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30

Studies of Muscle Juice

Report of work done at St. Andrews Biological Station  
during the summer of 1920

By

G. G. Benson.

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By C. C. Benson

At the present time the best known system for preserving fish for distribution at a distance from the place where it is taken and for use some time after it is caught, is that of freezing and cold storage. Unfortunately, in some cases, these processes cause changes in the flavour and texture of this food. It is, therefore, important to have information as to the causes of these difficulties and as to their chemical nature; but, with regard to flavour, our knowledge is so slight, that we have not even a nomenclature, and in regard to texture, we need much more information of certain branches of colloid chemistry, before we can have a clear picture of its nature.

Investigators have followed various possible means of study, trying to gain information in regard to changes due to freezing. A number of investigations have been made to determine the gross chemical changes due to cold storage. Pennington (1917) made an exhaustive chemical and biological study of chicken in cold storage; and Clark and Almy (1920) have determined changes in moisture content and in the nitrogenous constituents of fish kept under

different conditions of temperature. Such analyses have shown that only very slight changes occur in the constituents studied so long as the fish is good for food. Other investigations of the same kind have shown similar results and analyses made by Mrs. Ruth Neff McCamus (1920) for the Biological Board on fresh and frozen hake, failed to show variations in general chemical composition, even when the frozen tissues were of so spongy a form that the fish were totally unfit for food.

It seemed possible that the unfortunate changes due to freezing might be related to other earlier changes in the proteins, which in the case of fish make up almost the whole of the nutritious material. This seemed the more likely, because much more objection is taken to frozen fish than to frozen meat, and, while we commonly hang meat to make it "tender", allowing it to become rigid and then to soften again, we prefer fish in as fresh a condition as possible.

A study of the literature of the chemistry of rigor mortis was, therefore, made and this present report forms a preliminary communication of a study of certain chemical changes occurring in the proteins of muscle juice as affected by the stiffening and softening following death.

The muscle juice or plasma undergoes a peculiar form of clotting, which is apparently related to rigor and the juice should, therefore, show variations in composition related to the time since the animal's death.

It seemed probable also that the insoluble material of the muscle - the stroma - would vary and investigations were consequently undertaken with both plasma and stroma to see if there

were noticeable variations in these constituents in fish which was fresh and in that which had been kept.

The various methods of earlier investigators were examined and in some cases repeated with fresh fish muscle. Some lines of study proved apparently fruitless, as, for example, a number of attempts to separate the proteins of the plasma by extraction with different salt solutions and by dialysis according to methods of Kühne (1868), Halliburton (1887) and others.

Halliburton's (1887) method of separation of the proteins by heat coagulation likewise proved useless for this purpose but von Fürth's (1895) test-tube method of estimating the amounts of the proteins soon showed differences that it seemed worth while to investigate more fully.

In order to determine the amounts of the proteins in the plasma, von Fürth measured into a series of test-tubes, 5 cc. lots of the filtered juice and then heated these test-tubes to different temperatures to coagulate the different proteins. The plasma is, however, a peculiar protein solution not only in that it coagulates on standing but also in that it shows peculiarities of heat coagulation. Von Fürth, therefore, chose certain temperatures at which to produce the clots and kept the fluids at these temperatures for exactly five minutes in each case. The clots, as he formed them, were, he knew, mainly composed of certain proteins but were largely mixtures; but by this arbitrary arrangement he had always the same material in each set of experiments. The first clot was formed at 40°C, the second, at 50°C, the third, at 70°C and a fourth at 100°C. The second, third and

fourth coagula were thus certainly mixtures but the actual amounts of proteins precipitated during 5 minutes at each temperature were obtained by subtractions. The temperatures were chosen as a result of previous knowledge of the coagulation temperatures of the proteins present, but this aspect of the question does not concern us here.

The fish muscle plasma used in my experiments was obtained by the method described by von Fürth, and is similar to the methods used by Kühne and Halliburton for their studies with frogs and other animals.

The fish were decapitated and in most cases, were injected through the jugular vein, with a 0.6% solution of sodium chloride, until the fluid flowing from the sectioned blood vessels and from the body cavity, was no longer coloured red. It is difficult to find the jugular vein in fish of the size available, and in some experiments, the injection was omitted. Experiments made with fish, part of whose muscle was cut off above the point of injection and part below, showed that the injection made no change in the osmotic pressure of the juice as is shown in the following table where the depression of the freezing point is compared with that of muscle juice from the mutton fish and with that of sea water.

Table No. I.

$\Delta$ 's for sera and sea water from tank.				
Haddock	Haddock	Mutton Fish	Water from tank	& lab.
0.875	injected		1.777	1.803
0.835	0.875	0.985	1.772	1.801

In later experiments, a 1% solution of salt was used as these freezing point determinations indicate a higher salt content than had been expected.

So soon as the fish reached the laboratory, or immediately after injection, they were cut into steaks, which were used at once or put into the refrigerator, or left in the warm room, or frozen, and examined later as required.

The muscle was freed from bones, skin etc. and put through a meat mincer, using one of the finer knives. With the fresh fish this was done as quickly as possible, so as to avoid as far as could be, the chemical changes of rigor and the subsequent softening. Many investigators stress the need of grinding with sand in order to be able to squeeze out the juice. The sand undoubtedly serves to more completely break up the tissues, but it required more time and trouble for its use and for comparative results. I, therefore, thought it wiser to use the quicker and easier method. The quicker method is certainly the better as a means of preventing loss of time during which changes can occur.

The minced muscle was quickly put into squares of muslin of about 25 - 30 fibres to the centimeter and these were folded over to wrap the muscle. These pads were then put into a strong iron lemon squeezer and the juice pressed out into clean glass dishes.

It was usually filtered through fine muslin, in a few cases through paper but in all cases, material free of solid particles was used and was subjected to the same kind of filtration for comparative tests.

Undoubtedly a more accurate method of squeezing, with definite knowledge of the pressure used, would be better, but the results

so far obtained go to show that, even with this rough method of expressing the juice, valuable information can be gathered.

The volume of the juice was usually measured and, as the quantity allowed, it was used for as many tests as possible.

Bottazzi (1913), who used a Buchner press of 350 atmospheres pressure, was able to squeeze out from 40 to 63% juice but with the cruder method, much less was obtained (See Table II, p. 6).

The early work was all done with haddock and this was undoubtedly fortunate for not only was more juice obtained from this fish but its muscle was much more satisfactory to work with, than that of other fish. It was easily minced and squeezed out readily. The hake muscle became gummy and gave very little juice, and the cod, in one experiment, was nearly as bad.

These peculiarities are not due to the water content of the muscle as is shown in Table II.

Table II.

Water Content and Plasmas of Muscle of Fish.

Fish.	% of water in muscle	% of juice squeezed out of muscle.
Haddock	80.3 79.7	16% 18%
Hake	81.1	4%

The explanation is to be found in the colloidal nature of the material and not in the amounts of water and solids. It is rather the relation of the water to the special protein here present, which gives such marked differences of consistency. Colloidal studies indicate lines of investigation, and inform-

ation which is being gathered as to the power which proteins have of imbibing water under different conditions, and the swelling which so results, should help to elucidate this part of the problem. This is especially true in regard to the muscle of the hake.

With the filtered juice, determinations were made of total solids by drying 5 cc. lots to constant weight in a boiling water oven, and of total proteins coagulated at 40°C, 50°C, 70°C and 100°C, according to the method of von Fürth. For the 40° coagulation the test tubes containing the 5 cc. lots of juice were put into a beaker which contained water, previously brought to the required temperature, and which was surrounded by another which contained water at a temperature 2° higher. The test tubes were kept in the beaker for exactly 5 minutes and then immediately cooled under the cold water tap. When the cold test tubes were put into the water bath, the temperature of the water fell slightly but soon rose again from the heat of the outer jacket but in no case was the temperature allowed to rise above the point of coagulation. For this clotting no other heat was supplied. The clots at 50° and 70° were prepared in the same way, except that the outer jacket was heated to 50° or 70° by means of a small gas flame. The coagula for 100°C were formed by keeping the tubes in a bath of water which had been heated to boiling and was kept boiling to the end of the 5 minute period. The precipitates were filtered on dry weighed papers, washed and dried to constant weight. The results of these determinations are given in Tables III and IV.

The determinations were all made in duplicate, except in the

one case noted, where the quantity of juice available was too small to allow of this. The duplicates agreed very closely in all cases but the one noted - that for the total coagulable protein of the fish of August 5th.

The coagulated proteins have been calculated as percentages of juice and of solids of the juice but could not be calculated as percentages of the muscle because the method of obtaining the juice caused so much loss of material, that the calculations of percentage of juice are only approximate.

Table III.

## Information of Haddock, furnishing juice.

Date caught 1920	% solids of muscle	% of juice		% solids of juice		Condition of fish when sample taken.		
		Fresh	Kept	Fresh	Kept	Time since caught	where kept	
July 22	19.42	-	-	-	-	1 hr.	Lab.	Mag. made 12 hrs. later
" 27	19.72	18		11.14		2 hrs.	"	
" "	20.00				19.71	20 "	"	Good but soft did not squeeze well.
" 30	20.77	18		11.28		2-4 hrs.		
" "			13			60 hrs.	Refrig.	Good but soft.
Aug. 5		10.1		9.45		1-2 "		
" "						3-4 "	on ice	Rigid parts frozen
" "						40 hrs.	Refrig.	good, soft.
" 9						2 "		
" "						24 "	"	
" "	20.9					48 "	"	good soft.

Table IV.

## Coagulable Proteins of Muscle Juice of Haddock.

Date Caught	Proteins in % of Juice coag'd at				Prots. coag'd at 40° as % of		Proteins coag'd at 100° as % of solids of juice
	40°		70°		Solids of Juice	Prots. coag'd at 100°	
	Fresh Kept	Fresh Kept	Fresh Kept	Fresh Kept			
July 28	3.55	4.35 (1)	6.61	6.61		54.2	
" 27	3.72	4.80	7.12	7.22	23.4	51.5	64.8
" "	1.71 (2)	3.93	6.46	6.63	16.0	55.7	61.9
" 30	3.98	5.01	7.76	7.66	26.2	59.1	67.5
" "	1.73 (2)	4.17	6.93	7.22	-	54.2	-
Aug. 5	2.95	4.12 (4)	5.75	5.65	39.5	52.2	59.3
" "	2.85	6.05	4.93	4.85	-	60.2	
" "	2.2			5.7 (5)			
" 9	3.72			6.22		50.7	
" "	1.50	3.30		6.22		54.1	
" "	1.60	3.30	6.24	6.15		56.0	

Notes. (1) Coag'n at 34° by mistake. (2) Filtration of clot delayed, probably high. (3) Filtration delayed. (4) No duplicate - (5) Duplicates poor 5.85 and 5.55.

The determinations of proteins coagulated at 40°C give the most interesting results. This clot, no doubt, consists largely of that muscle clot which is comparable to the fibrin clot of blood and in its amount evidently varies with the condition of the fish. Its amount is practically 3% or more of the juice in all cases where the haddock was fresh i.e., not more than 9 hours old - but is always less and usually much less, - 2% or below, when the fish has been kept. There are indications, too, that the amount of this coagulation is related to the condition of rigidity of the muscle. This point will be investigated later.

Determinations of coagula at 50°C seem to give but little information of value in regard to changes due to keeping.

The clots obtained at 70° apparently contain the whole of the coagulable protein and since it is much easier to boil the juice than to keep the water bath at 70°, the coagula at 100° are the better ones to use in later experiments. The values obtained for these clots seem to suggest a conversion of the proteins coagulated at 40° in the juice from fresh fish to proteins of a form coagulated above 50° and so appearing in these 70° and 100° coagula, for the diminution of amounts of total coagulable proteins as a result of keeping are not nearly so great as are those in the amounts of the proteins coagulated at 40°C. This aspect of the investigation also needs farther study.

The few experiments made with the stroma of the muscle gave so indecisive results that this work should be repeated before any conclusions can be drawn from them. The most hopeful line of attack is certainly that of the heat coagulation of the juice.

Two experiments are recorded in Table V. which were made

with the muscle of hake which had been badly frozen and so made spongy in the one case, and well frozen in the other. The well frozen material shows the same considerable amount of protein clot for 40°C as does the fresh haddock muscle but the clots at the higher temperatures are not comparable to those from the juice of haddock muscle and farther study should be made of this. Unfortunately the hake muscle gives so little juice that it is very difficult to obtain sufficient material for working.

Two experiments with cod muscle juice are included in this table, though the results are indefinite owing to the difficulty of obtaining material.

Table V.  
Coagulable Proteins of Hake and Cod.

Hake caught	Proteins coag'd at				Condition of fish when sampled	
	40°	50°	70°	100°	Time since caught	Kept in
July 29	1.55	3.23	3.55	3.65	24 hrs.	Spongy, frozen in air
" "	3.28	3.60	4.47	4.28	" "	Not " " quickly
Cod						
Aug. 5	1.0	-	-	4.5	4-5 "	Rigid
"	2.5			4.6	48 "	Refrig. Soft - good

The greatest variation in the factors here studied, seems to be in the amounts of the proteins coagulated at 40°C. Determinations of these amounts with fresh and frozen fish should thus assist us in noting delay in chemical change produced by cold storage. This line of study will also be followed at a later date.

## REFERENCES.

- Bottazzi (1913) *Biochem. Bull.* II. p. 379
- Clark and Almy (1918) U. S. Department Agriculture Bull. 635.
- Clark and Almy (1920) *J. Ind. and Eng. Chem.* 12, p. 656.
- Emmett and Grindley (1909) *Journal of Biol. Chem.* 6, p. IX.
- V. Fürth (1895) *Arch F. Expt. Path u. Pharm.* 36, p. 231.
- V. Fürth (1896) " " " " " 37, p. 389.
- Halliburton (1887) *Journal of Physiol.* 8, p. 133.
- Kühne (1868) *Lehrbuch d. Physiol. Chem.* p. 272.
- McCamus (1920) Report to Biol. Bd. of Can.
- Pennington et al (1917) *Journal of Biol. Chem.* 29. p XXXIII.
- Perlzweig and Gies (1913) *Bioch. Bull* 3, p. 69.
- Smith (1913) " " " 3, p. 54.

