FISH PROTEIN EXTRACTIBILITY & ITS DETERMINATION

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INTRODUCTION

Fish proteins gradually become denatured in cold storage. The rate of denaturation depends largely upon storage temperature.

Badly frozen stored fish are easily recognizable. The appearance of the thawed product, instead of being glossy and translucent, is dull and opaque and the texture, no longer firm and elastic, becomes soft and spongy. The cooked flesh loses its succulence and becomes dry, fibrous and tasteless.

The main proteins of fish flesh are called myosin and actin. They are responsible for the mechanism of contraction and relaxation of muscles and are called myofibrillar proteins. Muscle also contains many other proteins, the sarcoplasmic protein which are soluble in tissue fluid and in any salt solution. During freezing and cold storage, the proteins are affected, especially the myofibrillar protein, resulting in the textural changes of fish flesh.

The myofibrillar protein extractibility, therefore is used as a quality index for the assessment of frozen fish. It is expressed as follows:-

$$\text{Extractibility (\%)} = \frac{\text{MPN} - \text{SPN}}{\text{TN} - \text{NPN}} \times 100 \qquad \text{(A)}$$

where:

MPN	=	myofibrillar protein-nitrogen	(N mg/100 g sample)
SPN	=	sarcoplasmic protein-nitrogen	(N mg/100 g sample)
TN	=	total nitrogen	(N mg/100 g sample)
NPN	=	non proteinous nitrogen	(N mg/100 g sample)

The protein extractibility is applicable to fish and its product in general and can be used as an indicator of the degree of protein denaturation for demersal and pelagic species during cold storage.

I SAMPLING AND SAMPLE PREPARATION

Take a representative sample of 22 g or more from the product. Place the sample in a polyethylene bag and store in a refrigerator or in ice so as to maintain sample integrity before preparation for analysis.

The dark meat, if any, should be removed prior to homogenisation of fish flesh.

Comminute the sample until homogeneous and place the homogenate in a polyethylene bag. Store the sample in a refrigerator or in ice until required. Ensure that the prepared sample is still homogeneous prior to weighing.

II APPARATUS

Chopper or mincer
Analytical balance, decimal to 0.1 mg
Spatula
Bottom-drive homogeniser (Nihon Seiki SN-103) or equivalent
Refrigerated centrifuge, capable of centrifuging at 12,500 g
Beakers, 100 and 250 ml
Bulb pipettes, 10, 20 & 40 ml
Glass funnels 60 mm ø
Whatman filter paper, No. 41

III REAGENTS

a) Phosphate buffer solution

0.03 M potassium di-hydrogen phosphate, 1 litre

0.03 M di-sodium hydrogen phosphate, 1 litre.

Mix the above solutions into a 2 litre beaker.

Adjust the pH to 6.85 using these solutions.

Store in a refrigerator.

b) 0.1 M potassium chloride solution

Weigh KCl required accurately, use distilled water as solvent.

c) 0.5M potassium chloride buffered solution

Accurately weigh KCl required. Dissolve the weighed KCl in the required phosphate buffer solution.

d) Trichloroacetic acid solution (25%, w/v)

Dissolve 25 g TCA in 75 ml distilled water.

IV PROCEDURE

1. Total nitrogen. Accurately weigh a duplicate of 1 g of the homogeneous fish sample for protein digestion (refer to protein determination by Kjeldahl method B-1).

2. Sarcoplasmic protein nitrogen. Accurately weigh 10 g of the homogeneous fish sample. Blend the sample with 200 ml of 0.1 M KCl solution with the homogeniser for 4 mins (speed set at scale 2). Leave to stand in iced water for 2 hrs. Centrifuge the blended sample at 12,500 g or 9,000 rpm at 5°C for 20 mins. Pipette 20 ml supernatant (sarcoplasmic protein fraction) for digestion (refer to Kjeldahl method).

3. Myofibrillar protein nitrogen. Accurately weigh 10 g of the homogeneous fish sample. Blend sample with 200 ml 0.5 M KCl phosphate buffered solution with the homogeniser for 4 mins (speed set at scale 2). Leave to stand in iced water for 3 hrs. Centrifuge the blended sample at 12,500 g or 9,000 rpm at 5°C for 20 mins. Pipette 20 ml supernatant (myofibrillar protein aliquot) for digestion (refer to Kjeldahl method).

4. Non proteinous nitrogen. Pipette 40 ml of sarcoplasmic protein aliquot (see Section IV 2) into 100 ml beaker. Add 10 ml 25% TCA and leave to stand in iced water for 30 mins with occasional swirling. Filter the content of the beaker with Whatman No. 41 ashless filter paper. Pipette 40 ml of filtrate for digestion (refer to Kjeldahl method).

V CALCULATIONS

Ws in formula (1) in the protein determination by Kjeldahl method (B-1 Section IV) has to be replaced by the meat weight (g) in each of the protein aliquots used as follows:-

1. For myofibrillar protein nitrogen (MPN)

$$W_{MPN} = W_1 \times \frac{20}{W_1 + 200}$$

where W_1 = weight of fish flesh (g) used for myofibrillar protein extraction

20 is the volume (ml) of sarcoplasmic protein aliquot used for digestion

200 is the volume (ml) of 0.5 M KCl - buffered solution used for the extraction of myofibrillar protein

2. For sarcoplasmic protein nitrogen (SPN)

$$W_{SPN} = W_2 \times \frac{20}{W_2 + 200}$$

where W_2 = weight of fish flesh (g) used for sarcoplasmic protein extraction

20 is the volume (ml) of sarcoplasmic protein aliquot used for digestion

200 is the volume (ml) of 0.1 M KCl solution used for the extraction of sarcoplasmic protein

3. For non-proteinous nitrogen (NPN)

$$W_{NPN} = 40^a \times \frac{40^b}{50} \times \frac{W_2}{200 + W_2}$$

where 40^a is the volume (ml) of supernatant of sarcoplasmic protein aliquot used for non-proteinous nitrogen

40^b is the volume (ml) of filtrate used for digestion taken from the sarcoplasmic protein aliquot after precipitation by 25% TCA

50 is the total volume (ml) of sarcoplasmic protein aliquot after addition of 10 ml 25% TCA

W_2 = weight of fish flesh (g) used for sarcoplasmic protein extraction

200 is the volume (ml) of 0.1 M KCl solution used for the extraction of sarcoplasmic protein

Use the above equivalent meat weights and the formula (I) in protein determination by Kjeldahl method (B-1 Section IV) for calculation of the respective protein nitrogen aliquot, express in mgN/100 g and calculate the extractibility by formula (A).

N.B. The pH plays an important role in the extraction of fish protein. The optimum pH is between 6.5-7.0. Adjustment of the pH of the buffer solution is important and can be achieved by using di-sodium hydrogen phosphate, potassium di-hydrogen phosphate or sodium carbonate etc.