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Storage Quality of Fresh and Frozen-thawed Fish in Ice

HANNES MAGNÚSSON and EMILÍA MARTINSDÓTTIR

ABSTRACT

The objective was to determine whether traditional quality indexes of fresh (unfrozen) fish like sensory analysis, bacterial counts and trimethylamine content could be applied to thawed whole cod, cod fillets and ocean perch fillets kept in ice. Freezing and short-term freezer storage (≤5 wk at −25°C) had very little effect on bacterial counts. During long-term freezer storage (≥14 wk at −25°C) total counts were reduced as well as counts of trimethylamine oxide-reducing bacteria in cod fillets but not in ocean perch fillets. When the thawed fish was unacceptable the trimethylamine was <1 mgN/100g. Trimethylamine as a spoilage indicator was of no value when evaluating spoilage of thawed whole cod, cod fillets and ocean perch fillets kept in ice.

Key Words: fresh fish, thawed fish, cod, ocean perch, trimethylamine

INTRODUCTION

FROZEN-THAWED FISH, especially fillets are commonly marketed chilled. Such frozen-thawed fish may be kept for varying times in ice. Two very important species in this respect are cod (*Gadus morhua*) and ocean perch (*Sebastes marinus/S. mentella*)

Trimethylamine oxide (TMAO) is generally present in seawater fish. Trimethylamine (TMA) is formed from TMAO by bacterial reduction during iced storage. However during frozen storage TMAO can in gadoid fish species like cod be broken down to dimethylamine (DMA) and formaldehyde (FA) by endogenous enzymes (Hebard et al., 1982). This reaction is very temperature-dependent; the enzyme activity being inhibited if storage temperature is near −29°C (Castell et al., 1974). Relatively few reports have been published on the storage quality of thawed fish in comparison to fresh (unfrozen) fish in ice. Experiments done by Luijpen (1958) on cod fillets with skin kept at different temperatures showed that TMA was produced slower in thawed fillets than in unfrozen ones. Those results were not in accordance with sensory analysis and not explained by differences in bacterial counts. No information was found regarding this difference in TMA formation in whole fish.

Our main objective was to determine whether a difference in TMA formation occurred in thawed cod, kept whole or as fillets in comparison to unfrozen cod, and if so to find the reason. In addition, fillets of ocean perch were examined. Another objective was to examine the effect of freezing and freezer storage on microbiological flora and TMAO. We also evaluated whether methods for assessing spoilage and keeping quality of unfrozen fish like sensory analysis, bacterial counts and chemical indexes (TMA and total volatile bases, TVB) could be applied to thawed fish kept in ice.

MATERIAL & METHODS

Whole cod

On board a trawler cod was bled, gutted and iced into 90 L boxes. Four days from catch some of the cod were frozen whole in a plate freezer and kept at −25°C for 8 wk. At the same time the rest of the cod were iced for a storage trial at 0 to 1°C. The frozen cod was thawed at 15°C and iced in boxes as soon as the core temperature reached 0°C. Both unfrozen and thawed cod were kept iced for ≈3 wk. On each day of sampling, four cods were individually examined.

Authors Magnússon and Martinsdóttir are with Icelandic Fisheries Laboratories (IFL), P.O. Box 1405, Skúlagata 4, 121 Reykjavík, Iceland.

Cod fillets in ≈2.25 kg cartons

When examining the effect of freezing and freezer storage on microorganisms and TMAO, fully trimmed cod fillets in 2.25 kg cartons, each containing six units in a plastic envelope, were collected at a freezing plant. Unfrozen cod were examined microbiologically at exactly the same time as comparable cod was placed in a plate freezer. Frozen cartons were kept at −25°C for 18, 23 and 50 wk in three individual experiments. In all experiments samples were also examined after 1 day in freezer. Frozen units were kept for 1 hr at room temperature prior to examination. Each of the six units in the ≈2.25 kg cartons were examined individually. Cod samples from this experiment were not stored in ice after thawing.

Cod fillets

For ice storage experiments on cod fillets, fresh longline cod were used. The cod were bled onboard and gutted ashore. The cod were filleted and skinned within 24 hr from time of catch. Some fillets were iced in boxes while others were tunnel-frozen at the same time. Frozen fillets were kept at −25°C for 1 day, and for 5, 14, 27 and 52 wk. These fillets were thawed at 15°C until core temperature reached 0°C, and then iced immediately and kept as unfrozen fillets at 0 to 1°C for up to 3 wk. Samples for initial bacterial analysis were examined within 1 hr after removal from freezer. During iced storage, three separate fillets were taken on every sampling day for bacteria and chemical studies and an additional three for sensory evaluation.

Ocean perch fillets

Ocean perch were obtained from a local trawler. The ocean perch were kept unbled and ungutted in ice. On arrival on shore, one day from catch, the fish were filleted and skinned. Some fillets were iced in boxes while others were tunnel-frozen at the same time. Frozen fillets were kept at −25°C for 1 day, 7 wk and 25 wk. Fillets were thawed at 15°C until core temperature reached 0°C, then iced immediately and kept as unfrozen fillets at 0 to 1°C up to 3 wk. Samples for initial bacterial analysis were examined within 1 hr after removal from freezer. During ice storage three separate samples were taken on every sampling day for bacterial and chemical studies and an additional three for sensory evaluation. Each sample consisted of three fillets.

Bacterial counts

When examining whole cod, 3 × 7.5 cm² skin samples were cut along the lateral line (behind gills, mid region, tail region) and placed in a stomacher bag containing 60 mL Butterfield's buffer solution (pH 7.2). Blending was done in a Stomacher 400 for 1 min. Prior to taking samples of flesh, the skin was washed with 70% ethanol and then removed aseptically. The underlying muscle was removed and after mincing, 25g were weighed into a stomacher bag containing 225 mL Butterfield's buffer solution. Blending was done in a Stomacher for 1 min. All cod and ocean perch fillets were handled in the same way as muscle samples. Butterfield's buffer was used for all dilutions.

When examining whole cod and cod fillets, bacterial counts were done by the pour plate technique on plate count agar (PCA-Difco) with 0.5% NaCl (w/v) added. The plates were incubated at 35°C for 2 days when counting mesophilic bacteria and for 22°C for 3 days when counting psychrotrophic bacteria. Counts of coliform bacteria were done by the MPN-technique (FDA, BAM, 1992). When examining ocean perch fillets, total viable counts and selective counts of H_2S-producing bacteria were done on iron agar (IA-Oxoid) as described by Gram et al. (1987). The plates were incubated at 22°C for 3 days. Bacteria forming black colonies on this agar produce H_2S from sodium thiosulphate and/or cysteine.

Composition of bacterial flora

On each occasion, 25 randomly selected colonies were picked off the PCA plates (cod fillets) and IA plates (ocean perch fillets). The following tests were used for determination of purified strains: Gram-staining (Hucker's modification) and morphology on young PCA cultures, KOH-

test (Gregersen, 1978), motility test on fresh cultures in Nutrient broth by the "hanging-drop" method, production of oxidase (Kovacs, 1956) and catalase (3% H_2O_2) and oxidation-fermentation test for glucose metabolism in MOF medium (Leifson, 1963) with 0.5% NaCl used instead of seawater salts. The tubes were incubated at 22°C and acid formation recorded after 7 and 14 days. When testing for TMAO-reduction, each strain was inoculated into 50 mL of 0.5% TMAO-broth and incubated at 22°C for 7 days. The TMAO-medium was prepared by adding 1.25 mL of 20% filter-sterilized trimethylamine N-oxide (Sigma) solution to 48.75 mL of sterile Nutrient broth. Reduction of TMAO was detected by measuring TMA (described below) and by sensory evaluation (smell). Identification of Gram-negative bacteria was based on the determinative scheme of Shewan et al. (1960) but updated according to *Bergey' Manual* Vol. 1 (Krieg and Holt, 1984). Gram-positive strains were classified according to *Bergey's Manual* Vol. 2 (Sneath et al., 1986).

Chemical analysis

TMA (as mgN/100g muscle) was measured according to AOAC (1990) except that KOH was used instead of K_2CO_3. TMAO (as mgN/100g muscle) was measured as described by Bystedt et al. (1959) where available TMA is first measured and then TMAO reduced to TMA by $TiCl_3$. Total volatile bases (TVB, as mgN/100g muscle) were measured according to Antonacopoulus (1968) with a Struer automatic distillation unit. TVB was only measured in ocean perch fillets.

Sensory evaluation

Sensory evaluation was by a trained panel of 7 to 10 people on samples of cooked fillets. Fillets were cooked in a steam oven (6 min at 98°C) and the smell and taste were judged on a rating scale from 9 (highest freshness) to 1 (lowest). The scale is based on the Torryscale as originally described by Shewan et al. (1953) with slight modifications. The fish were judged unfit for consumption when the mean value for sensory score was below 4.5.

Statistical analysis

Statistical analysis was done using SYSTAT 5.0 statistical package run on a PC computer. Analysis of variance was used and when appropriate Tukey test was used to find mean separation (Wilkinson, 1990). Linear regression was used on sensory data vs storage time in ice. Results from all bacterial counts were shown as geometric means of individual measurements but all other tests as arithmetic means.

RESULTS & DISCUSSION

Whole cod

TMA measurements in unfrozen and frozen-thawed cod during 3 wk storage in ice showed TMA formation was much slower in thawed whole cod (Fig. 1). On the 11th day and onwards there was a significant difference ($p < 0.05$) between means. The sensory panel gave a significant one point higher average score ($p < 0.05$) for the unfrozen cod at the beginning of storage than for thawed cod. Thereafter similar scores were given for both groups and after 15 to 16 days storage in ice, both unfrozen and thawed cod were unacceptable. At that time TMA in unfrozen cod was about 10 mgN/100g while TMA in thawed cod was around 1. Cod containing more than 10–15 mgN/100g is considered unsuitable for most uses (Connell, 1990). Growth curves of psychrotrophic bacteria for unfrozen and thawed cod were very similar. The difference between counts in skin (cm^2) and flesh (g) was in the range of 3 to 4 log numbers (Fig. 2).

Cod fillets in ≈2.25 kg cartons

Bacterial counts showed that the freezing (1 day in freezer) did not result in reduction of mesophilic and psychrotrophic bacteria or coliforms. However, with increasing storage time in freezer, the reduction in bacterial numbers became greater. Thus 50 wk storage resulted in 70–91% kill of bacterial flora (Table 1).

The percentage composition of psychrotrophic flora in fillets kept frozen for 23 wk (Table 2) showed the freezing process and 1 day storage in a freezer did not have a notable effect on composition. However, long term freezer storage (23 wk) resulted in an increased proportion of Gram-positive bacteria as expected (Shewan, 1961). The mean total count of six samples (Log no./g) was 5.1 (SD ± 0.1) in unfrozen fillets, 5.1 (SD ± 0.1) in fillets kept frozen 1 day and 4.8 (SD ± 0.2) in comparable fillets kept for 23 wk in a freezer. From the % proportion of TMAO-reducing bacterial strains the number of such bacteria was calculated and found to be Log 4.3 in unfrozen fillets, 4.0 in fillets kept frozen 1 day, and 3.7 in fillets kept 23 wk.

Table 1—Reduction in bacterial numbers in cod fillets in 2.25 kg cartons after different keeping time at −25°C

	% Reduction in bacterial numbers		
Storage time	Mesophiles	Psychrotrophs	Coliforms
1 day	0	0	0
18 wk	28	37	60
23 wk	53	43	80
50 wk	70	90	91

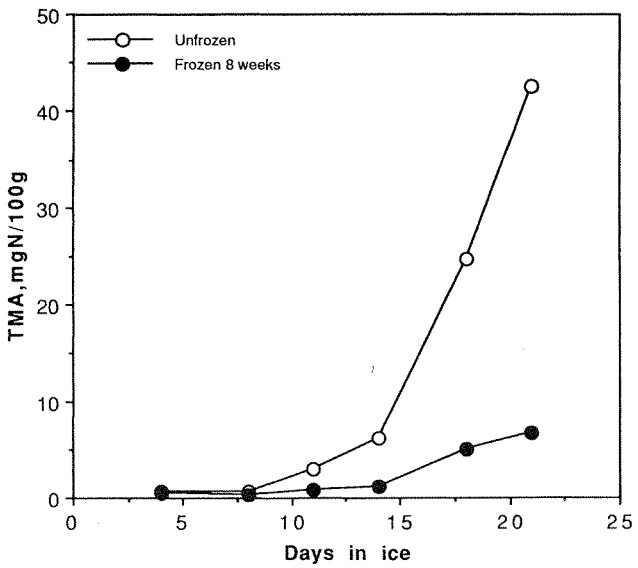

Fig. 1—Changes in trimethylamine (TMA) during iced storage of unfrozen and thawed whole cod (frozen 8 wks). Means of 4 samples.

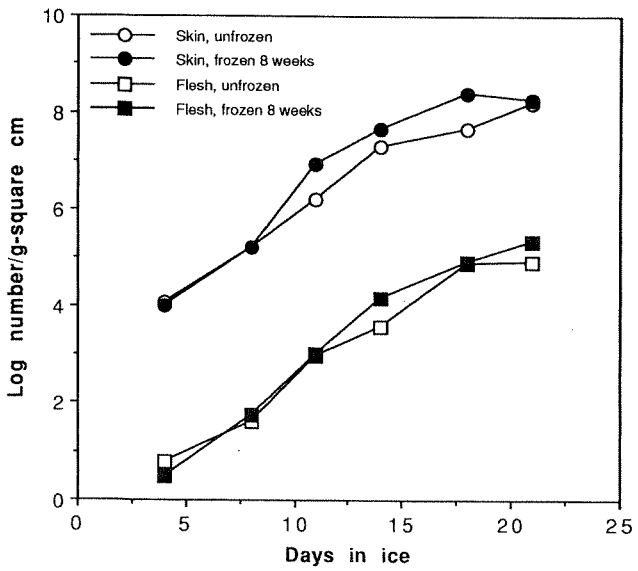

Fig. 2—Growth of bacteria on plate count agar with 0.5% NaCl (total count at 22°C) during iced storage of unfrozen and thawed whole cod (kept frozen 8 wks). Counts in both skin and flesh samples are shown. Means of 4 samples.

Table 2—Composition of the bacterial flora in cod fillets in ≈2.25 kg cartons (brackets show % proportion of TMAO reducing strains)

	% Composition of the psychrotrophic bacterial flora on PCA + 0.5% NaCl		
	Unfrozen	Frozen 1 day	Frozen 23 weeks
1. GRAM-positive	20	20	40
*Mic/Staph**	8(4)	8(4)	20(8)
Coryneforms	12(4)	12	20
2. GRAM-negative	72	80	52
*Mor/Aci**	52(8)	36(4)	32
*Flav/Cyt**	20	40	20
*Ps/Alt/Alc**	0	4	0
3. Unidentified	8	0	8

* Mic/Staph = *Micrococcus/Staphylococcus,* Mor/Acin = *Moraxella/Acinetobacter,* Flav/Cyt = *Flavobacterium/Cytophaga,* Ps/Alt/Alc = *Pseudomonas/Alteromonas/Alcaligenes*

Results from TMAO measurements in fillets kept up to 50 wk in a freezer indicated that TMAO was not broken down during freezer storage. Thus, TMAO (as mg N/100g) was 82.4 (SD ± 7.1) in unfrozen fillets, 82.1 (SD ± 8.5) in fillets kept frozen 1 day, 85.1 (SD ± 7.6) in fillets kept frozen 23 wk and 81.2 (SD ± 0.8) in fillets kept frozen 50 wk. Differences in means were not significant ($p > 0.05$).

Cod fillets

No significant difference was found between the initial number of psychrotrophic bacteria in unfrozen cod fillets, those kept frozen 1 day or 5 wk. However numbers of bacteria were lower ($p < 0.05$) when fillets had been kept in frozen storage for 14, 27 and 52 wk (Fig. 3). Most of the time in iced storage, numbers of bacteria were lower in those fillets that had been kept longest in a frozen state (Fig. 3). Differences in bacterial counts after 7, 10 and 14 days in ice were significant between fillets that had been kept for < 14 wk in a frozen state and those kept > 14 wk. After 17 days iced storage the difference was not significant.

The proportion of Gram-positive bacteria did not increase with increasing frozen storage as had been found for cod fillets in cartons (Table 3). However, during storage in ice, Gram-negative bacteria became predominant with the highest proportion of the genera *Pseudomonas/Alteromonas/Alcaligenes.* No marked difference in bacterial flora of unfrozen fillets occurred during storage in ice and frozen-thawed fillets.

From the % proportion of TMAO-reducing bacterial strains the number of such bacteria after 1 day storage in ice was calculated to be Log 4.3 in unfrozen fillets, 4.2 in those kept frozen 1 day and 3.4 in fillets kept frozen 27 wk. This corresponded to the TMAO-reducing bacteria in thawed fillets (kept frozen 27 wk) being 12% of the number of such bacteria in unfrozen fillets. During prolonged iced storage the number of TMAO-reducing bacteria was lower in thawed fillets than in unfrozen ones. Thus, after 10 days storage in ice, the number of TMAO-reducing bacteria was log 5.3 in unfrozen fillets but 4.7 in those kept frozen 27 wk. Calculated as percentages this corresponded to the TMAO-reducing bacteria in thawed fillets (kept frozen 27 wk) being 25% of the number in unfrozen fillets.

TMA measurements showed that average TMA had not exceeded 2 mgN/100g after 13 days storage in ice, even in unfrozen fillets. At the end of storage or after 21 days in ice the TMA level in unfrozen fillets was 16.3 (Fig. 4). The longer the

Table 3—Composition of the bacterial flora in cod fillets (numbers in brackets show % proportion of TMAO reducing strains)

	% Composition of the psychrotrophic bacterial flora on PCA + 0.5% NaCl								
	Unfrozen			Frozen 1 day			Frozen 27 wk		
	1 day in ice	10 days in ice	21 days in ice	1 day in ice	10 days in ice	21 days in ice	1 day in ice	10 days in ice	21 days in ice
1. GRAM-positive	48	16	12	24	24	12	44	12	0
*Mic/Stap**	8(4)	8	8	8(8)	12(4)	8	24(4)	0	0
Coryneforms	40(8)	8	4	16(4)	12	4	20(4)	12(4)	0
2. GRAM-negative	48	80	80	76	76	88	44	88	100
*Mor/Acin**	12	72(4)	28	20	40	8	20	56(4)	24
*Flav/Cyt**	28	0	8	48	0	4	20	4	0
*Ps/Alt/Alc**	8(4)	8	44(16)	8	36	76(16)	4	28	76
3. Unidentified	4	4	8	0	0	0	8	8	0

* Mic/Staph = *Micrococcus/Staphylococcus,* Mor/Acin = *Moraxella/Acinetobacter,* Flav/Cyt = *Flavobacterium/Cytophaga,* Ps/Alt/Alc = *Pseudomonas/Alteromonas/Alcaligenes.*

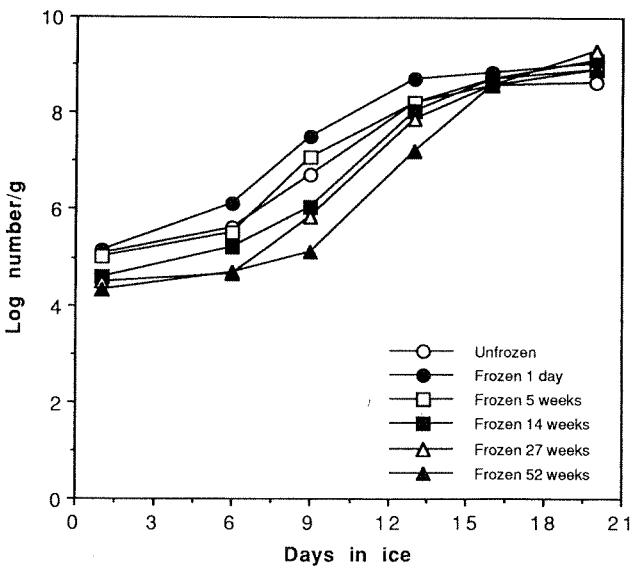

Fig. 3—Growth of bacteria on plate count agar with 0.5% NaCl (total count at 22°C) during iced storage of unfrozen and thawed cod fillets (kept frozen 1 day and 5, 14, 27 and 52 wks). Means of 3 samples.

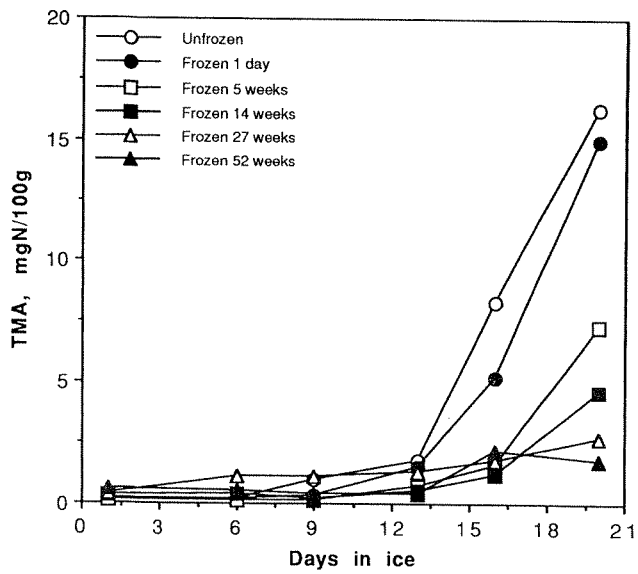

Fig. 4—Changes in trimethylamine (TMA) during iced storage of unfrozen and thawed cod fillets (kept frozen 1 day and 5, 14, and 27 and 52 wks). Means of 3 samples.

fillets had been kept frozen the slower the TMA formation during iced storage. Much more TMA was formed in the flesh of whole cod than in fillets kept on ice, reaching values > 40 after 21 days iced storage (Fig. 1). The reduction of TMAO to TMA was expected to proceed at a faster rate at conditions of low oxygen tension (Huss, 1972). The spoilage bacteria use TMAO as an electron acceptor instead of oxygen (anaerobic respiration) when oxygen is in low concentration. This leads to more TMA production in whole cod than in iced fillets where oxygen concentration would be expected to be much higher. We also expected that the skin would prevent leakage of TMAO from the flesh with the melt water from the ice.

Results from TMAO measurements in fillets kept up to 52 wk in a freezer showed that it was not broken down during freezer storage. Thus, TMAO (as mg N/100g) was 90.1 (SD ± 6.6) in unfrozen fillets, 83.3 (SD ± 9.4) in fillets kept frozen 5 wk, 85.1 (SD ± 12.3) in fillets kept frozen 14 wk, 78.2 (SD ± 3.0) in those kept frozen 27 wk and 94.9 (SD ± 4.3) in fillets kept frozen 52 wk.

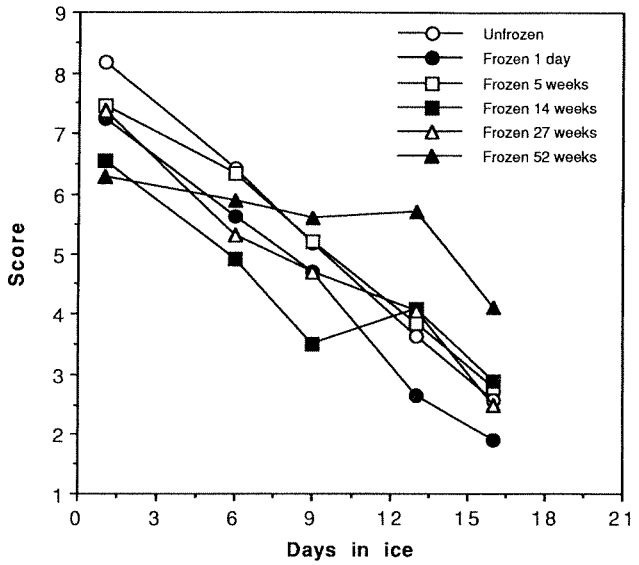

Fig. 5—Sensory evaluation during iced storage of unfrozen and thawed cod fillets (kept frozen 1 day and 5, 14, 27 and 52 weeks). Means of 3 samples.

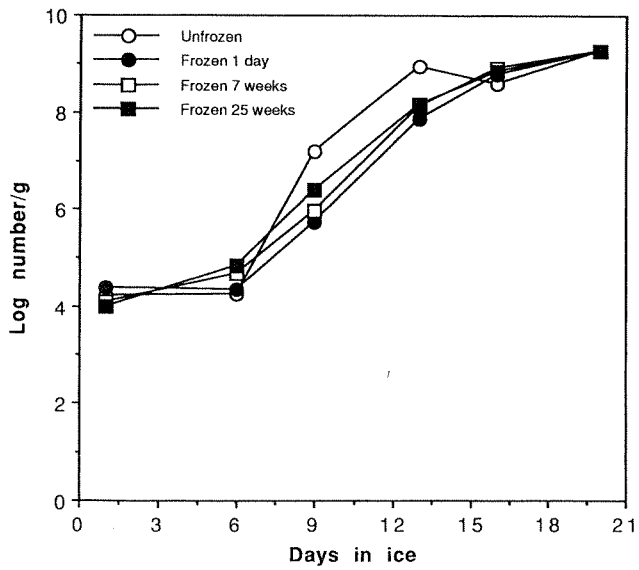

Fig. 6—Growth of bacteria on iron agar (total count at 22°C) during iced storage of unfrozen and thawed ocean perch fillets (kept frozen 1 day, 7 weeks and 25 weeks). Means of 3 samples.

Table 4—Linear regression of sensory scores for cod fillets vs days in ice (Y = a + b X (days in ice))

Storage time	Intercept (a)	Slope (b)	Coefficient of determination (r^2)
Unfrozen	8.33	−0.36	0.995
Frozen 1 day	7.49	−0.35	0.983
Frozen 5 wk	7.76	−0.30	0.983
Frozen 14 wk	6.63	−0.21	0.875
Frozen 27 wk	7.27	−0.28	0.971

Sensory evaluation showed that at the beginning of iced storage the unfrozen fillets had highest ($p < 0.05$) freshness scores (Fig. 5). Fillets kept 52 wk in frozen state were lower. Assuming linear regression (except for fillets kept 52 wk) between scores and storage time in ice the equations for the lines were calculated (Table 4) and maximum storage time in ice found using 4.5 as a borderline. In all groups except fillets kept for a year, the keeping time in ice was around 10–12 days. The fillets kept frozen for 52 wk had average scores between 5 and 6 most of the storage time. On the 14th day in ice those fillets had higher ($p < 0.05$) scores than those in all other experimental groups. By the time the panel judged the cod fillets unacceptable the TMA had not reached 2 mgN/100g. This applied both to unfrozen and thawed fillets.

Ocean perch fillets

Total counts on IA showed there was no significant difference in initial numbers of psychrotrophic bacteria in unfrozen and thawed fillets. Over 20 days storage the increase in numbers of bacteria were similar in all groups except that for 9 and 13 days in ice higher numbers were obtained on the unfrozen fillets (Fig. 6). Numbers of H_2S-producing bacteria on IA were low during storage. On average they were about 1% of the total count. No significant difference occurred in initial numbers of H_2S-producing bacteria in unfrozen and thawed fillets. However, the number of these bacteria was higher in unfrozen fillets on days 6, 9 and 13 of iced storage (Fig. 7).

A slight increase in proportion of Gram-positive bacteria was noticed during frozen storage (Table 5). In the beginning of iced storage, bacteria belonging to *Vibrio/Aeromonas* were most predominant in unfrozen fillets while in thawed fillets *Micrococcus/Staphylococcus* were most predominant. However, during iced storage, Gram-negative bacteria became predominant with high-

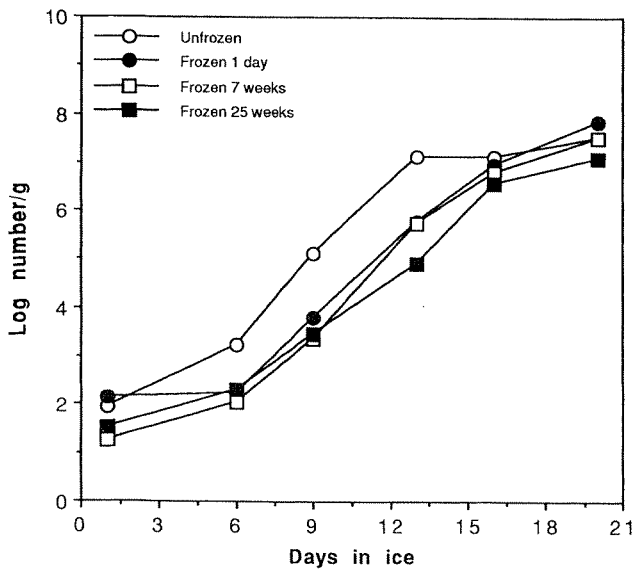

Fig. 7—Growth of H_2S-producing bacteria on iron agar (count at 22°C) during iced storage of unfrozen and thawed ocean perch fillets (kept frozen 1 day, 7 weeks and 25 weeks). Means of 3 samples.

Table 5—Composition of the bacterial flora in ocean perch fillets (numbers in brackets show % proportion of TMAO reducing strains)

	% Composition of the psychrotrophic bacterial flora on iron agar					
	Unfrozen		Frozen 1 day		Frozen 25 wk	
	1 day in ice	16 days in ice	1 day in ice	16 days in ice	1 day in ice	16 days in ice
1. GRAM-positive	44	0	60	16	52	8
*Mic/Staph**	4(4)	0	28(20)	0	40(20)	0
Coryneforms	16	0	12	12	8	8
Lactobacillus	12	0	4	4	4	0
*Ped/Strep/Leu**	12	0	16	0	0	0
2. GRAM-negative	52	100	36	80	40	92
*Mor/Acin**	16	0	4	0	20	20
*Flav/Cyt**	4	0	12	0	12	0
*Ps/Alt/Alc**	0	100(8)	0	80(12)	0	72(4)
Enterobacteriaceae	4	0	20	0	4(4)	0
Vibrio/Aeromonas	28(4)	0	0	0	4	0
3. Unidentified	4	0	4	4	8	0

* Mic/Staph = *Micrococcus/Staphylococcus*, Ped/Strep/Leu = *Pediococcus/Streptococcus/Leuconostoc*, Mor/Acin = *Moraxella/Acinetobacter*, Flav/Cyt = *Flavobacterium/Cytophaga*, Ps/Alt/Alc = *Pseudomonas/Alteromonas/Alcaligenes*.

est proportion of the genera *Pseudomonas/Alcaligenes/Alteromonas*. From the % proportion of TMAO-reducing bacterial strains the number of such bacteria after 1 day storage in ice was calculated to be Log 3.2 in unfrozen, 3.7 in fillets kept frozen 1 day and 3.4 in those kept frozen 25 wk. The number of these bacteria was not lower in thawed fillets than in unfrozen fillets during prolonged iced storage.

Thirteen of the isolated colonies from IA were black (H_2S-producing). These were identified as *Pseudomonas/Alteromonas/Alcaligenes* (8), *Vibrio/Aeromonas* (4) and *Enterobacteriaceae* (1). Only 6 of these 13 strains or 46% were able to reduce TMAO. These were *Pseudomonas/Alteromonas/Alcaligenes*. Members belonging to these genera were 62% of the total number of black colonies. This shows that not all strains which form black colonies on IA reduce TMAO and that other species than those belonging to *Pseudomonas/Alteromonas/Alcaligenes* can form black colonies. Furthermore 75% of bacteria identified as *Pseudomonas/Alteromonas/Alcaligenes* reduced TMAO. Some strains which belong to these genera therefore do not reduce TMAO. We could assume that at least some of the strains referred to as *Pseudomonas/Alteromonas/Alcaligenes* belong to the species *Alteromonas putrefaciens*, now commonly named *Shewanella putrefaciens* (MacDonell and Colwell, 1985). This species has frequently been reported as a main spoilage organism in fish.

After 16 days iced storage TMA had not yet reached 1 mgN/100g in any experimental groups. It was not until after 20 days in ice that difference was noticed in TMA values. Highest average values were found in unfrozen fillets or 6.8 (SD ± 1.4). Comparable values were 4.1 (SD ± 1.1) in fillets kept frozen 1 day, 3.5 (SD ± 0.3) in fillets kept frozen for 7 wk and 1.9 (SD ± 0.3) in fillets kept frozen 25 wk (Fig. 8). This difference was significant ($p < 0.05$) between unfrozen and thawed fillets. Results were in agreement with earlier results when examining TMA formation in frozen-thawed cod.

Analysis of TVB (mgN/100g, Fig. 9) showed in all experimental groups the TVB during the first 16 days storage had an average reduction of 28%. The explanation could be that some of the volatile bases leaked away with the melt water from the ice. Not until the 20th day of iced storage was an increase in TVB observed. The highest increase was in unfrozen fillets and there was a significant difference ($p < 0.05$) in TVB between unfrozen fillets (33 ± 3.2) and those kept frozen for 25 wk (20.8 ± 4.5).

TMAO (Fig. 10) showed initial values from 87.9 to 96.5 mgN/100g and were not significantly different ($p > 0.05$) between experimental groups. This indicated that TMAO had not broken down in the fillets during frozen storage. In all experimental groups TMAO decreased during storage in ice by up to 81%. This loss could not be related to bacterial reduction of TMAO as TMA was measured under 1 mgN/100g most of the storage time. The most likely explanation is that TMAO had leaked away with the melt water from the ice. The TMAO loss was not significantly lower in frozen-thawed fillets than in un-

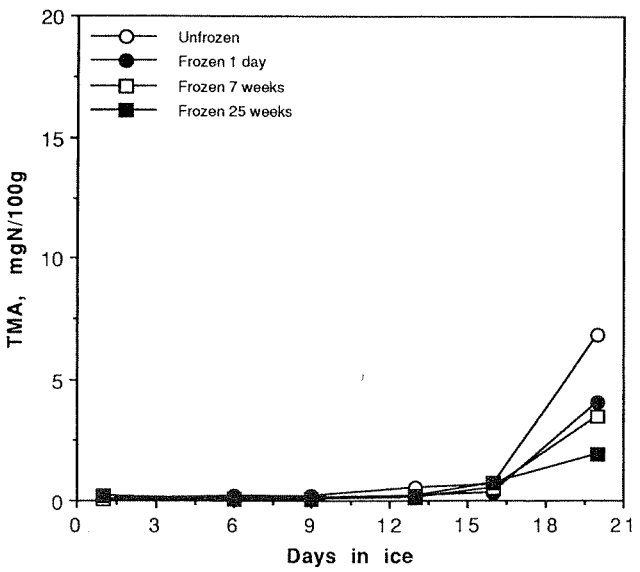

Fig. 8—Changes in trimethylamine (TMA) during iced storage of unfrozen and thawed ocean perch fillets (kept frozen 1 day, 7 weeks and 25 weeks). Means of 3 samples.

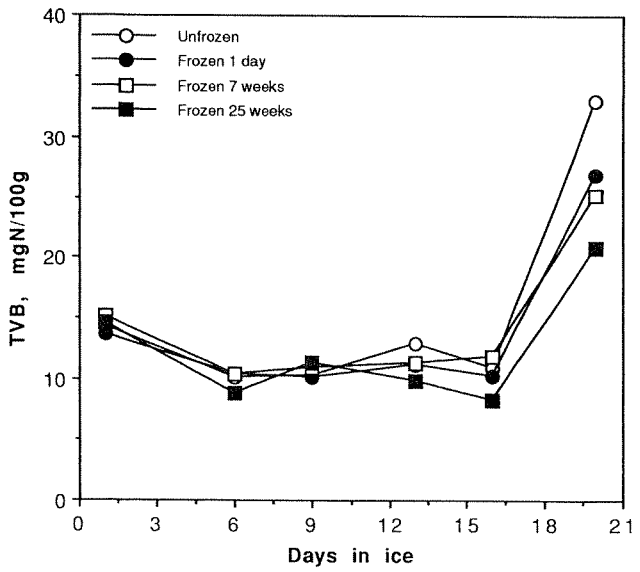

Fig. 9—Changes in total volatile bases (TVB) during iced storage of unfrozen and thawed ocean perch fillets (kept frozen 1 day, 7 weeks and 25 weeks). Means of 3 samples.

Table 6—Linear regression of sensory scores for ocean perch fillets versus days in ice (Y = a + b X (days in ice))

Storage time	Intercept (a)	Slope (b)	Coefficient of determination (r^2)
Unfrozen	8.76	−0.43	0.888
Frozen 1 day	7.71	−0.33	0.893
Frozen 7 wk	7.49	−0.33	0.917
Frozen 25 wk	6.56	−0.20	0.580

frozen fillets (as might have been expected) due to possible cell damage during freezing, freezer-storage and thawing.

Sensory evaluation showed that at the beginning of iced storage unfrozen fillets had significantly highest scores (Fig. 11). The unfrozen samples obtained higher scores throughout most of the storage period. Assuming linear regression (except for fillets kept 52 wk) between scores and storage time in ice equations were calculated (Table 6) and maximum storage time in ice was found using 4.5 as a borderline. On the 9th to 10th day all experimental groups had reached about the limit of edibility. By the time the panel judged the ocean perch fillets unacceptable the TMA was < 1 mgN/100g. This applied both to unfrozen and thawed fillets.

CONCLUSIONS

FREEZING AND SHORT-TERM FREEZER storage (≤5 wk) has little effect on bacterial counts. Thus, bacterial counts obtained from frozen samples kept for a short time in freezer reflect bacterial numbers just prior to freezing. However, during long-term freezer storage (≥14 wk) there was a reduction in total counts and counts of TMAO-reducing bacteria in cod fillets but not ocean perch fillets. The proportion of Gram-positive bacteria did not increase distinctly with increasing frozen storage as expected except in cod fillets kept in 2.25 kg cartons. However, during storage in ice, Gram-negative bacteria became predominant in both cod and ocean perch fillets. The numbers of H_2S-producing bacteria in ocean perch fillets were low throughout storage. Sensory evaluation showed that thawed fillets never had as high scores at the beginning of iced storage as unfrozen fillets. However, similar scores were found for both unfrozen and thawed fillets after 10–12 days in ice when they were unacceptable. The longer the fillets had been kept frozen, the slower the TMA and TVB formation in the fillets during iced storage. Use of TMA as a spoilage indicator is of no value when evaluating spoilage state of unfrozen and frozen-thawed cod and ocean perch fillets kept in ice.

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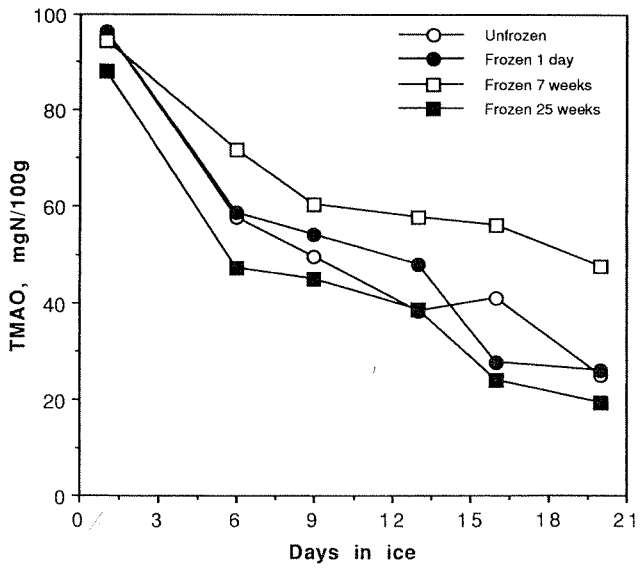

Fig. 10—Changes in trimethylamine oxide (TMAO) during iced storage of unfrozen and thawed ocean perch fillets (kept frozen 1 day, 7 weeks and 25 weeks). Means of 3 samples.

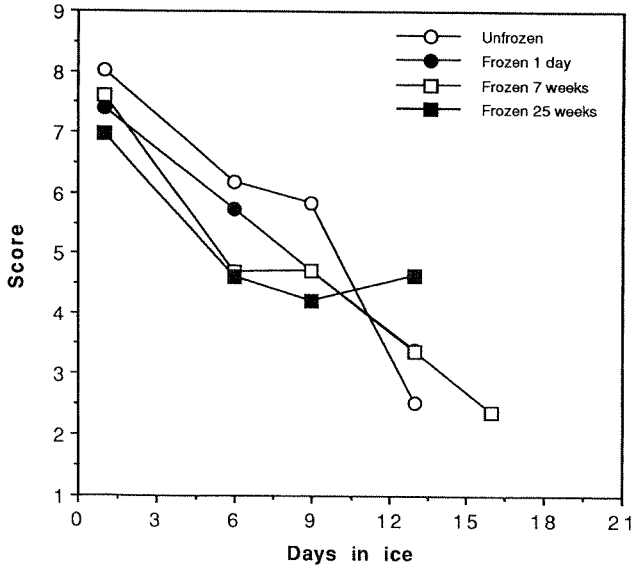

Fig. 11—Sensory evaluation during iced storage of unfrozen and thawed ocean perch fillets (kept frozen 1 day, 7 weeks and 25 weeks). Means of 3 samples.