CHALLENGES OF THE USAGE OF ULTRA-LOW TEMPERATURES FOR FISH FREEZING AND STORAGE

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ABSTRACT

The physical states of frozen fish (muscles and oils) was analysed in the temperature range between -150.0 and 20.0 °C. The information of the glass transition temperature, the end of freezing point, the amount of unfrozen water, the amount of liquid fraction of oil was obtained by the method of Differential Scanning Calorimetry (DSC). Five commercial fish species were investigated: Atlantic Cod, Atlantic Herring, Atlantic Mackerel, Atlantic Salmon and Rainbow Trout. The glass transition was detected in the temperature range between -84.2 °C and -67.7 °C in fish muscles. The amount of unfreezable water was calculated in the range between 5.1 and 8.6 %. A significant fraction of fish oil (between 40.0 and 60.0%) remained unfrozen at ultra-low temperatures. The packaging materials with medium barrier properties were considered to be of a great importance for the long-term storage of fatty fish due to a high amount of liquid fats at these temperatures. The two temperatures provided the high quality storage were proposed: below -35 °C and below -86 °C.

1. INTRODUCTION

Frozen food is defined as a product with a temperature below –10.0 °C, which is maintained during storage and sales. Approximately 80% of water in the product is converted into ice at such conditions. Fish, with a temperature of less than –18.0 °C is considered to be “deep frozen”(Bøgh-Sørensen, 2006). Traditionally, the storage temperature of frozen fish is between –18.0 and –30.0 °C (FAO/WHO, 2003). The shelf-life of fish is limited to several months at these temperatures (depending on the type of fish), which can be not sufficient for retail market. The production and the storage of high quality frozen fish is an energy demanding process (package, temperature decreasing, antioxidants, etc.). Thus, the fish industry requires an efficient process, where a balance between the long-term shelf-life and the resources is optimal.

The maintenance of High Quality shelf-Life (HQL) is preferable for frozen products, due to the reducing of wastes. The decreasing of freezing and storage temperatures is one of the options to increase the shelf-life of frozen fish. The following dependence was observed for HQL of fish by Bengtsson et.al. (1972), Figure 1.

Figure 1. The HQL-data systemized for fatty and lean fish, adopted from Bengtsson et al., (1972)
At the same time several investigation, which were devoted to the influence of low and ultra-low temperatures on the shelf-life of Atlantic Salmon and Atlantic Cod, did not observe any difference in quality parameters of fish at the storage temperatures of -40.0 and of -70.0 °C (Burgaard and Jørgensen, 2011; Mørkøre and Lilleholt, 2007). However, no final conclusion for these results was found. But, information concerning a physical state of fish and its components may give excessive information about the stability of fish during storage. Such knowledge can improve significantly the validation of the application of ULT (ultra-low temperature).

The study summarizes several studies which investigated the phase transition and stability of fish with respect to lipid oxidation at low and ultra-low temperatures.

2. MATERIALS AND METHODS

2.1. Raw Material

Five fish species were used for the experiments: Atlantic Cod (Gadus morhua); Atlantic Herring (Clupea harengus); Atlantic Mackerel (Scomber scombrus); Rainbow Trout (Oncorhynchus mykiss) and Atlantic Salmon (Salmo salar). The Cod, Salmon and Trout were aquaculture products. All the fish were obtained from a local fish-market, and stored in ice between 24 and 48 hours before the experiments (post-mortem fish). White muscles of fish from the middle part of a fillet were used for DSC (Differential Scanning Calorimetry) experiments. Fat was extracted from the whole fish’s fillets. The lipid fraction was following: Rainbow Trout – 6.7 (0.1) %, Atlantic Salmon – 6.8 (0.1) %, Atlantic Mackerel – 1.9 (0.1) %, Atlantic Herring – 10.0 (0.1) %, Atlantic Cod < 0.2 (0.1) %.

Herring fillets (preserved 2 days in ice before freezing) were used for the determination of oxidation stability during a long-term storage with respect to the temperature and the type of packaging material. The fillets were frozen at temperatures of -25.0 and -45.0 °C and vacuum packed in two types of packaging materials: polyolefin and polyamide/polyethylene (PA/PE) (Tolstorebrov et al., 2014c)

2.2. DSC Methods

The fish muscles were analysed by an ordinary DSC method using the DSC Q2000 which was equipped by the Liquid Nitrogen Cooling System (TA instruments, USA). The mass of a sample was in the range between 3.0 and 10.0 mg. Aluminium pans with hermetic lids were used (50.0 mg). A scanning procedure was done in the temperature range from -150.0 to 20.0 °C. The heating rate during the scanning was 5.0 °C min⁻¹. The equilibration time at -150.0 °C was 60 min. Helium was used as a purge gas (flow rate 25.0 mL min⁻¹). Some of the samples were dehumidified using vacuum freeze dryer Alpha 2-4 LSC Plus (Martin Christ GmbH, Germany). The desirable humidity was achieved knowing sorption isotherms by use of climate chamber KMB 115 (Binder®, Germany) (Tolstorebrov et al., 2014b)

The fish oils were analysed by DSC methods using the same device as for the analysis of fish muscles. The mass of a sample was between 2.0 mg and 3.0 mg. The cooling rate of 0.2 °C min⁻¹ was used for all the DSC experiments to avoid cold crystallization during heating. The full method is described by Tolstorebrov et al. (2014a).

2.3. Determination Of Unfrozen Water Content, Glass Transition And Freezing Point

A freezing point was determined as the extremum of a melting peak. A glass transition was characterized with the following parameters of the endothermic baseline shift: the onset of glass transition, the insipient point of glass transition and the end of glass transition. The unfrozen water content was determined by the partial integration of a melting peak with respect to the latent heat of fusion of water, which is a function of temperature (Riedel, 1978), eq 1.:

\[ \Delta H_w = 333.5 + 2.05 \times T - 4.19 \times 10^{-3}T^2 \]  

where

\[ \Delta H_w = \text{the latent heat of fusion of water [kJ kg}^{-1}] \], \( T= \text{the actual temperature [°C]} \).

The methods of determination are described by Tolstorebrov et al. (2014b).

2.4. Determination Of Liquid Fraction Of Fish Oil
The onset and the end points of melting are defined as a temperature at which a change in the slope of the heating curve occurs. The melting energy was obtained by the integration of the DSC melting peaks (the linear baseline function integration).

A liquid fraction in fish oil was measured by the integration of a melting peak with respect to the melting energy, \( \Delta H \) [kJ kg\(^{-1}\)], of different fraction using the following dependence, eq. 2:

\[
\Delta H_{fat} = 2.08 \times 10^{-6} \times \left( \frac{1}{273.02 + T} \right)^{-3.61}
\]  

(2)

### 2.5. Statistical Analysis

All experiments were made in five replicates. The average values are introduced in the article, standard deviation is introduced in brackets. The statistically significant difference was analysed using ANOVA one factor experiment. The difference was considered significant at \( p<0.05 \).

### 3. RESULTS AND DISCUSSION

#### 3.1. Thermal Transitions In Fish Muscles At Low And Ultra-low Temperatures

A freezing point was found in the range between -2.0 and -1.1 °C, Table 1, which was in an agreement with the previous studies. An extensive ice formation (melting) was observed in the temperature range between -15.0 °C and the freezing point, Figure 2. Approximately 95% of all freezable water was frozen at this temperature diapason. The equilibrium end of freezing (onset of melting, \( T_m \)) was detected in the temperature range between -33.5 and -29.3 °C. The heat flow curves did not show any endothermic processes below these temperatures. At the same time, a certain amount of unfrozen water was detected by integration of the melting peak on the heat flow curve. The unfreezable water content does not correlate with initial amount of water in fish muscles, but a good correlation was observed with the content of proteins (R\(^2\)=0.8).

<table>
<thead>
<tr>
<th>Fish</th>
<th>Moisture, % w.b.*</th>
<th>Glass transition temperatures, °C</th>
<th>Freezing point, ( T_f ), °C</th>
<th>Onset of melting, ( T_m ), °C</th>
<th>Unfreezable water, % w.b.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod</td>
<td>85.0</td>
<td>-84.2 (1.5) -76.3 (1.0) -69.6 (1.3)</td>
<td>-1.1 (0.1) -33.5 (0.6)</td>
<td>5.1 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Salmon</td>
<td>70.6</td>
<td>-80.0 (1.3) -76.5 (1.2) -68.1 (1.5)</td>
<td>-1.5 (0.1) -29.3 (0.4)</td>
<td>6.6 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Trout</td>
<td>70.5</td>
<td>-83.8 (1.4) -75.0 (1.1) -68.1 (1.2)</td>
<td>-2.0 (0.2) -30.8 (0.5)</td>
<td>6.7 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Herring</td>
<td>70.2</td>
<td>-84.2 (1.3) -76.4 (1.1) -69.2 (1.3)</td>
<td>-1.6 (0.2) -32.8 (0.7)</td>
<td>8.6 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Mackerel</td>
<td>80.4</td>
<td>-82.9 (1.5) -75.9 (1.7) -67.7 (1.6)</td>
<td>-1.6 (0.1) -31.7 (0.4)</td>
<td>8.5 (0.2)</td>
<td></td>
</tr>
</tbody>
</table>

* The standard deviation for moisture content equals 0.1 %. For other values standard deviation is introduced in brackets.

The end of freezing point refers to the formation of a so-called maximal freeze-concentrated solution, when the viscosity of the system is higher than 10\(^8\) Pa s (Roos, 1995). Such conditions are critical for the formation (propagation) of ice crystals or this process takes long time and cannot be detected during experiments. The further decreasing of temperature results in the increasing of the system’s viscosity. There is a second critical level of viscosity which refers to significant decreasing of molecular mobility. The glass transition occurs when the viscosity of the binary system reaches value of 10\(^12\) Pa s, (Champion et al., 2000; Roos, 1995). Glass transition is characterized by the lowest molecular mobility and it refers to the highest stability of a product during storage. However, several relaxation processes were observed at temperatures below the glass transition region (Champion et al., 2000).

In this study the glass transition occurred in the temperature range between -84.2 and -67.7 °C. A statistically significant difference in glass transition temperatures between fish species was not found (p>0.05). The other investigations reported the glass transition in fish muscles in the same temperature range (Nesvadba, 1993; Rahman et al., 2003; Shi et al., 2009). The glass transition shift of fish muscles on the heat flow curve was relatively low, when compared, for example, with binary mixtures. Also, a temperature difference between the onset and the end of a glass transition was approx. 15.0 K. The process was more visible on the derivation of the heat flow curve. Any other thermal processes were not detected in the temperature range between -150.0 and -86.0 °C.
Figure 2. Equilibrium ice formation in fish muscles at different temperatures, the curves were obtained by integration of melting peaks with respect to latent heat of fusion.

The absolute solid state of fish muscle occurred at temperatures below -86.0 °C. The system of maximal freeze-concentrated solution and ice crystals appeared in the temperature range between -86.0 and -33.0 °C. The intensive ice formation was determined at the temperatures higher than -33.0 °C. These statements are valid only for protein-water-ice systems. Thermal transitions of fats should be taken into account.

3.2. Physical Properties Of Fish Oil At Low And Ultra-low Temperatures

The determination of physical state of fish oils is complicated due to the polymorphism of fat crystal formation, as soon as fish fat is represented mostly by triacylglycerides (TAG). This study revealed that fish fat remains in a liquid phase at ultra-low temperatures. The onset of melting was detected in the temperature range between -90.0 and -80.0 °C for fatty fish species (Atlantic Cod oil was not analysed), Figure 3. The similar results were obtained by the DSC investigation of Salmon oil, which was extracted from salmon’s heads (Sathivel, 2005). It should be noted that the onset of fish oil’s crystallization is hard to detect using a DSC technology, because the melting process consists of several melting peaks and a heating process is inertial. Thus, one can use the end of melting point or the extremum of high temperature melting peak as a reference.

The TAGs, which include polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), did not show any crystallization. The glass transition was observed for TAGs, which consisted of tree molecules of DHA or EPA (Tolstorebrov et al., 2014a). The glass transition was observed in all the investigated samples, as soon as these fatty acids are common in fish oils Figure 3 (T_g - shift on the heat flow curve from the left side).

A significant amount of liquid fraction was detected at low and ultra-low temperatures for the samples of fish oils, Figure 4. Only insignificant amounts of fish oils (between 10.0 and 20.0 %) were frozen at storage temperatures, which are traditionally accepted (between -24.0 and -18.0%).

The question of fat solidification can be an important issue, because fat crystals do not contain dissolved oxygen or other gases in their structure (Hemmingsen, 1959). Thus, this form is the most stable during a long-term storage. It should be noted that the «unfreezable» part of fish oils is represented by poly unsaturated fatty acids. The degree of unsaturation decreases the freezing temperature significantly.

The plots of liquid fraction vs. temperature, which are introduced on Figure 4, show that a significant part of fish oils (between 40.0 and 60.0 %) was unfrozen at ultra-low temperatures. The full solidification was detected at glass transition temperatures (below -120.0 °C).
Figure 3. Heat flow curves for different fish oils (Equilibrium formation of ice crystals)

Figure 4. Liquid fraction in fish oils at different temperatures.
3.3. Stability Of Herring Fillets To Oxidation At Different Temperatures

The presence of significant amounts of unfrozen oils in fish at low and ultra-low temperature rises up a question concerning benefits of the temperature decreasing. The concentrated amount of highly oxidative TAGs in a liquid state will be exposed to oxygen; this can be a significant issue of oxidation at ultra-low temperatures (considering also a BET theory).

The herring fillets were vacuum packed (506.5 Pa) in Polyolefin and PA/PE and stored at -25.0 and -45.0 °C for one year. The polyolefin package has low O₂ – barrier properties, the PA/PE has medium barrier properties.

The formation of peroxides and TBARS was relatively high at -25.0 °C when the fillets were packed in polyolefin packaging material, Figure 5 A and B. The other three groups of fillets showed a significantly lower rate of peroxides and TBARS formation during long-term storage, when compared with the first group (p<0.05). The influence of the packaging materials on oxidation was the same as the influence of temperature decreasing. The statistically significant difference was not observed for herring fillets, which were packed in polyolefin bags at -45.0 °C, and fillets, which were packed in PA/PE bags at -25.0 °C (p>0.05).

![Figure 5. Oxidation of vacuum-packed herring fillets during a long-term storage with respect to storage temperature and packaging material.](image)

A simple model, which is based on the Fick’s law and takes into account properties of packaging material and a product, was made to understand the amount of oxygen, which penetrated through the package during a long-term storage at different temperatures, eq. 3.

\[
dC = -kC \, dt
\]

where
\( dC \) = the ratio of differences of oxygen concentrations in the beginning of a process and at any time, \( k \) = the coefficient, which takes into account the oxygen transmission rate, the thickness of material, the surface of penetration and the volume of package.

The results of modelling are introduced on Figure 6. The curves of oxygen accumulation show that packaging material with medium barrier qualities prevented accumulation of O₂ at low and ultra-low temperatures. Oxygen accumulated slowly in the PA/PE packages. The accumulation of oxygen was at a higher rate in Polyolefin bags. The oxygen concentration reached 55 % at -25.0 °C and 25 % at -45.0 °C (from its normal concentration in the air). The accumulation of oxygen in the package correlated with fat oxidation.
It should be noted that slow oxidation of lipids took place at low temperatures, even when the permeation of oxygen through the packaging material was insignificant. This occurred due to the presence of dissolved oxygen in fish tissues, which cannot be removed by existing vacuum packaging methods and/or by micro-damage of the packaging material during a long-term frozen storage. However, the shelf life of the herring fillets, which were packed into the PA/PE bags, exceeded 2 years at -25.0 °C. At the same time, storage of herring fillets, which were packed in Polyolefin bags, was approx. 208 days at -25.0 °C.

Other fish species, which contain natural antioxidants, showed weaker correlation of oxidation with packaging materials. For example, Atlantic Salmon packed in packaging materials with different O\textsubscript{2} permeability and stored in the temperature range between -60.0 and -25.0 °C showed low rate of oxidation, but general trends were similar as for herring fillets (Indergård et al., 2014).

4. CONCLUSIONS

The investigations of the physical properties of proteins at ultra-low temperatures revealed that the end of ice formation happened at temperatures below -33.5 °C. At the same time, the amount of unfreezable water was in the range between 5.1 and 8.6 %. The glass transition happened at temperatures below -69.0 °C. Based on this, we can suggest the two levels of stability of fish proteins at low temperatures: below the end of ice formation and below the glass transition. The first is more appropriate for the industry due to lower investment costs. At the same time, the shelf-life provided the high quality will be shorter, when compared with the storage below a glass transition temperature.

The most important information was obtained for lipids at ultra-low temperatures. A significant amount of fish oil is in a liquid state between -120.0 and -30.0 °C. This allows chain reactions in the system. The decreasing of temperature has a simultaneous negative and positive effect, due to the decreasing of the reaction rate and the increasing of the chemically active substrate in the liquid phase. This data was supported by the study of lipid oxidation in salmon and herring during a long-term storage at different
temperatures. The application of packaging material with a medium oxygen barrier caused a significant decrease of lipid oxidation and a significant extension of shelf-life.

We can conclude that the highest level of stability of frozen fish was situated far below the traditionally used temperature. Thus, a balance between the high-quality and a possible length of storage should be designed individually depending on the type of the fish and the process. For fatty fish, we would recommend storage between $-30.0$ and $-35.0$ °C when all of the freezable water is frozen, while at the same time, the application of vacuum and packaging material with a medium oxygen barrier would be beneficial. For lean fish, the packing material is not so essential, but a storage temperature should also be in the range between $-30.0$ and $-35.0$ °C. This can significantly decrease the deteriorative reaction without increasing of running costs and investments.

5. REFERENCES


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