Muscle temperature at point of filleting - Subsequent effect on storage quality of pre rigor filleted raw- and cold-smoked Atlantic salmon

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Abstract

The impact of increased muscle temperature at point of filleting on fillet quality of raw- and cold-smoked Atlantic salmon was investigated. Commercially reared fish (5.65 Kg, Kf: 1.23, pH: 7.29, muscle temperature: 6.68 °C) were killed and immediately tempered in three different containers. Muscle temperatures after filleting (< 3 hours post mortem) of the three groups were 2.08 °C (herby named T-2); 9.07 °C (herby named T-9,) and 14.09 °C (herby named T-14), respectively. The pH after filleting was significantly lowest for T-14 (6.93) followed by T-9 (7.06) and T-2 (7.22). Raised temperature at point of filleting was found to significantly alter development of rigor mortis, which subsequently affected muscle pH and the reflective properties of the fillet surface during 14 days ice storage. Of cold-smoked fillets however, a more distinct effect of raised temperature was observed on visual perception resulting in lighter and more yellowish cold-smoked fillets after 14 days storage. In addition, raised temperature also affects development of muscle pH in cold-smoked fillets during refrigerated storage. No effects of raised muscle temperature were found regarding drip loss, water holding capacity or fillet firmness neither for raw- or cold smoked fillets throughout the storage period.

Introduction

Pre- and post mortem muscle temperature are among several factors which affect the quality of farmed Atlantic salmon. It is accepted that high sea water temperature at time of slaughtering results in decreased shelf-life, poor fillet quality and increased gaping (LavÉTy et al., 1988; Love et al., 1969; Sikorski, 1989; Kiessling et al., 2006). These findings resulted in a considerably focus, in the late 90ties, to reduce muscle temperature before slaughtering of Atlantic salmon (Skjervold et al., 2002; Skjervold et al., 2001a; Skjervold et al., 1996). Today's practice includes either live chilling and/or chilling during exsanguination to secure a low muscle temperature during primary processing which is especially important during the growth season where sea water temperatures can reach up to 16-18°C. Live-chilling of salmon designated for pre rigor filleting is expensive due to a higher input of energy to reduce temperature of a whole salmon as compared to only the fillets. It is therefore interesting to show if a high muscle temperature at time of pre rigor filleting influences quality aspects, in the same pattern, as it do to whole fish or post rigor fillets.

The onset and strength of *rigor mortis* is dependent on several pre- and post mortem factors such as temperature and handling before harvest, metabolic activity of the fish, pre-slaughtering stress, post mortem temperature and shows large individual variation (Azam et

al., 1989; Sigholt et al., 1997; Jerrett et al., 1998; Mørkøre et al., 2008; Skjervold et al., 1999; Roth et al., 2012; Roth et al., 2006). These factors all results in accelerated autolysis and provide a rapid drop of muscle pH, which is related to flesh softening and reduced water holding capacity (Roth et al., 2002; Aursand et al., 2010). High temperature or short periods of high temperature during processing and/or transport will in all likelihood influence the drip loss. It has also been demonstrated that super chilling can have a negative effect on the water holding capacities in salmon (Hansen et al., 2009).

Surface colour and appearance are important decision-makers for consumers when purchasing raw- and smoked salmon products (Gormley, 1992; Sylvia, 1996; Anderson, 2000). Colour of salmon flesh is affected by many different parameters, among others; composition and amounts of carotenoids in the feed (Bjerkeng, 2000; Bjerkeng, 2008), genetic background (Torrissen and Naevdal, 1988), seasonal variations (Mørkøre and Rørvik, 2001), starvation and stress prior to slaughtering (Robb et al., 2000; Einen and Thomassen, 1998; Erikson and Misimi, 2008; Mørkøre et al., 2008), slaughtering procedures (Kiessling et al., 2004; Roth et al., 2010), ice chilling and temperature during frozen storage (Espe et al., 2004), muscle fibre density (Johnston et al., 2000), and salting and smoking procedures (Birkeland et al., 2004; Lerfall et al., 2011).

The joint focus of mostly all research so far about effects of relatively high temperature has been performed on whole salmon or post rigor fillets. Therefore; the aim of this study was to investigate the effects of increased muscle temperature on rigor mortis, drip loss, textural and reflective properties of raw pre-rigor filleted Atlantic salmon. In addition, subsequent effects on quality of cold-smoked fillets were investigated.

Material and methods

Fish material and experimental design

In this study a total of 63 Atlantic salmon (*Salmo salar* L.) were sampled in February 2014 at a commercial slaughterhouse in the middle of Norway. All fish (5.65±0.95 Kg, Kf: 1.23±0.08, pH: 7.29±0.11, muscle temperature: 6.68±0.19) were taken from the pre-slaughtering netpen after approximately 48 h resting and instantly killed by a blow to the head. Muscle pH and temperature were measured continuously before the fish was exsanguinated by gill cutting and tempered in three different containers (1000L) containing ice slurry or fresh water (temperature of: 0, 8 and 16 °C, respectively, n=21 salmon at each temperature). Muscle temperature was followed during exsanguination, and at temperature equilibrium, length and gross weight of the salmon were measured. The fish was thereafter gutted, weighted and

machine filleted pre rigor according to standard procedures. The muscle temperatures after filleting of the three groups were 2.08±0.47 °C (herby named T-2); 9.07±0.08 °C (herby named T-9,) and 14.09±0.19 °C (herby named T-14), respectively.

The right and left fillets were split into two different experiments. In *experiment 1*, the left fillets (n=21 of each group) were stored on ice in a refrigerated room (4.56±0.38 °C) for 14 days to show effects of increased muscle temperature at point of filleting on drip loss, water holding capacity (WHC), texture and reflection of light from the fillet surface in the range between 405-970 nm. In *experiment 2*, right fillets (n=7 of each group), were used to follow rigor mortis contractions during 144 hours ice storage. At day 6, these fillets used for rigor measurements were salted, cold-smoked and vacuum packaged. Weight changes, colorimetric characteristics (CIE, 1994) and pH were followed at each step in the cold-smoke process and during 28 days refrigerated storage (4.82±0.43 °C). After 28 days storage, dry matter (DM)- and sodium chloride (NaCl) content, texture and reflection properties were measured.

Chemical composition of the raw material

Chemical composition of the raw material was determined in the left fillet of salmon exsanguinated in water at 0 °C (T-2) 6 days post mortem (n=5). A cylinder (diameter 31 mm) was punched out from the dorsal part in front of the Norwegian Quality Cut (NQC) and stored at -80 °C until further analyses (Figure 1A). The muscle samples were thereafter homogenized individually and the dry matter was estimated gravimetrically after drying at 105 °C for 24 hours (ISO, 1983). Total fat was extracted and calculated by the method of Bligh and Dyer (1959) with slight modifications. Nitrogen content was measured on a Tecator Kjeltec system (Model 2020 Digestor and 1026 Distilling unit, Tecator, Höganäs, Sweden) (NCFA, 2003). Protein content was calculated from nitrogen measurements using the formula: %protein = %nitrogen × 6.25. Astaxanthin in tissue were extracted (Bligh and Dyer, 1959) and analyzed by HPLC using an Agilent1100 liquid chromatograph (Agilent Technologies, Paolo Alto, CA, USA) connected to an Agilent photodiode array UV-VIS detector. Astaxanthin was analyzed by the method of Vecchi et al. (1987) using a Lichrosorb SI60-5, 125*4.0 mm, 5 µm, Hichrom, Reading, UK, HPLC column modified with orthophosphoric acid (0.1% in CH_3OH).

Muscle pH and temperature

Muscle pH and temperature was measured right after death and after filleting in the anterior part of the dorsal muscle using a Mettler Toledo SevenGo pro^{TM} pH-meter (Mettler Toledo

Inc, USA) connected to an Inlab puncture electrode. During the exsanguination step the muscle temperature was followed in 3-4 fish at each temperature (0, 8 and 16 °C, respectively) using an E-Val Flex temperature system connected to seven thermocouples, (Ellab A/S, Hilleroed, Denmark). Moreover, during storage of the raw fillets (experiment 1), muscle pH and temperature was measured anterior to the dorsal fin at each sampling day (6, 10 and 14 days post mortem, Figure 1A). Of the right fillets (*experiment 2*), pH and temperature was measured at the end of the rigor measurements (initial smoking pH), after smoking and after 14 and 28 days refrigerated storage.

Rigor mortis measurements

The right fillets (n=7 of each group, in total 21 individuals, experiment 2) were used to follow *rigor mortis* during ice storage over a period of 144h. *Rigor mortis* were followed with an interval of 6 hour by measuring the length between 6 needles (3 in the dorsal- and 3 in the belly part of the muscle, respectively, Figure 1B).

Cold-smoking procedure

All fillets used to measure rigor contractions (n=7 of each group, in total 21 individuals, *experiment 2*) were dry salted on grids (22 hours, 4 °C, fine refined salt, minimum 99.8% Sodium Cloride (NaCl), GC Rieber, Norsal, Trondheim, Norway) at day 6 post mortem. Before drying and smoking all fillets were rinsed in cold water (~8 ºC) to remove excess of NaCl. Salt-cured salmon fillets were thereafter randomized on grids and dried at 22 °C for 180 minutes, then cold-smoked for 180 minutes (22-24 °C) in a Kerres smoke-air® showsmoker CS700 EL MAXI 1001 smoking cabinet (Germany).

Drip loss, dry matter and water holding capacity

Drip loss (DL) from the fillets was calculated as the difference in fillet weight between day 0 and day X of both raw and cold-smoked fillets. In addition, the mass transfer during salting and smoking was followed.

DL = $\frac{m_0 - m_x}{m_0} \times 100\,\%$, where

m_0: fillet weight at t_0

m_x: fillet weight at t_x

Water holding capacity (WHC) and dry matter (DM) of raw fillets (*experiment 1*) was measured in the belly part of NQC after a method described by Skipnes et al. (2007). WHC was measured at each sampling day (6, 10 and 14 days post mortem) on a defined area of the fillet (diameter 31mm, high 6 mm, approximately 5 g, Figure 1A). DM of cold-smoked fillets was measured at the end of the storage period (day 28) on a defined area of the fillet (diameter 31mm, high 6 mm, approximately 5 g, Figure 1B).

Texture

Instrumental textural analyses were performed using a Texture Analyser TA-XT2 (SMS Ltd., Surrey, England) equipped with a 25 kg load cell. A flat-ended cylinder probe (20 mm diameter, type P/1SP) was used. The force-time graph was recorded by a computer equipped with the Texture Exponent light software for windows (version 4.13, SMS), which was also used to analyze the data. Analyses were performed in duplicates (average values were used in data analysis) of each raw fillet (*experiment 1*) 6, 10 and 14 days post mortem (Figure 1A). Moreover, textural properties of the cold-smoked fillets (*experiment 2*) were measured at the end of the storage period (day 28, Figure 1B). The resistance force (N) in raw fillets were recorded with a constant speed of 5 mm sec^{-1}, and the surface breaking force (BF) and the force required to press the cylinder down to 60 % of fillet thickness (F60%) was used to describe firmness. However, on smoked fillets the resisting force was recorded at 30% of fillet thickness and presented as F30% (N).

Reflective- and colorimetric assessments

Multispectral imaging was carried out on a VideometerLab (Videometer A/S, Hoersholm, Denmark) system measuring the light reflected from the surface of raw fillets (*experiment 1*, Figure 1A) at day 6, 10 and 14 post mortem and of cold-smoked fillets (*experiment 2*, Figure 1B) at day 28. This system is based on a high-intensity integrating sphere illumination featuring light emitting diodes (LED) together with a high-resolution monochrome grayscale camera (Dissing et al., 2011). The data acquisition was done by imaging the fillet surface at 18 different wavelengths ranging from 405 to 970 nm. Before use, the system was calibrated radiometrically using both a diffuse white and a dark target followed by a light setup optimized to fit the object of interest. The data collected from the image at each wavelength was an average of all pixels recorded in the area of interest of each sample.

Colorimetric assessments (CIE, 1994) were performed in *experiment 2*, to follow colorimetric changes during salting, smoking and vacuum storage (at day 14 and 28) of the cold-smoked

fillets. The measurements were taken in triplicates (Figure 1B) with a Minolta Chroma meter, CR200 Minolta, Japan. L^* describes the lightness of the sample, a^* intensity in red ($a^* > 0$) and b^* intensity in yellow ($b^* > 0$).

Sodium chloride content in smoked fillets

Sodium chloride (NaCl) content was measured in cold-smoked fillets by a Chloride Analyser (Model 926 Sherwood Scientific Ltd.) after 28 days storage. Samples (1-1.5 g) were taken from the anterior part of the dorsal muscle (Figure 1B) and added hot deionised water (30 ml), homogenized (9500 rpm, 45 sec.) by an Ultra-Turrax T25, Janke & Kunkel IKA®-Labortechnik, Staufen, Germany and heated in a water bath (100 ºC, 10 min), cooled to room temperature and diluted to 100 ml in a volumetric flask before analyses.

Statistics

Data were analyzed by a univariate- or multivariate general linear model (GLM), one-way ANOVA, regression (R) or correlation (Pearson`s correlation coefficient, r) analyses using IBM SPSS statistics software (release 21, IBM corporation, US). To compare different groups Tukey`s pairwise comparison test were used. The alpha level was set to 5% ($P<0.05$). All results are given as mean ± SD, unless otherwise is stated.

Results and discussion

Chemical composition of the raw material

Averaged muscle dry matter (DM), protein and lipid content in the raw material sampled for analyses were 34.44±2.58%, 22.33±0.82% and 10.35±2.84%, respectively. Comparable white muscle DM content (Lerfall and Østerlie, 2011; Skjervold et al., 2001b), protein (Shearer et al., 1994) and lipid (Aursand et al., 1994) content in farmed Atlantic salmon have been reported elsewhere. The total content of muscle carotenoids was found to be 6.49±0.40 mg kg^{-1} where astaxanthin contributed with 5.75±0.44 mg kg^{-1}. In addition to astaxanthin, significant amounts of lutein were found (0.74±0.07 mg kg^{-1}).

Experiment 1: Quality characteristics of raw salmon fillets stored on ice for 14 days

Muscle pH, water holding capacity (WHC), rigor mortis and drip loss of raw fillets during ice storage

The development of muscle pH and muscle WHC of the different groups T-2, T-9 and T-14 during 14 days ice storage are presented in Table 2. No significant difference in initial muscle pH indicates an equal origin. However, adjusted muscle temperatures during exsanguination resulted in a significant reductions of muscle pH after bleeding related to increased temperature ($R = 0.716$, $P < 0.001$), which can be explained by increased reaction rates and acceleration of autolysis (Jerrett et al., 1998). Because of *rigor mortis*, a significant (GLM, $P < 0.001$) drop in muscle pH was observed for all groups from day 0 to day 6. At day 6, pH of group T-14 was numerically but insignificantly lower as compared to group T-2 ($P = 0.068$) and T-9 ($P = 0.361$). This tendency became significant ($P < 0.05$) at day 10, whereas no significant differences in muscle pH were observed between the groups at day 14. The WHC in muscle foods is known to be affected by pH, where pH close to the isoelectric point is known to lowering the WHC (Huff-Lonergan and Lonergan, 2005). In this study however, the differences observed in muscle pH are probably too small to give significant effects on WHC of the salmon muscle between the actual groups sampled for analyses.

At point of filleting, none of the salmon had visible signs on rigor mortis contractions, which means that all salmon were regarded as filleted pre rigor. However, significantly decreased pH in fillets of groups T-14 and T-9 during exsanguination indicates faster start of autolysis in those salmon. The development of *rigor mortis* was significantly affected by the fillet temperature at point of filleting ($P < 0.001$, Table 1), which is in line with earlier findings by Kiessling et al. (2006) who concluded that reduced storage temperature always prolongs the rigor process. In our study, shortest time from point of filleting to maximum contraction was observed in the groups T-9 and T-14 (30.4±5.1 hours and 30.9±6.3 hours, respectively). In group T-2 maximum rigor contraction occurs after 44.9±5.3 hours. Moreover, 96 hours post filleting, all fillets were regarded as post rigor.

No significant differences (GLM, $P > 0.523$) in drip loss (DL) were observed between the groups T-2, T-9 and T-14 during 14 days ice storage (Figure 2). Isolated from other days however, T-2 showed significantly lower DL at day 6 as compared to T-9 and T-14 (one-way ANOWA, $P < 0.05$). The DL was however affected by storage time as a result of muscle degradation (Ofstad et al., 1996; Ofstad et al., 1995). The linearity of the DL during storage, was found to be better in the group T-2 ($R^2 = 0.97$) as compared to group T-9 and T-14 ($R^2 =$ 0.86 and 0.80, respectively). Moreover, a low but significant correlation between muscle pH

and DL during storage was observed ($r = -0.311$, $P < 0.05$). The DL from salmon fillets consist of mainly water, proteins and lipids and is affected by a drop in muscle pH owing to anaerobic glycolysis (Ofstad et al., 1995), and by ultra-structural changes post mortem (Ofstad et al., 1996). However, other factors than pH are of major significance and there is a requirement for more research in order to understand the underlying mechanisms (Mørkøre et al., 2008).

Textural properties of raw fillets during ice storage

The breaking strength (BF) and firmness (F60%) of raw salmon fillets is presented in Table 2. Neither breaking strength nor firmness was significantly (GLM, $P > 0.451$ and $P > 0.404$, respectively) affected by any of the design variables. The texture of fish fillets is related to the diameter of the muscle fibers (Sigurgisladottir et al., 1999), inversely related to the water content (Jittinandana et al., 2002; Indrasena et al., 2000) and myofibril-myofibril attachments (Taylor et al., 2002). It is known to decrease during *post mortem* storage (Espe et al., 2004). In addition, seasonal variations occur. In a study by Espe et al. (2004), seasonal variation in fillet softness was found to be most pronounced in the tail region of the fillet, and salmon harvested in February, as done in the present study, were found to be softest after 14 days of storage. However, the softening of fish sampled in February was not distinct between day 6 and 14 (Espe et al., 2004), which may explain why we in our study did not found any significant differences in fillet firmness as an effect of storage time.

Reflective properties of the fillet surface of raw fillets during ice storage

Reflective properties of the fillet surface in the visible- (405-700nm) and the near infrared spectra (700 to 970nm) are presented in Figure 3. The fillet surface of fillets from group T-2 reflect numerically less light after 6 days ice storage as compared to fillets from the groups T-9 and T-14 (significantly at 570 nm (yellow), 940nm (UV) and 970 nm (UV) ($P < 0.05$), otherwise insignificant). It is likely to believe that this difference at 570 nm (in the yellow area) is related to faster degradation of fillets from group T-9 and T-14 as compared to T-2. At day 10 the differences was smaller and insignificant but numerically still in the same order (reflection of light: T-2 < T-9 < T-14). After 14 days storage this order had however equalized, and numerically equal reflective properties were observed between the different groups. The reflection properties of the salmon muscle show high reflection above 570 nm as well as low reflection properties between 405 and 570 nm. This is in match with a high

absorbance of light in the violet, blue and green area, while the yellow, red and dark area is highly reflected, giving the salmon muscle its characteristic pink colour (Dissing et al., 2011).

Experiment 2: Processing and quality characteristics of cold-smoked salmon fillets stored for 28 days

Mass transfers during salting, cold smoking and storage of cold-smoked salmon fillets

The dry salting procedure resulted in an average weight loss of 5.0±0.5% whereas the total loss after drying and smoking ended at 10.3±1.0%. In addition, during 28 days refrigerated storage a drip loss (mostly lipids) of 2.3±0.3% was observed. The flux of salt into the fillet (on average 51.2±6.9 g kg^{-1}) resulted in a total loss of 176.8±13.4 g kg^{-1} of the original muscle components (mostly water) during processing and 28 days storage. No significant differences in mass transfer (water out, NaCl in) during salting, cold smoking or storage was observed between the respective groups sampled for analyses (group T-2, T-9 or T-14).

Physiological- and chemical parameters of cold-smoked salmon fillets

The initial pH (raw fillets, day 6, Table 3) found in *experiment 2* confirmed that the groups T-2, T-9 or T-14 did not differ in pH at day 6 (*experiment 1*, Table 1). After smoking and 14- and 28 days storage however, significantly lower pH was observed in group T-14 as compared to group T-2 and T-9 ($P < 0.01$ and $P < 0.05$, respectively). This lowering in pH during storage of cold-smoked fillets of group T-14 is not explainable with autolytic mechanisms. It is therefore likely to believe that this distinct decrease in muscle pH during storage of fillets from group T-14 is a result of faster growth of lactic acid bacteria, normally accelerated after approximately 2 weeks storage (Leroi et al., 1998). This faster growth of lactic acid bacteria is probably related to increased temperature during primary processing which accelerate autolysis (Jerrett et al., 1998) and consequently microbiological growth (Hansen et al., 1996).

After 28 days storage the contents of DM and NaCl were found to be on average 43.4±2.4% and 28.9±3.7 g kg DM^{-1}, respectively. Significantly higher contents of DM were found in the group T-14 as compared to T-2 and T-9 (Table 3). Observed differences in DM was not explainable with neither contents of NaCl nor drip loss during processing and storage, and might therefore be a result of an analytical artefact. The textural properties of the cold-smoked fillets after 28 days storage did not show any significant differences between the groups. The group T-2 shows however, numerically but insignificantly ($P > 0.404$) lower firmness as compared to group T-9 and T-14.

Colorimetric- and reflective properties of cold-smoked fillets

Before and during processing all colorimetric parameters (*L*a*b**) of the respective groups were insignificant (P > 0.251). Salting and cold smoking however, resulted in darker (15.4% reduction of *L**) and less reddish (26.2% reduction of *a**) fillets (P < 0.001 and P < 0.001, respectively) as compared to the raw material. Moreover, yellowness (*b*>0*) decreased significantly during salting whereas increased yellowness as a result of the cold smoking process resulted in an insignificant change in yellowness between raw and smoked fillets (P > 0.816). After smoking, all colorimetric parameters (*L*a*b**) increased significantly, which resulted in lighter, more reddish and more yellowish fillets after 28 days of storage compared to freshly cold-smoked fillets (GLM: P < 0.001, Table 4). Lightness (*L**) were found to be significantly higher in the group T-14, both after 14 and 28 days refrigerated storage, as compared to T-2 (P > 0.008 and P > 0.004, respectively). Fillet redness (*a**) was however found to be significant higher in group T-2 after smoking as compared to the other groups (P < 0.01). After storage, this difference disappeared which resulted in an equal perception of redness between the groups after 14 and 28 days storage. Moreover, yellow perception was found to be significantly higher in the group T-14 after 14 days storage as compared to the other groups (P < 0.001). After 28 days however, this difference became insignificant because of a more distinct increase of yellowness in group T-2 and T-9 between day 14 and 28 as compared to group T-14.

Significantly highest reflection of light were measured in fillets from group T-14 (Figure 4, GLM: P < 0.001, Corrected model (405-525nm): P < 0.05; (570-970nm): P > 0.084-0.778). Between the groups T-2 and T-9 no significant differences in reflection of light was observed. This indicated that changes in the surface properties first occurs when the temperature during primary processing exceed a specific limit (in this study a short period of muscle temperature above 14 ˚C). Moreover, a significant correlation in both the visible- and the near infrared spectra (r = 0.48-0.63 and r = 0.44-0.56, respectively) between reflection of light from the fillet surface and fillet lightness (*L**) indicate a distinct effect of temperature on visual perception of cold-smoked salmon fillets.

Conclusion

The effect of increased muscle temperature (T=14, T=9 and T=2 °C) during filleting on various quality parameters was observed during 14 days ice storage. Significantly effects

were observed in a faster drop in pH and development of rigor mortis with increasing temperature, and an observed increase in reflection of light after 6 days storage from the fillet surface of salmon filleted with a muscle temperature above 9 ˚C. Insignificantly alterations were observed regarding DL, WHC and fillet firmness as an effect of temperature. Moreover, it is concluded that small differences observed in raw fillets expanded after cold-smoking which resulted in more distinct effects of temperature on visual perception of cold-smoked salmon fillets. In addition, temperature at time of filleting affects the development of muscle pH in cold-smoked fillets during refrigerated storage.

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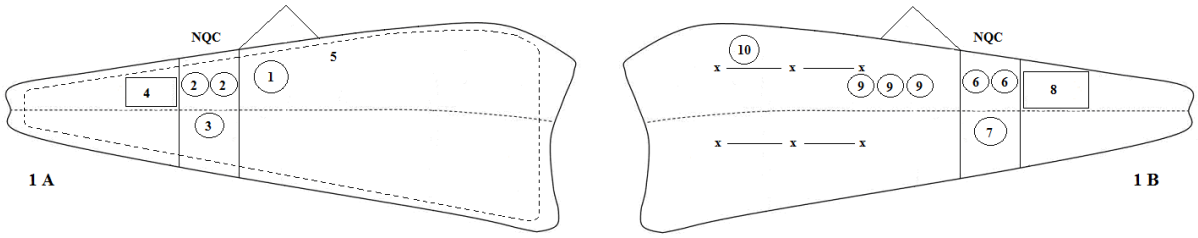


Figure 1. **A)** Schematic illustration showing the areas upon the left fillet from which analyses were conducted. 1: Chemical analysis of the raw material, 2: Textural properties of raw fillets, 3: Dry matter (DM) and water holding capacity (WHC) of raw fillets, 4: Reflection properties of raw fillets, 5: Muscle temperature and pH of raw muscel. **B)** Schematic illustration showing the areas upon the right fillet from which analyses were conducted. X: Rigor measurements of raw fillets, 6: Textural properties of smoked fillets, 7: Dry matter (DM) of smoked fillets, 8: Reflection properties of smoked fillets, 9: Colorimetric measurements of smoked fillets, 10: Analyses of sodium chloride content in smoked fillets.

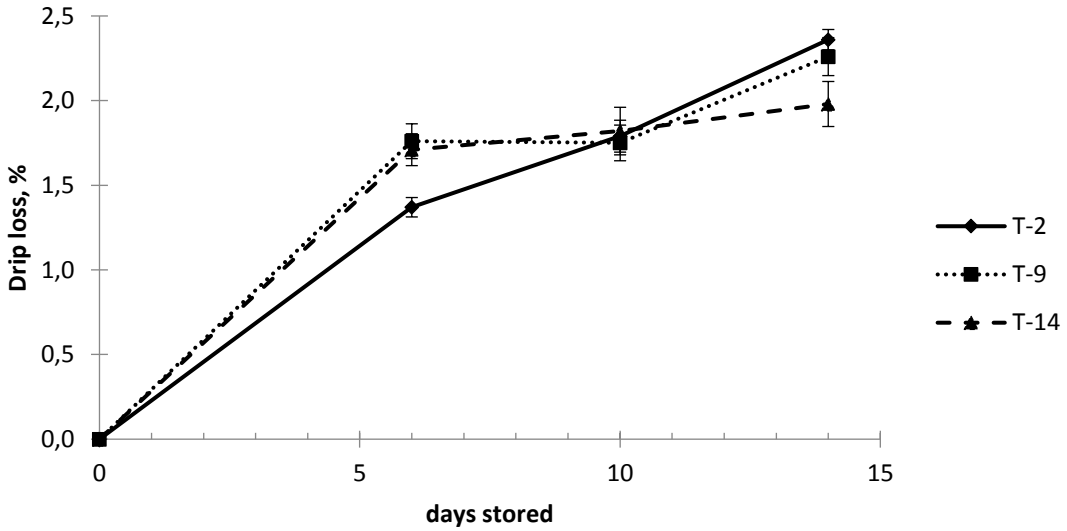
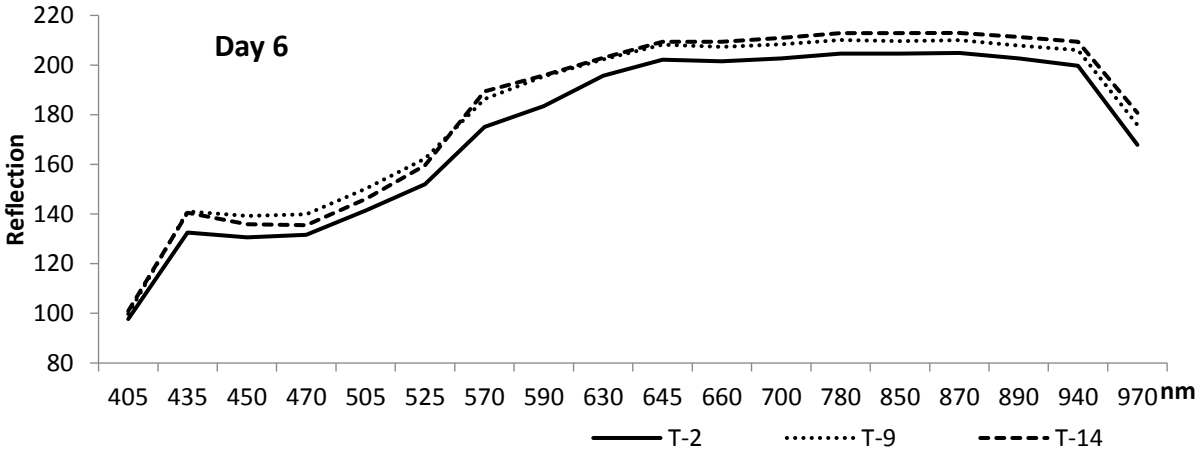


*Figure 2. Drip loss (DL, mean±SE) of raw salmon fillets during 14 days ice storage (GLM; Model: P<0.001; group: P>0.523; days stored: P<0.001; group*days stored: P<0.05).*



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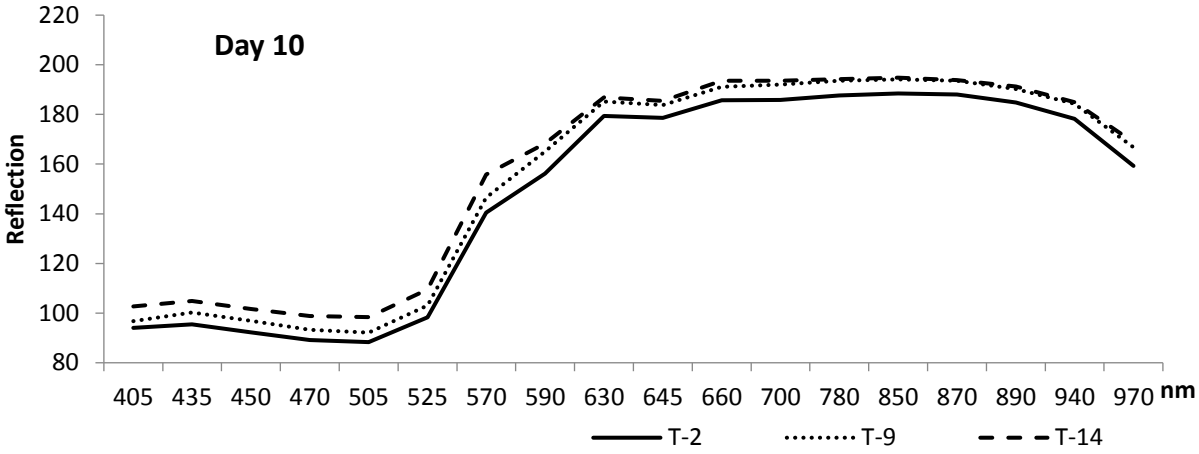

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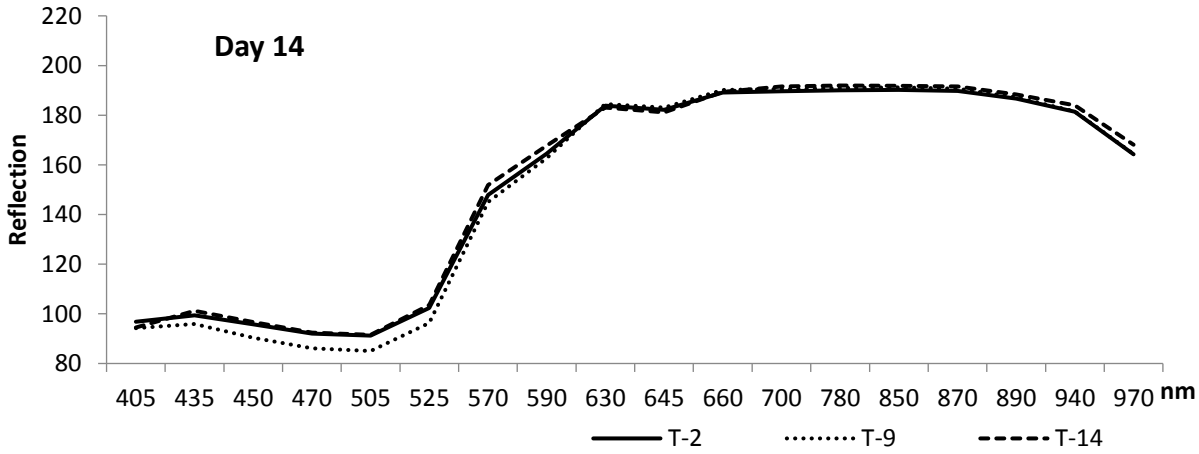

21

22 *Figure 3. Reflective properties of the fillet surface of raw salmon fillets during 14 days ice storage (GLM*
23 *multivariat; Model: P<0.001; group: P<0.001; days stored: P<0.001; group*days stored: P<0.001).*
24

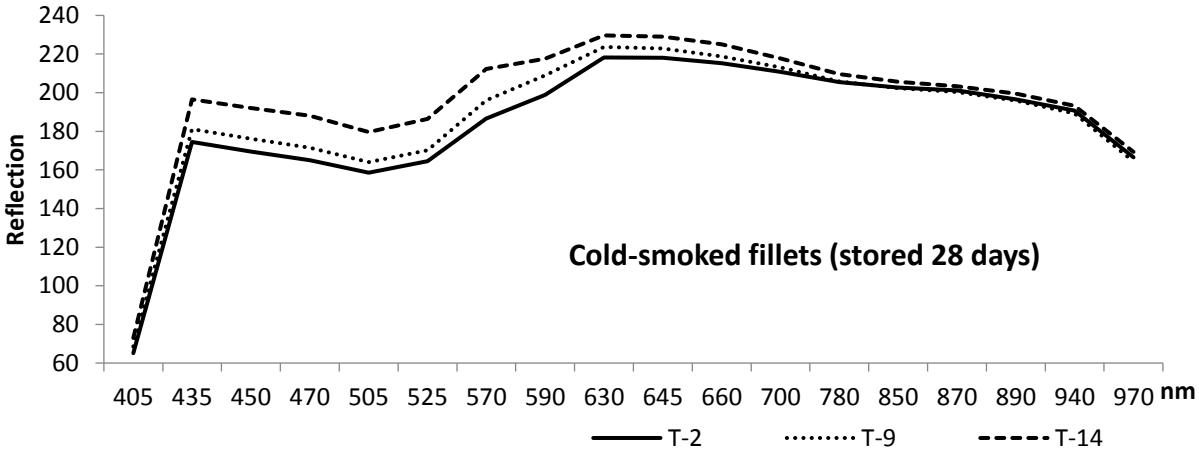

Figure 4. Reflective properties of the fillet surface of cold-smoked salmon fillets measured after 28 days refrigerated storage (GLM multivariat; Model: P<0.01; Corrected model (405-525nm): P=0.037-0.042); (570-970nm): P>0.05.

Table 1. Muscle pH during primary processing, maximum *rigor mortis* contraction (hours), and pH and water holding capacity (WHC) of raw salmon fillets stored on ice for 14 days

		Group[1]			
Parameter	**Day**	**T-2**	**T-9**	**T-14**	*Effect*[2]
pH (initial)	0	7.26±0.13	7.30±0.11	7.32±0.11	*ns*
pH (after bleeding)	0	7.22±0.10[a]	7.06±0.15[b]	6.93±0.08[c]	*P<0.001*
Rigor maximum (hours)		44.9±5.3[a]	30.4±5.1[b]	30.9±6.3[b]	*P<0.001*
pH (storage)	6	6.41±0.07[B]	6.38±0.06[B]	6.29±0.11	*ns*
	10	6.54±0.04[Aa]	6.53±0.10[Aa]	6.29±0.07[b]	*P<0.05*
	14	6.28±0.02[C]	6.29±0.07[B]	6.30±0.04	*ns*
	Effect[2]	*P<0.05*	*P<0.05*	*ns*	
WHC	6	93.4±1.1	93.9±1.3	93.2±3.1	*ns*
	10	91.9±2.8	94.5±1.4	94.0±2.1	*ns*
	14	92.5±1.1	90.5±3.1	90.5±1.2	*ns*
	Effect[2]	*ns*	*ns*	*ns*	

All values presented except for initial pH and pH after bleeding are an average ± SD of 6-7 fillets of each group at each sampling day. Initial pH and pH after bleeding represents an average ± SD of 21 fillets of each group.

[1] T-2 (salmon exsanguination at 0 ˚C), T-9 (salmon exsanguination at 8 ˚C) and T-14 (salmon exsanguination at 16 ˚C)

[2] Different lower case superscripts within each row (a,b,c) indicate significant differences between the groups whereas different capital letter superscripts within each column (A,B,C) indicate significant differences between days for each response by GLM and Tukeys pairwise comparison test. Level of significance was set to $P < 0.05$ (ns = not significant)

Table 2. Textural properties of raw salmon fillets during 14 days ice storage

		Group[1]			
Parameter	**Day**	**T-2**	**T-9**	**T-14**	*Effect*[2]
BF (N)[3]	6	18.3±1.4	17.9±1.4	20.0±2.6	*ns*
	10	17.5±2.5	16.8±1.8	17.9±3.3	*ns*
	14	18.8±2.7	17.5±3.0	17.9±1.9	*ns*
	Effect[2]	*ns*	*ns*	*ns*	
F60% (N)[3]	6	22.2±2.8	21.4±2.3	21.2±1.8	*ns*
	10	19.5±2.3	19.9±2.1	18.2±1.8	*ns*
	14	22.6±3.0	22.6±2.9	22.2±3.2	*ns*
	Effect[2]	*ns*	*ns*	*ns*	

All values presented are an average ± SD of 6-7 fillets of each group at each sampling day.

[1] T-2 (salmon exsanguination at 0 ˚C), T-9 (salmon exsanguination at 8 ˚C) and T-14 (salmon exsanguination at 16 ˚C)

[2] Different lower case superscripts within each row indicate significant differences between the groups by GLM and Tukeys pairwise comparison test. Level of significance was set to $P < 0.05$ (ns = not significant)

[3] BF (force (N) required to brake the fillet surface) and F60% (force (N) at 60% compression of fillet high).

Table 3. Physiological- and chemical properties of cold-smoked salmon fillets.

		Group[1]			*Effect*[2]
Parameters	**Processing step**	**T-2**	**T-9**	**T-14**	
pH	Raw (day 6)	6.36±0.06	6.41±0.06	6.34±0.08	*ns*
	Smoked	6.30±0.11	6.29±0.04	6.28±0.06	*ns*
	Stored 14 d	6.10±0.4[a]	6.11±0.04[a]	6.03±0.06[b]	*P<0.01*
	Stored 28 d	6.07±0.4[a]	6.07±0.06[a]	5.96±0.10[b]	*P<0.05*
Dry matter (DM)	Stored 28 d	42.9±1.3[ab]	42.2±2.6[b]	45.2±2.3[a]	*P<0.05*
NaCl (g kg DM^{-1})	Stored 28 d	30.7±2.8	29.6±1.7	26.4±4.7	*ns*
F30% (N)[3]	Stored 28 d	23.7±3.4	26.1±8.8	28.2±4.3	*ns*

All values presented are an average ± SD of 6-7 fillets of each group at each sampling day.

[1] T-2 (salmon exsanguination at 0 ˚C), T-9 (salmon exsanguination at 8 ˚C) and T-14 (salmon exsanguination at 16 ˚C)

[2] Different lower case superscripts (a,b,c) within each row indicate significant differences between the groups by one-way ANOVA and Tukeys pairwise comparison test. Level of significance was set to $P<0.05$ (ns = not significant)

[3] F30% (force (N) at 30% compression of fillet high)

Table 4. Colorimetric parameters (CIE, 1994) for raw, salted, cold-smoked and cold-smoked fillets stored for 28 days

		Group[1]			*Effect*[2]
Parameters	**Processing step**	**T-2**	**T-9**	**T-14**	
*L**	Raw	46.9±1.2^{A}	46.1±1.8^{A}	47.3±1.1^{A}	*ns*
	Salted	40.6±1.1^{C}	40.9±0.8^{C}	41.4±1.1^{C}	*ns*
	Smoked	38.3±1.4^{D}	40.8±2.0^{C}	39.6±2.2^{C}	*ns*
	Stored 14 d	41.3±1.2^{BCb}	42.3±2.0^{BCab}	44.3±1.4^{Ba}	*P<0.01*
	Stored 28 d	42.6±1.2^{Bb}	44.1±1.4^{ABab}	45.6±1.7^{ABa}	*P<0.01*
Effect[2]		*P<0.001*	*P<0.001*	*P<0.001*	
*a**	Raw	10.4±1.0^{A}	9.8±0.7^{A}	9.9±0.7^{A}	*ns*
	Salted	7.3±0.9^{B}	6.8±0.5^{C}	7.0±0.8^{B}	*ns*
	Smoked	8.1±0.5^{Ba}	6.9±0.4^{Cb}	7.2±1.0^{Bab}	*P<0.05*
	Stored 14 d	10.0±1.2^{A}	8.8±0.4^{B}	9.9±1.2^{A}	*ns*
	Stored 28 d	10.6±1.2^{A}	9.7±0.5^{A}	10.0±0.9^{A}	*ns*
Effect[2]		*P<0.001*	*P<0.001*	*P<0.001*	
*b**	Raw	18.0±1.9^{C}	17.0±0.7^{C}	17.5±1.5^{B}	*ns*
	Salted	12.5±1.2^{D}	11.7±0.9^{D}	12.2±1.4^{C}	*ns*
	Smoked	19.5±1.0^{C}	18.6±1.4^{C}	18.6±1.1^{B}	*ns*
	Stored 14 d	22.6±1.7^{Bb}	21.1±1.1^{Bb}	25.2±2.0^{Aa}	*P<0.01*
	Stored 28 d	25.4±1.3^{A}	24.4±1.0^{A}	26.2±2.1^{A}	*ns*
Effect[2]		*P<0.001*	*P<0.001*	*P<0.001*	

All values presented are an average ± SD of 6-7 fillets of each group at each sampling day.

[1] T-2 (salmon exsanguination at 0 ˚C), T-9 (salmon exsanguination at 8 ˚C) and T-14 (salmon exsanguination at 16 ˚C)

[2] Different lower case superscripts within each row (a,b,c) indicate significant differences between the groups whereas different capital letter superscripts within each column (A,B,C,D) indicate significant differences between each processing step by GLM and Tukeys pairwise comparison test. Level of significance was set to $P < 0.05$ (ns = not significant)