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Detailed experimental iced-storage characteristics of Barents Sea cod (*Gadus morhua*)

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An iced-storage experiment was performed with Barents-Sea cod in August 1997 onboard FRV "Walther Herwig III". Cod was investigated in daily intervals for its chemical, physical, sensory and microbiological properties. Analytical data were correlated with days in ice. Fischtester VI readings, RT freshness tester readings, DMA-N and TMAO-N content, grading according to the EU quality grading scheme, sensory assessment of cooked fillet samples and cfu on skin proved to correlate best with days in ice. Good correlation between sensory data and analytical data allow to some extent to substitute sensory methods by analytical ones.

Experimental controlled iced-storage experiments with marine fish have to be performed in order to determine the species-specific storage characteristics (Oehlenschläger 1995a). These characteristics are complex but can be explained to a great extent by a set of physical, chemical, sensory and microbiological data obtained by the experiments. Shelf life and freshness or spoilage of marine fish are today mostly determined by sensory assessment of either the outer appearance and the odour of the fish (EU grading scheme, Quality index method) or by a sensory assessment of the cooked sample by a QDA (Quantitative Descriptive Analysis) or other descriptive or hedonic methods (Olafsdottir et al. 1997). The aim of iced-storage experiments is to correlate the results obtained by analytical methods with those obtained by sensory methods and to try to substitute the expensive, time consuming and personnel intensive sensory tests by analytical methods. The Institute for Biochemistry and Technology in the Federal Research Centre for Fisheries has been carrying out iced-storage experiments since 1987

Beschreibung des Eislagerverhaltens von Kabeljau (*G. morhua*) aus der Barentssee

Im August 1997 wurde an Bord des FFS „Walther Herwig" ein Eislagerversuch mit Kabeljau aus der Barebtssee durchgeführt. Die Fische wurden in täglichem Abstand auf ihre chemischen, physikalischen, sensorischen und mikrobiologischen Eigenschaften hin untersucht. Die analytischen Daten wurden jeweils mit den Tagen in Eis korreliert. Es erwies sich, daß die Werte vom Fischtester VI sowie RT Frischetester, von Dimethylamin- und Trimethylaminoxidstickstoff, die Qualitätseinstufung anhand des EU-Qualitätsbewertungsschemas, die sensorische Bewertung von gegarten Filetproben und die Gesamtkeimzahl auf der Haut am besten mit den im Eis verbrachten Tagen korrelierten. Die guteKorrelation zwischen sensorischen und instrumentell ermittelten Daten läßt in gewissem Umfang einen Ersatz von Sensorikdaten durch instrumentell ermittelten zu.

(e.g. Rehbein et al. 1994, Oehlenschläger 1995b). Some recommendations for the use of analytical methods for freshness/spoilage determinations have been presented earlier (Oehlenschläger 1992).

Material and Methods

Fish

The cod used for the experiments was caught on August 1, 1997 by bottom trawling during the 187th research cruise of the FRV "Walther Herwig III". The cod was trawled on the Central Bank in the Barents Sea between 75° 10,41'N 031° 37,11'E and 75° 14,69'N 031° 39,67'E in 354-350 m depth from 15:15 to 16:15. Environmental conditions were: wind 10 m/sec (280°), T_{air} 4,1 °C, T_{water} 5,6 °C (surface).

After hauling the cod was killed by a cut through the throat, allowed to bleed for 15 min and then gutted by hand and thoroughly washed with seawater. The cod was then packed in PE-boxes (approx. 20 kg each) in flakeice with a fish:ice ration of 2:1. The boxes were stored in a hold kept throughout the whole storage period at a temperature of +1 °C with a fluctuation of ±1 °C. The weight of the gutted fish was 2857 ± 924 g (range 800 g - 6900 g), its length was 73,4 ± 8,1 cm (range 47-99 cm).

Chemical and physical methods

Sampling was performed each day. Five fish were taken at random from the hold and used for the chemical and physical analyses and for sensory assessments. pH was measured with a glass electrode in muscle tissue, between the gills and in aqueous muscle homogenate (muscle:water ratio 1:2). Electric properties of the fish were determined by using two intruments, the Intellectron Fischtester VI (Hamburg, Germany) and the RT Freshness tester (Reykjavik, Iceland).

A perchloric acid extract (Rehbein and Oehlenschläger 1982) was prepared from the left fillet muscle using 20 g comminuted fish muscle with 180 mL 6% (m/m) perchloric acid. This perchloric acid extract was used on board for the determination of total volatile basic nitrogen (TVB-N) (Rehbein et al. 1994), ammonia and creatine (Oehlenschläger and Rehbein 1990). Later it was used at land for the gas chromatographic determination of dimethylamine (DMA-N), trimethylamine (TMA-N) and trimethylamine oxide (TMAO-N) (Rehbein et al. 1994).

Microbiology

For the microbiological tests 3 different specimen of fish were taken and treated as described earlier (Meyer and Oehlenschläger 1996).

Sensory assessment

Immediately after being taken from the boxes the 5 specimens used later for chemical tests and sensory assessment, were graded into EU quality grades (E, A and B) by outer visual inspection including odour evaluation using the multilingual guide to EC freshness grades for fishery products (Nielsen et al. 1992). For later statistical treatment of data the grades E, A and B were converted into figures: E = 9, A = 8 (high A) or 7(low A), B = 6 (high B) or 5 (low B).

The right fillet muscle of the fish (left fillet was used for the chemical tests) was assessed by sensory tests. The use of the same fish for both, chemical tests and sensory assessment allowed a later correlation between the results obtained by the different methodologies. The skin-off fillet was put into a microwave suitable jar covered by a lid andcooked without salt and seasonings in a microwave oven. The microwave oven was adjusted to cook the sample to a core temperature of approx. 75 °C. The cooked samples were assessed immediately after cooking by a mixed panel consisting of 7 members: 2 were very experienced in assessing cooked ground fish samples, 2 were educated in sensory tests but not very familiar with fish and three were students who made the test on a voluntary basis after a short basic practical course in sensory assessment of fish. 4 members of the panel were smokers, 2 non-smokers, 4 male, 3 female. For the scoring a scale ranging from 0 to 100 was used. 100 denominated the properties associated with perfect fresh fish and 0 the opposite. Outer appearance and colour, odour and flavour, taste and texture had to be assessed. The panellists gave a score for their individual assessment of the sample indicated by a mark on the scale.

Results and Discussion

Physical tests

The electrical properties of the cod tissue as measured with the Intellectron Fischtester or the RT Freshness tester show an almost linear function with time in ice (Fig.1 and Fig. 2).

The initial readings of the Fischtester were 80 ± 1,3 (range 78 - 82). A slight increase was observed when fish entered rigor mortis, then a linear decrease of readings with a small scattering followed be observed. Close to the end of shelf life the readings started to become more scattered and at the end of the experiment a reading of approx. 20 remained probably due to the electric properties of the skin.

The pattern observed with the RT Freshness meter are similar starting with 12,3 ± 0,3 (range 11,8-12,6),
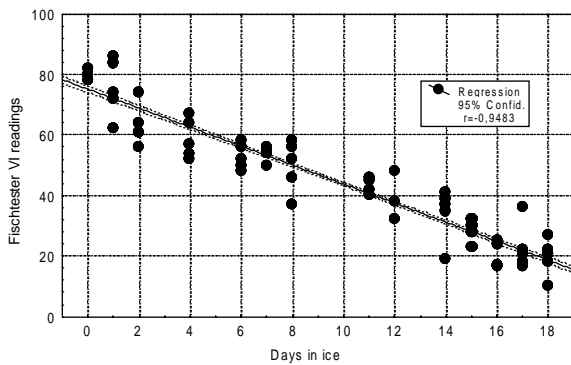
Fig. 1: Intellectron Fischtester VI readings during experimental iced-storage of Barents-Sea cod
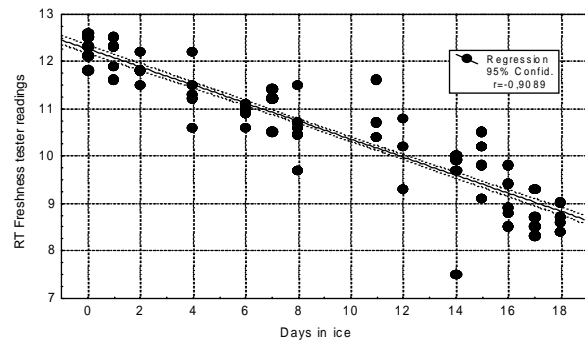

Fig. 2: RT Freshness tester readings during experimental iced-storage of Barents-Sea cod
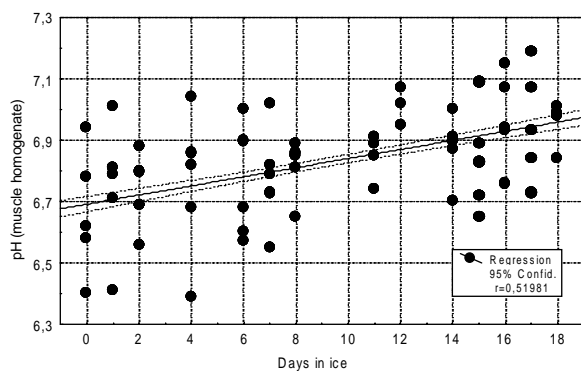

Fig. 3: pH value measured in aqueous homogenate of Barents Sea cod muscle during iced storage
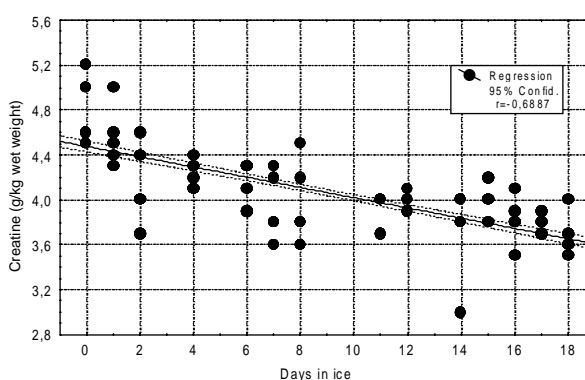

Fig. 4: Degradation of creatine in muscle tissue during experimental iced-storage of Barents Sea cod

however, on a lower scale because this instrument works with a reduced scale ranging from 0 to 14. It can be seen that the readings throughout the whole storage period are more scattering compared with the Fischtester readings, which can also be seen from the lower coefficient of regression with days in ice (r = 0,9483 and r = 0,9089, respectively, both significant p<0,05).

The pH measurements in intact muscle tissue and between the gills gave results that could not be used for a description of the iced-storage history. The coefficients of regression with days in ice were extremely low (r=0,23 and r=0,05, respectively, both not significant p<0,05). The pH measured in an aqueous fish muscle homogenate (6,66±0,19, range 6,4-6,94 on day 0) exhibited a better correlation with days in ice, shown in Fig. 3 (r=0,51981, p<0,05). However the pH-values measured in single specimen varied considerably, which makes it necessary to measure a large number of fish to get a reliable result.

Chemical analyses

Creatine
Creatine, which was shown to be a useful indicator for the time spent in ice in general (Oehlenschläger and Rehbein 1990) and especially in gadoids like whiting (*Merlangius merlangus*) (Oehlenschläger 1995c), did

not change dramatically during the iced-storage of Barents-Sea cod. From a mean initial content of 4,9 ± 0,3 g /kg wet weight (range 4,5-5,2) it was reduced after 18 days of iced-storage to only 3.7 g/kg. Furthermore, values obtained from different fishes were scattered, so that it has to be concluded that for this species – when stored under the conditions employed – creatine is not a suitable indicator for days passed in ice or remaining shelf life. The iced-storage behaviour of creatine in cod is demonstrated in Fig. 4.

Volatile amines and TMAO
The volatile amines, ammonia, DMA, TMA and TVB-N as well as TMAO can all be used as efficient indicators either for the freshness of fish (which is restricted to DMA) or its spoilage (extensive literature review: Oehlenschläger 1997). Ammonia-N, TMA-N and TVB-N exhibit an almost identical pattern throughout the whole storage period, however, on a different level of nitrogen. This pattern can be described as follows: The initial values found for the three amines (ammonia-N: 7,3 ± 1 mg/100 g (range 6,6-9,2) wet weight, TMA-N: 0,28 ± 0,12 mg/100 g (range 0,2-0,5) wet weight, TVB-N: 12,8 ± 1,4 mg/100 g (range 10,5-14,8) wet weight) do not change remarkably or at all during the first storage period, which ranges from day 0 to approx. day 11. After this period a slight increase can be observed for TMA-N and TVB-N from day 11 to day 16, when
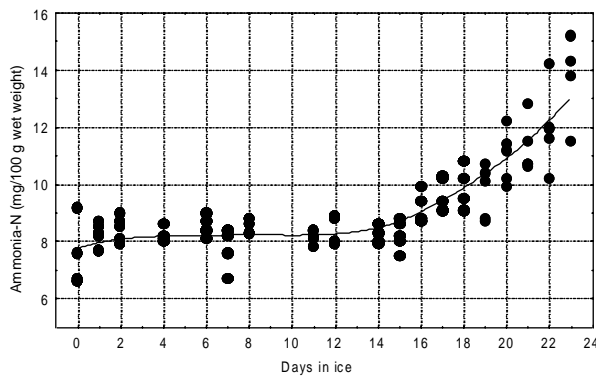
Fig. 5: Ammonia-N content in muscle tissue during experimental iced-storage of Barents Sea cod
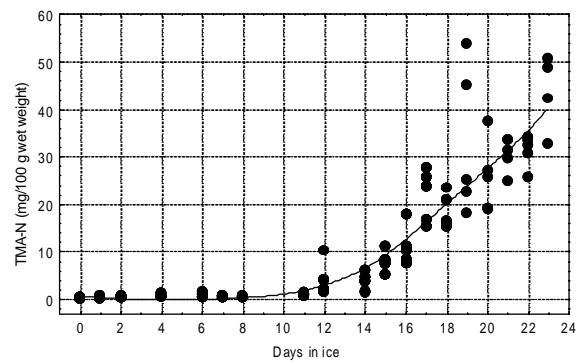

Fig. 6: Trimethylamine-nitrogen content in muscle tissue during experimental iced-storage of Barents Sea cod
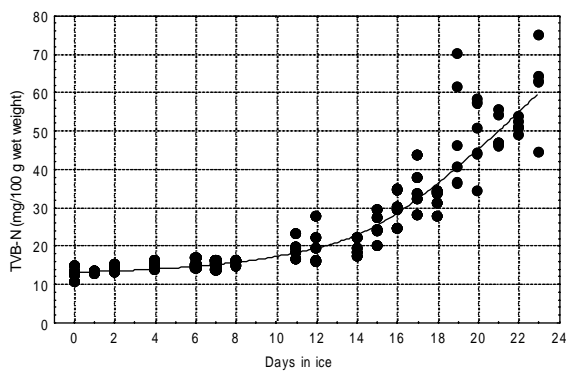

Fig. 7: Total volatile basic nitrogen (TVB-N) in muscle tissue during experimental iced-storage of Barents Sea cod
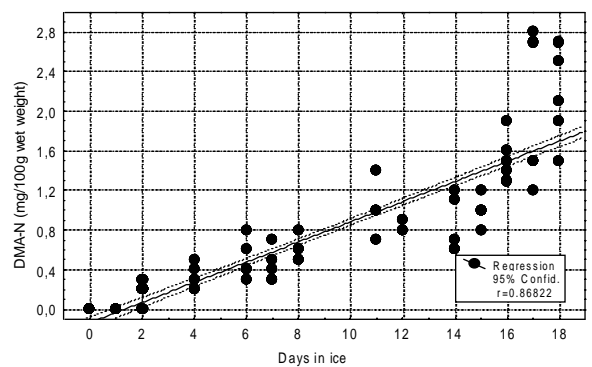

Fig. 8: Formation of Dimethylamine-nitrogen (DMA-N) in muscle tissue during
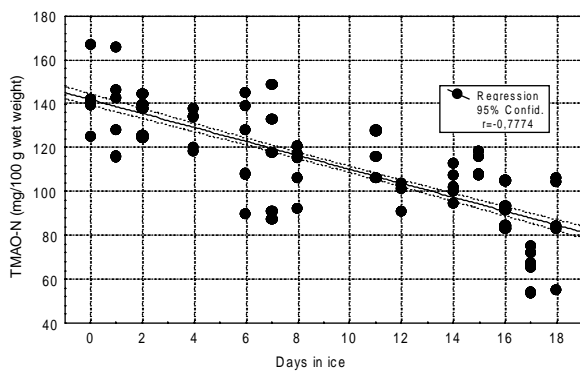

Fig. 9: Degradation of trimethylamine oxide (TMAO) in muscle tissue during experimental iced-storage of Barents Sea cod
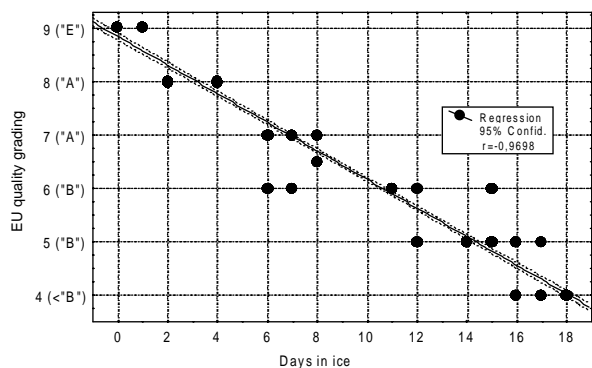

Fig. 10: Grading of Barents Sea cod according to EU quality grading scheme for fresh fish during experimental iced-storage

TVB-N reached the legal limit set for TVB-N by the EU Commission (30 mg/100 g wet weight) and TMA-N is around 10 mg/100 g wet weight, while ammonia-N is still on a constant level. The last storage period can be described by a steep increase of ammonia-N, TVB-N and TMA-N, which reach very high values in a short time period compared to the values observed in period 1 and 2. The fact that these three amines keep almost constant before noticeable signs of decomposition are reached makes them unfit for freshness indicators in cod. On the other hand, the increase of these three substances almost simultaneously with the onset of spoilage makes

them excellent spoilage indicators for cod. The iced-storage pattern of ammonia-N, TMA-N and TVB-N are shown in Figs. 5-7.

These three amines show no linear function with time. However, when a linear correlation with days in ice is calculated TVB-N has quite a good correlation ($r = 0,86822$, $p<0,05$).

DMA-N and TMAO-N show in contrast to the other three amines a linear relationship with days in ice. At the start of the iced-storage experiment the content of
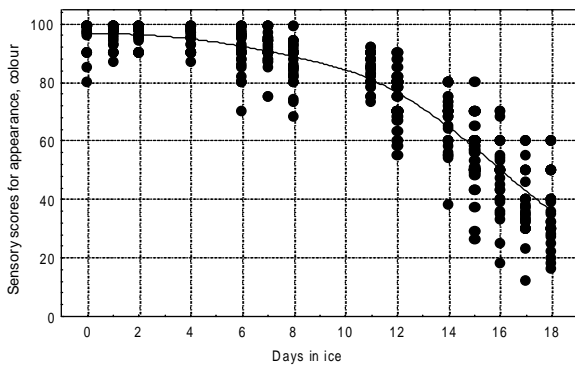
Fig. 11: Sensory scores for appearance/colour of cooked fillets of iced-stored Barents Sea cod
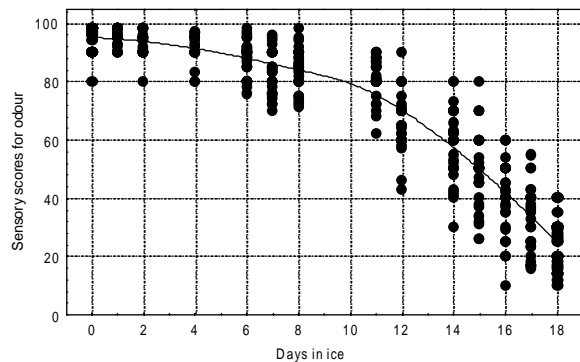

Fig. 12: Sensory scores for odour of cooked fillets of iced-stored Barents Sea cod
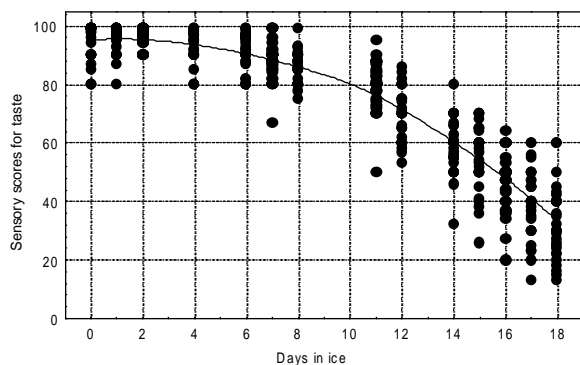

Fig. 13: Sensory scores for taste of cooked fillets of iced-stored Barents Sea cod
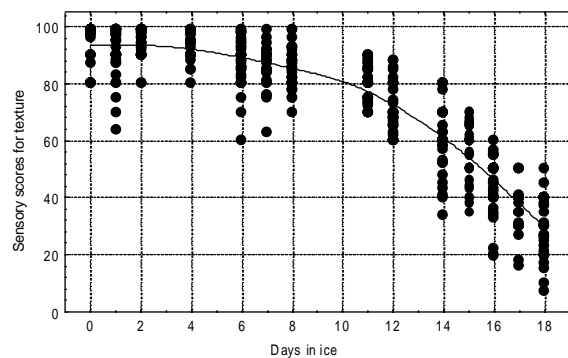

Fig. 14: Sensory scores for texture of cooked fillets of iced-stored Barents Sea cod

DMA-N is below the detection limit (<0,01 mg/100 g wet weight). From day 2 on there is a formation of DMA-N, which continues linear with time up to day 8. After this date there is a further increase in DMA-N formation. The linearity with time, however, is not as good as before and the values are much more scattered around the calculated graph. The correlation with time over the whole storage period is r = 0,86822 (p<0,05) in Fig. 8. this behaviour of DMA-N is demonstrated. Fig. 8 also demonstrated that DMA-N is an excellent freshness indicator for cod. Its absence in freshly caught fish and the linear development during the first storage period makes it an ideal tool for the determination of the early storage history of cod.

TMAO-N is in contrast to DMA-N, decreasing with progressing time in ice. The initial values found in freshly caught Barents Sea cod (134 ± 13,7 mg/100 g (range 125 - 167) wet weight) are very high compared to cod from other locations. The degradation of TMAO starts immediately after the death of the fish. It is linear throughout the whole time of iced-storage but the values are affected by a large scattering around the calculated function. When the fish is unfit for human consumption, TMAO-N concentrations of around 60 - 70 mg/100 g wet weight are still present (Fig. 9). This is an exception

compared to other fish species, which have only very low TMAO-N concentrations at the end of their storage life, and can be only explained by the high initial TMAO contents found in Barents-Sea cod.

Sensory assessment

The grading according to the EU-quality grading scheme showed that the cod was only 1 - 2 days of E (excellent) quality (Fig. 10). It was then graded A-quality up to day 8 and entered B-quality on day 9-10. B-quality was kept until day 14 - 15 and after this date the fish was found to be of substandard quality (<B). These figures show that cod can be marketed with a quality conform to EU-regulations as fresh fish stored in ice for approx. 2 weeks.

Figures 11 to 14 show the scatterplots for the sensory assessment of the cooked fillet samples for outer appearance and colour (11), odour and flavour (12), taste (13) and texture (14). The patterns of all four attributes are similar, although the scattering in the values is higher in texture. The scores given at the beginning of the experiment are just below 100 (appearance: 96,8 ± 4,6 range 80 - 99; odour: 95,1 ± 4,3 range 80 - 98; taste: 95,1 ± 6,2 range 80 - 99; texture: 94,9 ± 6,9 range 80 - 99). Approx. up to day 8 the scores remained high being above or around 90. Then a decline
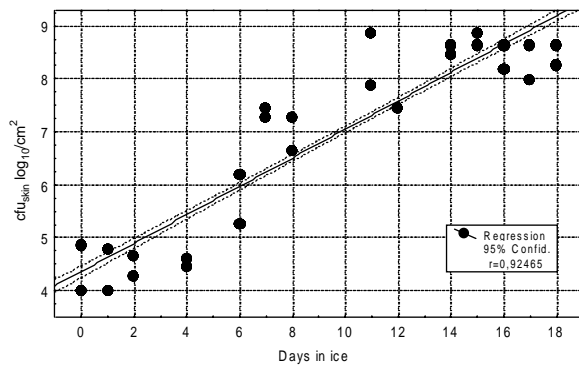
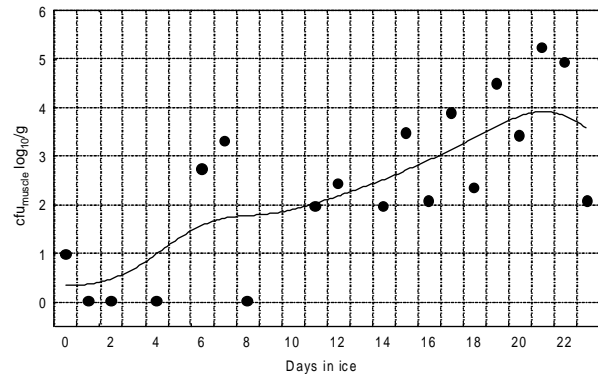Fig. 15: cfu on skin during iced-storage of Barents Sea cod

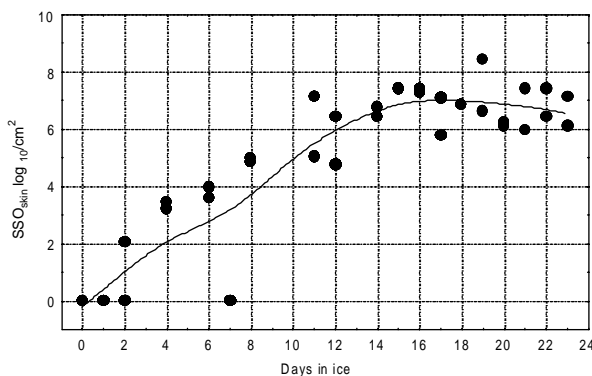Fig. 16: cfu in muscle tissue during iced-storage of Barents Sea cod

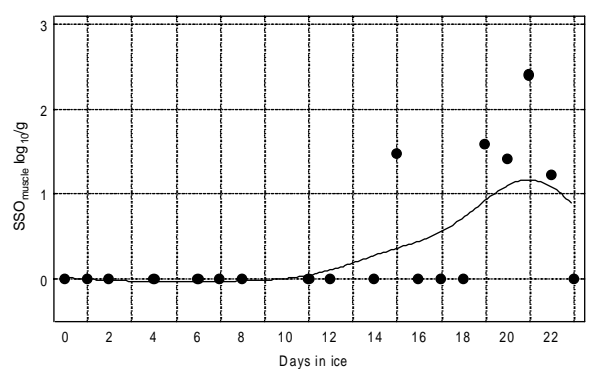Fig. 17: Specific spoilage organisms (SSO) on skin during iced-storage of Barents Sea cod

Fig. 18: Specific spoilage organisms (SSO) in muscle tissue during iced-storage of Barents Sea cod

started, which became steeper towards the end of the storage period reaching the limit of saleability (characterised by a score of 40) at day 17 for appearance, day 16 for odour, day 17 for taste and day 17 for texture. These results are in satisfying agreement with the results obtained with the outer inspection using the EU-quality grading scheme. It is, however, remarkable that the assessment of cooked samples always results in 2 or 3 days more shelf life compared to the EU-scheme. An explanation for this phenomenon, which have been observed in a number of iced-storage experiments with different species before, could be that the outer assessment is based to a great extent on the odour of e.g. gills and belly cavity. These two body parts are affected in the latter stages of iced-storage (but before the fish is spoilt) by unpleasant odours, which influence assessors to give poor grades, while in cooked fillet samples this odours are no more present (gills and peritoneum are removed during filleting) and - if having been present in muscle tissue - can be reduced or removed by the cooking process.

Microbiology

The total counts (CFU) of micro-organisms found on skin and in muscle tissue were low at the start of the experiment: cfu_{skin}: 4,26 ± 0,41 range 3,98 - 4,86;

cfu_{muscle}, SSO_{skin} and SSO_{muscle} 0 (the cfu found in muscle tissue on day 0 is an artefact). While there were found 10^4 to $10^5/cm^2$ on skin during the first 4 days in ice, the muscle tissue was sterile up to day 5. A maximum of 10^8 to $10^9/cm^2$ was reached at day 14, which did not change until the end of the experiment. cfu on skin (calculated log_{10}) grew according to a linear function up to day 14 (Fig. 15). The correlation of log_{10} cfu on skin with days in ice over the whole storage time gave a very satisfying coefficient of correlation r = 0,92465 (p<0,05). This means that cfu on skin can be used as a measure to predict days passed in ice or shelf life left. In muscle tissue the figures of cfu were generally lower reaching at the end of the storage time only $10^5/g$. There were muscle samples of single fishes present, which remained sterile until day 8. The decadic logarithms of cfu in muscle showed no acceptable linear function with time in ice (Fig. 16).

The specific spoilage organisms (SSO, prevailing *Shewanella putrefaciens*) on skin (Fig. 17) were present from day 2 on. They grew almost linear up to day 14 when a maximum of $10^7/cm^2$ was reached, which did not change up to day 22. The SSO in muscle tissue appeared first on day 15. They are from that date on present on a very low level (100/g), however, until the end of the experiment was reached, samples were found,

Tab. 1: Correlation coefficients for linear correlation of physical, chemical, sensory and microbiological parameters with days in ice (p<0,05)

Parameter	cc	Parameter	cc	Parameter	cc
Fischtester	-0,95	Sensory scores appearance	-0,88	Ammonia-N	0,57
Freshness meter	-0,91	Sensory scores taste	-0,90	creatine	-0,69
pH muscle homogenate	0,52	Sensory scores texture	-0,88	EU grading scheme	-0,97
TVB-N	0,86	cfu (skin)	0,93	Sensory scores odour	-0,89
TMA-N	0,77	cfu (muscle tissue)	0,50		
DMA-N	0,87	SSO (skin)	0,90		
TMAO-N	-0,78	SSO (muscle tissue)	0,18		

which contained no specific spoilage organisms. This is a surprising result because the cod spoilt normally. It could be possible that the micro-organisms responsible for the spoilage of Barents Sea cod are not or not fully detected by the assessment system used.

Correlations

In Table 1 the correlation coefficients for the correlation of most important parameters with days in ice are shown. The correlations are significant on a probability level of p<0,05.

The table demonstrates that 11 parameters used as freshness and/or spoilage indicators have a linear correlation coefficient better than 0,85. This means that they can be used with some restrictions in a linear model. Besides the correlation with days in ice some other significant linear correlations (r = 0,85) between parameters have been found: **Fischtester**: EU quality grade 0,93, sensory scores odour 0,85, cfu_{skin} –0,86, SSO_{skin} –0,86; **RT Freshness tester**: EU quality grade 0,90, sensory scores odour 0,85; **TVB-N**: TMA-N 0,95, DMA-N 0,89, sensory scores odour –0,87, sensory
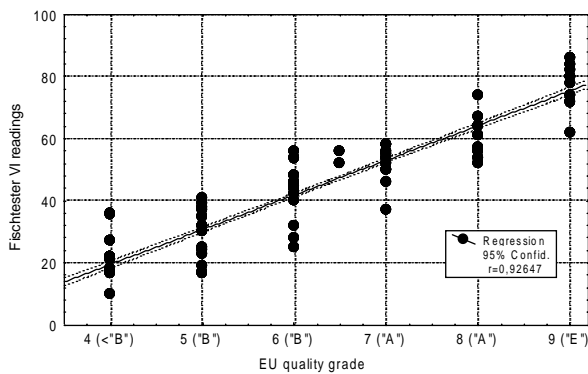

Fig. 19: Correlation between Intellectron Fischtester VI readings with EU quality grades during iced-storage of Barents Sea cod
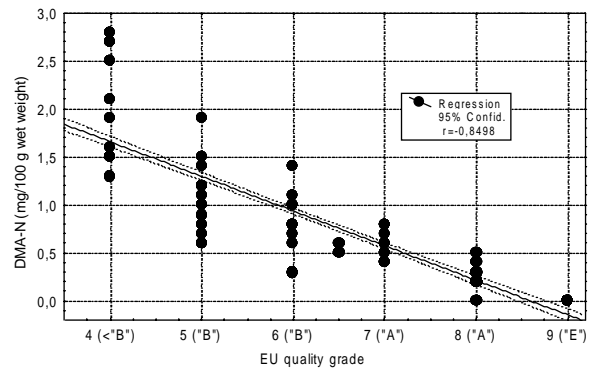

Fig. 20: Correlation between dimethylamine-nitrogen (DMA-N) and EU quality grades during iced-storage of Barents Sea cod
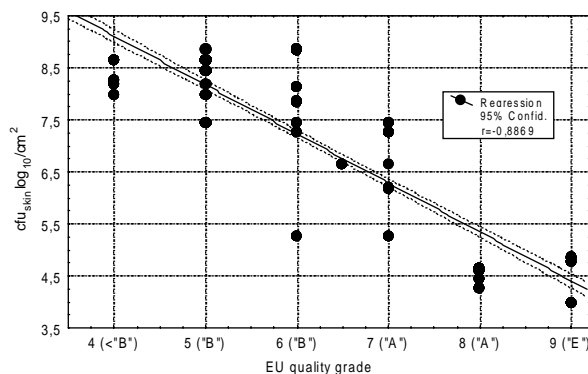

Fig. 21: Correlation between cfu_{skin} and EU quality grades during iced-storage of cod
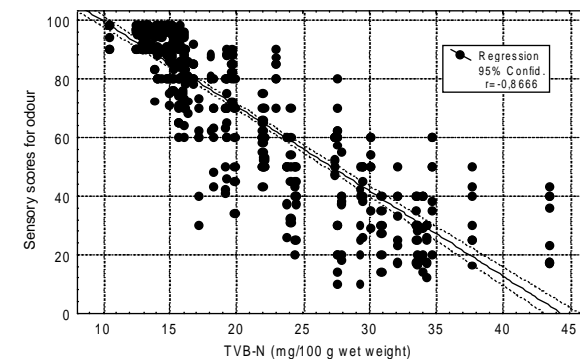

Fig. 22: Scatterplot of sensory scores for odour of cooked fillet samples and corresponding TVB-N values during iced-storage of Barents Sea cod

scores appearance –0,88, sensory scores taste –0,86, sensory scores texture –0,87; **TMA-N**: DMA-N 0,87, sensory scores odour –0,85, sensory scores appearance –0,86, sensory scores texture –0,85; **DMA-N**: EU quality grade –0,85, sensory scores appearance 0,85, sensory scores texture –0,85; **EU quality grade**: sensory scores odour 0,87, sensory scores appearance 0,85, sensory scores taste 0,86, sensory scores texture 0,85, cfu_{skin} –0,90; **sensory scores odour**: sensory scores appearance 0,94, sensory scores taste 0,94, sensory scores texture 0,93; **sensory scores appearance**: sensory scores taste 0,94, sensory scores texture 0,94; **sensory scores taste**: sensory scores texture 0,95.

In the preceding four figures a few examples for linear correlation between different freshness and/or spoilage indicators are given. Fig. 19 shows the correlation between Fischtester VI readings and the EU quality grade. It is obvious that high readings correlate with the grades E and A, while low readings (<45) are associated with quality grade B or less. The correlation of DMA-N with EU quality grade (Fig. 20) explains that low DMA-N contents (<0,5) are typical for the good grades (E and A) and that elevated DMA-N levels (>1) can be found only in samples graded B or lower. The cfu on skin correlates well with the EU quality grades, indicating that the good grades E and A go always together with a relative low cfu on skin and that the lesser grades are characterised by high cfu on skin (Fig. 21). The correlation for the scatterplot of sensory scores for odour with TVB-N levels explains simply that good scores for odour (no off-odours or unpleasant odours) correlate with low TVB-N values, whereas low sensory scores are found only when TVB-N is high (Fig. 22). This proves the experience that high TVB-N values are responsible for some off-odour components.

Conclusion

It was demonstrated that Barents-Sea cod could be characterised by a number of parameters. Some gave linear functions for the whole storage time, others only for certain periods of the total storage time. Chemical and microbiological parameters correlate well with sensory findings. This means that these parameters could be used alone or better in combination in order to substitute sensory assessment methods.

Acknowledgement

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