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ANALYTICAL METHODS AND RESULTS IN THE INVESTIGATION OF THE SEASONAL CHANGES IN THE CHEMICAL COMPOSITION OF HERRING

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ANALYTICAL METHODS AND RESULTS IN THE INVESTIGATION OF THE SEASONAL CHANGES IN THE CHEMICAL COMPOSITION OF HERRING

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In the following report on the work on the condition of herring with locality and season the report has been divided into three main sections:

1. Methods of analyses
2. A complete list of analyses to date
3. Recommendations concerning the future of the work.

1. METHODS OF ANALYSES

(a). Samples canned with water (wet samples)

Sampling

The markings and serial number of the can are recorded and then the can and contents are weighed to the nearest gram (wt. A). Two holes are then punched in the top of the can and the liquid is drained off into a litre beaker; the can is opened and the drained meat is put through a food chopper with a bread crumber attachment. The minced solids are collected in a litre beaker and the mincer is cleaned off with a scalpel, or knife, and then any small pieces of meat and the adhering oil are washed off with water into the liquids beaker. The solids are then stirred into the liquids, care being taken that no large lumps are formed and then the inside of the empty can is rinsed into the mixture. After being well stirred, the mixed contents are

poured from one beaker to the other 12 times to ensure good mixing and 4 samples are immediately poured out into petri dishes which have been weighed to the nearest 0.01 g. (wt. B). If any time is lost during the taking of the samples, the contents of the beaker are remixed by pouring to and fro several times into the other beaker. The petri dishes and contents are then weighed to the nearest 0.01 g. (wt. C). The empty cans are dried and weighed (wt. D).

Drying

The samples are dried for 24 hours in an oven at 98° C. They are then desiccated and weighed to the nearest 0.01 g. (wt. E).

Extraction

The dried samples are ground in a glass mortar or evaporating dish and transferred to extraction thimbles, the dish and pestle being rinsed off into the thimble with carbon tetrachloride. The thimbles are then extracted for 4 hours with carbon tetrachloride, drained, and then dried for 1 hour at 98°C. The contents of the thimbles are then transferred to weighing bottles, which have been weighed to the nearest 0.001 g. (wt. F), any meal adhering to the thimble being removed with the aid of a scalpel or a camel's hair brush. The bottles are dried for 3 hours at 98° C., desiccated, and weighed (wt. G). The contents are then stored for protein and ash determinations.

Calculations

i. Wt. A (can + contents) - wt. D (can) = contents of can.

This acts as a rough guide as to whether leakage has occurred. If the weight of contents is much under the average the results should be considered carefully before including in the data.

ii. Wt. C (petri dish + wet contents) - wt. B (petri dish
= contents wet.

Wt. E (petri dish + dry contents - wt. B (petri dish)
= contents dry.

$\frac{\text{Contents dry}}{\text{Contents wet}} \times 100$ = percentage of solids in wet sample. This should be constant for any series of 4 samples. If any discrepancies occur this is indicative of poor sampling and the results in question should be rejected.

iii. Wt. E (dried contents + petri dish) - wt. B (petri dish)
= wt. of dried sample.

Wt. G (extracted contents + bottle) - wt. F (bottle)
= wt. of extracted sample.

$\frac{\text{Weight of extracted sample}}{\text{Weight of dry sample}} \times 100$ = percentage of solids (as oil free solids) in dried sample

100 per cent - percentage of solids = percentage of oil in sample.

Remarks

The initial stage of sampling should give no trouble and good checks are obtained for the amount of solids in the wet sample (calculation ii) (see list of analytical results). Such close agreement was obtained, during the last summer, in the oil and meal values of dried samples of a series that only

3 samples per can were taken, since this allowed more samples to be done per day. At first it is suggested that 4 samples per can be taken in case of any accidents, but later, if wished, 3 samples per can should be ample.

In weighing the dried samples in the petri dishes, weighing is to the nearest decigram and this should be done rapidly since the samples increase slowly in weight, on exposure to air. As a result, samples should be kept in a desiccator, except while being weighed, and unless extracted soon afterward, should be stored in a desiccator. If covers are used for the petri dishes this objection is largely removed, but the weight of dishes plus samples plus covers is rather large for a balance weighing accurately to 0.01 g. If weighing bottles are used, the samples taken are too small to give consistent results, and also due to the depth of the bottles, drying is not always complete in 24 hours, as it is in the case of the shallow petri dishes.

The oven is maintained at 98° C. (just below boiling point of water) to prevent spattering of samples which occurs if the oven is over 100° C.

Extraction is straightforward but the tops of the thimbles must be above the siphon level of the soxhlets, as otherwise, due to the high specific gravity of the carbon tetrachloride, meal will be floated over with the oil.

Removal of the dried meal from the thimbles introduces the most serious error in the procedure, due to the difficulty in removing all of the meal without removing part of the thimble. It has been found that by exercising care, consistent results are obtained but the technique is capable to improvement at this point.

(b). Samples canned dry (dry samples)

As in the case of wet samples, the can and contents, and later the cleaned can, are weighed as a check on leakage, although more variation is found in these figures than in the water filled can. Two holes are then punched in the top, the liquid is drained into a litre beaker; the tin is opened, and the contents are put through the mincer using a bread crumber attachment. The cutter and surface of the mincer are cleaned with a scalpel and then wiped off with a little ground flesh, as is the can. The liquid and solids are mixed and then turned into the liquids beaker and remixed. Samples are taken by inserting a scalpel to the bottom of the mixture and lifting the scalpel to obtain a cross-section of the sample. Approximate 40-g. samples are taken and placed in weighing bottles, with lids, and weighed to 0.01 g. Since evaporation is a serious factor, every operation should be considered from a point of view of time as well as from other aspects. It is found that this method gives consistent results.

The drying and subsequent treatment is the same as in the case of wet samples.

Protein and ash (for both wet and dry canned samples)

The dried extracted samples for each can are stored in a common container. This sample is ground until all passes through an 80-mesh seive. This is accomplished by grinding the sample in an iron mortar till most of it has passed through the seive and the remaining screenings are ground in a small agate mortar. The seived material is mixed and sampled by the ordinary method of rolling on glazed paper, quartering, and remixing.

Protein determinations are run by drying part of the ground sample for 1 hour at 98° C. and cooling in a desiccator and then weighing out approximately 0.40 g. to the nearest 0.1 mg. Approximately 7 g. of potassium sulphate are placed in a Kjeldahl flask and shaken around the sides to remove any moisture and then the weighed meal sample is introduced on the tip of a piece of glazed paper which reaches down past the neck of the flask. The potassium sulphate is put in first, since it absorbs any moisture and thus prevents the meal from sticking to the sides of the flask. A glass bead to prevent lumping, a piece of copper wire 1 cm. long (about the same thickness as bell wire) are added and then 25 cc. of concentrated sulphuric are put in to digest the mixture till it has become colourless and then for half an hour longer. The flask is cooled, diluted with 200 cc. distilled water, a teaspoon of powdered pumice added and 85 cc. of 40 per cent soda poured down the side of the flask to form a layer at the bottom. The distillation apparatus is prepared by placing the condenser tip in $\frac{N}{14}$ HCl containing methyl red as an indicator, the gas is lighted, the flask connected to the rubber stopper and then the contents are mixed by shaking. If this procedure is not followed, the following errors occur:

(1). If the soda is not added to form a lower layer, ammonia is lost at this stage. Similarly, if the flask is connected to the condenser and the contents mixed before the receiving flask is in place, ammonia is lost.

(2) If the apparatus is set up and the contents of the flask mixed before the gas is turned on, the heat of reaction of the acid and base cause the flask to suck back the standard in the receiver and spoil the determination.

Titrate with $\frac{N}{14}$ soda to a definite yellow.

Ash

Two 1-g. samples are accurately weighed (to 0.1 mg.) from the dried desiccated meal which has been ground and well mixed. The weighed samples are placed in weighed crucibles and then heated cautiously until gases are no longer evolved and the mass does not bubble any more. (This step requires some time and patience.) The contents are then heated at dull red heat for half and hour and then the crucibles are completely cooled. The ash and carbon adhering to the sides is removed, as much as possible, to the bottom of the crucible by using a scalpel, then 4 to 5 drops of concentrated nitric acid are added and the contents carefully heated to dryness and then heated for 3 minutes at dull red heat, desiccated, and weighed. If the ash is not white, it is cooled and retreated with nitric acid, but in all the samples done so far the ash became white on the first treatment.

List of samples with serial number of cans

Series	Place caught	Date of catch	Corresponding cans in table	Remarks (Note: can number underlined means canned dry)
C	Cowichan gap	Nov. 17/31	1,2,3,4	Series complete
D	Shelter arm	Dec. 7/31	5,6,7,8	Series complete
E	Vernon bay	Dec. 8/31	9,10,11,12	Series complete
F	Nootka sound	Jan. 13/32	13,14,15,16	Only one analysis made on can 15 but results used
G	Barkley sound	Feb. 9/32	17,18,19	Can 20 leaking on arrival, rejected
No mark	Point Grey	July ?/32	21,22,23,24	Series complete
H	Point Grey	Oct. 13/32	25,29,30	One wet sample lost - not fit for analyses
I	Trincomali channel	Oct. 22/32	37,38,39,40	Series complete
J	Pender harbour	Oct. 24/32	41,42,43,44	Series complete
K	Pender island	Nov. 13/32	45	Cans 46,47 rejected, can 48 had been destroyed
L	Pender island	Nov. 20/32	33,34,35,36	Series complete
M	Barkley sound	Nov. 22/32	50,51	Can 49 destroyed, can 52 missing
N	Pender harbour	Nov. 21/32	53,54,56	Can 55 missing
O	Barkley sound	Dec. 1/32	57,58,60	Can 59 missing
P	Pender harbour	Dec. 1/32	61,62,64	Can 63 missing
Q	Sydney inlet	Dec. 5/32	65,66,67,68	Series complete
R	Kyuquot sound	Dec. 21/32	69,70,72	Can 71 missing
S	East coast (Trincomali channel)	Dec. 24/32	73,76	Cans 74,75 missing

Can 31 belongs to series AB (AB7). There was another can AB6 but the contents were not good so it was destroyed.

2. A COMPLETE LIST OF ANALYSES TO DATE

Regarding the tables of results

Cans 1 - 24 inclusive were analysed during the summer of 1932. These analyses include wet and dry samples, and a synopsis of the results were submitted in a previous report.

Cans 25 - 73 represent samples done during the summer of 1933. In order to facilitate the work, only wet samples were done, the intention being to do all the dry samples in one series later. Protein and ash determinations remain to be done on all the samples. In comparing the results of wet samples for 1932 and 1933, it will be seen that the results for 1933 (when samples were dried in petri dishes) are more consistent than those of the previous year.

As is explained in the next section of the report, the protein values reported for samples 1 - 24 are probably incorrect since it is doubtful whether the factor N x 6.25 represents the protein value. As soon as possible a table giving the percentage N_2 found in the oil free dried meal for these samples will be submitted.

In completing the analyses the serial numbers on the remaining cans should be used in recording the data, as the previous data have been recorded in an index system arranged to include these numbers.

Results of analyses (wet samples)

Can no. and marks	Wt. of contents of can g.	Wt. of wet sample taken g.	% solids in wet sample	% solids in dry sample	% oil in dry sample	% protein dry, oil free sample (N x 6.25)	% ash in dry, oil free sample
2 (CB7)	850	51.5	27.7	48.8			
		50.4	24.5	53.3		81.8	12.87
		42.7	23.2	56.0		80.1	12.88
		42.6	22.8	56.3			
Av.			24.5	53.6	46.4	81.0	12.9
3 (CB7)	824	51.7	21.7	54.2			
		51.1	22.1	56.5		82.9	12.27
		47.8	21.7	54.4		82.4	12.29
Av.			21.8	55.0	45.0	82.6	12.3
4 (CB8)	851	47.0	23.4	55.0			
		51.2	23.8	53.7		81.1	13.20
		49.7	22.8	53.6		81.2	13.58
		48.3	22.9	55.3			
Av.			23.2	54.4	45.6	81.2	13.4
6 (BD5)	842	45.7	21.1	57.9			
		41.4	20.3	56.9		83.1	10.94
		47.4	20.9	56.9		84.1	10.80
		42.8	20.2	60.7			
Av.			20.6	58.1	41.9	83.6	10.9
7 (BD5)	836	49.6	20.9	55.7			
		48.2	20.5	56.6		82.9	11.74
		43.0	20.1	60.3		81.9	11.90
		48.5	20.1	59.4			
Av.			20.4	58.0	42.0	82.4	11.9
8 (BD6)	811	43.2	24.0	56.3			
		46.6	23.3	57.3		85.3	12.16
		51.1	24.3	56.2		85.6	12.28
		47.6	23.6	56.8			
Av.			23.8	56.6	43.4	85.4	12.2

Results of analyses (wet samples)

Can no. and marks	Wt. of contents of can g.	Wt. of wet sample taken g.	% solids in wet sample	% solids in dry sample	% oil in dry sample	% protein dry, oil free sample (N x 6.25)	% ash in dry, oil free sample
10 (BE7)	856	42.6	22.8	55.1			
		50.9	22.8	56.0		82.4	11.83
		43.9	22.6	53.7		80.2	11.98
		44.0	22.5	56.1		81.3	
Av.			22.7	55.2	44.8	81.3	11.9
11 (BE7)	826	57.6	20.2	61.0			
		44.4	20.2	58.3		79.2	12.11
		43.7	21.1	59.3		79.2	12.09
Av.			20.5	59.5	40.5	79.2	12.1
12 (BE7)	854	42.6	20.2	58.5			
		48.6	20.4	54.6		80.9	
		48.4	20.2	56.8		82.2	12.10
		54.7	19.9	56.3		81.7	12.16
Av.			20.2	56.6	43.4	81.6	12.1
14 (BF6)	?	51.7	18.0	68.9			
		40.2	17.2	67.4		85.4	11.76
		41.6	17.4	67.0		84.0	11.66
		47.4	17.3	66.9			
Av.			17.5	67.6	32.4	84.7	11.7
15 (BF6)	843	spoiled samples 1,2,3				85.9	11.83
						85.9	11.78
		55.0	15.9	69.9	30.1	85.9	11.8
16 (BF6)	791	30.0	15.3	73.4			
		25.5	16.5	74.4		85.4	12.82
		26.0	16.5	73.5		85.4	12.68
Av.			16.1	73.8	26.2	85.4	12.8
18 (BG6)	857	41.3	15.5	72.7		82.3	
		49.2	15.4	73.4		80.6	11.91
		50.5	16.4	69.7		83.1	12.08
Av.				71.9	28.1	82.0	12.0

Results of analyses (wet samples) cont'd

Can no. and marks	Wt. of contents of can g.	Wt. of wet sample taken g.	% solids in wet sample	% solids in dry sample	% oil in dry sample	% protein dry, oil free sample (N x 6.25)	% ash, in dry, oil free sample
19 (BG6)	857	44.3	15.3	71.1			
		49.3	18.1	67.5		83.4	
		48.8	15.4	71.6		82.8	11.12
		54.8	17.1	68.6		82.3	10.99
Av.			16.5	69.7	30.3	82.8	11.1
21 Pt. Grey, no marks	822	26.3	45.5	47.8		86.0	11.28
		34.8	37.0	47.9		86.0	11.35
Av.			41.2	47.8	52.2	86.0	11.3
22 Pt. Grey no marks	834	37.7	34.8	41.7			
		40.1	36.5	40.0		80.2	11.46
		29.4	37.0	41.7		80.4	11.36
Av.			36.1	41.1	58.9	80.3	11.4
23 Pt. Grey no marks	851	34.3	29.7	50.5			
		36.6	30.1	51.3			
		38.0	32.2	47.8		83.1	11.18
		33.4	31.0	50.5		83.3	11.16
		22.3	38.4	50.4		83.2	
Av.			30.8	50.1	49.9	83.2	11.2
25 (BH)	861	83.0	22.4	52.9			
		76.5	22.4	51.3			
		86.0	22.4	51.4			
Av.			22.4	51.9	48.1		
29 (BH5)	858	60.8	20.7	47.5			
		78.9	20.7	48.6			
		62.9	20.6	48.2			
		78.0	20.7	48.4			
Av.			20.7	48.2	51.8		

Results of analyses (wet samples)

cont'd

Can no. and marks	Wt. of contents of can g.	Wt. of wet sample taken g.	% solids in wet sample	% solids in dry sample	% oil in dry sample	% protein dry, oil free sample (N x 6.25)	% ash in dry, oil free sample
31 (AB7)	854	66.1	20.3	53.3			
		81.1	20.3	54.0			
		55.1	20.3	54.2			
		76.8	20.3	54.0			
Av.			20.3	53.9	46.1		
33 (BL6)	837	76.3	20.4	53.7			
		47.2	20.4	53.7			
		74.7	20.0	53.8			
		85.4	20.4	54.0			
Av.			20.3	53.8	46.2		
34 (BL5)	845	60.1	18.1	53.5			
		62.5	18.0	53.8			
		57.7	17.9	53.4			
		61.4	18.0	53.7			
Av.			18.0	53.6	46.4		
35 (BL6)	849	67.0	19.1	54.6			
		71.1	19.1	54.3			
		71.2	18.9	54.3			
		66.3	19.1	61.2 (rejected)			
Av.			19.0	54.4	45.6		
37 (BI6)	848	64.2	19.1	49.4			
		68.8	19.1	49.5			
		67.8	19.1	49.2			
		69.4	19.3	49.7			
Av.			19.2	49.4	50.6		
38 (BI6)	850	54.4	17.3	54.3			
		71.5	17.3	53.8			
		61.7	17.3	53.8			
		71.4	17.3	54.3			
Av.			17.3	54.0	46.0		

Results of analyses (wet samples) cont'd

Can no. and marks	Wt. of contents of can g.	Wt. of wet sample taken g.	% solids in wet sample	% solids in dry sample	% oil in dry sample	% protein dry, oil free sample (N x 6.25)	% ash in dry, oil free sample
39 (BI6)	854	77.6	19.4	53.0			
		74.4	19.4	53.6			
		54.8	19.4	53.3			
		59.0	19.4	53.3			
Av.			19.4	53.3	46.7		
41 (BJ5)	856	65.8	26.2	49.7			
		69.9	26.2	48.2			
		59.7	26.2	49.9			
		55.8	26.6	48.3			
Av.			26.3	49.0	51.0		
42 (BJ6)	855	83.4	27.7	46.5			
		62.5	27.6	46.9			
		75.0	26.8	47.0			
		70.7	27.6	46.8			
Av.			27.4	46.8	53.2		
43 (BJ5)	824	61.3	25.2	48.2			
		61.3	25.2	47.0			
		72.8	25.1	47.0			
		78.9	25.2	47.1			
Av.			25.2	47.3	52.7		
45 (BK5)	850	81.1	20.4	55.8			
		62.2	20.3	55.8			
		77.1	20.2	56.9			
		80.7	20.2	56.0			
Av.			20.3	56.1	43.9		
50 (BM5)	858	86.4	19.6	54.4			
		70.9	19.7	55.1			
		73.8	19.6	53.7			
		75.5	19.6	54.8			
Av.			19.6	54.5	45.5		

Results of analyses (wet samples) cont'd

Can no. and marks	Wt. of contents of can g.	Wt. of wet sample taken g.	% solids in wet sample	% solids in dry sample	% oil in dry sample	% protein dry, oil free sample (N x 6.25)	% ash in dry, oil free sample
51 (BM5)	851	76.1	17.9	56.0			
		66.0	17.8	56.0			
		67.1	17.8	56.4			
		64.4	17.8	56.2			
Av.			17.8	56.2	43.8		
53 (BN5)	866	72.2	———	———			
		81.1	21.4	50.8			
		84.0	21.4	49.9			
Av.			21.4	50.4	49.6		
54 (BN5)	837	65.7	20.4	52.0			
		74.1	20.4	50.6			
		88.1	20.3	51.8			
Av.			20.4	51.4	48.6		
57 (BO7)	779 oil stains on top and sides	67.6	19.3	60.0			
		83.9	19.4	60.1			
		90.6	19.3	59.5			
Av.			19.3	59.7	40.3		
58 (BO6)	743	65.7	20.4	52.9			
		68.4	20.4	52.8			
		?	?	53.0			
Av.			20.4	52.9	47.1		
61 (BP6)	803 oil stains on top and sides	70.2	21.7	61.3			
		68.0	21.6	61.5			
		78.9	21.6	61.4			
Av.			21.6	61.4	38.6		
62 (BP5)	834	78.9	1912	57.6			
		83.8	19.2	59.4			
		70.4	19.2	60.0			
Av.			19.2	59.0	41.0		

Results of analyses (wet samples) cont'd

Can no. and marks	Wt. of contents of can g.	Wt. of wet sample taken g.	% solids in wet sample	% solids in dry sample	% oil in dry sample	% protein dry, oil free sample (N x 6.25)	% ash in dry, oil free sample
65	856	79.1	19.4	60.8			
(BQ5)		77.9	19.4	61.2			
		72.0	19.1	61.2			
Av.			19.3	61.1	38.9		
66	857	76.5	21.8	59.8			
(BQ7)		83.3	21.8	58.8			
		77.1	21.8	59.5			
Av.			21.8	59.4	40.6		
67	845	79.5	18.8	57.9			
(BQ7)		73.2	18.7	58.5			
		80.4	19.1	57.8			
Av.			18.9	58.1	41.9		
69	827	78.9	16.9	59.6			
(BR7)		69.0	16.9	58.8			
		72.4	17.0	60.0			
Av.			16.9	59.5	40.5		
70	840	84.5	19.0	60.1			
(BR6)		87.5	19.0	59.9			
		90.5	19.0	60.2			
			19.0	60.1	39.9		
		79.3	17.9	56.5			
		83.2	17.9	56.0			
		93.6	17.9	56.2			
Av.			17.9	56.2	43.8		

Results of analyses (dry samples)

Can no. and marks	Wt. of contents of can g.	Wt. of wet sample taken g.	% solids in sample	% water	% meal (solids) in dry sample	% oil in dry sample	% protein dry, oil free sample (N x 6.25)	% ash in dry, oil free sample
1 (CA8)	637	29.02	32.9		54.9			
		32.73	33.0		54.5			
		32.04	33.1		57.3		81.5	13.01
		32.60	33.1		58.1		81.4	12.99
		38.75	33.0		54.3			
Av.			33.1	66.9	55.4	44.6	81.4	13.0
5 (AD6)	647	31.78	32.1		54.3			
		37.78	33.3		56.9		84.2	12.10
		35.59	32.1		57.1		86.7	12.10
		38.34	31.1		56.8		83.9	
		34.68	32.2		59.2			
		32.86	32.2		56.8			
Av.			32.2	67.8	56.8	43.2	84.2	12.1
9 (AE7)	641	28.85	30.3		59.9			
		35.89	30.4		58.8		83.0	12.19
		36.85	30.6		60.6		82.6	12.07
		37.68	30.5		59.7			
		29.36	30.3		63.0			
		30.69	30.3		64.1			
Av.			30.4	69.6	61.0	39.0	82.8	12.13
13 (AF)	536	31.81	27.3		73.0			
		35.21	27.5		72.8		85.1	12.80
		33.88	27.4		70.9		85.0	12.78
		35.19	27.5		72.0			
		32.82	27.4	70.0				
		31.55	27.4		70.0			
Av.			27.4	72.6	71.4	28.6	85.0	12.8

Results of analyses (dry samples)

Can no. and marks	Wt. of contents of can g.	Wt. of wet sample taken g.	% solids in sample	% water	% meal (solids) in dry sample	% oil in dry sample	% protein dry, oil free sample (N x 6.25)	% ash in dry, oil free sample
17	532.	32.10	26.6		73.9			
(AG)		32.20	26.6		72.5		81.4	11.72
		34.08	26.6		75.4		81.8	11.70
		35.98	26.6		74.9		81.2	
		34.82	26.6	~~75.4~~	75.4			
		26.55	26.7		76.3			
Av.			26.6	73.4	74.9	25.1	81.5	11.7
24	585	28.20	35.6		45.9			
		32.10	35.9		46.1			
Pt. Grey		28.69	35.9		46.8		81.9	11.29
no marks		35.20	35.8		45.8		82.0	11.35
		35.18	35.9		45.4			
		31.95	36.0		44.9			
Av.			35.8	64.2	45.8	54.2	82.0	11.3

3. RECOMMENDATIONS CONCERNING THE FUTURE OF THE WORK

Besides completing the samples collected in the intended manner, there are three points to be checked in the analytical procedure. They are:

(1) The loss of nitrogen during the drying of samples

(2) The effect of air on the oil value of dried samples due to oxidation

(3) The effect of various solvents on the value obtained for the oil content.

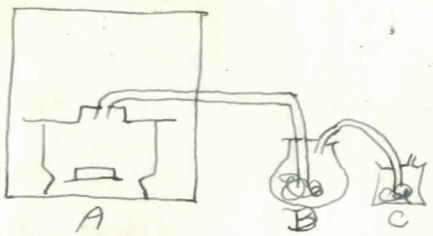
The following notes are pertinent to the points listed.

(1) G. Buglia and A. Constantino Z. f. physiol. chem. 86: 137-140, in a supplement to their series of papers on muscle chemistry, note that when muscle tissues of certain marine animals were dried at 100 - 102° C. there was a loss of some volatile alkaline-reacting substance originating from the decomposition of the muscle extractives. At a temperature 110 - 112° C. a loss of substances, which were acid in reaction, was noticed. S. Yamatmato and S. Masuda, J. Imp. Fish. Inst., (Japan) 22: 53-55, 188-197, 1926, note that when fish muscle is dried by heating for a long time monoamino acid N is, in the most cases, gradually decomposed and is lost.

In the results of the 1932 analyses, protein values were obtained by multiplying the Kjeldahl nitrogen by the factor 6.25. When this was done and the value of ash for the dry extracted meal added, the total was 92 - 96 per cent. Since all the fat had been extracted and since the carbohydrate content of fish is negligible, it follows that the dry oil free meal must be composed of nitrogenous material and inorganic substances and that the total of

protein and ash should be 100 per cent unless some nitrogen has been lost. The papers referred to above point to a loss during the drying process. Another place where losses probably occur is in the extraction of the oil since some nitrogenous materials (such as lecithin or allied substances) are probably extracted. This point is discussed by J. Johnstone, Report Lancashire Sea Fisheries Lab. 1917 and he points out that it is doubtful whether the protein factor should be taken as 6.25 x Kjeldahl N.

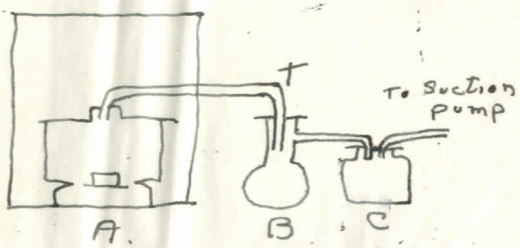
To study these points it was intended to dry samples in a vacuum desiccator fitted with glass tubing leading to a receiving flask containing enough 1N sulphuric acid to absorb any nitrogenous products which were driven off. The weights of the samples, before and after drying, are determined so the amount of moisture lost could be calculated. By concentration of the contents of the receiving flask and by a Kjeldahl determination (run in triplicate on samples of the concentrate) the amount of nitrogen could be found and hence the loss per sample calculated. If this loss were found to be appreciable it would be well to run experiments on several samples in order to obtain enough data to calculate the correction needed for the protein factor.



A = dessiccator at atmospheric pressure.
B = flask containing H_2SO_4.
C = trap containing H_2SO_4 to prevent absorption of NH_3 from the air by flask B and thus prevent erroneous results.

To study the loss of nitrogen during extraction, Kjeldahls should be run in the usual manner on weighed samples of the extracted oil (1- 2-g. samples).

(2) In many investigations of marine products, the procedure has been to dry the samples in a vacuum oven to prevent oxidation of the oil. In this investigation, a vacuum oven was not available, and since Johnstone points out, that while air drying does introduce an error in the oil value that this error is small, all samples were air dried. One experiment conducted in 1932 showed that on drying the samples in a current of CO_2 the values for the dry material were the same as air dried samples. It was intended, however, to carry out some experiments by drying samples in the air and samples, from the same can, in a vacuum desiccator, extracting the dried materials and seeing if there was any marked difference in the oil values. For this experiment it is recommended that 6 samples, from a can containing water, be dried as usual in petri dishes and then 6 samples be dried in petri dishes in a vacuum desiccator and the oil and meal of both series be determined as usual. Six samples are recommended in place of the usual 4 since the increase in number should be sufficient to rule out any differences due to errors in sampling.



A = desiccator pumped down with water pump.
B = distilling flask to receive moisture.
C = Winchester to take up any changes in pressure and prevent the water from sucking back.

The tube T should not extend too far in the flask B or otherwise if there is any sucking back, the condensed moisture will run back into the desiccator and ruin the samples.

(3) <u>The effect of various solvents on the oil values</u>

The two most complete pieces of research in the literature concerned with the composition of herring are that of Johnstone and a paper by J.R. Bruce,

Biochem. J., 18: 469-85, 1924, and both these workers used carbon tetrachloride for oil extractions. For this reason, together with the fact that it is a cheap solvent, non-inflammable, and has a high enough boiling point to prevent great losses during extraction and repurification, it was used. Many workers on other kinds of fish have used ether or petroleum ether. Since some literature has appeared suggesting that the type of solvent will affect the oil value, it was intended to dry samples in the air (and if possible in a vacuum desiccator) and then extract with either carbon tetrachloride, ether, or petroleum ether, and determine the differences obtained for the value of dry oil free meal and hence the oil content. While this is not essential, it will add greatly to the completeness of the paper if it can be included, since it will furnish a basis of comparison to workers who have used solvents other than carbon tetrachloride.