Journal of Coastal Development
Volume 3, Number 3, June 2000 : 647-654

ISSN: 1410- 5217
Accredited: 69/Dikti/Kep/2000

A STUDY OF CHANGES IN THE PROTEIN QUALITY OF RAINBOW TROUT (*Salmo gairdneri*) IN FROZEN STORAGE

Eko Nurcahya Dewi[1)]

[1)]Faculty of Fisheries and Marine Science,
University of Diponegoro, Jl. Hayam Wuruk 4A Semarang Indonesia

ABSTRACT

Freezing is an effective method of preservation which can produce high quality foods. However, low temperature applied during processing could result in serious product quality deterioration in particular functional properties of protein. During the freezing process salt concentration increases and the change in pH may cause extensive denaturation of muscle protein. The process of denaturation can be accelerated with a resulting increase in thaw drip loss of tissue. In order to examine protein quality, parameters such as soluble protein, drip loss, and changes in the electrophoretic pattern of sarcoplasmic protein of fish muscle were studied. The fish used in this experiments was rainbow trout (Salmo gairdneri). The fish was filleted and frozen in an airblast freezer for an hour at -30 ℃. They were called "fresh quality samples", whereas the "poor quality" samples were fish fillets that have been kept on ice for 3 days before freezing. The samples were analysed for salt soluble protein , thaw drip, water holding capacity and isoelectric focusing of sarcoplasmic protein at the freezing day and the finished day storage (5 weeks). There was a decrease of 7% on the "fresh quality " sample from the initial value of 82.8% and 7.5% of the "poor quality "sample from 82.5% on the salt soluble protein. Although the differences were small and there were no significant difference between the two samples , the result suggests that some of the protein became denatured during frozen storage (5 weeks). There was an increase from 4.3% to 11.6% in "fresh quality" and 6.9 %to 12% in "poor quality "sample. The drop of these values reflect the extent of protein denaturation due to ice crystal formation and cell rupture. Sarcoplasmic protein pattern of both samples were not much different before freezing, however there was a disappearance of some light sarcoplasmic bands in the sample after freezing process. On further storage (5 weeks) the pattern obtained were still nearly the same, but light bands did not correspond exactly in their position.

Keywords : fish fillet , freezing, frozen storage, salt soluble protein, drip loss , isoelectric focusing sarcoplasmic protein pattern

I. INTRODUCTION

Freezing of fish is an effective process for preserving the quality of fish for up to 6 months frozen storage. However it is difficult to obtain high quality product, since the initial fish quality have to be good , the temperature of storage at least -20°C and also during the freezing process quality retention or loss during freeze preservation of animal tissue can relate to how the freezing process is conducted.

A major problem arising from improper freezing and storage procedures is excessive exude followed by a change in texture. During the freezing process salt concentration increases and the concomitant change in pH may cause extensive denaturation of muscle protein properties that is not accompanied by cleavage of peptide bonds. In practice the protein denaturation in fish muscle is

ISSN: 1410- 5217
Accredited: 69/Dikti/Kep/2000

mainly manifested by a decrease in extractability of the myofibrillar fraction due to aggregation or no longer soluble/ extractable by 5 % salt (sodium chloride) solution under condition in which the native protein is soluble or extractable.[1,11] The process of denaturation can be accelerated with a resulting decrease in water holding capacity of tissue.

Many previous researches have largely concentrated on investigating the chemical composition of the fish, the structure of the tissue as a consequence of freezing and the structure of frozen tissue during storage. But very little has been done on farmed fish such as trout. Furthermore, because of their abundance and their possible utilization as a source of food, fresh water fish species are being studied for the manufacture of new products.

The objectives of the present study were to investigate the effect of the freezing process and frozen storage on the quality of protein changes , in particular salt soluble protein, and any changes in the pattern of isoelectric focusing (IEF) of sarcoplasmic protein.

II. MATERIALS AND METHODS

2.1. Sample Preparation

The fishes used in the experiment were rainbow trout (*Salmo gairdneri*) just harvested from Louth Aquafarm, U.K. The fishes were deheaded beheaded and gutted manually. The dressed fishes were then washed, filleted and blast frozen for one hour to - 30°C (measured at center of fillets and stored at - 20°C ±2° C). These were called *"fresh quality"* samples, *whereas* the *"poor quality"* samples were fish fillets that have been sealed in a polyethylene bag and kept on ice for 3 days at -20°C ± 2°C prior to freezing. Samples were analyzed on the frozen time, weekly interval and finished, storage at week 5. All determination were made in duplicate.

2.2. Chemical Analysis

The salt soluble protein (SSP) determination was adapted from Cowie and Little [2] where soluble nitrogen (SN), non-protein nitrogen (NPN) and total nitrogen (TN) in cold neutral 5% sodium chloride solution were determined to obtain salt soluble protein. The method modified by Deb [3] was followed to measured Drip loss where 10 g sealed samples were thawed and drained overnight in a refrigerator at 4°C in a muslin bag and the weight loss was measured. The water holding capacity (WHC) was performed measured using the methods described by Wang [13] and expressed as the percentage of water expelled in the fish fillet after 10 g sample centrifugation in 0-5°C for 15 minutes at 20000 rpm. PH was measured using digital Corning pH meter.The identification of sarcoplasmic protein was established as mentioned in the Phast System Separation Technique File No. 100 . The pH of gel used ranges between 3-9 and they were brought ready made from Pharmacia [10].

2.3. Statistical Analysis

The data was analyzed statistically using the Analysis of Variance (Anova) at 1% and 5% significancy level .[12]

ISSN: 1410- 5217
Accredited: 69/Dikti/Kep/2000

III. RESULTS AND DISCUSSION

3.1. Salt Soluble Protein (SSP)

The changes of protein solubility in fillets rainbow trout muscle protein in 5% sodium chloride are presented in figure 1. The figure indicates Salt soluble protein value for *"fresh quality"* samples and *"poor quality"* samples on the day immediately after frozen process have a high extractable protein value of 82.8% and 82.5 % respectively. The *"poor quality"* sample salt soluble protein values appear to be similar in amount to the *"fresh quality"* samples. Since even the *"poor quality"* samples have been kept for 3 days on ice at - 20°C, the result may be explained by considering that the fish were filleted really fresh directly after harvesting. This is consistent with earlier work done by Conell [1] that there remain a fairly constant level of protein solubility on cod fillets for the ice storage of time of 6 - 8 days. The low initial value of % SSP for fish fillet indicates that these fish may have been chilled for sometime before purchase.

The amount of salt soluble protein in fish is an indicator that myofibrillar protein has decreased in its solubility. Myofibrillar protein is expected to have values around 70% -80%. Conell's [1] study on fresh cod observed that almost all of the muscle protein are soluble in 5% sodium chloride solution. During prolonged storage in ice the total extractable protein has shown to either fall progressively or remain virtually constant. Reducing the amount of salt soluble protein after frozen storage is subject to many factors such as temperature storage, freezing process, fish species, the condition of fish at the time of catch and the quality of the fish which is frozen.

Figure 1 also displayed that on the following week after freezing there was still a small difference in the extractability between the two samples. That was a drop of about 4% in the *"fresh quality"* material and 5% in the *"poor quality"*. The drop on these values in the first 7 days was similar to those on common carp reported by Wang [13] and cod muscle protein by Montero and Borderias. [9] The former observed a drop of about 5% in SSP of whole carp during frozen storage at -20°C, while the latter also found that fish muscle protein soluble in 5 % NaCl and the solubility only decreased rapidly over 90 days. The salt soluble values were not much changed until finished storage (5 weeks) for both samples, there were 76.9 % and 76.3 % for the " *fresh and poor*" quality samples. Some studies have shown that the quality deterioration was increased if fish have been held on ice prior to freezing. However, in these experiments salt-soluble protein values were not much different for fresh and sample that have been held on ice prior to freezing. It may be related to depletion of TMAO and co-factor for the TMAO demethylase reaction and formation of trimethylamine (TMA). Since the samples were really fresh there was no difference about the depletion of TMA .[5]

The finished values do not differ considerably from the initial concentration of salt-soluble protein in both two samples. Protein solubilisation proceeded slowly during the experiment and in general still continuing at the end of the experiment. Although the differences were small and they were not statistically different ($P > 0.01$), the result suggests that some of the protein denatured during frozen storage. This indicates that more proteins became insoluble as the fish aged and or that the insoluble proteins had increased their capacity to retain water and salt. Protein tend to aggregate and lose solubility mainly due to the formation of non-covalent bonds among molecules.

ISSN: 1410- 5217
Accredited: 69/Dikti/Kep/2000

3.2. Changes in Drip loss

In general an increasing value of drip loss or expressible moisture was found during this study for both samples (figure 2). There was an increase from 4,23% to 11,6% in the *"fresh quality"* sample and from the initial of 6,9 to 12% in other samples. The value from *"fresh quality"* sample remained consistently lower than the *"poor quality"* sample from the freezing process until finished storage.

The drip loss at both two samples is also statistically significantly different ($P < 0.01$) from the freezing process to finished storage. The result suggests that all the samples lost their ability to hold water in the muscle tissue due to denaturation protein the denaturation of the protein. Volume of drip is still used to indicate general rough changes in the quality of fish muscle. As is well known 10% of the total water in fish muscle is possibly bound strongly to polar group of solutes. Bound water as it is called is not freezable, whereas water that is mobilised by being held in membrane muscle channels is freezable. Upon freezing fish, some of the immobilised water will be converted to ice which may cause cell wall damage resulting in drip upon thawing. These are several moisture related changes including damage due to the removal of protein water of hydration and damage due to increase in salt concentration.

This theory is in accordance with previous studies on fish that attributed the increased hydration of the myofibrillar protein to the increased pH. In the *"fresh quality"* sample the pH was slightly raised from the initial to finished storage from 6.23 to 6.50 whereas in the *"poor quality"* sample pH range was 6.54 -6.61. The fluctuation in pH during storage may be due to fish variation, such as post mortem condition before death. In this experiment samples used were slaughtered immediately after capture in the farm. Hence the rigor is prolonged when the fish was immediately frozen and glycogen could not be converted into lactic acid.[1)] However, rigor might have occurred in fillets that have been stored on ice before being frozen, and it resulted in higher pH value on raw material for fillets that were kept on ice for 3 days before frozen rather than the fresh one. The isoelectric point of fish protein is around 4.5 - 5.5. At this pH the protein are electrically neutral and less hydrophilic than in the ionized state which means that their water binding capacity and solubility are at maximum. If the pH is higher than isoelectric point the solubility will increase. [(7)] It can be explained that both sample have high solubility of protein and water holding capacity since their pH was above their isoelectric point.

If drip loss indicated excess bound water fluid which escaped from originally held within the cell wall is therefore drained out from thawed product under natural condition, water holding capacity indicates the ability of muscle to retain its bulk phase bound water during the application of various stresses such as pressure.

In fish that have been properly stored on ice the value of 5 % to 10% of drip loss can be found during the first week storage [(4)]. However various factors influence the degree of thaw drip. These may be inherent factors in the fish used, pre-freezing treatment, type of freezing process, storage condition and condition during thawing.

As well as drip loss, basically a decreasing trend has been found for both samples during frozen storage with an original value of 85.5% for the *"fresh quality"* and about 84.4% for *"poor quality"*. There were similarities in the amount for both samples in the freezing day proceeds, whereas at the end of the five-week storage the first sample was found to be higher on the value of WHC than the other although there were not statistically different.

ISSN: 1410- 5217
Accredited: 69/Dikti/Kep/2000

3.3. Isoelectric Focussing (IEF)

It can be seen from figure 3 that in raw fish fillet samples at least 12 heavy bands were present and besides, there are many light bands. At the freezing day, in the *"fresh quality"* sample was no remarkable change in the pattern of sarcoplasmic protein. One of the sharp bands that was laid in the middle of the gel disappeared. Some of the light bands also did not correspond exactly on their position. This can be assumed that some amino acid which have pI near neutral might have decreased in the solubility.

The" *fresh quality*" samples showed difference in the band pattern compared to the *"poor quality"* sample after being kept 3 days before freezing. Some of light bands in the cathode position and in the middle of the gel have disappeared on the *"poor quality"*. Samples that have been frozen also showed the decrease of sharpness intensity of the bands. In general the *"fresh quality"* and the *"poor quality"* showed minor changes in pattern. The differences are mainly in the intensity of the bands sharpness. The changes in the sarcoplasmic pattern during the three-day storage in ice is still consistent with research done by Mackie [6] who stated that during iced storage changes in the composition of the sarcoplasmic protein may occur as a consequence of proteolysis by endogenous and bacterial enzymes. In addition, protein can be leached out by melting ice. He studied that the profile remain remarkably constant even to the stage of spoilage when the fish has become unfit for human consumption.

On further storage (at week 4th and 5th) both patterns obtained were nearly the same, but some light bands did not correspond exactly in their position. At finished storage the pattern showed less band sharpness than previous bands. The patterns showed that the effect of frozen storage at 5 weeks time has been shown to have a minimal effects on the sarcoplasmic patterns. This has some relevance to Mackie's [7] research that concluded that he found sarcoplasmic protein more resistant to denaturation during frozen storage compared with the myofibrillar proteins

IV. CONCLUSIONS

From this study it can be concluded that :

1. Storage on ice for 3 days before the fillets were frozen seems not to have significant influence on the solubility of protein but have significant influence on thaw drip loss.
2. Freezing process of fillets rainbow trout have some influence on the protein solubility, thaw drip loss and isoelectric focusing (IEF) pattern of the sarcoplasmic protein.
3. Protein denaturation occurred during freezing process and frozen storage of rainbow trout and this were demonstrated by the loss of protein solubility, thaw drip loss and the changes in the IEF patterns of sarcoplasmic protein.

ACKNOWLEDGMENTS

The author, Eko Nurcahya Dewi, would like to express her gratitude to ODA (Overseas Development Administration) for financial support during her study in UK, and to Diponegoro University for permission to study in UK. Special thanks go to Dr. J. Ahmad for his guidance.

ISSN: 1410- 5217
Accredited: 69/Dikti/Kep/2000

Figure 1 : Changes in Salt Soluble Protein During Frozen Storage (%)

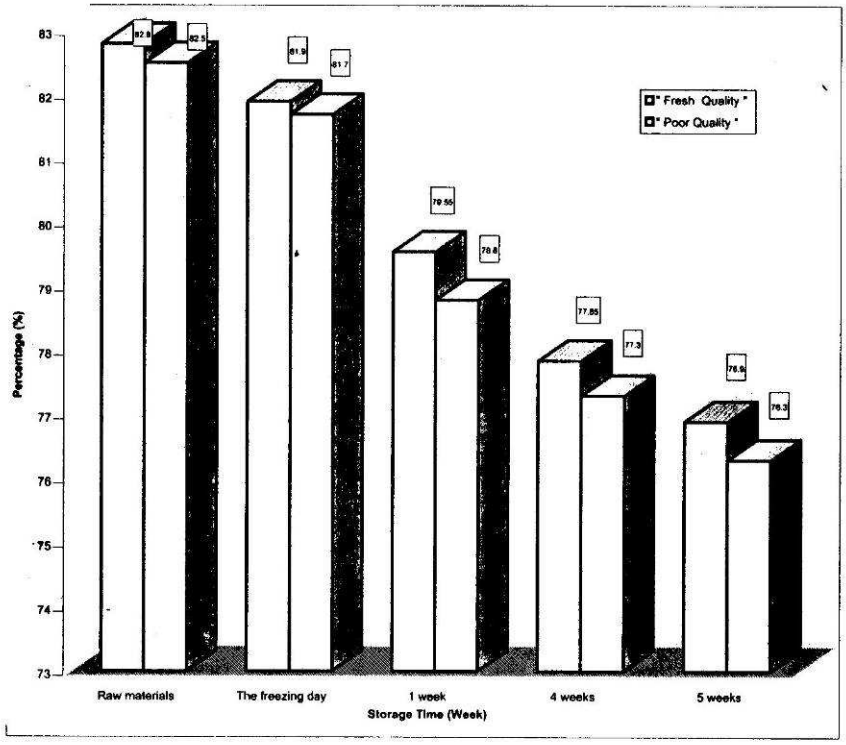
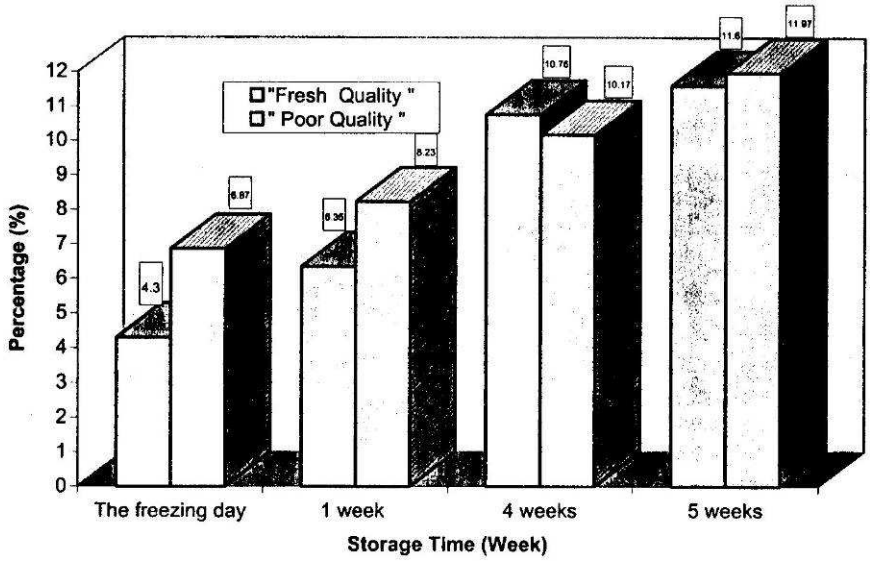


Figure 2. Drip Loss Measurement of Rainbow Trout during Frozen Storage (%)



ISSN: 1410- 5217
Accredited: 69/Dikti/Kep/2000

Figure 3: Isoelectric focusing of Rainbow trout

Note : Left to right, 1-4 "*poor quality*", 5-8 "*Fresh quality*" for raw materials
Left to right, 1-2 "*poor quality*", 3-6 "*Fresh quality*" for the freezing day
Left to right, 1-4 "*poor quality*", 5-8 "*Fresh quality*" for the 1,4 and 5 weeks storage

ISSN: 1410- 5217
Accredited: 69/Dikti/Kep/2000

REFERENCES

1.Conell, J.J. (1980). The effect of freezing and frozen storage on the protein of fish muscle, In : ***Low Temperature Biology of Foodstuffs*** (edited by J.Hawthorn and J.E Rolfe), pp. 337-355, Pergamon Press London.

2.Cowie, W.P and Little, W.T.(1966). The relationship between the toughness of cod stored at 29°C and its muscle protein solubility and pH.,***J.of Fd Technology***, 1, 335-343.

3.Deb,A.K. (1988). Influence of freezing methods and storage temperature on the quality of Norway lobster (*Nephrops norvegicus*), ***M.Phil. Thesis***. Humberside College of Higher Education.

4.Dyer W.J. (1969). Fish proteins with special reference to freezing, In : ***Freezing and Irradiation of Fish***, (edited by R.Kreuser).Fishing News Book.London

5.Haard, Norman F.(1992). Biochemical reaction in fish muscle during frozen storage, In : ***Seafood Science and Technology*** (edited by : Graham Bligh), pp.176-209, Fishing News Book, New York.

6.Mackie, I.M. (1980). A review of some recent application of Electro-phoresis and Iso electric focusing in the identification of species of fish and fish product, In: ***Advances in Fish*** *Science and Technology*, (edited by : J.J. Conell), Fishing News Book. London.

7.Mackie, I.M. (1997). Identifying Species. *In Fish Processing Technology* (edited by G.M.Hall) Blackie Academic and Professional, London

8.Matsumoto,J.J (1980).Chemical deterioration of muscle protein during frozen storage. *In Chemical Deterioration of Protein* (edited by J.R Whitaker and Masao Fujimaki) American Society, Washington DC.

9.Montero, P. and Borderias J. (1992). Influence of myofibrillar protein and collagen aggregation on the texture of frozen Hake muscle, In : Quality Assurance in The Fish Industry, (edited by H.H Huss, M.Jacobsen and J.Liston). Elsevier Science Publisher, Amsterdam.

10.Pharmacia (1986). Phast System Separation Technique File No.100. IEF and Electrophoretic Titration Pharmacia, Sweden

11.Shenouda, S.Y.K. (1980). Theories of protein denaturation during frozen storage of fish flesh, In : Advances in Food Research, 26 (edited by J.Chichester). Academic Press. London.

12.Steel RGD and J.H Torrie. 1970. Principle and Procedure of Statistic. Mc Graw Hill Company. New York

13.Wang, Qiukian.(1990).Quality changes during frozen stored of whole carp. M.Phil ThesisSchool of Food, Humberside College of Higher Education.