The solubility and aggregation of fish muscle proteins during freezing and refrigeration (from "Processing of fishery products")

by V.P. Bykov

Original title: O rastvorimosti i agregatsii myshechnykh belkov pri kholodil'noi obrabotke ryby ("Tekhnologiya rybnykh produktov")


Translated by the Translation Bureau (IH) Foreign Languages Division Department of the Secretary of State of Canada

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B. C.
Halifax Laboratory, Halifax, N. S.
Freshwater Institute, Winnipeg, Man.

1971

36 pages typescript
The solubility and aggregation of fish muscle proteins during freezing and refrigeration

Reference IN ENGLISH - RéFÉRENCE EN ANGLAIS

Reports of the All-Union Scientific Research Institute of Marine Fishery and Oceanography

Translator (initials) - TRADUCTEUR (INITIALES)

Date completed - DATE ACHEVÉ E

Unedited Draft Translation - TRADUCTION NON REVISÉE

Only for information - Information seulement
Translation of an article published in 'Trudy vsesoyuznogo nauchno-issledovatel'skogo Instituta morskogo rybnogo Khozvaistva i Okeanografii (VNIRI)', Vol. 73, 1970, p. 7 - 35.

On the solubility and aggregation of fish muscle proteins during freezing and refrigeration.

(Ob, rastvorimosti i agregatsii myshechnykh belkov pri khолодil'noi obrabotke ryby)

By

V.P. Bykov.

The freezing and cold storage of fish substantially affects its quality, therefore the properties of frozen fish flesh may differ markedly from the properties of fresh fish.

As a result of freezing, fish flesh becomes dry, tough and fibrous. The muscle fluid easily escapes from fish that has been frozen and then defrosted. The taste properties of fresh meat are, of course, better than those of frozen fish.

*This work was carried out in May-Dec. 1964, in the Chemical Technical Institute of the Fish Directorate, Bergen, (Norway) under the guidance of Dr. Iens. W. Iensen, to whom the author would like to express thanks. The author thanks the Director of the Institute E. Heen, for enabling him to carry out this study.
Studies over the last three decades of the characters of cod fish have shown that the properties of the fish tissues subjected to freezing deteriorate as a result of the denaturation of proteins of the actomyosin complex which form the most labile protein fraction of the muscles and compose 60 - 80% of the proteins of fish meat. The denaturation of actomyosin is shown, in particular, by the loss of the ability to dissolve in a saline solution.

Many works have indicated that an increase in the degree of toughness of fish meat corresponds with a decrease in the solubility of actomyosin. However, in some later studies, no correspondence was found between an increase in the degree of toughness of the meat and a decrease in the solubility of actomyosin.

It was recently determined that the solubility of actomyosin is influenced not only by its denaturation during freezing of the fish, but also by the physiological processes that occur during lifetime and the mechanical and chemical processes occurring after death in the muscles of the fish.

In order to investigate the denaturation of actomyosin during freezing and particularly cold storage of the fish, as well as observations on its solubility, other methods began to be applied, e.g. measuring the average weight of protein particles, the constant of their sedimentation and diffusion, and likewise the optical rotation of the protein solution together with determining the content of the SH group, the ATPase activity of actomyosin.
It was noted that even before the denaturation of actomyosin, expressed in the loss of its solubility, the structure of its molecules was somewhat altered and this was accompanied by the aggregation of molecules, which appears to influence the change in the native properties of protein, in particular the ability to retain moisture.

In 1958 Seaaran assumed that freezing produces some changes in the structure of actomyosin that cannot be traced by the usual methods and with no noticeable effect on its solubility; the subsequent cold storage of fish is accompanied by changes that are so far unknown and which lead to the gradual, irreversible aggregation of actomyosin molecules.

Japanese research workers (Suzuki, Kanka, Tanaka 1965), using ultracentrifuging, determined that though the solubility of actomyosin did not change when the fish was frozen in liquid nitrogen and then kept in cold storage at -20°C for 3-5 weeks, the constant of sedimentation of the actomyosin increased, which indicates the aggregation of the actomyosin molecules.

Connell (1960) studying the aggregation of actomyosin came to the conclusion that it has two stages: 1) a change in the structure of actomyosin molecules, which does not affect the coefficient of sedimentation, and 2) the aggregation itself which is accompanied by a corresponding increase in the coefficient of sedimentation.

It is known from classical biochemistry (Poglazov, 1965) that myosin, actin and actomyosin have a high aggregational
capacity. However in studying the properties of these proteins one must distinguish between the aggregation ability of native and denatured protein. The aggregation of denatured protein, as against that of native protein, is an extremely widespread occurrence and one that has been studied many times (Bressler, 1965).

Poglazov shows that in dilute solutions of myosin, the degree of dispersion of its particles changes as a result of the aggregation ability of the myosin, and this depends on the pH of the medium and the ionic strength of the solution. These indicators also change in fish tissues during storage and freezing, and they appear to influence the aggregation of molecules of proteins of the actomyosin complex and the change in the properties of fish meat.

Little attention has been paid to the aggregation of proteins of the actomyosin complex and it is not clear what influence it has on the change in the properties of fish meat during freezing.

The Norwegian scientists (Heen, Karst 1965) have shown that insufficient research has been done on protein aggregation and the accompanying decrease in the ability to retain moisture and its loss of solubility. As the aggregation of proteins during the freezing of fish is of interest in understanding denatured protein transformations, observations were made of protein aggregation at all stages of freezing, taking into account the condition after death of the original fresh fish. At the same time, as well as observations on the possible aggregation of proteins, their solubility was also determined.
In studying protein phases - in aggregation and solubility (10) - the initial point of departure was as follows. It is known that there are three protein fractions in fish meat: myofibrillar, sarcoplasmic and stroma.

The myofibrillar fraction is composed of myosin, actin, the complex protein actomyosin, tropomyosin, from which the myofibrils are formed; the sarcoplasmic fraction has up to 50 protein enzymes which take part in metabolic processes; stroma is for the most part composed of collagen. Myofibrillar proteins form nearly 60 - 80%, sarcoplasmic - 20 - 30%, collagen 3 - 5% and its content only rises to 10% in sharks and rays.

In addition fish meat contains nitrogenous extractive substances. The first two protein fractions, as well as the nitrogenous extractives, are easily soluble in a saline solution.

If the muscles are ground up in a blender in a salt solution (0.5M KCl + 0.03M NaHCO₃, pH = 7.4), the soluble protein fractions (myofibrillar and sarcoplasmic) and likewise the nitrogen extractives pass into the solution. At the same time, the amount of proteins extracted is influenced by their condition. Denaturation as a result of cold storage brings about a decrease in the amount of protein extracted.

If the protein solution is centrifuged then a certain amount of protein will be precipitated. The protein precipitated by centrifuging is influenced by the size of protein particles and the length of centrifuging. The larger the particles the less force required to precipitate them and the quicker they are precipitated. With an increase in pressure for the same length of time
of centrifuging, finer and finer particles are precipitated.

Whereas freezing fish causes the aggregation of protein molecules, one can therefore, by comparing the results of centrifuging protein solutions obtained before and after freezing treatments, assess the increase in size of particles for any given freezing treatment from the amount of protein precipitated, and consequently aggregation.

Methods of determining aggregation were as follows. Samples (1) 10 g. in weight were taken from a fillet and mixed in a blender (Waring Blender), and to this was added 350 ml. of a solution of potassium chloride (0.5M KCl + 0.03M NaHCO₃ · pH = 7.4). The samples were ground up in the blender for 30 sec. at 20,000 rev./min. in an atmosphere of nitrogen by Dyer's method (Dyer 1950), in order to prevent denaturation of the proteins during grinding.

As a result of this treatment the proteins dissolved and formed a homogeneous opalescing solution. To separate the undisolved connective tissue, 250 ml. of solution were centrifuged at 2,000 rev./min. for 10 min., after which the solution was filtered through mineral wool. Two parallel samples of 5 ml. each were taken from the original solution and the total content of soluble proteins in them was determined by microkjeldal. Eight samples from the remaining solution, each of 10 ml. were put in centrifuge test-tubes. Two samples each were centrifuged at 7,000, 12,000 and 17,000 rev./min. for 15 min., and also at 17,000 rev./min. for 180 min.

Two parallel samples, each of 5 ml., were taken from the solutions that had been centrifuged in different conditions
and then filtered through mineral wool and the protein content of them was then determined by microkjeldahl. Then the amount of protein was calculated that had been precipitated under the given conditions of centrifuging. The difference between the total content of soluble protein in the original solution and the protein content of the solution that was centrifuged was used to determine the amount of precipitated protein and express the amount of precipitated protein under the differing conditions of centrifuging in % of the total content of soluble protein in the original solution. As well as determining the total content of soluble protein, the total content of protein (nitrogen) in fish meat was also determined, and the amount of total soluble protein expressed in % of the total content of protein in fish meat, as accepted by many investigators.

As the method of determining nitrogen content (protein) in fish meat usual in Norway (Howe, 1943) differs somewhat from the method generally accepted in our country, it seems relevant to give a short description.

Two g. of meat each were taken from near the head and near the tail of the fish. From these two determinations one obtains the average content of total nitrogen in the meat of the fish in question. This method of testing for total nitrogen in lean fish, to which cod belongs, is the accepted method in the Chemical Technical Institute of the Fish Directorate.

It is the accepted practice of many research workers to express the total content of soluble protein as a % of the total protein content of the fish meat.
To determine nitrogen content, 2 g. of meat or 5 ml. of solution are placed in a Kjeldahl flask, to which is added 10 ml. of concentrated sulphuric acid. To this is also added circa lg. (to the eye) of a catalyst formed by a mixture of 90 g. Na₂SO₄, 1.5 g. CuSO₄ and 1 g. powdered selenium. The catalyst enables the sample to be digested in 2-3 hours.

After the sample is digested the Kjeldahl flask is cooled and the contents transferred to flasks with a measured capacity of 50 ml., and distilled water is added up to the measured level. Five ml. of the test solution together with 10 ml. of a 30% solution of NaOH are taken from the measuring flasks and put into the apparatus for distilling nitrogen. There is also put into the receptacle 5 ml. of a 4% solution of H₃O₄ and 8 drops of an indicator (the content of the indicator is 100 ml. of a solution of bromo-cresol green at a concentration of 0.1% and 20 ml. of a solution of methyl red at a concentration of 0.1%).

From the moment the solution starts to boil, nitrogen is distilled for only 2 min. The contents of the flask are titrated with a 0.01n. solution of HCl.

As well as determining the total content of soluble protein in the original solution containing the various protein fractions, the content of both the soluble fractions was also determined (i.e. the sarcoplastic with the nitrogenous extractive substances, and actomyosin), and the first was determined chemically whereas the second was determined from the difference between the total content of soluble proteins and the content of the
sarcoplasmic fraction with nitrogenous extractive substances.*

The sarcoplasmic fraction with nitrogenous extractives was determined in the following way. Two parallel samples of 3 ml. each were taken from the original solution and to them was added 27 ml. each of distilled water. Then the solution was centrifuged at 12,000 rev./min. for 15 min. to precipitate the actomyosin. From the centrifuged material 20 ml. of the solution were taken to determine the content of nitrogen in it by micro-kjeldahl.

Observations were made on the solubility of the different fractions of protein and also on the possible aggregation of proteins in the fish both while it was stored fresh, and during freezing, cold storage and defrosting.

For the experiments live fjord cod was used, about 2kg. in weight, drained and gutted. The dismembered fish was delivered from the market to the laboratory within 15 min., and here a fillet was taken from it and sent immediately for analysis.

A total of 10 fish were used for the experiments. Data about protein content \((N \times 6.25)\) in the meat of the fish studied is presented in Table 1.

From the table it can be seen that the protein content of different fish specimens taken from near the head varies from 16.7 to 20.8%, and from near the tail from 16.7 to 20.4%.

* In the rest of the report, whenever the sarcoplasmic protein fraction is being discussed, it should be born in mind that this protein fraction contains nitrogenous extractive substances.
The average protein content of fish near the head was 18.6%, while near the tail it was 18%. The average protein content of fish meat of all the fish, putting together the data from near the head and near the tail, amounted to 18.3%.

The results of observation on the solubility of proteins in the original fresh fish showed that it varies sharply with the individual specimens (table 2).

**Table 1.**

<table>
<thead>
<tr>
<th>No. of specimen</th>
<th>Near head</th>
<th>Near tail</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20,8</td>
<td>20,4</td>
<td>20,6</td>
</tr>
<tr>
<td>2</td>
<td>19,1</td>
<td>17,7</td>
<td>18,4</td>
</tr>
<tr>
<td>3</td>
<td>18,8</td>
<td>18,0</td>
<td>18,4</td>
</tr>
<tr>
<td>4</td>
<td>18,7</td>
<td>17,9</td>
<td>18,3</td>
</tr>
<tr>
<td>5</td>
<td>17,8</td>
<td>17,5</td>
<td>17,7</td>
</tr>
<tr>
<td>6</td>
<td>16,7</td>
<td>16,7</td>
<td>16,7</td>
</tr>
<tr>
<td>7</td>
<td>18,4</td>
<td>18,3</td>
<td>18,4</td>
</tr>
<tr>
<td>8</td>
<td>17,7</td>
<td>17,7</td>
<td>17,7</td>
</tr>
<tr>
<td>9</td>
<td>19,2</td>
<td>-</td>
<td>19,2</td>
</tr>
<tr>
<td>10</td>
<td>18,3</td>
<td>17,7</td>
<td>18,0</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>18,6</strong></td>
<td><strong>18,0</strong></td>
<td><strong>18,3</strong></td>
</tr>
</tbody>
</table>

**Table 2.**

<table>
<thead>
<tr>
<th>No. of specimen</th>
<th>Date of study</th>
<th>Total content of soluble proteins in % of total content of proteins in fish meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29/IV</td>
<td>74,1</td>
</tr>
<tr>
<td>2</td>
<td>20/V</td>
<td>57,6</td>
</tr>
<tr>
<td>3</td>
<td>14/VII</td>
<td>39,5</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>57,2</strong></td>
</tr>
</tbody>
</table>
From the table it can be seen that the content of soluble proteins in the fish specimens investigated varied from 39.5 to 74.1% and averaged 57.2%. Moreover, in fish studied in spring (April - May), the content of soluble proteins (57.6 - 74.1%) is significantly higher than in fish studied in July (39.5%). The reduction in the content of soluble proteins in fish meat in summer may be related to a higher rate of metabolism, as a result of which fish in fishponds are more tired in summer than in spring. It is known (Ganelina, 1962) that as animals get tired the solubility of muscle protein decreases.

Change of proteins in the meat of fresh fish.

In order to determine the change of solubility in proteins after rigor mortis sets in when fresh fish are being stored, three series of experiments were carried out.

First series of experiments. Six fish were delivered to the laboratory immediately after being killed, gutted and decapitated. Two of them were immediately sent for analysis (before the onset of rigor mortis) and four were put in polyethylene bags and placed in the refrigerator at a temperature of 0 ± 2 C., and of these 4, two were stored until full rigor mortis and two until they relaxed*, after which they were sent for investigation. From each fish pieces of meat were cut from the back towards the head and the total content of soluble proteins in them was determined. The results of the analysis is shown in table 3.

From the data in the table it can be seen that protein solubility in fish immediately after being killed is

* i.e. until they reached the post-rigor relaxation phase. Translator's note.
Table 3.

<table>
<thead>
<tr>
<th>Condition of fish</th>
<th>Total content of soluble proteins</th>
<th>In % of protein content of fish meat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In g./100g. meat</td>
<td>I</td>
</tr>
<tr>
<td>(1) Fresh from kill</td>
<td>10,6</td>
<td>7,0</td>
</tr>
<tr>
<td>(b) Full rigor</td>
<td>14,1</td>
<td>9,5</td>
</tr>
<tr>
<td>(c) Relaxation after rigor</td>
<td>12,4</td>
<td>10,4</td>
</tr>
</tbody>
</table>

significantly lower (39.5 - 57.6%) than in fish in a state of rigor mortis (53.7 - 76.5%).

If the protein solubility of fish immediately after being killed is taken as 100%, then it is 34% higher in fish in a state of rigor mortis, and 29% higher in fish that have become relaxed.

So if fresh fish are stored, protein solubility increases significantly when rigor mortis sets in, and decreases somewhat again during further storage.

Second series of experiments. The results of the first series (16) of experiments could be affected by individual variations in the amounts of protein solubility in different fish. Taking this into account, a second series of experiments was carried out to confirm the findings in the first series of experiments governing the changes in protein solubility in relation to the condition of the fish after death.

Samples were taken from the same cod specimen immediately after slaughter and every 24 hours during storage at a temperature of 0 + 2 C. to determine the total content of soluble proteins.
Experiments were carried out for 5 days. Results of the experiments are shown below.

<table>
<thead>
<tr>
<th>Time of storage of fish, in days.</th>
<th>Total content of soluble proteins, in g./100g. meat.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.3</td>
</tr>
<tr>
<td>1</td>
<td>10.6</td>
</tr>
<tr>
<td>2</td>
<td>11.9</td>
</tr>
<tr>
<td>3</td>
<td>11.2</td>
</tr>
<tr>
<td>4</td>
<td>10.9</td>
</tr>
<tr>
<td>5</td>
<td>10.3</td>
</tr>
<tr>
<td>Average</td>
<td>10.9</td>
</tr>
</tbody>
</table>

From these data it can be seen that as the fish is stored the amount of soluble protein in its meat increases by 15% and then gradually decreases again to the initial amount. So these data confirm those obtained in the first series of experiments.

It would seem natural to assume that the change in solubility is a result of rigor mortis occurring in the fish. To prove this experimentally special experiments were set up.

Third series of experiments: Immediately after slaughter fillets were cut from both sides of cod, packed in polyethylene and put into storage in the refrigerature at a temperature of 0°+2°C. In one fillet the contraction in length characteristic of rigor mortis was observed, while from the other pieces of meat were cut out to determine the solubility of the proteins. The results of these experiments are presented in table 4.

As seen from the table, the gradual onset of rigor mortis while the fillet is being stored is accompanied by a corresponding increase in the solubility of actomyosin, which
may indicate the interrelationship of these two phenomena.

So it has been shown by observations of the change in protein solubility that solubility increases as a result of rigor mortis setting in and decreases somewhat during further storage of the fish.

To elucidate the role of the two fractions of soluble proteins (myofibrillar and sarcoplasmic) in this change in protein solubility, the solubility of these fractions was determined separately. For this a cod specimen was used which was stored at 0/±2°C, and samples were taken from it over five days. The content of soluble proteins of the myofibrillar (actomyosin) and sarcoplasmic fractions was determined by the method described above (Fig. 1).

As Fig. 1 shows, protein solubility during rigor mortis is increased in the myofibrillar fraction (proteins of the actomyosin complex) and this initial increase in solubility during rigor mortis is accompanied by a subsequent decrease to approximately the original amount.

As for the sarcoplasmic fraction, its solubility does not change with the occurrence of rigor mortis. The
subsequent slight increase in the amount of the sarcoplasmic  
fraction should apparently be ascribed to the nitrogenous 
extractives formed as a result of autolytic processes and 
isolated together with the proteins of this fraction.

So the series of experiments on fresh fish described above 
showed that when fresh fish is stored the onset of rigor mortis 
brings about a reversible increase in the solubility of proteins 
of the actomyosin complex. At the same time the solubility of 
the sarcoplasmic protein fraction remains almost unchanged.

The results of experiments centrifuging protein solutions 
obtained from fish in varying conditions after death are shown 
in table 5 and on Fig. 2.

From the data shown in Fig. 2 it is clear that a smaller 
amount of protein (4 - 42.5%) is deposited from a protein 
solution taken from fish immediately after killing under any 
conditions of centrifuging than from a protein solution obtained 
from fish that are in rigor mortis (8.9 - 55.9%) or after relaxation, 
(8.1 - 50%).

At the same time, the amount of protein deposited from 
a solution obtained from fish meat in rigor mortis is roughly 
equal to the amount of protein deposited from a solution 
obtained from fish meat in the relaxed condition after rigor.

The relative increase of the amount of protein 
deposited from the solution obtained from the fish in rigor, 
and the absence of further changes in the amount of protein 
deposited from the solution obtained from fish in a relaxed 
condition may be explained by the formation of actomyosin
Protein, g./100g. meat

Length of storage, in days.

Fig. 1. The change of solubility of proteins of fish meat during storage at a temperature of 0 ±2°C.: 1 - total content of soluble proteins; 2 - proteins of the actomyosin complex; 3 - proteins of the sarcoplasmic fraction with nitrogenous extractives.

Deposited protein, %

Original solution

Fig. 2. Precipitation of proteins from solution obtained from fresh fish: 1 - fish before rigor mortis; 2 - fish in a state of rigor; 3 - fish in a state of relaxation.
from actin and myosin as rigor mortis takes place in the fish and the absence of its dissociation during further storage of the fresh fish.

To sum up all the observations of protein change in fresh fish, one may conclude that when it is stored there is a reversible increase of solubility of the actomyosin complex when rigor mortis starts, and this is accompanied by an increase in the average size of particles of soluble proteins of fish meat.

The change of proteins in fish meat during freezing

Two series of experiments were made to study the changes in fish meat proteins during freezing.

In the first series we observed the change in solubility in different post-mortem phases of protein fractions influenced by
freezing, and in the second series of experiments, the possible aggregation of proteins during freezing was studied.

In the first series of experiments fillets were cut from both sides of cod. A sample was taken from one fillet to determine the solubility of the protein fractions, and from the other a piece of meat was cut, packed in aluminium foil and put in the freezer at a temperature of \(-25^\circ C\). Without air circulation for freezing.

After 24 hours a sample was taken from the frozen meat to determine the solubility of the protein fractions. The remainder of the two halves of the fillets were packed in polyethylene and stored in the refrigerator at 0 to +2\(^\circ\)C. After every 24 hours storage samples were taken from one half of the fillet to determine the solubility of the protein fractions (21) and from the other, pieces of fillet were cut, frozen and then sent to be studied.

These experiments were repeated for a duration of 5 days. It can be seen from Fig. 3 that when fish that has been stored for 2 days (i.e. from the moment of slaughter to the onset of rigor mortis) is frozen, the solubility of the protein fractions remained virtually unchanged.

However when fish kept more than 48 hours is frozen, protein solubility decreases significantly in the actomyosin fraction.

To observe possible protein aggregation in fish that
that have been frozen, taking into account the condition after death of cod stored at a temperature of 0°/+2°C, samples were taken immediately after slaughter, in rigor, and in the relaxation phase.

Pieces of meat were cut from the fish at every stage after death and these were packed in aluminium foil and frozen in the freezer as described above.

Proteins were extracted from samples of fish meat both before and after freezing and the protein solutions were centrifuged.

The results of the experiments are presented in table 6 and on Fig. 4. The data show that when protein solutions obtained from fish samples taken immediately after killing were centrifuged, the amounts of proteins precipitated were significantly higher for frozen than for fresh fish. So when a solution obtained from fresh fish was centrifuged the
proteins isolated amounted to from 4.3 to 34.3%, while for a solution from frozen fish it was 7.2 to 55.4%.

These data indicate that in a solution obtained from fresh fish the soluble protein particles are not as big as in the solution obtained from frozen fish. The difference in the size of protein particles in solutions obtained from fish before and after freezing may indicate aggregation of the protein particles when the fish is frozen.

However, the picture was completely different in the experiments carried out on fish frozen while in rigor. When protein solutions taken from meat samples were centrifuged, precipitation from them was greater before freezing (9.4 - 25.2%) than after it (3.1 - 15.8%).

This difference in the average size of protein particles in solutions taken from fish before and after freezing indicates the decrease in the average size of particles i.e. their disaggregation. The decrease in the average size of particles may be explained, e.g., by the dissociation of actomyosin to actin and myosin.

In experiments with fish frozen while in a relaxed condition the same general principle was observed as in experiments with fish frozen in a state of rigor. However, the average size of protein particles does not decrease significantly as a result of freezing; protein isolated from the solution was 8.2 - 23.1% before freezing, 6.5 - 20.6% after freezing.

So protein solubility did not change uniformly when the fish was frozen in varying conditions after death. Fish
Table 6.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Conditions of centrifuging</th>
<th>Original</th>
<th>7,000</th>
<th>12,000</th>
<th>17,000</th>
<th>17,000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>180</td>
<td></td>
</tr>
</tbody>
</table>

| Fish fresh from killing | Soluble g./100g. meat | 7.0 | 6.7 | 6.4 | 5.8 | 4.6 |
|                        | Precipitated g./100g. meat | 8.3 | 7.7 | 7.3 | 5.8 | 3.7 |
|                        | % of content in original solution | 0.3 | 0.6 | 1.2 | 2.4 |
|                        |                           | 0.6 | 1.0 | 2.5 | 4.6 |
| Fish in rigor mortis    | Soluble g./100g. meat | 9.5 | 8.6 | 8.0 | 7.0 | - |
|                        | Precipitated g./100g. meat | 9.5 | 9.2 | 8.6 | 8.0 |
|                        | % of content in original solution | 0.9 | 1.5 | 2.5 | - |
|                        |                           | 0.3 | 0.9 | 1.5 |
| Fish in post-rigor relaxation | Soluble g./100g. meat | 10.4 | 2.5 | 9.2 | 8.0 | - |
|                        | Precipitated g./100g. meat | 9.2 | 8.6 | 8.0 | 7.3 |
|                        | % of content in original solution | 0.5 | 1.5 | 2.2 |
|                        |                           | 0.6 | 1.2 | 1.9 |

Note. In the fractions, the numerator represents the figure before freezing; the denominator, after freezing.
frozen immediately after being killed or in complete rigor did not show a decrease in protein solubility, but when frozen in a state of relaxation there was a significant decrease in proteins of the actomyosin complex.

At the same time there is considerable variation in the average size of particles of the soluble proteins when fish is frozen either immediately after being killed or in a state of rigor, and the nature of this variation is not uniform when there is a difference in the post mortem condition of the fish before freezing. The average size of protein particles increases in fish frozen immediately after being killed, while in fish frozen in rigor or after relaxation it decreases, but in this

Fig. 4. Precipitation of proteins obtained from solution of (a) fresh and (δ) subsequently frozen fish. I - fish immediately after killing; II - fish in rigor; III - fish in relaxed state.
latter case, not to any significant amount.

The change in soluble proteins in fish meat during cold storage.

To study the changes in the proteins of fish meat during cold storage, observations were made of the solubility and also the possible aggregation of proteins. A total of three series of experiments were made. In the first series of experiments, observations were made of the change in solubility of proteins during cold storage in relation to the time of storage, in the second series the change of protein solubility was observed in relation to the time of storage and the post mortem condition of the fish before freezing. In the third series, protein aggregation was studied in relation to the length of time the frozen fish was stored.

First series of experiments. Cod was selected for the experiments which was removed for study immediately after being killed. Fillets were cut from the fish and frozen.

Samples were taken from the fillet to determine the total content of protein, and the content of soluble proteins, among them proteins of the actomyosin and sarcoplasmic fraction. The remaining part of the fillet was packed in aluminium foil and frozen at a temperature of -25°C, and then stored at the same temperature for 29 weeks.

Samples were taken periodically from the frozen fillet to determine the indices mentioned above.

The results of the experiments are shown in table 7 and Fig. 5.
### Table 1

<table>
<thead>
<tr>
<th>Length of storage, in weeks</th>
<th>Content of proteins in solution</th>
<th>Amount of proteins deposited from solution in % of total content of soluble proteins after centrifuging at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Original</td>
<td>After centrifuging at: g./100g. meat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15.3</td>
<td>14.4</td>
</tr>
<tr>
<td>2</td>
<td>13.5</td>
<td>12.7</td>
</tr>
<tr>
<td>4</td>
<td>11.6</td>
<td>11.3</td>
</tr>
<tr>
<td>6</td>
<td>8.9</td>
<td>8.7</td>
</tr>
<tr>
<td>8</td>
<td>8.0</td>
<td>7.6</td>
</tr>
<tr>
<td>10</td>
<td>7.3</td>
<td>6.4</td>
</tr>
<tr>
<td>14</td>
<td>7.7</td>
<td>6.4</td>
</tr>
<tr>
<td>29</td>
<td>5.6</td>
<td>4.9</td>
</tr>
</tbody>
</table>

**Fresh fish before freezing**

**Frozen fish**
The data show that when the frozen fish is stored for 10 weeks the amount of soluble proteins gradually decreases from 15.3 to 7.3 g./100 g. meat, affecting both the actomyosin and the sarcoplasmic protein fractions.

Thus in 10 days of storage the solubility of the actomyosin fraction decreases from 9.9 to 6.4 g./100 g. meat, and the sarcoplasmic from 5.4 to 0.9 g./100 g. meat.

However, during continued storage of the fish, starting from the 10 - 14 th. weeks, the amount of soluble protein increases from 7.3 to 7.7 g./100 g. meat because of the sarcoplasmic fraction, the content of which increases from 0.9 to 2.7 g./100 g. meat.

* This appears to be an author's error for 'weeks'. Translator's note.
During further storage of the fish, from 14 to 29 weeks, the protein solubility of the fish meat gradually decreased from 7.7 to 5.6 g./100 g. meat exclusively at the expense of the actomyosin fraction, the solubility of which decreases from 6.4 to 2.3 g./100 g. meat. At the same time the solubility of the sarcoplasmic fraction gradually increased (from 2.7 to 3.3 g./100 g. meat).

So the solubility of the muscle proteins gradually decreases during storage of the frozen fish. In this regard the solubility of the actomyosin fraction decreases during the entire storage period while the sarcoplasmic fraction decreases initially and then increases again.

The second series of experiments was carried out on three fish dispatched for study in the three post-mortem phases, (straight after being killed, in rigor, and after relaxation) and the content of soluble proteins in them was determined as in the original samples (before freezing), and also after different lengths of storage.

The results of the investigation are presented in Table 8, which shows that independently of the post-mortem condition of the fish originally sent to be frozen, the protein solubility of the frozen fish gradually decreases during storage.

This decrease is however significantly greater in fish that are frozen when they have reached the relaxation stage than in fish frozen immediately after being killed or in a state of rigor. So, in fish frozen after relaxation the amount of soluble protein dropped to 21.3%, and for fish frozen
immediately after being killed and in rigor to 34.2 and 38% respectively.

The third series of experiments was carried out on the same fish (28) as the first and consisted of centrifuging the protein solutions obtained from fish after it had been stored for varying lengths of time.

The results of the experiments are presented in Fig. 6, and it can be seen that the amount of protein precipitated from the solution increases continually up to 10 weeks of storage, which indicates that the average size of protein particles in the solution gets bigger i.e. aggregation occurs.

The change in the amount of deposited proteins varies in accordance with the variation in the decrease of actomyosin solubility.

The processes of aggregation of the protein particles and the decrease of protein solubility appear to follow one another when frozen fish is stored.

The amount of deposited protein starts to decrease after the frozen fish has been stored for 10 weeks and this coincides with an increase in solubility of proteins of the sarcoplasmic fraction, in which there is a decrease in the average size of protein particles.

So when frozen fish is stored for a certain period (up to 10 weeks), the average size of particles of the soluble proteins continually increases. This aggregation is accompanied by a further loss in the solubility of part of the aggregated
Table 8

<table>
<thead>
<tr>
<th>Length of storage of fish, in weeks</th>
<th>Content of soluble protein in fish</th>
<th>Fresh from</th>
<th>In rigor</th>
<th>After relaxation kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fresh fish</td>
<td>10.6</td>
<td>14.1</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>Frozen fish</td>
<td>57.6</td>
<td>76.5</td>
<td>66.7</td>
</tr>
<tr>
<td>2</td>
<td>10.3</td>
<td>9.5</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>55.9</td>
<td>51.8</td>
<td>51.8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8.0</td>
<td>9.5</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46.7</td>
<td>51.6</td>
<td>25.1</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>6.3</td>
<td>7.0</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34.2</td>
<td>38.0</td>
<td>21.3</td>
<td></td>
</tr>
</tbody>
</table>

Note. In the fractions, the numerator shows g./100 g. meat, the denominator, %

Fig. 6. Precipitation of proteins from solutions obtained from frozen fish stored for varying lengths of time: I - 1,000, II - 12,000, III - 17,000, IV - 17,000

*This appears to be an author's error for 7,000.*

Translator's note
proteins, chiefly actomyosin which has the highest molecular weight.

After 10 weeks of storage, the relative content of proteins with a high molecular weight decreases sharply, while the amount of sarcoplasm proteins passing into the solution increases significantly. This may explain the decrease in the relative amount of proteins deposited from the solution obtained from fish after 10 weeks of storage.

The change in the proteins of fish meat after defrosting

Experimental procedure to observe the solubility of proteins in fish meat after defrosting was as follows.

Frozen fillets prepared from fish in different post mortem conditions (immediately after slaughter, in rigor and after relaxation) were stored for 6 and 29 weeks and pieces of meat were taken from them and immediately ground up in the blender in a salt solution.

The remaining part of the frozen fillet was defrosted in the atmosphere at a temperature of 18°C+20°C and from it pieces were taken immediately after defrosting and sent for analysis.

Results of observations of the change in protein solubility during defrostation are presented in Table 9.

The table shows that independently of the post mortem condition of the fish before freezing and the length of the storage period from freezing to defrosting, the amount of
soluble proteins in defrosted fish was higher in all cases than in frozen fish before defrosting. The only exception was for fish frozen immediately after being killed and defrosted after 6 weeks storage, when the content of soluble proteins in the frozen fish was somewhat higher (46.7%), than in the defrosted (43.5%).

It may be assumed, based on our studies with carp (Bykov 1963), that the change in protein solubility during defrosting takes place when the temperature in the meat corresponds to the temperature zone of maximum crystal formation. The experiment with cod confirmed this assumption. Fish was studied that had been stored for 14 weeks and defrosted in the atmosphere at a temperature of +18 /+ 20°C.

Before defrosting started and during it as the temperature increased, pieces of meat were taken from the fish and immediately sent for observation to determine the total content of soluble proteins.

Results of observations of the change in protein solubility in relation to temperature increase in the body of the fish being defrosted are presented below.

<table>
<thead>
<tr>
<th>Temperature in body of fish, °C.</th>
<th>Protein solubility, g./100 g. meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>-22</td>
<td>6.1</td>
</tr>
<tr>
<td>-4.5</td>
<td>6.4</td>
</tr>
<tr>
<td>-3</td>
<td>7.3</td>
</tr>
<tr>
<td>-2</td>
<td>8.3</td>
</tr>
<tr>
<td>0</td>
<td>10.1</td>
</tr>
<tr>
<td>+15</td>
<td>8.3</td>
</tr>
</tbody>
</table>
The above data show that the change in temperature in the body of the fish from -22 to -4.5°C was not accompanied by any significant change in protein solubility; it increased from 6.1 to 6.4 g./100 g. meat. However, when passing the temperature zone from -4.5 to 0°C, solubility gradually rose from 6.4 to 10.1 g./100 g. meat. With a further increase in temperature to 15°C, protein solubility again decreased somewhat (to 8.3 g./100 g. meat).

It was observed that when proteins were deposited from solutions obtained from fish frozen immediately after killing, both before and after defrosting (Fig. 7), the average particle size of the soluble proteins increased when the fish was defrosted, if defrosted immediately after freezing or after two weeks storage in the frozen state.

Table 9.

<table>
<thead>
<tr>
<th>Length of storage of frozen fish, in weeks</th>
<th>Content of soluble protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in g./100 g. meat</td>
</tr>
<tr>
<td></td>
<td>fresh in frozen fish</td>
</tr>
<tr>
<td></td>
<td>from rigor kill</td>
</tr>
<tr>
<td></td>
<td>fresh in relaxed</td>
</tr>
<tr>
<td></td>
<td>killed</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
</tr>
</tbody>
</table>

Note. In fractions, the numerator shows the figure before defrosting, the denominator after defrosting.
However when fish that has been stored for 12 weeks is defrosted, the amount of proteins deposited by centrifuging is virtually unchanged.

So defrosting fish is generally accompanied by an increase in protein solubility and at the same time the average size of particle of the soluble proteins increases when the fish is stored for a short length of time before being defrosted, (0 - 2 weeks), but when the frozen fish was stored for a longer period (up to 12 weeks) before being defrosted, no change was noted in the average size of particle of soluble proteins.

---

Fig. 7. Precipitation of proteins from solution obtained from (a) frozen fish, and (b) defrosted at varying lengths of time after freezing: I - at once; II - after 2 weeks storage; III - after 12 weeks storage.
Conclusion

When fish (cod) are subjected to refrigeration - stored in chilled condition, frozen, stored when frozen, and defrosted - changes take place in the properties of the muscle proteins (solubility, average size of particles of protein extracts obtained by dissolving fish-meat proteins in a saline solution).

The total content of soluble proteins in fish meat increases as a result of the onset of rigor mortis when fish is stored in chilled condition.

In addition, the size of particles in a solution obtained from fish in a state of rigor increases in comparison with the average size of particles in a solution obtained from fish immediately after being killed before the start of rigor mortis.

This increase in the average size of particles as a result of rigor mortis occurring in the fish may be explained by the formation of actomyosin from actin and myosin which has a higher molecular weight than its composite parts, actin and myosin.

During the freezing process the total content of soluble proteins hardly changes when it is frozen immediately after being killed or in a state of rigor mortis, and it decreases when the fish is frozen in a state of relaxation after rigor.

The average size of protein particles in solution increases when fish is frozen immediately after slaughter and on the other hand decreases when fish is frozen in rigor.

However when fish is frozen in the post-rigor relaxation phase, only a slight increase in the average size of protein
particles is observed. The increase in the average size of protein particles which is observed when fish is frozen immediately after being killed appears to be related to the formation of actomyosin from actin and myosin. The decrease in the average size of protein particles when the fish is frozen in a state of rigor may be explained by the dissociation of actomyosin to actin and myosin.

This is confirmed by the experiment of freezing fish in a state of relaxation when in the fresh fish before freezing actomyosin is known to exist in the dissociated state, and during freezing the average size of particles of soluble proteins hardly changes. In this instance a slight decrease in the average size of protein particles may be explained by the proteolytic processes of decomposition of the protein molecules.

So the aggregation and disaggregation of proteins soluble in salt solutions, which is observed when fish are frozen, may be attributed to the same processes taking place as in fresh fish, namely the formation of the actomyosin complex from actin and myosin and its dissociation to actin and myosin, just as in fresh fish, when rigor mortis sets in and is suspended.

When frozen fish is stored for 24 weeks, solubility of the muscle proteins gradually decreases. In this case the solubility of the actomyosin fraction decreases throughout the entire period while the sarcoplasmic decreases at first (for up to 10 weeks storage) and then increases again somewhat. The decrease in protein solubility coincides with a corresponding increase in the average size of soluble protein particles.
The storage of frozen fish for up to 10 weeks is accompanied by a continual increase in the average size of particles of soluble proteins, i.e. their aggregation. This appears to be brought about by the actomyosin which has the highest molecular weight of these proteins.

After 10 weeks storage when a large amount of proteins of the actomyosin fraction has become insoluble while the number of soluble proteins in the sarcoplasmic fraction has started to increase, the average size of particles of soluble proteins in solution started to decrease, because of the relative increase in the amount of proteins of the sarcoplasmic fraction, which have a smaller molecular weight than that of proteins of the actomyosin fraction.

Defrosting fish was generally accompanied by an increase in protein solubility, regardless of the post-mortem condition of the fish before freezing.

When the frozen fish was stored for a short length of time (up to two weeks) before being defrosted, then the average size of protein particles increases after defrosting, but when it is stored for a long period (12 weeks), there was no change in the average size of particles of soluble proteins.

Literature


Bykov, V.P. Vliyanie defrostatsii ryby TVD na ee kachestvo.

I don't know what TVD stands for.


