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**Heat denaturation of proteins from  
Atlantic Salmon (*Salmo salar*)**



**Master Thesis  
by Sari Savitri**

**Biological Chemistry  
Stavanger University  
2011**

## **Abstract**

Foods preserved by heat treatment have to meet several food safety criteria. These criteria requirements are not easy to meet, as heat causes protein denaturation which is considered to be one of the main reasons for quality changes in fish muscle. On the other hand consumers demand fresh or fresh-like minimally processed foods but there is a requirement also to consider the microbial safety and shelf life are increasing.

Denaturation of proteins in Atlantic salmon was studied by differential scanning calorimetry (DSC) at a constant heating rate of 5 °C/min, for five different heating times with the range of temperatures of 26-72 °C and this gave four proteins denaturation peaks. The denaturation enthalpy is decreasing with increasing temperature and the time required for denaturation of all proteins is decreasing with increasing processing temperature.

Cook loss and loss in water holding capacity (WHC) was explained by denaturation of the proteins and it was shown that the protein denaturation occur in a lower temperature range (30-60 °C) when salmon is heated. At 50 °C, cook loss is maximized while at the same time WHC is minimized and myosin protein of salmon is in the process of denaturation.

## Acknowledgment

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# 1. Introduction

## 1.1. Background

Norwegian Salmon is the best known fish product from Norway. Norwegian Salmon is known scientifically as *Salmo salar* (Atlantic salmon) which has good nutritional value (high amount plenty of omega-3 fatty acids). The market for chilled ready meals has grown strongly in recent years with good quality and contains no additives. Thermal processing or pasteurization is commonly used in the food industry to extend the shelf life. Pasteurization is a mild or moderate heat treatment, usually performed on fishery products after the product is placed in the hermetically sealed finished product container [1].

During thermal processing operation, protein denaturation is considered to be one of the main reasons for quality changes in fish muscle. The high temperature may cause severe quality deterioration, such as degradation in color and texture, nutrient loss, cook loss (weight loss) and area shrinkage, rendering the products less attractive to most consumers. In particular, denaturation of heme proteins and oxidation of carotenoid (e.g. astaxhantin) pigments darken the products [2]. The denaturation of the proteins also leads to reduced water holding capacity and shrunken muscle fibers, subsequently leading to a harder and more compact tissue texture [3].

Heating causes progressive shrinkage and disintegration of the myofibril; and water, soluble proteins, and fats are expelled from the tissue [4-6]. The expelled water soluble proteins may after cooling form a curd on the fish surface, causing a yellow or white appearance.

Heating improves digestibility of some nutrients but also causes loss of others [7]. To avoid non-intended changes it is desired to use moderate temperatures and short processing times, but microbial safety and shelf life must also be considered.

## ***1.2. Scope of the study***

The objective of this study is to provide information which can be used to improve quality of Atlantic salmon (*Salmo salar*).

To achieve the objective of this study, the following tasks were included for this master thesis project :

- Develop an understanding of how to quantify the kinetics of protein denaturation during thermal processing of farmed Atlantic salmon using differential scanning calorimetry (DSC).
- Compare the protein denaturation to cook loss and loss of water holding capacity (WHC).
- Estimate optimization of mild thermal processing of vacuum packed convenience fish products.

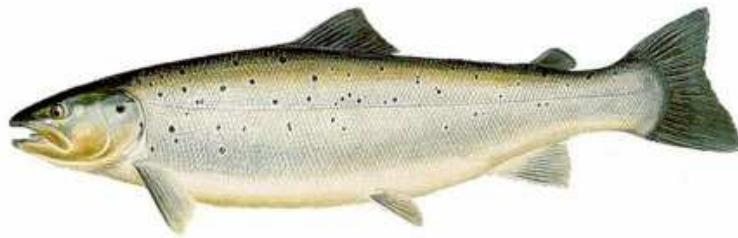
### ***1.3. Report Outline***

The next chapter of this report is dedicated to the description of theories relevant to the background of this study including some concepts in protein denaturation of Atlantic salmon and description of thermal processing. The methodology of Atlantic salmon using differential scanning calorimetry (DSC) and comparison of protein denaturation to cook loss and loss of water holding capacity (WHC) is described in chapter 3. The results and discussion are presented in chapter 4 while the conclusions are shown in chapter 5.

## 2. Theoretical Background

### 2.1. *Atlantic Salmon (Salmo salar)*

Atlantic salmon, also known scientifically as *Salmo salar* commonly farmed in large scale is a species of fish in the family Salmonidae. Numerous species of salmon are found in the wild, and a several are cultured. The Atlantic salmon (Figure 1), though, is the most important salmon species produced by aquaculture. Morphologically, mature Atlantic salmon are compressed laterally and have a streamlined body. Color and shape of salmon, in general, will change at different life stages [8].



**Figure 1. The Atlantic salmon (*Salmo salar*).**

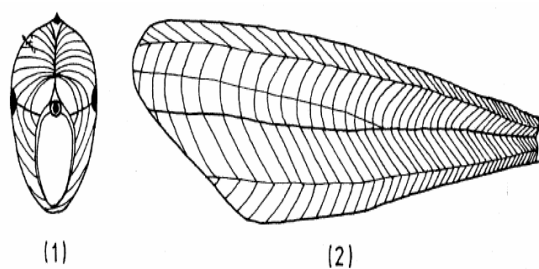
**Source : Atlantic Salmon Federation**

Salmon spawns in fresh water and develops through several stages before becoming a smolt, the stage at which it migrates to the sea to feed. The big changes of habitat requires physiological, morphological and behavioural changes to prepare atlantic salmon for its new environment. These changes are called the parr-smolt transformation or smoltification, and pre-adapt the salmon for survival and growth in the marine environment. The development of hypo-osmotic regulatory ability plays an important part in facilitating the transition from rivers to the sea.

The morphology of Atlantic salmon is almost similar to brown trout (*Salmo trutta*) because they often occur in the same areas. Juvenile Atlantic salmon has a small mouth, pointed head, long pectoral fins, narrow tail stalk and deeply forked, sharp-ended tail, whereas juvenile brown trout is more robust and has a blunter head and tail. The differences between them in growth and ontogeny, and Atlantic salmon has a more fusiform body and fewer spots than brown trout. Hybridization between the two species may occur, often as a consequence of human activities, such as stocking and translocation [9]. The connective tissue forms (Figure 2) is a supporting network through the whole fish muscle.



Source : <http://www.callandermcdowell.co.uk>



**Figure 2. The metameric structure of fish muscles. The pattern of lines on the cross (1) and longitudinal (2) section represents the arrangement of sheets of connective tissue in the muscles [10]**

## **2.2. *Main components in Atlantic Salmon***

### **2.2.1. Water**

The main constituent of fish flesh is water, which usually accounts for about 80% of the weight of a fresh white fish fillet. Whereas the average water content of the flesh of fatty fish is about 70%. The water is bound to approximately 95% within the muscle cells. “Binding” means that the water is restricted in its molecular movements; it is immobilized by charged or hydrophilic side chains of amino acids and capillary forces. Approximately 80% water is immobilized by the myofibrillar and cytoskeletal proteins [11].

In the sarcoplasm with its soluble (sarcoplasmic) proteins between the fibres, approximately 15% of the water is partially immobilized by the protein surfaces, water-solute, and water-water interactions. A part of this water is “free”, it means unbound by protein side chains, ions, or capillary forces. Nevertheless, this water is inhibited from free flowing out of the cell by the cellular and sub cellular lipid bilayer membranes [11]. The approximate moisture content of fresh salmon is usually between 66% and 72% [12].

### **2.2.2. Protein**

All proteins sources are chains of chemical units linked together to make one long molecule of which there are about twenty types. These types are called amino acids, and certain of them are essential in the human diet for the maintenance of good health. Two essential amino acids called lysine and methionine are generally found in high concentrations in fish proteins. Thus fish and cereal protein can supplement each other in the diet. Fish protein provides a good combination of amino acids which is highly suited to man’s

nutritional requirements and compares favourably with that provided by meat, milk and eggs [13].

After water, protein is the most important component of fish fillet and this justifies the relevance of studying their denaturation, particularly that of myofibrillar proteins. Salmon muscle contains approximately 20-22% protein [14]. Proteins from skeletal muscle of Atlantic salmon can be separated into three group, based on solubility properties (Table 1) [14, 15].

**Table 1. Distribution of Protein fractions in fish Muscle [15]**

Source	Sarcoplasmic Protein (%)	Myofibril Protein (%)	Stroma Protein (%)
Fish, general	10-25	70-90	3-10
Atlantic Cod	21	76	3
Carp	24	71	5
Flounder	21	76	3
Beef	16-28	39-68	16-28
Atlantic salmon	30	75-80	10

In functional and technological terms, the myofibrillar proteins are the more important proteins, constituting 50% of the muscle proteins. Among them, the contractile proteins, myosin and actin, correspond to 75-80% of myofibrillar proteins [16-19].

### ***2.2.2.1. Sarcoplasmic proteins***

Sarcoplasmic proteins, referred to as 'myogen', are soluble in the muscle sarcoplasm. They include a large number of proteins such as myoglobin, enzymes and other albumins. Sarcoplasmic proteins may be used to fingerprint fish species using electrophoretic and isoelectric focusing technique [20].

The content of sarcoplasmic proteins is generally higher in pelagic fish species as compared with demersal fish. Dark muscles of some species contain less sarcoplasmic proteins than their white muscle counterpart [21]. The sarcoplasmic proteins, mainly albumins, account for approximately 30% of the total muscle proteins. A large proportion of sarcoplasmic proteins may be composed of haemoproteins [22].

### ***2.2.2.2. Myofibrillar proteins***

The largest amount of myofibrillar proteins present in muscle of aquatic species are myosin, actin, tropomyosin and troponins C, I and T [21].

#### ***2.2.2.2.1. Myosin***

Myosin is the most abundant myofibrillar fraction of fish muscles and contributes 50 to 60% to its total protein amount (Figure 3). The myosin molecule consists of two heavy chains, associated non-covalently with two pairs of light chains.



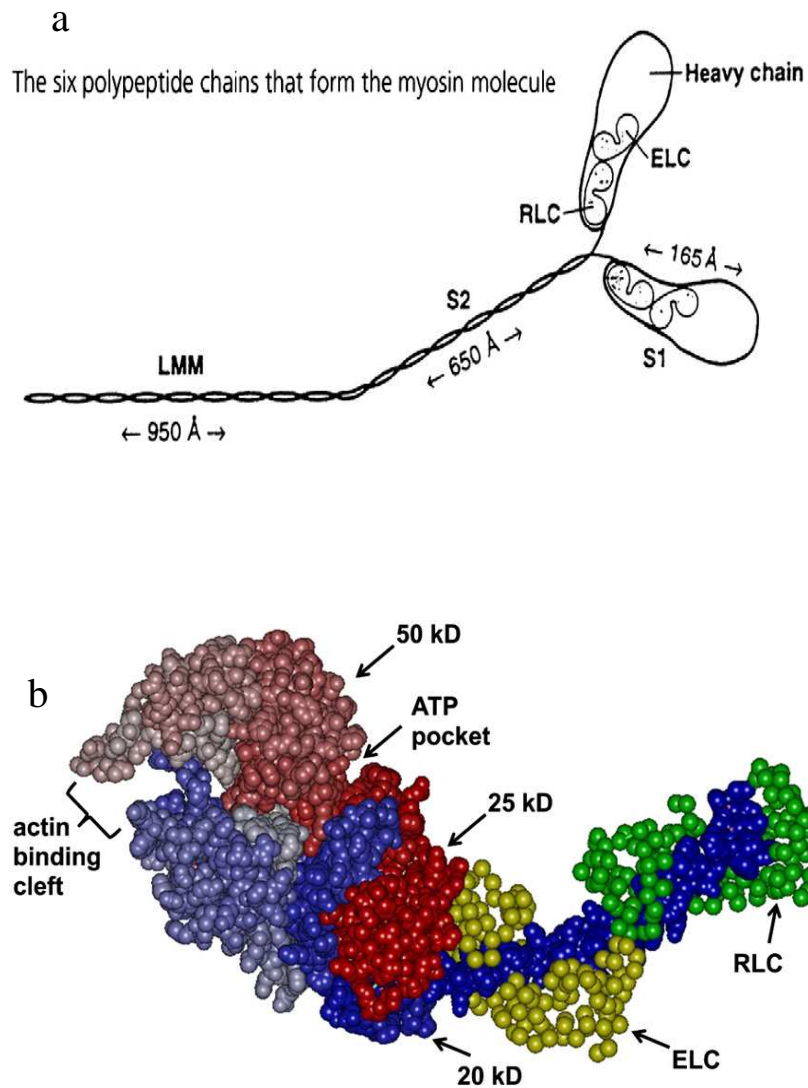


Figure 3. a. Myosin molecule [23], b. S1 unit of a myosin molecule [24]

In contrast to most proteins of mammalian origin, the loss of ATPase activity occurs at a faster rate in fish muscle [25, 26]. Myosin consists of two heavy chains and four light chains. Each of the two S-1s contains a

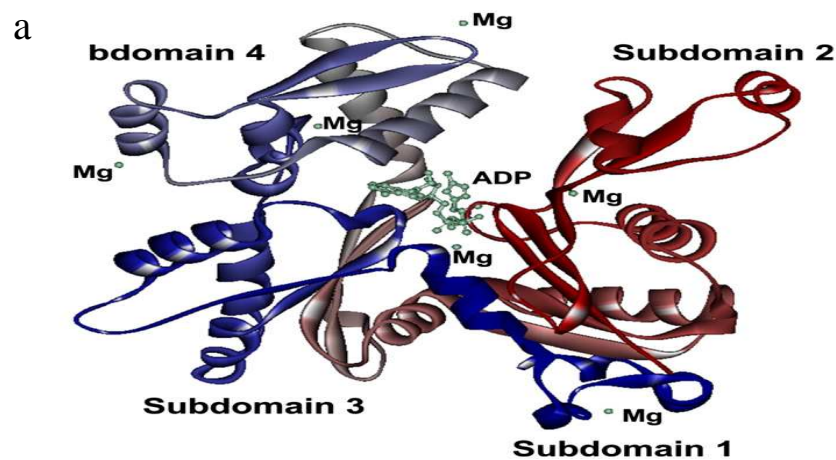
party of heavy chain and two light chains. Together, they comprise the head of myosin, which has ATPase activity and actin-binding ability. The rod, which forms an  $\alpha$ -helical coiled-coil structure in heavy chains, has filament-forming ability [27].

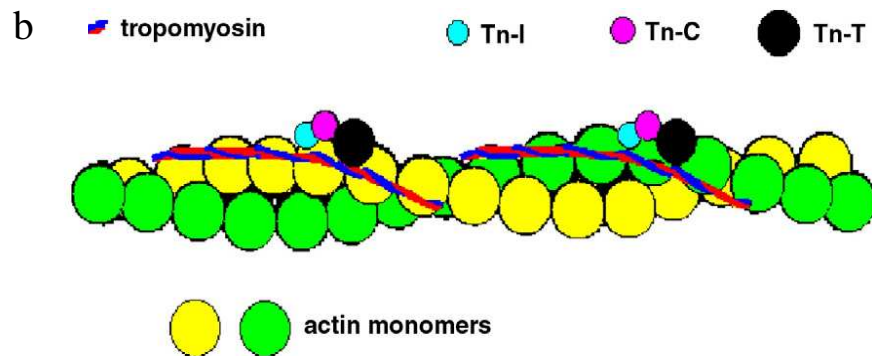
#### 2.2.2.2.2. Paramyosin

Paramyosin is a protein found in invertebrates and is present in quantities ranging from 0.1 to 10 times that of myosin [28]. Paramyosin molecules constitute a core of the thick myofibrils of invertebrate muscles which is covered by a layer of myosin. Paramyosin is known to maintain the tension in the muscle tissues.

#### 2.2.2.2.3. Actin

Actin, the second most abundant myofibrillar protein, constitutes approximately 20% of the total content of these proteins in fish muscles. G-actin is the monomeric form of the molecule and in the presence of neutral salts polymerizes to F-actin (Figure 4).





**Figure 4.a. G-actin subdomains [29], b. Actin filament (F-actin)**  
 (<http://www.cryst.bbk.ac.uk/PPS2/course/section11/assembli.html>)

Similar to myosin, actin shows  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  activated ATPase characteristics. Other constituents of myofibrillar proteins include tropomyosin and troponins which account for 10% of the total amount. Several isoforms of tropomyosin have been described for different sources of muscle foods. Their number depends on the species from which the muscle is originated, however, sequence of the homogenous isoforms from different species are nearly identical. Similarly troponins exist in several isoforms [30].

### 2.2.2.3. Stroma proteins

The residue after extraction of sarcoplasmic and myofibrillar proteins is known as stroma. It is composed of collagen and elastin from the connective tissues. Stroma is soluble in dilute solutions of HCl or NaOH and contributes up to 10% of the crude muscle proteins. The collagen

content in muscle depends on the species, feeding regime and state of maturity of the fish. In general, fish muscle contain approximately 0.2 to 2.2% collagen [31].

### **2.2.3. Fat**

In animals in general, increasing dietary fat tends to increase whole body fat [32]. In fish, the location of the deposition of the additional fat appears to differ depending on the species and fish size [33, 34]. Einen et al. showed that fat deposition, when a given diet was fed, is fish size dependent [35]. Hillestad et al. reported that ration affected fillet fat in Atlantic salmon but that increasing dietary fat affected visceral but not fillet fat [36]. Salmon muscle contains approximately 15-18% fat [14].

The fat content and polyunsaturated fatty acid (PUFA) composition in salmon are important quality parameters [37], which affect the taste, texture and colour of the fillet [38] and are influenced by environmental factors such as diet, season, ranching condition, temperature and biological differences such as stage of maturity, age, sex and size [39-44].

## **2.3. *Denaturation***

### **2.3.1. Definition**

Denaturation is understood as being any modification of its structure (secondary, tertiary or quaternary) without necessarily breaking the peptidic chains in their primary structure. The denaturation may be produced by physical means (heat and cold, mechanical treatment, irradiation, etc.) and/or

chemical agents (acids, alkalis, salts, metals, organic solvents, etc.) and should be considered as an irreversible endothermic transition [45].

### **2.3.2. Thermal denaturation**

As the name implies, thermal denaturation is brought about by increasing the temperature. It is also the oldest mode since it has become part of man's preparation of food. [46]. Heating is one of the most important treatments of food processing. Heat denaturation or thermal denaturation and aggregation of proteins are therefore the most typical events in food processing. The heat denaturation involves a cooperative or non cooperative transition of a protein from its folded to its unfolded state.

It is related to some structural disorganization of the three-dimensional structure of native molecules. The unfolding changes the interaction of the protein with aqueous medium and induced aggregation governs the structure, flavour, texture, and other qualities of food. It also contributes to the nutritional qualities and physical stability of the foods during storage .

The heat effects of denaturation and aggregation of proteins are usually small and have the opposite sign, namely, heat absorption (endothermic) and release (exothermic), respectively [47].

## ***2.4. Analysis for heat denaturation of protein***

### **2.4.1. Differential Scanning Calorimetry**

Differential scanning calorimetry (DSC) is a powerful technique to characterize the energetics and mechanisms of temperature-induced conformational changes of biological macromolecules [48]. DSC is also a very useful means of studying the thermal properties of muscle proteins (Figure 5) [49].

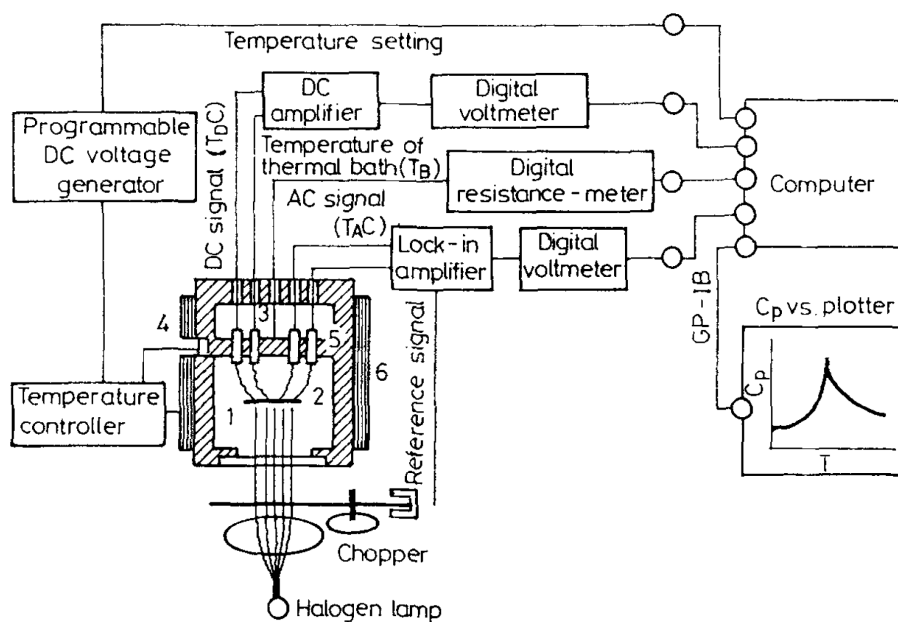


**Figure 5. The DSC machine and Crucible sealing press (Mettler Toledo)**

Modern differential scanning calorimeters are designed to determine the enthalpies of these processes by measuring the differential heat flow required to maintain a sample of the material and an inert reference at the same temperature. This temperature is usually programmed to scan a temperature range by increasing linearly at a predetermined rate. The apparatus can also be used to measure heat capacity, thermal emissivity and

the purity of solid samples. In addition, it can be used to yield phase diagram information and to provide kinetic data [50].

The DSC thermograms obtained as a result of thermal denaturation of muscle proteins during measurement are usually interpreted in terms of peak temperatures and corresponding enthalpy changes for the different subfragments with myosin and actin as most dominant in determination of the enthalpy changes (Figure 6) [51, 52].



**Figure 6. Schematic of the principle of calorimeter. 1 Specimen, 2 Thermocouple, 3 P1 Thermometer for temperature measurement, 4 P1 Thermometer for temperature control, 5 Thermal bath, 6 Heater [53]**

### 2.4.2. Water holding capacity and cook loss

Water holding capacity (WHC) is the ability of muscle to resist water loss, and it is very important from both commercial and consumer acceptance points of view. The water is found in both free and bound forms. The water-binding ability of myosin has been related to polar amino acids, especially the negatively charged aspartic acid and glutamic acid residues [54].

WHC equipment used in this thesis project (Figure 7) has even been used as measure for quality [55] and for characterising protein denaturation [56]. Several methods can be used for measuring WHC, the first one reported was developed by Childs and Baldelli in 1934 [57].



**Figure 7. Benchtop Centrifuge ROTINA 420 R Without rotor.**

**Source :** <http://www.hettichlab.com>



### **3. Material and Methods**

#### **3.1. Material**

Farmed Atlantic salmon (*Salmo salar*) were obtained from a fish farm (Bremnes Seashore AS, Bremnes Norway), with a fat content of 14%, protein content of 19,9 gr/100gr and water 67 gr/100gr. The fish were kept on ice during transportation and 4 days storage. The 40 loins were divided into 30 loins for ground frozen and 10 loins for fresh sample (5 for ground and 5 for whole sample).

#### **3.2. Methods**

##### **3.2.1. Sample preparation**

The 30 loins were coarsely ground in a grinder (Food Processor TC RF Forniture Sirman) and mixed manually. The mixture was packaged in plastic food beakers (510 x 360 x 392 mm, Polimoon AS, Kristiansand Norway) and sealed under vacuum (vacuum machine Webomatic) before freezing. The beakers were stored in a freezer at  $-80\pm 1$  °C until further use, while the fresh samples were analyzed on the fifth day after transportation. After 1 month of storage at  $-80$  °C, the material was used over a period of 3 months.

### **3.2.2. DSC Analysis**

For differential scanning calorimetry the protocol of Skipnes et al. [58] was used except instrument, pan (medium pressure crucibles) , heating rate and the amount of water in reference pan (75% of salmon samples). DSC was performed at heating rate of 5 °C/min over the range from 2 °C to 110 °C on Mettler Toledo DSC-1/200. Pans containing water were used as reference and 2 min equilibration at 0 °C was done before each run. Samples were measured in triplicate. The residual denaturation enthalpy ( $\Delta H$ ), was defined as the area under the denaturation peak using a straight base line as shown in Figure 8.

### **3.2.3. Screening Test**

Protein denaturation was screened by heating in the water bath (Water Bath Grant GR150) at temperatures 26, 40, 46, 54, 64, 68, 70, 71 and 72 °C every 4, 5, 6, 8 and 10 minute. All samples were cooled in ice water immediately after heat treatment and scanning by DSC and scanned once again to investigate if the denaturation was irreversible and to establish a base line.

### **3.2.4. Water Holding Capacity and Cook Loss**

The methods of Water holding capacity and cook loss used in this experiments was developed by Skipnes et al [56].

#### ***3.2.4.1. Preparation of the fish sample***

Raw fish sample was cutted into pieces or cut samples so that they have a diameter of 31 mm. Weight out approx. 5 g of muscle tissue with a total height of approx. 6 mm. The muscle tissue should be homogeneous and free of connective tissue.

#### ***3.2.4.2. Preparation of the samples cups***

The sample cups should be kept on ice until used. Before filling with the sample cup, the top lid is screwed onto the sample cup. The sample cup was weighed with the top lid (weight g1).

#### ***3.2.4.3. Filling the cup with the sample***

The sample cups (the top lid now forms the bottom) was inverted, and prepared sample material was filled into the cup and weighed once again (weight g2). The filter was screwed in until it makes contact with the sample. The bottom lid was screwed as far as it will go (until it is in contact with the filter) and weigh the sample cups again (weight g3).

#### ***3.2.4.4. Heating of the sample***

The sample cup was placed for a specific period of time in a water bath preheated to the required temperature (e.g., 10 minutes at 80 °C). Note: Ensure that no air bubbles form on the sample cup in the water bath as they would prevent uniform heat exchange.

#### ***3.2.4.5. Removal of the exudate***

The sample cup was removed from the water bath and allow it to cool in ice water at 0 – 1.8 °C and then dry the outside of the cup inside wall the sample cup by blowing with pressurised air. The remainder of the exudate from the bottom lid and the inside wall of the sample cup was wiped. Ensure that not to touch the filter during the drying procedure. The sample in the sample cups was weighed together with the dried lid (weight g4).

#### ***3.2.4.6. Centrifugation***

The bottom lid was screwed back on, this time in its base position and centrifuge for 15 minutes at 1800 min<sup>-1</sup> and 4 °C.

#### ***3.2.4.7. Removal of the exudate***

After centrifugation, the bottom lid was unscrew in which the liquid that was lost during centrifugation has collected, and blowing with pressurised air. The remainder of the exudate from the bottom lid and the inside wall of the sample cup was wiped. When drying the inside of the cup ensure again that the filter is

not touched. The sample in the sample cup was weighed together with the dried lid.

Step 3.b.4.d and 3.b.4.e are omitted for the determination of the WHC of raw samples. The samples were measured in quadruplicate.

### **3.2.5. Water content and cook loss**

Cook loss was measured as weight of sample before and after draining for 30 s. The surface of the cup lid was dried with a cloth. The dry matter content of fish muscle was determined gravimetrically after drying at 105 °C for 16 h in drying ovens termaks (NKML 23, 1991). The dry matter content of cook loss was determined the same way as for the fish [56].

## **3.3. Calculations**

### **3.3.1. Determination of cook loss**

The cook loss is the difference between the  $g_3$  (part 3.2.4.3) value and the  $g_4$  (part 3.2.4.5) value . The weight of the sample is given by  $g_2$  (part 3.2.4.3) minus  $g_1$  (part 3.2.4.2). The percentage weight loss can therefore be calculated from these values.

### 3.3.2. Determination of WHC of raw samples

The WHC of raw samples is calculated as the ratio of the water remaining after centrifugation to the initial water content of the sample, using the following formula :

$$WHC = \frac{W_o - \Delta W}{W_o} \times 100\%$$

$$W_o = \frac{V_o}{V_o + D_o} \times 100\%$$

$$\Delta W = \frac{\Delta V_o}{V_o + D_o} \times 100\%$$

$V_0$  = Initial water content of the sample

$\Delta V_0$  = Difference in water content of the sample, before and after centrifugation

$D_0$  = Initial dry mass of the sample. The dry mass can be determined gravimetrically, for instance by drying it for 16 hours at 105 °C.

### 3.3.3. Determination of WHC of heated samples

The fish sample loses liquid when it is cooked. The liquid comprises water, dissolved protein, ash, salt and fat. The remaining dry material D1 is therefore somewhat less than the initial dry mass D0. The sample will lose not just water but also additional dry mass during the centrifugation procedure. As a result, the remaining dry mass D2 after heating and after centrifugation will be significantly lower than D0.

This method calculates the water holding capacity on the basis of water content of the raw sample and takes into account the weight loss on cooking as the total loss WHCTot.

$$WHCTot = \frac{W_o - \Delta WTot}{W_o} \times 100\%$$

Where

$$WTot = \frac{\Delta V1 + C1}{V1 + C1} \times 100\%$$

V0 = Initial water content of the sample

D0 = Initial dry mass of the sample

V1 = Water loss from the heated sample through centrifugation

C1 = Weight loss on cooking of the sample

This leads to the following new definition of water holding capacity:

$$WHCT_{ot} = \frac{VO - (\Delta V1 - C1)}{VO} \times 100\%$$

This calculation method describes the change in water holding capacity from raw samples to cooked samples. The percentage of dry mass in the exudate is also taken into consideration in the calculation.

### ***3.4. Statistical Analysis***

The data for quality attributes of raw and heated samples were compared using analysis of variance (ANOVA). Statistical significance was set at  $P < 0.050$ .

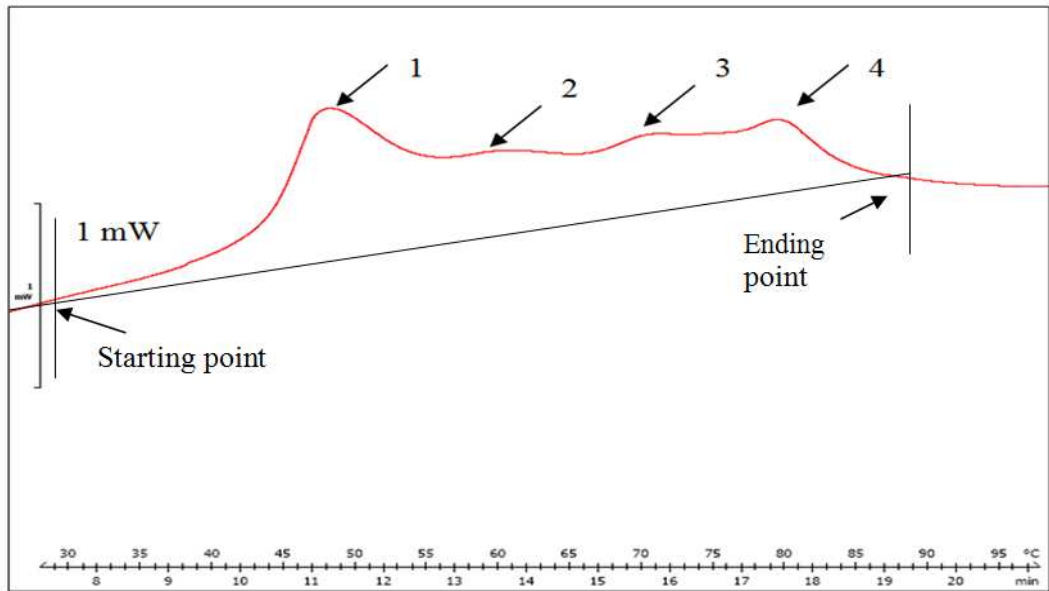


## **4. Result and discussion**

To meet the needs of premium quality of minimally processed convenience salmon, it is important to prevent undesired changes (i.e. protein denaturation, flaking, tenderness and juiciness). To avoid the changes, the measurement results of protein denaturation during thermal processing of farmed Atlantic salmon using DSC have been done. Further, cook loss and loss of WHC has been quantified and explained by protein denaturation.

### ***4.1. Differential scanning calorimetry***

The DSC curve for untreated salmon muscle with four peaks as shown in Figure 8. According to the literature, denaturation of myosin is expected to occur at temperature from 44 °C and 54 °C with any possibilities other protein like collagen will denature in this region [49] as well. The last peak which occur around 73 °C – 74 °C is due to actin denaturation, while others peaks between myosin and actin are due to sarcoplasmic denaturation [49, 51]. This corresponds well with peak in the thermogram of figures.

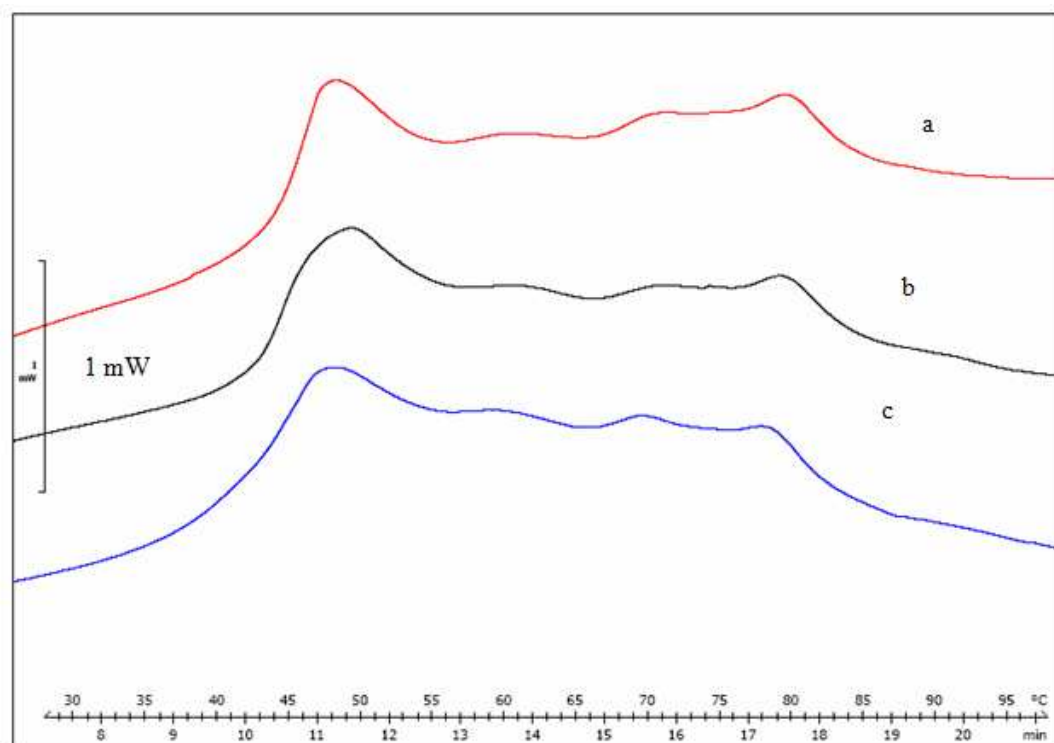


**Figure 8. DSC thermogram of untreated Atlantic salmon muscle (whole) obtained at a scanning rate of 5 °C/min**

The temperature range of endothermic peaks correspond with the temperature range in which the  $\alpha$ -helix unfolded greatly, although the number of denaturation peaks are different between salmon and with other fish species [27]. The type of DSC machine and heating rate also cause differences in the thermograms. Ofstad et.al [59] who analyzed fresh and frozen Atlantic salmon (*Salmo salar*) using a Setaram micro Calorimeter with 1 °C/min heating rate found 5 peaks.

Figure 9 shows DSC thermograms for the fresh whole (a), fresh ground (b) and frozen ground (c) samples. The peak positions are listed in Table 2 (together with the literature data). The peak maximum temperature,  $T_m$  of myosin from whole fresh salmon is higher (2.1 °C) than the ground fresh salmon and 0.9 °C higher than the ground frozen salmon but there is no significant differences between those three samples myosin peaks ( $P > 0.050$ ). Valeria et. al [60] also found no significant

differences between ground fresh and ground frozen of Tm myosin peak of Sea salmon (*Pseudoperca semifasciata*). It has also been confirmed by literature that freezing followed by immediate thawing has little effect on the characteristic thermal transitions of fish muscle [49].



**Figure 9. Comparison DSC-thermogram between fresh whole (a/red line), fresh ground (b/black line) and frozen ground samples (c/blue line)**

**Table 2. Average value and standard deviation for peak maximum temperature ( $T_m$ ) for untreated salmon.**

Material	Apparatus/ Heating rate	Peak 1 (°C)	Peak 2 (°C)	Peak 3 (°C)	Peak 4 (°C)	References
Atlantic salmon ( <i>Salmo salar</i> )	DSC-1 5 °C/Min					Present work (n=4)
Whole Fresh		48,9±1,1	60,6±0,3	70,3±0,2	79,5±0,2	
Ground Fresh		46,8±0,4	59,3±0,2	68,6±0,4	77,3±0,2	
Ground Frozen		48,0±0,2	59,4±0,2	69,1±0,1	78,2±0,1	
Atlantic salmon ( <i>Salmo salar</i> )	Setaram Micro 1 °C/min					Ofstad et al. (1996)
Ground frozen		44-45		65	76	
Sea Salmon ( <i>Pseudoperca semifasciata</i> )	Polymer Laboratories calorimeter 10 °C/Min					Valeria et.al. (2010)
Ground Fresh		50,7±0,1			76,8±0,2	
Ground Frozen		49,8±0,7			77,4±0,6	
Atlantic Cod ( <i>Gadus morhua</i> )	DSC-7 10 °C/min					Skipnes et.al (2008)
ground frozen		44.1±0.2	57.3±0.1	69.5±0.3	76.1±0.7	

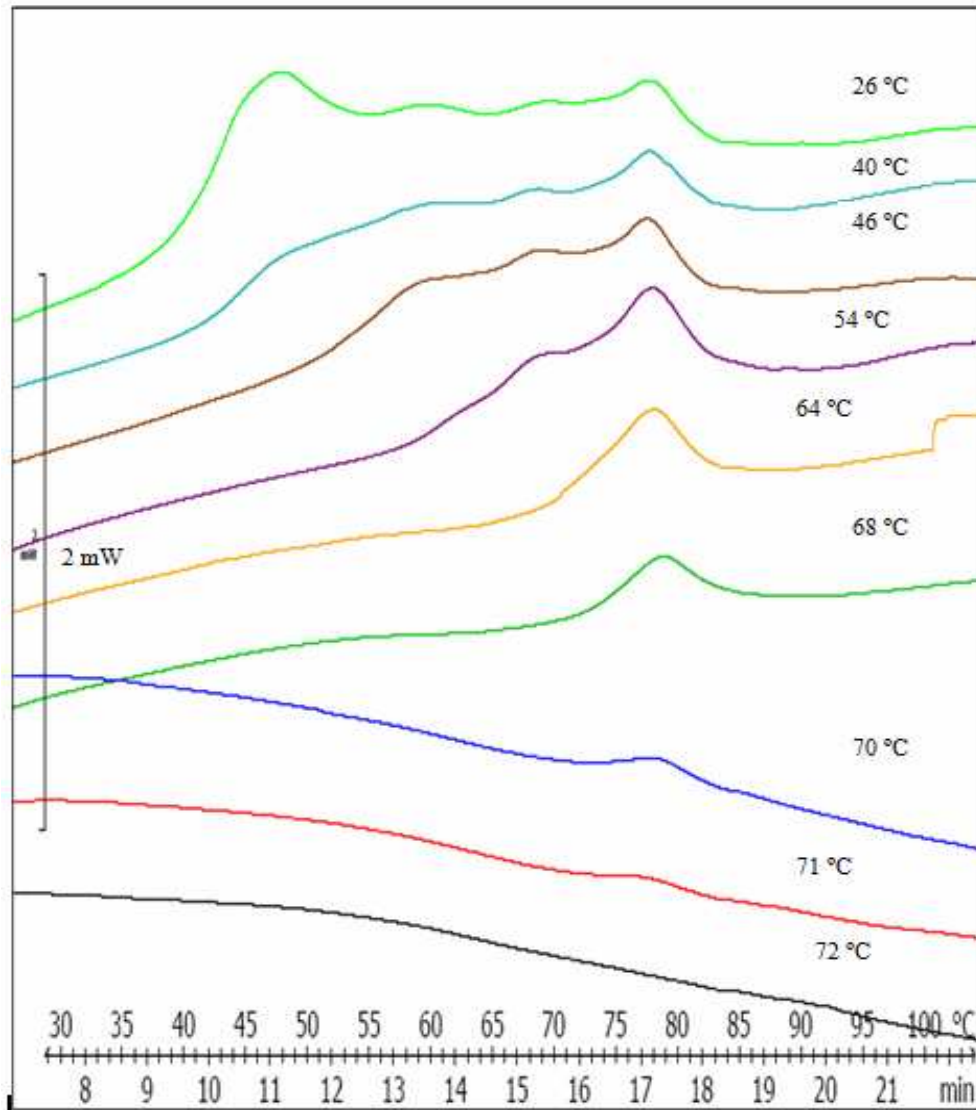
The whole fresh salmon also have a higher heat absorption of a sarcoplasmic and actin proteins among three samples. The  $T_m$  for each peak in the present study was quite high compared to same fish species measured by other apparatus and different heating rate [59], but it is quite similar compared to other species even it

was measured by different apparatus and different heating rate [60]. Atlantic salmon (present study) have higher  $T_m$  for each peaks compared to Atlantic cod except for peak 3, the differences between cod and salmon also found in Ofstad et al. [59] that salmon had higher  $T_m$  than cod. This fact shows that salmon muscle is more heat stable than cod.

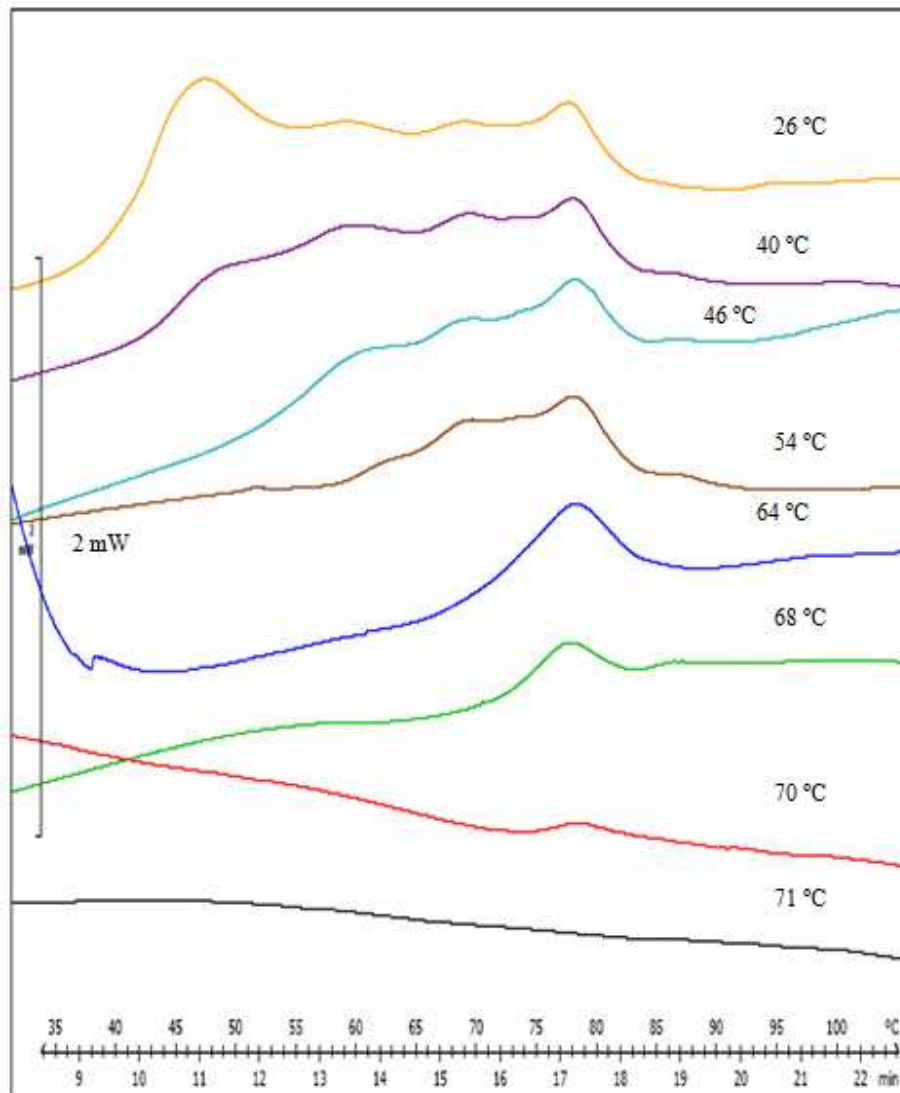
The differences were also found in denaturation enthalpy Table 3 for each peak. Peak 1 and peak 2 for whole fresh and ground frozen samples was found significantly different ( $P < 0.050$ ). The differences between whole and ground fresh was found in peak 2 and 3. Whole and ground fresh salmon was found significant different in peak 4 and both also different statistically with ground frozen salmon ( $P < 0.050$ ).

**Table 3. Mean  $\pm$  SD of denaturation enthalpy for each peak of Atlantic salmon (*Salmo salar*) (n=5)**

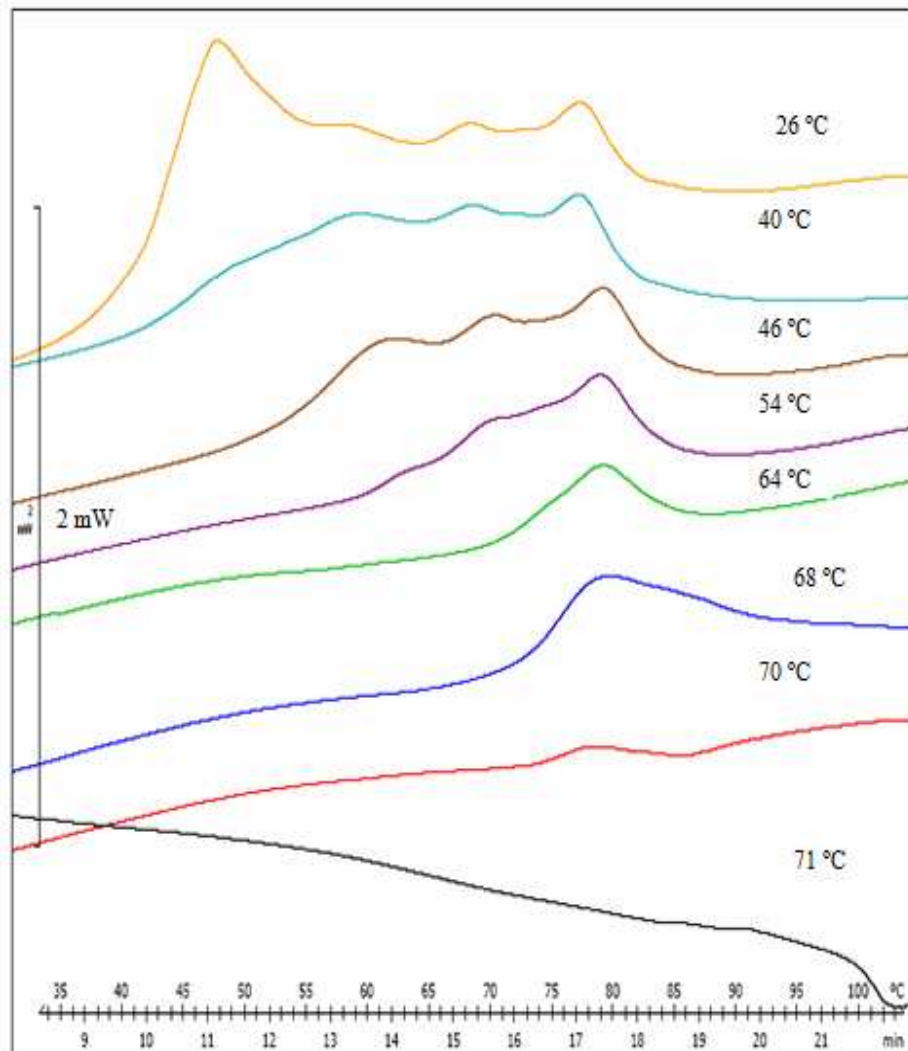
Type of salmon	Denaturation Enthalpy (J/g)			
	Peak 1	Peak 2	Peak 3	Peak 4
Fresh whole	0.87 $\pm$ 0.001	0.04 $\pm$ 0.001	0.05 $\pm$ 0.001	0.11 $\pm$ 0.001
Ground fresh	0.86 $\pm$ 0.020	0.02 $\pm$ 0.001	0.03 $\pm$ 0.001	0.09 $\pm$ 0.001
Ground frozen	0.83 $\pm$ 0.001	0.03 $\pm$ 0.001	0.04 $\pm$ 0.001	0.08 $\pm$ 0.001



**Figure 10. DSC thermograms for Atlantic salmon (*Salmo salar*) for 4 minute pre-heating time obtained at a scanning rate of 5 °C/min**

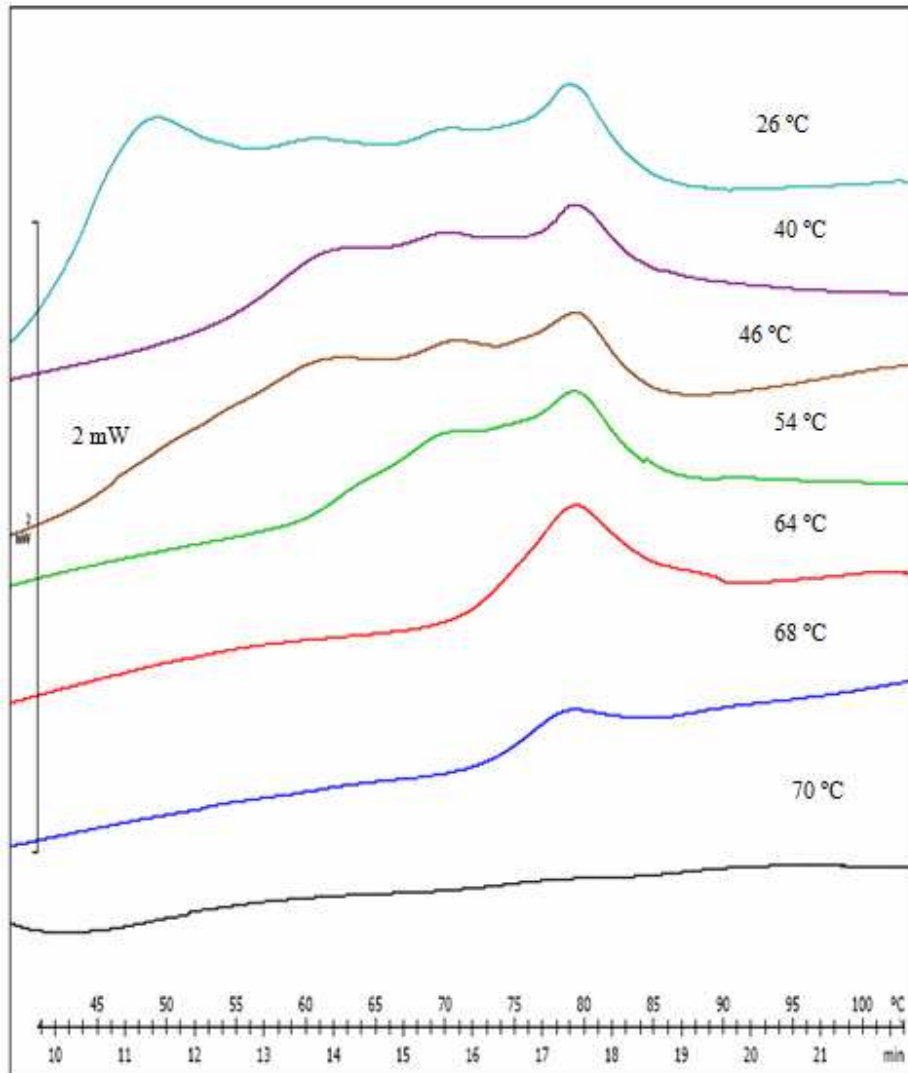


**Figure 11. DSC thermograms for Atlantic salmon (*Salmo salar*) for 5 minute pre-heating time obtained at a scanning rate of 5 °C/min**

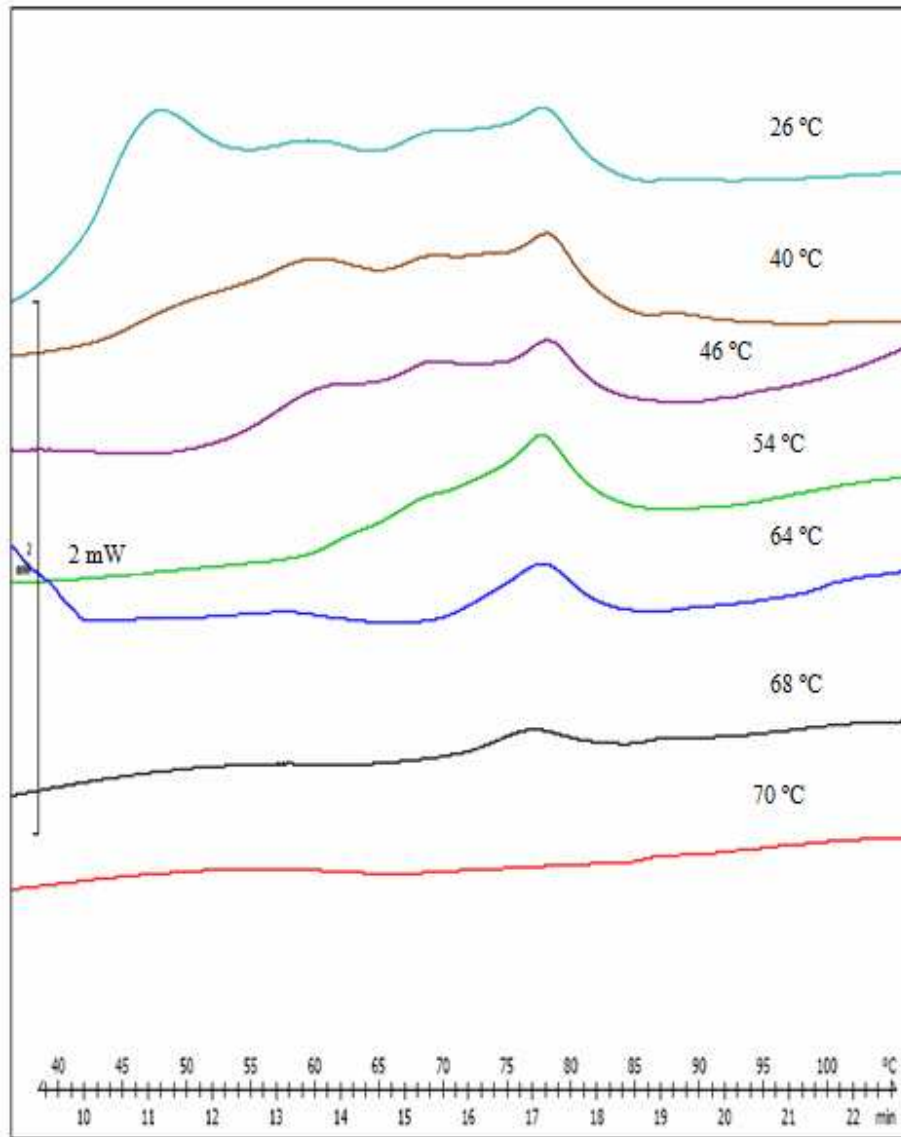


**Figure 12. DSC thermograms for Atlantic salmon (*Salmo salar*) for 6 minute pre-heating time obtained at a scanning rate of 5 °C/min**





**Figure 13. DSC thermograms for Atlantic salmon (*Salmo salar*) for 8 minute pre-heating time obtained at a scanning rate of 5 °C/min**

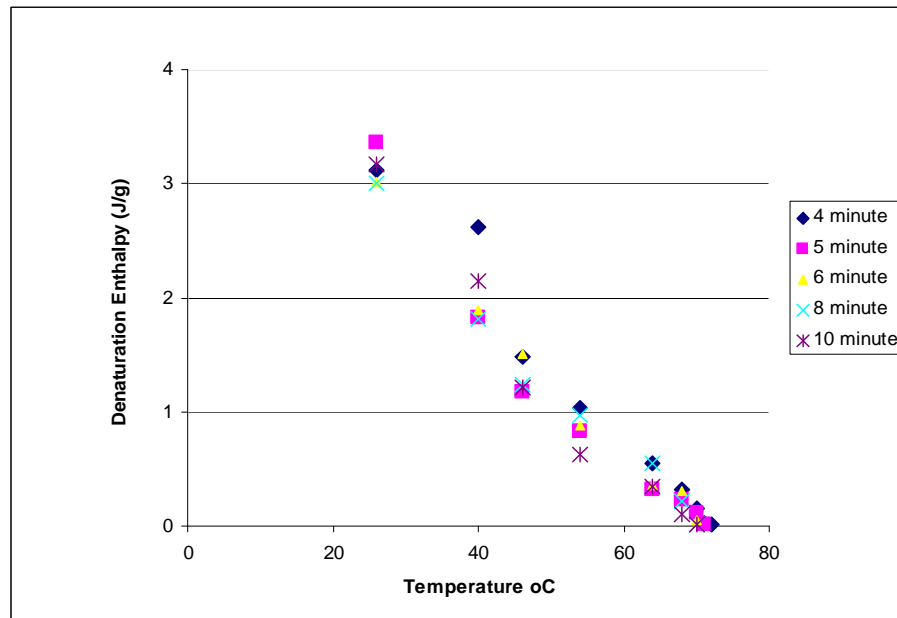


**Figure 14. DSC thermograms for Atlantic salmon (*Salmo salar*) for 10 minute pre-heating time obtained at a scanning rate of 5 °C/min**

The thermograms in Figure 10 to Figure 14 are obtained by DSC of Atlantic salmon at pre-heating time 4, 5, 6, 8 and 10 minute respectively. The peak heights of the endothermic curve as well as total denaturation enthalpy of all peaks in Figure 15 decreased with increasing temperature. The values for  $T_m$  and areas of the curve from samples pre-heated at 26-39 °C for each heating time were the same as for untreated samples. For each heating time the peak 1 (myosin) were strongly reduced after 40 °C. After 54 °C, peak 2 and 3 starts to reduced and the peaks disappeared at higher temperature.

Salmon myosin proteins can be denatured by heating. Chan et al. [61] found that some regions of the myosin molecule are less thermo stable than others and have a tendency to denature before the whole myosin molecule is completely denatured. Actin, on the other hand is not denatured by freezing and more heat stable than myosin [49].

The time required for denaturation of all proteins in question is decreasing with increasing processing temperature. At 70 °C, 8 min heating is required for completing the denaturation, at 71 °C it is sufficient to heat for 6 min while on 4 and 5 min heating is enough at 72 °C. This behaviour make it feasible to determine inactivation parameters for thermal denaturation of salmon proteins.

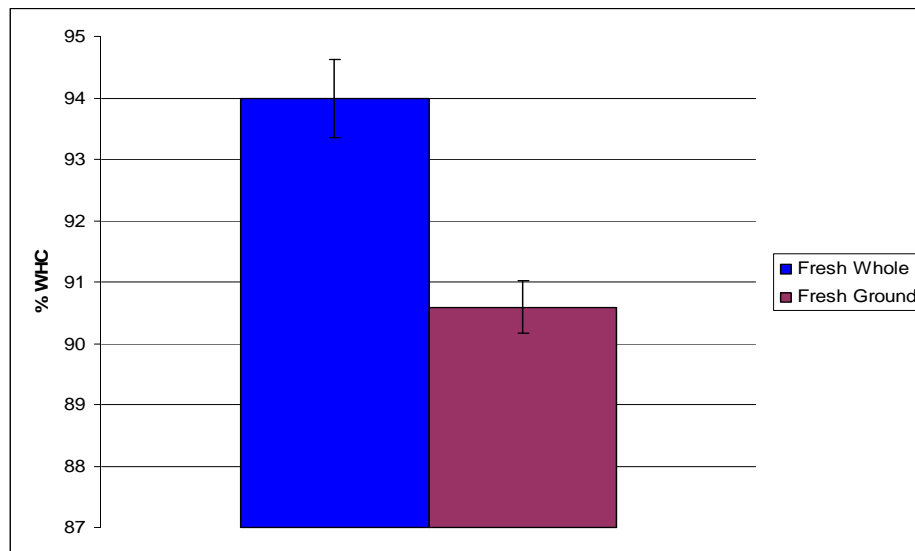


**Figure 15. Average denaturation Enthalpy of salmon for each heating time and temperature investigated ( n=3)**

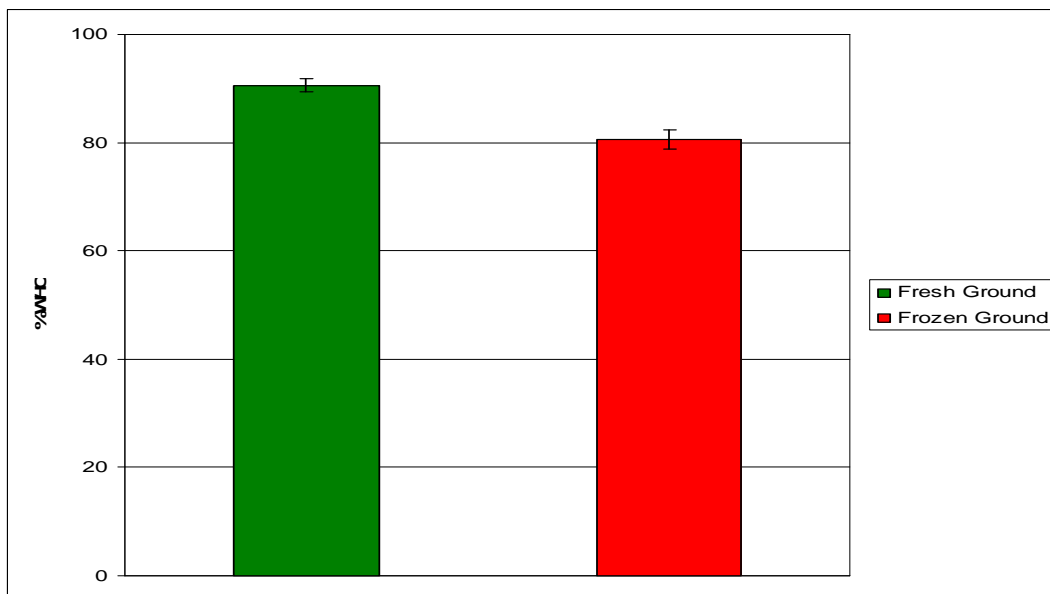
#### ***4. 2. Water Holding capacity***

The WHC of fresh whole samples was found to be significantly different ( $P < 0.050$ ) from the ground ones (Figure 16), this is in agreement with Skipnes et.al [56], who found a significant difference between whole and coarsely minced Atlantic cod (*Gadus morhua*).

In whole meat muscle, very little drip loss occurs, while evaporative losses from the surface of the carcass may occur. However, once the muscles are cut the opportunity for drip to escape exists. The size of the piece of fish can affect the percentage of the product that is lost as drip. Smaller cuts of fish lose relatively more drip than the larger one [62]. The drip tends to flow along the length of the fibers [63].

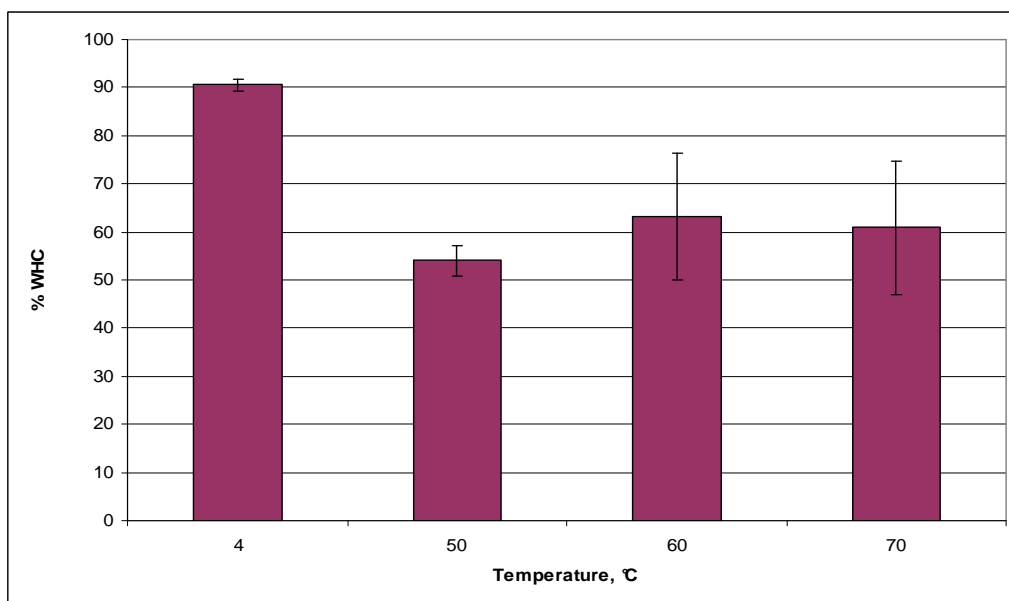


**Figure 16. WHC of fresh whole and fresh ground Atlantic salmon (*Salmo salar*) and standard error (n = 8)**



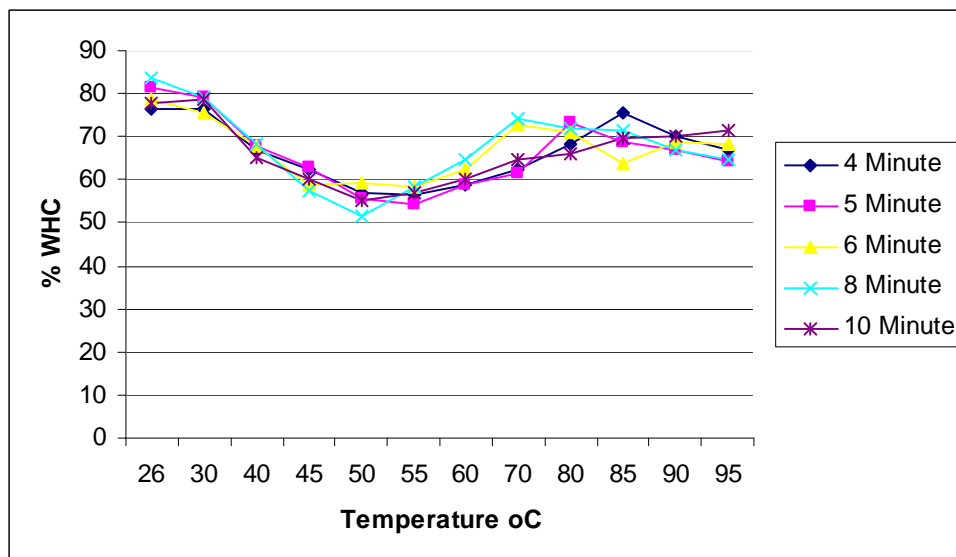
**Figure 17. WHC of fresh ground and frozen Atlantic salmon (*Salmo salar*) and standard deviation (n = 8)**

There is a significant difference ( $P < 0.050$ ) in WHC between the fresh and frozen ground fish, as shown in Figure 17. Ground fish is more vulnerable to freezing damage than fish fillets [64]. If freezing damage to ground fish could be reduced, it would increase flexibility in the manufacture of added-value fish products. The loss of additional liquid when a force is applied to the fish has relevance for fish products made from compressed blocks of fillets or ground. The water content for thawed fish are likely to be lower than for fresh fish due to water migration during frozen storage and drip loss during thawing [65].



**Figure 18. WHC of fresh ground Atlantic salmon (*Salmo salar*) heated at 50 °C, 60 °C, and 70 °C (n = 8) and standard deviation compared to raw salmon**

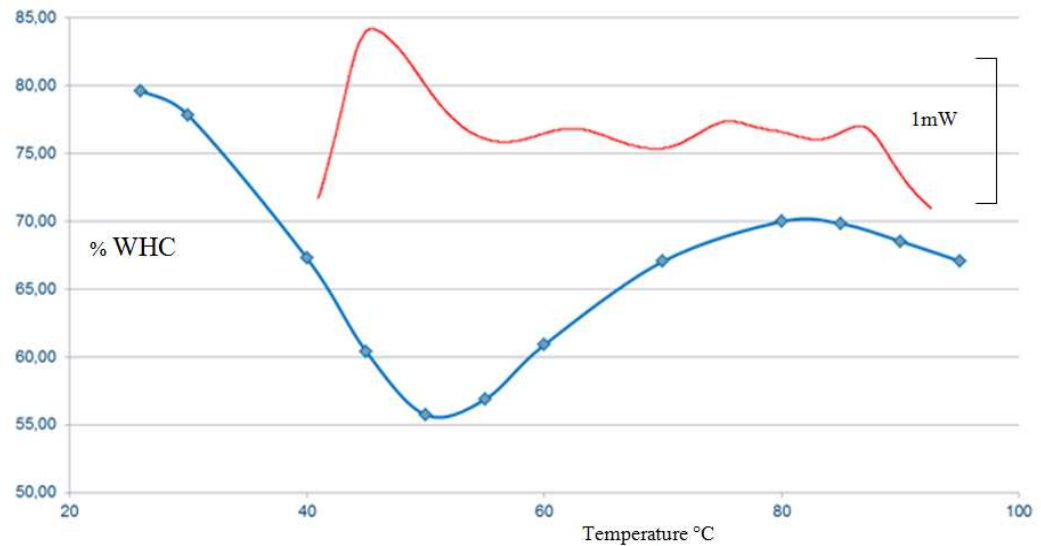
The differences in WHC of fresh ground salmon after pre-heat treatment at temperature 4, 50, 60 and 70 °C can be seen in Figure 18. A significant difference was found between the WHC of salmon at 4 °C compared to others temperature ( $P < 0.001$ ). The WHC seemed to decreased when it was pre-heated at 50 °C, 60 °C and 70 °C but no significant differences were found ( $P > 0.050$ ).



**Figure 19. WHC of frozen ground Atlantic salmon (*Salmo salar*) as a function of temperature plotted for each pre-heating time (4, 5, 6, 8 and 10 minute)**

Figure 19 shows the results from measurement of WHC of frozen ground salmon after heat treatment at temperatures 26-95 °C. The WHC showed similar pattern for the frozen salmon as seen in Figure 18 for fresh fish, decreasing WHC

until the temperature reaches 50 °C and increasing WHC with increasing temperature until 70 °C to 85 °C depending on processing time.

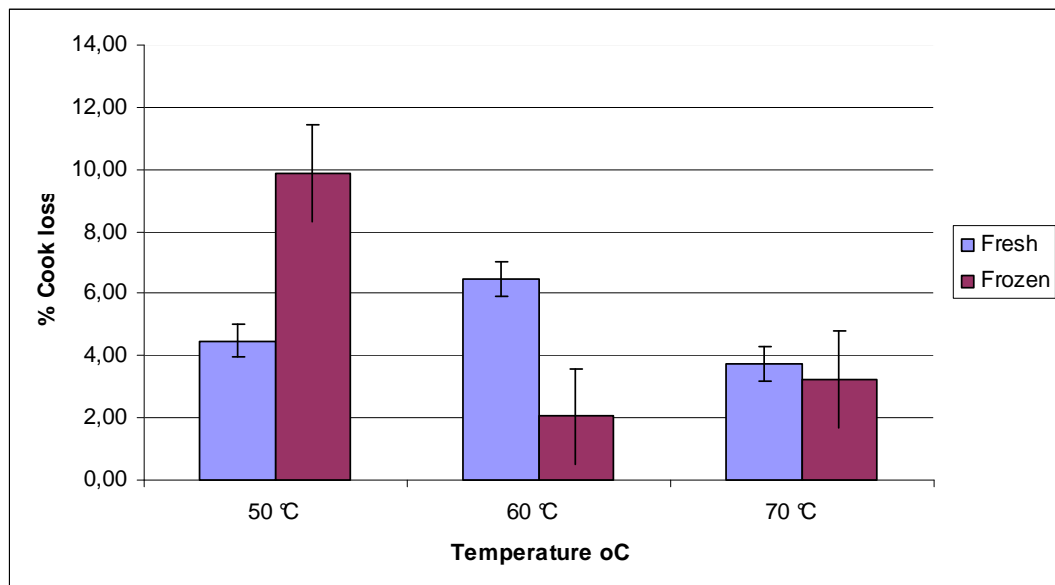


**Figure 20. Thawed ground Atlantic salmon (*Salmo salar*) WHC in blue line (Mean) and DSC-thermogram in red line obtained at scanning rate 5 °C/min**

Figure 20 shows both protein denaturation and WHC, the protein-water interaction is essential for protein solubility as well as WHC. Aspartic acid and glutamic acid have the highest water-binding ability, and myosin ability has been related to those amino acids [54, 66]. A minimum of WHC is reached at temperature where the myosin denaturation has been completed.



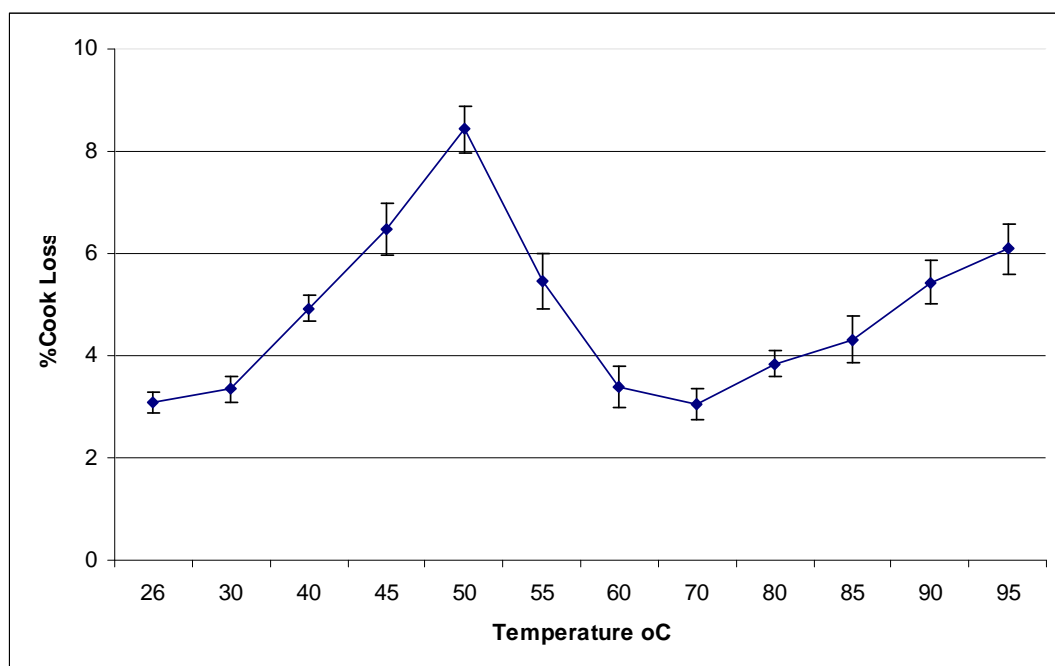
### 4.3. Cook Loss



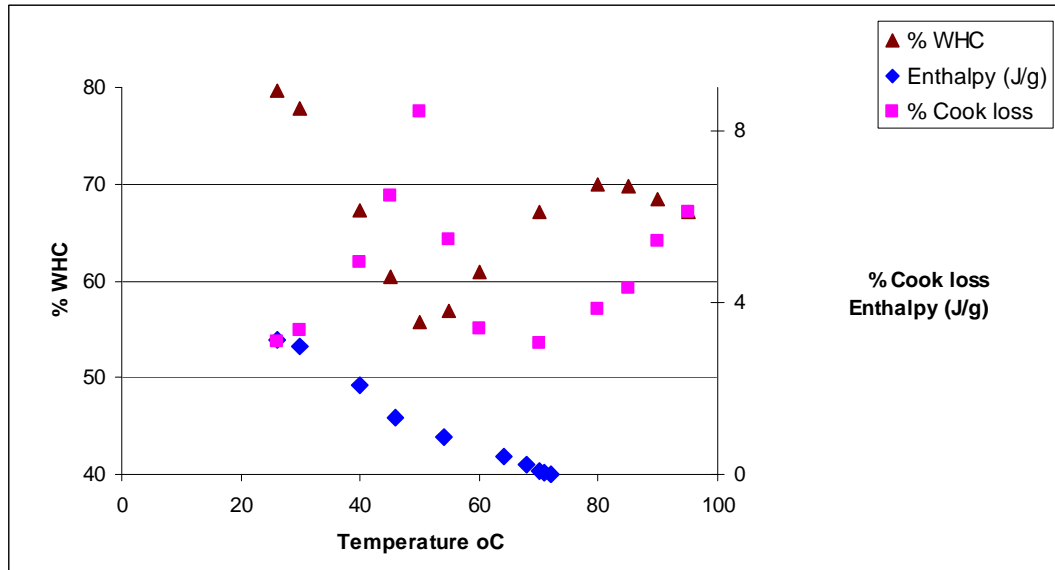
**Figure 21. Comparison of cook loss between fresh and frozen ground Atlantic Salmon (*Salmo salar*) after 10 min heating at 50 °C, 60 °C and 70 °C (n=8) and SE**

Fresh and frozen salmon was found significantly different in cook loss at 50 °C ( $P < 0.050$ ). Figure 21, cook loss at 50 °C for frozen salmon with others temperature was also found significantly different ( $P < 0.050$ ). The differences between fresh and frozen ground salmon in cook loss at 50 °C related with the  $T_m$  of myosin peak of DSC thermograms Table 2. Frozen ground salmon has higher  $T_m$  than fresh ground salmon in all peaks, and myosin for fresh ground salmon was denatured earlier than frozen ground salmon.

As shown in Figure 22, cook loss is strongly dependent on processing temperature and increases when heating from 26 up to 50 °C. Cook loss is reaching a maximum at 50 °C and then decreased rapidly to the minimum cook loss at 70 °C. The previous study about salmon Ofstad et al. [67], found that the water loss increased from 30 °C until maximum temperature 45-50 °C which is similar pattern with the cook loss in the Figure 21.



**Figure 22. Cook loss of thawed Atlantic salmon (*Salmo salar*) average over heating time and determined for each temperature investigated (n=20) and standard error**



**Figure 23. Denaturation Enthalpy (J/g), % WHC and % Cook loss of Atlantic salmon (*Salmo salar*) average over heating time and determined for each temperature investigated**

The relation between WHC, cook loss and denaturation enthalpy can be seen in Figure 23. When salmon was heated at 50 °C, WHC reached a minimum peak and cook loss reached a maximum. At 30 °C cook loss start to increase to the high level at 50 °C, at the same time WHC decrease rapidly until reached minimum temperature which also coincident with the denaturation enthalpy that drop for the first time from 30 °C to 40 °C. Above 60 °C WHC was moderate until 95 °C and cook loss start to increase from 70 °C with increasing temperature.

WHC in fish tissue is strongly related to myofibril proteins. The increased cook loss might be explained by the reduced water-imbibing capacity of the tightly bound myofibrillar proteins, which causes the immobilized cellular water to move and flow out at lower pressure [68, 69]. Increase of expressible moisture is a sign of reduction of WHC due to denaturation of proteins [70].

The reduction of water loss may be caused by aggregates of sarcoplasmic proteins and collagen are able to hold water and/or plug the intercellular capillaries, thus preventing water from being released during centrifugation [67]. Ofstad et al. [67] found at increased temperatures, the extracellular spaces had increased and breakage of the pericellular layers concomitant with shrinkage of the myofibrils has occurred and at maximum temperature intracellular gaps appeared.

Water is a dipolar molecule and as such is attracted to charged species like proteins. In fact, some of the water in muscle cells is very closely bound to protein [71]. One amino acid of an ionic chain has been claimed to bind 4-7 water molecules in aspartic acid, glutamic acid and lysine [54].

As proteins are denatured they begin to open up and expose their hydrophobic core. Consequently, they begin to interact with other hydrophobic regions on the same protein (intra) or with other denatured proteins (inter). The hydrophobic regions of the proteins are excluded from water, which leads to a stronger interaction between them. It has been suggested that the exposure of hydrophobic domains is a prerequisite for the formation of large myosin aggregates [72]

## 5. Conclusion

Protein denaturation obtained by DSC at a constant heating rate of 5 °C/min, cook loss and WHC of Atlantic salmon have been quantified. There were found four proteins denaturation peaks of salmon muscle. These proteins are myosin, sarcoplasmic and actin protein. The time required for denaturation of all proteins is decreasing with increasing processing temperature.

Actin protein of salmon muscle is more heat stable than myosin protein. Myosin denaturation of salmon muscle occurs at temperature from 46.8 and 48.9 °C and actin protein denatures from 77.3 – 79.5 °C.

The cook loss and changes in WHC has been expected to be a result of protein denaturation by several authors. The major cook loss occurs at the temperature where the myosin had been denatured when heating salmon. Increasing cook loss and decreasing WHC at 50 °C was attained correspondingly with the appearance of transverse shrinkage of the muscle cells, intercellular gaps and widening of the extracellular spaces.

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