Quality characteristics and consumer acceptance of diploid and triploid cold smoked Atlantic salmon reared at 5, 10 and 15 °C

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Abstract

This study determined the processing characteristics, textural and colorimetric properties, NaCl content and consumer's acceptability of dry salted cold smoked triploid Atlantic salmon (average weight of 1.6±0.3 kg) reared at different temperatures (5, 10 and 15 °C). As a reference, diploid siblings kept and processed under equal conditions was used. Ploidy did not affect the raw material biometrics but increased holding temperature gave increased blood lactate and decreased muscle pH at point of death. Triploid Atlantic salmon was found to be suitable for cold smoke processing but the differences in quality between diploid and triploid was significant. Cold smoked triploid salmon have on average lower processing yield, higher weight loss throughout processing and storage, and was softer as compared to diploids. Ploidy did however not affect the NaCl content. A consumer test did also distinguish between cold smoked diploid and triploid salmon originally kept at 10 °C. In addition, increased holding temperature was found to give a step-wise lower weight loss during processing and significant darker fillets after cold smoking and storage.

Keywords: Triploid Atlantic salmon; holding temperature; cold smoking; yield; color quality

1. Introduction

Because of the 45 “green production concessions” in Norwegian aquaculture (FOR-2013-06-24-754) the raw material used in production of cold smoked Atlantic salmon (*Salmo Salar* L.) today includes triploids. The use of sterile triploids (O'Flynn, et al., 1997) in aquaculture is supported by several conservation and management organizations including North Atlantic Salmon Conservation Organization (NASCO) and Food and Agricultural Organization (FAO) (Taranger and Albretsen, 2014). The use of triploids will therefore probably increase in the future. Due to the scant knowledge about flesh quality of triploid salmon this may be an unknown challenge for the processing industry. The triploid genetic setup (2n+1) (Benfey, 1999) gives all triploid cells one extra set of chromosomes. This leads to increased nuclear volumes and cell size to accommodate the extra genetic material (Benfey, 1999). Consequently, triploid cells are 30% larger than diploids. Larger cells may induce new challenges related to drip loss and textural properties during processing and storage.

The quality of the raw material is an important factor to produce a high quality smoked product. Triploid Atlantic salmon is known to have lower proportions of superior quality as compared to diploids at slaughter (Fraser et al., 2013; Taylor, Preston, Guy, & Migaud, 2011). The flesh quality of triploid Atlantic salmon is however not well documented, where only a few studies deal with the topic. In a recent study by Lerfall et al. (2017) triploids were characterized by lower blood hematocrit (Hct) and rigor index (Ir), and higher fillet drip loss (DL) and collagenase activity. They were moreover found to be paler and less yellowish compared to diploids. Bjørnevik, Espe, Beattie, Nortvedt, and Kiessling (2004) reported triploids to have more gaping and softer fillets, which can be related to the muscle cellularity (Johnston et al., 2000) where diploid salmon have one third fewer muscle fibers than triploids (Johnston, Strugnell, McCracken, & Johnstone, 1999). The colorimetric characteristics are affected by several parameters including ploidy, genetic variations, variation in muscle density and different seasonal factors (Bjørnevik et al., 2004; Choubert, Blanc, & Vallée, 1997; Johnston et al., 2000). The literature is however not sure about which of the mentioned discriminants that are of

highest significance for the flesh color. Significant differences in growth, and differences in flesh properties between diploid and triploid Atlantic salmon, shows the importance of increased knowledge about processing characteristics of triploid Atlantic salmon in a cold smoke process.

Cold smoke processing of Atlantic salmon consists of several steps including salting, drying and smoking and the quality of the end product is both affected by raw material characteristics and all processing steps applied (Bencze Rørå et al., 1998; Birkeland, Bencze Rørå, Skåra, & Bjerkeng, 2004; Birkeland & Bjerkeng, 2005; Cardinal et al., 2001; Espe, Nortvedt, Lie, & Hafsteinsson, 2002; Lerfall, Akse, Østerlie, & Birkeland, 2011; Lerfall & Rotabakk, 2016). Salt is usually added to fillets by dry salting or injection of brine where dry salting is driven by diffusion (Dyer, 1942; Rørå, Furuhaug, Fjæra, & Skjervold, 2004). Triploid cells contain by definition 50% more DNA than diploids, which results in increased nuclear volume and cell size compared to diploids (Benfey, 1999). Larger muscle cells in triploid salmon raise questions about how this will affect factors such as product yield, DL, color, salt diffusion and sensory properties throughout dry salting, cold smoking and refrigerated storage. It is important that the technological properties of diploid and triploid Atlantic salmon is as equal as possible. Hence, the aim of the present study was to investigate the processing characteristics, textural and colorimetric properties, salt content and consumer's acceptability of dry salted and cold smoked triploid Atlantic salmon reared at different temperatures. As reference, diploid siblings reared and processed under equal conditions was used.

2. Material and methods

2.1. Fish material and experimental design

The salmon used were of the same selection as presented in Lerfall et al. (2017). In short, triploidy was induced by subjecting fertilized eggs for approximately 6 min to a hydrostatic pressure of 65,500 kPa. Diploid eggs were not pressurized. All eggs were then incubated at 5.8 ˚C. Following smoltification, both groups (diploid and triploid smolts less than a year old) were transferred to an Institute of Marine Research, Matre, Norway (IMR) sea-pen system (seawater, mass salinity 34 g/kg)

92 in Smørdalen (Masfjord, Norway). At an average weight of 1 kg, both groups were hauled and
93 transported to the experimental facilities at IMR, Matre. The fish were evenly distributed into six 3m
94 in diameter tanks (9m^3) with three tanks for each ploidy. The temperature was then adjusted to 5, 10
95 and 15 ˚C over 30 d and thereafter held constant over 27-29 d until the fish were slaughtered. After
96 four d of starvation, 60 farmed Atlantic salmon (50% diploid and 50% triploid, average weight of
97 1.6±0.3 kg) were slaughtered between the 19th and 21st of August 2014. The fish were killed one by
98 one by a sharp blow to the head (approximately 3 min between each fish).

99 The sampling procedure resulted in a full factorial design with six groups of salmon with different
100 ploidy and water temperature: Group 1, Diploid salmon kept at 5 °C; Group 2, Triploid salmon kept at
101 5 °C; Group 3, Diploid salmon kept at 10 °C; Group 4, Triploid salmon kept at 10 °C; Group 5, Diploid
102 salmon kept 15 °C and Group 6, Triploid salmon kept at 15 °C.

103 Immediately after killing, the first five salmon from each group (n = 10) were sampled for a blood
104 analysis of the lactate. All the fish were analyzed for muscle pH, temperature at death, length and
105 whole body weight before the fish was stored, on ice during rigor mortise (60 h). All fillets were
106 thereafter hand filleted, frozen individually, and kept frozen (-30 °C) for 60 d before processing.

107 *2.2. Raw material control*

108 Muscle pH and temperature was measured right after death in the anterior dorsal muscle close to
109 the gills by using a Mettler Toledo SevenGo proTM pH-meter (Mettler Toledo International Inc., USA)
110 connected to an Inlab puncture electrode. Blood samples were immediately extracted from the
111 caudal vein (n = 30). The blood lactate was measured immediately using a Lactate Pro 2 analyzer
112 (Arkray Factory Inc., Koka-Shi, Japan).

113 *2.3. Salting and smoking procedure*

114 After thawing (48 h, 2 °C), all fillets were covered with sodium chloride (fine-refined salt, minimum
115 99.8% NaCl) and stored on grids in a refrigerated room (20 h, 2 °C). All fillets were thereafter rinsed

in cold water (approximately 8 °C) to remove excess of NaCl. Salt-cured fillets of all six groups were then randomized on grids, dried separately for 60 min, followed by four circles of 50 min smoking (beech chips) and 10 min drying (23 °C, relative humidity: 75-83%, air velocity: 0.4-0.8 m/s) according to Birkeland, Skåra, Bjerkeng, and Rørå (2003). Vacuum packaged fillets from all protocols were stored in a refrigerated room (2 °C) for 28 d.

2.4. Processing yield, weight loss and NaCl content

The weight loss (WL) at each processing step and throughout storage were calculated as the difference in fillet weight between raw, and salted and smoked fillets, respectively (Lerfall, et al., 2016). Moreover, the WL during 28 d refrigerated vacuum storage was calculated as the difference in fillet weight between smoked fillets and fillets stored 28 d. The processing yield was moreover calculated as % smoked fillet compared to the initial fillet weight.

Content of NaCl was analysed on samples of minced smoked salmon. The salt content was determined conductivimetrically after a method described by Birkeland, et al. (2004) and analyzed on a Dicromat 11-6 Salt Analyser (PCL Control Instrumentation Ltd., Leicester, UK).

2.5. Textural properties

Instrumental textural analyses were performed in the dorsal part of the Norwegian quality cut (NQC) using a Texture Analyzer TA-XT2 (SMS Ltd., Surrey, England) equipped with a 30 kg load cell. A flat-ended cylinder probe (10 mm diameter, type P/1SP) was used. The force-time graph was recorded by a computer equipped with the Texture Exponent software for windows (version 6.1.7.0, SMS), which was also used for the data analyses. The analyses were performed in duplicates (average values were used for data analyses) of each fillet at the end of the storage period (28 d post smoking). The resistance force (N) was recorded with a constant speed of 5 mm/s, and the force required to press the cylinder down to 80% of fillet thickness was used to describe firmness.

2.6. Colorimetric properties

Surface color (CIE, 1994) was measured on a DigiEye full system, VeriVide Ltd., Leicester, UK of the raw material, after each processing step, and after 28 d refrigerated storage. The software Digipix (version 2.7) was used to calculate *L*a*b** values from RGB values obtained from the fillet image

2.7. Consumer acceptance

Participants in the consumer test were recruited in the canteen of IPARK, Stavanger, Norway. Before testing, they were told that they would taste a cold smoked salmon product produced from diploid and triploid Atlantic salmon. The average age of the participants was 43±11 years ranging from 23 to 68 years old and 34% was females.

Sensory evaluation of vacuum packaged, diploid and triploid cold smoked salmon, consisting of a triangle test that were performed 35 d post smoking (Table 1). A total of 144 participants were divided into three groups (46, 48, and 48 participants) testing salmon kept at 5, 10, and 15 °C, respectively. All panelist was served two tringles each where both triangles consists of three coded clear 50 mL cups containing a slice of smoked salmon (~10 g). Before testing, all samples were equilibrated to ambient temperature in order to avoid any possible effect of the product temperature during evaluation. Sample presentation was randomized and water was provided for rinsing between samples. Triangle discrimination tests were conducted in order to determine if a perceived difference existed between diploid and triploid cold smoked salmon. Panelists were asked to identify the odd sample. In addition, all participants were asked to answer if they prefer cold smoked salmon with high or low intensity of redness, salty taste and smoke aroma. They were moreover asked about how often they consume cold smoked salmon products.

2.8. Statistics

The data were analyzed by a general linear model (GLM) with the ploidy and holding temperature as fixed factors. Multivariate GLM with *L**, *a** and *b**, and different processing steps as multiple Y were used to analyze fillet appearance and weight loss, respectively. To compare different groups, GLM and Duncan`s comparison test was used. Pearson`s correlation coefficient (r) was used to calculate

the linearity dependence between the variables X and Y. The consumer test was analyzed with a Z-test approximation of the binomial test. All statistical analyses were performed using an IBM Statistical Package for the Social Sciences statistics software (release 23, IBM corporation, New York, USA). The alpha level was set to 5% ($P < 0.05$). All results are given as an average ± standard deviation (SD), unless otherwise stated.

3. Results

3.1. Biometrics and raw material characteristics

The raw material characteristics of diploid and triploid salmon from the experimental design is thoroughly documented in Lerfall et al. (2017) whereas biometrics and raw material characteristics of the selection used in the smoking trial is presented in Table 2. Whole body weight was only found affected by holding temperature (GLM, P=0.028) where significantly highest body weight was found of salmon kept at 10 °C. No effect of ploidy was however found regarding body weight (GLM, P>0.46). A similar effect of holding temperature was found for the condition factor, cf (GLM, P=0.002). The cf was however close to be affected by ploidy (GLM, main effect) where diploid salmon have numerically higher cf as compared to triploids (GLM, P>0.067, cf on average 1.1 and 1.0, respectively).

The average death temperature of the fish from each group reflected the experimental holding temperature of the respective tank. Muscle pH at point of death decreased and blood lactate increased as a function of increased holding temperature (Table 2). A significant correlation was found between muscle temperature, and muscle pH and blood lactate at point of death (r=-0.40, P=0.002 and r=0.61, P<0.001, respectively). Muscle pH and blood lactate at point of death were moreover found to correlate (r=-0.57, P=0.001).

3.2. Processing yield, weight loss and NaCl content

188 The salting step was found to be the major contributor to the weight loss from fillets during
189 processing (on average: 9.3±1.1%) followed by the smoking step (on average: 4.2±0.7%) and 28 d
190 refrigerated storage (on average: 2.6±0.6%). The total weight loss after smoking and storage ended
191 at 13.1±1.5% and 15.3±1.7%, respectively.

192 The weight loss for all groups at each processing step is presented in Table 3. All processing steps
193 were significantly affected by the experimental design (Multivariate GLM: $P<0.001$) where holding
194 temperature were found to be the main discriminant (Multivariate GLM: $P<0.001$, $F=11.8$) followed
195 by ploidy (Multivariate GLM: $P=0.007$, $F=4.2$). Main effects of ploidy and holding temperature on the
196 weight loss after each step are shown in Fig.1.

197 The processing yield (Table 3) was significantly affected by the experimental design (GLM: $P=0.008$)
198 where significantly higher yield was observed of diploid as compared to triploid salmon (on average:
199 87.2% and 86.7% respectively, GLM: $P=0.045$). A significant effect of holding temperature was
200 moreover found, where lower yield was found of salmon kept at 5 °C as compared to those kept at
201 10 and 15 °C, respectively (GLM: $P<0.001$).

202 The NaCl content (Table 3) of the cold smoked salmon was significantly affected by the experimental
203 design (GLM: $P<0.041$). The salt content did however not differ between ploidy ($P>0.36$). The main
204 discriminant related to salt content was found to be temperature where cold smoked salmon
205 originally kept at 5 °C was found to be salter compared to those originally kept at 15 °C (Table 3,
206 $P<0.013$).

207 *3.3. Textural properties*

208 The fillet firmness (N) was significantly lowest in cold smoked triploid salmon kept at 10 °C whereas
209 diploid salmon kept at 5 °C were found to be firmest (Fig.2). Cold smoked diploid salmon was
210 moreover found to be significantly firmer as compared to triploids (on average: 12.4N and 11.3N
211 respectively, GLM: $P=0.024$, $F=5.2$). The most discriminant factor was however, holding temperature,
212 where significant lowest firmness was observed in cold smoked fillets of salmon kept at 10 °C (on

213 average 10.5N GLM: P<0.001, F=10.1). As a comparison, cold smoked fillets of salmon kept at 5 and
214 15 °C showed a firmness of 12.9 and 12.3N, respectively.

215 *3.4. Colorimetric properties*

216 The fillet appearance (CIE, 1994) was found to be affected by both ploidy (diploid versus triploid) and
217 holding temperature (5, 10 or 15 °C) (Multivariate GLM, P<0.001, Fig. 3A-F).

218 Raw diploid salmon was found to be darker compared to triploids (P=0.001, Fig. 3A). This pattern
219 continued after salting (L^*= 53.5±1.8 (diploid) versus 54.0±1.9 (triploid), P=0.042) whereas after
220 smoking and 28 d refrigerated storage these differences were found to be insignificant although
221 statistical tendencies towards significance were still observed (P = 0.074 and 0.174, respectively). No
222 significant main effects of ploidy was however found related to fillet redness (a^*, Fig. 3C) or
223 yellowness (b^*, Fig 3E).

224 Holding temperature was found to be the main discriminant related to fillet appearance. Raw fillets
225 of salmon kept at 5 °C was found to be significantly palest (higher L^*-value), followed by salmon kept
226 at 10 °C. Salmon kept at 15 °C were found to be significantly darkest (Fig. 3B). This pattern continued
227 after salting, smoking and 28 d refrigerated storage. The fillet redness (a^*) of the raw material was
228 moreover found to increase with increased holding temperature (Fig. 3D). On average, this trend
229 continued during processing, and throughout 28 d refrigerated storage. The intensity of b^*
230 (yellowness) followed the same pattern as observed for the fillet redness (Fig. 3F). An equalization of
231 yellow perception was however observed after smoking and 28 d refrigerated storage showing
232 smaller effects of holding temperature on the end product as compared to the raw material.

233 *3.5. Consumer acceptance*

234 The majority of the participants (63%) consumed cold smoked salmon products 2-3 times per month
235 or more often. Among the participants, 60% prefer cold-smoked salmon with high intensity of
236 redness, whereas 66% and 70% prefer high intensity of smoke aroma and low salt content,

237 respectively. The sensory evaluation of diploid and triploid cold smoked salmon is presented in Table
238 4. The participants managed to distinguish between diploid and triploid cold smoked salmon kept at
239 10 °C (P=0.008). It was however not possible to distinguish between diploid and triploid salmon kept
240 at 5 and 15 °C (P>0.263 and >0.344, respectively).

241 **4. Discussion**

242 All the fish examined in the present study were of the Aquagen strain (Aqua Gen AS, Trondheim,
243 Norway) but differed in ploidy, and in holding temperature throughout the last period of the life
244 cycle. Feeding and rearing strategies together with the pretreatment before salting and smoking
245 were on the other hand equal. Hence, observed differences in biometrics and measured parameters
246 throughout salting, cold smoking and 28 d refrigerated storage were likely caused by differences in
247 ploidy and holding temperature.

248 Dry salting of salmon fillets results in a salting out process where salt diffuse into the muscle
249 structure whereas solutes leaking out (Dyer, 1942; Horner, 1997). This process is affected by several
250 factors such as lipid content (Gallart-Jornet et al., 2007), freezing prior to salting (Deng, 1977) and
251 the ratio between the surface area and fillet thickness. In the present study, small salmon (1.6 kg)
252 with a relatively high surface to flesh ratio (thin fillets) were processed. This resulted in a relatively
253 high weight loss during dry salting (on average: 9.3%) and after cold smoking (total weight loss of
254 13.1%). Other studies on fresh unfrozen commercial sized salmon (±5 Kg) have shown lower weight
255 loss during dry salting (±20 h) and cold smoking (5-6% and 10-11%, respectively) (Lerfall, Bendiksen,
256 Olsen, & Østerlie, 2016; Lerfall & Rotabakk, 2016). All fillets used in the present study were on the
257 other hand frozen prior to processing. Freezing is known to affect the muscle structure of smoked
258 salmon fillets (Sigurgisladottir, Ingvarsdottir, Torrissen, Cardinal, & Hafsteinsson, 2000) but only small
259 effects on yields and weight loss during processing is reported (Cardinal et al., 2001; Sigurgisladottir,
260 Ingvarsdottir, et al., 2000). Cardinal et al. (2001) reported moreover that lean salmon fillets were
261 more affected by freezing compared to salmon with higher fat content.

Holding temperature is known as a significant factor to manage the growth rate of Atlantic salmon (Austreng, Storebakken, & Åsgård, 1987; Brett, 1979; Hevrøy et al., 2013). In a controlled experiment reported by Hevrøy et al. (2013), diploid salmon were fed (45 d) at 13 °C, 15 °C, 17 °C and 19 °C, respectively. The most efficient growth was achieved in water temperature of 13 °C. Furthermore, salmon reared at 15 °C and 17 °C grew efficiently the first two wk but exhibited reduced feed intake and growth in the last part of the study. The weight loss of raw salmon fillets is known to be affected by the temperature in the sea (Nordgarden, et al., 2003), where increased growth rate (*e.g.* in summer) is known to increase the fillet drip loss during storage (Mørkøre, et al., 2010; Roth, et al., 2006).

In the present study a significant relationship between weight loss during processing and 28 d storage, and fish size was observed ($r=-0.719$ to -0.772, $P<0.001$). This indicate small fishes to lose more weight during processing, probably because of a higher surface to flesh ratio and lower fillet thickness. The weight loss is also related to the diffusion of salt into the muscle tissue (Dyer, 1942). In the present study, the fish size correlated significantly with the salt content ($r=-0.80$, $P<0.001$) showing small fish with higher surface to flesh ratio to have higher salt content. Larger cell size of triploids does however not affect the salt diffusion ($P>0.36$) or induce any growth advantages to triploids (Benfey, 1999).

Triploid salmon is earlier reported to be softer compared to diploids which has been explained with less small muscle fibers and 23% larger mean cross-sectional fibers area in triploid than diploid salmon (Bjørnevik et al., 2004). There is also found indications on an inverse relationship between average fiber diameter and flesh firmness (Hurling, Rodell, & Hunt, 1996). Lerfall et al. (2017) reported differences in fillet firmness to be dependent on the holding temperature. At low temperatures (5 and 10 °C), triploid salmon tends to have firmer tissue as compare to diploids. Significantly higher fillet firmness in cold smoked fillets of triploids, as compared to diploids, is therefore likely to be a result of increased effect of the salting and smoking process on factors

287 affecting the textural properties in the salmon muscle. *i.e.* dehydration, increased ionic strength and
288 changed microstructure (Birkeland et al., 2004; Jittinandana, Kenney, Slider, & Kiser, 2002;
289 Sigurgisladottir, Sigurdardottir, Torrissen, Vallet, & Hafsteinsson, 2000).

290 The color of salmon fillets is mainly due to the carotenoid concentration in the muscle tissue (Skrede
291 & Storebakken, 1986) whereas decomposition of carotenoids during salting and smoking has little
292 influence on the color changes during processing (Birkeland, 2004; Lerfall et al., 2011). The
293 development of color during smoking is caused by a series of chemical reactions such as protein and
294 lipid oxidation (Hidalgo & Zamora, 2000) as well as Maillard reactions (Martins, Jongen, & van Boekel,
295 2000). Ploidy is earlier reported to affect the flesh color in rainbow trout (Choubert et al., 1997), and
296 Bjørnevik et al. (2004) reported a darker (lower *L** value) and a more reddish color (higher *a** value)
297 of raw triploid salmon. In the present study, main effects of ploidy indicate raw and salted triploids to
298 be paler than diploids, whereas only tendencies of paler triploid were observed after smoking and 28
299 d refrigerated storage. Differences in colorimetric characteristics between diploid and triploid cold
300 smoked salmon is most likely affected by the fish growth where a significant correlation between the
301 fish weight and *a** and *L** were observed ($r=0.419$, $P<0.001$ and $r=-0.276$, $P=0.002$, respectively). The
302 intensity of redness (increased *a** value) was moreover found to increased stepwise with increased
303 holding temperature independent of ploidy.

304 In the present study largest effects of ploidy was observed for salmon kept at 10 °C (data not shown).
305 Triploid salmon kept at 10 °C were found to be softer (lower firmness), less reddish and paler as
306 compared to respective diploids. Color is a key attribute of food items (Francis, 1995) and an
307 important decision maker for consumers when purchasing smoked salmon products (Gormley, 1992;
308 Røra, Monfort, & Espe, 2004). In the presented study, the majority of the consumers was attracted
309 by the red color of the smoked product which probably affected the consumers to distinguishes
310 between diploid and triploid cold smoked salmon originally kept at 10 °C. Of the other selections (5
311 and 15 °C), only minor differences in quality was found (only small differences in fillet firmness and

312 colorimetric characteristics). Hence, it was harder for the consumers to distinguishes between the
313 diploid and triploid cold smoked product.

314 **5. Conclusion**

315 It is concluded that triploid Atlantic salmon is suitable for cold smoke processing but the differences
316 in quality between diploid and triploid cold smoked salmon is significant. Triploid cold smoked
317 salmon have on average lower processing yield, higher weight loss throughout processing and
318 storage, and was softer as compared to diploids. The ploidy did however not affect the NaCl content.
319 It is moreover concluded that a consumer test distinguishes between cold smoked diploid and
320 triploid salmon originally kept at 10 °C. In addition, increased holding temperature gives a step-wise
321 lower weight loss during processing and significant darker fillets after cold smoking and storage.

322 **Acknowledgment**

323 This work was supported by Norwegian research council (project 233689) and by funds from the
324 Institute of Marine Research (IMR, Matre), Norwegian University of Life Science (NMBU, Ås), Nofima
325 AS, Stavanger and the Norwegian University of Science and Technology (NTNU, Trondheim). The
326 authors wish to thank staff at Institute of Marine Research (IMR, Matre), Norwegian university of life
327 Science (NMBU, Ås), Nofima AS (Stavanger) and Norwegian University of Science and Technology
328 (NTNU, Trondheim) for excellent technical support.

References

Austreng, E., Storebakken, T., & Åsgård, T. (1987). Growth rate estimates for cultured Atlantic salmon and rainbow trout. *Aquaculture, 60*, 157-160.

Bencze Rørå, A. M., Kvåle, A., Mørkøre, T., Rørvik, K.-A., Hallbjoørn, S., Thomassen, S., & Magny, S. (1998). Process yield, colour and sensory quality of smoked Atlantic salmon (Salmo salar) in relation to raw material characteristics. *Food Research International, 31*, 601-609.

Benfey, T. J. (1999). The physiology and behavior of triploid fishes. *Reviews in Fisheries Science, 7*, 39-67.

Birkeland, S. (2004). *Cold-smoking of Atlantic salmon (Salmo salar) – influence of processing conditions and raw material characteristics on quality and yield.* (dr. Sci. Thesis), Agricultural University of Norway.

Birkeland, S., Bencze Rørå, A. M., Skåra, T., & Bjerkeng, B. (2004). Effects of cold smoking procedures and raw material characteristics on product yield and quality parameters of cold smoked Atlantic salmon (Salmo salar L.) fillets. *Food Research International, 37*, 273-286.

Birkeland, S., & Bjerkeng, B. (2005). The quality of cold-smoked Atlantic salmon (Salmo salar) as affected by salting method, time and temperature. *International Journal of Food Science & Technology, 40*, 963-976.

Birkeland, S., Skåra, T., Bjerkeng, B., & Rørå, A. M. B. (2003). Product Yield and Gaping in Cold-smoked Atlantic Salmon (Salmo salar) Fillets as Influenced by Different Injection-salting Techniques. *Journal of Food Science, 68*, 1743-1748

Bjørnevik, M., Espe, M., Beattie, C., Nortvedt, R., & Kiessling, A. (2004). Temporal variation in muscle fibre area, gaping, texture, colour and collagen in triploid and diploid Atlantic salmon (Salmo salar L). *Journal of the Science of Food and Agriculture, 84*, 530-540.

Brett, J. R. (1979). 10 - Environmental Factors and Growth. In D. J. R. W.S. Hoar & J. R. Brett (Eds.), *Fish Physiology* (Vol. 8, pp. 599-675), Academic Press.

Cardinal, M., Knockaert, C., Torrissen, O., Sigurgisladottir, S., Mørkøre, T., Thomassen, M., & Luc Vallet, J. (2001). Relation of smoking parameters to the yield, colour and sensory quality of smoked Atlantic salmon (Salmo salar). *Food Research International, 34*, 537-550.

Choubert, G., Blanc, J. M., & Vallée, F. (1997). Colour measurement, using the CIELCH colour space, of muscle of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed astaxanthin: effects of family, ploidy, sex, and location of reading. *Aquaculture Research, 28*, 15-22.

CIE. (1994). Survey of reference materials for testing the performance of spectrophotometers and colorimeters *Publication CIE nr. 114.1*. Vienna, Austria: Central bureau of the CIE.

Deng, J. C. (1977). Effect of freezing and frozen storage on salt penetration into fish muscle immersed in brine. *Journal of Food Science, 42*, 348-351.

Dyer, W.J., 1942. The salting of codfish, Progress report of Atlantic Coast Stations. Fishery research Board of Canada.

Espe, M., Nortvedt, R., Lie, Ø., & Hafsteinsson, H. (2002). Atlantic salmon (*Salmo salar*, L) as raw material for the smoking industry. II: Effect of different smoking methods on losses of nutrients and on the oxidation of lipids. *Food Chemistry, 77*, 41-46.

Francis, F. J. (1995). Quality as influenced by color. *Food Quality and Preference, 6*, 149-155.

Fraser, T. W. K., Hansen, T., Skjæraasen, J. E., Mayer, I., Sambraus, F., & Fjelldal, P. G. (2013). The effect of triploidy on the culture performance, deformity prevalence, and heart morphology in Atlantic salmon. *Aquaculture, 416–417*, 255-264.

Gallart-Jornet, L., Barat, J. M., Rustad, T., Erikson, U., Escriche, I., & Fito, P. (2007). Influence of brine concentration on Atlantic salmon fillet salting. *Journal of Food Engineering, 80*, 267-275.

Gormley, T. R. (1992). A note on consumer Preference of smoked salmon colour. *Irish Journal of Agricultural and Food Research, 31*, 199-202.

Hevrøy, E. M., Hunskår, C., de Gelder, S., Shimizu, M., Waagbø, R., Breck, O., Takle, H., Sussort, S., & Hansen, T. (2013). GH–IGF system regulation of attenuated muscle growth and lipolysis in

379 Atlantic salmon reared at elevated sea temperatures. *Journal of Comparative Physiology B,*
380 *183*, 243-259.
381 Hidalgo, F. J., & Zamora, R. (2000). The role of lipids in nonenzymatic browning. *Grasas y Aceites, 51*,
382 35-49.
383 Horner, W. F. A. (1997). Preservation of fish by curing (drying, salting and smoking). In G. M. Hall
384 (Ed.), *Fish Processing Technology* (second ed., pp. 32-73). London: Blackie Academic &
385 Professional.
386 Hurling, R., Rodell, J., & Hunt, H. (1996). Fiber diameter and fish texture. *Journal of Texture Studies,*
387 *27*, 679-685.
388 Jittinandana, S., Kenney, P. B., Slider, S. D., & Kiser, R. A. (2002). Effect of Brine Concentration and
389 Brining Time on Quality of Smoked Rainbow Trout Fillets. *Journal of Food Science, 67*, 2095-
390 2099.
391 Johnston, I., Strugnell, G., McCracken, M. L., & Johnstone, R. (1999). Muscle growth and
392 development in normal-sex-ratio and all-female diploid and triploid Atlantic salmon. *Journal*
393 *of Experimental Biology, 202*, 1991-2016.
394 Johnston, I. A., Alderson, R., Sandham, C., Dingwall, A., Mitchell, D., Selkirk, C., Nickell, D., Baker.
395 R.,Robertson, B., Whyte, D., & Springate, J. (2000). Muscle fibre density in relation to the
396 colour and texture of smoked Atlantic salmon (*Salmo salar* L.). *Aquaculture, 189*, 335-349.
397 Lerfall, J., Akse, L., Østerlie, M., & Birkeland, S. (2011). Salting method affects the retention of
398 carotenoids in the fillet surface of cold-smoked Atlantic salmon (*Salmo salar* L.). *International*
399 *Journal of Food Science & Technology, 46*, 2218-2223.
400 Lerfall, J., Bendiksen, E. Å., Olsen, J. V., & Østerlie, M. (2016). A comparative study of organic- versus
401 conventional Atlantic salmon. II. Fillet color, carotenoid- and fatty acid composition as
402 affected by dry salting, cold smoking and storage. *Aquaculture, 451*, 369-376.
403 Lerfall, J., & Rotabakk, B. T. (2016). Muscle temperature at the point of filleting-Subsequent effect on
404 storage quality of prerigor filleted raw- and cold-smoked Atlantic salmon. *Food Science &*
405 *Technology Internatinal*, *22*, 153-163
406 Lerfall, J., Hasli, P. R., Skare, E. F., Olsen, R. E., Rotabakk, B. T., Roth, B., Slinde, E., & Egelandsdal, B.
407 (2017). A comparative study of diploid versus triploid Atlantic salmon (Salmo salar L.). The
408 effects of rearing temperatures (5, 10 and 15 °C) on raw material characteristics and storage
409 quality. *Food Chemistry*, *225*, 37-44.
410 Martins, S. I. F. S., Jongen, W. M. F., & van Boekel, M. A. J. S. (2000). A review of Maillard reaction in
411 food and implications to kinetic modelling. *Trends in Food Science & Technology, 11*, 364-
412 373.
413 Mørkøre, T., et al., 2010. Relevance of season and nucleotide catabolism on changes in fillet quality
414 during chilled storage of raw Atlantic salmon (Salmo salar L.). Food Chemistry. 119, 1417-
415 1425.
416 Nordgarden, U., et al., 2003. Seasonally changing metabolism in Atlantic salmon (Salmo salar L.) I –
417 Growth and feed conversion ratio. Aquaculture Nutrition. 9, 287-293.
418 O'Flynn, F.M., et al., 1997. Comparisons of cultured triploid and diploid Atlantic salmon(Salmo salar
419 L.). *ICES Journal of Marine Science: Journal du Conseil.* 54, 1160-1165.
420 Roth, B., et al., 2006. Pre or post mortem muscle activity in Atlantic salmon (Salmo salar). The effect
421 on rigor mortis and the physical properties of flesh. Aquaculture. 257, 504-510.
422 Røra, A. M. B., Monfort, M. C., & Espe, M. (2004). Effects of Country of Origin on Consumer
423 Preference of Smoked Salmon Collected in a French Hypermarket. *Journal of Aquatic Food*
424 *Product Technology, 13*, 69-85.
425 Rørå, A. M. B., Furuhaug, R., Fjæra, S. O., & Skjervold, P. O. (2004). Salt diffusion in pre-rigor filleted
426 Atlantic salmon. *Aquaculture, 232*, 255-263.
427 Sigurgisladottir, S., Ingvarsdottir, H., Torrissen, O. J., Cardinal, M., & Hafsteinsson, H. (2000). Effects
428 of freezing/thawing on the microstructure and the texture of smoked Atlantic salmon (Salmo
429 salar). *Food Research International, 33*, 857-865.

Sigurgisladottir, S., Sigurdardottir, M. S., Torrissen, O., Vallet, J. L., & Hafsteinsson, H. (2000). Effects of different salting and smoking processes on the microstructure, the texture and yield of Atlantic salmon (Salmo salar) fillets. *Food Research International, 33*, 847-855.

Skrede, G., & Storebakken, T. (1986). Characteristics of Color in Raw, Baked and Smoked Wild and Pen-Reared Atlantic Salmon. *Journal of Food Science, 51*, 804-808.

Taranger, G.l., & Albretsen, J. 2014. Risikovurdering norsk fiskeoppdrett 2013, pp. 155.

Taylor, J. F., Preston, A. C., Guy, D., & Migaud, H. (2011). Ploidy effects on hatchery survival, deformities, and performance in Atlantic salmon (Salmo salar). *Aquaculture, 315*, 61-68.

Figure captions:

Figure 1. Main effects (GLM) of (**A**) ploidy (■ diploid and ■ triploid) and (**B**) holding temperature (■ 5 °C, ■ 10 °C and ■ 15 °C) on the weight loss (average±SE) of salmon fillets during cold smoke processing and 28 d refrigerated storage. Different letters indicate significant variation (P<0.05) between the respective groups by GLM and Duncan's comparison test.

Figure 2 Firmness (average±SE) of ■ diploid and ■ triploid smoked salmon kept at 5, 10 and 15 °C determines instrumentally as the force at 80% compression of the fillet height (GLM; model: P<0.001; ploidy: P=0.024; holding temperature: P<0.001).

Figure 3 Main effects (GLM) of ploidy (**A, C, E,** ■ diploid and ■ triploid) and holding temperature (**B, D, F,** ■ 5 °C, ■ 10 °C and ■ 15 °C) on CIE L*a*b* values (average±SE) (CIE, 1994) of raw, salted, smoked and stored (28d) diploid and triploid salmon kept at 5, 10 and 15 °C, respectively. Different letters (abc) indicate significant variation (P<0.05) between respective groups within each processing step by GLM and Duncan's comparison test.

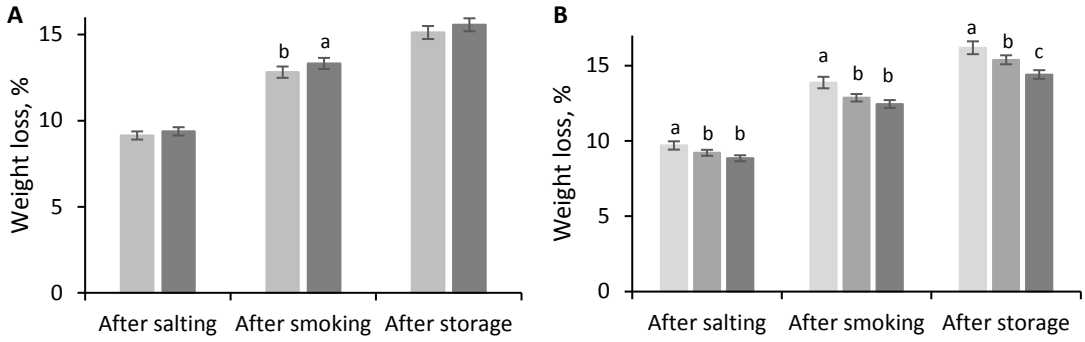
A
Weight loss, %
15
10
5
0
b
a
After salting
After smoking
After storage
B
Weight loss, %
15
10
5
0
a
b
b
a
b
b
a
b
c
After salting
After smoking
After storage

Figure 1.

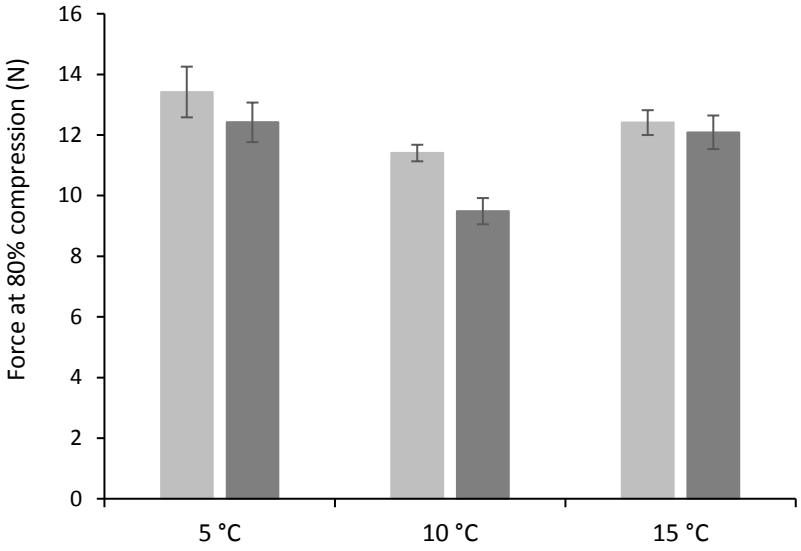

Figure 2.

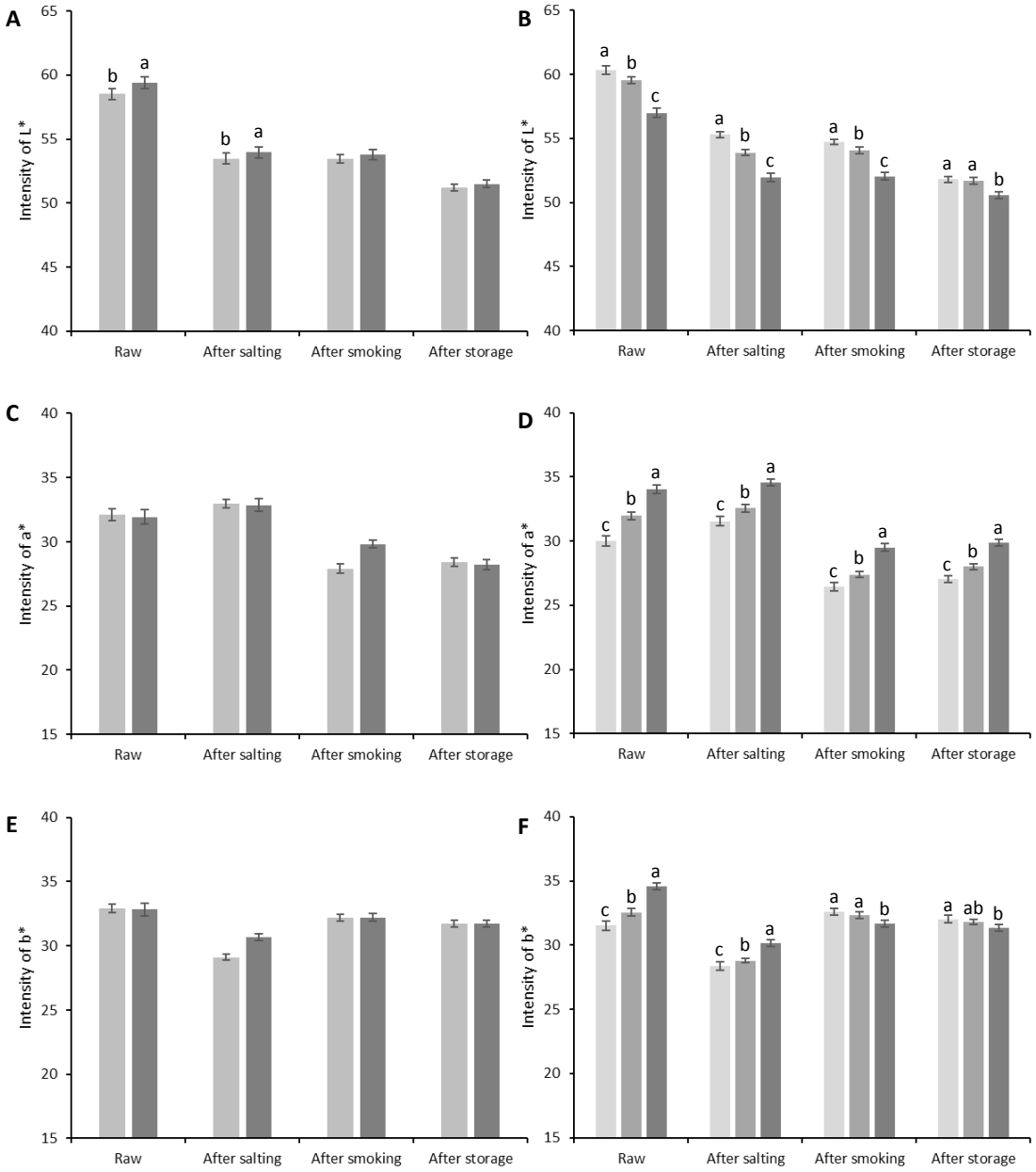
A
B
C
D
E
F
Intensity of L*
Intensity of a*
Intensity of b*
Raw
After salting
After smoking
After storage

Figure 3.

Table 1
Sensory evaluation of cold smoked salmon discriminated by a triangle test

Group	Triangle number	Triploid salmon		Diploid salmon	
5°C	1.triangel	215		321	973
	2.triangel	786	587	611	
10°C	1.triangel	486	904	553	
	2.triangel	853		351	178
15°C	1.triangel	228	538	824	
	2.triangel	176		711	315

Table 2

Raw material characteristics. Average biometrics, death temperature, pH and blood lactate of diploid and triploid Atlantic salmon kept at 5, 10 and 15°C

	5 °C		10 °C		15 °C		GLM [c]			
	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid	P_M	P_P	P_T	$P_{P\times T}$
Whole weight, kg [a]	1.5±0.4	1.4±0.3	1.7±0.2	1.7±0.4	1.6±0.3	1.5±0.3	*0.16*	*0.46*	*0.028*	*0.95*
Condition factor [a]	1.0±0.1	1.0±0.1	1.1±0.1	1.1±0.1	1.0±0.1	1.0±0.1	*0.007*	*0.067*	*0.002*	*0.86*
Death temp., °C [a]	6.1±0.2	6.0±0.3	11.2±0.2	11.1±0.1	15.9±0.1	15.8±0.2	*<0.001*	*0.18*	*<0.001*	*0.92*
Muscle pH [a]	7.2±0.1	7.3±0.2	7.1±0.1	7.1±0.3	7.0±0.2	7.1±0.2	*0.032*	*0.20*	*0.008*	*0.63*
Lactate, mmol/L [b]	0.9±0.3	1.1±0.7	1.7±0.8	2.1±1.4	2.5±1.0	2.6±1.1	*0.031*	*0.52*	*0.004*	*0.94*

[a] Average values of 10 individuals per group, in total 60 individuals.

[b] Average values of 5 individuals per group, in total 30 individuals

[c] General Linear Model (GLM) analyses of variance, where P_M, P_P, P_T, and $P_{P\times T}$ are the significance levels for the effects of the model, ploidy, holding temperature and the interaction between ploidy and holding temperature, respectively.

Table 3

Processing yield, weight loss and content of NaCl (% of wet weight) after each processing step during cold smoke processing of diploid and triploid Atlantic salmon kept at 5, 10 and 15°C

	5 °C		10 °C		15 °C		GLM [b]			
	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid	P_M	P_P	P_T	$P_{P\times T}$
Weight loss salting, % [a]	9.5±1.3	9.9±1.2	9.1±0.9	9.3±0.9	8.8±0.9	8.9±0.9	*0.008*	*0.20*	*0.001*	*0.62*
Weight loss smoking, % [a]	4.5±0.9	4.7±0.6	3.8±0.4	4.3±0.4	3.8±0.5	4.1±0.5	*<0.001*	*0.040*	*<0.001*	*0.84*
Weight loss storage, % [a]	2.5±0.4	2.9±0.4	3.2±0.8	2.6±0.3	2.3±0.5	2.1±0.4	*<0.001*	*0.13*	*<0.001*	*0.33*
Yield, after smoking, % [a]	86.4±1.8	85.8±1.5	87.4±1.0	86.8±1.1	87.7±1.2	87.4±1.2	*<0.001*	*0.040*	*<0.001*	*0.84*
NaCl content, % [a]	6.2±1.2	6.6±1.1	5.3±0.6	5.8±0.8	6.3±0.7	6.1±0.8	*0.041*	*0.36*	*0.013*	*0.35*

[a] Average values of 10 individuals per group, in total 60 individuals. An average of the left and right fillet was used for statistical analyses.

[b] General Linear Model (GLM) analyses of variance, where P_M, P_P, P_T, and $P_{P\times T}$ are the significance levels for the effects of the model, ploidy, holding temperature and the interaction between ploidy and holding temperature, respectively.

Table 4

Sensory evaluation of cold smoked salmon discriminated by a triangle test

Group	Number of answers	Correct answer	P-value*
5°C	92	33	0.263
10°C	98	44	0.008
15°C	97	34	0.344

* The consumer test was analyzed with a Z-test approximation of the binomial test