Changes in Texture, Water Holding Capacity, Colour and Thermal Stability of Frozen Cod *(Gadus morhua)* Fillets: Effect of Frozen Storage Temperature

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Summary
Frozen storage of individually packed cod fillets for up to 13 months caught in the Barents Sea revealed differences in quality caused by storage temperature. While at −14 °C already after 7 months pronounced changes in texture parameters and water holding capacity were observed, changes of quality parameters at −28 °C after 13 months storage were negligible. Changes in same parameters of single and double frozen samples stored at −20 °C were between both. Numerous linear correlations could be observed between instrumentally measured parameters and storage time. However, frequency of correlations was decreasing with decreasing storage temperature. Deviating from this behaviour, changes in redness with storage time were more wave-like instead of linear. Changes in DSC curves were found for the myosin peak, whereas the actin transition was almost unaffected. Compared with earlier results obtained on Baltic Sea cod the transition temperatures for both myosin and actin shifted to lower temperatures indicating the influence of environmental conditions on thermal stability of muscle proteins.

Zusammenfassung
Die TK-Lagerung bis zu 13 Monaten von einzeln verpackten Dorschfilets aus der Barentssee machte temperaturbedingte Qualitätsunterschiede deutlich. Während die bei −14 °C gelagerten Filets bereits nach 7 Monaten merkliche Unterschiede in den Texturmerkmalen und der Wasserbindung aufwiesen, waren entsprechende Veränderungen bis −28 °C geringfügig. Auch die Unterschiede in den untersuchten physikalischen Parameters zwischen einfach und doppelgefrorenen Filets bei einer Lagertemperatur von −20 °C waren gering. Zahlreiche lineare Korrelationen zwischen den einzelnen Parametern und der Lagerzeit wurden nachgewiesen, deren Häufigkeit und Ausprägung sich allerdings mit abnehmender Lagertemperatur verringerten. Eine Ausnahme stellten die wellenartigen Veränderungen des Rotwertes mit zunehmender Lagerzeit unabhängig von der Lagertemperatur dar. In den DSC-Kurven zeigten sich beim Myosin-Peak Veränderungen, die auf eine Denaturierung dieser Proteinfraktion zurückzuführen sind und deren Ausprägung mit steigender Lagertemperatur zunahm. Verglichen mit den an Ostseedorsch erhalten DSC-Mustern

weisen niedrigere Umwandlungstemperaturen für Myosin und Aktin beim Dorsch aus der Barentssee auf eine verminderte thermische Stabilität hin, die durch die Lebensbedingungen begründet ist.

Keywords: Fish fillet, frozen storage, instrumental measurement, colour, texture, water holding capacity, differential scanning calorimetry / Fischfilet, Gefrierlagerung, Instrumentelle Messungen, Farbe, Textur, Wasserbindung, Differential Scanning Calorimetry

Introduction

Although frozen storage prevents microbial spoilage of fish tissue, it is accompanied by changes in protein solubility, protein functionality, texture, and nutritional quality. Fish can undergo a number of alterations, largely affecting texture in lean species such as cod. This means that by the end of frozen storage the product is hard and fibrous and has lost juiciness, so that it is liable to be rejected by the consumer. These changes, which are attributed largely to alterations in the myofibrillar proteins, depend not only on species but also on technological factors such as processing prior to freezing, or storage conditions.

Recently two papers have been published in this journal dealing with the influence of frozen storage at different temperatures on quality attributes of Baltic cod fillets[1,2]. It has been found that cod stored at −20 and −30 °C showed the lowest drip losses during one year storage period while cod stored at −10 °C exhibited highest drip losses although storage period was only 6 months. It has been reported furthermore that unwanted changes in texture, colour and water binding capacity were most pronounced at −10 °C while those at −30 °C were negligible. Changes at −20 °C were in between. One disadvantage in these studies was that the fish had been caught at both different times (within a time period of 4 months) and fishing grounds in the Baltic Sea in spring 2002. Therefore, a direct comparison of the results proved to be difficult. To overcome these shortcomings, in the present investigation raw material was caught within a narrow time frame and at almost same catching ground. The aim of this work was to examine the effect of 3 different storage temperatures (−14, −20 and −28 °C) whereby the fish fillets stored at −20 °C were both single and double frozen. Storage experiments were accompanied by physical methods for texture, water holding capacity and colour as well as thermal analysis to follow the changes of these functional properties during the storage period.

Material and methods

Material

During the 256th cruise of the research vessel "Walther Herwig III" cod was caught in the Barents Sea. Cod for the storage experiment at −14 °C and for the double freezing experiment at −20 °C was caught (74° 22,82'N 16°27,60'E and 74°22,68'N 16°33,06'E, respectively) on 23rd of September 2003. Fish used for the −14 °C experiment was stored in ice, filleted and skinned manually next morning being still in rigor, vacuum-packed (*WEBOMATIC* C20, Bochum, Germany), air-blast frozen (Sabroe tunnel freezer) and stored at −34 °C until the end of the cruise.

The cod for the storage experiment at −20 °C and −28 °C was caught (75°06,63'N 32°16,09'E and 75°06,71'N 32°17,01'E, respectively) on 1st of October 2003, filleted and skinned in pre rigor condition and processed as aforementioned. The cod used for the double freezing experiment was headed and gutted, air-blast frozen overnight and stored at −34 °C until further treatment. After 2 weeks of frozen storage cod was thawed overnight by immersion in sea water, filleted and skinned. Fillets were packed in commercial packaging material (cardboard boxes) and block-frozen overnight using a Sabroe plate freezer. Fillet blocks (7.5 kg each) were stored at −34 °C until the end of the cruise. At the research department in Hamburg the blocks with double frozen cod fillets were sawed into appropriate portions and packed in pouches with zip-fasteners. At the research department all samples were stored frozen at the respective temperature whereby the actual temperatures were monitored by temperature loggers.

Methods

The determination of the different properties was performed every month, every second month and every third month on cod fillets stored at −14 °C, at −20 °C (single and double frozen) and at −28 °C, respectively. Methods applied were the same as described in detail previously in ref.[2] with one exception; for colour measurement a spectral colour meter spectro pen® (*Dr. Lange*, Düsseldorf, Germany) was used which is a genuine grating colorimeter measuring the visible spectral range (400 to 700 nm) at intervals of 10 nm. It operates on the spectral method described in DIN 5033 using 45/10° viewing geometry and D65 as illuminant. Additionally proximate composition of the cod was determined by using the respective German § 35 methods[3–5]. The results were statistically evaluated using the software package STATISTICA (*StatSoft*, Inc. (1996), Tulsa, OK, USA).

Results

The proximate composition of the cod was found as to be expected with water in the range from 80.2 to 80.9 %, crude protein in the range from 19.7 to 19.9 % and ash with 1.3 %.

Texture measurements

The texture of the intact thawed muscle was characterised by instrumental Texture Profile Analysis (TPA) which is an imitative test that compresses a bite-size piece of food two

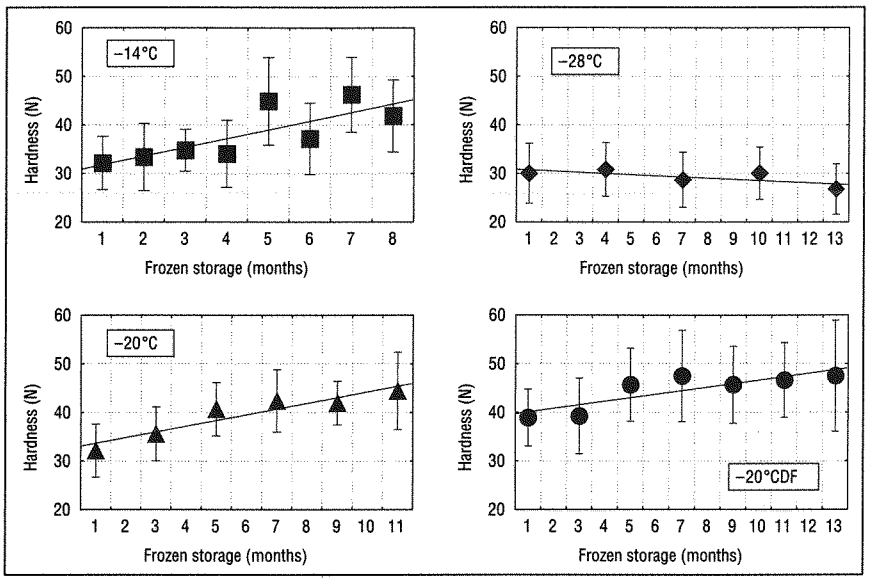

Fig. 1 Hardness of cod fillet during frozen storage as affected by time and temperature

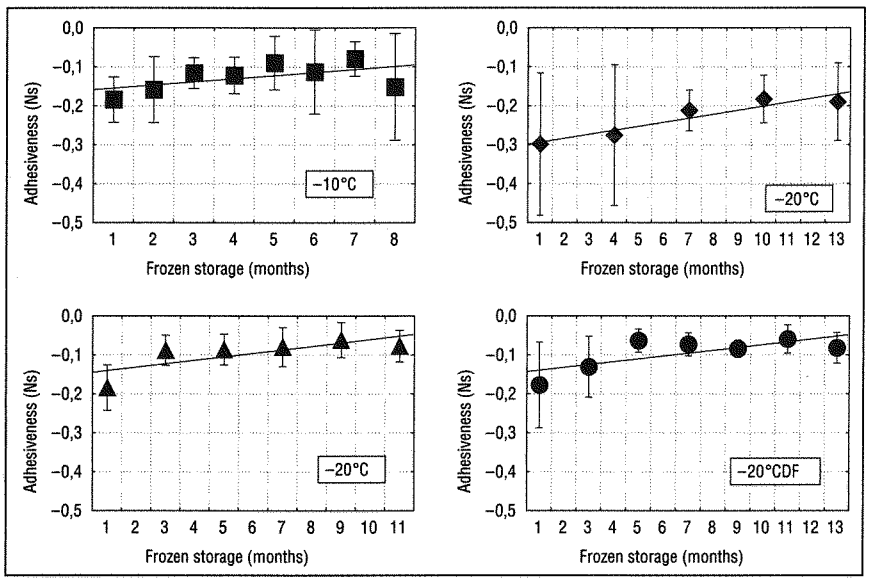

Fig. 2 Adhesiveness of cod fillet during frozen storage as affected by time and temperature

times in a reciprocating motion that imitates the action of the jaw and extracts from the resulting force-time curve a number of textural parameters that correlate well with sensory evaluation of these parameters[6], e.g. hardness, chewiness, adhesiveness, cohesiveness and springiness.

Fig. 1 shows the changes of hardness during frozen storage at different temperatures and makes clear that hardness increased during storage at all temperatures investigated with the exception of storage at −28 °C. At this storage temperature the hardness did not change significantly over the whole storage period of 13 months. The initial hardness at the start of the storage trial was almost identical with exception of the double frozen sample which evidenced that repeated freezing causes increasing hardness. However, the further increase in consequence of storage at −20 °C did not seem to be markedly different between single and double frozen samples. When storage was performed at the highest temperature of −14 °C, the increase in hardness was most pronounced causing hardness values after half a year of storage comparable with those after one year at −20 °C.

The evolution of chewiness (not shown) was comparable to hardness. At −28 °C storage almost no changes in chewiness were observable while chewiness during −14 °C storage increased heavily indicating pronounced toughness of these samples. At −20 °C irrespective of single or double frozen samples were stored, a comparable increase in chewiness to −14 °C storage was to be seen, however, only after one year. Adhesiveness that can be seen as the joining of surfaces of different composition developed during frozen storage as shown in Fig. 2. While at −14 °C almost no changes in adhesiveness were detectable during storage, at −28 °C adhesiveness decreased slightly with prolonged storage time. However, it was striking that during first months of storage a relatively high standard deviation became obvious. Single and double frozen samples behaved similarly in adhesiveness.

In both cohesiveness (Fig. 3) and springiness (not shown), the further 2 textural parameters obtained by TPA, changes at −28 °C storage were only small and evidenced that at this low temperature even one year of frozen storage did not exert a remarkable influence. In contrast, high storage temperature at −14 °C caused within a relatively short time span comparably strong changes in both parameters causing a tough and gummy texture of samples.

Other than TPA described above, the penetration force was measured on minced muscle. This kind of tests is a so-called puncture test[6]. The puncture test measures the force required to push a probe into a food. Results are shown in Fig. 4 and it becomes obvious that minced fillet stored at −28 °C is least resistant against penetration of cylindrical plungers and this behaviour did not change significantly during the 13 months storage period. Contrary to that the penetration force needed for −14 °C samples is markedly higher during the whole storage period and characterised by a marked increase with progressive storage. The same behaviour was observed at −20 °C storage although the increase with storage time was less. Differences between single and double frozen fillets were negligible.

Water holding capacity

The expressible moisture of approximately 7–8 % was lowest in samples stored at –28 °C and did not change during the whole 13 months storage period. Small differences were to be seen when the other storage experiments were compared. However, taking into account that storage period at –14 °C was shortest; the rise in expressible moisture was steepest. Surprisingly, double freezing of cod fillets did not cause increasing water loss during frozen storage at –20 °C when compared with that of single frozen fillet stored at same temperature (Fig. 5).

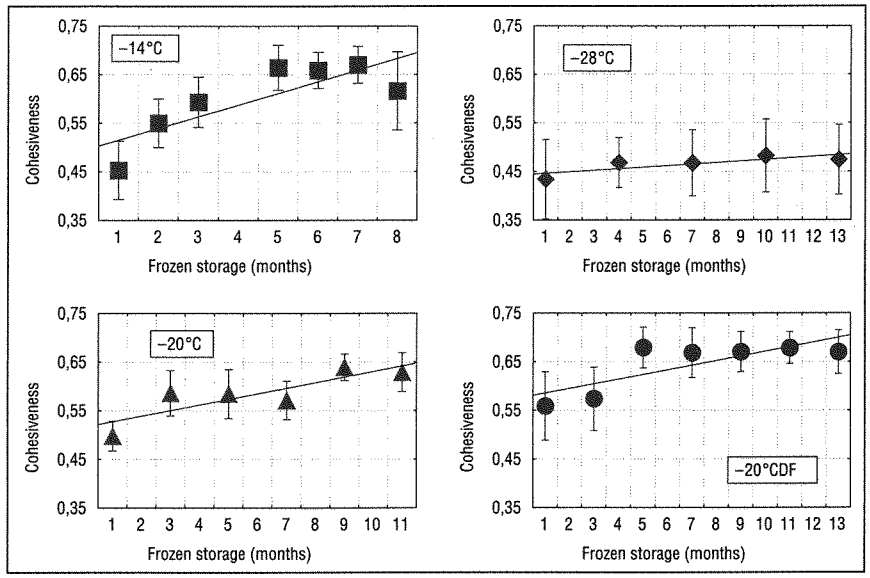

Fig. 3 Cohesiveness of cod fillet during frozen storage as affected by time and temperature

Colour measurement

Colour is usually considered the most important attribute of any food's appearance especially if it is associated with other aspects of food quality for example the visible deterioration which occurs when a food spoils[7]. In Fig. 6 and 7 changes in lightness (L*) and redness (a*) were displayed measured on intact muscle as well as on mince during frozen storage at different condition. Particularly in L* and b* (not shown) it became evident that mincing caused a marked increase in both colour values because smaller particles scatter and reflect more incident light and the sample therefore appears lighter[8]. Only during storage at –14 °C and somewhat lower at –20 °C for the single frozen sample an noticeable increase in L* could be observed whereas L* during storage at –28 °C and at –20 °C for the double frozen sample did not change (Fig. 6). A comparable behaviour was found in b* (not shown). While this both colour values showed an almost linear relationship with storage time, changes in a* could not be described by a linear equation (Fig. 7). Changes in a* seemed to be more wave-like and most pronounced at the –14 °C storage. In all cases, a* increased up to month five and decreased during further storage until the initial level had been met.

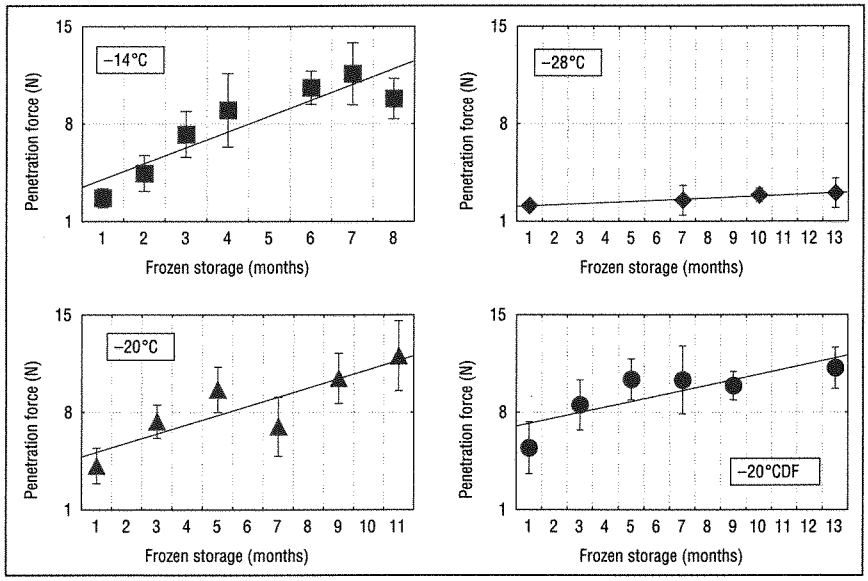

Fig. 4 Penetration force of minced cod fillet during frozen storage as affected by time and temperature

Thermal analysis

The DSC curves taken on cod fillets during frozen storage at different temperatures are shown in Fig. 8, while the respective transition temperatures and enthalpies could be seen in Table 1. In general DSC curves obtained from cod fillet were characterised by two main peaks comprising myosin, the lower temperature peak, and actin, the higher temperature peak. In between both peaks a minor peak can be found originating from sarcoplasmic and/or connective tissue proteins. During storage at –14 °C the T_{max} of the myosin peak decreased obviously indicating a loss of thermal stability at this high frozen storage temperature. But the transition enthalpy as an indication of the degree of denaturation of this protein was only slightly decreasing during the 7 months storage period (Table 1). However, almost no changes were found for actin in regard to T_{max} and ΔH. The minor peak between myosin and actin was sometimes difficult to detect and did not show significant changes. The same tendencies as discussed above were also seen at the single frozen fillet stored at –20 °C. The decrease of T_{max} of the myosin fraction, however, was less pronounced. On the double frozen fillets, thermal stability of myosin decreased much stronger indicating in connection with the measured reduction in enthalpy a more pronounced denaturation un-

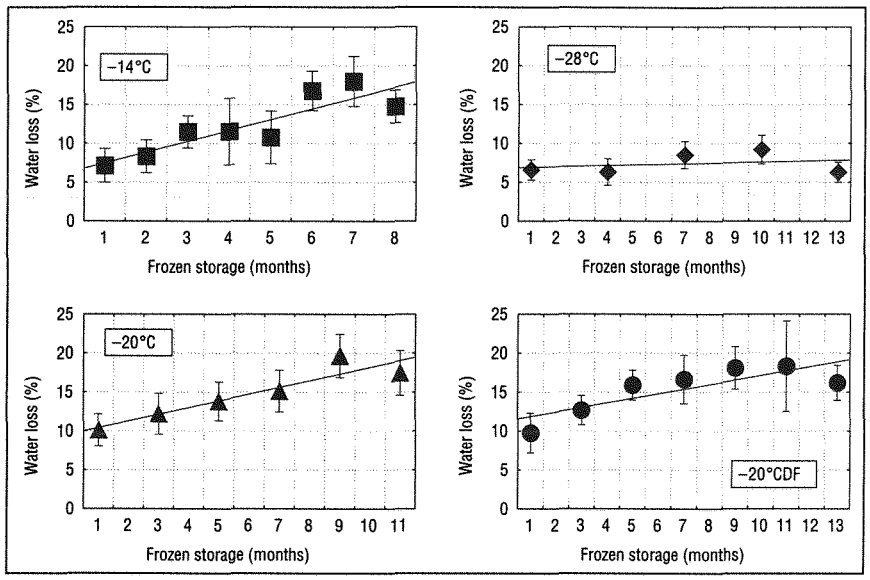

Fig. 5 Water holding capacity of cod fillet during frozen storage as affected by time and temperature

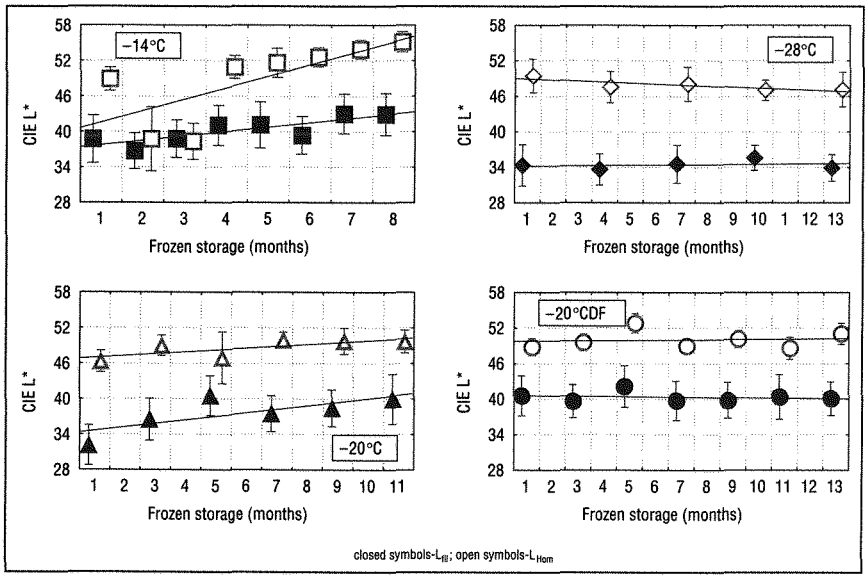

Fig. 6 Lightness of cod fillet during frozen storage as affected by time and temperature

der these conditions. The T_{max} of actin, on the other hand, shifted slightly to higher temperature. As expected, during storage of cod fillet at −28 °C proteins were least influenced. Almost no changes were to be seen regarding to T_{max} and ΔH for all peaks detectable in the curve indicating the stability of the muscle proteins against denaturation during prolonged storage at low temperature. Most of the textural and water binding behaviour shown above are supported and explained by the results of DSC measurements.

Correlation between instrumentally measured parameters and storage time

For statistical evaluation, the instrumentally measured results were correlated with storage time. There were numerous significant ($P < 0.05$) correlation between different parameters (Table 2). In Fig. 9 the most significant correlations ($P < 0.05$) at −10 °C were plotted. It could be seen that penetration force, water loss, lightness and cohesiveness were strongly positively correlated with storage time. That means with rising storage time these parameters increased. Comparable strong correlation could be seen at −20 °C storage as shown for penetration force, water loss, chewiness and cohesiveness measured on single frozen samples with storage time (Fig. 10). Correlation of same parameters with storage time for double frozen samples were however weaker (Fig. 11), but still significant ($P < 0.05$). At −28 °C the most pronounced correlations accounted for redness and yellowness were quite weak and showed reverses premises (Fig. 12). In contrast, correlations of hardness and water loss with storage time were not significant ($P > 0.05$).

Discussion

In general, the main conclusions were comparable with the results of the first study using Baltic Sea cod[2]. All parameters investigated during storage time at different temperatures were most influenced at a temperature of −14 °C and least influenced by using −28 °C as storage temperature. Changes observed when using −20 °C as storage temperature were found to be in between both extremes. Storage of double frozen cod fillet at −20 °C did obviously not cause marked differences in the behaviour of the physical parameters investigated compared to the single frozen trial at same temperature. This is in agreement with earlier results in respect of the influence of double freezing on quality attributes of coated cod fillet portions[9].

Concerning the differences in texture attributes as result of differing storage conditions it has been reported that the force required for compression of pieces of both cod and haddock fillet were higher at −10 °C compared with −30 °C whereby an increase in hardness with time of storage was observed[10]. Pronounced differences in formaldehyde and dimethylamine formation have been found in cod and haddock fillets which were, however, not reflected in protein solubility, amino acid content and aggregate formation, which were comparable for both species. This was in contrast to previously held views that formaldehyde is responsible for toughening in frozen fish fillets. Textural changes

may be attributed according to *Badii* and *Howell*[11] mainly to ice crystal formation and the action of lipid oxidation products on proteins. The resulting protein denaturation involves hydrophobic groups and exposure of other polar groups due to the unfolding of the molecules accompanied by changes in the secondary structure[12]. Atomic force microscopy revealed that the muscle structure of frozen cod fillet at −30 °C is uniform with an even layer of small granules whereas that of −10 °C stored cod fillet is characterised by uneven and irregular-shaped particles forming holes in the surface[13]. Comparing the influence of storage temperature (−20 and −30 °C) on the formation of aggregates in cod fillets and possible relation to texture and functionality it was found that the nature of the aggregates changes considerably according to the storage temperature, time and the intactness of the muscle[14,15]. The texture was found to be related to the type of salt-insoluble aggregate formed, shear resistance being significantly higher where these aggregates contained a larger amount of myosin linked by covalent bounds. Comparable results were reported concerning the influence of different storage temperatures (−10 °C vs. −30 °C) on the apparent viscosity of homogenates from hake fillets[16]. Samples stored at −10 °C for 30 weeks showed a large decrease of this functional property, whereas at −30 °C there was only a slight decrease throughout storage. On intact hake muscle measured Kramer shear resistance increased significantly only in samples stored at −10 °C, whereas −30 °C samples did not present significant variations in this parameter. It has been concluded very recently that increasing muscle hardness during frozen storage is accompanied by aggregation of fish muscle at two different levels: (a) cementation of myofibrils and (b) increased fusion of myofilaments[17]. In view of time/temperature differences among changes in structure, ultrastructure, and functionality, it seems likely that cementation of the myofibrils is the main cause of increased muscle hardness. Lipid deterioration during frozen storage of cod, measured on the basis of free fatty acids, peroxide value, thiobarbituric acid index and fluorescent compounds, revealed that most lipid damage indices showed significant correlations with storage time at −30 °C

Tab. 1 Transition temperatures and enthalpies measured by DSC on cod fillet during frozen storage at different temperatures

		Peak I		Peak II		Peak III	
$T_{storage}$ [°C]	$t_{storage}$ [months]	T_{max} [°C]	ΔH [Jg^{-1}]	T_{max} [°C]	ΔH [Jg^{-1}]	T_{max} [°C]	ΔH [Jg^{-1}]
−14	1	44.3	0.62	56.3	0.01	74.4	0.50
	2	43.6	0.80	58.1	0.01	74.2	0.70
	3	43.8	0.74	0	0	75.2	0.46
	4	42.9	0.80	0	0	74.4	0.51
	5	41.7	0.41	0	0	74.3	0.52
	6	42.4	0.60	57.6	0.02	74.8	0.57
	7	40.5	0.55	57.0	0.01	75.0	0.66
−20	1	44.9	0.78	0	0	74.9	0.50
	3	43.6	0.93	57.9	0.02	74.3	0.66
	5	43.5	0.67	56.8	0.01	73.9	0.54
	7	43.2	0.59	57.7	0.01	74.7	0.43
	11	43.2	0.55	57.5	0.03	74.2	0.60
−20DF	1	44.1	0.77	57.9	0.01	73.9	0.58
	3	43.2	0.72	57.0	0.02	74.9	0.53
	5	41.8	0.70	56.6	0.02	74.4	0.46
	7	43.4	0.76	58.1	0.02	74.5	0.53
	11	39.1	0.69	0	0	74.7	0.44
	13	42.1	0.52	0	0	75.2	0.53
−28	1	44.7	0.91	58.9	0.01	73.9	0.59
	4	44.0	0.85	58.1	0.02	73.3	0.57
	7	44.3	0.78	60.2	0.02	73.8	0.54
	10	44.5	0.67	58.2	0.02	74.3	0.58
	13	44.2	1.01	59.3	0.01	74.1	0.53

Tab. 2 Significant correlations ($P < 0.05$) between the variables investigated and storage time

$T_{storage}$ [°C]	No of variables	No of correlations
−14	13	13
−20	13	12
−20DF	13	8
−28	13	7

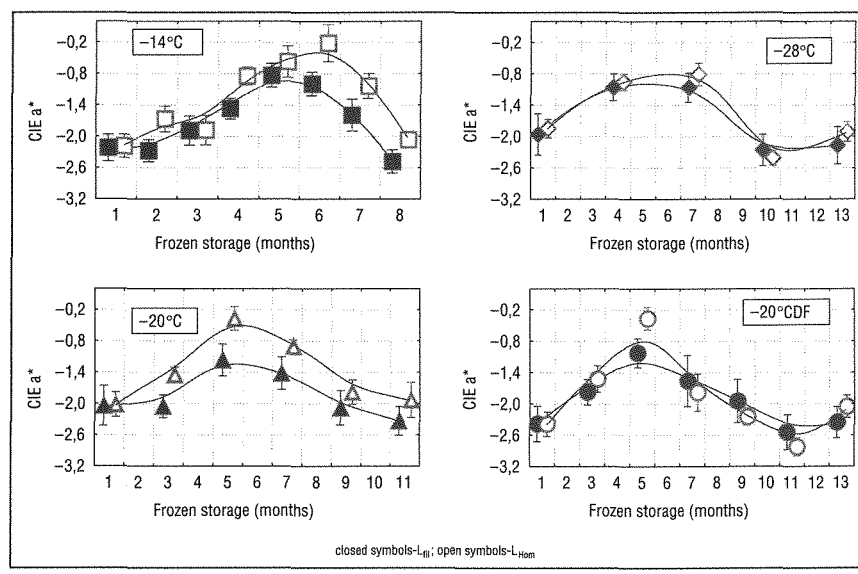

Fig. 7 Redness of cod fillet during frozen storage as affected by time and temperature

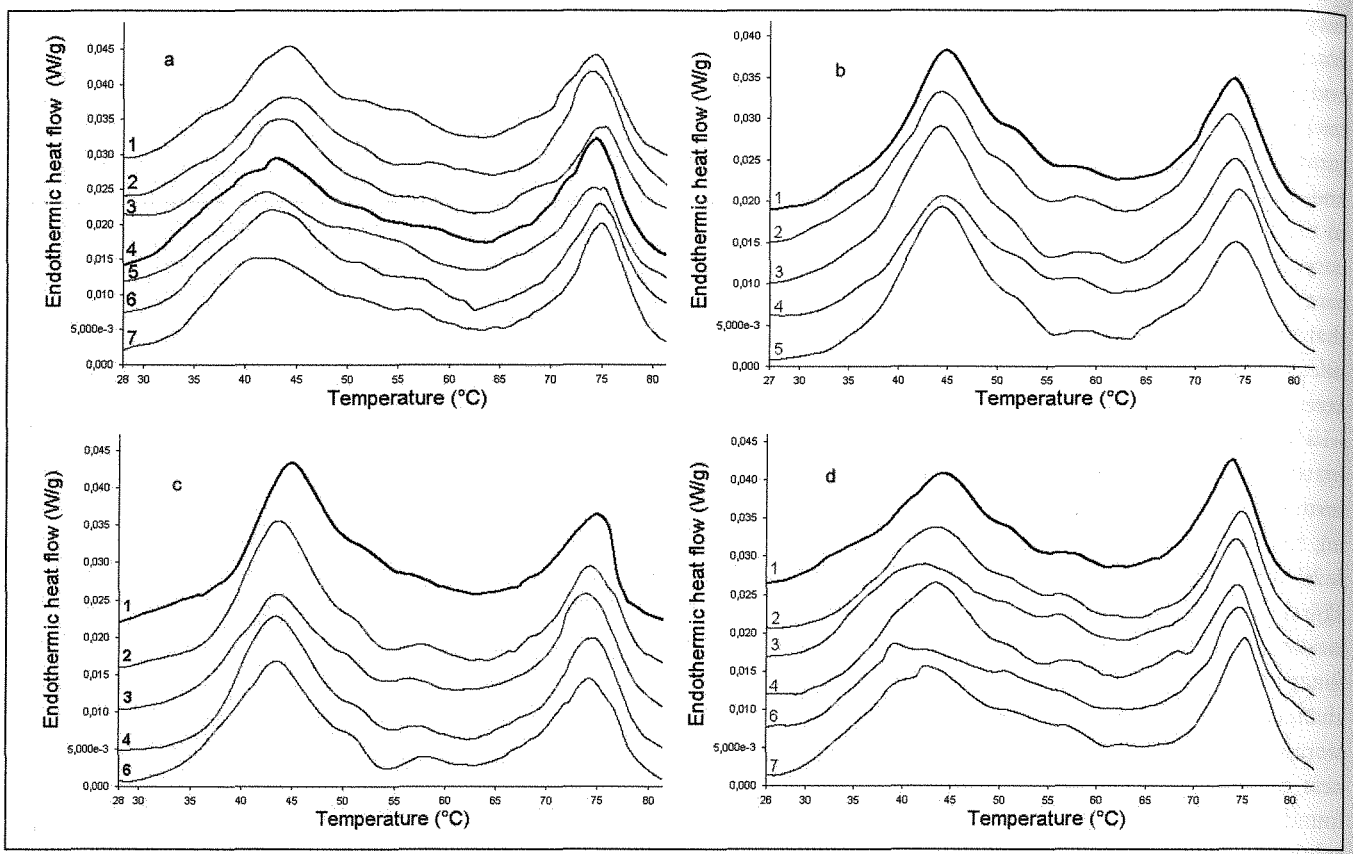

Fig. 8 DSC curves taken from cod fillet after distinct times of frozen storage at −14 °C (a), −28 °C (b), −20 °C, single frozen (c) and −20 °C, double frozen (d); all curves are average curves from at least 4 measurements

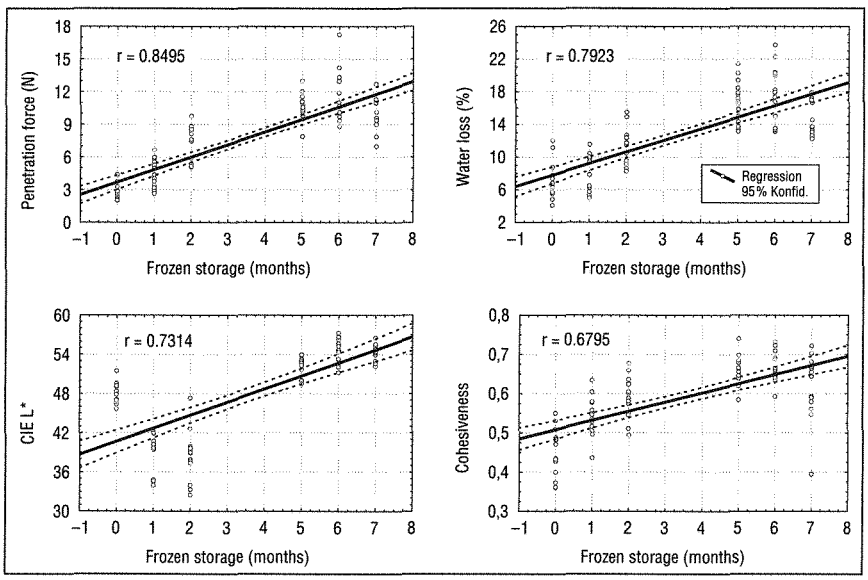

Fig. 9 Regression between penetration forces, water loss, cohesiveness, lightness measured on cod fillet and storage time at −14 °C

however, at −10 °C only free fatty acids and fluorescence detection provided significant correlations with storage time[18]. According to *Kim* and *Heldman*[19], the rate of textural deterioration of cod muscle during frozen storage can be described by a first-order relationship. The increased toughness of frozen fish with storage time (up to 30 days) at −7, −10 and −18 °C results from protein denaturation of cod muscle, while the slightly decreased cohesiveness results in the loss of water holding capacity. This does not agree with present findings which showed a general increase of cohesiveness during prolonged frozen storage. Despite the findings that no differences could be observed between frozen cod muscle, which produces formaldehyde, and frozen haddock fillets, which produce negligible formaldehyde, that could be seen as confirmation that formaldehyde is not a major factor in fish protein aggregation[13], the relationship between trimethylamine oxide demethylase (TMAOase) activity and the formaldehyde accumulation during frozen storage of gadiform fish was investigated recently[20]. Enzyme activities in the major white muscle of gadiform fish showed large variation between species as well as between individuals. Similar large variations in the rate of formaldehyde accumulation during frozen storage were detected, which could be accounted for by the endogenous white muscle in situ TMAOase activity. This TMAOase activity was also correlated with the rate of insolubilisation of otherwise high ionic strength soluble proteins.

On cod fillets after different iced storage times (1 and

8 days) stored frozen at −20 and −30 °C for 6 weeks the water holding capacity was found not to be significantly different ($P > 0.05$). However, water holding capacity for cod fillets before freezing was significantly higher ($P < 0.001$) than after freezing[21]. The water holding capacity measured on frozen stored hake muscle decreased significantly over the first 7 weeks in samples stored at −10 °C and over the first 16 weeks at −30 °C; there were no major differences thereafter. Decrease was lower in −30 °C samples[17]. It was concluded that water holding capacity seems to relate more to protein aggregation at the myofilament lattice level than to cementation of myofibrils. When cod fillets frozen at −45 °C were stored frozen at −9 °C and at −40 °C for 2 weeks the drip loss was significantly lower for −40 °C samples whether they were thawed at 5 °C or 25 °C[22]. These findings supporting the fact of preserving the water holding capacity by low storage temperatures are in agreement with present results.

During frozen storage only moderate colour changes could be observed. Particularly regarding to the −14 °C storage this is in contrast to the previous study where changes in all colour values were more pronounced[2]. Also this wave-like behaviour in a* was not observed earlier. Observations could not be supported which stating that the appearance of both cod and haddock fillets kept at −10 °C for 65 weeks was yellowish, unlike the fillets stored at −30 °C which remained white[11].

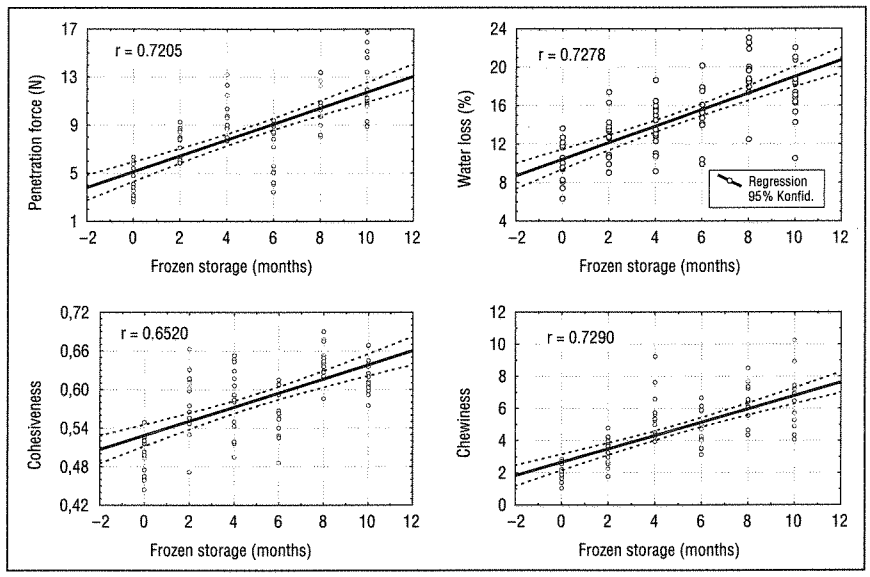

Fig. 10 Regression between penetration forces, water loss, chewiness, cohesiveness measured on single frozen cod fillet and storage time at −20 °C

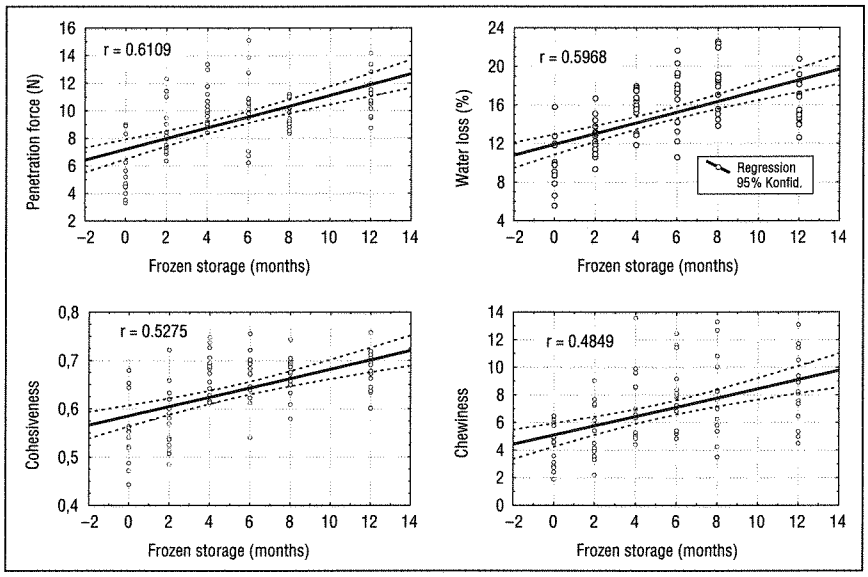

Fig. 11 Regression between penetration force, water loss, chewiness, cohesiveness measured on double frozen cod fillet and storage time at −20 °C

In the DSC pattern, the freezing process itself causes T_{max} to slightly decreasing compared with fresh fish muscle[23]. It was found by *Hastings* et al.[24] that freezing followed by immediate thawing has little effect on the characteristic thermal transition of cod muscle. However, after 2 weeks at −10 °C it was apparent that myosin had undergone some partial denaturation. Further storage caused little subsequent effect on the myosin transition after 2 weeks at −10 °C, whereas actin, collagen and sarcoplasmic proteins are largely unaffected by frozen storage. Results obtained on Baltic sea cod[2] evidenced higher T_{max} for myosin (~5 to 6 °C) as well as for actin (~4 to 5 °C) indicating that thermal stability of muscle proteins is highly dependent on the environmental conditions. Averaged water temperature in the Baltic sea is reportedly approximately 10 °C higher than the one of the Barents Sea[25]. Myosin was found to be more thermostable in species adopted to warm water[23,24]. This was also found to be valid for isolated myosin possibly in part due to differences in the mode of self-association of the myosin molecules[26]. According to *Howell* et al.[27] the onset temperature of myofibrillar protein denaturation occurred up to 11 °C higher for tropical species than cod at pH 7 and low ionic strength. *Davis* et al.[28] summarised their results as follows: (1) Actin and its associated proteins may undergo some modification during prolonged frozen storage, but the effect is minima compared with myosin. (2) There appears to be a correlation between the extent of freeze denaturation of the muscle (mainly the myosin) and the habitat temperature of the fish. (3) The rate of unfolding of myosin in frozen muscle appears to be similar for both warm (red snapper) and cold water (cod) fish. Unfolding of the cod

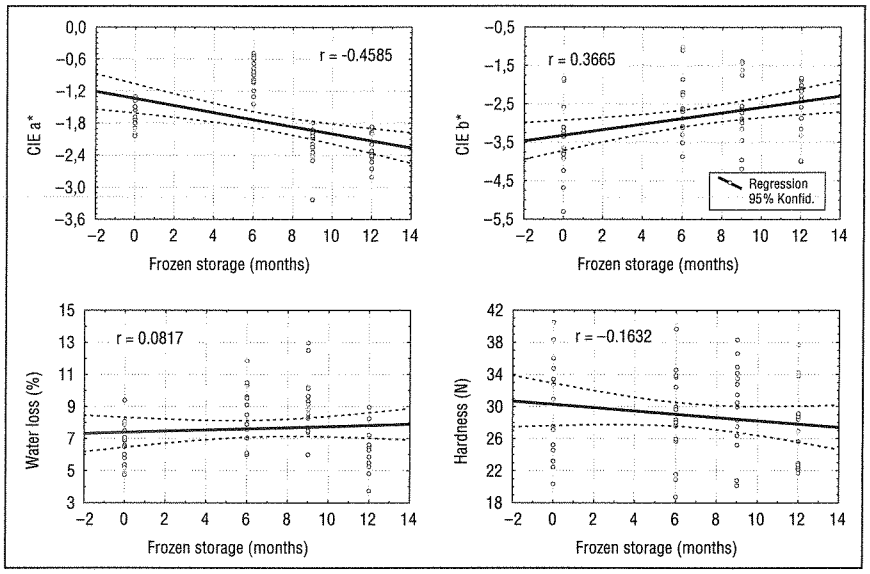

Fig. 12 Regression between redness, yellowness, hardness, water loss measured on cod fillet and storage time at −28 °C

myosin seems to be accompanied by a more drastic breakage of bonds than the unfolding of red snapper and after about 10 weeks frozen storage of cod muscle, there was an increase in the number of heat-labile endothermic bonds associated with the myosin.

The shape of DSC curves depends on the instrument used. Because instrument used by *Careche* et al.[16] were comparable with the own one, DSC curves taken from hake fillet stored at −10 °C und −30 °C can be confronted with the presented results. Both studies show that a considerably high fraction of muscle proteins remained in the native-like condition even at the higher frozen storage temperature. It is well-known that DSC traces are the resultant of energetically opposed effects, such as melting of hydrogen bonds during the thermal process (endothermic) and hydrophobic interactions (exothermic), the former being the highest contributor to the DSC profile. From their study on the extractability and thermal stability of frozen hake fillets stored at −10 and −30 °C it is concluded by the authors[16] that during frozen storage there are protein structural changes that occur mainly in the myosin molecule and which affect thermal transition but with little loss of functionality. They stated furthermore, when severe inextractability and apparent viscosity loss are present, there are qualitative changes in the thermal transition, but small reduction in enthalpy of denaturation as compared with unfrozen muscle. This is compatible with hydrogen bond reformation. Actin inextractability in NaCl, when DSC profiles show no sign of denaturation could be explained by myosin provoking inextractability of actin through actomyosin complex. The comparable low influence of frozen storage on the DSC pattern were observed earlier[29] when single and double frozen samples were compared.

On DSC curves obtained from cod stored at −10 °C and −30 °C for 30 weeks it was observed that T_{max} and ΔH decreased for the myosin peak and increased slightly for the second and third peak at −10 °C compared with that of −30 °C, due to protein aggregation of fish fillets, especially myosin[10]. In further investigation it was found that for myosin stored at −10 °C compared with that stored at −30 °C the T_{max} changes were not significant, but ΔH decreased from 0.67 to 0.54 J/g indicating protein denaturation and exposure of polar groups. In contrast, ΔH for actin increased from 0.29 at −30 °C to 0.37 J/g at −10 °C; the increase may be indicative of actin polymerisation or dehydration effects, in which case the denaturing effect on myosin is underestimated[12]. When mackerel fillet were frozen stored at −20 °C and −30 °C for up to 2 years ΔH decreased significantly ($P < 0.05$) in line with extent of denaturation due to increased time of storage at −20 °C compared with that of −30 °C. T_{max} values only decreased significantly for samples stored at −20 °C compared with those stored at −30 °C after 2 years. This decrease in T_{max} and ΔH reportedly suggests an alteration of the native structure of myosin due to the presence of lipid oxidation products as well as the formation of ice crystals, dehydration and an increase in salt concentration, which subsequently requires less thermal energy for denaturation[30].

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Zusammenfassung

Salze der 2-Ethylhexansäure (2-EHA) finden als Stabilisatoren für PVC-Dichtungen bei Metalldeckeln für Glasgefäße Verwendung. Diese können

PVC, Deckeldichtungen

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