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Effects of controlled thawing media temperatures on quality and safety of *pre-rigor* frozen Atlantic cod (*Gadus morhua*)

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ABSTRACT

Novel strategies for thawing of *pre-rigor* frozen Atlantic cod (*Gadus morhua*) in water with air circulation, applying different and controlled temperatures are presented. After thawing (day 0) and after six days of storage at 2.9 ± 0.6 °C (day 6), quality parameters (thawing- and drip loss, cooking yield, sensory evaluation, and textural properties), chemical (pH, water content, total volatile basic nitrogen (TVB-N)) and microbiological analyses (total viable counts (TVC-IA), H_2S-producing bacteria (H_2S-IA), coliforms, thermo-tolerant coliforms and presumptive *E. coli*, and *Listeria monocytogenes*) were performed. The results obtained were compared statistically. Both thawing strategies, thawing at 10 °C and −0.5 °C or at constant 10 °C, preserved good quality fish. The hygienic conditions during the thawing processes were satisfactory and there were no indications of impaired food safety during any of the thawing strategies. No pathogens were detected in any of the cod samples, nor in the thawing media. The results showed that water thawing at −0.5 to 10 °C is suitable for frozen cod, without compromising quality and safety, and that no significant difference were seen between the selected thawing temperature regimes.

1. Introduction

Because of their biological composition, fish are amongst the most highly perishable food products, and even at normal refrigeration storage conditions, the shelf life is limited by oxidation, and enzymatic reactions, as well as microbiological spoilage (Adams & Moss, 1995, pp. 119–125; Sampels, 2015). The main specific spoilage bacteria (SSB) reported in cold water marine fish are *Pseudomonas* spp., *Shewanella putrefaciens*, and *Photobacterium phosphoreum* (Gram & Huss, 1996; Ólafsdóttir, Lauzon, Martinsdóttir, Oehlenschläuger, & Kristbergsson, 2006). *P. phosphoreum* is CO_2-tolerant and is a spoilage organism of fish stored under modified atmosphere, whereas *Pseudomonas* spp. and *Shewanella putrefaciens* produce hydrogen sulphide (H_2S) and are the predominant SSBs of fresh marine fish from temperate waters stored at aerobic conditions (Gram & Dalgaard, 2002).

The catching of whitefish in Northern waters is typically seasonal, as can be seen during the short and intensive period where migrating spawning cod ("skrei") are caught in Northern Norway during the winter and spring each year (Standal & Bouwer Utne, 2007). A substantial part of the caught whitefish is received, and further processed by the land-based industry relying on raw materials from the fishing fleet. As the land-based industry is aiming to level out the seasonal character of their activity, strategies to extend the period of supply of high quality and safe raw materials are highly needed. This challenge can be met by applying fish frozen at sea, under the premise that the characteristics of the raw material are not negatively altered during freezing, storage, and thawing.

Freezing is a common method of preservation, important for shelf life extension and conservation of quality. Hence, freezing on board immediately after capture (*pre-rigor*), before autolysis and bacteria-driven degradations come into play, would be an optimal situation (MacCallum, Jaffray, Churchill, & Idler, 1968). However, the quality of the product is closely related to the freezing and thawing conditions, as they influence chemical reactions and muscle degradation (Baygar, Alparslan, & Çaklı, 2013; Ersoy, Aksan, & Ozeren, 2008; Genc, Esteves, Anibal, & Diler, 2015; Li & Sun, 2002).

There are many potential methods for thawing fish, the water thawing methods being most commonly applied by the industry

Abbreviations: H/G, Headed and gutted

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(Archer, Edmonds, & George, 2008; Backi, Leth, & Gravdahl, 2016; Haugland, 2002). Commercially, media temperatures applied for thawing are usually 12–25 °C (Archer et al., 2008). However, mostly the fish is thawed in uncontrolled manner using batch thawing in running water, without temperature control (Haugland, 2002). Methods applicable at an industrial scale were recently reviewed by Backi (2017).

In a recent study by Roiha, Jónsson, Backi, Lunestad, and Karlsdóttir (2018), it has been concluded that water thawing with air circulation provided efficient thawing and that the quality of the fish was good, with a shelf life of up to 14 days post-filleting. As a follow-up of the trial by Roiha et al. (2018), the aim for the present thawing trial was to investigate the effects of different controlled temperatures of thawing media during water thawing on the quality and safety of cod fillets. Thawed fresh fillets were further evaluated during chilled storage for up to six days.

2. Materials and methods

2.1. Experimental design

Atlantic cod (*Gadus morhua*) were caught by a commercial trawling vessel in the Norwegian Sea, in February 2015. The cod were headed and gutted (H/G) and frozen *pre-rigor* in blocks at −40 °C using a vertical plate freezer on-board the vessel. To avoid thaw *rigor*, the fish blocks were stored at −28 °C for nine weeks before thawing to ensure that the fish had passed *rigor mortis* (Stroud, 1969, p. 12). Four blocks of cod were randomly divided into two groups (T10 and T10–0.5), with approximately 20 fish in each group. The average gutted weight of the cod was 3.0 ± 1.1 kg. Using two different temperature regimes, the two groups were thawed in 1000-L fish tubs with an air diffusion element at the bottom, generating circulation to secure a homogenous water temperature. Additional turbulence was induced as the thawing medium (potable water) was exchanged and re-used after being heated in a heat exchanger. The first group (T10) was thawed with air circulation and continuous water flow at constant-value controlled temperature of 10 °C (4 h). The second group (T10–0.5) was thawed at 10 °C for 2 h before the water temperature was lowered to −0.5 °C (for 26–27 h), also at constant-value controlled levels.

2.2. Temperature profiling

For temperature profiling of the cod during thawing, temperature data loggers (iButton DS1922F, Thermochron, Maxim Integrated, San Jose, USA) recording temperature at 1 h intervals, were placed in the muscle of three fish per group after making an incision with a scalpel just below the first dorsal fin before freezing on-board. The temperature of the thawing water was recorded at 5 min intervals according to the graphical outline in Fig. 1.

2.3. Sampling

After thawing, the H/G cod were manually filleted skin on and pre-weighed before packing into Styrofoam boxes, covered with flake-ice of potable quality, and with plastic film between the ice and the fish. Quality and safety evaluations of fillets were performed 2–3 h after thawing (day 0) and after six days of storage at 2.9 ± 0.6 °C (day 6).

Fillets from the right side of the fish were washed in cold tap water for 10–15 s, and the surface water was wiped off using tissue paper. The fillets were weighed, and T10 (n = 20) and T10–0.5 (n = 20) fillets were subjected to sensory evaluation and evaluation of muscle redness and blood spots. Fillets from the left side of the fish were analysed for physicochemical- and microbiological parameters. A schematic drawing of a fillet and how sampling was conducted for each parameter is given in Fig. 2. Analysis of physicochemical parameters included water content, total volatile basic nitrogen (TVB-N), muscle pH, texture, cooking yield, and drip loss during storage. Microbiological parameters included total viable counts (TVC-IA), H_2S-producing bacteria (H_2S-IA), coliforms, thermo-tolerant coliforms, and *Listeria monocytogenes*.

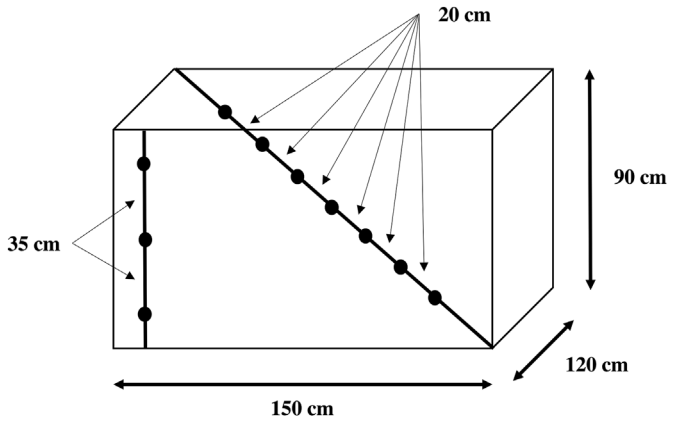

Fig. 1. Graphical outline of fish tubs, circles indicating location of temperature loggers in the water column.

2.4. Chemical analyses

Two sections of the fillet (Fig. 2) were used for measuring muscle pH and water content. The pH-measurements were performed according to the manual of the producer (Weilheim, WTW, pH3110, Germany). The muscle water content was determined by drying samples of approximately 2 g at 105 °C for 24 h (n = 6). The difference in weight before and after drying was taken as the total water content of the sample, and expressed as percentage (%).

For evaluation of cod freshness, total volatile basic nitrogen (TVB-N) was determined in duplicate by the Conway micro-diffusion method described by Conway and Byrne (1933), after extracting the fish muscle with 7.5% aqueous trichloroacetic acid solution (Malle & Tao, 1987). The results were expressed as mg TVB-N/100 g muscle.

2.5. Thawing and drip loss

Thawing loss of the thawed blocks was determined from the known weights of the fish blocks before and after thawing and expressed as:

$$\text{Thawing loss (\%)} = \frac{\text{weight of frozen fish block (g)} - \text{weight of thawed fish block (g)}}{\text{weight of frozen fish block (g)}} \times 100$$

The fillets were weighed at day 0 and at day 6 to evaluate drip loss during cold storage, according to following equation:

$$\text{Drip loss (\%)} = \frac{\text{weight of fillets before storage (g)} - \text{weight of fillets after storage (g)}}{\text{weight of fillets before storage (g)}} \times 100$$

2.6. Cooking yield

The cooking yield of the fillets was determined from 150 g of section 3 of each fillet (Fig. 2). The samples were cooked for 6 min in a pre-heated oven (Rational, SelfCookingCenter, Canada) at 95 °C and full steam (100%). After cooking, excess water was separated from the material and the cooked samples were allowed to cool to room temperature (20 °C) for 15 min before additional weighing. Percentage cooking yield was determined by the following equation:

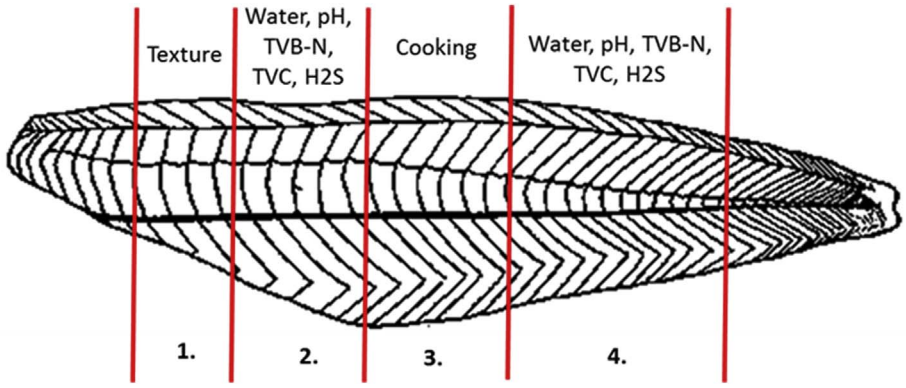

Fig. 2. Schematic drawing of a cod fillet and how sampling was conducted. Whole fillets (opposite side of fish) were used for sensory evaluation and evaluation of muscle redness and blood spots. Samples for microbiological parameters were taken from sections 2 and 4. TVB-N = total volatile basic nitrogen, TVC = total viable counts, H2S = H_2S-producing bacteria, and pH = muscle pH.

$$Cooking\ yield\ (\%) = \frac{weight\ of\ cooked\ muscle\ (g)}{weight\ of\ uncooked\ muscle\ (g)} \times 100$$

2.7. Sensory evaluation

The quality index method (QIM) scheme for cod, based on the work of Bonilla, Sveinsdottir, and Martinsdottir (2007) was applied for sensory evaluation of the fillets. Attributes were adjusted to meet the scope of the current study, and scores were based on colour, texture, gaping, and smell. Total score shows the quality index and gives an indication of storage time and remaining shelf life. Each sampling day, ten fillets from each experimental group were placed on a white board and evaluated according to the scheme described in Table 1. Four panellists participated in the evaluation. They had all been trained according to international standards (ISO., 1993) and were experienced in the sensory method used in this evaluation.

Evaluation of muscle redness and blood spots of fillets was conducted using a scoring system, ranging from 0 to 4, where 0 = no visible, 0.5 = marginal, 1 = trace, 2 = small, 3 = obvious, and 4 = pronounced muscle redness according to Stone and Sidel (1985). Muscle blood spots were evaluated according to a range of 0–3, where 0 = no blood spots, 1 = minimum (1–3 very small or 1 small), 2 = few spots (many small or 1–3 large spots), and 3 = many blood spots according to Arason, Þórðarson, Karlsdóttir, Högnason, and Flosason (2014).

Table 1
The quality index method (QIM) scheme used to evaluate the cod fillets for sensory evaluation, adopted from Bonilla et al. (2007) with modifications.

Quality attribute	Description	Grade
Texture	Firm, springy	0
	Firmness gained slowly after pressure	1
	Soft texture, no springiness	2
Colour	Shining, bright colour according to specie	0
	Matte colour, characteristic for specie	1
	Small yellow dots, colour very matte/dull	2
	Large yellow dots, characteristic colour vanishing	3
	Yellow and mucous	4
Smell	Fresh, seaweedy, metallic	0
	Neutral	1
	Fishy, trace of thawing odour	2
	Obvious thawing odour, sour, trace of ammonia	3
	Strong ammonia, off-odour	4
Gaping	No visible gaps	0
	Gaping less than 20% (1–3) longitudinal cracks	1
	Minor gaping on one area (20%) or > 3 longitudinal cracks	2
	Some gaping, 25–75% of the fillet	3
	Deep cracks or gaping in more than 75% of the fillet	4
	Grade (0–14)TOTAL SCORE	

2.8. Fillet texture measured by shear force (toughness)

Textural properties of the right fillets were measured with a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with 5 kg load cell and a Warner-Bratzler shear blade, as described by Jonsson, Sigurgisladottir, Hafsteinsson, and Kristbergsson (2001). Each sample was cut according to Fig. 2 and were 8 cm in diameter, 2 cm wide and 2 cm in thickness.

2.9. Microbial analyses

Microbiological assessments of the fillets, and of the thawing media before and after thawing, included analyses of total viable counts (TVC-IA), H_2S-producing bacteria (H_2S-IA), coliforms, thermo-tolerant coliforms, and *Listeria monocytogenes*. Six fillets were analysed per sampling point, 24 fillets in total. Tissues from the cod filets were taken from section two and four of the fillets as shown in Fig. 2.

Total viable counts (TVC-IA) of cod fillets were examined by aerobic cultivation on Iron Agar Lyngby (Oxoid), which also gives the number of H_2S-producing bacteria as black colonies, due to precipitation of iron sulphide (FeS) (Gram, 1992; Gram, Trolle, & Huss, 1987). Sample preparation was done according to the Nordic Committee on Food Analysis (NMKL) method 184 (NMKL., 2006). Black colonies and all colonies were enumerated, and the results were reported separately as log cfu/g. Analyses for coliforms and thermo-tolerant coliforms were performed from a homogenate of 10 g muscle tissue in peptone water, by a Petrifilm™ (3M™ coliform Count Plates) method, according to the protocol supplied by the producer. The results were reported as log cfu/g and the detection limit was 10 cfu/g. From water samples, 100 ml were filtered through a 0.45 µm membrane filter, and the filter was transferred to appropriate agar plates. For coliforms, *m*-Endo agar LES (Difco) was incubated at 37 °C for 24 h. For thermo-tolerant coliforms, m−FC− agar (Difco) was incubated at 44.5 °C for 24 h. Blue colonies on m−FC− agar were to be confirmed as *E. coli* by inoculation and incubation in EC-broth, prior to indole testing.

For detection of *L. monocytogenes*, 25 g of muscle tissue was homogenised with 225 ml Half-Frazer broth (BioRad), incubated at 30 °C for 24 h, followed by analyses using chromogenic agar RAPID'L.mono (Bio-Rad), performed according to the protocol supplied by the producer. For analyses of water samples, 100 ml were filtered through 0.45 µm membrane filters, before the filter was transferred to a stomacher bag, and analysed as done for cod samples.

2.10. Statistical analyses

Statistical analyses were performed using Microsoft Office Excel 2010 (Microsoft Inc., Redmond, WA., USA). For evaluation of fillet shear forces, a one-factor analysis of variance (ANOVA) was performed. Where significant differences were indicated ($P < .05$), Tukey's *post hoc* test was run. Discrete variables data (blood in muscle and

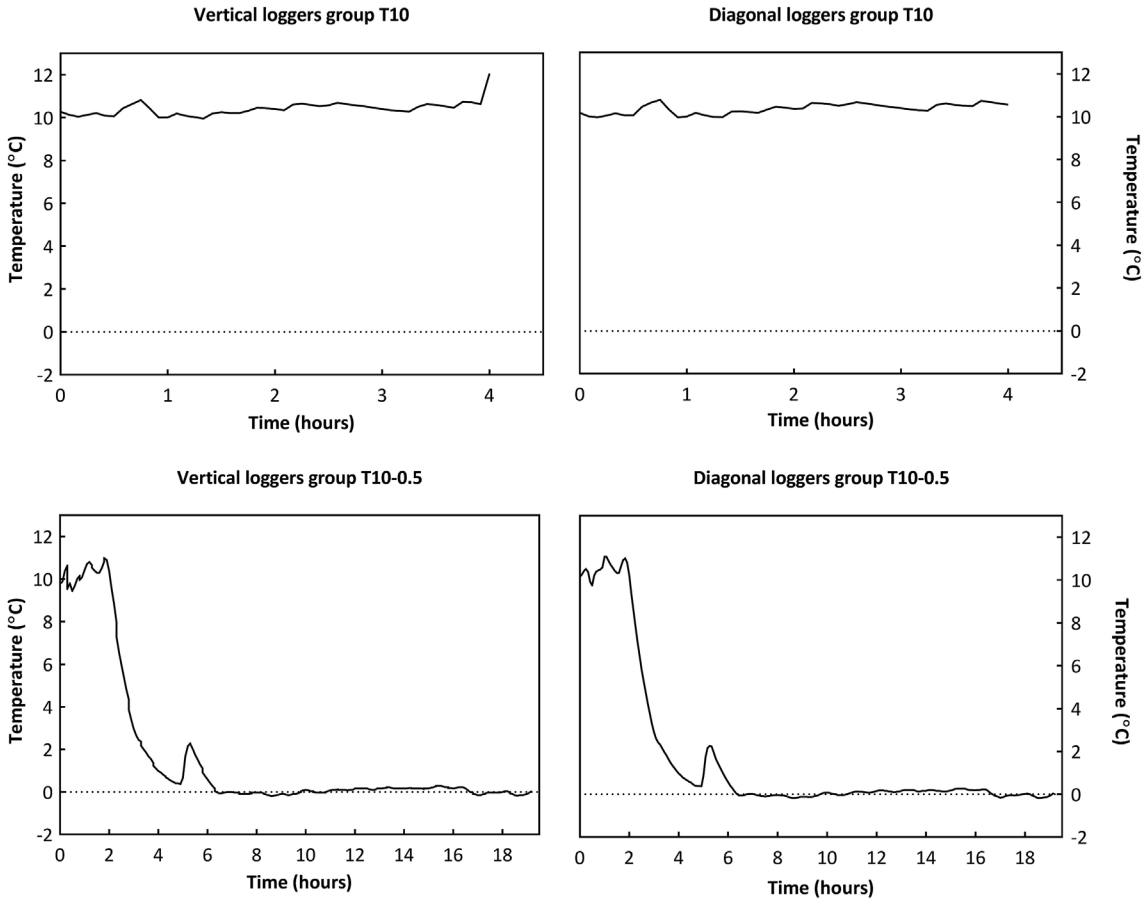

Fig. 3. Temperature profiles from diagonal and vertical loggers of the water of group T10 and T10–0.5 during thawing.

bloodstains) were tested using non-parametric statistics (Kruskal Wallis or Mann Whitney). If not stated otherwise, results are reported as mean values ± standard deviation (SD).

3. Results and discussion

3.1. Temperature profiling

Thawing frozen food correctly is important for retaining food safety and quality, and important factors during thawing include adequate temperature control, hygiene, and handling practices. Temperature profiles of the water, of group T10 and T10–0.5, respectively, during thawing are shown in Fig. 3. The air circulation and continuous water flow during thawing resulted in homogenised temperature distribution, both horizontally and vertically.

The temperatures of the cod muscle were monitored during thawing. The average temperature of the fish muscle before thawing was −13.6 °C. The muscle reached a temperature of 0 °C after 5–6 and 28–29 h, respectively, for the T10 and T10–0.5 group. After thawing, the cod was kept at cold storage (2.9 ± 0.6 °C) prior to filleting and analysing.

3.2. Muscle chemistry

The values of pH, water content, and TVB-N with means ± SD are given in Table 2. The average pH and water content of thawed cod muscle was 6.7 and 81.7%, respectively. The pH-value was within the reported range of cod frozen at sea before *rigor* (Martinsdóttir & Magnússon, 2001). A small difference in muscle pH between the thawing methods was found, where fillets thawed at 10 °C had a slightly higher muscle pH, both at day 0 and day 6 ($P < .05$). No change in muscle pH during cold storage was observed, which has previous been reported for thawed fillets kept at 4 °C (Martinsdóttir & Magnússon, 2001).

The water content of the muscle ranged from 80.3% to 83.0%, which is normal for wild cod caught in February. After spawning and during summer, the water content tends to increase followed by decrease again (to its normal level) in October–November (Love, 1960). No significant difference was observed between the experimental groups. Neither did the water content decrease significantly after cold storage for 6 days after thawing (Table 2).

Total volatile basic nitrogen (TVB-N) was used for evaluation of cod freshness, and the values obtained directly post thawing ranged between 9.0 mg N/100 g and 14.6 mg N/100 g. After six days of storage, one of the samples in the T10 group had TVB-N of 45 mg N/100 g, which is above the threshold limit of 35 mg N/100 g for acceptability of consumption of gadoid species (Ólafsdóttir et al., 2006). However, no significant difference between the two thawing regimes could be detected.

To detect differences between the thawing methods, and a detection of limit of shelf life, the fillets should have been stored for a longer period. Roiha et al. (2018) obtained a shelf life of up to 14 days of frozen-thawed cod thawed in water with air circulation, which could also be expected in the current study. Previous studies have found a shelf life of fresh cod fillets (processed from gutted, iced cod) in the usual range between 10 and 13 days (Lauzon, Magnússon, Sveinsdóttir, Gudjónsdóttir, & Martinsdóttir, 2009; Lauzon et al., 2010).

3.3. Thawing loss, drip loss and cooking yield

The ability of the fish muscle to retain its natural water and therefore its juiciness is one of the quality criteria of fresh fish, especially

Table 2
Fillet quality of frozen-thawed Atlantic cod (*Gadus morhua*). Parameters included thawing loss (%) during block thawing, drip loss (%), and cooking yield (%); sensory evaluation of texture, colour, smell, gaping, and total quality index (TQI), along with muscle redness and blood spots; shear force (mean ± SD) of cod fillets and muscle pH and water content (%); total viable counts (TVC), hydrogen sulphide producing bacteria (H_2S), and total volatile basic nitrogen (TVB-N). Parameters were determined after thawing (day 0) and after storage for six days (day 6) at 2.9 ± 0.6 °C: Comparisons of thawing in water with air circulation at two different temperature regimes (T10, T10–0.5). Each parameter is expressed as a mean and standard deviation (SD) of at least six replicates.

Quality parameter	Day 0		Day 6	
	T10	T10–0.5	T10	T10–0.5
Thawing loss (%)	1.7 ± 1.2^{X}	3.6 ± 0.0^{Y}	n.a.	n.a.
Drip loss (%)^{NS}	n.a.	n.a.	2.9 ± 1.1	3.3 ± 1.0
Cooking yield (%)^{NS}	85.6 ± 1.9	85.2 ± 3.6	85.3 ± 2.9	87.0 ± 3.2
Texture (range: 0–2)	1.0 ± 0.3	1.0 ± 0.3	1.3 ± 0.4	1.4 ± 0.4
Colour (range: 0–4)	1.0 ± 0.4	1.2 ± 0.4	1.4 ± 0.5	1.4 ± 0.5
Smell (range: 0–4)	1.1 ± 0.3	1.2 ± 0.4	1.7 ± 0.7	1.9 ± 0.7
Gaping (range: 0–4)	1.3 ± 0.4	1.3 ± 0.7	1.5 ± 0.6	1.6 ± 0.6
TQI (range: 0–14)	4.3 ± 0.8	4.7 ± 1.3	5.9 ± 1.4	6.3 ± 1.3
Muscle redness (scale 0–4)	0.2 ± 0.1*	0.1 ± 0.0*	0.3 ± 0.1*	0.4 ± 0.1*
Blood spots (scale 0–3)	0.2 ± 0.1^{X}	0.5 ± 0.1^{Y}	0.2 ± 0.1^{X}	0.5 ± 0.1^{Y}
Shear force (N)^{NS}	28.4 ± 1.6	30.3 ± 6.0	26.0 ± 1.5	28.3 ± 3.3
Muscle water (%)^{NS}	82.1 ± 0.1*	81.8 ± 0.2	81.5 ± 0.2*	81.3 ± 0.1
Muscle pH	6.6 ± 0.2^{X}	6.8 ± 0.2^{Y}	6.7 ± 0.1^{X}	6.8 ± 0.2^{Y}
TVC (Log cfu/g)	2.8 ± 0.2	1.8 ± 0.4	4.8 ± 0.4	4.2 ± 0.8
H_2S (Log cfu/g)	n.d.	n.d.	2.3 ± 0.6[a]	1.0[b]
TVB-N (mg N/100 g)	12.5 ± 2.3	12.6 ± 1.8	18.8 ± 12.9	15.0 ± 5.2

Different letters (X, Y) indicate significant differences between the thawing treatments ($P < .05$) (n = 6). * Significant difference between storage times (day 0, day 6, $P < .05$). NS = no significant differences between groups ($P > .05$).

[a] Average of two samples above detection limit.

[b] One sample only above detection limit. n.a. = not available (i.e. not measured), n.d. = not detected (i.e. below detection limit).

from a consumer perspective. Therefore, drip loss during storage and after cooking are important parameters that should be included when evaluating seafood quality. The drip loss during cold storage and cooking yield is presented in Table 2. At day 0, significant differences were found between thawing methods ($P < .05$), with respect to thawing loss, where the loss was less for the T10 (1.7%), compared to T10–0.5 (3.6%).

After six days of chilled storage, no differences between the thawing methods were found concerning drip loss (%) or cooking yield (%) of the fillets ($P > .05$). For comparisons, Erikson et al. (2016) reported 1.9–2.9% drip loss of fillets of Atlantic cod, frozen *pre-rigor* in Cell Alive Systems, just after thawing, and this was increased to 4.8–5.5% of body weight after storage on ice for 6 days.

3.4. Sensory evaluation

The scores of texture, smell, colour, gaping, and total quality index (TQI), of the thawed fillets, evaluated at day 0 and day 6, are presented in Table 2. As expected during storage, all parameters increased. However, the increase was not significant and no differences were observed between the experimental groups. The results are comparable to the findings by Roiha et al. (2018), with no significant differences in scores for colour, smell, and gaping from the current study. On the other hand, the texture parameter at day 0 (1.0 ± 0.3) and at day 6 (1.3 ± 0.4) in the current study are somewhat higher than seen for water thawing with air circulation in the previous trial at day 0 (0.2 ± 0.4) and day 6 (0.3 ± 0.5) (Roiha et al., 2018).

Fillet redness and the amount of blood spots in the fillets were assessed visually (Table 2). No differences were observed in muscle redness between the treatments. However, the visual assessment did detect an increase in fillet redness after cold storage for both treatments ($P < .05$). Assessing the amount of blood spots, the fillets of the T10–0.5 group had more blood spots than those of the T10 group ($P < .05$). Further, no difference was detected in the amount of blood spots at day 0 and day 6 for the two treatments. The prevalence of muscle redness and blood spots were low, indicating that fillets from all thawing treatments were of good quality, and also indicating gentle capture with little bruising along with sufficient bleeding after capture. In the current experiment, a number of factors may have affected the fish quality, positively or negatively, and thereby diminishing the effect of the different thawing procedures. Such factors include the different hauls (fishing depth and thawing time) from which the fish were selected, and additionally the on-board handling of the fish. In commercial trawl fisheries, the process of gutting all fish in a haul can take hours. Wagner (1978) found that cod quality (external appearance and consistency), was reduced as trawling time increased, and Botta and Bonnell (1988) showed that quality reduction of fresh Atlantic cod was largely due to external factors such as delayed bleeding. However, in the current experiment, the holding time was low, and the average time to gut all fish in the dry bin was equal to the mean haul duration.

3.5. Fillet texture

The texture of fish is an important contributor to palatability, and thus influences its consumer value. In this study, shear force (toughness) was measured with a Warner-Bratzler blade (Table 2). There were no differences in shear forces observed between the two thawing methods T10 and T10–0.5. Even though the T10–0.5 fillets seemed to have a softer texture compared to the T10 fillets, both at day 0 after thawing and at day 6 after chilled storage; however, the difference was not significant ($P > .05$). Furthermore, chilled storage for 6 days at 2.9 ± 0.6 °C had no effect on the measured shear force (N) of the samples. Neither did haul, nor fishing depth ($P > .05$). The measured shear force of 26–30 N for the cod fillets complies with studies of whole cod fillets kept on ice for 3 days, reporting total shear force values between 25 and 28 N (Bjørnevik, Karlsen, Johnston, & Kiessling, 2003).

3.6. Microbial analyses

Water thawing is frequently applied in the seafood industry and is used to thaw a variety of products such as whole, H/G fish, fish blocks and shellfish (Archer et al., 2008). During thawing, fish and equipment may be exposed to human contact, or other sources of microbial contamination, therefore proper hygiene is crucial. In addition, the fish is in direct contact with the thawing medium, which can increase the risk of cross-contamination. Coliform bacteria mainly originate from the intestines of warm-blooded animals, and include genera that originate from faeces of such animals and humans. Hence, assays for coliforms are often used as an indicator of the hygienic standards during food production. Such indicator organisms of faecal contamination include coliforms, thermo-tolerant coliforms, *E. coli*, and enterococci (Noble, Lee, & Schiff, 2004). Based on current and former legislations and guidelines, Svanevik, Roiha, Levsen, and Lunestad (2015) outlined and proposed an assessment scheme for fish-, surface-, and water samples for the pelagic fish industry, which could also be applied for the white fish industry. These guidelines were set for quality, hygiene, and safety by limits of heterotrophic plate counts (HPC), or total viable counts (TVC), faecal indicator organisms (thermo-tolerant coliforms and enterococci), and *Listeria monocytogenes*, respectively. The presences of coliforms in foods are undesirable, however, no limits were proposed for coliforms, because coliform counts are inadequate to differentiate between faecal and non-faecal contamination. In the present study, coliforms and thermo-tolerant coliforms in the thawing media and in the cod fillets were examined. Additionally, the presence of *L. monocytogenes* in the thawing media and in the fillets was analysed. No coliforms nor *L. monocytogenes* were detected in any of the fish samples, nor in the thawing media. Prior to thawing, the initial water TVC was 3.7 ± 0.04 log cfu/g. After thawing, the TVC of the water was

4.1 ± 0.07 and 3.3 ± 0.07 log cfu/g for the T10 and T10–0.5 experimental groups, respectively. Hence, the viable counts increased significantly with higher temperatures during thawing, whereas when reducing the temperature below 0 °C during thawing, the viable counts were significantly reduced. This may not necessarily imply impaired food safety, but rather shows that the thawing water temperature may affect the quality of the final product, as high bacterial counts are often related to increased number of spoiling bacteria, possibly reducing the shelf life of the product (Gram & Dalgaard, 2002; Gram & Huss, 1996). According to the Council Directive on the quality of water intended for human consumption (EU., 1998), all kinds of water involved in food production should hold potable quality. Considering the above mentioned assessment scheme by Svanevik et al. (2015), the hygiene conditions in the current study during the thawing processes were considered good and there were no implications of impaired food safety.

Total viable counts (TVC-IA) and H_2S-producing bacteria (H_2S-IA) on iron agar of fillets thawed in water bath kept at 10 °C, or in water bath at 10 °C for 2 h in water bath, followed by 18 h at −0.5 °C, are shown in Table 2. As expected, both TVC-IA and H_2S-IA increased after six days of chilled storage. However, the differences between the two thawing methods were not significant. Microbial spoilage of fresh fish, stored aerobically at chilled conditions, is commonly seen if the number of SSBs exceeds 8.0 log cfu/g (Gram & Huss, 1996), of which neither samples exceeded in the current study. Values of H_2S-IA at day zero after thawing were all below detection limit of 10 cfu/g. After six days of chilled storage, one of six fillets from the fish thawed in water bath kept at 10 °C had TVC-IA > 300 000 cfu/g (> 5.5 log cfu/g) and H_2S-IA of 600 cfu/g (2.8 log cfu/g). This was the above-mentioned sample in the T10 group with a TVB-N of 45 mg N/100 g. According to the assessment scheme by Svanevik et al. (2015) the microbial quality of the fish samples was good, and the results from the microbial analyses confirmed the findings of the chemical analyses of a shelf life of at least six days. Comparing the findings of the recently published study by Roiha et al. (2018), the microbial counts in the current study was significantly lower both directly after thawing and after six days storage. This can be explained by the lower water temperatures applied in this study (10 °C and −0.5 °C) compared to the study by Roiha et al. (2018), where the initial water temperature was 18 °C.

Freezing the fish directly after catch will not kill the bacteria present, but will arrest their growth, which again may prolong the shelf life of the thawed product. Bonilla et al. (2007) found an estimated shelf life of 7–10 days post filleting, based on counts of H_2S-producing bacteria and sensory evaluation, and suggested that the storage time of 3–5 days of the whole fish, from catch until filleting, could explain the short shelf life in that study compared to previous studies. For comparison, Lorentzen, Heide, Tobiassen, and Ageeva (2016) reported a shelf life of 12 days of cod stored at 0 °C.

4. Conclusions

In the present study, thawing of *pre-rigor* frozen Atlantic cod in water with air circulation and at the two controlled thawing temperature regimes preserved good quality fish, as determined by TVB-N, thawing- and drip loss, cooking yield, sensory evaluation, textural properties, and microbiological analyses. No indications of impaired food safety were found during any of the thawing strategies, as assessed by the presences of *Listeria monocytogenes* and coliforms.

This study shows that proper handling of the fish, with immediate freezing upon catch, adequate thawing procedures, and proper post-filleting handling and storage can provide the industry with an all-year supply of raw material, without compromising quality and safety of the final product.

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