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Published in:
LWT

Link to article, DOI:
10.1016/j.lwt.2020.109473

Publication date:
2020

Document Version
Peer reviewed version

Journal Pre-proof

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PII: S0023-6438(20)30462-X

DOI: https://doi.org/10.1016/j.lwt.2020.109473

Reference: YFSTL 109473

To appear in: *LWT - Food Science and Technology*

Received Date: 10 February 2020

Revised Date: 3 April 2020

Accepted Date: 22 April 2020

Please cite this article as: Sørensen, J.S., Ørnfeld-Jensen, O., Bøknæs, N., Mejlholm, O., Jessen, F., Dalgaard, P., Thawed and chilled Atlantic cod (*Gadus morhua* L.) from Greenland - Options for improved distribution, *LWT - Food Science and Technology* (2020), doi: https://doi.org/10.1016/j.lwt.2020.109473.

Jonas Steenholdt Sørensen: Conceptualisation, Investigation, Writing - Original Draft, Writing - Review & Editing **Oliver Ørnfeld-Jensen:** Investigation **Niels Bøknæs:** Conceptualisation, Resources **Ole Mejlholm:** Investigation, Resources **Flemming Jessen:** Writing - Review & Editing and **Paw Dalgaard:** Resources, Conceptualisation, Supervision, Writing - Review & Editing.

Thawed and chilled Atlantic cod (*Gadus morhua* L.) from Greenland - Options for improved distribution

Jonas Steenholdt Sørensen [1,2], Oliver Ørnfeld-Jensen[1], Niels Bøknæs[2], Ole Mejlholm[2], Flemming Jessen[1] and Paw Dalgaard[1]

[1]National Food Institute (DTU Food), Technical University of Denmark, Kgs. Lyngby, Denmark

[2]Royal Greenland Seafood A/S, Svenstrup J, Denmark

* Corresponding author: Food Microbiology and Hygiene, National Food Institute, Technical University of Denmark, Kemitorvet, Building 202, 2800, Kgs. Lyngby, Denmark. E-mail: jonsor@food.dtu.dk

Abstract

Frozen Atlantic cod can have a long shelf-life, but some markets demand convenience products and thawed and chilled (refreshed) fish may fulfil this demand. Sensory, chemical and microbiological changes for refreshed cod from Greenland were studied to determine shelf-life and potential indices of spoilage. Aerobic sensory shelf-life was 13 days at 2.9 °C and 19 days at 0.4 °C, with modified atmosphere packaging (MAP: 40% CO_2 and 60% N_2) extending shelf-life to >32 days. Low drip loss during chilled storage of 2.3-2.5% for refreshed cod in air and 3.4-3.6% in MAP suggested the studied fish material was suitable for a combination of frozen and chilled distribution. *Pseudomonas* spp. and *Psychrobacter* spp. dominated the spoilage microbiota of chilled cod in air, while *Carnobacterium maltaromaticum* and *Rahnella aquatilis* dominated the microbiota of chilled MAP cod. A specific spoilage organism, that limited sensory shelf-life and caused the observed chemical product changes, including the formation of total volatile basic nitrogen (TVBN), was not identified.

Keywords: Sensory shelf-life, Modified atmosphere packaging (MAP), Microbial changes, H_2S-producing bacteria, *Pseudomonas*.

1. Introduction

Food waste and losses must be reduced within all food industries to meet the UN Sustainable Development Goals (FAO, IFAD, UNICEF, WFP, & WHO, 2018). 27 % of all landed fish has been estimated to be wasted or lost (FAO, 2018). Microbial spoilage was a considerable cause of seafood losses and this may be reduced by improved hygiene, food preservation, packaging and management of conditions in distribution (Dalgaard, 2000; Ghaly, Dave, Budge, & Brooks, 2010; Svanevik, Roiha, Levsen, & Lunestad, 2015).

Frozen cod with shelf-life of 8-12 months at -24 to -30 °C (Bøgh-Sørensen, 2006) allowed catching of cod at high season and spreading the sales and distribution globally throughout the year (Hermansen & Dreyer, 2010; Kearney, 2010). Capture-based aquaculture (CBA), including live storage prior to processing and filleting of fish in *pre-rigor mortis* state, has been shown to improve the colour of the cod fillet, by decreasing discolouration and may improve other sensory attributes (Martinsdottir & Magnusson, 2001; Olsen, Tobiassen, Akse, Evensen, & Midling, 2013). Furthermore, live fish in net enclosures can be kept close to a processing facility and time from slaughter to freezing can be as short as two hours which was beneficial for the sensory quality of the fish (Martinsdottir & Magnusson, 2001). After distribution of frozen cod it can be marketed frozen or alternative as thawed and chilled (refreshed) products for catering or in consumer sizes packaging.

Compared to chilled fresh cod, the shelf-life of refreshed and aerobically stored cod has been extended marginally by less than 3-4 days. In contrast, shelf-life of refreshed chilled cod fillets in modified atmosphere packaging (MAP) has been extended by more than one to two weeks compared to unfrozen products (Guldager, Bøknæs, Østerberg, Nielsen, & Dalgaard, 1998). For refreshed MAP cod, markedly reduced production of both trimethylamine (TMA) and total volatile basic nitrogen (TVBN) was observed, and this was due to inactivation of the spoilage bacterium *Photobacterium* spp. by freezing and frozen storage (Bøknæs, Østerberg, Nielsen & Dalgaard, 2000; Bøknæs et al., 2002; Guldager et al., 1998). Although, refreshed MAP cod had relatively long chilled

shelf-life this product was challenged by high drip loss (Bøknæs et al., 2000; 2002; Guldager et al., 1998).

The objective of the present study was to determine shelf-life and indices of spoilage for thawed Atlantic cod from CBA in Greenland. Firstly, the effect of two different bleeding methods on microbial contamination of cod fillets was evaluated. Secondly, sensory, chemical and microbial changes of frozen, thawed and chilled cod fillet pieces were studied in a storage trial with four treatments including chilled storage at 0 °C and 3 °C in air or MAP (40% CO_2 and 60% N_2). Finally, and independent storage trial with cod in air was performed at ~1.5 °C to evaluate the results of the first storage trial.

2. Materials and methods

2.1 Effect of bleeding methods on microbial quality of cod fillets

The effect of two different bleeding methods on the microbial quality of cod fillets was evaluated. For method (I) stunned cod was double cut at the dorsal aorta and washed for three minutes in circulating refrigerated water (CRW). Then, fish were transferred to a larger tank with CRW at 4-8 °C where they were bled for 30 minutes. With method (II) the stunned cod was decapitated and eviscerated manually followed by washing and bleeding as for method (I). Evaluation of the concentration of microorganisms in nine cod fillet for both bleeding methods was performed during a two hour full-scale production in Maniitsoq, Greenland. When the fish was filleted knives and workbench were cleaned with ethanol between each fish. Samples were kept in individual plastic containers and transported in styrofoam boxes, cooled with ice from Maniitsoq to a laboratory in Nuuk, Greenland for enumeration of bacteria as described in section 2.4.1.

2.2 Storage trial with thawed Atlantic cod from capture-based aquaculture (Batch A).

88 **2.2.1 Raw material, packaging and storage conditions.**

89 Atlantic cod (*Gadus morhua* L.) were captured inshore by pound net and transported
90 alive to a fish factory in Maniitsoq, Greenland. The fish was handled by method II (See 2.1) and
91 machine filleted. Fillets were individually quick frozen (IQF). The studied fish raw material was
92 produced on the 27th of November of 2017 and transferred to DTU Food, Kgs. Lyngby, Denmark, in
93 March 2018. Storage and transport were at -20 °C. One-hundred fillets were thawed overnight at +2
94 °C. The thawed fillets were cut by hand into 300 pieces of approximated 100 g each. In between the
95 cutting of each fillet, the cutting board and knives were rinsed with 96 % ethanol to avoid cross-
96 contamination of microorganism between fillets.

97 A storage trial was performed with four treatments including (i) aerobic storage in ice;
98 (ii) aerobic chilled storage at 3 °C; (iii) MAP (40 % CO_2 and 60% N_2) storage in ice and (iv) chilled MAP
99 storage at 3 °C. Pieces of cod were packed as previously described (Sørensen et al., 2020). The iced
100 samples, both aerobic and MAP were entirely covered with flake ice, which was regularly refilled
101 during storage, as the ice melted. The temperature was recorded every 30 minutes (TinyTaq Plus,
102 Gemini Data Loggers Ltd., Chichester, UK).

103 After thawing of the cod, before dividing the pieces of fillet meat into the four
104 treatments, samples to determine the initial sensory, chemical and microbiological quality attributes
105 were analysed using methods described in the sections 2.2.2-2.2.4. During storage and for each
106 treatment, sampling was performed with intervals of three to five days with a total storage period of
107 up to 26 days for aerobic storage and of up to 32 days for MAP storage. At each sampling time, three
108 randomly picked bags, from each treatment, were analysed for microbiological and chemical
109 changes. Five other randomly selected bags, from each treatment, were chosen for sensory
110 evaluation.

111

2.2.2 Sensory changes of refreshed and chilled cod

Sensory evaluation of batch A cod was performed by using the Quality Index Method for thawed Atlantic cod fillets as previously described (Sørensen et al., 2020).

2.2.3 Chemical changes

Chemical changes as potential indices of spoilage were determined throughout the storage trial: Concentrations of trimethylamine-oxide (TMAO), trimethylamine (TMA) and total volatile basic nitrogen (TVBN) were determined in duplicate for each bag by a modified Conway and Byrne method (Conway & Byrne, 1933). pH was recorded in 25 g fish mixed with 75 mL H_20 for each sample as part of the first step in the Conway and Byrne protocol, lactic acid was quantified by HPLC and the headspace gas composition was determined on each bag for microbiological and chemical analysis by using a gas analyser as previously described (Sørensen et al. 2020). Drip loss was measured by gravity draining of liquid in each bag for one minute and calculated as the percentage loss of the total weight (Guldager et al., 1998).

2.2.4 Microbiological changes

The microbiota was quantified by diluting 20.0 grams of cod flesh without skin tenfold in chilled physiological saline with 0.1 % peptone (PSP) (NMKL, 2006) followed by homogenisation for 60 seconds in a Stomacher 400 (Seward Medical, London, UK). Total viable counts (TVC) was determined by spread plating on chilled Long and Hammer (LH) ager (NMKL, 2006), *Pseudomonas* spp. was determined by spread plating on Pseudomonads agar (CM0559, Oxoid, Basingstoke, UK) with CFC selective supplement (SR0103, Oxoid, Basingstoke, UK), H_2S-producing bacteria were determined as black colonies by pour plating in Iron Agar (IA) Lyngby (CM0964, Oxoid, Basingstoke, UK) with L-cysteine hydrochloride, *Photobacterium* spp. was enumerated by using a conductance

method and Lactic acid bacteria (LAB) were quantified by pour plating in nitrite actidione polymyxin (NAP) agar using methods and incubation as described by Sørensen et al. (2020).

To identify the dominating microbiota for treatments of the storage trial, all countable colonies on LH plates were divided into groups based on colony characteristics (size, profile, elevation, boundary, colour) and for each group of colonies, their proportion of the concentration of countable colonies was calculated. To identify the groups of colonies present for each treatment, a total of 30 colonies with five to nine colonies for each treatment were isolated from LH plates (highest dilutions) at the time of sensory spoilage or at the end of the storage period. To identify isolates, these were pure-cultured and their *16S rRNA gene* was sequenced as as previously described (Sørensen et al.,2020).

2.3 Additional storage trial with refreshed cod in air (Batch B)

An independent storage trial was performed with cod pieces which were produced, packed and analysed as described above in section 2.2 with the following modifications. The cod was processed on 27th July 2017 and transferred to DTU Food in October 2017. The additional storage trial included a single treatment (v) with aerobic storage at 1.5 °C to fill the gap between treatment i and ii. Sensory evaluation was performed in triplicate with a minimum of five assessors to evaluate off-odours by using a simple scale with three grades (Dalgaard, 2000). An average score of 2.5 or above was used as the point of spoilage. Cod pieces were stored and evaluated during 18 days and at start and end of storage pH was measured (See 2.2.3). Ten colonies were isolated at the end of the storage trial and their *16S rRNA* gene was sequenced to characterise the dominating microbiota as described in section 2.2.4.

2.4 Statistical analyses

To evaluate differences between the microbial concentrations resulting from the two studied bleeding methods, differences in product pH and in lactic acid concentrations a two-tailed homoscedastic distribution t-Test was preformed using Excel 2016 (Microsoft Corp., Redmond, WA, USA). A most-probable-number technique was used to determine low concentrations of H_2S-producing bacteria in IA (Jarvis, Wilrich, & Wilrich, 2010). To evaluate drip loss an one-way ANOVA was performed using GraphPad Prism 8.3.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1 Effect of bleeding methods on concentrations of microorganisms in cod fillets

TVC and concentrations for *Pseudomonas* spp. in cod fillets did not differ ($p > 0.05$) for the two studied bleeding methods. However, the bleeding method II with decapitation resulted in significantly lower concentrations in IA ($p < 0.0001$) and of H_2S-producing bacteria ($p < 0.0001$) in the cod fillets (Table 1). Irrespective of the studied bleeding methods, the microbiota in cod fillets, was dominated (> 96.8%) by psychrotolerant microorganisms unable to grow in IA after pour plating but with the ability to grow on the surface of chilled LH-agar plates at 15 °C (Table 1).

3.2 Storage trial with cod from batch A

3.2.1 Storage conditions

After freezing in Greenland, the cod fillets were stored for 4.5 months at -20 °C and after thawing and packaging at DTU Food the fish was stored at 2.9 ± 0.4 °C (Chilled) and at 0.4 ± 0.1 °C (Iced). The equilibrium CO_2 concentration for headspace gas in MAP decreased from 36% to 29% during storage in ice but remained constant at 2.9 °C (Table 2).

3.2.2 Sensory changes

Refreshed cod in air at 2.9 °C had a sensory shelf-life of 13 days, determined by total QI scores, with a cut-off level of 5 (Fig. 1). Refreshed MAP cod had shelf-life above 32 days at both 2.9 °C and 0.4 °C as total QI scores did not increase during storage (Fig. 1).

3.2.3 Chemical changes

Average drip loss for the four treatment ranged from 2.3% to 3.6% and did not change significantly during storage ($p > 0.4$, linear regression). There was a small but significant difference in drip loss between the treatments ($p < 0.01$) with the highest drip for MAP cod fillets (Table 2). An increase in pH of 0.3 units from the initial value was evaluated as an index of spoilage and this resulted in shelf-life for refreshed cod in air of 14 days at 2.9 °C and 22 days at 0.4 °C (Table 3). The EU critical limit of 35 mg-N TVBN/100 g (EC, 2008) was suitable as an index of spoilage for refreshed cod in air from batch A but TVBN concentrations did not increase for refreshed MAP cod (Fig. 2; Table 3; Table 4). The formation of TVBN could not be explained by a formation of TMA. In fact, TMA concentrations remained below 7.5 ± 2.9 mg-N/100 g of cod flesh for all treatments and the initial TMAO concentration of 74 ± 11 mg/100 g remained close to this value during storage (Results not shown). The initial lactic acid concentration for refreshed cod was 2177 ± 89 ppm. For cod in air, the lactic acid concentrations decreased towards the end of the storage period (Table 4). Dry matter was on average for all treatment 19.6 ± 1.0 %.

3.2.3 Microbiological changes

The time for TVC to reach 7.0 log CFU/g underestimated sensory shelf-life and was not suitable as an index of spoilage (Table 3). The initial concentration of *Pseudomonas* spp. in cod after thawing was 1.4 log CFU/g. For refreshed cod in air *Pseudomonas* spp. grew to 9.0 log CFU/g after 13

days at 2.9 °C and after 19 days at 0.4 °C. When stored in MAP, growth of *Pseudomonas* spp. was slower and reached 5.9 log CFU/g at 2.9 °C and 3.5 log CFU/g at 0.4 °C after 32 days of storage (Fig. 3). For refreshed MAP cod concentrations of *Pseudomonas* spp. were approximately two log CFU/g lower than concentrations of TVC (Fig. 3). H_2S-producing bacteria, determined in IA as black colonies, was detected after 11 days of storage and never reached more than 3.0 log CFU/g in any of the treatments (data not shown). *Photobacterium* spp., determined by a Malthus conductance method, was not detected nor showed any growth during the storage for any of the four treatments (data not shown).

3.2.4 Identification of isolates from the dominating microbiota

Pseudomonas spp. dominated the microbiota with other identified species being *Rahnella aquatilis*, *Carnobacterium maltaromaticum* and *Serratia conticola* for refreshed cod in air, when stored in MAP, *C. maltaromaticum* and *R. aquatilis* dominated the microbiota (Table 5).

3.3 Additional storage trial with refreshed cod in air (Batch B)

The cod in batch B was stored in air at 1.4 ± 1.0 °C (Table 2) and had a sensory shelf-life of 13 days based on average odour scores exceeding 2.5. pH was lower than observed with cod from batch A (Table 4). TVBN and lactic acid concentrations did not change significantly during the storage period and TVBN concentrations remained below the EU critical limit (Table 3; Fig. 2). With 7.0 log CFU/g for TVC as a potential index of spoilage, the corresponding shelf-life for refreshed batch B cod in air was ten days at 1.4 °C and concentrations of TVC or *Pseudomonas* spp. never reached 9.0 log CFU/g (Fig. 3). Thus, the studied potential indices of spoilage (pH, TVBN, TVC and *Pseudomonas* spp.) did not corresponded to the observed sensory shelf-life of 13 days. Concentrations of TVC on LH and of *Pseudomonas* spp. on CFC agar were similar during the storage

231 trial (Fig. 3) and the isolated microbiota was dominated by *Pseudomonas* spp. and *Psycrobacter* spp.
232 *Photobacterium* spp. was not detected and no growth of H_2S producing bacteria was observed (data
233 not shown).

234

235 **4. Discussion**

236 The observed drip losses (Table 2) was markedly lower than previously observed with
237 cod from other regions and production methods. Frozen-at-sea cod from the Norwegian Sea had a
238 drip loss in the range of 1.7 – 3.3 % for refreshed fillets in air (Roiha, Jónsson, Backi, Lunestad, &
239 Karlsdóttir 2017; Roiha et al. 2018). Whiting had drip losses of, respectively, 6.0 – 9.0 % and 9.4 –
240 16.4 % for refreshed fish in air and MAP (Fagan, Gormley & Uí Mhuircheartaigh, 2003; Fagan,
241 Gormley & Uí Mhuircheartaigh, 2004). Cod from the Baltic Sea and frozen *post-rigor mortis* had drip
242 loss of 13 – 19 % for refreshed MAP fillets, which was much higher than the 4.6 – 5.4 % observed for
243 fresh MAP fillets (Bøknæs et al., 2002; Guldager et al., 1998). Bøknæs et al. (2002) found drip losses
244 of 11.4 – 12.8 % for frozen-at-sea refreshed MAP cod from the Barents Sea. The pronounced
245 difference in drip loss for refreshed MAP cod from CBA in Greenland (3.4 - 3.6 %) and frozen-at-sea
246 refreshed MAP cod from the Barents Sea (11.4 - 12.8 %) was not due to differences in product pH of
247 6.8 - 7.0. Low drip loss for refreshed cod from CBA in Greenland suggest this fish is suitable for a
248 combination of frozen and chilled distribution. Further studies are relevant to determine if the
249 difference in drip loss was due to production method, region and sub-group of Atlantic cod.

250 Frozen storage for less than one months extended shelf-life of chilled refreshed cod
251 marginally whereas sensory shelf-life was extended 3-4 days in ice following frozen storage periods
252 up to twelve months (Magnússon & Martinsdóttir, 1995; Vyncke, 1983). After frozen storage during
253 one to 12 months at –20 °C to –28 °C, several studies with cod from Belgium, Iceland and Norway
254 found sensory shelf-life of 7 – 15 days in ice for refreshed cod in air (Hansen et al., 2015;
255 Martinsdottir & Magnusson, 2001; Roiha et al., 2017; Vyncke, 1983). Fresh cod from CBA in

256 Greenland had sensory shelf-life of 15 days in air (Sørensen et al., 2020). Thus, the sensory shelf-life
257 extension from 15 days to 19 days for iced refreshed cod in air (Table 3) was similar to cod from
258 other regions and production methods. In contrast, sensory shelf-life of refreshed MAP cod from
259 CBA in Greenland of > 32 days at both 0.4 °C and 2.9 °C (Table 3) was markedly longer than
260 previously observed with cod from other regions. Baltic Sea cod had sensory shelf-life extended from
261 11 - 12 days at 1.6 °C for fresh MAP fish to more than 20 days at 1.6 °C for refreshed MAP cod,
262 previously frozen at -21 °C during eight weeks (Guldager et al., 1998). For Barents Sea MAP (13 %
263 CO_2, 83 % O_2) refreshed cod, sensory shelf-life was 19 days at 0 °C after frozen storage at -23 °C for
264 ten months (Hansen et al., 2015). Also for Barents Sea MAP (34 % CO_2 and 66 % N_2) refreshed cod
265 Bøknæs et al. (2002) found sensory shelf-life of 21 days at 2.1 – 2.5 °C after frozen storage at -20 °C
266 during six to twelve months.

267 Refreshed cod will typically be cooked before consumption and pathogenic
268 microorganisms will then be inactivated. Raw refreshed cod can be used for ready-to-eat dishes like
269 ceviche where the occurrence of *L. monocytogenes* can be a challenge (Fuchs & Sirvas, 1991). To
270 avoid more than 100-fold growth of *L. monocytogenes* we suggest limiting the safe shelf-life of
271 refreshed cod to 15 days at 2°C in air and 20 days at 2°C in MAP. These suggestions were based on
272 product characteristics for refreshed cod (Table 2; Table 4) and predictions by the *L. monocytogenes*
273 growth model of Mejlholm & Dalgaard (2009), as included in the Food Spoilage and Safety Predictor
274 software (http://fssp.food.dtu.dk).

275 Sørensen et al. (2020) pointed out *Photobacterium carnosum* as the specific spoilage
276 organism (SSO), that limited the sensory shelf-life of fresh cod from CBA in Greenland. The absence
277 of *Photobacterium* spp. in the refreshed cod (See 3.2.3 and Table 5) showed *P. carnosum* to be
278 inactivated by freezing and frozen storage as previously observed for other species from the *P.*
279 *phosphoreum* clade (Bøknæs et al., 2000, 2002; Dalgaard et al., 2006; Emborg et al., 2002; Guldager
280 et al., 1998). The absence *Photobacterium* spp. explained the limited TMA formation for refreshed

cod. However, Bøknæs et al. (2002) found *Photobacterium* spp. to survive 12 months frozen storage of Barents Sea cod at -30 °C, and this resulted in pronounced TMA formation in the refreshed MAP fish. If kept at -30 °C, a similar survival of *Photobacterium* spp. with associated TMA formation and the shelf-life limitation must be expected for refreshed cod from Greenland.

Growth of H_2S-producing bacteria to more than 7.0 log CFU/g has been observed for aerobically stored refreshed cod from Iceland and Norway (Magnússon & Martinsdóttir, 1995; Martinsdottir & Magnusson, 2001; Roiha et al., 2017, 2018). With cod from CBA in Greenland, no or very limited growth of H_2S-producing bacteria was observed and this was probably explained by the production method (Table 1). Furthermore, H_2S-producing *Shewanella* was to some extend inactivated by freezing and frozen storage. Based on decimal reduction times and frozen storage of 4.5 months, a reduction of 3.2 log would be expected (Emborg et al., 2002).

The observed long sensory shelf-life (Table 3) seems related to the absence or very low concentrations of *Photobacterium* spp. and H_2S-producing bacteria in the studied cod. Avoiding contamination of the thawed cod therefore becomes important to maintain the long shelf-life. Process contamination can markedly reduce the sensory product shelf-life, as shown with thawed shrimp that was contaminated prior to chilled distribution (Mejlholm et al., 2008). Contamination with H_2S-producing bacteria may be more problematic to avoid than contamination with *Photobacterium* spp. as H_2S-producing bacteria was shown to be present in a fish processing environment after sanitation, while *Photobacterium* spp. were more likely to originate from the fish being processed (Møretrø, Moen, Heir, Hansen, & Langsrud, 2016).

In the present and some previous studies with refreshed chilled cod in air, TVC reached 8 - 9 log CFU/g during storage and the spoilage microbiota was dominated by *Pseudomonas* spp. unable to produce TMA (Fig. 3; Table 3; Magnússon & Martinsdóttir, 1995; Roiha et al., 2017). However, a dominating microbiota, including *Psychrobacter* spp., as observed for batch B, was also previously reported (Hansen et al., 2015). The measured concentrations of *Pseudomonas* spp. in refreshed cod

306 could not alone explain the observed formation of TVBN based on their spoilage activity and yield
307 factor for TVBN formation (log (Y_{TVBN}/CFU)) of -10.2 (Table 5; Fig. 2; Sørensen et al., 2020). The
308 remaining formation of TVBN must have been formed by other members of the dominating
309 microbiota including *C. maltaromaticum*, *R. aquatilis*, and *S. conticola* (Fig. 2; Table 5). *C.*
310 *maltaromaticum* has a high resistance to freezing and frozen storage and it was previously
311 determined as a dominating part of the spoilage microbiota in refreshed seafood including MAP cod,
312 garfish and salmon (Dalgaard et al., 2006; Emborg et al., 2002; Guldager et al., 1998). *R. aquatilis* was
313 previously found as part of the spoilage microbiota for chilled cold-smoked salmon (Paludan-Müller,
314 Dalgaard, Huss, & Gram, 1998). Indices of spoilage or an SSO responsible for spoilage
315 and TVBN formation was not identified for refreshed cod from CBA in Greenland neither for storage
316 in air or MAP (Table 3). For refreshed cod in air 35 mg-N TVBN/100 g corresponded to the
317 determined sensory shelf-life for batch A. However, this was not the case for batch B (Fig. 2; Table
318 3). The EU critical limit of 35 mg-N/100 g (EC, 2008) therefore could not be confirmed as spoilage
319 index for chilled refreshed cod in air although Roiha et al., (2017) found this index of spoilage
320 appropriate. The absent or very limited TMA-formation for refreshed cod in air (See 3.2.3) previously
321 kept 4.5 months at -20 °C corresponded to previous studies with cod from other regions. Magnússon
322 & Martinsdóttir (1995) found <8.0 mg-N TMA/100g in aerobically stored refreshed cod from Iceland
323 when previously kept from 5 to 52 weeks at -25 °C. Martinsdottir & Magnusson, (2001) confirmed
324 this effect of frozen storage time on TMA formation and showed markedly less TMA development in
325 chilled refreshed cod when frozen in the *pre-rigor mortis* state compared to freezing *post-rigor*
326 *mortis*. For refreshed MAP cod previously stored at close to -20 °C, the observed absence of TVBN
327 and TMA formation (Fig. 2) has previously been observed with cod for other regions as well as for
328 refreshed whiting, mackerel and salmon (Bøknæs et al., 2000, 2002; Fagan et al., 2004)

329

330 **5. Conclusions**

The long sensory shelf-life and the low drip loss for refreshed cod fillets from CBA in Greenland makes this fish raw material particularly suitable for a combination of frozen and chilled distribution. Sensory shelf-life of refreshed MAP cod was above 32 days at 2.9 °C, however since the product is frozen within most of the distribution chain, this long chilled shelf-life after thawing is not needed. A safe shelf-life of no more than 15-20 days at 2°C is recommended, to prevent more than 100-fold potential growth of *Listeria monocytogenes* in these products.

Acknowledgements

Innovation Fund Denmark (grant no. 5189- 00175B) funded this research. We thank laboratory technician Mia Laursen for help with the storage trials and senior research scientist Grethe Hyldig and food technician Rie Sørensen for input on sensory evaluation. We thank the sensory panel for their contribution.

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Table 1: Concentrations of microorganisms in fresh Atlantic cod after production with two difference bleeding method.

		Long and Hammer (LH)[a]	Iron agar, total count[b]		Iron agar, black colonies[b]		Pseudomonads agar[b]	
Bleeding method	n	log(CFU/g)	log(CFU/g)	% of LH	log(CFU/g)	% of LH	log(CFU/g)	% of LH
(I) Double cut[c]	9	4.8 ± 0.6^{A}	3.3 ± 0.2^{A}	3.2	2.8 ± 0.6^{A}	1.3	2.0 ± 0.4^{A}	0.2
(II) Decapitation[d]	9	4.1 ± 0.9^{A}	2.5 ± 0.4^{B}	2.5	$0.4 \pm 0.3^{B\ e}$	0.02	2.1 ± 0.3^{A}	1.0

$^{A-B}$ Avg. ±SD Upper case letters indicate significant differences between (I) and (II) by Student's t-Test.
[a] Samples analysed after storage at 0 °C for 48 hours.
[b] Samples analysed after storage at 0 °C for 120 hours.
[c] Manual cutting of the left and right dorsal aorta.
[d] Machine decapitation and manual removal of viscera prior to bleeding.
[e] Quantified by most probable numbers (Jarvis, Wilrich & Wilrich, 2010).

Table 2: Storage conditions and drip loss of Atlantic cod during storage in air or modified atmosphere packaging (MAP).

	Temperature (°C)	Gas composition (% CO_2)		Drip loss (%)
	Avg. ± SD	Start	End of storage trial	Avg ± SD
Batch A				
Chilled cod in air	2.9 ± 0.4	-[a]	-[a]	2.4 ± 0.4
Iced cod in air	0.4 ± 0.1	-[a]	-[a]	2.5 ± 1.0
Chilled cod in MAP	2.9 ± 0.4	35.5 ± 0.1	35.2 ± 0.5	3.6 ± 0.7
Iced cod in MAP	0.4 ± 0.1	36.2 ± 2.4	29.3 ± 0.8	3.4 ± 1.3
Batch B				
Chilled cod in air	1.4 ± 1.0	-[a]	-[a]	-[b]

[a] CO_2 was not determined due to packaging in atmosphere air and used of highly permeable bags.
[b] Drip loss were not determined for batch B.

Table 3: Shelf-life of refreshed Atlantic cod based on sensory evaluation and indices of spoilage.

	Sensory shelf-life and shelf-life determined from indices of spoilage (days)				
	Batch A				Batch B
	Chilled cod in air 2.9 °C	Iced cod in air 0.4 °C	Chilled cod in MAP 2.9 °C	Iced cod in MAP 0.4 °C	Chilled cod in air 1.4 °C
Sensory shelf-life	13	19	> 32	> 32	13
Shelf-life from indices of spoilage					
pH ≥ 7.1	14	22	> 32	> 32	>18
TVBN ≥ 35mg-N/100g	13	19	> 32	> 32	> 18
TVC ≥ 7.0 log CFU/g	9	13	24	> 32	10
CFC ≥ 9.0 log CFU/g	13	19	> 32	> 32	> 18

Table 4: Changes in pH and lactic acid concentrations during storage of chilled and iced cod in air or modified atmosphere packaging (MAP).

	pH (Avg. ± SD)			Lactic acid in the fish (ppm; Avg. ± SD)	
	Start	Sensory spoilage	End of storage trial	Start	End of storage trial
Batch A					
Chilled cod in air[a]	6.8 ± 0.1	7.0 ± 0.3	7.2 ± 0.2 *[f]	2177 ± 89	959 ± 637 *[f]
Iced cod in air[b]		6.9 ± 0.2	7.4 ± 0.2 **[f]		950 ± 611 *[f]
Chilled cod in MAP[c]		-[d]	6.8 ± 0.04		2483 ± 317
Iced cod in MAP[c]		-[d]	7.0 ± 0.1		2196 ± 234
Batch B					
Chilled cod in air[a]	6.5 ± 0.3	-[e]	6.7 ± 0.3	2686 ± 886	2438 ± 425

[a] Storage trial ended after 18 days.
[b] Storage trial ended after 26 days.
[c] Storage trial ended after 32 days.
[d] Products did not reach end of sensory shelf-life.
[e] pH were exclusively determined at the start and at the end of the storage trial.
[f] * indicate $p < 0.05$; ** $p < 0.01$, tested between start and end of storage trial (Student's t-Test).

Table 5: Microbiota as characterised by the *16S rRNA* gene sequencing of isolates from batch A.

	Microbiota: Percentage of isolates on LH			
	Chilled cod in air	Iced cod in air	Chilled cod in MAP	Iced cod in MAP
Number of isolates	8	9	8	5
log CFU/g	9.4	8.7	7.7	5.4
Pseudomonas spp.	75	78	7	6
Serratia conticola	11	-[a]	-[a]	-[a]
Carnobacterium maltaromaticum	14	-[a]	93	23
Rahnella aquatilis	-[a]	22	-[a]	71

[a] No isolates identified.

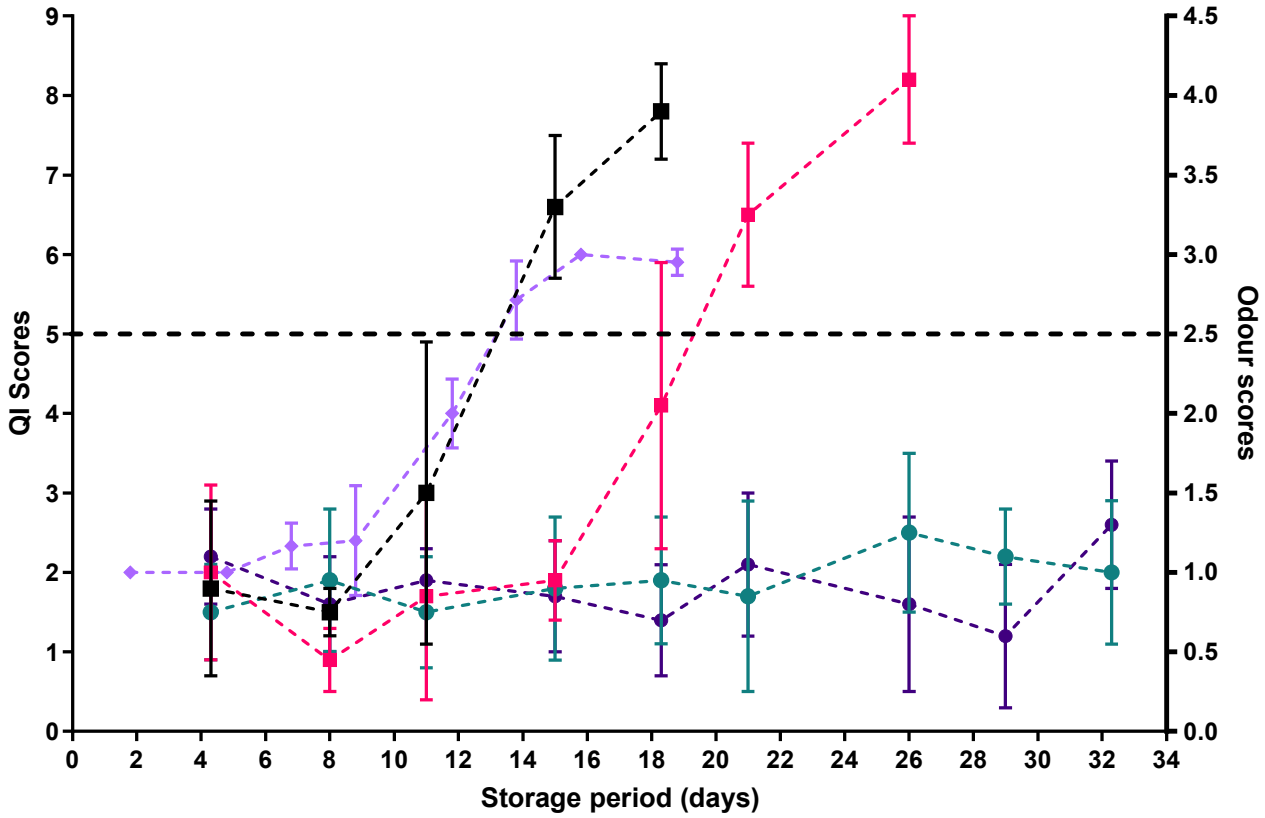


Fig. 1. Total Quality Index (QI) scores during storage of refreshed cod at difference storage conditions: (■) Chilled cod in air batch A, (■) iced cod in air batch A, (●) chilled cod in MAP batch A, (●) iced cod in MAP batch A, (♦) chilled cod in air batch B. Symbols and error bars indicate Avg. ± SD. Dashed black line indicate limit for sensory spoilage.

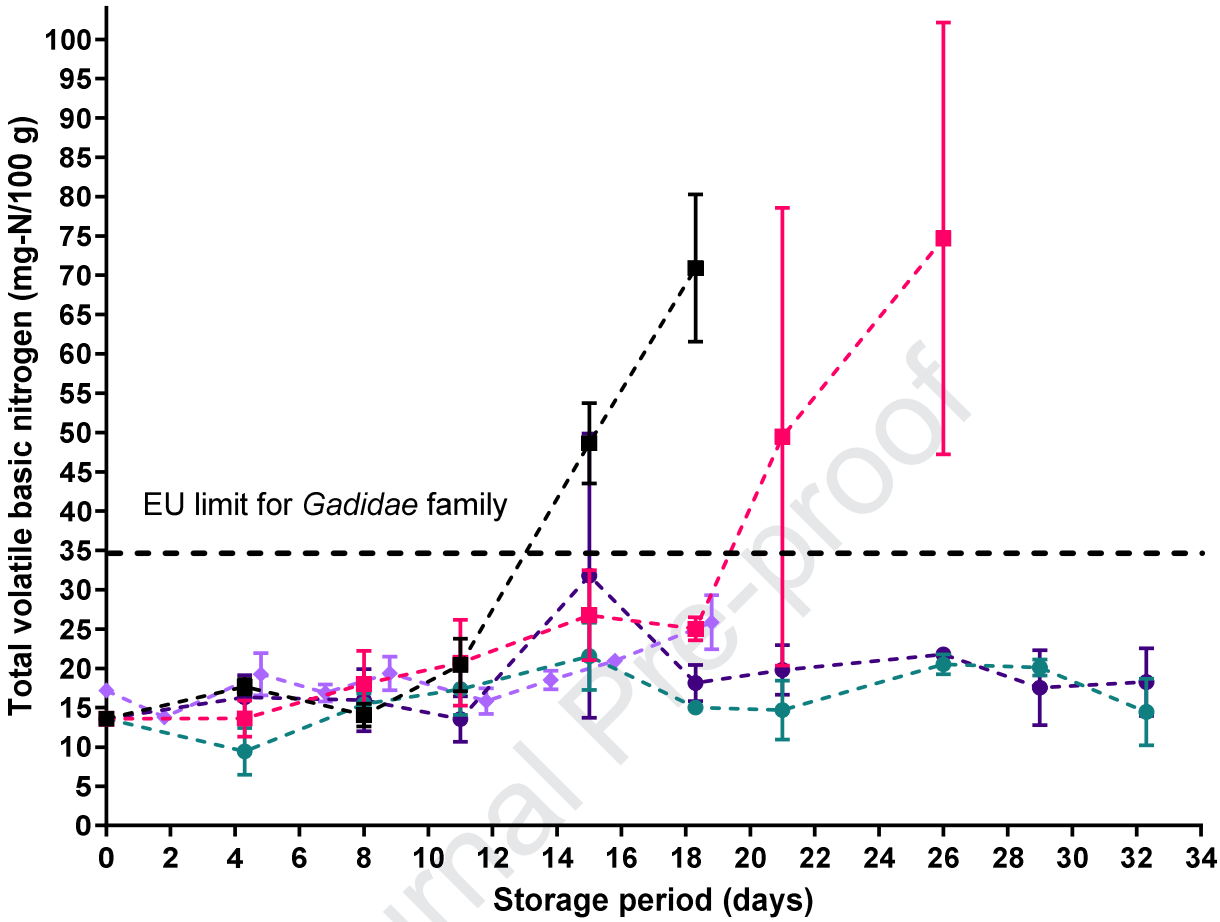
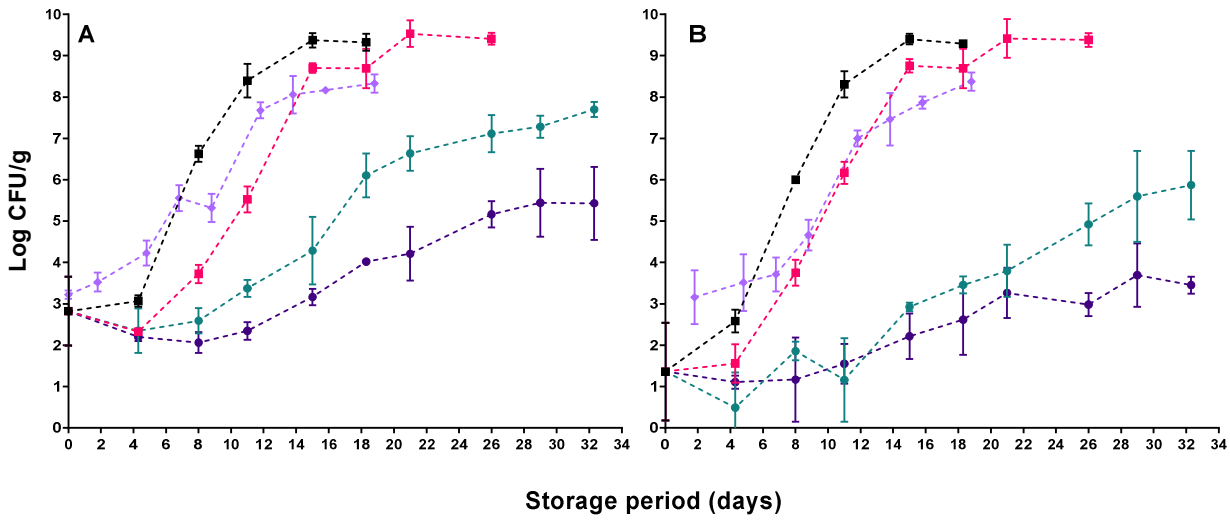


Fig. 2. Formation of total volatile basic nitrogen (TVBN) during storage of refreshed cod at different storage conditions: (■) Chilled cod in air batch A, (■) iced cod in air batch A, (●) chilled cod in MAP batch A, (●) iced cod in MAP batch A, (♦) chilled cod in air batch B. Symbols and error bars indicate Avg. ± SD. The dashed line represent the critical EU limit of 35 mg-N TVBN/100g (EC, 2008).



12

13 **Fig. 3.** Microbial changes determined by total viable counts (A) and on selective media (Pseudomonas CFC Agar)

14 for *Pseudomonas* spp. (B) at different storage conditions: (■) Chilled cod in air batch A, (■) iced cod in air batch

15 A, (●) chilled cod in MAP batch A, (●) iced cod in MAP batch A, (♦) chilled cod in air batch B. Symbols and error

16 bars indicate Avg. ± SD.

- Shelf-life of refresh MAP Atlantic cod was more than 32 days
- Spoilage of refresh cod correlates with the formation of TVN
- Low drip loss, 3.4-.3.6%, during storage of refresh cod in MAP
- *Pseudomonas* and *Psycrobacter* dominated the microbiota of refresh cod in air

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jonas Steenholdt Sørensen, Niels Bøknæs and Ole Mejlholm are employed by Royal Greenland.