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The effect of freeze-chilling on quality changes of cod loins (*Gadus morhua)* during chilled storage

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ABSTRACT

Fish is highly perishable and has a short shelf-life. Freeze-chilling involves freezing and frozen storage followed by thawing and chilled storage. It offers more sustainable logistic benefits as it enables the products to be held frozen and released into the chill chain as required. Freeze-chilling can offer consumers high quality fish products throughout the year. The study was performed in the industry and included industrial methods for thawing, filleting, packaging, and freezing. The effect of freeze-chilling and chilling on vacuum packed cod loins was evaluated by sensory evaluation, drip loss, and total viable counts (TVC). Loins were stored at 4°C for up to 14 days after thawing. Analyses were performed on day 0, 6, 10 and 14. The results indicated that cod loins worked well as freeze-chilled vacuum-packed loins during short-term chilled storage after thawing. Freeze-chilled samples kept acceptable quality based on the sensory score but had higher drip loss.

Keywords: Freeze-chilling, chilling, cod, quality.

1. INTRODUCTION

Fish are amongst the most perishable food products, and even at normal refrigerated storage conditions the shelf life is limited by oxidative, enzymatic and microbiological spoilage. Freezing is becoming a more common preservation technology in global fish processing. The proportion of fish produced for human consumption that has been frozen has increased from 30% in 2014 to 35% in 2018 (FAO, 2020, 2016). In the future, it is expected that the Norwegian fishing industry carry out more filleting rather than export of whole fish. This will increase the need to include freeze-chilling in the so-called 'cold chain' (Ndraha et al., 2018).
The concept of freeze-chilling refers to freezing of food products and frozen storage followed by thawing and chilled storage. Freeze-chill technology has advantages in making markets more accessible (O'Leary et al., 2000). It offers logistic benefits in distribution and retail as packaged fillets can be held frozen and released into the marked as required. Thawing in consumer packs, prior to retail, enables the possibility of extended shelf-life for groceries and consumer, compared to cold chain transportation and retail. In the case of whitefish, for which availability is seasonally dependent (Standal and Utne, 2007), freeze-chilling during processing can be beneficial to both the industry and consumers.

The effect of freezing, thawing, and chilling of white fish is well described in the literature (Bøknæs et al., 2002, 2001, 2000; Erikson et al., 2021; Hedges, 2002; Roiha et al., 2018, 2017). However, freezing, thawing and subsequent chilling of consumer packed fish as representing a typical "Refresh"-chain have received less attention. Some studies are performed on raw whiting (*Merlangius merlangus*), mackerel (*Scomber scombrus*), salmon (*Salmo salar*) (Fagan et al., 2004, 2003), grass carp (*Ctenopharyngodon idellus*) (Yin et al., 2014) and cod (*Gadus morhua*) (Bøknæs et al., 2002; Guldager et al., 1998). Most of these studies have a greater emphasis on laboratory activities rather than on *industrial processes.*

To our knowledge, the effect on quality parameters of different raw materials of cod is not addressed in previous studies on freeze-chilling. The objective of the current trials was to assess the effect on freeze-chilling, in comparison with fresh, on some quality parameters for vacuum packed cod loins. The quality parameters included sensorial score, total viable counts (TVC), drip loss, water holding capacity and texture.

The study was performed in the industry and included industrial methods for thawing, filleting, packaging, and freezing.

2. MAIN SECTION

2.1. Measurements and methods

The study was performed in the industry and included industrial methods for filleting, packaging, and two different freezing methods of cod loins. Three process treatments for vacuum packed loins were compared: T1) chilled, T2) freeze-chilled, fresh raw material, and T3) freeze-chilled, frozen raw material. T1 group was stored in refrigerated chambers at 3.3±0.5°C for 14 days.

Freeze chilled samples made from fresh cod (T2) were frozen in a spiral freezer to core temperature -18°C and stored between -18°C and -25°C for 16 days. The samples were then thawed overnight at 3.3±0.5°C and stored in refrigerated chambers at 3.3±0.5°C for 14 days after thawing.

For freeze chilled samples made from frozen cod (T3), frozen cod blocks (20 kilos) were thawed in temperature-controlled seawater to 0°C (single fish) before producing the samples. The freeze chilled samples were then frozen in a spiral freezer for 80 min to a core temperature -18°C and stored at -22°C to -26°C for 30 days, thawed overnight at 3.3±0.5°C and stored in refrigerated chambers at 3.3±0.5°C for 14 days after thawing.

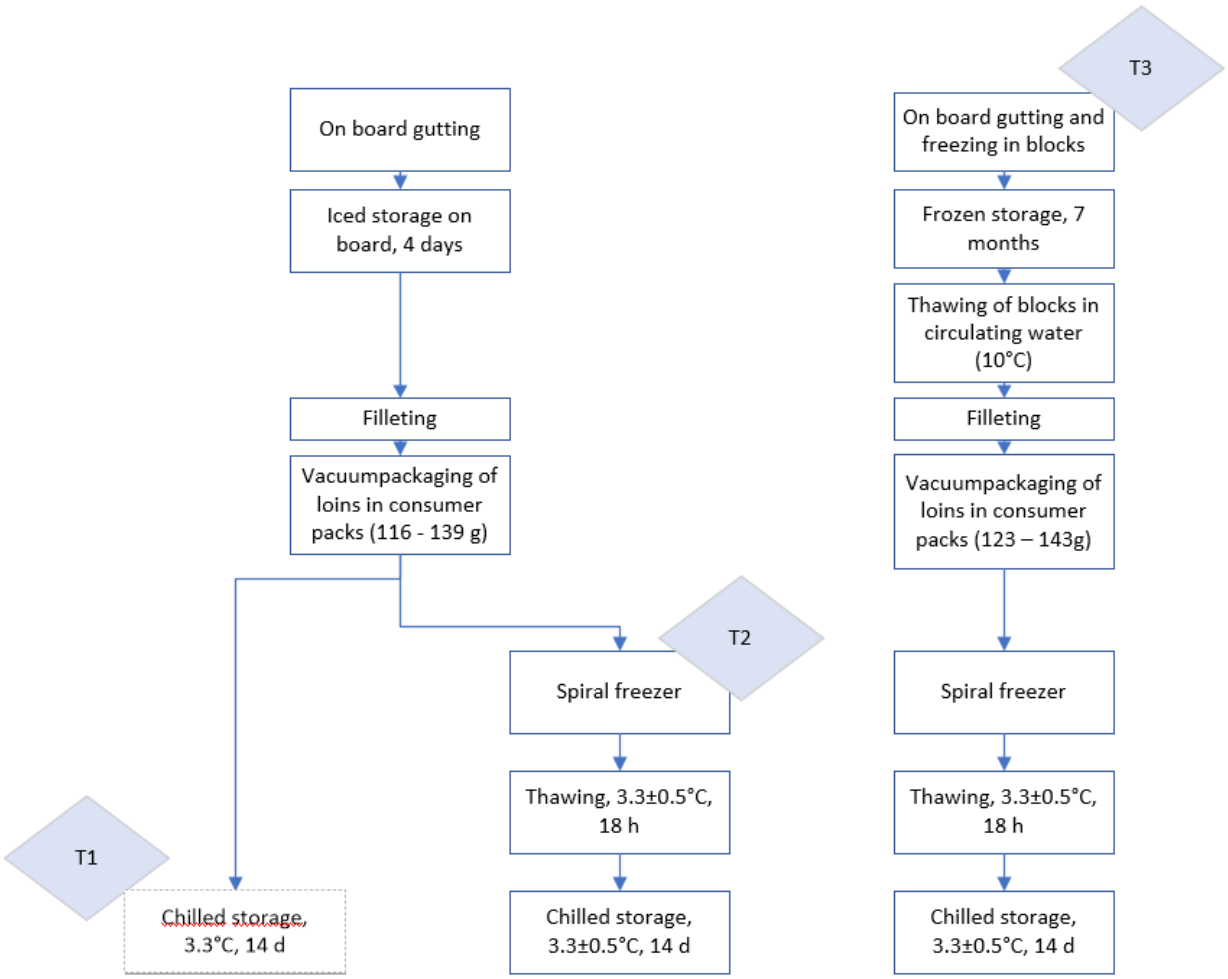

Figure 1: Process treatments in the trials

During freezing, frozen storage, thawing and chilled storage temperature loggers *(IButton DS1922L Thermochron Data Logger, UK)* recording temperature at 10-minute intervals were placed in the package of two random products from each processing treatment. In addition, the temperature was monitored in the centre on two products from T2 and T3 group during thawing (*Testo 176 T4 m/TC type K,Testo Ltd, Hampshire, United Kingdom*). Ambient temperature was monitored (*HOBO 64K Pendant®*, Onset.com) in the freezer, thawing chamber and refrigerated chambers.

Analyses were performed after chilled storage at 3.3±0.5°C on day 0, 6, 10 and 14, as shown in Fig. 2.

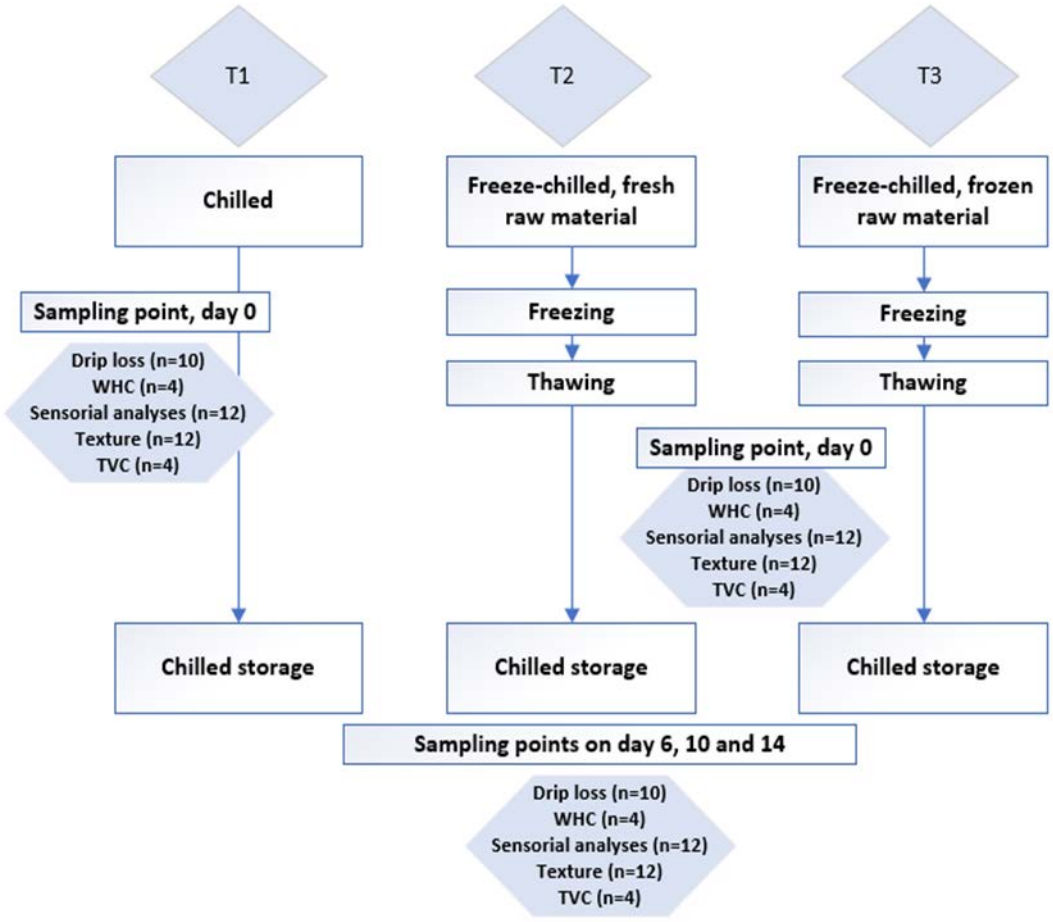

Figure 2: Sampling points and analyses in the trials

For quantification of *drip loss*, the samples were removed from the vacuum bag after the designated storage period and placed on a filter paper (*Whatman International Ltd., Maidstone, UK, No 1*) to dry. The samples were weighed, and drip loss was calculated from initial weight. Drip loss is expressed as % weight loss. For the freeze-chilled samples, the drip loss on day 0 represent thawing loss.

The *water holding capacity* (WHC) was determined by a centrifugation method as described by Eide and co-workers (1982). The cod muscle was coarsely minced in a mixer (*Quick foodmaster, Tefal*) for 10–15 s, and 2 g of the sample was weighed in a glass sample holder. Samples were centrifuged at 210 × g for 5 min at 4°C (*Jouan KR 22i*). Centrifugation loss of water was calculated as the difference in weight before and after centrifugation. The WHC (expressed as %) was calculated as the ratio of remaining water compared with the water content in the sample before centrifugation. For analyses of water content, samples (n=4) were dried at 105°C overnight to constant weight in a sterilizer (*Termaks, series TS 8000*). The moisture content is shown in Table 2.

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 loins. Attributes and the scheme were adjusted to meet the scope of the current study.

Each sampling day, twelve raw loins from each experimental group were placed on a light grey board and evaluated according to the scheme described in Table 1. The samples were evaluated by a panel consisting of three semi-trained assessors from the scientific staff. Total score is the sum of scores from each attribute and gives an indication of the quality. A high score is indicating low quality.

Table 1: The quality index method (QIM) scheme used to evaluate the cod loins 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, brightness	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
Colour, blood spots	No blood spots or redness	0
	Redness, blood spots small area	1
	Redness, blood spots large area	2
	Brown, small area	3
	Brown, large area	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 gaps in 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
Total score		0 - 18

Colour was evaluated sensorially as brightness and redness or blood spots in the loins. Both attributes were evaluated on a pre-defined scales ranging from 0-4 as described in Table 1.

Instrumental texture analyses were performed on a Texture Analyser Plus TA XT.2 (Stable Micro System UK) with a cylindrical probe 12 mm diameter which had a flat bottom were used. The speed of the probe during the measurements was 1.0 mm / second. The end point for the measurement was 30% of the initial loins' height and the results are expressed as hardness (g). The measurements were performed on the thickest part of the loins (n=12). Three measurements were made on each loin. By using specific macros in the Exponent software (Exponent, Stable Micro Systems, Surrey, UK) included with the Texture Analyzer, the force at 30% of the initial loins height was assessed.

Microbiological assessments of the loins included analyses of total viable counts (TVC). Four loins were analysed per sampling point. Total viable counts were examined by aerobic cultivation on Iron Agar, (Gram, 1992; Gram et al, 1987). Sample preparation was done according to the Nordic Committee on Food Analysis (NMKL) method 184 (NMKL, 2006). The results are reported as log cfu/g.

Statistical analyses were performed using SPSS statistical software v.27 (IBM SPSS statistics, New York, US) and Microsoft Office Excel 2010 (Microsoft Inc., Redmond, WA. USA). Mean values and standard deviation of n=12 samples for each regime and day are given unless otherwise stated. The data were subjected to analysis of variance (ANOVA), to assess whether there were significant differences for different quality parameters at different storage times between and within the groups. Significance was defined as $p<0.05$.

2.2. Results and discussion

Adequate temperature control and handling practices during freezing, thawing and chilled storage of fish is important for food safety and quality. The temperatures of the cod loins were monitored during freezing (Fig. 3), thawing (Fig. 4) and chilling (Fig. 5).

The freezing methods in the present study resulted in a rapid freezing rate. During freezing, the cod loins reached a temperature of -18°C within 100 minutes and 70 minutes, respectively, for the T2 and T3 group. Rapid and even freezing results in small crystals and less damage to the fish muscle (Fellows, 2009; Jessen et al., 2014).

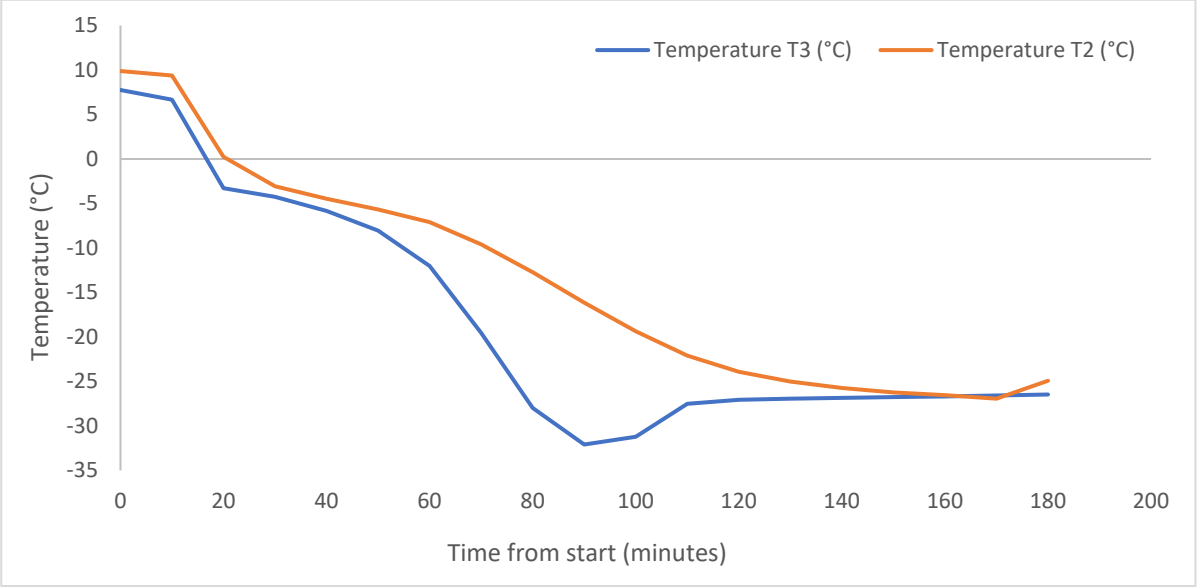

Figure 3. Temperature profile during freezing of T2 and T3. The temperature was monitored with temperature loggers placed in the vacuum pack of five packages. During freezing the packages was turned so the logger did not touch the belts.

The temperature in the refrigerated chamber was 3.3±0.5°C during thawing and subsequent chilled storage. During thawing, the products reached a temperature of 0°C after 11.2 (T2) and 17.0 h (T3). The size of the loins was on average 11.1 x 6.2 x 2 cm (l x b x h) and average weight was 117±10 g, but the variation in size and weight of the loins were large and might explain the differences before the temperature reached 0°C. Others have argued that the thawing rate should be quick to minimize drip loss and dry texture but maintaining a low temperature to avoid bacterial reactions (Cai et al., 2019). The relatively long thawing period in the present study might have influenced the results.

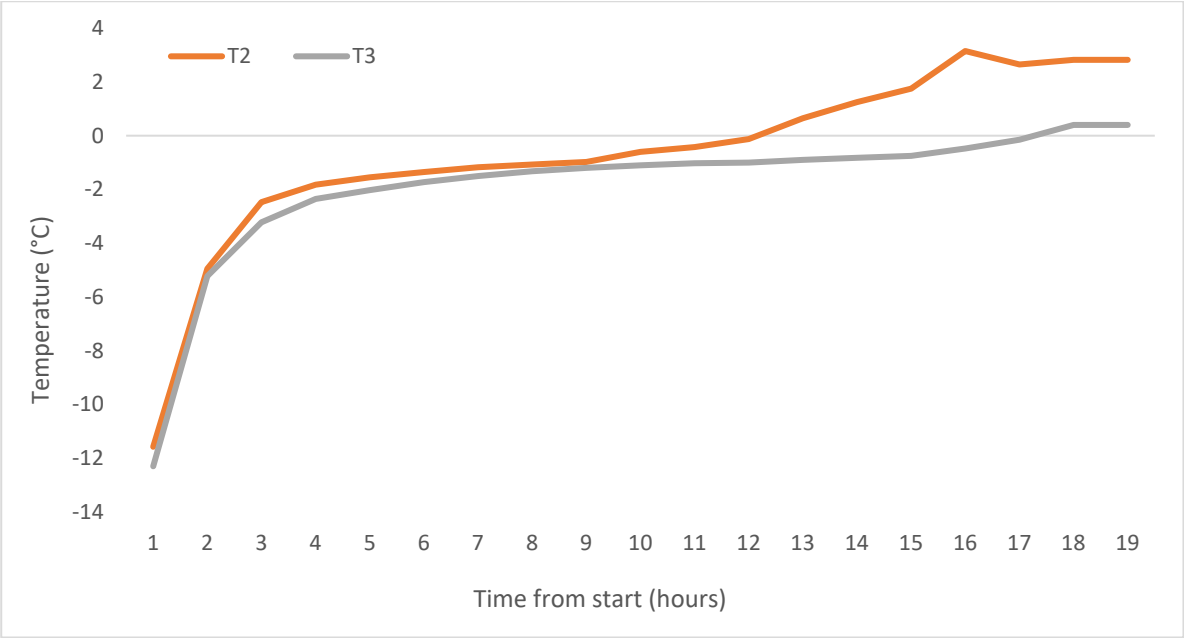

Figure 4. Temperature during thawing of cod loins. The temperature was monitored in the centre of four products from T2 and T3 group during thawing.

As described in the review by Svendsen and co-workers (2022) both freezing and thawing have effects on the quality of the product because they influence chemical reactions and muscle degradation. For lean fish the most important degradation processes are due to denaturation of proteins which can result in tough and dry texture. Decreased water-holding capacity and liquid losses upon thawing are also effects that can be induced due to freezing and frozen storage and is dependent on the formation of ice crystals.

In the current experiment, several factors, may have affected the fish quality, and thereby diminishing the effect of the different processing treatments. The different fishing grounds, and season from which the fish were selected, and additionally the onboard handling of the fish may have affected the results. The raw material for T1 and T2 was four days old at time of processing, while the raw material for T3 was frozen at the boat upon catch. Further, the raw material to T3 was caught in January while the fish used in T1 and T2 was caught in October. In addition, the packaging method differed between T3 and T1 and T2.

The samples for experimental group T3 were double frozen, first as whole fish blocks which was thawed in circulating water prior to processing and a subsequent second freezing in consumer packs. Thus, the product had a second stage of thawing in the laboratory before the final product was tested in the shelf life tests.

Table 2: Quality of chilled and frozen-thawed cod loins. Parameters were examined at day 0 and after storage for 6, 10 and 14 days.

Quality parameter	Storage time (days)	T1	T2	T3
Drip loss (%)	0	na	4.4 ± 1.6^{A}	12.0 ± 1.4^{A}
(n=10)	6	2.8 ± 1.2^{A}	5.2 ± 2.2^{AB}	13.8 ± 1.6^{AB}
	10	2.9 ± 0.9^{A}	5.7 ± 1.1^{AB}	12.0 ± 1.5^{AB}
	14	3.1 ± 0.9^{A}	4.8 ± 1.1^{AB}	12.1 ± 1.8^{AB}
WHC (%)	0	92.3 ± 2.3^{Aa}	86.7 ± 1.2^{AB}	65.6 ± 2.2^{ABa}
(n=4)	6	95.8 ± 2.0^{A}	86.3 ± 2.8^{AB}	67.1 ± 4.6^{AB}
	10	97.3 ± 2.3^{Aa}	95.9 ± 1.5^{B}	68.9 ± 1.6^{AB}
	14	95.0 ± 2.4^{A}	90.0 ± 3.7	78.8 ± 8.3^{Aa}
Sensory score (0 - 18)	0	3.9 ± 1.5^{a}	3.1 ± 1.1^{Aa}	4.8 ± 1.5^{A}
(n=12)	6	7.6 ± 1.6^{ABa}	5.0 ± 1.7^{Aa}	4.4 ± 1.0^{ABa}
	10	8.1 ± 2.1^{Aa}	6.4 ± 2.1^{a}	5.5 ± 1.8^{A}
	14	6.8 ± 1.5^{a}	6.6 ± 1.6^{a}	6.2 ± 1.3^{a}
Colour sensory score	0	0.5 ± 0.3^{Aa}	0.2 ± 0.3^{ABab}	1.0 ± 0.0^{ABa}
Brightness (0-4)	6	0.8 ± 0.2^{Ab}	0.8 ± 0.4^{Bab}	1.1 ± 0.2^{AB}
(n=12)	10	1.2 ± 0.5^{ab}	1.1 ± 0.5^{a}	1.0 ± 0.1^{b}
	14	1.3 ± 0.3^{ab}	1.4 ± 0.4^{ab}	1.2 ± 0.2^{ab}
Hardness (g)	0	311 ± 92^{a}	233 ± 85^{a}	317 ± 105
(n=12)	6	491 ± 174^{Aa}	307 ± 70^{A}	358 ± 102^{A}
	10	422 ± 139	359 ± 120^{a}	416 ± 191
	14	495 ± 102^{Aa}	379 ± 122^{a}	356 ± 168^{A}
TVC (log cfu/g)	0	4.4 ± 0.1^{a}	4.4 ± 0.4^{Aa}	3.8 ± 0.1^{Aa}
	6	4.8 ± 0.1^{Ab}	5.7 ± 0.4^{ABa}	5.1 ± 0.2^{Bb}
	10	4.8 ± 0.2^{Ac}	5.3 ± 0.4^{B}	6.8 ± 0.4^{ABc}
	14	5.6 ± 0.2^{Aabc}	5.6 ± 0.4^{Ba}	8.2 ± 0.3^{ABabc}

Each parameter is expressed as a mean and standard deviation (SD). Comparisons were made between the different treatment groups. Treatments sharing a capital letter (A, B, C) indicate significant differences between the treatment groups at the day of sampling. Small letters (a, b, c) indicate significant differences between storage times for the same treatment group. n.a.= not available (i.e not measured)

The ability of the fish muscle to retain its natural water and thereby its juiciness is one of the quality criteria of fish. The drip loss during thawing and chilled storage are shown in Table 2. At day 0, significant differences were found between the two raw materials ($p<0.01$), with respect to thawing loss, where the loss was less for the T2 group (4.4±1.6%) compared to the T3 group (12.0±1.4%). Chilled samples had least drip loss at all sampling points during the storage period of 14 days ($p<0.05$). In contrary to other studies, who have shown that the drip loss significantly increases as a function of time (Bøknæs et al., 2001; Jensen et al., 2010; Kristoffersen et al., 2007), only a slight trend was observed in this study. Most of the drip loss occurred during the first six to ten days for the freeze-chilled samples. The results agree with other studies showing that maximum drip loss for fresh cod was observed after 10 days of chilled storage (Aune et al., 2014; Kristoffersen et al, 2007; Jensen et al, 2010).

The water content of the cod fillets ranged from 80.5% (T3) to 82.6 % (T1), with no significant difference between the experimental groups and during storage time. These findings indicate that no water uptake occurred during the water thawing where the fish was in direct contact with the thawing medium.

The different processing treatments affected the water holding capacity. Treatment group T3 (based on frozen raw material) had lower WHC compared to the treatment groups based on fresh raw material (T1 and T2) ($p<0.05$). The WHC was affected throughout the storage period and processing treatment. The WHC of samples in T1 and T3 increased with time, which was the opposite of what would be expected. The T1 and

T2 groups had probably lost most of its loosely bound water during the iced storage before packaging, with mainly the intracellular water remaining. Alternatively, it could be linked to protein denaturation, cross-linking and textural changes.

The total quality index score is presented in Table 2. As expected, the total score increased during storage for all experimental groups with a maximum score on day ten (T1) and 14 (T2 and T3). T1 samples received higher total quality score (indicating lower quality) than T2 and T3 after six days of chilled storage ($p<0.001$). At sampling day ten, T3 still received higher acceptability than T1 ($P<0.05$). These observations do not support previous studies on freeze-chilling on whiting and grass carp where a higher acceptability of fresh fish was observed (Fagan et al., 2004, 2003; Yin et al., 2014).

Development of frozen storage odour was most pronounced for T2 with five days of iced storage prior to freezing *(data not presented)* compared to T3 which was frozen on the boat upon catch. After six days of chilled storage in vacuum bags, samples made from fresh raw material (T1 and T2) had higher scores (indicating lower quality) for odour compared to samples based on frozen raw material (T3).

The results from the sensorial evaluation of surface colour are shown in Table 2. Loins from experimental groups T1 and T2 were brighter ($p<0.05$) than the loins from treatment group T3 both at sampling day 0 and sampling day 6. The colour tended to be more matte with storage time, however it was not significant for all treatment groups. There were no significant differences in redness or blood spots between the experimental groups and during storage time. Colour is one of the major attributes, which affect the consumers perception of quality (Francis, 1995), and it is generally accepted that whiteness is positive, and redness is negative when evaluating cod loins. Yellow discoloration can occur if the fish have been incorrectly frozen and thawed (Archer et al., 2008).

The texture of fish is an important contributor to palatability, and thus influences consumer perceptions. In this study, hardness was measured with a probe imitating a finger. Different treatments had effects on textural characteristics (Table 2). However, the differences in hardness were only significant at the sampling point after six days of chilled storage ($p<0.05$) between T1 and T2 and T1 and T3 and at day 14 between T1 and T3. The standard deviation in the hardness measurements is large and are related to the shape and size of the loins. Even though the refreshed loins (T2) seemed to have a softer texture, at day 0 just after thawing, compared to the fresh loins (T1), the difference was not significant ($P>0.05$). Surprisingly, hardness values for T1 and T2 showed a significant increase with storage time ($p<0.05$). The results are inconsistent with other studies on freeze-chilled fish (Yin et al., 2014), reporting a decrease in hardness with storage time. The toughening of fish during freezing is well documented (IIR, 1986). Freezing and frozen storage are associated with protein denaturation and aggregation (Mackie, 1993). These reactions may cause the changes in texture observed for the freeze-chilled samples.

The results of total viable counts (TVC) are presented in Table 2. The count represents the number of colony forming units (cfu) per g (or per ml) of the sample. As expected, TVC increased during chilled storage ($p<0.05$). Several parameters such as initial microbial quality, time before packaging, processing treatment, packaging method and material as well as the temperature conditions during storage, will impact on the microbial growth (Bøknæs et al., 2000, 2001, 2002; Magnússon and Martinsdóttir, 1995). In this study, T3 had the highest values of TVC at the end of the storage period. However, at sampling day 0, both T1 and T2 group had higher viable counts compared with the T3 group. Water thawing is frequently applied in the seafood industry to thaw fish blocks (Archer et al., 2008). During thawing, fish may be exposed to human contact, or other sources of microbial contamination. In addition, the fish is in direct contact with the thawing medium, which can increase the risk of cross contamination. The raw material for T3 was thawed in water prior to further filleting and packaging and this may explain the higher TVC-values during storage in this group.

High viable counts of fish are often related to an increased number of SSOs (specific spoilage organisms), causing off-flavours associated with seafood spoilage (Gram and Dalgaard, 2002; Gram and Huss, 1996). It is well known that processing- and packaging parameters can cause changes in the development and

composition of the spoilage bacteria and different types of spoilage (Gram and Huss, 1996). In this study, compliance between the odour scores and the TVC-values was not observed. The odour scores of fish from treatment T3, was better throughout the storage period compared to T1 and T2 (data not shown). The results indicate that even for the same type of product, off-odours may develop differently, depending on process treatment and other unknown factors interacting with the microbial development.

As a former recommendation, TVC should not exceed $5.0x10^5$ CFU/g in raw fish fillets, chilled or frozen (Tobiassen et al., 2006). According to the proposed limits, all treatments kept relatively good quality during short term storage of six days. However, based on these limits a longer storage period after thawing is not recommended. The results agree with a study by Roiha and coworkers (2018) showing a shelf-life of at least six days of thawed cod. As mentioned, the shelf life of freeze-chilled fish will vary depending on the microorganisms present in the fish at the time of freezing, the storage time and temperature, and processing conditions (DeWitt and Oliveira, 2016; Duarte et al., 2020). Moreover, packaging method will also influence the shelf life of the final product (Duarte et al., 2020). MAP in combination with freeze-chilling seems to be an important strategy to increase the shelf life of fish (Fagan et al., 2004).

3. CONCLUSIONS

Freeze-chilled samples made from frozen raw material showed higher sensory scores, but lower water holding capacity, higher drip loss and higher values for TVC than the chilled samples. Considering all parameters, including the evaluation of sensory and microbial quality, a shelf life of the freeze-chilled loins during chilled storage between 6 and 10 days, is obtainable if the raw material are frozen immediate upon catch. The present study clearly showed the need for fresh raw material when producing thawed chilled cod loins packed in vacuum. 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.

This study was performed in the industry and gives a brief overview about how industrial methods for freezing and thawing affect some quality parameters of raw cod loins. However, the effects of other freezing and thawing rates or production processes should be investigated in addition to different storage temperatures after thawing. Factors of significance for the product and the how the consumer perceive the quality after cooking is also a topic for further research.

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