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Post mortem changes in fish muscle from the view point of cell structure research.

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Post mortem changes in fish muscle from the view point of cell structure research.

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In 1958, Ironside and Love (1) showed that there is a difference in the amount of soluble protein in the various parts of the cod fillet, when they are extracted with a neutral salt solution (5 % NaCl, 0°C).

They further showed that the amount of extractable protein was dependent on the size of the fish and on the different seasons of the year.

The solubility of muscle protein has been studied by a series of skilled scientists. (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 21, 22).

According to our experience it is difficult to get reproducible results; but this work of Ironside and Love (1) has been done very carefully and with many parallels.

Let us therefore see which circumstances may produce a variation in the amount of soluble protein in the muscle.

1) A different proportion between the soluble proteins (myofibrillar proteins and sarcoplasmic proteins) and the insoluble proteins (stroma-proteins), for instance due to the influence of myocommata, the fibrous septa between the about 50 segments of the cod fillet.

2) A change in the composition and physical properties of the soluble proteins. (2, 10, 11, 23, 24).

3) Variations in the condition of the fish pre and post mortem, e.g. food condition, exhaustion and temperature a short time after capture.

Argument no 1 was used by Ironside and Love (1) to explain variations between the different parts of the fillet; (25) a few experiments made by us, however, only showed a difference between the samples taken at the end of the tail and the rest of the fillet (respectively ca. 93 % and 75 % soluble protein of the total protein).

Besides the work mentioned (1), it is remarkable how little the myocommata has been studied from a technological point of view. (12, 13, 14).

The argument no. 2 was used by Ironside and Love (1) to account for the variations in amount of soluble protein in the different seasons, and in fish caught on the different fishing-grounds. They supposed that the fish mobilized the essential

amino acids for the development of the gonads during shortage of food. (15, 16, 17). A change in the amino acids composition of the contractile proteins would probably cause a serious convertion in the properties of this protein. (26).

The unsoluble acto-myosin is difficult to examine properly. (10).

The argument no. 3 is not taken into consideration by Ironside and Love (1), but the change in solubility may be due to an aggregation of the actomyosin, (10, 23, 62) which may be caused by conditions in the muscle before and after the animal is dead. (18, 19, 9 , 27).

Studying the recent progress in the meat industry, we find some papers about: Protein solubility, influenced by physiological condition in the muscle. In mammals at high temperature and low pH shortly after death not only the solubility of the myofibrillar proteins is reduced, but also the sarcoplasmic protein decreases remarkably. (6, 28, 29, 30, 31).

The influence on the amount of sarcoplasmic proteins by shorter storage of the fillet at room temperature, we just reasently have begun to study.

It is not clear if variations in the low fat contents of lean fish may influence the stability and solubility of proteins and the water binding capacity of the fillet. (32, 33, 34, 35, 36).

As the amount of soluble proteins seems to be related with the juice-retaining properties (6) and consistency of the muscle, the variations may have the following consequences for the fish technology:

It may complicate the continous and reproducible experiments with fish muscle, at which one uses different parts of the same muscle for treatments or storage experiments, or continous takes out samples of the same fillet. (35, 37, 38).

If the season and the fishing-grounds are of importance for the properties of the fillets of lean fish, (1, 35) it will be difficult to co-ordinate results made in different laboratories.

These observations make it clear, that fish technologists seem to forget that a fish fillet is not a homogeneous material with constant properties. (35). It is necessary to study the cell structure. But the literature has become so overwhelming that it nearly paralyses our judgment.

I will just pick out some points which I suppose will be of interest to the fish technology.

Let us consider what we will see by a fixation of a cross-section of the muscle fiber.

The lipids and the glycogen are visible only by special methods, and the salts are mostly not observed by the ordinary fixation reagents.

The sarcoplasma lays around the myofibrillar proteins in the muscle cell. It contains the nuclei and the sarcosomes or mitochondria. The structure of the sarcoplasma with its tubular system has lately been investigated in electron micrograph by several scientists. (39, 40).

By treatment of sarcoplasma with a fixation reagens, a skrinkage occurs, due to coagulation and dehydration. The structure is dependent on the amount of water and on the amount of myogen. It has been shown in the interesting work of P. Krüger (41) that in the muscles there are two different sorts of muscle fiber, each with specific properties of technological importance. (42, 43, 44).

One type of fibres has a low myogen contents. Here the myogen sediments uniformly on the myofibrils, called "Fibrillen Struktur". By insufficient fixation of the fibres one gets irregular skrinkage, called the fields of Cohnheim.

The other type of fibre has a thicker sarcoplasma with a higher myogen contents. In this type there are less fibrils, and they are united into greater coagulates during fixation. The interspaces look like a net work of band, called "Felder Struktur".

The identification demands experience and a rapid, acid fixation reagent, such as "Susa" (Sublimat - trichloracetic acid acetid acid - formol), or one gets lump formation.

In fish muscle we find the "Felder Struktur" in the red muscle (Musculus lateralis superficialis), and this structure may be of greater significance than just the red colour of myoglobin. (45).

This muscle was already studied by W. Stirling (1886) (46) in whiting, haddoc and mackerel, further in the interesting work of Knoll (1891), 47, 48).

The extension of the red muscle is important. It differs in the various species of fish; sometimes the muscle is situated as a broad or more narraw band along the horisontal septum, sometimes it lies as a thin layer below the skin. (49, 13, 12).

In tuna the contents of myoglobin in white and red muscle are 0,37 - 1,28 mg/g wet weight and 5,3 - 24,4 mg/g wet weight respectively. (50, 51, 52).

According to our own results there is no relation between the myoglobin composition and the size of the tuna. (53).

The observation of Knoll (47) that the red muscle contains myoglobin and a rather high contents of fat and phospholipids has special interest, as the intimate contact between myoglobin and fat contributes both to rancidity and discoloration. (54, 55).

This is particularly disagreeable, as the red muscle is often situated on the surface of the skinned fillet. Myoglobin oxidises to the brown ferric compound fourteen to sixteen times as rapidly as hemoglobin when exposed to atmospheric oxygen. (56).

Myoglobin may serve for storing of oxygen in the muscle. But the chief reason for the higher amount of myoglobin in the red than in the white muscle we suppose, lies in the different metabolism. The red muscle is distinguished from the white one by the accumulations of relatively large mitochondria in the sarcoplasma.

In the red muscle dominates the aerobic oxidative phosphorylating system (the enzymes of the citric acid cycle) which is localised in the mitochondria; in the white muscle dominates the anaerobic glycolytic enzyme system, which is soluble. The myoglobin is necessary for the transport of oxygen to the mitochondria. (44, 43, 57, 58).

A series of works has been done on the electrophoretic pattern of the myogen, the enzymes and the vitamins in red and white muscle. (59, 49, 60, 61, 62, 63, 64, 65, 66).

The red muscle fibres are able to sustain activity (ionic contraction). They are called tonic muscle fibres, in contrast to the white muscle fibres, which are able to convulsive activity, but soon becomes tired (tetanal muscle fibres). (41, 44).

In the cold-blooded fishes, for whom the supply of muscle energy is more limited than in the warm-blooded animals who breathe with lungs, the white muscle is faster exhausted than the white muscles in mammals. (67, 68).

We supposed that the red muscle ought to contain a higher amount of tropomyosin, as this protein is supposed to possess tonic properties, e.g. in the Molluscan "Paramyosin Smooth Muscle", and as it occurs as a complex with nucleic acid. (69, 9, 70). But our analysis in the red muscle of herring showed a lower content of tropomyosin than in the white muscle. (ca. 60 % less).

The contents of tropomyosin is interesting because it is the only muscle protein which remains undenatured at over 100°C. And thus in spite of its low concentration (ca. 0,4 %) it may to a certain degree influence e.g. the water-holding capacity in heated fish-products. (59, 9).

In his work, Krüger (41) points out that the influence of temperature on the muscle activity is a sol-gel transformation.

One might then assume that at lower temperature, e.g. + 2°C, the muscle of a mammal will be in a more stiff gel-condition than in a cold-blooded fish.

The lower viscosity of fish sarcoplasma corresponds with our examinations of plaice-myogen in the ultracentrifuge. We found a fraction of smaller myogen-molecules with far lower sedimentation rate of 1,5 S in addition to those two fractions which are common to fishes and mammals of about 5,5 S and 7,1 S. (71, 72, 59).

A lower viscosity of the sarcoplasma of fish muscle may cause a greater turnover number of the proteolytic enzymes in fish muscle post mortem.

Studying the interesting works of de Duve (73, 74) on the intracellular hydrolyses by the lyso-somal enzymes, we may thus assume a higher effect in fish fillets than in mammals.

As the water-binding capacity of a fillet may change during "fish saving" (91) it is interesting to study some of those factors which influences it. (75).

Parallell to the disappearence of adenosinetriphosphate (ATP) in the muscle fibre, the myosin also loses its bound potassium. (76, 77, 78).

The myogen, which to a smaller extent also binds K, may release it by decrease of pH, and K is changed into a diffusible state. (41, 79).

The difference in concentration inside and outside the fibre cell gives a resting potential of the muscle near the theoretical value. (77).

In 1958 prof. Meessen (80) showed that in heart muscle by a decrease in potassium there occurs a loosening and disorganisation of the myofilament.

There are thus certain indications that potassium influences the consistency and the water retention of the muscle.

In an interesting work, however, Hamm (81) points out that potassium has a free mobility in the tissue, and therefore has no significance for changes in moisture retention. (67).

The curious fact that potassium at the same time is bound to the muscle proteins inside the fibre, and on the other hand is freely movable in the tissue, has been discussed by Ussig. (77).

In contrast to the alkali-ions, the bivalent cations Ca, Mg and Zn influence the consistency and water-retention by decreasing the water-binding capacity.

Which of Ca, Mg and Zn is most important, is still discussed. (66, 67, 68, 69, 70).

Grau and Hamm succeeded in finding a method to measure the bound water in muscle, which has later been somewhat modified. (85, 86, 29, 87).

But this method shows only the total amount of bound water. We should like to find a method which gives a picture of how the water is bound in the muscle, and which indicated the changes in solubility of the proteins inside the muscle. But there are so many factors influencing the results that it is difficult to find a better method.

As the water-binding capacity of the muscle is of importance to the technology of fish and meat, there has been done a lot fo find chemicals to improve the water-retention. (82, 88, 89). Accepted for use are polyphosphates. (90).

The water-binding capacity of a muscle containing 1 % phosphate and 1,7 % NaCl is dependent on the following factors. (84):

The effect decreases by increasing storage temperature of the muscle tissue 0° - 10° - 20°C.

The effect on grinded muscle increases by time of storage during 1 to 16 hours, after which it remains nearly constant.

The effect rises by increasing the ionic strenght of the polyphosphate NaCl-mixture in the muscle in the range 0,30 - 0,36 and by pH in the range pH 6 to 8,5.

The effect depends on specific action of the different salts.

The explanation of the moisture-retention effect of these phosphates seems to be a swelling and solubility effect due to a rise in ionic strength and particularly in pH and the depolymerising action on actomyosin.

The use of water-binding agents has some technological objections. The bacterial growth may be increased by a rise in pH and by infection from the brine. The addition of polyphosphates into the fillets is time-consuming, and may be misused.

I have here tried to give a survey of some of the problems of the fish as raw material, and I hope it also shows how much work is still left.

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