AN ESTIMATION OF THE AMOUNT OF BOUND WATER IN COD MUSCLE

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Lyophilic colloids play a large part in the make up of biological tissue. The proteins belong to this system of colloids. As the name of this group implies, the colloidal particles have a very strong attraction for water. Many investigators have conducted experiments in the attempt to differentiate between that part of the total water in the system which is not associated with the particle, and that portion of the water which is linked up with the colloidal particle to form the micelle. Out of these researches has arisen the term "bound water", which can be defined in almost as many ways as there have been different methods of attacking this problem.

Undoubtedly there is a specific field of force which holds the water and the particle together. The exact mechanism of this binding is not completely understood. Hardy (1928) considers it as a surface phenomenon, while Moran (1926) favours the idea of the formation of a molecular complex between the water and the particle.

In this report the bound water will be defined as that part of the water in the system which is incapable of being frozen.

The object of the work was to try to determine the state of the water in cod muscle. The phase of the problem done during the summer of 1931 can be divided into two sections; first, the amount of bound water in cod muscle at -20°C.; secondly, the effect of the time of storage at -20°C. on the amount of bound water.

A calorimetric method was used to determine the amount of bound water in these experiments. Previous workers had used this method to determine the amount of bound water in

lyophilic colloidal systems. Thoenes (1925) worked with gelatine gels. Robinson (1928) adopted Thoenes method for his experiments on the relationship of the hydrophilic colloids to the winter hardness of insects. J. L. St. John (1931) modified Robinson's experimental procedure to obtain the temperature at which unbound water is completely frozen in a biocolloid. All these methods are based on the idea that the amount of the water frozen out at any temperature may be determined by measuring the heat required to raise the system from that temperature to some temperature above the freezing point f the system, provided that the specific heats, latent heat of fusion of water, and the freezing point of the system are known. Thus knowing the amount of the water in the state of ice at a definite temperature, and the total water content of the system, the bound water, being the difference between these two values, may be calculated. The method of calculating the amount of bound water will be clearly shown later in this report.

APPARATUS

The method used is a calorimetric one, the heat measurements were made in a Richards adiabatic calorimeter. The calorimeter proper was a copper vessel holding about 750 grams of water stirred by an up and down stirrer. The outer container was an earthenware vessel about six gallons capacity stirred by four two-bladed propellors.

The temperature change in the inner bath was read by a Beckmann thermometer placed in the outer bath. The temperature adjustment between the inner and outer bath was obtained by means of a thermocouple system of such sensitivity that a difference of 0.001°C. between the two baths produced a deflection of one millimeter on the galvanometer scale. The absolute temperature of the outer bath was read by a tenth degree thermometer placed directly in it.

The sample of fish was held in a brass container fitted with a tight screw cap. The weight of the container was about 35 grams. It could hold from 10 to 15 grams of fish muscle. The heat capacity of the container was determined over a range required for these experiments.

The low temperature bath used to bring the sample to the initial temperature, about -20°C., was a Dewar flask. Suspended in this flask was a copper tube into which fitted the container holding the sample. This kept the container from direct contact with the cooling solution. The bath liquid was an ether-CO_2 mixture, which could be controlled to 0.1°C. The liquid was stirred by a stream of dried air.

The temperature of the low temperature bath was determined by a toluene thermometer graduated in degrees. It was calibrated against a platinum resistance thermometer.

The initial temperature of the sample was not known to better than 0.5°C. The error in this measurement may be even greater than this, as the toluene thermometer could not be totally immersed in the cooling solution. A cork fitted into the mouth of the Dewar flask and the bath liquid was up to within $\frac{1}{8}$ to $\frac{1}{2}$ inch of the cork. The thermometer extended through the cork so that the meniscus of the toluene was just above the surface of the cork. The thermometer could not be calibrated until the writer had returned to McGill University, where it was possible through the kindness of Mr. W. F. Hampton to use a calibrated platinum resistance thermometer to standardize the toluene thermometer. The thermometer was found to have a correction of -0.9°C. at -20.0°C. when it was set up in the above manner.

The sample of fish, weighed into the container, was allowed to remain in the low temperature bath for one hour; during this time the temperature of the bath was controlled to 0.1°C.

The calorimeter was then assembled, the water in the calorimeter being at such a temperature that it would be close to 20°C. at the end of the run. The inner and outer baths were then balanced so that the change in the Beckmann thermometer reading did not vary more than 0.002° over a period of ten minutes. The container was placed in the calorimeter and the heat change followed by keeping the thermal balance between the two baths. The large initial temperature drop could be followed to within ±0.5°. After the initial change the two baths could be held constant to 0.02°. When the temperature of the baths remained constant to 0.002° for ten minutes the run was considered as completed.

EXPERIMENTAL MATERIAL

Fresh cod muscle was used as the experimental material. The fish was filleted after rigor had set in. The center portion of the fillets from several fish were taken. These parts were chopped up in a meat chopper and mixed thoroughly. This chopped muscle was rapidly frozen in cake forms in a small brine freezer. The frozen fish cakes that were used for storage experiments were wrapped in wax paper, sealed in a tin box, which was stored in the inner low temperature room at -20°C. The variation in the temperature in the room over the period of storage was about one degree.

When the sample of fish was required, one of the frozen cakes was ground up and sampled. At the same time a sample was taken for a moisture determination. These operations were carried out in the low temperature room.

METHOD OF DETERMINING THE MOISTURE CONTENT OF THE SAMPLE

The sample of the fish was put into a weighed weighing bottle, fitted with a tight ground glass stopper. The stoppered weighing bottle containing the sample was placed in a desiccator in the low temperature room. After the desiccator and the contents had warmed up to the temperature of the balance room, the weight of the sample could be determined without the inconvenience of moisture condensing on the cool surface of the weighing bottle. The sample was dried to a constant weight in an air oven operating at 105°C. It required about 48 hours for a sample weighing 2 to 3 grams to come to a constant weight.

METHOD OF CALCULATING THE AMOUNT OF BOUND WATER

J. L. St. John used the expression

$$X = \frac{FN\,(T_3-T_4) - (SW+S_1W_1)\,(T_4-T_2)}{80 + 0.5\,(T_2-T_1)}$$

to calculate X, the weight of the free water (that water which is frozen) in a biocolloid. The reader is referred to the original paper for the derivation and the significance of the symbols used in the expression.

In the derivation of this expression St. John assumed that the specific heat of bound water was "one" over the entire temperature range. Unfortunately experimental values for the specific heat of bound water are not available, but from the theoretical considerations the specific heat of bound water should be expected to lie between the values of the specific heat of water in the liquid state and the solid state, as the number of degrees of freedom of the molecule of bound water will be less than the number of degrees of freedom of the molecule of water in the liquid state, if the present idea of bound water is valid, that is, that bound water is some type of a surface phenomenon.

St. John failed to consider that the latent heat of fusion of water changes with the temperature. As the freezing point of a biocolloid will be lower than the freezing point of pure water this factor must be considered in the derivation of the above expression.

The derivation of the expression to calculate X used in this work is based on the expression of St. John; but a correction has been made for the variation of the latent heat of fusion of water with the temperature. The correction to the specific heat of bound water cannot be made, but in this work the values obtained for the bound water in cod muscle will only be comparative among themselves, as at present the writer is only interested in the effects which certain controllable factors have on the amount of the bound water found in cod muscle.

The following values, from the data of Chipman and Langstroth (1929), for the specific heat of cod muscle were used.

Temperature Range	Specific Heat
25° to 0.0°	0.89
-0.8° to -10°	0.77 *
-10° to -20°	0.70
-20° to -30°	0.63

* This value was obtained by extrapolating the heat capacity curve of cod muscle to 0.8°, assuming in the calculation of X that the sample is warmed up to -0.8° before the ice melts. At -0.8° the ice melts absorbing λ calories per gram, the latent heat of fusion.

The average freezing point of cod muscle was taken as -0.8°C from Chipman and Langstroth (1929).

The latent heat of fusion of water at -0.8° was calculated from the equation

$$\frac{d\lambda}{dt} = C_w - C_I \quad \text{(Clausius)}$$

which gives the variation of the latent heat with temperature, when

λ is the latent heat of fusion
C_w is the average specific heat of water
C_I is the average specific heat of ice.

At zero the latent heat of fusion of water is given by W. H. Barnes and O. Maass (1930) to be 79.4 calories per gram.

The average specific heat of water between 0° and -3° is 1.01 calories / C° (H. T. Barnes and H. L. Cook).

The average specific heat of ice between 0° and -3° is .48 calories / C° (W. H. Barnes and O. Maass, 1930).

Then $\frac{d\lambda}{dT} = 1.01 - 0.48 = 0.53$ cal. /gram degree.

Therefore λ at 0.8° is 75.9 calories per gram.

The specific heat of the container was determined experimentally over the range from -20° to 20° and the mean value from three experiments gave the specific heat as 0.086 calories per gram degree.

The following expression was used to calculate X, the amount of water in grams that was frozen in cod muscle at -20°C.

$$X = \frac{(C+W)(T_3 - T_4) - SW(T_4 - T_2) - sw(T_4 - T_2)}{\lambda + 0.5(T_2 - T_1)}$$

To illustrate the use of this expression the following experimental data is given -

The average freezing point of the sample, T_1 = 0.8°C.
" initial temperature of the sample, T_2 = -20.9°C.
" " " " " calorimeter, T_3 = 22.67°C.
on the absolute thermometer and 4.904° by the Beckmann.
The final temperature of the calorimeter, T_4 = 20.97°C.
on the absolute thermometer and 2.210° by the Beckmann.
The water equivalent of the calorimeter, C = 21.6 calories per degree.
The weight of water in the calorimeter, W = 515.9 grams.
" " " the container w = 32.94 "
" " " " sample A = 9.200 "
" specific heat of the container s = 0.086 calories per gram degree.
The specific heat of the sample S.

The value for X from this data is 6.952 grams. This sample contained 81.60% water - so the percentage of the total water that was frozen at -20° was 92.60%. The percentage of the total water in the state of bound water at -20° was 7.40%.

The following table shows the experimental results obtained. The results have been calculated from data similar to the above using the above expression.

TABLE 1.

Expt. No.	Percentage of Total Water in Sample as Free Water (frozen at -20°C.).			Time of Storage in Days
	1	2	Mean	
10A	95.2	95.5	95.3 *	0
10B	91.3	91.9	91.6	5
10C	95.2	92.7	93.9	7
10D	90.9	92.4	91.6	14
10E	90.2	91.5	90.9	28
11A	91.0	89.8	90.5	0
11B	91.5	--	91.5	4
11C	90.7	90.3	90.5	10
11D	92.0	92.6	92.3	17
11E	90.8	--	90.8	24

* The initial temperature of the sample in this case was -25°C. This may be the cause of the high value recorded.

DISCUSSION OF RESULTS

The experimental error in each individual result is about 4%. The uncertainty in determining the initial temperature of the sample by means of the toluene thermometer was the cause for such a large error in the experimental technic.

From the results the percentage of the total water in cod muscle that is frozen at -20°C. is between 90% to 94%. Therefore the amount of bound water in cod muscle at -20°C. is between 10% to 6% of the total water. This value agrees to within the experimental error with the results of Moran (1930) who reports that the bound water in mammalian muscle at -20°C. is not more than 6% of the total water.

It can be seen from the above table that this method does not indicate that the amount of water frozen in cod muscle varies with the time of storage over a period of four weeks. This point will be useful to the investigator in further work on this problem, as samples can be stored at -20°C. without having the state of the water in the muscle undergoing any change.

RECOMMENDATIONS FOR FURTHER WORK

For further work on this problem it would be desirable to investigate a system, related to fish muscle, but more stable and less complex. This should lead to information concerning the influence of

morganic salts present in fish muscle upon the amount of bound water.

Such a series of investigations would require the services of a permanent member of the staff, who would have more time at his disposal to appreciate all the possibilities arising out of this research.

Also to perform calorimetric work of any type at the Experimental Station, I strongly recommend the purchase of a platinum resistance thermometer so that measurements at low temperatures can be made with some confidence.

SUMMARY

By using a calorimetric method from 90% to 94% of the total water in fresh cod muscle could be accounted for as ice at -20°C. Therefore 10% to 6% of the water in cod muscle can be considered as bound water. This agrees to within the experimental error, about 4%, with Moran's work on the state of water in beef tissue.

No change in the amount of bound water in cod muscle could be detected by this method after storage at -20°C. for four weeks.

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