OAEP--1-120.

พปส / OAEP -1-120

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OFFICE OF ATOMIC
ENERGY FOR PEACE

พปส
OAEP -1-120

การปรับปรุงคุณภาพทางจุลินทรีย์ของกุ้งแช่แข็งด้วยรังสีแกมมา

IMPROVEMENT OF BACTERIOLOGICAL QUALITY OF FROZEN SHRIMP BY GAMMA RADIATION

โกวิทย์ นุชประมูล   เสาวพงศ์ พึ่งศิลป์
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DECEMBER 1985

สำนักงานพลังงานปรมาณูเพื่อสันติ

OFFICE OF ATOMIC ENERGY FOR PEACE

ISBN 974-7399-29-6

บทคัดย่อ

ได้ทำการศึกษาการปรับปรุงคุณภาพทางจุลินทรีย์ของกุ้งแช่แข็ง โดยการใช้รังสีแกมมาปริมาณ 2 และ 3 กิโลเกรย์ และได้ทำการศึกษาผลของรังสีแกมมาที่มีต่อเชื้อซัลโมเนลลา ในกุ้งแช่แข็ง และที่มีต่อคุณภาพทางประสาทสัมผัสของกุ้งแช่แข็ง การฉายรังสีกุ้งแช่แข็งที่ 3 กิโลเกรย์ สามารถลดจำนวนบักเตรีทั้งหมดลงได้ 2 log cycles และทำลายเชื้อ Enterobacteriaceae, *E. coli*,*V. parahaemolyticus* และ *S. aureus* ที่มีอยู่ในกุ้งในระดับปกติได้หมด ยกเว้น coliform ซึ่งยังคงพบในบางตัวอย่าง แต่มีปริมาณน้อยกว่า 10 เซลล์ต่อกรัม สำหรับเชื้อ ซัลโมเนลลา นั้น ตรวจไม่พบทั้งในกุ้งแช่แข็งฉายรังสีและไม่ฉายรังสี ค่าความต้านทานต่อรังสีของเชื้อซัลโมเนลลาทั้ง 5 สายพันธุ์ที่ศึกษา มีความแตกต่างกัน เชื้อ *S. lexington* มีความต้านทานต่อรังสีสูงสุด และเชื้อ *S. anatum* มีความต้านทานต่อรังสีต่ำสุด ค่าความต้านทานต่อรังสีของเชื้อซัลโมเนลลาในกุ้งแช่แข็ง สูงกว่าในกุ้งแช่เย็น การทำลายเชื้อซัลโมเนลลาในกุ้งแช่แข็งให้ลดลง 7 log cycles จะต้องใช้รังสีปริมาณ 4.2 กิโลเกรย์ กุ้งแช่แข็งที่ผ่านการฉายรังสีที่ 4.2 กิโลเกรย์ จะมีคุณภาพด้านกลิ่นรสเปลี่ยนแปลงไปบ้าง แต่การเปลี่ยนแปลงดังกล่าว จะลดลงในระหว่างการเก็บรักษาที่อุณหภูมิเยือกแข็ง อย่างไรก็ตาม คุณภาพของกุ้งแช่แข็งฉายรังสีหลังจากเก็บรักษาไว้นาน 4 เดือน ก็ยังเป็นที่ยอมรับของผู้บริโภค รังสีปริมาณ 3 กิโลเกรย์ น่าจะพอเพียงสำหรับใช้ปรับปรุงคุณภาพทางจุลินทรีย์ของกุ้งแช่แข็ง.

ABSTRACT

The possible use of gamma irradiation at doses of 2 and 3 kGy to improve bacteriological quality of frozen shrimp was investigated. The effects of gamma irradiation on Salmonella viability in frozen shrimp and on sensory quality of frozen shrimp were also evaluated. Treatment of frozen shrimp at 3 kGy reduced bacterial load by 2 log cycles and eliminated Enterobacteriaceae, *E. coli*, *V. parahaemolyticus*, and *S. aureus*. Coliform was still present in a 3 kGy treated samples but in a very small percentage and the count was less than 10 cell/g. Salmonella was not detected in either irradiated or non-irradiated frozen shrimp. A difference in radiation resistance was noted among five Salmonella serotypes tested. *S. lexington* was the most resistant and *S. anatum* was the least resistant. Salmonella resistance was higher in frozen inoculated shrimp than in refrigerated inoculated shrimp. A dose of at least 4.2 kGy is required for a seven log cycle reduction of Salmonella contamination in frozen shrimp. Off-flavors were produced in frozen shrimp irradiated at 4.2 kGy but diminished during subsequent frozen storage. However, irradiated frozen shrimp was of acceptable quality for at least four months. Dosage at 3 kGy appeared to be sufficient for improving bacteriological quality of frozen shrimp.

1. INTRODUCTION

Shrimp are known to be the food articles of great nutritional value for human consumption and are of economic importance. The frozen shrimp industry of Thailand has grown in recent years and shrimp now represents one of an important export item. The quantity and value of the export of Thai shrimp to Japan, U.S.A., Hongkong, etc. are 22,647 tons for 2,764 million bahts in 1982.[1] So far, the market for frozen shrimp has expanded with increased production.

The trade in shrimp is confronted with strict hygiene regulations and the problem of health hazards resulting from foodborne microorganisms. Salmonellosis is recognized as a public health problem in both industriallized and developing countries. It must be emphasized that peeling, cleaning, and packaging of shrimp are entirely done by hand. Consequently, the risk of contamination and cross contamination with microorganisms of public health concern are high. Samples taken from frozen shrimp in 1981 and 1982 showed 2.2 and 1.3 per cent positive for Salmonella.[2] The evidence of Salmonella in frozen shrimp had caused great concern to the shrimp industry and led to the economic losses due to the product failed to meet the bacteriological standard which was made more stringent by most importing countries. High rejection of exported frozen shrimp by US FDA recently amounted to 83 and 17 million bahts in 1981 and 1982.[3] Rejection was due to Salmonella and Arizona contamination and decomposition of products.

The application of ionizing radiation to poultry and meats has been shown to be useful in eliminating Salmonella and extending the shelf-life of the product.[4-7] Radiation treatment of frozen shrimp will provide greater assurance that exports will be acceptable and minimize economic losses.

This paper was designed to study the effect of irradiation on Salmonella viability in view of their elimination in frozen shrimp. Included in this work also, are bacteriological analysis of irradiated and non-irradiated frozen shrimp and sensory evaluation of the quality of irradiated frozen shrimp.

2. EXPERIMENTAL

2.1 Bacteriological analysis of irradiated and non-irradiated frozen shrimp.

Samples of frozen shrimp from fish processing plants were collected and irradiated at 0, 2, and 3 kGy. Irradiation was carried out at room temperature in a Gammabeam 650 (Atomic Energy Canada Ltd.), at a dose rate of 1.96 kGy per hour on May 30, 1982. Corrections for decay were made after each exposure. The samples were analyzed for Total viable bacterial counts, Enterobacteriaceae counts, Coliform, Faecal coliform, Escherichia coli, Salmonella, Vibrio parahaemolyticus, and Staphylococcus aureus.

The total viable bacterial counts were determined by drop plate technique using tryptone-glucose-yeast extract agar. The plates were incubated for 48 hours at 35°C. The most probable number (MPN) of coliform, faecal coliform, and Escherichia coli in each sample were determined according to procedure described in the International Atomic Energy Agency - Microbiological Specifications and Testing Methods for Irradiated Foods[8].

The Enterobacteriaceae counts were estimated on violet red bile dextrose agar after one hour of resuscitation in trypticase soy broth at 25°C. The plates were incubated for 24 hours at 35°C. For quantitation of Salmonella by the most probable numbers methods, groups of five tubes of selenite cystine broth were inoculated with the proper serial dilution, incubated at 37°C for 24-48 hours, then streaked on brilliant green agar. The plates were incubated at 37°C for 24 hours. Suspected Salmonella colonies were examined biochemically in triple sugar iron agar and serologically by Salmonella O- serum polyvalent. The number of positive tubes was determined and the Salmonella count was estimated from a table of most probable numbers.

The MPN of Vibrio parahaemolyticus and Staphylococcus aureus in each sample were determined according to procedure described in Compendium of Methods for the Microbiological Examination of Foods[9].

2.2 Radiation survival of Salmonella in shrimp.

Salmonella lexington, *Salmonella agona*, *Salmonella typhimurium*, *Salmonella derby*, and *Salmonella anatum* supplied by Division of Clinical Pathology, Department of Medical Sciences were used in the artificial contamination process of shrimp. Cultures were maintained on nutrient agar slants. To maintain similar populations of the cultures to be used for inoculation, an inoculum from the pure culture was transferred to 50 ml of nutrient broth and incubated for 18-24 hours at 35°C (shaking). In every case, 2 ml of cell suspension from the above mentioned cultures were aseptically taken into a pipette and dispersed in the same medium (300 ml in each flask). The inoculated flask was incubated at 35°C for 20 hours in a Lab-Line Orbit Environ Shaker (150 rev/min). After incubation, the inoculated broth was added to the inoculation bath containing sterile distilled water to get 10^8 cells per ml.

Before use in the inoculation process, frozen shrimp (peeled and shell on headless) previously irradiated at 10 kGy were thawed for 24 hours at 4°C. The thawed samples were then dipped into the inoculation bath for 20 minutes at 20°C. After dipping, the inoculated samples were drained for 2 minutes. Approximately 100 g of inoculated samples were put in the sterile bottle and kept at 4°C (refrigerated inoculated shrimp) and at -18°C (frozen inoculated shrimp) till irradiation. The inoculated samples were irradiated in triplicate to a maximum dose of 1.8 kGy in increments of 0.3 kGy.

A quantitative count of Salmonella in refrigerated inoculated shrimp was made immediately after irradiation, whereas with frozen inoculated samples counts were made the following day, with an interim storage of 4°C. Approximately 80-100 g of irradiated shrimp were taken aseptically from each sample and placed into a sterile plastic bag. A threefold quantity of sterile phosphate peptone water was added and mixed for 30 seconds in a Lab-Blendor Stomacher-400. Starting from this first dilution, further decimal dilutions were prepared using sterile phosphate peptone water. Amount of 0.1 ml of aliquots from each dilution was plated on nutrient agar in petridishes, using drop-plate techniques. The non-irradiated shrimp handled exactly the same way was used as a control in each experiment. The inoculated plates were incubated at 37°C for 24-48 hours and then the visible colonies developed on them were counted as survivors. Each viable count was the average number of cells from duplicate platings. The survivors/ml of irradiated and non-irradiated shrimp was determined from a particular dilution. The logarithmic mean of the surviving Salmonella counts was estimated. From these logarithmic mean values, except the values of the control treatment, a regression line was calculated by the method of least squares. From the regression line, $y = a + bx$, the D_{10}-value was calculated.

2.3 <u>Effect of irradiation in combination with frozen storage on the viability of Salmonella in shrimp</u>.

Shell on headless shrimp were inoculated with *Salmonella lexington* in the manner previously described. The inoculated samples were placed in the sterile bottle (approximately 100 g per unit) and kept at -18°C till irradiation. The frozen inoculated samples were irradiated in triplicate at 0, 2, 3, and 4 kGy and stored for a period of four months at -18°C. Salmonella counts were estimated after 1, 16, 30, 60, 90, and 120 days of frozen storage, using the most probable number technique.

2.4 <u>Sensory evaluation of the quality of irradiated frozen shrimp</u>.

Frozen shell on headless shrimp were prepared at fish processing plant using fresh shrimp of less than one-day-old shrimp from commercial source. The frozen samples were irradiated at 0.0, 2.2, and 4.2 kGy at ambient temperature. After irradiation, the frozen samples were stored at -18°C for a period of four months.

Flavor panel evaluations were carried out after 1, 2, 3, and 4 months using 13 staff members of the Division of Biological Science, Office of Atomic Energy for Peace and 14 staff members and students of the Department of Food Science, Kasetsart University. Frozen samples were thawed overnight at 4°C, soaked in 2.5 % brine solution for 5 minutes, and cooked in steam for 4 minutes. Cooked shrimp were served in coded dishes to judges. Each judges received three coded samples and one control sample as a reference. The control sample was also included as a coded sample. The judges were asked to evaluate the cooked shrimp for color, taste, odor, and texture by comparing each coded sample with reference sample according to multiple comparisons test described by Larmond[10]. The judges were also asked to evaluate the reference sample for color, odor, taste, and texture on a nine point desirability scale ranging from "9", highest affirmative value to, "1", lowest affirmative value.

The organoleptic test of irradiated frozen shrimp was also done by distributing the cooked meat derived from irradiated and non-irradiated frozen shrimp to about 32-46 panelists at Chieng Mai University in Chieng Mai, 30-45 panelists at Khon Kaen University in Khon Kaen, 38-47 panelists at Prince of Songkhla University in Songkhla, and 37-42 panelists at Srinakharinwirot University in Chonburi. The panelists were taken from students and staff members of the universities concerned. Each panelists received three coded samples and one reference sample to be evaluated whether there is any difference in color, odor, taste, and texture by comparing each coded sample with reference sample according to multiple comparisons test. This organoleptic test was done twice. The first trial was done using frozen samples after one month of storage. For the second trial, four month frozen storage samples were used.

3. RESULTS AND DISCUSSION

3.1 Bacteriological analysis of irradiated and non-irradiated frozen shrimp.

Results of bacteriological examination of 326 samples of frozen shrimp are shown in Table 3.1.1. Total bacterial counts of the samples from frozen shrimp ranged from 8.30×10^4 to 6.50×10^7 organisms per g, and the highest distribution frequency was between 10^5 and 10^6 organisms per g. Irradiation at 2 and 3 kGy reduced the count by 1-2 log cycles. 93 per cent of frozen shrimp samples was contaminated with Enterobacteriaceae, most of them containing less than 10^4 per g. The Enterobacteriaceae flora of frozen shrimp consisted of *Escherichia coli*, Enterobacter, Klebsiella, Citrobacter, Providencia, and Proteus spp. Irradiation at 2 kGy eliminated Enterobacteriaceae as high as 96 per cent of the samples. A dose of 3 kGy is required for the complete elimination of Enterobacteriaceae in frozen shrimp.

Coliforms, *E. coli*, *V. parahaemolyticus*, and *S. aureus* were detected in 94, 71, 9 and 35 per cent of the frozen samples, respectively. However, only 1, 2, and 11 per cent of the samples had *V. parahaemolyticus*, *S. aureus*, and *E. coli* counts of over 10 per g. After irradiation with 3 kGy, no detectable number of *E. coli*, *V. parahaemolyticus*, and *S. aureus* was found in frozen shrimp. *V. parahaemolyticus* is known to be common contaminants in seafood. It was found that a dose of 2 kGy was sufficient for the elimination of this organism.

Salmonella was not detected in all samples examined. However, an examination of frozen shrimp by Department of Medical Sciences during 1981-1982 showed 1-2 per cent positive for Salmonella. The prevalent species of Salmonella were found to be *S. typhimurium* (4,5,12 : i; 1,2), *S. lexington* (3,10 : Z_{10}, 1,5), and *S. agona* (4,12 : f,g,s).[2]

3.2 Radiation survival of Salmonella in shrimp.

A number of experiments on cell survival were carried out with *S. lexington*, *S. agona*, *S. typhimurium*, *S. derby*, and *S. anatum*. The data on cell survival in the artificially inoculated shrimp at refrigerated and frozen state before and after irradiation with various doses is given in Tables 3.2.1 and 3.2.2. The effect of gamma radiation on Salmonella in refrigerated and frozen shrimp followed the linear log number/dose relationship. D_{10} - values ranged from 0.30 to 0.48 kGy for refrigerated contaminated shrimp and from 0.42 to 0.59 kGy for frozen inoculated shrimp.

It is obvious from the survival data that Salmonella is more resistant in frozen shrimp than in refrigerated shrimp. A greater destruction of cell was also observed when comminuted chicken inoculated with *S. oranienburg* was irradiated in the non frozen state (0°C) compared with the frozen state (-18°C).[11] Among Salmonella serotypes found in frozen shrimp, *S. lexington* was the most resistant, *S. typhimurium* and *S. agona* were intermediate, and *S. anatum* was the least resistant. Taking the resistance of *S. lexington* as a reference, an irradiation dose of at least 4.2 kGy is required for a seven log cycle reduction of Salmonella contamination in frozen shrimp. Since the number of viable Salmonella occurring in most foods and animal feeds was seldom found to exceed 1/g,[4] the calculated dose of 4.2 kGy would be amply safe for the elimination of Salmonella in frozen shrimp.

3.3 Effect of irradiation in combination with frozen storage on the viability of Salmonella in shrimp.

Since in actual practice it was more likely that a frozen shrimp would be stored frozen for some time, it was decided to compare the effect of storage on the viability of Salmonella in irradiated and non-irradiated frozen shrimp. Frozen inoculated shrimp were irradiated at several dose levels and then stored at -18°C for four months. The result of a study using *S. lexington* as a test organism is shown in Table 3.3.1.

Irradiation doses of 3 and 4 kGy reduced the Salmonella count from an initial number of 10^7/g to less than 10/g. A further decrease in the count was observed during an extended frozen storage at -18°C. There also appeared to be a decrease in the Salmonella count for non-irradiated samples. Therefore, irradiated Salmonella are not more susceptible to freezing injury during frozen storage than non-irradiated Salmonella. However, this result demonstrates an added benefit of frozen storage in reducing the numbers of Salmonella in irradiated frozen shrimp to a level beyond that can be detected by the normally accepted procedures.

3.4 Sensory evaluation of the quality of irradiated frozen shrimp.

Flavor panels were carried out after 1,2,3, and 4 months of frozen storage to evaluate the effects of gamma irradiation and the interrelationship of gamma irradiation and storage time on frozen shrimp quality. Mean scores for the color, odor, taste, and texture of frozen shrimp with or without radiation treatment are listed in Tables 3.4.1, 3.4.2, 3.4.3 and 3.4.4, respectively.

Treatment of frozen shrimp with 2.2 kGy did not affect the color, taste, and texture but did affect the odor of frozen shrimp. The difference in quality was more pronounced after a 4.2 kGy treatment. Mean scores for the odor and taste of cooked meat derived from the treated samples were significantly ($p \leq 0.05$) lower than those observed for non-treated samples after 1 and 2 months of frozen storage. However, no significant difference in scores was observed during subsequent frozen storage. Radiation-induced changes in odor[12] and its diminishment during storage[13] were also observed in shrimp irradiated at dose above 2.5 kGy. Although factorial analysis showed no significant difference in color and texture between treated and non-treated shrimp, scores for treated samples were somewhat reduced from those observed for non-treated samples at every periods of frozen storage. Loss in color and change in texture have been found in irradiated shrimp and were brought about by the degradation of astaxanthine and proteins.[14-15] Storage of frozen shrimp at -18°C for various time periods did not affect the quality of the product. Doses of irradiation did not interact significantly with storage time with regard to all sensory factors.

Cooked meat derived from control samples did not show a significant difference in mean scores for the color, odor, taste, and texture during a 4-month frozen storage period (Table 3.4.5). However, the sensory scores decreased with increasing storage time.

Result of organoleptic evaluation of cooked meat derived from irradiated and non-irradiated frozen shrimp conducted in Chieng Mai, Khon Kaen, Songkhla, and Chonburi is shown in Table 3.4.6. This organoleptic test reflects more or less consumer quality. Again, significant differences in odor and taste scores between treated and non-treated samples were observed. This was particularly true for a 4.2 kGy treatment. It is obvious from the result that variation in the acceptability of irradiated shrimp exists among consumer from different localities. Therefore, consumer acceptance of the product should be relied on marketing trial at areas where the irradiated frozen shrimp are to be introduced.

4. CONCLUSIONS

Frozen shrimp samples from fish processing plants were irradiated at 2 and 3 kGy. Irradiated and non-irradiated samples were analyzed for their bacteriological quality. Irradiation dose of 3 kGy appeared to be effective in eliminating Enterobacteriaceae, Escherichia coli, Vibrio parahaemolyticus, and Staphylococcus aureus from naturally contaminated frozen shrimp and, in addition, reduced the total viable bacterial count by 2 log cycles. Coliform was not completely eliminated. The remaining count was less than 10 cell/g and considered to be non-significant in term of bacteriological quality. Salmonella was not detected in either irradiated or non-irradiated frozen shrimp.

A number of experiments on cell survival were carried out with several Salmonella serotypes in refrigerated inoculated shrimp and frozen inoculated shrimp. Salmonella resistance was lower in refrigerated inoculated shrimp than in frozen inoculated shrimp. D_{10}-values ranged from 0.30 to 0.48 kGy for refrigerated inoculated shrimp and from 0.42 to 0.59 kGy for frozen inoculated shrimp. Based on the D_{10}-value obtained, it was calculated that an irradiation dose of at least 4.2 kGy is required for a seven log cycle reduction of Salmonella in frozen shrimp. Frozen storage reduced the viability of Salmonella in inoculated shrimp by 2 log cycles within 120 days of storage at -18°C. Irradiated Salmonella are not more susceptible to freezing injury during frozen storage than non-irradiated Salmonella.

The sensory quality of frozen shrimp tended to decrease during frozen storage but was within the acceptable range on a nine point desirability scale even after four months of frozen storage. Application of gamma irradiation at 2.2 kGy did not significantly affect the color, taste, and texture but did affect the odor of frozen shrimp. The difference in quality was more pronounced after a 4.2 kGy treatment but it dissipated during subsequent storage at -18°C. Storage of irradiated frozen shrimp at -18°C for four months did not significantly affect the quality of the product. A difference in acceptance of irradiated frozen shrimp was noted among consumer at different localities.

ACKNOWLEDGEMENTS

This work was supported in part by the International Atomic Energy Agency, Research Contract No. 2809/R1/RB and No. 2809/R2/RB. The authors are indebted to the colleagues of the Biological Sciences Division and students and staff members of Kasetsart University, Chieng Mai University, Khon Kaen University, Prince of Songkhla University and Srinakharinwirot University, who voluntarily served as panel members for the sensory evaluation. Special thanks are given to the Division of Radiation Measurement for dose measurement and to the Division of Clinical Pathology, Department of Medical Sciences, for the identification of *V. parahaemolyticus* and Salmonella serotyping.

TABLE 3.1.1 Bacteriological examination of non-irradiated and irradiated frozen shrimp samples.

Organisms	Dose (kGy)	No. of samples	No. of organisms per g
Total bacterial count	0	127	8.30×10^4 - 6.50×10^7
	2	107	1.44×10^3 - 1.68×10^6
	3	38	2.65×10^2 - 5.60×10^5
Enterobacteriaceae	0	159 (11)*	ND - 5.80×10^5
	2	129 (124)	ND - 2.75×10^2
	3	38 (38)	ND
Coliform	0	157 (10)	ND - 2.40×10^4
	2	129 (95)	ND - 2.30
	3	38 (34)	ND - 2.30
Faecal coliform	0	157 (26)	ND - 2.40×10^4
	2	129 (124)	ND - 0.90
	3	38 (38)	ND
Escherichia coli	0	157 (45)	ND - 2.80×10^2
	2	129 (127)	ND - 0.90
	3	38 (38)	ND
Salmonella	0	159 (159)	ND
	2	129 (129)	ND
	3	38 (38)	ND
Vibrio parahaemolyticus	0	153 (139)	ND - 1.10×10^2
	2	129 (129)	ND
	3	38 (38)	ND
Staphylococcus aureus	0	144 (93)	ND - 7.00×10^1
	2	107 (87)	ND - 2.80
	3	38 (38)	ND

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

ND = Not detectable

TABLE 3.2.1 Survival of various Salmonella serotypes in artificially contaminated shrimp at refrigerated state.

Type		Log number of Salmonella per g							D_{10}-value (kGy)
		Radiation dose (kGy)							
		0.0	0.3	0.6	0.9	1.2	1.5	1.8	
S. agona	1	8.06	7.02	6.28	5.52	4.76	4.09	3.23	0.40
	2	8.16	6.23	5.26	4.85	4.06	3.39	2.94	0.46
S. anatum	1	8.09	6.37	5.75	4.87	4.18	3.37	2.63	0.36
	2	8.09	6.14	5.21	4.69	3.82	3.00	2.15	0.38
S. derby	1	8.90	8.44	7.36	6.34	5.29	4.45	3.35	0.30
	2	7.90	6.20	5.41	4.56	3.69	3.01	2.39	0.39
S. lexington	1	8.25	6.82	6.02	5.38	4.66	3.68	2.97	0.39
	2	8.08	6.23	5.36	4.52	3.84	3.11	2.48	0.40
S. typhimurium	1	8.59	7.47	6.75	5.93	5.28	4.82	3.80	0.42
	2	7.95	6.25	5.58	4.87	4.27	3.69	3.18	0.48

1 Peeled shrimp.

2 Shell on headless shrimp.

TABLE 3.2.2 Survival of various Salmonella serotypes in artificially contaminated shrimp at frozen state.

Type		Log number of Salmonella per g							D_{10}-value
		Radiation dose (kGy)							
		0.0	0.3	0.6	0.9	1.2	1.5	1.8	(kGy)
S. Agona	1	8.31	6.39	5.74	5.18	4.71	3.98	3.61	0.53
	2	8.08	7.02	6.55	6.20	5.31	4.50	-	0.48
S. anatum	1	8.00	7.25	6.48	5.53	4.74	4.51	4.20	0.48
	2	7.84	6.94	5.94	5.45	4.50	4.20	3.24	0.42
S. derby	1	8.06	6.96	6.20	5.72	4.84	4.47	3.75	0.47
	2	8.55	8.10	7.83	7.08	5.98	5.35	4.99	0.43
S. lexington	1	8.57	7.26	6.65	6.15	5.61	5.19	4.53	0.56
	2	7.85	7.06	6.41	6.13	5.99	5.11	4.30	0.59
S. typhimurium	1	8.58	7.65	7.23	6.92	5.96	5.23	4.70	0.48
	2	8.27	7.19	6.48	6.13	5.52	4.94	4.39	0.55

1 Peeled shrimp.

2 Shell on headless shrimp.

TABLE 3.3.1 Survival of *Salmonella lexington* in irradiated and non-irradiated frozen shrimp during storage at -18°C.

Storage time (days)	Survivors per g			
	0 kGy	2 kGy	3 kGy	4 kGy
1	2.29×10^7	2.50×10^2	2.85	1.34
16	4.32×10^6	6.68×10	0.90	ND
30	1.34×10^6	4.50×10	ND	ND
60	2.36×10^6	6.05	0.75	ND
90	1.11×10^5	1.05	ND	ND
120	1.55×10^5	1.20	ND	ND

ND = Not detectable

TABLE 3.4.1 Mean[1] color scores for cooked meat derived from non-irradiated and irradiated frozen shrimp stored at $-18^{\circ}C$ for various time periods.

Storage time	Irradiation dose (kGy)		
(months)	0	2.2	4.2
1	4.96	4.82	4.70
2	5.52	4.82	4.74
3	5.11	5.30	5.26
4	5.11	5.00	4.56

	Factorial analyses	
	F-value	Ranking of level means
Irradiation dose (D)	2.230 [2]	0 > 2.2 > 4.2
Storage time (T)	1.568 [2]	3 > 2 > 4 > 1
D x T	0.948 [2]	

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[1] n = 27

[2] N.S. $p \geq 0.05$

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

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

TABLE 3.4.2 Mean[1] odor scores for cooked meat derived from non-irradiated and irradiated frozen shrimp stored at -18°C for various time periods.

Storage time	Irradiation dose (kGy)		
(months)	0	2.2	4.2
1	5.59^{a}	4.63^{b}	4.18^{b}
2	5.44^{a}	4.85^{ab}	4.74^{b}
3	5.41^{a}	4.56^{b}	4.85^{ab}
4	5.11^{a}	4.70^{a}	4.56^{a}

	Factorial analyses	
	F-value	Ranking of level means
Irradiation dose (D)	14.547 [2]	$\underline{0} > \underline{2.2 > 4.2}$
Storage time (T)	0.659 [3]	$\underline{2 > 3 > 1 > 4}$
D x T	1.013 [3]	

[1] n = 27 [2] Sig. $p \leq 0.01$ [3] N.S. $p \geq 0.05$

Mean scores in a row (irradiation dose) with the same exponent letter did not vary significantly ($p = 0.05$).

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

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

TABLE 3.4.3 Mean[1] taste scores for cooked meat derived from non-irradiated and irradiated frozen shrimp stored at -18°C for various time periods.

Storage time (months)	Irradiation dose (kGy) 0	2.2	4.2
1	5.22^{a}	4.78^{ab}	4.41^{b}
2	5.48^{a}	5.00^{ab}	4.70^{b}
3	4.96^{a}	4.41^{a}	4.48^{a}
4	4.70^{a}	5.04^{a}	4.70^{a}

	Factorial analyses F-value	Ranking of level means
Irradiation dose (D)	5.045 [2]	0 > 2.2 > 4.2
Storage time (T)	1.865 [3]	2 > 4 > 1 > 3
D x T	1.113 [3]	

[1] n = 27

[2] Sig. $p \leq 0.01$

[3] N.S. $p \geq 0.05$

Mean scores in a row (irradiation dose) with the same exponent letter did not vary significantly ($p = 0.05$).

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

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

TABLE 3.4.4 Mean[1] texture scores for cooked meat derived from non-irradiated and irradiated frozen shrimp stored at -18°C for various time periods.

Storage time (months)	Irradiation dose (kGy)		
	0	2.2	4.2
1	5.04	4.96	5.04
2	5.37	5.22	5.22
3	5.07	5.00	4.74
4	5.15	4.89	4.85

	Factorial analyses	
	F-value	Ranking of level means
Irradiation dose (D)	0.818 [2]	$\underline{0 > 2.2 > 4.2}$
Storage time (T)	1.438 [2]	$\underline{2 > 1 > 4 > 3}$
D x T	0.175 [2]	

[1] n = 27

[2] N.S. $p \geq 0.05$

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

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

TABLE 3.4.5 Mean[1] and analysis of variance of flavor panel scores for cooked meat derived from frozen shrimp stored at -18°C for various time periods.

Sensory factor	Storage time (months)				F-value	Factor means ranking[2]
	1	2	3	4		
Color	7.56	7.33	7.52	7.11	1.684[3]	1 > 3 > 2 > 4
Odor	7.41	7.07	7.11	6.93	1.356[3]	1 > 3 > 2 > 4
Taste	7.48	7.00	7.04	6.96	1.824[3]	1 > 3 > 2 > 4
Texture	7.70	7.11	7.22	6.96	2.594[3]	1 > 3 > 2 > 4

[1] n = 27 judgements.

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

[3] Non significant $p \geq 0.05$.

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

TABLE 3.4.6 Mean flavor panel scores for cooked meat derived from non-irradiated and irradiated frozen shrimp.

Duration (months) / Dose (kGy)		color		odor		taste		texture	
		1	4	1	4	1	4	1	4
Chieng Mai	0	$4.91^{a}\pm1.07$	$5.59^{a}\pm1.34$	$4.96^{a}\pm1.01$	$5.19^{a}\pm1.26$	$4.63^{a}\pm0.95$	$5.12^{a}\pm1.62$	$4.93^{a}\pm1.08$	$5.19^{a}\pm1.42$
	2.2.	$5.06^{a}\pm1.10$	$5.19^{a}\pm1.47$	$4.91^{a}\pm0.98$	$5.41^{a}\pm1.04$	$5.02^{a}\pm1.00$	$5.06^{a}\pm1.19$	$5.09^{a}\pm1.09$	$4.97^{a}\pm1.31$
	4.2	$5.17^{a}\pm1.50$	$4.94^{a}\pm1.46$	$4.63^{a}\pm1.68$	$4.22^{a}\pm1.54$	$4.83^{a}\pm1.40$	$4.50^{a}\pm1.63$	$4.61^{a}\pm1.02$	$5.06^{a}\pm1.68$
Khon Kaen	0	$4.95^{a}\pm1.36$	$5.37^{a}\pm1.59$	$4.89^{a}\pm1.40$	$5.37^{a}\pm1.38$	$5.13^{a}\pm1.16$	$5.13^{a}\pm1.68$	$4.96^{a}\pm0.77$	$5.13^{a}\pm1.33$
	2.2	$4.84^{a}\pm1.69$	$4.83^{a}\pm1.15$	$4.36^{ab}\pm1.54$	$4.70^{ab}\pm1.29$	$4.51^{b}\pm1.12$	$4.57^{a}\pm1.14$	$4.33^{b}\pm1.02$	$4.50^{b}\pm1.17$
	4.2	$4.80^{a}\pm1.55$	$4.70^{a}\pm1.42$	$4.09^{b}\pm1.73$	$4.53^{b}\pm1.65$	$4.64^{ab}\pm1.40$	$4.63^{a}\pm1.38$	$4.87^{a}\pm1.18$	$4.60^{ab}\pm1.22$
Chonburi	0	$5.31^{a}\pm1.52$	$5.38^{a}\pm1.77$	$5.02^{a}\pm1.50$	$5.27^{a}\pm1.58$	$5.29^{a}\pm1.42$	$5.27^{a}\pm1.30$	$5.21^{a}\pm1.58$	$5.57^{a}\pm1.52$
	2.2	$4.76^{a}\pm1.94$	$5.57^{a}\pm1.62$	$4.29^{a}\pm1.49$	$5.24^{a}\pm1.26$	$4.76^{a}\pm1.72$	$5.11^{a}\pm1.31$	$4.69^{a}\pm1.46$	$5.22^{ab}\pm1.55$
	4.2	$4.98^{a}\pm1.52$	$5.11^{a}\pm1.52$	$4.50^{a}\pm1.82$	$5.05^{a}\pm1.65$	$4.67^{a}\pm1.75$	$4.95^{a}\pm1.73$	$5.05^{a}\pm1.70$	$4.86^{b}\pm1.64$
Songkhla	0	$5.06^{a}\pm1.39$	$5.34^{a}\pm1.80$	$4.70^{a}\pm1.59$	$4.95^{a}\pm1.75$	$4.26^{a}\pm1.55$	$4.76^{a}\pm1.58$	$4.42^{a}\pm1.62$	$4.74^{a}\pm1.20$
	2.2	$4.79^{a}\pm1.27$	$5.03^{a}\pm1.82$	$4.40^{a}\pm1.41$	$4.50^{a}\pm1.27$	$4.87^{a}\pm1.31$	$4.60^{a}\pm1.60$	$5.15^{a}\pm1.28$	$4.76^{a}\pm1.48$
	4.2	$4.55^{a}\pm1.52$	$5.31^{a}\pm1.79$	$4.36^{a}\pm1.52$	$4.47^{a}\pm1.97$	$4.49^{a}\pm1.59$	$4.84^{a}\pm1.40$	$4.47^{a}\pm1.57$	$4.89^{a}\pm1.54$

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

Mean scores in a column (irradiation dose) with the same exponent letter did not vary significantly ($p = 0.05$).

REFERENCES

1. Thailand. Ministry of Commerce. Department of Business Economics. Directions and Trend of Export of Frozen Shrimp in 1983-4, 1983.

2. Thailand. Ministry of Public Health. Department of Medical Sciences. 1982.

3. Thailand. Ministry of Commerce. Department of Foreign Trade. Foreign Trade Policy Division. 1982.

4. MOSSEL, D.A.A.; VAN SCHOTHORST, M. and KAMPELMACHER, E.H. (1968). Prospects for the Salmonella radicidation on some foods and feeds with particular reference to the estimation of the dose required. In Elimination of harmful organisms from food and feed by irradiation, p. 43-57, IAEA, Vienna.

5. KAHAN, R.S. (1978). Low-dose irradiation of fresh, non frozen chicken and other preservation methods for shelf-life extension and for improving its public health quality. In Food preservation by irradiation Vol. 2, p. 221-242, IAEA, Vienna.

6. OUWERKERK, T. (1981). Salmonella control in poultry through the use of gamma irradiation. In Combination process in food irradiation, p. 335-345, IAEA, Vienna.

7. LEY, F.J. (1968). Ionizing radiation for the elimination of Salmonella from frozen meat. In Elimination of harmful organisms from food and feed by irradiation, p. 29-35, IAEA, Vienna.

8. INTERNATIONAL ATOMIC ENERGY AGENCY. (1970) Microbiological specifications and testing methods for irradiated food, Technical Report Series No. 104, IAEA, Vienna.

9. SPECK, M.L. (1976). Compendium of methods for the microbiological examination of foods, 701 p., American Public Health Association, Inc., Washington, D.C.

10. LARMOND, E. (1977). Laboratory methods for sensory evaluation of foods, Publication 1637, Canada Department of Agriculture, Canada.

11. LICCIARDELLO, J.J.; NICKERSON, J.T.R. and GOLDBLITH, S.A. (1968). Elimination of Salmonella in poultry with ionizing radiation. In Elimination of harmful organisms from food and feed by irradiation, p. 1-28, IAEA, Vienna.

12. KUMTA, U.S.; MAVINKURVE, S.S.; GORE, M.S.; SAWANT, P.L.; GANGAL, S.V. and SREENIVASAN, A. (1970). Radiation pasteurization of fresh and blanched tropical shrimps. J. of Food Sci. 35 : 360-363.

13. AWAD, A.A.; SINHUBER, R.O. and ANDERSON, A.W. (1965). Radiation pasteurization of raw and chlortetracycline-treated shrimp. Food Technol. 19 : 864.

14. LUKTEN, A. and MACKINNEY, G. (1956). Effect of ionising radiation on carotenoid stability. Food Technol. 10 : 630.

15. KUMTA, U.S. and SREENIVASON, A. (1967). Radiation preservation of seafood. Food Irrad. 7(4) : 10.