Study of Microflora in Malaysian Dried Fishes and Their Decontamination by Gamma-irradiation

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The distribution of microorganisms in 10 samples of salted dried fish and the effects of irradiation of them were studied. The total aerobic bacteria in commercial dried fish were determined to be from 2×10^4 to 3×10^6 per gram. Mold counts were 1×10^2 to 7×10^3 per gram with a lower amount of yeasts. In spoiled dried fish, total aerobic bacteria were determined to be 4×10^6 or 1×10^7 per gram with a few yeasts. Coliforms were not isolated on MacConkey agar plates from any of the samples. The predominant bacteria occurring in spoiled dried fish were *Pediococcus halophilus*, *Vibrio costicola* and *Planococcus* sp. More than 50% of the molds consisted of the *Aspergillus niger* group, whereas lower amounts of the *A. flavus*, *A. fumigatus* and *A. ochraceus* groups, *Penicillium chrysogenum* series, *etc.* were also isolated from many samples of dried fish. All kinds of putrefactive microorganisms were radiation sensitive, and a dose of *ca.* 500 krad appears to be sufficient for extension of the shelf-life of dried fish from 2 to 4 times.

Salted dried fishes are one of the important protein sources for local people in South-east Asia.[1] The total landing of fish in Malaysia is about 500,000 tons per year, and approximately 70% of the fish is consumed fresh, and approximately 13% as a salted dried form. Salted dried fishes are consumed mainly by local people, and they have long been consumed as a traditional food by Malay people. Salted dried fishes contain 10 to 13% salt and 35 to 50% moisture, and are easily spoiled by the propagation of microorganisms and fly-maggots. When salted dried fishes are spoiled by microorganisms, they become covered with molds and red spots accompanied with an unpleasant odour.

This paper presents preliminary data on the effect of irradiation on the distribution of microorganisms in salted dried fish which affect the shelf life, and the reduction of microorganism counts to a hygenic level.

MATERIALS AND METHODS

1. Materials. Eight samples of salted dried fish (A, B, C, D, E, F, G and H) were obtained from a local market in the vicinity of the University of Agriculture, Malaysia. Two samples of spoiled dried fish (I and J) were obtained after one month storage in plastic film pouches at room temperature (*ca.* 30°C).

2. Enumeration of microorganisms. Total aerobic bacteria were determined on 5% salt-nutrient agar which contains Gibco-nutrient agar 23 g, crude salt 50 g, yeast extract 50 g, glucose 2 g, and K_2HPO_4 1 g per liter (pH 7.2). Molds and yeasts were determined using 7.5% salt-malt agar containing malt extract 50 g, crude salt 75 g, and agar 20 g per liter (pH 6.5). Each of the dried fish samples was cut into small pieces under sterile conditions, 20 g of which was mixed with 5% salt-sterile water. The resulting mixture was homogenized with a Coloworth stomacher Lab-Blender-400 for one minute and diluted 10^2 times or 10^4 times with 5% salt-sterile water, and then 0.2 ml aliquots were spread on the surface of agar plates. Colonies were counted and identified after 3 to 5 days incubation at 30°C. In this study, total aerobic bacteria were also enumerated using nutrient agar without the addition of salt.

3. Gamma-irradiation. The gamma-ray source used was 8 k-curie cobalt-60 in a Gamma-cell 220 at the National University of Malaysia. The dose rate in the chamber was estimated to be 0.6 Mrad per hr by determination with a Fricke dosimeter.

4. Storage of samples. Two pieces of dried sardine were packed in polyethylene pouches, and irradiated with doses of 300 and 500 krad. After irradiation, the samples were stored for 2 months at room temperature (28~32°C) at the University of Agriculture, Malaysia.

5. Microbiological identification. After enumeration of microorganisms on petri-dish cultures, typical strains of bacteria and molds were selected and transferred to suitable agar slants. Identification to the level of genus and species was confirmed mainly by reference to "Bergey's Manual of Determinative Bacteriology"[2] and "The Genus Aspergillus."[3]

RESULTS

1. Distribution of microorganisms in dried fish

As shown in Table I, total aerobic bacteria in commercial dried fish were determined to be 2×10^4 to 3×10^6 per gram. Mold counts were 1×10^1 to 7×10^3 per gram, and yeast counts 4×10^1 to 2×10^3 per gram. Total aerobic bacteria in spoiled dried fish were determined to be 4×10^6 or 1×10^7 per gram with a few yeasts. Coliforms were not isolated on Gibco–MacConkey agar plates from any samples. In this study, putrefactive bacteria in dried fishes were not detected on nonsalt-nutrient agar plates even after prolonged incubation.

TABLE I. DISTRIBUTION OF MICROORGANISMS IN DRIED FISHES (per gram)

Sample	Fish	Total bacteria	Coliforms	Molds	Yeasts
A	Sardine	4.9×10^4	0	5.6×10^2	7.5×10^1
B	Anchovy	1.7×10^4	0	4.7×10^2	1.5×10^3
C	Malayan flounder	5.8×10^4	0	6.7×10^3	6.3×10^1
D	Scad	6.3×10^4	0	6.0×10^2	8.8×10^1
E	Threadfin	3.8×10^4	0	1.0×10^2	4.4×10^1
F	Jewfish	2.6×10^6	0	3.4×10^2	5.1×10^2
G	Snakeskin gourami	8.6×10^5	0	5.3×10^2	8.8×10^1
H	Golden striped snapper	1.7×10^4	0	3.4×10^2	2.3×10^3
I*	Jewfish	4.3×10^6	0	1.3×10^1	1.3×10^3
J*	Jewfish	1.0×10^7	0	0	3.4×10^2

* Spoiled dried fish.

TABLE II. SOME CHARACTERISTICS OF REPRESENTATIVE BACTERIA IN DRIED FISHES

	Pediococcus halophilus	*Vibrio costicola*	*Planococcus* sp.	*Micrococcus varians*	Halophilic red cocci
Morphology	Cocci or diplococci	Rods	Cocci or diplococci	Cocci	Diplococci
Gram-reaction	+	−	+	+	+
Cell size (μ)	0.6~0.8	0.8×1~1.5	0.8~1	0.8~1	0.8~1
Motility	−	±	+	−	−
Oxidase	−	+	+	−	+
Catalase	−	+	+	+	+
Glucose	Fermented	−	−	Oxidized	−
Nitrate reduction	−	− or +	−	+	−
H_2S produced	+	−	+	−	+
Arginine hydrolysis	+	±	−	−	−
Gelatine liquefaction	−	−	−	+ or −	+
GC content of DNA	*	50%	*	*	62%
No. of test strains	12	4	1	3	2
Typical isolates	D17, DF1, DF30	D23, D28, DF2	D18	D22, DF26	D26, DF4

* Not tested.

TABLE III. GROWTH OF ISOLATED BACTERIA WITH DIFFERENT CONCENTRATIONS OF NaCl

	NaCl (%)				
	0	2	6	10	15
Pediococcus halophilus	−	+	+	++	±
Vibrio costicola	−	−	++	+++	+++
Planococcus sp.	±	+++	+++	+++	+++
Micrococcus varians	+++	+++	+++	+++	+++
Halophilic red cocci	−	+++	+++	++	++

TABLE IV. THE COMPOSITION OF MICOFLORA IN DRIED FISHES

Sample	*Pediococcus halophilus* (%)	*Vibrio costicola* (%)	*Planococcus* (%)	*Micrococcus* (%)	Halophilic red cocci (%)	*Bacillus* (%)	Total bacteria
A	10	—	—	80	—	—	5×10^4
B	—	—	—	30	—	30	2×10^4
C	—	—	—	50	—	20	6×10^4
D	—	—	—	70	5	—	6×10^4
E	—	—	—	80	20	—	4×10^4
F	70	—	30	—	—	—	3×10^6
G	80	—	—	—	—	—	9×10^5
H	—	—	20	70	—	—	2×10^4
I	20	60	—	15	5	—	4×10^6
J	10	80	—	10	—	—	1×10^7

The predominant bacteria occurring in spoiled dried fish were identified as *Pediococcus halophilus*, *Vibrio costicola* and *Planococcus* sp., as shown in Table II. All of these bacteria are halophiles, as shown in Table III. The most predominant bacteria in samples A, B, C, D, E and H which had counts below 6×10^4 per gram were *Micrococcus* with lower amounts of *Bacillus* or halophilic red cocci, as shown in Table IV. In this study, halophilic red cocci were isolated from the some dried fishes. The morphological and physiological characteristics of this bacterium resembled those of the genus *Micrococcus* or *Deinococcus*[4,5] except for the halophilic character. However, some of its characteristics were different from those of *M. roseus*. For example, the cell wall of this bacterium was lysed as in *Deinococcus* strains.[5] In the spoiled samples, I and J, which had a strong unpleasant odour, the predominant bacteria consisted of *Vibrio costicola* with lower amounts of *Pediococcus halophilus*, halophilic red cocci and *Micrococcoccus*. The predominant bacteria in samples F and G which had counts of more than 9×10^5 per gram of total aerobic bacteria, were *Pediococcus halophilus* with a lower amount of *Planococcus* sp. in sample F. From these results, it is concluded that the spoilage of salted dried fish is caused mainly by propagation of halophilic bacteria such as *V. costicola*, *P. halophilus* or *Planococcus* sp.

TABLE V. THE COMPOSITION OF MICOFLORA IN DRIED FISHES

A: *A. niger* group 30%, *A. fumigatus* group 40%, *Penicillium* sp. 20%, *etc.*
B: *A. niger* group 60%, *A. flavus* group 30%, *etc.*
C: *A. niger* group 60%, *A. flavus* group 10%, *A. ocharaceus* group 5%, *etc.*
D: *A. niger* group 50%, *P. chrysogenum* series 10%, *A. flavus* group 5%, *A. tamarii* 5%, *etc.*
E: *Rhizopus* sp. More than 50%.
F: *A. niger* group 50%, *A. fumigatus* group 20%, *etc.*
G: *A. niger* group 50%, *A. flavus* group 40%, *etc.*
H: *A. ochraceus* group 50%, *A. flavus* group 30%, *A. flavipes* 10%, *etc.*

TABLE VI. STORAGE EFFECT ON IRRADIATED DRIED SARDINE (counts/gram)

	After irradiation			After 2 months storage		
	Total bacteria	Molds	Yeasts	Total bacteria	Molds	Yeasts
Non-irrad.	4.5×10^3	7.3×10^3	0	5.6×10^6	1.0×10^2	1.9×10^5
300 krad	1.7×10^2	0	0	3.3×10^5	0	3.5×10^2
500 krad	4	0	0	0	0	0

More than 50% of molds on the dried fishes consisted of the *Aspergillus niger* group, whereas less amounts of the *A. flavus*, *A. fumigatus* and *A. ochraceus* groups, *Penicillium chrysogenum* series and *Rhizopus* were also isolated with a few *A. versicolor*, *etc.*, as shown in Table V. Yeasts consisted mainly of halophiles which were not identified in this study. Some samples contained *ca.* 40% of the *A. flavus* group which consisted mainly of *A. flavus* and *A. flavus* var. *columnaris*, and there is a possibility of mold propagation on dried fishes under unfavorable conditions.

2. Storage effect on irradiated dried fish

For the storage study, fresh salted dried sardine (moisture content, 36%) was obtained from the local market. On irradiation of samples in polyethylene pouches, the total bacterial count decreased with the increase in dose, and *P. halophilus* was isolated only from an unirradiated sample. The results of storage for 2 months are shown in Table VI. The total bacterial count reached 6×10^6 per gram in the unirradiated sample, with increases of two groups of halophilic yeasts to 2×10^5 per gram. The main bacterial flora consisted of *P. halophilus*. With 300 krad irradiation, *P. halophilus* and one group of halophilic yeasts also increased to 3×10^5 per gram. However, with 500 krad irradiation, no increases in the counts of microorganisms were observed even after 2 months storage.

Microbial reduction in aged dried sardine on irradiation is shown in Fig. 1. With irradiation below 200 krad, the survivors decreased rapidly with increasing dose, and residual microorganisms became more radiation-resistant in the higher-dose region.

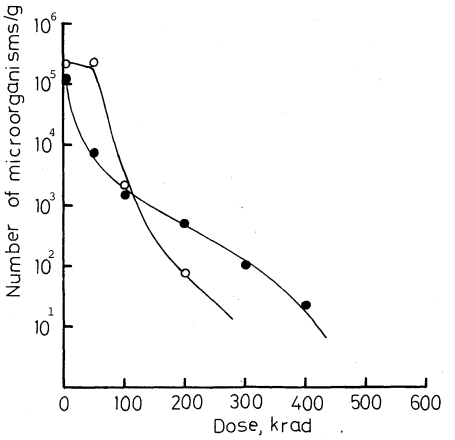

FIG. 1. Inactivation Curves for Total Bacteria in Dried Fishes on Gamma-radiation.

●, aged sardine; ○, Jewfish (J).

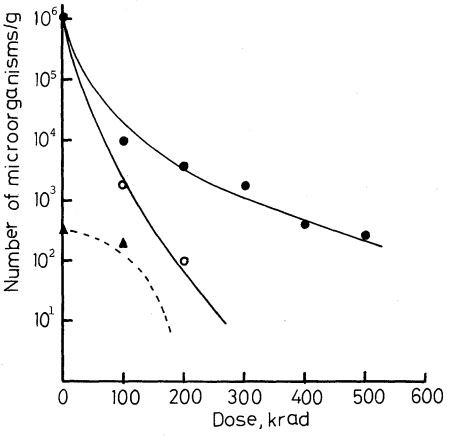

FIG. 2. Microbial Flora Changes in Dried Jewfish (I) as a Result of Gamma-radiation.

●, total bacteria; ○, *Pediococcus halophilus* and *Vibrio costicola*; △, yeasts.

The total bacteria in spoiled sample J decreased more rapidly in the same dose region. Microbial flora changes as a result of irra-

diation in spoiled sample I were also seen as shown in Fig. 2. The survivors of *V. costicola*, *P. halophilus* and halophilic yeasts decreased rapidly below 300 krad irradiation, and residual bacteria at more than 300 krad irradiation consisted of *Pseudomonas* and *Flavobacterium*. In this study, the sensitivity of *V. costicola* appeared to be higher than that of *P. halophilus*.

From these results, an irradiation dose of about 500 krad appears to be sufficient for shelf-life extension of dried fishes *i.e.* more than 2 months storage without putrefaction due to microorganisms.

DISCUSSION

Dried fish products are the main protein source in many South-east Asian countries. Many researchers have studied the extension of the shelf-life of dried fishes,[6~9] and radiation treatment, as expected, has been found to be the most promising method for this purpose. However, there have only been a few reports on microflora changes in dried fishes after irradiation. In this study, putrefaction of salted dried fishes was found to be caused by propagation of *P. halophilus, V. costicola* and *Planococcus* with lower amounts of halophilic yeasts. However, these microorganisms were eliminated by irradiation below 500 krad. In this study, storage effects were observed only for 2 months in polyethylene pouches, and a dose of *ca.* 500 krad appeared to be sufficient for shelf-life extension of dried fish of from 2 to 4 times. Organoleptic testing was not performed. However, many reports have described that radiation treatment with 500 krad would not adversely influence the flavor or taste,[10] and we also could not detect any unpleasant odour after 500 krad irradiation. This report is a preliminary one on irradiation effects on salted dried fishes, and further pilot scale studies are required.

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