Nýsköpun & neytendur
Innovation & Consumers

Vinnsla, virðisaukning & eldi
Value Chain, Processing & Aquaculture

Mælingar & miðlun
Analysis & Consulting

Líftækni & lífefni
Biotechnology & Biomolecules

Öryggi, umhverfi & erfðir
Food Safety, Environment & Genetics

Addition of collagen to heavy salted and lightly salted, chilled and frozen cod fillets

Kristín Anna Þórarinsdóttir
Hannes Magnússon
Irek Klonowski
Ásbjörn Jónsson
Frank Hansen
Egil Olsen
Sigurjón Arason

Vinnsla, virðisaukning og eldi

Skýrsla Matís 12-11
Maí 2011

ISSN 1670-7192

Skýrsluágrip Matís ohf
Icelandic Food and Biotech R&D
Report summary

ISSN: 1670-7192

Titill / Title	Addition of collagen to heavy salted and lightly salted, chilled and frozen cod fillets		
Höfundar / Authors	Kristín Anna Þórarinsdóttir[1], Hannes Magnússon[1], Irek Klonowski[1] Ásbjörn Jónsson[1], Frank Hansen[2], Egil Olsen[3], Sigurjón Arason[1] Affiliation: [1]Matis, [2]Hansen & Øye Consulting, [3]Delta Seafood/MVL		
Skýrsla nr. / Report no.	12-11	Útgáfudagur / Date:	Maí 2011
Verknr. / project no.	2002-2026		
Styrktaraðilar / funding:	NORA		
Ágrip á íslensku:	Áhrif viðbætts gelatíns sem unnið var úr fiski, voru könnuð á nýtingu, efnasamsetningu og gæði kældra, frystra og saltaðra þorskflaka. Gelatíninu var blandað í saltpækil sem síðan var sprautað í flökin. Söltuð flök voru pækluð eftir sprautun, síðan þurrsöltuð í 3 vikur og að lokum útvötnuð. Til samanburðar voru notuð flök sem sprautuð voru eingöngu með saltpækli. Meginniðurstöður voru þær að áhrif gelatíns á nýtingu og efnasamsetningu væru óveruleg. Breytingar voru fyrst og fremst af völdum hækkaðs saltinnihalds. Öðru máli gegndi um skemmdarferla í kældum afurðum. Örveruvöxtur og myndun niðurbrotsefna var meiri í þeim flökum sem sprautuð voru með gelatíni. Ekki var þó hægt að greina sjónrænan mun á útliti flaka eftir samsetningu pækils.		
Lykilorð á íslensku:	*Þorskur, gelatine, sprautun, nýting, gæði*		
Summary in English:	The effects of added fish gelatine on yield, chemical composition and quality of chilled, frozen and salted cod fillets were evaluated. The gelatine was mixed with salt brine and injected to the fillets. Salted fillets were brined after injection, dry salted for 3 weeks and finally rehydrated. Fillets injected only with salt brine were used as control. Effects of added gelatine on yield and chemical composition were not significant. Alterations were primarily due to the increased salt content by injection. Conversely, the growth of microorganisms and degradation within chilled fillets was accelerated by addition of gelatine. However, no significant differences were observed in visual appearance of the fillets.		
English keywords:	*Cod, gelatine, injection, yield, quality*		

© Copyright Matís ohf / Matis - Food Research, Innovation & Safety

Contents

Objectives 1

Material and methods 2

Experimental design 2

Raw material 3

Processing 3

Weighing 4

Sampling 4

Weight changes and cooking yield 4

Analysis of chemical composition 5

Water holding capacity 5

Microbial analysis 5

Analysis of TVB-N and TMA 6

Analysis of TBARS 6

Measurement of pH 6

Statistical analysis of data 6

Results and discussion 7

Raw fillets 7

Chilled fillets 7

Frozen fillets 12

Salted fillets 13

Conclusion 15

Acknowledgement 15

References 16

Objectives

The main objective of this study was to investigate if and how gelatine produced from by-products of cold water fish species could be used for injection to fish products. The gelatine was injected into cod fillets and the effects on weight and quality of chilled, frozen and salted cod fillets investigated in relation to following questions:

- Does the addition of gelatine increase weight yields?
- Are influences on chemical composition significant?
- How is the shelf life and quality affected (microbial and chemical analysis)?
- Are there visual differences in the appearance of the products?

The aim was to use the results described in this report to identify possibilities in utilisation of the gelatine for processing of commercial fish products, with regard to potential buyers, prices and markets.

Material and methods

Experimental design

Fillets were injected with brine without (salt) and with addition of gelatine (salt+gelatine). The fillets were then stored as chilled, frozen and heavy salted (Figure 1).

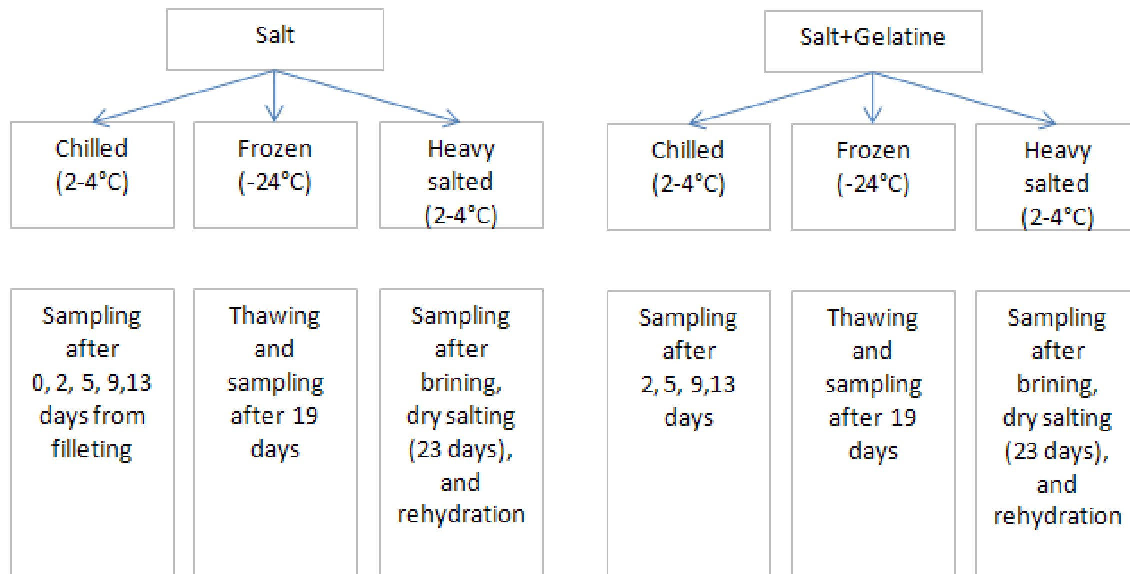


Figure 1. Experimental plan, fillets were injected with salt or with brine containing both salt and gelatine. After that different preservation methods were applied, chilling, freezing and further salting.

Parameters evaluated in fillets treated by different methods are listed in Table 2. Microbial growth was evaluated under aerobic and anaerobic conditions.

Table 1. Parameters evaluated in the experiments

Parameters	Chilled	Frozen	Salted
Weight changes during processing and storage	x	x	x
Cooking yield		x	x
Fat content (Soxhlet, Bligh and Dyer), PV, TBA		x	c
Microbial growth (TVC, H_2S), TVB, TMA	x		

Raw material

Cod (*Gadus morhua*) was caught on the 8th of March, 2011, in Ísafjarðardjúp on long-line. The fish was bled on board and stored in ice, in tubs during transport to Reykjavík. On 9th of March, the fish was gutted, beheaded and filleted. The average weight of the fillets was 264.8 ± 37.5.

Coarse salt ESCO Fishery salt K 3.2-0.4, European Salt Company GmbH & Co.KG, Hannover, Germany) was used for the procedures applied. Specifications are shown in Table 2.

Dried fish gelatine (high molecular weight) was used in the process for injection to the fish fillets. The collagen was extracted from cod skin and coarsely filtered. Thereafter, ultrafiltered and vacuum dried.

Table 2. Product data sheet for ESCO Fishery salt K 3,2-0,4 (European Salt Company GmbH & Co.KG, Hannover, Germany).

Chemical Analysis:	**Typical**	**Methods**
• Sodiumchloride	99 %	ASTM 534-98
• Calcium	0,3 %	ISO 2482
• Magnesium	0,02 %	ISO 2482
• Sulphate	0,7 %	ISO 2480
• H_2O-insoluble	0,15 %	ISO 2479
• Iron	5 mg/kg	ESPA/CN-115-00
• Copper	0,08 mg/kg	ESPA/CN-115-00
Granulometry:	**Typical**	**Methods**
• > 3,15 mm	10 %	EN 1235
• 0,40 - 3,15 mm	85 %	
• < 0,40 mm	5 %	
Physical Properties:		**Methods**
• Bulk density	1.050 - 1.250 kg/m³	EN 1236
On request:		
• anti caking agent E 535, calculated as $K_4[Fe(CN)_6]$ anhydr. and E 500	10 mg/kg	ESPA/CN-111-96

Processing

The brine used for injection contained salt only (20% w/w) or salt (20% w/w) and gelatine (2% w/w). The fish was processed under chilled conditions (5-10°C). In this temperature range, the solubility of the gelatine is reduced, which caused some difficulties regarding the injection procedure. The gelatine already solubilised, precipitated when getting in contact with cold parts of the machine and fish. The salt concentration of the brine did not cause problems in solubilisation of gelatine.

The fillets were injected using a multi-needle brine injector (Dorit INJECT-O-MAT, Model No. PSM-42F-3.0l, Dorit Fleischereimaschinen GmbH, Ellwangen, Germany) under 45 strokes per min and 20 mm belt advance per stroke. The diameter of the needles (n=42) was 2 mm and the distance between needles was approximately 20 mm. The injection dept of the needles was 5.5 mm from getting through the fillets. The pressure was 2.2 bars.

After injection, the fillets were divided in three groups for different preservation methods: Chilled, frozen and heavy salted.

Chilled fillets: The fillets were packed in plastic bags and stored in polystyrene boxes at 2°C until sampling took place.

Frozen fillets: The fillets were packed in plastic bags in one layer and frozen in air and stored at -24°C. After 19 days the fillets were thawed for 3 hours at room temperature (20°C) and over night at 2°C, covered with a plastic film.

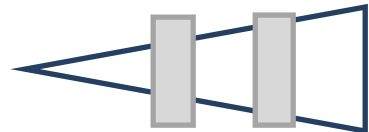
Salted fillets: The fillets were brine salted in a fish to brine ratio of 1:2 for 2 days. Then, they were dry salted for 21 days. The fillets were stacked with alternating layers of salt in plastic boxes, where the liquid was allowed to drain away from the fillets. After salting, the fillets were rehydrated in a ratio of 1:5 for 16 hours, and then the water was replaced with fresh water (ratio 1:5), for further rehydration for 72 hours.

Weighing

The fillets were weighed fresh and at each sampling point for evaluation of weight changes (yield). Additionally, weight changes during cooking of frozen, thawed and salted fillets were recorded.

Sampling

Chilled fillets: Samples were collected from the raw material and after 2, 5, 9, 13 days of storage. Two slices from different parts of the fillet (n=3) were pooled for analysis of water holding capacity (WHC), water and salt content. The rest of the fillet was pooled for analysis of microbial growth, total volatile base nitrogen (TVB) and trimethylamine (TMA).



Frozen fillets: Two slices from different part of the fillet (n=3) were pooled for analysis of water holding capacity (WHC), water and salt content. The rest of the fillet was pooled for analysis of fat content, peroxide value (PV), and thiobarbituric acid reactive substances (TBARS).

Salted fillets: Two slices from different part of the fillet (n=3) were pooled for analysis of water holding capacity (WHC), water and salt content. The rest of the fillet was pooled for analysis of fat content, PV, TBARS, TVB and TMA.

Weight changes and cooking yield

Fillets were allowed to drain for 10 min after injection and then weighed before storage or further processing. Weight changes were followed by recording the weight at each sampling point. The **weight yield (%)** was calculated as the relative mass of the weight of raw fillets (n=12).

Chilled fillets: The weight of fillets (n=3) at each sampling point was recorded.

Frozen fillets: The weight of frozen fillets (n=6) was recorded before and after thawing.

Salted fillets: The weight was recorded after brining (n=9), after dry salting (n=6) and after rehydration (n=3).

Drip loss (%) was expressed as relative weight reduction during thawing. **Cooking yield (%)** of frozen (n=3) and salted rehydrated fillets (n=3) was calculated from weight changes during cooking. Each fillet was placed on baking paper on a grid and cooked at 95-100 °C for 10 min in a preheated conventional steam oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany). After cooking, samples were allowed to cool at room temperature for 20 min, and then reweighed. The cooking yield (%) was calculated as the ratio of retained weight after cooking compared to the weight before cooking.

Analysis of chemical composition

Water content (%) of the fresh fish was determined according to ISO 6496 (1999). The **salt content** (%) was determined according to the AOAC Official Methods of analysis (AOAC 2000). The chemical analyses were carried out in a laboratory where the procedures have been accredited by Swedish Board for Accreditation and Conformity Assessment (SWEDAC). The measurement uncertainty was ± 0.4% and ± 0.1%, for analysis of water and salt content, respectively.

Lipid content (%) was evaluated after extraction of lipids by two different solvent systems, Soxhlet (AOCS, 1998) and Bligh & Dyer (1959) with some modifications (Hanson and Olley, 1963). In Bligh & Dyer, extraction of lipids was carried out by chloroform/methanol extraction system and with butylated hydroxytoluene (BHT) admixed into all solvents (50-100 mg/L).

Water holding capacity

The WHC was determined by a centrifugation method (Eide and others 1982). The saithe samples (n=3) were coarsely minced in a mixer (Braun Electronic, Type 4262, Kronberg, Germany) for approximately 20 s at speed 4. Approximately 2 g of the minced saithe muscle was weighed accurately into sample glass and centrifuged at 210 * g for 15 min. The weight of liquid expelled from the muscle during centrifugation was subtracted from the weight of water in the sample before centrifugation. The WHC was calculated as the ratio of the water remaining after centrifugation compared to the initial water content of the sample before centrifugation and expressed as % WHC.

Microbial analysis

Samples of 3 cod fillets were minced and 20 g weighed from each fillet into 180 g of cooled Maximum Recovery Dilutent (MRD, Oxoid) and blended in a Stomacher® Lab Blender 400 (Seward, UK) for 1 min to obtain 1/10 dilution. Cooled MRD was used for all subsequent dilutions. Total viable psychrotrophic counts (TVC) and counts of H2S-producing bacteria were evaluated on iron agar (IA) as described by Gram and others (1987) with the exception that 1% NaCl was used instead of 0.5% with no overlay. Surface-plating was used. Plates were incubated at 17°C for 5 days both aerobically and anaerobically in an anaerobic jar (Oxoid). Bacteria forming black colonies on IA produce H_2S from sodium thiosulphate and/or cysteine.

Analysis of TVB-N and TMA

Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) were determined by the methods described by Malle and Poumeyrol (1989). The TVB-N measurement was performed by direct distillation into boric acid using a Kjeldahl-type distillatory (Struer TVB-N distillatory, STRUERS, Copenhagen, Denmark). The acid was back-titrated with diluted H_2SO_4 solution. To determine TMA, the same method was used as for TVB but adding 20 mL of 35% formaldehyde to the distillation flask to block the primary and secondary amines. The TVB and TMA contents were expressed in mg N/100 g saithe tissue.

Analysis of TBARS

The quantity of secondary oxidation products was evaluated by analysis of **TBARS** (2-thiobarbituric acid reactive substances). TBARS were determined as described by Vyncke (1970, 1975) with modifications by Sörensen and Jörgensen (1996). The sample size was reduced to 15 g of sample and homogenised with 30 mL of 7.5% trichloroacetic acid (TCA) with 0.1% propylgallate and EDTA. Absorbance of samples and standards was measured at 530 nm in UV/VIS-spectrometer. TBARS, expressed as µmol malondialdehyde per kilogram of sample (µmol MDA/kg) was calculated using malondialdehyd-bis-(diethyl acetate) as standard.

Measurement of pH

The pH was measured in 5 grams of minced loins mixed with 5 mL of deionised water using the Radiometer PHM 80. The pH meter was calibrated using the buffer solutions (Orion, Thermo Fisher Scientific, Beverly, MA 01915, USA) buffer of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25°C) (Radiometer Analytical A/S, Bagsvaerd, Denmark).

Statistical analysis of data

Results are presented as average values ± standard deviation. Statistical analyses were carried out by t-test (two-way, assuming equal variances), general linear modelling (GLM) and Duncan's Multiple-Comparison test to evaluate effects of different treatments and storage times at each condition. The statistical analysis was performed using Microsoft Office Excel 2007 and NCSS 2000 (NCSS, Kaysville, Utah, USA).

Results and discussion

Raw fillets

The water content in fresh fillets was 82.8% (± 0.2), salt content 0.8% (± 0.1), lipid content-Bligh & Dyer) 0.54 (± 0.05), lipid content-Soxhlet 0.14 (±0.05), WHC 89.9% (± 2.0), pH 6.9 (±0.1), TBARS 2.4 µmole/kg (± 0.4), TVB 8.04 mg N/100g (± 0.51) and TMA 1.13 mg N/100g (± 0.07). The fillets were chilled in the processing line with slurry ice, resulting in slightly values for salt content than usually observed in fresh fish (0.2-0.3%).

Chilled fillets

Yield

The result indicated that the gelatine addition resulted in lower uptake and that weight yields were on average lower in comparison to fillets with only salt (GLM: p=0,000259).

Fillets injected with salt (S) had higher uptake ($p<0.05$) after injection, than fillets injected with salt and gelatine (Figure 2). During storage the salt injected fillets tended to have higher yield ($p>0.05$) except after 13 days similar values were obtained. Effects of storage time were not significant.

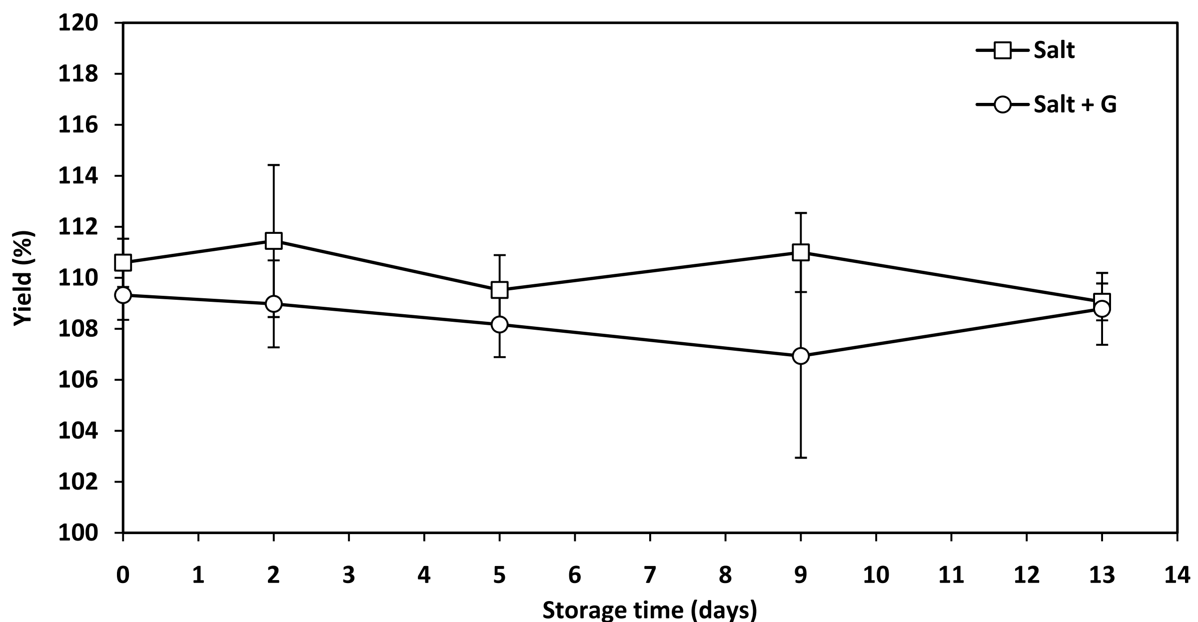


Figure 2. Weight changes of chilled fillets from injection (n=12) and at different sampling points (n=3) during chilled storage.

Chemical composition

Higher salt uptake ($p<0.05$) was obtained in the fillets without gelatine than in fillets injected with salt and gelatine (Salt + G). This was expressed by differences in salt content, which was on average

3.3–3.6% and 3.9-4.3%, in Salt + G and Salt, respectively (Table 3). Changes in salt content with storage time were not significant ($p > 0.05$).

The water content was slightly higher ($p = 0.08$) in fillets with added gelatine. Higher content was determined with longer storage time ($p < 0.05$) which was assumed to result from degradation of the muscle, increasing values as obtained from removal of water during measurement (drying of the sample).

Water holding capacity (WHC) was significantly increased by addition of salt to the fillets by injection, from 90% in raw fillets to 98-99% in chilled fillets. However, the effects of gelatine were not significant ($p > 0.05$). The increases in WHC by higher salt content were in harmony with previous findings in muscle food. It is known that the WHC increases with increasing salt concentration within the muscle up to approximately 5%, due to binding of salt to muscle proteins and denaturation. At higher salt concentrations (>10-12%), the proteins are known to aggregate, resulting in reduction of WHC (Duerr and Dyer, 1952; Hamm, 1975; Offer and Knight, 1988; Offer and Trinick, 1983).

Table 3. Chemical composition and water holding capacity of chilled fillets (n=3) at different sampling points (n=3) during chilled storage.

	Group	Storage time (days)											
		2			5			9			13		
Salt (%)	Salt	4.3	±	0.3	4.3	±	0.0	3.8	±	0.4	3.9	±	0.1
	Salt + G	3.3	±	0.1	3.3	±	0.0	3.3	±	0.2	3.6	±	0.1
Water (%)	Salt	81.7	±	0.6	81.5	±	0.1	82.3	±	0.2	82.3	±	0.7
	Salt + G	81.9	±	0.3	82.1	±	1.0	82.5	±	0.2	83.0	±	0.7
WHC (%)	Salt	98,7	±	0,4	99,3	±	0,2	98,8	±	0,1	98,8	±	0,6
	Salt + G	98,8	±	0,6	99,3	±	0,1	98,7	±	0,1	98,2	±	0,4

Microbial counts

Results from total viable counts (TVC) and counts of H_2S-producing bacteria are shown in Figure 3 and Figure 4. Very similar TVC were found whether plates were cultivated under aerobic or anaerobic conditions. This might indicate that the microbial flora was facultative anaerobic in nature, i.e. able to grow both in the presence and absence of oxygen. This was however not the case with H_2S-producing bacteria. The initial number of these bacteria under anaerobic conditions gradually decreased from about log 2-3/g to levels below detection limits (<log 1.3/g) on day 9. On day 13 the levels rose to about log 5/g. One of the main spoilage bacterium of marine fish which produces H_2S and reduces trimetylamine oxide (TMAO) to trimethylamine (TMA) is *Shewanella putrefaciens.* This bacterium is aerobic in nature meaning it needs oxygen for growth. It is therefore probable that this bacterium was initially present in low numbers and was gradually killed at anaerobic incubation on IA during the first 9 days of anaerobic storage by the absence of oxygen. What happened on day 13 is

not clear. It is possible that some anaerobic bacteria recovered in the later days of storage in both experimental groups reaching numbers up to log 5/g as stated before. Under aerobic conditions, H_2S-producing bacteria increased in numbers in both groups during the storage time.

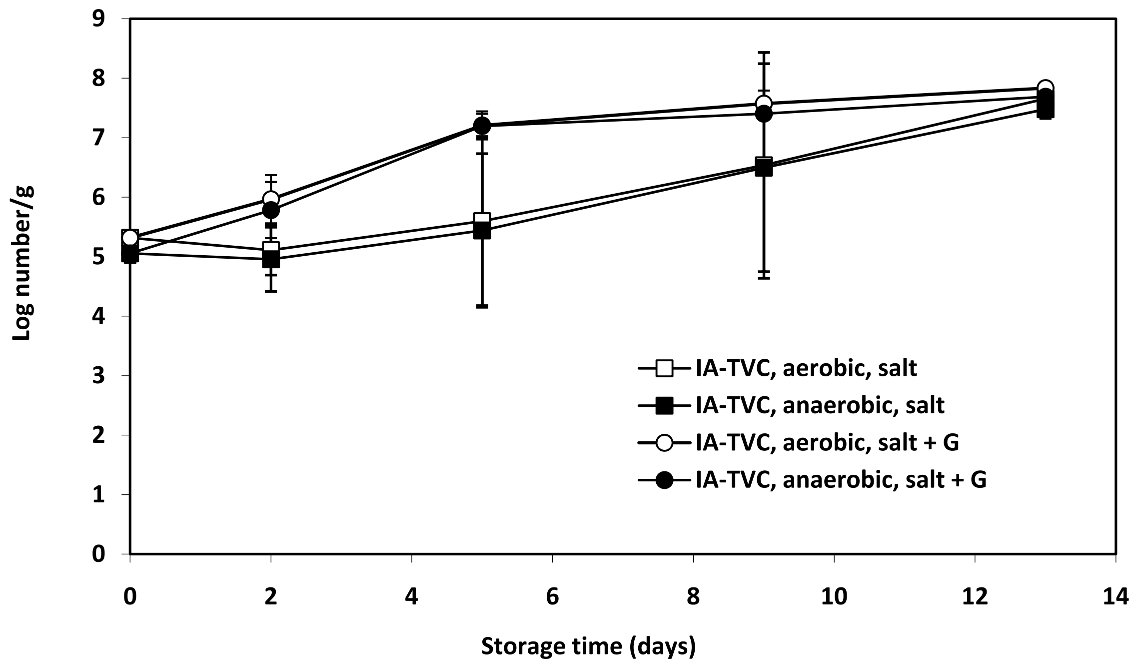


Figure 3. Changes in total viable counts (TVC) chilled fillets, from injection (n=12) and at different sampling points (n=3) during chilled storage.

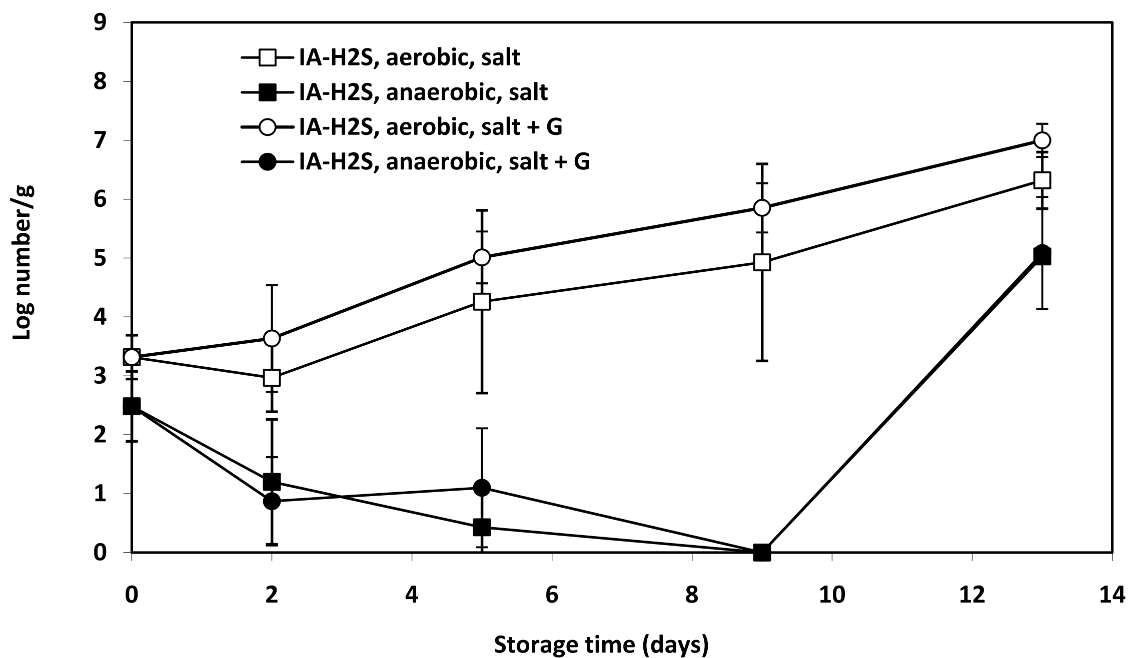


Figure 4. Changes in counts of H_2S-producing bacteria chilled fillets, from injection (n=12) and at different sampling points (n=3) during chilled storage.

The brine used for injection of fillets contained salt only (S) or salt and gelatine (Salt + G). The microbial counts were generally higher where gelatine was used (GLM results for aerobic conditions: p_{TVC}=0.10, p_{H2S}=0.06; GLM results for anaerobic conditions: p_{TVC}=0.02, p_{H2S}=0.27). This difference was noticeable in TVC on days 2, 5 and 9 at both aerobic and anaerobic conditions and at aerobic conditions during the whole storage period with regard to H_2S-producing bacteria. Similar but much lower counts were however found under anaerobic conditions in both experimental groups (salt with and without gelatine).

The higher microbial counts in fillets with added gelatine may have resulted from microbial contamination in the gelatine and/or that the gelatine was an easily accessible substrate for the microorganisms, facilitating their growth. Further treatments to reduce microbial counts within the gelatine might be needed.

TVB, TMA, pH

Results from TVB and TMA measurements are shown in Figure 5. The results were in good harmony with microbial counts apart from anaerobic counts of H_2S-producing bacteria. As with microbial counts higher values were generally obtained in the samples which contained salt and gelatine in comparison with samples without gelatine (GLM p_{TVB}=0.001; p_{TMA}= 0.00008). The results indicated that addition of the gelatine, had negative effects on the shelf life and quality of the products.

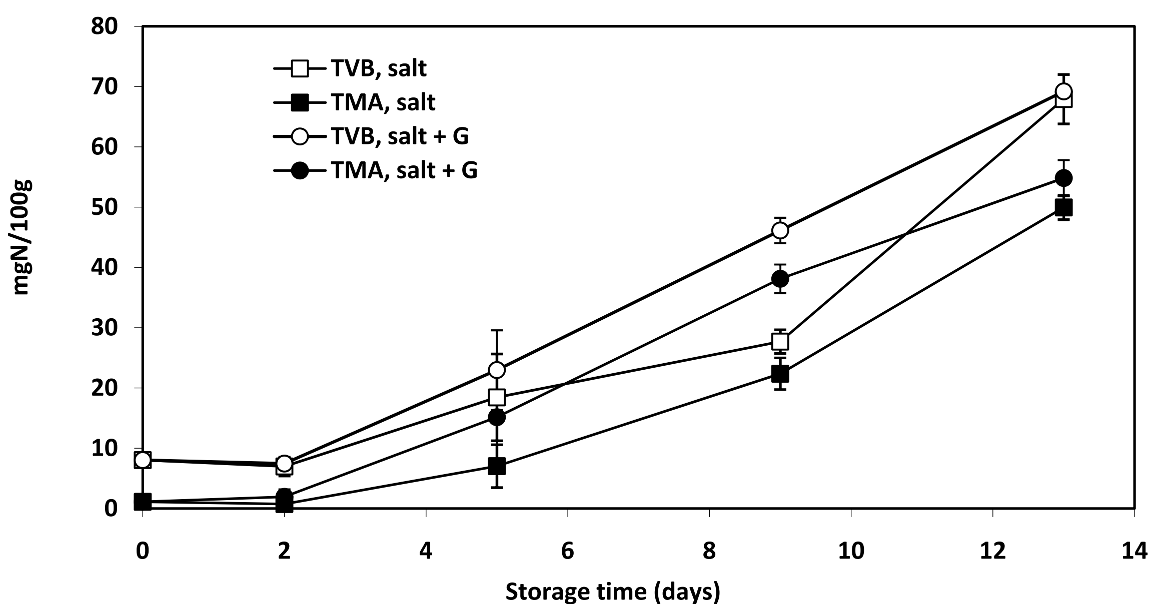


Figure 5. Changes in TVB and TMA content in chilled fillets from injection (n=12) and at different sampling points (n=3) during chilled storage.

The pH values were in good conformity with microbial and chemical measurements Figure 6. After day 5 the pH values increased considerably. As expected from the microbial and chemical data, higher values were usually found in the experimental group containing gelatine (GLM p=0.20).

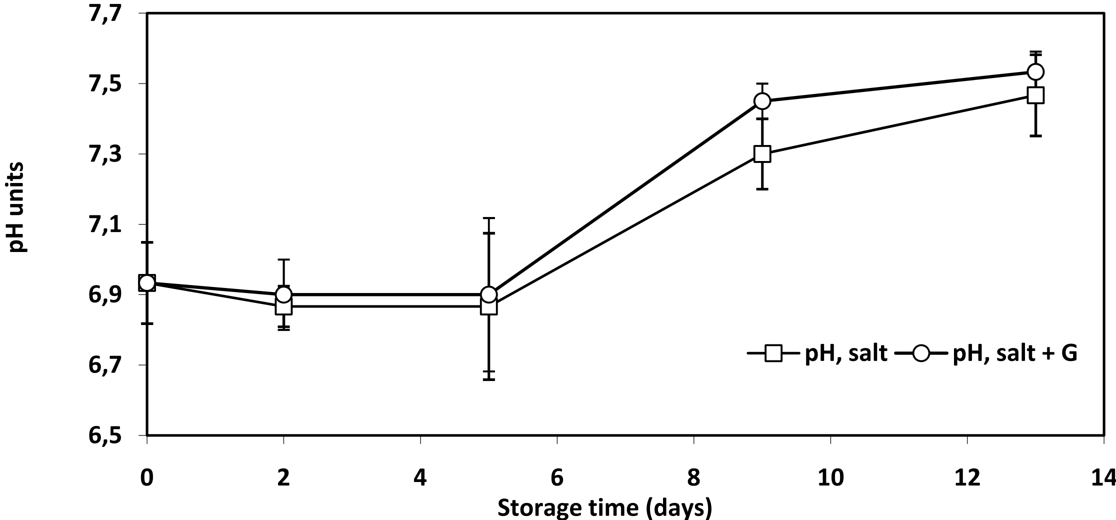


Figure 6. Changes in pH of chilled fillets from injection (n=12) and at different sampling points (n=3) during chilled storage.

Frozen fillets

Fillets injected with salt gained more weight by injection and maintained yield better through freezing, thawing and cooking. Differences between groups were significant at all stages ($p<0.05$).

Comparison of frozen and injected fillets showed that weight reduction of fillets with added gelatine was significant but not for fillets that were injected with salt only.

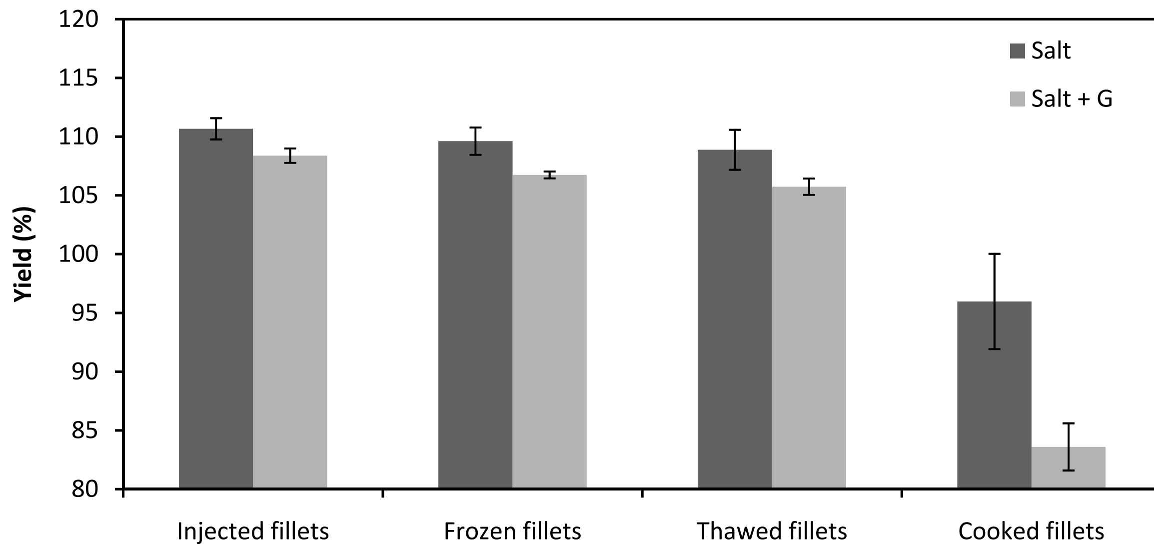


Figure 7. Weight changes of frozen fillets from injection (n=6) to cooking (n=3).

Changes in water and fat content were not significant by injection or freezing (Table 4). The increases in salt content resulted in higher water holding capacity like in the chilled fillets. The high water holding capacity was well maintained through freezing and thawing, as was reflected in result for weight yields. Higher values were obtained for TBARS in fillets with salt only, indicated that the gelatine had some retarding effects on lipid oxidation, possible by forming of film on the surface of the fillets, limiting the access of oxygen to muscle lipids. However, further studies are needed to confirm this.

Table 4. Chemical composition and water holding capacity of raw material and frozen, thawed fillets (n=3)

Group	Fat (%) Bligh & Dyer	Fat (%) Soxhlet	Water (%)	Salt (%)	WHC (%)	TBARS µmole/kg
Raw	0.5 ± 0.1	0.1 ± 0.0	82.8 ± 0.2	0.8 ± 0.1	89.9 ± 2.0	2.4 ± 0.4
Salt	0.4 ± 0.0	0.1 ± 0.0	81.8 ± 1.0	4.0 ± 0.0	99.1 ± 0.1	5.3 ± 0.4
Salt + G	0.4 ± 0.1	0.1 ± 0.1	82.3 ± 0.1	3.1 ± 0.1	98.7 ± 0.7	3.7 ± 0.4

Salted fillets

Higher uptake was obtained by injection of salt only but during brining and dry salting the yields of the two groups became similar (Figure 8). After rehydration, higher yields were obtained for fillets with added gelatine.

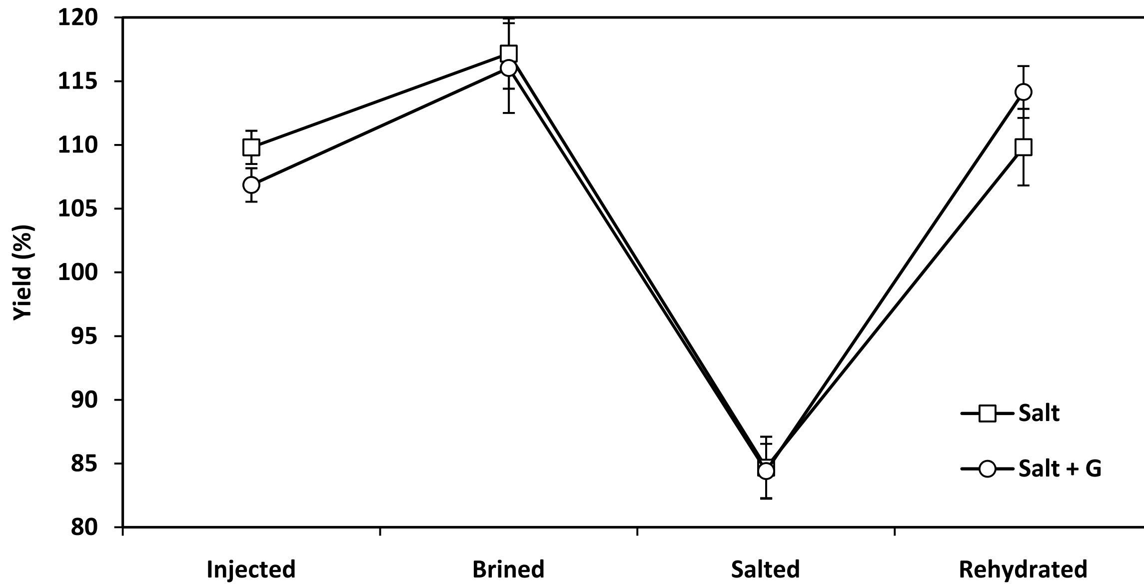


Figure 8. Weight changes of salted fillets from injection (n=6) to rehydration (n=3).

Higher salt uptake was obtained after brining in fillets that were injected with salt only but during dry salting, differences levelled off (Table 1). After rehydration similar values were obtained. Water contents in dry salted and rehydrated fillets were similar in both groups. The water holding capacity was also similar for the different treatments after brining and rehydration. However after dry salting the WHC of gelatine added fillets was higher. The amount of lipids was similar in both groups through the process. The level of oxidation was generally low, probably due to the short curing time. Stronger oxidation would have reduced the amount extracted by Blight & Dyer due to lipid degradation. Influences of gelatine addition on TVB and TMA were not significant. The contents in the salted fish were similar as in the raw material but decreased during rehydration. This has been explained be extraction or washing out of the nitrogenous compounds when the muscle is soaked in water.

Table 5. Chemical composition and water holding capacity of injected, brined fillets, dry salted and rehydrated fillets (n=3)

	Group	Brined			Dry salted			Rehydrated		
Salt (%)	Salt	11.5	±	0.6	22.6	±	0.1	2.8	±	0.7
	Salt + G	9.9	±	0.1	22.0	±	0.2	2.9	±	0.1
Water (%)	Salt	75.4	±	0.4	60.5	±	0.4	84.6	±	0.4
	Salt + G	76.8	±	0.3	60.4	±	0.3	84.6	±	0.7
WHC (%)	Salt	97.8	±	0.5	59.2	±	2.5	97.5	±	2.1
	Salt + G	97.1	±	0.3	65.9	±	2.4	97.6	±	1.1
Lipid (B&D) (%)	Salt	0.4	±	0.1	0.4	±	0.0	0.4	±	0.0
	Salt + G	0.4	±	0.0	0.4	±	0.1	0.3	±	0.0
Lipid (Soxhlet) (%)	Salt	0.1	±	0.0	0.1	±	0.0	0.1	±	0.0
	Salt + G	0.1	±	0.1	0.1	±	0.0	0.1	±	0.0
TBARS (µmól/kg)	Salt	2.6	±	0.4	4.0	±	0.7	3.3	±	1.0
	Salt + G	3.8	±	0.9	4.7	±	0.5	3.1	±	0.6
TVB (mg N/100g)	Salt	4.1	±	0.9	8.4	±	1.5	2.2	±	0.2
	Salt + G	5.3	±	0.3	7.1	±	0.2	2.0	±	0.1
TMA (mg N/100g)	Salt	0.3	±	0.4	0.9	±	0.0	N.D.		
	Salt + G	0.8	±	0.2	0.8	±	0.2	N.D.		

N.D. quantities of TMA to low to be detected

Conclusion

In this experiment, the gains of gelatine addition were minor and its use had certain limitations in practice. The addition of gelatine to the cod fillets did not improve weight yields of the fillets. On the contrary, the uptake during injection was lower which was assumed to result from higher viscosity of the brine. Furthermore, the solubility of the gelatine at the low temperature (0-5°C) is not high enough, which also causes problems. In processing of fish it is important to limit any temperature fluctuations as much as possible to reduce the risk of spoilage and shortening of shelf life. The salt concentration of the brine did not cause problems in solubilisation of gelatine.

The main changes in chemical composition of the fillets were due to injection of the salt but not due to injection of gelatine.

The addition of gelatine led to increases in microbial growth and in that aspect the use is negative for quality of the products. Visual comparison of the appearance of the fillets did not reveal differences between the groups. Further treatments to reduce microbial counts within the gelatine might be needed to obtained better results for the injected products.

Acknowledgement

The authors gratefully acknowledge the financial support of this work by NORA (Journal nr. 210-047; Project: "Injeksering av kollagen i fersk, frossen og saltede fiskefileter fra torsk og sej (CIF)").

References

AOAC 2000. 17th ed no.976.18. Measurement of salt in fish w/Tritrino ISO 6496 (1999). Method for analysing water in fish meal or fish. Official Methods of the Association of Official Chemists. Official Analytical Int, Arlington VA.

AOCS, American Oil Chemists Society. 1998. Official method BA 3-38. Application note Tecator nr AN 301. In: Firestone D, editor. Official methods and recommended practices of the American Oil Chemists Society. 5th ed. Champaign, Ill.: AOCS, 4p.

Bligh, E.G., Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37: 911-917.

Duerr, J.D., Dyer, W.J. 1952. Proteins in fish muscle. IV. Denaturation by salt. Journal of the Fisheries Research Board of Canada, 8:, 325-331.

Eide O., Borresen T., Strom T., 1982. Minced fish production from capelin (*Mallotus villosus*). Journal of Food Science 47: 347–54.

Gram L., Trolle G., Huss HH. 1987. Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. International Journal of Food Microbiology, 4:65-72.

Hamm, R. 1975. Water holding capacity of meat. In: Meat: proceedings of the twenty-first Easter School in Agricultural Science, University of Nottingham, 1974 (pp. 321-338): London: Butterworths.

Hanson W.C. 1950. The photometric determination of phosphorus in fertilizers using the phosphovanadomolybdate complex. Journal of the Science of Food and Agriculture, 1:172-173.

Hanson, S.W.F., Olley, J. 1963. Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. Journal of Biochemistry, 89: 101-102P.

ISO, Intl. Organisation for Standardization, 6496-1999(E), mod. Determination of moisture and other volatile matter content. Genf, Switzerland: The Int'l Organization for Standardization.

Malle, P., and S.H. Tao, 1987. Rapid quantitative determination of Trimethylamine using steam distillation. Journal of Food Protection, 50: 756-760.

Offer, G., Knight, P. 1988. The structural basis of water-holding in meat. Part 1: General principles and water uptake in meat processing. In: R. Lawrie, Developments in Meat Science - 4 (pp. 63-171). London: Elsevier Applied Science.

Offer, G., Trinick, J. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. Meat Science, 8:, 245-281.

Sørensen, G., Jørgensen, S.S. 1996. A critical examination of some experimental variables in the thiobarbituric acid (TBA) test of lipid oxidation in meat products. Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 202: 205-210.

Vyncke, W. 1970. Direct determination of the thiobarbituric acid value in trichloroacitic acid extracts of fish as a measure of oxidative rancidity. Fette, Seifen, Anstrichmittel, 77: 239-240.

Vyncke, W. 1975. Evaluation of the Direct Thiobarbituric Acid Extraction Method for Determining Oxidative Rancidity in Mackerel (*Scomber scombrus L.*). European Journal of Lipid Science and Technology, 77: 205-244.

Contents

Objectives 1
Material and methods 2
Experimental design 2
Raw material 3
Processing 3
Weighing 4
Sampling 4
Weight changes and cooking yield 4
Analysis of chemical composition 5
Water holding capacity 5
Microbial analysis 5
Analysis of TVB-N and TMA 6
Analysis of TBARS 6
Measurement of pH 6
Statistical analysis of data 6
Results and discussion 7
Raw fillets 7
Chilled fillets 7
Frozen fillets 12
Salted fillets 13
Conclusion 15
Acknowledgement 15
References 16

Objectives

The main objective of this study was to investigate if and how gelatine produced from by-products of cold water fish species could be used for injection to fish products. The gelatine was injected into cod fillets and the effects on weight and quality of chilled, frozen and salted cod fillets investigated in relation to following questions:

- Does the addition of gelatine increase weight yields?
- Are influences on chemical composition significant?
- How is the shelf life and quality affected (microbial and chemical analysis)?
- Are there visual differences in the appearance of the products?

The aim was to use the results described in this report to identify possibilities in utilisation of the gelatine for processing of commercial fish products, with regard to potential buyers, prices and markets.

Material and methods

Experimental design

Fillets were injected with brine without (salt) and with addition of gelatine (salt+gelatine). The fillets were then stored as chilled, frozen and heavy salted (Figure 1).

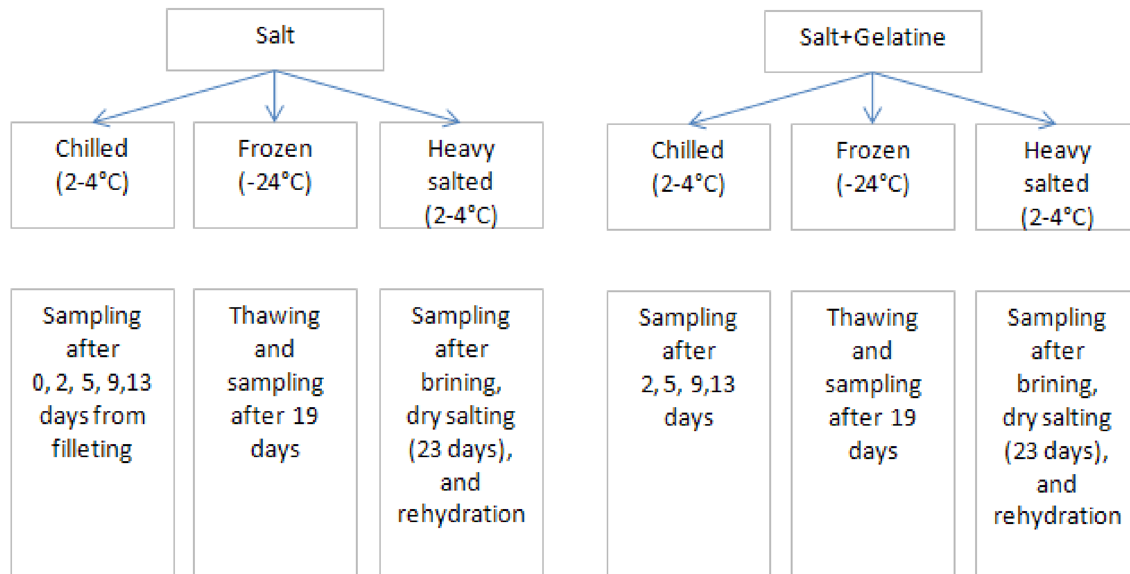


Figure 1. Experimental plan, fillets were injected with salt or with brine containing both salt and gelatine. After that different preservation methods were applied, chilling, freezing and further salting.

Parameters evaluated in fillets treated by different methods are listed in Table 2. Microbial growth was evaluated under aerobic and anaerobic conditions.

Table 1. Parameters evaluated in the experiments

Parameters	Chilled	Frozen	Salted
Weight changes during processing and storage	x	x	x
Cooking yield		x	x
Fat content (Soxhlet, Bligh and Dyer), PV, TBA		x	c
Microbial growth (TVC, H_2S), TVB, TMA	x		

Raw material

Cod (*Gadus morhua*) was caught on the 8th of March, 2011, in Ísafjarðardjúp on long-line. The fish was bled on board and stored in ice, in tubs during transport to Reykjavík. On 9th of March, the fish was gutted, beheaded and filleted. The average weight of the fillets was 264.8 ± 37.5.

Coarse salt ESCO Fishery salt K 3.2-0.4, European Salt Company GmbH & Co.KG, Hannover, Germany) was used for the procedures applied. Specifications are shown in Table 2.

Dried fish gelatine (high molecular weight) was used in the process for injection to the fish fillets. The collagen was extracted from cod skin and coarsely filtered. Thereafter, ultrafiltered and vacuum dried.

Table 2. Product data sheet for ESCO Fishery salt K 3,2-0,4 (European Salt Company GmbH & Co.KG, Hannover, Germany).

	Typical	Methods
Chemical Analysis:	**Typical**	**Methods**
• Sodiumchloride	99 %	ASTM 534-98
• Calcium	0,3 %	ISO 2482
• Magnesium	0,02 %	ISO 2482
• Sulphate	0,7 %	ISO 2480
• H_2O-insoluble	0,15 %	ISO 2479
• Iron	5 mg/kg	ESPA/CN-115-00
• Copper	0,08 mg/kg	ESPA/CN-115-00
Granulometry:	**Typical**	**Methods**
• > 3,15 mm	10 %	EN 1235
• 0,40 - 3,15 mm	85 %	
• < 0,40 mm	5 %	
Physical Properties:		**Methods**
• Bulk density	1.050 - 1.250 kg/m³	EN 1236
On request:		
• anti caking agent E 535, calculated as $K_4[Fe(CN)_6]$ anhydr. and E 500	10 mg/kg	ESPA/CN-111-96

Processing

The brine used for injection contained salt only (20% w/w) or salt (20% w/w) and gelatine (2% w/w). The fish was processed under chilled conditions (5-10°C). In this temperature range, the solubility of the gelatine is reduced, which caused some difficulties regarding the injection procedure. The gelatine already solubilised, precipitated when getting in contact with cold parts of the machine and fish. The salt concentration of the brine did not cause problems in solubilisation of gelatine.

The fillets were injected using a multi-needle brine injector (Dorit INJECT-O-MAT, Model No. PSM-42F-3.0I, Dorit Fleischereimaschinen GmbH, Ellwangen, Germany) under 45 strokes per min and 20 mm belt advance per stroke. The diameter of the needles (n=42) was 2 mm and the distance between needles was approximately 20 mm. The injection dept of the needles was 5.5 mm from getting through the fillets. The pressure was 2.2 bars.

After injection, the fillets were divided in three groups for different preservation methods: Chilled, frozen and heavy salted.

Chilled fillets: The fillets were packed in plastic bags and stored in polystyrene boxes at 2°C until sampling took place.

Frozen fillets: The fillets were packed in plastic bags in one layer and frozen in air and stored at -24°C. After 19 days the fillets were thawed for 3 hours at room temperature (20°C) and over night at 2°C, covered with a plastic film.

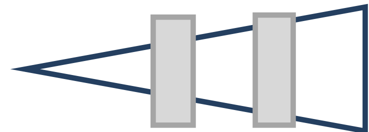
Salted fillets: The fillets were brine salted in a fish to brine ratio of 1:2 for 2 days. Then, they were dry salted for 21 days. The fillets were stacked with alternating layers of salt in plastic boxes, where the liquid was allowed to drain away from the fillets. After salting, the fillets were rehydrated in a ratio of 1:5 for 16 hours, and then the water was replaced with fresh water (ratio 1:5), for further rehydration for 72 hours.

Weighing

The fillets were weighed fresh and at each sampling point for evaluation of weight changes (yield). Additionally, weight changes during cooking of frozen, thawed and salted fillets were recorded.

Sampling

Chilled fillets: Samples were collected from the raw material and after 2, 5, 9, 13 days of storage. Two slices from different parts of the fillet (n=3) were pooled for analysis of water holding capacity (WHC), water and salt content. The rest of the fillet was pooled for analysis of microbial growth, total volatile base nitrogen (TVB) and trimethylamine (TMA).



Frozen fillets: Two slices from different part of the fillet (n=3) were pooled for analysis of water holding capacity (WHC), water and salt content. The rest of the fillet was pooled for analysis of fat content, peroxide value (PV), and thiobarbituric acid reactive substances (TBARS).

Salted fillets: Two slices from different part of the fillet (n=3) were pooled for analysis of water holding capacity (WHC), water and salt content. The rest of the fillet was pooled for analysis of fat content, PV, TBARS, TVB and TMA.

Weight changes and cooking yield

Fillets were allowed to drain for 10 min after injection and then weighed before storage or further processing. Weight changes were followed by recording the weight at each sampling point. The **weight yield (%)** was calculated as the relative mass of the weight of raw fillets (n=12).

Chilled fillets: The weight of fillets (n=3) at each sampling point was recorded.

Frozen fillets: The weight of frozen fillets (n=6) was recorded before and after thawing.

Salted fillets: The weight was recorded after brining (n=9), after dry salting (n=6) and after rehydration (n=3).

Drip loss (%) was expressed as relative weight reduction during thawing. **Cooking yield (%)** of frozen (n=3) and salted rehydrated fillets (n=3) was calculated from weight changes during cooking. Each fillet was placed on baking paper on a grid and cooked at 95-100 °C for 10 min in a preheated conventional steam oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany). After cooking, samples were allowed to cool at room temperature for 20 min, and then reweighed. The cooking yield (%) was calculated as the ratio of retained weight after cooking compared to the weight before cooking.

Analysis of chemical composition

Water content (%) of the fresh fish was determined according to ISO 6496 (1999). The **salt content** (%) was determined according to the AOAC Official Methods of analysis (AOAC 2000). The chemical analyses were carried out in a laboratory where the procedures have been accredited by Swedish Board for Accreditation and Conformity Assessment (SWEDAC). The measurement uncertainty was ± 0.4% and ± 0.1%, for analysis of water and salt content, respectively.

Lipid content (%) was evaluated after extraction of lipids by two different solvent systems, Soxhlet (AOCS, 1998) and Bligh & Dyer (1959) with some modifications (Hanson and Olley, 1963). In Bligh & Dyer, extraction of lipids was carried out by chloroform/methanol extraction system and with butylated hydroxytoluene (BHT) admixed into all solvents (50-100 mg/L).

Water holding capacity

The WHC was determined by a centrifugation method (Eide and others 1982). The saithe samples (n=3) were coarsely minced in a mixer (Braun Electronic, Type 4262, Kronberg, Germany) for approximately 20 s at speed 4. Approximately 2 g of the minced saithe muscle was weighed accurately into sample glass and centrifuged at 210 * g for 15 min. The weight of liquid expelled from the muscle during centrifugation was subtracted from the weight of water in the sample before centrifugation. The WHC was calculated as the ratio of the water remaining after centrifugation compared to the initial water content of the sample before centrifugation and expressed as % WHC.

Microbial analysis

Samples of 3 cod fillets were minced and 20 g weighed from each fillet into 180 g of cooled Maximum Recovery Dilutent (MRD, Oxoid) and blended in a Stomacher® Lab Blender 400 (Seward, UK) for 1 min to obtain 1/10 dilution. Cooled MRD was used for all subsequent dilutions. Total viable psychrotrophic counts (TVC) and counts of H2S-producing bacteria were evaluated on iron agar (IA) as described by Gram and others (1987) with the exception that 1% NaCl was used instead of 0.5% with no overlay. Surface-plating was used. Plates were incubated at 17°C for 5 days both aerobically and anaerobically in an anaerobic jar (Oxoid). Bacteria forming black colonies on IA produce H_2S from sodium thiosulphate and/or cysteine.

Analysis of TVB-N and TMA

Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) were determined by the methods described by Malle and Poumeyrol (1989). The TVB-N measurement was performed by direct distillation into boric acid using a Kjeldahl-type distillatory (Struer TVB-N distillatory, STRUERS, Copenhagen, Denmark). The acid was back-titrated with diluted H_2SO_4 solution. To determine TMA, the same method was used as for TVB but adding 20 mL of 35% formaldehyde to the distillation flask to block the primary and secondary amines. The TVB and TMA contents were expressed in mg N/100 g saithe tissue.

Analysis of TBARS

The quantity of secondary oxidation products was evaluated by analysis of **TBARS** (2-thiobarbituric acid reactive substances). TBARS were determined as described by Vyncke (1970, 1975) with modifications by Sörensen and Jörgensen (1996). The sample size was reduced to 15 g of sample and homogenised with 30 mL of 7.5% trichloroacetic acid (TCA) with 0.1% propylgallate and EDTA. Absorbance of samples and standards was measured at 530 nm in UV/VIS-spectrometer. TBARS, expressed as µmol malondialdehyde per kilogram of sample (µmol MDA/kg) was calculated using malondialdehyd-bis-(diethyl acetate) as standard.

Measurement of pH

The pH was measured in 5 grams of minced loins mixed with 5 mL of deionised water using the Radiometer PHM 80. The pH meter was calibrated using the buffer solutions (Orion, Thermo Fisher Scientific, Beverly, MA 01915, USA) buffer of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25°C) (Radiometer Analytical A/S, Bagsvaerd, Denmark).

Statistical analysis of data

Results are presented as average values ± standard deviation. Statistical analyses were carried out by t-test (two-way, assuming equal variances), general linear modelling (GLM) and Duncan's Multiple-Comparison test to evaluate effects of different treatments and storage times at each condition. The statistical analysis was performed using Microsoft Office Excel 2007 and NCSS 2000 (NCSS, Kaysville, Utah, USA).

Results and discussion

Raw fillets

The water content in fresh fillets was 82.8% (± 0.2), salt content 0.8% (± 0.1), lipid content-Bligh & Dyer) 0.54 (± 0.05), lipid content-Soxhlet 0.14 (±0.05), WHC 89.9% (± 2.0), pH 6.9 (±0.1), TBARS 2.4 µmole/kg (± 0.4), TVB 8.04 mg N/100g (± 0.51) and TMA 1.13 mg N/100g (± 0.07). The fillets were chilled in the processing line with slurry ice, resulting in slightly values for salt content than usually observed in fresh fish (0.2-0.3%).

Chilled fillets

Yield

The result indicated that the gelatine addition resulted in lower uptake and that weight yields were on average lower in comparison to fillets with only salt (GLM: p=0,000259).

Fillets injected with salt (S) had higher uptake ($p<0.05$) after injection, than fillets injected with salt and gelatine (Figure 2). During storage the salt injected fillets tended to have higher yield ($p>0.05$) except after 13 days similar values were obtained. Effects of storage time were not significant.

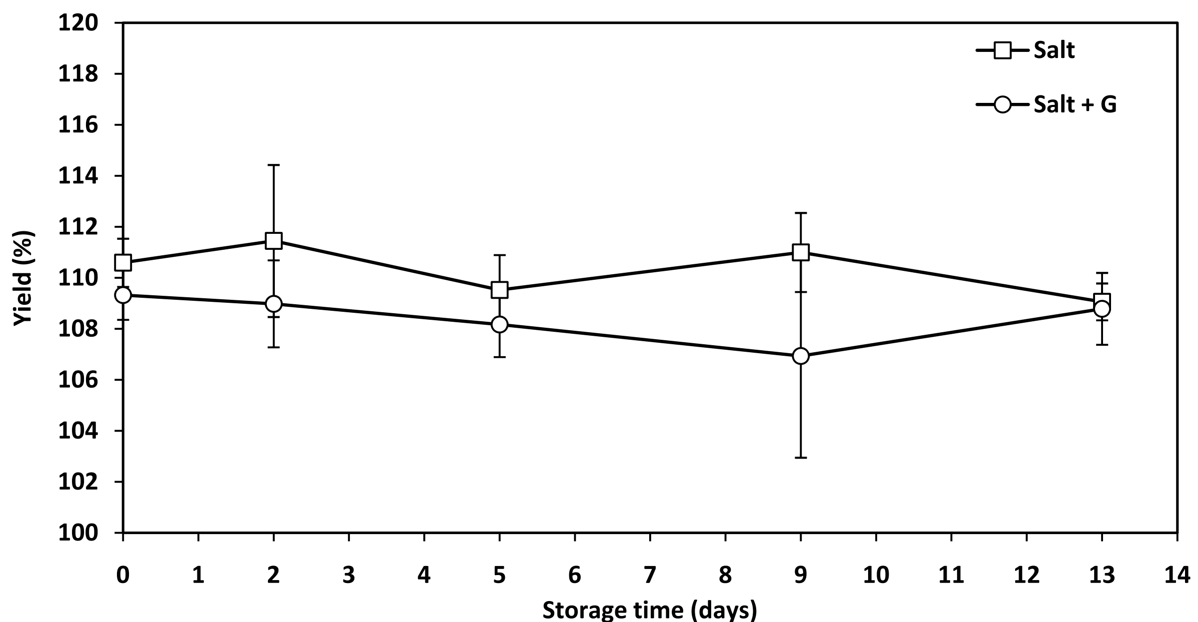


Figure 2. Weight changes of chilled fillets from injection (n=12) and at different sampling points (n=3) during chilled storage.

Chemical composition

Higher salt uptake ($p<0.05$) was obtained in the fillets without gelatine than in fillets injected with salt and gelatine (Salt + G). This was expressed by differences in salt content, which was on average

3.3–3.6% and 3.9-4.3%, in Salt + G and Salt, respectively (Table 3). Changes in salt content with storage time were not significant (p >0.05).

The water content was slightly higher (p=0.08) in fillets with added gelatine. Higher content was determined with longer storage time (p<0.05) which was assumed to result from degradation of the muscle, increasing values as obtained from removal of water during measurement (drying of the sample).

Water holding capacity (WHC) was significantly increased by addition of salt to the fillets by injection, from 90% in raw fillets to 98-99% in chilled fillets. However, the effects of gelatine were not significant (p>0.05). The increases in WHC by higher salt content were in harmony with previous findings in muscle food. It is known that the WHC increases with increasing salt concentration within the muscle up to approximately 5%, due to binding of salt to muscle proteins and denaturation. At higher salt concentrations (>10-12%), the proteins are known to aggregate, resulting in reduction of WHC (Duerr and Dyer, 1952; Hamm, 1975; Offer and Knight, 1988; Offer and Trinick, 1983).

Table 3. Chemical composition and water holding capacity of chilled fillets (n=3) at different sampling points (n=3) during chilled storage.

		Storage time (days)											
	Group	**2**			**5**			**9**			**13**		
Salt (%)	Salt	4.3	±	0.3	4.3	±	0.0	3.8	±	0.4	3.9	±	0.1
	Salt + G	3.3	±	0.1	3.3	±	0.0	3.3	±	0.2	3.6	±	0.1
Water (%)	Salt	81.7	±	0.6	81.5	±	0.1	82.3	±	0.2	82.3	±	0.7
	Salt + G	81.9	±	0.3	82.1	±	1.0	82.5	±	0.2	83.0	±	0.7
WHC (%)	Salt	98,7	±	0,4	99,3	±	0,2	98,8	±	0,1	98,8	±	0,6
	Salt + G	98,8	±	0,6	99,3	±	0,1	98,7	±	0,1	98,2	±	0,4

Microbial counts

Results from total viable counts (TVC) and counts of H_2S-producing bacteria are shown in Figure 3 and Figure 4. Very similar TVC were found whether plates were cultivated under aerobic or anaerobic conditions. This might indicate that the microbial flora was facultative anaerobic in nature, i.e. able to grow both in the presence and absence of oxygen. This was however not the case with H_2S-producing bacteria. The initial number of these bacteria under anaerobic conditions gradually decreased from about log 2-3/g to levels below detection limits (<log 1.3/g) on day 9. On day 13 the levels rose to about log 5/g. One of the main spoilage bacterium of marine fish which produces H_2S and reduces trimetylamine oxide (TMAO) to trimethylamine (TMA) is *Shewanella putrefaciens.* This bacterium is aerobic in nature meaning it needs oxygen for growth. It is therefore probable that this bacterium was initially present in low numbers and was gradually killed at anaerobic incubation on IA during the first 9 days of anaerobic storage by the absence of oxygen. What happened on day 13 is

not clear. It is possible that some anaerobic bacteria recovered in the later days of storage in both experimental groups reaching numbers up to log 5/g as stated before. Under aerobic conditions, H_2S-producing bacteria increased in numbers in both groups during the storage time.

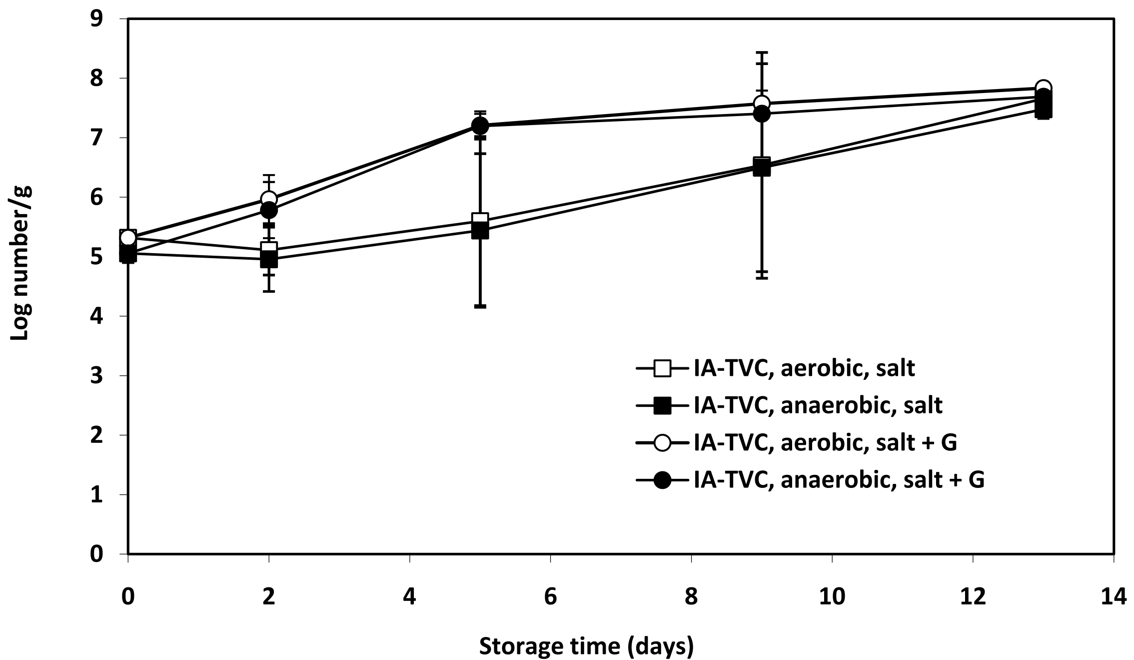


Figure 3. Changes in total viable counts (TVC) chilled fillets, from injection (n=12) and at different sampling points (n=3) during chilled storage.

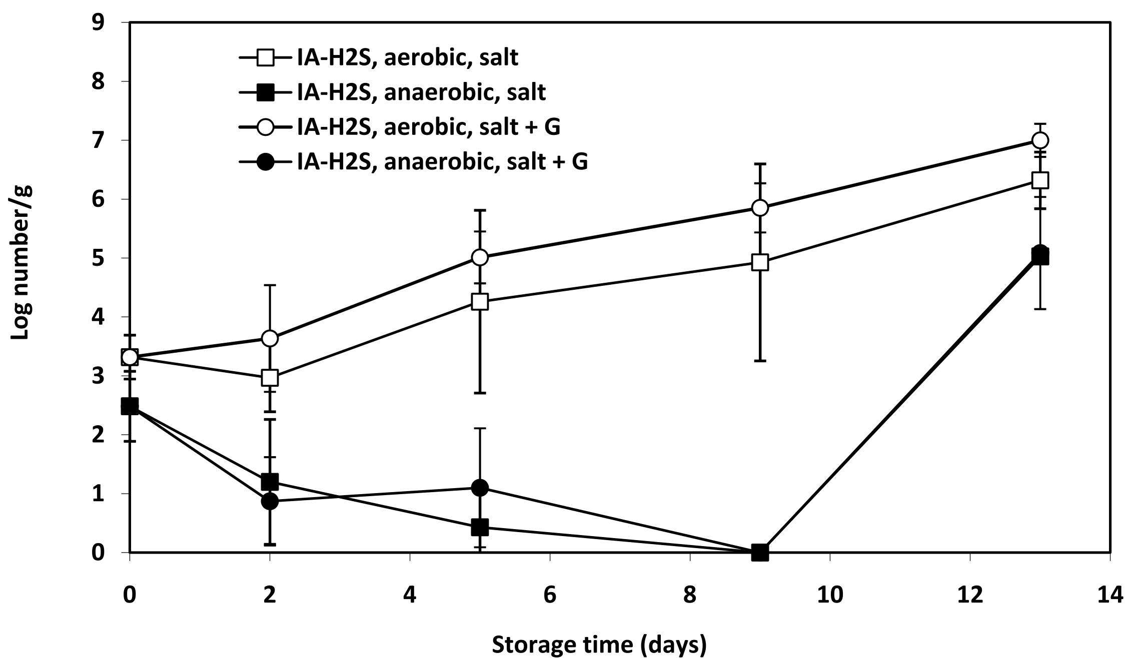


Figure 4. Changes in counts of H_2S-producing bacteria chilled fillets, from injection (n=12) and at different sampling points (n=3) during chilled storage.

The brine used for injection of fillets contained salt only (S) or salt and gelatine (Salt + G). The microbial counts were generally higher where gelatine was used (GLM results for aerobic conditions: p_{TVC}=0.10, p_{H2S}=0.06; GLM results for anaerobic conditions: p_{TVC}=0.02, p_{H2S}=0.27). This difference was noticeable in TVC on days 2, 5 and 9 at both aerobic and anaerobic conditions and at aerobic conditions during the whole storage period with regard to H_2S-producing bacteria. Similar but much lower counts were however found under anaerobic conditions in both experimental groups (salt with and without gelatine).

The higher microbial counts in fillets with added gelatine may have resulted from microbial contamination in the gelatine and/or that the gelatine was an easily accessible substrate for the microorganisms, facilitating their growth. Further treatments to reduce microbial counts within the gelatine might be needed.

TVB, TMA, pH

Results from TVB and TMA measurements are shown in Figure 5. The results were in good harmony with microbial counts apart from anaerobic counts of H_2S-producing bacteria. As with microbial counts higher values were generally obtained in the samples which contained salt and gelatine in comparison with samples without gelatine (GLM p_{TVB}=0.001; p_{TMA}= 0.00008). The results indicated that addition of the gelatine, had negative effects on the shelf life and quality of the products.

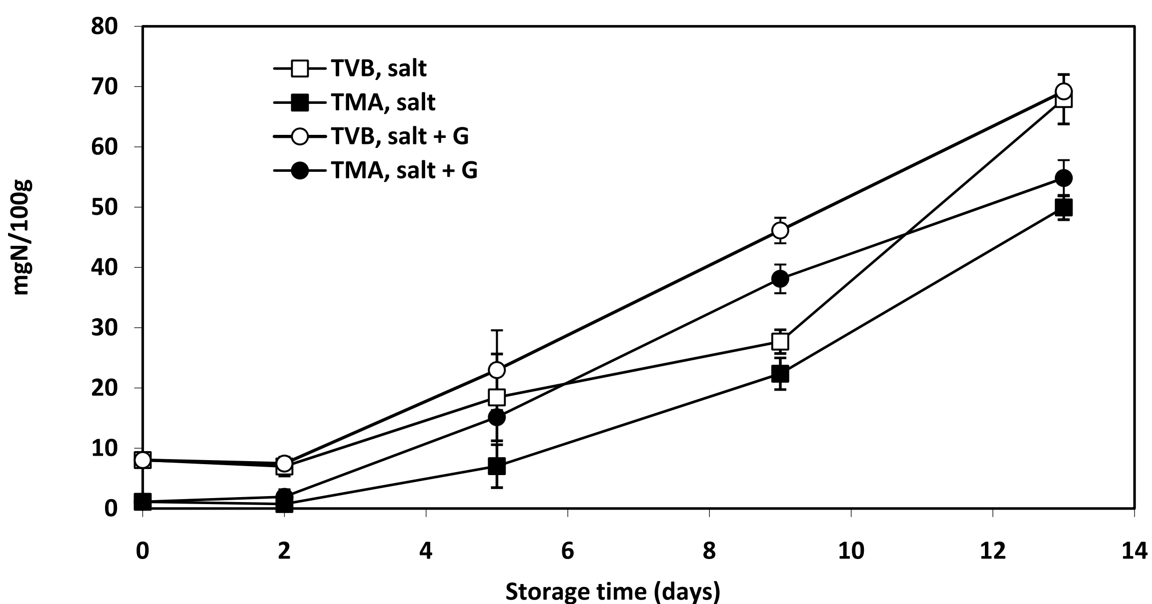


Figure 5. Changes in TVB and TMA content in chilled fillets from injection (n=12) and at different sampling points (n=3) during chilled storage.

The pH values were in good conformity with microbial and chemical measurements Figure 6. After day 5 the pH values increased considerably. As expected from the microbial and chemical data, higher values were usually found in the experimental group containing gelatine (GLM p=0.20).

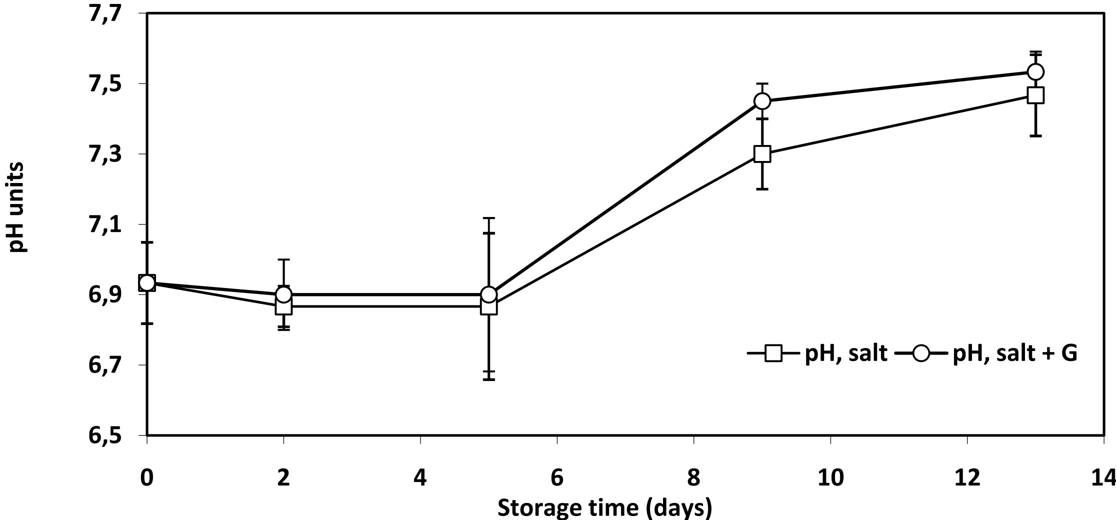


Figure 6. Changes in pH of chilled fillets from injection (n=12) and at different sampling points (n=3) during chilled storage.

Frozen fillets

Fillets injected with salt gained more weight by injection and maintained yield better through freezing, thawing and cooking. Differences between groups were significant at all stages ($p<0.05$).

Comparison of frozen and injected fillets showed that weight reduction of fillets with added gelatine was significant but not for fillets that were injected with salt only.

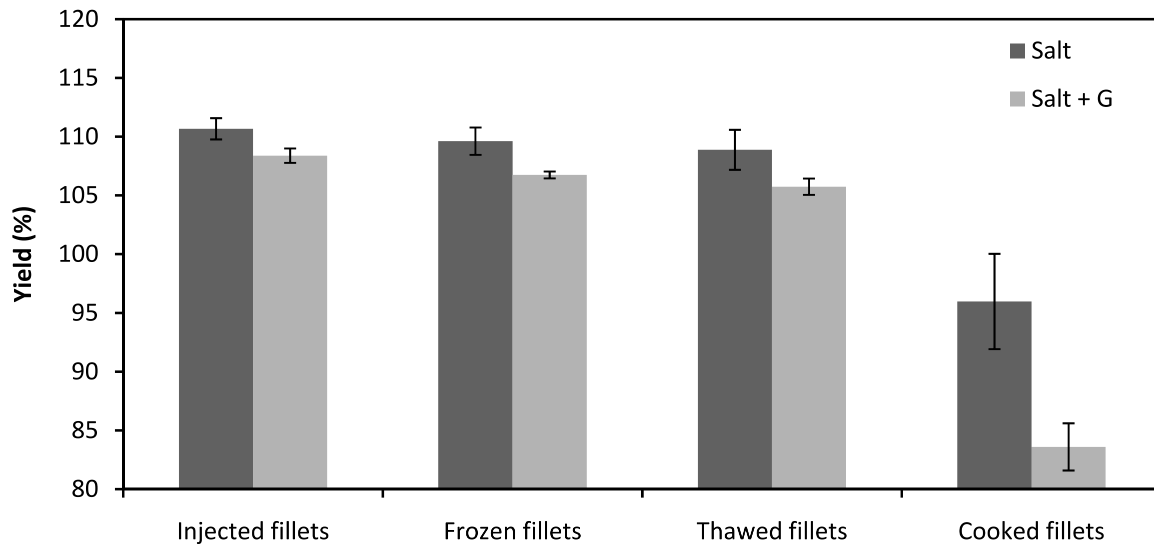


Figure 7. Weight changes of frozen fillets from injection (n=6) to cooking (n=3).

Changes in water and fat content were not significant by injection or freezing (Table 4). The increases in salt content resulted in higher water holding capacity like in the chilled fillets. The high water holding capacity was well maintained through freezing and thawing, as was reflected in result for weight yields. Higher values were obtained for TBARS in fillets with salt only, indicated that the gelatine had some retarding effects on lipid oxidation, possible by forming of film on the surface of the fillets, limiting the access of oxygen to muscle lipids. However, further studies are needed to confirm this.

Table 4. Chemical composition and water holding capacity of raw material and frozen, thawed fillets (n=3)

Group	Fat (%) Bligh & Dyer	Fat (%) Soxhlet	Water (%)	Salt (%)	WHC (%)	TBARS µmole/kg
Raw	0.5 ± 0.1	0.1 ± 0.0	82.8 ± 0.2	0.8 ± 0.1	89.9 ± 2.0	2.4 ± 0.4
Salt	0.4 ± 0.0	0.1 ± 0.0	81.8 ± 1.0	4.0 ± 0.0	99.1 ± 0.1	5.3 ± 0.4
Salt + G	0.4 ± 0.1	0.1 ± 0.1	82.3 ± 0.1	3.1 ± 0.1	98.7 ± 0.7	3.7 ± 0.4

Salted fillets

Higher uptake was obtained by injection of salt only but during brining and dry salting the yields of the two groups became similar (Figure 8). After rehydration, higher yields were obtained for fillets with added gelatine.

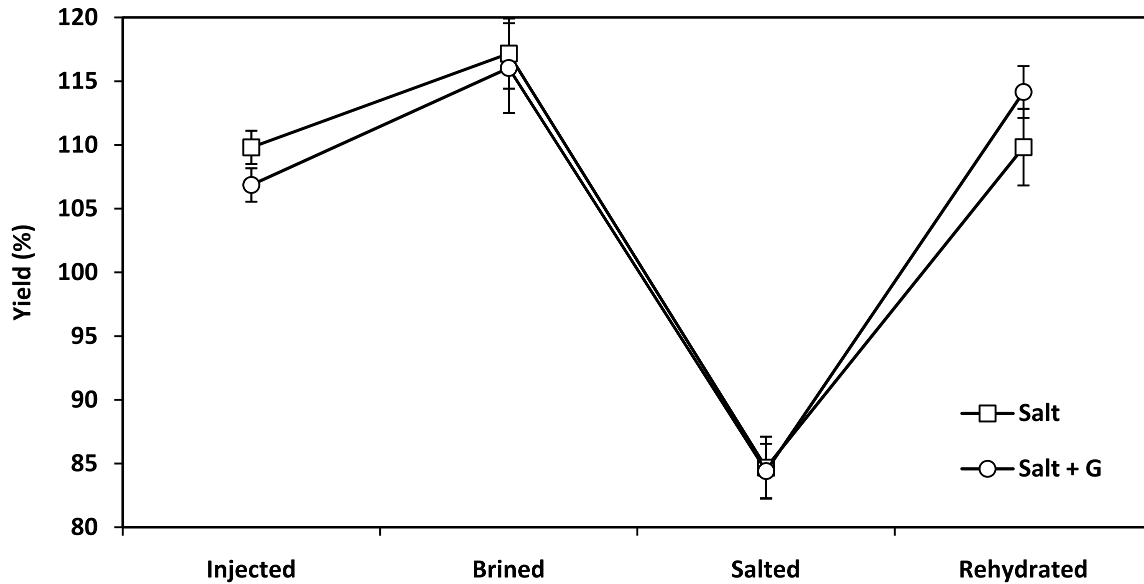


Figure 8. Weight changes of salted fillets from injection (n=6) to rehydration (n=3).

Higher salt uptake was obtained after brining in fillets that were injected with salt only but during dry salting, differences levelled off (Table 1). After rehydration similar values were obtained. Water contents in dry salted and rehydrated fillets were similar in both groups. The water holding capacity was also similar for the different treatments after brining and rehydration. However after dry salting the WHC of gelatine added fillets was higher. The amount of lipids was similar in both groups through the process. The level of oxidation was generally low, probably due to the short curing time. Stronger oxidation would have reduced the amount extracted by Blight & Dyer due to lipid degradation. Influences of gelatine addition on TVB and TMA were not significant. The contents in the salted fish were similar as in the raw material but decreased during rehydration. This has been explained be extraction or washing out of the nitrogenous compounds when the muscle is soaked in water.

Table 5. Chemical composition and water holding capacity of injected, brined fillets, dry salted and rehydrated fillets (n=3)

	Group	Brined			Dry salted			Rehydrated		
Salt (%)	Salt	11.5	±	0.6	22.6	±	0.1	2.8	±	0.7
	Salt + G	9.9	±	0.1	22.0	±	0.2	2.9	±	0.1
Water (%)	Salt	75.4	±	0.4	60.5	±	0.4	84.6	±	0.4
	Salt + G	76.8	±	0.3	60.4	±	0.3	84.6	±	0.7
WHC (%)	Salt	97.8	±	0.5	59.2	±	2.5	97.5	±	2.1
	Salt + G	97.1	±	0.3	65.9	±	2.4	97.6	±	1.1
Lipid (B&D) (%)	Salt	0.4	±	0.1	0.4	±	0.0	0.4	±	0.0
	Salt + G	0.4	±	0.0	0.4	±	0.1	0.3	±	0.0
Lipid (Soxhlet) (%)	Salt	0.1	±	0.0	0.1	±	0.0	0.1	±	0.0
	Salt + G	0.1	±	0.1	0.1	±	0.0	0.1	±	0.0
TBARS (µmól/kg)	Salt	2.6	±	0.4	4.0	±	0.7	3.3	±	1.0
	Salt + G	3.8	±	0.9	4.7	±	0.5	3.1	±	0.6
TVB (mg N/100g)	Salt	4.1	±	0.9	8.4	±	1.5	2.2	±	0.2
	Salt + G	5.3	±	0.3	7.1	±	0.2	2.0	±	0.1
TMA (mg N/100g)	Salt	0.3	±	0.4	0.9	±	0.0	N.D.		
	Salt + G	0.8	±	0.2	0.8	±	0.2	N.D.		

N.D. quantities of TMA to low to be detected

Conclusion

In this experiment, the gains of gelatine addition were minor and its use had certain limitations in practice. The addition of gelatine to the cod fillets did not improve weight yields of the fillets. On the contrary, the uptake during injection was lower which was assumed to result from higher viscosity of the brine. Furthermore, the solubility of the gelatine at the low temperature (0-5°C) is not high enough, which also causes problems. In processing of fish it is important to limit any temperature fluctuations as much as possible to reduce the risk of spoilage and shortening of shelf life. The salt concentration of the brine did not cause problems in solubilisation of gelatine.

The main changes in chemical composition of the fillets were due to injection of the salt but not due to injection of gelatine.

The addition of gelatine led to increases in microbial growth and in that aspect the use is negative for quality of the products. Visual comparison of the appearance of the fillets did not reveal differences between the groups. Further treatments to reduce microbial counts within the gelatine might be needed to obtained better results for the injected products.

Acknowledgement

The authors gratefully acknowledge the financial support of this work by NORA (Journal nr. 210-047; Project: "Injeksering av kollagen i fersk, frossen og saltede fiskefileter fra torsk og sej (CIF)").

References

AOAC 2000. 17th ed no.976.18. Measurement of salt in fish w/Tritrino ISO 6496 (1999). Method for analysing water in fish meal or fish. Official Methods of the Association of Official Chemists. Official Analytical Int, Arlington VA.

AOCS, American Oil Chemists Society. 1998. Official method BA 3-38. Application note Tecator nr AN 301. In: Firestone D, editor. Official methods and recommended practices of the American Oil Chemists Society. 5th ed. Champaign, Ill.: AOCS, 4p.

Bligh, E.G., Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37: 911-917.

Duerr, J.D., Dyer, W.J. 1952. Proteins in fish muscle. IV. Denaturation by salt. Journal of the Fisheries Research Board of Canada, 8:, 325-331.

Eide O., Borresen T., Strom T., 1982. Minced fish production from capelin (*Mallotus villosus*). Journal of Food Science 47: 347–54.

Gram L., Trolle G., Huss HH. 1987. Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. International Journal of Food Microbiology, 4:65-72.

Hamm, R. 1975. Water holding capacity of meat. In: Meat: proceedings of the twenty-first Easter School in Agricultural Science, University of Nottingham, 1974 (pp. 321-338): London: Butterworths.

Hanson W.C. 1950. The photometric determination of phosphorus in fertilizers using the phosphovanadomolybdate complex. Journal of the Science of Food and Agriculture, 1:172-173.

Hanson, S.W.F., Olley, J. 1963. Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. Journal of Biochemistry, 89: 101-102P.

ISO, Intl. Organisation for Standardization, 6496-1999(E), mod. Determination of moisture and other volatile matter content. Genf, Switzerland: The Int'l Organization for Standardization.

Malle, P., and S.H. Tao, 1987. Rapid quantitative determination of Trimethylamine using steam distillation. Journal of Food Protection, 50: 756-760.

Offer, G., Knight, P. 1988. The structural basis of water-holding in meat. Part 1: General principles and water uptake in meat processing. In: R. Lawrie, Developments in Meat Science - 4 (pp. 63-171). London: Elsevier Applied Science.

Offer, G., Trinick, J. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. Meat Science, 8:, 245-281.

Sørensen, G., Jørgensen, S.S. 1996. A critical examination of some experimental variables in the thiobarbituric acid (TBA) test of lipid oxidation in meat products. Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 202: 205-210.

Vyncke, W. 1970. Direct determination of the thiobarbituric acid value in trichloroacitic acid extracts of fish as a measure of oxidative rancidity. Fette, Seifen, Anstrichmittel, 77: 239-240.

Vyncke, W. 1975. Evaluation of the Direct Thiobarbituric Acid Extraction Method for Determining Oxidative Rancidity in Mackerel (*Scomber scombrus L.*). European Journal of Lipid Science and Technology, 77: 205-244.