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Influence of chilled and frozen storage on the stability of trout and herring fillet

Einfluss von Kühl- und Gefrierlagerung auf die Stabilität von Forellen- und Heringsfilets

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Abstract

Effects of chilled and frozen storage on specific enthalpy (ΔH) and transition temperature (T_d) of protein denaturation as well as on selected functional properties of muscle tissue of rainbow trout and herring were investigated. The T_d of myosin shifted from 39 to 33 °C during chilling of trout post mortem, but was also influenced by pH. Toughening during frozen storage of trout fillet was characterized by an increased storage modulus of a gel made from the raw fillet. Differences between long term and short term frozen stored, cooked trout fillet were identified by a compression test and a consumer panel. These changes did not affect the T_d and ΔH of heat denaturation during one year of frozen storage at −20 °C. In contrast the T_d of two myosin peaks of herring shifted during frozen storage at −20 °C to a significant lower value and overlaid finally. Myosin was aggregated by hydrophobic protein-protein interactions. Both thermal properties of myosin and chemical composition were sample specific for wild herring, but were relative constant for farmed trout samples over one year. Determination of T_d was very precise (standard deviation <2 %) at a low scanning rate (≤ 0.25 K·min^{-1}) and is useful for monitoring the quality of chilled and frozen stored trout and herring.

Kurzfassung

Der Einfluss der Kühl- und Gefrierlagerung von Regenbogenforelle und Hering auf die spezifische Enthalpie (ΔH) und die Übergangstemperatur (T_d) und ausgewählte funktionelle Eigenschaften der Muskelproteine wurde untersucht. Die Lagerung von Forelle auf Eis führt post mortem zu einer von 39 auf 33 °C fallenden Td von Myosin, wobei ein Einfluss des pH-Wertes beobachtet wird. Die Verfestigung des Forellenfilets während der Gefrierlagerung wird mittels steigender Werte des Speichermoduls in einem aus dem Filet hergestellten Gel gemessen. Texturunterschiede zwischen langzeitig und kurzzeitig gefroren gelagerten Forellenfilets werden im Kompressionstest und mit einem Konsumenten-Panel analysiert. Diese Veränderungen beeinflussten nicht die T_d und ΔH der Hitzedenaturierung der Proteine während der Lagerung von einem Jahr bei −20 °C. Im Gegensatz dazu verschieben sich die Td von zwei Myosinpeaks in Heringsfilet während der Lagerung bei −20 °C auf geringere Werte und überlagern sich schließlich. Myosin wird in Proteinagglomeraten hydrophob gebunden. Sowohl die thermischen Eigenschaften von Myosin, als auch die chemische Zusammensetzung von Hering sind probenspezifisch, während diese Eigenschaften bei Zuchtforelle aus einer Aquakultur im Beobachtungszeitraum von einem Jahr relativ konstant sind. Die Bestimmung von T_d ist sehr genau (Standardabweichung < 2%), wenn mit einer geringen Heizrate (≤ 0,25 K·min^{-1}) gemessen wird. Die Messung der T_d ist geeignet, um die Qualität von Forelle und Hering während der Kühl- und Gefrierlagerung zu überwachen.

Introduction

Freezing is an effective method for conservation of fish. Unfavourable is the simultaneous deterioration of muscle proteins resulting in toughening of the texture as well as in changes of several functional properties (Matsumoto 1980; Mackie 1993; Zayas 1997).

The aggregation of myosin has been shown to be a plausible reason for the toughening in frozen stored fish (Matthews et al. 1980; Kitazawa et al. 1997; Careche et al. 1998; Torrejón et al. 1999; Ramírez et al. 2000). The influence of lipid oxidation on toughening of fish fillet has been investigated at salt water fish species (Careche et al. 1990; Aubourg 1999; Saeed and Howell 2004) and the role of formaldehyde for texture changes has been discussed controversially (Sotelo et al. 1995; Badii and Howell 2001). Though the influencing parameters on texture changes are not the same for different species of fish but the muscle proteins are always the target of texture changing agents.

Protein stability under thermal stress could be described by means of the heat capacity (ΔH) and the transition temperatures (T_d) and will be used to classify the protein composition of muscle tissue and deterioration during frozen storage. ΔH and T_d of proteins have been shown to vary with fish species, to depend on the habitat conditions and on time of frozen storage (Hastings et al. 1985;

Davies et al. 1994). Badii and Howell (2002) studied the effect of frozen storage on cod and haddock and detected a tendency of decreasing T_d and ΔH of the myosin peak at –10 °C accompanied by a firmer texture of the fillet. Schubring (2004) measured hardening of cod fillet stored at –10 °C; nevertheless T_d and ΔH were not affected. Decreasing T_d and ΔH of myosin are linked to a firmer texture in mackerel which had been stored at –20 °C up to 2 years (Saeed and Howell 2004).

The aim of this work was to examine, whether the thermal properties of muscle proteins are reliable markers for monitoring deterioration of muscle protein during chilled and frozen storage of trout and herring. Thermal properties of the tissue were measured by dint of a high sensitive Calvet DSC to get at best possible partition of peaks with a low scanning rate. Farmed rainbow trout was used to exemplify fresh water fish and because the all-season availability, and herring from the Baltic Sea to exemplify salt water fish with special features at processing in consequence of frozen storage. The texture of a gel was investigated by a small deformation test and cooked fillets were investigated by a large deformation test and a sensory panel.

Materials and methods

Materials

Rainbow trout (*Oncorhynchus mykiss*) had been raised at an aquaculture farm (Uhthoff, Neubrandenburg, Germany). Live weight of the fish was 300 to 400 g. The fish were stunned after capture, gutted and washed immediately. The fish were then kept on ice until post rigor stadium (~48 hours), vacuum packed and frozen in a chamber freezer at –20 °C. Analyses were done after 1 week of storage (short-term-storage – sts) and various intervals of up to 50 weeks (long-term-storage – lts). To minimise fish-to-fish variation, a minimum of three fish were thawed over night at 2 °C to reach a temperature of about 0 °C. The fish were filleted and kept on ice until analysis.

Herring (*Clupea harengus*) from the southern Baltic Sea was supplied by Eurobaltic AG (Sassnitz, Germany). The landed herring had attained post rigor stadium and was classified with a quality index of 1 to 2. The fish were immediately machine filleted and transported on ice to the laboratory within 2 hours. The fillets were vacuum packed and frozen in a chamber freezer at –20 °C. Time between capture and freezing was about two days. For analysis a minimum of 5 fillets of herring were thawed at 10 °C for one hour.

Differential scanning calorimetry (DSC)

25 g of muscle tissue were taken from the middle part of the chilled fillets observing a minimum distance of 3 cm from the head and tail end. The tissue was homogenised by a mill fitted with a star-shaped cutter for 10 s (A10, IKA-Werke, Staufen, Germany). DSC studies were performed in a Micro DSC VII (Setaram, Caluire, France). 850 ± 20 mg of the sample were transferred to a stainless steel batch vessel. Heating rate was 0.25 °C·min^{-1} over the range of 15 to 90 °C; cooling rate was 1 °C·min^{-1}. A baseline was installed once a day and subtracted from the first heating curve of each sample. A linear baseline was installed from the first to the last point of a peak. Specific enthalpy (ΔH) is the area under the peak divided by the sample mass. The transition temperature (T_d) is the tip of the peak, which was evaluated by Setsoft 2000 (Setaram, Caluire, France), but sometimes a manual correction was necessary. Three replicates of each sample were tested and every replicate was prepared from the frozen fillet.

Extraction of proteins

Homogenised fillet was prepared as described for DSC and was diluted with 3 volumes of 3 mM $NaHCO_3$ for water soluble protein (wsp), at pH 6.5, or additional 0.6 M NaCl for salt soluble protein (ssp) and homogenized with a Ultra-Turrax. Extraction was performed by soft shaking with 50 volumes of the same buffer as before at 2 °C for 30 min. The extract was centrifuged at 12 000 × g for 10 min at 4 °C. The supernatant was used for SDS-PAGE and for the determination of protein concentrations.

Protein solubility

The protein concentration was determined by the method of Bradford (1976) using a Roti-Quant assay (Roth, Germany).

Sample composition

Protein content was determined according to Kjeldahl, dry substance by drying in sand at 103 °C and fat content by ether extraction (AOAC, 1990). The pH was determined after homogenizing the sample with 50 % of distilled water and with an inoLab Level 3 measuring system equipped with a SenTix Sp electrode (WTW, Weilheim, Germany).

Large deformation testing

Fish fillet was cut into 5 × 5 cm pieces, wrapped in aluminium foil and heated to 75 °C. Core temperature was controlled at three pieces of each charge. The fillet was further cooked at 75 °C for further 5 min and annealed at 40 °C in a climate chamber until compression test and sensory analysis but not longer than 30 min.

Fillets were compressed to an intrusion of 4 mm with a 12 mm cylindrical probe (P/0.5) on a Texture Analyser (TA-XT2i; Stable Micro Systems, GB). Test speed was 0.5 mm·s^{-1}. The area under the curves of 5 fillets each compressed twice at different spots were measured and evaluated as work of compression (Software: Texture Expert, Stable Micro Systems, GB).

Small deformation testing

Homogenised fillet was prepared as described for DSC. 13.5 g of the paste like sample were mixed with 1.5 g distilled water. The mixture was transferred to a rotation rheometer (MC 100, Paar Physica, Germany) equipped with a covered 50 mm plate-plate system (MP 31) with a gap of 3 mm. After 5 min relaxation measurement was carried out at 6 °C to a deformation of 2 % and at a frequency of 3 Hz. A total time of measurement of 120 min was used to evaluate the swelling capacity of the protein. Storage modulus was the mean of all values measured between 110 to 120 min and of three replicates.

Triangle test

To control texture differences a discriminative test (triangle test) was carried out by a consumer panel (ISO 1983). The samples were prepared like described for large deformation testing. The panel tested the samples by chewing.

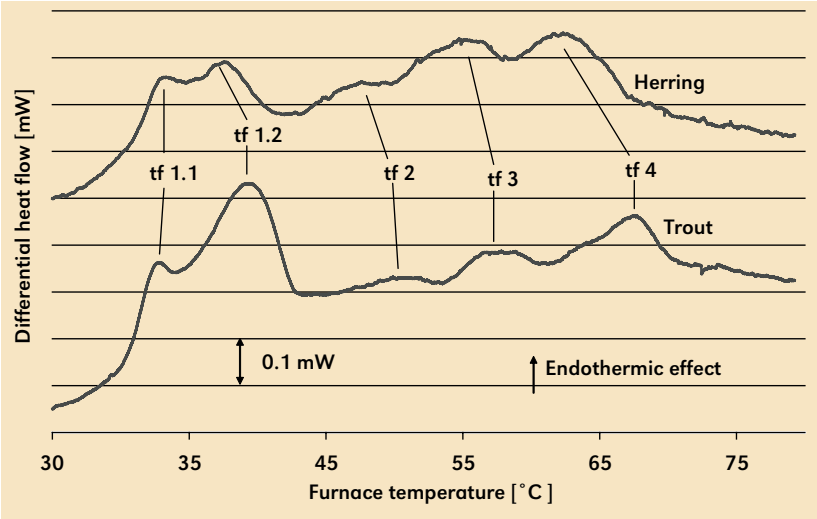

Figure 1: Typical DSC thermograms of short term, frozen stored trout and herring fillet and indication of thermal fractions (tf). Other transition temperatures may occur depending on the sample and time of storage.

Abbildung 1: Typische DSC-Thermogramme von kurzzeitig gefroren gelagerten Forellen- und Heringsfilets. Ausgewiesen sind die Bezeichnungen der thermischen Fraktionen (tf). In Abhängigkeit von der Probe und der Lagerzeit können andere Übergangstemperaturen auftreten.

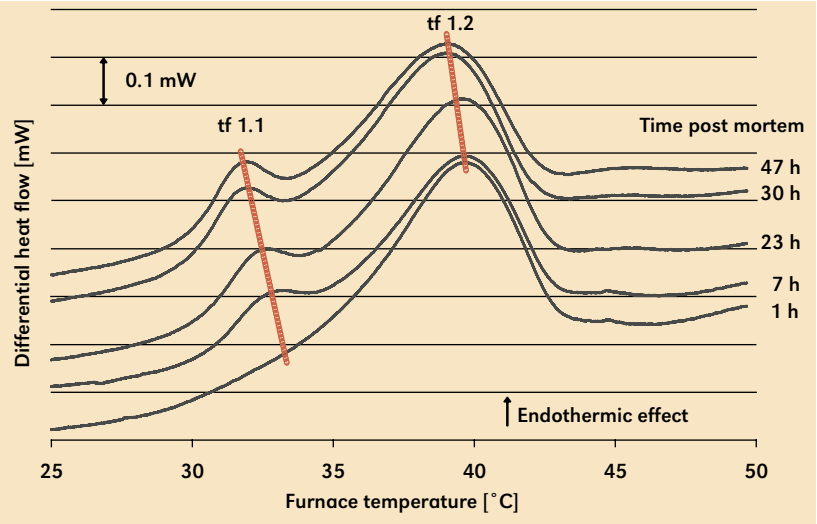

Figure 2: DSC thermogram of post mortem chilled trout fillet: Shift of transition temperatures of thermal fractions 1.1 and 1.2.

Abbildung 2: DSC-Thermogramme von post mortem auf Eis gelagertem Forellenfilet: Veränderungen der Übergangstemperaturen der thermischen Fraktionen 1.1 und 1.2.

Results and discussion

Changes in thermal stability of myosin post mortem during chilled storage of trout.

Up to 5 thermal fractions of proteins could be clearly recognized at muscle tissue of chilled or frozen stored trout and herring (Figure 1).

The thermally most unstable protein fraction (tf 1) of trout fillet splits post mortem into two thermal fractions: tf 1.1 and tf 1.2. The transition temperature (T_d) of tf 1.1 decreased, whereas T_d of tf 1.2 stagnated and subsequently decreased (Figure 2). We conclude from the observed shift of T_d a lower cooperativety of the proteins and the disintegration of tf 1.1 from the initial intact muscle fibre.

The thermal fraction tf 1.1 of trout has been identified as a ~200 kDa protein and for that is likely to present the myosin heavy chain. The tf 1.2 of trout presents proteins, which could not be detected by SDS-PAGE because of the high molecular weight or poor insolubility (Beyrer 2006). From the splitting of tf 1 into tf 1.1 and 1.2 during the rigor stadiums we conclude tf 1.2 to be a complex of proteins which includes myosin.

The splitting of the thermal fraction 1 followed the same pattern at four samples of trout derived from the same aquaculture and were handled equally. Nevertheless, the time course of the T_d shift of tf 1.1 and tf 1.2 was different for every sample (Table 1).

Table 1: Transition temperature of the thermal fraction 1.1 and 1.2 at chilled trout fillet (post mortem).
n. d. = not detected; – = tf 1.1 runes together with tf 1.2.

Tabelle 1: Übergangstemperatur der thermischen Fraktionen 1.1 und 1.2 in Forellenfilet (post mortem, auf Eis gelagert).
n. d. = nicht ermittelt; – = für tf 1.1 und tf 1.2 ist nur eine Übergangstemperatur nachweisbar.

Time *post mortem* [h]	Sample 2		3		4		15	
	Transition temperature [°C]							
	tf 1.1	tf 1.2	tf 1.1	tf 1.2	tf 1.1	tf 1.2	tf 1.1	tf 1.2
1 – 2	–	38,8	–	39,5	33,1	39,9	–	38,7
6 – 7	n. d.	n. d.	32,9	39,5	33,2	39,6	n. d.	n. d.
22 – 24	–	38,8	32,5	39,5	32,2	39,4	–	38,9
29 – 30	–	38,5	32,3	39,5	32,6	39,7	–	39,0
46 – 48	32,7	39,0	32,4	39,3	n. d.	n. d.	–	38,9
54	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	–	39,1
216	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	33,2	39,1

T_d of myosin of cod is pH-dependent (Ofstad et al. 1996; Jensen and Joergensen 2003) as well as the T_d of isolated myofibrils of cod and trout at the ionic strength of 0.05 (Howell et al. 1991). Chilled storage of trout is accompanied by a pH shift from 6.1 to 6.5 at sample 4 (Table 1) and T_d of tf 1.1 and 1.2 can be similarly shifted in an emulsion of trout fillet and 0.1 M Sörensen phosphate buffer (data not shown). Hence the authors suppose the T_d of tf 1.1 as a sample specific marker for the quality of post mortem chilled stored trout. It is necessary to characterise the influences of farming and handling of trout on the pH and on the shift on T_d of tf 1.1 by further investigations.

Protein stability of long term stored (lts) and short term stored (sts) trout at –20 °C

The quality of frozen trout has been analysed monthly over one year and compared to the quality of one week (short term) frozen stored trout.

The average protein content of all samples was 202 $g \cdot kg^{-1}$ of fresh substance and changed within the range of 4 $g \cdot kg^{-1}$ or 2 % respectively. Reproducibility of the method is about 1 % of the final protein amount. All trout samples collected from the same aquaculture (Tab. 2, 3 and 4) showed a very similar protein content over one year. Because the fish-to-fish and catch-to-catch variations are so low, we conclude a sufficient comparability of the samples at thermal analysis.

T_d of tf 1.1 (myosin) and tf 1.2 and ΔH of all protein transitions of long term stored (lts) trout did not shown a statistically proved trend over one year (Table 1). Single values, which are different from the one year average, need to be revised and shouldn't focus attention for interpretation. A relative constant T_d had been measured even for tf 4 (Table 1). Similarly, T_d of myosin, actin and sarcoplasmic proteins of mackerel had been constant over one year of frozen storage at –20 °C, but T_d and ΔH of myosin declined at prolonged frozen storage or at a storage temperature of –10 °C (Saeed and Howell 2004). We conclude the protein of trout is thermodynamically quite stable over one year at –20 °C.

Standard deviation of the average T_d is < 1 % at most cases (Table 2 and 3). This exceptionally low value might be due to the low heating rate of 0.25 $K \cdot min^{-1}$ at DSC measurement.

8 of 10 sts samples of trout showed the same average T_d as the lts samples for tf 1.1; tf 1.2 and tf 4 (Table 2 vs. Tab 3). The observation supports the assumption of the thermal stability of trout protein at long term frozen storage. Two out of ten sts trout samples have shown a lower T_d for tf 1.2, but only if tf 1.1 was not detectable and melts into the peak of tf 1.2 (Table 3, sample 22/9 and 35/11). That indicates thermally interacting protein-filaments and a high quality of these samples at the time of freezing (see preceeding section on post rigor changes).

The elastic properties of a water-protein-fat emulsion are attributed mainly to the protein. Badii and Howell (2002) and Saeed and Howell (2004) interpreted the storage modulus (G') of comminuted haddock, cod and mackerel muscle as the elasticity of the broken protein fibres and showed that the elasticity of the muscle fibres had been changed as a result of frozen storage.

G' will be influenced by the swelling of comminuted muscle fibres. We decided to evaluate the G' of the samples after two hours of swelling and examined swelling by a small deformation test. Time depended swelling increased G' of the gel and expanded G'-differences but at no case changed the order of the G' of two samples (data not shown).

The G' of homogenated muscle of trout increased after a storage time longer than 13 weeks at –20 °C (Table 4).

The protein solubility in lts trout fillet declined to 69 % over 13 weeks (Table 4) and to 60 % over 30 weeks. The subsequent increase up to over 100 % is unexpected

Table 2: Influence of storage time at –20 °C on thermal properties of trout fillet: Transition temperature (T_d) and specific enthalpy (ΔH). The values are given as mean ± standard deviation (sd). n = 3

Tabelle 2: Einfluss der Lagerzeit bei –20 °C auf die thermischen Eigenschaften von Forellenfilet: Übergangstemperatur (T_d) und spezifische Enthalpie (ΔH). Angegeben sind Mittelwerte und Standardabweichungen (sd). n = 3.

Weeks of storage	T_d [°C]			ΔH [J·g^{-1}]	
	tf1.1	tf1.2	tf4	tf1.1 ± 1.2	tf1-4
1	32.2 ± 0.1	39.4 ± 0.0	67.6 ± 0.2	0.8 ± 0.06	2.1 ± 0.22
5	32.4 ± 0.2	39.1 ± 0.2	68.1 ± 0.2	0.7 ± 0.05	1.9 ± 0.10
9	31.6 ± 0.1	39.3 ± 0.6	68.0 ± 1.0	0.8 ± 0.06	2.3 ± 0.46
13	31.8 ± 0.6	38.6 ± 0.4	67.8 ± 0.6	0.8 ± 0.07	2.0 ± 0.23
17	31.9 ± 0.1	39.6 ± 0.4	68.3 ± 0.3	0.8 ± 0.5	1.8 ± 0.10
22	31.8 ± 0.2	39.3 ± 0.3	69.1 ± 0.6	0.7 ± 0.02	1.8 ± 0.29
30	31.0 ± 0.1	40.3 ± 0.1	69.5 ± 0.4	0.7 ± 0.03	1.6 ± 0.18
35	31.5 ± 0.5	39.4 ± 0.3	67.8 ± 0.3	0.6 ± 0.04	2.1 ± 0.04
41	31.8 ± 0.5	38.8 ± 0.6	68.2 ± 1.2	0.7 ± 0.6	2.1 ± 0.13
50	32.1 ± 0.2	39.2 ± 0.3	69.4 ± 0.1	0.7 ± 0.04	2.2 ± 0.09
mean	31.8	39.3	68.4		
sd [% of mean]	1	1	1		

Table 3: Influence of the sample on thermal properties of short term stored trout fillet at –20 °C: Transition temperature (T_d) and specific enthalpy (ΔH). The values are given as mean ± standard deviation (sd). n = 3; n. d. = not detected.

Tabelle 3: Einfluss der Probe (Fang) auf die thermischen Eigenschaften von kurzzeitig, bei –20 °C gelagertem Forellenfilet: Übergangstemperatur (T_d) und spezifische Enthalpie (ΔH). Angegeben sind Mittelwerte und Standardabweichungen (sd). n = 3; n. d. = nicht ermittelt.

Weeks of storage / Sample no.	T_d [°C]			ΔH [J·g^{-1}]]	
	tf 1.1	tf 1.2	tf 4	tf 1.1+1.2	tf 1-4
1 / 1	32.2 ± 0.1	39.4 ± 0.0	67.6 ± 0.2	0.8 ± 0.06	2.1 ± 0.22
5 / 2	33.2 ± 0.1	38.8 ± 0.1	68.0 ± 0.3	0.7 ± 0.17	1.9 ± 0.36
9 / 4	32.5 ± 0.1	39.0 ± 0.1	67.7 ± 0.7	1.0 ± 0.02	2.6 ± 0.43
13 / 5	31.9 ± 0.3	39.6 ± 0.1	67.5 ± 0.2	1.1 ± 0.02	2.3 ± 0.01
17 / 7	32.3 ± 0.1	39.0 ± 0.2	68.1 ± 0.1	1.2 ± 0.07	1.9 ± 0.16
22 / 9	n. d.	38.3 ± 0.2	68.9 ± 0.5	0.6 ± 0.15	1.5 ± 0.66
30 /10	31.5 ± 0.5	39.4 ± 0.3	67.8 ± 0.3	0.6 ± 0.04	2.1 ± 0.04
35 /11	n. d.	37.2 ± 0.1	68.6 ± 0.0	0.8 ± 0.00	2.2 ± 0.03
41 /14	32.7 ± 0.1	38.6 ± 0.2	68.1 ± 0.3	0.9 ± 0.10	2.2 ± 0.11
50 /16	33.4 ± 0.7	38.6 ± 0.1	68.2 ± 0.1	0.9 ± 0.03	2.2 ± 0.22
mean	32.5	39.1	67.9		
sd [% of mean]	2.0	1.0	0.4		

(Table 4). Nevertheless, the changes of protein solubility as well as the changes of G' indicate the instability of proteins at long-term frozen storage of trout. Matsumoto (1980) proposed a model for the agglomeration of different myofibrillar proteins on the basis of protein changes of fish detected by numerous authors. From the increased G' and declined protein solubility in muscle tissue of trout we conclude the agglomeration of the proteins during frozen storage at –20 °C.

After parallel steam cooking the long term stored (lts) and short term stored (sts) fillets had been directly compared by a consumer panel in a triangle test and by a compression test. The cooked lts fillets differed from the sts fillets only, if the lts fillets stored longer than 35 weeks at –20 °C (Table 4). Texture changes measured at the cooked trout fillets are in agreement with the increasing G' at the small deformation testing of raw trout fillet homogenates.

Nevertheless, the moderate texture changes of frozen stored trout fillet are not correlated with stable thermal properties T_d and ΔH of the proteins (Table 1 vs. Table 4). Saeed and Howell (2004) studied the effect of storage at –20 °C on the DSC thermograms and G' at 80 °C of comminuted fillets of cod. G' reflected differences of the fillets after shorter storage times than the thermal analysis. Schubring (2004) determined no significant changes of T_d and ΔH as a consequence of the storage of cod at –20 °C although the texture changed significantly. At this state of art thermal analysis reflects changes of myofibrillar proteins, which are likewise measurable by established physical, sensory and chemical methods. But investigations on the influence of sample handling prior to the thermal analysis might lead to a higher sensitivity of the measuring. Furthermore, we suggest the thermal analysis of muscle tissue which had not been heat denatured during analysis. For example effects of freezing

Table 4: Influence of storage time on muscle tissue of trout: protein solubility and storage modulus (G') of the comminuted fillet, compression and sensory test on the cooked fillet. The values are given as mean ± standard deviation. n = 4 for protein solubility and G', n = 10 for compression and sensory test; values with subsequent letters and samples characterised as "different" (>) are significantly different ($p < 0.05$).

Tabelle 4: Einfluss der Lagerzeit auf das Muskelgewebe von Forelle: Proteinlöslichkeit und Speichermodul (G') des homogenisierten Filets, Kompressionstest und sensorische Analyse des gegarten Filets. Angegeben sind Mittelwerte und Standardabweichungen. n = 4 für die Proteinlöslichkeit und G', n = 10 für den Kompressionstest und die sensorische Analyse; Werte mit nachfolgenden unterschiedlichen Buchstaben gekennzeichnet sind und als „unterschiedlich" (>) markierte Proben unterscheiden sich signifikant ($p < 0,05$).

Duration of storage of lts samples (Weeks)	Protein olubility in 0,6 M NaCl [%]		G' [10^3 Pa]		Work of compression [N·mm]		Sensory test
	lts	sts	lts	sts	lts	sts	lts vs sts
1	100 ± 1 a	100 ± 1	10.8 ± 1.2 a	n.d.	5.3 ± 0.3	n.d.	n.d.
5	100 ± 0 a	99 ± 1	11.6 ± 0.3 a	10.4 ± 0.8 a	7.2 ± 0.8 =	7.0 ± 0.5	lts = sts
9	97 ± 1 c	97 ± 1	11.1 ± 0.3 a	9.9 ± 0.6 a	10.9 ± 1.9 =	8.2 ± 1.2	lts = sts
13	69 ± 1 d	77 ± 1	10.6 ± 0.6 a	10.6 ± 0.8 a	8.0 ± 1.2 =	7.9 ± 1.2	lts = sts
35	102 ± 0 a	104 ± 1	12.7 ± 0.7 b	10.0 ± 0.2 a	9.8 ± 0.8 =	8.9 ± 1.8	lts ≠ sts
41	111 ± 0 b	102 ± 1	12.3 ± 0.3 b	7.0 ± 0.1 d	10.9 ± 0.9 e >	6.9 ± 1.4 f	lts = sts
50	79 ± 0 f	128 ± 1	15.4 ± 0.6 c	10.7 ± 0.4 a	7.8 ± 0.9 g >	5.0 ± 0.5 h	lts ≠ sts

on muscle tissue might be identified by the specific heat capacity of the not heat denatured tissue (Beyrer and Ruesch gen. Klaas 2006).

Protein stability during frozen storage of herring at –20 °C

In contrast to trout the myosin correlated fraction tf 1 in herring is thermodynamically not stable at frozen storage. T_d of tf 1.1 and tf 1.2 decreased significantly ($p < 0.05$) during 16 weeks of frozen storage (Table 5). After prolonged storage only tf 1.2 was detected, and tf 1.1 melted into tf 1.2. The T_d of tf 1.1 + 1.2 declined further significantly ($p < 0.05$), which indicates an ongoing process of destabilisation of the proteins.

At SDS-PAGE we identified tf 1.1 as the ~200 kDa-myosin (Beyrer 2006). At the same time tf 1.1 was not detectable at thermal analysis, the ~200 kDa-myosin was missed at SDS-PAGE. If 8 M urea was supplemented to the 0.6 M NaCl extraction buffer, tf 1.1 appeared again at SDS-PAGE (Beyrer 2006). For example, cold induced, apolar, hydrophobic protein-protein interactions are likely to be dissolved by the urea in sardine fillets (Gómez-Guillén et al. 1997). The cross-linking of tf 1.1 of herring with other proteins is probable. This cross-linking might lead to a thermodynamically cooperative behaviour at DSC analysis and to the observed melting of tf 1.1 into tf 1.2.

Over 16 weeks of frozen storage the concentration of salt soluble and water soluble proteins declined monotonic to about 54 % and 64 % respectively (Table 5). This is likely to be caused by the agglomeration of proteins. Afterwards the protein solubility showed an unsuspected local maximum. Determination of the concentration of soluble proteins bases on the protein concentration of the solution and on the affinity of the proteins to the anionic Coomassie Brilliant Blue (Bradford reagent). It is im-

Table 5: Influence of storage time at –20 °C on properties of herring fillet: Protein solubility, transition temperature (T_d) and specific enthalpy (ΔH). The values are given as mean ± standard deviation. Values in the same row, with subsequent letters are significantly different ($p < 0.05$). n = 3.

Tabelle 5: Einfluss der Lagerzeit bei 20 °C auf die Eigenschaften von Heringsfilet: Proteinlöslichkeit, Übergangstemperatur (T_d) und spezifische Enthalpie (ΔH). Angegeben sind Mittelwerte und Standardabweichungen (sd). Werte in der gleichen Reihe mit nachfolgenden unterschiedlichen Buchstaben unterscheiden sich signifikant ($p < 0,05$). n = 3.

Storage time	Protein solubility [%]		T_d [°C]			ΔH [J·g^{-1}]	
[weeks]	ssp	wsp	tf 1.1	tf 1.2	tf 4	tf 1.1 + 1.2	tf 1–4
1	100 ± 2	100 ± 1	38.0 ± 0.1 a	39.9 ± 0.0 a	65.3 ± 0.2	0.7 ± 0.04 a	2.4 ± 0.08 a
5	79 ± 1	52 ± 1	37.9 ± 0.1 a	39.8 ± 0.1 b	66.4 ± 0.1	0.6 ± 0.03 b	2.2 ± 0.01 b
9	58 ± 2	68 ± 2	37.4 ± 0.2 b	39.2 ± 0.1 c	65.4 ± 0.4	0.6 ± 0.04 b	2.2 ± 0.17 b
16	54 ± 2	64 ± 4	37.1 ± 0.2 b	39.2 ± 0.2 c	65.6 ± 0.1	0.5 ± 0.05 b	2.0 ± 0.12 b
21	119 ± 2	122 ± 1		38.6 ± 0.2 d	65.3 ± 1.1	0.6 ± 0.04 b	2.1 ± 0.16 b
27	133 ± 2	122 ± 3		38.2 ± 0.2 d	66.2 ± 0.8	0.6 ± 0.06 b	2.1 ± 0.07 b
34	96 ± 2	85 ± 4		37.6 ± 0.3 e	66.1 ± 0.1	0.6 ± 0.02 b	2.0 ± 0.01 c

possible to value the influence of affinity changes of the proteins on the solubility measured by a Bradford protein assay and without additional analysis. Nevertheless the authors conclude the instability of muscle proteins at frozen storage of herring.

Specific enthalpy of all thermal fractions (ΔH of tf 1-4) of herring had a tendency to decrease within the storage time of 34 weeks (Table 5). In agreement with that, Davies et al. (1994) described the myosin peaks of herring disappeared immediately during storage of the fillet over 54 weeks at –20 °C, but numerical details are not given. In contrast this trend couldn't be shown for trout (Table 1) or mackerel (Saeed and Howell 2004) if stored at –20 °C over one year. Decreasing ΔH of protein of herring can be attributed to a lower stability of the protein at frozen storage in comparison to trout or mackerel.

The ΔH of the thermally most unstable fractions tf 1.1 and 1.2 was relative stable during frozen storage. The determination of ΔH of tf 1.1 and 1.2 is influenced by changes of the specific heat capacity (c_p) of the native muscle tissue during frozen storage of herring and that for couldn't be valued as a criterion of protein stability determined by heat denaturation (Beyrer and Ruesch gen. Klaas 2006). We conclude a need to consider c_p changes which influence the baseline at determination of ΔH of the heat denaturation of proteins.

T_d and ΔH of herring fillet with a different chemical composition differed significantly (Table 6). The control of protein stability at frozen storage demands an individual data basis, if T_d of tf 1.1 and 1.2 should be used as markers.

Table 6: Influence of the chemical composition on the transition temperature (T_d) and specific enthalpy (ΔH) of herring fillet stored at –20 °C for 1 week. The values are given as mean ± standard deviation. If sd is not given sd = ± 1 %. Values with following letters are significantly different ($p < 0.05$). n = 3.

Tabelle 6: Einfluss der chemischen Zusammensetzung von Heringsfilet auf die Übergangstemperatur (T_d) und die spezifische Enthalpie (ΔH). Die Filets wurden 1 Woche bei –20 °C gelagert. Angegeben sind Mittelwerte und Standardabweichungen (sd). Falls sd nicht angegeben wird, gilt: sd = ± 1 %. Werte, die mit unterschiedlichen Buchstaben gekennzeichnet sind, sind signifikant unterschiedlich ($p < 0,05$).

Feature		Month of catching	
		April	November
T_d [°C]	tf1.1	38.0 ± 0.1 a	33.9 ± 0.7 b
	tf1.2	39.9 ± 0.0 c	37.6 ± 0.3 d
	tf4	65.3 ± 0.2 e	62.6 ± 0.5 f
ΔH [J·g^{-1}]	tf1.1 + 1.2	0.7 ± 0.04 g	0.4 ± 0.06 h
	tf1-4	2.4 ± 0.08 i	2.0 ± 0.21 j
Protein [g·kg^{-1}]		153	191
Water [g·kg^{-1}]		797	743
Fat [g·kg^{-1}]		34	42

Conclusions

Thermal analysis of heat denaturation of muscle proteins reflects the changes of protein stability during storage of herring at –20 °C but not at trout. Thereby the myosin heavy chain is the instable protein at freezing of herring but also at chilling of trout. This could be measured by the denaturation temperature (T_d) at heating of the proteins.

Thermal properties of muscle tissue of farmed rainbow trout had been shown to be relative stable at different charges with similar chemical composition, but had been different at wild herring with different chemical composition. Control of protein stability during frozen storage of wild fish demands an individual data basis.

Measurement of thermal properties of heat denaturation of fish muscle proteins gives additional information's on protein denaturation during frozen storage but alternative parameters, like pH, protein solubility and rheological parameters of muscle tissue, indicate protein changes earlier during storage. To improve the sensibility and information content of thermal analysis of muscle tissue the authors suggest investigations on the influence of sample preparation on thermal analysis and detection of thermal properties of the not heat denatured material.

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