Kvalitet af optøet, kølet modificeret atmosfære-pakket torskefilet; modellering med teknologiske parametre

Ph.d.-afhandling

Erhvervsforskerprojekt EF 707

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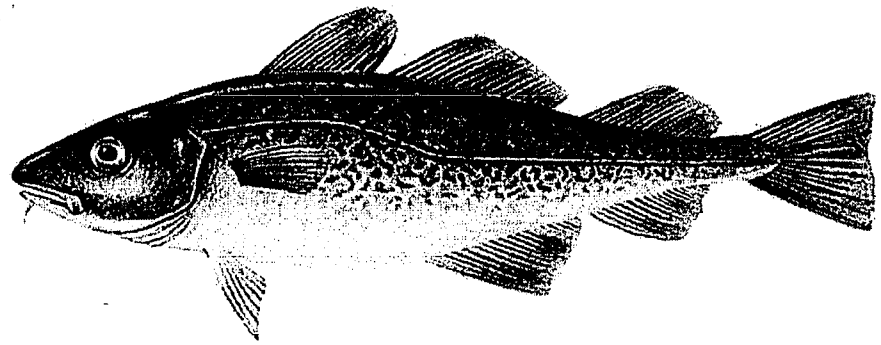

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Denne afhandling er resultatet af en gennemført erhvervsforskeruddannelse, et ph.d.-studium under Akademiet for de Tekniske Videnskaber, ATV.

The present thesis is the result of an industrial Ph.D. study from the Academy for the Technical Sciences, ATV.

Titel/Title:

Kvalitet af optøet kølet modificeret atmosfære pakket torskefilet;
modellering med teknologiske parametre.
Quality of thawed chilled cod fillets packed in modified atmosphere;
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Afhandlingen er baseret på følgende manuskripter, som der refereres til i teksten som artikel I–V:

The present thesis includes the following manuscripts, which are referred to in the text as paper I–V:

Artikel I:

Bøknæs, N., Guldager, H.S., Østerberg, C. & Nielsen, J. (2001). Production of high quality frozen cod (*Gadus morhua*) fillets and portions on a freezer trawler. *Journal of Aquatic Food Product Technology*, **10**, 33-47.

Artikel II:

Bøknæs, N., Østerberg, C., Nielsen, J. & Dalgaard, P. (2000). Influence of freshness and frozen storage temperature on quality of thawed cod fillets stored in modified atmosphere packaging. *Food Science and Technology*, **33**, 244–248.

Artikel III:

Bøknæs, N., Østerberg, C., Sørensen, R., Nielsen, J. & Dalgaard, P. (2001). Effects of technological parameters and fishing ground on quality attributes of thawed, chilled cod fillets stored in modified atmosphere packaging. *Food Science and Technology*, **34**, 513–520.

Artikel IV:

Bøknæs, N., Jensen, K.N., Guldager, H.S., Østerberg, C., Nielsen, J. & Dalgaard, P. (2002). Thawed chilled cod fillets in modified atmosphere packaging – application of multivariate data analysis to select key parameters in good manufacturing practice. *Food Science and Technology*, **35**, 436–443.

Artikel V:

Bøknæs, N., Jensen, K.N., Andersen, C.M. & Martens, H. (2002). Freshness assessment of thawed and chilled cod fillets packed in modified atmosphere using near-infrared spectroscopy. *Food Science and Technology*, **35**, 628–634.

Under erhvervsforskeruddannelsen er der tillige deltaget i udarbejdelsen af følgende artikler:
Other papers, which have been prepared during the industrial Ph.D. study are listed below:

Bechmann, I.E., Jensen, H.S., Bøknæs, N., Warm, K. & Nielsen, J. (1998). Prediction of chemical, physical and sensory data from process parameters for frozen cod using multivariate analysis. *Journal of the Science of Food and Agriculture* **78**, 329–336.

Guldager, H.S., Bøknæs, N., Østerberg, C., Nielsen, J. & Dalgaard, P. (1998). Thawed cod fillets spoil less rapidly than unfrozen fillets when stored under modified atmosphere at 2°C. *Journal of Food Protection* **9**, 1129–1136.

Warm, K., Bøknæs, N. & Nielsen, J. (1998). Development of quality index methods for evaluation of frozen cod (*Gadus morhua*) and cod fillets. *Journal of Aquatic Food Product Technology* **1**, 45–59.

INDHOLDSFORTEGNELSE

FORORD

Nærværende erhvervsforskerprojekt EF 707 benævnt "Kvalitet af optøet kølet modificeret atmosfære pakket torskefilet; modellering med teknologiske parametre" er gennemført i samarbejde med Danmarks Fiskeriundersøgelser, Afd. for Fiskeindustriel Forskning, Royal Greenland Overseas A/S og Mejeri- og Levnedsmiddelinstituttet, KVL i perioden 1. oktober 1997 til 1. oktober 2000. Akademiet for de tekniske videnskaber (ATV) har bidraget med økonomisk støtte til projektets gennemførelse.

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Niels Bøknæs

Aalborg, september 2000

SAMMENDRAG

Ved konsumering af ferske eller frosne detailpakkede fiskeprodukter i Danmark opleves der ofte store kvalitetsforskelle. Disse forskelle kan relateres direkte til for høj lagringstemperatur i produktions- og distributionskæden, for lang lagringstid eller anvendelse af en råvare, der ikke er frisk.

Det overordnede formål med nærværende erhvervsforskerprojekt har været at udvikle "good manufacturing practice" (GMP) til produktion af frosne højkvalitets torskeprodukter. Endvidere udvikles GMP til produktion af optøede kølede modificeret atmosfære pakkede (MAP) torskefileter, der udgør et alternativ til detailsalg af fiskeprodukter i Danmark.

Det videnskabelige formål med denne ph.d.-afhandling "Kvalitet af optøet kølet modificeret atmosfære pakket torskefilet; modellering med teknologiske parametre" har været at etablere viden om kemiske, fysiske, mikrobiologiske og sensoriske processer for torskefileter i relation til fryselagring samt efterfølgende kølelagring i MAP. Forståelse af disse processer er nødvendige for at kunne opstille GMP procedurer for henholdsvis produktion af frosne højkvalitets torskeprodukter og produktion af optøede kølede MAP torskefileter.

Nærværende forsøg med søfrosne torskeprodukter viste, at det var muligt at producere frosne produkter af højkvalitet. Der blev opstillet en optimeret GMP forarbejdningskæde til produktion af interleaved-pakkede benfri torskefileter samt benfri torskeudskæringer ombord på frysetrawlere i Barentshavet.

Ligeledes blev det vist, at produktion af optøede kølede MAP torskefileter er et brugbart alternativ til detailsalg af ferske fiskeprodukter. Vækst af *Photobacterium phosphoreum* og dannelse af trimethylamin (TMA) under kølelagring er meget mere udtalt for optøede MAP torskefileter fra Barentshavet sammenlignet med Østersøen. Med anvendelse af torskefileter fra Østersøen er der observeret en meget kraftig hæmning af *P. phosphoreum* vækst og TMA-dannelsen under kølelagring af torskefileter efter fryselagring ved (–20°C eller –30°C). Med anvendelse af torskefileter fra Barentshavet blev der fundet en kraftig *P. phosphoreum* vækst og TMA-dannelse under kølelagring med forudgående fryselagring ved –30°C. Derimod blev

der observeret en kraftig hæmning af *P. phosphoreum* under efterfølgende kølelagring i MAP efter fryselagring af torskefileter fra Barentshavet ved –20°C i mindst 3 måneder. Der er fundet højere dryptab under kølelagringen for optøede torskefileter fra Østersøen sammenlignet fra Barentshavet. Dette betyder, at torsk fra Østersøen dermed har en begrænset anvendelighed til produktion af optøede kølede MAP produkter. Det må derfor anbefales, at anvende en søfrossen råvare fra Barentshavet fryselagret i mindst 3 måneder ved –20°C til produktion af optøede kølede MAP torskefileter. Med dette set-up kan der produceres et detailprodukt, som inden for 14 dages kølelagring ved 2°C ikke udvikler aminlugt.

Søfrosne torskefileter fra Barentshavet har et meget højere TMAO- og NaCl-indhold i torskefileter sammenlignet med torskefileter fra Østersøen. Højt indhold af TMAO og NaCl i søfrosne torskefileter beskytter formentlig *P. phosphoreum* under fryselagring ved –30°C. Dette medfører, at *P. phosphoreum* efterfølgende vokser frem i optøede kølede MAP torskefileter som observeret i ferske MAP torskefileter.

De traditionelle bedømmelsesmetoder til ferske fiskeprodukter som TVN, TMA og sensorik har en begrænset anvendelighed til kvalitetsbedømmelse af optøede kølede MAP torskefileter, da disse produkter udviser meget forskellige fordærvelsesforløb. Disse forskellige fordærvelseskarakteristika kan relateres til forskelle i inaktiveringen af *P. phosphoreum* afhængig af fangstområde og fryselagringstemperatur. Anvendelse af nær infrarød reflektans (NIR) spektroskopi og multivariabel dataanalyse udgør en lovende metode til bestemmelse af kølelagringstid (dage ved 2°C) for optøede kølede MAP torskefileter med en prædiktiv korrelations koefficient på 0.9 og en RMSEPCV på 3.4 dage.

Denne afhandling har vist, at det er muligt kommercielt at producere frosne torskeprodukter af høj kvalitet med en opstillet GMP produktionsmetode, hvor der anvendes en søfrossen råvare. Endvidere er der opstillet GMP for produktion af optøede kølede MAP torskefileter med anvendelse af en søfrossen råvare. Derfor virker kommerciel afsætning af optøede kølede MAP torskefileter som et interessant alternativ til eksisterende kølede fiskeprodukter i Danmark. Desuden er det vist, at NIR har et potentiale som en mulig metode til bedømmelse af optøede kølede MAP torskefileter.

ABSTRACT

By consuming fresh or frozen retail packed fish products in Denmark, big differences in quality are often observed. These can be related directly to high storage temperature in production- and distribution chain, too long storage period or using of non-fresh raw material.

The overall objective of the present industrial Ph.D.-project has been to develop "good manufacturing practice" (GMP) for production of high quality frozen cod products. Furthermore, developing of GMP for production of thawed chilled modified atmosphere packed (MAP) cod fillets is an alternative for retail sale of fish products in Denmark.

To meet this goal, the scientific objective of the present study "Quality of thawed chilled cod fillets packed in modified atmosphere packaging; modelling with technological parameters" has been to establish knowledge about chemical, physical, microbiological and sensory changes for cod fillets in relation to frozen storage and subsequent chill storage in MAP. Understanding of these changes is necessary for setting up GMP procedures for production of high quality frozen cod products and production of thawed chilled MAP cod fillets, respectively.

The present study with frozen at sea cod products showed that it was possible to produce frozen cod products of high quality. An optimized GMP production chain was established for production of interleaved packed boneless cod fillets and also boneless cod portions on board freezer trawlers in the Barents Sea.

Besides, it was shown that production of thawed chilled MAP cod fillets is an useable alternative for retail sale of fresh fish products. *Photobacterium phosphoreum* growth and trimehylamine (TMA) production during chill storage is much more characteristics for thawed chilled MAP cod fillets from the Barents Sea compared with the Baltic Sea. During chill storage, cod fillets from the Baltic Sea, a very strong inhibition of *P. phosphoreum* growth and TMA production were observed after frozen storage (–20°C or –30°C). By using of cod fillets from the Barents Sea, a strong *P. phosphoreum* growth and TMA production were observed during chill storage after frozen storage at –30°C. Furthermore, for Barents Sea cod

fillets frozen stored in at least three months at –20°C, a strong inhibition of *P. phosphoreum* during chill storage was observed. Higher drip loss during chill storage was found for thawed MAP cod fillets from the Baltic Sea compared with the Barents Sea. It means that cod from the Baltic Sea has a limited application for production of thawed chilled MAP products. Therefore, it can be recommended to use sea frozen Barents Sea raw material frozen stored in at least three months at –20°C for production of thawed chilled MAP cod fillets. By using this set-up a retail product can be produced which after 14 days of chill storage at 2°C does not produce amine odour.

Frozen at sea cod fillets from the Barents Sea have much higher content of TMAO and NaCl compared with cod fillets from the Baltic Sea. High content of TMAO and NaCl in sea frozen cod fillets probaly protects *P. phopsphoreum* during frozen stoarge at –30°C. This results in subsequent growth of *P. phosphoreum* in thawed chilled MAP cod fillets as observed in fresh MAP cod fillets.

The traditional quality methods for fresh fish products such as TVN, TMA and sensory analysis have a limited application for quality assessing of thawed chilled MAP cod fillets as these products shows big difference in progress of spoilage. These different spoilage characteristics can be related to inactivation of *P. phosphoreum* depending on fishing ground and frozen storage temperature. Using of near infrared reflectance (NIR) spectroscopy and multivariate data analysis make up a promising method for determination of chill storage period (days at 2°C) for thawed chilled MAP cod fillets with a predictive correlation on 0.9 and a RMSEPCV on 3.4 days.

The present study has shown that it commercially is possible to produce high quality frozen cod products by setting up a GMP production chain using frozen at sea raw material. Furthermore, GMP for production of thawed chilled MAP cod fillets was set up by using sea frozen raw material. Therefore, commercial sale of thawed chilled MAP cod fillets seems to be an interesting alternative to existing chill fish products in Denmark. Besides, it was found that NIR has a promising potential as a possible method for evaluation of thawed chilled MAP cod fillets.

FORKORTELSER

AOAC	Association Official Analytical Chemists
APLSR	Anova partial least squares regression
ATP	Adenosin-tri-phosphat
ATV	Akademiet for de tekniske videnskaber
DFU	Danmarks Fiskeriundersøgelser
DMA	Dimethylamin
DPLSR	Diskriminant partial least squares regression
EDTA	Ethylendiamintetraacetat
FA	Frit formaldehyd
FAO	Food and Agriculture Organization
FFA	Frie fedtsyrer
GMP	Good manufacturing practice
HACCP	Hazard analysis critical control point
IQF	Individually quick frozen
KVL	Kongelig Veterinær- og Landbohøjskole
MAP	Modificeret atmosfære pakning
MSC	Multiple scatter correction
NIPALS	Non linear iterative partial alternating least squares
NIR	Nær infrarød reflektans
NMR	Nuclear magnetic resonance
PBO	Pinbone out
PCA	Principal component analysis
PLSR	Partial least squares regression
RMSEPCV	Root mean square error prediction of cross validation
TMA	Trimethylamin
TMAO	Trimethylaminoxid
TVC	Totalkim
TVN	Total flygtige baser
VP	Vacuum-pakning
QIM	Quality index method

1. INDLEDNING OG FORMÅL

1.1 Indledning

Afsætningen af fiskeprodukter i Danmark er steget gennem de seneste år, som følge af en betydelig reklamekampagne af foreningen Fiskebranchen i bl.a. TV. I den sammenhæng bliver modificeret atmosfære pakning (MAP) af fisk i stadig højere grad brugt til distribution af ferske fiskeprodukter i detailhandlen. Anvendelsen af MAP er ligeledes meget udbredt i Vesteuropa ved distribution og salg af andre fødevarer såsom kød, pålæg, brød og pasta. Dette skyldes en betydelig forlængelse af holdbarheden for kødprodukter med flere uger (Dalgaard, 1995a; Farber, 1991). Forøgelsen af holdbarhed ved MAP af ferske fiskeprodukter er dog relativ lille (Dalgaard, 1995a; Davis, 1993), men anvendes alligevel i praksis, da det er en bekvem pakkeform for fiskeprodukter uden lugtgener og drypvand i detailhandlens kølemontre.

Ved produktion af ferske MAP fiskeprodukter i Danmark anvendes der som regel benfri fileter med torsk, sej, rødspætte, laks, ørred eller skrubbe som råvare. Disse produkter har en meget begrænset holdbarhed, som bliver forkortet betydeligt, hvis fisken bliver opbevaret ukorrekt ved for høje og fluktuerende kølelagringstemperaturer. Lovgivningskravet påbyder en opbevaringstemperatur på maksimalt 2°C for salg af kølede fiskeprodukter. Dette temperaturkrav bliver imidlertid ikke altid opfyldt. En europæisk forbrugerundersøgelse har vist, at ferske fiskeprodukter ofte er fordærvede og har udviklet kraftige fiskelugte, hvilket kan relateres direkte til for høje opbevaringstemperaturer, for lang lagringstid eller en råvare, der ikke er frisk (Anon., 1995).

Salget af frosne fiskeprodukter i Danmark har ligeledes været stigende inden for de seneste år. De frosne fiskeprodukter, som sælges i Danmark, er imidlertid af meget vekslende kvalitet (Guldager, pers. komm.), og forbrugeren opfatter oftest frossen fisk som et sekundært produkt i forhold til fersk fisk (Juhl & Poulsen, 1999). Dette er i overensstemmelse med resultatet af en dansk forbrugerundersøgelse specifikt rettet mod frosne rødspættefileter, hvor disse produkter betegnes som værende af dårlig og forskelligartet kvalitet (Nielsen et al., 1997). Undersøgelser af andre markeder end det danske har vist, at bl.a. amerikanske forbrugerne også har meget negative holdninger til køb af frosne fiskeprodukter, hvor frossen fisk bliver

forbundet med dårlig lugt og smag samt grødagtig konsistens (Peavey et al., 1994). Desuden er det fundet, at mere end 30% af frosne fiskeprodukter på det europæiske marked beskrives som værende af dårlig kvalitet (Anon., 1995).

Den store variation i kvaliteten af frosne fiskeprodukter kan skyldes en eller flere faktorer. Fryselagringstiden er ofte meget lang, idet hovedparten af frosne fiskeprodukter, der sælges i danske butikker har en deklareret holdbarhed på over 18 måneder. Ligeledes er fluktuerende og for høje fryselagringstemperaturer for fiskeprodukterne under distribution og i detailhandlen almindelige (Persson & Löndahl, 1993). Endvidere produceres frosne fiskeprodukter med en optøet eller en iset råvare af meget vekslende kvalitet ved indfrysningstidspunktet. Sammenfattende kan det udledes, at der er et stort potentiale for udvikling af nye produktionskæder til frosne fiskeprodukter af høj kvalitet.

I bl.a. England er optøede fiskeprodukter blevet solgt i detailledet med succes (Herborg, 1986; Howgate, pers. komm.). Dette kombineret med udbredelsen af MAP for fiskeprodukter gør det relevant at afprøve mulighederne for som noget nyt i Danmark at producere optøede kølede MAP torskefileter, hvor råvaren er baseret på søfrosne fileter. Ved produktion af søfrosne torskefileter indfryses torsken umiddelbart efter fangst og forarbejdning ombord på fabriksskibe (frysetrawlere). Kombinationen af frossen råvare og MAP er en fleksibel og bekvem måde til forarbejdning, distribution og detailsalg af optøede kølede fiskeprodukter (Davis, 1993; Lanier & Korhonen, 1981). Til produktion af optøede kølede torskefileter kan der anvendes frossen råvare fra fjernfiskerier i Barentshavet, hvilket udjævner sæson-, pris- og vejrmæssige variationer i råvaretilgangen fra de kystnære fiskerier.

Det er vist, at optøede kølede MAP torskefileter har en længere holdbarhed sammenlignet med ferske MAP torskefileter lagret ved 2°C med anvendelse af råvare fra Østersøen (Guldager et al., 1998). I ferske MAP torskefileter er *Photobacterium phosphoreum* den specifikke fordærvelsesbakterie ansvarlig for produktion af trimehylamin (TMA) (Dalgaard, 1995b; Dalgaard et al., 1993). *P. phosphoreum* bliver imidlertid inaktiveret efter 8 ugers forudgående fryselagring ved –20°C, hvilket bevirker, at optøede MAP torskefileter udviser et andet bakteriologisk fordærv sammenlignet med traditionelle ferske torskefileter. De

traditionelle sensoriske fordærvelseskarakteristika (f.eks. aminlugt) bliver ikke observeret ved optøede kølede MAP torskefileter efter 20 dages kølelagring ved 2°C (Guldager et al., 1998).

Anvendelse af frossen råvare til optøede kølede MAP produkter er imidlertid ikke fuldstændig uproblematisk. Der er fundet signifikant højere dryptab for optøede kølede MAP sammenlignet med ferske MAP torskefileter (Dalgaard et al., 1993; Guldager et al., 1998). Endvidere er en lang række procesparametre ved produktion af optøede kølede MAP torskefileter ikke tidligere blevet undersøgt. Disse procesparametre omfatter: fryselagringstid, fryselagringstemperatur, kølelagringstid, kølelagringstemperatur, fangstområde, MAP under fryselagring, indfrysnings- og optøningsmetoder samt gassammensætning under kølelagring med henblik på kommerciel fremstilling af optøede kølede MAP torskefileter.

Endvidere skal det bemærkes, at i henhold til lovgivning i Danmark, skal det bekendtgøres ved mærkning, at der er tale om salg af optøede fisk. Det er på nuværende tidspunkt uvist, hvorledes forbrugerne vil stille sig til køb af optøede kølede MAP fiskeprodukter.

1.2 Formål

Det nuværende videngrundlag gør det vanskeligt at fastlægge den bedste praksis i hele produktionsforløbet fra fangst til forbruger for (i) frosne højkvalitets torskeprodukter og (ii) optøede kølede MAP torskefileter. Det overordnede formål med projektet har derfor været at undersøge muligheden for at opstille en optimeret produktionskæde til fremstilling af frosne højkvalitets torskeprodukter, hvor råvaren baseres på søfrosne torskefileter. Endvidere er det interessant at få undersøgt en række forskellige teknologiske procesparametre ved produktion af optøede kølede MAP torskefileter. Endelig er en evaluering af eksisterende metoder til kvalitetsbedømmelse påkrævet, da fordærvelsesforløbet for optøede kølede MAP produkter adskiller sig fra ferske torskefileter. I den forbindelse undersøges muligheden for at anvende nær infrarød reflektans (NIR) spektroskopi til kvalitetsbestemmelse af optøede kølede MAP torskefileter.

For at opfylde de overordnede formål med projektet skal følgende delmål opfyldes:

1) ***Opstilling af optimalt produktionsforløb (GMP) for søfrosne torskeprodukter.***

Der udføres to kommercielle fryselagringsforsøg, der opstartes ombord på frysetrawlere i Barentshavet for at klarlægge GMP for produktion af søfrosne torskefileter samt alternative produkttyper (**Artikel I og IV**).

2) ***Bestemmelse af relevante procesparametre for produktion af optøede kølede MAP torskefileter.***

Procesparametre for produktion af optøede kølede MAP torskefileter klarlægges med anvendelse af torskeråvare fra henholdsvis Østersøen og Barentshavet (**Artikel II og III**).

3) ***Opstilling af optimalt produktionsforløb (GMP) for optøede kølede MAP torskefileter med anvendelse af søfrossen råvare.***

Der udføres et kommercielt fryselagringsforsøg, der opstartes ombord på frysetrawler i Barentshavet for at klarlægge GMP for produktion af optøede kølede MAP torskefileter (**Artikel IV**).

4) ***Evaluering af eksisterende metoder samt en potentiel hurtigmetode (NIR) til kvalitets-differentiering af optøede kølede MAP torskefileter.***

De traditionelle mikrobiologiske, kemiske, fysiske og sensoriske metoder til bedømmelse af ferske MAP fiskeprodukter og NIR undersøges m.h.t. kvalitetsdifferentiering af optøede kølede MAP torskefileter (**Artikel IV og Artikel V**).

1.3 Afhandlingens opbygning

Baggrunden for projektet vil blive gennemgået i kapitel 2–4. Disse tre kapitler indeholder beskrivelser af råvare og forarbejdning samt ændringer i kvalitetsparametre for torskefileter under fryse- og kølelagring. I kapitel 5 præsenteres de anvendte metoder til kvalitetsbedømmelse samt multivariable dataanalysemetoder. Hovedresultater med tilhørende diskussion er indeholdt i kapitel 6. I kapitel 7 er der en sammenfattende konklusion for erhvervsforskerprojektet og endvidere en række overvejelser vedrørende fremtidigt arbejde. Afhandlingen er baseret på fem artikler, som er vedlagt efter referencerne.

2. RÅVARE OG FORARBEJDNING

Torsk (*Gadus morhua*) er globalt en af de vigtigste konsumfisk, og afsættes hovedsageligt som enten hel fersk fisk eller som saltet, tørret, fersk eller frosset filetprodukt. I dette kapitel angives torskens fangstområder og mængder med tilhørende fangstmetoder og fangstbehandling. Desuden præsenteres de forskellige alternative forarbejdningskæder til produktion af torskefileter. Afslutningsvis gennemgås forskellige aspekter ved produktion af søfrosne torskeprodukter.

2.1 Torsk som råvare

Torsk, der tilhører de gadoide fisk, kaldes også for atlanterhavstorsken. Der findes over 25 forskellige torskearter (Ryan, 1979), hvor de kommercielt mest betydende er følgende arter: sej (*Pollachius virens*), kuller (*Melanogrammus aeglefinus*), alaska pollack (*Theragra chalcogramma*), stillehavstorsk (*Gadus macrocephalus*) samt de forskellige kulmulearter (*Merluccius*). Torsken er meget udbredt i Atlanterhavet med tilstødende havområder. Fangstmængderne for torsk (Tabel 1) viser, at torsken er en meget vigtig konsumfisk både i national og global sammenhæng.

Tabel 1 Samlede danske og globale fangster af torsk (*Gadus morhua*) angivet i 1000 tons.

	1990	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**
Danske fangster[a]	130	111	75	59	99	95	102	91	73	74
Globale fangster[b]	1485	1337	1180	1139	1243	1268	1329	1375	1241	[c]

[a] Fangstdata fra (Anon., 1999).
[b] Fangstdata fra (Anon., 1998).
[c] Data ikke tilgængelig.

Torskens fangstområder har historisk varieret en del, og der er tidligere fanget store mængder af torsk ved Grønland og New Foundland. I dag fanges der store mængder af torsk i Barentshavet samt i farvandene omkring Island, Færøerne, Norge og Danmark. I de danske farvande fanges der store mængder af torsk både i Nordsøen og Østersøen. De torsk, der lever i Østersøen, er en underart af *Gadus morhua* (Ryan, 1979; Whitehead et al., 1986), som er tilpasset til at leve i Østersøens relativt lave saltindhold. Normalt angives saliniteten i

Østersøen til at være under 20‰, hvilket er en lav saltkoncentration sammenlignet med omkring 35‰ i de havområder, hvor torsken normalt lever.

2.2 Fangst og fangstbehandling

Torsk er en demersal fisk, der primært finder føden nær ved bunden. Derfor fanges torsk overvejende i bundtrawl samt med garn eller line sat ved bunden. Den sensoriske kvalitet af torsk afhænger af fangstmetoden, og langline angives generelt at være den mest skånsomme metode, der giver den bedste kvalitet (Botta et al., 1987). Efter fangsten skal torsken renses og afblødes hurtigst muligt for at opnå den højeste sensoriske kvalitet (Botta et al., 1986; Jones, 1965a; Kelly, 1969a; Olsen, 1986; Valdimarsson et al., 1984). Der har historisk været brugt to processer til fangstbehandlingen (i) strubeskæring, afblødning og efterfølgende rensning i to separate enhedsoperationer og (ii) strubeskæring og rensning med efterfølgende afblødning i en enhedsoperation. De ovennævnte studier viser dog, at tiden (fra fangst til fangstbehandling) er den klart vigtigste faktor i fangstbehandlingen m.h.t. den sensoriske kvalitet af fisken, og angiver, at afblødningen foregår mest effektiv i vand.

Umiddelbart efter fangst og fangstbehandling er torsken blød og fleksibel, og torsken betegnes som præ *rigor*. Efter et stykke tid vil torsken gennemløbe *rigor mortis* (dødsstivheden). Tidsrummet for *rigor mortis* gennemløb afhænger af faktorer som lagringstemperatur, art og fiskens fysiologiske tilstand (Tabel 2).

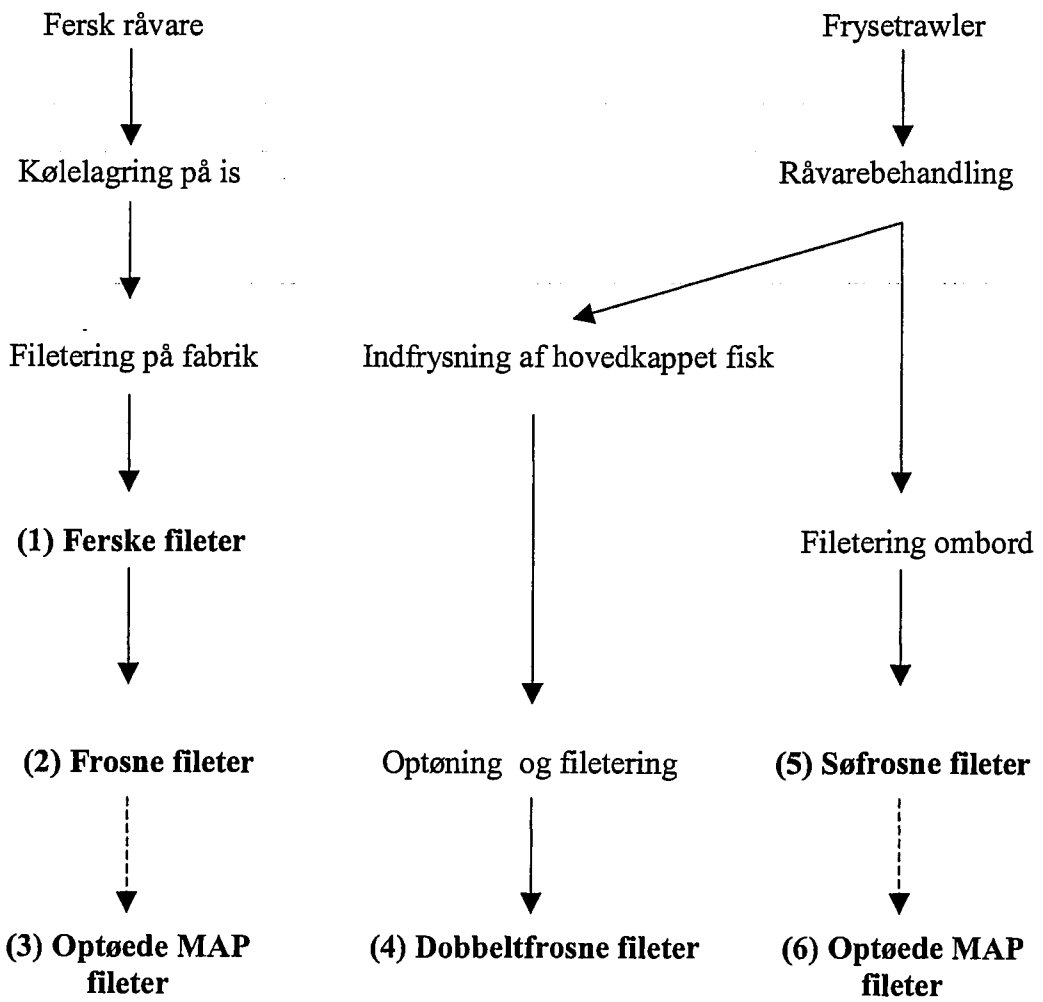
Tabel 2 Varighed for *rigor mortis* i torsk afhængig af forskellige parametre (Stroud, 1969).

Temperatur (°C)	**Betingelser**	**Tid fra død til begyndelse af *rigor* (timer)**	**Tid fra død til opløsning af *rigor* (timer)**
0	Trawlet	2–8	20–65
2.8	Trawlet	4.5–8.5	54–64
2.8	Ikke stresset	14–15	72–96
30	Trawlet	–	1–2

Endvidere er det fundet, at differencen mellem fiskens naturlige omgivelsestemperatur og den efterfølgende lagringstemperatur har stor betydning for *rigors* udvikling og varighed (Abe & Okuma, 1991; Hwang et al., 1991). Muskelkontraktionerne er stærkere, og *rigor* gennemløbes hurtigere, når differencen mellem omgivelsestemperatur og lagringstemperatur stiger.

2.3 Forarbejdningskæder for torskefileter

I dag findes der veldokumenterede forarbejdningskæder til filetprodukter med både fersk og frossen råvare. Disse er skitseret i Figur 1, hvor (1) og (2) angiver eksisterende filetprodukter baseret på fersk råvare med produktion på landbaserede fabrikker. (4) og (5) angiver eksisterende filetprodukter baseret på søfrossen råvare indfrosset ombord på frysetrawlere (Dore, 1989; Merritt, 1988). Punkterne (3) og (6) med optøede kølede MAP torskefileter angiver produkter, som henholdsvis kan baseres på fersk råvare fra landbaserede fabrikker eller søfrossen råvare fra frysetrawlere.



Figur 1 Flowdiagram for 6 alternative forarbejdningskæder til produktion af torskefileter til detailsalg. Optøede kølede MAP torskefileter repræsenterer en ny distributionsmetode i Danmark for detailsalg af torskefileter. Pilene betegner henholdsvis køle- og fryselagringsforløb, og de stiplede pile angiver de nye optøede kølede MAP torskefileter.

De øvrige punkter viser produktionsforløbet for eksisterende filetprodukter, som findes på markedet i dag. Ferske torskefileter (1) produceres på landbaserede fabrikker med en fersk iset råvare, der er landet af fiskebåde. Ferske torskefileter produceres primært som benfri PBO (pinbone out) torskefileter, der distribueres på is i flamingokasser. Frosne torskefileter (2) produceres primært med ferske benfri fileter eller udskæringer som loins, tails eller center cuts som råvare. Frosne torskefileter kan pakkes som filetblok (16,5 lbs.), der primært anvendes til videre forarbejdning i form af opsavning af portioner til detailpakning eller panerede produkter. Ligeledes kan frosne torskefileter detailpakkes som fileter eller filetudskæringer. Disse pakkes hovedsageligt løsfrosne i papæsker, vacuum-pakkede (VP) poser eller som enkeltfrosne torskeudskæringer, der pakkes i masterkartoner fortrinsvis til catering.

Ved produktion af dobbeltfrosne torskefileter (4) anvendes der som regel en hovedkappet renset råvare, der er indfrosset i blokke ombord på frysetrawlere. Fisken er hovedkappet og renset umiddelbart efter fangsten. Efter fryselagring, optøning og efterfølgende filetering på landbaserede fabrikker produceres der primært dobbeltfrosne benfri torskefileter eller torskeudskæringer. Dobbeltfrosne torskefileter kan pakkes på samme måde som enkeltfrosne fileter. Ved produktion af søfrosne torskefileter (5) fileteres råvaren ombord på frysetrawlere umiddelbart efter fangsten. Fileterne fryses herefter umiddelbart efter pakningen. Søfrosne torskefileter pakkes hovedsageligt som interleaved-pakkede filetblokke (torskefileterne adskilles med plastic i blokken før indfrysning således, at de enkelte torskefileter kan separeres i frossen tilstand) eller som 16,5 lbs. filetblokke. Inden for de seneste år er der endvidere blevet foretaget mindre produktioner af enkeltfrosne torskefileter og torskeudskæringer ombord på færøske, islandske og norske frysetrawlere. Disse enkeltfrosne torskeprodukter pakkes i masterkartoner ombord og anvendes overvejende til cateringsalg eller ompakning på land i detailemballage.

2.4 Produktion af søfrosne torskeprodukter

Det kommercielle fiskeri med produktion af søfrosne torskeprodukter ombord på frysetrawlere begyndte efter anden verdenskrig af nationer som bl.a. England, Sovjet, USA, Norge og Tyskland (Heen & Karsti, 1965; Watermann, 1987). Dette fiskeri har siden primært foregået på fjerne fiskefelter med indfrysning ombord på frysetrawlere af enten

hovedkappede hele torsk til optøning på landbaserede fabrikker eller torskefileter. Søfrosne torskefileter pakkes fortrinsvis i blokemballage til catering eller ompakning på landbaserede fabrikker. Søfrosne fiskeprodukter sælges ofte under betegnelsen "frozen at sea". Der er foretaget mange undersøgelser vedrørende produktion og kvalitet af søfrosne fiskeprodukter specielt ved Torry Research Station, Skotland (Burt et al., 1974; Jones, 1965a; Jones, 1965b; Jones, 1969; Kelly, 1969a; Love, 1966). I den forbindelse er der tidligere observeret kvalitetsproblemer m.h.t. misfarvning af søfrosne torskefileter som følge af dårlig afblødning i fangstbehandlingsprocessen. Ligeledes kan der optræde problemer med filetering af meget ferske torsk før og i *rigor mortis* ombord på frysetrawleren (Sørensen et al., 1997). Ved produktion af søfrossen torsk sker forarbejdningen af torsk ombord på frysetrawlerne som regel før *rigor* indtræder, da forarbejdning af torsk i *rigor* er meget problematisk. Dette underbygges i et screeningsforsøg udført af Cappeln & Jessen (2000), hvor op mod 50% af hel hovedkappet søfrossen torsk fra Barentshavet havde præ *rigor* status ved optøningstidspunktet på en landbaseret fabrik i Danmark.

3. KVALITETSFORRINGELSER UNDER FRYSELAGRING

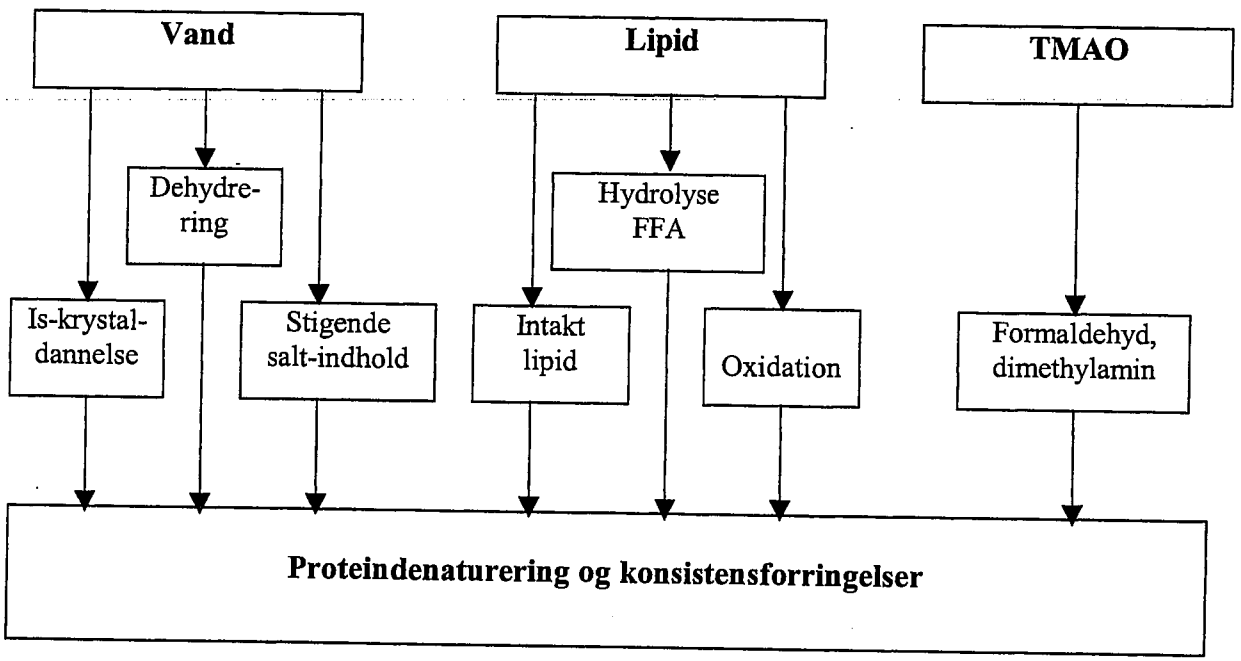
Frysning er en af de mest udbredte konserveringsformer for fisk og fiskeprodukter, da det er muligt at lagre store mængder af fisk over længere tid. Såfremt fisken behandles optimalt før og under fryselagring optræder der minimale sensoriske forandringer sammenlignet med råvaren ved indfrysningstidspunktet (Lavety, 1991). Frysning af fisk anvendes af mange årsager bl.a. for at udligne årstidsvariationer i fangsten af de enkelte fiskearter, vejrmæssige variationer samt udnyttelse af fiskefangster fra fjerne fangstområder som eksempelvis Barentshavet. Fisken fryses enten som råvare til senere forarbejdning eller som færdigvare direkte til konsum både i detail- såvel som cateringemballage. I dette kapitel angives de væsentligste sensoriske kvalitetsforandringer under fryselagring af torsk med de tilhørende biologiske og teknologiske kvalitetsparametre. Desuden præsenteres effekten af frysning på torskens mikrobiologi samt potentielle metoder til detektion af ferske kontra optøede torskefileter.

3.1 Frysning

Industriel indfrysning af torskefisk foregår i vertikal pladefryser, horisontal pladefryser eller frysetunnel afhængig af, hvilken produkttype, der skal indfryses (Dore, 1989; Morrison, 1993). Hel hovedkappet torsk indfryses blokvis i vertikal pladefryser, og filetblokke eller detailpakninger indfryses i horisontal pladefryser. Indfrysning af udskæringer eller enkeltfrosne fileter (IQF) foregår i frysetunnel. Disse tre indfrysningsmetoder repræsenterer alle en "hurtig indfrysning", som er anbefalet praksis for indfrysning af fiskeprodukter. Torsk fryser, når temperaturen er under –0.8°C (Sikorski & Kolakowska, 1994), og den mængde af vand, der fryser til is, stiger med faldende fryselagringstemperatur. Ved –30°C vil omkring 90% af vandet være blevet til is, og når temperaturen sænkes yderligere, vil denne andel øges langsomt, men ikke alt vandet vil fryse (Mackie, 1993). Det ufrosne vand kaldes ofte "ikke frysbart vand" og betragtes som bundet til proteinerne. Effekten af frysning er således, at der fjernes vand fra proteinerne ved isdannelse, men at en betydelig del af vandet forbliver ufrosset, hvorved der sker en opkoncentrering af salte og enzymer (Shenouda, 1980). Diffusion af salte og enzymer er endvidere stadig mulig med deraf følgende kemiske reaktioner.

3.2 Kvalitetsforringelser under fryselagring

I torsk, som er en mager fisk, udgør protein-, fedt- og vandindhold henholdsvis omkring 18%, 0.1% og 81% (Waterman, 1968). Adskillige studier viser, at torskefisk efter længere tids fryselagring får en sej og tør konsistens med tab af vandbindingsevne samt tilhørende udvikling af bismag (Giannini et al., 1993; Gill et al., 1979; Haard, 1992a; Kim & Heldman, 1985; Love, 1962; Love, 1992; Mackie, 1993; Matsumoto, 1980; Shenouda, 1980; Sikorski & Kolakowska, 1994; Sikorski et al., 1976). Disse kvalitetsforandringer kan henføres til proteindenaturering og konsistensforringelser (Figur 2), som kan relateres til ændringer i muskelcellernes vandfase, lipidindhold eller enzymatisk nedbrydning af TMAO i torskefiskene samt evt. en kombination af disse faktorer:



Figur 2 Forskellige faktorer, som medfører denaturering af fiskeproteiner og konsistensforringelser under fryselagring (modificeret efter Shenouda, 1980).

Der er principielt tre måder, hvorpå ændringer i vandfraktionen under frysning fører til proteinforandringer (Shenouda, 1980; Sikorski et al., 1976): (i) de dannede iskrystaller kan medføre fysisk ødelæggelse af cellemembranerne, men de kan også ødelægge de dominerende proteinstrukturer, der hovedsageligt udgøres af proteinerne myosin og actin, (ii) proteinernes naturlige struktur er normalt afhængig af, at der er bundet vand til dem. Når dette vand fryser

til is, sker der en dehydrering af proteinerne, som derved denaturerer samt (iii) under fryseprocessen sker der en kraftig opkoncentrering af opløselige stoffer i det ufrosne vand. Specielt har høje koncentrationer af salte en stor effekt på proteinerne, således at disse omlejres og danner nye bindinger.

Lipidindholdet i fisken findes dels i cellernes membranstruktur, men også som depotfedt lige under skind, i buglap, i lever og i bindevævet (Ackman & Hardy, 1980). Membranlipiderne er hovedsageligt fosforlipider bestående af lange umættede fedtsyrer (Ackman & Hardy, 1980). Hos de fleste fiskearter består depotfedtet hovedsageligt af triglycerider. Lipid kan nedbrydes enzymatisk ved en hydrolytisk spaltning af lipiderne, hvorved der dannes frie fedtsyrer (FFA) (Olley & Lovern, 1960). Lipidernes effekt på proteinerne afhænger af deres tilstand. Intakt lipid, der ikke er hydrolyseret eller oxideret, har en beskyttende effekt på proteinerne under fryselagringen. Dette er observeret ved fryselagring af laks (*Salmo salar*) med holdbarheder på helt op til 24 måneder ved –30°C (Sørensen et al., 1996). I torskefisk kan den oxidative lipidnedbrydning give anledning til dannelse af den såkaldte "frysehussmag og -lugt", der skyldes stoffet cis-4-heptenal (Hardy et al., 1979; McGill et al., 1974; McGill et al., 1977). Udviklingen af "frysehussmag og -lugt" under fryselagring af torsk afhænger således af temperatur og biologiske parametre som stamme, sæson og fangstområde (Love et al., 1975).

Under fryselagring af torskefisk nedbrydes trimethylaminoxid (TMAO) via TMAO dimethylase enzymatisk til formaldehyd (FA) og dimethylamin (DMA) (Castell, 1971; Mackie, 1974; Yamada & Amano, 1965). FA krydsbinder proteinerne, hvorved denatureringen af proteinerne accelleres (Castell et al., 1973; Rehbein, 1988). Den enzymatiske DMA- og FA-dannelse er kraftigst ved temperaturer mellem –1°C og –5°C. Dannelsen af DMA og FA kan reduceres ved at anvende lave fryselagringstemperaturer, effektiv rensning samt god afblødning, da enzymet TMAOase findes i store koncentrationer i blod, nyrevæv og indvolde (Tokunaga, 1970; Tokunaga, 1980). Det er dog fundet, at TMAO virker stabiliserende for proteiner under fryselagring (Owosu-Ansah & Hultin, 1984). Desuden er det vist, at et øget TMAO-indhold i dybvandsfisk forøger enzymstabiliteten mod høje tryk, og derfor er TMAO angivet som en universel stabilisator (Gillett et al., 1997; Yancey & Siebenaller, 1999).

TMAO findes hovedsageligt i saltvandsfisk og optages primært via føden (Ágústsson & Strøm, 1981). Der er dog fundet TMAO i ferskvandsfisken Nile aborre (*Lates niloticius*), som også kan leve i brakvand (Anthoni et al., 1990; Gram et al., 1989). Det menes, at TMAO virker som en osmose-regulerende-mekanisme, men TMAO-systemets virkemåde er i dag endnu ikke helt klarlagt. Saltvandsfisk optager dog tilsyneladende mere TMAO via føden sammenlignet med ferskvandsfisk, og begrænser samtidig TMAO-udskillelsen for at modvirke det omgivende saltvands højere osmotiske tryk (Hebard et al., 1982). Indholdet af TMAO i torskefamilien angives gennemsnitligt til 83 mg TMAO-N/100g (Hebard et al., 1982). TMAO-indholdet for fisk varierer dog betragteligt afhængig af art, sæson, størrelse og alder (Hebard et al., 1982). Shewan (1951) har fundet meget højere TMAO-indhold i arktiske torsk sammenlignet med torsk af samme art fra Nordsøen. Dette er i overensstemmelse med fund af TMAO-indhold på over 160 mg TMAO-N/100g i torsk fra Barentshavet (Oehlenschläger, 1998). Til sammenligning er der målt meget lavere TMAO-indhold i torsk fra Østersøen på omkring 50 mg TMAO-N/100g (Guldager et al., 1998). Endvidere er der i forskellige hvidfisk fundet, at TMAO-indholdet er meget højere i den hvide muskel sammenlignet med den røde muskel (Tokunaga, 1980). I et nyt studie har Yancey & Siebenaller (1999) fundet et meget højt TMAO-indhold i dybvandsfisk.

3.3 Biologiske og teknologiske parametre for kvalitet af frosne torskeprodukter

Kvaliteten af frossen torsk afhænger af biologiske og teknologiske parametre under fangst, forarbejdning og transport. De vigtigste biologiske parametre omfatter årstid, gydeperiode, alder, fødeindtag, og fangstområde (Castell, 1971; Love, 1975; Love, 1980; Love, 1988; Love, 1992). Desuden indvirker musklens pH på kvalitet af torsken under fryselagring, hvor højere pH-værdier giver en bedre konsistens og dermed en bedre lagringsstabilitet under fryselagring (Kelly, 1969b). Filetspaltning (gaping) afhænger af forskellige parametre som art, sæson og behandling af fisken efter fangsten (Love, 1988; Bremner, 1999). Det angives, at indfrysning før *rigor mortis* giver den mindste gaping af torskefileter efter optøning (Love et al., 1969). Cappeln & Jessen (2000) angiver dog en øget gaping af torskefileter ved anvendelse af for høje temperaturer i optøningsmediet.

De teknologiske parametre, der påvirker kvaliteten af frosne fiskeprodukter er angivet i Tabel 3.

Tabel 3 Teknologiske parametre, der påvirker kvaliteten af frosne torskeprodukter med tilhørende referencer.

Teknologiske parametre	Referencer
Fangstmetode	(Botta et al., 1987)
Fangstbehandling	(Botta et al., 1986; Jones, 1965a; Valdimarsson et al., 1984)
Friskhed ved indfrysning	(Connell, 1969; Connell & Howgate, 1968)
Fryselagringstemperatur	(Anon., 1971; Auborg & Medina, 1999; Bechmann et al., 1998; Careche et al., 1998; Leblanc et al., 1988)
Fryselagringstid	(Burt et al., 1974; Haard, 1992a; Jones, 1969; Kelly, 1969b; Sikorski & Kolakowska, 1994)
Dobbeltfrysning	(Dyer et al., 1962; Hurling & McArthur, 1996; MacCallum et al., 1969; Peters et al., 1968; Sørensen, 1986)
Emballering	(Dyer & Peters, 1969; Josephson et al., 1985; Nilsson & Ekstrand, 1994; Santos & Regenstein, 1990)
Optøning	(Cappeln et al., 1999; Hurling & McArthur, 1996; Jason, 1992)

Fryselagringstemperatur og -tid er de vigtigste af de teknologiske parametre for kvaliteten af frosne torskeprodukter. Generelt giver den laveste og mest stabile fryselagringstemperatur den længste holdbarhed. Ved –18°C, hvilket er lovgivningskravet for opbevaring af frosne fisk i den danske detailhandel, er holdbarheden af frosne torskeprodukter opgivet til at være 12 måneder (Mackie et al., 1986). Holdbarheden af frosne torskeprodukter kan dog forlænges op til 24 måneder såfremt temperaturen sænkes til –30°C (Anon., 1971).

3.4 Mikrobiologi under fryselagring

Ved fryselagringstemperaturer under –10°C stopper den mikrobiologiske aktivitet (Simmonds & Lamprecht, 1985). Generelt gælder det, at bedste praksis for frysning (hurtig indfrysning efterfulgt af lagring ved en stabil og lav temperatur) har den mindste effekt på inaktiveringen af mikroorganismer under indfrysning/fryselagring (Jay, 1991). Mikroorganismer bliver beskadiget eller inaktiveret under frysning. Det kan formodentligt relateres til enten denaturering eller koagulering af essentielle celle-proteiner eller enzymer som følge af en stigende opløsning i det "ufrosne" vand eller fysisk beskadigelse forårsaget af iskrystaller (Davies & Obafemi, 1985; Simmonds & Lamprecht, 1985). Overlevelsesgraden for

mikroorganismer under fryselagring afhænger af, hvilken mikroorganisme, det drejer sig om (Davies & Obafemi, 1985; Georgala & Hurst, 1963; Straka & Stokes, 1959). Mikrofloraen ved fangsttidspunktet (skind og gæller) for koldtvandsfisk fra salte farvande består primært af Gram-negative bakterier som *Pseudomonas, Moraxella, Acinetobacter, Shewanella, Flavobacterium* og *Vibrionaceae* (*Vibrio* og *Photobacterium*) (Liston, 1980). Disse Gram-negative bakterier er generelt følsomme overfor frysning og fryselagring, og deres overlevelse er afhængig af bl.a. lagringstemperatur og -tid (Shewan, 1961; Simmonds & Lamprecht, 1985). *Vibrio* angives til at være den mest følsomme m.h.t. fryselagring (Thampuran & Gopakumar, 1993).

Photobacterium phosphoreum, der tilhører de marine *Vibrios*, er den specifikke fordærvelsesbakterie for ferske MAP torskefileter (Dalgaard, 1995b; Dalgaard et al., 1993). Denne fordærvelsesbakterie er ligeledes meget følsom overfor frysning (Fujii et al., 1994; Guldager et al., 1998; van Spreekens, 1971; van Spreekens, 1974; van Spreekens, 1987). Inaktivering af andre marine *Vibrios* er mere tydelig efter fryselagring omkring –20°C sammenlignet med omkring –30°C (Chou et al., 1999; Dykes, 2000; Golden et al., 1988; Simmonds & Lamprecht, 1985; Shewan, 1961). Den svovlbrinte producerende *Shewanella putrefaciens* er blevet identificeret som den hovedansvarlige fordærvelsesbakterie for iset torsk (Gram et al., 1987; Jørgensen og Huss, 1989). *S. putrefaciens* er ligeledes meget følsom overfor frysning og fryselagring, og reduceres væsentligt allerede efter 1 måned ved –18°C (Licciardello & D'Entremont, 1987) og længere end 7 uger ved –25°C (Magnússon & Martinsdóttir, 1995).

3.5 Detektion af ferske kontra optøede torskefileter

Det er ikke altid sensorisk muligt at skelne mellem fersk og frossen-optøet fisk. Derfor har der inden for de seneste år været en stigende udvikling i metoder til detektion af fersk kontra frossen-optøet fisk med anvendelse af enzymatiske (Kitamikado et al., 1990; Rehbein, 1979; Salfi et al., 1985) eller elektriske/spektroskopiske metoder (Howell et al., 1996; Kim et al., 1987; Nott et al., 1999). Den lettest tilgængelige metode i dag til at skelne fersk fra optøet fisk er dog anvendelse af enten et Torrymeter, Fischtester eller RT-Freshtester, der alle tre er baseret på måling af fiskekødets dielektriske egenskaber (Dalgaard, 2000).

4. KVALITETSFORRINGELSER UNDER KØLELAGRING

Anvendelsen af MAP til holdbarhedsforlængelse af kommercielle fiskeprodukter begyndte i England omkring 1930 (Coyne, 1932). Men det er først i løbet af de sidste 10 år, at MAP af fiskeprodukter til detailsalg globalt er blevet meget udbredt. I dette kapitel uddybes anvendelsen af MAP for fiskeprodukter. Desuden gennemgås kvalitetsforringelser under kølelagring for ferske og optøede torskefileter lagret henholdsvis på is eller i MAP.

4.1 Generelt om MAP

I kommercielle sammenhænge anvendes O_2, N_2 og CO_2 som gasser ved pakning af fiskeprodukter i modificeret atmosfære. Væksten af aerobe bakterier og hæmmingen af anaerobe bakterier vil generelt blive fremmet af O_2. På grund af den lave opløselighed i vand bruges N_2 for at undgå sammenklapning af den anvendte emballering af fiskeproduktet. Derimod er CO_2 både opløselig i vand og fedt. Der er flere teorier vedrørende virkningen af CO_2 på bakterielle celler som: (i) forandringer i cellemembranernes struktur, (ii) hæmning af enzymatiske reaktioner, (iii) pH-ændringer samt (iv) fysiske-kemiske proteinforandringer (Daniels et al., 1985; Dixon & Kell, 1989). Den hæmmende effekt af CO_2 på mikrofloraen i MAP fisk er afhængig af mange faktorer såsom CO_2-koncentration, temperatur, vandaktivitet, og type af mikroorganisme (Farber, 1991). For at opnå den maksimale antimikrobielle effekt skal lagringstemperaturen for MAP produktet holdes så lav som muligt, da opløseligheden af CO_2 falder med stigende lagringstemperatur.

Der er de seneste 20 år lavet mange oversigtsartikler vedrørende teknologiske aspekter omhandlende MAP af fiskeprodukter (Ashie et al., 1996; Cann et al., 1984; Church, 1998; Gill & Molin, 1991; Haard, 1992b; Pedrosa-Menabrito & Regenstein, 1988; Pedrosa-Menabrito & Regenstein, 1990a; Stammen et al., 1990). Som det fremgår af Tabel 4, forlænger vacuum-pakning (VP) og MAP med CO_2 holdbarheden for kødprodukter med adskillige uger eller måneder. Med fiskeprodukter forholder det sig modsat, idet VP ikke forlænger holdbarheden, og hvor MAP kun giver nogle få dages længere holdbarhed ved 2°C. Disse forskelle kan primært relateres til forskelle i mikroflora og pH mellem fiske- og kødprodukter.

Tabel 4 Effekt af pakkemetode på holdbarhed for kølede fiske- og kød produkter (Dalgaard, 1995a).

Produkttype	Lagrings-temperatur (°C)	Holdbarhed (uger)		
		Luft	VP[a]	MAP[b]
Kød; oksekød, lam og fjekræ	1.0–4.4	1–3	1–12	3–21
Mager fisk; torsk og sej	0.0–4.0	1–2	1–2	1–3

[a] VP angiver vacuum-pakning.
[b] MAP angiver modificeret atmosfære pakning med CO_2-koncentrationer i intervallet 25%–100%.

For MAP af torskefileter anbefales det at anvende CO_2-indhold mellem 30% og 60% samt et gas/fisk forhold på mindst 2 (Cann et al., 1984; Davis, 1993). Anvendelsen af O_2 sammen med CO_2 giver en mindre holdbarhedsforlængelse på nogle få dage for MAP ferske torskefileter (Debevere & Boskou, 1996; Guldager et al., 1998).

Sammenlignet med fiskeprodukter lagret i luft forøger MAP ikke sundhedsrisikoen fra patogene bakterier som *Salmonella, Staphylococcus* and *Vibrio parahaemolyticus* (Farber, 1991; Hintlian & Hotchkiss, 1986; Reddy et al., 1992; Statham, 1984). Det skal dog bemærkes, at en begrænset vækst af de normale fordærvelsesbakterier i MAP fiskeprodukter kan fremme væksten af *C. botulinum* type E. Denne patogene bakterie kontamineres naturligt fra det omgivende miljø, og vokser helt ned til 3.3°C (Reddy et al., 1992). I nærværende afhandling er det fravalgt at undersøge aspekter vedrørende patogene bakterier i relation til optøede kølede MAP torskefileter.

4.2 Kølelagring af ferske MAP torskefileter

P. phosphoreum er den specifikke fordærvelsesbakterie for ferske MAP torskefileter (Dalgaard, 1995b; Dalgaard et al., 1993) og hovedansvarlig for produktion af store mængder trimethylamin (TMA) i fordærvede produkter. Ved fordærvelsestidspunktet indeholder produktet over 10^7 cfu/g *P. phosphoreum* og udgør langt hovedparten af mikrofloraen (Dalgaard, 1995b). *P. phosphoreum* kan vokse under høje CO_2-indhold i modsætning til den mest betydende fordærvelsesbakterie *S. putrefaciens* for isede kølede torskefileter. *P. phosphoreum* findes naturligt i det marine miljø (Baumann & Baumann, 1981), og er en saltafhængig bakterie med et vækstoptimum ved 1% NaCl (Dalgaard, 1993). *P. phosphoreum* tilhører de marine *Vibrios*, og er nært beslægtet med *Vibrio cholerae* og *Escherichia coli*

(Baumann & Baumann, 1981). Ved DFU, Lyngby er der de seneste år lavet adskillige publikationer med ferske MAP torskefileter omhandlende fangstområder (Dalgaard et al., 1997a), forlængelse af holdbarhed med EDTA (Dalgaard et al., 1998) samt prædiktion af restholdbarhed (Dalgaard & Huss, 1994; Dalgaard et al., 1997b; Dalgaard, 2000). Med baggrund i ovennævnte publikationer er der hos DFU, Lyngby udviklet et PC-baseret program "Seafood Spoilage Predictor" til prædiktion af restholdbarhed for ferske MAP torskefileter (http://www.dfu.min.dk/micro/ssp/) udfra startniveauer for *P. phosphoreum*, CO_2-indhold og kølelagringstemperatur.

4.3 Kølelagring af optøede torskefileter

Optøet og aerob lagret fisk har generelt en lidt længere holdbarhed sammenlignet med ferske produkter (Liston, 1980; Shewan, 1961; Simmonds & Lamprecht, 1980; Simmonds & Lamprecht, 1985). Holdbarhedsforlængelser under 2 dage ved temperaturer mellem 0°C og 2°C er sædvanligvis blevet observeret, dog viser et enkelt studie 3 dages ekstra holdbarhed ved 5°C (Simmonds & Lamprecht, 1980). Ligeledes er der observeret lavere TMA-værdier ved fordærvelsestidspunktet for optøede sammenlignet med ferske torskefileter (Magnússen & Martinsdóttir, 1995; Vyncke, 1983).

Det er meget sparsomt, hvilke tidligere studier, som vedrører produktion af optøede MAP fiskeprodukter (Esaiassen & Akse, 1997; Guldager et al., 1998; Lanier & Korhonen, 1981). I et amerikansk studie angives dog fordele ved detailsalg af optøede MAP ørreder m.h.t. udjævning af råvaretilgangen samt minimering af tid- og temperaturbelastning på færdigvaren sammenlignet med en traditionel produktion af ferske detailpakkede ørredfileter (Lanier & Korhonen, 1981). I et nyere studie (Guldager et al., 1998) er det endvidere vist, at *P. phosphoreum* er blevet inaktiveret under frysning (8 uger ved –20°C) af torskefileter fra Østersøen idet fordærvelsesbakterien ikke kunne detekteres i fisken under den efterfølgende kølelagring som MAP produkt (Tabel 5).

Tabel 5 Kemiske, fysiske og mikrobiologiske karakteristika efter 20 dages kølelagring ved 2°C i MAP for torskefileter fra Østersøen med en forudgående fryselagring ved –20°C i 8 uger (Guldager et al., 1998).

Analysemetode	Niveau
P. phosphoreum, log cfu/g	n.d.[a]
TVC, log cfu/g	5.7
TMA, mg TMA-N/100 g	4.2
DMA, mg DMA-N/100 g	1.9
pH	6.7
Drip loss, %	14.5

[a] n.d. angiver under detektionsgrænsen på 0.6 log cfu/g

Inaktivering af *P. phosphoreum* medførte, at optøede MAP torskefileter havde meget lavere værdier for totalkim (TVC), TMA og pH under kølelagringen sammenlignet med ferske torskefileter (Dalgaard, 1995b). Dette blev underbygget af de sensoriske bedømmelser, der selv efter 20 dage ved 2°C ikke viste signifikante stigninger for aminlugt og -smag (Guldager et al., 1998). Denne meget lave produktion af TMA i optøede kølede MAP torskefileter er ligeledes blevet observeret i et norsk studie, hvor der dog blev fundet TMA-værdier ved fordærvelsestidspunktet på niveau med ferske MAP torskefileter, hvis torskefileterne forudgående var lagret ved –30°C (Esaiassen & Akse, 1997). Der er generelt blevet fundet højere dryptab for optøede torskefileter sammenlignet med ferske MAP torskefileter under kølelagringen (Dalgaard et al., 1993; Guldager et al., 1998).

5. KVALITETSBEDØMMELSE OG MULTIVARIABEL DATAANALYSE

Kvalitet af fiskeprodukter er et koncept, som kan forklares ved hjælp af forskellige sensoriske, mikrobiologiske, kemiske og fysiske parametre, og derved defineres som en objektiv egenskab (Botta, 1995; Bremner, 1998; Gill, 1990; Ólafsdóttir et al., 1997; Pedrosa-Menabrito & Regenstein, 1990b). Traditionelt har fiskeindustrien tidligere primært målt kvalitet af fiskeprodukter via stikprøve-inspektion af færdigvaren, hvor eksempelvis totale flygtige baser (TVN), TVC samt sensoriske bedømmelser globalt har været almindeligt udbredt til måling af kvalitet. Indførelsen af HACCP (Hazard analysis critical control point)- og egenkontrolsystemer i fiskeindustrien de seneste 10 år har dog medført en stigende efterspørgsel for udvikling af hurtigmetoder, der evt. kan anvendes on-line i den daglige produktion. Udviklingen af disse hurtigmetoder er bl.a. sket inden for de spektroskopiske analysemetoder såsom NIR og "nuclear magnetic resonance" (NMR). Til udviklingen af disse hurtigmetoder er det nødvendigt at anvende multivariable databehandlingsteknikker som "principal component analysis" (PCA) og "partial least squares regression" (PLSR). I dette kapitel gives der en oversigt over metoder til kvalitetsbedømmelse af optøede kølede MAP torskefileter. Disse dækker et bredt udsnit af de potentielle metoder til henholdsvis fersk og frossen torskefisk. Endvidere præsenteres forskellige teknikker til multivariabel dataanalyse, der er anvendt i afhandlingen.

5.1 Sensoriske metoder

Sensorik er defineret som den videnskabelige disciplin, der anvender de menneskelige sanser som syn, lugt, smag, følelse og hørelse til f.eks. at karakterisere kvalitet af fiskeprodukter (Connell, 1980; Connell & Shewan, 1980; Hyldig & Nielsen, 1998; Nielsen, 1998; Nielsen & Jessen, 1997). De sensoriske metoder kan opdeles i henholdsvis forskelstest, beskrivende test eller affektive test. Forskelstest og beskrivende test er begge objektive metoder. Forskelstest anvendes til at bestemme forskelle mellem prøver v.h.a. triangel-test eller rangordentest. Beskrivende test anvendes til kvalitativt at bestemme forskellen mellem prøver ved hjælp af profilering, kvalitetsindeksmetoden eller struktureret skalering. De affektive test er subjektive og baseres primært på forbrugertest ved brug af markedsundersøgelser, accept-test eller præference-test. Der anvendes kun beskrivende test som sensorisk metode i nærværende

afhandling. Den første metode til struktureret skalering af fiskeprodukter blev udarbejdet ved Torry Research Station, Skotland for omkring 50 år siden (Ehrenberg & Shewan, 1953; Shewan et al., 1953). Der blev udvalgt forskellige kvalitetsparametre som udseende, konsistens, lugt og smag til Torry-metoden. Denne metode anvender en struktureret skala eks. fra 0–10 point, hvor der er relateret beskrivende termer og korresponderende point til de forskellige kvalitetsparametre. 10 point angiver den højeste kvalitet og i intervallet 4–6 kasseres fiskeproduktet. EU-skemaet er i dag den mest anvendte strukturerede skaleringsmetode i Europa i forbindelse med kvalitetskontrol hos fiskeritilsyn og fiskeindustri (Howgate et al., 1992). Alternativt kan kvalitetsindeksmetoden (QIM) anvendes som en beskrivende test, der indeholder en enkel, men nøjagtig beskrivelse af de enkelte kvalitetskriterier på en objektiv og uafhængig måde (Bremner, 1985; Bremner et al., 1987). QIM er oprindeligt udviklet i Hobart, Australien, men er blevet videreudviklet i Europa i de seneste 10 år. Der er udviklet QIM til fersk sild, sej, torsk, rødspætter, rødfisk, sardiner og laks (Hyldig & Nielsen, 1998; Jónsdóttir, 1992; Larsen et al., 1992; Luten & Martinsdóttir, 1998). Ligeledes er der udviklet QIM til kvalitetsbedømmelse af frossen torsk som henholdsvis optøet hel, fileteret og kogt torsk (Warm et al., 1998). I Tabel 6 vises de parametre, som bedømmes ved anvendelse af QIM til optøet kogt torskefilet (Artikel I).

Tabel 6 QIM til optøet kogt torsk, hvor 16 angiver den bedste kvalitet og 0 angiver den dårligste kvalitet (Warm et al., 1998).

Parameter	Niveau
Lugt	0–4
Farve	0–4
Smag	0–4
Konsistens	0–4
Samlet kvalitetsindeks	0–16

Profilering er tidligere anvendt til beskrivelse af kvalitetsegenskaber i videnskabelige studier af forskellige fiskeprodukter (Guldager et al., 1998; Jakobsen, 1999; Rørbæk, 1994). Ved profilering er det påkrævet, at den enkelte dommer kan detektere og kvantificere de forskellige produktegenskaber. Derfor kræves der et meget trænet panel til profilering (Botta, 1995; Meilgaard et al., 1999). I nærværende afhandling er der til sensorisk kvalitetsbestemmelse anvendt en profileringsanalyse (Artikel II og IV), hvor følgende udvalgte produktegenskaber for optøede kogte MAP torskefileter er blevet bedømt: aminlugt,

aminsmag, frysehuslugt, frysehussmag og saftighed på en intensitetsskala fra 0–15 (Guldager et al., 1998).

5.2 Mikrobiologiske metoder

P. phosphoreum, som er meget udbredt i det marine miljø, reducerer TMAO til TMA og er en meget CO_2 resistent bakterie. Der blev i 1996 hos DFU, Lyngby udviklet en følsom og selektiv Malthus-metode til kvantitativ bestemmelse af antal *P. phosphoreum* i fiskeprodukter (Dalgaard et al., 1996). Med den opstillede Malthus-metode kan der efter inkubering ved 15°C bestemmes antallet af *P. phosphoreum*. Detektionsgrænsen for den udviklede Malthusmetode ligger på 0.6 log (cfu/g). Denne metode er baseret på, at der eksisterer en sammenhæng mellem måling af den tid, som det tager ændringen i ledningsevnen at nå en bestemt værdi og antallet af *P. phosphoreum* i en opløsning, hvori væksten og selektionen af den specifikke bakterie er understøttet. Stoffet TMAO er en elektronacceptor, der vil udnyttes af bakterier under anaerobe forhold. TMA bruges til at producere ledningssignalet, idet den bakterielle reduktion af TMAO til TMA bevirker en ændring i opløsningens ledningsevne, som derved giver et udtryk for antallet af *P. phosphoreum*.

Ved sensorisk fordærv overstiger antallet af *P. phosphoreum* 10^7 cfu/g i ferske MAP torskefileter og udgør hovedparten af den samlede bakterieflora (TVC) (Dalgaard, 1995a). Ved direkte mikroskopering kan tilstedeværelsen af *P. phosphoreum*, der er en stor lysende bakterie med et meget "karakteristisk" udseende ligeledes observeres ved tidspunktet for sensorisk fordærv (Dalgaard, 1995b). TVC repræsenterer det totale antal synlige bakterier, der vokser frem på et medie ved en given temperatur. TVC bestemmes som overflade- spredning med "Long and Hammers medium" indeholdende 1% NaCl. Pladerne inkuberes aerobt ved 15°C i 7 dage som anvendt i tidligere studier (Dalgaard et al., 1996; van Spreekens, 1974). *P. phosphoreum* er varme-følsom og kræver mineraler under væksten. Derfor bliver *P. phosphoreum* ikke detekteret på et medie uden salt og ved inkubationstemperaturer over 20–25°C (Dalgaard, 1998). Begge mikrobiologiske analysemetoder er anvendt i Artikel II, III og IV.

5.3 Kemiske metoder

TMA er en flygtig amin, som associeres med typisk fiskelugt ved fordærv af kølede torskefisk. TMAO nedbrydes bakterielt i kølet fisk til TMA. *P. phosphoreum* producerer 10 til 100 gange så stor mængde af TMA sammenlignet med den velkendte fordærvelsesbakterie *Shewanella putrefaciens* i isede torskefisk (Dalgaard, 1995b). Derfor observeres der store forskelle m.h.t. kassation af kølede torskeprodukter udfra TMA-indholdet. Normalt kasseres torskeprodukter til konsum, når TMA-indholdet overstiger 10–30 mg TMA-N/100 g (Cann et al., 1984; Dalgaard et al., 1993). Til bestemmelse af TMA-, TMAO- og TVN-indhold anvendes en modificeret Conway-mikrodiffusions-metode (Conway & Byrne, 1933). DMA og FA dannes enzymatisk i ækvivalente mængder under fryselagring i gadoide fisk, der indeholder enzymet TMAO dimethylase. Den samlede mængde af FA kan dermed bestemmes udfra DMA-indholdet. I analytisk henseende skelnes der mellem frit formaldehyde, reversibelt bundet formaldehyd og bundet formaldehyd (Bechmann, 1998a). Det frie formaldehyd bestemmes efter Nash-metoden (Nash, 1953). Det frie og reversibel bundne formaldehyd bestemmes ved en damp-destillation (Anon., 1964), og er anvendt i industrien primært rettet mod det italienske marked, hvor grænseværdien for kassation er 60 ppm for frosne torskeprodukter. Til bestemmelse af DMA-indholdet i fiskeprodukter anvendes der i dag primært en gas chromatografisk metode (Manthey, 1988). I nærværende afhandling anvendes imidlertid en nyudviklet kapillar-elektroforese-metode, som endnu ikke er publiceret (Timm, pers. komm.). Saltindholdet i torskefileterne bestemmes udfra "sølvnitratsmetoden", hvor der anvendes analysemetoden fra AOAC (1995). TMAO og TMA er bestemt i Artikel II, III og IV, mens indholdet af frit formaldehyd og DMA er bestemt i Artikel IV.

5.4 Fysiske metoder

Vandbindingsevnen er bestemt efter en centrifugeringsmetode (Eide et al., 1982) i Artikel I og IV. Dryptabet er målt som det frivillige dryp under optøning og kølelagring (Artikel II, III og IV). Dette betyder, at dryptabet udregnes som en relativ vægtdifference som tidligere anvendt (Dalgaard et al., 1993; Guldager et al., 1998). pH i fiskekødet bestemmes med et autocal pH meter (Radiometer, Danmark) i Artikel IV.

5.5 Spektroskopiske metoder

I de senere år har der været en stor forskningsmæssig udvikling i anvendelse af spektroskopiske hurtigmetoder til kvalitetsbedømmelse af fiskeprodukter. Disse spektroskopiske metoder omfatter bl.a. NMR (Aursand et al., 1997; Jepsen et al., 1999) og NIR (Bechmann & Jørgensen, 1998; Jørgensen & Jensen, 1997; Pink et al., 1998; Pink et al., 1999; Wold & Isaksson, 1997; Wold et al., 1996). Bølgelængderne for det nær infrarøde område ligger mellem 800 nm (synlig lys) og 2500 nm (infrarød lys). I de senere år er NIR blevet anvendt til kvalitetsbedømmelse af mange forskellige fødevarer (Osborne et al., 1993). NIR er i dag en veletableret metode til bestemmelse af fedt- og vandindhold i laks (Sollid & Solberg, 1992; Wold & Isaksson, 1997; Wold et al., 1996). Ydermere viser nye studier, at der er et potentiale for anvendelse af NIR til bestemmelse af vandbindingsevne (Bechmann & Jørgensen, 1998; Jørgensen & Jensen, 1997) og DMA-indhold (Pink et al., 1998; Pink et al., 1999) for torskefisk. Desuden har norske undersøgelser vist lovende resultater for anvendelse af NIR til bestemmelse af kølelagringstid ved 0°C for fersk torsk (Sigernes et al., 1998). NIR har imidlertid ikke tidligere været anvendt til kvalitetsbedømmelse af optøede kølede MAP torskefileter. Med baggrund i dette undersøges potentialet for anvendelse af NIR som en hurtigmetode til kvalitetsbedømmelse af optøede kølede MAP torskefileter i Artikel V.

5.6 Multivariabel dataanalyse

Naturen er multivariabel, hvilket medfører, at et fænomen sædvanligvis afhænger af forskellige faktorer (Esbensen, 1994). Derfor anvendes multivariabel dataanalyse i nærværende afhandling som supplement til de klassiske statistiske uni-variable metoder. De anvendte multivariable metoder omfatter PCA og PLSR. Begge multivariable metoder er baseret på empirisk data-modellering, og dækker henholdsvis klassifikation og kalibrering. For yderligere uddybning af multivariabel dataanalyse henvises der til Martens & Næs (1989) og Bro (1996). Både PCA og PLSR er begge metoder, som er blevet anvendt meget inden for fødevareforskningen de seneste år (Barroso et al., 1998; Bechmann, 1998b; Bechmann et al., 1998; Bechmann & Jørgensen, 1998; Girard & Nakai, 1993; Horimoto & Nakai, 1998; Jensen & Jørgensen, 1997; Jepsen et al., 1999; Pino et al., 1999; Rosenfeld et al., 1997; Shaw et al., 1999; Sivertsen et al., 1999).

PCA er en meget vigtig matematisk metode, som kan bruges til at komprimere store datamængder. I praksis har det vist sig, at antallet af variable ofte kan reduceres fra flere hundrede til nogle få afledte variable uden, at der mistes væsentligt information i datamaterialet. Datakompression er meget vigtig af flere grunde:

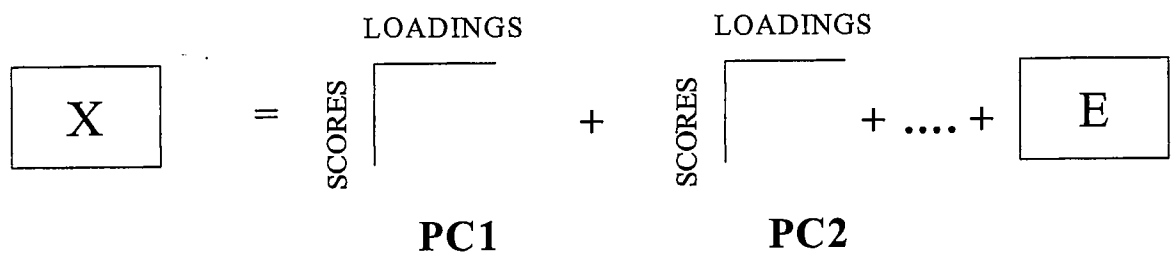
1. Mennesker kan kun forholde sig til nogle få fænomener eller dimensioner af gangen. Når et stort datamateriale er reduceret til et mindre antal variable (eller prøver) er det oftest betydelig lettere at tolke, finde strukturer, ligheder mellem prøver og variable eller mellem grupper af prøver og variable.
2. Datasæt med mange variable er ofte korrelerede (høj korrelation mellem variable), som gør det vanskeligt at anvende dem til eksempelvis klassisk regressionsanalyse. PCA fjerner kollineariteten i datamaterialet.
3. PCA er et vigtigt værktøj til at identificere evt. afvigende prøver ("outliers") i datamaterialet.

Før der laves en PCA til "eksplorativ" dataanalyse, opstilles data i en såkaldt "data-matrice" (Figur 3). Dette er en tabel, hvor hver række i tabellen refererer til en prøve, og hver kolonne refererer til en variabel.

$$\begin{bmatrix} x_{11} & x_{12} & \dots & x_{1K} \\ . & . & \dots & . \\ . & . & \dots & . \\ x_{N1} & x_{N2} & \dots & x_{NK} \end{bmatrix}$$

Figur 3 Opstilling af en data-matrice til PCA, hvor variable er angivet med 1, 2, ... , K (vandret), og antallet af prøver er N (lodret).

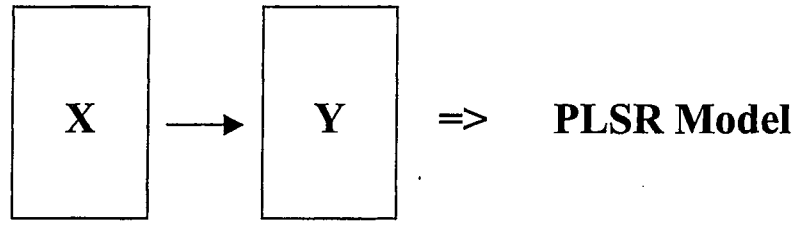
PCA består i en konstruktion af latente faktorer (principale komponenter) fra de underliggende strukturer i de originale data. Matematisk set er principale komponenter linear kombinationer af variable, som har specielle egenskaber m.h.t. varians. I en PCA bliver datamatricen dekomponeret til henholdsvis systematisk variation og støj som illustreret i Figur 4.



Figur 4 En grafisk fremstilling af PCA dekomponering af en data-matrice X i henholdsvis systematisk variation (repræsenteret ved de principale componenter PC1 og PC2) og støj (E).

Den systematiske variation er beskrevet med de principale componenter (PC1, PC2 o.s.v.), som repræsenterer henholdsvis scores og loadings. Scores er relateret til prøver og loadings er relateret til variable. Scores og loadings kan begge præsenteres i et "bi-plot" (Artikel I og II). NIPALS-algoritmen (Wold, 1966) anvendes til PCA i software programmet "Unscrambler", der anvendes til den multivariable dataanalyse i nærværende afhandling. Det skal bemærkes, at PCA er en gammel og veletableret teknik, som er godt beskrevet (Esbensen, 1994; Martens & Næs, 1989; Wold et al., 1987).

Hvis formålet med analysen er at beskrive sammenhængen mellem to sæt af variable X og Y (Figur 5), optræder de principale komponenter oftest ikke som optimale latente variable. I stedet for at lade de latente variable forklare så meget som muligt af variansen i X, bør man trække den information ud i X, som varierer mest mulig med information i Y. Til dette formål anvendes PLSR. Detaljer vedrørende PLSR-algoritmen findes uddybet hos (Martens & Martens, 1986; Martens & Næs, 1989). PLSR dekomponerer den kollineære X til et sæt ukorrelerede latente variable, som regressionen udføres på. I Artikel V undersøges om det er muligt med anvendelse af PLSR på NIR-målinger at prædiktere kølelagringstid (dage ved 2°C) for optøede MAP torskefileter. Der blev anvendt en "Jack-knifing" metode til udvælgelse af signifikante NIR-bølgelængder (Martens & Martens, 1999).



Figur 5 Der laves en model ud fra X og Y-matricen, som begge indeholder flere variable og prøver.

PLSR-modeller kan ligeledes anvendes til fortolkning af designede forsøg med henholdsvis Anova PLSR (APLSR) eller Diskriminant PLSR (DPLSR) (Martens & Martens, 1986; Wold et al., 1983). For anvendelse af disse multivariable teknikker henvises til følgende studier (Bechmann, 1998b; Bechmann et al., 1998; Jacobsen et al., 1999; Martens, 1986; Martens & Jørgensen, 1998). I Artikel IV anvendes APLSR med designvariable (fryselagringstemperatur, fryselagringstid og kølelagringstid) som X-matrice (1 eller 0 som indikatorer for niveauer for de respektive design variable) og 11 sensoriske, kemiske, fysiske og mikrobiologiske kvalitetsparametre som Y-matrice. I Artikel V anvendes DPLSR, hvor 459 NIR bølgelængder anvendes som X-matrice og design variable (fryselagringstemperatur, fryselagringstid og kølelagringstid) som Y-matrice (1 eller 0 som indikatorer for niveauer for de respektive design variable). Ved validering af PLSR-modellerne anvendes der i nærværende afhandling fuld krydsvalidering (Martens & Næs, 1989). Der blev anvendt "Jack-knifing" metoden til estimering af parameter usikkerheder i PLSR (Martens & Martens, 1999).

6. HOVEDRESULTATER OG DISKUSSION

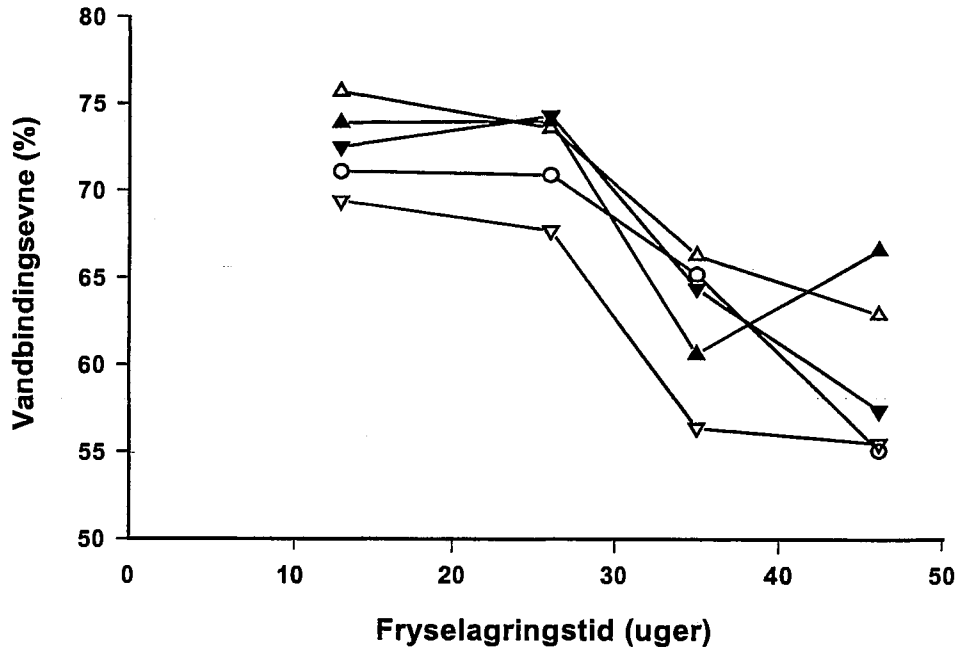
Det overordnede formål med dette erhvervsforskerprojekt har været at undersøge hele produktionskæden for frosne højkvalitetsprodukter med henblik på afsætning af optøede kølede MAP torskefileter i Danmark som et alternativ til detailsalg af kølede fiskeprodukter. Produktionen af forskellige former for søfrosne torskefileter og torskeudskæringer optimeres ligeledes med henblik på at producere frosne højkvalitets torskeprodukter. Forskellige teknologiske og biologiske parametre ved produktion af optøede kølede MAP torskefileter undersøges. Endvidere sammenlignes indfrosset råvare fra Østersøen med søfrosne torskefileter fra Barentshavet m.h.t. produktion af optøede kølede MAP torskefileter. Endelig vurderes anvendeligheden af forskellige mikrobiologiske, fysiske, kemiske, sensoriske og spektroskopiske metoder til kvalitetsbedømmelse af optøede kølede MAP torskefileter.

Det eksperimentelle arbejde i nærværende afhandling kan opdeles i fire dele. Første del vedrører produktion af søfrosne højkvalitets torskeprodukter, samt potentialet for anvendelse af søfrosne torskefileter som råvare til optøede kølede MAP produkter (Artikel I og IV). Den anden del vedrører produktion af optøede kølede MAP torskefileter m.h.t. effekt af råvarekvalitet ved indfrysning, fryselagringstemperatur, fryselagringstid, fangstområde, kølelagringstemperatur, MAP under fryselagring, indfrysnings- og optøningsmetoder samt gassammensætning under kølelagring (Artikel II, III og IV). Effekten af TMAO og NaCl for kvaliteten af optøede kølede MAP torskefileter er behandlet i tredje del (Artikel III). Den fjerde del, der vedrører NIR som en alternativ metode til kvalitetsbedømmelse optøede kølede MAP torskefileter er vist i Artikel V.

6.1 Produktion af søfrossen torsk (Artikel I og IV)

Det første fryselagringsforsøg vedrørende torskeprodukter blev udført ombord på den grønlandske frysetrawler "Sisimiut" i Barentshavet (Artikel I). De søfrosne torskefileter omfattede glaserede torskeudskæringer, glaserede VP torskefileter, uglaserede torskeudskæringer, interleaved-pakkede torskefileter og dobbeltfrosne torskefileter. De søfrosne torskeudskæringer repræsenterede en ny form for kommercielle frosne torskeprodukter. Temperaturen under fryselagring i 46 uger var –28.0±3.2°C dog med

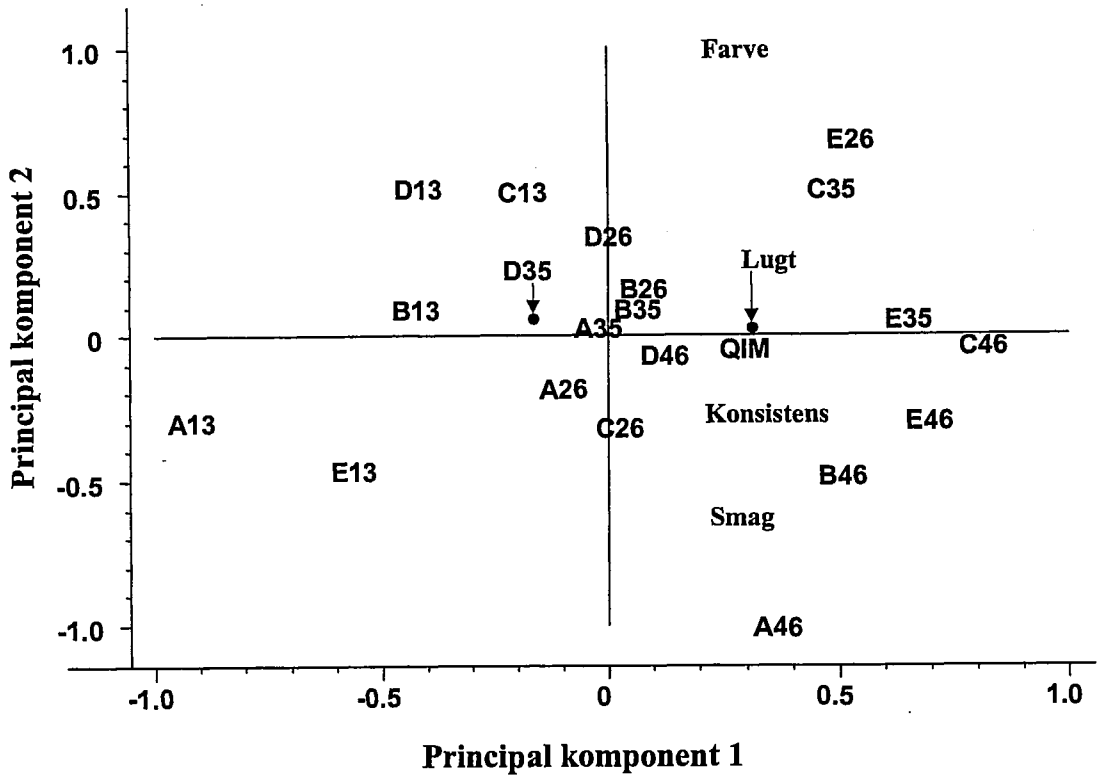
realistiske fluktuationer under transport og lagring i forhold til et kommercielt forløb. Vandbindingsevnen var lavere for de dobbeltfrosne torskefileter under hele fryselagringsforløbet (Figur 6). Det ses endvidere, at vandbindingsevnen faldt efter 26 ugers fryselagring for alle fem produkttyper. Desuden var kogesvindet betydelig højere for de dobbeltfrosne sammenlignet med de enkeltfrosne torskeprodukter under hele fryselagringen (Artikel I).



Figur 6 Vandbindingsevne som funktion af fryselagringstid for de fem forskellige søfrosne torskeprodukter. Kode A (τ), glaserede torskeudskæringer; kode B (μ), glacerede VP torskeudskæringer; kode C (Δ), uglacerede torskeudskæringer; kode D (σ), interleaved-pakkede torskefileter og kode E (∇), dobbeltfrosne torskefileter.

Effekten af fryselagringstiden på den sensoriske kvalitet af fisken kan ses af Figur 7. Koderne fryselagret 13 og 26 uger er placeret til venstre i bi-plottet modsat QIM-bedømmelserne, som er placeret til højre. En høj QIM-værdi er ensbetydende med en dårlig sensorisk kvalitet. Det betyder, at koderne, som er fryselagret kort tid, har en bedre sensorisk kvalitet sammenlignet med koderne, som er fryselagret 35 og 46 uger og korrelerer med QIM-bedømmelserne. Koderne 35 og 46 ugers fryselagring for dobbeltfrosne torskefileter samt 46 uger for uglaserede torskeudskæringer er placeret længst til højre i bi-plottet, hvilket betyder, at disse tre koder har den dårligste sensoriske kvalitet af alle koderne. Forsøget viser, at det er muligt at producere søfrosne torskeprodukter med høj sensorisk og fysisk kvalitet selv efter 46 ugers fryselagring, såfremt produktet frysebeskyttes. Frysebeskyttelsen kan f.eks. bestå i glasering,

interleaved-pakning eller glasering kombineret med VP, som der blev anvendt i dette forsøg. Det er således muligt at producere frosne højkvalitets torskeprodukter til det danske marked til erstatning for de eksisterende (ofte dobbeltfrosne) torskeprodukter af meget varierende kvalitet. Søfrosne torskeudskæringer er tilmed et nemt og bekvemt produkt, som kan anvendes til catering eller ompakkes til detailsalg.



Figur 7 PCA af sensoriske data med QIM, Lugt, Farve, Smag og Konsistens for søfrosne torskeprodukter. Bi-plot med principal komponent 1 og principal komponent 2. Principal komponent 1 og 2 forklarer henholdsvis 77% og 7% af den samlede variation i datasættet. Glaserede torskeudskæringer (A); glacerede VP torskeudskæringer (B); uglacerede torskeudskæringer (C); interleaved-pakkede torskefileter (D) og dobbeltfrosne torskefileter (E). Numrene henviser til fryselagringstiden i uger.

Produktion af højkvalitets produkter som søfrosne torskeudskæringer kræver yderligere installering af frysetunnel med glaseringsudstyr ombord på frysetrawlerne. Søfrosne torskeudskæringer afregnes idag ikke højere end de traditionelle interleaved-pakkede torskefiletblokke. Dermed er der på nuværende tidspunkt intet incitament til at omstille produktionen ombord på de eksisterende frysetrawlere, hvortil der kræves store investeringer og udvidelse af antal produkttyper og produktionsprocessen ombord. Derfor er de

efterfølgende forsøg i nærværende afhandling baseret på søfrosne interleaved-pakkede filetblokke, som udgør et standard filetprodukt ombord på eksisterende frysetrawlere.

Det andet fryselagringsforsøg blev påbegyndt ombord på den grønlandske/russiske frysetrawler "Karelia" i Barentshavet (Artikel IV). I dette forsøg blev kvaliteten af interleaved-pakkede torskefileter m.h.t. fryselagringstid og -temperatur undersøgt. Vandbindingsevnen for torskefileter lagret omkring –30°C var signifikant højere sammenlignet med lagring ved –24°C (Tabel 7). Under 12 måneders fryselagring ved –30°C blev der ikke observeret forandringer i vandbindingsevnen. Derimod resulterede fryselagring i mindst 3 måneder ved –24°C i en meget lavere vandbindingsevne sammenlignet med lagring ved –30°C. Indholdet af FA var højere ved –24°C sammenlignet med –30°C. Det skal bemærkes, at FA-indholdet var meget lavt selv efter 12 måneders fryselagring ved –24°C sammenlignet med et tidligere studie (Leblanc et al., 1988).

Tabel 7 Forsøgskarakteristika og kvalitetsparametre (gennemsnit±standardafvigelse) for søfrosne interleaved-pakkede torskefileter lagret op til 12 måneder ved to forskellige fryselagringstemperaturer.

Kode	Fryselagrings-tid (måneder)	Fryselagrings-temperatur (°C)	Vandbin-dingsevne (%)	Frit formaldehyd (ppm)
A	3	–29.0±2.7	73.5±6.9	3.3±1.7
B	6	–31.5±2.9	76.4±3.6	1.5±0.7
C	6	–24.6±4.3	63.9±2.5	6.6±1.8
D	9	–32.2±2.6	76.5±2.6	1.5±0.9
E	9	–23.5±4.0	62.5±2.6	8.0±2.4
F	12	–32.4±3.4	76.1±6.0	2.3±0.2
G	12	–23.4±3.6	62.9±2.8	9.7±1.2

Erfaringerne opnået ved gennemførelsen af de to lagringsforsøg ombord på frysetrawlere i Barentshavet kan sammenfattes i følgende opstilling af GMP for produktion af søfrosne torskefileter:

1. Korte trawltider (under 6 timer) med ikke for store mængder hel fisk (max. 20 tons).
2. Hurtig maskinel hovedkapning og rensning ombord med afblødning i havvand (mindst ½ time).
3. Maskinel filetering og afskinding før indtræden af *rigor mortis*.
4. Manuel trimning af torskefileterne.
5. Manuel pakning af interleaved pakkede torskefiletblokke.

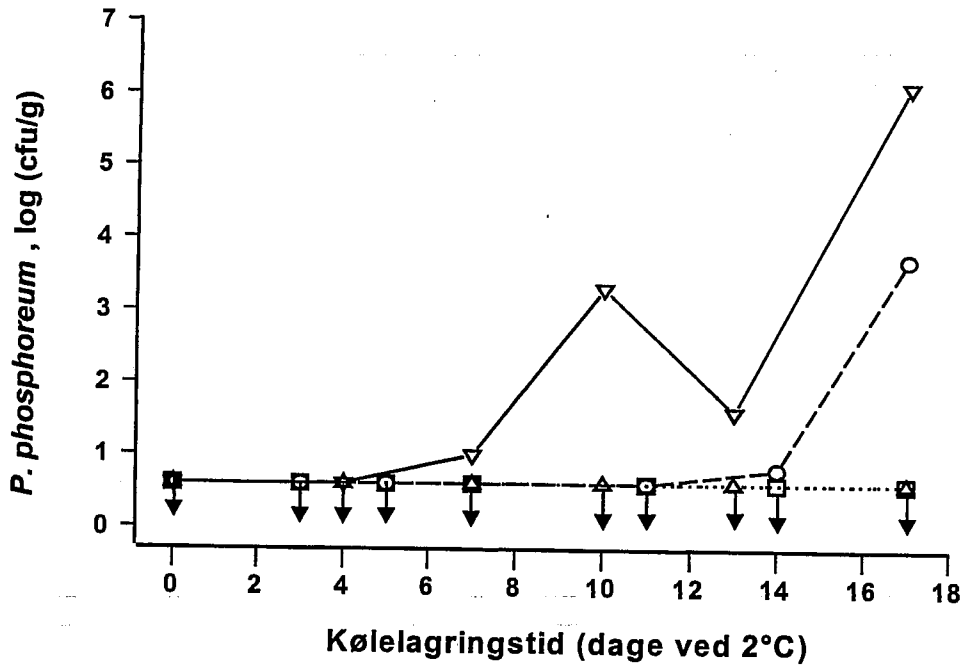
6. Indfrysning i pladefryser med en kernetemperatur på under –25°C.
7. Placering på fryselager ved –30°C og under transport ikke over –20°C.
8. Maksimal fryselagringstid på 12 måneder før salg.

Den foreslåede produktionspraksis betyder, at der går mindre end 6 timer fra fangsten hives ombord til de frosne torskefileter er placeret på trawlerens fryselager. Denne tid- og temperaturbelastning på råvaren før indfrysning skal sammenlignes med den traditionelle landbaserede produktion af frosne torskefileter, hvor råvaren enten er ferske isede torsk opbevaret ofte adskillige dage på is eller optøede hovedkappede torsk.

Tidligere studier har vist store problemer m.h.t. misfarvning af søfrosne fileter (Burt et al., 1974; Jones, 1965a; Jones, 1969; Kelly, 1969a). I nærværende forsøg med søfrosne produkter blev der imidlertidig ikke observeret problemer med misfarvning af torskefileterne (Artikel I og IV). Dette kan formodentligt relateres til en forbedret fangst og fangstbehandling i den opstillede GMP for produktionen af søfrosne torskefileter. Denne GMP er udformet med baggrund i studier og observationer ombord på nyere frysetrawlere. Søfrosne torskefileter er som regel indfrosset før indtrædelse af *rigor mortis* ombord på frysetrawlerne. Forsøg hos DFU, Lyngby efter 3 måneders fryselagring af søfrosne torskefileter viste ligeledes, at over halvdelen af de søfrosne torskefileter havde potentiale udfra indholdet af ATP- og glycogen til at udvikle optøningsrigor (resultater ikke vist). I disse forsøg blev der dog ikke observeret optøningsrigor for søfrosne torskefileter i form af filetkrympning eller øget dryptab (Jones, 1965b; McDonald & Jones, 1976). Dette skyldes formentlig, at der er anvendt en relativ langsom optøning, som har minimeret effekten af optøningsrigor (Bito, 1986; McDonald & Jones, 1976).

6.2 Produktion af optøede kølede MAP torskefileter (Artikel II, III og IV)

Den første forsøgsrække med optøede kølede MAP torskefileter vedrører effekten af råvarekvalitet ved indfrysning (1 dag ved 0°C kontra 8 dage ved 0°C) og forskellige fryselagringstemperaturer (–20°C kontra –30°C) med en råvare fra Østersøen (Artikel II). Der blev kun observeret signifikant vækst for *P. phosphoreum* og TMA-dannelse under kølelagring ved 2°C for torskefileter lagret 8 dage ved 0°C før frysning ved –30°C (Figur 8).



Figur 8 Gennemsnitlig antal af *P. phosphoreum* i optøede MAP torskefileter kølelagret ved 2°C. □, kode A (1 dag i luft ved 0°C før frysning og –20°C i 6 uger); μ, kode B (1 dag i luft ved 0°C før frysning og –30°C i 6 uger; Δ, kode C (8 dage i luft ved 0°C før frysning og –20°C i 6 uger) og ∇, kode D (8 dage i luft ved 0°C før frysning og –30°C i 6 uger). Pilene indikerer under detektionsgrænsen (< 0.6 log cfu/g).

Inaktiveringen af *P. phosphoreum* under kølelagring ved 2°C efter en forudgående fryselagringstemperatur ved –20°C bekræfter et tidligere studie omhandlende optøede kølede MAP torskefileter (Guldager et al., 1998). Yderligere blev der for torskefileter lagret 8 dage ved 0°C før indfrysning sensorisk detekteret frysehussmag og -lugt i optøede kølede MAP torskefileter efter fryselagring ved både –20°C og –30°C (Artikel II). Dette viser helt klart nødvendigheden af at anvende en helt frisk råvare til produktion af optøede kølede MAP torskefileter med en kort tid- og temperaturbelastning før indfrysning af råvaren.

Den anden forsøgsrække med optøede kølede MAP torskefileter omfatter forskellige fryselagringsforløb og -tider med en råvare fra Østersøen (Artikel III). Efter 21 dage ved 2°C blev det fundet, at *P. phosphoreum* var blevet inaktiveret efter både 4 og 12 ugers fryselagring ved –20°C eller ved fluktuerende temperatur mellem –20°C og –30°C (Tabel 8). Ved –30°C fryselagring blev der efter både 4 og 12 ugers fryselagring ligeledes observeret en meget kraftig hæmning af *P. phosphoreum* under kølelagring ved 2°C. Som ventet blev der ikke

udviklet TMA under kølelagringen, da *P. phosphoreum* var inaktiveret eller væksten meget kraftigt hæmmet i alle koderne.

Tabel 8 Produktkarakteristika og kvalitetsparametre (gennemsnit±standardafvigelse) for optøede kølede MAP torskefileter (Østersøen) med forskellige fryselagringstemperaturer og -tider.

Fryselagringstemperatur (°C)	–20°C		–30°C		–20°C/–30°C	
	0 d[a]	21 d	0 d	21 d	0 d	21 d
4 ugers fryselagringstid[b]						
P. phosphoreum, log (cfu/g)	i.d.[d]	i.d.	i.d.	2.7±2.2	i.d.	i.d.
TVC, log (cfu/g)	4.4±0.1	6.8±0.3	4.3±0.3	7.8±0.5	4.1±0.3	7.5±0.1
TMA, mg TMA-N/100 g	3.1±0.1	0.7±0.2	2.3±2.0	i.d.	0.3±2.7	i.d.
Dryptab, %	2.9±1.8	13.2±2.1	2.9±1.3	13.5±1.0	3.4±1.5	13.7±2.3
12 ugers fryselagringstid[c]						
P. phosphoreum, log (cfu/g)	i.m.[e]	i.d.	i.m.	0.7±0.2	i.m.	i.d.
TVC, log (cfu/g)	i.m.	7.3±0.6	i.m.	7.7±0.1	i.m.	7.9±0.7
TMA, mg TMA-N/100 g	1.4±1.8	2.0±0.4	1.2±0.4	1.4±0.3	1.9±1.6	0.4±0.5
Dryptab, %	1.7±0.6	18.8±2.3	3.0±1.6	18.1±0.9	2.0±0.6	18.8±0.4

[a] d viser antal dage i MAP ved 2°C.
[b] Kølelagringstemperaturen var 2.6±1.1°C.
[c] Kølelagringstemperaturen var 2.5±0.8°C.
[d] i.d. indikerer ikke detekteret.
[e] i.m. indikerer ikke målt.

Dryptabet under kølelagringen var betydelig højere efter fryselagring i 12 uger sammenlignet med 4 uger. Det bemærkes, at der ikke blev observeret forskelle i dryptab for de tre opstillede fryselagringsforløb. Efter de første 8 dages kølelagring steg dryptabene fra ~11% til ~16% for henholdsvis 4 og 12 ugers forudgående fryselagring (Artikel III). Disse relativt høje dryptab under kølelagringen af optøede kølede MAP produkter betyder, at torsk fra Østersøen har en begrænset anvendelighed til produktion af optøede kølede MAP torskefileter.

Forsøg med optøede kølede MAP torsk fra Østersøen viste hverken effekt af MAP under fryselagring eller forskellige opstillede indfrysnings- og optøningsmetoder på væksten af *P. phosphoreum* og TMA-dannelsen (resultater ikke vist). Endvidere blev der ikke observeret effekter af O_2 (0% eller 20%) under kølelagring af optøede MAP torskefileter fra Barentshavet på vækst af *P. phosphoreum* og TMA-dannelse (resultater ikke vist).

I den tredje forsøgsrække med optøede kølede MAP torskefileter blev betydningen af fangstområde og kølelagringstemperatur undersøgt (Artikel III). Der observeres en kraftig

hæmning af *P. phosphoreum* og TMA-dannelse i optøede kølede MAP torsk fra Østersøen under kølelagring (Tabel 9), hvilket stemmer overens med tidligere studier (Guldager et al., 1998; Artikel II). I torsk fra Barentshavet blev der imidlertidig observeret en signifikant vækst af *P. phosphoreum* og TMA-dannelse under kølelagring som optøet kølet MAP produkt.

Tabel 9 Kvalitetsparametre (gennemsnit±standardafvigelse) for optøede kølede MAP torskefileter produceret med råvare fra henholdsvis Barentshavet og Østersøen.

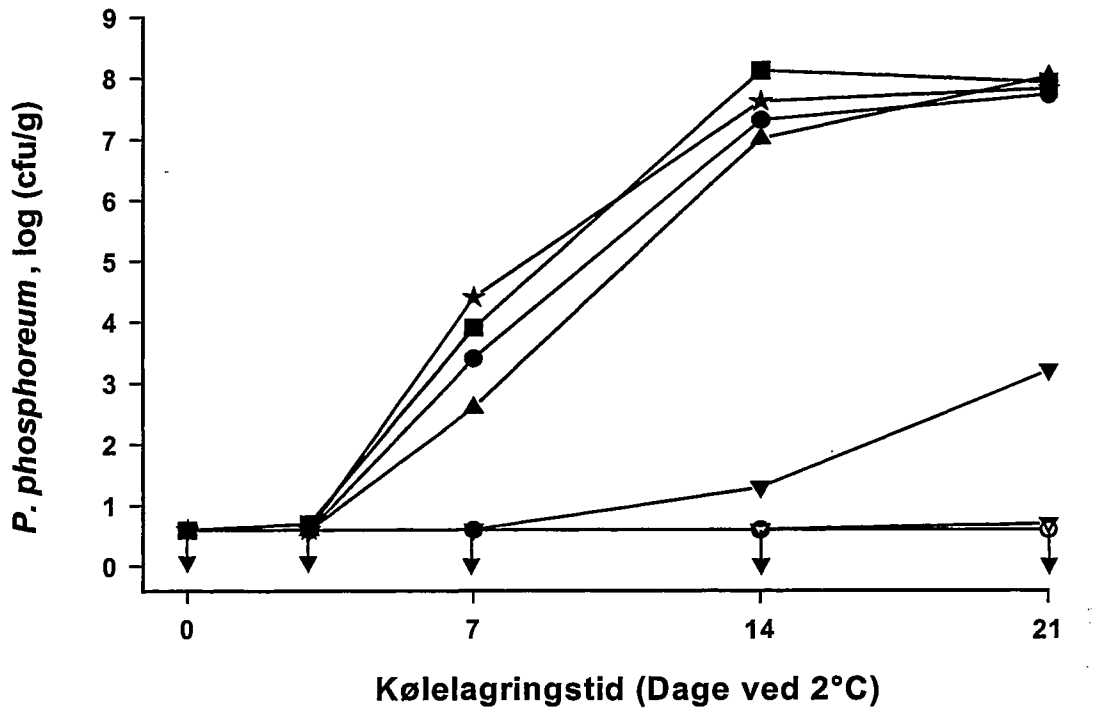
	2°C		5°C	
	0 dage	**21 dage**	**0 dage**	**14 dage**
Torsk fra Barentshavet[a]				
P. phosphoreum, log (cfu/g)	i.d.[c]	8.2±0.1	i.d.	8.1±0.1
TVC, log (cfu/g)	3.1±0.3	8.6±0.8	3.1±0.3	8.3±0.2
TMA, mg TMA-N/100 g	0.9±0.2	72.5±17.2	0.9±0.2	86.9±14.8
Dryptab, %	3.7±1.7	10.6±1.2	3.7±1.7	9.2±2.0
Torsk fra Østersøen[b]				
P. phosphoreum, log (cfu/g)	i.d.	2.7±1.8	i.d.	5.1±0.2
TVC, log (cfu/g)	6.0±0.2	8.2±0.7	6.0±0.2	9.2±0.3
TMA, mg TMA-N/100 g	i.d.	2.8±3.7	i.d.	40.6±10.2
Dryptab, %	5.6±0.1	12.2±0.8	5.6±0.1	11.3±2.5

[a] Produktkarakteristika for torsk fra Barentshavet; TMAO = (102.3±5.1 mg TMAO-N/100 g), NaCl = (0.47±0.04%), fryselagring = (15 uger ved –29.9±3.1°C).
[b] Produktkarakteristika for torsk fra Østersøen; TMAO = (43.8±7.5 mg TMAO-N/100 g), NaCl = (0.14±0.01%), fryselagring = (7 uger ved –26.8±0.6°C).
[c] i.d. indikerer ikke detekteret.

P. phosphoreum i torsk fra Barentshavet inaktiveres dermed ikke under frysning ved –30°C og ved den efterfølgende kølelagring som optøet MAP produkt dannes der store mængder af TMA som observeret i fersk MAP torsk (Dalgaard, 1995b; Dalgaard et al., 1993). Det er ikke tidligere undersøgt, hvorfor *P. phosphoreum* beskyttes under fryselagringen af torsk fra Barentshavet. Søfrosne torskefileter fra Barentshavet indeholdt imidlertidig signifikant højere TMAO- og NaCl-indhold sammenlignet med torsk fra Østersøen (Artikel III). TMAO-indholdet i torsk varierer med havdybden, saliniteten og vandtemperaturen (Gillet et al., 1997; Hebard et al., 1982; Shewan, 1951) og dette kan være årsag til det høje TMAO-indhold i torsk fra Barentshavet (Oehlenschläger, 1998). Det højere saltindhold i søfrosne torskefileter fra Barentshavet skyldes sandsynligvis anvendelse af havvand under forarbejdningen ombord på frysetrawleren med tilhørende saltoptag i torskefileterne. Der blev observeret generelt mindre dryptab under kølelagringen af optøede kølede MAP torskefileter med råvare fra Barentshavet sammenlignet med Østersøen. Dette kan skyldes, at et højere TMAO-indhold virker

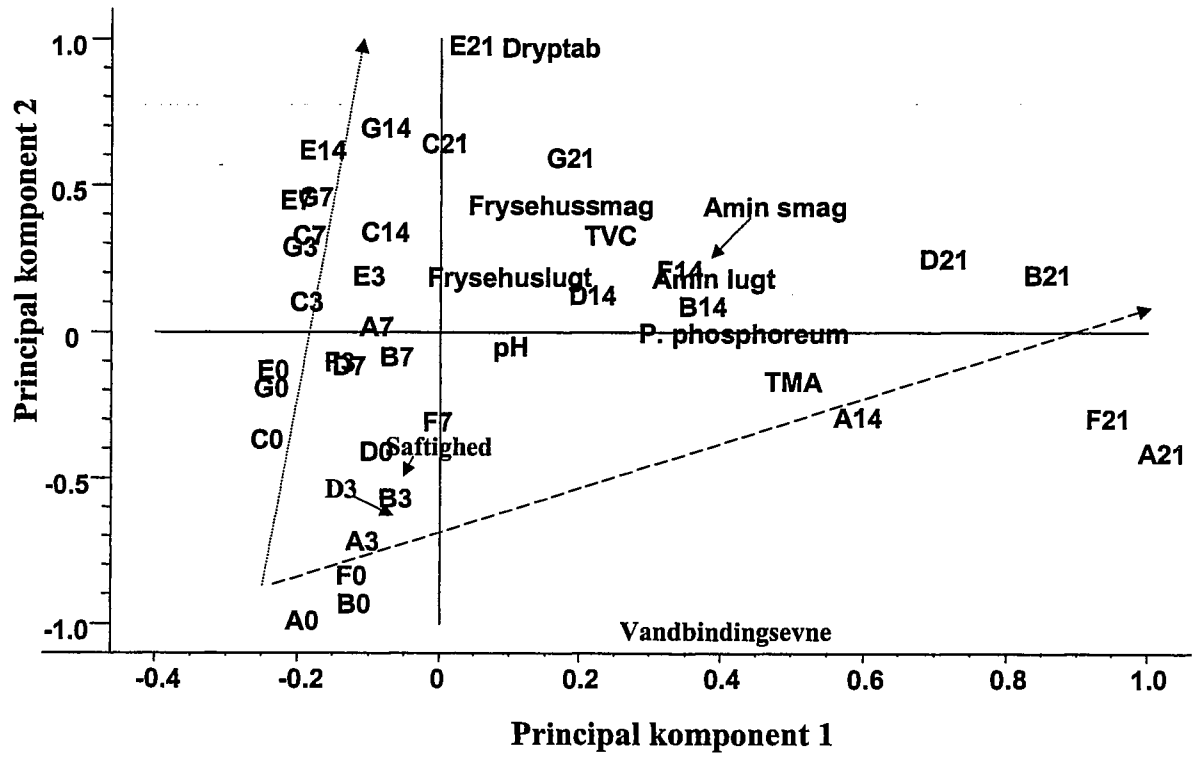
beskyttende mod proteindenaturering (Owuzu-Ansah & Hultin, 1984) under fryselagringen af torsk fra Barentshavet.

Den fjerde forsøgsrække vedrører udvælgelsen af de vigtigste procesparametre for opstilling af GMP til produktion af optøede kølede MAP torskefileter med råvare fra Barentshavet. I forsøget varieres følgende procesparametre: (i) fryselagringstemperatur (–20°C og –30°C), (ii) fryselagringstid (3, 6, 9 og 12 måneder) og (iii) kølelagring (0, 3, 7, 14 og 21 dage ved 2°C). Efter 14 dages kølelagring af optøede MAP torskefileter ved 2°C var antallet af *P. phosphoreum* omkring 10^7 cfu/g for alle koder fryselagret omkring –30°C (Figur 9). I modsætning til fryselagring ved –20°C, hvor *P. phosphoreum* blev hæmmet meget kraftigt under den efterfølgende kølelagring.



Figur 9 Gennemsnitlig antal *P. phosphoreum* i optøede MAP torskefileter fra Barentshavet efter forskellige fryselagrings- og kølelagringstider. ν, kode A (–30°C i 3 måneder); λ, kode B (–30°C i 6 måneder); τ, batch C (–20°C i 6 måneder); σ, batch D (–30°C i 9 måneder); ∇, batch E (–20°C i 9 måneder); H, batch F (–30°C i 12 måneder); O, batch G (–20°C i 12 måneder). Pilene indikerer under detektionsgrænsen (<0.6 log cfu/g).

Multivariabel dataanalyse blev anvendt til at klarlægge sammenhænge mellem de forskellige procesparametre. Det fremgår af bi-plottet fra PCA af mikrobiologiske, kemiske, fysiske og sensoriske målinger af optøede kølede MAP torskefileter (Figur 10), at den første principale komponent forklarer variationen i kølelagringstiden for de koder, der har været fryselageret ved –30°C (kode A, B, D og F). Disse koder er umiddelbart efter optøning (dag 0) placeret til venstre i bi-plottet og er karakteriseret ved en høj sensorisk saftighed og vandbindingsevne. Som kølelagringstiden øges, trækker koderne mod højre i bi-plottet efterhånden som aminlugt, aminsmag, TMA-dannelse samt vækst af *P. phosphoreum* bliver mere og mere dominerende. Koderne fryselagret ved –20°C (kode C, E og G) er derimod placeret til venstre i bi-plottet og har meget lave værdier for disse almindelige fordærvelseskarakteristika for ferske MAP torskefileter.



Figur 10 PCA på mikrobiologiske, kemiske, fysiske og sensoriske målinger af optøede kølede MAP torskefileter. Bi-plot fra principal komponent 1 versus principal komponent 2. Principal komponent 1 og 2 forklarede henholdsvis 60% og 19%, af den totale variation i datasættet. Bogstaver henviser til fryselagringstid og -temperaturer (se Tabel 7). Numrene indikerer antallet af kølelagringsdage. Pilene indikerer de to forskellige profiler for optøede MAP torskefileter relateret til forudgående fryselagringstemperatur ved henholdsvis –20°C (prikket pil) og –30°C (linieret pil).

Den anden principale komponent forklarer forskelle mellem de to anvendte fryselagringstemperaturer. Det kan ses, at koderne fryselagret ved –20°C (C, E og G) har større dryptab og lavere vandbindingsevne sammenlignet med koder fryselagret ved –30°C, efterhånden som kølelagringstiden øges.

Dryptabet under kølelagring af optøede MAP torskefileter var generelt lavere for torsk fra Barentshavet sammenlignet med Østersøen. Dryptabet var op til ~9% efter 21 dages kølelagring af optøede MAP torskefileter fra Barentshavet fryselagret i 3 måneder ved –30°C. Det skal sammenlignes med dryptab op til ~18% for torsk fra Østersøen med tilsvarende frysebetingelser. Ifølge disse resultater har torskene fra Barentshavet en højere fysisk kvalitet sammenlignet med torsk fra Østersøen.

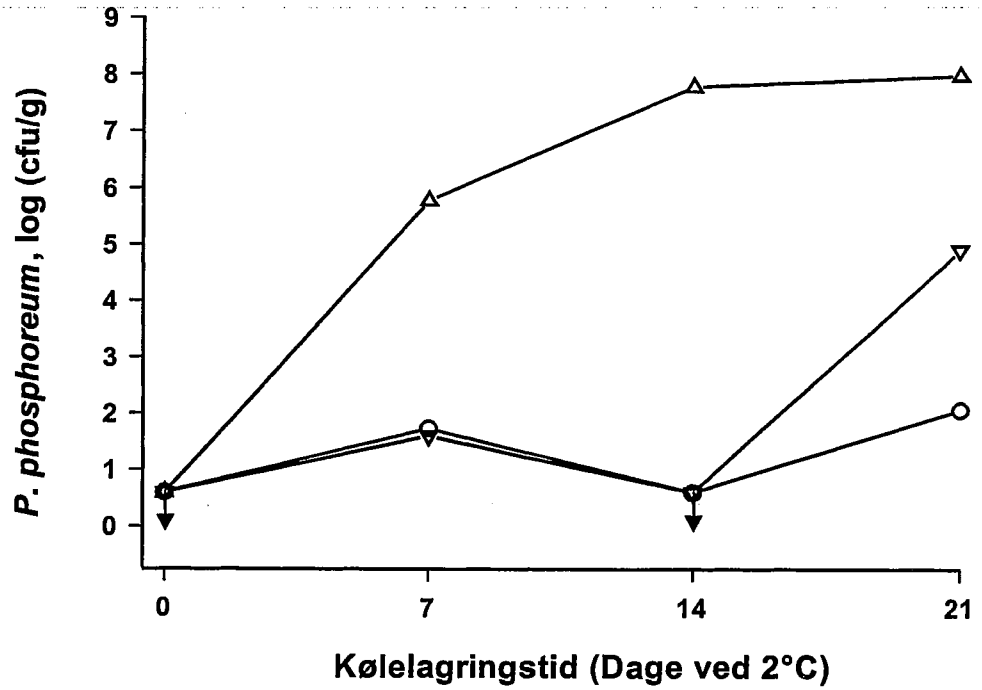
DMA-indholdet i optøede MAP torskefileter fra Barentshavet var under 1.5 mg DMA-N/100 g efter 21 dage ved 2°C for samtlige koder (Artikel IV). Et tidligere studie omhandlende fersklagring af torskefileter fra Barentshavet ved 0°C viser DMA-værdier omkring 2 mg DMA-N/100 g efter 18 dage (Oehlenschläger, 1998). Dette viser, at DMA-dannelsen ikke er en kritisk faktor ved afsætning af optøede kølede MAP torskefileter sammenlignet med ferske torskefileter, hvilket stemmer overens med et tidligere studie (Guldager et al., 1998).

Erfaringerne opnået ved gennemførelsen af de fire forsøgsrækker kan sammenfattes i følgende opstilling af GMP for produktion af optøede kølede MAP torskefileter:

1. Råvaren skal være søfrosne benfri interleaved-pakkede torskefileter fra Barentshavet, der er indfrosset mindre end 6 timer efter fangsten.
2. Fryselagring i op til 12 måneder ved –30°C med opbevaring mindst 3 måneder ved –20°C.
3. Opsavning af frosne benfri torskefileter og derefter detailpakning i modificeret atmosfære ($40\%CO_2/40\%N_2/20\%O_2$).
4. Transport enten som frosset MAP færdigvare eller som optøet kølet MAP produkt.
5. Langsom optøning (ved 5°C) og detailsalg ved 2°C, hvor der slet ikke udvikles aminlugt under 14 dages kølelagring.

6.3 Effekt af TMAO og NaCl for optøede kølede MAP torskefileter (Artikel III)

I dette forsøg blev effekten af TMAO og NaCl under fryselagring og kølelagring for optøede kølede MAP torskefileter undersøgt (Artikel III). Inaktivering af *P. phosphoreum* under frysning af torsk fra Østersøen blev modvirket ved at tilsætte TMAO og NaCl før indfrysning. Dette resulterede i højere vækst for *P. phosphoreum* og TMA-dannelse for koden tilsat TMAO og NaCl før indfrysning (Figur 11 og Tabel 10) med en observeret vækst for *P. phosphoreum* på 5.7 log cfu/g efter 7 dages kølelagring. Ved at anvende en vækstmodel (Dalgaard et al., 1997b) estimeres der en vækst for *P. phosphoreum* på 7.2 log cfu/g efter 7 dage ved 2.1°C på omtrent samme niveau som den behandlede kode. Det betyder, at de observerede forskelle mellem *P. phosphoreum* vækst og TMA-dannelse i optøede køede MAP torsk fra henholdsvis Østersøen og Barentshavet (Artikel III) formentlig kan relateres til forskelle i torskefileternes TMAO- og NaCl-indhold.



Figur 11 Gennemsnitlig antal af *Photobacterium phosphoreum* i optøede MAP torskefileter fra Østersøen lagret ved 2°C. Kode A (μ), optøede ubehandlede MAP torskefileter; kode B (Δ), optøede MAP torskefileter behandlet med 5% NaCl og 7% TMAO i fersk tilstand før indfrysning; kode C (∇), optøede MAP torskefilet behandlet med 5% NaCl og 7% TMAO efter frysning og optøning. Pilene indikerer ingen detektion af *P. phosphoreum* (<0.6 log cfu/g).

Det relativt lave TMAO- og NaCl-indhold i torsk fra Østersøen kan formodentligt forklare den fundne forlængelse af holdbarheden for optøede kølede MAP torskefileter fra Østersøen med forudgående fryselagring ved –30°C (Artikel II og III). Det vil derfor være usikkert om torsk fra nordatlantiske fangstområder også har længere holdbarheder som optøede kølede MAP produkter med anvendelse af forudgående fryselagring omkring –30°C.

Tabel 10 Effekt af TMAO og NaCl på TMA-dannelse (gennemsnit±standardafvigelse) i naturlig kontamineret optøede MAP torsk fra Østersøen under kølelagring ved 2.1±0.8°C. TMAO- og NaCl-indhold er begge målt ved kølelagringens begyndelse (dag 0).

Batch	TMAO (mg TMAO-N/100 g)	NaCl (%)	TMA (TMA-N/100g) 0 dage	7 dage	14 dage	21 dage
A[a]	61.1±6.6	0.08±0.03	i.d.[e]	1.6±0.9	1.8±0.6	2.5±0.1
B[b]	124.8±7.4[d]	0.42±0.05[d]	0.1±0.2	1.4±1.3	31.6±4.3	96.2±13.6
C[c]	134.8±12.4[d]	0.47±0.03[d]	i.d.	0.0±0.1	1.5±0.5	26.7±18.3

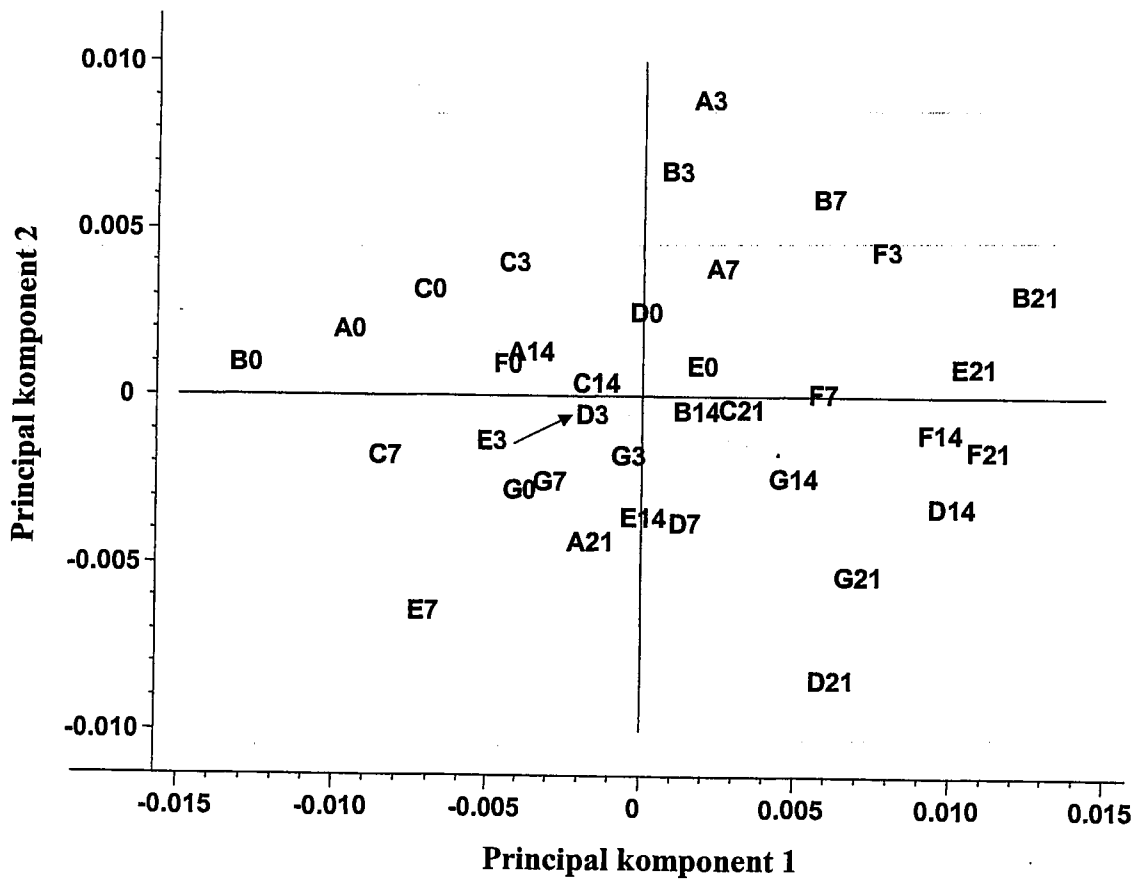
[a] Optøet ubehandlet MAP torskefilet.
[b] Optøet MAP torskefilet behandlet med 5% NaCl og 7% TMAO i fersk tilstand før indfrysning.
[c] Optøet MAP torskefilet behandlet med 5% NaCl og 7% TMAO efter fryselagring og optøning.
[d] Signifikant (P<0.001) højere TMAO og NaCl indhold for begge behandlede koder sammenlignet med den ubehandlede kode.
[e] i.d. indikerer ikke detekteret.

Den beskyttende effekt af TMAO er observeret i tidligere studier omhandlende proteiner og enzymer (Brown et al., 1997; Gillet et al., 1997; Lin og Timasheff, 1994; Timasheff, 1992; Yancey og Siebenaller, 1999; Yancey & Somero, 1979). Det er derfor sandsynligt, at TMAO stabiliserer celleproteinerne i *P. phosphoreum* og derved beskytter fordærvelsesbakterien under fryselagringen. TMAO og NaCl havde tilsyneladende en svag effekt på væksten for *P. phosphoreum* og TMA-dannelsen, når torskefileterne blev behandlet efter frysning og optøning. Dette kan skyldes effekten af NaCl, da væksten for *P. phosphoreum* er optimal ved ca. 1% (Dalgaard, 1993).

6.4 Kvalitet af optøede kølede MAP torskefileter med NIR (Artikel V)

Potentialet for at anvende NIR som en hurtigmetode til kvalitetsbedømmelse af optøede kølede MAP torskefileter blev undersøgt ved at anvende forsøgsopstillingen fra fjerde forsøgsrække (afsnit 6.2, side 36). NIR-målingerne blev udført på homogeniserede farsprøver (Artikel V) sideløbende med de øvrige fysiske, kemiske, sensoriske, mikrobiologiske

analyser. I den multivariable dataanalyse blev DPLSR først anvendt for at vise mulig kvalitativ information i NIR-spektrene m.h.t. til de valgte procesparametre. Det fremgår af scoresplottet fra DPLSR, at forskellen i kølelagringstid mellem 0 og 21 dage ved 2°C udstrækker variationen i første principale komponent (Artikel V). Koder med kort og lang kølelagringstid ligger henholdsvis til venstre og højre i scoresplottet (Figur 12). Endvidere observeres der ingen systematiske forskelle m.h.t. fryselagringstid og -temperatur. DPLSR-modellen viser, at optøede kølede MAP torskefileter kan klassificeres i klasser med 0, 3, 7, 14 og 21 dage ved 2°C (Figur 12). Dette indikerer, at NIR-spektrene indeholder noget information vedrørende kølelagringstiden for optøede MAP torskefileter.



Figur 12 Scoresplot af 1. principale komponent versus 2. principale komponent fra DPLSR-model med MSC behandlede NIR-spektre (reflektansdata) og alle tre design variable. Kode A (–30°C og 3 måneder), kode B (–30°C og 6 måneder), kode C (–20°C og 6 måneder), kode D (–30°C og 9 måneder), kode E (–20°C og 9 måneder), kode F (–30°C og 12 måneder) og kode G (–20°C og 12 måneder). Numrene indikerer kølelagringsperioden i dage ved 2°C.

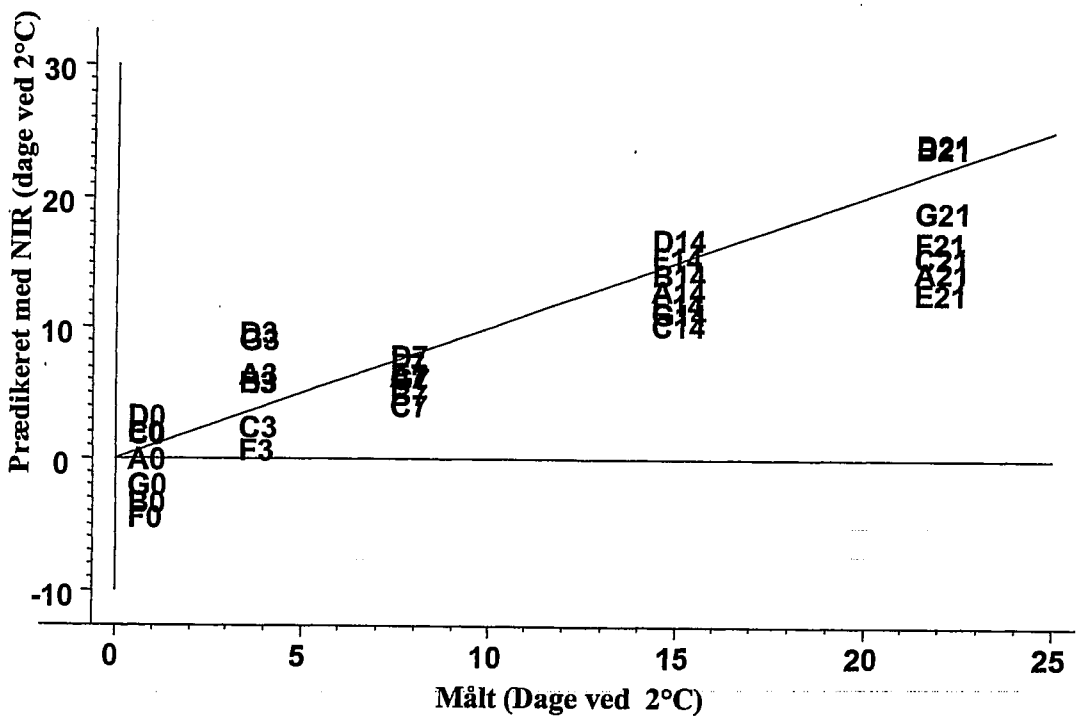
Til prædiktion af kølelagringstiden (dage ved 2°C) opstilles derfor kvantitative PLSR-modeller, som baseres på udglattede "multiple scatter correction" (MSC) behandlede NIR-spektre. Alle 35 koder anvendes til modellerne uafhængig af fryselagringstid og -temperatur. Antallet af bølgelængder reduceres til 230, og der anvendes en teknik til udglatning af NIR-data (Artikel V). Udvælgelsen af signifikante bølgelængder udføres v.h.a. en Jack-knifing metode (Martens & Martens, 1999).

Tabel 11 Validering af PLSR-modeller for prædiktion af kølelagringstid (dage ved 2°C) for optøede kølede MAP torskefileter med NIR med anvendelse af henholdsvis udglattede MSC behandlede NIR-spektre eller signifikante bølgelængder udvalgt med Jack-knifing.

X-values	Antal PLSR faktorer	RMSEPCV[a]	Korrelation koefficienter	% Y var. forklaret[b]
Fuld spektrum	4	4.9	0.77	61
Jack-knifing bølgelængder[c]	3	3.4	0.90	81

[a] "root mean square error prediction of cross validation" (RMSEPCV) er angivet i samme enhed som kølelagringstiden (dage ved 2°C).
[b] Varians i y-matricen forklaret af de respektive modeller.
[c] Signifikante bølgelængder udvalgt med Jack-knifing.

Den bedste PLSR-model gav en prædiktiv korrelations koefficient på 0.9 og en RMSEPCV på 3.4 dage (Tabel 11 og Figur 13). Prædiktionen af kølelagringstiden (dage ved 2°C) blev fundet mere eller mindre uafhængig af forudgående fryselagringstemperatur og -tid. Dette betyder, at NIR har et lovende potentiale til kvalitetsbedømmelse af optøede kølede MAP torskefileter. Der er observeret store forskelle i inaktiveringen af *P. phosphoreum* afhængig af fangstområde og fryselagringstemperatur (Artikel III og IV). Dette betyder, at fordærvelsesforløbet for optøede kølede MAP torskefileter er vidt forskellige med enten inaktivering af *P. phosphoreum* og dermed ingen TMA-dannelse eller en minimal hæmning af *P. phosphoreum* og TMA-dannelse som observeret for ferske MAP torskefileter (Dalgaard, 1995b; Dalgaard et al., 1993). Traditionelle bedømmelsesmetoder til ferske torskefileter som TVN, TMA og sensorik har derfor kun en begrænset anvendelighed til kvalitetsbestemmelse af optøede kølede MAP torskefileter.



Figur 13 Relationen mellem kølelagringstid ved 2°C for optøede MAP torskefileter (abscissen) og udglattede MSC behandlede NIR-spektre (ordinaten) med anvendelse af en PLSR-model (fuld krydsvalideret med 3 principale komponenter) baseret på signifikante Jack-knifing bølgelængder. Den fuld optrukne line viser y = x. Kode A (–30°C og 3 måneder), kode B (–30°C og 6 måneder), kode C (–20°C og 6 måneder), kode D (–30°C og 9 måneder), kode E (–20°C og 9 måneder), kode F (–30°C og 12 måneder) og kode G (–20°C og 12 måneder). Numrene viser kølelagringsperioden i dage ved 2°C.

Det skal dog bemærkes, at før NIR kan introduceres som en industriel hurtigmetode til kvalitetsbestemmelse af optøede kølede MAP torskefileter, er det nødvendigt at udvikle en større kalibreringsmodel, som indeholder biologiske variationer såsom årstid, fangstområde og torskestørrelse. Desuden er det oplagt at foretage NIR-målingen på hel torskefilet fremfor på fars således, at selve målingen bliver endnu hurtigere.

7. KONKLUSION

7.1 Konklusion

I nærværende afhandling undersøges potentialet for produktion af frosne højkvalitets torskeprodukter som et alternativ til de eksisterende frosne torskeprodukter på det danske marked. Endvidere undersøges potentialet for produktion af optøede kølede MAP torskefileter, som et nyt produkt til detailsalg i Danmark.

Nærværende forsøg med søfrosne torskeprodukter viste, at det var muligt at producere frosne højkvalitetsprodukter. Der blev opstillet en optimeret GMP forarbejdningskæde til produktion af interleaved-pakkede benfri torskefileter samt benfri torskeudskæringer ombord på frysetrawlere i Barentshavet. Dette betyder, at der i praksis kan produceres frosne højkvalitets torskeprodukter til både detail- og cateringmarkedet. Søfrosne torskeprodukter har derfor en mulighed for at erstatte en række af de torskeprodukter, med varierende kvalitet, som findes på det danske marked i dag. De eksisterende produkter er ofte dobbeltfrosne eller produceret med en "ældre" iset råvare. En afgørende forudsætning for at bibeholde den høje kvalitet for søfrosne torskeprodukter er at holde fryselagringstemperaturen så konstant som muligt og under –18°C i hele frysekæden (fra indfrysning ombord på frysetrawler til salg i detailhandlens frysemontre). Samtidigt bør deklarerede holdbarheder for frosne torskeprodukter nedsættes til 12 måneder.

Nærværende forsøg viste ligeledes, at afsætning af optøede kølede MAP torskefileter er et brugbart alternativ til detailsalg af ferske fiskeprodukter. Det er vist, at vækst af *P. phosphoreum* og TMA-dannelse under kølelagring var meget mere udtalt for optøede MAP torskefileter fra Barentshavet sammenlignet med Østersøen. Med anvendelse af torsk fra Østersøen er der observeret en meget kraftig hæmning af *P. phosphoreum* vækst og TMA-dannelse under kølelagring af torskefileter efter fryselagring ved (–20°C eller –30°C). Med anvendelse af torskefileter fra Barentshavet blev der fundet en kraftig *P. phosphoreum* vækst og TMA-dannelse under kølelagring med forudgående fryselagring ved –30°C. Hvorimod, der efter fryselagring af torskefileter fra Barentshavet i mindst 3 måneder (–20°C) blev observeret en kraftig hæmning af *P. phosphoreum* under efterfølgende kølelagring i MAP. Forsøgene viste dog klart højere dryptab under kølelagringen for optøede torskefileter fra Østersøen sammenlignet med råvare fra Barentshavet. Da dryptabet er en vigtig parameter

ved afsætning af fiskeprodukter, har torskefileter fra Østersøen en begrænset anvendelighed til produktion af optøede kølede produkter. Derfor anbefales, at anvende en søfrossen torskeråvare fra Barentshavet fryselagret i mindst 3 måneder ved –20°C til produktion af optøede kølede MAP torskefileter. Med dette set-up kan der produceres et detailprodukt, som inden for 14 dages kølelagring ved 2°C ikke udvikler aminlugt.

Søfrosne torskefileter fra Barentshavet havde et højere TMAO- og NaCl-indhold i torskefileter sammenlignet med Østersøen. Nærværende forsøg viste, at et højere TMAO- og NaCl-indhold i torskefileterne formentlig beskyttede *P. phosphoreum* under fryselagring ved –30°C, således at *P. phosphoreum* efterfølgende voksede frem i optøede MAP torskefileter som tidligere observeret i ferske MAP torskefileter.

De traditionelle bedømmelsesmetoder til ferske fiskeprodukter som TVN, TMA og sensorik har en begrænset anvendelighed til kvalitetsbedømmelse af optøede kølede MAP torskefileter, da disse produkter ikke viser samme fordærvelseskarakteristika. Der er observeret store forskelle i inaktiveringen af *P. phosphoreum* afhængig af fangstområde og fryselagringstemperatur. Nærværende forsøg viser et potentiale for anvendelse af NIR kombineret med multivariabel dataanalyse som en analysemetode til bestemmelse af kølelagringstid (dage ved 2°C) for optøede kølede MAP torskefileter med en prædiktiv korrelations koefficient på 0.9 og en RMSEPCV på 3.4 dage.

Ved afsætning af optøede kølede MAP torskefileter opnås der en meget højere fleksibilitet i produktionen m.h.t. vejr-, mængde-, sæson- og prismæssige faktorer sammenlignet med produktion af ferske MAP torskefileter. Det bliver dermed lettere at styre produktion/logistik af detailpakkede kølede fiskeprodukter sammenlignet med nuværende praksis for produktion og afsætning af ferske MAP torskefileter. Fordelen er, at der opnås en bedre forsyningssikkerhed for råvaren sammenlignet med produktion med fersk råvare til MAP fiskeprodukter. Ved anvendelse af frossen råvare til produktion af MAP fiskeprodukter, kan detailpakningerne evt. først optøs i forretningerne. Dermed vil tid- og temperaturbelastningen på kølelagrede fiskeprodukter til detailsalg blive minimeret betragteligt sammenlignet med den nuværende praksis ved afsætning af ferske MAP fiskeprodukter i dag.

7.2 Fremtidige forsøg

I fremtidige forsøg vil det være vigtigt at undersøge potentialet for minimering af et forholdsvis højt dryptab for optøede kølede MAP torskefileter med tilsætning af eksempelvis fosfater etc. til torskefileterne. Desuden kan der anvendes absorberende bakker således, at de optøede produkter ser indbydende ud i detailpakningerne.

Der bør ligeledes laves forsøg vedrørende effekten af korttidsfrysning ved –20°C for torskefileter fra Barentshavet med henblik på inaktivering af *P. phosphoreum* og tilhørende reduktion i TMA-dannelsen. Desuden bør de separate effekter af højere TMAO- og NaCl-indhold i torskefileter undersøges med henblik på at klarlægge inaktiveringen af *P. phosphoreum* for fryselagrede torskefileter.

Desuden ligger der fremtidige forsøg med videreudvikling af NIR som en målemetode til kvalitetsbestemmelse af optøede kølede MAP torskefileter. I den forbindelse bør der opbygges en større kalibreringsmodel, hvor der bl.a. anvendes mere sofistikerede forbehandlingsteknikker til NIR-data. Ligeledes bør potentialet for at måle med NIR på hel torskefilet undersøges i fremtidige forsøg.

Endvidere vil det være nødvendigt via forbruger- og markedsundersøgelser i Danmark at klarlægge forbrugernes holding til køb af optøede kølede MAP torskefileter som erstatning for ferske torskefileter før produktet introduceres på markedet.

8. REFERENCER

Abe, H. & Okuma, E. (1991). Rigor-mortis progress of carp acclimated to different water temperatures. *Nippon Suisan Gakkaishi* **57**, 2095–2100.

Ackman, R.G. & Hardy, R. (1980). Fish lipids. In *Advances in fish science and technology* ed. Connell, J.J. London: Fishing News Books, pp. 86–111.

Ágústsson, I. & Strøm, A.R. (1981). Biosynthesis and turnover of trimethylamine oxide in the teleost cod, *Gadus morhua*. *J. Biolog. Chem.* **256**, 8045–8049.

Anonymous (1964). *Determination of formaldehyde in foods*. Nordic Comittee on the Food Analysis No. 54.

Anonymous (1971). *Cold storage of frozen fish*, Torry Advisory Note, No. 28. Aberdeen: Torry Research Station.

Anonymous (1995). *Parallel food testing in the European Union: Fish*. London: International Consumer Research & Testing.

Anonymous (1998). *FAO yearbook, fishery statistics, capture production*. Rome: Food and Agriculture Organization of the United Nations.

Anonymous (1999). *Konsumlandinger i Danmark*. København: Fiskeridirektoratet, Ministeriet for Fødevarer, Landbrug og Fiskeri.

Anthoni, U., Børresen, T., Christophersen, C., Gram, L. & Nielsen, P.H. (1990). Is trimethylamine oxide a reliable indicator for the marine origin of fish ? *Comp. Biochem. Physiol.* **97B**, 569–571.

AOAC (1995). Salt (chlorine as sodium chloride) in seafood. In *Official methods of analysis of AOAC International*, Official Method No. 937.09, Sect. 35.1.18. 16th edit. Arlington: Association of Official Analytical Chemists.

Ashie, I.N.A., Smith, J.P. & Simpson, B.K. (1996). Spoilage and shelf-life extension of fresh fish and shellfish. *Crit. Rev. Food Sci. Nutr.* **36**, 87–121.

Auborg, S.P. & Medina, I. (1999). Influence of storage time and temperature on lipid deterioration during cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) frozen storage. *J. Sci. Food Agric.* **79**, 1943–1948.

Aursand, M., Gribbestad, I., Skjetne, T., Grasdalen, H. & Jørgensen, L. (1997). Assessment of lipid quality in salmon and trout by quantitative NMR techniques. In *Seafood from producer to consumer, integrated approach to quality* eds. Luten, J.B., Børresen, T. & Oehlenschläger, J. Amsterdam: Elsevier, pp. 367–378.

Barroso, M., Careche, M., Barrios, L. & Borderias, A.J. (1998). Frozen hake fillets quality as related to texture and viscosity by mechanical methods. *J. Food Sci.* **63**, 793–796.

Baumann, P. & Baumann, L. (1981). The marine gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In *The Prokaryotes* eds. Starr, M.P., Stolp, H., Truper, H.G., Balows, A. & Schlegel H.G. Berlin: Springer Verlag, pp. 1302–1330.

Bechmann, I.E. (1998a). Comparison of the formaldehyd content found in boiled and raw mince of frozen saithe using different analytical methods. *Food Sci. Technol.* **31**, 449–453.

Bechmann, I.E. (1998b). *Multivariate methods for the assessment of quality parameters for frozen fish*, Ph.D. Thesis. Lyngby: Danish Institute of Fisheries Research, Department of Seafood Research.

Bechmann, I.E., Jensen, H.S., Bøknæs, N., Warm, K. & Nielsen, J. (1998). Prediction of chemical, physical and sensory data from process parameters for frozen cod using multivariate analysis. *J. Sci. Food Agric.* **78**, 329–336.

Bechmann, I.E. & Jørgensen, B.M. (1998). Rapid assessment of quality parameters for frozen cod using near infrared spectroscopy. *J. Food Technol.* **31**, 648–652.

Bito, M. (1986). The influence of freshness of the fish, freezing temperature, thawing rate and thawing temperature on thaw rigor. *Bull. Tokai Reg. Fish. Res. Lab.* **119**, 25–31.

Botta, J.R. (1995). *Evaluation of seafood freshness quality*. New York: VCH Publishers.

Botta, J.R., Kennedy, K. & Squires, B.E. (1987). Effect of method of catching and time of season on sensory quality of fresh raw atlantic cod (*Gadus morhua*). *J. Food Sci.* **52**, 928–931, 938.

Botta, J.R., Squires, B.E. & Johnson, J. (1986). Effect of bleeding/gutting procedures on the sensory quality of fresh raw atlantic cod (*Gadus morhua*). *Can. Inst. Food Sci. Technol. J.* **19**, 186–190.

Bremner, H.A. (1985). Estimating time-temperature effects by a rapid systematic sensory method. In *Seafood quality determination* eds. Kramer, D.E. & Liston, J. Amsterdam: Elsevier, pp. 59–70.

Bremner, H.A. (1998). If freshnesss is lost, where does it go? In *Methods to determine the freshness of fish in research and industry* eds. Ólafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E. & Heia, K. Paris: International Institute of Refrigeration, pp. 297–305.

Bremner, H.A. (1999). Gaping in fish flesh. In *Extracellular matrix of fish and shellfish* eds. Sato, K., Sakaguchi, M. & Bremner, H.A. Trivandrum: Research Signpost, pp. 81–94.

Bremner, H.A., Olley, J. & Vail, A.M.A. (1987). A convenient easy-to-use system for estimating the quality of chilled seafood. *Fish Proces. Bull.* **7**, 59–70.

Bro, R. (1996). *Håndbog i multivariabel kalibrering* (på dansk). København: Mejeri- og Levnedsmiddelinstituttet, KVL.

Brown, C.R., Hong-Brown, L.Q. & Welch, W.J. (1997). Correcting temperature-sensitive protein folding defects. *J. Clin. Inv.*, **99**, 1432–1444.

Burt, J.R., Dreosti, G.M., Jones, N.R., Kelman, J.H., McDonald, I., Murray, J. & Simmonds, C.K. (1974). Technological investigations into cape hake II. Freezing at sea. *J. Food Technol.* **9**, 235–245.

Cann, D.C., Smith, G.L. & Houston, N.C. (1984). *Further studies on marine fish stored under modified atmosphere packaging*. Aberdeen: Torry Research Station.

Cappeln, G. & Jessen, F. (2000). ATP and glycogen content related to gaping in *pre rigor* cod (*Gadus morhua*) frozen in blocks at sea. *J. Food Aquat. Prod. Technol.* (under trykning).

Cappeln, G., Nielsen, J. & Jessen, F. (1999). Synthesis and degradation of adenosine triphosphate in cod (*Gadus morhua*) at subzero temperatures. *J. Sci. Food Agric.* **79**, 1099–1104.

Careche, M., Mazo, M.L.D., Torrejon, P. & Tejada, M. (1998). Importance of frozen storage temperature in the type of aggregation of myofibrillar proteins in cod (*Gadus morhua*) fillets. *J. Agric. Food Chem.* **46**, 1539–1546.

Castell, C.H. (1971). Some fundamental problems in the quality assessment of fishery products. In *Fish inspection and quality control* ed. Kreuzer R. London: Fishing News Books, pp. 9–13.

Castell, C.H., Neal, W.E. & Dale, J. (1973). Comparison of changes in trimethylamine, and extractable protein in iced and frozen gadoid fillets. *J. Fish. Res. Board Canada* **30**, 1246–1248.

Chou, C.C., Cheng, S.J., Wang, Y.C. & Chung, K.T. (1999). Behaviour of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in tryptic soy broth subjected to various low temperature treatments. *Food Res. Int.* **32**, 1–6.

Church, N. (1998). MAP fish and crustaceans – sensory enhancement. *Food Sci. Technol. Today* **12**, 73–83.

Connell, J.J. (1969). Changes in the eating quality of frozen stored cod and associated chemical and physical changes. In *Freezing and irradiation of fish* ed. Kreuzer, R. London: Fishing News Books, pp. 323–338.

Connell, J.J. (1980). *Advances in fish science and technology*. London: Fishing News Books.

Connell, J.J. & Howgate, P.F. (1968). Sensory and objective measurements of the quality of frozen stored cod of different initial freshnesses. *J. Sci. Food Agric.* **19**, 342–354.

Connell J.J. & Shewan, J.M. (1980) Sensory and non-sensory assessment of fish. In *Advances in fish science and technology* ed. Connell, J.J. Farnham: Fishing News Books, pp. 56–65.

Conway, E.J. & Byrne, A. (1933). An absorption apparatus for the micro-determination of certain volatile substances. *Biochem. J.* **27**, 419–429.

Coyne, F.P. (1932). The effect of carbon dioxide on bacterial growth with special reference to the preservation of fish. Part I. *J. Soc. Chem. Ind.* **51**, 119T–121T.

Dalgaard, P. (1993). *Evaluation and prediction of microbial fish spoilage*, Ph.D. Thesis. Lyngby: Technological Laboratory, Ministry of Fisheries, Technical University.

Dalgaard, P. (1995a). The effect of anaerobic conditions and carbon dioxide. In *Quality and quality changes in fresh fish* ed. Huss, H.H. Rome: FAO, pp. 78–83.

Dalgaard, P. (1995b). Qualitative and quantitative characterization of spoilage bacteria from packed fish. *Int. J. Food Microbiol.* **26**, 319–333.

Dalgaard, P. (1998). *Photobacterium phosphorerum* – a microbial parameter for prediction of remaining shelf life in MAP cod fillets. In *Methods to determine the freshness of fish in research and industry* eds. Ólafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E. & Heia, K. Paris: International Institute of Refrigeration, pp. 166–174.

Dalgaard, P. (2000). Fresh and lightly preserved seafood, In *Shelf life evaluation of foods* eds. Man, C.M.D. & Jones, A.A. Maryland: Aspen Publishers, pp. 110–139.

Dalgaard, P., Gram, L. & Huss, H.H. (1993). Spoilage and shelf-life of cod fillets packed in vacuum or modified atmospheres. *Int. J. Food Microbiol.* **19**, 283–294.

Dalgaard, P. & Huss, H.H. (1994). *Mathematical modelling used for evaluation and prediction of microbial fish spoilage.* Paper presented at the 37th annual conference of the Canadian Institute of Food Science and Technology (CIFST). New Developments in Seafood Science and Technology Symposium, 11–14 May. Vancouver: CIFST, pp. 73–89.

Dalgaard, P., Mejlholm, O. & Huss, H.H. (1996). Conductance method for quantitative determination of *Photobacterium phosphoreum* in fish products. *J. Appl. Bacteriol.* **81**, 57–64.

Dalgaard, P., Mejlholm, O., Christiansen, T.J. & Huss, H.H. (1997a). Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. *Lett. Appl. Microbiol.* **24**, 373–378.

Dalgaard, P., Mejlholm, O. & Huss, H.H. (1997b). Application of an iterative approach for development of a microbial model predicting the shelf-life of packed fish. *Int. J. Food Microbiol.* **38**, 169–179.

Dalgaard, P., Munoz, L.G. & Mejlholm, O. (1998). Specific inhibition of *Photobacterium phosphoreum* extends the shelf life of modified-atmosphere-packed cod fillets. *J. Food Prot.* **61**, 1191–1194.

Daniels, J.A., Krishnamurthi, R & Rizvi, S.S.H. (1985). A review of effects of carbondioxide on microbial growth and food quality. *J. Food Prot.* **48**, 532–537.

Davies, R. & Obafemi, A. (1985). Response of micro-organisms to freeze-thaw stress. In *Microbiology of frozen foods* ed. Robinson, R.K. pp. 83–107. London: Elsevier.

Davis, H.K. (1993). Fish. In *Principles and applications of modified atmosphere packaging of foods* ed. Parry, R.T. London: Blackie Academic, pp. 189–228.

Debevere, J. & Boskou, G. (1996). Effect of modified atmosphere packaging on the TVB/TMA-producing microflora of cod fillets. *Int. J. Food Microbiol.* **31**, 221–229.

Dixon, N.M. & Kell, D.B. (1989). The inhibition by CO_2 of the growth and metabolism of micro-organisms. *J. Appl. Bacteriol.* **67**, 109–136.

Dore, I. (1989). *The new frozen seafood handbook, a complete reference for the seafood business*. New York: Van Nostrand Reinhold.

Dyer, W.J., Fraser, D.I., Ellis, D.G. & Idler, D.R. (1962). Quality changes in stored refrozen cod fillets. *Bull. Int. Froid* **1**, 515–524.

Dyer, W.J. & Peters, J. (1969). Factors influencing quality changes during frozen storage and distribution of frozen products, include glazing, coating and packaging. In *Freezing and irradiation of fish* ed. Kreuzer, R. London: Fishing News Books, pp. 317–322.

Dykes, G.A. (2000). The effect of freezing on the survival of *Escherichia coli* O157:H7 on beef trimmings. *Food Res. Int.* **33**, 387–392.

Ehrenberg, A.S.C. & Shewan, J.M. (1953). The objective approach to sensory tests of food. *J. Sci. Food Agric.* **6**, 207–217.

Eide, O., Børresen, T. & Strøm, O. (1982). Minced fish production from capelin (*Mallotus villosus*). A new method for gutting, skinning and removal of fat from fatty species. *J. Food Sci.* **47**, 347–349, 354.

Esaiassen, M. & Akse, L. (1997). *Lagring av torskefilet pakket i modifisert atmosfære, sammenlignende forsøg med fersk og tint filet*, Rapport No. 21 (på norsk). Tromsø: Fiskeriforskning.

Esbensen, K. (1994). *Multivariate analysis – in practice*. Trondheim: Camo.

Farber, J.M. (1991). Microbiological aspects of modified-atmosphere packaging technology – a review. *J. Food Prot.* **54**, 58–70.

Fujii, T., Kurihara, K. & Okuzumi, M. (1994). Viability and histamine decarboxylase activity of halophelic histamine-forming bacteria during frozen storage. *J. Food Prot.* **57**, 611–613, 942.

Georgala, D.L. & Hurst, A. (1963). The survival of food poisoning bacteria in frozen foods. *J. Appl. Bact.* **26**, 346–358.

Giannini, D.H., Ciarlo, A.S., Boeri, R.L. & Almandos, M.E. (1993). Relationship between texture and some objective indices in frozen blocks of argentine hake (*Merluccius hubbsi*) fillets. *Food Sci. Technol.* **26**, 111–115.

Gill, C.D. & Molin, G. (1991). Modified atmospheres and vacuum packaging. In *Food preservatives* eds. Russell, N.J. & Gould, G.W. London: Blackie, pp. 172–199.

Gill, T.A. (1990). Objective analysis of seafood quality. *Food Rev. Int.* **6**, 681–714.

Gill, T.A., Keith, R.A. & Smith Lall, B. (1979). Textural deterioration of red hake and haddock muscle in frozen storage as related to chemical parameters and changes in the myofibrillar proteins. *J. Food Sci.* **44**, 661–667.

Gillett, M.B., Suko, J.R., Santoso, F.O. & Yancey, P.H. (1997). Elevated levels of trimethylamine oxide in muscles of deep-sea gadiform teleosts: A high-pressure adaption ? *J. Exper. Zoo.*, **279**, 386–391.

Girard, B. & Nakai, S. (1993). Species differentiation by multivariate analysis of headspace volatile patterns from canned pacific salmon. *J. Aquat. Food Prod. Technol.* **2**, 51–68.

Gram, L., Oundo, J.O. & Bon, J. (1989). Storage life of Nile perch (*Lates niloticius*) in relation to temperature and initial bacterial load. *Trop. Sci.* **29**, 221–236.

Gram, L., Trolle, G. & Huss, H.H. (1987). Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. *Int. J. Food Microbiol.* **4**, 65–72.

Golden, D.A., Beuchat, L.R. & Brackett, R.E. (1988). Inactivation and injury of *Listeria monocytogenes* as affected by heating and freezing. *Food Microbiol.* **5**, 17–23.

Guldager, H.S. (2000). *Personlig kommunikation*. Lyngby: Danish Institute for Fisheries Research, Department of Seafood Research.

Guldager, H.S., Bøknæs, N., Østerberg, C., Nielsen, J. & Dalgaard, P. (1998). Thawed cod fillets spoil less rapidly than unfrozen fillets when stored under modified atmosphere at 2°C. *J. Food Prot.* **61**, 1129–1136.

Haard, N.F. (1992a). Biochemical reactions in fish muscle during frozen storage. In *Seafood science and technology* ed. Bligh, E.G. London: Fishing News Books, pp. 176–209.

Haard, N.F. (1992b). Technological aspects of extending prime quality of seafood: a review. *J. Aquat. Food Prod. Technol.* **1**, 9–27.

Hardy, R., McGill, A.S. & Gunstone, F.D. (1979). Lipid and autooxidative changes in cold stored cod (*Gadus morhua*). *J. Sci. Food Agric.* **30**, 999–1006.

Hebard, C.E., Flick, G.J. & Martin, R.E. (1982). Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In *Chemistry and biochemistry of marine food products* eds. Martin, R.E., Flick, G.J., Hebard, C.E. & Ward, D.R. Westport: AVI, pp. 149–304.

Heen, E. & Karsti, O. (1965). Fish and shellfish freezing. In *Fish as food, vol. 4, Processing, part 2* ed. Borgstrom, G. New York: Academic Press, pp. 355–418.

Herborg, L. (1986). Can frozen fish be sold as fresh. *Infofish Marketing* **2**, 37–38.

Hintlian, C.B. & Hotchkiss, J.H. (1986). The safety of modified atmosphere packaging. A review. *Food Technol.* **40**, 70–76.

Horimoto, Y. & Nakai, S. (1998). Classification of pasteurized milk using principal component similarity analysis of off-flavours. *Food Res. Int.* **31**, 279–287.

Howell, N., Shavila, Y., Grootveld, M. & Williams, S. (1996). High-resolution NMR and magnetic resonance imaging (MRI) studies on fresh and frozen cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). *J. Sci. Food Agric.* **72**, 49–56.

Howgate, P. (2000). *Personlig kommunikation.* London: Institute of Food Science & Technology.

Howgate, P., Johnston, A. & Whittle, K. (1992). *Multilingual guide to EC freshness grades for fishery products.* Aberdeen: Torry Research Station.

Hurling, R. & McArthur, H. (1996). Thawing, refreezing and frozen storage effects on muscle functionality and sensory attributes of frozen cod (*Gadus morhua*). *J. Food Sci.* **61**, 1289–1296.

Hwang, G., Ushio, H, Watabe, S., Iwamoto, M. & Hashimoto, K. (1991). The effect of thermal acclimation on *rigor mortis* progress of carp stored at different temperatures. *Nippon Suisan Gakkaishi* **57**, 541–548.

Hyldig, G. & Nielsen, J. (1998). A rapid sensory method for quality management. In *Methods to determine the freshness of fish in research and industry* eds. Ólafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E. & Heia, K. Paris: International Institute of Refrigeration, pp. 297–305.

Jacobsen, C. (1999). *Oxidation mechanisms in fish oil enriched emulsions*, Ph.D. Thesis. Lyngby: Technical University of Denmark.

Jacobsen, C., Hartvigsen, K., Lund, P., Meyer, A.S., Adler-Nissen, J., Holstborg, J. & Hølmer, G. (1999). Oxidation in fish-oil-enriched mayonnaise. 1. Assessment of propyl gallate as an oxidant by discriminant partial least squares regression. *Eur. Food Res. Technol.* **210**, 13–30.

Jason, A.C. (1992). Thawing. In *Fish handling and processing* eds. Aitken, A., Mackie, I.M., Merritt, J.H. & Windsor, H.M. Edinburgh: H.M. Stationary Office, pp. 88–97.

Jay, J.M. (1991). Low-temperature food preservation and characteristics of psychrotrophic microorganisms. In *Modern food microbiology*. New York: Chapman & Hall, pp. 314–334.

Jensen, H.S. & Jørgensen, B.M. (1997). A sensometric approach to cod-quality measurement. *Food Qual. Prefer.* **8**, 403–407.

Jepsen, S.M., Pedersen, H.T. & Engelsen, S.B. (1999). Application of chemometrics to low-field ^{1}H NMR relaxation data of intact fish flesh. *J. Sci. Food Agric.* **79**, 1793–1802.

Jones, N.R. (1965a). Freezing fillets at sea. In *Fish quality at sea, the official proceedings of the conference on the design of fishing vessels and their equipment in relation to the improvement of quality, London May 31 and June 1 in conjunction with the World Fishing Exhibition*. London: Grampain Press, pp. 81–95.

Jones, N.R. (1965b). Problems associated with freezing very fresh fish. In *Fish handling and preservation: proceedings at a meeting on Fish Technology, Scheveningen, September 1964.* Paris: OECD, pp. 31–55.

Jones, N.R. (1969). Fish as a raw material for freezing: factors influencing the quality of products frozen at sea. In *Freezing and irradiation of fish* ed. Kreuzer, R. London: Fishing News Books, pp. 31–38.

Jónsdóttir, S. (1992). Quality index method and TQM-system. In *Quality issues in the fish industry* eds. Ólafsson, R. & Ingphórsson, A.H. Reykjavik: The Research Liasion Office, University of Iceland, pp. 81–94.

Josephson, D.B., Lindsay, R.C. & Stuiber, D.A. (1985). Effect of handling and packaging on the quality of frozen whitefish. *J. Food Sci.* **50**, 1–4.

Juhl, H.J. & Poulsen, S. (1999). Fersk fisk – kvalitet i detailhandelen. Resultater af en markedsundersøgelse af forbrugernes opfattelse og anvendelse af fersk fisk. *Fødevarekvalitet fra A til Ø* **1**, 36–39.

Jørgensen, B.R. & Huss, H.H. (1989). Growth and activity of *Shewanella putrefaciens* isolated from spoiling fish. *Int. J. Food Microbiol.* **9**, 51–62.

Jørgensen, B.M. & Jensen, H.S. (1997). Can near-infrared spectroscopy be used to measure quality attributes in frozen cod ? In *Seafood from producer to consumer, integrated approach to quality* eds. Luten, J.B., Børresen, T. & Oehlenschläger, J. Amsterdam: Elsevier, pp. 491–496.

Kelly, T.R. (1969a). Discolouration in sea-frozen fish fillets. In *Freezing and irradiation of fish* ed. Kreuzer, R. London: Fishing News Books, pp. 64–67.

Kelly, K.O. (1969b). Factors affecting the texture of frozen fish. In *Freezing and irradiation of fish* ed. Kreuzer, R. London: Fishing News Books, pp. 339–341.

Kim, J.-B., Murata, M. & Sakaguchi, M. (1987). A method for the differentiation of frozen-thawed from unfrozen fish fillets by a combination of Torrymeter readings and K values. *Nippon Suisan Gakkaishi* **53**, 159–164.

Kim, Y.J. & Heldman, D.R. (1985). Quantitative analysis of texture change in cod muscle during frozen storage. *J. Food Process Eng.* **7**, 265–272.

Kitamikado, M., Yuan, C.-S. & Ueno, R. (1990). An enzymatic method designed to differentiate between fresh and frozen-thawed fish. *J. Food Sci.* **55**, 74–76.

Lanier, T.C. & Korhonen, R. (1981). Modified atmosphere and conventional packaging of fresh and previously-frozen fish. In *Proceedings first national conference on modified and controlled atmosphere packaging of seafood products in San Antonio* ed. Martin, R.E. Washington DC: The National Fisheries Institute, pp. 80–97.

Larsen, E., Heldbo, J., Mosby, C. & Nielsen, J. (1992). Development of a method for quality assessment of fish for human consumption based on sensory evaluation. In *Quality assurance in the fish industry* eds. Huss, H.H, Jakobsen, M. & Liston, J. Amsterdam: Elsevier, pp. 351–358.

Lavety, J. (1991). Physio-chemical problems associated with fish freezing. In *Food freezing, today and tomorrow* ed. Bald, W.B. London: Springer, pp. 123–131.

Leblanc, E.L., Leblanc, R.J. & Blum, I.E. (1988). Prediction of quality in frozen cod (*Gadus morhua*) fillets. *J. Food Sci.* **53**, 328–340.

Licciardello, J.J. & D'Entremont, D.L. (1987). Bacterial growth rate in iced fresh or frozen-thawed atlantic cod, *Gadus morhua. Mar. Fish. Rev.* **49**, 43–45.

Lin, T.-Y. & Timasheff, S.N. (1994). Why do some organisms use a urea-methylamine as osmolyte ? Thermodynamic compensation of urea and trimethylamine N-oxide interactions with proteins. *Biochem.* **33**, 12695–12701.

Liston, J. (1980). Microbiology in fishery science. In *Advances in fish science and technology* ed. J.J. Connell. London: Fishing News Books, pp. 138–157.

Love, R.M. (1962). Protein denaturation in frozen fish. VI.-Cold storage studies on cod using the cell fragility method. *J. Sci. Food Agric.* **13**, 269–278.

Love, R.M. (1966). Bending, shrinkage and texture of cod frozen at sea. *J. Food Technol.* **1**, 137–140.

Love, R.M. (1975). Variability in atlantic cod (*Gadus morhua*) from the northeast atlantic: a review of seasonal and environmental influences on various attributes of the flesh. *J. Fish. Res. Board Canada* **32**, 2333–2342.

Love, R.M. (1980). *The chemical biology of fishes with a supplementary key to the chemical literature*. London: Academic Press.

Love, R.M. (1988). *The food fishes, their intrinsic and practical implications*. London: Farrand Press.

Love, R.M. (1992). Biochemical dynamics and the quality of fresh and frozen fish. In *Fish processing technology* ed. Hall, G.M. New York: Chapman & Hall, pp. 1–30.

Love, R.M., Hardy, R. & Nishimito, J. (1975). Lipids in the flesh of cod (*Gadus morhua* L.) from Faroe Bank and Aberdeen Bank in early summer and autumn. *Mem. Fac. Fish., Kagoshima Univ.* **24**, 123–126.

Love, R.M., Lavety, J. & Steel, P.J. (1969). The connective tissues of fish. II. Gaping in commercial species of frozen fish in relation to rigor mortis. *J. Food Technol.* **4**, 39–44.

Luten, J. & Martinsdóttir, E. (1998). QIM: a European tool for fish freshness evaluation in the fishery chain. In *Methods to determine the freshness of fish in research and industry* eds. Ólafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E. & Heia, K. Paris: International Institute of Refrigeration, pp. 287–296.

MacCallum, W.A., Jaffray, J.I., Chalker, D.A. & Idler, D.R. (1969) Condition of Newfoundland offshore fish and its influence on quality after single and double freezing. In *Freezing and irradiation of fish* ed. Kreuzer, R. London: Fishing News Books, pp. 56–63.

Mackie, I.M. (1974). Decomposition of trimethylamine oxide during iced and frozen-storage of whole and comminuted tissue of fish. *Proc. IV Int. Congress Food Sci. and Technol.* **1**, 243–250.

Mackie, I.M. (1993). The effects of freezing on flesh proteins. *Food Rev. Int.* **9**, 575–610.

Mackie, I.M., Howgate, P., Craig, A., Laird, W.M. & Ritchie, A.H. (1986). Acceptability of frozen stored consumer fish products. *Rev. Int. Froid* **9**, 169–173.

Magnússen, H. & Martinsdóttir, E. (1995). Storage quality of fresh and frozen-thawed fish in ice. *J. Food Sci.* **60**, 273–278.

Manthey, M. (1988). Gaschromatographische bestimmung von dimethyl- und trimethylamin in fisch und fischerzeugnissen. *Inf. Fischwirtsch.* **35**, 131–135.

Martens, M. (1986). Sensory and chemical quality criteria of stored versus not-stored frozen peas studied by multivariate data analysis. In *The shelf life of foods and beverages* ed. Charalamboos G. Amsterdam: Elsevier, pp. 775–790.

Martens, M. & Jørgensen, B.M. (1998). Multivariate data analysis used for investigation of the sensory quality of fish. In *Methods to determine the freshness of fish in research and industry* eds. Ólafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E. & Heia, K. Paris: International Institute of Refrigeration, pp. 325–332.

Martens, M. & Martens, H. (1986). Partial least squares regression. In *Statistical procedures in food research* ed. Pigott, J.R. New York: Elsevier, pp. 293–359.

Martens, H. & Martens, M. (1999). Modified Jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). *Food Qual. Pref.* **11**, 5–16.

Martens, H. & Næs, T. (1989). *Multivariate calibration.* Chichester: John Wiley & Sons.

Matsumoto, J.J. (1980). Chemical deteriorations of muscle proteins during frozen storage. In *Chemical deterioration of proteins* ed. Whitaker, J.R. Washington D.C.: American Chemical Society, pp. 95–124.

McDonald, I. & Jones, N.R. (1976). Control of thaw rigor by manipulation of temperature in cold store. *J. Food Technol.* **11**, 69–71.

McGill, A.S., Hardy, R. & Burt, J.R. (1974). Hept-*cis*-4-enal and its contribution to the off-flavour in cold stored cod. *J. Sci. Food Agric.* **25**, 1477–1489.

McGill, A.S., Hardy, R. & Gunstone, F.D. (1977). Further analysis of the volatile components of frozen cold stored cod and the influence of these on flavour. *J. Sci. Food Agric.* **28**, 200–205.

Meilgaard, M., Civile, G.V. & Carr, B.T. (1999). *Sensory evaluation techniques*, third edition. Bocan Raton: CRC Press.

Merritt, J.H. (1988). Freezing at sea. In *Refrigeration on fishing vessels*. Farnham: Fishing News Books, pp. 133–159.

Morrison, C.R. (1993). Fish and shellfish. In *Frozen food technology* ed. Mallet, C.P. London: Chapman & Hall, pp. 196–236.

Nash, T. (1953). The colorimetric estimation of formaldehyde by means of the Hantzch reaction. *Biochem. J.* **55**, 416–421.

Nielsen, J. (1998). Sensory analysis of fish. In *Methods to determine the freshness of fish in research and industry* eds. Ólafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E. & Heia, K. Paris: International Institute of Refrigeration, pp. 279–286.

Nielsen, J. & Jessen, K. (1997). New developments in sensory analysis for fish and fishery products. In *Seafood from producer to consumer, integrated approach to quality* eds. Luten, J.B., Børresen, T. & Oehlenschläger, J. Amsterdam: Elsevier, pp. 537–547.

Nielsen, N.A., Sørensen, E. & Grunert, K.G. (1997). Consumer motives for buying fresh or frozen plaice – a means-end chain approach. In *Seafood from producer to consumer, integrated approach to quality* eds. Luten, J.B., Børresen, T. & Oehlenschläger, J. Amsterdam: Elsevier, pp. 31–43.

Nilsson, K. & Ekstrand, B. (1994). Refreezing rate after glazing affects cod and rainbow trout muscle tissue. *J. Food Sci.* **59**, 797–798, 838.

Nott, K.P., Evans, S.D. & Hall, L.D. (1999). The effect of freeze-thawing on the magnetic resonance imaging parameters of cod and mackerel. *Food Sci.Technol.* **32**, 261–268.

Oehlenschläger, J. (1998). Detailed experimental iced-storage characteristics of Barents Sea cod (*Gadus morhua*). *Inf. Fischwirtsch.* **45**, 35–42.

Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I.M., Henehan, G., Nielsen, J. & Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. *Trends Food Sci. Technol.* **8**, 257–265.

Olley, J. & Lovern, J.A. (1960). Phospholipid hydrolysis in cod flesh stored at various temperatures. *J. Sci. Food Agric.* **11**, 644–652.

Olsen, K.B. (1986). European fish handling and holding methods. In *New directions in fisheries technology* ed. Roache, J.R. Ottawa: Department of Fisheries and Oceans, pp. 46–56.

Osborne, B.G., Fearn, T. & Hindle, P.H. (1993). *Practical NIR spectroscopy with applications in food and beverage analysis*, second edition. Harlow: Longman Scientific & Technical.

Owusu-Ansah, Y. & Hultin, H.O. (1984). Trimethylamine oxide prevents insolubilization of red hake muscle proteins during frozen storage. *J. Agric. Food Chem.* **32**, 1032–1035.

Peavey, S., Work, T. & Riley, J. (1994). Consumer attitudes toward fresh and frozen fish. *J. Aquat. Food Prod. Technol.* **3**, 71–87.

Pedrosa-Menabrito, A. & Regenstein, J.M. (1988). Shelf-life extension of fresh fish – a review part I – spoilage of fish. *J. Food Qual.* **11**, 117–127.

Pedrosa-Menabrito, A. & Regenstein, J.M. (1990a). Shelf-life extension of fresh fish – a review part II – preservation of fish. *J. Food Qual.* **13**, 129–146.

Pedrosa-Menabrito, A. & Regenstein, J.M. (1990b). Shelf-life extension of fresh fish – a review part III – fish quality and methods of assessment. *J. Food Qual.* **13**, 209–223.

Persson, P.O. & Löndahl, G. (1993). Freezing technology. In: *Frozen food technology*. London. Blackie Academic & professional, pp. 20–58.

Peters, J.A., MacCallum, W.A., Dyer, W.J., Idler, D.R., Slavin, J.W., Lane, J.P., Fraser, D.I. & Laishley, E.J. (1968). Effect of stage of *rigor* and of freezing-thawing processes on storage quality of refrozen cod. *J. Fish. Res. Board Canada* **25**, 299–320.

Pink, J., Naczk, M. & Pink, D. (1998). Evaluation of the quality of frozen minced red hake: use of Fourier transform infrared spectroscopy. *J. Agri. Food Chem.* **46**, 3667–3672.

Pink, J., Naczk, M. & Pink, D. (1999). Evaluation of the quality of frozen minced red hake: use of Fourier transform near-infrared spectroscopy. *J. Agri. Food Chem.* **47**, 4280–4284.

Pino, J.A., Marbot, R., Perez, J. & Villavicencio, M.N. (1999). Application of multivariate statistical methods to differentiation of rum aged in two types of oak barrels. *J. Food Qual.* **22**, 183–191.

Reddy, N.R., Armstrong, D.J., Rhodehamel, E.J. & Kautter, D.A. (1992). Shelf-life extension and safety concerns about fresh fishery products packaged under modified atmospheres: a review. *J. Food Saf.* **12**, 87–118.

Rehbein, H. (1979). Development of an enzymatic method to differentiate fresh and seafrozen and thawed fish fillets. I. Comparison of the applicability of some enzymes of fish muscle. *Z. Lebensm. Unters. Forsch.* **169**, 263–265.

Rehbein, H. (1988). Relevance of trimethylamine oxide demethylase and haemoglobin content to formaldehyde production and texture deterioration in frozen stored minced fish muscle. *J. Sci. Food Agric.* **43**, 261–276.

Rosenfeld, H.J., Baardseth, P. & Skrede, G. (1997). Evaluation of carrot varieties for production of deep fried carrot chips-IV. The influence of growing environment on carrot raw material. *Food Res. Int.* **30**, 611–618.

Ryan, J.J. (1979). The cod family and its utilization. *Mar. Fish. Rev.* **41**, 25–36.

Rørbæk, K. (1994). *Oxidation and flavours in fish oil*, Ph.D. Thesis. Lyngby, Technical University of Denmark.

Salfi, V., Fucetola, F. & Pannunzio, G. (1985). A micromethod for the differentiation of fresh from frozen fish muscle. *J. Sci. Food Agric.* **36**, 811–814.

Santos, E.E.M. & Regenstein, J.M. (1990). Effects of vacuum packaging, glazing, and erythorbic acid on the shelf-life of frozen white hake and mackerel. *J. Food Sci.* **55**, 64–70.

Shaw, P.E., Moshonas, M.G., Buslig, B.S., Barros, S.M. & Widmer, W.W. (1999). Discriminant and principal component analyses to classify commercial orange juices based on relative amounts of volatile constituents. *J. Sci. Food Agric.* **79**, 1949–1953.

Shenouda, S.Y.K. (1980). Theories of protein denaturation during frozen storage of fish flesh. In *Advances in food research* eds. Chichester, C.O., Mrak, E.M. & Stewart, G.F. New York: Academic Press, pp. 275–311.

Shewan, J.M. (1951). The chemistry and metabolism of the nitrogenous extractives in fish. In *The biochemistry of fish* ed. Williams, R.T. Cambridge: University Press, pp. 28–49.

Shewan, J.M. (1961). The microbiology of sea-water fishes. In *Fish as food, vol. 1* ed. Borgstrøm G. London: Academic Press, pp. 487–560.

Shewan, J.M., Macintosh, R.C., Tucker, C.G. & Ehrenberg, A.S.C. (1953). The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. *J. Sci. Food Agric.* **4**, 283–298.

Sigernes, F., Esaiassen, M., Heia, K., Wold, J.P., Eilertsen, G. & Sørensen, N.K. (1998). Assessment of fish (cod) freshness by VIS/NIR spectroscopy. In *Methods to determine the freshness of fish in research and industry* eds. Ólafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E. & Heia, K. Paris: International Institute of Refrigeration, pp. 279–286.

Sikorski, Z.E. & Kolakowska, A. (1994). Changes in proteins in frozen stored fish. In *Seafood proteins* eds. Sikorski, Z.E., Pan, B.S. & Shahidi, F. New York: Chapman & Hall, pp. 99–112.

Sikorski, Z., Olley, J. & Kostuch, S. (1976). Proteins changes in frozen fish. *Crit. Rev. Food Sci. Nutr.* **8**, 97–129.

Simmonds, C.K. & Lamprecht, E.C. (1980). Spoilage patterns of wet and thawed frozen cape hake during chilled storage. In *Advances in fish science and technology* ed. Connell, J.J. Farnham: Fishing News Books, pp. 417–421.

Simmonds, C.K. & Lamprecht, E.C. (1985). Microbiology of frozen fish and related products. In *Microbiology of frozen foods* ed. Robinson, R.K. London: Elsevier, pp. 169–208.

Sivertsen, H.K., Holen, B., Nicolaysen, F. & Risvik, E. (1999). Classification of french red wines according to their geographical origin by the use of multivariate analyses. *J. Sci. Food Agric.* **79**, 107–115.

Sollid, H. & Solberg (1992). Salmon fat content estimation by near infrared transmission spectroscopy. *J. Food Sci.* **57**, 792–793.

Stammen, K., Gerdes, D. & Caporaso, F. (1990). Modified atmosphere packaging of seafood. *Crit. Rev. Food Sci. Nutr.* **29**, 301–331.

Statham, J.A. (1984). Modified atmosphere storage of fisheries products: the state of the art. *Food Technol. Aust.* **36**, 233–239.

Straka, R.P. & Stokes, J.L. (1959). Metabolic injury to bacteria at low temperatures. *J. Bacteriol.* **78**, 181–185.

Stroud, G.D. (1969). *Rigor in fish. The effect on quality.* Torry Advisory Note, No. 36, pp. 3–11. Aberdeen: Torry Research Station.

Sørensen, N.K. (1986). *Dobbelfrysing av fisk. Litteraturstudie fra inn- og utland*, rapport fra Fiskeriteknologisk Forskningsinstitut (på norsk). Tromsø: FTFI.

Sørensen, N.K., Brataas, R., Nyvold, T.E. & Lauritzen, K. (1997). Influence of early processing (*pre-rigor*) on fish quality. In *Seafood from producer to consumer, integrated approach to quality* ed. Luten, J.B., Børresen, T. & Oehlenschläger, J. Amsterdam: Elsevier, pp. 253–263.

Sørensen, N.K., Gundersen, B., Nyvold, T.E. & Elsevoll, E. (1996). Evaluation of sensory quality during freezing and frozen storage of whole, gutted atlantic salmon (*salmo salar*). In *Refrigeration science and technology, proceedings*, 1996–2, pp. 307–315.

Thampuran, N. & Gopakumar, K. (1993). Freezing temperature and freezing menstruum on the survival of selected marine bacteria. *Fish. Technol.* **30**, 139–146.

Timasheff, S.N. (1992). A physicochemical basis for the selection of osmolytes by nature. In *Water and life: a comparative analysis of water relationships at the organismic, cellular, and molecular levels* eds. Somero, G.N., Osmond, C.B. & Bolis, C.L. Berlin: Springer-Verlag, pp. 70–84.

Timm, M. (2000). *Personlig kommunikation*. Lyngby: Danish Institute for Fisheries Research, Department of Seafood Research.

Tokunaga, T. (1970). Trimethylamine oxide and its decompostion in the bloody muscle of fish–I. TMAO, TMA and DMA contents in ordinary and bloody muscles. *Bull. Jpn. Soc. Sci. Fish.* **36**, 502–509.

Tokunaga, T. (1980). Biochemical and food scientific study on trimethylamine oxide and its related substances in marine fisheries. *Bull. Tokai Reg. Fish. Res. Lab.* ***101***, 1–129.

Valdimarsson, G., Matthiasson, A. & Stefansson, G. (1984) The effect of on board bleeding and gutting on the quality of fresh, quick frozen and salted products. In *Papers presented at the jubilee seminar of the Icelandic Fisheries Laboratories September 23–24* ed. Moller, A., Reykjavik: Icelandic Fisheries Laboratories, pp. 61–72.

van Spreekens, K.J.A. (1971). *Quality assessment of iced and prepacked cod fillets*, FAO Fish. Tech. paper No. 115. Rome: Food and Agriculture Organization of the United Nations, pp. 85–94.

van Spreekens, K.J.A. (1974). The suitability of a modification of Long and Hammer's medium for the enumeration of more fastidious bacteria from fishery products. *Arch. Lebensmittelhyg.* **25**, 213–219.

van Spreekens, K.J.A. (1987). Histamine production by the psychrophilic flora. In *Seafood quality determination* eds. Kramer, D.E. & Liston, J. Amsterdam: Elsevier, pp. 309–318.

Vyncke, W. (1983). Shelf life of thawed cod fillets kept in ice. *Z. Lebensm. Unters. Forsch.* **177**, 19–21.

Warm, K., Bøknæs, N. & Nielsen, J. (1998). Development of quality index methods for evaluation of frozen cod (*Gadus morhua*) and cod fillets. *J. Aquat. Food Product Technol.* **7**, 45–59.

Waterman, J.J. (1968). *The cod*, Torry Advisory Note, No. 33. Aberdeen: Torry Research Station, pp. 3–17.

Waterman, J.J. (1987). *Freezing fish at sea a history*. Edinburgh: H.M. Stationary Office.

Whitehead, P.J.P., Bauchot, M.-L., Hureau, J.-C., Nielsen, J. & Tortonese, E. (1986). Fishes of the north-eastern Atlantic and the Mediterranean. Paris: Unesco.

Wold, H. (1966). Estimation of principal components and related models by iterative least squares. In *Multivariate analysis* ed. Krishnaiah, P.R. Academic Press: New York.

Wold, J.P. & Isaksson, T. (1997). Non-destructive determination of fat and moisture in whole Atlantic salmon by near-infrared diffuse spectroscopy. *J. Food Sci.* **62**, 734–736.

Wold, J.P., Jakobsen, T. & Krane, L. (1996). Atlantic salmon average fat content estimated by near-infrared transmittance spectroscopy. *J. Food Sci.* **61**, 74–77.

Wold, S., Albano, C., Dunn III, W.J., Esbensen, K., Hellberg, S., Johansson, E. and Sjöström, M. (1983). Recognition: finding and using regularities in multivariate data. In *Food research and data analysis* eds. Martens, H. & Russwurm, H. London: Applied Science Publishers, pp. 147–188.

Wold, S., Esbensen, K, & Geladi, P. (1987). Principal component analysis. *Chem. Intel. Lab. Sys.* **2**, 37–52.

Yamada, K. & Amano, K. (1965). Studies on the biological formation of formaldehyde and dimethylamine in fish and shellfish. V: On the enzymatic formation in the pyloric caeca of alaska pollock. *Bull. Jpn. Soc. Sci. Fish.* **31**, 60–64.

Yancey, P.H. & Siebenaller, J.F. (1999). Trimethylamine oxide stabilizes teleost and mammalian lactate dehydrogenases against inactivation by hydrostatic pressure and trypsinolysis. *J. Experiment. Biol.* **202**, 3597–3603.

Yancey, P.H. & Somero, G. (1979). Counteraction of urea destabilization of protein structure by methylamine regulatory compounds of elasmobrach fishes. *Biochem. J.* **183**, 317–320.

Artikel I

Bøknæs, N., Guldager, H.S., Østerberg, C. & Nielsen, J. (2001). Production of high quality frozen cod (*Gadus morhua*) fillets and portions on a freezer trawler. *Journal of Aquatic Food Product Technology,* **10**, 33–47.

Production of High Quality Frozen Cod (*Gadus morhua*) Fillets and Portions on a Freezer Trawler

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ABSTRACT. Five batches of "frozen at sea" cod products processed on a freezer trawler in the Barents Sea were studied: (i) glazed portions, (ii) glazed vacuum packed portions, (iii) unglazed portions, (iv) interleaved packed fillets, and (v) double frozen cod fillets. All batches were produced from a single catch on a freezer trawler. Changes in product quality during frozen storage were evaluated by measuring water holding capacity, water loss on cooking and sensory quality indices of cooked samples. The glazed cod portions, glazed vacuum packed cod portions and interleaved packed cod fillets remained with high sensory and physical quality after 46 weeks of frozen storage. In contrast, unglazed cod portions and double frozen cod fillets developed cold

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storage odor and flavor coupled with very dry and fibrous texture during frozen storage. It was concluded the improved handling and processing technique used in this present study made it possible to produce high quality "frozen at sea" cod products from the Barents Sea on freezer trawlers over a storage period of 46 weeks. This provides the opportunity to introduce high quality "frozen at sea" cod products in place of the traditional single or double frozen cod products on the retail or catering markets.

KEYWORDS. Frozen at sea, cod portions, sensory evaluation, freezer trawler, double freezing

INTRODUCTION

In 1995, a European survey revealed that frozen fish products mostly were described as tasting neutral and insipid (Anon., 1995). An American investigation also showed negative consumer attitudes to the purchase of frozen fish products (Peavey et al., 1994). Frozen fish were associated with bad smell, tough and dry texture and inferior taste. Participants believed frozen fish were less nutritious and bonier than fresh (Peavey et al., 1994). Clearly new methods for production of frozen fish products of high consumer quality and value are required. The quality of frozen cod depends on biological and technological parameters during catching, processing and distribution. The primary biological parameters include fish, size, season of catch, sexual maturation and fishing grounds (Castell, 1971; Love, 1988). The primary technological parameters are catching methods (Botta et al., 1987), catch handling (Jones, 1965; Valdimarsson et al., 1984; Botta et al., 1987), freshness at the point of freezing, rate of freezing, temperature of cold storage, period of cold storage (Jones, 1969; Kelly, 1969; Burt et al., 1974; Haard, 1992; Sikorski and Kolakowska, 1994), double freezing (Dyer et al., 1962; Peters et al., 1968; MacCallum et al., 1969; Hurling and McArthur, 1996), packaging methods (Josephson et al., 1985) and thawing methods (Hurling and McArthur, 1996; Cappeln et al., 1999). During frozen storage, protein denaturation, enzymatic reactions and lipid oxidation take place in fish muscle. Protein denaturation is related to textural changes with dry and fibrous fish flesh after the frozen storage period (Shenouda, 1980). During frozen storage, TMAOase in gadoid fish causes enzymatic formation of formaldehyde and dimethylamine leading to an acceleration of protein denaturation of fish flesh (Castell, 1973; Rehbein, 1988). Development of oxida-

tive flavors and odors during frozen storage depends on frozen storage temperature, duration of frozen storage and biological factors like species of fish and season (McGill et al., 1974; McGill et al., 1977). As an alternative to traditional commercial frozen fish products manufactured ashore, "frozen at sea" products are recently being more widely distributed. "Frozen at sea" products are produced on freezer trawlers at sea where the fish are gutted, filleted and frozen in blocks in a pristine state within a short time after catch. Normally, manufacturing of "frozen at sea" products represented an improved handling and processing technique. For many years, "frozen at sea" products have been (i) interleaved packed fillet in blocks including various fillet products with pinbone in/pinbone out and skinless/skin-on, (ii) boneless fillet in blocks or (iii) headed and gutted whole fish in blocks (Merritt, 1988; Morrison, 1993). Interleaved packed cod fillets are particularly marketed for catering and further processing, primarily for European and American markets. The gutted and whole cod blocks are thawed at land-based plants and processed primarily for double frozen cod products. Recently, this has been a widespread processing method worldwide for producing commercial double frozen cod products. "Frozen at sea" technology (Jones, 1965; Jones, 1969; Kelly, 1969; Burt et al., 1974) may overcome several of the problems related to varied quality of frozen cod products. However, today it is not a common practice to produce frozen cod fillet portions on commercial freezer trawlers at sea.

The objectives of this present study were (i) to evaluate sensory attributes and physical properties during frozen storage of different traditional and new frozen cod products from a freezer trawler, and (ii) to study the potential for manufacturing "frozen at sea" cod portions on a freezer trawler. The sensory attributes and physical properties of the frozen cod products were assessed by several different methods including sensory evaluation, water holding capacity and water loss on cooking.

MATERIALS AND METHODS

Processing and Storage Characteristics

Five batches of frozen cod (*Gadus morhua*) products from the Greenlandic freezer trawler "Sisimiut" were studied: (i) glazed cod portions (batch A), (ii) glazed vacuum packed cod portions (batch B), (iii) unglazed cod portions (batch C), (iv) interleaved packed cod fillets (batch D), and (v) double frozen cod fillets (batch E). The cod were caught, processed and frozen on the Greenlandic freezer trawler "Sisimiut" in December 1996, in Russian zone (72°N, 43°E) at the Barents Sea. The tow duration was four hours and the

catch volume was about six metric ton, primarily cod. Cod from this single catch were used for all batches and represented optimal catch and on board handling.

Cod of 2-4 kg were headed and gutted immediately after catch using a Baader 424 machine. After bleeding in seawater with a temperature of 4°C for about 1/2 hour, the headed and gutted cod were filleted before *rigor mortis* using a Baader 190 filleting machine and skinned using a Baader 51 skinning machine. Interleaved packed cod fillets and headed and gutted whole cod were frozen in a horizontal plate freezer and packed in cardboard boxes. Cod fillets were cut by hand into loins and tails portions and individually quick-frozen (IQF) in a blast freezer until the core temperature reached −28°C (after 70 min). Frozen cod portions were packed in polyethylene bags and cardboard boxes. The five batches were placed in the cold store on "Sisimiut." Before storage on board, batch A and batch B were glazed by dipping portions once for 10 sec in 1°C freshwater and placed in cardboard boxes. The applied glazing of the portions constituted between 5% and 7% of portion weight with tail portions having greater percentage uptake than loins portions, because of a larger surface to volume ratio. Storage on "Sisimiut" lasted nine weeks and all frozen cod products were transported to a fish processor in Denmark and subsequently to DIFRES at the end of February 1997.

Headed and gutted whole cod (batch E) were industrially water thawed in batches by a processor in Denmark with an initial water temperature of 30°C equalized overnight to 0°C before filleting. Thawed cod were machine filleted and frozen again in a blast freezer as double frozen fillets and afterwards glazed with fresh water before frozen storage (processing of double frozen cod fillets only lasted a few hours). At the same time, cod portions from batch B were vacuum packed. Cod portions used in batches A, B and C were skinless and boneless. Cod fillets used in batch D were interleaved packed with skin-on and pinbone in. Batch A, B, C and D all represented single frozen cod products. Double frozen cod used in batch E were skinless fillets with pinbone in. The average weight of the cod fillets (batch D and E) was between 504 gram and 598 gram and of cod portions (batch A, B and C) between 117 gram and 143 gram. Samples from all the five batches were collected after 13, 26, 35 and 46 weeks of frozen storage. Cod fillets and portions were packed in polyethylene bags and thawed overnight in air at 5°C before assessing by sensory and physical analyses. Temperatures during transport and storage were recorded by loggers (Tinytag, Gemini Data Loggers, UK) placed between the cod portions in the cardboard boxes on "Sisimiut" until time for thawing of cod fillets and cod portions at DIFRES.

Physical Analyses

At each sampling period, the water holding capacity (WHC) was determined in quadruplicate as relative water loss during a mild centrifugation (1500 × g, 5 min, 10°C) of minced cod muscle (Eide et al., 1982). Seven cod fillets or portions from each batch were analyzed for WHC. The water loss on cooking (WLC) was determined as relative weight loss during steaming at 90°C in polyethylene bags for seven min. Seven cod fillets or portions from each batch were analyzed.

Sensory Evaluation and Statistical Analysis

Sensory evaluation of thawed cooked cod fillets was performed by a panel of 11 trained panelists using the Quality Index Method (QIM) for thawed cooked cod samples (Warm et al., 1998). Loins of cod fillets were packed in porcelain bowls, wrapped in foil and heated at 100°C for 20 min in a hot air oven and immediately served to the panelists. At every sampling time each panelist evaluated two fish pieces from each of the five batches. Samples were served in randomized order within duplicates, batches and with respect to each panelist.

The QIM method is based on a selected number of independent parameters describing the quality of the thawed cooked cod fillets. The sensory QIM method for assessing thawed cooked cod fillets describes the attributes: Odor, color, flavor and texture, which are evaluated on a score scale from 0 to 4, where 0 is the best. The scores for all parameters were added to give the total quality index, where 0 represented the quality of freshly caught, appropriately handled cod, and a quality index of 16 represented cod with the lowest sensory quality. In this present study, the working definition of high quality frozen cod fillets and portions is material that exhibited no freezer burn, is not discolored and after thawing and cooking, has a low QIM score (below 8), a characteristic neutral pleasant taste, a succulent and flaky texture, and has a high water holding capacity (above 70%). Conversely, low quality material may exhibit freezer burn, or be discolored, has a high QIM score (above 10), showed evidence of cold storage odor and flavor, has a dry and tough texture and a low water holding capacity (below 60%).

The sensory data obtained from the experiments were analyzed using the multivariate method Principal Component Analysis (PCA). PCA is a method for extracting the systematic variations in an understandable and clear form (Martens and Næs, 1989; Esbensen, 1994). In this study QIM responses averaged over duplicates and panelists were used in a PCA performed on a matrix with 20 objects (five batches and four frozen storage periods) and values for the five sensory variables: QIM, odor, color, flavor and texture. In a PCA, the data matrix is decomposed into two smaller matrices consisting of

scores and loadings (structure part) and residuals (noise part). In this way systematic variations are extracted by reducing the original number of variables (here the five QIM responses) to a smaller set of factors called principal components. The first principal component (PC1) explained the largest variance and PC2 the second largest variance, and so on (Martens and Næs, 1989). Sensory data were analyzed using Unscrambler® (Anon., 1996). The effects according to frozen storage periods and batches in this present study were determined using two-way analysis of variance. After finding significant effects of frozen storage period and batches, one-way analysis of variance and Tukey-Kramer multiple comparison test (Sokal and Rohlf, 1985) were further used to test the significant differences of frozen storage periods and batches, respectively. These statistical tests were done by using Graphpad Instat (Anon., 1993).

RESULTS

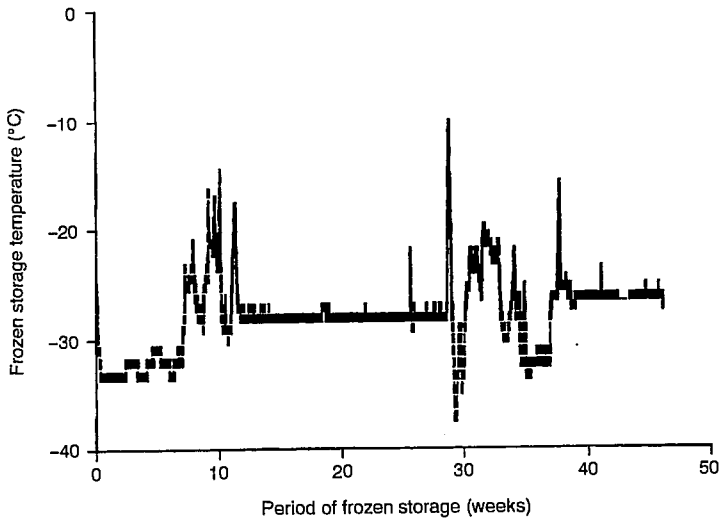
Processing and Storage Characteristics

By freezing on board in a horizontal plate freezer or individually quick-freezing (IQF) in a blast freezer, a core temperature of $-28°C$ was reached in less than two hours. Figure 1 shows the profile of temperature during frozen storage of all products from the time of freezing and during the 46 weeks of storage. Temperature fluctuated between $-11°C$ and $-38°C$ and average temperature was $-28.0°C \pm 3.2°C$ (S.D.). Constant low storage temperatures on the freezer trawler as well as the fluctuating and higher temperatures seen during transportation reflect practically obtainable conditions for distribution of "frozen at sea" cod products from catch to consumer.

Physical Analyses

Highest water loss on cooking (WLC) was observed for double frozen cod fillets during storage (Figure 2). Using two-way analysis of variance, effects of both storage periods and batches on WLC were significant ($p < 0.05$). A one-way analysis showed significant ($p < 0.05$) differences in WLC among batches of all 13, 26, 35 and 46 weeks of storage. Generally, higher values for WLC for double frozen cod fillets (batch E) compared with the other batches were observed at equal storage periods (Table 1 and Figure 2). Furthermore, one-way analysis and Tukey-Kramer multiple comparison test showed no significant ($p > 0.05$) differences in WLC for glazed cod portions (batch A) except between 13 and, respectively, 35 and 46 weeks of storage. Significant ($p < 0.05$) differences in WLC for both glazed vacuum packed portions

FIGURE 1. Profile of temperature during frozen storage of cod products over 46 weeks. Temperature profile for production of double frozen cod fillets (batch D) including extra thawing, processing and freezing was not shown in this figure.



(batch B) and unglazed portions (batch C) were found except between 13 to 26 weeks, and 35 to 46 weeks of storage. For interleaved packed cod fillets (batch D), no significant ($p > 0.05$) differences were found among the respective storage periods. For double frozen cod fillets (batch E), significant ($p < 0.05$) differences were found in WLC except comparing 13 to 26, 26 to 35 and 26 to 46 weeks of storage.

Lowest water holding capacity (WHC) was observed for double frozen cod fillets during storage (Figure 3). Using two-way analysis of variance, effects for both storage periods and batches on WHC were significant ($p < 0.05$). A one-way analysis showed significant ($p < 0.05$) differences in WHC among batches for all 13, 26, 35 and 46 weeks of storage. Overall significantly lower WHC for double frozen cod fillets (batch E) was observed (Table 1 and Figure 3). Furthermore, one-way analysis and Tukey-Kramer multiple comparison test showed significant ($p < 0.05$) differences in WHC for glazed portions (batch A), glazed vacuum packed portions (batch B) and interleaved packed cod fillets (batch D) except comparing storage of 13 and 26 weeks. Significant ($p < 0.05$) differences in WHC for unglazed portions (batch C) and double frozen cod fillets (batch E) were found except comparing storage of respective 13 to 26 weeks and 35 to 46 weeks.

FIGURE 2. Changes in water loss on cooking (%) for the five batches of frozen cod products during frozen storage of 46 weeks. ▼, glazed cod portions (batch A); ○, glazed vacuum packed cod portions (batch B); Δ, unglazed cod portions (batch C); ▲, interleaved packed cod fillets (batch D) and ▽, double frozen cod fillets (batch E). Each point is an average of seven measurements.

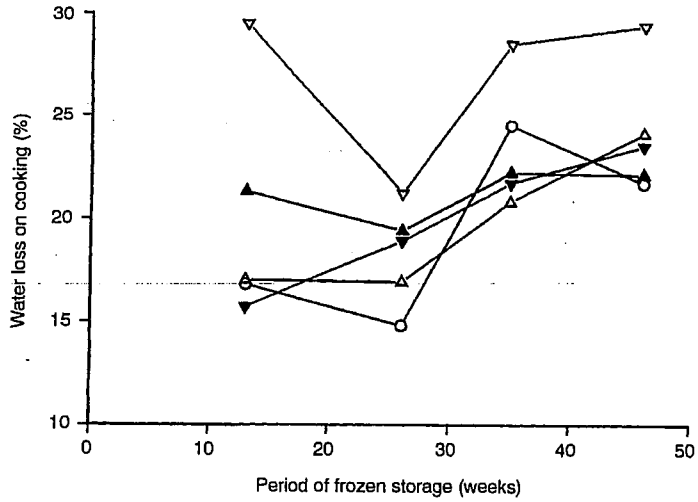
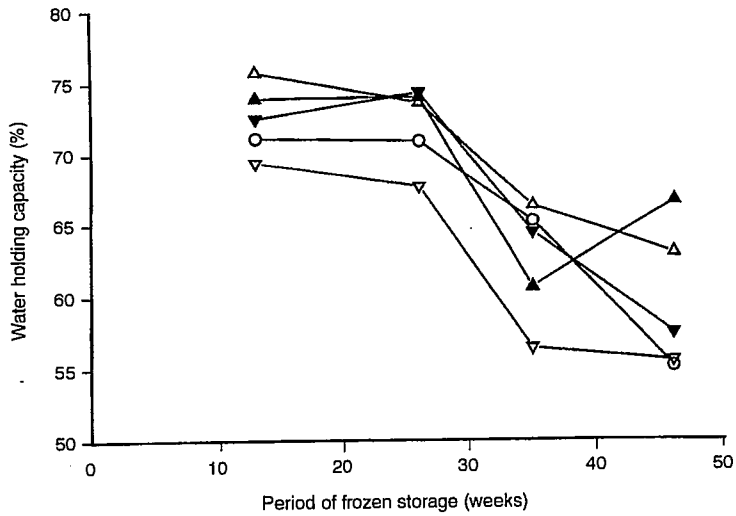


TABLE 1. Tukey-Kramer multiple comparison test for detecting differences between batches for water holding capacity (WHC) and water loss on cooking (WLC) at equal periods of frozen storage in weeks (wk). Glazed cod portions (batch A), glazed vacuum packed cod portions (batch B), unglazed cod portions (batch C), interleaved packed cod fillets (batch D), and double frozen cod fillets (batch E).

	Water loss on cooking (WLC)				Water holding capacity (WHC)			
Comparisons	**13 wk**	**26 wk**	**35 wk**	**46 wk**	**13 wk**	**26 wk**	**35 wk**	**46 wk**
Batch A *vs.* batch B	NS[a]	NS	NS	NS	NS	NS	NS	NS
Batch A *vs.* batch C	NS	NS	NS	NS	NS	NS	NS	S[b]
Batch A *vs.* batch D	S	NS	NS	NS	NS	NS	NS	S
Batch A *vs.* batch E	S	NS	S	S	NS	S	S	NS
Batch B *vs.* batch C	NS	NS	NS	NS	S	NS	NS	S
Batch B *vs.* batch D	NS	NS	NS	NS	NS	NS	S	S
Batch B *vs.* batch E	S	S	NS	S	NS	NS	S	NS
Batch C *vs.* batch D	NS	NS	NS	NS	NS	NS	S	NS
Batch C *vs.* batch E	S	NS	S	S	S	S	S	S
Batch D *vs.* batch E	S	NS	S	S	S	S	NS	S

[a] NS, no significant difference between batches using Tukey-Kramer multiple comparison test ($p > 0.05$).
[b] S, significant effect between batches using Tukey-Kramer multiple comparison test ($p < 0.05$).

FIGURE 3. Changes in water holding capacity (%) for the five batches of frozen cod products during frozen storage of 46 weeks. ▼, glazed cod portions (batch A); ○, glazed vacuum packed cod portions (batch B); Δ, unglazed cod portions (batch C); ▲, interleaved packed cod fillets (batch D) and ▽, double frozen cod fillets (batch E). Each point is an average of seven measurements.



Sensory Evaluation and Multivariate Analysis

After 13 weeks of storage all five batches had high sensory quality with neutral odor, sweet flavor, white color and succulent and flaky texture (Table 2). These desirable sensory properties remained longer for glazed portions (batch A), glazed vacuum packed portions (batch B) and interleaved packed fillets (batch D) than for unglazed portions (batch C) and double frozen fillets (batch E). A high degree of freezer burn on the unglazed portions occurred after 26 weeks of storage, causing a poor appearance. Cold storage odor and flavor was formed in the double frozen fillets and unglazed portions and they developed a very dry and fibrous texture. The raw flesh color of all batches was, however, assessed as being white with no blood marks throughout storage.

A bi-plot (Figure 4) of the scores characterizing samples and of the loadings characterizing sensory variables indicated that principal component 1 accounted for the variation due to storage period. Principal component 1 and principal component 2 explained 77% and 7%, respectively, of the total variation in the sensory data set. Samples with 13 weeks of storage were characterized by low QIM scores for all sensory parameters. This indicated

TABLE 2. Sensory characteristics during frozen storage of the five frozen cod products. Glazed cod portions (batch A), glazed vacuum packed cod portions (batch B), unglazed cod portions (batch C), interleaved packed cod fillets (batch D), and double frozen cod fillets (batch E) were stored for 46 weeks.

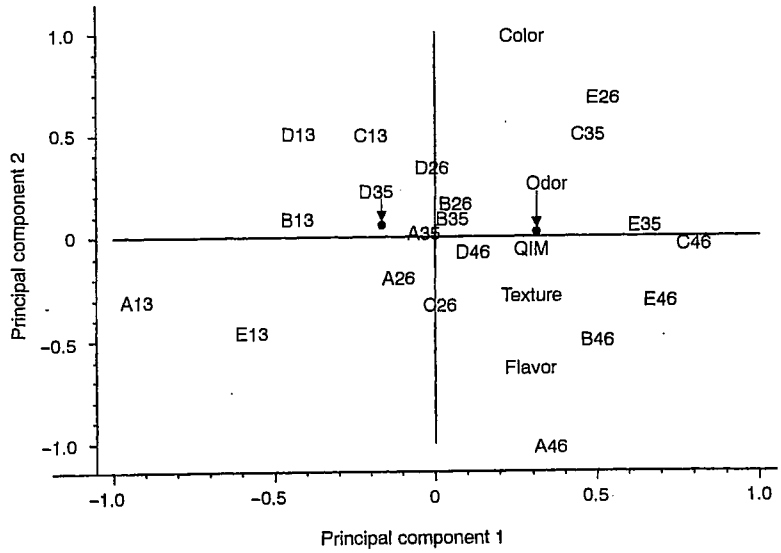
Period	Sensory characteristics
13 weeks	All batches: Neutral odor, sweet flavor, white color, succulent and flaky texture.
26 weeks	Batch A, B and D: Neutral odor and flavor, succulent and flaky texture.
	Batch C: Slightly cold storage odor and flavor, freezer burn, dry and fibrous texture.
	Batch E: Slightly cold storage odor and flavor, dry and fibrous texture.
35 weeks	Batch A, B and D: Neutral odor and flavor, succulent and flaky texture.
	Batch C: Slightly cold storage odor and flavor, freezer burn, dry and fibrous texture.
	Batch E: Slightly cold storage odor and flavor, dry and fibrous texture.
46 weeks	Batch A, B and D: Neutral odor and flavor, succulent texture.
	Batch C: Cold storage odor and flavor, freezer burn, very dry and fibrous texture.
	Batch E: Cold storage odor and flavor, very dry and fibrous texture.

that all batches had high sensory quality after 13 weeks of frozen storage. Double frozen cod fillets after 35 and 46 weeks of storage and unglazed cod portions after 46 weeks of storage were characterized by higher sensory QIM scores. This indicates that these samples had lower sensory quality compared to the other frozen products. After 13 weeks of storage, glazed cod portions were of highest sensory quality. However, after longer storage periods, glazed cod portions, glazed vacuum packed cod portions and interleaved packed fillets were placed near each other in the bi-plot indicating equal sensory quality (Table 2 and Figure 4).

DISCUSSION

This present study showed that high quality "frozen at sea" cod products can be produced from the Barents Sea on freezer trawlers having a high sensory and physical quality during a commercial frozen storage of 46 weeks. Glazed portions, glazed vacuum packed portions and interleaved packed fillets represented products of high sensory and physical quality during 46 weeks of frozen storage. The poorest texture was observed for the double frozen cod fillets that had a dry and fibrous texture (Table 2 and Figure 4). The significant textural changes of the double frozen cod fillets and the unglazed cod portions were, respectively, recognized by sensory analysis. This findings are in agreement with other studies comparing single

FIGURE 4. Principal component analysis of sensory data from QIM, odor, color, flavor and texture. Bi-plot of principal component 2 versus principal component 1. Principal Component 1 and 2 explained 77% and 7%, respectively, of the total variation in the data set. Glazed cod portions (batch A), glazed vacuum packed cod portions (batch B), unglazed cod portions (batch C), interleaved packed cod fillets (batch D), and double frozen cod fillets (batch E). Numbers indicate period of frozen storage in weeks.



and double freezing of cod fillets (Dyer et al., 1962; MacCallum et al., 1969). This provides the opportunity to replace some of the traditionally commercial frozen fish products of low quality on the retail or catering markets with high quality "frozen at sea" cod products. However, in other studies (Peters et al., 1968; Hurling and McArthur, 1996), no significant quality differences between single and double frozen cod products were observed. The variable results reported comparing single and double frozen might be due to a number of different factors including initial quality of raw material before freezing, frozen storage, glazing or thawing methods.

Sensory changes in this present study depended on frozen storage conditions and corresponded with a former study of our own (Bechmann et al., 1998). The sensory changes observed in this present study for the five frozen cod products are also supported by findings of other studies concerning quality of frozen cod products (Baines et al., 1969; Warm et al., 1998). Unglazed cod portions and particularly double frozen fillets developed cold storage flavor and odor during the frozen storage resulting in low quality of

the frozen cod products as observed by others (McGill et al., 1974; McGill et al., 1977). The unglazed cod portions developed a high degree of freezer burn with very dry surfaces during storage. During frozen storage glazed portions, glazed vacuum packed portions and interleaved packed fillets remained of equal sensory and physical quality. This means that both glazing, vacuum packaging and interleaved packaging protected effectively against dehydration during frozen storage. The importance of glazing cod portions immediately after freezing to protect against dehydration was also previously reported (Josephson, 1985; Nilsson and Ekstrand, 1994). The results for water loss on cooking for cod products (Figure 2) corresponded to those of a previous study (Leblanc et al., 1988). Significantly higher water loss on cooking and significantly lower water holding capacity (Figure 3) of the double frozen cod products was expected due to denaturation of proteins during repeated freezing (Sikorski, 1994).

Earlier studies showed discoloration of the fillets produced on board "frozen at sea" trawlers (Jones, 1965; Jones, 1969; Kelly, 1969; Burt et al., 1974). The improvements in our "frozen at sea" cod products with no discoloration of cod fillets was probably due to effective catch handling including a new bleeding technique where cod raw material are headed and gutted immediately after catching. This findings agreed with later studies concerning improved catch handling of white fish (Valdimarsson et al., 1984; Botta et al., 1986). Cod products in this present study were frozen before the onset of *rigor mortis* on the freezer trawler "Sisimiut" and thaw rigor, which is related to muscle contraction, shrinkage and gaping of the fillets during the freezing and thawing process (Cappeln et al., 1999). The applied thawing of cod products was, however, relatively slow as recommended (Cappeln, Pers. Comm.) and this may have minimized the effects of thaw rigor.

Producing "frozen at sea" cod portions on a freezer trawler requires installation of a blast freezer with glazing equipment. Furthermore, the production on the freezer trawlers demands a lot of manual work including hand cutting of the portions. However, production of portions provides the opportunity to produce value-added products on a freezer trawler especially during catching periods with small tow sizes. In addition, the current demand for food that is easy to prepare (Peavey et al., 1994) is met by "frozen at sea" cod portions which are portioned, boneless and only need thawing before cooking.

CONCLUSIONS AND RECOMMENDATIONS

This present study showed that an improved handling and processing technique made it possible to produce "frozen at sea" cod products of high sensory and physical quality from the Barents Sea on a freezer trawler. It is

recommended that this improved manufacturing technique on freezer trawlers include short tow duration, small catch size, quick gutting with subsequent bleeding and a period shorter than six hours between catching and freezing of the cod portions and cod fillets on board. In addition, frozen cod products must be stored at temperatures below $-20°C$ during storage.

This present study also showed that producing glazed cod portions on a commercial freezer trawler is possible in practice. The "frozen at sea" glazed cod portions, vacuum packed glazed cod portions and interleaved packed cod fillets remained of high sensory and physical quality during a commercial frozen storage of 46 weeks. A great potential for commercial application of "frozen at sea" cod products compared with the traditional double frozen cod products were observed. We believe that "frozen at sea" cod products manufactured with improved handling and processing techniques are a highly acceptable superior product alternative to the existing commercial frozen cod products on the retail or catering markets.

REFERENCES

Anonymous. 1993. *The Graphpad Instat 2.04a Users Guide*, Graphpad Software, San Diego.

Anonymous. 1995. *Parallel Food Testing in the European Union: Fish*, International Consumers Research & Testing Limited, London.

Anonymous. 1996. *The Unscrambler 6.0 Users Guide*, Camo A/S, Trondheim.

Baines, C.R., Connell, J.J., Gibson, D.M., Howgate, P.F., Livingston, E.I., and Shewan, J.M. 1969. A taste panel technique for evaluating the eating quality of frozen cod. In: *Freezing and Irradiation of Fish*. Kreuzer, R. (Ed.) pp. 361-366. Fishing News Books, London.

Bechmann, I.E, Jensen, H.S., Bøknæs, N., Warm, K., and Nielsen, J. 1998. Prediction of chemical, physical and sensory data from process parameters for frozen cod using multivariate analysis. J. Sci. Food Agric. 78: 329.

Botta, J.R., Squires, B.E., and Johnson, J. 1986. Effect of bleeding/gutting procedures on the sensory quality of fresh raw atlantic cod (*Gadus morhua*). Can. Inst. Sci. Technol. 19: 186.

Botta, J.R., Bonnell, G., and Squires, B.E. 1987. Effect of method of catching and time of season on sensory quality of fresh raw atlantic cod (*Gadus morhua*). J. Food Sci. 52: 928.

Burt, J.R., Dreosti, G.M., Jones, N.R., Kelman, J.H., McDonald, I., Murray, J., and Simmonds, C.K. 1974. Technological investigations into Cape hake II. Freezing at sea. J. Food Tech. 9: 235.

Cappeln, G., Nielsen, J., and Jessen, F. 1999. Synthesis and degradation of adenosin triphosphate in cod (*Gadus morhua*) at subzero temperatures. J. Sci. Food Agric. 79: 1099.

Cappeln, G. 2000. *Personal communication*, Danish Institute for Fisheries Research, Department of Seafood Research, Lyngby.

Castell, C.H. 1971. Some fundamental problems in the quality assessment of fishery products. In: *Fish Inspection and Quality Control.* Kreuzer, R. (Ed.) pp. 9-13. Fishing News Books, London.

Castell, C.H., Smith, B., and Dyer, W.J. 1973. Effects of formaldehyde on salt extractable proteins on gadoid muscle. J. Fish. Res. Board Can. 30: 1205.

Dyer, W.J., Fraser, D.I., Ellis, D.G., and Idler, D.R. 1962. Quality changes in stored refrozen cod fillets. Bull. Int. Froid 1: 515.

Eide, O., Børresen, T., and Strøm, O. 1982. Minced fish production from capelin (*Mallotus villosus*). A new method for gutting, skinning and removal of fat from fatty species. J. Food Sci. 47: 347.

Esbensen, K. 1994. *Multivariate Analysis–In Practice*, CAMO A/S, Trondheim.

Haard, N.F. 1992. Biochemical reactions in fish muscle during frozen storage. In: *Seafood Science and Technology.* Bligh, E.G. (Ed.) pp. 176-209. Fishing News Books, Oxford.

Hurling, R. and McArthur, H. 1996. Thawing, refreezing and frozen storage effects on muscle functionality and sensory attributes of frozen cod (*Gadus morhua*). J. Food Sci. 61: 1289.

Jones, N.R. 1965. Freezing fillets at sea. In: *Fish Quality at Sea: The Official Proceedings of the Conference on the Design of Fishing Vessels and Their Equipment in Relation to the Improvement of Quality.* Organised by the White Fish Authority, Olympia, May 31 and June 1, 1965 in conjunction with the WORLD-FISHING Exhibition. pp. 81-95. White Fish Authority, London.

Jones, N.R. 1969. Fish as a raw material for freezing: Factors influencing the quality of products frozen at sea. In: *Freezing and Irradiation of Fish.* Kreuzer, R. (Ed.) pp. 31-38. Fishing News Books, London.

Josephson, D.B., Lindsay, R.C., and Stuiber, D.A. 1985. Effect of handling and packaging on the quality of frozen whitefish. J. Food Sci. 50: 1.

Kelly, T.R. 1969. Discolouration in sea-frozen fish fillets. In: *Freezing and Irradiation of Fish.* Kreuzer, R. (Ed.) pp. 64-67. Fishing News Books, London.

Leblanc, E.L., Leblanc, R.J., and Blum, I.E. 1988. Prediction of quality in frozen cod (*Gadus morhua*) fillets. J. Food Sci. 53: 328.

Love, R.M. 1988. *The Food Fishes, Their Intrinsic Variation and Practical Implications.* Farrand Press, London.

MacCallum, W.A., Jaffray, J.I., Chalker, D.A., and Idler, D.R. 1969. Condition of Newfoundland offshore fish and its influence on quality after single and double freezing. In: *Freezing and Irradiation of Fish.* Kreuzer, R. (Ed.) pp. 56-63. Fishing News Books, London.

Martens, H. and Næs, T. 1989. *Multivariate Calibration*, John Wiley & Sons Ltd., Chicester.

McGill, A.S., Hardy, R., Burt, J.R., and Gunstone, F.D. 1974. Hept-*cis*-4-enal and its contribution to the off-flavour in cold stored cod. J. Sci. Food Agric. 25: 1477.

McGill, A.S., Hardy, R., and Gunstone, F.D. 1977. Further analysis of the volatile components of frozen cold stored cod and the influence of these on flavour. J. Sci. Food Agric. 28: 200.

Merritt, J.H. 1988. Freezing at sea. In: *Refrigeration on Fishing Vessels.* pp. 133-159. Fishing News Books, Surrey.

Morrison, C.R. 1993. Fish and shellfish. In: *Frozen Food Technology.* Mallet, C.P. (Ed.) pp. 196- 236. Chapman and Hall, Surrey.

Nilsson, K. and Ekstrand, B. 1994. Refreezing rate after glazing affects cod and rainbow trout muscle tissue. J. Food Sci. 59: 797.

Peavey, S., Work, T., and Riley, J. 1994. Consumer attitudes toward fresh and frozen fish. J. Aquat. Food Product Technol. 3: 71.

Peters, J.A., MacCallum, W.A., Dyer, W.J., Idler, D.R, Slavin, J.W., Lane, J.P., Fraser, D.I., and Laishley, E.J. 1968. Effect of stage of rigor and of freezing-thawing processes on storage quality of refrozen cod. J. Fish. Res. Board Can. 25: 299.

Rehbein, H. 1988. Relevance of trimethylamine oxide demethylase activity and hemoglobin content to formaldehyde production and texture deterioration in frozen stored minced fish muscle. J. Sci. Food Agric. 43: 261.

Shenouda, S.Y.K. 1980. Theories of protein denaturation during frozen storage of fish flesh. In: *Advances in Food Research.* Chichester, C.O., Mrak, E.M. and Stewart, G.F. (Eds.) pp. 275-311. Academic Press, New York.

Sikorski, Z.E. and Kolakowska, A. 1994. Changes in proteins in frozen stored fish. In: *Seafood Proteins.* Sikorski, Z.E., Pan, B.S., and Shahidi, F. (Eds.) pp. 99-112. Chapman & Hall, New York.

Sokal, R.R. and Rohlf, F.J. 1985. *Biometry*, W.H. Freman and Co., New York.

Valdimarsson, G., Matthiasson, A., and Stefansson, G. 1984. The effect of on board bleeding and gutting on the quality of fresh, quick frozen and salted products. In: *Paper Presented at the Jubilee Seminar of the Icelandic Fisheries Laboratories, Sept. 23-24.* Möller, A. (Ed.) pp. 61-72. Icelandic Fisheries Laboratories, Reykjavik.

Warm, K., Bøknæs, N., and Nielsen, J. 1998. Development of quality index methods for evaluation of frozen cod (*Gadus morhua*) and cod fillets. J. Aquat. Food Product Technol. 7: 45.

Artikel II

Bøknæs, N., Østerberg, C., Nielsen, J. & Dalgaard, P. (2000). Influence of freshness and frozen storage temperature on quality of thawed cod fillets stored in modified atmosphere packaging. *Food Science and Technology*, **33**, 244–248.

Research Note

Influence of Freshness and Frozen Storage Temperature on Quality of Thawed Cod Fillets Stored in Modified Atmosphere Packaging

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The effects of keeping cod fillets in air at 0 °C for 1 or 8 d prior to modified atmosphere packaging (MAP) and subsequent frozen storage at − 20 °C and − 30 °C for 6 wk were studied. Quality attributes of thawed MAP cod fillets stored at 2 °C for up to 17 d were evaluated. The known specific spoilage organism of fresh MAP cod, Photobacterium phosphoreum, *was found in levels of 2.3 and 5.8 Log (cfu/g) after 1 and 8 d of chill storage in air at 0 °C, respectively. Frozen storage at both − 20 °C and − 30 °C for 6 wk reduced numbers of* P. phosphoreum *to below the limit of detection. After chill storage at 2 °C,* P. phosphoreum *was not detected in cod fillets stored frozen at − 20 °C. Significant growth of* P. phosphoreum *and production of TMA during chill storage at 2 °C were only observed in cod fillets kept for 8 d in air at 0 °C prior to frozen storage at − 30 °C. Frozen storage odour and taste developed in thawed MAP cod fillets during chill storage but they were substantially more pronounced in cod fillets stored 8 d in air at 0 °C before freezing. The present study clearly showed the need for fresh raw material when producing thawed chilled cod fillets packed in modified atmosphere. Consequently, it was concluded that 'frozen at sea' raw material in combination with MAP seems to be a promising technology, combining the inhibitory effect on microbial growth and TMA production for the manufacture of prime quality thawed MAP cod fillets for retail.*

Keywords: MAP; cod; thawing; *Photobacterium phosphoreum*; TMA

Introduction

In recent years, consumer demands for conveniently packaged boneless fish fillets have been met, in some countries, by the use of modified atmosphere packaging (MAP). In Denmark, for example, the sale of these products has increased substantially since 1995 (unpublished data). However, complaints from consumers have been that fresh MAP fish products are at times spoiled and exhibit a typical amine-like odour. This is most probably due to temperature abuse, too long storage times or use of raw fish material of an insufficient degree of freshness (1). All these factors are known to result in a high number of bacteria causing amine odour and spoilage in fresh fish retail products. Clearly, freezing prevents microbial growth in seafood but shelf life of thawed and aerobically stored fish is only extended by a few days as compared to unfrozen fish products (2–4). For MAP cod, however, where *Photobacterium phosphoreum* is responsible for trimethylamine (TMA) production and product deterioration (5–7) of fresh products, shelf life of thawed chilled MAP fillets was extended from 11 to more than 20 d at 2 °C (8). *Photobacterium phosphoreum* was totally inactivated in thawed chilled MAP cod fillets after storage at − 20 °C for 8 wk and, in agreement with the shelf life extension determined by sensory evaluation, no TMA and very little amine odour and taste were detected during 20 d of chill storage of thawed MAP cod fillets (8). Consequently, frozen fish seem most interesting as raw material for chilled MAP fish products but few studies have been carried out on the use of thawed MAP fish for retail (8–10). Today, frozen fish products are manufactured on-shore at factories or at sea on freezer trawlers. The duration between time of catch and processing of fish raw material in on-shore factories can be considerable, in some cases, up to 10 d in ice. However, production at sea allows freezing of fish in a pristine state.

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The objectives of the present study were to evaluate the effects of chill storage duration of fish before freezing (1 or 8 d in air at 0 °C) and frozen storage temperature (− 20 °C or − 30 °C) on quality attributes of thawed cod fillets stored at 2 °C in MAP. Temperatures of − 20 °C and − 30 °C were selected because 'frozen at sea' fish products during processing, distribution and sale usually fluctuate between − 18 °C and − 30 °C (unpublished data). Quality attributes were assessed by several different methods, including sensory evaluation, production of TMA, specific counts of *P. phosphoreum* and total viable counts.

Materials and Methods

Product and storage characteristics

A simple two factor, two level factorial design was used to evaluate the effects of chill storage duration before freezing and of frozen storage temperature (**Table 1**).

Four sub-batches of cod fillets (A, B, C and D) were packed from a single batch of cod (*Gadus morhua*) caught in the Baltic Sea in September 1997. The fish were filleted by a local fishing company and transported in ice to the Danish Institute for Fisheries Research (DIFRES) the day after catching and filleting. Each fillet from batch A and B was weighed and placed in a plastic tray including drip pads. Each tray containing one cod fillet of approximately 250–300 g was packed in a modified atmosphere of about 60% CO_2 and 40% N_2 in Riloten 40/70X bags of low gas permeability (5). Fish to gas ratio was 1:2 (wt/vol; 250 g cod fillets and 500 mL gas). Cod fillets used in batch C and D were wrapped with polyethylene and stored aerobically in boxes at 0 °C for a further 7 d before packaging in modified atmosphere and freezing. The MAP cod fillets in the four batches were frozen in a blast freezer until centre temperature reached − 30 °C. After freezing, batches A and C were stored at − 20 °C and batches B and D were stored at − 30 °C. After 6 wk of frozen storage, the cod fillets were thawed in air at 5 °C for 14 h and transferred for chill storage at 2 °C. Seven times during chill storage (after 0, 3, 5, 7, 11, 14 and 17 d) seven packs with cod fillets from batch A and batch B were used for analysis. Six times during chill storage (after 0, 4, 7, 10, 13 and 17 d) seven packs with cod fillets from batch C and batch D were used for analysis. At each sampling time four packs of each batch were used for sensory evaluation. In addition, three separate packs were used for microbiological, chemical and physical analyses. Temperatures were recorded by loggers (Tinytag, Gemini Data Loggers, U.K.) during frozen and chill storage. The gas composition was determined for all packs in this study using a gas analyser (PBI, Dansensor, Denmark).

Table 1 Codes of the four sub-batches studies with different duration of chill storage before freezing and frozen storage for 6 wk

	Duration of aerobic chill storage at (0 °C)	
Frozen storage temperature	1 d	8 d
− 20 °C	Batch A	Batch C
− 30 °C	Batch B	Batch D

Sensory evaluation

Sensory evaluation of thawed cooked cod was performed by a panel of seven to nine trained assessors used in several earlier studies treating quality of chilled thawed MAP cod fillets. Sensory quality changes during chill storage of thawed MAP cod fillets were described by using a category scale from 0 to 10 corresponding to low and high scores of each attribute, respectively (8). The applied sensory attributes, frozen storage odour and taste, amine odour and taste together with juiciness were chosen in a recently published study (8). Loins from cod fillets were placed in porcelain bowls and heated at 100 °C for 20 min in a hot air oven and immediately served to the assessors. At each sampling time each assessor evaluated one piece of cod from each of the four batches. Fizz Network software (11) was used for collection and evaluation of sensory data.

Chemical and physical analyses

Trimethylamine oxide (TMAO) and TMA were determined in triplicate by a modification of the method of Conway (12). Trimethylamine oxide was determined at first day of the chill storage period only whereas TMA was determined at each sampling time. Before freezing and at first day of chill storage of thawed cod fillets, water holding capacity (WHC) was determined in quadruplicate as the relative water loss during a mild centrifugation of minced fish muscle (13).

Microbiological analyses

Numbers of *P. phosphoreum* were determined in triplicate by a conductance method. Total viable counts (TVC) were determined in triplicate by spread plating on Long and Hammer's agar containing 1% NaCl (14) incubated aerobically at 15 °C for 7 d. Both *P. phosphoreum* and TVC were determined as previously described (7,15).

Statistical analyses

To detect significant differences between batches and periods of chill storage, one-way analysis of variance was used. Furthermore, the Tukey–Kramer multiple comparison test was used to find significant differences between batches. These statistical tests were done using Graphpad Instat (16). Sensory data obtained in this experiment were analysed using multivariate Principal Component Analysis (PCA) previously described (17). Sensory responses averaged over assessors were used in PCA performed on a data matrix with 26 objects (the four batches at each period of chill storage) and five variables (sensory attributes). Sensory data were analysed using Unscrambler® (18).

Results

Raw material and storage characteristics

The average storage temperature of cod fillets before freezing was 0.0 ± 0.2 °C. Temperatures in the centre of cod fillets from all sub-batches reached − 30 °C after 3.5 h during blast freezing. The temperature equalization from − 30 °C to − 20 °C for batches A and C took approximately 6 h (results not shown). Frozen storage temperature for batches A and C was − 21.2 ± 0.4 °C (s_x) with no significant difference ($P > 0.05$) between the two frozen storage periods. Batches B and D were kept at − 30.2 ± 1.5 °C and again no significant difference ($P > 0.05$) between the two frozen storage periods was observed. The average chill storage temperature was 1.9 ± 0.4 °C with no significant difference between the four batches ($P > 0.05$). After storage at 0 °C and just prior to freezing, the cod contained 48.2 ± 4.4 mg TMAO-N/100 g of TMAO and had WHC of 86.1 ± 1.8%. This relatively low concentration of TMAO for cod was previous observed in raw material from the Baltic Sea (8). Initial numbers of *P. phosphoreum* before freezing were 2.3 ± 1.7 and 5.8 ± 1.1 Log (cfu/g) for batches A and B and batches C and D, respectively. The corresponding values of TVC were 5.8 ± 0.2 and 7.0 ± 0.4 Log (cfu/g). The initial TVC level in cod raw material of 5.8 Log (cfu/g) corresponded to several previous studies with newly caught cod from the Baltic Sea and the North Sea (7,8,19).

Microbiological changes

After frozen storage, numbers of *P. phosphoreum* in thawed cod fillets were below the detection limit of 0.6 Log (cfu/g) in all four batches (**Fig. 1**). It was not detected at all during chill storage of fish previously kept at − 20 °C for 6 wk. After 17 d at 2 °C, however, significantly higher numbers of *P. phosphoreum* ($P < 0.01$) were detected for batch D (8 d in air at 0 °C and frozen storage at − 30 °C) compared to batches A and C both stored at − 20 °C. During chill storage at 2 °C of thawed fillets, no significant differences in TVC ($P > 0.05$) were observed in the four batches (**Table 2**).

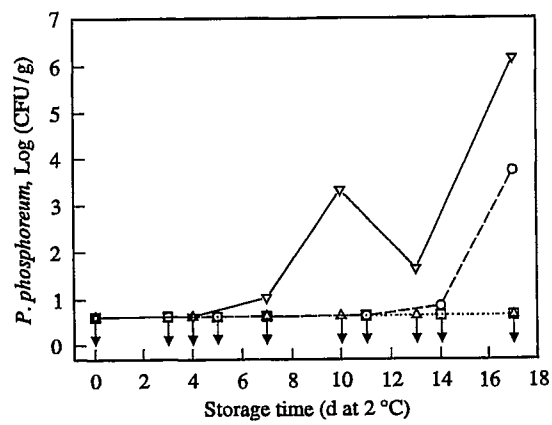

Fig. 1 Average numbers of *P. phosphoreum* in thawed chilled MAP cod fillets stored at 2 °C. □, batch A (1 d in air at 0 °C before freezing and − 20 °C for 6 wk); ○, batch B (1 d in air at 0 °C before freezing and − 30 °C for 6 wk); △, batch C (8 d in air at 0 °C before freezing and − 20 °C for 6 wk); and ▽, batch D (8 d in air at 0 °C before freezing and − 30 °C for 6 wk)

Chemical and physical changes

Significantly higher TMA production ($P < 0.01$) was only observed for batch D (8 d in air at 0 °C before freezing and frozen storage at − 30 °C) chill stored between 11 and 17 d at 2 °C (**Table 2**). No significant ($P > 0.05$) differences for WHC between the four batches were found after freezing and storing for 6 wk, the average WHC after freezing was 70.5 ± 5.2%. However, WHC for cod fillets before freezing was significantly higher ($P < 0.001$) than after freezing. During chill storage, average CO_2 concentration in the four batches was 56.7 ± 7.3%. Average pH value was 6.7 ± 0.1 with no significant difference between the four batches during chill storage.

Sensory changes

A biplot of scores characterizing samples and of loadings characterizing sensory attributes indicated that principal component 1 accounted for most of the variation due to the chill storage of thawed cod fillets attributes (**Fig. 2**). Principal components 1 and 2 explained 58% and 10%, respectively, of the total variation of the sensory data. Samples from raw cod material that had been stored 1 d in air at 0 °C before freezing (batches A and B) were, after chill storage of 0 and 3 d at 2 °C, characterized by a juicy texture, very low scores for amine odour and taste, together with very low scores for frozen storage taste and odour. After chill storage of 13 and 17 d at 2 °C, samples from raw cod material that had been stored 8 d in air at 0 °C before freezing (batches C and D) were given higher scores for amine and frozen storage taste and odour. At equal days of chill storage, batches C and D were given lower scores for juiciness and higher scores for amine taste and odour together with frozen storage taste and odour compared to batches A and B. It should be noted

Table 2 Values for TMA (mg TMA-N/100 g) and TVC Log (cfu/g) during chill storage of thawed MAP cod fillets at 2 °C. Data were averaged over 0 to 10 d and 11 to 17 d at 2 °C, respectively, for values from the four batches

	TMA mg TMA-N/100 g		TVC Log (cfu/g)	
Batch	0–10 d	11–17 d	0–10 d	11–17 d
A	1.3 ± 3.3[a]	3.5 ± 1.8[b]	6.3 ± 0.5[a]	6.6 ± 0.2[b]
B	2.0 ± 4.2[a]	3.7 ± 0.8[b]	6.3 ± 0.3[a]	6.9 ± 0.3[b]
C	1.7 ± 1.8[a]	3.0 ± 1.4[c]	6.7 ± 0.1[a]	7.4 ± 1.2[c]
D	0.4 ± 3.0[a]	16.6 ± 5.1[c]	6.3 ± 0.2[a]	7.0 ± 1.0[c]

[a] The average ± s_x of four replicates
[b] The average ± s_x of three replicates
[c] The average ± s_x of two replicates

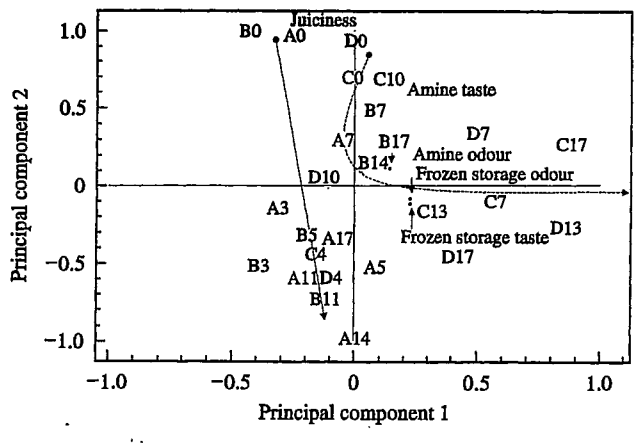

Fig. 2 Principal component analysis of data from sensory evaluation of thawed chilled MAP cod fillets. Biplot of principal component 1 *vs.* principal component 2. Principal components 1 and 2 explained 58% and 10%, respectively, of the total variation for the sensory data. Batch A (1 d in air at 0 °C before freezing and − 20 °C for 6 wk); batch B (1 d in air at 0 °C before freezing and − 30 °C for 6 wk); batch C (8 d in air at 0 °C before freezing and − 20 °C for 6 wk); and batch D (8 d in air at 0 °C before freezing and − 30 °C for 6 wk). Letters indicate batch and numbers indicate days of chill storage for thawed MAP cod fillets. Curves with arrows indicate development of different sensory profiles depending on chill storage duration before freezing. Dotted arrow indicates cod fillets stored 1 d in air at 0 °C before freezing and dashed arrow indicates cod fillets stored 8 d in air at 0 °C before freezing, respectively

that on a category scale from 0 (low) to 10 (high), all average scores did not exceed 5.4.

Discussion

Short term frozen storage for 6 wk at − 20 °C or − 30 °C substantially reduced numbers of *P. phosphoreum* and this specific spoilage organism was not detected during chill storage of cod fillets previously frozen stored at − 20 °C (**Fig. 1**). This inactivation of *P. phosphoreum* is in agreement with previous studies (8,20) and explains why TMA and amine odour and taste were not produced in thawed chilled MAP cod fillets previously stored at − 20 °C (**Table 2**). Significant growth of *P. phosphoreum* and production of TMA were observed in thawed chilled MAP cod fillets kept for 8 d in air at 0 °C prior to frozen storage at − 30 °C (**Table 2, Fig. 1**), however. Today, temperatures below − 20 °C are frequently used during manufacturing and distribution of commercial frozen cod fillets. Consequently, the substantially different effect of − 20 °C and − 30 °C on inactivation of *P. phosphoreum* is of considerable practical importance as it explains TMA development and differences in shelf life of thawed MAP cod previously kept at the two different frozen storage temperatures. Interestingly, reports in Norwegian trade journals indicate that studies from Norway also observed longer shelf life and reduced TMA production in thawed MAP cod when frozen at − 20 °C as opposed to − 30 °C. However, we have found no data in the literature for the effect of different frozen storage temperatures on inactivation of *P. phosphoreum* in naturally contaminated seafood. In fact, a sensitive and specific method for detection of *P. phosphoreum* became available only recently (15). As observed for *P. phosphoreum* at − 20 °C and − 30 °C, inactivation of some other microorganisms has been previously shown to be more pronounced at higher freezing temperatures (2,21–23). The inactivation of *P. phosphoreum* during frozen storage of cod could be related to differences in ice crystal formation, enzymatic activity or cell damage after storing at the various frozen temperatures. Sensory changes of thawed MAP cod fillets including texture changes and frozen storage odour and taste (**Fig. 2**) correspond to what has been observed previously for frozen (24–26) and thawed chilled MAP cod fillets (8). Development of frozen storage odour and taste was most pronounced for thawed chilled MAP fillets with 8 d of aerobic chill storage prior to freezing. These observations support previous suggestions that compounds associated with frozen storage odour and taste are products of lipid oxidation (24). Storage in air before freezing may allow these processes to be initiated. Furthermore, higher juiciness was detected in cod with 1 d of aerobic chill storage before freezing, as compared to that stored for 8 d in air at 0 °C before freezing. This could be related to a precedent drip loss of cod fillets during 8 d in air at 0 °C before freezing (results not shown). Significantly lower WHC for frozen cod fillets corresponded to results of other studies (25,26).

The present study documented the requirement for raw material with a high degree of freshness when used for thawed MAP cod fillet production. Such fish could be obtained by using 'frozen at sea' fillets and, as compared to fresh MAP cod, this technology allows a raw material supply to be less dependent on fishing ground and season of catch. The combined use of 'frozen at sea' and MAP technology delayed microbiological spoilage of thawed products and this approach seems promising for prevention of the spoilage problems previously encountered with fresh MAP fish in retail.

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References

1 ANONYMOUS. *Parallel Food Testing in the European Union: Fish.* London: International Consumers Research and Testing Limited (1995)

2 SHEWAN, J. M. The microbiology of sea-water fishes. In: BORGSTRØM, G. (Ed.), *Fish as Food.* London: Academic Press, pp. 487–560 (1961)

3 LISTON, J. Microbiology in fishery science. In: CONNELL, J. J. (Ed.), *Advances in Fish Science and Technology.* London: Fishing News Books Ltd, pp. 138–157 (1980)

4 MAGNÚSSON, H. AND MARTINSDÓTTIR, E. Storage quality of fresh frozen-thawed fish in ice. *Journal of Food Science*, **60**, 273–278 (1995)
5 DALGAARD, P., GRAM, L. AND HUSS, H. H. Spoilage and shelf life of cod fillets packed in vacuum or modified atmospheres. *International Journal of Food Microbiology*, **19**, 283–294 (1993)
6 DALGAARD, P. Qualitative and quantitative characterization of spoilage bacteria from packed fish. *International Journal of Food Microbiology*, **26**, 319–333 (1995)
7 DALGAARD, P., MEJLHOLM, O., CHRISTIANSEN, T. J. AND HUSS, H. H. Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. *Letters of Applied Microbiology*, **24**, 373–378 (1997)
8 GULDAGER, H. S., BØKNÆS, N., ØSTERBERG, C., NIELSEN, J. AND DALGAARD, P. Thawed cod fillets spoil less rapidly than unfrozen fillets when stored under modified atmosphere at 2 °C. *Journal of Food Protection*, **61**, 1129–1136 (1998)
9 LANIER, T. C. AND KORHONEN, R. Modified atmosphere and conventional packaging of fresh and previously-frozen fish. In: MARTIN, R. E. (Ed.), *Proceedings First National Conference on Modified and Controlled Atmosphere Packaging of Seafood Products, San Antonio*. Washington DC: The National Fisheries Institute, pp. 80–97 (1981)
10 LØKKEN, G. B. Personal communication. Tromsø, Norway: Norwegian Institute of Fisheries and Aquaculture Ltd (1997)
11 ANONYMOUS. *Fizz. Sessions Generation, Data Collection and Computation for Sensory Analysis*. Couternon, France: Biosystems (1995)
12 CONWAY, E. J. AND BYRNE, A. An absorption apparatus for the micro-determination of certain volatile substances. *Biochemical Journal*, **27**, 419–429 (1933)
13 EIDE, O., BØRRESEN, T. AND STRØM, O. Minced fish production from capelin (*Mallotus villosus*). A new method for gutting, skinning and removal of fat from small fatty species. *Journal of Food Science*, **47**, 347–349, 354 (1982)
14 VAN SPREEKENS, J. J. A. The suitability of a modification of Long and Hammer's medium for enumeration of more fastidious bacteria from fresh fishery products. *Archiv für Lebensmittelhygiene*, **25**, 213–219 (1974)
15 DALGAARD, P., MEJLHOLM, O. AND HUSS, H. H. Conductance method for quantitative determination of *Photobacterium phosphoreum* in fish products. *Journal of Applied Bacteriology*, **81**, 57–64 (1996)
16 ANONYMOUS. *The Graphpad Instat 2.04a Users Guide*. San Diego: Graphpad Software (1993)
17 MARTENS, H. AND NÆS, T. *Multivariate Calibration*. Chichester, U.K.: John Wiley and Sons Ltd. (1989)
18 ANONYMOUS. *The Unscrambler 6.0 Users Guide*. Trondheim, Norway: Camo A/S (1996)
19 CANN, D. C., SMITH, G. L. AND HOUSTON, N. C. *Further Studies on Marine Fish Stored Under Modified Atmosphere Packaging*. Aberdeen, U.K.: Torry Research Station (1984)
20 FUJII, T., KURIHARA, K. AND OKUZUMI, M. Viability and histidine decarboxylase activity of halophilic histamine-forming bacteria during frozen storage. *Journal of Food Protection*, **57**, 611–613, 942 (1994)
21 SIMMONDS, C. K. AND LAMPRECHT, E. C. Microbiology of frozen fish and related products. In: ROBINSON, R. K. (Ed.), *Microbiology of Frozen Foods*. London: Elsevier, pp. 169–208 (1985)
22 GOLDEN, D. A., BEUCHAT, L. R. AND BRACKETT, R. E. Inactivation and injury of *Listeria monocytogenes* as affected by heating and freezing. *Food Microbiology*, **5**, 17–23 (1988)
23 CHOU, C. C., CHENG, S. J., WANG, Y. C. AND CHUNG, K. T. Behaviour of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in tryptic soy broth subjected to various low temperature treatments. *Food Research International*, **32**, 1–6 (1999)
24 MCGILL, A. S., HARDY, R., BURT, J. R. AND GUNSTONE, F. D. Further analysis of the volatile components of frozen cold stored cod and the influence of these on flavour. *Journal of the Science of Food and Agriculture*, **28**, 200–205 (1977)
25 SHENOUDA, S. Y. Theories of protein denaturation during frozen storage of flesh. In: CHICHESTER, C. O., MRAK, E. M. AND STEWART, G. F. (Eds), *Advances in Food Research*. New York: Academic Press, pp. 275–311 (1980)
26 MACKIE, I. M. The effects of freezing on flesh proteins. *Food Reviews International*, **9**, 575–610 (1993)

Artikel III

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Effects of Technological Parameters and Fishing Ground on Quality Attributes of Thawed, Chilled Cod Fillets Stored in Modified Atmosphere Packaging

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Effects were studied of various technological parameters and fishing ground on quality attributes of thawed, chilled cod fillets stored in modified atmosphere packaging (MAP). Frozen fillets of Baltic Sea and Barents Sea cod, representing two commercial fishing grounds, were used as raw material. The parameters investigated were: (1) packaging in modified atmosphere during frozen storage, (2) frozen storage period and temperature, (3) fishing ground and chill storage temperature, together with (4) the addition of trimethylamine oxide (TMAO) and sodium chloride (NaCl) to cod fillets before freezing or after freezing and thawing. Application of MAP during frozen storage resulted in a significant increase in the drip loss of thawed, chilled MAP cod fillets but none of the other quality attributes studied were influenced by this treatment. This implies that packaging cod fillets without MAP during frozen storage is more appropriate for manufacturing of thawed chilled MAP cod fillets. During chill storage of thawed MAP Barents Sea fillets previously kept at −30 °C for 15 weeks, significant growth of Photobacterium phosphoreum *and production of trimethylamine were observed. On the contrary,* P. phosphoreum *growth and trimethylamine production in thawed and chill-stored MAP Baltic Sea cod fillets were strongly inhibited after as little as 4 weeks of frozen storage at −30 °C. Contents of trimethylamine oxide and NaCl were substantially higher in fillets of Barents Sea cod compared to fillets of Baltic Sea cod. Therefore, addition of trimethylamine oxide and NaCl to Baltic Sea cod fillets was evaluated and shown to protect* P. phosphoreum *against frozen storage inactivation and this explained the observed differences in growth of the spoilage bacteria and trimethylamine production between thawed and chill stored MAP fillets from the two fishing grounds. Despite modest production of trimethylamine in Baltic Sea fillets, this cod raw material was less suitable for production of thawed MAP products due to high drip losses during chill storage.*

Keywords: drip; frozen storage; NaCl; photobacterium; trimethylamine and trimethylamine oxide

Introduction

Sale of fresh fish products in modified atmosphere packaging (MAP) is increasing on the European retail market. The popularity of these products is related to the fact that conveniently packed fresh fish can be sold from chill cabinets e.g. in supermarkets in the same way as many other foods. However, the shelf-life of fresh MAP seafood is very short in comparison with that of fresh MAP meat products (Farber, 1991; Dalgaard, 1995a) and a European consumer study reported problems with off-flavours in fresh MAP seafood in retail (Anon., 1995). These off-flavours most likely resulted from spoilage bacteria having reached high numbers due to inappropriate time-temperature storage conditions, insufficient degree of freshness of the fish raw material before packaging, or insufficient hygienic practice prior to packaging.

To obviate problems with shelf-life and off-flavour for fresh MAP fish, marketing of previously-frozen thawed MAP products is an interesting technology that has been used e.g. by the seafood sector in Great Britain (Howgate, pers. comm.) and Australia (Lanier and Korhonen, 1981). Frozen fish raw material can be processed at sea in the pristine state (Bøknæs *et al.*, 2001) and thereby difficulties of reliable supplies of high quality fresh fish can be circumvented, resulting in more flexible manufacturing and distributing of thawed chilled MAP fish products (Lanier and Korhonen, 1981; Davis, 1993). Furthermore, shelf-life of thawed MAP fish can be extended substantially as compared to corresponding fresh MAP products. In fact, it was shown recently for Baltic Sea cod that shelf-life of fresh MAP fillets was extended from 11–12 days at 2 °C to >20 days for the thawed MAP product. The extension of shelf-life was caused by inhibition of the

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specific spoilage organism *Photobacterium phosphoreum* and by a corresponding reduction in trimethylamine (TMA) production in thawed MAP cod fillets previously kept frozen at −20 °C or −30 °C for 6 or 8 weeks (Guldager *et al.*, 1998; Bøknæs *et al.*, 2000). Thus, modified atmosphere packaging of thawed fish is interesting with respect to both management of supply chains and shelf-life of products. Nevertheless, there are only a small number of studies that look at the effects of technological parameters like packaging in modified atmosphere during frozen storage, period and temperature of frozen storage or biological parameters like fishing ground on important quality attributes and product shelf-life of thawed chilled MAP fish products.

Objectives of the present study were to evaluate the effects of technological parameters and fishing ground on quality attributes of thawed, chilled MAP cod fillets. Effects of MAP during frozen storage, frozen storage period and temperature and content of trimethylamine oxide (TMAO) and NaCl were evaluated in four series of storage experiments with cod from the Baltic Sea or the Barents Sea. During chill storage of thawed MAP cod fillets at 2 °C and 5 °C drip loss, TMA production, numbers of *P. phosphoreum*, total viable counts, TMAO, NaCl and sensory profiling were measured.

Materials and Methods

Storage experiments—general approach

Experiments with thawed, chilled MAP fillets were carried out using cod (*Gadus morhua*) from Baltic Sea or Barents Sea (**Table 1**). These two types of cod represented fish raw material from shallow or profound fishing grounds, respectively. The Baltic Sea cod were filleted at a local fishing company and transported in ice to the Danish Institute for Fisheries Research (DIFRES) where they arrived the day after catch and processing. Afterwards, the fresh Baltic Sea cod fillets were packed in a modified atmosphere using Riloten bags and trays including absorbent drip pads as previously described (Bøknæs *et al.*, 2000). The gas to fish ratio was always greater than 2 : 1 (vol/wt; 500 mL gas and 250-g cod fillets). The packed Baltic Sea cod fillets were frozen in a blast freezer for 4 h in MAP until core temperature had reached −30 °C. The Barents Sea cod fillets were produced on the Russian/Greenlandic freezer trawler 'Karelia'. These fish were headed and gutted immediately after catch and bled in seawater at 4 °C for about 30 min before being filleted in pre rigor-mortis state. Cod fillets were packed in interleaved blocks, frozen in a horizontal plate freezer for 2 h and placed in the cold store (−30 °C) on board the freezer trawler. After 10 weeks the 'frozen at sea' cod blocks were transported to DIFRES where they were stored at −30 °C for a further 5 weeks. Prior to chill storage, approximately 250-g portions of the frozen Barents Sea cod fillets were prepared by sawing. The frozen portions were packed in modified atmosphere as described above for fresh cod fillets. All MAP cod fillets were thawed by storage at 5 °C for 20 h. In all experiments, temperatures for frozen and chill storage were

Table 1 Outline of experiments

Experiment	Fish raw material and time of catch	Frozen storage period and temperature range	Chill storage period and temperature range
Effects of MAP during frozen storage on quality attributes of thawed chilled MAP Baltic Sea cod	Baltic Sea cod (spring)	8 weeks: −30.5 ± 0.5 °C	21 days at 2 °C[a]
Effects of frozen storage period and temperature regimes on quality attributes of thawed chilled MAP Baltic Sea cod	Baltic Sea cod (summer)	4 and 12 weeks[b]: −22.7 ± 0.6 °C, −21.9 ± 0.5 °C; 4 and 12 weeks[b]: −31.4 ± 0.2 °C, −31.2 ± 0.3 °C; 4 and 12 weeks[b]: −27.4 ± 4.3 °C, −25.8 ± 4.6 °C	21 days at 2 °C[a]
Effects of fishing ground and related parameters on quality attributes of thawed chilled MAP cod	Baltic Sea cod (winter) Barents Sea cod (winter)	7 weeks: −26.8 ± 0.6 °C 15 weeks: −29.9 ± 3.1 °C	21 days at 2 °C[a] 14 days at 5 °C[a]
Effects of added TMAO and NaCl on quality attributes of thawed chilled MAP Baltic Sea cod fillets	Baltic Sea cod (spring)	7 weeks: −33.0 ± 0.9 °C	21 days at 2 °C[c]

[a] All batches were packed in atmospheres of 40%CO_2/40%N_2/20%O_2.
[b] No significant ($P > 0.05$) difference between frozen storage temperatures of 4 and 12 weeks.
[c] All batches were packed in atmospheres of 40%CO_2/60%N_2.

recorded by loggers (Tinytag Gemini Data Loggers Ltd, Chichester, U.K.).

Effects of MAP during frozen storage on quality attributes of thawed chilled MAP Baltic Sea cod. Baltic Sea cod fillets were packed either in air or MAP prior to frozen storage. After thawing and during subsequent MAP chill storage of 0, 8, 14 and 21 days at 2 °C, seven packs were removed from each batch and analysed. During chill storage of MAP samples, three separate packs were used for microbiological analyses including numbers of TVC and *P. phosphoreum* and chemical analysis including TMA. Four packs were used for sensory evaluation. Furthermore, seven packs were used for determination of drip loss and gas compostion of the modified atmosphere.

Effects of frozen storage period and temperature regimes on quality attributes of thawed chilled MAP Baltic Sea cod. The effects of 4 and 12 weeks of frozen storage at approximately −20 °C and −30 °C on quality attributes of thawed chilled MAP Baltic Sea cod fillets were evaluated (**Table 1**). Frozen storage temperatures of approximately −20 °C and −30 °C were chosen to reflect conditions used in commercial practice (Bøknæs *et al.*, 2001). In addition, fluctuating frozen storage regimes were obtained by moving cod fillets between −20 °C and −30 °C every week. Four times during chill storage at 2 °C on day 0, 8, 14 and 21, seven packs were removed from each batch for analysis. Three separate packs were analysed for TMA production, total viable counts (TVC) and numbers of *P. phosphoreum*. Drip loss and gas composition of the modified atmosphere were determined for all seven packs.

Effects of fishing ground and related parameters on quality attributes of thawed chilled MAP cod. The effect of fishing ground and frozen storage at approximately −30 °C on quality attributes of thawed MAP cod fillets stored at 2 °C and 5 °C were evaluated (**Table 1**). Three packs from each batch of thawed MAP fillets were analysed at the start and at the end of the experiment for numbers of *P. phosphoreum*, TVC, TMA, TMAO, NaCl, drip loss and gas composition of the modified atmosphere.

Effects of TMAO and NaCl addition on quality attributes of thawed chilled MAP Baltic Sea cod fillets. Effects of TMAO and NaCl addition on quality attributes of thawed chilled MAP Baltic Sea cod fillets were studied (**Table 1**). Particularly, addition of TMAO and NaCl prior to frozen storage was compared with addition after thawing and prior to chill storage. Uniformly sized cod loins of approximately 50 g were treated by dipping in water solutions (2 °C) containing 50 g/L NaCl and 70 g/L TMAO dihydrat (Fluka No. 92277) for 5 min. A batch of cod loins, without addition of TMAO and NaCl, was also studied. The three batches of cod loins were all vacuum packaged in Riloten bags before frozen storage at −30 °C for 7 weeks and after thawing all batches were packed in modified atmosphere and chill-stored at 2 °C (**Table 1**). Four times during chill storage at 2 °C on day 0, 7, 14 and 21, three packs from each batch were removed and analysed for TVC, *P. phosphoreum*, TMA, NaCl, TMAO and gas composition of the modified atmosphere.

Analyses and Statistics

Composition of the modified atmosphere was measured for each pack using a gas analyser (PBI Dansensor Ltd. Ringsted Denmark). Numbers of *P. phosphoreum* were determined by a conductance method and total viable counts (TVC) by spread plating on Long and Hammer's medium containing 10 g/L NaCl (incubated aerobically at 15 °C for 7 days) as previously described (Dalgaard *et al.*, 1996, 1997a). *Photobacterium phosphoreum* and TVC analyses were both determined in triplicate. Trimethylamine (TMA) and trimethylamine oxide (TMAO) were determined in triplicate by a modification of the method of Conway (Conway and Byrne, 1933). The percentage drip loss was determined from the drained weight of thawed cod fillets at each sampling period. NaCl was determined in duplicate by using the Chlorine as Sodium Chloride method (AOAC, 1995). Sensory evaluation of thawed cooked cod was performed by a panel of four to seven trained assessors as previously described (Guldager *et al.*, 1998). At each sampling, each assessor evaluated two heated cod loins from each batch. Sensory changes during chill storage of thawed MAP cod fillets were described by using a category scale corresponding to low and high intensity of sensory attributes like juiciness and amine odour. Very high and low levels of juiciness and amine odour were indicated by 10 and 0, respectively. Previous studies found MAP cod fillets to be sensory rejected when numbers of *P. phosphoreum* exceed 7 log cfu/g and TMA production were higher than 10–30 mg TMA-N/100 g (Cann *et al.*, 1984; Dalgaard *et al.*, 1993). These criteria used to determine the end of the shelf-life were also applied to the present study. To detect significant differences between batches and treatments, one-way analysis of variance and the Tukey-Kramer multiple comparison test were applied using the Graphpad software (Sokal and Rohlf, 1985; Anon., 1993).

Results and Discussion

Effects of MAP during frozen storage on quality attributes of thawed chilled MAP Baltic Sea cod

During chill storage, significant ($P < 0.05$) higher drip loss was detected for cod fillets packed in MAP as compared to air under frozen storage (**Table 2**). After 8 days of chill storage, drip loss for both types of packaging was above 10% indicating drip loss as a potential problem for commercial use of Baltic Sea cod in thawed MAP products. There was shown to be no significant ($P > 0.05$) effects of packaging, during frozen storage, on numbers

Table 2 Effects of MAP during frozen storage on levels of *Photobacterium phosphoreum*, TVC, TMA, drip loss, amine odour and juiciness for thawed chilled stored (1.3 ± 0.6 °C) MAP Baltic Sea cod fillets after frozen storage in either MAP or air (average ± standard deviation). During chill storage, average gas composition of packs was 34.2 ± 3.1% CO_2

	Packaging in MAP during frozen storage				Packaging in air during frozen storage			
Chill storage in MAP	0 d[a]	8 d	14 d	21 d	0 d	8 d	14 d	21 d
P. phosphoreum, log (cfu/g)	b.d[b]	1.1 ± 0.8	b.d.	3.7 ± 3.4	b.d.	b.d.	b.d.	2.3 ± 1.9
TVC, log (cfu/g)	5.8 ± 0.1	5.6 ± 0.2	5.9 ± 0.1	7.1 ± 0.3	5.5 ± 0.1	5.6 ± 0.2	5.8 ± 0.1	6.8 ± 0.3
TMA, mg TMA-N/100 g	2.3 ± 1.3	1.1 ± 0.8	0.0 ± 0.2	0.6 ± 0.2	2.6 ± 0.2	1.3 ± 0.6	0.6 ± 0.2	3.0 ± 3.4
Drip loss, %	2.5 ± 1.1	13.0 ± 1.4	12.8 ± 1.4	13.1 ± 1.5	4.9 ± 1.5	10.0 ± 1.1	10.5 ± 0.7	13.3 ± 1.6
Amine odour	0.1 ± 0.3	2.2 ± 2.4	2.6 ± 1.5	2.1 ± 1.3	0.3 ± 0.5	1.7 ± 1.4	2.5 ± 1.7	2.3 ± 1.6
Juiciness.	5.4 ± 1.8	3.0 ± 1.2	2.8 ± 1.4	3.4 ± 1.0	4.6 ± 2.5	3.9 ± 1.0	3.3 ± 1.4	2.8 ± 0.6

[a] Indicates days of chill storage in MAP.
[b] b.d. indicates below detection limit.

of *P. phosphoreum* and production of TMA in the thawed MAP products (**Table 2**). Consequently, retail packaging of sea-frozen fillets in MAP on-shore at a central factory seems more appropriate than at sea. Furthermore, the MAP products will take up substantial space on freezer trawlers.

Numbers of TVC increased significantly ($P < 0.001$) in thawed MAP fillets during 21 days at 2 °C. Packaging in air of MAP during frozen storage, however, did not influence TVC ($P > 0.05$) or sensory attributes i.e. amine odour and juiciness ($P > 0.05$) for thawed heated MAP fillets. The very low observed levels for amine odour, *P. phosphoreum* growth and TMA production agrees with recent studies concerning thawed chilled MAP cod products (Guldager *et al.*, 1998; Bøknæs *et al.*, 2000). Low levels (<5.4) for juiciness on heated cod products were found for all chill storage periods (**Table 2**). These low levels for juiciness corresponded to the relatively high measured drip loss for thawed MAP cod products during chill storage. Before freezing, the Baltic Sea fillets contained 41.4 ± 8.4 mg TMAO-N/100 g.

Effects of frozen storage period and temperature regimes on quality attributes of thawed chilled MAP Baltic Sea cod

Drip losses during chill storage of thawed MAP Baltic Sea cod fillets were significantly influenced ($P < 0.01$) by the frozen storage periods of 4 and 12 weeks but the three frozen storage temperature regimes had no clear effect on this quality attribute (**Table 3**). Drip losses after 8 days of chill storage of thawed MAP Baltic Sea cod increased to ~11% and ~16%, respectively, for fillets previously stored frozen during 4 or 12 weeks (**Table 3**). These values correspond to drip losses of ~13% and ~10%, respectively, observed after 8 days of chill storage at 2 °C for thawed Baltic Sea fillets previous kept for 8 weeks at 2 °C (**Table 2**). That drip losses increase with time of frozen storage suggests Baltic Sea cod as a less suitable raw material for production of thawed chilled MAP products. Guldager *et al.* (1998) found somewhat lower drip losses of ~8% after up to 10 days of chill storage at 2 °C for thawed MAP Baltic Sea cod caught in winter and kept at −20 °C for 8 weeks prior to thawing. These differences in drip losses might be related to seasonal variability in the physical properties of cod (Love, 1975; Love, 1988). Frozen storage for both 4 and 12 weeks at a constant temperature of −20 °C or by frozen storage temperatures fluctuating between −20 °C and −30 °C, prevented growth of *P. phosphoreum* during 21 days of chill storage at 2 °C (**Table 3**). With frozen storage at −30 °C, growth of *P. phosphoreum* was detected after 21 days at 2 °C but the increase in cell levels was not significant ($P > 0.05$). This frozen storage inhibition of *P. phosphoreum* at −20 °C and −30 °C is in agreement with recent studies (Guldager *et al.*, 1998; Bøknæs *et al.*, 2000). Following all three frozen storage regimes, numbers of TVC increased significantly ($P < 0.001$) (**Table 3**) as has been observed previously for thawed and aerobically stored seafood (Shewan, 1961; Davies and Obafemi, 1985; Simmonds and Lamprecht, 1985). No significant amounts of TMA ($P > 0.05$) were produced in the six batches of Baltic Sea fillets (**Table 3**). This was expected due to the frozen storage inactivation of *P. phosphoreum*; previous studies showing this specific spoilage organism to be responsible for production of TMA in fresh MAP cod fillets (Dalgaard *et al.*, 1993; Dalgaard, 1995b). Before freezing, the Baltic Sea fillets contained 38.4 ± 3.6 mg TMAO-N/100 g. There was no significant ($P > 0.05$) difference between the chill storage temperature of 4 and 12 weeks.

Effects of fishing ground and related parameters on quality attributes of thawed chilled MAP cod

Cod fillets from the Barents Sea contained significantly ($P < 0.001$) higher concentrations of TMAO and NaCl as compared to Baltic Sea cod fillets (**Table 4**). The TMAO content of gadoids has been suggested to increase with depth, salinity and temperature of the water where the fish lives (Shewan, 1951; Hebard *et al.*, 1982; Gillett *et al.*, 1997). This could explain the high TMAO content in Barents Sea cod as well as the high levels of TMA previously found to be produced in these products as compared to North Sea and Faroe Bank cod (Ehrenberg and Shewan, 1955; Reay, 1957; Oehlenschläger, 1998). A NaCl level of 1.5 g/L, and thus equal to cod raw

material from the Baltic Sea, has been determined in Barents Sea cod which were not processed at sea on freezer trawlers (Sørensen, pers. comm.). Consequently, the higher NaCl content of Barents Sea cod fillets found in the present study might be related to the use of seawater during processing of 'frozen at sea' fillets on board the freezer trawlers.

The present study showed drip losses of thawed chilled Barents Sea cod fillets after 15 weeks of frozen storage to be significantly ($P < 0.001$) lower than observed for similar products produced from Baltic Sea cod after 4–12 weeks of frozen storage (**Table 3** and **Table 4**). The high TMAO and NaCl content of Barents Sea cod most likely protects proteins against denaturation during frozen storage and thereby improves their water holding capacity therefore contributing to a low drip loss (Owusu-Ansah and Hultin, 1984; Fennema, 1990). Production of high quality frozen and aerobically stored products from Barents Sea cod is possible as recently documented (Bøknæs *et al.*, 2001) and Barents Sea 'frozen at sea' cod fillets appears as an interesting fish raw material for production of thawed chilled MAP products.

After thawing, initial numbers of TVC were significantly ($P < 0.001$) higher for Baltic Sea cod fillets as compared to Barents Sea cod fillets (**Table 4**). This difference probably reflects a different microbiology of the warmer Baltic waters. In fact, warmer waters usually contain higher microbiological loads (Shewan, 1977). Nevertheless, initial numbers of TVC are most unlikely to influence shelf-life and other quality attributes of thawed chilled MAP cod fillets (Guldager *et al.*, 1998).

Opposed to data with Baltic Sea cod, significant ($P < 0.001$) growth of *P. phosphoreum* and production of TMA were observed in thawed MAP Barents Sea fillets at 2 °C and 5 °C (**Table 4**). The lack of frozen storage inactivation of *P. phosphoreum* in Barents Sea cod resulted in production of very large amounts of TMA and thereby influenced both shelf-life and quality attributes of thawed MAP fillets. We have found no previous reports suggesting frozen storage inactivation of micro-organisms in cod fishes can be as different as observed in the present study for *P. phosphoreum* in Baltic Sea and Barents Sea cod. Therefore, possible reasons to explain the differences were evaluated.

Effects of TMAO and NaCl addition on quality attributes of thawed chilled MAP Baltic Sea cod fillets

Significantly ($P < 0.001$) higher values for *P. phosphoreum* (**Fig. 1**), TMA and TVC (**Table 5**) were detected during chill storage, for Baltic Sea fillets with TMAO and NaCl added prior to frozen storage (Batch B) as compared to fillets with addition of TMAO and NaCl after thawing (Batch A) or no addition of the two compounds (Batch C). Frozen storage inactivation of *P. phosphoreum* was clearly counteracted by addition of TMAO and NaCl to Baltic Sea cod fillets prior to freezing (**Fig. 1**). In fact, the level of *P. phosphoreum* of 5.7 log cfu/g in batch B after 7 days (**Fig. 1**) was similar to approximate 7 log cfu/g predicted by using a mathematical model for

Table 3 Effects of frozen storage period and temperature regimes on levels of *Photobacterium phosphoreum*, total viable counts (TVC), TMA and drip loss (average ± standard deviation) in thawed MAP Baltic Sea cod fillets stored at 2 °C. During chill storage, average gas composition of packs was 34.9 ± 3.2% CO_2

Frozen storage temperature (°C)	− 20 °C				− 30 °C			− 20 °C/ − 30 °C			
	0 d[a]	8 d	14 d	21 d	0 d	8 d	14 d	21 d	0 d	8 d	14 d	21 d
4 weeks of frozen storage[b]												
P. phosphoreum, log (cfu/g)	b.d.[c]	b.d.	b.d.	b.d.	b.d.	b.d.	0.9 ± 0.5	2.7 ± 2.2	b.d.	b.d.	b.d.	b.d.
TVC, log (cfu/g)	4.4 ± 0.1	4.6 ± 0.2	6.5 ± 0.1	6.8 ± 0.3	4.3 ± 0.3	4.8 ± 0.3	6.5 ± 0.1	7.8 ± 0.5	4.1 ± 0.3	5.0 ± 0.4	6.2 ± 0.1	7.5 ± 0.1
TMA, mg TMA-N/100 g	3.1 ± 0.1	4.6 ± 1.6	b.d.	0.7 ± 0.2	2.3 ± 2.0	1.1 ± 1.2	b.d.	b.d.	0.3 ± 2.7	0.1 ± 0.7	b.d.	b.d.
Drip loss, %	2.9 ± 1.8	11.0 ± 1.4	12.8 ± 2.5	13.2 ± 2.1	2.9 ± 1.3	10.5 ± 0.7	13.2 ± 1.6	13.5 ± 1.0	3.4 ± 1.5	10.8 ± 0.7	12.7 ± 1.6	13.7 ± 2.3
12 weeks of frozen storage[d]												
P. phosphoreum, log (cfu/g)	n.m.[e]	b.d.	b.d.	b.d.	n.m.	b.d.	b.d.	0.7 ± 0.2	n.m.	b.d.	b.d.	b.d.
TVC, log (cfu/g)	n.m.	5.0 ± 0.1	4.9 ± 0.2	7.3 ± 0.6	n.m.	5.0 ± 0.2	5.8 ± 0.1	7.7 ± 0.1	n.m.	4.5 ± 0.2	4.8 ± 0.3	7.9 ± 0.7
TMA, mg TMA-N/100 g	1.4 ± 1.8	2.0 ± 0.6	2.2 ± 0.7	2.0 ± 0.4	1.2 ± 0.4	0.7 ± 0.4	2.0 ± 0.4	1.4 ± 0.3	1.9 ± 1.6	2.0 ± 0.9	1.3 ± 1.3	0.4 ± 0.5
Drip loss, %	1.7 ± 0.6	15.6 ± 2.4	16.5 ± 1.6	18.8 ± 2.3	3.0 ± 1.6	16.8 ± 1.9	16.1 ± 1.5	18.1 ± 0.9	2.0 ± 0.6	17.7 ± 1.8	18.5 ± 2.3	18.8 ± 0.4

[a] d indicates days of chill storage in MAP.
[b] Chill storage temperature was 2.6 ± 1.1 °C.
[c] b.d. indicates below detection limit.
[d] Chill storage temperature was 2.5 ± 0.8 °C.
[e] n.m. indicates not measured.

Table 4 Quality attributes of chill stored thawed MAP cod fillets produced from Baltic Sea or Barents Sea cod raw material. Products were chill stored at 2 °C (0 and 21 days) and 5 °C (0 and 14 days), respectively. During chill storage at 2 °C and 5 °C respectively, average gas composition of packs during chill storage was (35.2 ± 2.2% CO_2) and (37.3 ± 3.1 CO_2). TMAO and NaCl contents were measured before chill storage

	2 °C		5 °C	
	0 d	21 d	0 d	14 d
Barents Sea cod[a]				
P. phosphoreum, log (cfu/g)	b.d.[b]	8.2 ± 0.1	b.d.	8.1 ± 0.1
TVC, log (cfu/g)	3.1 ± 0.3	8.6 ± 0.8	3.1 ± 0.3	8.3 ± 0.2
TMA, mg TMA-N/100 g	0.9 ± 0.2	72.5 ± 17.2	0.9 ± 0.2	86.9 ± 14.8
Drip loss, %	3.7 ± 1.7	10.6 ± 1.2	3.7 ± 1.7	9.2 ± 2.0
Baltic Sea cod[c]				
P. phosphoreum, log (cfu/g)	b.d.	2.7 ± 1.8	b.d.	5.1 ± 0.2
TVC, log (cfu/g)	6.0 ± 0.2	8.2 ± 0.7	6.0 ± 0.2	9.2 ± 0.3
TMA, mg TMA-N/100 g	b.d.	2.8 ± 3.7	b.d.	40.6 ± 10.2
Drip loss, %	5.6 ± 0.1	12.2 ± 0.8	5.6 ± 0.1	11.3 ± 2.5

[a] Product characteristics for Barents Sea cod: TMAO = 102.3 ± 5.1 mg TMAO-N/100 g; NaCl = 4.7 ± 0.4 g/L; chill storage temperatures = 1.7 ± 0.7 °C and 5.3 ± 0.5 °C; frozen storage conditions = 15 weeks at −29.9 ± 3.1 °C.
[b] b.d. indicates below detection limit.
[c] Product characteristics for Baltic Sea cod: TMAO = 43.8 ± 7.5 mg TMAO-N/100 g; NaCl = 1.4 ± 0.1 g/L, chill storage temperatures = 2.0 ± 0.4 °C and 5.8 ± 0.4 °C; frozen storage conditions = (7 weeks at −26.8 ± 0.6 °C).

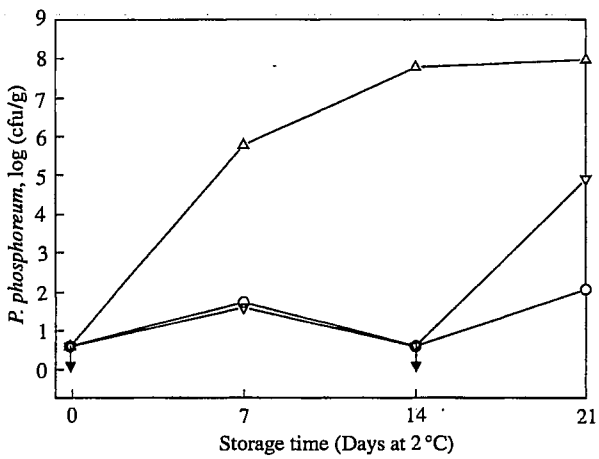

Fig. 1 Average numbers of *Photobacterium phosphoreum* in thawed MAP Baltic Sea cod fillets stored at 2 °C. Thawed untreated MAP cod fillets (○); thawed MAP cod fillets with added TMAO and NaCl prior to frozen storage (△) and thawed MAP cod fillets with added TMAO and NaCl after frozen storage and thawing (▽). Arrows indicate levels of *P. phosphoreum* below the limit of detection (<0.6 log cfu/g). This was observed at 0 days at 2 °C for all three batches (○, △, ▽) and after 14 days at 2 °C for two batches (○, ▽)

growth of this specific spoilage organism at 2.1 °C in fresh MAP cod fillets (Dalgaard *et al.*, 1997b).

As expected, growth of *P. phosphoreum* to high levels during chill storage resulted in production of high levels of TMA (**Table 5**). These results suggest the markedly different growth of *P. phosphoreum* and corresponding production of TMA during chill storage of thawed MAP Baltic Sea and Barents Sea fillets can be explained simply by the different contents of TMAO and NaCl in the two products (**Table 5**). These data are important as they provide a possible explanation as to why storage trials with thawed chilled MAP cod from different fishing grounds might not lead to the same conclusions with respect to effect of frozen storage on shelf-life. In fact, it might explain the recent reports of very long shelf-lives for thawed chilled MAP Baltic Sea fillets (Guldager *et al.*, 1998; Bøknæs *et al.*, 2000). Cod from the North Atlantic area is closely related to the Barents Sea cod and data presented here might be relevant to estimate effects of frozen storage on quality attributes of thawed MAP products produced from this fish raw material. Furthermore, our results suggest the use of freshwater instead of seawater for processing of thawed MAP Barents Sea cod might reduce growth of *P. phosphoreum* and therefore this would be interesting to evaluate in future studies.

Trimethylamine oxide is known to stabilize enzymes and other proteins (Owusu-Ansah and Hultin, 1984; Timasheff, 1992; Lin and Timasheff, 1994; Brown *et al.*, 1997; Gillett *et al.*, 1997; Yancey and Siebenaller, 1999). It is most likely that TMAO in the same way stabilizes proteins in cells of *P. phosphoreum* and thereby counteracts inactivation of the organism during frozen storage. TMAO and NaCl seem to have a weak stimulating effect on growth of *P. phosphoreum* when added to cod fillets after freezing and thawing (**Fig. 1**). This might result from an effect of TMAO on cell damage repair or the effect of salt as growth of this spoilage bacteria was shown to be optimal at approximately 10 g/L NaCl (Dalgaard, 1993). The present study showed growth of *P. phosphoreum* and production of TMA to be significantly more pronounced during chill storage of thawed MAP Barents Sea cod fillets as compared to thawed MAP Baltic Sea cod fillets. This difference was due to higher TMAO and NaCl contents in the 'frozen at sea' Barents Sea cod fillets. Nevertheless, Baltic Sea cod fillets were less suitable for production of thawed MAP products due to a substantial drip loss during chill storage. It seems most

Table 5 Effects of TMAO and NaCl on development of TMA and TVC (average ± standard deviation) in naturally contaminated thawed MAP Baltic Sea cod fillets during chill storage at 2.1 ± 0.8 °C. Average gas composition during chill storage was 37.0 ± 1.4% CO_2. TMAO and NaCl content were measured before chill storage (0 d)

			TMA (TMA-N/100 g)				TVC (log cfu/g)			
Batch	TMAO (mg TMAO-N/100 g)	NaCl (%)	0 d	7 d	14 d	21 d	0 d	7 d	14 d	21 d
A[a]	61.1 ± 6.6	0.08 ± 0.03	b.d.[b]	1.6 ± 0.9	1.8 ± 0.6	2.5 ± 0.1	5.8 ± 0.2	6.0 ± 0.1	5.7 ± 0.1	6.0 ± 0.5
B[c]	124.8 ± 7.4[d]	0.42 ± 0.05[d]	0.1 ± 0.2	1.4 ± 1.3	31.6 ± 4.3	96.2 ± 13.6	5.7 ± 0.0	6.1 ± 0.3	8.0 ± 0.2	8.3 ± 0.1
C[e]	134.8 ± 12.4[d]	0.47 ± 0.03[d]	b.d.	0.0 ± 0.1	1.5 ± 0.5	26.7 ± 18.3	5.7 ± 0.1	5.5 ± 0.4	6.3 ± 0.9	6.8 ± 0.9

[a] Thawed untreated MAP cod fillets.
[b] b.d. indicates below detection limit.
[c] Thawed MAP cod fillets treated with 50 g/L NaCl and 70 g/L TMAO in the fresh state before freezing.
[d] Significant ($P < 0.001$) higher TMAO and NaCl content for both treated batches compared with the untreated batch A was found.
[e] Thawed MAP cod fillets treated with 50 g/L NaCl and 70 g/L TMAO after freezing and thawing.

interesting in future studies to further evaluate shelf-life and quality attributes of thawed chilled MAP Barents Sea cod fillets obtained from 'frozen at sea' fish raw material.

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References

ANONYMOUS. *The Graphpad Instat 2.04a Users Guide.* San Diego: Graphpad Software (1993)

ANONYMOUS. *Parallel Food Testing in the European Union: Fish.* London: International Consumer Research & Testing Limited (1995)

AOAC (Association of Official Analytical Chemists). Official Method no. 937.09, Sect. 35.1.18, Salt (chlorine as sodium chloride) in seafood. In: *Official Methods of Analysis of AOAC International*, 16th Edn. Arlington: Association of Official Analytical Chemists, p. 7 (1995)

BØKNÆS, N., ØSTERBERG, C., NIELSEN, J. AND DALGAARD, P. Influence of freshness and frozen storage temperature on quality of thawed cod fillets stored in modified atmosphere packaging. *Lebensmittel-Wissenschaft und-Technologie*, **33**, 244–248 (2000)

BØKNÆS, N., GULDAGER, H. S., ØSTERBERG, C. AND NIELSEN, J. Production of high quality frozen cod (*Gadus morhua*) fillets and portions on a freezer trawler. *Journal of Aquatic Food Product Technology*, **10**, 33–47 (2001)

BROWN, C. R., HONG-BROWN, L. Q. AND WELCH, W. J. Correcting temperature-sensitive protein folding defects. *Journal of Clinical Investigation*, **99**, 1432–1444 (1997)

CANN, D. C., SMITH, G. L. AND HOUSTON, N. C. *Further Studies on Marine Fish Stored Under Modified Atmosphere Packaging.* Aberdeen: Torry Research Station, 61 pp. (1984)

CONWAY, E. J. AND BYRNE, A. An absorption apparatus for the micro-determination of certain volatile substances. *Biochemical Journal*, **27**, 419–429 (1933)

DALGAARD, P. *Evaluation and prediction of microbial fish spoilage.* Ph.D. thesis. Copenhagen: Royal Veterinary and Agricultural University (1993)

DALGAARD, P. The effect of anaerobic conditions and carbon dioxide. In: HUSS, H. H. (Ed.), *Quality and Quality Changes in Fresh Fish*, FAO Fisheries Technical Paper No. 348. Rome: FAO, pp. 78–83 (1995a)

DALGAARD, P. Qualitative and quantitative characterization of spoilage bacteria from packed fish. *International Journal of Food Microbiology*, **26**, 319–333 (1995b)

DALGAARD, P., GRAM, L. AND HUSS, H. H. Spoilage and shelf life of cod fillets packed in vacuum or modified atmosphere. *International Journal of Food Microbiology*, **19**, 283–294 (1993)

DALGAARD, P., MEJLHOLM, O., CHRISTIANSEN, T. J. AND HUSS, H. H. Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. *Letters in Applied Microbiology*, **24**, 373–378 (1997a)

DALGAARD, P., MEJLHOLM, O. AND HUSS, H. H. Conductance method for quantitative determination of *Photobacterium phosphoreum* in fish products. *Journal of Applied Bacteriology*, **81**, 57–64 (1996)

DALGAARD, P., MEJLHOLM, O. AND HUSS, H. H. Application of an iterative approach for development of a microbial model predicting the shelf-life of packed fish. *International Journal of Food Microbiology*, **38**, 169–179 (1997b)

DAVIES, R. AND OBAFEMI, A. Response of micro-organisms to freeze-thaw stress, In: ROBINSON, R. K. (Ed.), *Microbiology of Frozen Foods.* London: Elsevier, pp. 83–107 (1985)

DAVIS, H. K. Fish. In: PARRY, R. T. (Ed.), *Principles and Applications of Modified Atmosphere Packaging of Foods.* Glasgow: Chapman & Hall, pp. 189–228 (1993)

EHRENBERG, A. S. C. AND SHEWAN, J. M. Volatile bases and sensory quality-factors in iced white fish. *Journal of the Science of Food and Agriculture*, **6**, 207–217 (1955)

FARBER, J. M. Microbiological aspects of modified-atmosphere packaging technology—a review. *Journal of Food Protection*, **54**, 58–70 (1991)

FENNEMA, O. R. Comparative water holding properties of various muscle foods. A critical review relating to definitions, methods of measurements, governing factors, comparative data and mechanistic matters. *Journal of Muscle Foods*, **1**, 363–381 (1990)

GILLETT, M. B., SUKO, J. R., SANTOSO, F. O. AND YANCEY, P. H. Elevated levels of trimethylamine oxide in muscles of deep-sea gadiform teleosts: a high-pressure adaption? *The Journal of Experimental Zoology*, **279**, 386–391 (1997)

GULDAGER, H. S., BØKNÆS, N., ØSTERBERG, C., NIELSEN, J. AND DALGAARD, P. Thawed cod fillets spoil less rapidly than unfrozen fillets when stored under modified atmosphere at 2 °C. *Journal of Food Protection*, **61**, 1129–1136 (1998)

HEBARD, C. E., FLICK, G. J. AND MARTIN, R. E. Occurence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In: MARTIN, R. E., FLICK, G. J., HEBARD, C. E. AND WARD, D. R. (Eds), *Chemistry and Biochemistry of Marine Food Products*. Westport: AVI Publishing Company, pp. 149–304 (1982)

LANIER, T. C. AND KORHONEN, R. Modified atmosphere and conventional packaging of fresh and previously-frozen fish. In: MARTIN, R. E. (Ed.), *Proceedings first national conference on modified and controlled atmosphere packaging of seafood products, San Antonio*. Washington DC: The National Fisheries Institute, pp. 80–97 (1981)

LIN, T.-Y. AND TIMASHEFF, S. N. Why do some organisms use a urea-methylamine mixture as osmolyte? Thermodynamic compensation of urea and trimethylamine N-oxide inter-actions with proteins. *Biochemistry*, **33**, 12695–12701 (1994)

LOVE, R. M. Variability in atlantic cod (*Gadus morhua*) from the northeast atlantic: a review of seasonal and environmental influences on various attributes of the flesh. *Journal of Fisheries Research Board Canada*, **32**, 2333–2342 (1975)

LOVE, R. M. *The Food Fishes, Their Intrinsic Variation and Practical Implications*. London: Farrand Press, pp. 1–276 (1988)

OEHLENSCHLÄGER, J. Detailed experimental iced-storage characteristics of Barents Sea cod (*Gadus morhua*). *Information für Fischwirtschaft*, **45**, 35–42 (1998)

OWUSU-ANSAH, Y. AND HULTIN, H. O. Trimethylamine oxide prevents insolubilization of red hake muscle proteins during frozen storage. *Journal of Agricultural Food Chemical*, **32**, 1032–1035 (1984)

REAY, G. A. Improvement in quality of iced white fish. In: *Report of the Superintendent of the Torry Research Station and the Humber Laboratory*. Aberdeen: Torry Research Station, pp. 3–31 (1957)

SHEWAN, J. M. The chemistry and metabolism of the nitrogenous extractives in fish. In: WILLIAMS, R. T. (Ed.), *The Biochemistry of Fish*. Cambridge: University Press, pp. 28–49 (1951)

SHEWAN, J. M. The microbiology of sea-water fishes. In: BORGSTRØM, G. (Ed.), *Fish as Food*. London: Academic Press, pp. 487–560 (1961)

SHEWAN, J. M. The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In: *Proceedings of the Conference on Handling, Processing and Marketing of Tropical Fish*. London: Tropical Products Institute, pp. 51–56 (1977)

SIMMONDS, C. K. AND LAMPRECHT, E. C. Microbiology of frozen fish and related products. In: ROBINSON, R. K. (Ed.), *Microbiology of Frozen Foods*. London: Elsevier, pp. 169–208 (1985)

SOKAL, R. R. AND ROHLF, F. J. *Biometry: the Principles and practice of Statistics in Biological Research* (SOKAL, R. R. AND ROHLF, F. J. Eds.), 3rd Edn. San Francisco: W. H. Freeman and Co 887 pp. (1995)

TIMASHEFF, S. N. A physicochemical basis for the selection of osmolytes by nature. In: SOMERO, G. N., OSMOND, C. B. AND BOLIS, C. L. (Eds), *Water and Life: a Comparative Analysis of Water Relationships at the Organismic, Cellular and Molecular*. Berlin: Springer-Verlag, pp. 70–84 (1992)

YANCEY, P. H. AND SIEBENALLER, J. F. Trimethylamine oxide stabilizes teleost and mammalian lactate dehydrogenases against inactivation by hydrostatic pressure and trypsinolysis. *The Journal of Experimental Biology*, **202**, 3597–3603 (1999)

Artikel IV

Bøknæs, N., Jensen, K.N., Guldager, H.S., Østerberg, C., Nielsen, J. & Dalgaard, P. (2002). Thawed chilled cod fillets in modified atmosphere packaging – application of multivariate data analysis to select key parameters in good manufacturing practice. *Food Science and Technology,* **35**, 436–443.

Thawed Chilled Barents Sea Cod Fillets in Modified Atmosphere Packaging-Application of Multivariate Data Analysis to Select Key Parameters in Good Manufacturing Practice

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The purpose of the present study was to select key parameters in good manufacturing practice for production of thawed chilled modified atmosphere packed (MAP) cod (Gadus morhua) fillets. The effect of frozen storage temperature (−20 and −30 °C), frozen storage period (3, 6, 9 and 12 mo) and chill storage periods up to 21 d at 2 °C were evaluated for thawed MAP Barents Sea cod fillets. Sensory, chemical, microbiological and physical quality attributes were evaluated and multivariate data analysis (principal component analysis and partial least-squares regression) applied for identification of key parameters in good manufacturing practice for this product. Frozen storage of up to 12 mo had no significant effect on quality attributes and shelf-life at 2 °C was above 14 d irrespective of the time of frozen storage. As compared to a previous study with Baltic Sea, cod drip losses during chill storage was low for thawed MAP Barents Sea cod and this fish raw material seemed the more appropriate for production of thawed chilled MAP products. Frozen storage inactivation of the spoilage bacteria of Photobacterium phosphoreum was modest in Barents Sea cod, possibly due to high trimethylamine oxide (TMAO) and NaCl contents.

Keywords: frozen at sea; MAP; *Photobacterium phosphoreum*; sensory evaluation; TMA

Introduction

Modified atmosphere packaging (MAP) is an increasingly popular food preservation technique. Consumer demands for fresh and convenient foods free of chemical preservation has led to growth in the use of MAP and this technique may also reduce wastage and extend shelf-life of a range of seafoods (Farber, 1991; Haard, 1992; Church, 1998). At the same time the use of frozen seafood as raw material for chilled products is becoming more acceptable on the European fish market (Herborg, 1986). Frozen-at-sea technology allows fish raw material to be frozen in a pristine state and both supply and distribution of these products can be more regular and flexible than for fresh fish (Bøknæs *et al.*, 2001). As an example, frozen-at-sea fish fillets could be retail packed in modified atmosphere at centralized factories and distributed globally in the frozen state independent of e.g. season, weather or distance from fishing grounds. Then products could be thawed in shops and sold in the same way as fresh MAP fillets (Lanier and Korhonen, 1981). Furthermore, shelf-life of thawed MAP Baltic Sea cod was extended as compared to fresh MAP cod. This was due to inactivation of the specific spoilage organism (SSO) *Photobacterium phosphoreum* resulting in a very limited formation of trimethylamine (TMA) (Guldager *et al.*, 1998; Bøknæs *et al.*, 2000). However, application of frozen fish raw material cannot be used uncritically in production of thawed MAP fish. Unacceptable drip losses (DL) of 14–19% have been observed in thawed MAP fillets of Baltic Sea cod and with thawed MAP Barents Sea cod, previously kept at −30 °C, growth of *P. phosphoreum* and marked formation of TMA occurred (Bøknæs *et al.*, 2002). For production and distribution of thawed chilled MAP cod products, processing parameters resulting in least TMA formation, drip loss and sensory changes together with a maximal inactivation of spoilage microorganisms clearly remain to be determined.

The objectives of the present study were to determine effects of (i) frozen storage temperature, (ii) frozen storage period and (iii) chill storage period on quality attributes of thawed MAP cod fillets from the Barents

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Sea. Cod fillets were kept at −20 and −30 °C for 3, 6, 9 and 12 mo prior to thawing and MAP storage at 2 °C for 21 d. Quality attributes of the thawed MAP cod were assessed at regular intervals during chilled storage by using sensory, chemical, microbiological and physical analyses including amongst others the determination of TMA, drip loss and specific counts of *P. phosphoreum*. Multivariate data analysis and particularly partial least-squares regression (PLSR) was used to analyse relations between storage conditions and product quality attributes.

Table 1 Experimental design

Design variable	Level
Frozen storage temperature (°C)	−20, −30[a]
Frozen storage period (mo)	3, 6, 9 and 12
Chill storage period (d)	0, 3, 7, 14 and 21

[a]The first three months, all batches were stored at commercial temperatures of close to −30 °C on board the freezer trawler and at −25 to −30 °C during transport from the Barents Sea to DIFRES, respectively.

Materials and Methods

Storage trials and experimental design

Cods (*Gadus morhua*) were caught, processed and frozen on board a Russian/Greenlandic freezer trawler in February 1999 in the Norwegian zone (72 °N, 16 °W) of the Barents Sea. Cods from a single catch were processed and storage trials with thawed MAP cod fillets were later carried out by using a factorial design with two frozen storage temperatures, four frozen storage periods and five chill storage periods. Due to capacity onboard the freezer trawler, it was not possible in practice to study a sub-batch of cod fillets after 3 mo of frozen storage at −20 °C (**Table 1**).

Tow duration was 6 h and catch size about 6 metric tonne, primarily cods. Cods of 1.5–3 kg were deheaded and gutted immediately after catch. The fish were bled in seawater for about 30 min at 4 °C and then filleted and skinned by, respectively, a BAADER 190 and BAADER 51 (Nordischer Maschinenbau Rud. BAADER, Lübeck, Deutchland). Cod fillets were trimmed manually for bones, parasites and blood stains. Boneless fillets were manually interleave packed after separating cod fillets with plastic film in blocks. The packed fillet blocks each containing ca. 6.8 kg were frozen in a horizontal plate freezer until a core temperature of −25 °C was reached (ca. 2 h). Frozen cod blocks were packed in cardboard boxes and placed in the cold store on board the freezer trawler (ca. −30 °C). After 10 wk, the frozen blocks were landed and transported to the Danish Institute for Seafood Research (DIFRES) where they were randomly divided into two portions and kept at −20 and −30 °C, respectively. From each portion, frozen blocks were collected after 3, 6, 9 and 12 mo and sawed into pieces of less than 100 g. Afterwards, frozen cod pieces weighing altogether ca. 250 g were placed in trays with absorbent drip pads and packed in Riloten bags (Danisco Flexible, Lyngby, Denmark) containing a modified atmosphere with 40% CO_2, 40% N_2 and 20% O_2 (AGA, Copenhagen, Denmark). The fish to gas ratio was ca. 250 g cod to ca. 500 mL gas. Packed fillet pieces were then thawed for 20 h at 5 °C whereafter they were transferred for chill storage at 2 °C. After 0, 3, 7, 14 and 21 d of chill storage, 12 packs from each sub-batch were removed for analysis. Three separate packs were analysed by microbiological, chemical and physical methods, whereas, nine different packs were used for sensory evaluation. Gas composition (Combi Check 9800-1, PBI Dansensor Ltd., Ringsted, Denmark) and drip loss were determined for all 12 packs. Temperature during all periods of frozen and chill storage were recorded by loggers (Tinytag, Gemini Data Loggers Ltd., Chichester, U.K.).

Microbiological analyses

P. phosphoreum was enumerated specifically by a conductance method and total viable counts (TVC) were determined by spread plating on the Long and Hammer medium containing 1 g NaCl/100 g (15 °C, 7 d). Both microbiological analyses were carried out in triplicate as previously described (Dalgaard *et al.*, 1996; 1997*a*).

Chemical and physical analyses

TMA and trimethylamine oxide (TMAO) were determined in triplicate by a modified Conway microdiffusion method (Conway and Byrne, 1933). pH was measured on the Conway extract by using an Autocal pH meter (Radiometer, Copenhagen, Denmark). Water holding capacity (WHC) was determined with eight measurements as the relative water loss using a mild centrifugation of minced fish muscle (Eide *et al.*, 1982). The content of NaCl was determined in duplicate as chloride (AOAC, 1995). Free formaldehyde (FA) was determined on Conway extracts in duplicate by the Nash test as described by Bechmann (1998). Also dimethylamine (DMA) content was determined in Conway extracts and to do this a newly developed capillary electrophoresis method was applied (Timm and Jørgensen, 2002). The percentage drip loss was determined as the drained weight of thawed cod fillets at each sampling time. For each sub-batch of packs TMA, pH, drip loss, DMA, NaCl and WHC were determined at each sampling time, whereas, TMAO and FA were determined only on the first day of chill storage.

Sensory analyses

Sixteen assessors were used to evaluate the chilled products. The assessors were trained in profiling analysis of heated samples. Changes in quality attributes during chilled storage of MAP fillets were described using a category scale from 0 to 15 corresponding to low and high intensity, respectively. The sensory quality attributes were frozen storage odour and taste (FSO and FST), amine odour and taste (AO and AT) together

with juiciness (JCN). These attributes were selected in a recently published study concerning thawed chilled MAP cod fillets and sensory analyses were carried out as described in that study (Guldager *et al.*, 1998). At each sampling time, each assessor evaluated two heated cod loins from each sub-batch. Samples were served to each assessor in randomized order of duplicates and sub-batches to minimize possible carry-over effects between samples from different sub-batches. At each sensory session one extra sample was included in the randomized order to prevent the assessors from guessing the sensory scores of samples due to relative time of chilled storage. The degree of freshness of the extra sample differed between sensory sessions and data from these samples were not included in the study. Fizz Network software (Anonymous, 1995) was used for collection and evaluation of sensory results.

Data analysis

The significance of observed differences between initial storage characteristics was tested by one-way analysis of variance followed by the Tukey–Kramer post-test. Principal component analysis (PCA) was used for classification of samples on the basis of microbiological, sensory, chemical and physical data (Martens and Næs, 1989). The data matrix consisted of 35 samples (rows) differing with respect to frozen storage temperature, frozen storage period and chill storage period. The columns in the data matrix contained 11 quality attributes: TVCs, TMA content, pH, WHC, DL, number of *P. phosphoreum* and normalized values for assessments of the five sensory attributes: amine odour, amine taste, frozen storage odour, frozen storage taste and juiciness. All quality attributes were averaged over values within batches (chill storage periods) and 'standardized' by subtracting the column mean and dividing the result by the column standard deviation. The sensory values were normalized for panel performance using an assessor reliability test (Thybo and Martens, 2000). In that way, prior to the main data analysis differences in the levels of sensory score levels for the assessors were projected away by a preliminary ANOVA PLSR (Jacobsen *et al.*, 2001). In order to identify the effect of the various storage treatments on the measured quality attributes an ANOVA PLSR was calculated, as a mean of selecting key parameters in good manufacturing practice for this new retail fish product. For this ANOVA PLSR, the *X*-matrix consists of columns with indicator variables (values either 0 or 1), in this case representing the experimental design (frozen storage temperature, frozen storage period and chill storage period). The *Y*-matrix consists of response variables in this case the measured quality attributes. In both matrices, each row represents a sample. The ANOVA PLSR thus relates the given design parameters to structure in the response variables. All variables were standardized as described above for PCA. Full cross validation and a newly developed modified Jack-knife estimation of parameter uncertainty (Martens and Martens, 1999) was used for interpretation of the design parameters and quality attributes. All multivariate data analysis was performed using the software programme Unscrambler version 7.5 (Camo, Trondheim, Norway).

Results and Discussion

Storage conditions and product characteristics

Temperatures of both frozen and chilled storage were close to the target values (**Table 2**). The average percentage of CO_2 in the modified atmosphere was $33.7 \pm 3.5\%$ during chill storage and no significant difference between sub-batches was observed ($P > 0.05$). After thawing and prior to chill storage products contained 105.2 ± 8.6 mg TMAO-N/100 g and 0.32 ± 0.10 g NaCl/100 g, again no significant differences between batches were observed ($P > 0.05$).

Effect of storage conditions on quality attributes as determined by multivariate analysis

PCA of the 11 microbiological, chemical, physical and sensory quality attributes i.e. the *Y*-variables provided a first and a second principal component (PC1 and PC2) that explained 52 and 22%, respectively, of the total variation in these data. A bi-plot indicated PC1 accounted mainly for variation due to the chill storage period of fillets previously kept at −30 °C (sub-batches A, B, D and F). In fact, samples chilled stored for 0 d were placed to the left while samples stored for 14 and 21 d were placed to the right (**Fig. 1**). PC2 primarily explained variation between sub-batches previously stored at −20 or −30 °C. Furthermore, changes in

Table 2 Storage characteristics for all batches. Results were shown as average ± standard deviation (S.D.).

Batch	Storage period (mo)	Frozen storage temperature (°C)	Chill storage temperature (°C)
A (−30 °C)	3	-29.0 ± 2.7^a	2.1 ± 0.5^b
B (−30 °C)	6	-31.5 ± 2.9^a	2.5 ± 0.6^b
C (−20 °C)	6	-24.6 ± 4.3^c	2.5 ± 0.6^b
D (−30 °C)	9	-32.2 ± 2.6^a	2.1 ± 0.7^b
E (−20 °C)	9	-23.5 ± 4.0^c	2.1 ± 0.7^b
F (−30 °C)	12	-32.4 ± 3.4^a	2.5 ± 0.2^b
G (−20 °C)	12	-23.4 ± 3.6^c	2.5 ± 0.2^b

[a]No significant differences between frozen storage temperatures around −30 °C ($P > 0.05$).
[b]No significant differences between chill storage temperatures at 2 °C ($P > 0.05$).
[c]No significant differences between frozen storage temperatures around −20 °C ($P > 0.05$).

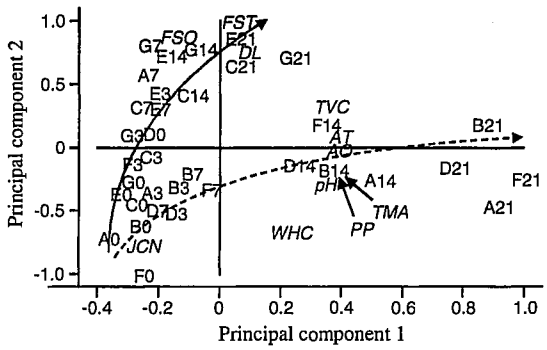

Fig. 1 Bi-plot of PCA scores and loadings characterizing cod samples and quality attributes, respectively. Letters indicate sub-batches exposed to different frozen storage temperatures and frozen storage periods (see **Table 2**). Numbers indicate the period at 2 °C in days. Abbreviations for quality attributes is provided in **Table 3**. Changes during chill storage were related to the previous frozen storage temperatures of −20 °C (solid line) and −30 °C (dashed line)

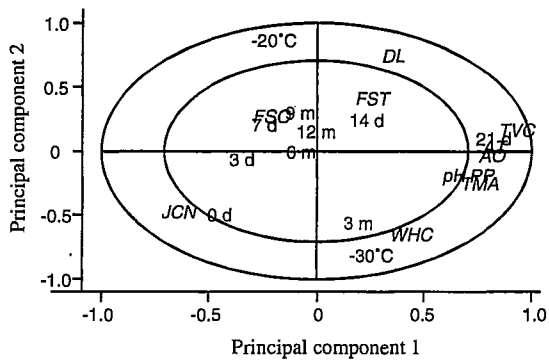

Fig. 2 Correlation loadings plot from ANOVA PLSR analysis of quality attributes. The design variables were frozen storage temperature (−20, −30 °C), frozen storage period months (3, 6, 9 and 12 mo) and chill storage period at 2 °C (0, 3, 7, 14 and 21 d). Abbreviations used are explained in **Table 3**. Inner and outer ellipses indicate 50 and 100% explained variance

FSO and FST were observed for samples stored at −20 °C. All samples stored for a short chill storage period (batch A0, B0, C0, D0, E0, F0 and G0) were characterized by relatively high values for JCN as they were placed near and thus positively correlated with this attribute. In contrast, samples frozen stored at −20 °C and subsequently chilled stored for 21 d (G21, E21 and C21) were negatively correlated to JCN. The opposite scenario was seen for DL. In addition, samples frozen stored at −20 °C from day 7 and 14 (batches C, E and G) were negatively correlated to WHC. The bi-plot also illustrated that microbiological changes during chill storage was different in samples frozen stored at −30 °C (dashed arrow in **Fig. 1**) and at −20 °C (solid arrow in **Fig. 1**). Samples frozen stored at −30 °C from day 14 (A14, B14, D14 and F14) were positively correlated to levels of *P. phosphoreum* (PP), content of TMA, AO, AT and pH. Whereas none of the samples frozen stored at −20 °C were placed near these attributes. Interestingly, no clear effect of frozen storage period was identified by the PCA.

Based on all quality attributes including nonnormalized sensory data, the ANOVA PLSR resulted in a model with five PCs. PC 1 and PC 2 explained 27% of the total variance in the design variables, describing storage conditions, and 66% of the total variance in quality attribute data. After using an assessor reliability test (Thybo and Martens, 2000; Jacobsen *et al.*, 2001) on sensory data for obtaining normalized sensory values, this ANOVA PLSR model was reduced to only two PCs that explained 27 and 64% of the total variance in *X*- and *Y*-variables, respectively.

The two concentric circles in **Fig. 2** indicated positions where 50% (inner ellipse) and 100% (outer ellipse) of variability in scores of the 11 quality attributes were explained by the ANOVA PLSR model with two PCs. Variables located near each other and close to the outer ellipse are thus positively correlated with a correlation coefficient of ~1.0. With the exception of scores for FSO and FST more than 50% of the variation in all sensory, physical, chemical and microbiological quality attributes were explained by the ANOVA PLSR model (**Fig. 2**). Therefore, this ANOVA PLSR model allowed the effect of the three design variables (frozen storage temperature, frozen storage period and chill storage period) on each of the quality attributes to be evaluated (as shown in **Fig. 3**) using the Jack-knife test with an uncertainty limit given as two times standard deviation (Martens and Martens, 1999).

Frozen storage temperatures of −20 or −30 °C have no significant effect on amine odour as average ±2S.D. for the *B*-coefficient did not differed from zero (**Fig. 3**). Furthermore, this figure showed frozen storage periods of 3, 6, 9 and 12 mo to have no systematic effect on amine odour whereas chill storage for 0, 3, 7, 14 and 21 d at 2 °C systematically influence scores for amine odour. In the same way, **Fig. 3** shows the influence of the design variables on the quality attributes drip loss and *P. phosphoreum*.

Table 3 summarizes the statistical effect of design variables on the 11 quality attributes. It is particularly noteworthy that frozen storage periods had no significant effects on any of the 11 quality attributes studied whereas the chill storage periods influenced eight of the 11 attributes and frozen storage temperature influenced five of the 11 attributes.

The present study showed quality changes in thawed MAP cod fillets during chill storage to be complex (**Figs. 1** and **2**). Therefore, a multivariate approach was appropriate or even necessary to obtain coherence between and overview of several quality changes observed during chill storage of thawed MAP cod products. Effects and interactions in the experimental design was evaluated successfully using multivariate analysis and we found these tools most useful for identification of key parameters in good manufacturing practice for thawed chilled MAP cod fillets. This is in agreement with other recent studies where partial least-squares regression was suitable for interpretation of experimental designs and including large sets of quality attribute data (Bechmann *et al.*, 1998; Jacobsen *et al.*, 1999, 2001; Jepsen *et al.*, 1999; Jørgensen *et al.*, 2000).

Sensory changes

The observed effects of chill storage period on amine odour and taste (**Fig. 3** and **Table 3**) resulted from increases of these scores whereas scores for juiciness decreased during chill storage (**Table 4**). The changes in FSO and FST showed by PCA (**Fig. 1**) were not found by ANOVA PLSR (**Fig. 2**). This contradiction could be related to high standard deviations for these measured sensory attributes. The high standard deviations for sensory attributes (**Table 4**) corresponds to what was observed in a previous study (Guldager *et al.*, 1998) and might be explained by the fact that quality changes of thawed MAP cod fillets resulted from both frozen and chill storage deterioration. It is, however, also possible that individual assessors scaled the sensory attributes differently after 0, 3, 7, 14 and 21 d of chill storage. By using an assessor reliability test, differences in scaling between assessors for each frozen storage period were eliminated because individual assessors scaled the sensory attributes differently. This resulted in a simple ANOVA PLSR model with only two PCs. The positive results obtained here with the assessor reliability tests are in agreement with Jacobsen *et al.* (1999; 2001) who also have successfully applied this method on sensory data related to lipid oxidation on fish-oil-enriched mayonnaise.

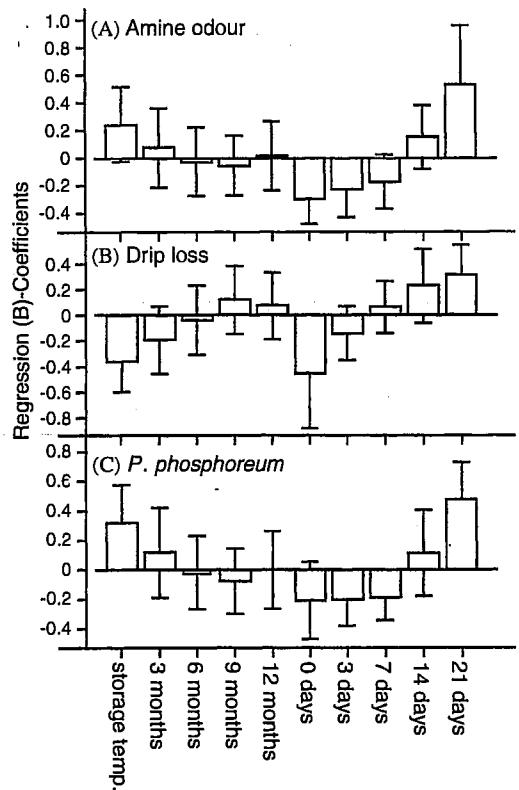

Fig. 3 Effect of design variables on average regression (B)—coefficients for three selected quality attributes (amine odour, drip loss and *P. phosphoreum*). Effects were determined by ANOVA PLSR and parameter uncertainty evaluated by the Jack-knife method. Error bars indicate two times S.D.

Physical, chemical and microbiological changes

Changes in physical, chemical and microbiological quality attributes supported sensory data and clearly documented spoilage reactions to be different for sub-batches of thawed fillets previously stored at −20 or −30 °C (**Table 4**).

The statistically significant effect of frozen storage temperature on TMA production, pH and levels of *P. phosphoreum* (**Table 3**) resulted from markedly increasing values during chill storage of fillets previously kept at −30 °C, whereas no clear changes were observed in fillets kept at −20 °C (**Table 4** and **Fig. 4**). Frozen storage at −30 °C did not inactivate *P. phosphoreum* and at 2 °C this spoilage bacteria grew to high levels in these sub-batches. The pronounced production of TMA thus resulted from *P. phosphoreum* activity, which was previously found to produce large amounts of TMA when present in cod in levels of 10^7 cfu/g or higher (Dalgaard *et al.*, 1993; Dalgaard, 1995; Bøknæs *et al.* 2002). The clear correlation between *P. phosphoreum*, TMA, pH and amine odour (**Fig. 2**) was expected. In

Table 3 Effects of design parameters determined by ANOVA PLSR and the Jack-knife method

	Effect of design variables		
Response variables	Frozen storage temperature	Frozen storage period	Chill storage period
Amine odour (AO)	No effect	No effect	Effect
Amine taste (AT)	No effect	No effect	Effect
Frozen storage odour (FSO)	No effect	No effect	No effect
Frozen storage taste (FST)	No effect	No effect	No effect
Juiciness (JCN)	No effect	No effect	Effect
Water holding capacity (WHC)	Effect[a]	No effect	No effect
Drip loss (DL)	Effect	No effect	Effect
Trimethylamine (TMA)	Effect	No effect	Effect
pH	Effect	No effect	Effect
P. phosphoreum (pp)	Effect	No effect	Effect
Total viable count (TVC)	No effect	No effect	Effect

[a]The Jack-knife method was used to determine if *B*-coefficients (average±2S.D.) obtained by ANOVA PLSR for different quality attributes differed from zero and thus had a significant effect.

Table 4 Changes in quality attribute scores during chill storage after 12 mo of frozen storage

	Quality attributes scores (AVG±S.D.)[a] (d)				
	0	3	7	14	21
Frozen storage at −20 °C[b]					
AO	2.1±2.2	1.3±1.4	0.8±0.9	2.5±2.7	4.1±4.6
AT	1.7±1.8	1.1±1.2	1.5±1.7	2.8±3.0	5.3±4.4
FSO	4.4±4.0	2.9±2.9	5.1±3.9	3.5±3.1	4.1±4.6
FST	2.7±1.8	3.4±2.8	5.9±3.9	4.6±4.2	4.9±4.8
JCN	3.3±2.8	3.4±2.0	1.9±1.3	1.6±1.6	3.2±3.4
WHC (%)	62.9±2.8	63.2±1.5	64.4±2.5	64.2±3.2	70.5±2.5
DL (%)	5.3±1.1	10.7±1.9	11.0±1.5	11.4±1.0	12.8±1.2
TMA (mg TMA-N/100 g)	1.8±0.2	2.4±0.2	2.0±0.7	3.1±0.7	10.7±4.6
pH	6.9±0.1	6.6±0.0	6.9±0.1	6.8±0.1	7.0±0.1
P. phosphoreum (log (cfu/g))	b.d.[c]	b.d.	b.d.	b.d.	b.d.
TVC (log (cfu/g))	4.1±0.2	5.2±0.1	5.3±0.3	6.9±0.7	8.5±0.7
Frozen storage at −30 °C[b]					
AO	1.6±1.8	1.2±1.6	1.4±1.9	5.0±4.1	10.9±3.6
AT	1.6±2.0	1.3±2.3	1.1±2.2	3.6±3.7	9.1±4.3
FSO	1.7±1.9	3.3±3.6	3.0±2.5	3.1±2.8	3.2±4.7
FST	1.8±1.6	2.9±3.6	3.6±2.6	3.3±3.1	3.7±3.4
JCN	5.5±4.8	4.2±3.4	4.1±3.8	1.2±1.2	1.6±1.7
WHC (%)	76.1±6.0	71.8±5.0	74.9±1.0	73.2±2.6	84.8±5.4
DL (%)	5.6±2.3	9.6±2.0	11.7±1.5	11.8±1.6	11.9±1.4
TMA (mg TMA-N/100 g)	0.2±0.6	0.7±0.7	1.0±0.7	21.6±12.7	93.3±7.0
pH	6.8±0.2	6.6±0.1	7.0±0.1	6.9±0.1	7.7±0.1
P. phosphoreum (log (cfu/g))	b.d.	b.d.	4.4±0.9	7.6±0.2	7.8±0.1
TVC (log (cfu/g))	5.0±0.2	4.8±0.4	6.1±0.1	8.1±0.1	8.6±0.1

[a]Frozen storage period had no effect on quality attributes as shown in **Table 3** and therefore quality attribute scores were shown only for 12 mo of frozen storage.
[b]Frozen storage temperature (see **Table 2**).
[c]b.d. indicated below detection limit of 0.6 log (cfu/g).

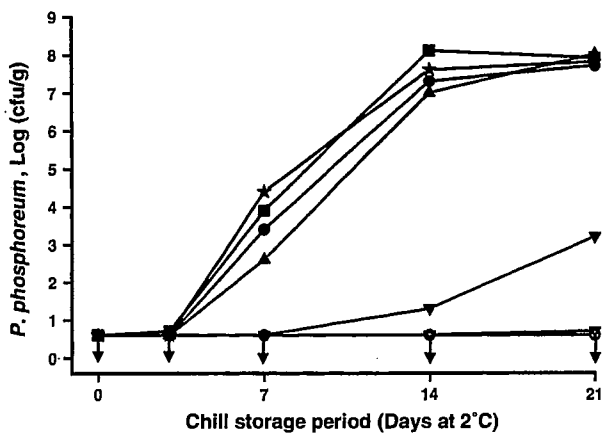

Fig. 4 Average numbers of *P. phosphoreum* in thawed MAP cod fillets after the various frozen and chill storage experiments. (■), batch A (3 mo at −30 °C); (●), batch B (6 mo at −30 °C); (▼), batch C (3 mo at −30 °C and 3 mo at −20 °C); (▲), batch D (9 mo at −30 °C); (▽), batch E (3 mo at −30 °C and 6 mo at −20 °C); (*), batch F (12 mo at −30 °C); (○), batch G (3 mo at −30 °C and 9 mo at −20 °C). Arrows indicate levels below the detection limit of 0.6 log (cfu/g)

fact, *P. phosphoreum* produced the TMA that caused pH to rise and with pH above ca. 6.7 the amine odour of TMA increases markedly (Castell and Triggs, 1955). With Baltic Sea cod it has also been observed that amine odour may be more pronounced for thawed MAP fillets previously kept at −30 °C as opposed to −20 °C. However, this was only found in cod with a modest degree of freshness when initially frozen (Bøknæs *et al.*, 2000).

The level of FA remained below 10 ppm for all sub-batches (results not shown). Much higher levels have earlier been found in frozen cod fillets (Leblanc *et al.*, 1988) mostly related to higher temperatures during frozen storage. Levels of DMA remained below 1.5 mg DMA-N/100 g during chill storage of all sub-batches (results not shown) and this corresponded to other studies with Barents Sea cod stored in ice (Oehlenschläger, 1998) and with thawed MAP Baltic Sea cod fillets (Guldager *et al.*, 1998). Consequently, formation of DMA seems of little importance in thawed chilled MAP cod products.

Drip losses increased during chill storage and reached levels of 10–12% (**Table 4**) corresponding to a previous less extensive study with thawed MAP Barents Sea cod fillets (Bøknæs *et al.*, 2002). These drip losses are higher than the 4–8% reported at the end of shelf-life of fresh MAP cod (Dalgaard *et al.*, 1993; Guldager *et al.*, 1998). However, drip losses of thawed MAP Baltic Sea cod have been as high as 14–19% (Guldager *et al.*, 1998; Bøknæs *et al.*, 2002). Consequently, Barents Sea cod seems more appropriate than Baltic Sea cod as raw material for production of thawed MAP products. The lower drip losses in thawed MAP Barents Sea cod could be related to relatively high TMAO and NaCl contents as these factors may stabilize proteins during freezing (Fennema, 1990; Bøknæs *et al.*, 2002).

As previously observed (Bøknæs *et al.*, 2002), frozen storage at −30 °C did not inactivate *P. phosphoreum* but after frozen storage at this temperature a substantial lag

phase was observed before levels of the organism increased in thawed MAP cod (**Fig. 4**). This growth pattern differs from fresh MAP cod where exponential growth of *P. phosphoreum* with no significant lag phase have been observed (Dalgaard *et al.*, 1997*b*; Guldager *et al.*, 1998). The lack of frozen storage inactivation of *P. phosphoreum* at −30 °C is most likely due to the higher contents of TMAO and NaCl in Barents Sea cod as compared to Baltic Sea cod (Bøknæs *et al.*, 2002). As expected and despite the stabilizing effect of these factors, *P. phosphoreum* was inactivated or at least strongly inhibited in Barents Sea cod stored at −20 °C (**Table 4** and **Fig. 4**) during 3 mo or more (Guldager *et al.*, 1998; Bøknæs *et al.*, 2000; 2002).

In thawed MAP cod previously kept at −30 °C, TVC increased to ca. 10^8 cfu/g (**Table 4**) and as previously observed for fresh MAP fish fillets (Dalgaard *et al.*, 1997*a*, *b*) this was due to growth of *P. phosphoreum* that dominated the spoilage microflora. In products kept at −20 °C prior to thawing, increases in TVC were due to microorganisms other than *P. phosphoreum* and these organisms remain to be identified.

Shelf-life of thawed MAP Barents Sea cod

Spoilage criteria previously suggested for fresh MAP cod fillets included sensory changes and particularly changes in amine odour corresponding to 50% of the scales used, 30 mg-N TMA/100 g and levels of *P. phosphoreum* (Cann *et al.*, 1984; Dalgaard *et al.*, 1993, 1997*b*; Guldager *et al.*, 1998). For thawed MAP Barents Sea cod previously kept at −30 °C, these spoilage criteria all resulted in shelf-life being between 14 and 21 d and most likely close to 14 d (**Table 4** and **Fig. 4**). After 3, 6, 9 and 12 mo of frozen storage, concentrations of TMA varied from 10 to 68 mg TMA-N/100 g after 14 d at 2 °C and from 46 to 93 mg TMA-N/100 g after 21 d. Shelf-life of MAP cod kept at −20 °C prior to thawing were above 21 d as judged by the spoilage criteria suggested for fresh MAP cod fillets. Levels of TMA after 3, 6, 9 and 12 mo of frozen storage at −20 °C varied from 2 to 11 mg TMA-N/100 g after 21 d at 2 °C. For inspection of thawed MAP cod e.g. by national or international authorities, spoilage criteria previously suggested for fresh MAP cod cannot be applied uncritically and further research is needed to identify efficient and generally valid indices of spoilage for thawed MAP cod.

Conclusion

In general, good manufacturing practice is established to ensure consistent production of safe and wholesome products with expected sensory characteristics (Anonymous, 1998; Jarvis, 2000). For this to be obtained, a number of key parameters in production and distribution typically need to be identified and controlled. The present study expanded existing knowledge about thawed MAP Barents Sea cod by showing that frozen storage of up to 12 mo had no significant effect on quality attributes and that shelf-life of thawed MAP products were 14 d or above at 2 °C. It was confirmed that interleaved packed fillets frozen *pre-rigor* were appropriate as raw material for this product and that blocks could be sawed into pieces and modified atmosphere packed prior to thawing. However, frozen storage of Barents Sea cod at around −30 °C inactivated *P. phosphoreum* much less than previously observed for Baltic Sea cod. Furthermore, lower drip loss was found for thawed chilled MAP cod fillets using Barents Sea cod as compared to Baltic Sea cod. Therefore, Barents Sea cod seems more appropriate than Baltic Sea cod as raw material for production of thawed chilled MAP cod products. Multivariate data analysis and particularly ANOVA PLSR was most valuable to assist the identification of important parameters to be taken into account in good manufacturing practice of thawed chilled MAP cod products. Finally, thawed MAP fish products have the obvious advantage that nematodes inside the fillets are killed by freezing and thus do not crawl up to the surface of the fillet as it may happen with fresh fillets. The Barents Sea is an important fishing bank for commercial production of frozen-at-sea cod raw material (FAO, 1999) and we believe the results of the present study can be of considerable practical importance.

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References

ANONYMOUS *Fizz, Sessions Generation, Data Collection And Computation for Sensory Analysis*. Couternon, France: Biosystems (1995)

ANONYMOUS *Food and Drink — Good Manufacturing Practice: a Guide to its Responsible management*. London: Institute of Food Science and Technology (1998)

AOAC, Official Method no. 937.09, Salt (chlorine as sodium chloride) in seafood. In: *Official Methods of Analysis of AOAC International*, 16th edn. Chapter 35, Section 35.1.18, Arlington: Association of Official Analytical Chemists, p. 7 (1995)

BECHMANN, I. E., Comparison of the formaldehyde content found in boiled and raw mince of frozen saithe using different analytical methods. *Lebensmittel-Wissenschaft und-Technologie*, **31**, 449–453 (1998)

BECHMANN, I. E., JENSEN, H. S., BØKNÆS, N., WARM, K. AND NIELSEN, J. Prediction of chemical, physical and sensory data from process parameters for frozen cod using multivariate analysis. *Journal of the Science of Food and Agriculture*, **78**, 329–336 (1998)

BØKNÆS, N., GULDAGER, H. S., ØSTERBERG, C. AND NIELSEN, J. Production of high quality frozen cod (*Gadus morhua*) fillets and portions on a freezer trawler. *Journal of Aquatic Food Product Technology*, **10**, 33–47 (2001)

BØKNÆS, N., ØSTERBERG, C., NIELSEN, J. AND DALGAARD, P. Influence of freshness and frozen storage temperature on quality of thawed cod fillets stored in modified atmosphere packaging. *Lebensmittel-Wissenschaft und-Technologie*, **33**, 244–248 (2000)

BØKNÆS, N., ØSTERBERG, C., SØRENSEN, R., NIELSEN, J. AND DALGAARD, P. Effects of technological parameters and fishing ground on quality attributes of thawed chilled cod fillets stored in modified atmosphere packaging. *Lebensmittel-Wissenschaft und-Technologie*, **34**, 513–520 (2002)

CANN, D. C., SMITH, G. L. AND HOUSTON, N. C. *Further Studies on Marine Fish Stored Under Modified Atmosphere Packaging*. Aberdeen: Torry Research Station, pp. 1–61 (1984)

CASTELL, C. H. AND TRIGGS, R. E. Spoilage of haddock in the trawlers at sea: the measurement of spoilage and standards of quality. *Journal of Fisheries Research Board Canada*, **12**, 329–341 (1955)

CHURCH, N. MAP fish and crustaceans — sensory enhancement. *Food Science Technology Today*, **12**, 73–83 (1998)

CONWAY, E. J. AND BYRNE, A. An absorption apparatus for the micro-determination of certain volatile substances. *Biochemical Journal*, **27**, 419–429 (1933)

DALGAARD, P. Qualitative and quantitative characterization of spoilage bacteria from packed fish. *International Journal of Food Microbiology*, **26**, 319–333 (1995)

DALGAARD, P., GRAM, L. AND HUSS, H. H. Spoilage and shelf life of cod fillets packed in vacuum or modified atmosphere. *International Journal of Food Microbiology*, **19**, 283–294 (1993)

DALGAARD, P., MEJLHOLM, O., CHRISTIANSEN, T. J. AND HUSS, H. H. Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. *Letters in Applied Microbiology*, **24**, 373–378 (1997*a*)

DALGAARD, P., MEJLHOLM, O. AND HUSS, H. H. Application of an iterative approach for development of a microbial model predicting the shelf-life of packed fish. *International Journal of Food Microbiology*, **38**, 169–179 (1997*b*)

DALGAARD, P., MEJLHOLM, O. AND HUSS, H. H. Conductance method for quantitative determination of *Photobacterium phosphoreum* in fish products. *Journal of Applied Bacteriology*, **81**, 57–64 (1996)

EIDE, O., BØRRESEN, T. AND STRØM, O. Minced fish production from capelin (*Mallotus villosus*). A new method for gutting, skinning and removal of fat from fatty species. *Journal of Food Science*, **47**, 347–349, 354 (1982)

FAO YEARBOOK. *Fishery Statistics Commodities*. Rome: FAO, UN (1999)

FARBER, J. M. Microbiological aspects of modified-atmosphere packaging technology — a review. *Journal of Food Protection*, **54**, 58–70 (1991)

FENNEMA, O. R. Comparative water holding properties of various muscle foods. A critical review relating to definitions, methods of measurements, governing factors, comparative data and mechanistic matters. *Journal of Muscle Foods*, **1**, 363–381 (1990)

GULDAGER, H. S., BØKNÆS, N., ØSTERBERG, C., NIELSEN, J. AND DALGAARD, P. Thawed cod fillets spoil less rapidly than unfrozen fillets when stored under modified atmosphere at 2 °C. *Journal of Food Protection*, **61**, 1129–1136 (1998)

HAARD, N. F. Technological aspects of extending prime quality of seafood: a review. *Journal of Aquatic Food Product Technology*, **1**, 9–27 (1992)

HERBORG, L. Can frozen fish be sold as fresh? *Infofish Marketing*, **2**, 37–38 (1986)

JACOBSEN, C., HARTVIGSEN, K., LUND, P., MEYER, A. S., ADLER-NISSEN, J., HOLSTBORG, J. AND HØLMER, G. Oxidation in fish-oil-enriched mayonnaise 1. Assessment of propyl gallate as an antioxidant by discriminant partial least squares regression analysis. *European Food Research Technology*, **210**, 13–30 (1999)

JACOBSEN, C., HARTVIGSEN, K., THOMSEN, M. K., HANSEN, L., LUND, P., SKIBSTED, L., HØLMER, G., ADLER-NISSEN, J. AND MEYER, A. S. Lipid oxidation in fish oil enriched mayonnaise: calcium disodium ethylenediaminetetra-acetate, but not gallic acid, strongly inhibited oxidative deterioration. *Journal of Agriculture and Food Chemistry*, **49**, 1009–1019 (2001)

JARVIS, B. Good manufacturing practice. In: ROBINSON, R., BATT, C. AND PATEL, P. (Eds.), *Encyclopedia of Food Microbiology*, New York: Academic Press Ltd., pp. 961–972 (2000)

JEPSEN, S. M., PEDERSEN, H. T. AND ENGELSEN, S. B. Application of chemometrics to low-field ^{1}H NMR relaxation data of intact fish flesh. *Journal of the Science of Food and Agriculture*, **79**, 1793–1802 (1999)

JØRGENSEN, L. V., HUSS, H. H. AND DALGAARD, P. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. *Journal of Agriculture and Food Chemistry*, **48**, 2448–2453 (2000)

LANIER, T. C. AND KORHONEN, R. Modified atmosphere and conventional packaging of fresh and previously-frozen fish. In: MARTIN, R. E. (Ed.), *Proceedings of the First National Conference on Modified and Controlled Atmosphere Packaging of Seafood Products in San Antonio*. Washington DC: The National Fisheries Institute, pp. 80–97 (1981)

LEBLANC, E. L., LEBLANC, R. J. AND BLUM, I. E. Prediction of quality in frozen cod (*Gadus morhua*) fillets. *Journal of Food Science*, **53**, 328–340 (1988)

MARTENS, H. AND MARTENS, M. Modified Jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). *Food Quality Preference*, **11**, 5–16 (1999)

MARTENS, H. AND NÆS, T. *Multivariate Calibration*. Chicester: John Wiley & Sons (1989)

MARTENS, M. AND MARTENS, H. Partial least squares regression, In: PIGGOTT, J. R. (Ed.), *Statistical Procedures*. London: Elsevier, pp. 293–359 (1986)

OEHLENSCHLÄGER, J. Detailed experimental iced-storage characteristics of Barents Sea cod (*Gadus morhua*). *Information für Fischwirtschaft*, **45**, 35–42 (1998)

THYBO, A. K. AND MARTENS, M. Analysis of sensory assessors in texture profiling of potatoes by multivariate modelling. *Food Quality and Preference*, **11**, 283–288 (2000)

TIMM, M. AND JØRGENSEN, B. Simultaneous determination of ammonia, dimethylamine, trimethylamine, and trimethylamine-N-oxide in fish extracts by capillary electrophoresis with indirect UV-detection. *Food Chemistry*, **76**, 509–518 (2002).

Artikel V

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Freshness Assessment of Thawed and Chilled Cod Fillets Packed in Modified Atmosphere Using Near-infrared Spectroscopy

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Near-infrared reflectance (NIR) spectra was recorded of 105 samples of cod mince prepared from chill stored thawed cod fillets of varying quality in modified atmosphere packaging (MAP). Traditional chemical, physical, microbiological and sensory quality methods developed for assessing fresh fish products were determined on the same cod fillets. The purpose was to evaluate the potential of NIR spectroscopy for estimating (i) frozen storage temperature, (ii) frozen storage period and (iii) chill storage period of thawed-chilled MAP Barents Sea cod fillets. Furthermore, the potential for measuring of selected quality attributes as drip loss, water holding capacity and content of dimethylamine by NIR was evaluated. The results of the investigation were presented using multivariate modelling methods such as partial least-squares regression (PLSR) and discriminant partial least-squares regression (DPLSR). Systematic differences in the NIR measurements on minced cod fillets were primarily due to the chill storage duration (days at 2 °C) on thawed-chilled MAP fillets. PLSR models based on wavelengths selected by a new Jack-knife method resulted in a correlation coefficient of 0.90 between measured and predicted duration of chill storage period (days at 2 °C). The root-mean-square error of cross-validation (RMSECV) was 3.4 d at 2 °C. NIR measurements provided promising results for evaluation of freshness for thawed-chilled MAP cod fillets completing the traditionally quality methods. However, it is necessary to study the effect of e.g. sample preparation, season, fishing ground and cod size together with more sophisticated pre-treatments of NIR spectra before the NIR method can be integrated as a method for evaluation of thawed-chilled MAP cod fillets.

Keywords: fish; frozen storage; MAP; multivariate analysis; NIR; thawing

Introduction

The use of 'frozen at sea' cod raw material instead of fresh cod fillets for the production of chilled products packed in modified atmosphere packaging (MAP) for retail sale seems promising (Bøknæs *et al.*, 2002). The quality of thawed MAP fish product depends on processing conditions as (i) frozen storage temperature, (ii) frozen storage period and (iii) chill storage period for cod fillets (Bøknæs *et al.*, 2000, 2001, 2002). The limiting factor for the shelf-life of fresh MAP cod products is *Photobacterium phosphoreum* growth and trimethylamine (TMA) production (Dalgaard *et al.*, 1993; Dalgaard, 1995) and the quality can be evaluated using a series of time-consuming physical, chemical and sensory parameters. However, near-infrared reflectance (NIR) spectroscopy for the estimation of processing conditions is an attractive possibility because it is fast, easy to handle, inexpensive to use and has the ability to carry out many samples as routine. NIR spectroscopy is already a well-established method for determining fat and water content in fish (Sollid and Solberg, 1992; Downey, 1996; Wold *et al.*, 1996; Wold and Isaksson, 1997). In addition, recent studies have shown good correlations between NIR spectroscopy and quality attributes of frozen gadoid fish (Jørgensen and Jensen, 1997; Bechmann and Jørgensen, 1998; Pink *et al.*, 1998, 1999). In recent Norwegian studies, NIR has also been used to assess freshness of cod, measured as days on ice (Sigernes *et al.*, 1998). However, the assessment of processing conditions for thawed–chilled MAP cod fillets by NIR spectroscopy has, to our knowledge, never been performed.

The objectives of the present study were to evaluate the potential of NIR spectroscopy to estimate (i) frozen

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storage temperature, (ii) frozen storage period, (iii) chill storage period and (iv) measurements of drip loss, water holding capacity (WHC) and dimethylamine (DMA) of thawed cod fillets from the Barents Sea packed in modified atmosphere and stored at 2 °C. Multivariate data analysis was used to determine the correlation between NIR spectroscopy and the selected storage conditions and physical/chemical measurements.

Materials and Methods

Experimental design

A factorial design with two frozen storage temperatures (−20 or −30 °C), four frozen storage periods (3, 6, 9 or 12 mo) as shown in **Table 1** and five chill storage periods (0, 3, 7, 14 or 21 d at 2 °C) was used as previously described by Bøknæs *et al.* (2002). Cod (*Gadus morhua*) were caught, processed and frozen on-board a Russian/Greenlandic freezer trawler in February 1999 in the Norwegian zone (72 °N, 16 °W) of the Barents Sea. Tow duration was 6 h and catch size about 6 metric tonnes, primarily cod for this single catch. Cod of 1.5–3 kg were deheaded and gutted immediately after catch. Cod were bled in seawater for about 30 min at 4 °C and then filleted and skinned by, respectively, a BAADER 190 and BAADER 51 (Nordischer Maschinenbau Rud. BAADER, Lübeck, Deutchland). Cod fillets were trimmed manually for bones, parasites and blood stains. Boneless fillets were manually interleaved packed when separating cod fillets with plastic film in the blocks. The packed fillet blocks, each containing ca 6.8 kg, were frozen in a horizontal plate freezer until a core temperature of −25 °C was reached (ca. 2 h). Frozen cod blocks were packed in cardboard boxes and placed in the cold store on-board the freezer trawler (ca. −30 °C). After 10 wk, the frozen blocks were landed and transported to the Danish Institute for Seafood Research (DIFRES) where they were randomly divided into two portions and kept at −20 and −30 °C, respectively. From each portion, frozen blocks were collected after 3, 6, 9 and 12 mo and sawed into pieces of less than 100 g. Afterwards, frozen cod pieces weighing altogether ca. 250 g were placed in trays with absorbent drip pads and packed in Riloten bags (Danisco Flexible, Lyngby, Denmark) containing a modified atmosphere with 40% CO_2, 40% N_2 and 20% O_2 (AGA, Copenhagen, Denmark). The fish-to-gas ratio was ca. 250 g cod and ca. 500 mL gas. Packed fillet pieces were then thawed for 20 h at 5 °C whereafter they were transferred for chill storage at 2 °C. After different days of chill storage, three packs from each batch were removed and homogenized for NIR analysis. The thawed MAP cod fillets (ca. 100 g) were homogenized for 15 s using a Speedy meat mincer (Krups, No. 7180-70, Ireland). Reference measurements of the selected quality attributes: drip loss, WHC and content of DMA were described in the corresponding study (Bøknæs *et al.*, 2002). For all experiments frozen and chill storage temperatures were recorded by loggers (Tinytag, Gemini Data Loggers Ltd., Chichester, U.K.).

Near-infrared measurements

For NIR analysis, minced cod was placed standardized in a tube (weight of 10 g cod mince, height of 3.5 cm and measured surface area of ca. 3 cm^2). Afterwards, the tubes were placed in a black cylinder and NIR reflectance spectra was recorded from the surface of the cod mince. During measuring, the cod samples were stored on ice. NIR reflectance spectra was measured in duplicate for each pack (three packs per batch) with an InfraProver, II Fourier transform spectrometer (Bran and Luebbe, Germany) using an optical fibre bundle as described by Bechmann and Jørgensen (1998). The spectra were sampled at 12 cm^{-1} intervals from 4,500 to 9,996 cm^{-1} (1,000–2,222 nm), i.e. 459 data points per spectrum. Preliminary data analysis revealed two extreme samples, which were removed as outliers before further data analysis. The six reflectance measurements for each batch were averaged before pre-treatment and modelling of the data. Different types of pre-treatment of the resulting NIR spectra were tested, and the following was chosen due to its simplicity and its

Table 1 Storage characteristics for all batches including frozen storage period and frozen storage temperature and chill storage temperature. All seven batches were stored at −29.0±2.7 °C for the first 3 mo of frozen storage. Results were shown as average ± S.D. CO_2 concentration during chill storage was 33.7±3.5% for all the seven batches

Batch	Storage period (mo)	Frozen storage temperature (°C)	Chill storage Temperature (°C)
A[a] (–30 °C)	3	–29.0±2.7[b]	2.1±0.5[c]
B[a] (–30 °C)	6	–31.5±2.9[b]	2.5±0.6[c]
C[d] (–20 °C)	6	–24.6±4.3[b]	2.5±0.6[c]
D[a] (–30 °C)	9	–32.2±2.6[b]	2.1±0.7[c]
E[d] (–20 °C)	9	–23.5±4.0[b]	2.1±0.7[c]
F[a] (–30 °C)	12	–32.4±3.4[b]	2.5±0.2[c]
G[d] (–20 °C)	12	–23.4±3.6[b]	2.5±0.2[c]

[a]Significant ($P<0.05$) *P. phosphoreum* growth and TMA production during chill storage in MAP.
[b]No significant ($P>0.05$) difference between frozen storage temperatures.
[c]No significant ($P>0.05$) difference in chill storage temperature.
[d]No significant ($P>0.05$) *P. phosphoreum* growth and TMA production during chill storage in MAP.

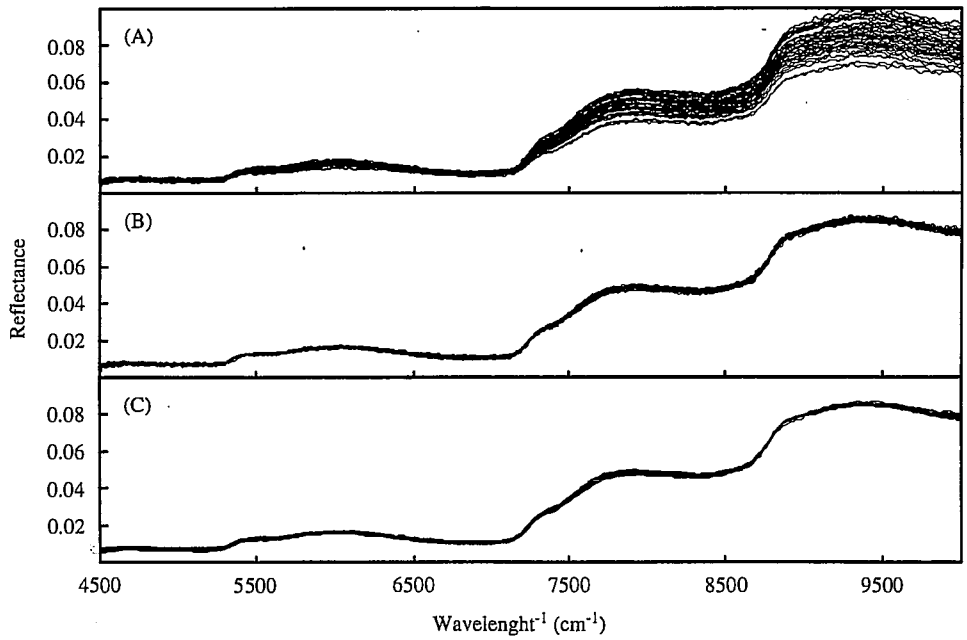

Fig. 1 NIR reflectance spectra measured on minced thawed MAP cod fillets showed: (**A**) all the 35 averaged raw NIR spectra; (**B**) all the 35 averaged and MSC NIR spectra and (**C**) averaging and smoothing of all the 35 averaged and MSC NIR spectra

successful modelling (see below). Each average reflectance spectrum was corrected for additive and multiplicative main effects using multiplicative scatter correction (MSC) (Geladi *et al.*, 1985) for eliminating the effects of physical phenomena like the light-scattering effects of particles with different sizes and shapes (Isaksson and Næs, 1988; Helland *et al.*, 1995). Since the MSC-treated and averaged reflectance spectra was rather noisy, they were averaged and smoothed in the final modelling. Batch mean of NIR spectra on minced cod fillets before and after pre-treatment (MSC, averaging and smoothing) was shown in **Fig. 1**. The number of wavelength channels was reduced down to 230 by averaging of adjacent wavelengths of the MSC-treated NIR reflectance spectra. Afterwards, the spectra was smoothed by a moving average filter based on three neighbouring wavelength channels.

Multivariate data analysis

Partial least-squares regression (PLSR) (Wold *et al.*, 1983) was used for the multivariate calibration in (i) qualitative modelling and (ii) quantitative modelling. In the qualitative modelling, discriminant partial least-squares regression (DPLSR) (Wold *et al.*, 1983) was used in order to gain overview of the data (see also Martens and Martens, 1986; Martens and Jørgensen, 1998; Jacobsen *et al.*, 1999, 2001). In order to investigate the effect of (i) frozen storage temperature, (ii) frozen storage period and (iii) chill storage period for thawed–chilled MAP cod fillets, DPLSR was employed to predict qualitative main effects of the experimental design (Y), from the averaged and smoothed MSC-treated NIR data (X). This means that each frozen storage temperature (−20 or −30 °C), each frozen storage period (3, 6, 9 or 12 m) and each chill storage period (0, 3, 7, 14 or 21 d at 2 °C) were represented by an indicator variable (with values 0 or 1). No standardization (weighting) was used on X and Y variables in the qualitative modelling. The model was validated by full cross-validation.

In the quantitative modelling, PLSR was employed to determine the predictive ability of the NIR spectra (X) and the duration of chill storage period (now represented as one single quantitative variable y, representing the number of days at 2 °C) for thawed MAP cod fillets. Furthermore, PLSR was used to determine the predictive ability of the NIR spectra and, respectively, (i) drip loss, (ii) WHC and (iii) content of DMA. For analysing PLSR results, a newly developed modified Jack-knife method for estimation of parameter uncertainty in PLSR was used (Martens and Martens, 1999). In this case, a manual selection of wavelengths with $P>0.1$ was used for elimination of nonsignificant wavelengths in the NIR spectra. In the quantitative modelling, the NIR data (X variables) was standardized (weighting with 1/standard deviation) since it improved the predictability. The single y-variables were not weighted. The PLSR models were validated by full cross-validation. Multivariate data analysis was performed using The Unscrambler® Ver. 7.6 (CAMO, Norway).

Results

Experimental design

The experimental batch characteristics including temperatures for frozen and chill storage were shown in **Table 1**. The growth of *P. phosphoreum* and TMA production in the thawed–chilled MAP cod fillets was showed in the corresponding study (Bøknæs *et al.*, 2002).

Multivariate data analysis

Qualitative modelling. The data set consisted of 35 samples (7 batches × 5 periods of chill storage) in an NIR spectra matrix (35 × 459) and a design matrix (35 × 10). The design matrix contained qualitative indicators for the main effects, i.e. 0 or 1 variables for (i) frozen storage temperature (−20 or −30 °C), (ii) frozen storage period (3, 6, 9 or 12 mo) and (iii) chill storage period (0, 3, 7, 14 or 21 d at 2 °C). The DPLSR model contained three factors explaining 64% of the variance in X and 22% of the variance in Y. The scores for the first two factors were shown in **Fig. 2**.

In the scores plot (**Fig. 2**), differences in chill storage between 0 and 21 d expanded the first factor for the DPLSR. Samples with few and many days of chill storage were mostly placed at the left and the right in the plot, respectively (**Fig. 2**). This indicated a weak tendency for the fact that the first factor accounted for most of the variation due to the chill storage period of thawed MAP cod fillets. No systematic differences in MSC-treated NIR spectra for frozen storage temperature and period were observed according to the second and third factor. However, this qualitative modelling indicated that NIR spectra could be used for estimating chill storage period of thawed MAP cod fillets.

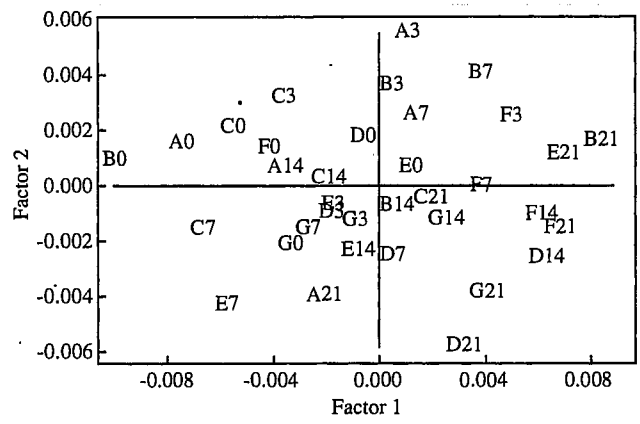

Fig. 2 Scores plot of factor 1 *vs.* factor 2 from DPLSR analysis including NIR spectra and all three design variables. Letters indicate different frozen storage temperatures and frozen storage periods (see **Table 1**). Numbers indicate the period of chill storage in days at 2 °C. Explained variance for X were 44 and 14% and for Y 6 and 7% for factors 1 and 2, respectively

Quantitative modelling. PLSR models using NIR for estimating the period of chill storage (y = days at 2 °C) and selected quality measurements of thawed MAP cod fillets were evaluated. The PLSR models were based on MSC-treated NIR spectra for all 35 samples independent of frozen storage temperature and period. In order to reduce the risk of chance correlations in the Jack-knifed variable selection, the number of wavelength channels was reduced down to 230 by averaging and smoothing wavelengths of the MSC-treated NIR reflectance spectra.

An initial PLSR model was developed, using all 230 wavelength channels of smoothed MSC-treated NIR reflectance spectra as X-variables and chill storage period (0, 3, 7, 14 or 21 d at 2 °C) as y-variable (**Table 2**). The Jack-knife method was used for selecting significant wavelengths in smoothed MSC-treated NIR spectra. All wavelengths that were found to give nonsignificant contributions ($P > 0.1$) estimated by full cross-validation using the Jack-knife method were eliminated in order to remove detrimental X-variables from the model. To further guard against incidental chance correlations, single significant wavelength channels, i.e. with non-significant neighbours on either side, were also eliminated. The regression coefficients for the PLSR models before and after elimination of nonsignificant wavelengths were shown in **Fig. 3**. The significant wavelengths in **Fig. 3B** were not directly connected to water or protein. However, the significant wavelength region between 7600 and 8000 cm^{-1} (connected with C–H groups) could possibly be related to protein changes during chill storage. The PLSR models were evaluated for their number of factors, root-mean-square error of cross-validation (RMSECV), correlation between predicted and measured values, and by the amount of explained validation variance of the Y data. These results were shown in **Table 2**.

When the Jack-knife method was used to select significant NIR reflectance wavelengths, a lower prediction error of the chill storage period by MSC-treated

Table 2 Validation of PLSR-models for the prediction of chill storage period (days at 2 °C), drip loss, DMA and WHC for thawed–chilled MAP cod fillets from NIR data using full spectrum (230 wavelengths) or selected wavelengths of averaged and smoothed MSC–treated NIR data

y-Value	X-values	Resulting range	Number of PLSR factors	RMSECV[a]	Correlation coefficient	% Y var. expl.[b]
Chill storage (d)	Full spectrum	0–21	4	4.9	0.77	61
Chill storage (d)	Selected wavelengths[c]	0–21	3	3.4	0.90	81
Drip loss (%)	Full spectrum	3.2–13.8	3	2.3	0.69	49
Drip loss (%)	Selected wavelengths[c]	3.2–13.8	2	2.0	0.78	63
DMA (mg DMA-N/100 g)[d]	Full spectrum	0–1.5	—	—	<0.1	–
WHC (%)[d]	Full spectrum	62.5–84.8	—	—	<0.1	–

[a]The root-mean-square error of cross-validation (RMSECV) is expressed in the same units as for the y-value.
[b]Amount of variance in the Y-matrix explained by the models (%).
[c]Significant wavelengths selected by the Jack-knifing method with $P < 0.1$.
[d]Since no correlation between NIR and y-value was established, no validation values were reported.

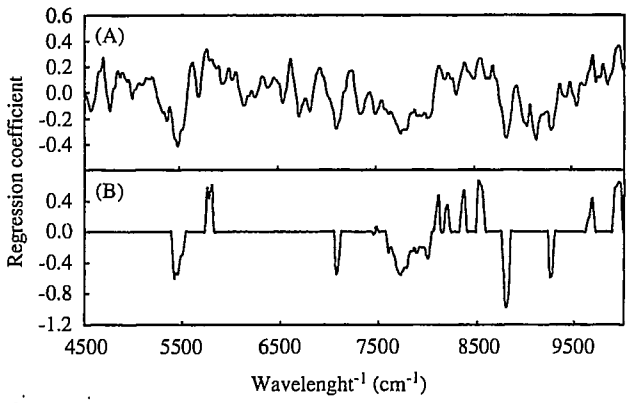

Fig. 3 Regressions coefficients from PLSR models for prediction of chill storage period (days at 2 °C). The regressions coefficients were shown for the optimal number of factors in the model: (**A**) before elimination of nonsignificant wavelengths in a four-factor model and (**B**) after elimination of nonsignificant wavelengths in a three-factor model

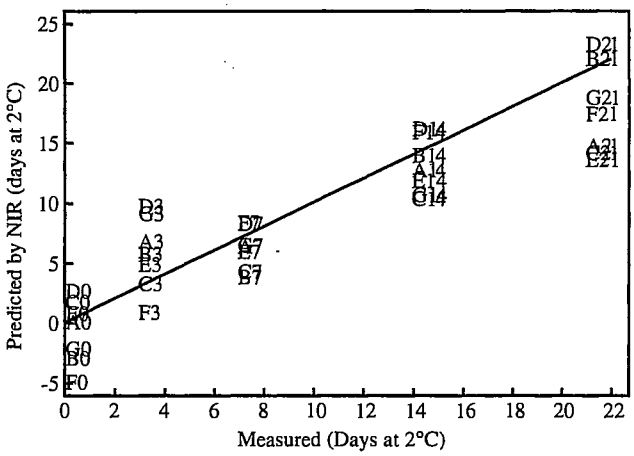

Fig. 4 The relation between duration of chill storage (days at 2 °C) of thawed MAP cod (abscissa) and predicted chill storage period (days at 2 °C) determined from the NIR measurements (ordinate) as obtained by a cross-validated PLSR model based on only significant Jack-knifing wavelengths. The solid line indicates $y = x$. Letters indicate different frozen storage temperatures and frozen storage periods (see Table 1). Numbers indicate the period of chill storage in days at 2 °C

NIR spectra was found (an increase from 61% to 81% of explained variance). The initial use of all NIR wavelengths may thus have included too many noisy and irrelevant *X*-variables in the model (**Table 2**). Results from PLSR modelling for prediction of WHC, DMA and drip loss were also shown in **Table 2**. These PLSR results showed the same tendency for a quite good prediction of drip loss by NIR as obtained for prediction of chill storage period. However, we obtained very low correlations (<0.1) between NIR and measurements of DMA or WHC (**Table 2**).

Discussion

Systematic differences in the NIR measurements on minced cod fillets, observed in the qualitative calibration models (**Fig. 2**) were primarily due to the chill storage duration (days at 2 °C) on thawed–chilled MAP cod fillets. In contrast, frozen storage temperature and frozen storage period did not influence the NIR measurements very much, at least not in a clear and systematic way. The final quantitative calibration model for prediction of chill storage period, after elimination of nonsignificant wavelength channels, showed quite good predictive ability in the cross validation (**Fig. 3**). Compared to the full spectrum model, the Jack-knifed-based variable selection apparently caused an appreciable improvement (from 61% to 81% of explained variance) in the prediction of the number of days of chill storage at 2 °C (y).

To what extent could this cross-validated improvement be an artefact? The cross-validation by itself is quite safe (Martens and Dardenne, 1998), since the only way the predicted y-variables of the samples have been used, other than for estimating the cross-validated prediction error (RMSECV) itself, is to determine one single parameter—the optimal number of factors. Hence, the initial result (61% prediction ability) is good enough.

However, the combination of Jack-knife-based variable selection and cross-validation needs to be scrutinized. In the Jack-knife-based testing, a high number of more or less independent wavelength channels are assessed with respect to their apparent effect on model stability, and only the best ones are retained in the model. Then, in the cross-validation, the prediction error estimation is based on more or less the same criterion—the X–y agreement between each individual sample and the other samples. Therefore, there is a risk of overfitting that is not detected by the cross-validation. If many incidentally significant X-variables survive the Jack-knife testing (we expect about 10% of nonsense variables to survive the chosen test level), then their combination may create an artificially high cross-validated gain in the predictive ability to be too high.

Preliminary quantitative modelling (results not shown), based on the original, unsmoothed NIR spectra with rather noisy 459 X-variable channels indeed indicated some over fitting even in the cross-validation, because the resulting regression coefficient vector was quite noisy. This was the reason why the number of X-variables was reduced to 230 and smoothed, and finally, that the few retained lonely individual wavelength channels were eliminated manually. With this procedure, we expect the estimated prediction error RMSECV of the final calibration model to be realistic for the models predictive ability in new samples of the same general kind.

This final PLSR model for prediction of chill storage period gave a correlation coefficient of 0.9 and RMSECV on 3.4 d. The prediction of chill storage (days at 2 °C) was obtained more or less independently of the previous frozen storage temperature and frozen storage period. In an earlier study concerning prediction of storage period for fresh cod fillets with no precedent frozen storage treatment, Sigernes *et al.* (1998) found a correlation coefficient of 0.95 and RMSECV on 28 h. Hence, it seems that NIR has a future potential of measuring chill storage duration for the thawed MAP

cod fillets (**Table 2**). This could be very useful in practice, because the application of traditional methods for the quality assessment of MAP cod fillets including number of *P. phosphoreum* and TMA production were difficult due to differences in microbiological spoilage of thawed–chilled MAP Barents Sea cod (**Table 1**) depending of the precedent frozen storage temperature (Bøknæs *et al.*, 2002).

During chill storage, significant changes in texture for thawed MAP cod fillets have previously been reported (Guldager *et al.*, 1998; Bøknæs *et al.*, 2000, 2001, 2002). However, textural changes obtained for thawed–chilled MAP cod fillets have been related to inevitable processes during freezing, thawing and chill storage such as protein denaturation and enzymatic reactions (Shenouda, 1980; Rehbein, 1988). Such textural changes might be influenced in NIR spectra shown in the present study. This is confirmed in the present study using averaged and smoothed MSC-treated NIR data for predicting drip loss of thawed–chilled MAP cod fillets with a correlation coefficient of 0.78 and RMSEPCV of 2.0%. In addition, a recent study also showed the potential for using visual/near-infrared reflectance spectroscopy for nondestructive estimating of texture in farmed Atlantic salmon (*Salmo salar*) (Isaksson *et al.*, 2001). Recently in Canadian studies, NIR spectroscopy has been used to predict DMA content in frozen minced Atlantic red hake (*Urophycis chuss*) with correlation coefficient higher than 0.95 (Pink *et al.*, 1998, 1999). In contrast, a very low correlation coefficient (<0.1) between NIR spectra and DMA content in thawed–chilled MAP cod fillets was obtained in the present study. This might be related to the very little formation of DMA during frozen and chill storage as DMA content was found to be below 1.5 mg DMA-N/100 g for all batches in the present study (Bøknæs *et al.*, 2002). Furthermore, a very low correlation coefficient (<0.1) on predicting WHC by NIR was also found in the present study. In contrast, recent studies (Jørgensen and Jensen, 1997; Bechmann and Jørgensen, 1998) showed higher correlation using NIR measured on the skin of whole thawed cod or on centrifuged cod mince for determining WHC in thawed cod fillets. However, it was also shown that the use of NIR spectra of mince as in this study was inferior to the use of spectra of the centrifuged mince (Jørgensen and Jensen, 1997). This might explain the very low correlation coefficient between NIR on raw cod mince and WHC obtained in the present study.

In conclusion, NIR is a promising method for estimating duration of chill storage period of thawed–chilled MAP cod fillets. However, before NIR can be introduced for the evaluation of thawed–chilled MAP cod fillets, it is necessary to develop a calibration model spanning the variations related to e.g. chill storage period, sample preparation, season, fishing ground and cod size. Furthermore, data modelling could be optimized using other more sophisticated pre-treatments for NIR measurements on raw thawed MAP cod fillets instead of measuring on minced cod.

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References

Bechmann, I. E. and Jørgensen, B. Rapid assessment of quality parameters for frozen cod using near infrared spectroscopy. *Lebensmittel-Wissenschaft und-Technologie*, **31**, 648–652 (1998)

Bøknæs, N., Østerberg, C., Nielsen, J. and Dalgaard, P. Influence of freshness and frozen storage temperature on quality of thawed cod fillets stored in modified atmosphere packaging. *Lebensmittel-Wissenschaft und-Technologie*, **33**, 244–248 (2000)

Bøknæs, N., Østerberg, C., Sørensen, R., Nielsen, J. and Dalgaard, P. Effects of technological parameters and fishing ground on quality attributes of thawed chilled cod fillets stored in modified atmosphere packaging. *Lebensmittel-Wissenschaft und-Technologie*, **34**, 513–520 (2001)

Bøknæs, N., Jensen, K. N., Guldager, H. S., Østerberg, C., Nielsen, J. and Dalgaard, P. Thawed chilled Barents Sea cod fillets in modified atmosphere packaging—application of multivariate data analysis to select key parameters in good manufacturing practice. *Lebensmittel-Wissenschaft und-Technologie*, **35**, 436–443 (2002)

Dalgaard, P. Qualitative and quantitative characterization of spoilage bacteria from packed fish. *International Journal of Food Microbiology*, **26**, 319–333 (1995)

Dalgaard , P., Gram, L. and Huss, H. H. Spoilage and shelf life of cod fillets packed in vacuum or modified atmospheres. *International Journal of Food Microbiology*, **19**, 283–294 (1993)

Downey, G. Non-invasive and non-destructive percutaneous analysis of farmed salmon flesh by near infra-red spectroscopy. *Food Chemistry*, **55**, 305-311 (1996)

Geladi, P., Macdougall, D. and Martens, H. Linearization and scatter-correction for near-infrared reflectance of meat. *Applied Spectroscopy*, **39**, 491–500 (1985)

Guldager, H. S., Bøknæs, N., Østerberg, C., Nielsen, J. and Dalgaard, P. Thawed cod fillets spoil less rapidly than unfrozen fillets when stored under modified atmosphere at 2 °C. *Journal of Food Protection*, **61**, 1129–1136 (1998)

Helland, I. S., Næs, T. and Isaksson, T. Related versions of the multiplicative scatter correction method for preprocessing spectroscopic data. *Chemometrics and Intelligent Laboratory Systems*, **29**, 233–241 (1995)

Isaksson, T. and Næs, T. The effect of multiplicative scatter correction (MSC) and linear improvement in NIR spectroscopy. *Applied Spectroscopy*, **42**, 1273–1284 (1988)

Isaksson, T. Swensen, L. P., Taylor, R. G., Fjæra, S. O. and Skervold, P. O. Non-destructive texture analysis of farmed Atlantic salmon using visual/near-infrared reflectance spectroscopy. *Journal of the Science of Food and Agriculture*, **82**, 53–60 (2001)

Jacobsen, C., Hartvigsen, K., Lund, P., Meyer, A. S., Adler-Nissen, J., Holstborg, J. and Hølmer, G. Oxidation in fish-oil-enriched mayonnaise, 1. Assessment of propyl gallate as an antioxidant by discriminating partial least squares regression analysis. *European Food Research Technology*, **210**, 13–30 (1999)

Jacobsen, C., Hartvigsen, K., Thomsen, M. K., Hansen, L. F., Lund, P., Skibsted, L., Hølmer, G., Adler-Nissen, J. and Meyer, A. S. Lipid oxidation in fish oil enriched mayonnaise: calcium disodium ethylenediaminetetraacetate,

but not gallic acid, strongly inhibited oxidative deterioration. *Journal of Agriculture and Food Chemistry*, **49**, 1009–1019 (2001)

JØRGENSEN, B. AND JENSEN, H. S. Can near-infrared spectrometry be used to measure quality attributes in frozen cod? In: LUTEN, J. B., BØRRESEN, T. AND OEHLENSCHLÄGER, J. (Eds), *Seafood from Producer to Consumer, Integrated Approach to Quality*. Amsterdam: Elsevier, pp. 491–496 (1997)

MARTENS, H. AND DARDENNE, P. Validation and verification of regression in small data sets. *Chemometrics and Intelligent Laboratory Systems*, **44**, 99–122 (1998)

MARTENS, M. AND JØRGENSEN, B. Multivariate data analysis used for investigation of the sensory quality of fish. In: OLAFSDÓTTIR, G., LUTEN, J. P., DALGAARD, P., CARECHE, M., VERREZ-BAGNIS, V., MARTINSDÓTTIR, E. AND HEIA, K. (Eds), *Methods to Determine the Freshness of Fish in Research and Industry*. Paris: International Institute of Refrigeration, pp. 369–375 (1998)

MARTENS, M. AND MARTENS, H. Partial least squares regression. In: PIGGOTT, J. R. (Ed.), *Statistical Procedures*. London: Elsevier, pp. 293–359 (1986)

MARTENS, H. AND MARTENS, M. Modified Jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). *Food Quality and Preference*, **11**, 5–16 (1999)

PINK, J., NACZK, M. AND PINK, D. Evaluation of the quality of frozen minced red hake: use of Fourier transform infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, **46**, 3667–3672 (1998)

PINK, J., NACZK, M. AND PINK, D. Evaluation of the quality of frozen minced red hake: use of Fourier transform near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, **47**, 4280–4284 (1999)

REHBEIN, H. Relevance of trimethylamine oxide demethylase activity and haemoglobin content to formaldehyde production and texture deterioration in frozen stored minced fish muscle. *Journal of the Science of Food and Agriculture*, **43**, 261–276 (1988)

SHENOUDA, S. Y. K. Theories of protein denaturation during frozen storage of flesh. In: CHICHESTER, C. O., MRAK, E. M. AND STEWART, G. F. (Eds), *Advances in Food Research*. New York: Academic Press, pp. 275–311 (1980)

SIGERNES, F., ESAIASSEN, K., HEIA, K., WOLD, J. P., EILERTSEN, G. AND SØRENSEN, N. K. Assessment of fish (cod) freshness by VIS/NIR spectroscopy. In: OLAFSDÓTTIR, G., LUTEN, J. P., DALGAARD, P., CARECHE, M., VERREZ-BAGNIS, V., MARTINSDÓTTIR, E. AND HEIA, K. (Eds), *Methods to Determine the Freshness of Fish in Research and Industry*. Paris: International Institute of Refrigeration, pp. 369–375 (1998)

SOLLID, H. AND SOLBERG, C. Salmon fat content estimation by near infrared transmission spectroscopy. *Journal of Food Science*, **57**, 792–793 (1992)

WOLD, S., ALBANO, C., DUNN III, W.J., ESBENSEN, K. HELLBERG, S., JOHANSSON, E. AND SJÖSTRÖM, M. Recognition: finding and using regularities in multivariate data. In: MARTENS, H. AND RUSSWURM, H. (Eds), *Food Research and Data Analysis*. London: Applied Science Publishers, pp. 147–188 (1983)

WOLD, J. P. AND ISAKSSON, T. Non-destructive determination of fat and moisture in whole Atlantic salmon by near-infrared diffuse spectroscopy. *Journal of Food Science*, **62**, 734–736 (1997)

WOLD, J. P., JAKOBSEN, T. AND KRANE, L. Atlantic salmon average fat content estimated by near-infrared transmittance spectroscopy. *Journal of Food Science*, **61**, 74–77 (1996)