ยังคงเหลืออยู่ในบางตัวอย่าง และมีปริมาณไม่มากกว่า 100 โคโลนีต่อกรัม การฉายรังสี จะทำให้สีของเนื้อไก่เปลี่ยนแปลงไปเป็นสีชมพู และสังเกตได้เมื่อฉายรังสีที่ 2 กิโลเกรย์ คุณภาพด้านสีและกลิ่นของเนื้อไก่แช่แข็งฉายรังสีที่ 3 และ 4 กิโลเกรย์ จะลดลงในระหว่างการเก็บรักษาที่อุณหภูมิเยือกแข็ง แต่ก็ยังเป็นที่ยอมรับของผู้บริโภค แม้ว่าจะเก็บไว้นานถึง 8 เดือน รังสีปริมาณ 3.2 กิโลเกรย์ น่าจะพอ-เพียงสำหรับใช้ปรับปรุงคุณภาพทางจุลินทรีย์ของเนื้อไก่แช่แข็ง

ABSTRACT

The possible use of gamma irradiation at doses of 1.6 to 4.0 kGy to improve bacteriological quality of frozen chicken was investigated. The effects of gamma irradiation on Salmonella viability in frozen chicken and on sensory quality of frozen chicken were also evaluated. D_{10}-values for different isolated strains of Salmonella in frozen chicken varied from 0.41 to 0.57 kGy. A dose of 4 kGy is required for a seven log cycles reduction of Salmonella contamination in frozen chicken. Approximately 21 per cent of frozen chicken examined were contaminated with Salmonella. Salmonella typhimurium, Salmonella virchow, and Salmonella java were predominant. Irradiation of frozen chicken at a minimum dose of 3.2 kGy eliminated Salmonella, Coliform, Escherichia coli, and Staphylococcus aureus and, in addition, reduced bacterial load by 2 log cycles. Faecal streptococci was still present in a 3.2 kGy samples but in a very small percentage and the count was not over 100 colonies per g. Discolouring of chicken meat was noted after a 2 kGy treatment. The sensory quality of frozen chicken irradiated at 3 and 4 kGy tended to decrease during frozen storage but was within the acceptable range on a nine point hedonic scale even after eight months of frozen storage. Dosage at 3.2 kGy appeared to be sufficient for improving bacteriological quality of frozen chicken.

1. INTRODUCTION

Poultry industries play an important role in the economy of the nation. They serve as a main source of protein and provide foreign exchange earnings. In 1982, Thailand exported more than 30,000 tons of frozen chicken valued at approximately Baht 1,310 million.[1]

The number of cases of Salmonellosis from man and feed has increased in recent years. Chicken is an important vector of this disease and bacteriological examination of chicken in Thailand have revealed that 6 per cent of frozen chicken may be contaminated. Bacteriological quality of frozen chicken did not always comply with the guideline, 22 per cent due to coliform, 20 per cent due to Faecal streptococci, and 5 per cent due to Staphylococcus aureus.[2] Apart from Salmonella contamination, the shelf-life of chicken is also short due to its perishable nature and microbial contamination. One method of reducing this Salmonella contamination of market chicken would be to treat the packaged chicken with ionizing radiation. Several investigations have shown the feasibility of using ionizing radiation in eliminating Salmonella and extending the shelf-life of poultry and meats.[3-6] Since the raising of poultry free from pathogenic microorganisms will not be accomplished in the near future and cross contamination is unavoidable, large scale irradiation of chicken would be a very important preventive method.

This paper reports the effect of irradiation on Salmonella and other pathogens in frozen chicken. Identification of Salmonella serotypes associated with frozen chicken, determination of their radiation resistance, and sensory evaluation of the quality of irradiated frozen chicken are also included.

2. EXPERIMENTAL

2.1 The effect of irradiation on the quality of frozen chicken

Commercial samples of frozen chicken (boneless breast, boneless leg, bone-in-leg, wing sticks, skin, fillet and yakitori) packed in plastic bags with a net weight of 2 kg per pack were obtained from 7 poultry processing plants. The samples were irradiated at room temperature in a Gammabeam 650 (Atomic Energy Canada Ltd.) at a dose rate of 0.46 kGy per hour using the dose range of 1.6 to 4.0 kGy and determined for Total viable count, Coliform, *Escherichia coli*, Faecal streptococci, *Staphylococcus aureus* and Salmonella.

2.1.1 Bacteriological evaluation

Total viable counts and most probable number of Coliform and *Escherichia coli* were determined by the method of ICMSF.[7] Isolation and identification of Salmonella serotypes were performed by the methods recommended by ICMSF and Horwitz.[8] Enumeration of *Staphylococcus aureus* and Faecal streptococci were made by the method of the Nordic Committee on Food Analysis.[9]

2.1.2 Sensory evaluation

The organoleptic properties of raw and cooked chicken meat made from non-irradiated and irradiated frozen chicken were judged subjectively by a trained panel of 13 members. Scoring was done according to multiple comparisons test described by Larmond.[10] Sensory evaluation of irradiated and non-irradiated frozen chicken was determined after 1,2,3,4 and 8 months. The judges were asked to evaluate cooked chicken meat for color and odor using a nine-point hedonic scale.

2.2 Radiation resistance of Salmonella in chicken

Radiation resistance of Salmonella serotypes associated with chicken was determined using artificial contaminated chicken. Half carcasses of chicken were immersed in Salmonella solutions containing 10^8 cells per ml. After draining, chicken carcasses were packed in plastic bags and kept frozen at -18°C till irradiation. The packed chicken carcasses were irradiated in triplicate to a maximum dose of 3 kGy in increments of 0.75 kGy. A quantitative count of Salmonella in irradiated and non-irradiated samples was determined by the most probable numbers methods as described by Licciardello.[11]

3. RESULTS AND DISCUSSION

3.1 The effect of irradiation on the quality of frozen chicken

3.1.1 Bacteriological effect

Results of bacteriological examination of 789 samples of frozen chicken are shown in Table 3.1.1.1. Total viable counts of non-irradiated samples ranged from 10^2 and 10^6 colonies per g, and the highest distribution frequency was between 10^4 and 10^5 organisms per g. Irradiation at 3.2 up to 4.0 kGy reduced the count by 2 log cycles.

Coliform, *Escherichia coli*, *Staphylococcus aureus*, Faecal streptococci and Salmonella were detected in 97, 54, 8, 47 and 21 per cent of the non-irradiated samples, respectively. After irradiation with 3.2 up to 4.0 kGy, no detectable number of Coliform, *Escherichia coli*, *Staphylococcus aureus*, and Salmonella was found in frozen chicken. Faecal streptococci was still present in a 3.2 kGy samples but in a very small percentage and the count was not over 100 colonies per g. Serotypes of Salmonella isolated from non-irradiated and irradiated frozen chicken were *S. typhimurium*, *S. krefeld*, *S. emek*, *S. virchow*, *S. senftenberg*, *S. anatum*, *S. panama*, *S. ohio*, *S. java*, *S. bovis-morbificans*, *S. ruiru*, *S. heidelberg*, *S. stanley*, *S. brandenberg*, and *S. bredeney* (Table 3.1.1.2). Microbiological standard for frozen chicken is shown in Table 3.1.1.3. If this standard is taken into account, roughly more than 20 per cent of the samples examined would fail to meet its limits. The application of radiation at dose of 3.2 kGy thus seems to be promising for reducing the bacterial load down to acceptable levels.

3.1.2 Sensory evaluation

Flavor panels were carried out to evaluate the effects of gamma irradiation, and the interrelationship of gamma irradiation and storage time on frozen chicken quality. Mean flavor panel scores for raw and cooked meat and mean scores for the color and odor of cooked meat derived from frozen chicken with or without radiation treatment are listed in Tables 3.1.2.1, 3.1.2.2, and 3.1.2.3, respectively.

Radiation at dose of 4 kGy did not affect the odor of frozen chicken but did affect the color. Mean scores for the color of raw meat derived from 3 and 4 kGy samples were significantly ($p < 0.05$) higher than those observed for non-irradiated samples. Change in color was observed after a 2 kGy treatment where the color became slight pink. After cooking, mean color scores for irradiated and non-irradiated samples did not differ significantly. Although analysis of variance showed no significant difference in odor between irradiated and non-irradiated chicken, scores of 3 and 4 kGy samples were somewhat reduced from those observed for non-irradiated samples. Radiation induced changes in color and odor was also observed in chicken carcasses irradiated at doses of 2.5 and 5.0 kGy.[4]

Storage of irradiated frozen chicken at -18°C up to 8 months did not affect the quality of the product (Tables 3.1.2.2 and 3.1.2.3). Mean scores for the color and odor did not differ significantly. Doses of irradiation did not interact significantly with storage time with regard to all sensory factors.

3.2 Radiation resistance of Salmonella in chicken

A number of experiments on cell survival were carried out with *S. typhimurium*, *S. senftenberg*, *S. panama*, *S. krefeld*, *S. infantis*, *S. agona*, *S. derby*, *S. thompson*, and *S. emek*. The effect of gamma irradiation on Salmonella followed the linear log number/dose relationship. D_{10}-values ranged from 0.41 to 0.57 kGy (Table 3.2.1). Among Salmonella serotypes found in frozen chicken, *S. senftenberg* was the most resistant and *S. emek* was the least resistant. On the basis of the 7D concept, the dose of 4 kGy is required for decontamination of Salmonella at 10^6 cells per g. in frozen chicken. Since the number of viable Salmonella occurring in most foods and animal feeds was seldom found to exceed 1/g[3], the calculated dose of 4 kGy would be amply safe for decontamination of Salmonella in frozen chicken.

4. CONCLUSIONS

Commercial samples of frozen chicken from 7 poultry processing plants were irradiated at doses from 1.6 to 4.0 kGy. Irradiated and non-irradiated samples were analyzed for their bacteriological quality. Approximately 21 per cent of frozen chicken examined were contaminated with Salmonella. 14 serotypes of Salmonella were isolated and *S. typhimurium*, *S. virchow*, and *S. java* were predominant. Irradiation of frozen chicken at a minimum dose of 3.2 kGy appeared to be effective in eliminating naturally contaminating Salmonella, Coliform, *Escherichia coli*, and *Staphylococcus aureus*. Total viable count was also reduced by 2 log cycles. Faecal streptococci was not completely eliminated. The remaining count was not over 100 colonies per g but still in the acceptable level of microbiological standard for frozen chicken.

A number of experiments on cell survival were carried out with different isolated strains of Salmonella in frozen inoculated chicken. D_{10}-values ranged from 0.41 to 0.57 kGy. On the basis of the 7D concept, the dose of 4 kGy is required for decontamination of Salmonella in frozen chicken.

No objectionable odor of frozen chicken irradiated at doses as high as 4 kGy was found by the sensory panel. Discolouring of chicken meat was noted at the dose of 2 kGy. The sensory quality of frozen chicken irradiated at 3 and 4 kGy tended to decrease during frozen storage but was within the acceptable range on a nine point hedonic scale even after eight months of frozen storage. Dosage at 3.2 kGy appeared to be sufficient for improving bacteriological quality of frozen chicken.

ACKNOWLEDGEMENTS

This work was supported in part by the International Atomic Energy Agency, Research Contract No. 2809/R3/RB. The authors are indebted to poultry processing plants for their co-operation and contribution of samples for analysis. Special thanks are given to the Division of Radiation Measurement for dose distribution measurement and to the Division of Clinical Pathology, Department of Medical Science for the identification of Salmonella serotypes.

Table 3.1.1.1 Bacteriological examination of non-irradiated and irradiated frozen chicken samples.

Organisms	Dose (kGy)	Number of samples	Number of organisms per g
Total bacterial count	0.0	210	1.0×10^2 - $>3.0 \times 10^6$
	1.6	69	3.0×10 - 5.2×10^4
	1.8	95	2.0×10 - 3.0×10^5
	2.4	66	1.0×10 - 1.5×10^4
	2.7	95	1.0×10 - 1.1×10^4
	3.0	62	1.2×10 - 5.1×10^4
	3.2	67(1)	<10 - 1.0×10^4
	3.6	65	3.0×10 - 5.8×10^4
	4.0	60(6)	<10 - 2.2×10^3
Coliform	0.0	210(6)	<3 - 2.4×10^4
	1.6	69(63)	<3 - 1.0×10^3
	1.8	95(91)	<3 - 4.3×10^2
	2.4	66(62)	<3 - 2.3×10^2
	2.7	95(93)	<3 - 2.3×10
	3.0	62(58)	<3 - 4.3×10
	3.2	67(67)	<3
	3.6	65(65)	<3
	4.0	60(60)	<3
<u>Escherichia coli</u>	0.0	210(97)	<3 - 1.3×10^3
	1.6	69(69)	<3
	1.8	95(94)	<3 - 4.0
	2.4	66(66)	<3
	2.7	95(95)	<3
	3.0	62(62)	<3
	3.2	67(67)	<3
	3.6	65(65)	<3
	4.0	60(60)	<3

- Number in parenthesis is the number of samples that organisms are not detected.

Table 3.1.1.1 (Continued)

Organisms	Dose (kGy)	Number of samples	Number of organisms per g
Staphylococcus aureus	0.0	210(193)	$<10^2$ - 2.4×10^3
	1.6	69(69)	$<10^2$
	1.8	95(95)	$<10^2$
	2.4	66(66)	$<10^2$
	2.7	95(95)	$<10^2$
	3.0	62(62)	$<10^2$
	3.2	67(67)	$<10^2$
	3.6	65(65)	$<10^2$
	4.0	60(60)	$<10^2$
Faecal Streptococci	0.0	210(112)	$<10^2$ - 2.5×10^4
	1.6	69(53)	$<10^2$ - 1.4×10^3
	1.8	95(91)	$<10^2$ - 9.0×10^2
	2.4	66(63)	$<10^2$ - 5.0×10^2
	2.7	95(94)	$<10^2$ - 7.0×10^2
	3.0	62(62)	$<10^2$
	3.2	67(64)	$<10^2$ - 1.0×10^2
	3.6	65(65)	$<10^2$
	4.0	60(60)	$<10^2$
Salmonella spp.	0.0	210(165)	Detectable
	1.6	69(65)	Detectable
	1.8	95(89)	Detectable
	2.4	66(64)	Detectable
	2.7	95(89)	Detectable
	3.0	62(48)	Detectable
	3.2	67(67)	ND
	3.6	65(65)	ND
	4.0	60(60)	ND

- Number in parenthesis is the number of samples that organisms are not detected.

- ND = Not detectable.

Table 3.1.1.2 Salmonella serotypes isolated from non-irradiated and irradiated frozen chicken samples.

Dose (kGy)	Serotypes
0.0	*S. anatum*, *S. krefeld*, *S. emek*, *S. virchow*, *S. panama*, *S. ohio*, *S. java*, *S. bovis-morbificans*, *S. typhimurium*, *S. ruiru*, *S. heidelberg*, *S. stanley*, *S. senftenberg* and *S. brandenberg*
1.6	*S. typhimurium* and *S. krefeld*
1.8	*S. krefeld*, *S. ohio*, *S. anatum* and *S. emek*
2.4	*S. krefeld* and *S. emek*
2.7	*S. typhimurium*, *S. bredeney*, *S. krefeld* and *S. ohio*
3.0	*S. typhimurium*, *S. senftenberg* and *S. virchow*
3.2	none
3.6	none
4.0	none

Table 3.1.1.3 Microbiological standard* for frozen chicken.

Types of organisms	Number of organisms per g
Total mesophilic count	5×10^6
Faecal streptococci	1×10^3
Staphylococcus aureus	3×10^2
Salmonella spp.	0

* Thai Industrial Standard (TIS. 590-1985).

Table 3.1.2.1 Mean[1] and analysis of variance of flavor panel scores for raw and cooked meat derived from non-irradiated and irradiated frozen chicken stored at -18°C.

Sensory factor		Dose (kGy)					F-value	Factor mean ranking[2]
		0	1	2	3	4		
Color	raw	5.07	5.50	6.07	6.29	6.29	2.84[4]	3>4>2>1>0
	cooked	4.92	4.77	4.54	5.00	4.85	1.83[3]	3>0>4>1>2
Odor	raw	4.93	5.00	4.64	4.29	4.21	2.04[3]	1>0>2>3>4
	cooked	5.00	4.77	5.00	4.62	4.15	2.77[3]	0>2>1>3>4

[1] Number of judges = 13

[2] Factor means with the same underline did not vary significantly ($p>0.05$)

[3] Non significant $p>0.05$

[4] Significant $p<0.05$

Nine point scale ranging from 9, extremely better than reference, 5 no difference, and 1, extremely worse than reference.

Table 3.1.2.2 Mean[1] color scores for cooked meat derived from non-irradiated and irradiated frozen chicken stored at -18°C for various time periods.

Storage time (months)	Irradiation dose (kGy)		
	0	3	4
1	7.33	7.17	7.08
2	7.33	7.42	7.50
3	7.17	7.00	6.75
4	6.92	7.42	7.42
8	6.67	7.33	7.25

	Factorial analysis	
	F-value	Ranking of level means
Irradiation dose (D)	0.734 [2]	3>4>0
Storage time (T)	1.453 [2]	2>4>1>8>3
D x T	1.201 [2]	

[1] Number of judges = 12

[2] Non-significant p>0.05

Level mean with the same underline did not vary significantly (p>0.05)

Nine point hedonic scale ranging from 9, highest affirmative value, to 1, lowest affirmative value.

Table 3.1.2.3 Mean[1] odor scores for cooked meat derived from non-irradiated and irradiated frozen chicken stored at -18°C for various time periods.

Storage time (months)	Irradiation dose (kGy)		
	0	3	4
1	7.33	7.17	6.92
2	7.08	7.25	6.75
3	7.00	6.25	6.50
4	7.00	6.58	7.08
8	6.83	6.58	6.50

	Factorial analyses	
	F-value	Ranking of level means
Irradiation dose (D)	1.319 [2]	0>3>4
Storage time (T)	1.616 [2]	1>2>4>8>3
D x T	0.497 [2]	

[1] Number of judges = 12

[2] Non-significant $p>0.05$

Level means with the same underline did not vary significantly ($p>0.05$)

Nine point hedonic scale ranging from 9, highest affirmative value, to 1, lowest affirmative value.

Table 3.2.1 D_{10} value of various Salmonella serotypes in artificially contaminated chicken at frozen state.

Salmonella serotypes	D_{10}-value (kGy)
S. typhimurium	0.46
S. senftenberg	0.57
S. panama	0.44
S. krefeld	0.54
S. infantis	0.49
S. agona	0.48
S. derby	0.49
S. thompson	0.50
S. emek	0.41

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