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Abstract

Fresh crab meat from swimming crab (Portunus pelagicus, Linn.) was irradiated at doses of 0.075, 0.15 and 0.25 Mrad. and held at 3°C. The storage life of nonirradiated crab meat was approximately 7 days compared with 14 days for crab meat irradiated at 0.075 Mrad and 28 days for samples receiving 0.15 or 0.25 Mrad treatment. Total aerobic count, trimethylamine nitrogen, total volatile basic nitrogen, and ammonia contents were used as objective indices of freshness in comparison with sensory evaluation of the crab meat. All objective indices correlated well with the sensory judgement of the samples. The crab meat used in the study was heavily contaminated with microorganisms. Irradiation at 0.15 and 0.25 Mrad reduced approximately 2 log cycles in the total count. Acinetobacter (Achromobacter) was predominated in irradiated crab meat, especially after prolonged storage. High coagulase positive staphylococci count was detected in only nonirradiated crab meat.

The total production of seafoods in Thailand is estimated to be approximately one million metric tons per year. A significant portion of the catch comprises of sea crabs of which the majority are swimming crabs (Portunus pelagicus, Linn) and Blue crabs (Scylla serrata, Forskal). Swimming crabs are usually caught by trawlers. The catch of swimming crabs by far outweight that of Blue crabs. As soon as swimming crabs are caught, they are dumped into the fish hold and mixed with ice. The crabs may remain in a trawler as long as 10 days before landing.

Locally, the crabs are cooked by boiling whole in a brine solution. After cooking, the meat is picked by hand and packed in the polyethylene bag at approximately one kilogram per bag. There is no classification of the meat picked into different grades in this country; in other words, the meat from different parts of the body are packed together in the same bag.

There is no regulation in the term of the sanitary practice in picking crab meat. The packed crab meat bags are chilled in ice during distribution and marketing. Fortunately, most of the crab meat picking places are relatively near to Bangkok. The time lapse between meat picking and marketing is not great. However, fresh crab meat has relatively short shelf-life of approximately 2-3 days in ice. Hence, there is practically no distribution of crab meat into inland markets i.e.to the North or to the Northeast.

For these reasons, this study was undertaken to search for means to increase the shelf-life of fresh crab meat by exposing it to gamma radiation.

Several workers, especially those in the U.S.A., reported that gamma irradiation could extend the storage life of fresh crab meat significantly. Miyauchi et. al. (1965) found that a dose range of 0.1 to 0.2 Mrad would produce a shelf-life of Dungeness crab meat at 33 F for 2-5 weeks in a laboratory scale studies. Goldblith and Nickerson (1966) indicated that irradiation of crab meat with low aerobic bacterial count at 125 Krad while holding at 32°F was a good method of extending the refrigerated storage life. In addition, they found that spoilage of lobster meat or crab meat because of the growth of anaerobic bacteria during refrigerate storage has never been observed during tests made on numerous samples and no significant increase in clostridia had been observed in these samples. These findings agree with that of Miyauchi et al. (1966) who found that vacuum packing and nonvacuum packing of crab meat in C-enameled cans were equally effective in extending the shelf-life of radiation-pasteurized crab meat. However, they found that storage life of the crab meat at 33°F was almost a double of that at 42°F. Reber et al. (1968) reforted that the protein efficiency ratio of crab meat irradiated at substerilizing doses was statistically equal to that of nonirradiated crab meat.

This paper reports the effect of gamma irradiation on the storage life of fresh crab meat held at 3°C. The following criteria were investigated :

1. The determination of the effect of low dose irradiation of 0.075, 0.15 and 0.25 Mrad on the spoilage characteristics of crab meat held at 3°C.

2. The determination of the bacteriological spoilage pattern of nonirradiated and irradiated crab meat through bacterial counts and characterization of the more prevalent bacteria in the product.

3. The determination of the correlation between bacterial count, trimethylamine nitrogen (TMA-N), total volatile basic nitrogen (TVB-N), ammonia content, and sensory evaluation in nonirradiated and irradiated crab meat.

Materials and Methods

Fresh crab meat packed in polyethylene bags, approximately one kilogram per bag, was obtained from the Fish Market Organization. The meat was transported in ice to the laboratory at the Biological Science Division where the meat was repacked into smaller polyethylene bags, each bag contained 200 g. of crab meat. The bags were then heat sealed at atmospheric pressure.

The crab meat in bags were divided into 4 lots : one lot was assigned as nonirradiated control; the remaining three lots were each subjected to gamma irradiation by the "Gamma-beam 650" at doses of 0.075, 0.15 and 0.25 Mrad. The samples were irradiated in ice in a rectangular metal container of $9\frac{1}{2}$ x 13 x 7 in dimension. The dose rate was determined by the Fricke dosimetry.

After irradiation, all 4 lots of samples were stored in a walk in cooler with constant temperature of 3°C. Every week, a sample was drawn from each lot to determine bacterial count, chemical analyses, and sensory evaluation.

Bacteriological analyses

(i) Quantitative bacteriological analyses

Approximately 20 g. of crab meat was taken aseptically from each sample and placed into a sterile blendor jar. A 180 ml of sterile 0.1 % peptone water was added and blended for a few minutes.

The total aerobic count was determined according to the procedure described in the American Public Health Association-Standard Method for the Examination of Dairy Products (1960). Serial dilutions were made from the crab meat in 0.1 % peptone water. Duplicate plates were plated in Tryptone glucose extract agar (Difco) using pour plate and were incubated at 20°C and 35°C.

In addition, the most probable number (MPN) of coliform, faecal coliform, and E. coli in each sample were determined according to procedure described in the International Atomic Energy Agency-Microbiological Specifications and Testing Methods for Irradiated Foods(1970).

(ii) Qualitative bacteriological analyses

Representative colonies to be identified were subsequently isolated from countable dilution plates. As many colonies showing different morphological characteristics and pigments were taken from each plate. Isolated colonies were subcultured and characterized by the following tests : gram stain, cell morphology, pigmentation, oxidative-fermentative test (Hugh and Leifson, 1953), spore stain, and gelatin liquefaction.

The number of coagulase positive staphylococci in each sample of crab meat was enumerated by surface plating of appropriate dilutions on Baird-Parker Agar. Suspected colonies of coagulase positive Staphylococci were confirmed by coagulase test using coagulase plasma (Difco).

Chemical Analyses

TMA-N and TVB-N contents of the crab meat were determined by Dyer's colorimetric method (Dyer, 1959) and by method described by Farber and Ferro (1956) using conway microdiffusion units respectively. Ammonia content of the samples was determined by the method of Burnett (1965).

Sensory evaluation

Organoleptic properties of cooked crab meat were judged subjectively by an experienced panel of 10 members. Each sample of crab meat was first cooked by dipping in boiling 1 % brine solution for 2 minutes. The samples were prepared and served to the panel according to the procedure described by Larmond (1967). The panel was then asked to evaluate the quality of the samples as to odor and taste. Nine point hedonic scale was used for the quality evaluation. A score of 9 being the best imaginable quality; 1 being the worst immaginable; and 5 being the borderline of acceptability.

Results

Sensory evaluation

Results of organoleptic properties as to odor and taste of nonirradiated crabmeat and of crabmeat irradiated at different doses are shown in Table 1. It was found that nonirradiated crab meat could be kept in good quality for about 7 days at 3°C. The crab meat irradiated at 0.075, 0.15 and 0.25 Mrad could be kept for approximately 14, 21 and 28 days respectively. When the crab meat were kept for these length of time, the odor and taste scores of the samples were in the likeness catagory as judged by the panel. It appears that, judging by sensory evaluation of the product alone, crab meat irradiated at 0.15 and 0.25 Mrad could be kept in good quality 3 times longer than that of nonirradiated counterpart.

Table 1. Organoleptic scores* of crab meat irradiated at different doses and stored at 3°C.

Storage Time (Days)	Dose (Mrad)							
	0 (control)		0.075		0.15		0.25	
	odor	taste	odor	taste	odor	taste	odor	taste
1	7.8	7.6	7.2	7.2	7.2	7.4	6.9	7.5
7	6.9	7.5	6.8	7.2	7.1	7.2	7.0	7.6
14	4.2	4.1	6.4	6.2	7.0	7.3	6.8	7.1
21	-**	-	5.7	5.5	6.9	6.3	6.7	7.0
28	-	-	5.5	5.3	6.7	7.0	6.6	6.7
35	-	-	4.9	4.2	5.3	4.2	5.5	5.8
42	-	-	-	-	3.7	4.2	5.0	4.5

* score of 6-9 : increasing ordor in likeness.
5 : borderline of acceptability.
4-1 : increasing ordor in dislikeness.

** sample was too spoiled.

Chemical Analyses

Results on determinations of TMA-N, TVB-N, and ammonia contents of each sample are shown in Tables 2, 3, and 4 respectively. The results indicated that TMA-N, TVB-N, and NH_3 contents serves as useful indices of quality of both nonirradiated and irradiated crabmeat. Their values increased with increasing storage time and with decreasing in quality attributes as judged organoleptically. In addition, the values of TMA-N, TVB-N, and NH_3 contents of the nonirradiated crab meat at the time of spoilage were within the same range as those of irradiated crab meat of the same quality.

Table 2. Trimethylamine content (mg/100g) of crab meat irradiated at different doses and stored at 3°C.

Storage Time (Days)	Dose (Mrad)			
	0 (Control)	0.075	0.15	0.25
1	1.4	2.9	1.6	1.0
7	23.5	4.0	2.1	7.7
14	63.7	15.5	12.2	14.3
21	-*	35.8	29.1	20.9
28	-	50.1	35.3	34.0
35	-	48.0	45.2	45.2
42	-	-	48.5	51.7

-* sample was too spoiled.

Table 3. Total volatile basic nitrogen content (mg/100g) of crab meat irradiated at different doses and stored at 3°C.

Storage Time (Days)	Dose (Mrad)			
	0 (Control)	0.075	0.15	0.25
1	21.3	35.1	19.4	13.7
7	64.0	35.6	26.0	35.0
14	127.0	47.9	34.5	32.9
21	-*	114.7	50.2	47.7
28	-	152.2	86.5	92.4
35	-	168.0	118.1	124.0
42	-	-	163.8	127.0

-* sample was too spoiled.

Table 4. Ammonia content (µg/g) of crab meat irradiated at different doses and stored at 3°C.

Storage Time (Days)	Dose (Mrad)			
	0 (Control)	0.075	0.15	0.25
1	14.4	25.1	14.0	15.5
7	33.8	19.0	14.8	19.8
14	87.7	24.1	27.2	19.0
21	-*	57.3	45.5	37.9
28	-	173.4	56.9	58.0
35	-	-	135.7	121.8
42	-	-	161.0	155.0

-* sample was too spoiled.

Bacteriological analyses

Results on total aerobic bacterial counts of crab meat irradiated at different doses and incubated at 20°C and 35°C are shown in Table 4 and 5 respectively.

Table 4. Total aerobic bacterial counts (colonies/g) of crab meat irradiated at different doses. Plates were incubated at 20°C.

Storage Time (Days)	Dose (Mrad)			
	0 (Control)	0.075	0.15	0.25
1	4.6×10^7	1.4×10^6	7.2×10^5	1.1×10^5
7	2.7×10^8	4.8×10^7	1.3×10^7	9.2×10^5
14	2.8×10^8	9.3×10^7	1.4×10^7	2.6×10^6
21	-*	5.4×10^8	7.6×10^7	1.2×10^7
28	-	1.1×10^9	1.3×10^8	6.2×10^7
35	-	-	2.6×10^8	1.2×10^8

-* sample was too spoiled.

Table 5. Total aerobic bacterial counts (colonies/g) of crab meat irradiated at different doses. Plates were incubated at 35°C.

Storage Time (Days)	Dose (Mrad)			
	0 (Control)	0.075	0.15	0.25
1	4.4×10^7	6.4×10^5	3.7×10^5	2.3×10^5
7	8.8×10^7	3.6×10^6	4.7×10^5	2.1×10^5
14	7.9×10^8	1.3×10^7	5.6×10^6	1.0×10^6
21	-*	1.8×10^8	1.7×10^7	1.0×10^7
28	-	4.3×10^8	1.7×10^8	9.1×10^7
35	-	-	1.4×10^9	3.1×10^8

-* sample was too spoiled.

It was found that fresh nonirradiated crab meat contained excessively high bacterial counts. Irradiation at 0.15 and 0.25 Mrad appeared to reduce the microbial load down approximately 2 log cycles. At the time of spoilage, as judged organoleptically, both nonirradiated and irradiated crab meat contained more than 10^8 bacteria per gram.

Results on the enumeration of coagulase positive staphylococci in crab meat are shown in Table 6. Only nonirradiated crab meat contained high coagulase positive staphylococci count. A small count of the staphylococci was found in 0.075 Mrad sample only at one day after irradiation. No coagulase positive staphylococci was detected in crab meat irradiated at 0.15 and 0.25 Mrad.

Table 6. Incidence of coagulase positive staphylococci (colonies/g) in crab meat.

Storage Time (Days)	Dose (Mrad)			
	0 (Control)	0.075	0.15	0.25
1	2.8×10^5	6.3×10^2	-	-
7	1.7×10^5	-	-	-
14	1.1×10^6	-	-	-

- not detectable.

Relatively few genera of bacteria were found to predominate in crab meat. Staphylococcus, Acinetobacter (Achromobacter), Corynebacterium, and Micrococcus were predominant in nonirradiated crab meat and in the samples irradiated at 0.075 and 0.15 Mrad. Only Acinetobacter (Achromobacter), Micrococcus, and Bacillus were predominant in the crab meat irradiated at 0.25 Mrad. The results are illustrated in Table 7.

Table 7. The predominated bacteria in crab meat irradiated at different doses and stored at 3°C.

Storage Time (Days)	Dose (Mrad)			
	0 (Control)	0.075	0.15	0.25
1	Staphylococcus Acinetobacter (Achromobacter) Corynebacterium Enterobacter	Acinetobacter (Achromobacter) Micrococcus Corynebacterium	Micrococcus Corynebacterium Acinetobacter (Achromobacter	Micrococcus Acinetobacter (Achromobacter)
7	Acinetobacter (Achromobacter) Micrococcus Enterobacter	Acinetobacter (Achromobacter) Corynebacterium	Acinetobacter (Achromobacter) Bacillus	Acinetobacter (Achromobacter) Bacillus
14	Corynebacterium Acinetobacter (Achromobacter) Bacillus	Acinetobacter (Achromobacter) Corynebacterium	Acinetobacter (Achromobacter) Bacillus Corynebacterium	Acinetobacter (Achromobacter) Bacillus
21	-	Acinetobacter (Achromobacter) Micrococcus	Acinetobacter (Achromobacter)	Acinetobacter (Achromobacter)
28	-	-	Acinetobacter (Achromobacter)	Acinetobacter (Achromobacter)

Coliform, faecal coliform, and E. coli were found in only nonirradiated crab meat. Relatively low number of coliform were detected in crab meat irradiated at 0.075 Mrad, as shown in Table 8.

Table 8. MPN/g of coliform, faecal coliform, and E. coli in crab meat.

Storage Time (Days)	Coliform				Faecal coliform				E. coli			
	C	0.075 Mrad	0.15 Mrad	0.25 Mrad	C	0.075 Mrad	0.15 Mrad	0.25 Mrad	C	0.075 Mrad	0.15 Mrad	0.25 Mrad
1	119	0.9	-	-	2.1	-	-	-	2.1	-	-	-
7	184	-	-	-	24	-	-	-	9.3	-	-	-
14	210	-	-	-	-	-	-	-	-	-	-	-

- not detectable

Discussion

Crab meat used in this studies was heavily contaminated with microorganisms which probably resulted from poor sanitary practice during processing, meat picking, and marketing. Large number of Acinetobacter (Achromobacter) was detected in crab meat both before and after irradiation. Crab meat irradiated at 0.15 and 0.25 Mrad contained this organism almost exclusively, especially after certain period of storage. This finding agrees with Sinnhuber and Lee (1966) who found that the predominated microflora of crab meat irradiated at 0.1 and 0.4 Mrad were Achromobacter. The presence of this organism appeared to have little effect on the spoilage of the crab meat during refrigerated storage.

Every sample of fresh nonirradiated crab meat in this experiment was found to be heavily contaminated with coagulase positive Staphylococci. This finding indicates that fresh crab meat is a potential health hazard product since the Staphylococci enterotoxin might have already been formed in the food, or the toxin could easily form if the food is mishandled. Usually, foods would be toxic when they contain more than one million cells of coagulase positive Staphylococci per gram. Although irradiation at 0.15 and 0.25 Mrad could eliminate the lived Staphylococci, but it causes no harm to the toxin that might already been produced in the food. It is, therefore, strongly recommended that strict sanitary practice should be used in every step of crab meat processing and marketing to prevent Staphylococci food poisoning.

The freshness of crab meat could well be measured by chemical tests used in this studies. Results on ammonia content determination in crab meat agree closely with that of Burnett (1965) who divided the freshness of crab meat into 4 stages. It was clear that the crab meat used in this studies belonged to stage 1 quality, i.e. fresh but with strong fishy odor. At the time of spoilage, the ammonia contents of both irradiated and nonirradiated crab meat were higher than 100 µg/100g which indicated that they were in the stage of advanced decomposition (stage 3).

All the chemical and bacteriological attributes used as indices of quality of crab meat in this studies correlate fairly well with that of sensory evaluation, i.e. the values increase when the organoleptic properties decrease.

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