

ANALYSES OF NEW BATCHES OF IMPORTED CANNED FISH IN FIJI

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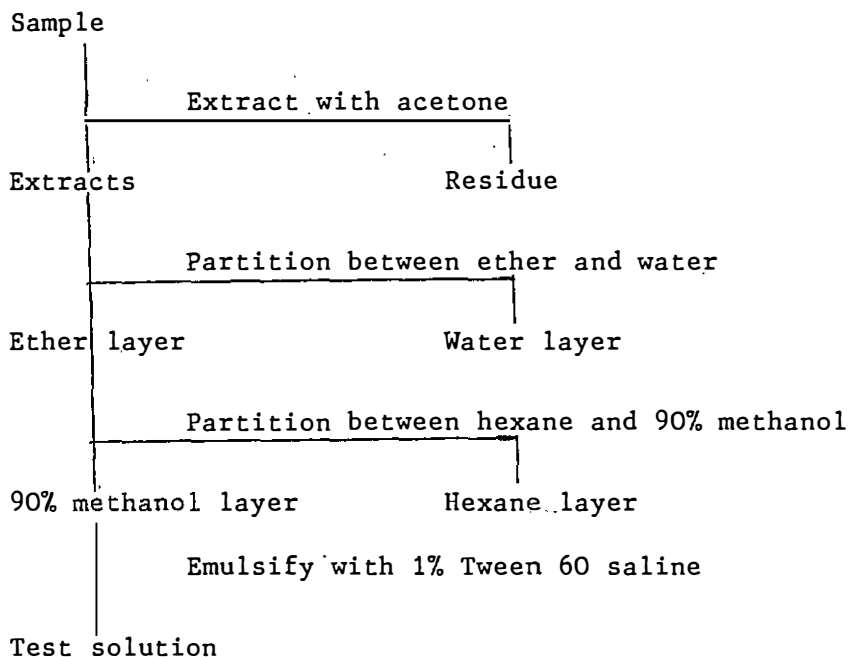
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EXTRACTION AND PARTIAL PURIFICATION

The known quantities of the prepared samples were placed in a homogenizer and 300mls of Acetone added. The sample was homogenized for 2 minutes. The homogenate was filtered (No. 1 filter paper) using a suction pump and buchner flask. To the residue, 200mls of Acetone was added and together with the used filter paper it was homogenized in the blender for 2 minutes. The homogenate was filtered again and the residue homogenized and filtered a third time. The total filtrate was evaporated using a Rotary evaporator (Rotavapor R 110) and a water bath. The extract was washed out with 200mls of diethyl ether into a separating funnel. 20mls of distilled water was added and after shaking, left for 5 minutes to form partition between ether and water. The bottom layer (i.e. water) was drained into a flask and the ether layer transferred to evaporating flask. To the drained water layer, 200mls of diethyl ether was added and the separation of water and ether layers repeated in the separating funnel.

The ether layer from the second separation was added to the evaporating flask and the diethyl ether evaporated in the Rotary evaporator. The extract was then washed out in a flask with 300mls of hexane and 100mls of 90% methanol. The two solvents were placed in a separating funnel to form partition layers. The bottom layer (methanol) was drained into a smaller evaporating flask while 20mls of 90% methanol and 100mls of hexane added to the top layer (hexane) and partitioning repeated. The methanol layer from this separation was added to the previously recovered methanol in the smaller evaporating flask. Methanol was then evaporated in the Rotary evaporator. The residue was washed from the evaporating flask into yet smaller evaporating flask using ethanol. Ethanol was then evaporated. The toxin extract was transferred to a graduated test tube, with a pipette using 1% tween 60 saline. The volume was made upto 2.5ml with 1% tween 60 saline.

The procedure outlined above is summarized in Figure 1.



MOUSE TEST

From the test solution, 3 laboratory mice weighing approximately 20 grammes each were injected intraperitoneally 1ml, 0.6ml and 0.4ml, respectively, of the toxin extract. The reaction of the mice was noted for 3 hours and the death time, if mice died, observed within 24 hours.

In the event of death of mice the toxicity level was calculated and expressed as mouse units. One mouse unit is defined as the minimum amount of toxin required to kill a mouse of 20 grammes body weight within 24 hours.

An example calculation:

If one injected 1ml, 0.6ml and 0.4ml, of toxin, respectively, in each of the three respective mice and only surviving mice was one which received 0.4ml injection, then the minimum amount of toxin to kill the mouse would be 0.6ml (i.e., out of these three dilutions).

Thus 0.6ml of extract will be attributed to contain 1 mouse unit (mu) of toxin. The total amount of toxin in 2.5ml extract will therefore be $\frac{2.5}{0.6} = 4.16$ mu.

Since the final extract (2.5ml) would have been prepared from 100 grammes of tissue the toxic score of the tissue sample will be 4.16 mu/100g of tissue.

RESULTS

1. Physical Examination

In Table 1, the amounts of fluid, flesh and viscera in each of the cans and batches tested are given. Average figures with standard deviation of each of the batches is also presented. We have also remarked on the relative abundance of fish pieces and various portions of the viscera, if present. From this Table the following is deduced:

- (a) Relative weights of fluid, flesh and viscera vary between different cans.
- (b) The amount of viscera varied from nothing in some cans to 3% of the total weight of contents.
- (c) Of the six batches tested three batches had viscera in them. These were two batches of sardines and one batch of mackerel, respectively. The viscera portions included liver, gonads and abdominal fats.

2. The results of toxicity tests carried out by us are given in Table II. No lipophilic toxin(s) were detected in any of the six batches tested.

GENERAL COMMENTS

- (1) The samples had a remarkably low viscera. Some cans had a total absence of viscera, indicating an efficient degutting process in the canning procedure.
- (2) No toxicity was detected in any of the batches tested.
- (3) These batches appear generally to be of far superior quality in comparison to other batches tested earlier by us.

BATCH/CAN	F L U I D		F L E S H		V I S C E R A			R E M A R K S
	WEIGHT (g)	% TOTAL WEIGHT	WEIGHT (g)	% TOTAL WEIGHT	WEIGHT (g)	% TOTAL WEIGHT	% TISSUE WEIGHT	
SAMPLE: SARDINES								
BATCH NO: SAN JYO 4202								
CAN NO: 1	35.00	21.00	131.40	78.82	0.30	0.18	0.23	4 pieces; liver
2	36.00	19.46	147.80	79.89	1.20	0.65	0.81	4 pieces; liver
3	37.30	21.22	135.90	77.30	2.60	1.48	1.88	4 pieces; liver
4	32.50	18.72	140.60	80.99	0.50	0.29	0.35	5 pieces; liver
5	28.80	16.74	142.20	82.63	1.10	0.64	0.77	4 pieces; liver
AVERAGE AV \pm SD	33.92 \pm 3.0.	19.43%	139.58 \pm 5.58	79.93%	1.14 \pm 0.81	0.65%	0.81%	4.2 \pm 0.4
SAMPLE: MACKEREL								
BATCH NO: MKN JYO 4201								
CAN NO: 1	39.40	23.51	128.20	76.49	0	0	0	2 pieces; no viscera
2	40.70	23.54	132.20	76.46	0	0	0	2 pieces; no viscera
3	37.00	21.35	136.30	78.65	0	0	0	2 pieces; no viscera
4	38.50	21.95	136.90	78.05	0	0	0	2 pieces; no viscera
5	42.40	23.79	135.80	76.21	0	0	0	2 pieces; no viscera
AVERAGE AV \pm SD	39.60 \pm 1.85	22.83%	133.88 \pm 3.28	77.17%	0	0	0	2 \pm 0
SAMPLE: SARDINES								
BATCH NO: SAN CBY 4224								
CAN NO: 1	128.70	28.19	327.80	71.81	0	0	0	8 pieces; no viscera
2	119.40	26.56	330.20	73.44	0	0	0	7 pieces; no viscera
3	126.70	27.23	338.60	72.77	0	0	0	7 pieces; no viscera
4	128.60	28.65	320.20	71.35	0	0	0	7 pieces; no viscera
5	129.70	28.83	317.20	70.51	3.00	0.67	0.94	7 pieces; gonads
AVERAGE AV \pm SD	126.62 \pm 3.74	27.89%	326.80 \pm 7.58	71.97%	0.60 \pm 1.20	0.13%	0.19%	7.2 \pm 0.4

TABLE 1: Continued

BATCH/CAN	F L U I D		F L E S H		V I S C E R A			R E M A R K S
	WEIGHT (g)	% TOTAL WEIGHT	WEIGHT (g)	% TOTAL WEIGHT	WEIGHT (g)	% TOTAL WEIGHT	% TISSUE WEIGHT	FLESH: VISCERA AND GONADS
SAMPLE: MACKEREL								
BATCH NO: MKN CBY 4214								
CAN NO: 1	49.40	11.12	379.80	85.50	15.00	3.38	3.80	2 pieces; fat only
2	56.50	12.55	378.20	84.03	15.40	3.42	3.91	3½ pieces; fat only
3	49.10	10.97	386.00	86.20	12.70	2.84	3.19	3 pieces; fat and gonads
4	63.60	13.59	393.60	84.12	10.70	2.29	2.65	4 pieces; fat and gonads
5	78.10	17.67	363.80	82.33	0	0	0	3 pieces; no viscera
AVERAGE AV ± SD	59.34 ± 10.79	13.18%	380.28 ± 9.86	84.44%	10.76 ± 5.64	2.39%	3.71%	3.1 ± 0.66 pieces
SAMPLE: SARDINES								
BATCH NO: SAN TYO 4128								
CAN NO: 1	117.80	26.96	318.20	72.83	0.90	0.21	0.28	7 pieces; liver only
2	119.70	26.61	324.20	72.08	5.90	1.31	1.79	8 pieces; liver, gonad and fat
3	111.30	25.45	323.00	73.86	3.00	0.69	0.92	7 pieces; liver only
4	105.90	24.16	328.50	74.93	4.00	0.91	1.20	8 pieces; liver and fat
5	112.80	25.43	325.80	73.45	5.00	1.13	1.51	7 pieces; liver and fat
AVERAGE AV ± SD	113.5 ± 4.90	25.72%	323.94 ± 3.41	73.43%	3.76 ± 1.73	0.85%	1.14%	7.40 ± 0.5 pieces
SAMPLE: MACKEREL								
BATCH NO: MKN TYO 4126								
CAN NO: 1	48.00	11.19	381.00	88.81	0	0	0	4 pieces; no viscera present
2	56.50	12.67	389.40	87.33	0	0	0	3 pieces; no viscera present
3	61.40	13.86	381.60	86.14	0	0	0	3 pieces; no viscera present
4	76.00	17.09	368.60	82.91	0	0	0	3 pieces; no viscera present
5	66.50	14.98	377.40	85.02	0	0	0	3 pieces; no viscera present
AVERAGE AV ± SD	61.68 ± 9.41	13.96%	379.6 ± 6.75	86.04%	0	0	0	3.2 ± 0.4 pieces

TABLE II: TOXICITY SCORE OF CANNED FISH BIOASSAYED FOR LIPOPHYLIC TOXIN(S)

SAMPLE	BATCH CAN NO	TISSUE	TOXICITY SCORE MJ/100g
SARDINES	SAN JYO 4202	FLESH + LIVER	N.D.
MACKEREL	MKN JYO 4201	FLESH	N.D.
SARDINES	SAN CBY 4224	FLESH + GONADS	N.D.
MACKEREL	MKN CBY 4214	FLESH + VISCERA	N.D.
SARDINES	SAN TYO 4128	FLESH + VISCERA	N.D.
MACKEREL	MKN TYO 4126	FLESH	N.D.

APPENDIX 1: COST OF ANALYSES OF CANNED FISH

<u>Chemicals</u>	\$	
Acetone	12.00	
Diethyl ether	12.00	
Hexane	10.00	
Methanol	15.00	
Ethanol	2.00	
	<hr/>	
Cost per analysis	51.00	
No. of analyses	6	\$306.00
<u>Mice</u>		
18 mice @ \$1.75		31.50
Technician time - 18 hrs @ \$2.50		45.00
Report production		<hr/> 20.00
		\$402.50
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