TRYPTIC HYDROLYSIS OF FRESH AND FROZEN FISH MUSCLE

JEAN R. PANTON

HALIFAX, 1930.

BIOLOGICAL BOARD OF CANADA

MANUSCRIPT REPORTS OF THE EXPERIMENTAL STATIONS

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Tryptic Hydrolysis of Fresh and Frozen Fish Muscle.

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Stage 2 gives a measure of the total amino acids present because amino acids behave similarly to ammonia in the presence of 85% alcohol. Potash or soda will displace ammonia in equivalent quantities from ammonium salts in alcoholic solution at ordinary temperatures and at the same time, the ammonia forms no compound with phenolphthalein used as indicator. The carboxyl groups of all the amino acids contained in an amino acid mixture, except that of arginine, are estimated by the total titration value obtained on completing stage 3.

Method of Preparation of Muscle, Sampling and Titrating. (Wynne: Contributions to Canadian Biology and Fisheries, Vol. IV, No.2, 1929.)

Three five hundred cc. flasks each containing 80 grams muscle, minced in a food chopper, and 350 cc. boric acid borate buffer solution (pH 7.5) were prepared. The frozen fillets were minced frozen. The flasks stood overnight at room temperature. Next morning, to two of them, 1.2 grams of papain were added. The solution was readjusted to pH 7.5 when necessary and the flasks were incubated at 37°C and at intervals samples were removed, filtered through No. 1 Whatmann paper and titrated according to the procedure used by Foreman. At the end of an experiment the pH of the remaining digest mixtures was determined and was found to be lowered slightly, varying from pH 7.5 to pH 7.2. Some typical results :

HADDOCK MUSCLE

Time	Fresh (Stage 2 and 3) cc.N/10 NaOH per 10cc. sample	Time	Fresh (Stage 2 and 3) cc.N/10 NaOH per 10cc. sample
½ hour	6.44	1 hour	8.64
24 "	14.94	20.5 "	13.62
50 "	24.22	68 "	23.64
96 "	29.64	97.5 ""	29.56
121 "	30.72	119 "	31.76

Frozen 7 months		Frozen 6 months	
Temp. of storage °C cc.N/10 NaOH per 10 cc. sample		Temp. of storage °C cc.N/10NaOH per 10 cc. sample	
Time		Time	
1 hour	7.44	½ hour	7.24
49.5 "	25.48	50 "	26.38
96.5 "	26.68	77.5"	29.42
120 "	31.52	100 "	30.98
144.5 "	31.30	145 "	33.58

Haddock muscle continued

Frozen less than 24 hours temp. of storage °C cc.N/10 NaOH per 10cc. Sample	
Time	
½ hours	6.24
24.5 "	14.76
71.5 "	25.56
96.5 "	28.28
118.5 "	27.68

Frozen less than 24 hours temp. of storage °C cc.N/10 NaOH per 10 cc. sample	
Time	
½ hour	7.14
24.5 "	15.44
71.5 "	26.28
96.5 "	31.38
118.5 "	31.50

COD MUSCLE

Fresh (Stages 2 and 3) cc.N/10 NaOH per 10 cc.	
Time	
1 hour	7.52
18.5 "	25.74
41.5 "	27.46
88.5 "	31.40
138.5 "	32.50
184.5 "	32.20

Fresh cc.N/10 NaOH per 10 cc.	
Time	
1 hour	8.54
18.5 "	24.34
41.5 "	30.06
88.5 "	31.60
138.5"	---
184.5"	32.80

Frozen 2 years Temp. of storage °C(Stages 2,3) cc.N/10NaOH per 10 cc.	
Time	
½ hour	6.04
24.5 "	18.32
68 "	26.16
120.5"	30.00

Frozen 2 years Temp. of storage °C(Stages 2,3) cc.N/10 NaOH per 10 cc.	
Time	
½ hour	6.82
24.5 "	19.52
68 "	28.28
120.5 "	29.50

MACKEREL MUSCLE

Time	Fresh (Stages 2, 3) cc.N/10 NaOH per 10 cc.	Time	Fresh (Stages 2,3) cc.N/ 10 NaOH per 10 cc.
½ hour	7.00	½ hour	8.04
24.5 "	23.98	24.5 "	25.98
69.5 "	35.08	69.5 "	37.78
97.5 "	37.78	97.5 "	38.80
140 "	37.70	140 "	40.30

Time	Frozen Temp. of storage °C (Stages 2,3) cc.N/10 NaOH per 10 cc.	Time	Frozen Temp. of storage °C (Stages 2,3) cc.N/10 NaOH per 10 cc.
1 hour	7.04	1 hour	7.14
25 "	25.44	25 "	25.24
37 "	29.76	37 "	29.26
66 "	35.26	66 "	35.66
85.5 "	36.26	85.5 "	37.98
131 "	37.58	131 "	40.38
180 "	37.16	180 "	39.78

Conclusions:

(1) For any one species of fish muscle, the freezing process does not alter the total digestibility of the muscle by trypsin, if it is kept stored under suitable conditions of temperature.

(2) In the case of the frozen cod muscle, as long a period as two years' storage has not altered the total digestion of the muscle by tyypsin.

(3) The total/maximum amino acid produced from the tryptic

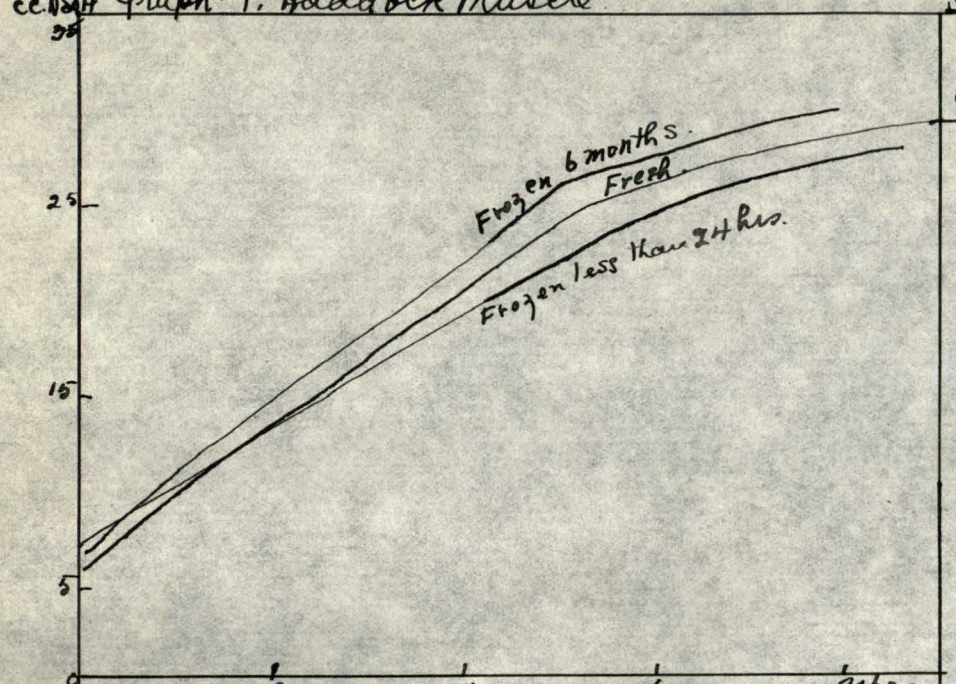
hydrolysis of mackerel (fresh or frozen) appears to be higher than for cod or haddock muscle.

Before finishing my full report, total protein determinations will be run on haddock, cod and mackerel muscle to determine any variations.

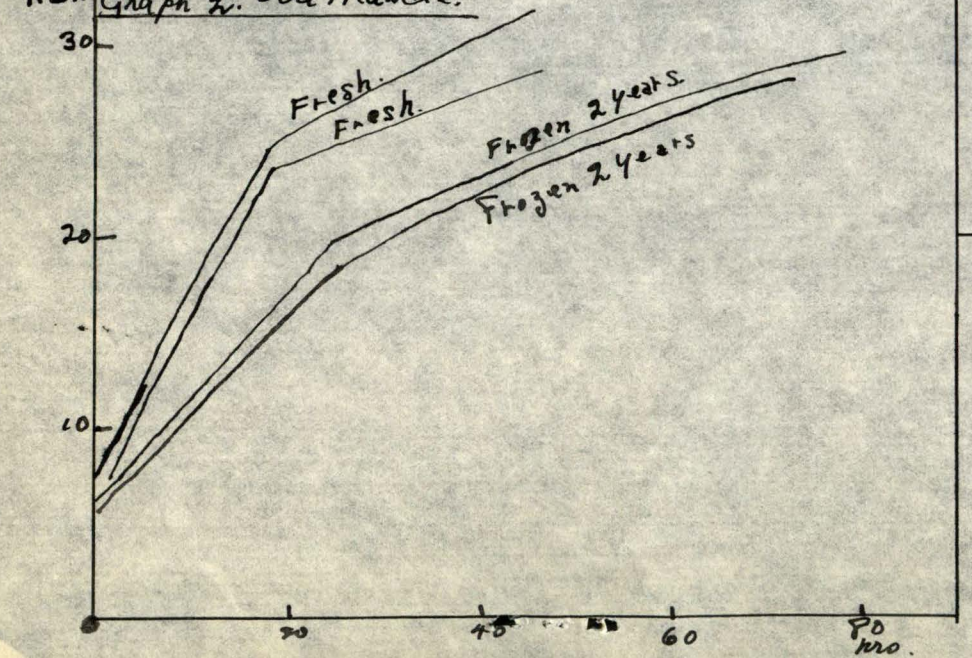
(4) By an examination of the accompanying graphs, the initial rate of digestion by trypsin shows no variation for fresh or frozen muscle, except in the case of cod muscle which was stored for two years . In this case, the initial rate of digestion is lowered.

Initial Rate of Digestion by Trypsin.

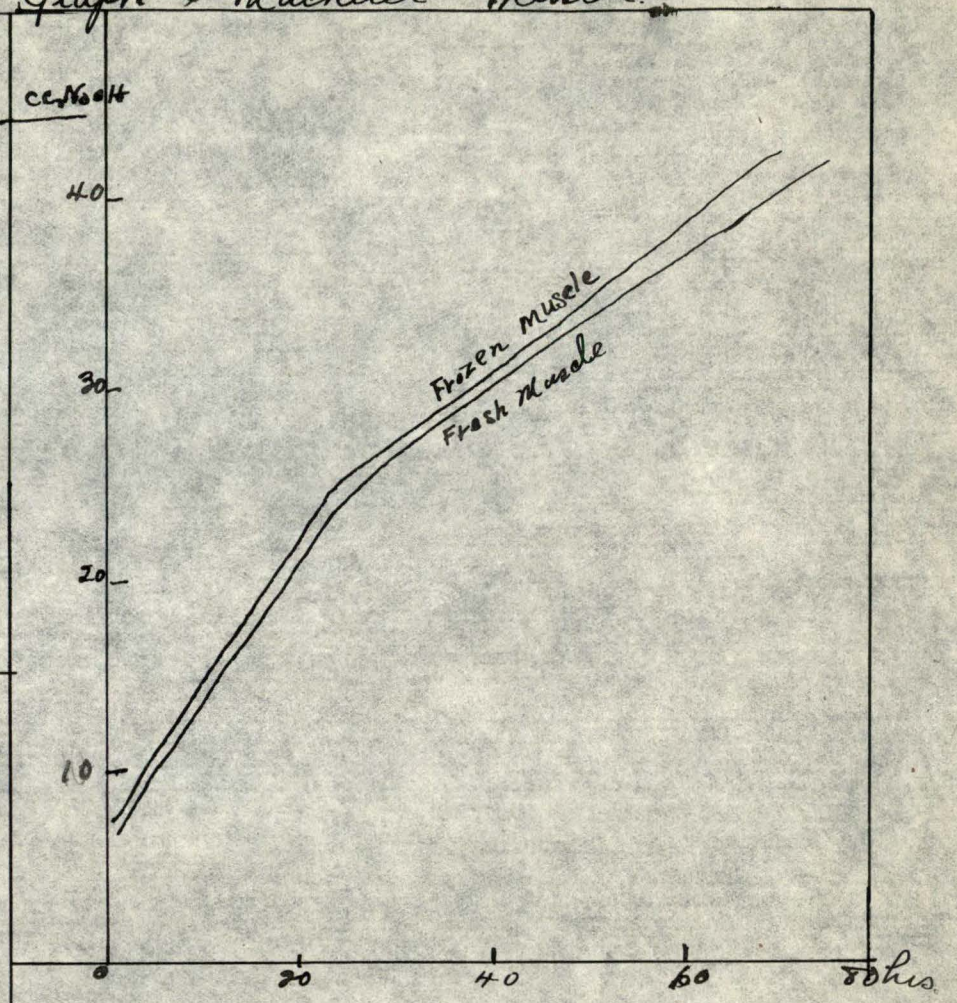
Graph #1. Haddock Muscle



Graph #2. Cod Muscle.



Graph #3 Mackerel Muscle.



cc. NaOH = cc. $\frac{N}{10}$ alcoholic NaOH for titration of stages 2. & 3.