Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins
Icelandic Fisheries Laboratories Report Summary

Titill / Title	**Bulk storage of cod. Comparison of ice, CSW and CO_2-saturated CSW storage.**		
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Ágrip á íslensku:

Heill hausaður þorskur var geymdur í þrem mismunandi kælimiðlum (ís, sjókrapa (CSW), og CO_2-mettuðum sjókrapa (CSW)). Samanburður á geymsluþoli milli kælimiðla var gerður með mælingum á efna- og örveruinnihaldi fisksins ásamt skynmati.
Geymsluþol var svipað í hópunum eða 13-14 dagar. Fiskur geymdur í krapa kom að jafnaði best út ef tillit er tekið til örveruinnihalds. Í skynmati var soðinn fiskur í krapa með kolsýru betri en hinir hóparnir. Ekki er hægt að mæla með einni geymsluaðferð umfram aðra af þeim sem prófaðar voru án þess að taka tillit til mismunandi forsenda.

Lykilorð á íslensku: Þorskur, geymsluþol, ker, ís, sjókrapi, ferskvatnskrapi, kolsýra, TMA, TVB-N, P-hlutfall, örverur, skynmat.

Summary in English:

Whole gutted cod (*Gadus morhua*) was stored in three different cooling media ice, chilled seawater (CSW) and CO_2 saturated seawater (CSW) in plastic fish tubs. Comparison of the keeping quality was made by measurements of chemical and bacteriological parameters as well as sensory evaluation.

The shelf life was found to be 13-14 days in all groups. Fish in CSW was on average judged best based on bacterial counts. Sensory analysis of cooked cod ranks CSW-CO_2 storage first place followed by cod in ice.
It is not possible to recommend one of the three tested methods without considering different aspects i.e. different quality criteria price of storage method etc.

English keywords: Cod, bulk storage, tubs, ice ,CSW, CO_2, TMA. TVN, P-ratio, bacteria, sensory evaluation.

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1 Introduction

Today the majority of bulk transport of whole fish in Iceland, domestic and export, is done by use of icing in isolated plastic tubs. The tubs are usually stored either in refrigerated areas (onboard catching vessels) or in containers (export). In 1996 some 35.700 tonnes were exported fresh (iced) in containers (Útvegur, 1996).

There are very few studies published about combination of ice and CO_2 in bulk and retail packaging of fresh fish. This kind of information would be very useful, e.g., when planning export of fish. For example, vacuum and modified atmosphere (MAP) packaging may be used both on bulk and in retail packages. The amount of ice might be reduced in bulk packages when using some packaging method that increases the shelf life of fish. It is not known whether it is economically reasonable to use packaging methods that increase the shelf-life both in bulk and in retail packages.

An increasingly common method in transport and packaging , although mostly used in retail, is the use of modified atmospheres (MA). Carbon-dioxide (CO_2) has been used to prolong the shelf life of fish in retail packaging but little for larger packages or bulk. CO_2 was used by Einarsson and Valdimarsson (1994) to store whole iced fish in a container and resulted in an increase in shelf-life of 4-7 days compared to traditional ice.

Chilled fresh and seawater (CFW and CSW respectively) has been used for storage of whole fish. However fish kept under these conditions has tended to foul and thus become unfit for production (Karlsdóttir, 1996).

It was therefore of interest to see if it was possible to prevent the fish kept in CFW and CSW from fouling by saturating the tanks with CO_2 and thus prevent the fouling organisms in growing. To simplify the experiment only CSW and traditional ice were tested.

The aim of this study was to compare different types of cooling media, as alternatives for transportation of gutted whole fresh fish in bulk. The methods used for comparison are documented in such a way that they can serve as guidelines for the industry in further comparison in the future. This will help both producers and users of tubs in the future in designing and choosing the right tub for their transport route.

2 Material and methods

2.1 Packing and storage

Iced fresh cod (*Gadus morhua*) 3-8 kg, was bought from a local fishmarket one day after catch. The fish was gutted and iced with head on in 660 l tubs.

i) Ice

Approximately 300 kilos of fish were iced with 180 kg of fresh ice in a 660 l tub. The lid was put on the tub and the tub stowed with the other groups.

ii) Chilled sea-water

The tub was filled with approximately 230 kg of fish and 170 kg of ice. Then the tub was filled with 180 liters of fresh seawater taken out at sea and then closed with a lid and stored.

iii) CO_2-saturated CSW

The tub was filled with fish, ice and seawater as above and the tub closed with a lid. Before the lid was fully closed gaseous CO_2 (Isaga hf) was bubbled for 60 min. (10 l/min) through a porous hose placed at the bottom of the tub. CO_2 bubbling was repeated at each sampling. The tubs were placed in a refrigerated storage with an ambient temperature of 3-4°C.

Table 1. Experimental set-up, showing distribution of cooling media between tubs.

Tub A	Tub B	Tub C
Ice	CSW	CSW+CO_2

2.2 Sampling and analysis

Samples were taken for microbial, chemical and sensory analysis at start (day 1) and on days 8, 11, 15 and 18.

2.2.1 Temperature. Temperature in the tubs was recorded continuously with automatic temperature-recorders (Hamster, ELBPO-BUCHS AG, Switzerland). Two recorders were placed in each tub, one near the bottom and another near the top in opposite corner sections.

2.2.2 Microbiology. From each group three fish were taken for analysis at each sampling date. A sample was taken from skin and muscle from each fish and analysed for psychrotrophic bacteria (colony forming units) and H_2S-reducing bacteria on Iron agar**.**

i) Skin samples

Three standardised samples of skin (3 x 7,5 cm^2) were taken along the lateral-line at one side of each fish. The samples were macerated in a stomacher (Lab 400) with 60 ml of Butterfield's buffer solution, inoculated onto petri plates and on iron-agar plates (pour-plate with overlay). The plates were incubated at 22±1°C for 3 days before counting. Total number of colony forming units (cfu), as well as the number of black colonies (H_2S-producing bacteria) were counted.

ii) Muscle samples
On the opposite side of the fish the skin was removed aseptically and the underlying muscle removed and minced aseptically. 25g of mince from each fish were diluted with Butterfield's buffer and analysed for bacteria in the same way as above.

2.2.3 Freshness. Freshness was evaluated by measuring total volatile base nitrogen (TVB-N) and trimethylamine (TMA) in fresh minced filet from each group.

TVB-N was measured on a Struer TVB-N instrument, (Struer, Denmark), based on the method of Antonacopulus (1968). Ten grams of minced fillet were distilled with 1 tsp. of MgO and 100 ml of water into 100 ml of 0,27% boric acid containing 5 drops of Tashiro indicator. After distillation the distillate was titrated with 0,1 N H_2SO_4 to the equivalence point.

TMA was measured on a Struer instrument (Struer, Denmark) based on a method by Malle and Tao (1987). One-hundred grams of minced fillet were homogenised with 200 ml of 7,5% TCA and filtered. Twenty five ml of filtrate, 10 ml of 10% NaOH and 20 ml of 35% formaldehyde were placed into a 500 ml boiling flask and the ammonia was distilled into a receiver flask containing 10 ml of 4% boric acid and 0,04 ml methyl red/bromocreosol green until about 80 ml of distillate had been collected. The distillate was titrated with 0,025 N H_2SO_4 to the equivalence point when the solution became gray.

The P-ratio was calculated from TVB-N and TMA as:

$$\text{P ratio} = \left[\frac{mg\,\text{TMA} - \text{N} / 100\,g\,\text{fish}}{mg\,\text{TVB} - \text{N} / 100\,g\,\text{fish}} * 100 \right]$$

Where [mg TVB-N/100 g fish = ml titrated x 14] and [mg TMA-N/100 g fish = ml titrated x 4,2].

2.2.4 Sensory evaluation.
Sensory evaluations were carried out using score schemes according to IFL (Martinsdóttir, 1995) which may be found in table 7.2 and 7.3 in the appendix. Three fish were evaluated at day one and two fish at day 8, 11 and 15. No evaluation was performed on day 18 as the fish was too spoiled.

i) Whole cod.
Whole fish was evaluated by 5-7 trained panelists according to a "Quality index method", where each parameter (e.g. smell of gills) was recorded separately and rated 0-3 or 0-2 according to the influence of the parameter (table 7.3). The scores were summed into a final score (i.e. quality index).

ii) Cooked cod.
The fish was filleted and cooked and freshness and appearance evaluated by 9-11 panellists according to a Torry scheme (table 7.1) ranging from 10 to 3, wher a score of 10 is given for very fresh fish. At IFL an average score of 5,5 is used as a minimum score for consumability (Martinsdóttir, 1995). Each sample was tested three times during one day.

3 Results

3.1 Temperature

Recordings of temperature in the tubs are presented in figure 7.1 in the appendix. In the Ice-group the lower recorder showed steady readings just below 0°C. The upper recorder showed a steady rise to approximately 4°C during the storage period (figure 7.1). In the CSW and CSW+CO_2 groups the temperature was stable around -1,5°C regardless of position in the tub (figure 7.3). The upper recorder in the CSW group had clearly failed, showing temperatures down to -30°C (figure 7.2).

3.2 Freshness (TMA and TVB-N)

The results of TVB-N and TMA analysis are presented in table 7.1 in the appendix.

In all groups the TVB-N showed similar development, little or no increase the first 11 days and and then a sharper increase towards day 18. At day 18 there was no difference between the ice and CSW groups, but the CSW+CO_2 group was slightly higher in TVB-N.

In the ice-group very little increase in TMA was detected until day 18, when a significant increase was detected. No significant difference was detected between the CSW and CSW+CO_2 groups, TMA started to rise at day 11 and was significantly higher than in the ice-group throughout day 18.

The ratio between TMA and TVB-N (P-ratio) was calculated and is presented in figure 6 and table 7.1. No significant change was found in the P-values for any group until day 11 when the value for the CSW and CSW+CO_2 groups began to rise. At day 18 there was no significant difference between the CSW and CSW+CO_2 groups, but they were significantly higher than the ice -group.

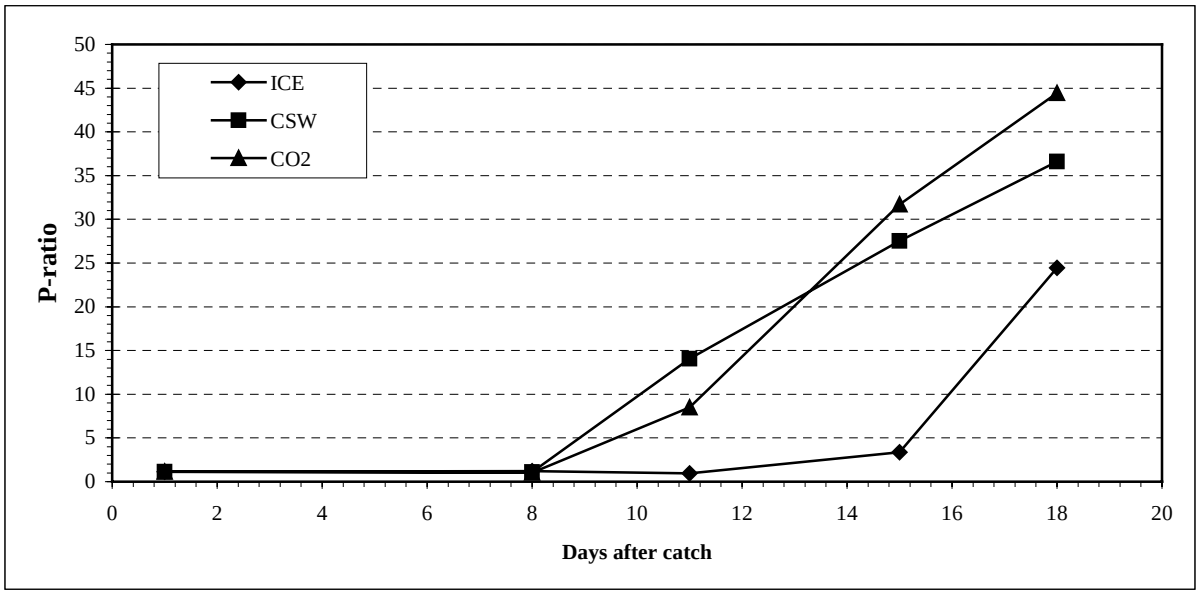

*Figure 1. Effect of storage in different cooling media on the ratio between TVB-N and TMA (P-ratio = (TMA/TVN)*100) in the muscle of cod.*

3.3 Microbiology

Results from the microbiological analyses are presented in figures 2 through 6 and in table 7.1 in the appendix.

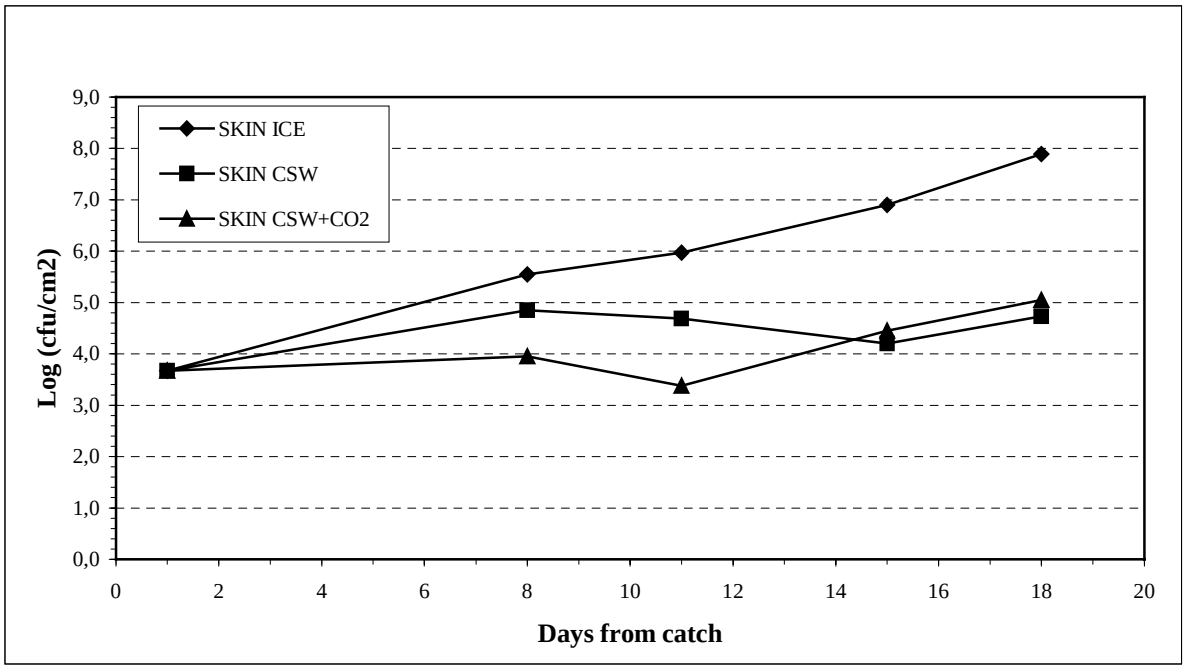

Figure 2. Effect of different cooling media on the number of colony forming units (cfu/cm^2) on skin of cod (Iron agar at 22°C).

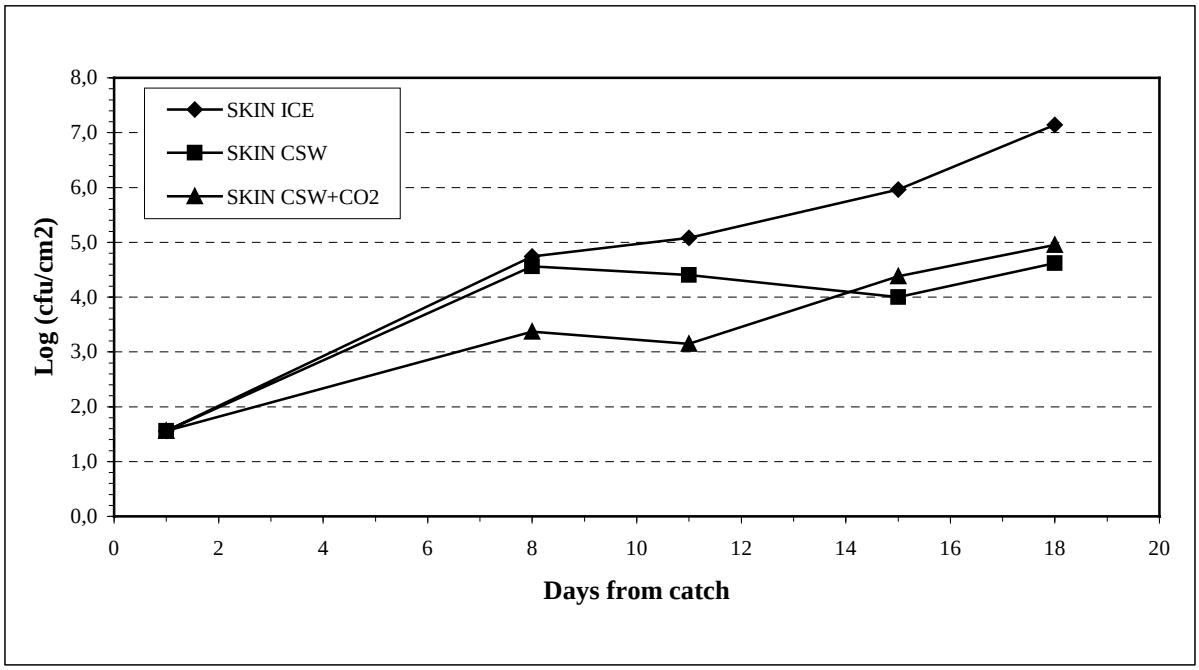

Figure 3. Effect of different cooling media on the number of H_2S-producing colony forming units (cfu/cm^2) on skin of cod (Iron agar at 22°C).

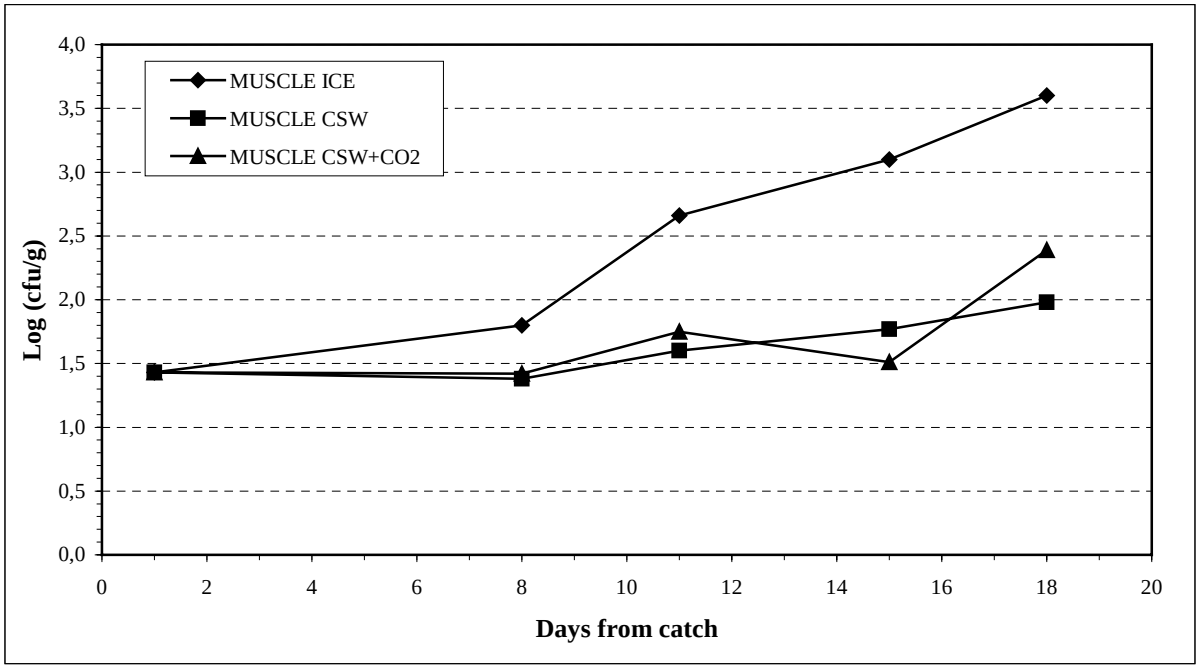

Figure 4. Effect of different cooling media on the number of colony forming units (cfu/cm^2) in muscle of cod (Iron agar at 22°C).

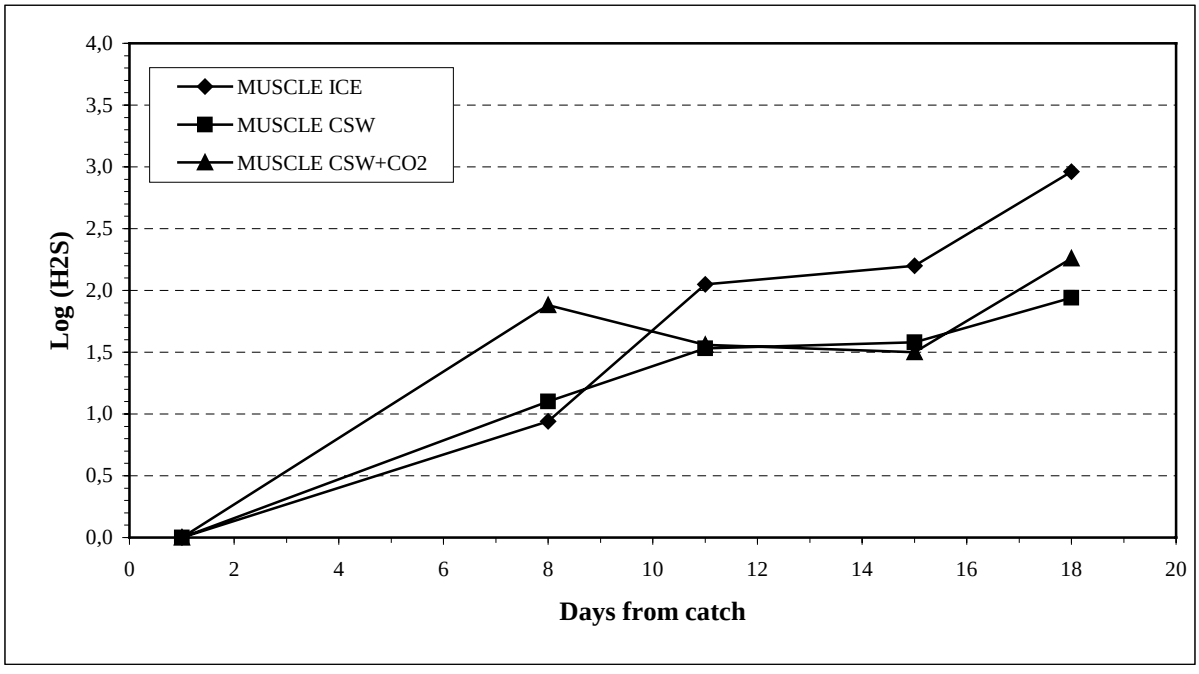

Figure 5. Effect of different cooling media on the number of H_2S-producing colony forming units (cfu/cm^2) in muscle of cod (Iron agar at 22°C).

3.4 Sensory evaluation

i) Whole cod.

For whole cod the ice-group got the highest sensory scores throughout the experiment followed by the CSW+CO_2 and CSW respectively (except on day 8). In the CSW and CSW+CO_2 groups the general appearance of the fish, especially the gills, was dull and whitish, resulting in lower score. The result of the evaluation is presented in table 2.

Table 2. Results of sensory evaluation of whole cod according to Quality index method (score 0-23). Lower score means better result.

Sample day	ICE	CSW	CSW+CO_2
1	1,1	1,1	1,1
8	7,8	9,8	11,0
11	11,0	14,7	13,2
15	14,3	16,9	15,9

ii) Cooked cod.

The results of the sensory analysis of cooked cod are presented in table 3. As all groups scored less than 5,5 points on day 15 no evaluation was performed at the last sampling date (day 18). On day 8 the CSW group scored significantly lower than the other groups. Both the CSW and ice-groups scored for significantly lower than the CSW+CO_2 group on day 11 although all groups were in fairly good condition. On day 15 all groups scored lower than 5,5 and were thus judged not fit for consumption, having distinct sour- and TMA-taste.

Table 3. Results from sensory evaluation of cooked cod according to Torry-scores. Average scores (SD). At IFL a value of 5,5 is used as a boundary for consumability.

Sample day	ICE	CSW	CSW+CO_2
1	9,5 (0,5)	9,5 (0,5)	9,5 (0,5)
8	8,4 (0,6)	7,3 (1,2)	8,6 (0,7)
11	7,3 (1,0)	7,0 (1,1)	7,9 (0,9)
15	5,4 (1,0)	4,7 (0,9)	5,0 (1,2)

4 Discussion

The fish used in this study was of a general good quality. Although there was some variation in size and appearance between the batches received, it was not considered necessary to mix them before setting up the experiment.

No real difference in storage time was detected between the methods tested in the current study. All groups had a storage time of 13-14 days which is normal. However, overall ranking (table 4) reveals that storage in CSW gives a better result than the other groups although some individual parameters rank higher in the ice-group. Use of CSW (preferrably with CO_2) may therefore be advantageous in some cases, e.g. where the appearance of the fish is not crucial.

Table 4. Evaluation of the storage methods based on ranking the results, the highest rank giving the best result (+++ is better, + is worse).

Parameter		ICE	CSW	CSW+CO_2
Skin	**Cfu**	+	+++	++
	H_2S	+	+++	++
Muscle	**Cfu**	+	+++	++
	H_2S	+	+++	++
Sensory	**Whole**	+++	+	++
	Cooked	++	+	+++
P-ratio		**+++**	**++**	**+**
SUM		**12**	**16**	**14**

The P-ratio (the ratio between TVB-N and TMA) has been suggested to better express the freshness of stored fish (Horner *et. al.*, 1994) as it is not affected by leaching of volatile substances from the fish due to run-off or washing out. In the current study this was found valuable as the fish was stored under different conditions and even submerged in seawater. No significant differences are found in TVB-N concentrations between the groups while a marked difference is found in TMA. This difference is enhanced by expressing the results as P-ratio, whereas most of the TVB-N measured at day 11 is derived from TMA.

During spoilage TMA is produced from TMA-O by bacteria. Low numbers of bacteria may therefore explain the low values of TMA during the first period of storage. The increase in TMA in the CSW, CSW- CO_2 groups without apparent increase in bacterial numbers must there fore be explained by bacteria not detected or (and) more active TMA producing bacteria.

Sensory analysis of whole cod reveals that storage in ice gives the best result, with the other two groups quite similar. Lower scores of the CSW and CSW+CO_2 groups may be explained by the development of a pale appearance, especially on the gills, caused by whitening of the mucus, as found also by Einarsson and Valdimarsson (1994). This mucus may easily be washed off with water before processing and thus “restoring” quality. Compared with the ice-group, the CSW and CSW-CO_2 groups had less bacteria (cfu and H_2S) both on skin and in flesh, which may be explained by the unfavorable conditions for bacterial growth: low temperature and low oxygen (in CSW+CO_2).

For cooked cod the CSW+CO_2 group gets the best sensory score overall, except at day 15 where the Ice-group gets a slightly higher score.

Sensory analysis suggests that by use of CSW+CO_2 one can obtain at least as good results as for the traditional icing-method. However, fish stored in CSW with or without CO_2, tends to lose some of its "visual-quality" but still maintains good "sensory-quality" although the CSW group scores lower than CSW+CO_2.

In general bacterial numbers (cfu/unit) were found to increase during the first 8 days of storage, followed by a slowdown period and a further slow increase from day 11 onwards, which may be caused by variation in the composition of the microflora. Higher numbers of bacteria, both TBC and H_2S producing, were detected on skin compared to muscle in all groups.

The bacterial growth (cfu and H_2S-producing bacteria) was higher in the Ice-group compared to CSW and CSW+CO_2 throughout the experiment with the exception of H_2S-producing bacteria in muscle, where numbers were lowest until day 11 when they surpassed the other groups.

As may be seen in figures 7.1 through 7.3, the temperature in the tubs was stable once it had stabilised after the fish was put in, with the exeption of the upper recorder in the Ice-tub, which showed a slow increase in temperature throughout the experiment. This may be explained by melting of the ice at the top. Sub-zero temperatures at the bottom of the same tub may be explained by accumulation of melting water, as the outlets at the bottom were partially obstructed by fish. However this should not have affected the results significantly as samples were taken in the upper part of the tub above the accumulated water. The readings of the upper recorder in the CSW-group were rejected as the recorder clearly showed foulty readings, down to –30°C.

Distribution of CO_2 into the tub may not have been ideal, as the tubs are not designed for this purpose. It may be possible to increase the effectiveness of CO_2-addition by means of tub-design, thus increasing the efficiency of CO_2 distribution. Also continuous addition of CO_2 throughout the storage period may be advantageous which would however increase cost and complicate the use of the system. Therefore this experiment was focused on using existing material with minor modifications.

No judgement is passed on the absolute quality of the storage methods tested, as the differences are not clear enaough. They are all good enough for short time storage and transport if the quality of the raw material is good. However, if the time from catching plus the transport time is near the keeping time of the fish it may be advantageous to use CSW, preferrably with CO_2, and well insulated tubs. By comparison of the sensoric, bacterial and chemical results it may be concluded that CSW with added CO_2 gives the best overall result, with low bacterial numbers and high sensory score of flesh, although TMA is high.

By use of these methods it may be possible to reach markets that are further away or to reach traditional markets using less expensive modes of transport e.g. ships instead of planes etc.

The method that gets your product to the right place at the right time with the right quality at the lowest cost is the best method for that particular application.

Further studies on the use of modified atmosperes should pay attention to the method of distribution into the tubs to ensure full effect of the gas used. Also one might consider to modify the ice itself by saturating the water with MA before produsing the ice or to use other gases than CO_2 (e.g. N_2, O_3) or mixtures thereof.

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7 Appendix

Table 7.1. Results of chemical and microbiological analysis of cod stored under different conditions.

					Skin (22°C)		Muscle (22°C)	
Group	**Day**[1]	**TVN**	**TMA**	**P ratio**[2]	**Log (TBC)**	**Log (H_2S)**	**Log (TBC)**	**Log (H_2S)**
ICE	**1**	17,9 (1.3)	0,2 (0,0)	1,12 (0,08)	3,7	1,6	1,4	0,0
	8	16,5 (0,4)	0,2 (0,0)	1,22 (0,03)	5,6	4,7	1,8	0,9
	11	22,1 (5,2)	3,1 (0,0)	0,94 (0,20)	6,0	5,1	2,7	2,1
	15	23,0 (4,3)	0,8 (0,8)	3,36 (3,50)	6,9	6,0	3,1	2,2
	20	32,5 (11,1)	9,1 (7,0)	24,46 (17,50)	7,9	7,1	3,6	3,0
CSW	**1**	17,9 (1,3)	0,2 (0,0)	1,12 (0,08)	3,7	1,6	1,4	0,0
	8	18,1 (1,2)	0,2 (0,0)	1,11 (0,07)	4,9	4,6	1,4	1,1
	11	21,6 (1,9)	3,2 (2,9)	14,06 (12,23)	4,7	4,4	1,6	1,5
	15	24,4 (5,1)	7,2 (4,5)	27,53 (14,25)	4,2	4,0	1,8	1,6
	20	34,2 (5,9)	12,8 (6,0)	36,59 (11,74)	4,7	4,6	2,0	1,9
CSW+CO_2	**1**	17,9 (1,3)	0,2 (0,0)	1,12 (0,08)	3,7	1,6	1,4	0,0
	8	19,4 (1,4)	0,2 (0,0)	1,03 (0,07)	4	3,4	1,4	1,9
	11	19,9 (2,4)	1,8 (2,49)	8,51 (9,27)	3,4	3,2	1,8	1,6
	15	27,8 (3,6)	7,2 (2,5)	31,7 (5,00)	4,5	4,4	1,5	1,5
	20	44,4 (10,0)	20,5 (8,4)	44,43 (10,03)	5,1	5,0	2,4	2,3

[1] Experiment started two days after catch.
[2] TMA/TVN*100

Table 7.2. Scale used for sensory evaluation of whole cod by Quality index method (Martinsdóttir 1995).

Quality aspect	***Parameter***	***Description***	***Score***
Appearance	*Skin*	*Bright, iridescent pigmentation*	*0*
		Rather dull, becoming discoloured	*1*
		Dull	*2*
	Stiffness	*In rigor mortis*	*0*
		Firm, elastic	*1*
		Soft	*2*
		Very soft	3
Eyes	*Cornea*	*Clear*	*0*
		Opalescent	*1*
		Milky	*2*
	Form	*Convex*	*0*
		Flat, slightly sunken	*1*
		Sunken, concave	*2*
	Colour of pupil	*Black*	*0*
		Opaque	*1*
		Grey	*2*
Gills	*Colour*	*Bright*	*0*
		Less coloured, becoming discoloured	*1*
		Discoloured, brown spots	*2*
		Brown, discoloured	*3*
	Smell	*Fresh, seaweedy, metallic*	*0*
		Neutral, grassy, musty	*1*
		Yeast, bread, beer, sour milk	*2*
		Acetuc acid, sulphuric, very sour	*3*
	Mucus	*Clear*	*0*
		Milky	*1*
		Milky, dark, opaque	*2*
Blood	*Colour*	*Red*	*0*
		Dark red	*1*
		Brown	*2*
Fillet, cuts	*Colour*	*Translucent, bluish*	*0*
		Waxy, milky	*1*
		Opaque, yellow, brown spots	*2*
Quality standard			***0-23***

Table 7.3. Criteria (Torry-scale) used for sensory evaluation of cooked cod (Martinsdóttir, 1995).

Description of odour	Taste	Score
Initially weak smell of sweet, boiled milk, starchy followed by strengthening of these odours	Watery, metallic, starchy. Initially no sweetness but meaty flavours with slight sweetness	10
Shellfish, seaweed, boiled meat	Sweet, meaty, characteristic for the species	9
Loss of odour, neutral odour	Sweet and characteristic flavours but reduced in intensity	8
Woodcarvings, woodsap, vanillin	Neutral	7
Condensed milk, boiled potatoes	Insipid	6
Milk jug odours, boiled laundry-like	Slight sourness, trace of off-flavours	5
Lactic acid, sour milk, TMA	Slight bitterness, sour, off-flavours, TMA	4
Lower fatty acids (e.g. acetic or butyric acids), composed grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide	3

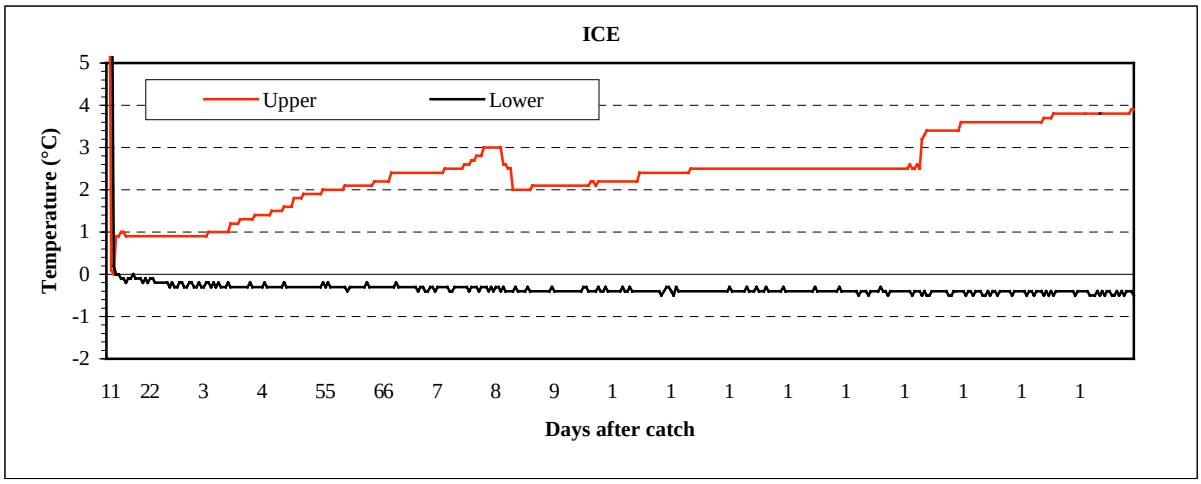

Figure 7.1. Temperature recordings in the Ice-tub during the experiment.

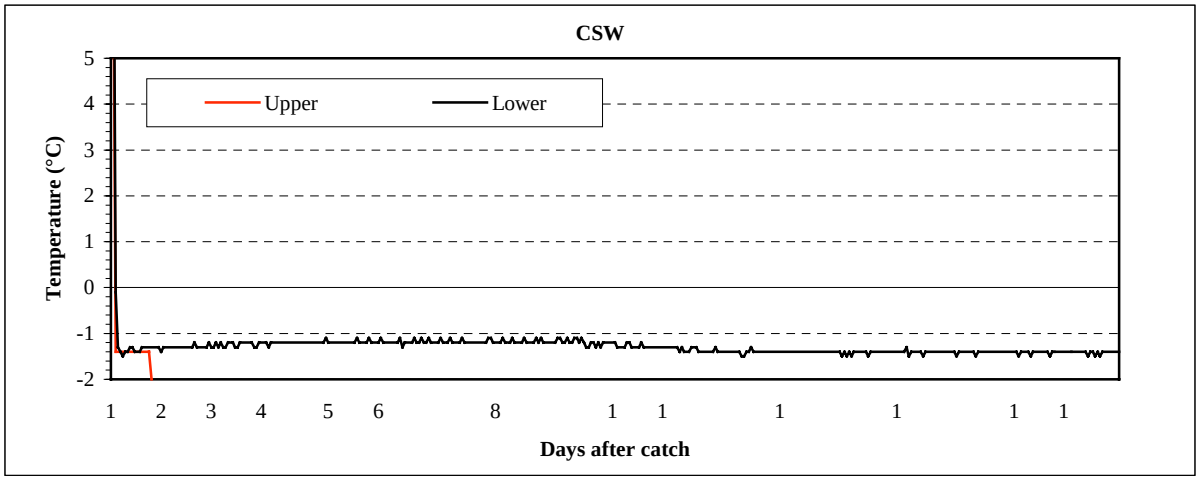

Figure 7.2. Temperature recordings in the CSW-tub during the experiment.

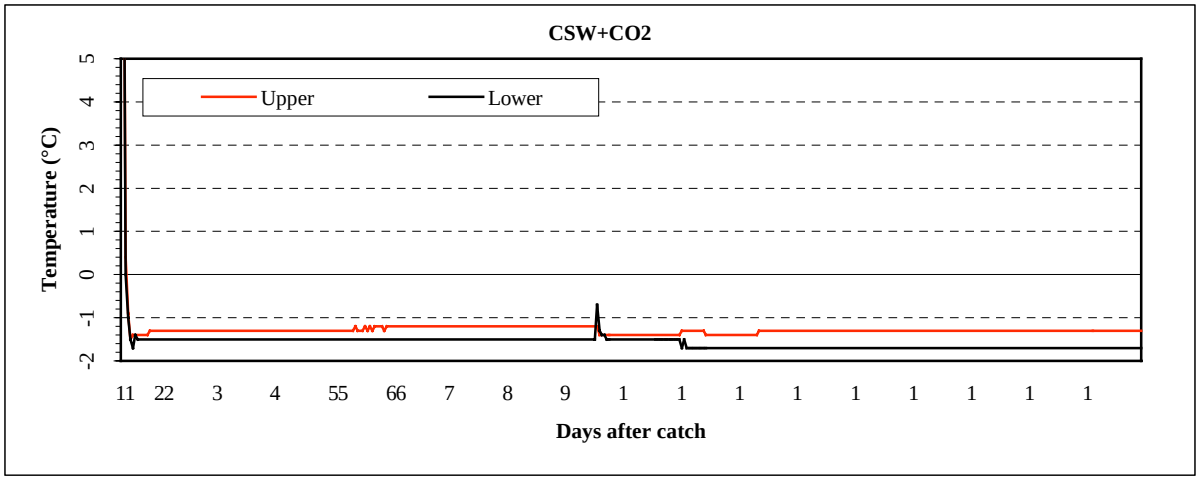

Figure 7.3. Temperature recordings in the CSW+CO_2-tub during the experiment.