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Iced Storage of Fish, New Aspects: Comparison between Flake Ice and StreamIce® – Part II: Horse mackerel *(Trachurus trachurus)*

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Summary
Comparative iced storage experiments using flake ice and an ice slurry called StreamIce® were performed on board ship using horse mackerel caught in early autumn as raw material.
Sensory assessment of freshness by quality index method (QIM) and the European grading scheme for fresh and refrigerated fish as well as determination of total viable count and specific spoilage bacteria, *Shewanella putrefaciens*, were performed on board. Furthermore, changes in TVB-N and volatile amines such as TMA were estimated using trichloroacetic acid extracts prepared on board. Instrumental colour measurements were performed on both iced stored samples on board and on frozen/thawed samples later ashore. Furthermore, instrumental measurements of texture parameters, water binding and thermal behaviour were conducted on samples frozen after distinct iced storage on board and thawed after 4 months of frozen storage.
No significant differences in the evolution of freshness of horse mackerels along iced storage in flake ice and slurry ice were found by sensory assessments. Also the microbial investigation and the determination of TVB-N and TMA-N did not reveal any significant differences between samples stored in both types of ice. Measurements of texture, water binding ability and colour did not reveal any significant differences between both ice types. On the other hand, thermal stability was found to be lowered in slurry ice samples. In these samples the transition temperature of the myosin peak decreased from day 12 of storage on and was paralleled by the transition enthalpy. In contrast, the flake ice batch did not show remarkable changes in thermal behaviour.

Zusammenfassung
Eislagerungsuntersuchungen mit im September gefangenem Stöcker wurden an Bord des Fischereiforschungsschiffs „Walther Herwig III" durchgeführt. Dabei wurde konventionelles Scherbeneis mit einem Eisslurry (StreamIce®) hinsichtlich der Entwicklung sensorischer, mikrobiologischer und chemisch-physikalischer Parameter von Stöcker während der Lagerung bei 0 bis 1 °C verglichen. Bei dieser Eisvariante (StreamIce®) handelt es sich um mikrokristalline Eiskristalle in einer wässrigen Lösung mit einer Temperatur leicht oberhalb des Gefrierpunktes des Fischmuskels.
Der Vergleich beider Eisvarianten hinsichtlich sensorisch bewerteter Qualität und mikrobiologischer Befunde ergab keine signifikanten Unterschiede während einer 15-tägigen Lagerung der Fische. Auch bezüglich der Entwicklung der TVB-N-Werte, der Farbe sowie der instrumentell bestimmten Textur und Wasserbindung erwiesen sich die Eislagerproben

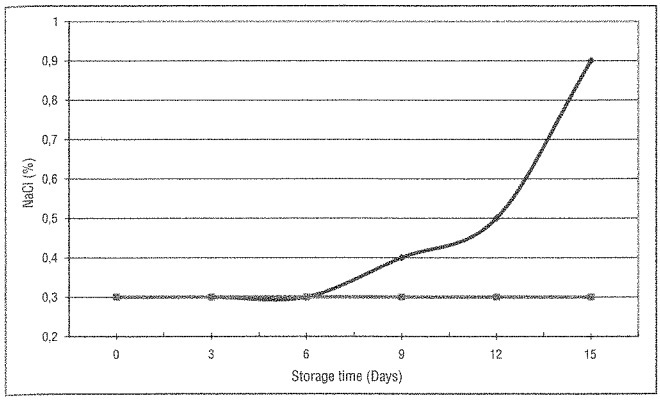

Fig. 1 Evolution of NaCl content in muscle of horse mackerel during ice storage under different condition (■ Flake ice, ◆ StreamIce)

als nicht signifikant verschieden. Die thermische Stabilität der mit dem Eisslurry gekühlten Fische zeigte sich dagegen als merklich beeinflusst. Dieses äußerte sich insbesondere in der Verringerung von Denaturierungstemperatur und -enthalpie des Myosinpeaks mit zunehmender Lagerdauer.

Keywords: Fish, horse mackerel, iced storage, quality, sensory assessment, TVB-N, texture, colour, DSC / Fisch, Stöcker, Eislagerung, Qualität, sensorische Beurteilung, TVB-N, Textur, Farbe, DSC

Introduction

Recently in this journal a report has been published on a comparative ice storage experiment using a new form of ice, a fine-crystalline ice slurry, and the traditional ice, well-known as flake ice (*Schubring* and *Meyer*, 2006). Sardine has been iced during this experiment performed on board the FRV "Walther Herwig III" at the 279th research cruise. In the first paper some information were given on application of ice slurries in general and more deeply on their use in fish processing. The present paper deals with the second experiment which was directed on another pelagic species, horse mackerel. Trial followed the same aim, icing the fish immediately after catching on board using both traditionally applied flake ice and an ice slurry called StreamIce® and to observe the quality changes in the differently iced fish during storage on board at 0 to 1 °C by using sensory, microbial and chemical methods. Measurements were completed by application of physical methods for texture, colour, water binding ability and thermal stability ashore using samples taken at predetermined time intervals and deep frozen.

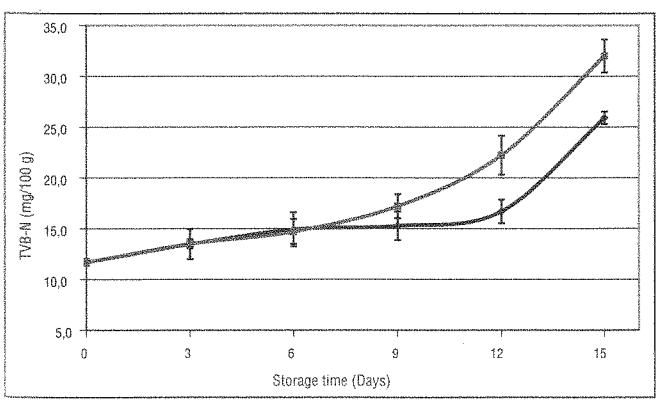

Fig. 2 Evolution of total volatile basic nitrogen (TVB-N) in muscle of horse mackerel along ice storage under different conditions (■ Flake ice, ◆ StreamIce)

Materials and methods

Refrigeration systems

Same refrigeration system consisting of a ZIEGRA StreamIce® machine SI 750 TW for installation on board (*ZIEGRA Eismaschinen*, Isernhagen/Germany) and the on board installed ice making equipment Buus Iceflaker Type SD 1000 M (*Buus Refrigeration*, Denmark) was used for processing StreamIce® (SI) and flake ice (FI), respectively. For processing SI seawater was used, whereas FI was prepared from fresh water. The composition of the ice slurry was 40 % ice and 60 % seawater. The fish specimens were surrounded by either FI or SI at a fish:ice ratio of 1:1 and stored for 15 days (according to duration of the cruise) in fish boxes in a refrigerated room at temperatures in the range 0 to 1 °C. Ice was renewed when required.

Fish material, processing and sampling

Horse mackerel (*T. trachurus*) was caught in the Lyme Bay, in the mid of September 2005. Lyme Bay is an area of the English Channel situated in the southwest of England between Torbay in the west and Portland in the east. Length of horse mackerels was mainly in the range from 23 to 26 cm and their weight was from 125 to 175 g. The fish was neither headed nor gutted prior to ice storage. Its temperature after catching was approximately 18.0 to 18.5 °C. Every third day five of the differently iced specimens were taken for sensory, chemical and microbiological analysis, respectively. Additionally, 10 fish per sampling day were air-blast frozen at −35 °C for later physical measurements ashore and stored at the same temperature on board and later in the institute until investigated.

Sensory analysis

Sensory analysis was conducted on whole fish by a panel of scientific crew members consisting of 10 experienced judges, according to official guidelines (Anonymus, 1996) concerning fresh and refrigerated fatty fish. Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and unacceptable quality (C). Sensory assessment of the fish included the following parameter: skin, skin mucus, consistency of flesh, gill covers, eye, gills, and smell of gills. Additionally, the QIM scheme developed for horse mackerel (*Inácio* et al. 2003) was used for sensory evaluation.

The following quality parameters were evaluated: general appearance (surface appearance, flesh firmness), eyes (pupil, shape), gill cover (bloodiness), gills (colour, smell), abdomen (postgill/belly-burst), vent (appeareance). Demerit points ranged from 0 to 17.

Microbiological analysis

For microbiological analysis aerobic total viable count (TVC) and specific spoiling bacteria (SSB) on skin and in tissue of horse mackerel were determined. On each sampling day skin and tissue of five randomly taken fishes were pooled. Always 24 cm^2 of skin and 10 g of tissue were prepared aseptically from every fish and collected. Pooled samples were homogenized for 3 min in sterile NaCl-peptone solution in a stomacher. After homogenization serial decimal dilutions were inoculated on modified plate count agar plates containing ferrous citrate according to Lyngby-Agar and incubated at room temperature for three days prior to counting colonies (*Gram* et al., 1987). The number of all colonies is TVC whereas numbers of black colonies gave counts of SSB, mainly *Shewanella putrefaciens*.

Chemical analysis

Proximate composition was determined using the respective German standard methods for protein, fat, dry matter, sodium chloride, pH and ash as well as the total volatile basic nitrogen (TVB-N) according to § 64 LFGB. Trimethylamine oxide (TMAO) and volatile amines (TMA, DMA) were determined according to *Oetjen* and *Karl* (1999).

Physical analysis

Physical measurements were performed on defrosted samples by thawing overnight in a refrigerator after 4 months of frozen storage. Colour measurements were performed using a spectral colorimeter spectro pen® (*Dr. Lange*, Düsseldorf/Germany), on both the skin (belly flaps) and on homogenates prepared from skin-less fillets on board and later ashore. Texture measurements were performed as Texture Profile Analysis at 60 % strain using a Texture Analyser TA.XT2. Additionally penetration force was measured using same equipment on the homogenates prepared for colour measurement. Water binding ability was measured as expressible moisture at 75 % strain using above mentioned texture analyser. The degree of denaturation of muscle proteins was characterised by determination of thermal stability using a SETARAM MicroDSC VII. Methods are described in the previous report (*Schubring* and *Meyer*, 2006).

Statistical analysis

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

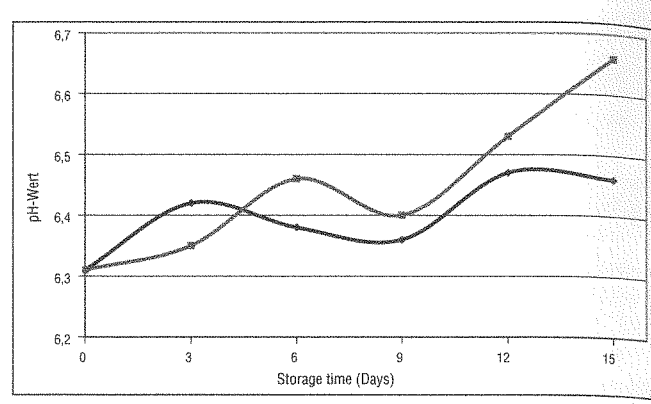

Fig. 3 Evolution of pH value in horse mackerel muscle along ice storage under different condition (■ Flake ice, ◆ Streamlce)

Results and discussion

Proximate composition

Proximate composition of horse mackerel was determined as follows: Protein 19.1 ± 0.28 %, fat 6.1 ± 0.3 %, water 75.3 ± 0.05 %, ash 0.7 ± 0.3 %. Seasonal variation of chemical composition of horse mackerel caught off the Portuguese coast was investigated by *Bandarra* et al. (2001) showing that protein content remaining fairly constant over the year while the amount of total lipids present showed a minimum in February. *Osaka* et al. (2002) compared chemical components and body colour of horse mackerel caught in different Japanese fishing areas and found no difference in quality among the catches with the exception of the crude lipid content of one catching ground. They further found consistently high levels of docosahexaenoic acids (DHA) in various fishing grounds which suggests that horse mackerel offers a stable source of DHA (*Osaka* et al., 2003).

The presence of NaCl in SI has led to a progressive increase of sodium chloride in the fish muscle after the 6th day of storage (Fig. 1). This increase continued until reaching 0.9 % NaCl at day 15, whereas fish samples treated with FI did not show any remarkable changes in NaCl content along storage. Comparable results have been found by *Losada* et al. (2005).

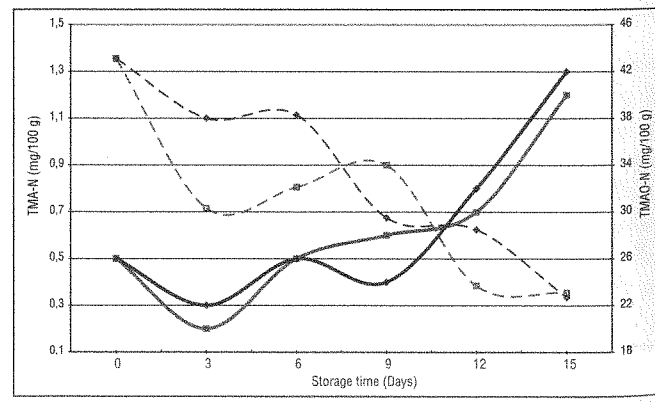

Fig. 4 Evolution of trimethylamine nitrogen (TMA-N) (■◆) and trimethylamine oxide nitrogen (TMAO-N) (❑❖) in horse mackerel muscle along ice storage under different conditions (■❑ Streamlce, ◆❖ Flake ice)

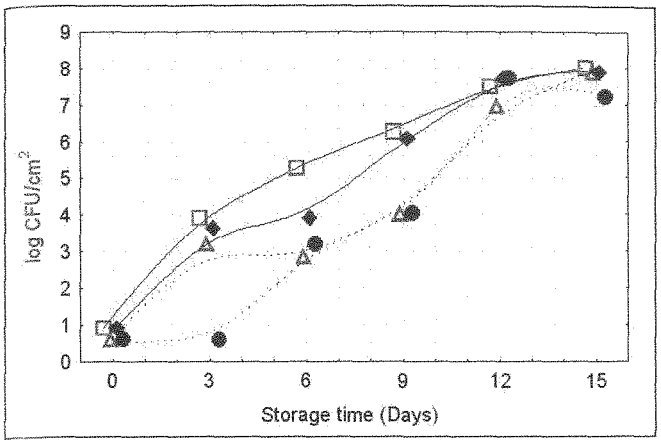

Fig. 5 Evolution of total viable counts (◆❑) and specific spoilage organisms (●△) on skin of horse mackerel along ice storage (Flake ice ■▲, Streamice ◆●)

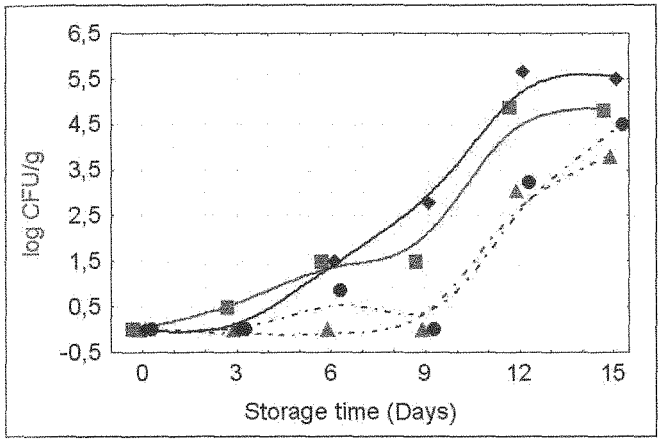

Fig. 6 Evolution of total viable counts (◆■) and specific spoilage organisms (●▲) in tissue of horse mackerel along ice storage (Flake ice ■▲, Streamlce ◆●)

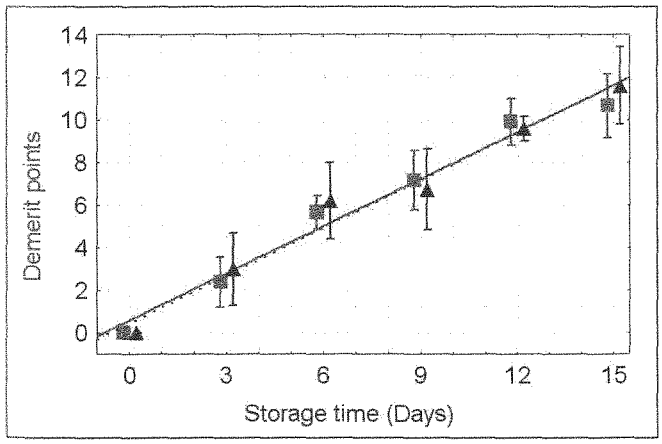

Fig. 7 Changes in freshness (mean and standard deviation) evaluated by QIM in horse mackerel along ice storage (Flake ice ▲, Streamlce ■)

The evolution of TVB-N contents in horse mackerel during storage in the different ice types is shown in Fig. 2. From day 6 on there is a significant difference to be seen between FI and SI. The moderate increase in TVB-N values with progressing storage time in FI samples became steeper while comparable changes in SI samples were only observable after day 12. TVB-N values of SI samples are remarkable lower (25.3 mg/100 g) than that of FI samples (31.9 mg/100 g) at the end of iced storage. In contrast to gadoids (Anonymus, 1996), for horse mackerel there is no official limit set indicating acceptable quality. When comparing our results with those reported by *Rodríguez* et al. (2005) for horse mackerel it becomes obvious that initial TVB-N values were much lower indicating the influence of freshness of fish at the beginning of ice storage experiment. This is explained by the fact that here fish was iced immediately after they have been caught whereas in the Spanish trial it took 10 hours after catching that the storage experiment started. However during this time fish was reportedly kept in ice (*Rodríguez* et al., 2005). This lower freshness also possibly explains the different results in regard to evolution of TVB-N. For FI samples a TVB-N of about 65 mg/100 g at day 15 of storage were reported, whereas values for SI were almost comparable with our results (*Rodríguez* et al., 2005). According to *Aubourg* (2001), TVB-N value measured at iced stored whole horse mackerel showed a gradual increase throughout the chilled storage and a significant increase compared to the raw sample was observed at day 12.

The pH increased slightly during iced storage (Fig. 3). In accordance with the more pronounced increase in TVB-N the increase in pH was stronger in FI compared with SI. However, pH values > 7.25 for FI samples after 15 days iced storage as reported by *Rodríguez* et al. (2005) could not be observed. *Simeomidou* et al. (1998) reported also no significant changes in pH during iced storage of horse mackerel.

There is only to be seen an increase in TMA-N with prolonged storage time (Fig. 4) and the values are very low. Differences between SI and FI were almost negligible and the raise of TMA-N is not as dramatic as reported by *Rodríguez* et al. (2005) who found TMA-N values of 10.5 and 22.5 mg/100 g in slurry ice batch and flake ice batch, respectively, after 19 days of storage. Higher TMA values were also reported by *Mendes* et al. (2005) for horse mackerel stored in ice at 3 °C. The "limit of acceptability" of 10–15 mg/100 g as it is called by the authors was attained near the 10th day of storage. The content of TMAO-N, the precursor of the TMA produced by bacterial action during ice storage, decreased with increasing storage time from 43 to 23 mg/100 g due to the formation of TMA and the leaching out effect of the melt water (Fig. 4). Differences between SI and FI were not obvious at the end of iced storage. The TMAO content of horse mackerel was about 4 times higher than that of sardine at the beginning of storage trial (*Schubring* and *Meyer*, 2006).

Aubourg (2001) observed in iced stored horse mackerel a significant increase in TMA-N at day 9 compared to the unstored samples. *Mishima* at al. (2005) investigated recently the relationship between storage temperature and freshness and found that the change in K-value in horse mackerel in-

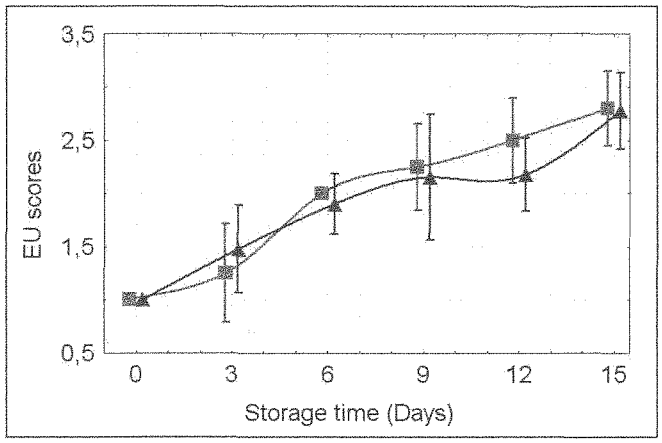

Fig. 8 Evolution of smell (mean and standard deviation) on horse mackerel along ice storage (Flake ice ■, Streamice ▲)

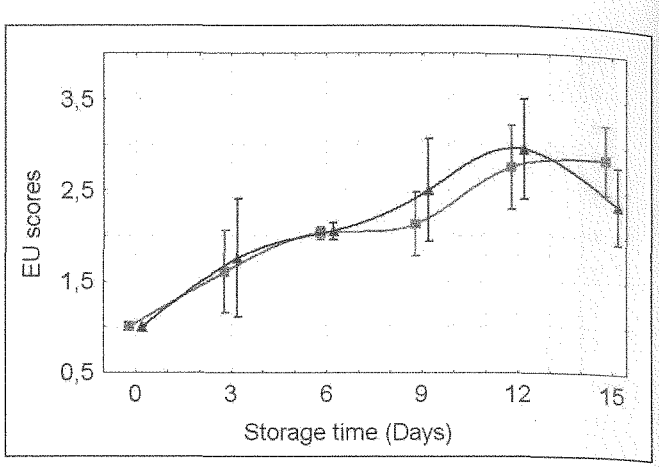

Fig. 9 Evolution of eyes (mean and standard deviation) on horse mackerel along ice storage (Flake ice ■, Streamice ▲)

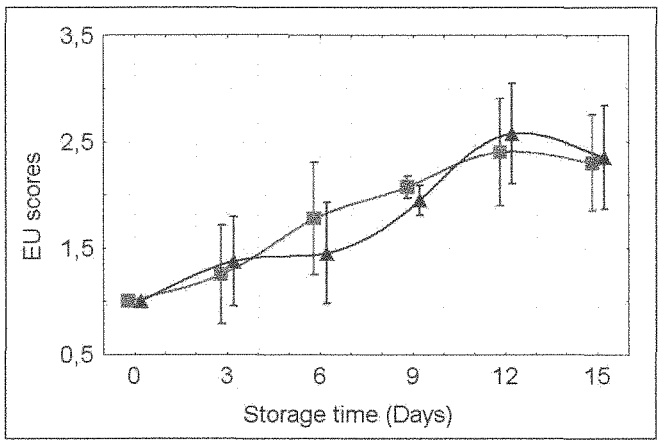

Fig. 10 Evolution of consistency (mean and standard deviation) on horse mackerel along ice storage (Flake ice ■, Streamice ▲)

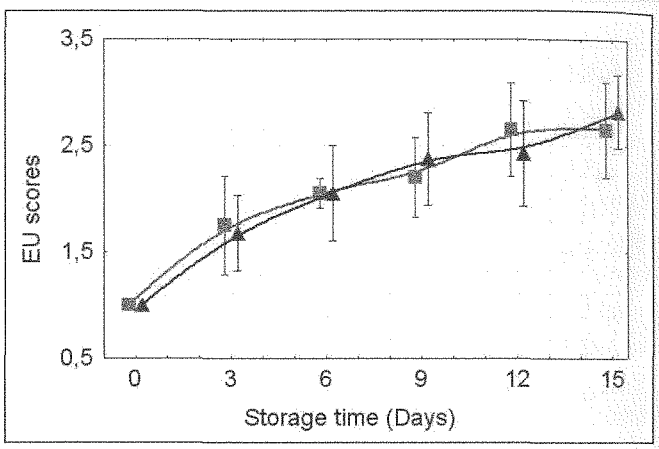

Fig. 11 Evolution of gill (mean and standard deviation) on horse mackerel along ice storage (Flake ice ■, Streamice ▲)

creased with higher storage temperature. However, at 0, 5 and 10 °C, K-values changed slowly and all reached about 3 % after 24 h. Temporal changes in energy-related compounds, such as ATP, IMP and lactic acid were found to be slower at 10 °C than at 0, 5, and 15 °C storage, suggesting a delay in progress of rigor mortis. Based on this results storing the horse mackerel in two stages is suggested, the first stage is storage at 5 or 10 °C in order to delay rigor mortis and the second stage is at 0 °C to prevent deterioration of freshness.

Microbiological analysis

Results of microbiological analysis of skin samples are shown in Fig. 5. Numbers of TVC increased gradually from 10^1/cm² to 10^8/cm² at day 12 and stayed at this level up to end of experiment. Except the range from day 4 to 7, when TVC in SI samples showed one log unit lower counts, there were no significant differences compared with FI samples. Counts of SSB increased from $0.5x10^1$/cm² after a lag phase of two days to about 10^8/cm² at the end of storage time with no significant differences between storage conditions. Growth of bacteria in tissue samples is shown in Fig. 6. TVC in the tissue increased from $0.5x10^1$ at day 3 to 10^5–$5x10^5$/g on day 12 staying stationary the last 3 days. Counts of SSB increased continuously from day 9 to $5x10^4$/g at the end of storage. Comparing counts in tissue samples no differences in bacterial growth due to storage conditions could be detected. These findings are in contrast to results published by *Rodríguez* et al. (2005). They found generally lower TVC in tissue stored in ice slurry compared to FI samples with differences ranging from 2 to 2.6 log units. TVC counts in tissue of horse mackerel stored in FI of $5x10^5$ and $5x10^6$/g were estimated on day 8 and 12 while the present experiments showed $5x10^2$/g and 10^5/g, respectively. During iced storage of horse mackerel a reduction in bacterial count in the first 2 days was found by *Inácio* et al. (2003) and they assumed that this could be due to change from seawater to freshwater ice. After day zero, all bacterial numbers decreased until day 6 after which the exponential growth began. However, a lack of correspondence between microbial counts and sensory benefits was noticed. The similarity of the total counts in iron agar and *Pseudomonas* agar showed that the *Pseudomonas* group of bacteria was always dominant and almost the only present during spoilage. *Mendes* et al. (2005) found a linear relationship between the increase of dose irradiation and the reduction

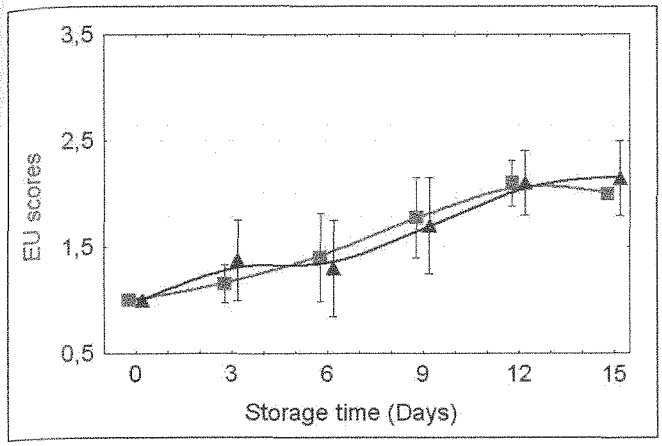

Fig. 12 Evolution of gill cover (mean and standard deviation) on horse mackerel along ice storage (Flake ice ■, StreamIce ▲)

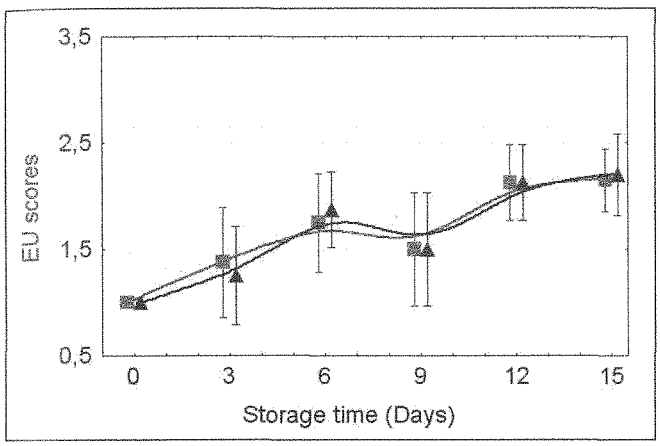

Fig. 13 Evolution of skin (mean and standard deviation) on horse mackerel along ice storage (Flake ice ■, StreamIce ▲)

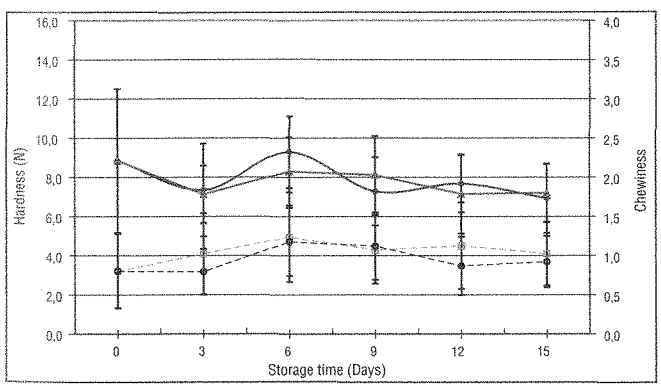

Fig. 14 Evolution of hardness (◆▲) and chewiness (○❑) in muscle of horse mackerel along ice storage under different conditions (◆❑ StreamIce, ○▲ Flake ice)

of viable micro organisms, with a correlation coefficient of 0.99 and 0.97, respectively, for skin and muscle of iced stored horse mackerel. Assuming the limit in acceptability in what concerns the TVC of irradiated fish is $1x10^6$ cfu bacteria/g, this was attained in the skin at day 13, while in the irradiated samples that threshold was only reached at day 23. TVC in the muscle was 1 to 1.4 log units lower than on the skin. According to *Kuda* et al. (2002) increased the aerobic bacterial count of horse mackerel skin from 10^3 to 10^7 cfu/cm² in 7 days at 4 °C and was connected with increasing alkaline phosphatase activity from 10 to 150 mmol *p*-nitrophenol/min/g tissue, that can therefore be seen as spoilage index in most red-flesh fishes.

Sensory evaluation

In recent years, the quality index method (QIM), a grading system for estimating the freshness and quality of seafood, has been established for several species and is widely used in the industry. Freshness is one of the most important quality criteria for fish, and storage time and temperature are the main factors affecting the rate of loss of quality and shelf life of fish (*Whittle*, 1997). The freshness of horse mackerel evaluated by QIM decreased with storage time (Fig. 7). However, of the 17 possible demerit points only 10 to 11 were reached after 15 days of storage. Freshness decreased almost linearly with storage time for both FI and SI with coefficients of correlation of r = 0.95 und 0.93, respectively. Icing conditions did not affect significantly the freshness of horse mackerel along 15 days storage as shown by the high correlation coefficient of r = 0.94 between FI and SI. It appeared that horse mackerel was not spoiled completely after 15 days of iced storage. Prolongation of iced storage was unfortunately impossible due to limited duration of research cruise.

This statement is supported by the results obtained when freshness was graded according to the EU grading scheme (Fig. 8 to 14). In the figures the freshness categories E, A, B, C were transformed in 1, 2, 3, 4, respectively and displayed on Y axis. Changes in smell along storage time (Fig. 8) were in the range from E to B and significant for both FI and SI with r = 0.88 and r = 0.83, respectively. Differences between FI and SI were not significant ($p > 0.05$). The eyes (Fig. 9) became flat, with blurred pupils and blood seepage around the eyes and their grading changed from E to B along iced storage with coefficients of correlation of r = 0.88 (FI) and 0.70 (SI). Differences between FI and SI were found to be insignificant. Almost the same was found for consistency (Fig. 10). Changes in consistency were characterised by an increase in softness and well correlated with storage time (r = 0.78 (FI), r = 0.80 (SI)) and were not significantly influenced by the type of ice used. Almost independent from ice used for cooling, gills became increasingly discoloured and the mucus of gills became opaque along storage time (Fig. 11). A correlation coefficient of r = 0.83 was calculated for both SI and FI. Changes in both skin (Fig. 12) and gill cover (Fig. 13) were less pronounced along iced storage. At the end of trial fish had lost somewhat of lustre and shine and the colour became dull, while the colour of gill cover became brownish and a partly pronounced seepage of blood became obvious. However, there were no significant differences between FI and SI. From the results of sensory evaluation it can be concluded, that horse mackerel was quite stable during iced storage. Its freshness after 15 days

of storage at 0 to 1 °C was mainly graded as B allowing the utilisation for further processing in industry. Thereby it was not important whether the fish was stored in FI or in SI; differences between both were not significant ($p > 0.05$).

These results of sensory evaluation do not fully agree with the ones published earlier (*Inácio* et al., 2003; *Rodríguez* et al., 2005). Storing horse mackerel in crushed tape water ice at 2 °C rejection was achieved at day 9 measured by the EU scheme (*Inácio* et al., 2003). On the other hand, the mean of the QIM value at day 12 was lower than 17 (maximum demerit points for this species), as some characteristics were not achieved. According to *Rodríguez* et al. (2005) horse mackerel stored in slurry ice maintained good quality (E and A categories) up to day 8, while the counterpart batch stored in flake ice only maintained such good quality up to day 2. As storage time progressed, sensory quality decreased and by day 8 (flake ice batch) and day 19 (slurry ice batch) the specimens were no longer acceptable. *Mendes* et al. (2005) reported also a sensory shelf life of 8 days for horse mackerel stored in ice at 0 ± 1 °C. The reasons for the reported differences of sensory shelf life of iced stored horse mackerel could be manifold. One of the main causes is seen in differences of initial quality of the raw material. The remarkable difference between FI and SI found by *Rodríguez* et al. (2005) can not be verified by our results. Reasons for that were possibly differences in initial quality and the delayed start of experiment.

Physical measurements

As mentioned earlier physical measurements were performed on samples frozen on board at the given sampling day and thawed after frozen storage by defrosting overnight in a refrigerator. On this way changes in the horse mackerel muscle caused by ice storage on board should be detectable due to same influence that freezing and frozen storage can possibly exert on all samples and can therefore be denied. As shown in Fig. 14, hardness and chewiness, two texture parameter derived from TPA, were almost not affected by the different ice types used for storage. While chewiness almost did not change during storage, hardness appeared slightly decreasing along ice storage. This was expected and

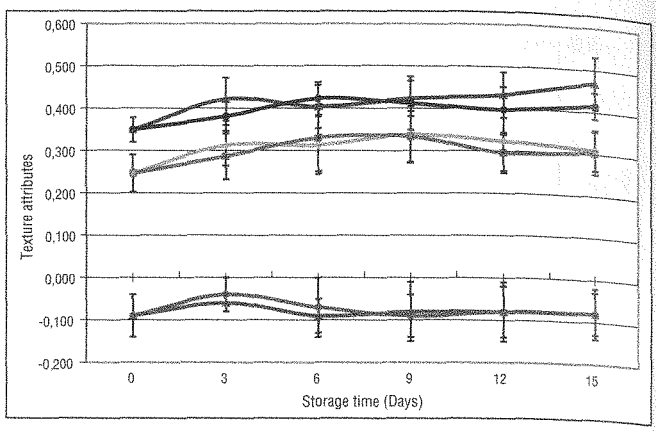

Fig. 15 Evolution of cohesiveness (○▲), springiness (❑❖) and adhesiveness (●Δ) in muscle of horse mackerel along ice storage under different conditions (StreamIce▲❖Δ, Flake ice ○❑●)

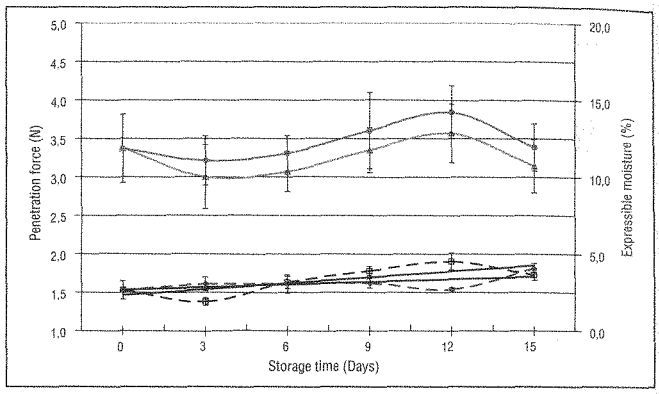

Fig. 16 Evolution of penetration force (◆❑) and expressible moisture (●Δ) in muscle of horse mackerel along ice storage under different conditions (◆Δ StreamIce, ●❑ Flake ice)

according to the sensory schemes used, because texture of flesh should become softer with progressing of iced storage. According to sensory evaluation consistence became slightly soft after 15 days of iced storage (Fig. 10). The other texture parameters measured by TPA, cohesiveness, springiness and adhesiveness, did not vary significantly by both ice type and storage time. However, there seems to be a tendency of slightly increasing in cohesiveness as well as springiness with progressing storage time while adhesiveness decreased slightly at day 3 turned back to the initial

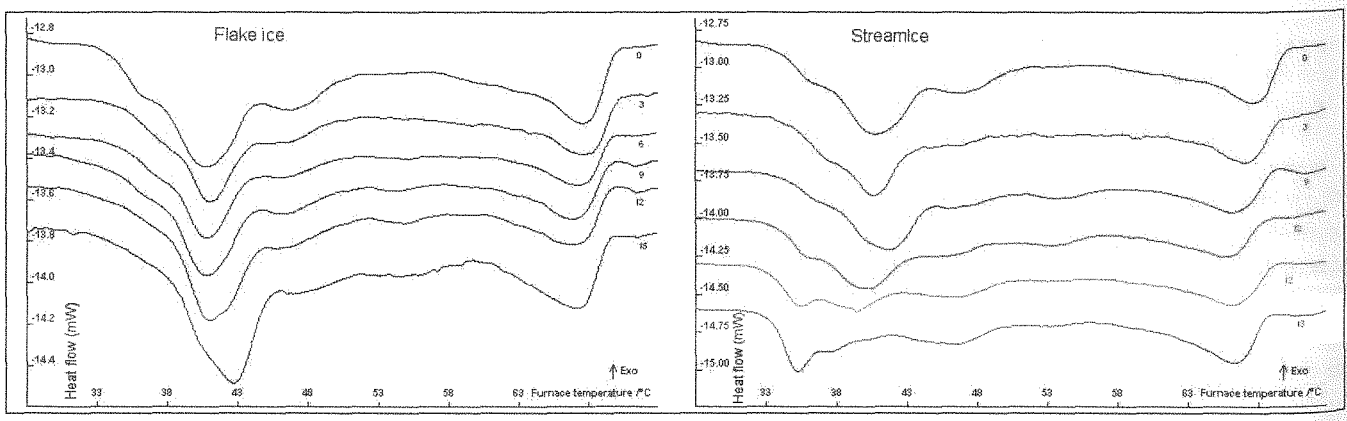

Fig. 17 DSC curves taken on horse mackerels dependent on storage time in Flake ice (left) and in StreamIce (right)

Tab. 1 Transition temperatures and -enthalpies of muscle proteins measured along the ice storage of horse mackerels in both Flake ice and Streamice

Day	Ice	Peak I			Peak II		
		T_{on}	T_{max}	ΔH	T_{on}	T_{max}	ΔH
0	–	36.1	40.6	2.270	62.4	67.7	1.047
3	SI	34.9	40.5	1.709	58.8	66.9	0.796
3	FI	37.6	40.9	2.051	63.0	68.1	1.107
6	SI	36.5	40.8	0.992	60.8	66.9	0.461
6	FI	37.4	40.7	1.906	57.4	62.6	0.712
9	SI	36.7	41.6	1.822	61.1	66.3	0.761
9	FI	37.0	40.7	0.906	62.6	66.9	0.329
12	SI	33.2	39.4	1.583	61.3	66.2	0.814
12	FI	37.7	41.0	2.288	62.5	67.2	0.789
15	SI	33.7	35.3	0.802	61.9	66.1	0.904

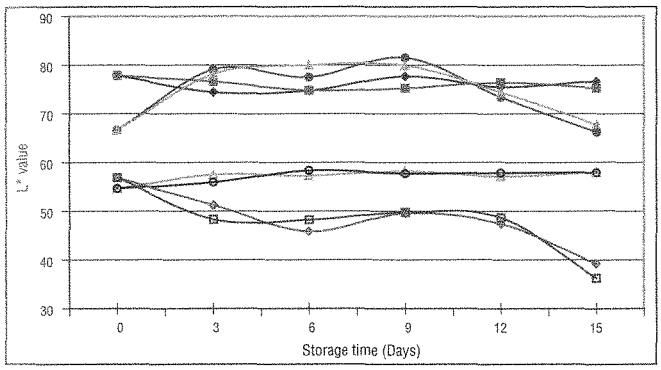

Fig. 18 Evolution of lightness measured on skin (◆■ ashore, ▲● on board) and on homogenised muscle (○△ ashore, ❖❑ on board) of horse mackerel along ice storage under different conditions (◆△❑▲ Streamice, ■●○❖ Flake ice)

value and stayed unchanged until end of storage (Fig. 15). After homogenising the intact muscle a probe consisting of eight small cylinders regularly arranged in two different squares was used to measure the resistance of the minced muscle against penetration by 99 % strain. Fig. 16 shows that the force necessary to penetrate the sample increased slightly with continuing storage. This behaviour is independent from ice type used for storage and characterised by $R^2 = 0.606$ and $R^2 = 0.404$ for FI and SI, respectively. This tendency could possibly be explained as follows. During homogenisation the particles from specimens stored longer in ice became finer than those processed from specimens at the beginning of ice storage. The finer the particles the denser the homogenate in the petri dish and the higher the penetration force. Besides, the water holding capacity (WHC) was measured, too (Fig. 16). It becomes obvious that pattern of changes are almost comparable between FI and SI. With progression of storage time up to day 12, WHC decreased as the expressible moisture measured increased. However at day 15, expressible moisture is almost at the same level as at the beginning of experiment. In general, changes in texture and water binding ability that could be detected by physical measurements on frozen/thawed samples after 4 months of frozen storage are comparably small. Available literature on measurements of texture and water binding ability of iced stored fish had already been discussed recently (*Schubring* and *Meyer*, 2006).

Using the DSC curves (Fig. 17) taken on specimens at the various storage times it became obvious that there are some differences between samples stored in FI and those stored in SI. An influence of the storage time in ice on DSC pattern was also detectable. This testifies to the fact that the muscle proteins have partly been denatured during iced storage. This is documented further by the transition temperatures (T_{max}) and transition enthalpies (ΔH) calculated from the DSC curves and shown in Table 1. Two main peaks are to be seen in the DSC curve comprising myosin (peak I) and actin (peak II). T_{max} of the raw material (day 0) have been calculated as high as 40.6 and 67.7 °C for myosin and actin, respectively. T_{max} of myosin in the FI batch did not change very much along storage. However, at the end of storage even a small increase was detectable. The calculated ΔH is almost at the same level with the exception of day 9. In contrast, in the SI batch T_{max} of myosin decreased from day 12 on, indicating a loss in thermal stability. Furthermore, it becomes obvious that in the SI batch ΔH parallels the behaviour of T_{max}. In contrast, actin peak in both batches, SI and FI, were almost stable and did not show marked changes in both T_{max} and ΔH. This behaviour does not agree with the results obtained when DSC measurements were performed along iced storage of sardine in FI and SI where almost no changes could be detected (*Schubring* and *Meyer*, 2006).

The conversion of a protein from a native to a denatured state by heat is a co-operative phenomenon and is accompanied by a significant uptake of heat, seen as an endothermic peak in the DSC curve. 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. A

successful approach to the study of the native conformation of proteins is the subjection of the protein to physical and chemical stresses, followed by a determination of the effect of these stresses on its thermal denaturation.

The reason for differences in thermal stability between FI and SI remains unclear up to now.

Chen (2003) as well as *Chen* et al. (2004) investigated thermal stability of horse mackerel myoglobin (Mb). It was found that T_{max} shifted to lower temperature with duration of refrigerated storage (*Chen*, 2003). The Mb of horse mackerel before storage showed main T_{max} at 51.8 °C and a shoulder peak at around 59.8 °C in the DSC curve. These 2 peaks shrank and T_{max} shifted to lower temperature around 50.2 and 58.3 °C, respectively, after 1 day refrigerated storage. The shoulder peak disappeared on the 3rd day, and the main peak shifted to 47.5 °C on the 7th day of refrigerated storage. These results indicated that the thermal stability of Mb decreased during refrigerated storage. Among 12 migratory fishes, horse mackerel Mb exhibited the lowest thermal stability (*Chen* et al., 2004). It seems possible that not only Mb of horse mackerel exhibits low thermal stability but also the muscle protein myosin. DSC curves of salt-ground horse mackerel surimi showed two clearly discernable endothermic peaks at 51.2 and 66.7 °C which are believed due, respectively, to the thermal denaturation of myosin and actin (*Chen*, 2006). For actomyosin of jack mackerel (*Trachurus murphyi*) stored frozen at –18 °C for 12 weeks two endothermic peaks at 46.8 °C and 68.9 °C were found and attributed to myosin and actin respectively (*Dondero* et al., 1996).

Colour measurements were performed on board as well as on defrosted samples after about 4 months of frozen storage ashore and taken on both skin of intact muscle in the range of belly flaps and on homogenised deskinned muscle are shown in Figures 18 to 20. Lightness (Fig. 18) did not change remarkably along the storage time when it was taken ashore. When measurements were performed on board, L* increased at day 3, remained steady until day 12 and decreased subsequently up to a value corresponding the initial L*. Lightness of skin is much higher than the one measured on homogenate. L* taken on homogenate ashore remained almost unchanged, while L* of homogenates prepared on board decreased markedly with prolonged storage time. In general, differences in L* between FI and SI were not significant.

When redness has been measured on homogenised muscle ashore it can be seen that a* decreased along storage time (Fig. 19). However, in freshly prepared homogenates on board a* increased with prolonged storage. Same tendency could also be observed, when measurement were taken on skin of the intact fish. Increase or decrease of a* means that after 3 to 6 days a* levelled of and remained unchanged until the end of storage. Differences between FI and SI which did not match the aforementioned trend could not be observed.

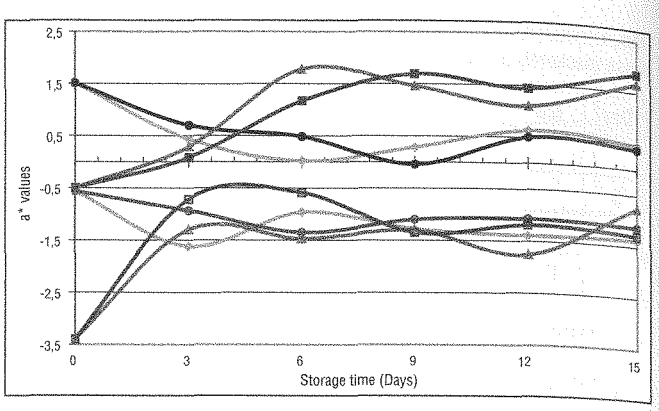

Fig. 19 Evolution of redness measured on skin (■▲ on board, ●◆ ashore) and on homogenised muscle (■▲ on board, ●◆ ashore) of horse mackerel along ice storage under different conditions (❖◆❑■ StreamIce, ▲Δ●○ Flake ice)

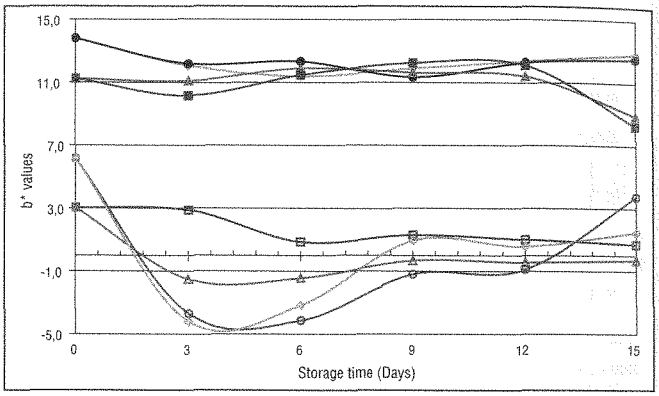

Fig. 20 Evolution of yellowness measured on skin (❑Δ ashore, ❖○ on board) and on homogenised muscle (◆● ashore, ▲■ on board) of horse mackerel along ice storage under different conditions (❑●❖■ StreamIce, ◆Δ○▲ Flake ice)

Homogenised samples and skin samples are strongly separated by yellowness being higher in the first. When b* was taken on homogenates on board a decrease could be observed at the end of storage while b* taken on homogenates ashore did not change very much. Homogenates prepared from SI and FI samples did not differ in b*. Measurements taken on skin on board mirrored obviously colour changes of skin observed along storage. Yellowness decreased at the beginning of iced storage, however after day 6 b* started to increase and this was stronger in FI samples. When measurements were taken ashore, changes in b* were lower with a slight trend toward a decrease. Available literature on colour measurements taken on iced stored fish has already been discussed recently (*Schubring* and *Meyer*, 2006).

Conclusion

Comparative iced storage experiments using flake ice and an ice slurry called StreamIce® were performed on board ship using horse mackerel caught in early autumn as raw

material. Due to performance of the trails on board highest initial quality possible of fish was ensured from the very early beginning with both ice types. Sensory assessment of freshness by quality index method (QIM) and the European grading scheme for fresh and refrigerated fish as well as determination of total viable count (TVC) and specific spoilage bacteria, *Shewanella putrefaciens*, were performed on board. Furthermore, changes in TVB-N and volatile amines such as TMA were estimated using trichloracetic acid extracts prepared on board. While instrumental colour measurements were performed on both ice stored samples and frozen/thawed samples the instrumental measurements of texture parameters, water binding and thermal behaviour were conducted on samples frozen after distinct iced storage on board and thawed after 4 months of frozen storage. No significant differences in the evolution of freshness of horse mackerel along iced storage in flake ice and slurry ice were found by sensory assessments. This statement is supported by microbial investigation and by the results of determination of TVB-N and TMA-N. Changes in texture parameters, colour and water binding ability along storage time were observed, however, they were not significantly different between both ice types. DSC curves taken from samples after different ice storage time revealed some signs of protein denaturation particularly in the SI batch at the end of storage by shifting T_{max} of myosin to lower temperature and decreasing the transition enthalpy. Under the conditions used in this trial which were close to those commercially applied, horse mackerel was not spoiled after 15 days of storage at 0 to 1 °C. Their quality was B graded thus allowing further industrial processing. Significant differences between flake ice and the ice slurry used were not obvious as in the previous experiment using sardine.

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