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Quality of Atlantic Cod Frozen in Cell Alive System, Air-Blast, and Cold Storage Freezers

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ABSTRACT

Gutted Atlantic cod, packed in cartons, were frozen immediately after killing in a magnetic field (cell alive system). The results were compared with traditional air-blast freezing or by putting the cartons directly in a cold storage room (without forced convection of air). After frozen storage, external and fillet properties were compared. In spite of differences in freezing rates, only minor differences were found among treatments. The mechanism for the freezing of fish in the magnetic field, under the current conditions, appeared to be similar to that of traditional freezing methods.

KEYWORDS

Cod quality; freezing; cell alive system; air-blast freezing; microstructure

Introduction

A major part of the commercial catch of Atlantic cod (*Gadus morhua*) in Norway is frozen at sea after bleeding and gutting. Plate freezers are commonly used, where typically the fish are frozen 1–3 h after the catch is taken on board. To obtain the best possible quality, cod fillets should be frozen in the prerigor state (MacCallum et al., 1968; Martinsdóttir and Magnússon, 2001). However, prerigor-frozen cod fillets can shrink rapidly during thawing (McDonald and Jones, 1976). Provided Atlantic cod are correctly frozen, stored, and thawed, the market quality product can be good and of comparable quality as fresh fish (Vyncke, 1983). Nevertheless, frozen storage of gadoid fish makes them susceptible to protein denaturation, and aggregation of contractile proteins can reduce quality due to toughening, dehydration, and reduced water holding capacity (Love, 1988). The rate of freezing is considered important since fast freezing produces small ice crystals, both extra- and intracellularly, resulting in less tissue damage than slow freezing processes where large crystals first form extracellularly and the concentration of solutes outside the cells increases (Bello et al., 1982; Alizadeh et al., 2007). In turn, the cells start to lose water by osmosis, and the cells will gradually shrink. Large crystals are then formed extracellularly. Furthermore, during frozen storage, temperature fluctuations should be avoided since repeated thawing and freezing cycles can damage cells in a similar way and dehydration will occur (Storey, 1980). Moreover, during frozen storage, the textural properties of cod can deteriorate (Badii and Howell, 2002), reducing the eating quality of the product (see Hedges, 2002). Concerning freezing and effects on specific flesh quality parameters, Mørkøre and Lilleholt (2007) studied the effect on lightness, thaw exudates, liquid leakage, gaping, and mechanical properties when farmed Atlantic cod fillets were frozen at −10, −25, −40, −55, or −70°C. The impact of freezing was complex, but no further beneficial effects of low temperature were found when temperature was lowered below −40°C. By freezing rested prerigor cod, the most pronounced changes in glycolytic and nucleotide metabolites occurred when the muscle passed through the critical temperature zone (CTZ; −0.8 to −5°C). Below −5°C, only minor changes occurred. During freezing of fish, the CTZ should be passed through as quickly as possible. For example, rapid freezing

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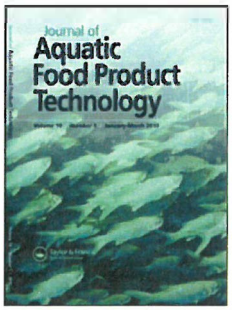

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where duration in the CTZ was 0.22 h, 39% of phosphocreatine (PCr) was dephosphorylated, whereas only small amounts of ATP and glycogen were lost. At 5.4 h in CTZ, half of the initial amount of glycogen was lost and almost all of PCr and about a third of the other high-energy phosphates (HEP) were converted to IMP. With slow freezing (20 h in CTZ), only traces of glycogen and HEP remained in prerigor frozen muscle (Nowlan and Dyer, 1969). Rapid thawing (by increasing ambient temperature too quickly) can also be detrimental. Slow thawing at low external temperature is considered the best option to preserve cod quality (Martinsdóttir and Magnússon, 2001).

Freezing of foods in magnetic fields has currently attracted much attention. However, scientific data to show the potential effectiveness of such freezers are scarce. Suzuki et al. (2009) carried out an experimental assessment of freezing of foods in a weak oscillating (50 Hz) magnetic field (0.5 mT). Among the products tested were bigeye tuna and yellowtail muscle samples (Ø 40 × 40 mm). No significant differences on freezing curves or product quality as indicated by drip amount, color, microstructure, and sensory evaluation were observed compared with control experiments. The cell alive system (CAS) freezing method represents commercially available freezers based on this technology. By using 60 Hz alternating magnetic fields of 0.1 mT with an induced electric field in a CAS freezer, ice crystal formation was inhibited (Kaku et al., 2012), which was helpful for the survival of periodontal ligament cells after cryopreservation (Abedini et al., 2011). However, the claimed freezing mechanism and effectiveness of such freezers has been questioned (Wowk, 2012) and debated (Kobayashi and Kirschvink, 2014). In contrast to conventional freezing methods, where propagation of ice occurs from the outside toward the core of the product, it is claimed that the advantage of using CAS is that a considerable supercooling of the product takes place. Homogenous nucleation and instantaneous freezing will then occur throughout the product, preventing ice crystal formation due to the magnetic field vibration function prohibiting water molecules to make clusters during the freezing process (Takeda, 1991).

A related technology intended for chilled storage was evaluated by Hsieh et al. (2011), where fresh tilapia (*Orechromis niloticus*) were stored at 4°C in a high-voltage electrostatic field (100 kV m^{-1}). Compared with traditional ice storage for up to a week, fish stored in the electrostatic field exhibited considerably fresher appearance and sensory characteristics, lower bacterial counts, lower K-values, and slower increase of volatile basic nitrogen.

Large supercooling can also be created by using pressure shift freezing where the chilled product is put under high pressure (for example 100 to 200 MPa). A rapid release of pressure produces supercooling, causing quick and uniform nucleation in the product due to the shift in the freezing point to normal conditions. Higher pressure and lower temperature lead to more intensive nucleation and the formation of smaller ice crystals. It is well-documented that under such conditions, the microstructure of Atlantic salmon (*Salmo salar*) (Alizadeh et al., 2007) and turbot (*Scophthalmus maximus*; Chevalier et al., 2001) fillets have considerably less freezing damage and drip loss than fillets frozen by using air-blast or direct-contact freezers.

To our knowledge, no independent scientific evaluation of the CAS freezing technology for the preservation of fish is available. If successful, CAS freezing technology could constitute a potential method for the production of a high-quality niche product where the cod are frozen immediately after capture on board while the muscle cells are still alive. In the present research, our goal was to study the effects of freezing method on cod quality by comparing CAS freezing of unstressed Atlantic cod (with aerobic tissues) with traditional freezing of the same product in an air-blast freezer or directly in a cold storage room.

Materials and methods

Experimental design, fish, and sampling before freezing

One of the potential usages of CAS freezers, marketed by MMC (Vigra, Norway), would be on fishing vessels where the fish can be frozen immediately after catching. To verify the claimed

advantages CAS technology might have compared with traditional freezing methods, MMC had therefore performed several tests with various food products without being able to produce evidence that CAS technology really retains product properties. However, proper control of actually being able to freeze live cells had not been studied in detail. The CAS parameters (set points, see below) used in this study were based on the company's experience of what might be the best choice for the freezing of fish. Regarding frozen storage period, we chose to simulate a commercial situation where the fishing vessel might be at sea for a few weeks before returning to a plant for thawing and processing before marketing. Moreover, we also did not want too long storage time in case any positive effects of CAS might be overshadowed by long storage time.

Particular emphasis was put on being able to freeze live muscle cells, since the very concept of the cell alive system implies that the cells are preserved intact to obtain a high-quality product after freezing and thus it assumes a high degree of cell integrity during and after thawing. Consequently, it was important to keep control of fish handling procedures and ante-mortem stress. Therefore, we used farmed Atlantic cod as a substitute for their wild counterparts normally frozen at sea. The fish were handled carefully to avoid excessive muscle activity (anaerobic metabolism). Atlantic cod were farmed at Solund Fiskefarm AS in sea cages at Langøy in the County of Sogn and Fjordane, Norway. The fish were offered Aller 54/17EX marine feed (Aller Aqua, Christiansfeld, Denmark) with a gross composition of 54% protein, 17% fat, 11% nitrogen-free extracts, 9.4% ash, 0.6% fiber, and with a gross energy content of 21.4 MJ. After fasting for 10 days, the fish were transported by a well-boat on October 5, 2011 for about 10 h to the Atlantic Cod Farms AS processing plant in Ålesund. Forty (weight 2.33 ± 0.54 kg and fork length 58 ± 4 cm; mean values ± *SD*) of the fish pumped from the well-boat to the processing plant were randomly collected from the hose outlet and transferred to a 1000-L tub filled with fresh seawater at 13.1°C. The tub, under constant oxygenation (pure oxygen gas connected to two diffusers), was transported at a fish density of about 186 kg m^{-3} in a van for 20 min to the premises of MMC Kulde AS. The fish were basically calm during the transport, although a few short bursts of activity were occasionally observed for some of the fish. After arrival, live fish were sampled and processed before they were assigned to three different freezing methods as shown in Table 1. Continuous sampling of single fish meant that the remaining fish had a greater period for recovery from transport stress, and the period between killing and freezing was affected accordingly (Table 1). In order to provide rested fish in as normal physiological condition as possible, the CAS group had two sample periods commencing after a 50 min period in the recovery tub (CAS1) and after a 430 min period (CAS2). The initial body temperature of all fish was within the range of 14.0 ± 0.4°C (Table 1).

To make sure the muscle cells were frozen alive in an aerobic state, it was of importance to minimize excessive struggling of fish, that is, avoid in vivo production of lactate from glycogen (anaerobic pathway). One fish at a time was quickly netted from the tub and killed by a percussive blow to the head. The stress level of each fish was immediately assessed, first by determining the lactate level in blood collected immediately after cutting the gill arches. Then, the state of the white muscle was determined by measuring the redox potential, initial pH, and ability of the muscle to

Table 1. Experimental groups, number of fish in group, freezing method, recovery period in oxygenated tub after live transport, period from point of death to start of freezing, body temperature at death, and frozen storage temperature.

Group[1]	No. of fish	Freezing method[2] and treatment label	Recovery period in oxygenated tub after live transport (min)	Period postmortem at start of freezing (min)	Body temperature at death (°C)	Frozen storage set temperature (°C)
1	10	CAS1	50–110	10–85	13.6 ± 0.2	−3υ
2	10	ABF	185–230	About 300	13.7 ± 0.1	(−35)[3] & −27
3	10	CSF	285–335	About 180	13.9 ± 0.1	−27
4	10	CAS2	430–485	10–65	14.4 ± 0.1	−30

[1]Groups 1 and 4 (CAS1 & CAS2) had the same freezing process but Group 4 had a longer recovery period in the tub. [2]CAS = cell alive system; ABF = air-blast freezing; CSF = cold store freezing. [3]Transferred to cold store freezer (used for CSF) after 15 h in ABF.

twitch. Subsequently, weight, fork length, and body temperature were determined before the fish were labeled, gutted, and briefly washed in chilled fresh water. Temperature loggers were placed inside 20 fish before each fish was wrapped in a plastic sheet and put in small cardboard cartons (two fish per carton). The CAS fish (n = 20) were frozen and stored on-site at the MMC premises, whereas two other groups of fish (n = 10) were transported to a fish processing plant (Brødrene Sperre AS, Ellingsøy) nearby for air-blast freezing (five cartons) before transfer to cold storage (ABF fish) or by placing five cartons directly in the cold storage (CSF fish). This experimental material was frozen along with the company's fish production on the same day.

Freezing methods

Cell alive system

The CAS freezer (ABI Co., Chiba, Japan) with freezing chamber dimensions (width, height, and depth) 1,700 × 1,650 × 1,000 mm^3 had a capacity to freeze a batch of about 30 kg h^{-1} of a given product. As in a conventional batch-type freezer, cold air is circulated by fans (two fans placed next to the shelves in the present model) from the evaporator toward the products placed on shelves. A magnetic field is generated by five electromagnetic generators surrounding the shelves. Rather than having the possibility to read the magnetic field strength and frequency directly, the magnetic field can be altered from the control panel on a scale from 0–100%. The principle of the system is to freeze a product under influence of a static and a pulsating magnetic field. The experiment was run under the following conditions (instrument set points): evaporation temperature −45°C, cooling fan speed 15% during initial freezing, then changed to 40% throughout storage, and magnetic field 50%. After the initial freezing (−45°C for 16 h 30 min), the wanted storage temperature was set to −30°C (the freezer automatically switches to the chosen "storage mode").

From a previous unpublished test by Vidar Hardarson (SINTEF), it was found that a 40% fan speed corresponded to an air speed of about 0.3–0.4 ms^{-1} as assessed without load. Furthermore, measurement of the magnetic field strength indicated a vertical curvilinear profile and that the magnetic field strength setting (0–100%) showed a complex nonlinear behavior. When set-points of 32, 40, 66, or 100% were used, the ranges of the broadband and harmonic components were in the range of 100–320 and 27–48 μT, respectively.

Additional CAS experiment

It is claimed that the given advantage of using CAS is that undercooling of the product takes place and that homogenous nucleation and instantaneous freezing will then occur throughout the product, preventing ice crystal formation since water molecules cannot cluster if they are in motion during freezing according to hypothesis put forward by Takeda (1991). In contrast, it is well-known that with conventional freezing methods, propagation of ice occurs from the outside of the product to the core.

In the main experiment, we did not measure temperature gradients through the cod during freezing. Because of this, as well as our conclusions from that experiment, we decided to carry out an additional test (October 2013) with the CAS freezer to check the temperature profiles of two products during initial freezing. The two products were freshwater and Atlantic salmon.

Freshwater from the tap (540 mL) was used to completely fill a plastic bottle (90 mm in diameter). An iButton temperature logger was attached to the end of a rigid plastic tube (9 mm in diameter). Two similar items were made, where one tube was submerged in the water to measure the temperature in the center of the bottle and the other one measured the water temperature at the bottom of the bottle (iButton logger pressed against the bottle wall). The tubes were firmly held in place by drilled holes in the bottle screw cap.

Two gutted farmed Atlantic salmon, weighing 3.65 and 3.70 kg with fork lengths 65 and 68 cm, were used. After commercial slaughter, the fish were kept overnight in ice before freezing. In each fish, one, respectively two, loggers were placed under the skin after cutting a notch with scalpel. Similarly, one or two loggers per fish were placed next to the backbone in the thickest part of the

fish. In addition, two thin needle-shaped temperature sensors per fish were inserted in a similar fashion (subcutaneous and core).

The iButtons were programmed to read temperature every 6th second. We used the same CAS settings as described above for the main experiment. Since we were only interested in measuring the cooling-down phase, the experiment was terminated after 4 h when the core temperatures had reached lower set point temperature levels.

Air-blast freezing (ABF)

After packing and transport to the processing plant nearby, five of our cardboard boxes (two fish per box) were frozen in a tunnel freezer along with the company's own production that day. The air-blast freezer has a fan (120 kW) circulating chilled air at a speed of 5 ms^{-1} (with load). The temperature set point was −35°C. After about 15 h, the boxes were moved to the cold storage freezer, see below.

Cold storage freezing (CSF)

Another five cardboard boxes were frozen directly in the cold storage room (load capacity: 18,000 tons) without forced convection (air speed ≈ 0 ms^{-1}). The freezer temperature set point was −27°C. It could be mentioned that Storey (1980) estimated the proportions of ice, freezable, and unfreezable (bound) water in Atlantic cod, stored at −30°C, as 72.2, 1.7, and 6.4 g water (100 g fish)$^{-1}$, respectively.

Frozen storage and thawing

All groups of fish were stored in CAS or CSF (including ABF after initial freezing) freezers for 46 days. Subsequently, all fish were transported in a van equipped with a freezer (−20°C) to our laboratory for thawing and quality assessment. Twenty-two hours after the fish left their original freezers, the cardboard boxes were opened, and the plastic sheets were discarded. Instead, each fish was put in a preweighed plastic bag before the fish were placed in a cold storage room (3.4°C). Since slow thawing is considered the best for frozen cod (Martinsdóttir and Magnússon, 2001), we chose to thaw our fish under such conditions. Twenty fish (10 CAS, five ABF, and five CSF fish) were thawed for quality assessment immediately after thawing, whereas the other half of the fish were placed on crushed ice in styrofoam boxes for quality assessment after one week (typical in-market quality). After 24 h, the fish, stored without ice, were still not entirely thawed. However, at this point it was considered advantageous to excise white muscle samples for histological studies while the muscle was still in a semifrozen state (24–28 h). Forty-seven hours after start of thawing, various analyses related to fish quality were carried out. The biggest fish, still containing minor amounts of ice, were assessed toward the end of the day when all ice had melted.

Fish and fillet quality

To retain as much free water (drip loss) as possible in the bag, care was exerted when the fish were lifted out of the plastic bags in which they had been stored during thawing. The bags were then re-weighed to determine the drip loss of each fish. On the left side of the fish where the initial pH had been measured just after killing, and where the histology samples had been excised, additional samples were excised for analysis of high-energy phosphates and ATP degradation products, water content, water holding capacity (WHC), and NMR proton relaxation times. Muscle pH and core temperatures were then measured. The ability of the muscle to twitch was determined on the intact (right) side of the fish. The external quality of the fish (right side) was assessed by using the quality index method (QIM) for cod. Before assessments, each fish was briefly washed in running tap water (8°C), and excess water was dried off with a paper towel. The fish were then filleted and briefly washed similarly. The temperature loggers were collected, and the data were later extracted. Fillet

quality was also assessed by using QIM ("color" and "presence of blood" are a part of the total QI score), gaping frequency, and texture (hardness).

After ice storage for 6 days, the remaining 20 fish were assessed in a similar fashion, except that the samples for histology were excised just before the rest of the assessments were done (twitch ability was not checked at this stage).

Analytical methods

Redox potential

Just after killing, the redox potential of the white muscle was measured using a SenTix® ORP electrode (WTW GmbH, Weilheim, Germany) connected to a WTW 330i pH meter. The values are reported relative to the hydrogen scale as $E_h = E_{measured} + E_{ref}$, where E_{ref} = 215 mV at 13–14°C (initial fish temperature) according to the WTW electrode manual.

Blood lactate

Whole blood lactate was measured by using the Lactate SCOUT test strip method (EKF-Diagnostic GmbH, Magdeburg, Germany). The test strip was briefly soaked in blood just after the gill arches were cut. It was then inserted into the test meter where the lactate concentration was read in mmol L^{-1} on the display. The measuring range is 0.5–25 mmol L^{-1}.

Body and core temperatures

The fish body temperature was measured after killing by using a Testo 110 thermometer (Testo AG, Lenzkirch, Germany). The probe was inserted in the epaxial muscle between the lateral line and the dorsal fin, and in the same location after thawing and subsequent ice store for 6 days. Before freezing, temperature loggers (Maxim Thermochron DS1921K iButtons, Sunnyvale, CA, USA) were pressed into the muscle next to the backbone in the thickest part of twenty fish. Half of them were programmed to record core temperature every min; whereas for the other half, temperature was recorded every 10 min. The loggers have a temperature cut-off at −40°C. In the CAS fish, six loggers of each type were used; whereas for the ABF and CSF fish, two loggers of each type were used.

White muscle pH

Just after killing, thawing, and subsequent ice storage, pH was measured directly in the white muscle in the same location as the temperature was determined. A shielded glass electrode (WTW SenTix 41) connected to a portable pH meter (Model WTW 330i WTW) was used.

Muscle twitches

Muscle contractions were determined just after killing using a Twitch Tester Quality Assessment Tool (AQUI-S Ltd., Lower Hutt, New Zealand). Since the fish were frozen shortly after killing in a rested state, with most of the high-energy phosphate (PCr and ATP) stores probably intact, we also wanted to check twitch ability just after thawing (related to potential thaw rigor issues). The instrument measures the electrical excitability of muscle tissues. An electrical pulse was generated (9V DC) by the instrument every 0.6 s. One, or a few (< 4), measurements were performed on one side of each fish. For each measurement, the electrodes were in contact with the fish for about 1–3 s. The following scale was devised: 3 = clear tail twitch (electrodes placed along the entire lateral line, behind the head and near the caudal fin); 2 = weak tail twitch (electrodes placed as above); 1 = minor muscle contractions in (small) restricted areas of the fish surface (electrodes placed a few cm apart); 0 = no contractions whatsoever.

Microstructure/histology

For muscle histology, two to three pieces (2 mm thickness) of white muscle tissues were cut transversally to the muscle fibers between the lateral line and the front part of the dorsal fin. Samples from five fish in each treatment were used.

The fresh tissues were fixed in 4% formalin in phosphate buffer. At 24–28 h after initiation of thawing, samples were fixed in Clarke's solution (absolute ethanol and glacial acetic acid, 3:1) that had been kept in the cold storage (3.4°C) for 24 h. They were then dehydrated in absolute ethanol at room temperature, embedded in paraffin, and 4 μm thick sections were cut transversally to the muscle fibers on an Autocut 2055 microtome (Leica Microsystems, Wetzlar, Germany). No difference was found between the fixation methods on muscle morphology and shrinkage (separate test). The sections were stained with "Orange G" and 1% blue aniline, after rehydration in "Tissue Clear." One section from each of the tissue blocks per fish (n = 5–6) was used for the histological analyses of muscle tissue from the various freezing methods.

A Zeiss Axioskop 2 Plus- light microscope (Zeiss GmbH, Jena, Germany) was used for tissue examination, and then morphometric analysis was performed on the software CAST 2 (Olympus Inc., Ballerupe, Denmark). A uniform point grid frame was used on each section to estimate the area fractions of muscle tissue and extracellular spaces left by the ice crystals in the tissue. For each section, the area fraction analyses were done from 3 point grid areas close to the surface (near the skin) and from 3 point grid areas located 7.5 mm interior to the surface (Figure 1). Counts were made of the number of points hitting muscle tissue or extracellular spaces. The mean ratio of the point totals (ΣPstruc/ΣPframe) from the surface and inner areas affords an unbiased estimate of the area fraction occupied by extracellular spaces in the tissue (see Gundersen et al., 1988). There was no significant difference between data from the two areas, and only data from the surface area was used in the further analyses. In order to compute the thickness of extracellular spaces between muscle fibers, pictures of the sections were converted to black and white binary images. The ImageJ (a public domain, Java-based image processing program developed at the National Institutes of Health, Bethesda, MD, USA) specialized implementation of Bob Dougherty's Local Thickness plugin (Dougherty and Kunzelmann, 2007) was used, as this defines the thickness at a point as the diameter of the greatest sphere that fits within the structure and contains the point. The plugin calculates mean and standard deviation, as well as maximum size, of the extracellular space directly from pixel values in the resulting thickness map. The plugin assumes that extracellular spaces are the foreground of a binarized image.

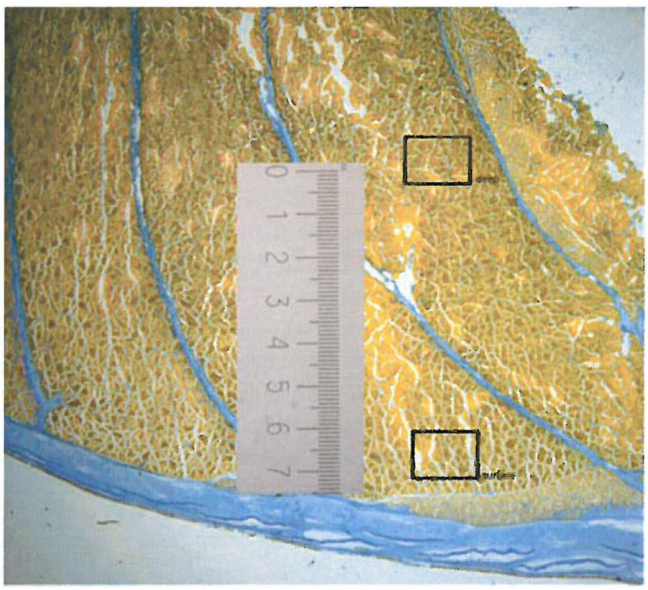

Figure 1. Histological section of cod white muscle, demonstrating location of the areas of morphometric analysis (black rectangles) of muscle fibers and extracellular spaces between fibers.

High-energy phosphates, ATP degradation products, and K-values

After thawing and after ice storage for another 6 days, white muscle samples (1–3 g) were excised between the lateral line and the first dorsal fin of all fish. The samples were freeze-clamped using aluminum tongs precooled in liquid nitrogen. The samples were immediately immersed in liquid nitrogen before they were transferred to a −80°C freezer for storage before analysis. After freeze-drying (Hetosicc, Model CD 13-1; Heto Lab Equipment A/S, Birkerød, Denmark), the samples were analyzed using the high performance liquid chromatography (HPLC) method of Sellevold and others (1986). The HPLC instrument used was a Waters Alliance 2695 with a 2487 dual wavelength UV/VIS detector. The column used was a Supelco Discovery HS C18, 569252-U (15 cm × 4.6 mm, 3 µm), operating at the following conditions: flow rate 1.0 mL min^{-1} at 4°C; UV monitoring at 254 nm for ATP and related degradation products, and at 206 nm for phosphocreatine. The microfiltered isocratic mobile phase, adjusted to pH 6.25, consisted of KH_2PO_4 (215 mmol L^{-1}), tetrabutylammonium hydrogen sulphate (TBAHS, 0.08%), and acetonitrile (3.5%). K-values were calculated as:

$$K = [(HxR + Hx)/(ATP + ADP + AMP + IMP + HxR + Hx)] \times 100\%.$$

Concentrations of all metabolites were calculated as µmol (g muscle dry wt) $^{-1}$.

Quality Index Method

After thawing and ice storage, the QIM was used to assess quality attributes of the cod (Martinsdóttir et al., 2001). The QI scheme lists how the quality attributes odor and appearance (skin, texture, eyes, gills, and fillet/blood color) change with storage time. For each quality attribute, a score from 0 (very fresh fish) to 2 or 3 is given. Finally, the scores for all 10 quality attributes are summarized and presented as a QI score. The maximal possible score is 23 (an alternative version of the QIM does not include assessment of fillet appearance and residual blood; in that case, the range of the QI scheme is 0–19).

Water content

The water content of the dorsal muscle was determined by drying a 2 g sample to constant weight at 105°C for 24 h. The difference in weight before and after drying was taken as the total water content of the sample. The mean of three replicates is reported here.

Water holding capacity

A piece of the dorsal muscle was minced and subjected to the low-speed centrifugation method described by Eide et al. (1982). A centrifugal force of 230 × *g* was used (Hultmann and Rustad, 2002). The water holding capacity is defined as the percentage of water retained in the mince after centrifugation for 5 min. Four parallel samples were run for each fish.

Drip loss

After transport to our laboratory at −20°C, each of the fish were immediately put in separate preweighed plastic bags. The amount of free water (drip loss) was determined after re-weighing the bags after thawing and after ice storage. The drip loss was normalized with respect to body weight.

Low-field ^{1}H NMR relaxation times

After thawing and ice storage, low-field (LF) ^{1}H NMR transversal relaxation (T_2) measurements were performed on five fish in each group. Five parallel samples were excised from white epaxial muscle below the first dorsal fin (1 × 1 × 2 cm^3, approximately 2 g) and placed in NMR tubes (diameter 10 mm). The tubes were immediately placed in ice and kept there until the LF NMR measurements were performed. The measurements were performed on a minispec mq 20 (Bruker Optik GmbH, Ettlingen, Germany) with a magnetic field strength of 0.47 T corresponding to a proton resonance frequency of 20 MHz. The instrument was equipped with a 10 mm temperature-variable probe.

A built-in heating element was connected to the temperature control unit (BVT3000; Bruker Optik GmbH). The temperature in the probe was regulated to 4°C by blowing compressed air through the sample holder. T_2 relaxation was measured using the Carr-Purcell-Meiboom-Gill pulse sequence (CPMG; Carr and Purcell, 1954; Meiboom and Gill, 1958). The measurements were performed with a time delay between the 90 and 180° pulses (τ) of 150 μs. Data from 4,000 echoes were acquired from 16 scan repetitions. The repetition time between two succeeding scans was set to 3 s. All even echoes were sampled.

The NMR transverse relaxation data were analyzed using biexponential fitting of the T_2 relaxation data, that is, by fitting of the following equation to the experimental CPMG curves:

$$Signal = A_{21}e^{-t/T_{21}} + A_{22}e^{-t/T_{22}},$$

where T_{21} and T_{22} were the two relaxation components, and A_{21} and A_{22} were the corresponding amplitudes. The calculations were made using MatLab (The Mathworks Inc., Natick, MA, USA) using 4,000 data points. Since the absolute relaxation amplitudes are proportional to the amount of water and fat in the sample, the relative amplitudes within samples were used. T_{21} populations were calculated as: $A_{21}/(A_{21} + A_{22})$. For a biexponential fitting, the T_{21} and T_{22} populations sum up to 100%.

Gaping

To assess fillet gaping, the method proposed by Andersen et al. (1994) was used. The scores are: 0 = no gaping; 1 = few small (< 2 cm) slits (< 5); 2 = some small slits (< 10); 3 = many slits (>10 small or a few large, > 2 cm); 4 = severe gaping (many large slits); 5 = extreme gaping (the fillet falls apart).

Hardness

Fillet hardness was measured after thawing by using the TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK). A flat-ended cylindrical plunger (½" in diameter) was pressed into the fillets perpendicular to the muscle fibers at a constant speed of 5 mm s^{-1} until it reached 60% of the original fillet height. The measurements were performed at three different locations along the thickest part of the epaxial muscle. The mean value was used as an estimate of fillet hardness for each fish.

Statistics

Initial white muscle pH was analyzed for possible significant differences by subjecting the data set to a one-way analysis of variance (ANOVA) test followed by a Tukey post hoc test where significance was indicated. Before analysis, it was confirmed that the data actually passed the normality (Shapiro-Wilk) and equal variance (Levene median) tests. Except for the initial muscle pH, microstructure (maximum thickness of extracellular spaces), NMR T_{22} relaxation times after thawing, as well as A_{21} and A_{22} populations after ice storage, CAS1 and CAS2 groups (showing effect of antemortem stress) were clearly not different. Therefore, the values of the other quality-related parameters were pooled, and the group is termed "CAS" (n = 20).

To compare histological means from the area fraction analyses, the group data were statistically tested using one-way ANOVA followed by a Student-Newman-Keuls test for more than two means. Data were tested for homogeneity of variances using the Levene test. The results from analyses of extracellular spaces (open spaces between muscle fibers) exhibited no normal distribution and was tested by a Kruskal-Wallis one-way analysis of variance on ranks, followed by a pairwise multiple comparison procedures (Dunn's method). A 5% level of confidence was used for all statistical testing.

Results and discussion

Condition of fish before freezing

Since our goal was to freeze live muscle cells, effort was made to minimize anaerobic metabolism (excessive muscle work) before freezing. It is evident that the fish gradually recovered after the handling stress associated with sampling from well-boat and transport to the MMC premises (Table 1). Blood lactate, redox potential, initial white muscle pH, and muscle twitches values as obtained just after killing are shown in Table 2. During the measurements of initial pH of the CAS1 group, it became clear that the fish were stressed (pH 7.05, Table 2). Therefore, is was decided to split this group into two where the CAS2 fish were the last ones to be sampled to, hopefully, achieve a recovery effect with a higher initial pH to increase the probability that aerobic muscle cells were indeed frozen in CAS2. Recovery to rested state did indeed take place (Table 2). Typical blood and muscle values of rested or anesthetized farmed cod are: lactate 0.3 to 0.4 mmol L^{-1} (Brown et al., 2010), initial white muscle pH about pH 7.6, and twitch ability 3.0 (Erikson et al., 2011). Thus, in terms of handling stress it is clear that the CSF and CAS2 fish were close to being in the rested state, whereas the CAS1 fish were stressed at the time of killing and freezing. The ABF fish were intermediate relative to the other groups. The white muscle redox potential of all fish were positive (range: 247 to 427 mV), meaning aerobic conditions prevailed as the fish were ready for freezing.

Freezing and frozen storage

Since all groups of fish were packed in cardboard cartons, the observed freezing times would be expected to be longer than if unpacked fish had been studied. This was due to the increased resistance to heat transfer caused by the external convective boundary layer, carton wall, and trapped air inside the carton. The mean initial freezing curves of the gutted fish frozen in CAS, ABF, and CSF are shown in Figure 2a. As expected a priori, the temperature drop in the CSF fish was the slowest, reaching −0.5°C after 1 h. The almost flat shape of the freezing curve for the CSF fish showed that ice formation (phase transition) in this fish took place during several hours (Figure 2b). In case of ABF and CAS, the mean temperatures reached the CTZ after about 20 and 12 min, respectively. After 1 h, the temperatures had dropped to about −2.7 and −4.0°C, respectively. The CAS fish had passed through the CTZ after about 80 min, whereas the ABF fish did so after 2 h (Figure 2b). Ten hours after the freezing process had started, the mean temperature in the CSF, ABF, and CAS fish were approximately −3, −31, and −38°C, respectively. The core temperature in the CSF fish dropped below the critical zone after about 15 h and reached the storage temperature of −27°C after about 30 h (Figure 2c). After 15 h, the ABF fish, frozen to −33°C, were transferred and subsequently stored in the −27°C freezer (along with the CSF fish). The CAS fish were initially frozen to −45°C before the storage temperature was gradually reduced to −32°C (after change to storage mode) after about 30 h. All groups were stored at practically constant temperatures throughout the storage period. The duration the fish stayed in the CTZ is shown in Table 3. During frozen storage, the core temperature of fish in all groups remained

Table 2. Blood lactate, white muscle redox potential, pH, and twitches as determined immediately after killing; the fish were frozen in CAS within 10 to 85 min postmortem.

Group[1]	Lactate (mmol L^{-1})	E_h (mV)[2]	pH	Twitch (range: 0–3)
CAS1	3.6 ± 1.0	427 → 275	7.05 ± 0.07[a]	2.0 ± 0.0
ABF	5.0 ± 1.1[3]	419 → 265	7.25 ± 0.11[a]	1.9 ± 0.1
CSF	≤ 0.5[4]	400 → 275	7.46 ± 0.02[b]	2.0 ± 0.0
CAS2	≤ 1.0[5]	387 → 247	7.42 ± 0.03[b]	2.0 ± 0.0

Mean values ± *SEM* (n = 10). Different letter, a or b, means significant differences in pH ($p < 0.05$). [1]The recovery period after transport increased for each group downward in the table (range: 50–485 min posttransport). [2]The redox potential initially decreased rapidly until a more stable level was reached (latter value). [3]Four fish had blood lactate contents below the detection limit of the instrument (< 0.5 mmol L^{-1}). [4]Lactate equal to, or lower than the detection limit of the instrument (all fish). [5]1.1 (n = 1), 0.8 (n = 1), and < 0.5 (n = 8) mmol lactate L^{-1}.

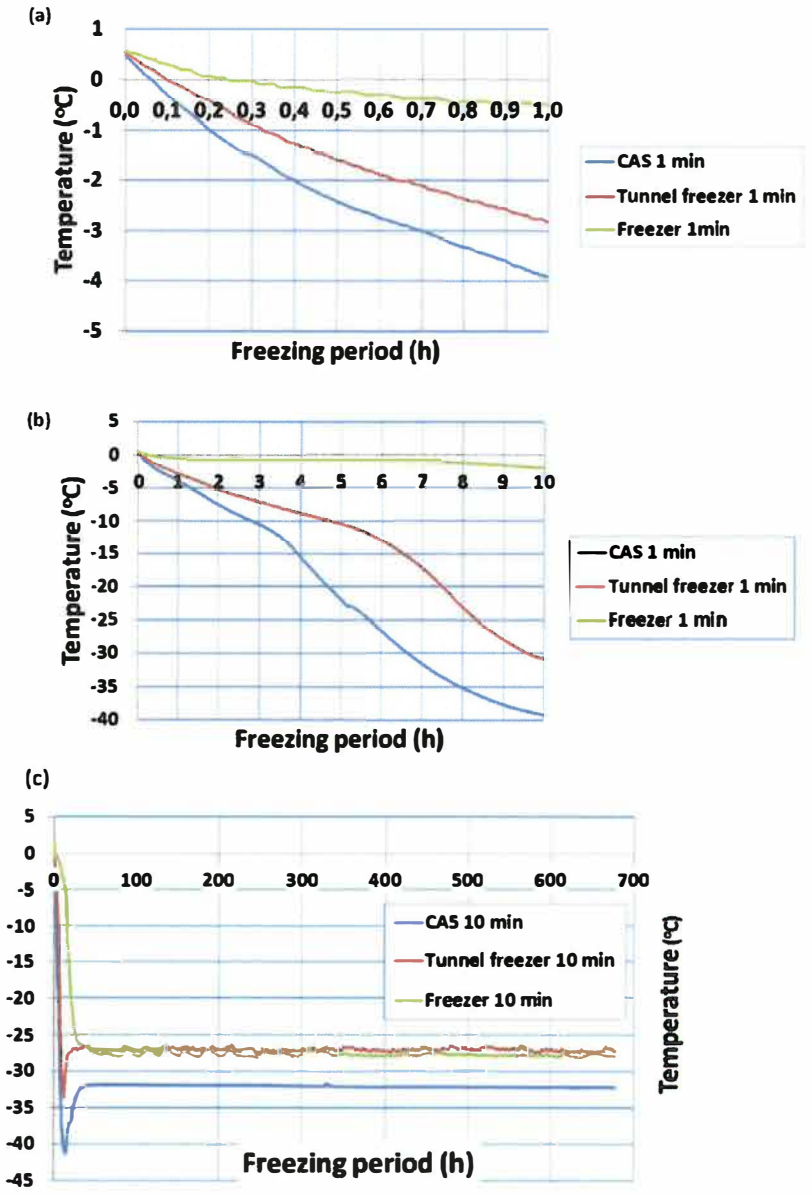

Figure 2. Mean core temperatures of gutted Atlantic cod during the 1st h (a), 10 h after start of freezing (b), and during the first 28 days of frozen storage (c). All fish were kept in the frozen state for 47 days. Temperature was logged every min in case of (a) and (b), whereas during storage period (c), temperature was logged every 10 min. Cell alive system (CAS1 and CAS2) freezing: $n = 6$, air-blast freezing (ABF or "tunnel freezer"), and cold storage freezing (CSF or "freezer"): $n = 3$.

quite stable at −27 and −32°C (see Figure 2c and Table 3), thus avoiding the possible detrimental effects of temperature fluctuations. Within the time and temperature resolutions used here (loggers and figures), we were not able to identify any signs of undercooling (would be apparent as spikes in Figure 2). Moreover, apart from a quicker chilling rate in case of CAS, the shape of the freezing curve was basically similar to those of the conventional freezing methods. The observed differences in freezing rates were probably due to differences in air circulation speeds, and to some extent, the somewhat different lower temperatures (set points). Since the fans were placed next to the fish in the small CAS freezer, the air circulation was probably more efficient than in the large commercial ABF,

Table 3. Mean duration of fish staying within the critical temperature zone (CTZ: −0.8 to −5.0°C) during the freezing process and actual core temperatures in fish during frozen storage.

Freezing method	Duration of fish in CTZ[1] (min)	Storage temperature[2] (°C)
Cell alive system (CAS)	67 ± 14	−32
Air-blast freezing (ABF)	98	−27
Cold storage freezing (CSF)	478	−27

[1]Mean values as measured by temperature loggers in the fish. Only data from the loggers programmed to read every 60 s was used for this purpose. CAS = pooled mean value ± *SEM* of six loggers placed in three CAS1 and three CAS2 fish are shown. In case of ABF and CSF, the average values of two fish per treatment are shown. [2]After reaching the temperature storage plateau (Figure 2c), the temperature variation from 30 h onward was always ≤ 0.6°C for all freezing methods as measured during the first 28 days of the 47 day frozen storage.

whereas there was no forced convection at all in the CSF. Our data could not reveal whether the magnetic field in the CAS contributed to the freezing process in any way.

Rigor mortis

Since rested fish were frozen, with high levels of glycogen and ATP, there could be a possibility that thaw rigor might occur. However, we did not observe any signs of thaw rigor during, or just after thawing, nor did we observe rigor during further storage on ice. One reason for this could be that it would hardly be possible to distinguish thaw rigor stiffness from cold stiffness during thawing. Alternatively, it could mean that rigor had already passed in the frozen state (Cappeln and Jessen, 2001). For example, when fish are stored for at least 8 weeks at −7°C, the flesh has time to pass through rigor in the frozen state (Stroud, 1968). Thaw rigor proceeds intensely and rapidly, where HEP decompose particularly rapidly between −3 and 0°C (Bito, 1986).

Microstructure of white muscle just after thawing

Newly slaughtered, fresh cod muscle had a very homogenous muscle fiber distribution, with very narrow extracellular spaces between the muscle fibers (Figure 3a). Observations of the histological sections of newly thawed muscle demonstrated that CAS freezing gave a more homogenous distribution of muscle fibers and extracellular spaces than the CSF samples, where muscle fibers seemed more compressed, and extracellular spaces were larger and seemed very unevenly distributed (Figures 3b–e). ABF also gave very large variations in the thickness of the extracellular spaces between muscle fibers (Figure 3d). This qualitative difference in appearance might be due to a different ice-crystal formation as a result of the differences in freezing rates between the three freezing methods (Figure 2 and Table 3).

The quantitative analyses of the relative area fractions of muscle fibers, connective tissue, and extracellular space demonstrated a significant difference between fresh and thawed tissue (Figure 4), where the mean muscle fiber area constituted 91% of the total area and extracellular space only 6% of the total area. Mean extracellular space area fraction varied between 25–34% of the total area in all thawed tissue samples, and no significant difference was found between any of the treatments in mean values of muscle, connective tissue, and extracellular space area fractions. Effects from freezing on the transverse microstructure of fish fillets can mainly be seen as increased extracellular space between the muscle fibers that have been frozen, as observed by—e.g., Liljemark (1969), Jarenback and Liljemark (1975) and Sigurgisladottir et al. (2000). The degree of muscle tissue shrinkage also seemed to vary between different types of Atlantic salmon (Sigurgisladottir et al., 2000), but this was not observed among our quite homogenously sized cod.

Further quantitative analyses of the extracellular space thickness, similarly gave no significant difference between freezing methods on the mean value of extracellular space between the fibers (Figure 5). However, the analysis showed a highly significant difference in the maximum thickness of the extracellular spaces between the CAS1 and the CSF treatments. The mean value of maximum

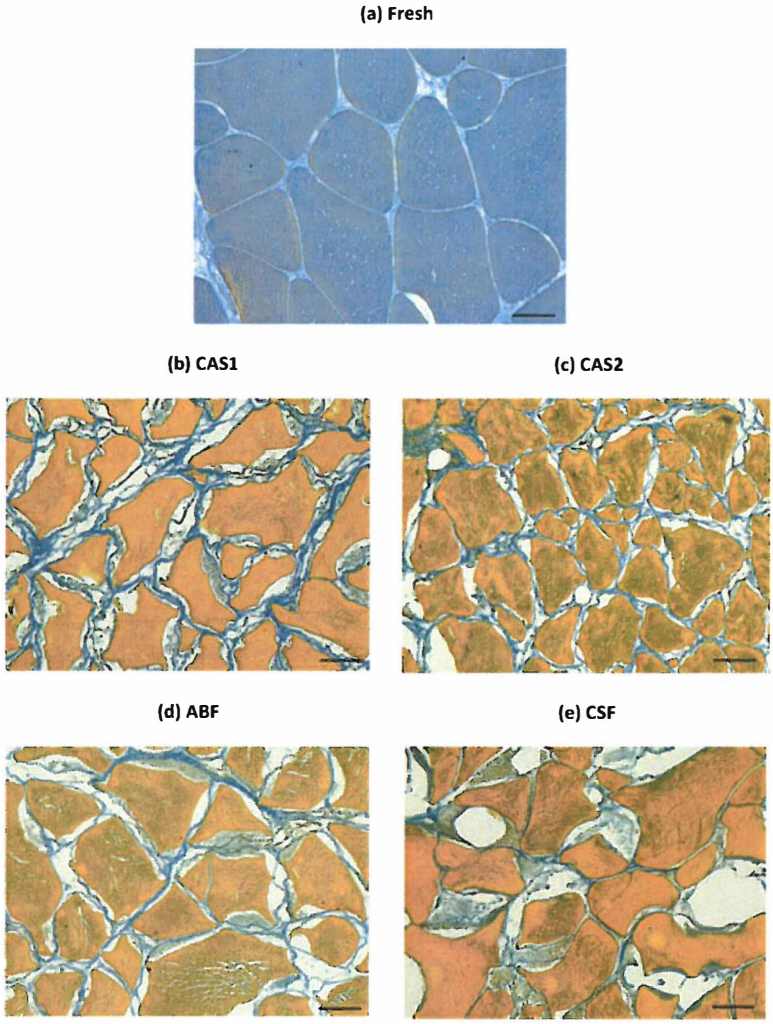

Figure 3. Muscle tissue of cod, fresh (a) and frozen by different methods (b–e). Fresh cod tissue was fixated by 4% formalin in phosphate buffer, and the newly thawed tissues were fixed in Clarke's fixative (more suitable for frozen tissue). The sections were stained with Orange G and Blue aniline. Scale bar = 100 µm.

thickness of extracellular spaces in tissues from CAS2 and ABF treatments were close to the CAS1 results, but had somewhat higher variation and were not statistically different from the CSF treated tissue.

External appearance of fish (Quality index)

Immediately after frozen storage for 47 days and thawing for 47 h at about 3°C, fish and fillet quality were assessed for five individuals in each group. The assessment was repeated for the remaining fish after further ice storage for 6 days. Upon assessments, the mean core temperatures on these two occasions (determined manually) were 4.6 ± 1.7 and 2.1 ± 0.5°C (n = 20), respectively. The external quality features of the fish are shown in Table 4. No differences between freezing methods were observed for the QI on either day of assessment. Ice storage for 6 days increased the overall QI score by approximately 4 demerit points. Generally, the appearance of the fish was good. No signs of rigor mortis (thaw rigor) were observed (see above). The fish were firm and elastic on both days. The eye

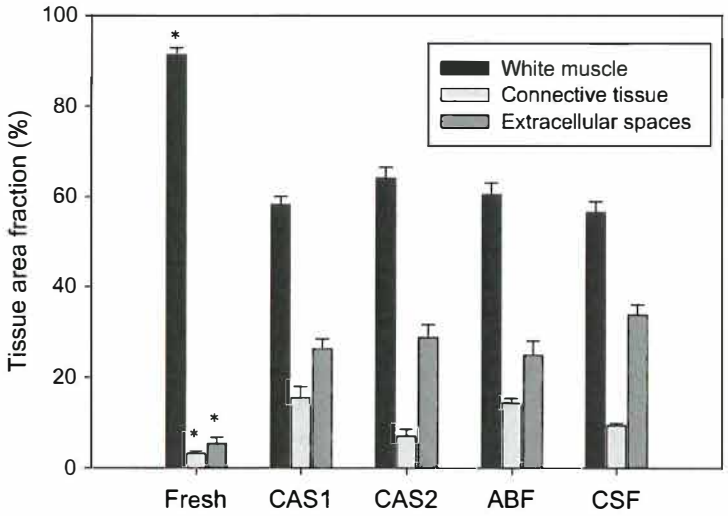

Figure 4. Area fraction distribution of muscle fiber tissue, connective tissue, and interstitial spaces in white muscle tissue from newly thawed cod after different freezing methods. The asterisk (*) denotes significant difference between fresh and frozen muscle tissues. No significant differences were found between freezing methods. Bars represent mean values (± *SEM*) of tissue from five fish per treatment.

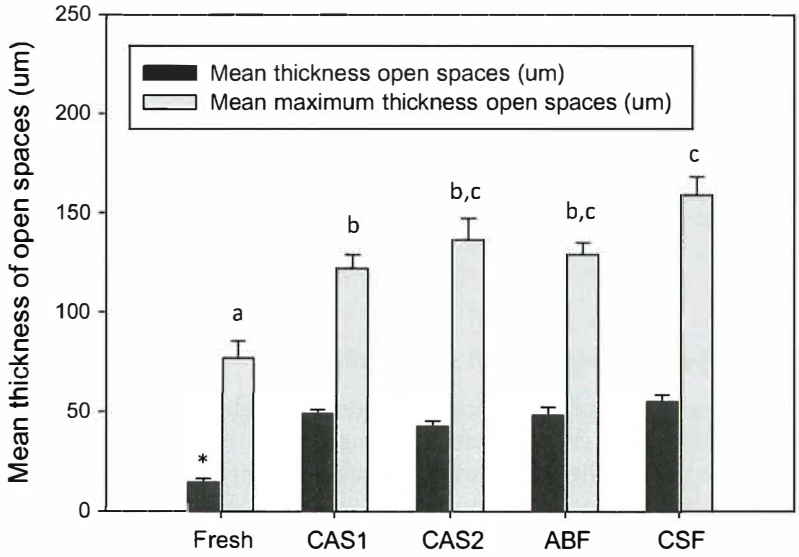

Figure 5. Quantitative analysis of mean and mean maximum thickness of extracellular spaces between muscle fibers in white muscle tissue from fresh and newly thawed cod after having been subjected to different freezing methods. Fresh muscle was significantly different from frozen, and CSF tissue had larger open spaces between muscle fibers than muscle fibers from the other treatments. However, variation was large in the muscle tissue from all treatments, and only CAS1 tissue had significantly smaller maximum thickness of the extracellular spaces between the muscle fibers than the CSF tissue.

form was flat or sunken, and pupils had turned grey in all 40 fish. Gill color was becoming discolored, and the odor was classified as "neutral, grassy, musty" (score 1) just after thawing. After 6 days on ice, the gills had a brownish color with a "yeast, bread, beer, sour milk" (score 2) odor. Over the same period of time, gill mucus score increased from "clear" to "milky" (scores 0–1), to "milky" to "dark, opaque" (scores 1–2).

Table 4. External appearance (QI) of Atlantic cod as determined just after thawing and after subsequent ice storage for 6 days: Comparison of cell alive system (CAS), air-blast (ABF), and cold storage (CSF) freezing methods.

	Quality index (QI range: 0–23)		
	CAS[1]	ABF	CSF
After thawing	9.9 ± 0.2	10.2 ± 0.4	10.0 ± 0.3
After ice storage	14.4 ± 0.6	13.7 ± 0.9	14.4 ± 0.6

Mean values ± *SEM* (CAS: n =10; ABF and CSF: n = 5). [1]Pooled values of CAS1 and CAS2.

The QIM is normally intended for quality assessment of nonfrozen fish, typically during ice storage. Since we froze our fish directly after killing and assessed QIM just after thawing, our results were not biased by storage other than in the frozen state. Even so, the fish did not have a fresh (unfrozen) appearance, and there were no significant differences in appearance with regard to freezing method (Table 4). Nor did we observe any particular signs of better appearance of the CAS fish such as the persistent eye clarity observed after storage of fish in a high-voltage electric field (Hsieh et al., 2011). Our mean QI values were approximately 10 immediately after thawing. By comparison, when farmed cod of similar size were stored on ice for 7 days, the QI values were about 7 to 8 when using the same version of the QIM with range 0–23 (Erikson et al., 2012). In other words, the effect of freezing and thawing alone produced fish with a somewhat less appealing appearance than fish stored fresh on ice for 7 days.

Fillet appearance

Quality indices related to flesh quality are shown in Table 5. Fillet color and color of possible residual blood in fillets was assessed visually as a part of the QI score shown in Table 4. On both days, fillet color was regarded as "waxy, milky" (score 1), whereas the minimal amounts of residual blood in the fillets had a bright red color (score 0) after thawing, changing to dark red (score 1) after ice storage for 6 days.

Degradation of high-energy phosphates and K-values

Since the fish most probably had considerable amounts of high-energy phosphates in the muscle when freezing was initiated, we checked before filleting whether twitches still could be observed just after thawing. No signs of twitches whatsoever were observed, probably because of lack of ability to transfer nerve signals and ATP.

Particularly in the case of CSF and CAS2, it was likely that the levels of PCr and ATP were high at the time of death, since the mean initial pH was 7.4 at the beginning of freezing (Table 2). However, after freezing and thawing, the high-energy phosphates were largely depleted and only traces of ATP were detected (Table 5). The levels of PCr were in the range of 2.0–3.9 μmol (g dry wt)$^{-1}$, where the loss of the phosphate moiety resulted in high levels of creatine (Cr) at 183–201 μmol (g dry wt)$^{-1}$. Since AMP and ADP do not accumulate in the ATP degradation pathway in cod, their levels (ranges) remained low at 2.1–2.3 and 0.2–0.3 μmol (g dry wt)$^{-1}$, respectively. Hence, just after thawing, IMP was the dominating catabolite, about 34–38 μmol (g dry wt)$^{-1}$, derived from ATP. Significant levels of HxR, about 7–10 μmol (g dry wt)$^{-1}$, were also present. Some amounts of Hx, 2.0–3.9 μmol (g dry wt)$^{-1}$, had already been formed at this stage. The mean K-values ranged from 18.4 to 25.5%. The main feature of further ice storage for 6 days was a shift from the flavor enhancer IMP, 9.1–17.5 μmol (g dry wt)$^{-1}$; to higher amounts of HxR, 22.1–24.3 μmol (g dry wt)$^{-1}$; and the bitter tasting Hx, 5.1–7.4 μmol (g dry wt)$^{-1}$. This resulted in increased K-values (58.7–75.8%). Due to

Table 5. Fillet quality (pH, water content, drip loss, water holding capacity, NMR relaxation times, ATP-related nucleotide catabolites, K-values, gaping, and hardness) of Atlantic cod as determined just after thawing and after additional ice storage for 6 days: Comparison of CAS, ABF, and CSF freezing methods.

Quality parameter	Cell alive system (CAS)[1]	Air-blast (ABF)	Cold storage (CSF)
	After	*thawing*	
Ultimate pH	6.43 ± 0.05	6.41 ± 0.14	6.48 ± 0.06
Water content (%)	77.8 ± 0.3	77.5 ± 0.4	77.7 ± 0.3
Drip loss (% of body weight)^{nsd}	1.9 ± 0.2	2.9 ± 0.6	2.2 ± 0.2
WHC (%)^{nsd}	80.8 ± 1.2	78.7 ± 1.7	81.2 ± 1.2
T_{21} relaxation time (ms)^{nsd}	46.9 ± 2.0	45.0 ± 1.2	45.6 ± 0.8
T_{22} relaxation time (ms)	CAS1: 144.7 ± 6.6^{a} CAS2: 129.8 ± 5.7^{b}	130.2 ± 5.3^{b}	139.3 ± 5.5^{a,b}
A_{21} population (%)^{nsd}	80.6 ± 0.7	80.1 ± 0.6	79.2 ± 0.6
A_{22} population (%)^{nsd}	19.4 ± 0.7	19.9 ± 0.6	20.8 ± 0.6
ATP (μmol/g dry wt)	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.1
IMP (μmol/g dry wt)	38.2 ± 3.2	34.4 ± 2.6	35.7 ± 2.8
HxR (μmol/g dry wt)	7.1 ± 1.2	9.6 ± 1.6	8.2 ± 1.2
Hx (μmol/g dry wt)	1.8 ± 0.2	3.1 ± 0.5	2.5 ± 0.6
K-value (%)	18.4 ± 3.3	25.5 ± 3.5	21.8 ± 3.4
Gaping (range: 0–5)^{nsd}	1.8 ± 0.3	2.2 ± 0.2	2.0 ± 0.3
Hardness (N)^{nsd}	9.8 ± 0.8	9.6 ± 0.5	10.6 ± 0.5
	After	*ice storage*	
Ultimate pH	6.65 ± 0.07	6.55 ± 0.08	6.63 ± 0.07
Water content (%)	78.6 ± 0.4	78.1 ± 0.4	79.0 ± 0.6
Drip loss (% of body weight)^{nsd}	4.8 ± 0.6	5.7 ± 0.9	5.3 ± 0.6
WHC (%)$^{2,\ nsd}$	72.1 ± 1.7	70.0 ± 1.2	70.1 ± 2.4
T_{21} relaxation time (ms)	45.7 ± 1.5^{a}	44.1 ± 1.4^{b}	45.9 ± 1.6^{a}
T_{22} relaxation time (ms)^{nsd}	136.4 ± 3.8	145.1 ± 6.0	156.9 ± 8.7
A_{21} population (%)	CAS1: 76.1 ± 1.3^{a} CAS2: 80.5 ± 1.3^{b}	78.5 ± 1.3^{a,b}	76.8 ± 1.6^{a}
A_{22} population (%)	CAS1: 23.9 ± 0.5^{a} CAS2: 19.5 ± 0.5^{b}	21.5 ± 0.5^{a,b}	23.2 ± 0.6^{a}
ATP (μmol/g dry wt)	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
IMP (μmol/g dry wt)	11.3 ± 4.9	17.5 ± 4.1	9.1 ± 6.0
HxR (μmol/g dry wt)	23.7 ± 3.5	22.1 ± 2.2	24.3 ± 3.4
Hx (μmol/g dry wt)	5.1 ± 0.6	5.6 ± 0.6	7.4 ± 2.0
K-value (%)	68.6 ± 10.3	58.7 ± 7.2	75.8 ± 10.6
Gaping (range: 0–5)^{nsd}	1.2 ± 0.4	1.2 ± 0.6	1.2 ± 0.6

Mean values ± *SEM* (CAS: n = 10; ABF and CSF: n =5). [1]Pooled values of CAS1 and CAS2 unless otherwise stated. [2]Determined after 7 days on ice. Where in doubt, statistical analyses were run: different superscript, a or b, denotes statistical difference ($p < 0.05$); nsd = no statistical difference ($p > 0.05$).

the comparatively large variation among individual fish in each group, freezing method did not produce significant differences in nucleotide catabolism.

By using the same methodology, typical initial values of PCr, ATP, and IMP in rested farmed Atlantic cod are in the ranges of 96.0–86.3, 30.6–31.3, and 1.1–0.8 μmol (g dry wt)$^{-1}$, respectively. After ice storage for 7 days postmortem (unfrozen fish), the levels of IMP, HxR, and Hx were 15.1–17.6, 16.1–19.5, and 2.3–3.3 μmol (g dry wt)$^{-1}$, respectively. The corresponding K-values were about 50% (Erikson et al., 2011). In another study, the K-values after 7 days on ice were 53–68% (Digre et al., 2011b). Degradation of glycogen occurs during freezing along with synthesis and degradation of ATP. If ATP was present after frozen storage, it would be depleted during thawing since ATP can disappear in a matter of a few minutes during the thawing process (Cappeln and Jessen, 2001). At temperatures ≤ −40°C, the level of ATP is stable for at least 12 weeks (Cappeln et al., 1999).

Ultimate pH

Typical ultimate pH values in farmed cod range from 6.3 (Erikson et al., 2011) to 6.5 (Erikson et al., 2012). As measured just after thawing, our present values were around pH 6.4, independent of

freezing method ($p > 0.05$). This shows that the glycolysis was completed somewhere between start of freezing and just after thawing (significant in vivo depletion of glycogen by excessive muscle work was not considered a factor since fish in CAS2 and CSF groups, in particular, exhibited initial pH values near those typical of rested fish, Table 2). Six days later after ice storage, the mean pH values had increased to around 6.6, but as before, no significant differences between groups were observed.

Water content, water holding capacity, and drip loss

The water content (78–79%) of the white muscle was not different among groups and did not increase significantly after storage for 6 days after thawing (Table 5). Our values are practically identical to the values reported by Mørkøre and Lilleholt (2007) after thawing of farmed cod frozen at −10, −25, −40, −55, and −70°C. Similar values were also determined in rested farmed cod after ice storage for 7 days (Erikson et al., 2011).

WHC, related to the properties of the protein network, was not affected by freezing method. The values were reduced from about 79–81% just after thawing to about 70–72% after subsequent ice storage (Table 5). Unfrozen farmed cod show a considerably higher ability to retain water after 7 days on ice, with WHC values of 80–83% (Digre et al., 2011a) and 86–87% (Erikson et al., 2011). Freezing and thawing of wild-caught Atlantic cod reduced WHC from about 85% (fresh fish) to 82% (Erikson et al., 2004). Thus, in the present study, it seems that the freezing and thawing process per se caused the rather low WHC values observed here.

Just after thawing, the drip loss ranged from 1.9 to 2.9% of body weight. Further ice storage increased drip loss to 4.8 to 5.5% of body weight. However, on both days after thawing, freezing method did not affect the susceptibility of muscle tissues to lose water ($p > 0.05$).

NMR proton relaxation behavior

NMR water proton relaxation behavior is thought to be related to the three-dimensional structure of tissues by a proton exchange between water and macromolecules (biopolymers). If, for example, changes in muscle tissues would occur as a result of fish processing, this can be detected as changes in proton relaxation times (Erikson et al., 2004). A correlation between NMR relaxation times and light microscopy of salmon muscle has been described by Aursand et al. (2009).

The T_{21} relaxation components did not differ between treatments ($p > 0.05$). Nor did relaxation behavior change after further ice storage except from a slightly lower value for the ABF fish (Table 5). The mean transversal relaxation time components ranged from T_{21} = 44.1–47.2 ms. For the longer relaxation component (T_{22}), there were significant differences just after thawing as ABF fish and unstressed CAS2 fish exhibited lower mean values (129.8 ms) than stressed CAS1 fish (144.7 ms). If we assume that T_{22} is associated with juice exudation (drip loss; Steen and Lambelet, 1997), this might suggest that water is lost more easily from stressed fish. After ice storage, significant differences between treatments could no longer be detected even though the mean values suggested the following order of the T_{22} values: 136.4 ms (CAS), 145.1 ms (ABF), and 156.9 ms (CSF). The corresponding proton populations of the two relaxation components did not differ between treatments as determined just after thawing ($p > 0.05$), and their values were approximately A_{21} = 79–80% and A_{22} = 20–21% (Table 5). After ice storage, the distribution of protons changed to about A_{21} = 76–79% and A_{22} = 22–24 %, indicating a tendency to increased drip loss.

T_{21} values of frozen-thawed Atlantic cod have been reported as 50 ms (Jepsen et al., 1999; Erikson et al., 2004) and 32–46 ms depending of frozen storage time and temperature (Steen and Lambelet, 1997). The corresponding T_{22} mean values in these studies were 306, 117, and 122–224 ms. Moreover, Hurling and McArthur (1996) demonstrated that T_2 relaxation times of cod, stored at −70 and −22°C, subjected to either slow or fast thawing and re-freezing, showed no clear changes in the amount of what they referred to as "free water" (T_{22}). Moreover, all treatments exhibited similar patterns of water distribution, even after 9 months of frozen storage. Even though refrozen cod were

less able to retain water on thawing and centrifugation, the NMR data did not indicate changes in the proportion of water related to the two "water phases" (A_{21} and A_{22} populations). Depending on storage time (2 or 4 months) and storage temperature (−10, −20, or −70°C), Steen and Lambelet (1997) reported A_{21} and A_{22} populations of 47–64 and 36–53%, respectively.

Gaping

Gaping scores were generally low, both after thawing and further ice storage. The mean values of all treatments did not differ ($p > 0.05$), and they were always within scores of 1.2 to 2.2. These scores correspond to < 5 small slits (< 2 cm) up to < 10 small slits. By using the same method, Mørkøre and Lilleholt (2007) reported similar values (1.4–2.5) after thawing of farmed cod. Rested farmed cod stored on ice for 7 days resulted in gaping scores of 1.9–2.9 (Erikson et al., 2011).

Hardness

Perhaps due to our comparatively low storage temperatures (−27 and −32°C) and short storage time of 47 days, our sensory impression of the fillets was that they were firm without any clearly apparent flaws. Instrumental measurements of hardness just after thawing did not reveal differences among treatments ($p > 0.05$). The fillet hardness values were then around 10 N (Table 5). Other reported hardness values of fillets cut from farmed cod stored on ice for 7 or 8 days, obtained by use of the same instrumental method, are 11–14 N (Digre et al., 2011a, 2011b; Erikson et al., 2012), 5–6 N (Erikson et al., 2011), and 12–17 N (Digre et al., 2010). Hardness values measured after ice storage were discarded because instrument set points were accidentally not stored.

Additional CAS experiment

In both tap water and gutted salmon, all measured temperatures approached −40°C after 3.5–4 h in the CAS freezer. The temperature profiles of both products exhibited similar behavior, as the temperature drop was faster near the bottom than in the center of the bottle and faster subcutaneously than in the core of the fish (data not shown). This indicates that the freezing mechanism was similar to traditional freezing methods where the freezing gradually proceeds from the outside towards the center (core) of a given product. This is basically in line with Watanabe et al. (2011), who concluded that weak oscillating magnetic fields have no apparent influence on temperature history during freezing of pure water, NaCl solution, and various foods within their experimental conditions.

Conclusions

Regardless of freezing method, the thawed cod were regarded as good-quality fish. Our study basically represented an ideal case, where the fish were frozen just after killing, at relatively high freezing rates (in two of three cases). A low and constant storage temperature regime prevailed, storage time was comparatively short, and the thawing process was slow. Under such conditions, changes in sensory attributes of thawed and cooked fish would be expected to be quite small (Hedges, 2002). In spite of the considerably slower freezing rate of fish frozen directly in the cold storage freezer, this did not result in inferior quality. Based on the measured temperature profiles during freezing of water and salmon in CAS, it seemed that the freezing mechanism was "traditional"; that is, from the outside toward the core of the products. We cannot, however, exclude the possibility that the magnetic field might have had some effect not measured by the battery of tests employed here. Differences in ante-mortem stress levels did not affect cod quality as observed after freezing and thawing (except from some minor differences in NMR relaxation parameters). This is in

line with previous studies, where handling stress before slaughter of farmed cod merely has a modest effect on quality characteristics after ice storage (see, for example, Erikson et al., 2011).

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