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Research article

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ABSTRACT

Smoked fish fillets are pre-salted as a food conservation and quality preservation measure. Here we investigated biochemical and sensory aspects of smoked salmon fillets. Left-side salmon fillets were dry-salted while the right-side fillets underwent a mixed salting method consisting of an injection of saturated brine followed by surface application of dry salt. After 6 h of salting, all the fillets were smoked. At each step of the process, quality was evaluated using instrumental measurements (pH, color, texture, water content, salt content, a_w), and lipid distribution was visualized by MRI. Mixed-salted fillets had a higher salt content than dry-salted fillets and variability in salt distribution was dependent on the salting process. However, these variations had no effect on pH, color or texture, which showed similar values regardless of salting method. Fatty areas had a lower salt content due to slower diffusion of aqueous salt solutions through them. Mixed salting speeds up the salting of the muscle without significantly affecting the quality traits of the salmon fillet.

1. Introduction

Salmon consumption has been on the rise since the 1980s. Around 90% of farmed salmon is Atlantic salmon (*Salmo salar*) that is mainly farmed in northern Europe and Chile. A substantial percentage of salmon fished in northern Europe gets exported to other European countries, where it is processed as cold smoked salmon for the European market. Smoked salmon processing involves filleting and deboning fresh salmon then salting and smoking the fillets. Salting can be done by brining (wet brine incubation or brine injection), by salting with dry salt, or by a combination of the two salting methods (brining + dry salt), and smoking can be carried out either hot or cold (Sampels, 2015).

Different salting methods are reported to affect yield, fillet color, texture and gaping in salmon (Birkeland and Bjerkeng, 2005; Birkeland et al., 2004a; Birkeland et al., 2003; Bjornevik et al., 2018; Cardinal et al., 2001; Martinez et al., 2012; Sigurgisladottir et al., 2000). Dry-salting the fillet-muscle surface tends to extract water through a phenomenon of osmosis. Salt penetration into the tissue is slow because it encounters a counter-current of intramuscular water exiting the muscle. Muscle fibers that lose water decrease in volume (Filgueras et al., 2016), which is visible at macroscopic scale by shrinkage of the muscle pieces. Conversely, salting by incubation in brine or by brine injection causes swelling of muscle cells that accumulate the brine (Offer and Knight, 1988). Mechanistically, the chloride ions intercalate between the myofilaments (Hamm, 1960) and/or between the myosin molecules (Offer and Knight, 1988; Offer and Trinick, 1983) and thus free up space that gets filled by water molecules, resulting in muscle swelling and increased water retention. It is this phenomenon that essentially explains the better yield of brined fillets compared to dry-salted fillets (Birkeland et al., 2004a; Bjornevik et al., 2018; Cardinal et al., 2001; Thorarinsdottir et al., 2004). However, salting with brine leads to more gaping, softer texture (Birkeland and Bjerkeng, 2005; Bjornevik et al., 2018), and paler color (Bjornevik et al., 2018) than dry-salting the fillets.

Mixed salting method is expected to promote better in-tissue salt distribution than dry salting alone while also achieving a satisfactory yield thanks to the brining injection step (Birkeland et al., 2007; Lerfall and Hoel, 2021; Thorarinsdottir et al., 2010). However, there is no published research on the effects of mixed salting method on the time–course of the physicochemical characteristics and quality of salmon fillets.

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The objective of this study was to characterize the physicochemical and textural changes in salmon fillets subjected to mixed salting (brine + dry salt) and dry salting, and to understand the underlying mechanisms involved by investigating muscle changes at each step of the process.

2. Materials and methods

1. Experimental design

Preparation of the fillets was carried out at an industrial site. The experiment used 15 fresh and graded Atlantic salmon (*Salmo salar*) sourced from the same supplier.

The fillets (1013 ± 19 g per fillet) were removed and deboned by professional staff on the production line and rigorously tagged for traceability toward the end product.

2. Processing steps

The experimental protocol is summarized in Figure 1.

2.1. Salting

For each salmon, the right-side fillet was salted with dry salt by sprinkling an even layer of fine salt across the inner surface of the fillet with an amount of salt calculated to 3.5% of the weight of the fillet, and then left at 4 °C for 6 h until measurements, sampling, and smoking.

The left-side (contralateral) fillet was first injected with a cold saturated brine solution (357 g of NaCl/L) using the industrial processor's brine injector, then covered with an even layer of fine salt with an amount of salt calculated to 2% of the weight of the fillet, and left in the same conditions as the right fillet at 4 °C for 6 h until measurements, sampling, and smoking. Therefore, the NaCl brought in mixed salting was 2% (dry salt) plus 1.6% (salt content in brine) resulting in 3.6% NaCl brought, which is similar to the 3.5% of the right dry salted contralateral fillets.

It was not possible for us to include in the experimental protocol fillets salted only by brine injection because of availability of the injector located on the production line, whose settings (injected volume) should have been modified only for the experimentation. Therefore, fillets salted

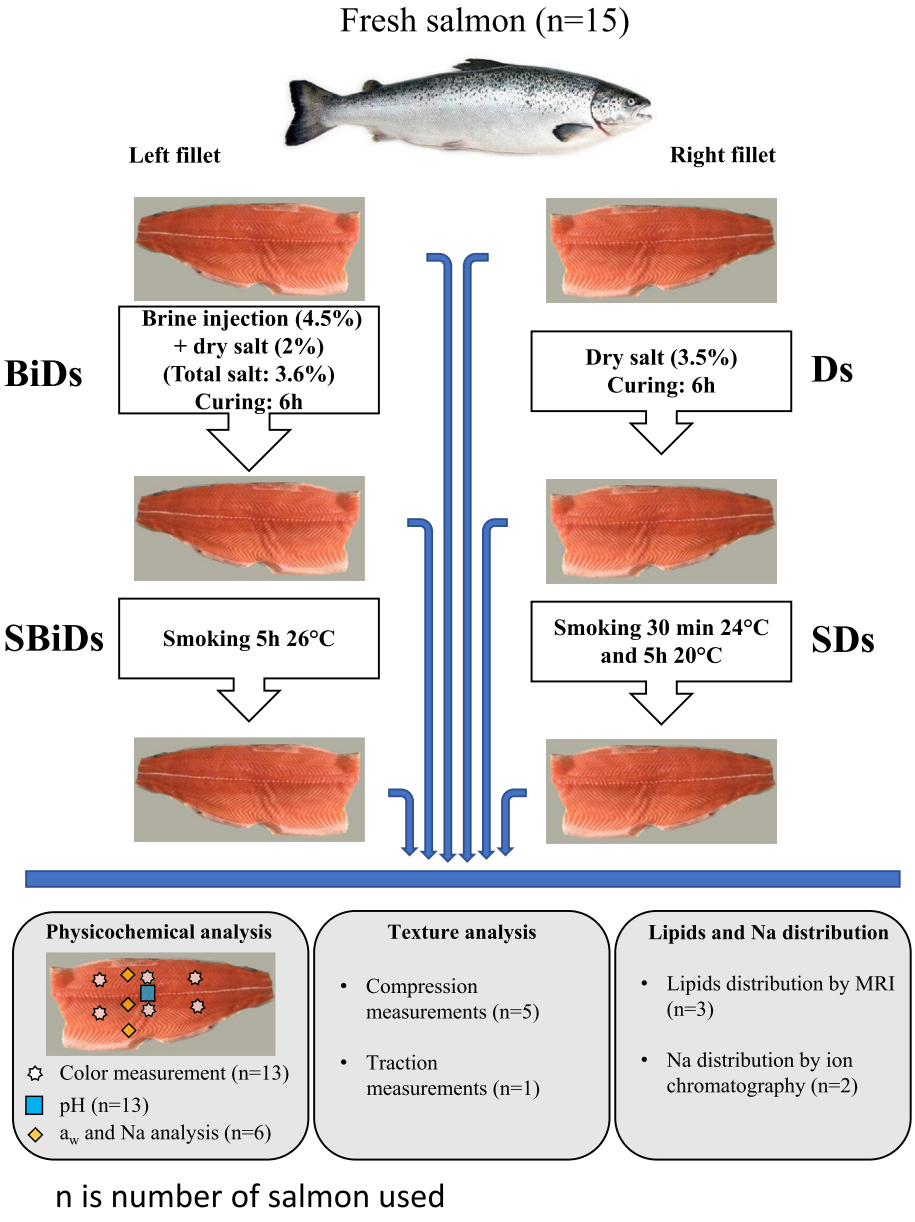

Figure 1. Experimental design and anatomical locations of measurements and samples.

with dry salt according to the factory's standardized protocol are the controls which are compared to fillets having undergone mixed salting.

2.2. Smoking

At the end of the salting process, the excess dry salt was removed from the surface and the fillets were smoked with beechwood at 60% humidity. To ensure that the fillets produced were compliant with sensory quality standards, the smoking protocol was adapted to the salting process: dry-salted fillets were smoked for 30 min at 24 °C and then 5 h at 20 °C, while the mixed-method (brine injection + dry salt) fillets were smoked for 5 h at 26 °C to consider the contribution of the water from the brine injection.

Fresh fillets were identified as 'Control', dry-salted fillets as 'Ds', mixed-salted (brine injection-plus-dry salting) fillets as 'BiDs', smoked dry-salted fillets as 'SDs', and smoked mixed-salted fillets as 'SBiDs'.

3. Physicochemical measurements

3.1. pH

pH measurements were performed at each stage of processing on both fillets of 13 salmon using 1 g of muscle ground in 10 mL of distilled water for 30 s at 15,000 rpm (Polytron PT 2100). pH was measured in the suspension using a Metrohm 654 pH-meter and a Metrohm 3M KCl pH electrode.

3.2. Color

Color measurements were carried out on the same salmon as used for the pH measurements (n = 13). Color was measured using a chromameter (Konica Minolta CM-2500d, Tokyo, Japan) based on the CIELab color space system after calibration with a standard reference white. CIE L*, a* and b* color coordinates were calculated using the CIE standard illuminant D65. Each measurement was an average of three consecutive acquisitions. For each fillet, 6 measurements distributed over the entire fillet (three at the back and three at the belly) were performed. L *, a * and b * data were recorded and transferred to Excel using the Konica Minolta SpectraMagic NX software, Tokyo, Japan.

3.3. Texture

Six salmon (i.e. 12 fillets) were reserved for texture measurements. In the processing plant, following color measurement and sampling for pH determination, a 10 cm portion was taken from each of the 12 raw fillets. The rest of the right fillets were dry-salted (Ds; 6 fillets) and the rest of the contralateral left fillets were brined and then dry-salted (BiDs, 6 fillets). At the end of the salting process, another 10 cm portion was taken from each fillet. The rest of the fillets were then smoked, and a final portion of 10 cm portion was taken from each smoked fillet (6 SBiDs and 6 SDs). Each 10 cm portion taken at each stage of the process was placed in code-identified plastic bag and kept in ice until measurements at the laboratory 48 h later. Mechanical measurements were performed using an Instron texture analyzer (Model 5543, Instron Worldwide Headquarters, Norwood, MA) coupled with dedicated acquisition software (Instron Bluehill software V2.27).

Compression measurements were carried out on 5 salmon at each stage of processing (fresh, salted, salted-smoked). For each of the 30 fillet pieces (10 raw control, 5 BiDs, 5 Ds, 5 SBiDs and 5 SDs), ten 3 cm × 1 cm × 1 cm cubes were cut away and run through a compression cycle test that measured deformation at 20% then at 80% at a constant speed of 1 $mm.s^{-1}$. Maximum yield stress was recorded. The final data for each fillet was the average measurement across the 10 repeats.

Because of the significant time required for this type of measurement, traction analysis was performed on a single salmon to avoid any bias linked to post-mortem change in muscle tissue. Eight 8 cm × 1 cm × 1 cm strips of muscle were taken from the two fillets at each stage of process (raw, BiDs, Ds, SBiDs and SDs; 8 repeats per condition), and their ends were attached to the traction stand using liquid nitrogen. The value of the ultimate tensile strength was recorded at each stage of the process (fresh, salted, salted-smoked) for each of the 8 repetitions.

3.4. Salt content, water content and a_w measurement

Samples of 0.5 g (for NaCl content determination) and 5 g (for water content and a_w measurements) were taken in triplicate from the fillets of 6 salmon, before salting (n = 12 control fresh fillets), after mixed-method salting (BiDs; n = 6 left-side fillets), dry-salting (Ds; n = 6 right-side fillets) and after and again after smoking (SBiDs; n = 6 fillets and SDs; n = 6 fillets).

3.4.1. Salt content

NaCl content was calculated from the sodium concentration measured by ion chromatography as previously described (Mirade et al., 2020). Briefly, a 0.5 g sample was homogenized in 10 mL of ultrapure water (PT 2100, Kinematica, Polytron) and centrifuged 20 min at 14,000 rpm. The NaCl content was measured in the supernatant by ion chromatography (850 professional IC, Metrohm, Switzerland) using a Metrosep C4-silica column grafted with carboxylic groups and a conductometric detector. NaCl content was calculated from a calibration curve obtained on standards of 1, 2, 5, 12.5 and 25 ppm.

3.4.2. Water content

Samples of 5 g were cut into small pieces and placed in a temperature-controlled chamber (Model FT127U, Firlabo, France) for 24 h at 105 °C, as per standard (NF EN 14346, 2007). Water content, *X*, expressed in kg water per kg^{-1} dry matter, was calculated as follows:

$$X = \frac{mi - mf}{mf} \quad (1)$$

where *mi* is initial mass of the analyzed sample and *mf* is final mass of sample after drying.

3.4.3. Water activity (a_w)

Samples of 3–5 g were cut into small pieces, placed in a measuring cup, and left for about 90 min to equilibrate. The a_w value was read through an a_w measurement device (a_w-Sprint TH-500 Novasina, Garches, France) calibrated with standards certified to 0.11, 0.33, 0.53, 0.75, 0.90 and 0.98 a_w.

4. In-fillet distributions of NaCl and water

Two fishes were used for the ion chromatography analysis to characterize NaCl distribution. The two fillets of one salmon were salted with the BiDs and Ds process respectively while the fillets from the other salmon were smoked after salting (SBiDs and SDs). The analyses were carried out between 18 and 22 days after the end of the process to allow time for the salt to diffuse into the fillets. A 10-cm-wide slice was removed from the head and a 20-cm-wide slice was removed from the tail (see supplementary data S1) to keep the most representative pieces of a commercial fillet (which averages 16 cm wide and 2 cm thick and weighs about 600 g). The piece was squared into 24 portions of about 8 cm^2 each (see supplementary data S1). Each spatially identified section was ground, then 0.5 g of ground material was used to determine NaCl content, and 5 g of ground material was used to determine water content as per sections 3.4.1 and 3.4.2 respectively.

5. Lipid MRI mapping

Magnetic resonance imaging (MRI) of lipid content was performed at 4.7 T on a Bruker imager (Bruker GmbH, Ettlingen, Germany) with a 26

cm-diameter bore equipped with a BGA-26 gradient system and a 20 cm-diameter volumic radiofrequency quadrature-polarized (1H/23Na) coil (RAPID Biomedical GmbH, Rimpar, Germany) for both emission and signal reception. A 3D diffusion-weighted MRI was performed on the whole volume of each portion of the salmon filets. A diffusion tensor imaging sequence was used to highlight lipids by exploiting the vast difference between the apparent diffusion coefficients of lipids and muscle fibers. The main acquisition parameters were as follows: TE/TR 26/500 m s; spatial resolution 1 mm^3; b value 21 s/mm^2; total acquisition time 3h50.

Averaged profiles were calculated from these 3D diffusion-weighted MRI. To illustrate, the profile presented in the part 7.4 of the Results and Discussion section corresponds to the mean value for a 5-mm slice through the thick center of the fillet. This mean slice was then averaged in head-to-tail direction to obtain the dorsal-belly profile.

6. Statistical analysis

Results are expressed as mean ± standard error of the mean. Statistical analyses of physicochemical data and image data were performed using Statistica software (Statistica, version 13.5). Analyses of variance and comparisons of means were performed using a Fisher test followed by a post-hoc Tuckey HST test. Differences were considered significant at $p < 0.05$.

7. Results and discussion

7.1. Evolution of pH

Compared to fresh salmon, salting did not cause a significant variation in pH regardless of the process applied, whereas smoking caused a significant drop of around 0.15 pH units (Figure 2). These results are consistent with previous studies reporting that smoking decreases pH by 0.2–0.3 pH units (Chan et al., 2020; Rizo et al., 2015). This decrease in pH is attributed to the deposition of acidic organic compounds such as carbonyls, phenols, organic acids or tars.

7.2. Color change

The results of the color analysis are presented in Table 1. The different processing steps were found to cause variations in luminance (L*), red index (a*) and yellow index (b*).

Luminance (L*) decreased at each step of the process regardless of salting method (dry salting or brining plus salting). Redness (a*) decreased during salting but remained stable during the smoking process, whereas yellow index (b *) decreased during salting but then increased after smoking.

The effect of salting alone (before the smoking process) on color parameters is consistent with (Simpson et al., 2018) who found a significant

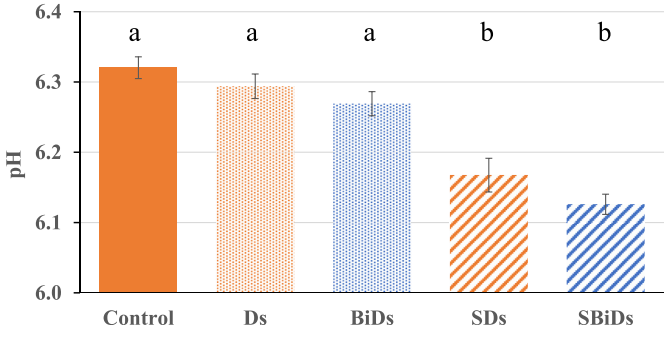

Figure 2. pH as a function of processing stage (n=13 salmon) Ds: dry-salted, BiDs: brine-injected and dry-salted, SDs: smoked after dry-salting, SBiDs: smoked after brine injection and dry-salting.

Table 1. Color as a function of processing stage.

fillet	process	L*	a*	b*
right	control	$43.47^{a} \pm 0.70$	$16.44^{a} \pm 0.23$	$14.99^{a} \pm 0.34$
	Ds	$40.75^{b} \pm 0.48$	$15.01^{b} \pm 0.22$	$11.30^{b} \pm 0.33$
	SDs	$35.35^{c} \pm 0.36$	$15.30^{b} \pm 0.24$	$16.08^{c} \pm 0.35$
left	control	$43.95^{a} \pm 0.65$	$16.52^{a} \pm 0.24$	$13.39^{a} \pm 0.32$
	BiDs	$40.49^{b} \pm 0.49$	$14.30^{b} \pm 0.27$	$10.52^{b} \pm 0.36$
	SBiDs	$35.53^{c} \pm 0.28$	$14.18^{b} \pm 0.29$	$15.77^{c} \pm 0.51$

Values are expressed as mean ± SEM (n = 13 salmon). Different superscript letters in the same column and for a given side (right or left) flag significant differences at $p < 0.05$.
Ds: dry-salted, BiDs: brine injection and dry-salting, SDs: smoked after dry-salting, SBiDs: smoked after brine injection and dry-salting.

decrease in L*, a* and b* following brining of salmon samples. The decrease in luminance after salting may be related to surface dehydration, as the color measurement was carried out after the fillets had been left for 6 h to equilibrate. The decreases in redness (a*) and yellowness (b*) may result from partial decomposition or extraction of astaxanthin during the salting process (Birkeland and Bjerkeng, 2004, 2005; Birkeland et al., 2004b; Lerfall et al., 2011; Lerfall et al., 2016). Indeed, a* and b* are positively correlated with in-fillet astaxanthin content (Bjerkeng, 2000), and decomposition or extraction of astaxanthin during the salting process (Birkeland and Bjerkeng, 2004) could be the cause of a decrease in redness and yellowness. In salmon muscle, astaxanthin is linked to alpha actinin (Matthews et al., 2006), which is a component protein of the Z disks delimiting sarcomeres in myofibrils (Boland et al., 2019). The addition of salt increases ionic strength, which leads to solubilization of myofibrillar proteins (Offer and Knight, 1988; Offer and Trinick, 1983) and promotes the release and extraction of astaxanthin.

The smoking step significantly decreased lightness and increased yellowness but had no effect on redness compared to the previous salting step. The decrease in luminance after smoking is consistent with previous findings (Birkeland et al., 2003; Cardinal et al., 2001; Choubert et al., 1992). Our results are close to those of Birkeland et al. (2003) who reported that mean L*, a* and b* values were 42.2 ± 2.0, 10.7 ± 0.9 and 13.5 ± 1.4, respectively, in unsalted controls progressing to 38.5 ± 2.3, 9.5 ± 1.2 and 17.9 ± 1.5, respectively, after dry salting and 40.6 ± 0.2, 9.2 ± 1.3 and 19.2 ± 1.5, respectively, after salting by brine injection.

The absorption of compounds during smoking (Arvanitoyannis and Kotsanopoulos, 2012) associated with the increase in NaCl content is likely to decrease luminance and redness. The increase in yellow index b* after smoking is probably due to uptake of organic molecules coming from components of the smoke and from reactions of smoke compounds

Table 2. Colour as a function of processing stage and anatomical location.

location	process	L*	a*	b*
back	control	$39.45^{a} \pm 0.47$	$15.94^{a} \pm 0.38$	$12.30^{a} \pm 0.32$
	Ds	$38.21^{b} \pm 0.44$	$14.72^{b} \pm 0.51$	$9.59^{b} \pm 0.58$
	BiDs	$38.38^{b} \pm 0.45$	$13.97^{b} \pm 0.41$	$9.03^{b} \pm 0.37$
	SDs	$34.17^{c} \pm 0.41$	$14.85^{ab} \pm 0.48$	$14.36^{c} \pm 0.66$
	SBiDs	$35.20^{c} \pm 0.35$	$13.72^{b} \pm 0.58$	$14.04^{ac} \pm 0.80$
belly	control	$46.96^{a} \pm 0.53$	$17.01^{a} \pm 0.43$	$16.08^{a} \pm 0.39$
	Ds	$43.30^{a} \pm 0.50$	$15.30^{b} \pm 0.35$	$13.02^{b} \pm 0.34$
	BiDs	$42.61^{a} \pm 0.55$	$14.64^{b} \pm 0.55$	$12.02^{b} \pm 0.58$
	SDs	$36.54^{b} \pm 0.35$	$15.74^{ab} \pm 0.49$	$17.80^{a} \pm 0.43$
	SBiDs	$35.81^{b} \pm 0.33$	$14.74^{b} \pm 0.43$	$17.63^{a} \pm 0.58$

Values are expressed as Mean ± SEM (n = 13 salmon). Different superscript letters in the same column and for a given side (back or belly) flag significant differences at $p < 0.05$.
Ds: dry-salted, BiDs: brine injection and dry-salting, SDs: smoked after dry-salting, SBiDs: smoked after brine injection and dry-salting.

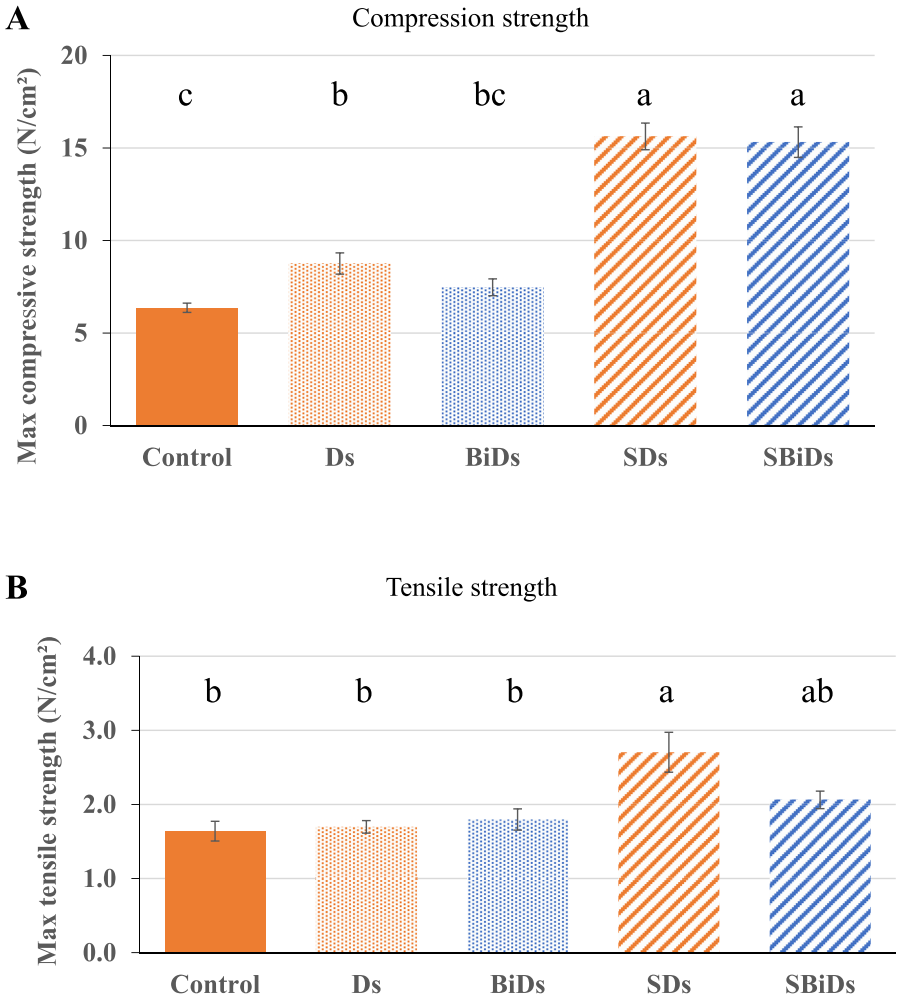

Figure 3. Time–course changes in compressive and tensile strength A: compressive strength. B: tensile strength. Different letters mean significant differences at $p<0.05$. Ds: dry-salted, BiDs: brine injection and dry-salting, SDs: smoked after dry-salting, SBiDs: smoked after brine injection and dry-salting.

with proteins (Toth and Potthast, 1984). Indeed, the typical smoke color is likely to come from condensation reactions between carbonyls and amines (Martinez et al., 2007).

We also observed that luminance (L*) varied significantly depending on location of the measurement point (Table 2). The higher luminance of the ventral part compared to the dorsal part may be related to the higher ventral part lipid content (see section 5: Link between salt content and fat distribution; Aursand et al., 1994). There was no salting-method effect on color of the smoked fillets in our experimental conditions.

7.3. Texture change

The results of mechanical measurements are reported in Figure 3. Compression-test results (Figure 3A) showed a slight increase in firmness of the flesh after salting (only significant for dry-salting). The force required to reach the maximum compressive strength was two-fold higher after smoking than in non-smoked salted samples, regardless of salting process.

The increase in firmness after dry-salting is a probably a consequence to the extraction of water by dry salt (Filgueras et al., 2016), highlighted in our experiment by the decrease in water content of salted samples compared to controls (Figure 4). Water transfers from muscle fibers to extracellular space and then to outside the fillet (as exudate) lead to a decrease in muscle-fibre cross-sectional area (Sigurgisladottir et al., 2000) and thus an increase in fiber density, which is likely to increase firmness (Johnston et al., 2000).

The increase in firmness observed in our samples after smoking agrees with previous studies (Birkeland et al., 2004a; Chan et al., 2020; Kong et al., 2015; Sigurgisladottir et al., 2001; Sigurgisladottir et al., 2000). Birkeland et al., 2004a observed a 70% and 125% increase in firmness after salting by injection and dry-salting respectively. However, unlike Birkeland et al., we found no between-method differences in texture, probably because the two methods tested here (dry-salting vs mixed brine-injection plus dry-salting) did not cause sufficient variations in structure and water content to significantly modify the texture. The greater firmness of smoked fillets is thought to be due to a decrease in moisture, activation of interactions between muscle proteins (Birkeland et al., 2004a), and interactions between muscle proteins and molecules contained in the smoke (Toth and Potthast, 1984).

The tensile-test results (Figure 3B) reflect the elasticity of the flesh. SDs fillets were significantly more elastic than both Ds and BiDs fillets, in line with previous results (Birkeland et al., 2004a; Martinez et al., 2012). Smoking lead to protein insolubilization, especially at high ionic strength (Gomez-Guillen et al., 2000) and a loss of water which explain the better

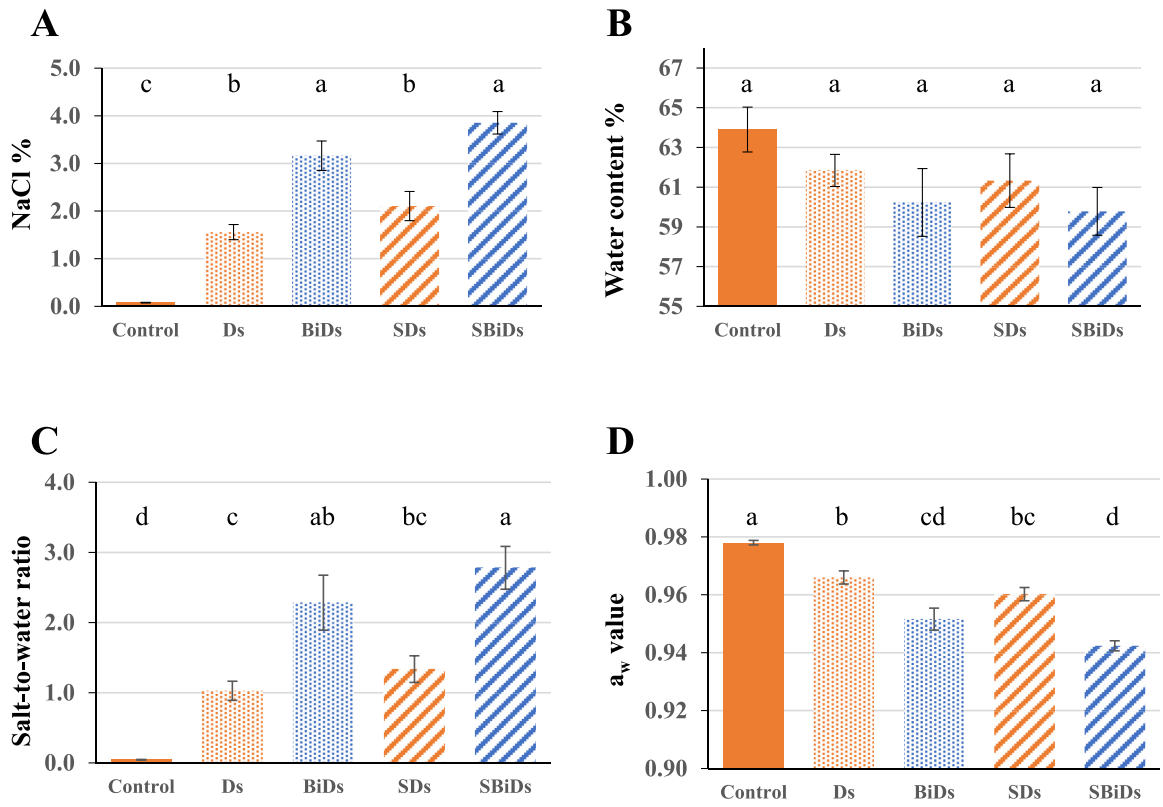

Figure 4. Physicochemical changes over the course of processing A: NaCl values as determined by HPLC. B: Water content determined by measuring weight loss after drying. C: Ratio of NaCl to water content after normalization by dry weight (water content (kg water/kg dry matter) per NaCl (%). D: a_w values. Ds: dry-salted, BiDs: brine injection and dry-salting, SDs: smoked after dry-salting, SBiDs: smoked after brine injection and dry-salting.

elasticity of smoked fillets. However, the elasticity of the SBiDs portion was not different from that of the unsmoked fillets. The lower tensile strength of the SBiDs compared to SDs samples could be due to the higher gaping rate of the BiDs fillets (Birkeland et al., 2004a; Bjornevik et al., 2018) and therefore a weakness in muscle cohesion.

7.4. Evolution of salt content and relation to water content

Fresh salmon contains less than 0.1% salt, which corresponds to the physiological sodium content in muscle. The salt content of the salted filets depended on salting method (Figure 4A). BiDs and SBiDs fillets had significantly higher salt concentrations compared to Ds and SDs fillets. Regardless of the salting process (Ds or BiDs) the salt rate initially added was similar (3.5% versus 3.6% see Materials and Methods section). After 6 h of salting, the dry salt that remained on the surface of the fillet was removed, which partly explains the higher salt content in the brine-injected BiDs fillets. Moreover, diffusion of dry salt into the tissue is much slower after dry salting than brining (Filgueras et al., 2016), especially when the brine is injected into the product. It is thus not surprising that BiDs and SBiDs had a higher salt concentration than Ds and SDs fillets (Figure 4A). The slight increase in NaCl concentration during smoking (Figure 4A) is a consequence of dehydration during the process (Carton et al., 2009). One of the roles of salting is to reduce water activity in order to limit bacterial development in the food (Oliveira et al., 2012). Here, the addition of salt led to a reduction (although not statistically significant) in water content (Figure 4B) but the salt/water ratio evolves in the same way as the salt content (Figure 4C). The water content was highly correlated with the decrease in a_w ($r = 0.93$) (Figure 4D), in agreement with the interpretation of Sampels (2015). The a_w values found here for fresh salmon (0.98) are consistent with data from the scientific literature (Rizo et al., 2015). However, for smoked salmon, Rizo et al. (2015) found an a_w of 0.936, which was significantly lower than the a_w here and lower than the a_w value of 0.96 reported by Lovdal (2015). These differences are due to process variations that can lead to relatively significant water losses, which has repercussions on key characteristics of the fillets, including a_w.

Complementary to the above analysis, this study also showed how salt content, as expressed per water content after normalization by weight, was distributed along the back to belly (Figure 5). Comparing the salt content against water content usefully compensates for the added water brought by the brine injection in some treatments. Compared to the samples salted with dry salt, the mixed salted samples show a more homogeneous salt distribution in the back-belly direction but more variable in the head-tail direction, revealed by the high standard deviations (Figure 5). This result suggests an easier diffusion of the brine in the back-belly direction than in the head-tail direction which can be explained by the difficulty of the brine to cross the myosepta between 2 injection points (see supplementary data for illustration). Figure 6 shows that overall, NaCl-to-water ratio was higher in the dorsal part than in the ventral part of the fillets. This is probably related to the lower lipid content of the white muscle of the dorsal part compared to that of the belly flap. However, salt content in the end-product also depends on multiple other factors, such as duration and temperature of salting, thickness of the fillets, and concentration of the brine (Gallart-Jornet et al., 2007a, 2007b).

7.5. Link between salt content and fat distribution

MRI was used to map the distribution of fat content in a piece of fish fillet. At the end of the mapping process, the piece of fish fillet was used to measure NaCl content by ion chromatography (Figure 6). The lipid content shows a fat decrease from the head to tail of the fish, in

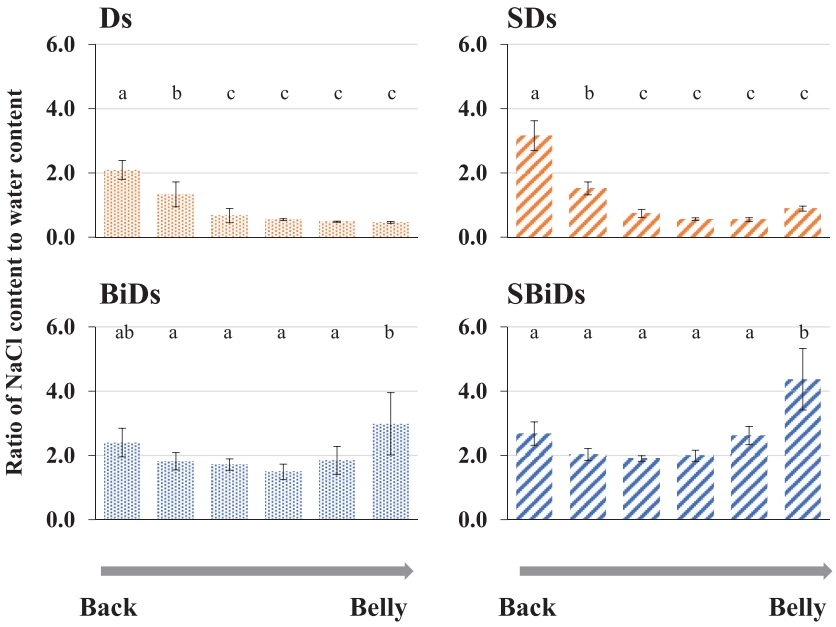

Figure 5. Differences between salting processes Values are expressed as NaCl content (%) normalized by water content (Kg water/Kg dry matter). Ds: dry-salted, BiDs: brine injection and dry-salting, SDs: smoked after dry-salting, SBiDs: smoked after brine injection and dry-salting.

accordance with the literature (Aursand et al., 2009; Bocker et al., 2008; Carton et al., 2009; Katikou et al., 2001; Segtnan et al., 2009). Along the dorsal-belly axis, the lipid content of the white muscle was high near the back and belly but progressively decreased approaching the backbone, in line with previous results (Aursand et al., 1994; Katikou et al., 2001; Segtnan et al., 2009). We studied the lipid profiles using an 'average profile' running from back to belly and along the head-to-tail sections (Figure 6). The results showed that myosepta revealed oscillations of lipid density on the head-to-tail lipid profiles. The profiles suggest that the myosepta are oriented differently at the back/belly in comparison to the area at the center of the fillet. We then cross-compared the MRI profiles to the ion chromatography-measured NaCl concentration datapoints from back to belly (Figure 6), and found that salt concentrations were the lowest in the middle of the fillet and the highest in the belly-flap areas. Cross-comparison of lipid profiles against NaCl profiles (Figure 6) showed that the high fat content in the back area limits salt uptake in the salmon muscle, as previously reported (Cardinal et al., 2001; Gallart-Jornet et al., 2007a), due to a decrease in salt diffusivity (Lebert and Daudin, 2014; Wang et al., 2000). However, as the belly flap is the thinnest part of the fillet, it is expected to have a higher relative salt content. Salt content was minimal in the inner part of the filet where deboning had caused muscle damage (red arrow on Figure 6). It is

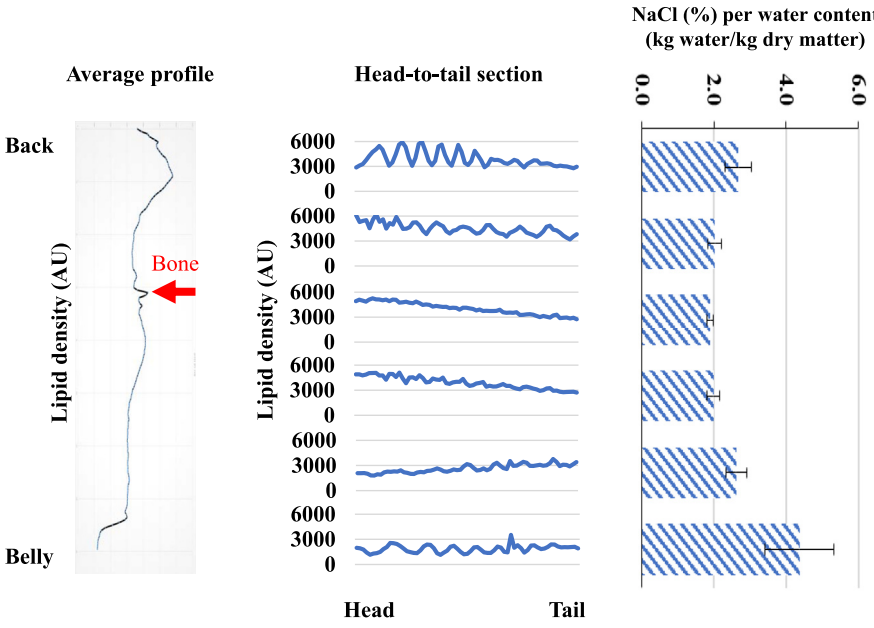

Figure 6. Lipid and sodium distribution profiles in salted salmon fillets Lipid distribution was assessed by MRI mapping. The same sample was then divided into pieces to measure NaCl (%) by ion chromatography (Figure 5). The left-hand column is the averaged back-to belly lipid profile, the middle column is the averaged head-to-tail lipid profiles for each back-to-belly, and the right-hand column is the salt content in these back-to-belly sections. The example below illustrates the profiles of SBiDs (smoked after brine+dry salting). The lipid profiles reveal how myosepta orientation (vertical/horizontal) can affect NaCl distribution.

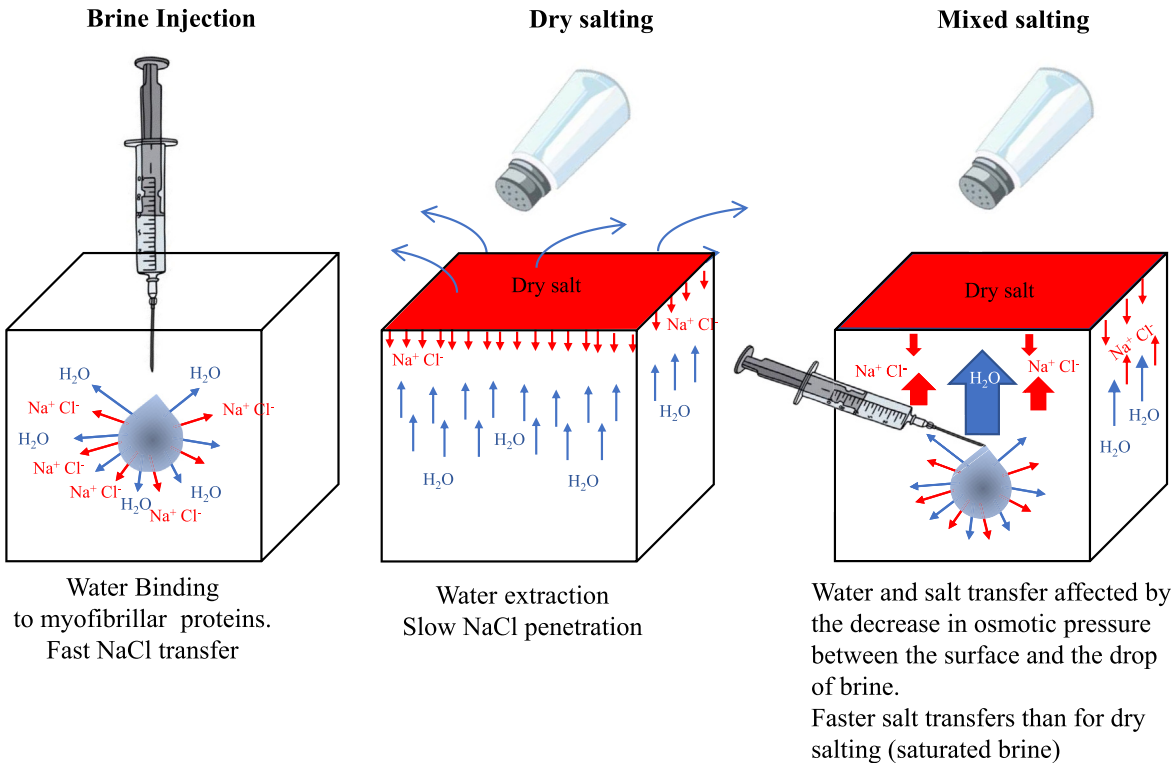

Figure 7. Water and NaCl transfers in brine, dry-salted and mixed-method samples.

possible that this muscle damage could have affected diffusion of the salt. That said, this part is also the thickest section of the fillet, which likely largely explains the lower salt intake in this area. Hypothetically, our data points to orientation or thickness of the myosepta as a further factor preventing salt diffusion, but further research is needed. Note that a number of previous studies using advanced imaging techniques have demonstrated that salt diffusion and distribution is highly affected by fat distribution (Aursand, Erikson and Veliyulin, 2010; Cardinal et al., 2001; Segtnan et al., 2009).

8. Conclusion

The objective of this study was to characterize the effect of a mixed salting method (brine injection + dry salting) on salt distribution and quality traits in salmon fillets. Under our experimental conditions, mixed-method salting led to a higher salt content than dry-salting, but there was no salting-method effect on color or texture of the fillets after the salting step and then after the smoking step. The fattiest areas of the fillet were also those that contained the least salt, which points to an impact of lipids as a barrier to in-fillet salt diffusion. The mixed salted fillets show a more homogeneous salt distribution in the back-belly direction but a more variable salt distribution in the head-tail direction than dry salted fillets. The head to tail NaCl content variability is probably due to the difficulty of the aqueous phase to cross fat-rich myosepta, but also because the injection of brine in tandem with surface-salting with dry salt disturbs the gradients and affects the in-fillet transfers of water and salt (Figure 7).

Compared to dry-salting, mixed-method salting accelerated salt uptake without significantly altering the instrumental traits of the fillets, but it also generated a more local variability in salt distribution that could have consequences on sensory properties during end-product storage. Further research is needed to better understand why the mixed-method salting causes more heterogeneous local salt profile in the flesh and to investigate whether this local variability in salt content leads to heterogeneity in muscle ultrastructure and protein unfolding that can affect the intrinsic quality of smoked salmon fillets.

Declarations

Author contribution statement

Thierry Astruc, Annie Vénien: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sylvie Clerjon, Stéphane Portanguen: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Raphael Favier: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Olivier Loison: Performed the experiments.

Pierre-Sylvain Mirade, Arno Germond: Analyzed and interpreted the data; Wrote the paper.

Jacques Rouel: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Mailys Lethiec: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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