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DSC studies on deep frozen fishery products

R. Schubring*

Institute for Biochemistry and Technology of the Federal Research Centre for Fisheries Palmaille 9, D-22767 Hamburg, Germany

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Abstract

In Germany frozen battered and breaded fish portions like ''fish fingers'' are important fishery products which account to more than 25% of the entire production of the German fish processing industry. The raw material for the production of these items consists of two kinds of frozen blocks of fillets — single frozen (SF) and double frozen (DF) ones. The possibility to differentiate between SF and DF fillets was investigated by applying two types of differential scanning calorimeters. The transition temperatures as well as the transition enthalpies of both fillet types were not significantly different. Furthermore, no influence of the rigor state on the thermal stability of the fish proteins could be detected in frozen fillets. The differences in the transition temperatures measured by using the various calorimeters could be explained by the differences in scanning rates applied.

Keywords: DSC; Fish; Protein; Freezing; Denaturation

1. Introduction

The German fish processing industry is counted as one of the most important processors for frozen battered and breaded portions of fish fillet world-wide. It is more and more dependent on import of the raw material. In 1996, approximately 170 000 tonnes of frozen lean fish fillet were imported. In the same year 120 257 tonnes of frozen battered and breaded products were made. This was an increase by 20.6% compared to 1995 [1]. The importance of this product range is underlined by the fact that it accounted to more than 25% of the entire production of the German fish processing industry.

In the international trade it is more and more common that beside frozen fish blocks produced onboard of fish processing trawlers also double frozen blocks of fish fillet are used. They are produced ashore by thawing, filleting and skinning the frozen round or gutted fish and by refreezing the fillets. Taking this into account the following two specifications can be seen as raw material for the production of frozen battered and breaded fish fillets or portions thereof:

> Frozen blocks of fish fillet produced from fresh fish using a continuous process. This process includes only SF.
> Frozen blocks of fish fillet produced from frozen fish using an interrupted process. This process includes DF.

Therefore the questions are: how is the quality of frozen fish fillet influenced by refreezing and is there a significant influence on the quality of the final product? Will it be possible to differentiate the refrozen raw material from the single frozen one by using

*Fax: +40-38905262
E-mail address: schubring.ibt@bfa-fisch.de (R. Schubring)

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analytical methods which are applicable by the processing industry?

Although freezing is widely distributed as a preservation method and is superior to almost all of the other preservation techniques regarding the quality assurance, freeze-induced changes of the proteins of meat and fish are detectable. They are a result of denaturation of the proteins mainly caused by aggregation of native molecules and by some other reactions. On that occasion formaldehyde, free fatty acids and intermediate products of the oxidation of fatty acids are at disposal as reaction partners. Further causes of the denaturation are the withdrawal of water by freezing, the increase of ion concentration and protein–protein interaction. The denaturation is defined as any modification in conformation (secondary, tertiary and quaternary) not accompanied by the rupture of peptide bonds [2].

Thermophysical properties of foods are very significant elements to consider during the study of preservation processes by means of cold such as freezing [3]. Differential scanning calorimetry has emerged as a technique of choice for the study of thermal transitions of food. In this technique, the substance of interest and an inert reference are heated at a programmed rate. Any thermal induced changes occurring in the sample are then recorded as a differential heat flow displayed normally as a peak on a experimental curve. The conversion of a protein from native to denatured state by heat is a co-operative phenomenon and is accompanied by a significant uptake of heat, seen as an endothermic peak in the DSC curve [4].

For proteins, the thermally induced process detectable by DSC is the structural melting or unfolding of the molecule. The thermal denaturation of proteins is attributed to the rupture of intramolecular hydrogen bonds. The denaturation temperatures are measures of the thermal stability of proteins. Their determination under controlled conditions can provide direct comparison of the thermal stability of the different proteins. The enthalpy value is correlated with the net content of the ordered secondary structure of a protein. It is actually a net value from a combination of endothermic reactions and exothermic processes, including protein aggregation and the break-up of hydrophobic interactions [5]. A successful approach to the study of the native conformation of proteins is the subjection of the protein to physical and chemical stresses, followed by a determination of the effect of theses stresses on its thermal denaturation [6].

Very recently, DSC investigations have been performed about the influence of post mortem states on the water holding capacity of various fish species [7], the suitability of shark minces for processing into surimi [8] and the influence of the freshness of the raw material in this respect [9]. Furthermore, the effect of processing steps on the quality of fishburger was investigated [10] and the ripening [11] of salted herring products was followed by monitoring changes of pyloric caeca [12]. The cryoprotective properties of proline in cod muscle was studied during freezing to $-60^{\circ}C$ and subsequent heating to $+10^{\circ}C$ by DSC [13]. The influencing of both myofibrillar proteins, myosin and actin, during the processing of fish gels using high pressure and heat followed by DSC was subject of very recently performed studies [14–17].

After reviewing the relevant literature [18] it became clear that the above mentioned situation-the possible influence of double frozen fillet blocks in comparison with single frozen ones on the quality of battered and breaded frozen portions of fish fillet-needs more investigation. Thus, to my knowledge, the influence of the state of rigor prior to filleting and freezing in this context was not the subject of research as yet, therefore, fillet blocks were processed and frozen in *pre-*, *in-* and *post-rigor* states, respectively. Therefore, the objective of the study was to evaluate the above mentioned influence, if any, under practice-like conditions using sensory, chemical and physical methods. Here, only the results of DSC investigations are reported. The outcome of the other measurement techniques is described in detail at an other place [18].

2. Experimental

2.1. Materials

The frozen fish blocks were processed onboard FRV ''Walther Herwig III'' during the 184th and 195th cruises in spring 1997 and 1998, respectively, using saithe (*Pollachius virens*) caught by a bottom trawl net in different fishing areas of the North Sea. The temperature of the water was approximately $6^{\circ}C$. Immediately after hauling the fish was killed by cutting the

throat, and skinless fillets were processed and frozen after bleeding out within 2–3 h (SF). An appropriate amount of headed and gutted (H and G) fish were also frozen for later processing of double frozen fillets *pre-rigor*. After an iced storage period of 14 h when the fish has entered rigor, the saithe was filleted, deskinned and frozen as mentioned above (SF) and an appropriate amount of H and G fish for processing double frozen fillets *in-rigor* was frozen as well. This sampling step was left out during the 195th research trip. After passing the rigor state in approximately 72 h the *post-rigor* SF fillet was processed, deskinned and frozen. The same procedure was used to prepare the gutted fish as raw material for the processing of double frozen *post-rigor* fillets. After the H and G fish has been frozen stored for 10 days it was thawed using tape water and processed into deskinned fillet dependent on rigor state (*pre-*, *in-* and *post-rigor*) and refrozen. The freezing process of the geometrically exactly sized fish blocks was performed under industrial conditions in a Sabroe plate freezer using packaging material commercially used. The H and G fish was frozen in a Sabroe tunnel freezer. The core temperature of the blocks after freezing was in both cases −30°C. The frozen storage was performed onboard at –30°C and later ashore at −24°C. After seven weeks of frozen storage battered and breaded portions of fish fillet were made on a production line of a German processor using both single and double frozen fillet blocks. These portions were packaged in cardboard boxes and stored at −24°C until investigation. Additionally, commercially processed single and double frozen fish fillet blocks prepared from cod (*Gadus morhua*) and Alaska pollock (*Theragra chalcogramma*) were used.

2.2. Differential scanning calorimetry

Differential scanning calorimetry was performed with a Perkin-Elmer DSC 7 (Überlingen, Germany) fitted with a Colora-cooling equipment (Kryo-Thermostat WK 5, Colora GmbH, Lorch, Germany) at ambient temperature [11]. The instrument was calibrated for temperature and enthalpy using indium and naphthalene as standards. The fish samples (15 ± 3 mg) were weighed accurately (0.1 mg) into 30 µl aluminium pans (BO 169 320) and sealed. At least triplicate samples were heated from 25°C to 95°C at a scanning rate of 10 K/min, with an empty sealed pan as a reference. The transition temperature (T_{max}) was recorded. The transition enthalpy was determined from the peak area and expressed in J/g sample material.

Additionally, preliminary investigations were performed on selected samples using a SETARAM Micro DSC III in the same temperature range as mentioned above. The fish samples (700–800 mg) were weighed accurately in closed, "batch" vessels and heated at scanning rates of 0.5 or 0.2 K/min. Distilled water was used as reference sample.

3. Results

From the PE-DSC 7 curves, a typical one is shown in Fig. 1, the transition temperatures and transition enthalpies were derived using the Perkin-Elmer software and are summarised in Tables 1–3. As musculature is a complex system comprising three classes of proteins: sarcoplasmic, myofibrillar and stromal, the DSC curves of whole muscle are also very complex. The first peak at lower temperature is connected with myosin and related proteins and the last one at higher temperature with actin, the other important myofibrillar protein. The peaks in between are connected with sarcoplasmic proteins and/or collagen [5,19–21]. As mentioned above during the 195th cruise the sampling includes only the *pre-rigor* and *post-rigor* states. That is why, in Table 2 only two rigor states are displayed. In general, there is no significant influence of double freezing as well as of the rigor states to be seen. Variations in transition temperatures and enthalpies are not significant. On the other hand, concerning the industrially produced samples from cod and Alaska pollock the double freezing could obviously be

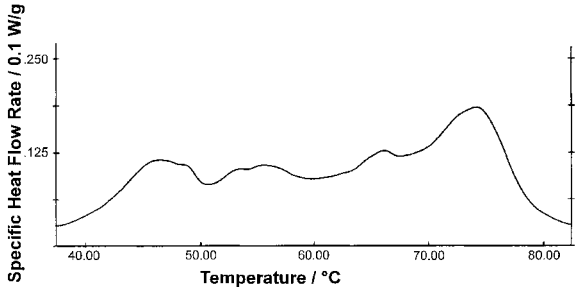

Fig. 1. PE-DSC 7 measured curve of frozen saithe fillet.

Table 1
T_{max} (°C) and ΔH (J/g ww) measured by PE-DSC 7 on SF and DF saithe fillets processed during the 184th cruise dependent on *rigor mortis* (PR-*pre-rigor,* IR-*in-rigor,* NR-*post-rigor*) after 15 months of frozen storage (*s* — standard deviation, $n = 3$ runs)

	T_{max}	*s*	T_{max}	*s*	T_{max}	*s*	T_{max}	*s*	ΔH	*s*
SFPR	46.39	0.26	54.15	0.29	62.98	0.98	73.53	2.26	2.12	0.57
DFPR	44.71	1.92	54.46	0.42	63.31	0.65	75.18	0.47	2.98	0.49
SFIR	44.19	1.62	53.15	1.03	62.32	0.42	74.82	0.29	1.75	0.71
DFIR	43.27	3.2	54.92	0.78	65.86	0.23	74.83	2.34	2.61	0.83
SFNR	44.97	0.95	53.19	0.56	66.42	2.2	74.87	0.46	1.64	0.42
DFNR	47.57	2.34	54.29	1.74	63.8	0.91	73.99	1.65	1.97	0.74

Table 2
T_{max} (°C) and ΔH (J/g ww) measured by PE-DSC 7 on SF and DF saithe fillets processed during the 195th cruise dependent on *rigor mortis* (PR-*pre-rigor*, NR-*post-rigor*) after three months of frozen storage (*s* — standard deviation, $n = 3$ runs)

	T_{max}	*s*	T_{max}	*s*	T_{max}	*s*	T_{max}	*s*	ΔH	*s*
SFPR	46.14	0.37	52.39	0.14	62.86	1.04	74.79	0.7	2.7	0.7
DFPR	46.44	0.18	54.26	1.06	63.29	0.91	74.39	0.4	1.6	0.8
SFNR	48.34	0.67	54.21	0.78	66.06	4.12	74.3	0.5	1.6	0.5
DFNR	45.98	1.31	56.14	0.54	63.94	2.27	73.59	1.2	1.8	0.6

Table 3
T_{max} (°C) and ΔH (J/g ww) measured by PE-DSC 7 on SF and DF fillets of Alaska pollock (AP) and cod (C) both commercially processed (*s* — standard deviation, $n = 3$ runs)

	T_{max}	*s*	T_{max}	*s*	T_{max}	*s*	ΔH	*s*
APSF	45.00	0.97	53.33	0.64	73.13	0.57	3.16	0.69
APDF	42.60	1.27	54.05	0.07	75.00	0.29	2.24	0.72
CSF	45.42	1.89	53.34	0.27	74.37	0.49	1.73	0.49
CDF	42.58	0.48	55.23	0.56	76.00	0.80	2.71	0.81

accompanied by a slight reduction of the first transition temperature (Table 3). But, this finding could not be verified using the other DSC (Table 4). The differences in T_{onset} as well as in T_{max} were not significant. Therefore, further research seems to be necessary to make clear if double freezing is connected with a shift of the myosin transition temperature in commercially processed products. The Micro DSC III curve (Fig. 2) shows only three distinct peaks. It was assumed before that Micro DSC would allow a better differentiation between the muscle protein components because the sample weight was approximately 50-fold of that used for DSC 7 measurement. As in the DSC 7 measurement no significant influence of double freezing as well as of rigor states could be detected when thermal analysis were performed using a Micro DSC III. In so far the results were consistent. Surprising is the shift towards lower transition temperatures compared with DSC 7 observed when a Micro DSC III was used. The only explanation for this distinct behaviour is to be

Table 4
T_{max} and T_{ons} (°C) and ΔH (J/g ww) measured by Micro DSC III on SF and DF fillets of Alaska pollock commercially processed

	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH	ΔH_{ges}
SFAP	31.17	35.34	0.36	46.55	49.86	0.14	59.12	67.53	0.47	0.96
DFAP	31.26	35.00	0.66	47.37	50.46	0.17	62.48	68.33	0.52	1.35

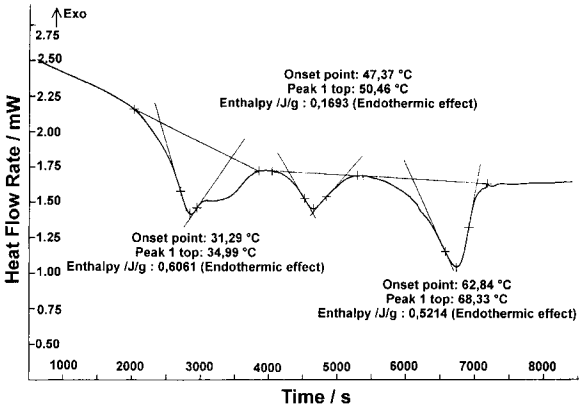

Fig. 2. Micro DSC III measured curve of frozen saithe fillet.

seen in the differences of the scanning rates. This assumption is supported by the data in Tables 5 and 6. They underline the influence of the scanning rate on both the transition temperature and enthalpy. In all transition peaks both T_{onset} and T_{max} are lowered, while the ΔH increased with decreasing scanning rate.

4. Discussion

The influence of freezing on the thermal stability of fish muscle proteins was subject of numerous studies [19,21–23]. Freezing followed by immediate thawing had little effect on the characteristic thermal transitions of cod muscle. However, after two weeks at $-10^{\circ}C$ it became apparent that myosin had undergone some partial denaturation. Continuous frozen storage resulted in little subsequent effect on the myosin transition after two weeks at $-10^{\circ}C$, while actin, collagen and sarcoplasmic proteins were largely unaffected by frozen storage [19]. Comparing the influence of frozen storage on different fish species it was noted that over frozen storage the first myosin transition evaluated by DSC on isolated fish myofibrils in cod became less defined as the transition became less cooperative. Trout myosin, however, showed a marked progressive increase in the relative size of the first peak, accompanied by a broadening of the myosin transition. Red snapper, red mullet, and catfish all exhibited broadening of the myosin transition over time, with the first myosin transition appearing more prominently after 6 weeks of frozen storage. The observed changes over frozen storage were similar in all species, also they occurred more slowly, i.e. to a lesser extent at a given time, in tropical than in cold-water species. Therefore it was concluded that tropical fish myosin are more stable than those of cold-water fish during frozen storage. The actin transition was usually not affected over iced or frozen storage [21]. In observing the thermal stability of proteins of a range of fish muscles of different habitat temperatures before and after frozen storage at $-20^{\circ}C$ the significant

Table 5
T_{max} and T_{ons} (°C) and ΔH (J/g ww) measured by Micro DSC III on SF and DF fillets of saithe processed during the 195th cruise dependent on *rigor mortis* (PR, *pre-rigor*; NR, *post-rigor*) after three months of frozen storage

	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH	ΔH_{ges}
SFPR	32.88	38.07	0.17	47.7	50.72	0.94	63.24	69.42	0.59	0.86
DFPR	33.59	38.26	0.16	46.38	50.22	0.18	62.1	68.26	0.44	0.78
SFNR	30.71	33.24	0.06	45.75	50.22	0.18	62.42	68.28	0.37	0.62
DFNR	30.2	34.28	0.13	46.63	50.14	0.17	62.61	67.94	0.38	0.68

Table 6
T_{max} and T_{ons} (°C) and ΔH (J/g ww) measured by Micro DSC III on *post-rigor* mortem SF and DF fillets of saithe processed during the 195th cruise after three months of frozen storage dependent on scanning rate (0.5 or 0.2 K/min)

	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH	ΔH_{ges}
SF(0.5)	30.71	33.24	0.06	45.75	50.22	0.18	62.42	68.28	0.37	0.62
SF(0.2)	29.30	34.93	0.48	44.28	49.68	0.26	61.66	67.04	0.52	1.26
DF(0.5)	30.20	34.28	0.13	46.63	50.14	0.17	62.61	67.94	0.38	0.68
DF(0.2)	28.78	31.25	0.40	45.45	48.85	0.20	59.81	65.32	0.30	0.89

dependence of myosin stability on preferred habitat temperature, with both T_{onset} and T_{max} of the myosin peak increasing with habitat temperature was confirmed. Prolonged frozen storage up to 54 weeks at $-20^{\circ}C$ had a marked effect on the myosin transition in all fish investigated. Sharp, narrow peaks became shallower and broader suggesting loss of cooperativity. Concerning enthalpy cold water species showed an increase on frozen storage but species from warmer habitats generally exhibited small decreases. There was little effect of frozen storage on the actin peaks [22]. Freezing and thawing of freshwater prawns caused a decrease of onset and peak melting temperatures corresponding to myosin denaturation. While the freezing rate caused no significant differences in the thermal properties, they were influenced by the rate of thawing [23]. Frozen storage of red meat at temperatures above $-20^{\circ}C$ led to reduction in the enthalpy of myosin without affecting the actin transition [24]. In a study about the influence of methods and operating conditions used to measure physical properties of food DSC was employed to determine the influence of freezing and frozen storage on the specific heat and enthalpy of pork and lamb [25].

The possibility of a marked influence of freezing and subsequent frozen storage on the thermal stability of muscle proteins has to be taken into account when discussing the own results. The thermal stability of muscle proteins seems to be influenced by post mortem ultrastructural changes [7]. In salted surimi sols, the enthalpy of transition decreased substantially after the development of *rigor mortis* from 0.41 cal/g (1.72 J/g) (*pre-rigor*) to 0.14 cal/g (0.59 J/g) (*post-rigor*), while the transition temperatures remained nearly constant [26]. It was further observed that the exotherm associated with muscle contraction in *pre-rigor* muscle disappeared gradually at the onset of rigor, while the denaturation endotherms of the muscle proteins remained unchanged [27]. While rigor state seems to have an influence on thermal stability of freshly caught unfrozen muscle, freezing obviously deletes differences caused by the various rigor states. Therefore no differences in thermal stability dependent on rigor mortis were detectable in both single and double frozen fillets.

The denaturation of proteins during freeze-thawing can be ascribed primarily to the increase of the ice–water interface during freezing. That is why surface denaturation plays in important role in the denaturation of proteins during freezing. 11 freeze/thaw cycles did not lead to an increased protein denaturation compared with a single freeze–thawing cycle [28]. In freshwater prawns subjected to multiple freeze–thaw cycles the enthalpy of protein denaturation was found to be decreased from 16.6 (fresh) to 13.5 J/g after one freeze–thaw cycle with minor changes thereafter [29]. When comparing cod fillets after 9 months of frozen storage at $-22^{\circ}C$ and at $-70^{\circ}C$ with those ones which were thawed slowly or fast after one week of frozen storage and additionally frozen at $-22^{\circ}C$ it was found that DSC analysis indicated that after the 9-month storage the thermal profile of the $-70^{\circ}C$ reference had not changed but treatments stored at $-22^{\circ}C$ exhibited a slight broadening of the complex endotherm between $30^{\circ}C$ and $50^{\circ}C$. The denaturation enthalpy did not differ between once frozen (ΔH = 2.57 J/g), ''slow thawed'' refrozen (ΔH = 2.78 J/g), and ''fast thawed'' refrozen (ΔH = 2.42 J/g) treatments. The $-70^{\circ}C$ reference showed a slightly higher denaturation enthalpy (ΔH = 2.94 J/g). It is concluded, therefore, that protein solubility decline observed was not caused by complete protein denaturation, as an endothermic event was clearly visible in samples with reduced protein solubility. The endotherm with $T_{max} \approx 75^{\circ}C$ was relatively unaffected by frozen storage and treatment. This would suggest that primarily the thermal stability of myosin changed on frozen storage, whereas actin was unaffected [30]. The aforementioned results on the effect of double freezing on thermal stability of muscle proteins are in good agreement with own results. Myosin seems to be the most susceptible protein fraction regarding to changes caused by freezing as well as refreezing. In contrast, in pressurised gels beside myosin also actin is affected dependent on the pressure and temperature regimes applied [14,15].

5. Conclusions

The results of this study deliver additional evidence that it is not possible to differentiate between single and double frozen fish fillets by the use of differential scanning calorimetry. These result is in a good agreement with the relevant literature and with the results of chemical and sensorial investigations performed using

the same samples. The rigor state of the raw material before freezing does not seem to affect the thermal behaviour of the finished product.

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