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## Effect of salting and cold-smoking procedures on Atlantic salmon originating from pre-or post rigor filleted raw material. Based on the measurement of physiochemical characteristics

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### Abstract :

Different methods in use for cold smoking of salmon affect product yield and quality. The combinations of filleting time (pre or post), salting methods (dry or injection), salting targets (2.5 or 4%) and smoking temperatures (15 or 25 °C) were studied during 6 weeks of 4 °C cold storage. Salting method had the greatest influence on flesh quality. Injection salting led to 15% higher fillet yield, moderate to strong gaping score, softer texture, and paler fish compared with dry salting. Pre-rigor filleting reduced fillet gaping from a moderate to minor score, but only in dry salted fillets. Smoking at 15 °C reduced yield by 0.5% in injected salted fish, compared with 25 °C. The combination of pre-rigor filleting, dry salting and 15 °C smoking temperature gave the lowest gaping incidence and highest shear force and flesh colour. Liquid loss increased by 240% and the L-value by 6.5 units during 6 weeks cold storage, whereas the other quality parameters measured showed only small changes during the storage period.

### Highlights

► Salting technique is more important for flesh quality than filleting time, salt target and smoking temperature. ► The combination of pre-rigor filleting, dry salting and 15 °C smoking temperature gave lower gaping, higher shear force and colour. ► Injection salting increased fillet yield. ► Liquid loss increased during cold storage.

**Keywords :** *Salmo salar*, Flesh quality, Texture, Liquid loss, Fillet yield, Cold storage

## 37 **1 Introduction**

38 In 2016, Norway produced 1233 000 metric tons of farmed Atlantic salmon (*Salmo salar*  
39 L.) (Statistics\_Norway, 2017). A substantial part of this is exported to other European  
40 countries, where it is processed as cold smoked salmon for the European market. Large  
41 quantities of Norwegian salmon is exported as whole gutted fresh fish on ice, arriving 3 to 5  
42 days post mortem at a processing company for further filleting and processing to produce cold  
43 smoked products. Modern slaughter technology reduces pre-slaughter stress allowing  
44 industrial pre rigor filleting. Pre rigor filleting has positive effects, increasing the freshness of  
45 fish at market, reducing total weight of fish handled to exported fillets only. Pre rigor filleting  
46 increases fillet thickness, reduces the incidence and severity of gaping and increases colour

47 score in raw Atlantic salmon compared with filleting post rigor (Skjervold et al., 2001b,  
48 Taylor et al., 2002).

49 The smoking process includes several steps, and for each the choice of control parameters  
50 such as salting method, concentration of brine, or smoking temperature determines specific  
51 finished product characteristics (Cardinal et al., 2001, Birkeland et al., 2003, Birkeland and  
52 Bjerkeng, 2005, Birkeland et al., 2007). Processing salmon fillets while still in pre or in-rigor  
53 state is possible, but studies have shown that salt uptake is reduced compared with post rigor  
54 fillets (Wang et al., 1998, Rørå et al., 2004). No work has addressed the effect of pre rigor  
55 filleting on the uptake of salt and smoke when processed post rigor, compared with post rigor  
56 filleting. The change in fillet shape might affect the microstructure and thereby the absorption  
57 rate of salt and smoke within the muscle, as well as gaping and colour of the final product.

58 Different salting methods are reported to affect the yield, fillet colour, texture and gaping in  
59 salmon (Sigurgisladottir et al., 2000, Cardinal et al., 2001, Birkeland et al., 2003, Birkeland et  
60 al., 2004a, Martinez et al., 2012). Cold smoked salmon on the European market typically  
61 contain between 1.7 % to 5.1% salt in the water phase (Cardinal et al., 2004). The uptake and  
62 distribution of salt in the fillet depends on the method used, brine concentration and brining  
63 time (Sigurgisladottir et al., 2000, Rørå et al., 2004, Birkeland et al., 2007). Salt can be added  
64 by dry salting, brine salting or injection salting. Traditionally the two first methods are  
65 commonly used by the salmon processing industry (Espe et al., 2001, Mørkøre et al., 2001).  
66 However injection salting is a rapid curing method which results in a significantly higher  
67 yield after both salting and smoking (Birkeland et al., 2003, Birkeland et al., 2007). This  
68 contrasts with dry salting where water leaks out of the fish, resulting in a lower yield after  
69 salting and smoking (Cardinal et al., 2001). The type of salting method chosen will therefore  
70 have an economic impact.

71 Most of the available literature on smoked salmon refers to smoking temperatures between  
72 20 and 30°C (Sigurgisladottir et al., 2000, Cardinal et al., 2001, Espe et al., 2001, Birkeland et  
73 al., 2004b, Hultmann et al., 2004, Rørå et al., 2004, Løje et al., 2007, Varlet et al., 2007).  
74 Smoke contains several phenolic compounds imparting taste to the product, the absorption of  
75 which is dependent on smoking temperature and smoking time (Cardinal et al., 2001, Sérot et  
76 al., 2004). Texture and liquid loss of Atlantic salmon changes with smoking temperatures  
77 between 20 and 30°C, higher temperatures increasing both texture as well as liquid loss  
78 (Cardinal et al., 2001, Hultmann et al., 2004). Although there is smoked salmon on the market  
79 processed below 20°C, literature about the effect of such low temperatures on absorption of  
80 smoke components and flesh quality is sparse.

81 Although there are many published studies on cold smoked salmon, few deal with the  
82 combined effects of different processing procedures. There is no published literature on  
83 differences in salt and phenolics uptake in pre and post rigor filleted salmon, when both are  
84 salted post rigor, or the effect of smoking temperatures below 20°C. There are also few  
85 studies dealing with the stability of liquid loss, colour and texture in smoked salmon during  
86 cold storage for more than 2 weeks. Therefore, this study aimed to examine the effects of time  
87 of filleting (pre or post rigor) and different aspects of the smoking process on smoked salmon  
88 quality. We assessed the combined effects of dry versus injection salting, 2.5 versus 4.0% salt  
89 concentration, and 15°C versus 25°C smoking temperature on yield, texture, fillet gaping,  
90 colour, chemical content and microstructure, during both the manufacturing process and cold  
91 storage for up to 6 weeks.

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## 96 **2 Materials and methods**

### 97 **2.1 Raw materials**

98 Farmed superior Atlantic salmon (*Salmo salar*, weight  $4267 \pm 247$ g, n=208) were  
99 slaughtered locally at Marine Harvest, Sotra, Norway. The fish had been fed commercial feed  
100 from Skretting (Atlantic XE 2000 and Orion XE 12mm, both containing 50 mg astaxanthin  
101 and 40% lipid), during the last 6 months before slaughter. All fish were dispatched on the  
102 same day using the slaughterhouse commercial procedure for slaughtering, bled in chilled  
103 seawater, gutted and washed. One half of the group (104 fish) was immediately filleted and  
104 trimmed manually at Institute of Marine Research (IMR), Matre, Norway. Before onset of  
105 *rigor mortis* (pre rigor), fillets from both sides were individually marked, packed in plastic  
106 and covered with ice. The remaining 104 fish were gutted, packed on ice, and shipped to  
107 IFREMER (French Research Institute for the Exploration of the Seas), Nantes, France. Here  
108 they were kept for 5 days post harvest, to ensure fish were out of *rigor mortis* (post rigor),  
109 before being filleted and trimmed manually and both fillets individually marked. All salting  
110 and smoking procedures took place at IFREMER.

111

### 112 **2.2 Processing**

#### 113 *Salting methods*

114 Two salting methods (dry or injection; DS or IS), two salt targets (2.5% or 4.0% in smoked  
115 fish muscle; S2.5% and S4.0%) and two smoking temperatures (15°C or 25°C; T15°C and  
116 T25°C) were trialled, resulting in 16 different treatment groups with 22 fillets per group. The  
117 two fillets from the same fish were processed using the same salting procedure, and thereafter  
118 right and left fillet were smoked randomly at either 15°C or 25°C. A total of 32 fish, 16 pre  
119 rigor and 16 post rigor, were left unprocessed and used for raw material analyses (Figure 1).

120 For dry salting fillets were placed on grids and covered with rock salt (Salinor) with E535 and  
121 ferronitrite (20 mg/kg). Fillets were dry salted for 5 hours to achieve 2.5% salt concentration  
122 in the smoked salmon muscle, and for 12 hours to achieve 4.0% salt concentration.  
123 Afterwards the fillets were rinsed briefly with water (15°C). Brine-injection, 160 g L<sup>-1</sup> or 300  
124 g L<sup>-1</sup> (to achieve 2.5% or 4% salt concentration in smoked salmon muscle respectively) was  
125 carried out with a Injecstar B 152/72 injector modified for fish (equipped with 72 needles, 2  
126 mm in diameter). Injection pressure was < 2 bar, and all fish were injected for 60 seconds.

127

### 128 *Cold smoking*

129 Smoking was carried out in a closed air-conditioned cabinet equipped with an auto-  
130 combustion independent generator using beech sawdust with 20% (w/w) water added to the  
131 wood, air speed 2 m/s. Fillets were randomly distributed on the cabinet's grids and smoked at  
132 15°C for 4h, or at 25°C for 2h. Brine-injected fillets were dried before smoking to control the  
133 final water content of the product: 3 hours at 15°C for the 15°C samples and 2 hours at 22°C  
134 for the 25°C samples.

135

### 136 *Slicing and storage*

137 All fillets were stored at 4°C overnight before one fillet from each fish was sliced and  
138 vacuum packed, three slices per packet, 4-5 packets per fillet. The other fillet was kept whole.  
139 Both vacuum-packed slices and whole fillets were cold stored at 4°C for 1, 2, 4 or 6 weeks  
140 before further analysis (Attachment 1). Five or six fish per group was analysed for each  
141 storage time.

142

## 143 **2.3 Analysis**

144 Each fillet was weighed and analysed for gaping and colour before and after processing.  
145 From the raw unprocessed fillets (64 fillets) and from the whole processed fillets (176 fillets),  
146 the entire section under the dorsal fin was sent to Technological Institute of Iceland, Iceland  
147 (IceTec), for analysis of texture shear force and microstructure. IMR received the remainder  
148 of each fillet for analyses of astaxanthin, water and fat.

149 Shear force was analysed in raw and processed fillets immediately on arrival at IceTec (4 days  
150 after smoking), and after 1, 2, 4 and 6 weeks of cold storage. Microstructure was analysed in  
151 injection salted fillets only, 32 raw and 32 processed fillets. Vacuum-packed slices with  
152 processed salmon were analysed for salt and phenol content after 1 week, pH after 2 weeks,  
153 and colour and liquid loss after 1, 2, 4 and 6 weeks of cold storage. Fat, water and astaxanthin  
154 content were analysed in raw fillets immediately after arriving IMR, and in vacuum packed  
155 slices of processed fish after 1 and 4 weeks of cold storage (Attachment 1).

156

### 157 **2.3.1 Fillet yield**

158 Fillet yield is a measure of the change in fillet weight during salting and smoking. Total  
159 fillet weight was measured on raw fillets (5 days post mortem) and after processing. Fillet  
160 yield was calculated according to the formula:

$$161 \text{ Fillet yield \%} = (W_{\text{processed}} - W_{\text{raw}}) \times W_{\text{raw}}^{-1} \times 100\%$$

162 Where  $W_{\text{raw}}$  = weight (g) of raw fillet and  $W_{\text{processed}}$  = weight (g) of processed fillets.

163

### 164 **2.3.2 Gaping score**

165 Gaping score was assessed visually on whole fillets, along the head-tail axis and was  
166 independent of fillet length. A scale from 1 to 4 was used; where 1 is no gaping, 2 minor  
167 gaping (gaping covering 1-5 cm longitudinally of the fillet), 3 moderate gaping (6-10 cm) and  
168 4 strong gaping (> 10 cm). Fillets were not subjected to removal of pin bones.

169

### 170 **2.3.3 Flesh colour**

171 Flesh colour was measured at six locations evenly spread over the whole raw and newly  
172 smoked fillet. During cold storage, colour was measured at three locations in each of three  
173 vacuum packages per treatment group and storage time. The colour was quantified using a  
174 portable Hunterlab Miniscan/EX instrument (10° standard observer, illuminant D65, Hunter  
175 Associates Laboratory Inc, 11491 Sunset Hills Road, Reston, Virginia, USA) calibrated to a  
176 white and a black standard. The L\* variable represents lightness (L\* = 0 for black, 100 for  
177 white), the a\* scale represents the intensity of red and the b\* scale the intensity of yellow.

178

### 179 **2.3.4 Shear force**

180 Shear force was assessed using a Texture Analyser (TA-XT2 Texture Analyser; Stable  
181 Micro system, Haslemere, Surrey, UK), equipped with a V-shaped blade with a thickness of  
182 3.2 mm, height 125 mm and width 70 mm. The blade cut into fillet samples at a speed of 3  
183 mm/s. The maximum peak force (N) required to shear through the sample was recorded as  
184 shear force, and was regarded as the toughness of the fillet.

185

### 186 **2.3.5 Microstructure**

187 Samples for microstructure analysis were collected from the white muscle below the dorsal  
188 fin. Preparation of samples, cryo-sectioning, staining and image viewing was as described by  
189 Sigurgisladottir et al. (2001). Samples were taken from the fillets using a cork knife and  
190 frozen in liquid nitrogen. Specimens were sectioned frozen at -27°C in a cryostat (Leica  
191 CM1800, Heidelberg, Germany) for transverse cuts, 10 µm thick, and stained with Orange G  
192 and then methyl blue. The samples were examined under a microscope (Leica DMRA2) at



193 100x and 200x magnification. A minimum of 300 cross sections of white muscle fibre were  
194 measured from each fish.

195

### 196 **2.3.6 Chemical analysis**

197 Duplicates of 1 g ( $\pm 0.0001$  g) fish muscle homogenate were weighed into extraction tubes  
198 for analysis of astaxanthin, and 1.5-2 grams of anhydrous  $\text{Na}_2\text{SO}_4$  added and adjusted to 10  
199 ml with acetone. Astaxanthin was separated from other carotenoids on an HPLC system  
200 (Hewlett Packard Series 1100, Hewlett Packard Company, Germany) using acetonitrile/  
201 dichloromethane/methanol/water/propionic acid (68:22:4:3:3, v/v/v/v/v) as the mobile phase,  
202 flow rate was  $1.0 \text{ ml min}^{-1}$ , temperature  $1^\circ\text{C}$ , and absorption of astaxanthin determined at 476  
203 nm. The concentration of astaxanthin was determined using external standards. Crystalline  
204 astaxanthin (Hoffmann-La Roche, Basle, Switzerland), was dissolved in an  
205 acetone/chloroform mixture (85:15) to produce a stock solution containing  $120 \mu\text{g ml}^{-1}$   
206 astaxanthin, and further diluted with acetone to provide working standards. The astaxanthin  
207 content of the standard solutions was determined with a spectrophotometer at 476 nm,  
208 assuming an extinction coefficient of 2100.

209 The other chemical analyses were performed in triplicate. Dry matter content was analysed  
210 by oven drying of 3 g salmon at  $105^\circ\text{C}$  until a constant weight was reached, while total fat  
211 was determined gravimetrically after extraction with ethyl acetate. Total phenol was  
212 quantified by the method described in the French standard for smoked salmon (NF V 45-065,  
213 1995). Salt content was measured with a Chloride Analyser 926 (Corning, Halstead UK).

214

### 215 **2.3.7 Liquid loss**

216 Slices of 16 g ( $\pm 0.0001$ g) processed salmon were placed on pre-weighed absorbent pads  
217 (13x9 cm), made of perforated plastic and filled with absorbent material (Cryovac Norway,

218 A/S, Oslo, Norway). The fish and pad were covered with aluminium foil, left at 20°C for 20  
219 hours, the aluminium foil and fish removed, and the pad weighed for calculation of liquid loss  
220 (Mørkøre et al., 2002). Liquid loss % = Increase in pad weight x (fish weight)<sup>-1</sup> x 100.

221

## 222 **2.4 Statistical analyses**

223 Analysis of variance was carried out using the General Linear Model (GLM) module of the  
224 IBM SPSS Statistics software package (Version 20, 2011) The time of filleting (pre or post  
225 rigor), salting method (dry or injection salting), salt target (2.5 or 4%) and smoking  
226 temperature (15 or 25°C) were entered as treatment factors into full factorial models. Separate  
227 univariate analyses were performed for each response variable. Storage time (1, 2, 4, and 6  
228 weeks) was used as a covariate in the model when analysing parameters measured during  
229 storage. Only significant interactions are reported. Data presented in figures and tables,  
230 except for fillet yield, are mean values based on those effects with highest significance in the  
231 GLM. Fillet yield was analysed using the non-parametric Mann Whitney U-test. Other data  
232 analysed using one-way anova, followed by a Student-Newman-Keul *post hoc* test.  
233 Correlation between selected parameters was analysed using a Pearson correlation test.  
234 Treatment group averages are reported as mean ± standard deviation in tables, figures and  
235 text. A significance level of p<0.05 was chosen.

236

## 237 **3 Results**

238 The raw fillet mean weight was 1408±91 g, and the mean values of astaxanthin, fat and  
239 water in the raw fillets 5.33±0.79 mg kg<sup>-1</sup>, 15.8±1.8 g kg<sup>-1</sup> and 64.2±1.6 g kg<sup>-1</sup> respectively.  
240 The mean colour of raw fillets were 55±1, 26±1 and 23±1 for L\*-, a\*- and b\*-values  
241 respectively. The salt target chosen (S2.5% and S4.0 %), was only achieved for IS fish,

242 whereas DS had 25 % and 40% lower salt content than the respective levels of IS fish (Tab.2).  
243 Mean pH values were  $6.1\pm 0.1$  after processing and  $6.3\pm 0.1$  after 2 weeks of storage.

244 Treatment effects analysed with anova and their interactions on processed fish are shown  
245 in table 1.

246

### 247 **3.1 Fillet yield**

248 Salting method had a strong influence on the fillet yield in smoked fish (Fig.2). Fillet yield  
249 was  $92.7\pm 0.7\%$  in DS groups, equivalent to a 7 % weight reduction compared with raw fillets.  
250 The yield for IS fillets was  $106.7\pm 1.8\%$ , or a 6-7% weight increase compared with raw fillets.  
251 In total, the weight differences between IS and DS fillets after smoking was 14-15% (Fig 2)  
252 (Independent Mann-Whitney U-test,  $p < 0.001$ ). There was a significant effect of salting target  
253 and smoking temperature on fillet yield, but this was small. S2.5% gave 1% higher fillet yield  
254 than S4.0%, and IS-T25°C fillets had 0.5% higher fillet yield than IS-T15°C (effect of  
255 temperature on yield in dry salted fish = NS).

256

### 257 **3.2 Gaping and microstructure**

258 Gaping score was significantly affected by filleting time and salting method (Fig.3A). IS  
259 resulted in 1-2 higher gaping score, independent of filleting time. In DS fish, pre-rigor  
260 filleting reduced the gaping incidence compared with post-rigor filleting, whereas no such  
261 effect was seen in the IS fish. Significantly larger white muscle fibre area characterised pre-  
262 rigor filleted fish (Fig. 3B).

263

### 264 **3.3 Shear force, liquid loss, L- and a\*-value)**

265 Shear force, liquid loss, L-value and a\*-value over 6 weeks of cold storage are shown in  
266 Fig.4. Shear force was affected by filleting time, salting method and smoking temperature

267 (Tab.1). Post rigor filleting DS-T15°C, was characterised by higher shear force than pre-rigor  
268 filleting IS-T25°C. Shear force was not affected by storage, remaining consistent throughout  
269 the 6 weeks of cold storage. There was a significant interaction between salting method and  
270 salt target, where a slightly higher shear force was apparent at S2.5% in DS fish, but this was  
271 reversed in IS fish. In the IS group, S4.0% resulted in 1 newton higher shear force compared  
272 with S2.5%. Liquid loss (LL) was affected by salting method and storage (Tab.1). LL  
273 increased during storage and was higher in DS than IS after 6 weeks of storage. DS resulted in  
274 significantly lower L-value and higher a-value, (Fig.4C, 4D, Tab.1). The L-value was also  
275 affected by salt target and smoking temperature. Approximately the L-value was one unit  
276 higher at S2.5% compared with S4.0% (mean values not shown), and similarly one unit  
277 higher at T25°C compared with T15°C.

278

### 279 **3.4 Chemical content**

280 Filleting time had no significant effect on salt and phenol content, but phenol was affected  
281 by salt target and smoking temperature. Phenol content was higher in fillets smoked at 15°C  
282 compared with 25°C. At T25°C phenol content was affected by salt target, being lower at  
283 S4.0% (Fig.3C).

284 Table 2 shows the result for astaxanthin, salt, fat and water in processed fillets. Retention  
285 of astaxanthin through the smoking process was affected by both salting method and smoking  
286 temperature (Tab.1). DS-T15°C had 1.5 mg kg<sup>-1</sup> higher astaxanthin content than IS-T25°C  
287 after the first week of storage. DS fillets had 2% higher fat content than IS fillets in the first  
288 week after smoking, but the differences disappeared during 4 weeks of storage. DS fillets also  
289 had significantly lower water content than IS. There was a significant effect of increased salt  
290 target, resulting in lower water content.

291

### 292 **3.5 Correlation**

293 Shear force showed a negative overall significant relationship with gaping ( $r=-0.33$ ,  
294  $p<0.001$ ,  $n=46$ ) and with the L-value ( $r=-0.44$ ,  $p<0.001$ ), which means firmer fish has less  
295 gaping, and darker flesh. Liquid loss was not related to fat or water content, but was  
296 positively related with the L-value.

297

## 298 **4 Discussion**

### 299 **4.1 Effect of filleting time**

300 Pre-rigor filleting reduced fillet gaping, which has been observed in raw salmon fillets  
301 (Skjervold et al., 2001b). The phenomenon of gaping, the process where a fillet separates into  
302 myocomata, renders the flesh unsuitable for production of high value products such as slices  
303 of cold-smoked salmon. Fillet gaping, texture, and colour are important issues for the smoked  
304 processing industry, and are reason for complaint. Earlier studies have shown that pre-rigor  
305 filleting results in firmer texture (Skjervold et al., 2001a). This was not the case in our study,  
306 as lower shear force was found in pre-rigor fillets after smoking. When muscles are going  
307 through rigor mortis without being attached to the backbone, the myofibres can shorten, with  
308 an increase in white muscle fibre area or diameter as a result, as apparent in our study.  
309 Skjervold comparing fresh pre and post rigor filleted Atlantic salmon 7 days post mortem  
310 found no differences in muscle fibre diameter between groups (Skjervold et al., 2001b).

311

### 312 **4.2 Effect of salting method**

313 The salting method, dry or injection, was the factor most strongly influencing quality of  
314 smoked salmon. IS resulted in 14-15% higher yield than DS. Yield is a crucial economic issue  
315 for smoking companies, and similar differences in product yields have been reported in  
316 previous studies (Sigurgisladottir et al., 2000; Cardinal et al., 2001; Torrissen et al., 2001;

317 Mørkøre et al., 2001; Birkeland et al., 2003; Birkeland et al., 2004). In DS the initial brine  
318 film on the fillet surface will transfer water from the fish, dissolving salt crystals near the  
319 surface. Water will leak from the fish reducing fillet weight. During IS, water and salt are  
320 injected into the fish muscle. With salt concentrations below 1M brine, as in our study, salt  
321 will bind to protein, so the water added will stay inside the muscle dissolving salt crystals and  
322 leading to an increase in fillet weight (Barat et al., 2003). This contention is supported by the  
323 higher water content of injection-salted fillets, which also led to a lower percentage of fat in  
324 this product.

325 Reduced gaping in DS compared with IS has previously been reported (Birkeland et al.,  
326 2004a). IS involves needles piercing the flesh, as well as brine being forced into the fillets. It  
327 is not unexpected that such treatment may affect the fine connection between muscle fibres  
328 and connective tissue, thereby increasing gaping score. A combination of pre rigor filleting  
329 and DS resulted in the lowest gaping score in our study, whereas post rigor filleting and IS  
330 increased gaping score by 1.5 - 2 units. Higher shear force in DS compared to IS is in  
331 agreement with other studies (Birkeland et al., 2004a). The shear force in DS was 25% higher  
332 than in IS.

333 Darker colour score and higher astaxanthin content in DS salmon has been reported  
334 previously (Choubert et al., 1992, Cardinal et al., 2001, Birkeland et al., 2004a, Birkeland et  
335 al., 2004b, Martinez et al., 2012). Colour is affected by changes in the fillet surface,  
336 contingent upon changes in surface structure or water content, making dry salted fish appear  
337 darker. A higher astaxanthin content may be due to higher preservation and less pigment  
338 oxidation in DS compared with IS. But it can also be a consequence of water loss during dry  
339 salting, as argued by Choubert et al. (1992). In contrast Birkeland et al. (2004b) found no  
340 influence of salting method (dry or injection) on astaxanthin in Atlantic salmon.

341 The leaking of liquid in vacuum-packed smoked salmon is a well-known issue and may  
342 lead to downgrading of the product. The higher liquid loss in DS fish after 6 weeks might be  
343 related to higher protein denaturation in this group. Increasing salt content has been shown to  
344 increase liquid loss in cooked salmon, but at higher salt content levels than in our experiment  
345 (Ofstad et al., 1995).

346

### 347 **4.3 Effect of salt target**

348 The lower salt concentration achieved in the DS groups may have prevented direct  
349 comparison of the groups. Salt target had little influence on salmon fillet quality, only  
350 affecting fillet yield, water content and L-value.

351 The lower water content and fillet yield with increasing salt content are in agreement with  
352 previous work (Jittinandana et al., 2002, Birkeland and Bjerkeng, 2005, Birkeland et al., 2007,  
353 Rizo et al., 2013). Increase in salt content decreases the stable hydrophilic surface and  
354 increases protein-protein interaction, leading to water leaking out of the fillet (Arason et al.,  
355 2014), explaining the decrease in yield with increasing salt target.

356 The salt content is comparable with what is normally found in French Manufacture, where  
357 2-3% NaCl are reported. Salt together with phenol content and storage temperature are  
358 important factors controlling microbiological growth, and thereby both sensory quality and  
359 the food safety (Hwang, 2009). Though microbiology data are not available, salt level below 6  
360 % in the water phase are not sufficient to prevent growth of the pathogen bacteria *Listeria*  
361 *monocytogenes* (Peterson et al., 1993, Truelstrup Hansen et al., 1995). But the higher salt  
362 content in the IS group should prevent any microbial growth more than in the DS group  
363 (Hwang, 2009). The salting method itself, dry or brine injection, is not found to effect growth  
364 of *L.monocytogenesis* (Niedziela et al., 1998).

365

#### 366 **4.4 Effect of smoking temperature**

367 The weight gain measured as fillet yield in IS fillets was lower at T15°C than at T25°C.  
368 This is in agreement with Cardinal et al (2001), who reported higher total weight loss after  
369 smoking at 20°C compared with 30°C. This can be related to lower moisture loss in fillets  
370 smoked at higher temperatures due to case hardening, which prevents effective drying. If this  
371 is so, we would have expected higher shear force with higher smoking temperature, whereas  
372 the reverse was observed. The difference could also be an effect of the longer smoking time at  
373 15°C.

374 The higher astaxanthin content at T15°C could reflect less breakdown and oxidation of  
375 astaxanthin at lower temperature, thus preserving colour. However, Birkeland and co-authors  
376 (2004b) found higher astaxanthin retention at higher temperature, 30 vs 20°C, suggesting  
377 higher temperatures cause firmer binding of astaxanthin to the protein binding sites, thereby  
378 reducing its degradation.

379 The higher phenol content in the T15°C group is counter to reports of phenol content  
380 increasing with smoking temperature, from 20 to 30°C for salmon (Cardinal et al., 2001), and  
381 from 16 to 32°C for herring, *Clupea harengus* (Sérot et al., 2004). The differences in phenol  
382 content between smoking temperatures in our experiments could also be affected by duration  
383 of exposure rather than the difference in temperature. This is supported by Sérot et al. (2004),  
384 who reported increased phenol content with longer duration, from 60 to 240 minutes in the  
385 smokehouse. Phenolic compounds are of considerable importance for the preservation and  
386 organoleptic properties of smoked products and increased phenol content will increase shelf  
387 life (Kjällstrand and Petersson, 2001, Hwang, 2009). The sensorial and microbiological shelf  
388 life would therefore be expected to increase with increased phenol content, as achieved with  
389 longer duration time in the smoke oven at 15°C.

390



#### 391 **4.5 Effect of storage**

392 The acceptable shelf life of commercial smoked salmon depends on the country of export,  
393 varying from 21 to 60 days (Cardinal et al., 2004). Acceptable shelf life is normally connected  
394 to sensory deterioration (Truelstrup Hansen and Huss, 1998, Leroi et al., 2001). But also  
395 other quality issues, such as stability of colour, texture, fillet gaping and liquid loss can  
396 change during storage and are important to the consumer. Our data showed that shear force,  
397 gaping and chemical content was stable up to 6 weeks, which covers the shelf life set in many  
398 European markets. Our results are in agreement with others, finding minor changes in salmon  
399 texture during cold storage (Schubring, 2006, Martinez et al., 2012). Liquid loss is a problem  
400 in vacuum packed smoked salmon, and, in agreement with earlier studies, we found liquid  
401 loss increased with storage time (Rørå et al., 2003, Løje et al., 2007). Liquid loss is also  
402 reported to increase with higher fat content (Mørkøre et al., 2001, Birkeland et al., 2004a), but  
403 our results did not support this. It has been reported that colour of vacuum packed salmon is  
404 stable during cold storage (Bugueño et al., 2003), but our results show an increase in both  
405 redness and lightness during storage.

406

#### 407 **5 Conclusion**

408

409 The combined analysis of effects of different filleting and processing methods showed that  
410 salting method has a greater impact on physio-chemical quality than filleting time, salt target  
411 or smoking temperature. Pre-rigor filleting reduces gaping in DS fillets, whereas in IS this  
412 effect was not visible. Filleting time had no effect on the absorption of phenol and salt in the  
413 fillet. IS increases yield, but leads to lower shear force, paler colour and more gaping. DS  
414 lowers yield, but gives higher shear force, more colour and less gaping. Smoking at 15°C  
415 results in lower yield and L-value, but higher shear force, phenol and astaxanthin compared  
416 with T25°C. The higher phenol content, possible due to longer exposure time at T15°C, can

417 increase shelf life. The combination of DS-T15°C maintained higher astaxanthin content than  
418 IS-T25°C. Liquid loss and L-value increases during cold storage up to 6 weeks, whereas only  
419 small changes are seen in other quality parameters.

420 Summing up, our results show injection salting is preferred if yield is the only economic  
421 issue for the producer. However, as texture, gaping and pale colour are responsible for  
422 consumer complaints about smoked salmon, the combination of pre-rigor filleting, dry salting  
423 and smoking at 15°C should be considered a viable option.

424

425  
426

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428

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Table 1. Results from analyses of variance of the data in Fig. 1, 2 and 3, where  $P_F$ ,  $P_M$ ,  $P_S$ ,  $P_T$  are the significance level for the effects of filleting time, salting method, salting target and smoking temperature, and the interaction between selected effects respectively. Gutted body weight and storage time is used as covariate in the model for all parameters, except for gaping, microstructure and phenol where only gutted body weight is used.

	$P_F$	$P_M$	$P_S$	$P_T$	$P_{\text{store as covariate}}$	Interactions			
						$P_{F \times M}$	$P_{M \times S}$	$P_{F \times S}$	$P_{S \times T}$
Gaping	<0.001	<0.001	ns	ns	-	<0.05	ns	ns	ns
Microstructure	<0.05	-	-	-	-	-	-	-	-
Phenol	ns	ns	<0.002	<0.001	-				
Shear force	<0.01	<0.001	ns	<0.001	ns	ns	<0.05	ns	ns
Liquid loss	ns	<0.001	ns	ns	<0.001	ns	ns	ns	ns
L-value	ns	<0.001	<0.001	<0.001	<0.001	ns	ns	ns	ns
a*-value	ns	<0.001	ns	ns	<0.001	ns	ns	ns	ns
Astaxanthin	ns	<0.005	ns	<0.001	ns	ns	ns	ns	ns
Fat content	ns	<0.001	ns	ns	ns	ns	ns	ns	ns
Water content	ns	<0.001	<0.001	ns	ns	ns	ns	ns	ns

Table 2. Chemical content of astaxanthin, salt, fat and water in Atlantic salmon fillet, 1 and 4 weeks after processing. DS=dry salting, IS= injection salting, S2.5 and S4.0 refers to 2.5% and 4.0% salt target in the muscle, T15 and T25 refers to smoking temperature 15 or 25 °C. Different small letters indicate differences between groups at the same week, analysed with one-way ANOVA.

	Week	DS-T15	DS-T25	IS-T15	IS-T25
Astaxanthin mg kg <sup>-1</sup>	1	4.6±0.8 <sup>ac</sup>	3.9±1.0 <sup>c</sup>	4.2±0.7 <sup>bc</sup>	3.0±0.7 <sup>d</sup>
	4	4.2±0.8 <sup>a</sup>	3.5±0.7 <sup>b</sup>	3.8±0.7 <sup>a</sup>	3.4±0.6 <sup>a</sup>
		DS-S2.5	DS-S4.0	IS-S2.5	IS-S4.0
Salt g/100g	1	1.8±0.2	2.4±0.3	2.4±0.2	4.0±0.4
Fat g/100g	1	12.4±1.7 <sup>a</sup>	12.1±2.1 <sup>a</sup>	10.3±2.3 <sup>b</sup>	10.4±1.8 <sup>b</sup>
	4	10.6±1.3 <sup>a</sup>	11.5±1.6 <sup>a</sup>	10.5±1.5 <sup>a</sup>	11.2±1.7 <sup>a</sup>
Water g/100g	1	62.9±1.0 <sup>a</sup>	62.1±1.1 <sup>a</sup>	68.2±1.6 <sup>b</sup>	66.6±1.5 <sup>b</sup>
	4	63.3±1.2 <sup>a</sup>	62.0±0.8 <sup>a</sup>	67.5±1.5 <sup>b</sup>	65.0±1.6 <sup>b</sup>

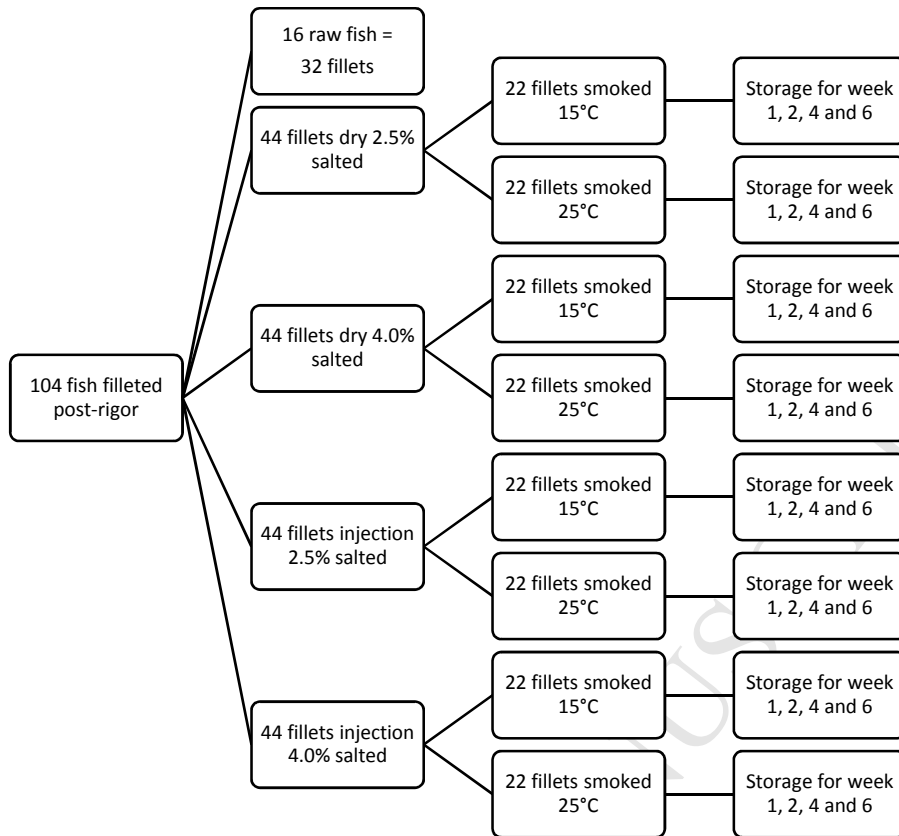
Figure 1. Flow chart for processing of post rigor filleted Atlantic salmon. Similar procedure was performed for 128 pre-rigor filleted Atlantic salmon.

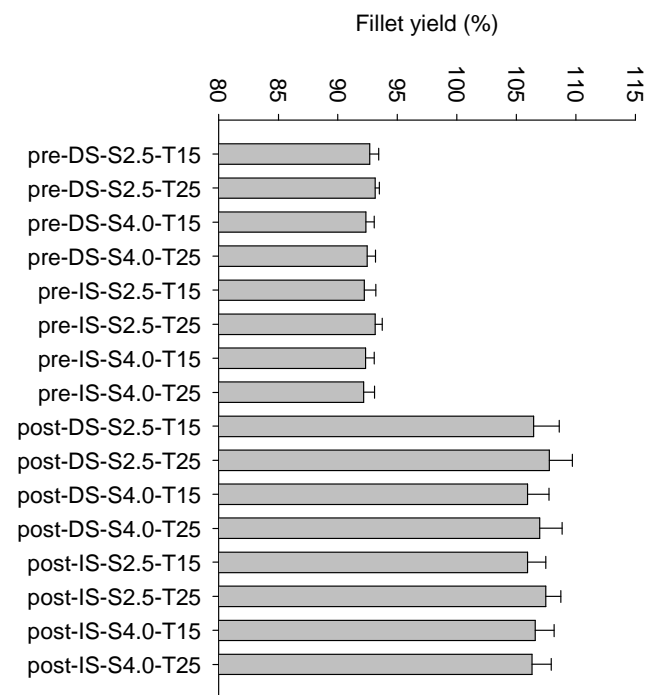
Figure 2. Fillet yield in Atlantic salmon introduced to different processing procedures; pre=pre rigor filleting and post=post-rigor filleting, DS=dry salting and IS=injection salting; S2.5=2.5% salt target and S4.0=4.0% salt target, T15=smoked at 15°C and T25= smoked at 25°C.

Figure 3. A) Gaping score, B) white muscle fibre cross sectional area and C) phenol content in fillets of Atlantic salmon introduced to different processing procedures; pre=pre rigor filleting and post=post-rigor filleting, DS=dry salting and IS=injection salting; S2.5=2.5% salt target and S4.0=4.0% salt target, T15=smoked at 15°C and T25= smoked at 25°C. Different letters indicate significant differences between means.

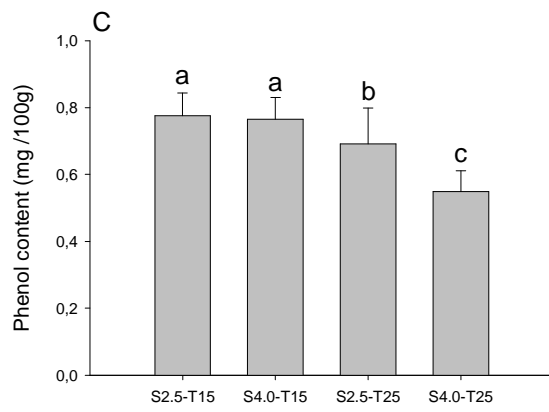
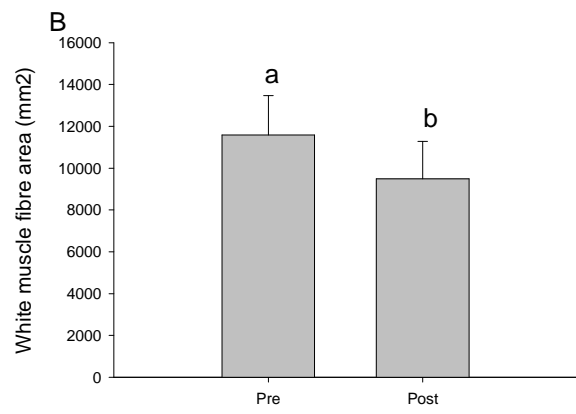
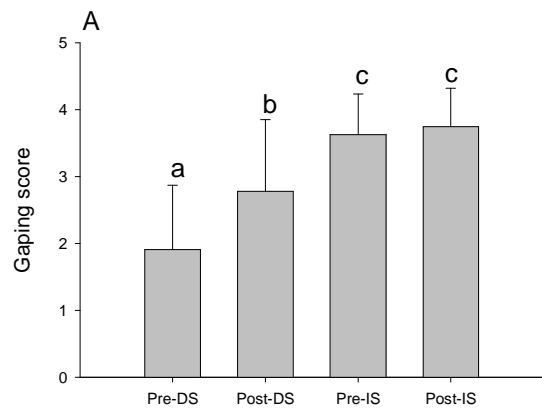
Figure 4. A) Shear force, B) liquid loss, C) L-value and D) a\*-value in fillets of Atlantic salmon introduced to different processing procedures: —●— dry salting 15°C smoking temperature, …○… dry salting 25°C smoking temperature, ---▼--- injection salting 15°C smoking temperature, ---△--- injection salting 25°C smoking temperature.







ACCEPT



ACCEPTED

