



วิธีกำจัดจุลินทรีย์จากปลาอินทรีรมควัน
(*Scomberomorus commersoni*-
Lacapede)

Elimination Pathogenic
Bacteria on Smoked Mackerel
(*Scomberomorus commersoni*.
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โดย

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ELIMINATION PATHOGENIC BACTERIA
ON SMOKED MACKEREL

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ABSTRACT

Hot smoked spanish mackerel (*Scomberomorus commersoni* LACAPEDE) and also the mackerel which was contaminated by microbail (*Vibrio parahemolyticus* (NC-10903), *Vibrio cholera*, *Listeria monocytogenes* (NC-11004), *E. coli* (NC-09001), *Salmonella typhimurium* (NC-0074), *Salmonella derby*, and *Staphylococcus aureus* (NC-08532) then all the fish sample with or without microorganisms contamination were irradiated at dose 0, 1, 2, 3, 4 and 5 kCy. and each of the treated samples were stored at ambient and refrigerated temperature. It was found that the radiated dose requirement to eliminate 7 microorganisms is 4 kCy is able to eliminate all of them even though at higher level of microbial contamination 10^7 to 10^9 cfu. and that radiated dose is able to extend shelf-life of the product one month longer on the product which was stored at low temperature $8 \pm 1^\circ \text{C}$, refrigerator). Peroxide Value (PV), TVB and the other were slightly increased on the low temperature treated sample.

OBJECTIVES :

- 1) To find a specific combination factors that are able to extend shelf-life of the hot smoked fish.
- 2) Looking for a certain radiated dose to eliminate pathogenic microorganisms.
- 3) To find the best solution or the best factor that is able to improve the quality of the smoked mackerel during storage.

EXPECTATIONS :

1) Raw material will be properly organized for better quality and produced a high value products.

2) New variety of the products under packing design will be identified in order to improve quality of the products.

INTRODUCTION

Spanish mackerel (*Scomberomorus Commersoni* LACAPEDE) is a pelagic fish the chemical composition is good and rich in nutritive value. The texture is good for minced making food for example fish ball. It is commonly utilized under fresh, and salted condition. It is also good for hot smoked product but Shelf-life is so short (2-3 days) after process it is impossible to introduce to the neighborhood city. After our one step of preservation studied we found that low temperature is able to maintain the quality of the product at least 1 to 2 weeks longer but there are still some question on pathogenic microorganisms for example *Vibrio sp.*, *Salmonella spp.*, *Listeria monocytogenes* and *Staph. aureus* which the products will be stored under low temperature. Radiation technique is another option that is possible used to combine with low temperature because all pathogenic that we mention above, is very sensitive to the gamma ray even at a very low dose. Then our studies more emphasis on microorganisms matter, what we would like to know how havey of each microorganisms level which has been accidentally contaminated on sea-food. How many kilogray of radiation dose need to be apply to this type of the product. What we expect not only domestic market, the smoked mackerel will be able to distribute to world wide market as well. Packaging material and packing technique is another matter that need more studying in optimum condition and is able to prolong quality of the product in order to economic point of views.

MATERIALS AND METHODS :

Spanish mackerel (*Scomberomorus commersoni* LACAPEDE) were cut into piece then seasoning overnight. Fish were dried for 1-2 hours in sundry before leave at 65°C then one more hour at 80°C in Torry kiln for one hour. Sugar cane was used as source of slight flavor. Smoked fish are divided into 2 parts. Firstly, 0.3 ml of 10^7 to 10^8 cell/gm stocked culture pathogenic microorganisms *Vibrio parahaemolyticus* (NC-10903), *Vibrio cholera*, *Listeria monocytogenes* (NC-11004), *E. coli* (NC-099001), *Salmonella typhimurium* (NC-0074), *Salmonella derby* and *Staphylococcus aureus* (NC-08532) at 10^6 cfu of each stock cultures were inoculated onto the smoked fish then they were irradiated at 0, 1, 2, 3, 4 and 5 kGy. Each of microorganisms on smoked fish were enumerated by plating count on TCBS agar, Listeria Oxford (formular) media and MacConkey (MC) agar or EMB Bismuth sulfite (BS) and Baird Parker (BP). All of stocked culture on alkaline peptone water, then microorganisms were pararely conducted the same time as the treated samples. The second experiment, smoked fish will be irradiated under frozen condition at 0, 1, 2, 3, 4 and 5 kGy then they were stored at ambient temperature ($28 \pm 5^\circ\text{C}$) and the other portions were stored at refrigerated ($8 \pm 1^\circ\text{C}$) condition. Both of specimens were periodically drawn for chemical-microbiological analysis also organoleptic evaluation the samples under frozen condition were irradiated doses at 0, 1, 3, 4 and 5 kGy with irradiation a dose rate 2.0 kGy/hr. The gamma ray source was used is Co-60 and located at Rungsit center.

MICROBIOLOGICAL ANALYSIS

Standard plate count for total viable aerobic plate count (TVC, cfu) will be followed BAM (Bacteria Annual Method for Foods FDA Standard). MacConkey Agar and EMB agar are used to identify *E. coli*, Baird Parker Agar (BP) with 1% tell urite and 5 ml of egg yolk solution, it was used to conduct for *Staphylococcus aureus*. Violet Red Bile sucrose salt agar (VR) was applied to identify Enterobacteriaceae, Oxytethacycline media are used to enumerate yeast and mould

Enumeration Pathogenic *Vibrios.* and *Salmoella spp.* were carried out using modifications of methods described in "Bacterial Analytical Manual for Food, FDA enumeration of *Listeria monocytogenes* was performed by Lovett (1989). Buchanan and Gibbons (1974).

CHEMICAL ANALYSIS :

Chemical composition of smoked mackerel will be analysed for instance moisture content, fat, salt content, water activity (Aw), Ammonical-N₂, Formaldehyde-N, Peroxide value (POV, meq/mg). All the methods have been followed A.O.A.C. 1980. Total Volatile Base Nitrogen (TVB-N, mgN/100 gm) is followed Beatty & Gibbons methods, (1963)

RESULTS AND DISCUSSIONS

It was found the TVC on the irradiated 0, 1, 2, 3, 4 and 5 kCy sample (smoked mackerel) were 1.59×10^8 , 1.76×10^6 , 6.15×10^4 , 4.90×10^3 , 0 and 0 kCy after 3 days stored at room temperature ($28 \pm 5^\circ\text{C}$) while the initial microorganisms number is 1.95×10^2 cell/gm. After microorganisms number reduced as the higher dose was applied (Table 4). The sample with 0, 1, 2, 3, 4 and 6 day while the treated sample but kept at refrigerator will spoile within 5, 7, 15, 25, 40 and 60 days respectively and it was found low count all the microorganisms on the samples which were applied higher doses (table 5). It was found that 4 and 5 kCy is able to extend shelf-life of the irradiated product. more than one month. For the pathogenic microbial contaminations have been studied by irradiated pure culture of (*E. coli* (NC-0900), *Vibrio parahemolyticus* (NC-10903, *Listeria monocytogenes* (NC-11004), *Sal. typhimurium* (NC-0074) and *Staph. aureus* (NC-08532). under cell suspension at a level 5.35×10^8 , 8.80×10^6 , 2.8×10^7 , 6.0×10^7 and 7.05×10^6 cell/gm respectively and specific microorganisms on the the treated samples were found 2.17×10^7 cell for *E. coli*, 2.50×10^6 cell for *Listeria monocytogenes*) 3.46×10^6 cell for *Salmonella Eypthimurium* and 2.26×10^6 cell/gm for *Staphylococcus aureus*. It was found that the radiated dose at 1 kCy is enough to eliminate

vibrio parahemolyticus and 2 kGy, for *Listeria monocytogenes* and *E. coli*. for *Salmonella typhimurium* and *Staphylococcus aureus* needed 4 kGy to eliminate the microorganism contamination on the sample at a high microbial contaminations as in table 3 and 4 on table 1 and 2 found higher microbial contamination but they required lesser radiated dose compare to the microorganism on the sample which were irradiated under frozen condition (table 3, 4).

CHEMICAL CHANGED DURING STORAGE :

Peroxide Value of the irradiate sample was slightly increased if stored the product under refrigerated condition and no significance difference ($p > 95\%$) on the irradiated or non-irradiated samples Graph 1 and 2.

Formaldehyde-N and Amonical-N were slightly increased at some degree on the product (sample) that stored under longer period of time and also they were stored at ambient temperature and not much difference of all treated samples and stored under both temperatures.

TVB was found :lightly difference at the first week but it made a big difference after 2 weeks later when the products stored under ambient and refrigerated condition (Table 7) also found bacterial growth on the treated samples which stored longer period of time (under refrigerated condition) is also reduce or slow down the TVB production on the products. Also found that the TVB number on higher radiated dose will develop smaller amount of TVB from smoked mackerel.

COUCLUSIONS:

1) Radiated dose at 4 kGy is enough to eliminate all pathogenic bacteria on a level of suspended up to 10^6 to 10^7 cfu.

2) Combination treated of radiation dose together with low temperature ($8-10^\circ\text{C}$) will able to extend shelf-life of the smoked mackerel one more month of the irradiated

product and stored at low temperature (8-10°C).

ACKNOWLEDGEMENTS

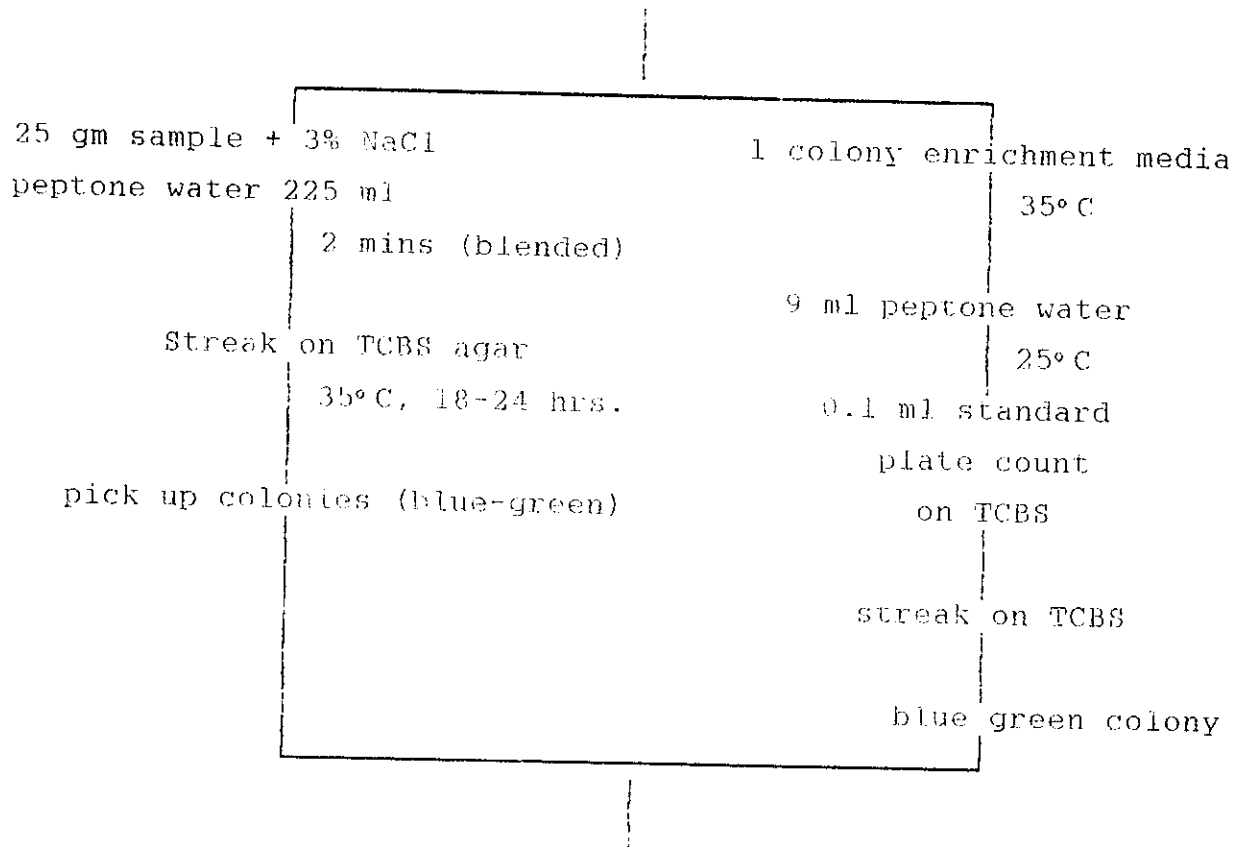
I would like to express my thank to OAEP and Training Irradiator center that took care dosemeter on my sample and Thank for IAEA that support our project not only financial but information and chance to meet many expertise an gain more new ideas and able to adjust our work for our country.

Finally I would like express my thank to Dr. M. Ahmed Who has been contributed a lot of up-date information and is very useful for my work. I also would lile to express my appreciation to my team work.

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Vibrio parahemolyticus



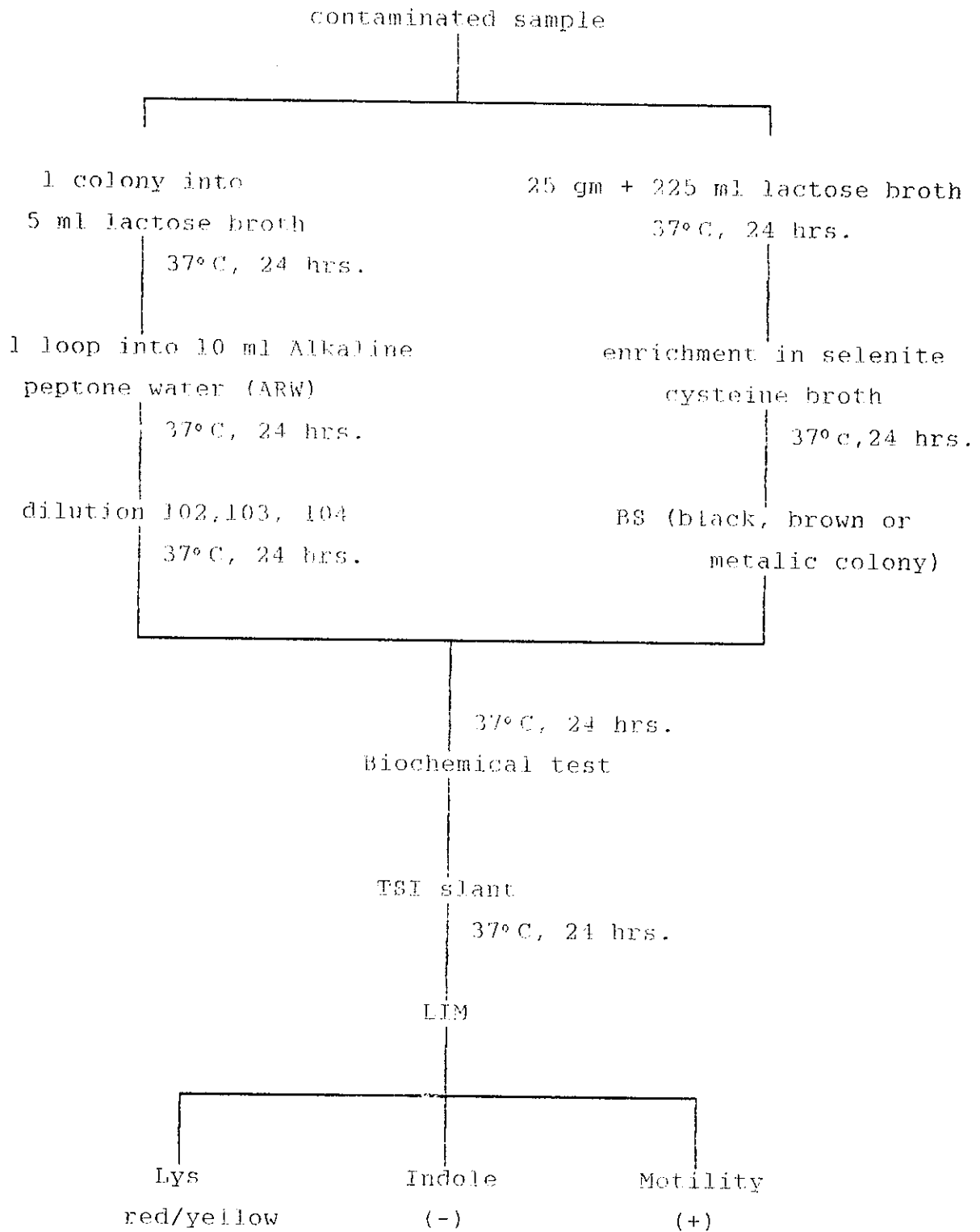
TSI slant

35° C, 18-24 hrs

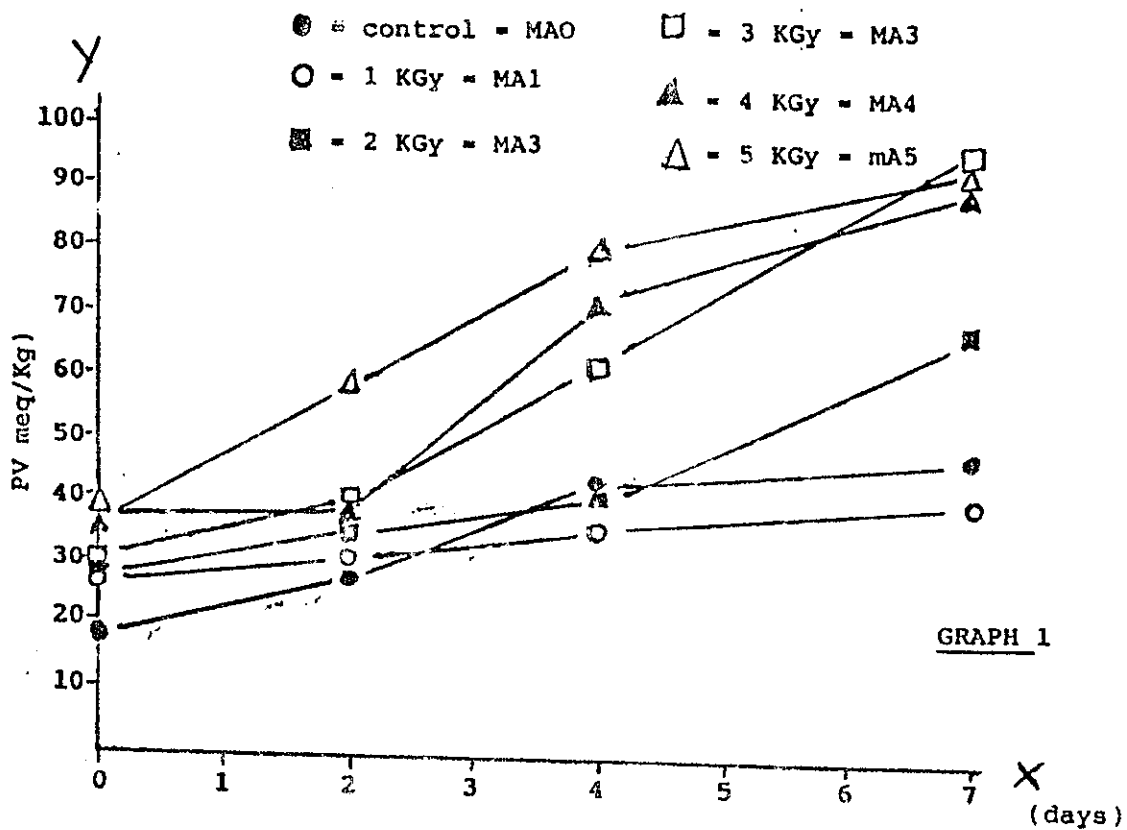
- morphology = gram-neg.
- cytochrom oxidase = (+)
- lysinedecarboxylase test = (+)
- Halophilism test = 0% NaCl = 0
= 3.5% NaCl = (+)
= 10% NaCl = (-)
- Hugh & Leifson test (glucose) = ferment +ve
- Growth (43° C) = ve (+)

Flow chart 1

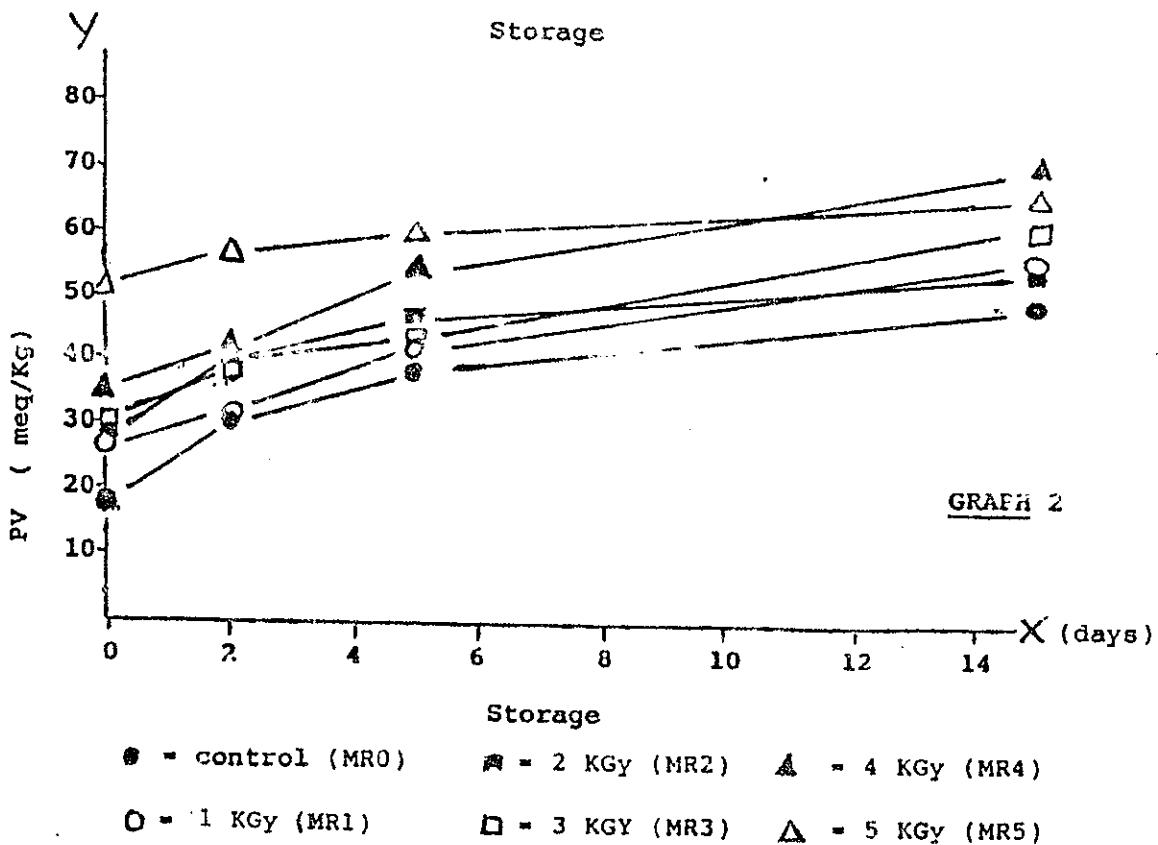
Salmonella, Shigella



Flow chart 2



GRAPH 1



GRAPH 2

Table 1 Reduction of Vibrio cholera and Vibrio parahemolyticus E.coli,
Listeria monocytogenes, Salmonella typhimurium and
Staphylococcus aureus after irradiation in peptone water

Samples	0	1 KGy	2 KGy	3 KGy	4 KGy	5 KGy
1. <u>E. coli</u>	++	+	+	-	-	-
2. <u>Vibrio cholera</u>	++	+	+	-	-	-
3. <u>Vibrio parahemolyticus</u>	++	+	-	-	-	-
4. <u>Listeria monocytogenes</u>	++	++	+	-	-	-
5. <u>Salmonella derby</u>	++	++	+	-	-	-
6. <u>Salmonella typhimurium</u>	++	+	+	-	-	-
7. <u>Staphylococcus aureus</u>	++	++	+	-	-	-

++ = were detected
+ = rarely detected
- = not detected

Table 2 Reduction of Vibrio parahemolyticus, Vibrio cholera, Listeria monocytogenes, E.coli, Salmonella typhimurium, Salmonella derby and Staphylococcus aureus after irradiation in peptone water

	KGy					
	0	1	2	3	4	5
<u>Vibrio parahemolyticus</u> (NC-10903) (on TCBS)	4.70x10 ⁸	2.64x10 ²	0	0	0	0
<u>Vibrio cholera</u> (on TCBS)	4.89x10 ⁹	8.45x10 ³	4.55x10 ¹	0	0	0
<u>Listeria monocytogenes</u> (NC-11004) (on Listeria Oxford media)	1.24x10 ⁷	2.25x10 ⁴	1.97x10 ¹	0	0	0
<u>E. coli</u> (NC-09001) (on EMB agar)	1.84x10 ⁸	9.26x10 ⁴	1.05x10 ¹	0	0	0
<u>Salmonella typhimurium</u> (NC-0074) (on Bismuth sulfite and Brilliant green Bile agar)	1.86x10 ⁷	1.08x10 ⁴	1.0x10 ²	0	0	0
<u>Salmonella derby</u> (On BS)	1.53x10 ⁶	2.40x10 ³	1.00x10 ¹	0	0	0
<u>Staphylococcus aureus</u> . (NC-00532) (Baird Parker)	7.30x10 ⁷	6.50x10 ⁴	1.02x10 ²	0	0	0

Table 3 Microbiological number on irradiated sample

Sample	Dose (KGy)	TVC (cfu) (35°C)	<u>Vibrio</u> <u>parahemo</u> <u>lyticus</u>	<u>E.coli</u>	<u>Listeria</u> <u>monocyto</u> <u>genes</u>	<u>Salmonella</u> <u>typhimu</u> <u>rium</u>	<u>Staphylo</u> <u>coccus</u> <u>aureus</u>	<u>Enterobac</u> <u>teriaceae</u>
fish with	0	8.80x10 ⁶	6.70x10 ³	x	x	x	x	2.75x10 ²
<u>Vibrio</u>	1	4.00x10 ²	0	x	x	x	x	0
<u>parahemo</u>	2	0	0	x	x	x	x	0
<u>lyticus</u>	3	0	0	x	x	x	x	0
	4	0	0	x	x	x	x	0
	5	0	0	x	x	x	x	0
smoked	0	2.81x10 ⁷	x	x	2.50x10 ⁶	x	x	x
fish with	1	4.45x10 ⁴	x	x	3.55x10 ³	x	x	x
<u>Listeria</u>	2	6.00x10 ⁴	x	x	0	x	x	x
<u>monocy-</u>	3	0	x	x	0	x	x	x
<u>rogenes</u>	4	0	x	x	0	x	x	x
	5	0	x	x	0	x	x	x
smoked	0	5.38x10 ⁸	x	2.17x10 ⁷	x	x	x	2.17x10 ⁷
fish with	1	5.55x10 ²	x	3.72x10 ⁴	x	x	x	0
<u>E.coli</u>	2	0	x	0	x	x	x	0
	3	0	x	0	x	x	x	0
	4	0	x	0	x	x	x	0
	5	0	x	0	x	x	x	0
Smoked	0	6.30x10 ⁶	x	x	x	3.46x10 ⁶	x	3.46x10 ⁶
fish with	1	4.35x10 ⁴	x	x	x	3.65x10 ³	x	3.65x10 ³
<u>Salmonella</u>	2	2.55x10 ²	x	x	x	4.60x10 ²	x	4.60x10 ²
<u>typhimurium</u>	3	7.50x10 ¹	x	x	x	1.50x10 ¹	x	1.50x10 ¹
	4	0	x	x	x	0	x	0
	5	0	x	x	x	0	x	0
smoked	0	7.05x10 ⁶	x	x	x	x	2.26x10 ⁶	x
fish with	1	6.20x10 ³	x	x	x	x	1.15x10 ²	x
<u>Staphylo</u>	2	2.05x10 ¹	x	x	x	x	4.55x10 ¹	x
<u>coccus</u>	3	0	x	x	x	x	1.00x10 ¹	x
<u>aureus</u>	4	0	x	x	x	x	0	x
	5	0	x	x	x	x	0	x

x = not determined

Table 4 Microbial changed on the irradiated sample (MA) during storage at ambient temperature for 1 month

Sample (KGy)	Date	TVC (cfu)	<u>E.coli</u> (44.5°C)	Enterobacteriaceae	<u>Staphylococcus aureus</u>	Yeast & Mould
non-irradiated smoked fish		1.95x10 ²	ND	0	1.50x10 ²	Mould (M)
MA0 (0 KGy)	1	ND	1.04x10 ³	3.29x10 ⁴	1.02x10 ⁴	6.00x10 ²
	3	1.59x10 ⁸	8.14x10 ⁷	3.39x10 ⁴	ND	6.00x10 ²
	4	1.96x10 ⁸	1.77x10 ⁸	3.49x10 ⁴	1.58x10 ⁸	6.00x10 ²
	5	8.50x10 ⁸	7.20x10 ⁸	3.90x10 ⁵	7.54x10 ⁸	6.00x10 ²
	7	1.96x10 ⁹	9.80x10 ⁸	2.36x10 ⁶	3.60x10 ⁷	0
MA1 (1 KGy)	1	ND	ND	ND	ND	ND
	3	1.76x10 ⁶	6.05x10 ⁵	0	0	0
	4	1.25x10 ⁶	1.45x10 ⁵	1.20x10 ²	2.80x10 ⁴	0
	5	5.21x10 ⁷	8.25x10 ⁷	0	1.52x10 ⁷	0
	7	3.38x10 ⁸	3.50x10 ²	2.81x10 ⁶	2.49x10 ⁷	0
	10 15					
MA2 (2 KGy)	1	ND	ND	ND	ND	ND
	3	6.15x10 ⁴	1.15x10 ⁴	0	0	0
	4	4.41x10 ⁷	6.85x10 ⁶	0	0	0
	5	6.25x10 ⁵	4.15x10 ⁵	0	0	0
	7	0	ND	0	0	0
	10	ND	ND	ND	ND	ND
	15 20					
MA3 (3 KGy)	0	ND	ND	ND	ND	ND
	3	4.90x10 ³	1.59x10 ³	0	0	0
	4	5.0x10 ⁹	0	0	0	0
	5	5.0x10 ⁹	0	0	0	0
	7	0	0	0	0	0
	10	0	0	0	0	0
	15 20					

Table 4 (continue)

Sample (KGy)	Date	TVC (cfu)	<u>E. coli</u> (44.5°C)	Entero- bacteriaceae	<u>Staphylococcus</u> <u>aureus</u>	Yeast & Mould
MA4 (4 KGy)	0	ND	ND	ND	ND	ND
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	7	0	0	0	0	0
	10	0	0	0	0	0
	15	0	0	0	0	0
	20	0	0	0	0	0
MA5 (5 KGy)	0	ND	ND	ND	ND	ND
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	7	0	0	0	0	0
	10	0	0	0	0	0
	15	0	0	0	0	0
	20	0	0	0	0	0

ND = not determined

Table 5 Microbial changed during stored the sample at refrigerator for 1 month

Sample of refrigerated temperature	Date (days)	TVC (cfu)	<u>Staphylo</u> <u>coccus</u> <u>aureus</u>	<u>E.coli</u>	Fitero- bacteriaceae	Yeast & Mould
MR0 (0 KGy)	1	4.50x10 ¹	0	0	0	0
	3	2.06x10 ²	0	0	0	0
	7	1.35x10 ³	0	0	0	0
	10	1.21x10 ⁶	1.62x10 ⁴	2.15x10 ⁴	0	+
	15	1.50x10 ⁴	2.50x10 ²	3.50x10 ³	0	+
	30	7.90x10 ⁵	3.10x10 ²	4.30x10 ²	0	+
MR1 (1 KGy)	1	0	ND	ND	ND	ND
	2	0	0	0	0	0
	3	0	0	0	0	0
	7	0	0	0	0	0
	10	5.65x10 ⁴	1.09x10 ⁴	0	0	0
	15	2.68x10 ⁵	5.20x10 ³	3.5x10 ⁴	0	0
30	2.21x10 ⁵	0	0	0	0	
MR2 (2 KGy)	1	ND	ND	ND	ND	ND
	2	0	0	0	0	0
	3	0	0	0	0	0
	7	0	0	0	0	0
	10	1.0x10 ²	0	0	0	0
	15	0x10 ¹⁰	0	0	0	0
30	0	0	0	0	0	
MR3 (3 KGy)	1	ND	ND	ND	ND	ND
	2	0	0	0	0	0
	3	0	0	0	0	0
	7	0	0	0	0	0
	10	ND	8.50x10 ⁴	0	0	0
	15	3.65x10 ²	1.40x10 ²	2.00x10 ⁴	0	0
30	0	0	0	0	0	

Table 5 (continue)

Sample of refrigerated temperature	Date (days)	TVC (cfu)	<u>staphylo</u> <u>coccus</u> <u>aureus</u>	<u>E.coli</u>	Entero- bacteriaceae	Yeast & Mould
MR4 (4 KGy)	1	ND	ND	ND	ND	ND
	2	0	0	0	0	0
	3	0	0	0	0	0
	7	0	0	0	0	0
	10	0	0	0	0	0
	15	0	0	0	0	0
	30	0	0	0	0	0
	MR5 (5 KGy)	1	ND	ND	ND	ND
2		0	0	0	0	0
3		0	0	0	0	0
7		0	0	0	0	0
10		0	0	0	0	0
15		2.00x10 ⁴	1.50x10 ⁴	3.20x10 ²	0	0
30		0	0	0	0	0

ND = not determined

Table 6 Formaldehyde-N and Ammonical-N changed of treated sample(0, 1, 2, 3, 4 and 5 KGy) during stored at ambient temperature (MA) (28±5°C) and refrigerator (MR) (8±1°C)

Date (days)	Formaldehyde (mgN%)											
	MA0	MA1	MA2	MA3	MA4	MA5	MR0	MR1	MR2	MR3	MR4	MR5
0	2.490	2.624	2.690	2.705	2.830	3.245	2.490	2.624	2.690	2.705	2.830	3.245
2	ND	ND	ND	ND	ND	ND	3.007	3.058	3.341	3.098	3.334	3.334
4	ND	4.556	4.880	3.824	3.610	3.306	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	4.581	3.544	3.216	3.114	3.091	2.468

Date (days)	Ammonical-N ₂ (mgN%)											
	MA0	MA1	MA2	MA3	MA4	MA5	MR0	MR1	MR2	MR3	MR4	MR5
0	0.524	0.492	0.517	0.473	0.536	0.484	0.526	0.492	0.547	0.473	0.536	0.484
2	0.542	0.560	0.515	0.649	0.636	0.667	0.457	0.542	0.585	0.656	0.669	0.721
4	0.543	0.617	0.597	0.667	0.628	0.730	0.543	0.617	0.597	0.667	0.628	0.630
7	0.670	0.840	0.609	0.861	0.831	0.889	0.479	0.543	0.573	0.670	0.875	0.857
15	-	-	-	-	-	-	0.504	0.538	0.567	0.648	0.856	0.993

MA0, MA1.... MA5 = irradiated (0, 1, 2, 3, 4 and 5 KGy) hot smoked mackerel stored at ambient temperature (28±5°C)

MR0, MR1.....MR5 = irradiated (0, 1, 2, 3, 4 and 5 KGy) hot smoked mackerel stored at refrigerated temperature (8±1°C), ND = not determined

Table 7 TVB of treated irradiated smoked fish during storage at ambient (28±5°C) and refrigerated (8±1°C)

Date (day)	TVB (mgN%)											
	MA0	MA1	MA2	MA3	MA4	MA5	MR0	MR1	MR2	MR3	MR4	MR5
0	24.633	23.496	23.496	23.117	23.875	23.496	24.633	23.496	23.496	23.117	23.875	23.496
2	26.528	26.528	25.769	26.149	25.769	25.769	28.801	28.043	27.285	24.254	24.633	23.496
4	31.045	30.332	28.801	27.286	27.286	23.117	-	-	-	-	-	-
7	41.686	35.623	35.244	30.317	29.180	28.804	35.623	35.623	30.317	29.559	27.286	28.043
11	57.695	36.302	-	30.468	-	27.875	-	-	-	-	-	-
12	75.199	-	-	-	-	-	-	-	-	-	-	-
13	80.385	44.730	-	40.841	-	40.012	-	-	-	-	-	-
15	81.245	80.385	-	77.792	-	74.446	57.139	26.381	34.865	33.349	32.591	30.317

MA0, MA1,..... MA5 = irradiated smoked fish at 0, 1, 2, 3, 4 and 5 KGY underfrozen condition then kept at ambient (A) temperature and refrigerator (R) and storage for 13 days

