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Research note

Influence of different cold storage temperatures during the *Rigor mortis* phase on fillet contraction and longer-term quality changes of Atlantic cod fillets

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ABSTRACT

Pre rigor produced fillets of Atlantic cod become shorter and more firm than *post rigor* produced fillets. In *pre rigor* excised muscle from warm-blooded animals and warm-water adapted fish, cold shortening, extensive contraction during cold storage, is known to occur. The aim of the present work was to study if the extent of fillet shortening in Atlantic cod could be reduced by a slight temperature increase during rigor contraction. The results demonstrate that fillets from this cold-water species showed no cold shortening. On the contrary, the fillets contracted the least when stored at 0 °C during *rigor* contraction.

1. Introduction

Industrial filleting of fish should be carried out *pre* or *post rigor mortis* (Huss, 1995; Love, 1988). *Pre rigor* filleting has several advantages. The fillets will arrive earlier to the markets, and the sales period may be extended compared to *post rigor* fillets (Skjervold et al., 2001; Tobiassen et al., 2006). Fillets produced *pre rigor* have firmer texture and less gaping than fillets processed *post rigor*, i.e. 3–4 days *post mortem* (Einen, Guerin, Fjaera, & Skjervold, 2002; Kristoffersen, Tobiassen, Esaiassen, et al., 2006; Skjervold et al., 2001; Tobiassen et al., 2006).

Pre rigor filleting may however have some disadvantages. A striking feature is the shrinkage caused by the non-hindered contraction of the muscle during *rigor mortis*. The change in fillet length varies according to species (Akse, Birkeland, Tobiassen, Joensen, & Larsen, 2008; Lee et al., 1998; Misimi, Erikson, Digre, Skavhaug, & Mathiassen, 2008), nutritional status and *pre* slaughter activity (Mørkøre, Mazo, Tahirovic, & Einen, 2008; Stien et al., 2005; Tobiassen et al., 2006). If the shortening is extensive, the fillets show a severely altered shape and a rough surface (Mørkøre, Hansen, & Rørvik, 2006). It has also been reported that *pre rigor* produced fillets may have a larger weight reduction due to increased drip loss during storage *post mortem* compared to *post rigor* produced fillets (Kristoffersen, Vang, Larsen, & Olsen, 2007).

For many animal and fish species the strength and extent of the *rigor* contraction of a *pre rigor* excised muscle is dependent on temperature. The shrinkage of *pre rigor* produced meat from warm blooded animal is reduced successively when temperature of the meat decreases to 10–20 °C below the physiological temperature. At lower temperatures the extent of shortening will increase again (Locker & Hagyard, 1963; Strasburg, Xiong, & Chiang, 2008). Several studies have shown that such “cold shortening” may occur in fish from temperate or tropical waters, *e.g.* fillets of plaice (*Paralichtys olivaceus*) contract more at 0 °C compared 5–15 °C (Iwamoto, Yamanaka, Watabe, & Hashimoto, 1987), and fillets from chinook salmon contract more at 0 °C than at 12 °C (Jerrett, Holland, & Cleaver, 1998). Such temperature dependent contractions have also been reported for *pre rigor* excised muscle of farmed red sea bream and Japanese flounder (Lee et al., 1998). There are very few studies regarding how temperature affects the development of *rigor mortis* in cold-water fish.

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The aim of the present work was therefore to study if the extent of fillet shortening in Atlantic cod, both wild and farmed, could be reduced by a slight increase in cold storage temperature during *rigor mortis* contraction.

2. Materials and methods

2.1. Fish material and storage

Wild Atlantic cod (*Gadus morhua* L.) ($n = 29$) were caught using Danish seine off the coast of Finnmark, Northern Norway in May. The fish were kept alive without feeding in net pens for three weeks at the Aquaculture Research Station, Tromsø. Farmed cod ($n = 14$), fed commercial cod feed, were raised at the same research station. The average weight and length for wild cod were 1800 ± 300 g and 63 ± 4 cm. For farmed cod the mean weight and length was 2400 ± 450 g and 64 ± 3 cm. The biological condition expressed as Fulton's condition factor ($K = 100^*(W/L^3)$, W = total weight (g), L = fork length (cm); Nash, Valencia, & Geffen, 2006) was 0.74 and 0.90 for the wild and the farmed cod, respectively.

The fish were carefully netted and stunned by a blow to the head, followed by cutting the isthmus and bleeding in sea water (8–9 °C) for approximately 30 min. After measuring weight and length, the fish were gutted, stored in ice and transported to the laboratory.

Within three hours after slaughter all fish were manually filleted and de-skinned. Individual fillet weights and lengths were measured. The wild and farmed cod fillets were divided into three groups each and put in individual and numbered plastic bags ensuring fillets from the same fish were in different groups. One group from wild cod fillets and one from farmed cod were stored in ice (0 °C), one group from each batch were placed at 4 °C, and the last two groups were stored at 7 °C for the first 48 h *post mortem*. During storage, fillet temperature was confirmed in two fillets at each storage temperature using temperature loggers (177-T4 loggers, Testo, Germany). The *rigor* contraction was completed after 48 h, and all groups were stored in ice (0 °C) for eight more days.

2.2. Analyses

Muscle pH was determined in the loin part of the fish through an incision in the skin two hours *post mortem* (before filleting) using a WTW 330/Set-1 pH-meter (Wissenschaftliche- Technische Werkstätten, Weilheim, Germany) equipped with a double pore glass electrode (Hamilton Bonaduz AG, Bonaduz, Switzerland).

Water content, liquid loss, pH and total volatile nitrogen (TVN) were determined at the end of the experiment, 10 days *post mortem*. Prior to muscle analysis the fillet was cut in smaller pieces and chopped in a kitchen blender for 25 s. Muscle pH was measured in minced muscle suspended in an equal volume 0.15 M KCl, using a glass electrode (Radiometer analytical, Standard pH Meter PHM 210). Water content was determined drying duplicates of minced muscle at 105 °C for 24 h. Liquid loss (%) was determined using a modified version of a centrifugation method described by Rørå, Regost, and Lampe (2003). Liquid was collected by means of a pre-weighted filter paper (filter paper circles, Whatman 589/1, Ø150 mm) which was folded and placed on top of the sample (10 g) in a disposable plastic tube (Greiner centrifuge tubes, 50 ml, Sigma–Aldrich). Samples were centrifuged for 10 min at 5 °C and 500 × *g* (Eppendorf Centrifuge 5810 R). Total Volatile Nitrogen (TVN) was determined by the Kjeldahl method (Tecator, 1983) and expressed as mg TVN per 100 g fish muscle.

2.3. Statistical analysis

The fillets of both wild and farmed cod were randomly distributed into three temperature groups ensuring that the two fillets from the same fish were stored at different temperatures, either 0 and 4 °C, 0 and 7 °C or 4 and 7 °C. This pairwise design is taken care of in the statistical analyses by including *cod ID* as one of the independent variables in the model. The changes in length and weight were transformed into per cent reduction of initial length (*Contraction*), and per cent *Weight loss* before carrying out the statistical analyses.

The effect of storage temperature on length reduction and weight loss was analyzed using repeated measurement models with storage *time*, *temperature* level and *cod ID* as independent variables, and either *Contraction* or *Weight loss* as the dependent variable. All independent variables were treated as categorical variables. Significance tests for *Contraction* and *Weight loss* have been carried out on data from both wild and farmed cod, and storage time less than two days and more than two days. In order to adjust for some of the effect of multiple testing on the simultaneous significance level, the significance level was reduced from 0.05 to 0.01. Only *p*-values below 0.01 were considered significant, which is the Bonferroni correction for a multiple of five significance tests. The statistical program *R* (Free Software Foundation: http://www.r-project.org) and in particular the function *lme* (Linear and Nonlinear Mixed Effects Models) from the *R*-library was used for data analysis. A two-way analysis of variance (ANOVA) was also performed on data registered 10 days *post mortem*, comparing storage temperatures and wild cod versus farmed cod concerning water content, liquid loss and pH.

3. Results and discussion

The pH in farmed cod muscle two hours *post mortem* was 7.5 and 7.7 in wild cod fillets, indicating non-stressful slaughtering (Kristoffersen, Tobiassen, Steinsund, & Olsen, 2006).

3.1. Contraction

As expected, *pre rigor* produced fillets contract during establishment of *rigor mortis*, and maximal shrinkage was achieved after approximately 36 h (Fig. 1). The wild cod contracted much less than

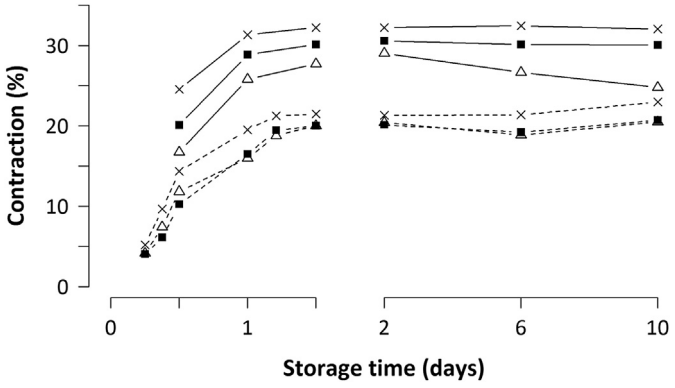

Fig. 1. Average contraction (% of initial fillet length) in fillets from wild (dotted line) and farmed (solid line) Atlantic cod during storage. *Pre rigor* produced fillets were stored at 0 (△), 4 (■) or 7 °C (×)for the first 48 h after slaughter. All fillets were stored at 0 °C from day 2–10 (for wild cod: $n = 19$ fillets at each temperature from day 0–6, and $n = 13$ at day 10. For farmed cod: $n = 6$ fillets at each temperature from day 0–6, and $n = 3$ at day 10).

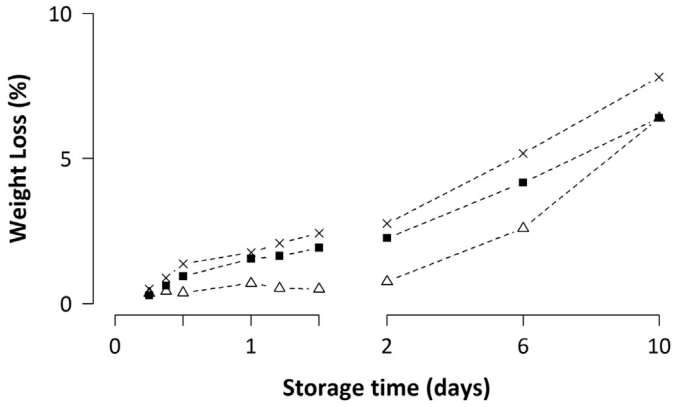

Fig. 2. Average weight loss (% of initial fillet weight) in fillets from wild cod during storage. Groups of fillets were stored at either 0 (△), 4 (■) or 7 °C (×) for the first 48 h after slaughter, and after that all fillets were stored at 0 °C from day 2–10.

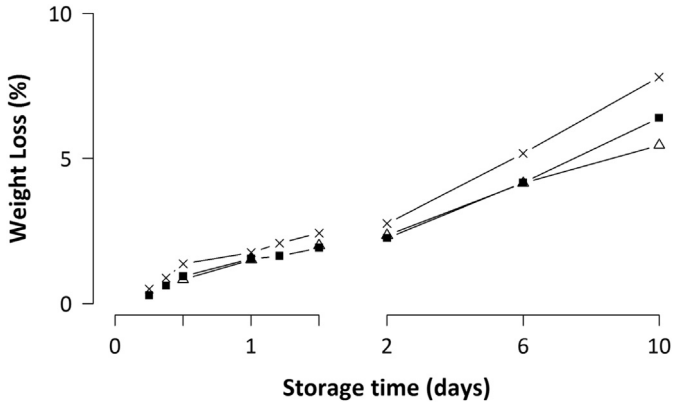

Fig. 3. Average weight loss (% of initial fillet weight) in fillets from farmed cod during storage. Groups of fillets were stored at either 0 (△), 4 (■) or 7 °C (×) for the first 48 h after slaughter, and after that all fillets were stored at 0 °C from day 2–10.

the farmed cod, and may be explained by higher condition factor and nutritional status of the farmed fish.

A maximum contraction of about 30% in the farmed cod fillets is generally higher than what is reported earlier for *pre rigor* filleted farmed cod. Kristoffersen et al., (2007) and Misimi et al., (2008) reported shortening of approximately 27 and 18%, respectively. Several factors may have contributed to these differences, like different time between slaughter and filleting (Akse et al., 2008; Strasburg et al., 2008) or if the fillets are stored with the skin on as appear to be the case in the work of Misimi et al., (2008).

Statistical analysis showed that both wild and farmed fish fillets stored at 7 °C for 48 h *post mortem* contracted significantly more than fillets stored at 0 °C ($p < 0.01$). Farmed fish fillets had shrunk 29, 31 and 32% when stored at 0, 4 and 7 °C, respectively, while the corresponding results from wild fish were 20, 20 and 21%. Mørkøre et al. (2006) did not find any difference in shrinkage of *pre rigor* excised fillets of farmed cod stored at 0 and 6 °C. However, those fillets were stored only 10 h *post mortem*, and *rigor mortis* was probably not fully developed at this stage. In the present study the difference in contraction remained during subsequent storage at 0 °C in the fillets from farmed fish. No significant difference was observed between the three temperature groups of wild fish fillets in this period. The statistical analysis verified that fillets from farmed cod stored at 0 °C from the time of filleting increased slightly in length when the *rigor mortis* was resolved (Fig. 1). Similar increase in fillet lengths after resolution of *rigor mortis* in *pre rigor* filleted farmed cod stored at 0 °C have been reported earlier (Kristoffersen et al., 2007; Misimi et al., 2008). However, the fish stored at 4 and 7 °C during the *pre rigor* phase did not show this increase in length after the resolution of *rigor*.

3.2. Weight loss

In general, the weights of the fillets decreased due to drip loss in all temperature groups of both wild and farmed cod fillets during storage (Figs. 2 and 3). However, the fillets from wild cod stored at 0 °C did not lose any appreciable weight during the first two days of storage (Fig. 2). Statistical analysis showed there was a significantly higher weight loss ($p < 0.001$) for the wild cod fillets stored at 4 and 7 °C compared to 0 °C during the initial 48 h. From day 2 to day 6 the rate of weight loss appeared similar in all three groups of the wild fish fillets, and at day 10 no significant difference could be found. The weight loss of the farmed fish fillets were not affected by the difference in storage temperature during the first two days *post mortem* (Fig. 3), and the weight loss were in the same range as the weight loss inn wild cod fillets stored at 4 and 7 °C.

The total weight loss after 10 days of storage was similar in fillets from wild and farmed cod, approximately 6–8%. This means that the more extensive contraction of 30% in the fillets of the farmed fish did not lead to a higher weight loss. The lower water content in the farmed fish fillets (Table 1) may have contributed to this. The ability of the fillets to retain liquid after 10 days of storage was investigated using a mild centrifugation test of minced muscle. The results expressed as liquid loss showed that the fillets from farmed fish had a higher liquid loss than the other group of fillets in spite of the lower water content. This is in accordance with previously reported findings for wild and farmed cod (Ofstad et al., 1996). Although the lower pH in the fillets of farmed fish may have contributed to the higher liquid loss other mechanisms are probably also responsible (Kristoffersen, Tobiassen, Esaiassen, et al., 2006; Olsson, Seppola, & Olsen, 2007; Rustad, 1992). One of these factors may be the strong muscle shortening which result in a more contracted state of the sarcomers. As reviewed by Huff-Lonergan and Lonergan (2005) such shortening may result in displacement of water from intramyofibrillar to extramyofibrillar and extracellular space. As a result, more liquid may be lost during the centrifugation test.

3.3. Storage stability

Increasing storage temperatures enhance degradation of fish, thus degradation products were analyzed to assess possible negative effects of the temporarily elevated temperature upon quality. Analysis of the total volatile bases (TVN) showed that the relatively small differences in the cold storage temperature during the *pre rigor* phase affected the quality of fillets at the end of the storage period (Table 1). Ten days *post mortem*, a significantly lower TVN value ($p < 0.001$) was detected in wild cod fillets stored at 0 °C compared to fillets stored at 4 and 7 °C (TVN values of 19.5, 38.2 and 44.8 mg/100 g, respectively). The corresponding values for the farmed cod fillets were 10.3, 13.5 and 16.3 mg/100 g. A possible explanation for the differences in TVN-levels between the farmed and wild cod fillets could be lower levels of trimethylamine oxide (TMAO) and reduced growth of microorganisms in farmed cod compared to wild cod, as previously reported by Herland, Esaiassen, and Olsen (2007), Herland, Esaiassen, Cooper, and Olsen (2009) and Olsson et al. (2007).

Table 1
Properties of *pre rigor* produced cod fillets 10 days *post mortem*. The fillets were initially stored at 0, 4 or 7 °C for 48 h and then at 0 °C for eight days. Different lowercase letters indicate significant differences within groups of wild ($n = 6$ in each group) and farmed cod ($n = 3$ in each group), while different capital letters indicate significant differences between wild and farmed cod. *P*-values from two-way ANOVA with factors '*temperature*' and '*cod type*'.

	Initial storage temperature	Water content (% wet weight)	Liquid loss (% wet weight)	Muscle pH	TVN (mg/100 g)
Wild cod	0 °C	81.8 ± 0.5	6.2 ± 0.9[a]	6.63 ± 0.04	19.5 ± 5.0[c]
	4 °C	81.3 ± 0.3	4.7 ± 0.73[b]	6.61 ± 0.07	38.2 ± 9.9[d]
	7 °C	81.3 ± 0.5	4.9 ± 1.2[a,b]	6.63 ± 0.07	44.8 ± 10.0[d]
	Mean	81.5 ± 0.5[A]	5.3 ± 1.1[A]	6.62 ± 0.06[A]	34.1 ± 13.7
Farmed cod	0 °C	80.1 ± 1.4	8.6 ± 2.0	6.41 ± 0.04	10.3 ± 4.1
	4 °C	80.5 ± 0.5	12.1 ± 0.6	6.16 ± 0.04	13.5 ± 2.3
	7 °C	80.4 ± 0.5	9.6 ± 0.3	6.24 ± 0.08	16.3 ± 2.4
	Mean	80.3 ± 0.8[B]	9.9 ± 2.0[B]	6.27 ± 0.12[B]	13.4 ± 3.7

[a] and [b] : *P*-value < 0.01.
[c] and [d], [A] and [B] : *P*-value < 0.001.

4. Conclusions

The results demonstrated that fillets from the cold-water species Atlantic cod, both farmed and wild, showed no cold shortening. On the contrary, the fillets contracted significantly less when stored at 0 °C during *rigor* contraction compared to 7 °C. Initial storage at 0 °C resulted in a lower weight loss in the wild cod fillets, compared to storage at 4 and 7 °C. The weight loss in farmed cod fillets were not significantly affected by the different temperatures. The total weight loss was similar, 6–8%, in both types of cod fillets despite the more extensive contraction in the farmed cod fillets.

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