FRACTIONATION AND DETERMINATION OF FISH PROTEINS

KATSUTOSHI MIWA AND LIM PANG YONG

INTRODUCTION

Myofibrillar proteins (MFP) such as myosin and actin are the major proteins in fish muscle and are soluble in 3-5% salt solution.

MFP solubility in salt solution and ATPase activity have been often used as an index for protein denaturation.

Ironside & Love (1958) reported that the determination of myofibrillar protein solubility, in 5% NaCl or 0.6 M KCl, was very useful for the studies of protein denaturation during freezing storage, and many scientists have since then used this method for the determination of MFP denaturation.

On the other hand the determined values by this method generally showed large deviations, due to the denaturation of MFP during extraction and the insufficient solubility of MFP. The deviation is caused, especially, by the foam formation during homogenization with a normal top driven homogenizer which accelerates the denaturation of MFP. Therefore, a bottom driven homogenizer with a cover immersed in chilled water is recommended. Multiple measurements with the same sample are necessary for the MFP determination by this method.

MFP can be determined not only by measuring the difference between salt soluble protein and water soluble protein (sacroplasmic protein), the precipitated actomyosin in the salt soluble protein solution which has been diluted with cold distilled water can also be measured. Therefore, this method is called myofibrillar protein or actomyosin determination. In this method the precipitated actomyosin is determined together with a small quantity of precipitated sarcoplasmic protein.

Sarcoplasmic proteins consists of regulatory proteins, glycolysis enzymes, myokinase, AMP-deaminase etc. and are more easily soluble in diluted salt solution than in distilled water. But MFP is not soluble in 0.01 - 0.1 M NaCl or KCl solution, therefore, sarcoplasmic protein can be determined by measuring the difference between the diluted salt soluble protein solution and non proteinous compounds.

There are some salt (0.6 M KCl) insoluble proteins in fish muscle, e.g. stroma protein, intercellular protein connected firmly with connective tissues and denatured protein. Stroma protein is not soluble in 0.1 N-NaOH solution, but the other proteins are soluble in it.

Using the differences between the various protein solubilities, the total nitrogen and non proteinous compounds nitrogen, sarcoplasmic protein, myofibrillar protein, residual intercellular protein & denatured protein and stroma protein can be determined.

APPARATUS

1. Chopper or mincer
2. Analytical balance
3. Homogenizer[*1,*2]
4. Refrigerated centrifuge, capable of centrifuging at 9,000 rpm and at 0°-5°C.
5. Beakers, 100 and 250 ml
6. Bulb pipettes, 10, 20 and 40 ml
7. Glass funnels 60 mm ø
8. Whatman filter paper, No. 41
9. Spatula

REAGENTS

1. Phosphate buffer solution.[*3]
   0.03 M potassium di-hydrogen phosphate, 1 litre.
   0.03 M di-sodium hydrogen phosphate, 1 litre.
   Mix the above two solutions and adjust the pH to 6.85 using these solutions.
2. 0.1 M potassium chloride solution.
   Weigh 7.455g KCl accurately, dissolve in 1 litre distilled water.
3. 0.6 M potassium chloride phosphate buffered solution.
   Weigh 44.73g KCl accurately, dissolve in 1 litre phosphate buffer solution.
4. 0.1 N sodium hydroxide solution.
   Weigh 4.0g NaOH accurately, dissolve in 1 litre distilled water.
5. Trichloroacetic acid solution (25%, W/V).
   Dissolve 25g TCA in 100 ml distilled water.

PROCEDURE

1. **Total nitrogen (tN)**

   Accurately weigh a duplicate of 1 g homogeneous fish meat for protein digestion. (refer to Protein determination by Kjeldahl method, Part B, B-1).

2. **Total sarcoplasmic protein nitrogen (tspN)**

   Accurately weigh 10g homogeneous fish meat. Blend the sample with 200 ml of 0.1 M KCl solution with the homogenizer for 4 minutes (speed set at scale 2). Leave to stand in iced-water for 2 hours. Centrifuge the blended sample at 12,500 g or 9,000 rmp at 0°-5°C for 20 minutes. Pipette 20 ml supernatant for digestion with H_2SO_4 (refer to Kjeldahl method).

3. **Non proteinous compounds nitrogen (npN)**

   Pipette 40 ml sarcoplasmic protein aliquot (from No. 2 above) into a 100 ml beaker. Add 10 ml 25% TCA and leave to stand in iced water for 30 minutes with occasional swirling. Filter the content of the beaker with Whatman No. 41 ashless filter paper. Pipette 40 ml of filtrate for digestion with H_2SO_4.

4. **Salt soluble protein nitrogen (sspN)**

   Accurately weigh 10g homogeneous fish meat. Blend sample with 200 ml 0.6 M KCl phosphate buffered solution with the homogenizer for 4 minutes (Speed set at scale 2). Leave to stand in iced-water for 2 hours. Centrifuge the blended sample at 12,500 g or 9,000 rpm at 0-5°C for 20 minutes. Pipette 20 ml supernatant for digestion with H_2SO_4.

5. **Residual intercellular protein and denatured protein nitrogen (rpN)**

   Wash the precipitate of salt soluble protein aliquot with 10 ml 0.6 M KCl phosphate buffered solution and centrifuge at 9,000 rpm for 10 minutes. Repeat this washing procedure twice. Discard the supernatant. Add 15 ml 0.1 N NaOH solution and stir with glass spatula and then centrifuge at 9,000 rpm for 10 minutes. Repeat this procedure three times. Transfer the supernatant into a 50 ml volumetric flask and adjust to 50 ml with 0.1 N NaOH. Pipette 10 ml supernatant for digestion with H_2SO_4.

6. **Stroma protein nitrogen (spN)**

   Suspend the precipitate from No. 5 above in water and transfer into flask for digestion with H_2SO_4.

7. Procedure Nos: 2, 3, 4, 5 and 6 must be repeated at least 3 times.

8. For digestion of the above fractionated protein nitrogen, refer to 'Protein determination by Kjeldahl method' in Part B, B-1.
   (See Fig.1)

CALCULATIONS

The following shows the calculations of equivalent meat weight of fish used in the extraction of various fish proteins:

1) **Meat weight used for total sarcoplasmic protein nitrogen (tspN)**

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

where W_1 is the weight of fish meat (g) used for estimated 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

2) **Meat weight for non proteinous compounds nitrogen (npN)**

$$W_{npN} = 40^a \times \frac{40^b}{50} \times \frac{W_1}{200 + W_1}$$

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 precipitating with TCA

50 is the total volume of sarcoplasmic protein aliquot after addition of TCA solution

W_1 is the weight of fish meat (g) used for sarcoplasmic protein extraction

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

3) **Meat weight for salt soluble protein nitrogen (sspN)**

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

where W_2 is the weight of fish meat (g) used for salt soluble protein extraction

20 is the volume of salt soluble protein aliquot used for digestion

200 is the volume (ml) of 0.6 M KCl phosphate buffered solution used for the sspN extraction

4) **Meat weight for residual intercellular protein & denatured protein nitrogen (rpN)**

$$W_{rpN} = W_2 \times \frac{50}{50}$$

W_2 is the weight of fish meat (g) used for salt soluble protein extraction

20 is the volume of residual intercellular protein and denatured protein aliquot used for digestion

50 is the total volume of residual intercellular protein and denatured protein aliquot

5) **Meat weight for stroma protein nitrogen (spN)**

$$W_{spN} = W_2$$

W_2 is the weight of fish meat (g) used for salt soluble protein extraction

Using the above respective calculated meat weight and the titration value for a particular protein nitrogen obtained by Kjeldahl method, calculate the respective protein nitrogen by the following formula:

$$\text{Protein nitrogen, mgN/100 g} = \frac{(b - a) \times 0.1 \times 14.00}{W} \times 100$$

where W is the weight of equivalent fish meat used for a particular protein extraction

a is the volume (ml) of 0.1 N H_2SO_4 used in blank titration

b is the volume (ml) of 0.1 N H_2SO_4 used in sample titration

14.00 is the atomic weight of nitrogen

To determine each protein nitrogen in fish meat, substitute the various calculated protein nitrogen(s) from the above into the following formula

1. **Actual sarcoplasmic protein nitrogen, aspN**

   The difference between total sarcoplasmic protein nitrogen and non proteinous compounds nitrogen:

   aspN = tspN - npN

2. **Myofibrillar protein nitrogen, mfpN**

   The difference between salt soluble protein nitrogen - total sarcoplasmic protein nitrogen:

   mfpN = sspN - tspN

3. **Compositions of various protein nitrogen:**

   i. $\text{aspN, \%} = \frac{\text{aspN}}{\text{tN}} \times 100\%$

   ii. $\text{mfpN, \%} = \frac{\text{mfpN}}{\text{tN}} \times 100\%$

   iii. $\text{rpN, \%} = \frac{\text{rpN}}{\text{tN}} \times 100\,\%$

   iv. $\text{spN, \%} = \frac{\text{spN}}{\text{tN}} \times 100\,\%$

REMARKS

*1 Nihon Seiki SN-03 type homogenizer. The homogenizer has a capacity of 400-500 ml and is bottom driven type, with a cover and a water jacket, so that foam formation can be prevented.

*2 It is very important to prevent the formation of foam during homogenization of fish meat, as denaturation of fish protein take place simultaneously with foam formation. Therefore, the use of the bottom driven homogenizer with a covered cup in water is recommended.

*3 The pH of the salt solution influences the extractibility and stability of fish proteins. The optimum pH is between 6.6 - 7.0. The phosphate buffer solution can be prepared by using di-sodium hydrogen phosphate, potassium di-hydrogen phosphate or sodium carbonate etc.

REFERENCES

J.I.M. Ironside & R.M. Love (1958). Studies on protein denaturation in frozen fish - I., Biological factors influencing the amounts of soluble and insoluble protein present in the muscle of the North Sea Cod. J. Sci. Food Agric., 9, 597-604.

Y. Shimizu & W. Simidu (1960). Studies on muscle of aquatic animals - XXVIII. Protein composition of fish muscle. Nippon Suisan Gakkaishi, Vol. 26, No. 8, p. 806-809.

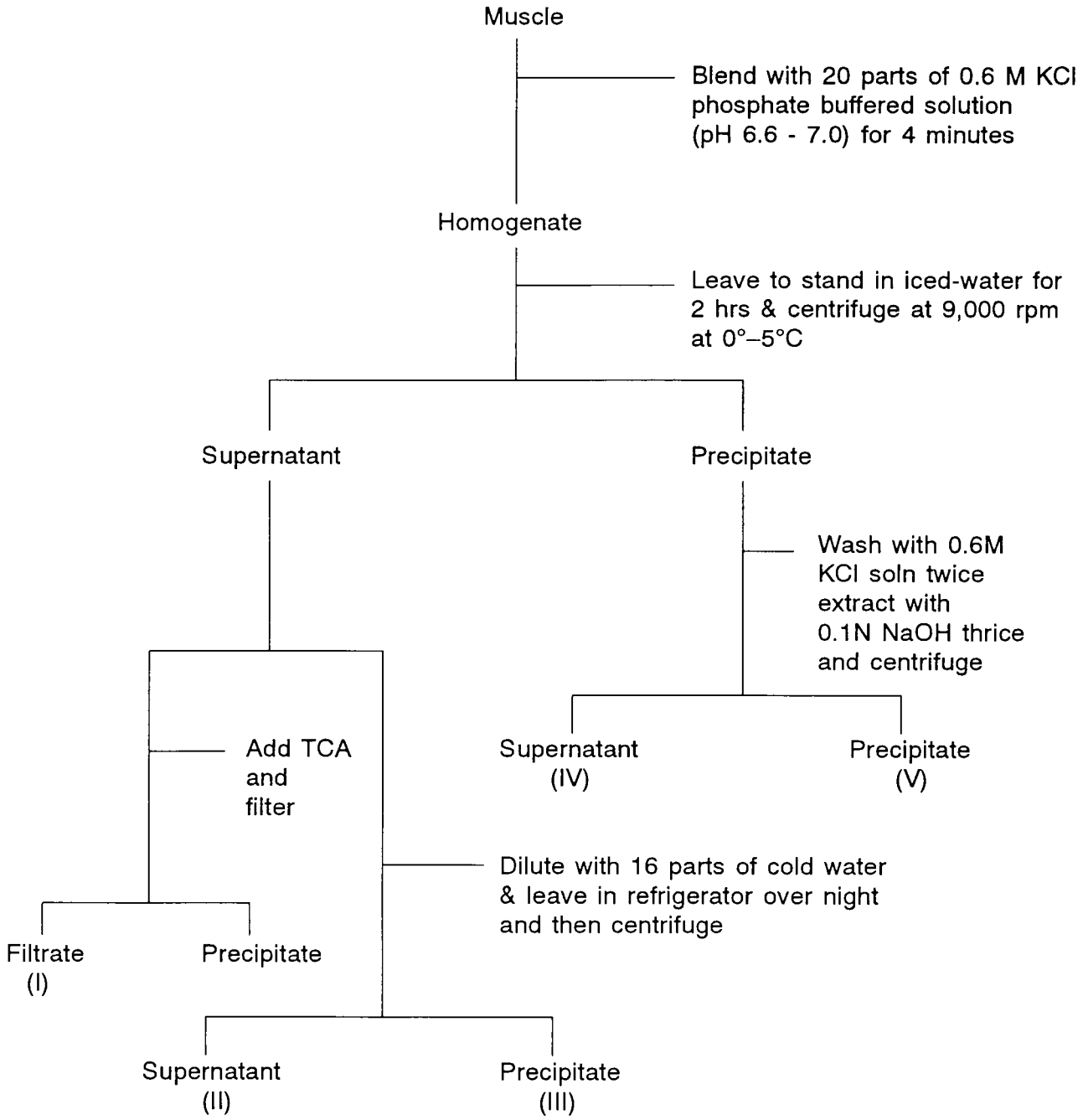

Fig. 1 Extraction and fractionation of protein in fish muscle

(I) Non proteinous compounds.

(II) Sarcoplasmic protein & non proteinous compounds.

(III) Myofibrillar protein or actomyosin protein

(IV) Residual intercellular protein and denatured protein

(V) Stroma protein