noch Spaltprodukte angezeigt werden, solange sich diese kompetitiv zum im Test verwendeten Sojamaterial verhalten. Bei einer Fermentation können jedoch auch neue antigene Abschnitte entstehen, so dass die Quantifizierung auch hier zunächst in Frage zu stellen wäre.

5 Beurteilung

Mit dem SoyResidue ELISA konnten bei dem hier verwendeten erhitzten Probenmaterial die darin enthaltenen prozessierten Sojabestandteile bis auf den Zusatz von Soja Sauce qualitativ nachgewiesen werden. Weitere Untersuchungen haben gezeigt, dass kommerziell erhältliche Sojaisolate, nativ oder teilhydrolysiert, und Sojafaserprodukte von diesem Test ausreichend erkannt werden. Es wurden keine falsch positiven Ergebnisse oder Matrixeffekte beobachtet. Die einfache Probenaufarbeitung und die kurze Extraktionszeit erlauben einen hohen Probendurchsatz bei geringem Arbeitsaufwand. Dies und die Konzeption der Mikrotiterplatte (abbrechbare Kavitäten) sowie das Mitführen von Negativ- und Positiv-Kontrollen gestatten, falls notwendig, auch die kostengünstige Durchführung von Einzeluntersuchungen. Für Fleischwaren ist ggf. die Extraktion am Ultra-Turrax der im Stomacher-Beutel vorzuziehen, wenn Gehalte unter 0,5 % Sojaprotein erwartet werden.

Dank

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Instrumental Colour, Texture, Water Holding and DSC Measurements on Frozen Cod Fillets *(Gadus morhua)* during Long Term Storage at Different Temperatures

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Summary

Individually packed Baltic cod, caught in the period from January to April 2003, has been frozen stored for 6 to 12 months at different temperatures (–10 to –30 °C). Beside single frozen fillet also double frozen fillet was stored at –20 °C. During storage changes in colour, texture, water holding capacity and thermoanalytical behaviour were assessed instrumentally. Colour, texture and water holding capacity during frozen storage were a function of temperature and time. Changes at –10 °C were most pronounced, while those at –30 °C were negligible. Changes at –20 °C were between both extremes. DSC curves were widely not affected by the time of storage. However, the storage temperature affected mainly the second transition peak that is ascribed to sarcoplasmic and connective tissue proteins. While at –10 °C this peak disappeared was the peak maximum temperature for double frozen fillets 7 °C lower compared to single frozen ones stored at both –20 and –30 °C. The frequency of significant linear correlations between instrumental data for colour, texture and water holding capacity and the respective storage time proved to be temperature-dependent and was highest at –10 °C. From the point of quality assurance, storage at –10 °C should be avoided, while storage at –30 °C should be strived for if ever possible.

Zusammenfassung

Individuell verpackte Filets von Ostseedorsch, gefangen in der Zeit von Januar–April 2003, wurden bei unterschiedlichen Temperaturen (–10 bis –30 °C) über einen Zeitraum von 6–12 Monaten gelagert. Während der Lagerung wurden Veränderungen der Farbe, Textur, Wasserbindung und des thermoanalytischen Verhaltens instrumentell bewertet. Die Veränderungen von Farbe, Textur und Wasserbindung während der Gefrierlagerung waren eine Funktion von Lagertemperatur und -zeit. Bei –10 °C waren sie am deutlichsten ausgeprägt, bei –30 °C dagegen teilweise zu vernachlässigen. Veränderungen während der Lagerung bei –20 °C bewegten sich in etwa in der Mitte zwischen beiden Extremen. Die DSC-Muster sind dagegen weitestgehend unbeeinflusst von der Lagerzeit d. h., zunehmende Lagerdauer bewirkte keine merkliche Veränderung der DSC-Muster. Die Lagertemperatur beeinflusste dagegen insbesondere die Umwandlungstemperatur des mittleren Peaks, der den Sarkoplasma- und Bindewebsproteinen zugeschrieben wird. Während bei –10 °C dieser Peak nicht verifiziert werden konnte, lag die Umwandlungstemperatur bei der Lagerung von zweifach gefrorenen Filets gegenüber einfach gefrorenen bei –20 wie auch gegenüber den bei –30 °C gelagerten um 7 °C niedriger. Die Häufigkeit der signifikanten linearen Korrelationen zwischen den instrumentellen Daten für Farbe, Textur und

Wasserbindung und der jeweiligen Lagerzeit erwies sich als temperaturabhängig und war für –10 °C am größten. Aus qualitativer Sicht ist daher –10 °C als Lagertemperatur zu vermeiden und möglichst eine Lagerung bei –30 °C anzustreben.

Keywords: fish fillet, frozen storage, instrumental measurements, colour, texture, water holding capacity, DSC measurement / Fischfilet, Gefrierlagerung, instrumentelle Messungen, Farbe, Textur, Wasserbindungsvermögen, DSC-Messungen

Introduction

Very recently, a paper has been published in this journal dealing with drip losses during long term frozen storage of cod at different temperatures[1]. The same material was used for experiments reported here, including colour and texture measurements as well as assessment of the water holding capacity (WHC) and performing differential scanning calorimetry (DSC). The aim of these studies was to monitor the influence of different treatments and frozen storage conditions on the physical properties of cod fillets. It could be expected that different temperatures of frozen storage will influence the functionality of the fish muscle proteins to various degrees and this should be detectable by evaluating colour, texture, WHC[2] and DSC. The influence of these treatments and conditions on the sensory evaluated quality of cod fillets is reported elsewhere[3].

Material and Methods

Fish

The Baltic cod used for the different storage experiments was caught at different times in the period from January to April 2002 in the western part of the Baltic Sea. Preparation of samples for the specific storage experiments was described in detail earlier[1]. Fish was manually filleted, packed in double PE pouches with zip-fasteners and subsequently shock frozen by liquid CO_2 using a freezer from the *Air Liquid GmbH*. The frozen, individually packed fillets were stored in deep freezing cabinets at –10, –20 and –30 °C, respectively. Beside immediately processed fillets, one sample consisted of twice frozen fillets stored at –20 °C. That means whole gutted cod was shock frozen and stored frozen for two weeks. Then it was thawed, filleted and the fillets handled as described above.

Methods

After distinct times of frozen storage, samples were taken out of the freezer, thawed overnight in a refrigerator and then directed to the different physical measurements.

Colour measurement

Colour measurement was performed on intact fillets using a tristimulus colorimeter CR 300 (*MINOLTA*, Ahrensburg, Germany) as described earlier[4,5]. On five fillets the colour was taken tenfold each. In the CIELab system L* denotes lightness on a 0 to 100 scale from black to white; a*, (+) red or (–) green; and b*, (+) yellow or (–) blue.

Texture measurement

Instrumental Texture Profile Analysis (TPA) was performed as described earlier[6,7] with slight modifications. The samples used for TPA measurement were cut out using a cork borer (Ø 1.5 cm), thawed at room temperature and brought to the measurement temperature (7 °C) using a refrigerator. This procedure allows the preparation of samples for TPA measurements with a minimum of distortion in sample size. The TPA measurements were performed at both 80 % and 40 % compression. Taking the influence of the measurement conditions on the result into account, the texture parameters hardness and chewiness were estimated by double compression of the sample to 80 %, whereas for evaluating springiness and cohesiveness the outcome of 40 % strain was used. In both cases a cylindrical probe of 5.0 cm diameter was used with a test speed of 0.8 mm/s. All TPA measurements were repeated 15 times using at least five portions of each sample. Instrumental TPA is a measurement method, which imitates the chewing process thus giving the possibility to observe and differentiate between single texture attributes and to characterise on this basis the very complex impression of food texture on humans. The texture attribute hardness is defined as the maximum force of the first compression. Chewiness, as the quantity to simulate the energy required masticating a sample to a steady state of swallowing, is calculated as the product of hardness, cohesiveness and springiness. Springiness gives an explanation of how well a product physically springs back after it has been deformed during the first compression. Cohesiveness is the attribute describing how well the product withstands a second deformation relative to how it behaved under the first deformation. Further, firmness was measured by compressing the samples (20 mm diameter) prepared by the use of a cork borer to 75 % using a flat-ended probe (50 mm diameter). The crosshead speed was set at 1.7 mm/s. That means, same conditions as for measuring WHC were used[8,9]. The measurements were repeated at least 15 times. The texture was additionally characterised by measuring the penetration force[10]. The penetration force indicates the resistance of the thawed ground fillet against the penetration of cylindrical probes. For this purpose the fillets were thawed overnight in a refrigerator and were comminuted (60 s) using a Krups3Mix 4000. The homogenate was filled bubble-free into petri dishes. The penetration force was measured at a test speed of 0.8 mm/s and applying a strain of 80 % using a spiked aeration plunger equipped with 8 small cylinders (Ø 3 mm) which are arranged in two different squares. The measurement was repeated 12 times. At least four portions were used. Instrumental texture measurements were carried out with a Stable Micro Systems Texture Analyser TA.XT2 (*Stable Micro Systems*, Godalming, England).

Water-holding capacity (WHC)

Expressible moisture was determined using a modification of the filter paper press method as described elsewhere[8,9]. Samples prepared from sliced fillet portions (20 mm diameter, 15 mm thick) were pressed between paired filter sheets (*Schleicher & Schuell* 2043 A, 7x7 cm) and parallel plates using a texture analyser TA.XT2 (*Stable Micro Systems*, Godalming, UK). A 25 kg load cell and a crosshead speed of 1.7 mm/s were used. Samples were pressed to 75 % deformation and held at that point for 15 s. WHC was defined as the expressible moisture, calculated as

% = 100 (initial weight – final weight) / initial weight.

Thermoanalytical behaviour

Protein denaturation was determined using differential scanning calorimetry[9,11,12] . The measurements were performed using a *Perkin Elmer* DSC 7 device equipped with *Perkin Elmer* Intra cooler II and *Pyris* software 3.81. The fish samples (15 ± 3 mg) were weighed (± 0.1 mg) into 60 µl stainless steel pans (*LVC* 0319-0218) and sealed. At least four samples were heated from 10 to 95 °C at a scanning rate of 10 K/min, with an empty sealed pan as reference. The transition temperature (T_{max}) was recorded. The instrument was calibrated for temperature and enthalpy using indium and naphthalene as standards. The transition temperature (T_{max}) was recorded and the transition enthalpy (ΔH) was calculated from the peak area using the *Pyris* software and expressed in J/g sample material. Results are displayed in the figures as average curves.

Statistical analysis

The results were statistically evaluated using the software package STATISTICA (*StatSoft, Inc.* (1996), Tulsa, OK, USA).

Results

Colour measurements

Fig. 1 shows the changes in lightness, redness and yellowness taken from cod fillets as a function of both storage temperature and time.

It is clearly to be seen that L* increases considerably and almost linearly with rising storage time at –10 °C. L* measured on fillets stored at –20 and –30 °C did not change remarkably, while for L* measured on twice frozen fillets (–20DF) stored at –20 °C even a slight decrease during storage was observed. Changes in a* are characterised by a pronounced decrease at –10 °C with increasing storage time, whereas at the other storage temperatures almost no changes were observed. Changes in b* were comparable to that in L*. A marked increase in b* with time of storage was observed at –10 °C, at the other temperatures b* did not change substantially. There are a number of reports on colour changes of fish flesh due to freezing and frozen storage[13–17]. Taking all these reports into account, it can be concluded that the results on colour changes of fish meat due to freezing and refreezing are not uniform. This is supported by results on the influence of double freezing on the colour of breaded and battered portions processed from fillet and minced fish flesh[18–23] as well as on shrimp[24].

Texture measurements

The changes in texture parameters (hardness, chewiness, cohesiveness and springiness) measured on cod fillets by TPA during frozen storage are shown in Fig. 2.

The increase in hardness during frozen storage is most pronounced at –10 °C followed by changes measured at –20 °C. Hardness measured at –30 °C remains almost unchanged during storage. Surprisingly, also hardness measured on DF fillets stored at –20 °C did not change and behaved therefore different compared to once frozen samples stored under same conditions. Comparable trends were also found for chewiness, cohesiveness and springiness. All these attributes increased markedly during storage at –10 °C, while only a slight increase, if ever, was observed at the other storage temperatures, indicating that almost no changes of these texture attributes are caused by these conditions. It becomes obvious that lowering the storage temperature reduces the degree of changes following the Q10 role for (bio) chemical reactions. Tendencies monitored for firmness are almost comparable to the TPA attribute hardness (Fig. 3). Differences in the absolute values are caused by different conditions of measurement. The resistance of minced fish fillet against penetration of eight small cylinders is to be seen in Fig. 3. After initially pronounced increase, the resistance (rise of penetration force) kept constant and

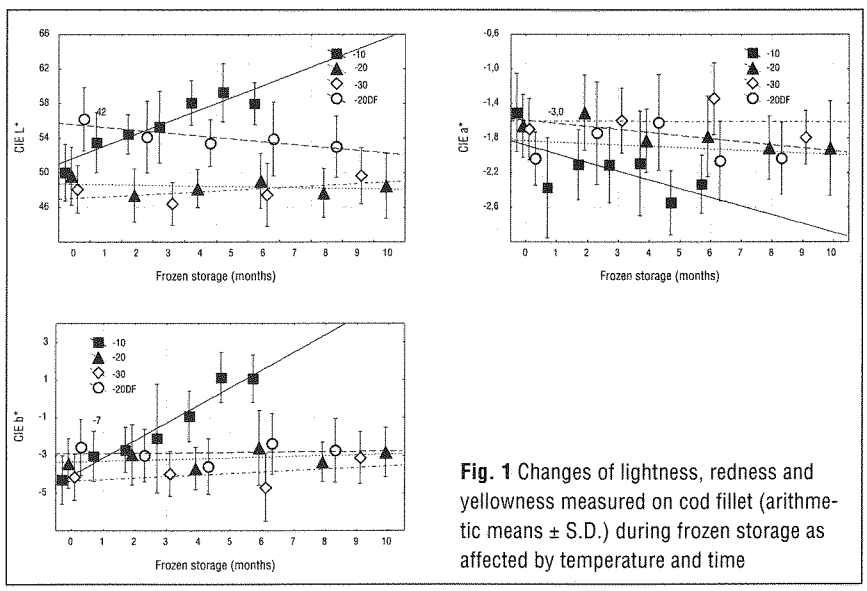

Fig. 1 Changes of lightness, redness and yellowness measured on cod fillet (arithmetic means ± S.D.) during frozen storage as affected by temperature and time

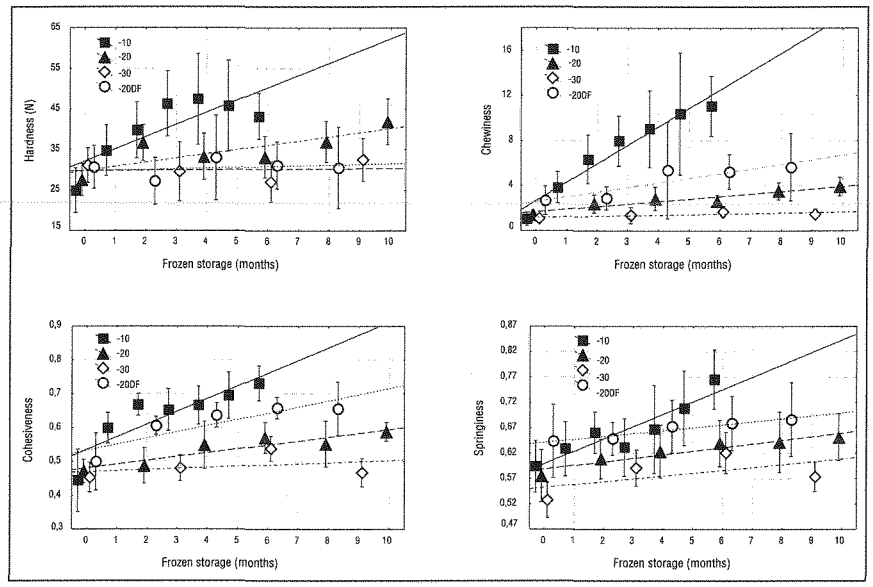

Fig. 2 Changes of texture parameters measured by TPA on cod fillet (arithmetic means ± S.D.) during frozen storage as affected by temperature and time

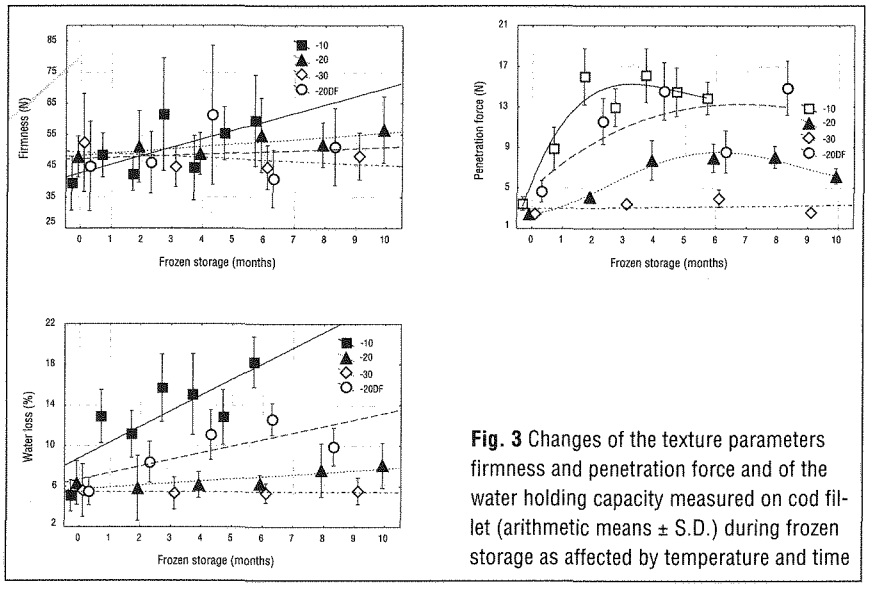

Fig. 3 Changes of the texture parameters firmness and penetration force and of the water holding capacity measured on cod fillet (arithmetic means ± S.D.) during frozen storage as affected by temperature and time

only very small changes occurred with proceeding storage at –10 and –20 °C. Only at –30 °C, the penetration force does not change over the entire storage period, indicating a quite stable situation. In heat- and high-pressure-induced gels of blue whiting, only minor changes in texture during frozen storage independent of the gel-forming processing step were found[25]. Texture changes of frozen stored cod and redfish minces were found to be more pronounced at –7 °C compared to –20 and –40 °C and faster in redfish than in cod mince[26]. When blocks containing fillet, mince or mixtures of both processed from pink salmon were stored at –18 °C for up to 12 months, chewiness tends to increase more for blocks made from unfrozen fish than for blocks made from frozen fish[27]. Hake fillets stored at –10 °C showed greater structural alterations than at –30 °C in terms of increase of β-sheets at the expense of α-helices. An increase of unordered structure was only found in fillets stored at –10 °C[28]. Increasing toughness with storage time was observed on air-blast frozen turbot fillet. Toughness was significantly higher than that of pressure shift frozen samples after 75 days of frozen storage[29]. When changes in texture of cod and haddock fillets stored at –10 and –30 °C for up to 30 weeks were measured, the toughness increased with higher storage temperature and prolonged time of storage for both species in a similar way[30]. Freeze-chilling involves freezing and frozen storage followed by thawing and chilled storage and offers logistic benefits for fish packers. Trials with whiting, mackerel and salmon fillets/portions indicated that the effects of the four treatments on the texture and colour of the raw samples were small in practical terms[31]. According to *Barroso* et al.[32], the methods for texture measurement reviewed did not provide sufficient information to assess fish quality unequivocally. It is therefore recommended to combine several methods by means of multivariate analysis.

Water holding capacity

The strongest increase in water loss is found during storage at –10 °C, followed by –20 °C, whereas WHC is almost unchanged when storage is performed at –30 °C (Fig. 3). The reduction in WHC of the twice frozen cod fillet is more pronounced than that of single frozen fillet. The method reported here was already used successfully to characterise quality changes caused by high pressure-supported thawing in comparison to conventionally defrosting[9] as well as to study the influence of double freezing on redfish quality[33]. There is obviously a good agreement with drip and cooking losses of same fillets as reported earlier[1], where it was found that cod stored at –30 and –20 °C showed the lowest drip losses during the storage period, while cod fillet stored at –10 °C exhibited the highest drip losses although the storage period was shortest (6 months versus 12 months). Drip loss of twice frozen fillet stored at –20 °C was as high as that of the –10 °C fillet.

Thermoanalytical behavior

Differential scanning calorimetry is frequently applied according to papers published recently, which deal with fish quality and processing[34–38]. DSC has emerged as a technique of choice for the study of thermal transitions of food. The conversion of a protein from a native to a denatured

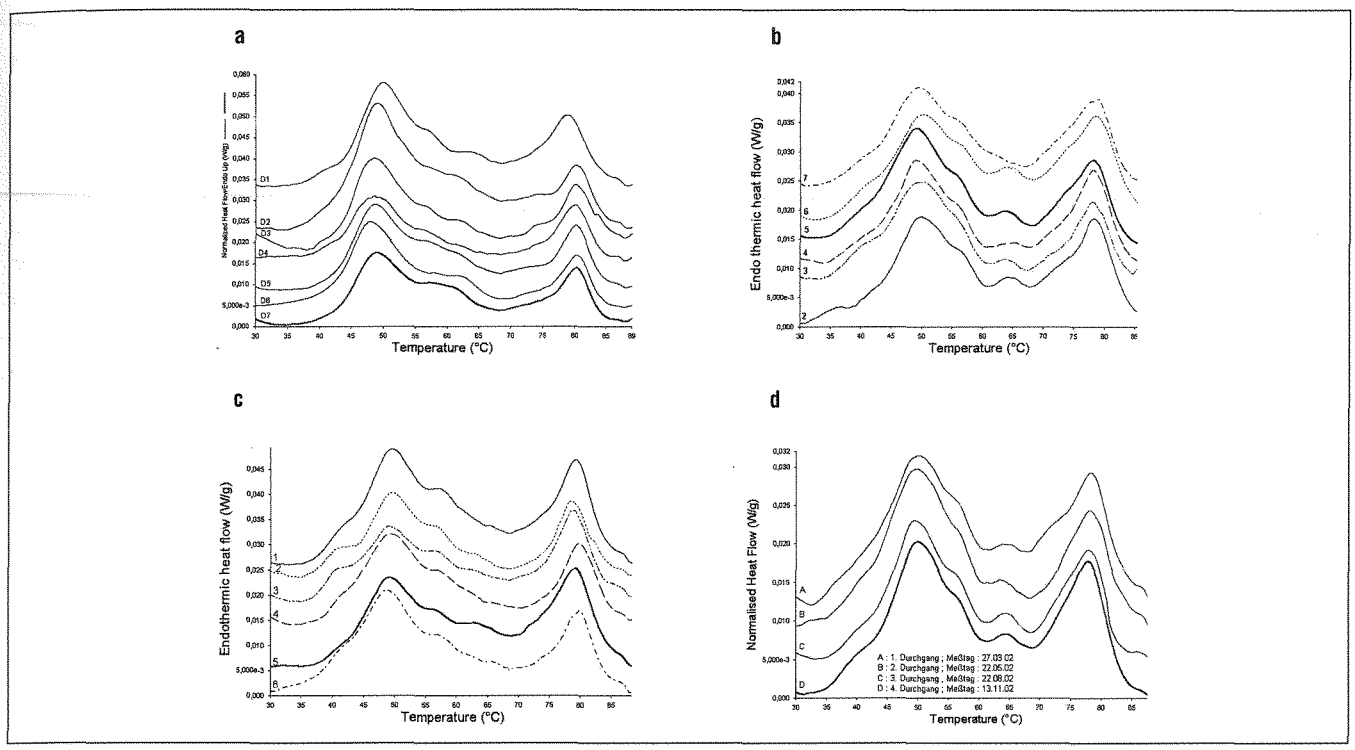

Fig. 4 DSC curves measured on cod fillet as affected by storage temperature (a: –10 °C, b: –20 °C, c: –20DF, d: –30 °C) and time; all curves are average curves from at least four measurements

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. For proteins, the thermally induced process detectable by DSC is the structural melting or unfolding of the molecule, thermal denaturation of proteins being attributed to the rupture of intermolecular hydrogen bonds, the temperatures at which the bonds rupture being a measure 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 which is correlated with the net content of the ordered secondary structure of a protein, is actually a net value obtained through the combination of endothermic reactions and exothermic processes, including protein aggregation and the break-up of hydrophobic interactions.

The DSC curves obtained from cod fillet stored at different temperatures are displayed in Fig. 4.

Within a given storage temperature all curves are comparable and mainly independent on the duration of storage. Small differences were observed between the different storage temperatures. This becomes also obvious from the tables displaying the transition temperatures for single peaks and the appertaining transition enthalpies to these peaks (Table 1–4). The lower temperature transition peak represents the myofibrillar protein myosin and the transition peak at higher temperature indicates actin. The peak in between, which is not so pronounced, represents the sarcoplasmic/connective tissue proteins. While transition temperatures for the first and third peak are quite comparable and almost independent of the storage temperature, the transition temperature of the second peak, if measurable,

Tab. 1 Transition temperatures and enthalpies measured by DSC on cod fillet during frozen storage at –10 °C

Frozen storage [months]	Peak I		Peak II		Peak III	
	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH
0	49.8	1.15	–	–	78.9	0.65
1	48.8	0.92	–	–	80.6	0.27
2	48.4	1.01	–	–	80.2	0.33
3	48.5	0.94	–	–	80.2	0.43
4	48.5	0.94	–	–	80.2	0.43
5	48.4	1.41	–	–	80.3	0.35
6	47.8	0.71	–	–	80.6	0.30
7	48.9	1.22	–	–	80.4	0.46

Tab. 2 Transition temperatures and enthalpies measured by DSC on cod fillet during frozen storage at –20 °C

Frozen storage [months]	Peak I		Peak II		Peak III	
	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH
0	46.8	0.80	61.7	0.02	75.6	0.45
2	49.8	0.87	64.2	0.03	78.4	0.65
4	49.9	1.20	64.0	0.02	78.3	0.53
6	49.1	1.10	65.3	0.01	78.2	0.65
8	49.1	1.20	64.1	0.03	78.2	0.60
10	49.9	0.94	65.1	0.03	78.8	0.65
12	49.4	1.15	–	–	78.9	0.65

Tab. 3 Transition temperatures and enthalpies measured by DSC on twice frozen cod fillet during frozen storage at –20 °C

Frozen storage [months]	Peak I		Peak II		Peak III	
	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH
0	49.5	1.5	57.7	0.03	79.3	0.70
2	49.6	1.3	57.2	0.03	78.5	0.59
4	48.9	1.1	57.1	0.02	78.9	0.66
6	49.1	1.2	57.4	0.01	79.3	0.58
8	49.0	1.2	56.9	0.01	79.3	0.69
10	48.7	1.2	57.3	0.02	80.0	0.59

Tab. 4 Transition temperatures and enthalpies measured by DSC on cod fillet during frozen storage at –30 °C

Frozen storage [months]	Peak I		Peak II		Peak III	
	T_{max}	ΔH	T_{max}	ΔH	T_{max}	ΔH
0	50.1	1.16	64.0	0.02	78.3	0.60
3	49.7	1.22	64.0	0.02	78.1	0.58
6	49.3	1.0	64.7	0.03	78.1	0.54
9	49.7	1.1	64.8	0.03	77.9	0.63

appears to be influenced by storage conditions (Table 1–4). At –10 °C, the second peak has almost disappeared, whereas at –20 and –30 °C, the peak is clearly visible and the same transition temperature was measured. For the twice frozen fillets however, the peak maximum has changed to lower temperatures by approximately 7 °C. This is not in agreement with results of earlier studies[11], which delivered evidence that single and double frozen fish muscle were not discernible. About the reason for these different results can only be speculated because as mentioned above the raw material used for processing into fillet was not identical. It seems that the second peak in the DSC curve of cod muscle is most sensitive against freeze denaturation. At a storage temperature of –10 °C which caused strongest changes in texture and WHC, the second peak was not detectable, presumable as result of pronounced protein denaturation. After refreezing and storage at –20 °C the transition maximum of the second peak is changed to lower temperatures and can be rated as sign of stronger denaturation compared to storage at –20 and at –30 °C for single frozen fillets. Further studies are necessary to verify this DSC results using identical raw material.

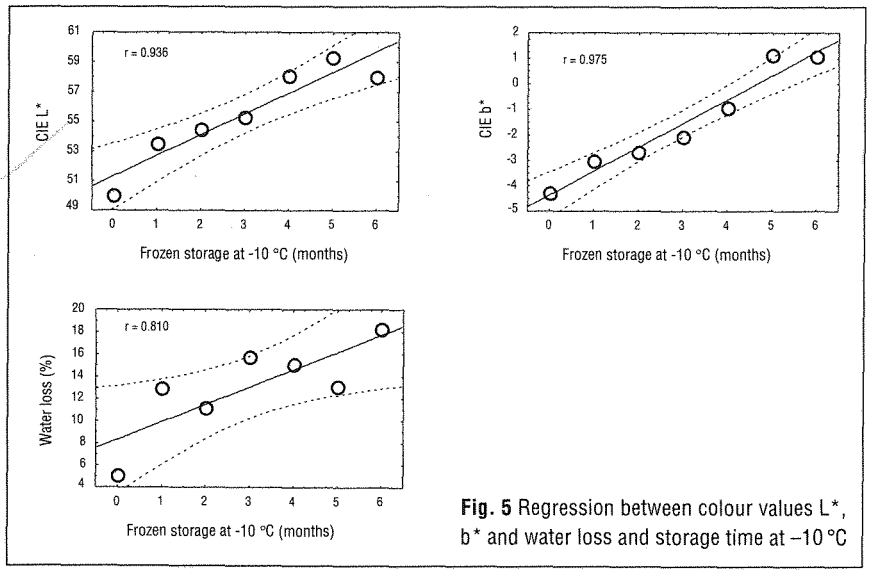

Fig. 5 Regression between colour values L*, b* and water loss and storage time at –10 °C

Correlation between instrumentally measured parameters and storage time

For statistical evaluation, the instrumentally measured results were correlated with the time of storage. There are numerous significant correlations between different parameters, but not all are displayed as figures. At –10 °C, the colour values L* and b* are significantly directly correlated with time of frozen storage (Fig. 5). Also, water loss is correlated. That means an increase in storage time caused a decrease in WHC, this relation is significant and follows a linear equation (Fig. 5). Also the texture parameters hardness, chewiness, cohesiveness and springiness are significantly correlated with storage time at –10 °C (Fig. 6). At –20 °C, the texture parameters cohesiveness, chewiness and firmness were directly correlated with storage time (Fig. 7), as also the water loss, while an inverse

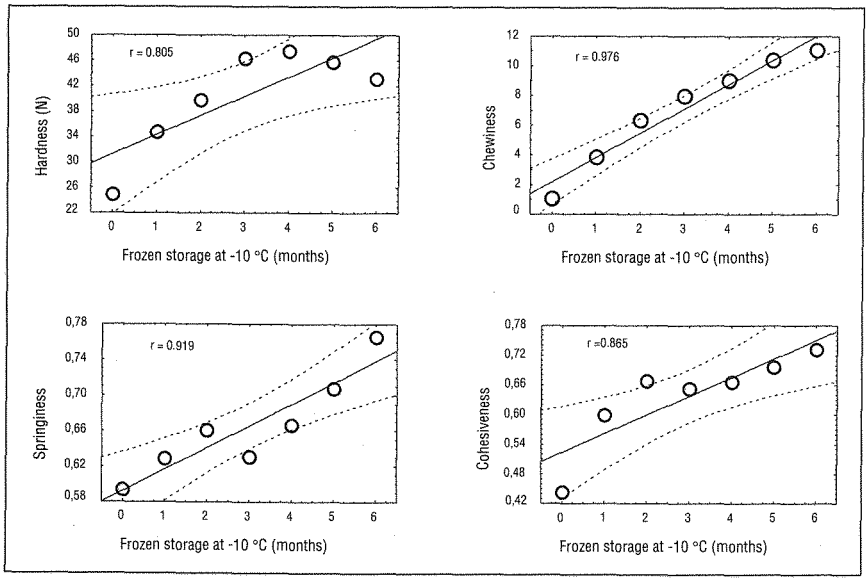

Fig. 6 Regression between texture attributes hardness, chewiness, cohesiveness, springiness measured by TPA and storage time at –10 °C

correlation between a*, the CIE redness, and the storage time was found (Fig. 8). In general, the number of significant linear correlations between instrumental measures and storage time decreases with decreasing storage temperature (Tab. 5). In terms of quality, this means the optimal frozen storage conditions resulted in a better WHC and almost constant texture and colour parameters. The extent of changes of physical parameters during frozen storage is determined by temperature and is expressed by the number of significant linear correlations between the parameter and the storage temperature.

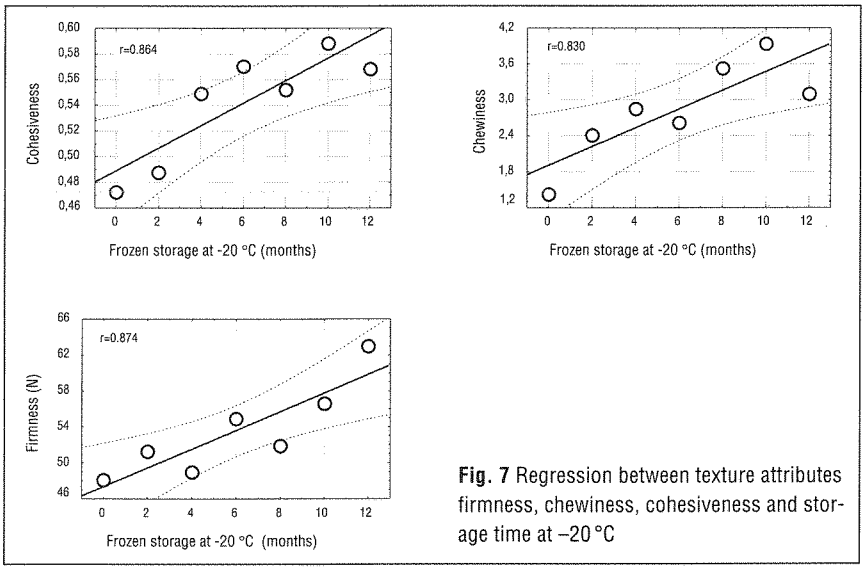

Fig. 7 Regression between texture attributes firmness, chewiness, cohesiveness and storage time at –20 °C

Conclusions

Experiments were performed to show the influences of different storage conditions on connected changes in colour, texture, and water holding capacity and thermoanalytical behaviour as quality indicators. Although results are affected to some extent by varying raw material due to fishing season, clear conclusions on changes in physical attributes in dependence of different storage conditions could be drawn. Changes of colour, texture and water holding capacity during frozen storage were a function of temperature and time. Changes at –10 °C were most pronounced, while those at –30 °C were almost negligible. Changes at –20 °C were between both extremes. In the DSC curves however, storage temperature affected mainly the second transition peak that is ascribed to sarcoplasmic and connective tissue proteins. The frequency of significant linear correlations between instrumental data for colour, texture and water holding capacity and the respective storage time proved to be temperature-dependent and was highest at –10 °C. From the point of quality assurance, storage at –10 °C should be avoided, while storage at –30 °C should be strived for if ever possible.

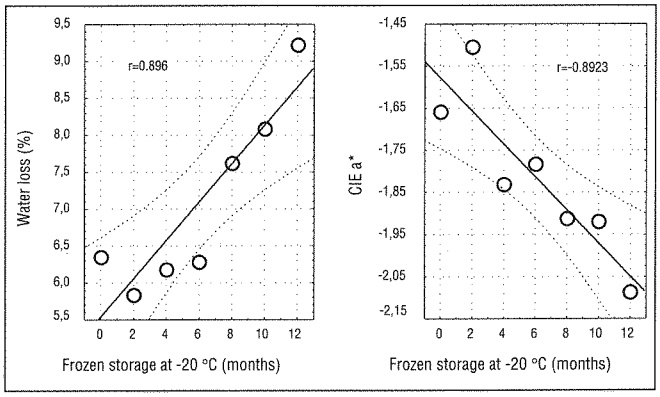

Fig. 8 Regression between redness, water loss and storage time at –20 °C

Tab. 5 Frequency of linear correlation of physical parameters and storage time as affected by storage temperature

Frequency of correlation	storage at °C
9	–10
6	–20
3	–20 df
1	–30

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