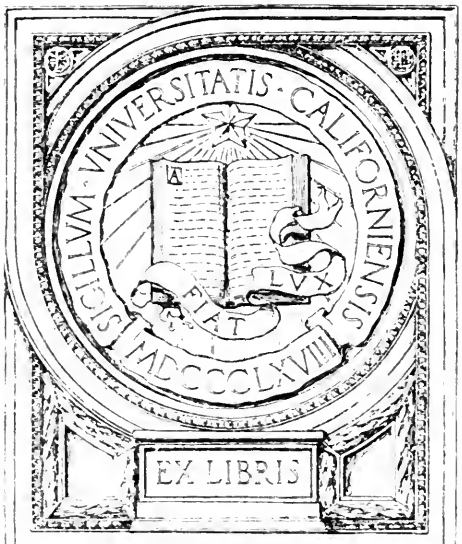
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A Study of the Influence of Cold Storage Temperatures Upon the Chemical Composition and Nutritive Value of Fish

BY

CLAYTON SIDNEY SMITH, B.Sc., M.Sc.

DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE OF COLUMBIA UNIVERSITY

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CONTENTS.

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A STUDY OF THE INFLUENCE OF COLD-STORAGE TEMPERATURES UPON THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF FISH

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I. INTRODUCTION

With the invention of the ammonia machine for the production of low temperatures, the cold-storage industry may properly date its beginning. Although the use of low temperatures for the preservation of foodstuffs has long been recognized, it is only recently that careful studies have been made of the effects upon food products of long periods of cold storage.

Among the first to note the effect of cold upon foods was Tellier,[1] who observed that meat stored at from —2° to +3° C. retained its freshness. Bouley[2] also found that at a temperature of —2° to +3° C., meat kept for an indefinite period so far as putrescence was concerned, but not from the standpoint of edibility. Grassman[3] found that meats kept for eight months at a temperature of —2° C. to —4° C. did not deteriorate. He claimed that refrigerated meats may be cooked in less time than the fresh materials.

A comparative study of the chemical composition of fresh and cold-stored foods was made by Girard,[4] who studied the phosphorus content of vegetables and some animal products, such as pork, mutton, beef, eggs and milk. Gautier[5] made a very detailed study of the difference between fresh and cold-stored beef and

[1] Tellier: *Rev. d. Hyg.*, 1897, xix, p. 298.
[2] Bouley: *Compt. rend.*, 1874, lxxix, p. 739.
[3] Grassman: *Landw. Jahrb.*, 1892, xxi, p. 467.
[4] Girard: *Compt. rend.*, 1896, cxxii, p. 1387.
[5] Gautier: *Rev. d. Hyg.*, 1897, xix, p. 289.

mutton. He found 1 per cent. less moisture in the cold-stored products than in the fresh, but no difference in digestibility.

The effect of cold-storage upon bacteria and enzymes in meat was studied by both Mai[6] and Müller,[7] who showed that putrefaction was prevented but that the action of the enzymes was not inhibited. In the more recent investigations of the effects of cold-storage, emphasis has been placed upon its influence on meat, poultry and game, and to some extent upon vegetables. Little, however, has been said concerning cold-stored fish. The gap in our knowledge at this point led to this investigation.

In 1861, Enoch Piper[8] established a plant in Beekman Street, New York City, for the freezing of fish by means of ice and salt. Davis,[9] in 1868, invented special pans for the freezing of fish, but used the same refrigerating agent. The first carload of frozen fish was shipped from Oregon to New York in 1883, but the quantity was very small compared with the large shipments today. As early as 1888, Russia had an important frozen-fish industry. Sturgeons and dolphins were the principal fish used and the freezing was generally conducted in cellars at the sea-shore.

The frozen-fish industry began in America in the early 90's. The first plant was established at Sandusky,[10] Ohio, in 1892. The industry progressed slowly at first because of a strong prejudice against cold-stored products and particularly against frozen fish. Salmon was at first practically the only fish frozen, but at the present time many varieties are refrigerated.

Each refrigerating concern may have its own particular method of freezing fish but the general practice seems to be to freeze the fish, dip them in water, and refreeze in order that they may be completely encased in ice. They are then stored at a temperature of — 16° C. The coating of ice prevents loss of water due to surface evaporation. This coating is renewed as occasion requires.

Much has appeared in the literature concerning processes for the production of low temperatures and methods for handling cold-

[6] Mai: *Zeit. Nahr. u. Genus.*, 1901, iv, p. 18.
[7] Müller: *Arch. f. Hyg.*, 1903, xlvii, p. 127.
[8] See Loverdo: *Le Froid Artificiel*, 1903, p. 401.
[9] Davis: *Ice and Refrigeration*, 1901, xxi, p. 93.
[10] *Ibid.*

stored products. Little, however, has been written concerning the effect of cold-storage upon the chemical composition of the flesh of fish.

The Report of the U. S. Commission of Fish and Fisheries, for 1888, contains data of analyses of American food fishes. The specimens were, for the most part, fresh fish, a few being preserved but none were cold-stored. More recent analyses of fresh fish have been made by Williams and by Ulrich.[11] Williams'[12] work was conducted from an economic standpoint, while that of Ulrich was a purely chemical study of the composition of fish flesh. It happens that both of these authors have analyzed specimens of fish belonging to species similar to those analyzed by us. Mention will be made of their results when our own are discussed.

The work here reported was undertaken with the hope of ascertaining what change, if any, fish muscle undergoes during long periods of cold-storage. In order that our experiments might be properly controlled, a preliminary study was made of the muscle of fresh fish.

II. EXPERIMENTAL

Preliminary handling of the fish. The fish used were the fluke, also known as summer flounder (*Paralithys dentatus* Linn.), and the winter flounder (*Pseudopleuronectes americanus* Walb.), both of which were furnished by a reliable dealer. These fish were selected because their habits imply that they might be particularly prone to bacterial decomposition in cold-storage. The flounder is peculiarly a "bottom fish," in fact is in the mud or sand most of the time. The various lots of fish were taken from the dealer's ordinary commercial products, which had been handled from the water to Fulton Market (N. Y.), and in the cold-storage plant, in accordance with the practical methods of the trade. As soon as a catch arrived at the wharf, three fish were sent to the laboratory and twenty-four others put into a cold-storage plant. At the plant the fish were suitably dipped, frozen and cold-stored as usual. Those which came to the laboratory arrived packed with cracked ice in an

[11] Ulrich: *Arch. Pharm.*, 1911, ccxlix, p. 68.
[12] Williams: *Chem. News*, 1911, civ, p. 273.

ordinary willow basket. The basket was lined with a water-proof paper, covered with burlap and the whole wrapped in heavy manila paper. The time in transit from the wharf to the laboratory was a little over an hour. Very little of the ice melted en route and, with one exception, the fish were never in contact with free water in the basket.

Upon requisition, fish were taken from storage, wrapped separately in paper, and sent at once to the laboratory, where they arrived in a short time. No appreciable thawing took place in transit. The storage samples, if received during the winter months, were kept over night under paper covers in shallow pans at room temperature. For the summer months this method of thawing was modified by placing the fish in an ordinary refrigerator over night and then allowing them to remain for an hour at room temperature the next morning. In either case, as soon as the fish were completely thawed, the analyses were begun.

Each lot of fish furnished by the dealer was given a number by him. The tag also bore the date and, in the case of stored fish, both the date of receipt at, and the date of delivery from, the cold-storage plant.

In every instance before the fish was prepared for analysis, the general external appearance was observed and, upon dissection, the color and texture of the muscle were also noted. Fresh fish which have been out of the water for some time often show "clots" of slime over their bodies, and in their mouths and gills. Under such conditions the gills may be pale and the fins slightly reddened. When a frozen fish is allowed to thaw at room temperature its skin becomes distinctly dry after the water has evaporated, no slime ever forming. Its gills may be pale but the fins are not red. A slight yellowish brown discoloration, just under the skin, was noted in the case of stored fish; in fresh fish there were certain gray subcutaneous areas. A difference in the consistency of the muscle of the stored fish was also noticed, that of the fresh fish being the firmer. These observations apply only to fresh fish kept on ice from 48–72 hours and to cold-stored fish immediately after thawing.

Analytic determinations and methods. The analytic determinations may be conveniently considered under four headings: *First*—

water, total solids, organic matter, inorganic matter; *second*—ammonium nitrogen, total nitrogen, "soluble nitrogen," "insoluble nitrogen," "coagulable nitrogen," "non-coagulable nitrogen;" *third*—lipins (per cent and acidity); *fourth*—reducing substances and acidity of aqueous extract.

1. The determination of *water* was made in the following manner: An accurately weighed sample, approximately 5 gm., was dried in a large porcelain crucible on a water bath for from three to four hours, after which it was placed in an air bath, at 110° C., and dried to constant weight.

In the determination of *ash,* the following precaution was taken to prevent volatilization of chlorides. After the organic matter had been completely charred over a low flame, an aqueous extract was made of the residue. This liquid was brought to the boiling point and allowed to cool somewhat, when it was decanted as carefully as possible through an ashless filter, most of the residue remaining in the crucible. After drying, the residue and filter were ignited over a bunsen burner to a white ash. The extract was then added to the residue in the crucible and evaporated to dryness on a water bath. Finally the crucible was heated over a low flame to remove residual carbonaceous matter. By this method the soluble salts were not subjected to the high heat of ignition.

Both *total solids* and *organic matter* were calculated from data obtained by the foregoing methods.

2. *Ammonium nitrogen* was determined by the following modification of Folin's method.[13] Fifty grams of fish muscle were ground in a mortar with sand. The finely ground meat was suspended in a mixture of equal volumes of 95 per cent alcohol and water in an aeration cylinder. The mixture of alcohol and water was used in order to prevent excessive frothing during aeration. The volume in each case was approximately 200 c.c. Fifty gm. of pure sodium chloride and 4 gm. of pure sodium hydroxide were added to each 200 c.c. of suspension. As soon as the alkali was added, very vigorous aeration was begun and continued for at least four hours.

Total nitrogen was determined by the Kjeldahl process. Oxidation was facilitated by the addition of a small piece of crystallin copper sulfate to the sulfuric acid.

[13] Steel and Gies: *Journal of Biological Chemistry,* 1908, v, p. 71; Steel: *Ibid.,* 1910, viii, p. 365; Shulansky and Gies: Biochemical Bulletin, 1913, iii, p. 45.

"*Soluble nitrogen*" was determined by the Kjeldahl method, in 10 c.c. of an aqueous extract prepared in the following manner: Twenty gm. of water were added to each gm. of fish taken, but in preparing the extracts, an allowance was made for the water content of the flesh, which was found to average 78.48 per cent. Approximately 50 gm. of flesh were used. After the extract-mixtures were prepared, they were shaken thirty times and allowed to stand over night. Then they were again shaken, allowed to settle and filtered. During filtration care was taken to prevent losses by evaporation.

"*Non-coagulable nitrogen*" was determined directly as follows: 100 c.c. of extract, prepared by the foregoing method, were heated gently to boiling and then treated with 2 c.c. of a 2 per cent solution of acetic acid. When the liquid had again been carried to the boiling point, the solution was filtered directly into a Kjeldahl flask. Into the beaker in which the precipitation had been made, were poured 50 c.c. of distilled water. This was brought to the boiling temperature and then used at once to wash the precipitate on the filter. The precipitate was washed twice in this manner, after which the total ("non-coagulable") nitrogen in the combined filtrate and washings was determined as usual.

From data obtained by the foregoing methods, the "*insoluble nitrogen*" and "*coagulable nitrogen*" were determined by difference.

3. To prepare flesh for the determination of its content of *lipins,* muscle was quickly removed from the fish, passed through a hashing machine, and the hash dried at room temperature before an electric fan, after which the residue was pulverized in a drug mill. Mixtures of aliquot portions of each powder were used in the extractions, which were made by the Soxhlet method upon 60 gm. charges, whenever practicable.

The *acidity* of the lipin mixture was determined by shaking the sample with 50 c.c. of 95 per cent alcohol to which 1 c.c. of 1 per cent phenolphthalein solution in 95 per cent alcohol had been added and titrating with $n/5$ alkali solution. A blank was always run simultaneously on the alcohol.

4. The *reducing power of aqueous extracts* of fish muscle, after removal of the protein, was determined by Benedict's[14] method. The extract was made by treating each gram of fish with 4 gm. of water. Coagulable protein was removed by the method described above.

We always ascertained the degree of *acidity of the aqueous extracts,* as prepared for the various nitrogen determinations. Fifth normal

[14] Benedict: *Journ. Am. Med. Assn.,* 1911, lvii, p. 1193.

sodium hydroxide solution was titrated against 100 c.c. of the extract, using phenolphthalein as the indicator.

Conduct of the examinations. Our examinations of the fish were conveniently divided into three series: (I) On *fresh* fish, (II) on fish

TABLE I

Series I. Fresh flounders. Percentage data, except as noted

Fish No.	Water	Total solids	Organic matter	Ash	Ammonium N	Fish No.	Nitrogen					Reaction of aqueous extract*
							Soluble	Insoluble	Coagulable	Non-coagulable	Total	
A1	78.35	21.65	20.36	1.29	0.014	.....	.....	.....	.....	.....	3.26	
A2	78.80	21.20	19.97	1.23	0.014	.....	.....	.....	.....	.....	3.21	
A3	79.01	20.99	19.77	1.22	0.012	.....	.....	.....	.....	.....	.....	
B4	78.74	21.26	19.97	1.29	0.025	.....	.....	.....	.....	.....	3.27	
B5	78.62	22.38	21.09	1.29	0.022	.....	.....	.....	.....	.....	3.51	
B6	79.10	20.90	19.62	1.28	.....	.....	.....	.....	.....	.....	3.32	
C7	76.81	23.19	21.96	1.23	0.011	.....	.....	.....	.....	.....	3.58	
C8	79.52	20.48	19.23	1.25	0.026	.....	.....	.....	.....	.....	3.21	
C9	79.76	20.24	19.04	1.20	0.023	.....	.....	.....	.....	.....	3.21	
D10	77.88	22.12	20.90	1.32	0.025	.....	.....	.....	.....	.....	3.49	
D11	75.78	24.22	22.66	1.56	0.029	.....	.....	.....	.....	.....	4.04	
D12	80.40	19.60	17.37	1.23	.....	.....	.....	.....	.....	.....	3.70	
L34	81.03	.....	.....	.....	.....	E13	1.079	2.361	0.705	0.374	3.44	1.75
L35	82.49	.....	.....	.....	.....	E14	1.084	2.716	0.711	0.373	3.80	1.85
L36	81.56	.....	.....	.....	.....	E15	0.924	2.336	0.552	0.372	3.26	1.68
M37	81.51	.....	.....	.....	.....	F16	1.011	2.409	0.612	0.399	3.42	1.45
M38	80.13	.....	.....	.....	.....	F17	0.959	2.511	0.501	0.458	3.47	1.20
M39	81.66	.....	.....	.....	.....	F18	0.958	2.282	0.562	0.396	3.24	1.05
N40	82.49	.....	.....	.....	.....	G19	1.118	1.752	0.772	0.346	2.87	1.35
N41	82.22	.....	.....	.....	.....	G20	1.065	1.695	0.724	0.341	2.76	1.30
N42	81.32	.....	.....	.....	.....	G21	0.746	1.774	0.429	0.317	2.52	2.25
O43	82.78	.....	.....	.....	.....	H22	0.799	1.921	0.485	0.314	2.72	1.30
O44	80.32	.....	.....	.....	.....	H23	1.012	2.018	0.629	0.383	3.03	1.35
O45	80.87	.....	.....	.....	.....	H24	1.173	1.727	0.737	0.436	2.90	1.00
P46	81.46	.....	.....	.....	.....	I25	0.852	1.838	0.426	0.426	2.69	1.00
P47	82.14	.....	.....	.....	.....	I26	0.852	1.958	0.453	0.399	2.81	1.20
P48	83.00	.....	.....	.....	.....	I27	0.759	2.121	0.351	0.408	2.88	1.10
R49	82.42	.....	.....	.....	.....	J28	0.852	1.998	0.447	0.405	2.85	1.35
R50	81.27	.....	.....	.....	.....	J29	0.746	2.244	0.363	0.383	2.99	1.10
R51	82.91	.....	.....	.....	.....	J30	0.932	2.238	0.554	0.378	3.17	1.15
.....	.....	.....	.....	.....	.....	K31	0.985	2.025	.....	.....	3.11	1.28
.....	.....	.....	.....	.....	.....	K32	1.518	1.562	1.137	0.381	3.08	1.63
.....	.....	.....	.....	.....	.....	K33	0.825	2.275	0.445	0.380	3.10	1.48
Average	80.15	21.52	20.16	1.28	0.0195	.....	0.964	2.084	0.579	0.383	3.18	1.37

Lipins—Fish L34–R51, in three groups; aliquot portions of the fish in each groups were extracted together:

Total amount of lipins in the fresh flesh (per cent)—0.386, 0.360, 0.392; *average, 0.379.*

Acidity (mg. of KOH to neutralize 1 gm. of lipins)—131, 147, 132; *average, 136.*

* Expressed as c.c. of *n*/5 sodium hydroxid solution required to neutralize 100 c.c. of extract.

stored for six months, and (III) on fish *stored for nine months.* The analytic determinations upon each series of fish were of the same kind, but for convenience they may be considered in three different groups.

TABLE 2

Series II. Flounders after six months of cold storage. Percentage data, except as noted

Fish No.	Water	Total solids	Organic matter	Ash	Ammonium N	Fish No.	Nitrogen					Reaction of aqueous extract*
							Soluble	Insoluble	Coagulable	Non-coagulable	Total	
A70	78.00	22.00	20.70	1.30	0.006	.....	.....	.....	.....	.....	3.57	
A71	78.28	21.72	19.47	1.25	0.011	.....	.....	.....	.....	.....	3.31	
A73	78.02	21.98	20.73	1.25	0.009	.....	.....	.....	.....	.....	3.47	
B74	77.06	22.94	21.59	1.35	0.034	.....	.....	.....	.....	.....	3.53	
B75	77.82	22.18	20.82	1.36	0.022	.....	.....	.....	.....	.....	3.43	
B76	77.80	22.20	20.93	1.27	0.010	.....	.....	.....	.....	.....	3.34	
C77	79.57	20.43	19.17	1.26	0.011	.....	.....	.....	.....	.....	3.19	
C78	79.72	20.28	.....	.....	0.016	.....	.....	.....	.....	.....	3.21	
C79	78.62	21.38	20.12	1.26	0.008	.....	.....	.....	.....	.....	3.41	
D80	79.29	20.71	19.41	1.30	0.015	.....	.....	.....	.....	.....	3.23	
D81	80.85	19.15	17.92	1.23	0.008	.....	.....	.....	.....	.....	3.09	
D82	79.33	20.67	19.39	1.28	0.008	.....	.....	.....	.....	.....	3.38	
D83	.....	.....	.....	.....	0.022	.....	.....	.....	.....	.....	.....	
D84	.....	.....	.....	.....	0.017	.....	.....	.....	.....	.....	.....	
D85	.....	.....	.....	.....	0.015	.....	.....	.....	.....	.....	.....	
L108	80.39	.....	.....	.....	.....	E86	1.145	2.395	0.714	0.431	3.54	1.33
L109	81.62	.....	.....	.....	.....	E87	0.799	2.581	0.430	0.469	3.38	1.43
L110	81.02	.....	.....	.....	.....	E88	0.945	2.485	0.572	0.373	3.43	1.38
M111	81.81	.....	.....	.....	.....	F99	0.959	2.101	0.586	0.373	3.06	1.25
M112	82.02	.....	.....	.....	.....	F100	0.959	2.251	0.597	0.362	3.21	1.10
M113	81.64	.....	.....	.....	.....	F101	0.907	2.313	0.545	0.362	3.22	1.20
N114	82.55	.....	.....	.....	.....	G102	0.746	2.244	0.437	0.309	2.99	1.30
N115	80.30	.....	.....	.....	.....	G103	0.825	2.015	0.483	0.341	2.84	1.10
N116	81.04	.....	.....	.....	.....	G104	.....	.....	.....	.....	2.95	1.10
O117	82.58	.....	.....	.....	.....	H105	0.864	1.926	0.566	0.298	2.79	1.00
O118	79.78	.....	.....	.....	.....	H106	0.770	1.870	0.474	0.296	2.54	1.00
O119	82.41	.....	.....	.....	.....	H107	0.833	1.777	0.588	0.245	2.61	0.90
P120	82.40											
P121	82.54											
P122	82.49											
R123	80.54											
R124	80.53											
Average	80.13	21.30	20.01	1.29	0.0142	.....	0.887	2.179	0.545	0.351	3.19	1.17

Lipins—Fish L108–R124, in three groups; aliquot portions of the fish in each group were extracted together:

Total amount of lipins in the undried flesh (per cent)—0.362, 0.458, 0.277; *average, 0.366.*

Acidity (mg. of KOH to neutralize 1 gm. of lipins)—150, 138, 120; *average, 136.*

* Expressed as c.c. of *n*/5 sodium hydroxid solution required to neutralize 100 c.c. of extract.

The *first group* of determinations included water, ash, ammonium nitrogen and total nitrogen. The determinations were made upon fish from four of the dealer's lots in each of the three series of observations.

The *second group* of determinations, comprising total, soluble and non-coagulable nitrogen, together with the reaction of the aqueous extract, was made upon fish from seven of the dealer's lots (Series I).

TABLE 3

Series III. Flounders after nine months of cold storage. Percentage data, except as noted

Fish No.	Water	Tota solids	Organic matter	Ash	Ammonium N	Fish No.	Nitrogen					Reaction of aqueous extract*
							Soluble	Insoluble	Coagulable	Non-coagulable	Total	
A125	78.06	21.99	20.59	1.40	0.027	.....	.....	.....	.....	.....	3.39	
A126	77.97	22.03	20.76	1.27	0.026	.....	.....	.....	.....	.....	3.44	
A127	78.55	21.45	20.31	1.14	0.013	.....	.....	.....	.....	.....	3.29	
B128	79.48	20.52	19.34	1.18	0.022	.....	.....	.....	.....	.....	3.34	
B129	77.82	22.18	20.89	1.29	0.010	.....	.....	.....	.....	.....	3.55	
B130	79.14	20.86	19.67	1.19	0.019	.....	.....	.....	.....	.....	3.31	
C131	78.70	21.30	20.05	1.25	0.024	.....	.....	.....	.....	.....	3.28	
C132	77.65	22.35	21.12	1.23	0.032	.....	.....	.....	.....	.....	3.48	
C133	79.37	20.63	.....	.....	0.032	.....	.....	.....	.....	.....	3.54	
D134	76.74	23.26	21.73	1.53	0.017	.....	.....	.....	.....	.....	3.79	
D135	79.00	21.00	20.73	1.27	0.021	.....	.....	.....	.....	.....	3.35	
D136	78.97	21.03	19.72	1.31	0.012	.....	.....	.....	.....	.....	3.33	
L149	82.45	.....	.....	.....	.....	E137	1.074	2.046	0.741	0.333	3.12	1.87
L150	84.24	.....	.....	.....	.....	E138	1.127	2.483	0.719	0.408	3.61	1.85
L151	80.55	.....	.....	.....	.....	E139	1.043	2.507	0.592	0.451	3.55	1.63
M152	73.98	.....	.....	.....	.....	F140	0.820	2.260	0.466	0.354	3.08	0.95
M153	80.87	.....	.....	.....	.....	F141	0.913	.....	0.542	0.371	.....	1.35
M154	81.49	.....	.....	.....	.....	F142	0.966	2.364	0.603	0.363	3.33	1.10
N155	83.02	.....	.....	.....	.....	G143	.....	.....	.....	.....	2.99	0.90
N156	81.76	.....	.....	.....	.....	G144	0.859	2.121	0.548	0.311	2.98	0.90
N157	81.68	.....	.....	.....	.....	G145	0.895	2.095	0.519	0.376	2.99	0.97
O158	81.82	.....	.....	.....	.....	H146	0.966	1.264	0.655	0.311	2.23	0.97
O159	81.69	.....	.....	.....	.....	H147	0.806	2.064	0.511	0.295	2.87	0.88
O160	82.38	.....	.....	.....	.....	H148	0.859	2.071	0.548	0.311	2.93	1.20
P161	81.90											
P162	82.18											
P163	82.51											
Average	80.24	21.55	20.45	1.28	0.0213	.....	0.937	2.128	0.586	0.353	3.11	1.23

Lipins—Fish L149–P163, in four groups; aliquot portions of the fish in each group were extracted together:

Total amount of lipins in the undried flesh (per cent)—0.442, 0.497, 0.391, 0.442; *average, 0.443.*

Acidity (mg. of KOH to neutralize 1 gm. of lipins)—111, 130, 139, 130; *average, 127.*

* Expressed as c.c. of *n*/5 sodium hydroxid solution required to neutralize 100 c.c. of extract.

When this group of determinations was repeated on Series II and III, four dealer's lots were used for each series.

The *third group* consisted of determinations of lipins, water and reducing power. Because of the small percentage of fat in the fish, all of the dealer's lots (six) in this group were drawn upon in each of the three series of observations.

In performing a particular set of analyses, samples of flesh were uniformly taken, as nearly as possible, from the same region. The selected parts were in the right angles made by a line running from the head to the tail and a line perpendicular to it about midway between the mouth and the tail. The skin on the back was opened, turned over, and flesh taken from all depths to the bone. Flesh near the viscera, as well as remote from them, were included. The skin was not analyzed.

For convenience in consulting the data, the following plan of tabulation was adopted. Each capital letter in the accompanying tables represents a dealer's lot of fish. The numeral following the capital letter indicates an individual fish. Thus, **B5** (Table 1) represents a particular fish that was *fresh* when analyzed. **B75** (Table 2) indicates a particular fish of the same lot which had been placed in cold storage and *analyzed six months later*. The capital letter also indicates, in terms of the appended summary, the date (in 1911) when a particular fresh fish was subjected to analysis (or placed in cold storage):

A–Sept. 9.	E–Oct. 18.	I–Nov. 27.	M–Dec. 6.
B–Sept. 29.	F–Nov. 1.	J–Nov. 29.	N–Dec. 8.
C–Oct. 3.	G–Nov. 22.	K–Dec. 1.	O–Dec. 11.
D–Oct. 10.	H–Nov. 24.	L–Dec. 4.	P–Dec. 13.
			R–Dec. 19.

III. DISCUSSION OF RESULTS

Water. From the tables it will be seen that, beginning with lot **L**, there is an increase in content of water. The fish in lots **A** to **D** were flukes or summer flounders, while those in lots **L** to **R** were winter flounders. Apparently the water-content of the winter flounder is about 3 per cent. higher than that of the summer variety. Williams finds the water-content of the plaice (*Pleuronectes platessa*) to be 79.86 per cent., while in the report of the U. S. Commissioner of Fish and Fisheries the value for the same fish is 77.39 per cent. The government analyst also reports 85.04 per cent. of water for the summer flounder and 84.35 per cent. for the winter

flounder (*Pleuronectes americanus*). It will be seen that the government figures accord neither with Williams' nor our own. The government values are based upon the analysis of but one fish in each case. Ulrich did not analyze a fresh specimen, but reports the water-content of smoked flounder to be 71.66 per cent.

On comparing the results for the three series, it will be noted that there was practically no change in the water-content. This would be naturally expected, because of the care taken to keep the fish completely encased in ice during the storage period. In the case of beef, however, Emmett and Grindley[15] report a loss of 1.3 per cent. of moisture after a forty-three days' period of storage.

Total solids. As the value for total solids was determined by difference, it varied inversely as the water-content. Our results show that there was no change in the water-content. Consequently, the value for total solids remained unchanged.

Inorganic matter. There was no reason to believe that possible changes in the flesh during cold-storage would affect the ash-yield, yet for an adequate analysis of the fresh specimen it was desirable to make this determination. By repeating the determination on the cold-stored products we were able to obtain a closer value for the ash-yield and at the same time detect any unexpected change. Ash determinations were made only upon summer flounders. The government analyst in the report already quoted gives the following percentage values for the ash content of three related fish: summer flounder, 1.29; winter flounder, 1.20; and plaice, 1.46. As was expected, the results obtained by us showed that cold-storage was without effect on the yield of ash.

Organic matter. As in the case of total solids, organic matter was determined by difference. Its variations were dependent upon variations in water-content and ash-yield. There being practically no variations in these, the percentage of organic matter remained unchanged.

Ammonium nitrogen. Without doubt this was our most important determination, especially from the standpoint of detection of bacterial influences. Our method, as already described, differs from that used by Pennington and Greenlee[16] in that we substituted

[15] Emmett and Grindley: *J. Ind. Eng. Chem.*, 1909, i, p. 413.

[16] Pennington and Greenlee: *Journ. Am. Chem. Soc.*, 1911, xxxii, p. 561.

sodium hydroxid for sodium carbonate. After a long series of experiments, Pennington and Greenlee found that the results obtained with the modified Folin method agreed very well with those obtained when the older magnesium oxid method was used. By using sodium hydroxid we were open to the criticism that our results might be higher than actual ammonium values. Yet granting this possibility, we found that the proportion of ammonium nitrogen was very low, even after a nine months' period of storage. If, therefore, by using a method which might give high results, no increase in ammonium nitrogen was found, after six months of storage and only a trivial increase after nine months of storage, it is fair to conclude that the fish were practically unchanged at the end of the last named period of storage. [See the preceding paper by Shulansky and Gies (p. 45) and the succeeding one by Perlzweig and Gies (p. 69).]

Pennington and Greenlee found the ammonium nitrogen content of *fresh* chicken meat to be 0.012 per cent. Houghton[17] reports 0.021 per cent. of ammonium nitrogen in *fresh* light chicken meat and 0.039 per cent. in the same kind of meat after a period of *five months of storage.* For dark chicken meat he reports 0.019 per cent. ammonium nitrogen in the *fresh* sample and 0.026 per cent. after a period of *five months of storage.* It would appear, then, that so far as the production of ammonium nitrogen is concerned, fish in cold-storage change more slowly than chickens.

Total nitrogen. Unless some ammonia was formed in the fish, and escaped into the air, there would be no chance for any diminution in the nitrogen content. Total nitrogen remained unchanged. It will be noticed that there is a difference of about 1 per cent. between the nitrogen content of flukes and flounders. This variation is partly explained when one takes into consideration the difference in contents of water.

"Soluble nitrogen." If any hydrolytic changes took place during the cold-storage period it would be natural to suppose that one or more of the nitrogenous constituents of the muscle became more soluble, or, in other words, that there was an increase in the "soluble nitrogen." Our results indicate that there was no in-

[17] Houghton: *J. Ind. Eng. Chem.*, 1911, iii, p. 497.

creased solubility of nitrogeneous substances. In the case of chicken, Houghton reports a slight increase in "soluble nitrogen" for light meat and a slight decrease for dark meat.

"Coagulable nitrogen" and "non-coagulable nitrogen." From the data in the tables it appears that there was a very slight increase in "coagulable nitrogen" and a corresponding decrease in "non-coagulable nitrogen." These differences are too slight to warrant any inferences.

Lipins. The term lipins is used to indicate the fats and fat-like substances in the "ethereal extract."[18] The flesh of the winter flounders that were used for the determinations was comparatively poor in ether-soluble constituents. The actual weight of lipins neutralized at any time was always less than one gram. This admits of relatively large degrees of experimental error; according to Allen,[19] 5 to 50 gm. of material should be used for this determination. Other authors recommend at least 4–5 gm. In our work such large samples were not available. While the values here reported are possibly somewhat too high, it is significant that there is no increase in acidity during a nine months' period of storage.

It is difficult to believe that the lipins would undergo any changes, significant of deterioration in nutritive value, which would not be shown more strikingly by the protein constituents of the flesh. The negative findings in this particular connection accord with such a view of the matter.

Reducing substances. We determined the reducing power of aqueous extracts, in order to detect any sugar which might have resulted from the hydrolysis of glycogen during the cold-storage period. A similar determination was made by Williams,[20] but her method involved the hydrolysis of all carbohydrate-yielding substances. She treated fish-powder with boiling dilute hydrochloric acid solution under a reflux condenser for three hours; proteins were removed by precipitation with lead acetate; then, after removal of the excess of lead, the reducing power of the filtrate was determined by the Fehling method and reported as percent. of glucose.

[18] Rosenbloom and Gies: Biochemical Bulletin, 1911, i, p. 51.
[19] Allen: Com. Organic Anal. (3 ed.), Vol. 2, pt. 1, p. 105.
[20] Williams: *Trans. Chem. Soc.*, 1897, lxxi, p. 651.

By this method a reducing power of 2.32 per cent. was reported for the plaice (*Pleuronectes platessa*).

Our own method failed to show any reducing power. Artificial hydrolysis was avoided. We were unable to get satisfactory responses to our qualitative tests for the presence of sugar in protein-free extracts, both from fresh and storage fish. For a comparative test, glucose, to the amount of 0.01 per cent., was added to one of the extracts, and a characteristic reduction obtained. For the quantitative determination of small amounts of sugar in meat, Bauer[21] recommends a spectroscopic method, since the polarimetric and titrimetric processes are unreliable for amounts less than 0.5 per cent. As no sugar was detected qualitatively, either before or after cold-storage, it seemed quite certain that no appreciable hydrolysis of glycogen had taken place.

Quantitative reaction of aqueous extracts. The reaction of aqueous extracts was always found to be acid to both litmus and phenolphthalein. From the data in the tables it is evident that the acidity of the aqueous extract was not materially affected by long periods of cold-storage. In several instances the same extract was titrated after standing for from two to four days in an ordinary house refrigerator. In each case the variation in acidity was within the limits of error of the method itself.

The general analytic results of our work are summarized in Table 4.

TABLE 4

Summary of general average data

Months in cold storage	Summer flounder (fluke)				Winter flounder.					
	Water	Nitrogen		Ash	Water	Nitrogen			Lipins	
		Total	Ammonium			Total	Soluble	Non-coagulable	Per cent	Acidity
	%	%	%	%	%	%	%	%		
None.....	78.56	3.44	0.0195	1.28	81.75	2.89	0.964	0.383	0.379	136
Six.....	78.69	3.33	0.0142	1.29	81.58	2.79	0.887	0.351	0.366	136
Nine.....	78.45	3.40	0.0213	1.28	82.04	2.83	0.937	0.353	0.443	127

[21] Bauer: *Arb. a. d. kais. Gesundhsmt*, 1908, xxx, p. 63.

IV. SUMMARY OF CONCLUSIONS

1. The proportions of water in, and yield of ash from, the flesh of flounders were unaffected by a nine months' period of cold-storage.

2. The changes in the proportions of soluble, coagulable and non-coagulable nitrogenous constituents were negligible.

3. During a nine months' period of cold-storage, there was practically no change in the content of ammonium nitrogen.

4. For fish with a low content of lipins, there was apparently no increase in the acidity of the muscle lipins during a nine months' period of cold-storage.

5. There was no production of reducing substance from any constituent of the flesh during any of the storage periods.

6. There was no evidence, whatever, of any depreciation in the nutritive value, or any change in the sanitary character, of the fish at any time during nine months of cold-storage.

The writer wishes to record his indebtedness and to express his thanks to Prof. William J. Gies under whose direction this work was done.[22]

[22] See the succeeding paper by Perlzweig and Gies (p. 69), for additional data on this subject.

BIOGRAPHICAL.

Clayton Sidney Smith was born in Newark, New Jersey, on April 23, 1887. He received his education in the public schools of Newark and at Rutgers College. In 1909 he was graduated with the degree of Bachelor of Science. In the summer of 1909 he matriculated in Columbia University for the degree of Master of Arts, but in the fall of 1910 changed his matriculation for that of the degree of Doctor of Philosophy. In 1912 he received the degree of Master of Science from Rutgers College.

During the year 1909–10 he was instructor in the Metuchen, New Jersey, High School. From 1910 to 1912 he was Assistant in the Department of Biological Chemistry of Columbia University. July 1, 1912, he was made Instructor, which position he held until November 1, when he resigned to become Assistant Pharmacologist in the Bureau of Chemistry, U. S. Department of Agriculture.

PUBLICATIONS.

Smith and Baker: On the Variations in the Occurrence of Nitrite in Saliva. *J. Allied (Dental) Soc.*, 6, 323 (1911).

***Smith:** Chemical Changes in Fish During Long Periods of Cold Storage. BIOCHEM. BULL., 2, 184 (1912).

***Salant and Smith:** Concerning the Pharmacological Action of the Tartrates. *J. Am. Chem. Soc.*, 35; Proc., p. xxix (1913).

Clark and Smith: Toxicological Studies on the Mushrooms *Clytocybe illudens* and *Inocybe infida*. Mycologia, 5, 224 (1913).

***Salant and Smith:** The Toxicity of Sodium Tartrate with Special Reference to Diet and Tolerance. *Proc. Soc. Exp. Biol. and Med.*, 10, 170 (1913).

* Preliminary communication.