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Liquid loss in thawed cod—Deconvoluting the effects of freezing-rate, freezing cycles, frozen storage time, and thawing-rate through a full factorial design

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Abstract

Fish is a highly perishable product and freezing is the obvious way to increase shelf life. The freezing process involves the initial freezing, a frozen storage period and thawing—all of which influence the quality of the end product. In this study, the quantity of liquid loss is used as an indication of the structural damage induced by these processes. A full factorial experiment design addresses the effects of freezing and thawing rates (fast vs. slow), number of freezing cycles (1 vs. 2) and frozen storage (1 year vs. 1 week). The results show strong evidence that fast processes of freezing and thawing reduce the subsequent liquid loss. However, 1 year frozen storage at −20°C induces high liquid loss independently of the freezing and thawing rates. Adding a second freezing cycle also adds to the liquid loss upon final thawing. By increasing the number of processing steps (additional freezing cycles) the strain put upon the samples progressively increases. This leaves samples at the end of long sequences of processing especially sensitive to damage caused by ice crystals. In this way, the thawing protocol might be of high importance, especially during the last freezing cycle of twice frozen samples.

Practical Application

In order to minimize liquid loss through production of frozen seafood, it is imperative that producers use the best practice at every stage. Liquid loss influences yield through production, but it also contributes to quality parameters relevant to both producers and consumers. Every stage of the production impacts liquid loss, and examining and classifying the different processing steps separately is the common approach to assess the effect. For the final product however, the impact of one isolated step is entangled in the combined effect off all the different processing steps. In this study, we have examined the processing continuum from the first freezing to the last thawing, including the effects of an extra freeze cycle and frozen storage period. In this way, we demonstrate the risk of pitfalls through such processing and also highlight the process combinations that are synonymous with low liquid loss.

1 | INTRODUCTION

The bulk part of the Norwegian of Atlantic cod (*Gadus morhua*) landings takes place during a 4-month period (Fiskeridirektoratet, 2018). This poses a major challenge with respect to western markets' demand for an all year delivery capacity. Furthermore, in the fresh state, cod fillets have a limited shelf life of 7 to 10 days (Bonilla, Sveinsdottir, & Martinsdottir, 2007). Freezing is, and has for a long time been a way for the seafood industry to increase shelf life of fish. But frozen fish products are often perceived by consumers as inferior to fresh ones (Peavey, Work, & Riley, 1994). This might limit the market potential of this product category. Since the freezing and thawing process will to some extent reduce the quality of the fish, a focus on raw material quality is imperative to elevate consumer's perception of frozen seafood.

To maintain the high quality of the fish produced during frozen storage it is crucial to keep the storage temperature stable and low (Burgaard, 2010; Leblanc, Leblanc, & Blum, 1988). The main mechanism of quality deterioration for frozen fish is related to ice crystal formation, which leads to cell rupture within the muscle. Protein denaturation during frozen storage can also be an issue, particularly for cod and other species that contain TMAO (trimethylamine oxide) (Shenouda, 1980; Sikorski, Olley, & Kortuch, 1976). For fatty fish exposed to oxygen, lipid oxidation will occur (Saeed & Howell, 2002; Undeland & Lingnert, 1999; Vazquez, Torres, Gallardo, Saraiva, & Aubourg, 2013). For cod and other lean species, however, the deterioration through frozen storage is closely linked to liquid loss.

There are few systematic studies available on the subject of thawing cod. Through the thawing process the cod muscle is kept in the temperature zone just below zero where phase transition occurs. This transition is similar to the phase transition during freezing, albeit in reverse direction. As heat conduction is much higher in solid water (ice) than in liquid water, the latent period of thawing (phase shift) is much longer than the latent period of freezing (Karel & Lund, 2003). Because crystals are formed during freezing, and recrystallization occurs during thawing, time spent in the latent zone can have detrimental effects on products. Despite such theoretical evidence in support of rapid thawing, what is generally recommended, and has been used in most scientific studies of frozen fish, is thawing the product overnight at refrigerated temperatures (Bøknæs, Jensen, Andersen, & Martens, 2002; Leduc et al., 2012; Sveinsdottir, Martinsdottir, Hyldig, & Sigurgisladottir, 2010).

The first important step in ensuring good quality is freezing the product as soon as possible after catch. For most fisheries this implies on-board freezing, something which limits processing capacity. Secondary processing of cod has been carried out in Asian countries for a number of years. Here, semi-thawed fish is manually processed and refrozen prior to distribution to western markets. Double freezing is thus already a common practice, even if the extended frozen storage time during transportation may affect the shelf life and the quality of the frozen cod product.

The key quality parameter for lean white fish species, liquid loss, affects both yield and sensory properties, and high amounts of exudates in product packages is known to have a negative impact on sales. Fish muscle normally expels liquid during chilled storage, and usually there is a correlation between the level of expelled liquid and the storage time of the product. Fish that has been previously frozen experiences higher liquid loss than fish kept fresh, and liquid expelled during thawing is often referred to as thawing loss. The liquid lost under thawing is a result of the way ice crystals have damaged the muscle by disrupting cells and tissues during freezing, frozen storage or thawing. The formation of large ice crystals is particularly damaging, and this process is more apparent during slow freezing than faster freezing (Sanz et al., 1999). Ice crystals may also grow in size during frozen storage, especially under fluctuating and/or elevated temperatures (Martino & Zaritzky, 1988). From an industrial point of view, any liquid loss during processing results in a corresponding loss of profit. For the consumer, liquid loss can be associated with a drier, less tender and less delicate product. Thus, monitoring the liquid loss reveals much about the quality of fish that has been frozen. Although liquid loss does not capture every aspect of quality, when measured directly after thawing, it provides a representative snapshot of the effect of ice crystals formation during the freezing process.

Hence, in this study we regard liquid loss as a quantitative indicator of the detrimental effects caused by ice crystals that are formed through freezing, frozen storage and thawing. Not to confuse the response parameter (loss of liquid) with the process ultimately leading up to this measurement (thawing), the expelled liquid is hereafter referred to as liquid loss rather than thawing loss. A full factorial experiment design addressed the effects of processing rates (fast vs. slow), number of freezing cycles (1 vs 2) and frozen storage (1 year vs. 1 week). The design allowed us to assess the influence of each processing procedure independently, or linked to a broader context. The samples were vacuum packed to limit non-temperature effects (freezer burn, sublimation, and oxidation) during the freezing process, and to better control the collection of liquid loss. Similarly, in order to limit typical chilled storage effects (from enzymes and bacteria), sampling was done immediately after thawing. This generated a comprehensive overview of the impact of ice crystals through the different stages of the freezing process.

2 | MATERIALS AND METHODS

2.1 | Raw material

Wild caught Atlantic cod, live stored for a year, were provided by Tromsø Aquaculture Research Station, Norway. The fish was harvested in late June; a couple of months after the spawning period. The main cod harvest season in Norway is the spawning period, and during this period the fish lose weight through starvation and loss of gonad mass (Black & Love, 1986). In the period after spawning the condition factor of the fish is relatively low (Mello & Rose, 2005) and the water-holding capacity of the fish muscle is reduced. The fish used in this study was still in a state of restitution, which means it might be more affected by freezing and thawing than it would have been prior

to spawning. Individual variation in fish condition tend to increase after spawning. This can make it more demanding to obtain statistical significance between groups for post-spawning cod. On the other hand, the post-spawning cod is more sensitive to the effects of processing and this more volatile state may boost the liquid loss. The individual variation in a cod population, is usually quite high, also for pre-spawning cod. Therefore, when the sample size (n) is low, the use of material with a high individual variation will to a larger degree display the outer boundaries of the sample population than material with low variation.

The fish (7.57 ± 1.87 kg, headed and gutted) were killed by a blow to the head and immediately gutted. It was bled for 30 min and kept on ice for 3 days for the rigor process to cease before filleting. After filleting, the back loin was separated from the fillet and used for the experiments. Each loin was cut in two or three portion sized samples (178.8 ± 30.1 g), depending on the size of the loin. The samples were vacuum-packed (99%) in sous vide plastic pouches (20 μm polyamide inside layer and 70 μm polyethylene outside layer, O_2 permeability: 45 $cm^3/[m^2\ d\ bar]^{-1}$) and kept on ice prior to freezing.

2.2 | Experiment design

The sample portions were subjected to fast (circulating air [3 m/s] at −40°C), which is compatible with an industrial blast freezing process. The slow freezing (still air at −20°C) is equivalent to freezing in a frozen storage facility with no air circulation or a domestic freezer. The temperature profiles for both fast and slow freezing regimes are shown in Figure 1. The samples that were thawed fast was placed in circulating water (~ 0.1 m/s at 4°C), something which resembles industrial or domestic thawing in circulating cold tap water. Slow air thawing with little circulation (~ 0.1 m/s at 4°C) corresponds to thawing in a refrigerator or a cold temperature controlled room. The progress of both fast and slow thawing is shown in Figure 2. All permutations of fast and slow freezing and fast and slow thawing were done in full factorial designs featuring both one freezing cycle (four groups) and two freezing cycles (16 groups). The experiment design for one and two freezing cycles is shown in Figure 3. Fish samples to be frozen twice were thawed 1 week after the first freezing cycle and then kept at 4°C for 12 hr before refreezing. The fish frozen slowly at −20°C were kept at this temperature until thawing. Frozen storage was done at −20°C for all groups. The fish frozen fast at −40°C were stored at −20°C for 24 hr prior to thawing, assuring that all groups had similar temperature prior to thawing. In addition to the two experiments described above, two parallel experiments were carried out in which the fish was kept 1 year in frozen storage at −20°C before the last thawing (Figure 3).

2.3 | Liquid loss

Liquid loss was collected directly from the vacuum packages after thawing. Liquid loss (LL, %) was determined according to the formula.

$$LL = [(m_0 - m_S)/m_0] \times 100\%$$

where m_0 is the initial weight of the loin, and m_S is the weight of the samples after thawing, when expelled liquid is removed.

2.4 | Statistics

Analysis of variance (general linear model) and student's *t* test were performed with Minitab 18 (Minitab, Coventry, UK). The general linear model was performed using Tukey's honestly significantly difference test at a *p* value of <.05 to obtain confidence intervals for all differences between level means. All results are given as the mean ±*SD* unless otherwise stated.

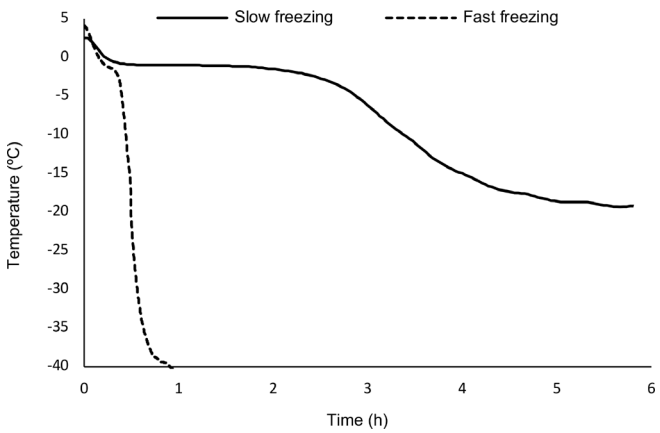

FIGURE 1 Core temperature of 30 mm thick cod muscle sample. For fast freezing air blast at −40°C was used. For slow freezing still air at −20°C was used

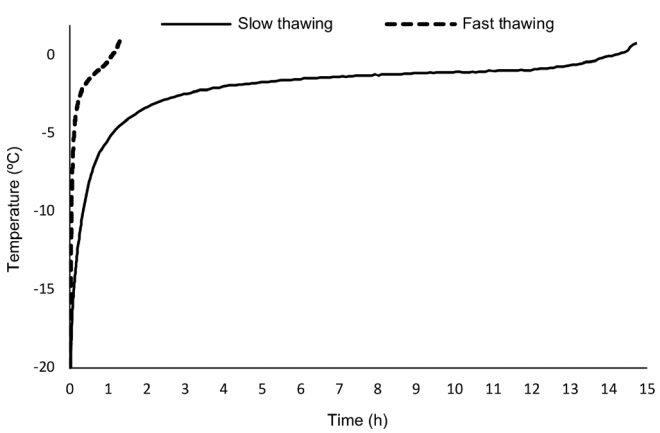

FIGURE 2 Core temperature of 30 mm thick cod muscle sample. For fast thawing circulating water at 4°C was used. For slow thawing air at 4°C with little circulation was used

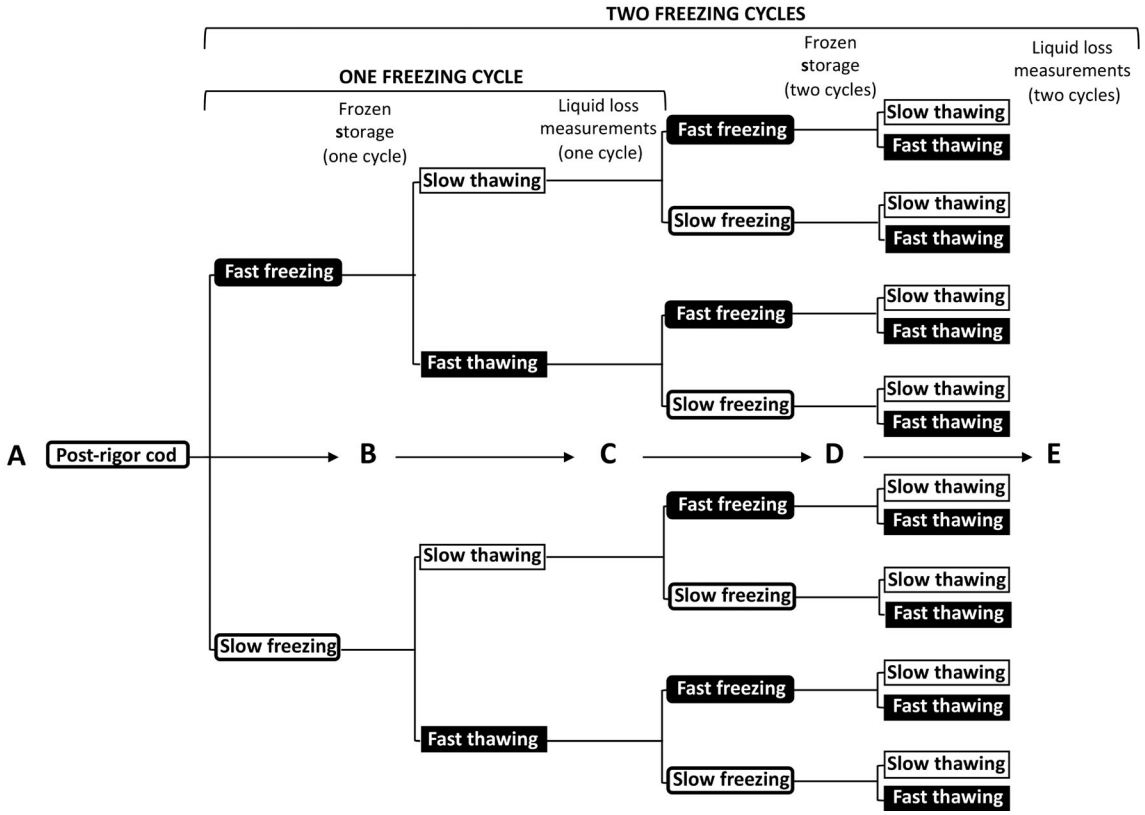

FIGURE 3 Schematic illustration of the full factorial design. Liquid loss measurement after one and two freezing cycles was done after the final thawing, at Stages C and E, respectively. In the first cycle, samples were kept frozen for a week or a year (Stage B) until thawing. The samples entering a second freezing cycle were stored for 12 hr at 4°C (Stage C) prior to refreezing. Similar to the one freezing cycle regime, samples were stored frozen for a week or a year (Stage D) until thawing

3 | RESULTS AND DISCUSSION

3.1 | One freezing cycle

Both the freezing and the thawing rate affect the liquid loss following the final thawing. Fast processes lead to shorter phase transition times, and therefore, less cell damage and subsequent liquid loss. A combination of fast freezing and fast thawing produced less than half the liquid loss than did the combination of slow processes (Figure 4). The vacuum packaging itself induced, on average (n = 10), a 2% liquid loss. This was measured as the package was opened and the liquid collected prior to freezing. The packaging-induced liquid loss was not subtracted from the subsequent liquid loss measurements. Thus, the difference between slow and fast rates were in reality greater than what the numbers suggest. In fact, if the average packaging loss (2%) is subtracted from all the samples, the slow processes would lead to a 220% increase in liquid loss (as opposed to 120% if this loss is included, as seen in Figure 4). However, keeping an eye to the product category as well as the processing, the packaging-induced drip loss is a realistic part of the product. For that reason packaging loss is included in the thawing loss data.

A closer look at the independent process of freezing and thawing, reveals that liquid loss is more influenced by the freezing rate than

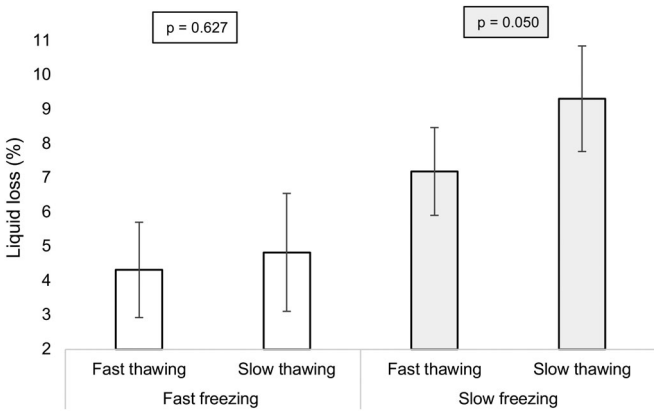

FIGURE 4 Liquid loss for samples after one freezing cycle. Samples were initially frozen fast (white bars) or slow (gray bars) and were frozen stored for 1 week at −20°C before thawing (fast or slow). t-test results (p-value) are shown in boxes between the two groups that were compared

the thawing rate (Figure 5). Our data show a very significant effect of fast freezing, and trading fast with slow freezing would increase the liquid loss from 4.6 to 8.4% (p < .001). A similar, but weaker trend was observed between fast and slow thawing, showing 5.8 versus 7.1%

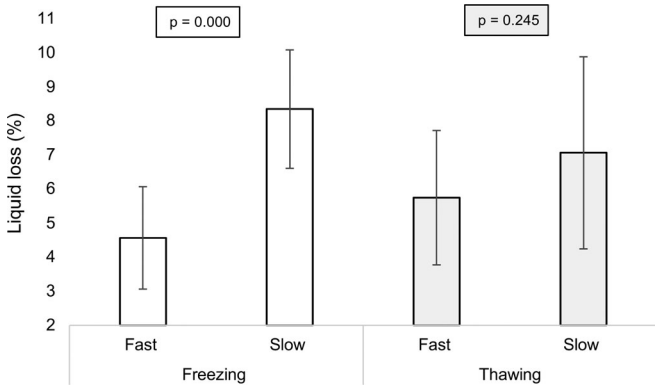

FIGURE 5 A comparison showing how the freezing rate (white bars) and thawing rate (gray bars) independently influence the liquid loss for samples treated with one freezing cycle and 1 week frozen storage at −20°C. *t*-test results (*p*-value) are shown in boxes between the two groups that were compared

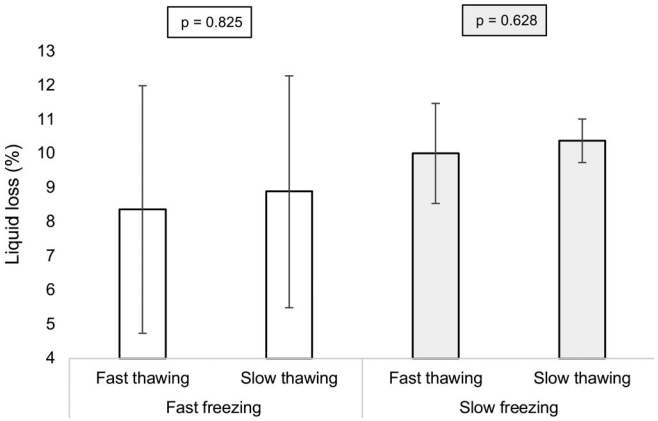

FIGURE 6 Liquid loss for samples after one freezing cycle. Samples were initially frozen fast (white bars) or slow (gray bars) and were frozen stored for a year at −20°C before thawing (fast or slow). *t*-test results (*p*-value) are shown in boxes between the two groups that were compared

liquid loss, respectively ($p < .07$). The freezing process seems to be of the greatest importance, and to a large extent it is the nature of the ice crystals that induce liquid loss. The damage that ice crystals do during the freeing process is presented in a recent study of frozen cod by the use of magnetic resonance imaging (MRI) analysis (Anderssen, Syed, & Stormo, 2020). This method enables an in-depth mapping of tissue damage and clearly links pronounced liquid loss and structural damage, visible through MRI, with slower freezing rates. Fast freezing produce many small ice crystals while slow freezing give rise to fewer but much bigger crystals (Ottestad, Enersen, & Wold, 2011). Our results in this study confirms this since most of the ice crystal formation takes place during the freezing process. Recrystallizations do also occur during thawing, and may, much like crystallizations during onset of freezing, recrystallizations also lead to structural damage (Cao et al., 2019). This process is manifested through a longer (slower) thawing process, which leads to increased liquid loss (Figure 5). However, when recrystallizations appear during thawing, they do so do in surroundings that, from a thermodynamical perspective, progressively favors the liquid state. Accordingly, the detrimental effects of slow thawing are less evident than those of slow freezing.

3.2 | One freezing cycle + frozen storage

One year of frozen storage at −20°C increased the liquid loss for all groups, but relatively more so for the fast frozen groups (Figure 6). This is because fast freezing, before frozen storage, showed the least liquid loss. Frozen storage for a year at −20°C seems to have an equalizing effect on liquid loss. Consequently, from a statistical point of view, the freezing- and thawing rate did not play a significant role after 1 year of frozen storage. Still, the impact of freezing rate and thawing rate, when compared independently (Figure 7), showed a trend where fast processes result in lower liquid loss than slow processes. This effect is much more pronounced for samples that had one only week of frozen storage (Figure 5), which strongly suggests that temperatures of −20°C are inadequate for long term frozen

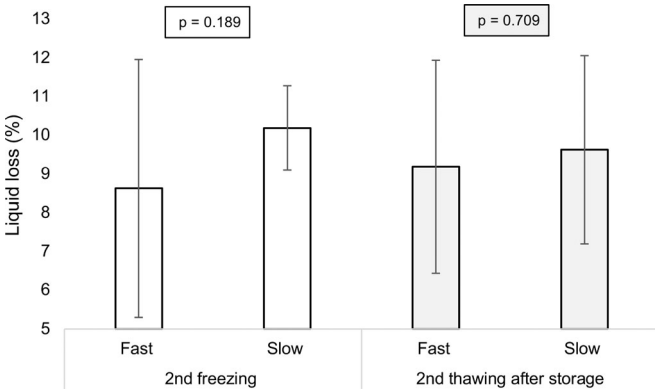

FIGURE 7 A comparison showing how the freezing rate (white bars) and thawing rate (gray bars) independently influence the liquid loss for samples treated with one freezing cycle and a year frozen storage at −20°C. *t*-test results (*p*-value) are shown in boxes between the two groups that were compared

storage of cod fillets. At this temperature the relatively high percentage (11%) of unfrozen water in the muscle (Hedges & Nielsen, 2000) may induce localized thawing and subsequent recrystallizations throughout the storage period. As shown here, the effects of recrystallizations, even at a very low rate, become noticeable over time. One should thus question the appropriateness of the industry norm of a deep-freezing temperature set at −18°C. The pooled data show that the freezing rate and the following frozen storage time significantly (both $p < .001$) influence the liquid loss (Figure 8). The thawing regime's lesser impact on liquid loss compared with the freezing regime may have a straightforward explanation. Structure damage due to penetrating ice crystals, both owing to their size and number, will to a large extent account for the ensuing liquid loss. Clearly, the freezing process is the starting point from where the ice crystals come into existence. It is also this process that determines the initial characteristics of the ice crystals (their size and number). Even if crystals are

transformed during frozen storage and recrystallized during thawing, the freezing process will, by virtue of being the primary step, govern the succeeding steps. In other words, the detrimental effects of poor freezing cannot be undone by optimal thawing.

3.3 | Double freezing cycle

A double freezing scheme involves two cycles of freezing and thawing. Double freezing is a commonly used practice for cod and other white fish species. The fish is typically frozen on-board followed by a frozen storage period until thawing, processing, and refreezing before distribution. Some studies are available on this topic, and they all conclude that double freezing can bring about good product for lean fish (Maccallum, Laishley, Dyer, & Idler, 1966; Schubring, 2001, 2002) and other fish species as well (Botta & Shaw, 1978; Schubring, 2010). This section mainly deals with the effects of the second freezing and the second thawing. The effects of the first cycle of freezing and thawing is already documented in preceding sections. Therefore, the effects of the first cycle will be limited to interaction effects between the first and second freezing cycle.

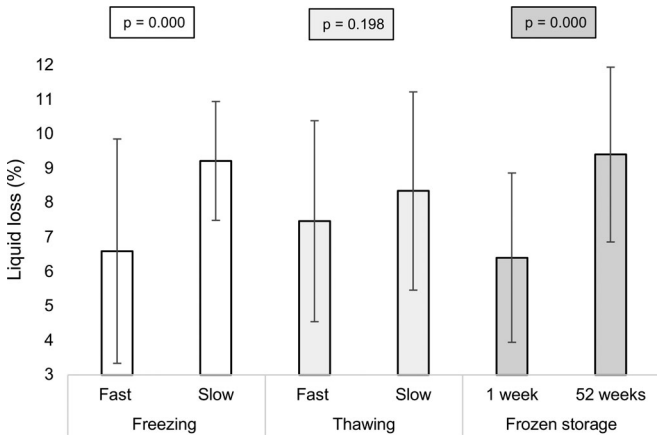

FIGURE 8 The main effects of one freezing cycle, taking into account freezing rate (white bars), thawing rate (gray bars) and the effect of frozen storage (dark gray bars)

All permutations of freezing and thawing for a double cycle (16 groups) are shown in Table 1, along with the resulting liquid loss. For the samples that were frozen stored for 1 week, only two (Groups 1 and 5) out of the 16 groups had a relative low liquid loss. These two groups shared the process combination of fast second freezing and fast final thawing. They also highlight an obvious interaction effect; that that the combination of fast first freezing and fast second freezing results in the lowest liquid loss (p = .037). When looking at the two processes independently, the second freezing and the second thawing, their rates (fast or slow) influenced the liquid loss (Figure 9). Compared to the once frozen samples the resulting liquid loss after two cycles is higher, obviously, because an extra cycle of freezing and thawing induces additional liquid loss. The pattern that a fast process will result in lower liquid loss than a slow process is analogous between once and twice frozen samples. A closer look shows that liquid loss cannot be low for double frozen samples unless the final thawing rate is fast. For the instances in which the final thawing is slow, the resulting liquid loss is typically higher than in the groups where the final thawing is fast. This is

TABLE 1 The liquid loss for all the groups treated with two freezing cycles

Group	First freezing	First thawing	Second freezing	Final thawing	Driploss (% ± *SD*)	Driploss* (% ± *SD*)
1	FAST	FAST	FAST	FAST	3,75 ± 0,82^{a}	9,41 ± 2,08^{ab}
2	FAST	FAST	FAST	SLOW	8,19 ± 3,81^{abc}	9,24 ± 1,28^{a}
3	FAST	FAST	SLOW	FAST	9,00 ± 2,06^{bc}	9,90 ± 2,33^{ab}
4	FAST	FAST	SLOW	SLOW	9,95 ± 3,43^{c}	10,77 ± 2,80^{ab}
5	FAST	SLOW	FAST	FAST	4,47 ± 0,84^{a}	12,18 ± 2,24^{ab}
6	FAST	SLOW	FAST	SLOW	10,23 ± 3,33^{c}	11,96 ± 1,99^{ab}
7	FAST	SLOW	SLOW	FAST	10,99 ± 1,58^{c}	11,50 ± 2,90^{ab}
8	FAST	SLOW	SLOW	SLOW	10,55 ± 1,12^{c}	12,11 ± 3,57^{ab}
9	SLOW	FAST	FAST	FAST	6,86 ± 1,68^{abc}	12,80 ± 2,66^{ab}
10	SLOW	FAST	FAST	SLOW	9,90 ± 1,55^{c}	14,60 ± 1,34^{b}
11	SLOW	FAST	SLOW	FAST	9,88 ± 1,77^{c}	11,22 ± 2,28^{ab}
12	SLOW	FAST	SLOW	SLOW	11,16 ± 3,38^{c}	10,22 ± 2,97^{ab}
13	SLOW	SLOW	FAST	FAST	8,51 ± 0,66^{abc}	14,33 ± 2,41^{ab}
14	SLOW	SLOW	FAST	SLOW	10,85 ± 3,70^{c}	12,77 ± 0,74^{ab}
15	SLOW	SLOW	SLOW	FAST	9,50 ± 1,66^{bc}	11,15 ± 2,64^{ab}
16	SLOW	SLOW	SLOW	SLOW	10,66 ± 1,80^{c}	11,13 ± 1,93^{ab}

Note: For each group, significant differences are indicated with different letters.
*After 1 year storage at −20°C.

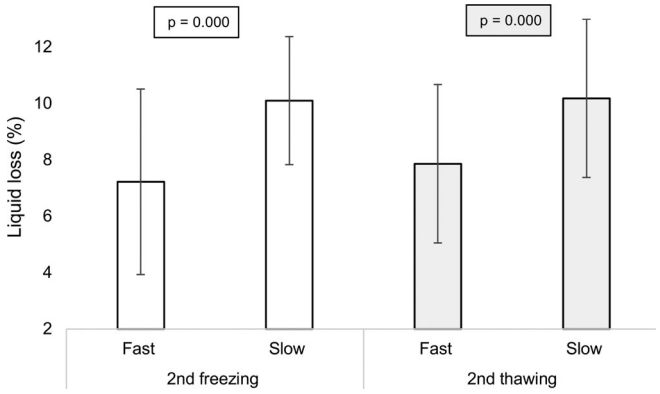

FIGURE 9 A comparison showing how the final freezing rate (white bars) and final thawing rate (gray bars) independently influence the liquid loss for samples treated with two freezing cycles and 1 week of frozen storage at −20°C. *t*-test results (*p*-value) are shown in boxes between the two groups that were compared

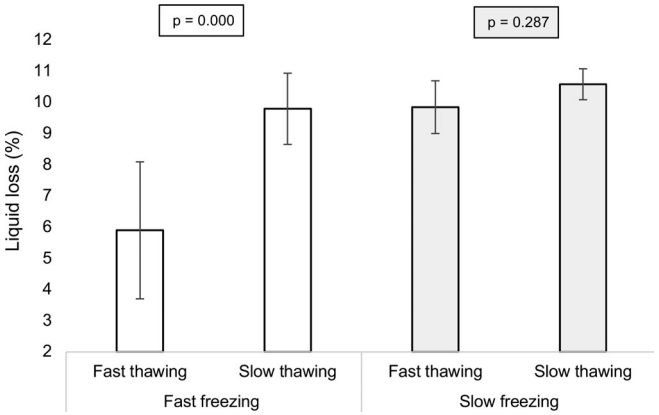

FIGURE 10 Liquid loss for samples treated with two freezing cycles and 1 week of frozen storage at −20°C. Here the focus is on how the thawing rate in the second cycle influence the liquid loss for samples that were frozen fast (white bars) or slow (gray bars) in the last cycle. *t*-test results (*p*-value) are shown in boxes between the two groups that were compared

clearly illustrated by the difference between Groups 1 and 2, which have identical processing steps until the final thawing, in which the liquid loss more than doubles when thawing is slow. It seems to be the rule that the last thawing determines the liquid loss. A similar trend was observed for the once frozen samples—that fast thawing results in less liquid loss than slow thawing. For the once frozen samples, the impact of the speed of the freezing process seems to be greater than the speed of the thawing process (Figure 5). For double frozen samples, on the other hand, the freezing rate does not appear to have a greater impact than the thawing rate (Figure 9). For instance, when perfect processing (all fast processes: Group 1, Table 1) is compared with almost perfect processing in which all the processes are fast except for the final thawing (Group 2, Table 1), the slow process at the end seems to have a devastating effect. To achieve a low liquid loss through double freezing, the process must not encompass several slow processes. One slow process, either freezing or thawing, have less damaging effects when it happens during the first cycle, as exemplified by Groups 5 and 9 (Table 1).

This does not necessarily mean that the final thawing is more crucial than the final freezing for double frozen samples. Reducing the liquid loss for double frozen samples is, as already mentioned, also closely linked to a final fast freezing process. The impact of varying the final thawing rate is only significant for samples that underwent a fast final freezing (Figure 10). This resonates with the observed interaction effect between the second freezing and second thawing (p = .003). In addition, when a slow process occurs in the first cycle and is followed by an optimal second cycle (fast freezing and fast thawing), liquid loss is higher for the group that underwent slow first freezing (i.e., Group 9, Table 1) compared with the group that underwent slow thawing (i.e., Group 5, Table 1). This suggests that the freezing process influences the liquid loss to a higher degree than the thawing process. Still, because freezing and thawing occur at different stages sequentially—first freezing and then thawing—it is difficult to compare the impact of the two. The data in this study show that processes that happen toward the end of the processing have greater impact than processes at an earlier stage. For double frozen samples, the last freezing is more detrimental than the first. An equivalent effect is observed for thawing. It is possible that the last thawing might be particularly decisive because it is the last process (out of four) in the entire processing sequence.

3.4 | Two freezing cycles + frozen storage

Double frozen samples stored 1 year at −20°C lost more liquid after thawing than samples frozen stored for 1 week. This is similar to the once frozen samples. Even if the liquid loss after a year of storage was overall quite high, an interaction effect between the first and second freezing was observed (p = .005). The positive effect of two successive fast freezing processes is retained also after 1 year storage. However, a slow freezing process leading up to frozen storage produces less liquid loss after thawing (Figure 11). This is in contrast to the once frozen and subsequently stored samples (Figure 7). In fact, this is the only instance where a slow process seems favorable. This result seems inexplicable, especially as a fast freezing seems to limit the liquid loss for all other instances. The main effects of the pooled data (second freezing, frozen storage, and final thawing) show that frozen storage and the following thawing most significantly (both p < .002) influence the liquid loss (Figure 12). This diverges from the results for the once frozen samples prior to storage. Here the freezing rate had greater impact than the thawing rate (Figure 7). It is reasonable to assume that the somewhat unexpected observation of how slow freezing reduces liquid loss for double frozen and stored samples will influence the statistics of the pooled data.

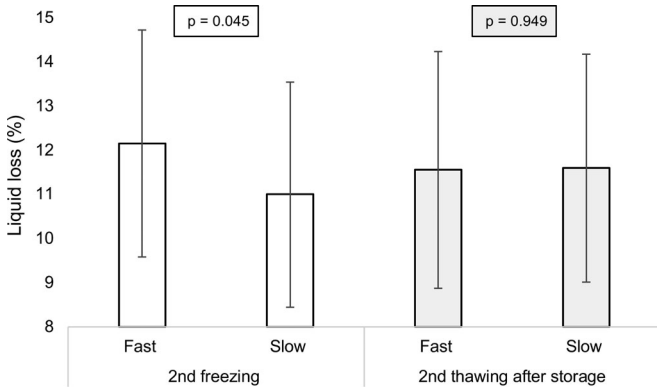

FIGURE 11 A comparison showing how the final freezing rate (white bars) and final thawing rate (gray bars) independently influence the liquid loss for samples treated with two freezing cycles and 1 year of frozen storage at −20°C. *t*-test results (*p*-value) are shown in boxes between the two groups that were compared

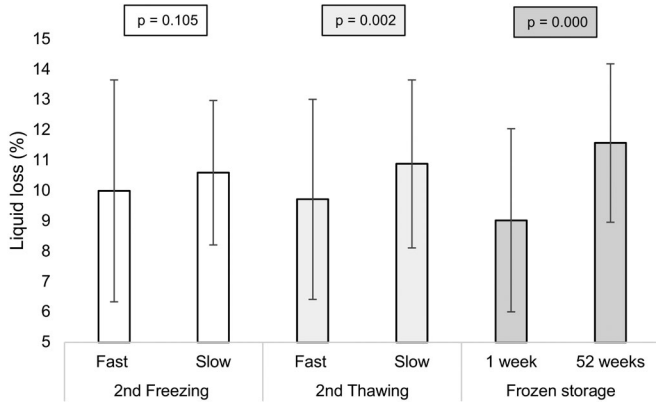

FIGURE 12 The main effects of two freezing cycles, showing the effect of the second freezing rate (white bars), the second thawing rate (gray bars) and the frozen storage (dark gray bars). *t*-test results (*p*-value) are shown in boxes between the two groups that were compared

4 | CONCLUSION

In this study, we present the results of four full factorial design experiments detailing the impact of freezing rate, thawing rate, freezing cycles, and frozen storage on liquid loss for cod. For once frozen samples there is strong evidence that fast processes of freezing and thawing reduce the subsequent liquid loss, and that the freezing process is the most critical of the two. One year frozen storage of cod at −20°C induces high liquid loss independently of the freezing and thawing rates. This should concern the seafood industry and distribution chains, with their widespread use of similar storage temperatures. Fast freezing and thawing reduces liquid loss also for fish frozen twice, and the liquid loss increases with an increasing number of processing steps. As a consequence, the detrimental effects of multiple processing steps increase toward the last step (final thawing). This means that despite the undisputable importance of ice crystal formation through the first part of the process, the freezing stage, the following recrystallizations that might occur during succeeding steps, such as frozen storage and thawing, might also have a significant effect. Such recrystallizations seem to exert a negative effect, especially during the last freezing cycle of twice frozen samples, and especially for final thawing that occurs as the last in a long sequence of processes. The pronounced impact of the final thawing might be related to the strain from the preceding processing steps, which makes the sample particularly vulnerable at this stage.

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AUTHOR CONTRIBUTIONS

Svein Kristian Stormo: Conceptualization; formal analysis; project administration; writing-original draft; writing-review & editing. **Torstein Skåra:** Conceptualization; formal analysis; writing-review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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