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The use of atomized purified condensed smoke (PCS) in cold-smoke processing of Atlantic salmon - Effects on quality and microbiological stability of a lightly salted product

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ABSTRACT

A novel technology for smoking of muscle foods is atomization of purified condensed smoke (PCS). The use of PCS is supported by the European Union and is considered healthier than traditional smoke-processing and is nowadays widely used in meat processing. However, scant information regarding the potential of PCS-processing of seafood is available. The aim of the present study was to study PCS-processing of lightly salted salmon added a gradient of potassium lactate (KL), and its effect on product quality. As a reference, traditionally cold smoked salmon (CSS) smoked with beech chips was used. The study stated that PCS-processing inhibit bacterial growth (aerobic plate count, and lactic acid bacteria) and thereby has potential to enhance the shelf life of CSS. Combination of barriers such as decreased water activity (a_w) and pH were of highly importance, whereas the effect of phenolic compounds and added KL were minor. PCS-processed salmon was moreover firmer, darker, and slightly less reddish and yellowish compared to those smoked traditionally. Fillet appearance and firmness were both found related to muscle a_w, showing the importance of optimizing the ratio between drying and atomization to obtain a PCS processed high-quality CSS.

1. Introduction

Half of the seafood eaten by humans originate from aquaculture, making nutritional and environmental aspects in aquaculture and seafood processing important (Boss, Overesch, & Baumgartner, 2016; Costa-Pierce, 2010). The worldwide production of farmed Atlantic salmon (*Salmo salar* L.) reached 2.4 million tons in 2011 (Asche, Roll, Sandvold, Sørvig, & Zhang, 2013) giving the marked a stable supply of the raw material, whereas 28% of the salmon products in the EU market are distributed as smoked (EC, 2016).

Cold smoke processing is a procedure making ready-to-eat (RTE) salmon as a lightly preserved fish product (Giménez & Dalgaard, 2004; Løvdal, 2015; Porsby, Vogel, Mohr, & Gram, 2008). Producing cold smoked salmon (CSS), protocols involving pyrolysis of wooden chips are commonly used, whereas dipping or spraying with liquid smoke is an alternative (Hattula, Elfving, Mroueh, & Luoma, 2001; Martinez, Salmerón, Guillén, & Casas, 2007; P.; Visciano, Perugini, Conte, & Amorena, 2008). Wooden smoke consists of several bioactive compounds, dispersed as an emulsion of droplets in an air and vapour phase, however often contaminated by undesirable polycyclic aromatic hydrocarbons (PAHs) (Visciano, Perugini, Amorena, & Ianieri, 2006), ash and soot (Wretling, Eriksson, Eskhult, & Larsson, 2010).

The use of atomized purified condensed smoke (PCSs) are an innovative production technology widely used to produce smoked meat products (Gedela, Gamble, Macwana, Escoubas, & Muriana, 2007; Lingbeck et al., 2014). Limited knowledge is however available about the product quality and industrial usage of PCS on seafood. Main benefits of the PCSs are that it is purified, and therefore appear as healthier without unhealth substances such as PAHs, ash and tar (Cardinal, Cornet, Sérot, & Baron, 2006; Lingbeck et al., 2014; Singh & Shalini, 2016). Varlet, Serot, Monteau, Bizec, and Prost (2007) stated moreover that salmon smoked with liquid smoke showed lover contents of PAHs compared to salmon smoked traditionally. PCS are regarded as environment-friendly, and to give better control and a more even distribution of the smoke flavour after being applicated. The smoking procedure using PCS are often performed by using compressed air in a closed chamber, where a liquid flow of atomized vapour is added in cycles combined with drying. Phenols, carbonyls and organic acids are the main compounds responsible for the flavour, colour and antimicrobial properties of smoked products (Lingbeck et al., 2014). PCS have demonstrated inhibiting potential against pathogenic bacteria occurring in seafood e.g. *Listeria monocytogenes* and *Aeromonas*

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hydrophila shown in vitro (Suñen, Fernandez-Galian, & Aristimuño, 2001) and in seafood systems (Suñen, Aristimuño, & Fernandez-Galian, 2003). Commercially, hundreds of PCSs are available, where aroma and flavour characteristics can be tailor made for its specific application (Martinez et al., 2007).

Shelf life and food safety of CSS is affected by its water activity (a_w), pH and contents of salts and polyphenols (Cornu et al., 2006; Løvdal, 2015). Smoking do moreover lead to firmer texture (Birkeland, Bencze Rørå, Skåra, & Bjerkeng, 2004) and darker and more yellowish colour (Birkeland et al., 2004; Birkeland, Haarstad, & Bjerkeng, 2004; Cardinal et al., 2001). The microbiota of CSS is complex (Joffraud et al., 2006), where spoilage bacteria degrade the product and restrict its shelf life, *i.e.* changing taste, smell and consistency of the product (Løvdal, 2015). To inhibit growth of unwanted microbiota, adequate levels of salt need to be added (traditionally 2.5-3-5% (w/w) for CSS) (Leroi, Joffraud, Chevalier, & Cardinal, 2001). High sodium intake for humans is however unhealthy (He & MacGregor, 2008; WHO, 2013) and the industry are therefore encouraged to reduce salt content of all food products, included seafood (WHO, 2013). To meet these challenges salt replacers such as potassium lactate, potassium diacetate and potassium acetate (Sallam, 2007) can be used.

The overall aim of the present study was to examine how the use of atomized PCS (SmokEz VTABB) combined with Opti.Form® PPA Plus as a NaCl substitute affect the overall quality and microbiological stability of a lightly salted CSS product. Opti.Form® PPA Plus (PURAC biochem bv, The Netherlands) is a commercial blend of 72.8% potassium lactate (KL), 5.2% potassium acetate diluted in deionized water, and was chosen due to its availability, its chemo-physical properties and that it can be used as an additive to seafood.

2. Material and methods

2.1. Raw material and experimental design

Atlantic salmon fillets (*Salmo salar* L.) used in the present study was produced under the Super Green™ label and purchased twice (n = 30 and 14) from Vikenco AS, Aukra, Norway. The raw material arrived NTNU, Trondheim, Norway, three days post mortem.

The salting procedure were performed in a two-stage process where injection of different concentrations of Opti.Form® PPA Plus was followed by traditional dry salting. After salting, fillets were either smoked traditionally by pyrolysis of wooden chips or by atomized PCS giving a factorial design consisting of salting and smoking procedures as factors (Table 1). After processing, the fillet part, posterior to the Norwegian quality cut (NQC) and det belly flap was removed before the experimental part was divided into four equally sized samples. Each sample was thereafter vacuum-packed (Webomatic packaging system, Webomatic®, Germany) and stored in a refrigerated room at 4 °C until sampling. Bacterial counts, pH and drip loss (DL) were analysed on day 1, 3, 5, 7, 9, 13, 17, 24 and 31 post processing (n = 3 on each sampling day). Total phenolic content (TPC, n = 6), NaCl (n = 3) and dry matter (DM, n = 6) were measured at day 9, whereas water activity was analysed at day 24 (n = 3), colour at each processing step (n = 7–8) and at day 0 (n = 7–8), 17 and 31 (n = 3), and texture were measured in duplicates of three samples at each sampling point at day 3, 17 and 31 post processing.

Table 1
Experimental design showing discriminant parameters between groups.

Group ID[b]	Number of fillets	[Opti.Form® PPA Plus], %[a]	Smoking technology
WC-0.0	7	0.0	Wooden chips
WC-2.6	7	2.6	Wooden chips
PCS-0.0	7	0.0	Atomized PCS
PCS-2.6	7	2.6	Atomized PCS
PCS-5.2	8	5.2	Atomized PCS
PCS-7.8	8	7.8	Atomized PCS

[a] Calculated concentration (w/w) of potassium lactate (KL) in the solution injected into fillets of the respective group.
[b] Abbreviates used: Wooden chips = WC and purified condensed smoke = PCS.

2.2. Salting and smoking procedure

Injections of diluted Opti.Form® PPA Plus (Table 1) were performed using a Dorit-DFT brine injector (Dorit-DFT Fleis-chereimaschinen GmbH, Germany) equipped with an array of 3 × 19 needles (3 mm diameter, 320 mm length, round needle tip with a pressure of 3.2 bar). After injection of either pure ion exchanged water (control) or diluted Opti.Form® PPA Plus (Table 1), fillets were dry-salted on grids with NaCl (fine-refined salt minimum 99.8% NaCl). After 3 h (4 °C), excess of NaCl were rinsed off with running water (approximately 6–8 °C).

Two different smoking protocols were used in the present study; traditional cold smoking by wooden chips, and atomized PCS, respectively. Traditional cold smoking was performed at 22 °C after a method described by Lerfall, Bendiksen, Olsen, and Østerlie (2016). In total, fillets were processed for 300 min, consisting of 120 min rapid drying and 180 min of smoking. For the atomized PCS protocol, a Red Arrow Powersmoker Model 100 BUDGET connected to a Kerres smoke-air® show smoker CS700 EL MAXI 1001 smoking cabinet, Kerres, Germany was used. Fillets were rapidly dried on grids for 150 min before they were processed by atomized PCS (SmokEz VTABB, 9–12% acetic acid, pH = 2.0–3.2, Red Arrow, USA) in cycles consisting of smoking and drying. Each cycle consists of four times atomization of PCS (30 s, liquid flow: 15 mL/min, pressure: 5.5 bar) followed by 6 min of air circulation after each period of 30 s atomization. This procedure resulted in a total cycle time of 57 min (30 and 27 min of drying and smoking, respectively). The PCS protocol was ended after a total of six cycles, giving a total processing time of 462 min consisting of 300 and 162 min drying and smoking, respectively.

2.3. Chemo-physical parameters

2.3.1. Processing yield, fillet dry matter and drip loss

Each fillet was weighed upon salting, and post smoking, to calculate processing yield for all experimental groups. The processing yield was expressed as per cent of initial sample weigh.

Drip loss, % (DL) from the samples as a function of storage time was measured as the difference in fillet mass between day 0 and respective sampling day throughout 31 storage, according to a method described by Rotabakk, Jørpeland, and Lerfall (2018). Fillet dry matter (DM) was analysed according to ISO.6496 (1983) by drying samples taken from the dorsal side of the lateral line at 105 °C for 24 h.

2.3.2. Muscle pH

Muscle pH was measured with a Hach HQ40d multi Portable Meter (Hach, USA) connected to a pH Selective Electrode Intellical™ PHC725 (Hach, USA) in the dorsal part of the raw material and at each sampling day throughout 31 days storage trial.

2.3.3. Colour measurement

The surface colour (CIE, 1994) was measured on a DigiEye full system, VeriVide Ltd., Leicester, UK of the raw material, and after 17 and 31 days refrigerated storage. The samples were placed in a standardized light-box (daylight, 6400 K) and photographed using a digital camera (Nikon D80, 35 mm lens, Nikon Corp., Japan) The software Digipix (version 2.8) was used to calculate $L^*a^*b^*$ values from RGB values obtained from the fillet image. L^* describes lightness of the sample ($L^* = 0$ = black, $L^* = 100$ = white), a^* the redness ($a^* > 0$) and b^* ($b^* > 0$) the yellowness.

2.3.4. Textural analysis

Instrumental textural analyses were performed in duplicates on each sample using a Texture Analyser TA-XT plus (Stable Micro Systems Ltd, England) equipped with a 25-kg load cell and a flat-ended cylindrical plunger (d = 20 mm, type P/1SP). Force-time graphs were recorded and analysed by Texture Exponent light software for Windows (version 4.12, SMS). Breaking force (B_f) was measured as the force (N) recorded when breakage of the sample surface was observed. Fillet firmness was measured as the force required to press the plunger down to 80% (F80) of the fillet thickness. Textural measurement was performed with a constant speed of 2 mm/s.

2.3.5. Sodium chloride (NaCl) content and water activity

Muscle samples for NaCl analyses (approximately 5 g) were taken above the lateral line of the smoked sample after nine days storage (4 °C). All samples were homogenized in heated deionized water (50 mL, 50 °C) using an electric blender (9000 rpm, 5 min, IKA ® T25 digital ULTRA TURRAX ®). After homogenization all samples were cooled to room temperature and diluted in a volumetric flask (250 mL). Contents of chloride (mg/L) was measured on a Hach HQ40d multi Portable Meter, Hach, USA connected to an Intellical™ (Cl^-) Ion Selective Electrode (ISE) (Hach, USA). The content of NaCl was moreover calculated based on the molecular weight and expressed as per cent NaCl of sample weigh. The water activity (a_w) was measured twice at the abdomen part of each sample 24 days post processing by using a top water activity measuring instrument (Lab Master-a_w neo, Novasina, Switzerland). The first measure was performed on the sample surface (circular piece, d = 40 mm, thickness 5 mm) whereas the second was done of the inner muscle sampled at the same area.

2.3.6. Total phenolic content (TPC)

Muscle samples of 2.5 g were analysed in duplicates of each sample after a method described by Singleton, Orthofer, and Lamuela-Raventós (1999). Before analysis all samples were added 10 mL of methanol:-water (100:80) and homogenized for 5 min (11000 rpm) with a IKA® T25 digital ULTRA TURRAX® homogenizer (GmBH & Co, Germany), thereafter centrifuged for 15 min at 5000 rpm before the supernatant was filtrated through a 0.2 μm Cellulose Acetate membrane (VWR International, USA). One mL of the filtrated supernatant was added Folin-Ciocalteu reagents (1 mL, VWR International, France) and diluted with deionized water (5 mL). After 8 min at room temperature, Na_2CO_3 (7%, 10 mL, Alfa Aesar, Germany) was added before the mixture was diluted to 100 mL in a volumetric flask (100 mL). Both sample solutions and standard solutions made from a serially diluted gallic acid (GA) monohydrate solution (CAS: 5995-86-8, Sigma Aldrich, USA) were stored dark at room temperature for 3 h before measured at 750 nm on a UV spectrophotometer (UV spectrophotometer UV-1800, Shimadzu Corp., Japan). TPC was expressed as mg GA equivalents/100 g muscle sample.

2.3.7. Microbiological analysis

A 10-g piece from lateral line area of the fish (n = 3) was aseptically transferred to a sterile stomacher bag and diluted 1:10 with sterile peptone water (8.5 g/L NaCl (Merck) and 1 g/L bacteriological peptone (Oxoide)) and homogenized vigorously for 60 s in a Stomacher 400 Lab Blender (Masticator Basic Panoramic, IUL Instruments, Spain). Appropriate serial dilutions were made in peptone water. Total aerobic plate count (APC) including H_2S-producing bacteria (black colonies) were quantified on Lyngby's iron agar (Oxoid) supplemented with 0.04% L-cysteine (Sigma-Aldrich). The plates were incubated at 22 °C for 72 ± 6 h. Lactic acid bacteria (LAB) were quantified using spread plates of de Man, Rogosa and Sharp agar (MRS, Oxoide) without Amphotericin B. The plates were incubated anaerobically at 25 °C for five days.

2.4. Statistics

The data were analysed by a general linear model (GLM) with smoking technology and amounts KL added as fixed factors, whereas time of storage was tested as a covariate. A multivariate MANOVA was used to analyse fillet appearance and textural properties where L^*, a^* and b^* were used as multiple Ys for fillet appearance and B_f and F80 for textural properties, respectively. To compare different groups, one-way ANOVA and Tukey's comparison test was used. Statistical analysis of microbiological plate counts was done on log-transformed data, and results are presented as average ± standard error (SE). For correlations between dependent parameters, Pearson's correlation coefficients (r) were calculated. All statistical analyses were performed using the Minitab 19 Statistical Software for Windows (Minitab Inc., USA). The alpha level was set to 5% ($P < 0.05$) and all results except for microbiological plate counts are given as average ± standard deviation (SD).

3. Results and discussion

3.1. Processing yield, dry matter and drip loss

The processing yield was significantly affected by the experimental design ($P < 0.001$) where traditionally smoked samples showed on average significantly higher processing yield than those treated with PCS with an average value of 94.5 ± 0.9% and 91.1 ± 1.0%, respectively (Table 2). Lower yield in PCS treated fillets was well correlated to the more intensive drying applied in PCS protocols to remove unwanted condense in the smoking cabinet. The effect of KL added, on the processing yield, was however minor and not significant (Table 2, $P > 0.058$). Higher yield of traditional smoked samples as compared to those treated with PCS, was supported by lower content of DM observed in CSS processed traditionally ($P = 0.040$). The insignificant effect of KL in the present study could be related to the low content of

Table 2
Chemical parameters of cold smoked salmon fillets smoked with smoke generated by wooden chips (WC) and fillets treated with purified condensed smoke (PCS). All values are presented as an average ± standard deviation (SD).

Group ID	Processingyield [% w/w]	TPC [mg/100 g fish]	NaCl [% w/w]	KL [% w/w]	DM[% w/w]	a_w muscle	a_w surface
WC-0.0	94.8 ± 1.1^{a}	0.60 ± 0.14^{c}	2.9 ± 0.1	–	35.9 ± 2.0	0.96 ± 0.009^{a}	0.94 ± 0.012^{a}
WC-2.6	94.3 ± 0.7^{a}	0.62 ± 0.03^{bc}	2.8 ± 0.5	0.04 ± 0.01	37.6 ± 4.1	0.94 ± 0.003^{a}	0.94 ± 0.008^{ab}
PCS-0.0	91.3 ± 1.1^{bc}	0.81 ± 0.07^{ab}	2.3 ± 0.4	–	37.7 ± 1.0	0.94 ± 0.004^{ab}	0.94 ± 0.015^{ab}
PCS-2.6	92.0 ± 0.9^{b}	0.87 ± 0.03^{a}	2.2 ± 0.2	0.05 ± 0.01	38.1 ± 3.7	0.92 ± 0.010^{bc}	0.92 ± 0.006^{abc}
PCS-5.2	90.2 ± 1.5^{c}	0.90 ± 0.04^{a}	2.5 ± 0.4	0.12 ± 0.02	41.4 ± 1.3	0.92 ± 0.009^{bc}	0.91 ± 0.008^{c}
PCS-7.8	91.0 ± 0.6^{bc}	0.80 ± 0.06^{abc}	2.4 ± 0.2	0.16 ± 0.03	40.1 ± 1.0	0.92 ± 0.005^{c}	0.91 ± 0.006^{bc}
P-value	*< 0.001*	*< 0.001*	*> 0.168*	*< 0.001*	*> 0.189*	*< 0.001*	*= 0.004*

Means ± SD of n = 3 for salt (NaCl) and water activities, n = 6 for total phenolic content (TPC) and dry matter (DM), n = 7–8 for processing yield. Concentrations of potassium lactate (KL) was calculated based on the increase in weigh after injection of the different solutions). Different lower letter superscripts indicate significant difference ($P < 0.05$) by One-Way ANOVA and Tukey's Pairwise Comparisons test (^{abc}).

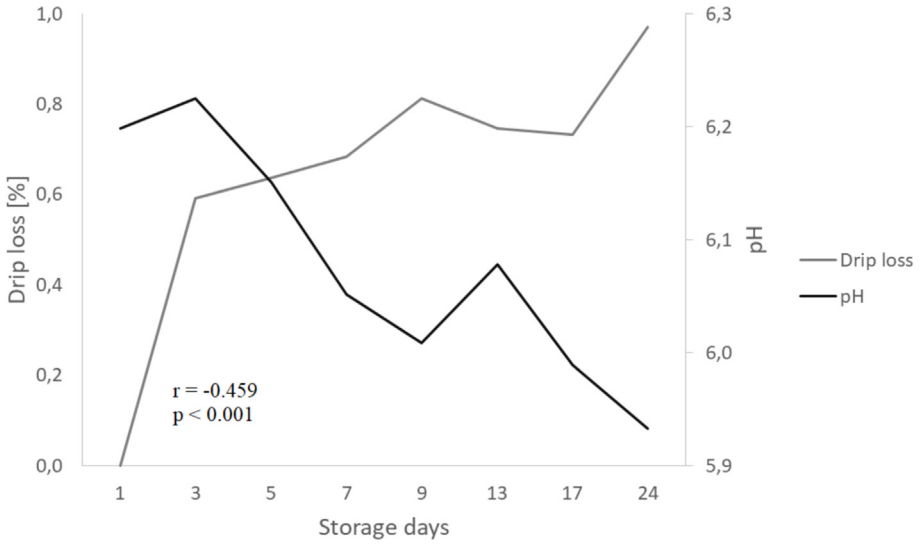

Fig. 1. Overall relation between drip loss, % and pH as a function of storage time independent of smoking technology used (average of samples smoked traditionally and with purified condensed smoked (PCS)).

KL absorbed in the product (Table 2). Previous studies on other muscle foods have stated that the yield is dependent on the amount KL added. Guàrdia, Guerrero, Gelabert, Gou, and Arnau (2008) and Fulladosa, Serra, Gou, and Arnau (2009) showed that higher content of KL decreased moisture content in dry cured ham and small calibre fermented sausages, respectively. In the present study, no such effect was observed in CSS, neither for traditionally nor PCS treated samples ($P > 0.569$ and $P > 0.190$).

In the present study, significantly higher DL was observed throughout storage for PCS samples as compared to those smoked traditionally ($P < 0.001$, DL of 0.84 ± 0.04% and 0.55 ± 0.06%, respectively). No such effects were observed regarding added levels of KL, neither for PCS treated samples ($P > 0.166$), nor those smoked traditionally ($P > 0.199$). A moderate negative correlation was moreover found between the overall average DL and pH as shown in Fig. 1 ($r = -0.459$, $P < 0.001$). Splitting the correlation based on smoking technology, a significant effect of PCS on the sample pH was observed ($r = -0.417$, $P < 0.001$) whereas no such effect was observed among those smoked traditionally ($r = -0.142$, $P > 0.336$). This supports a higher transmission of organic acids (e.g. acetic acid) from the smoke to the product when the smoke is generated by atomized PCS compared to pyrolysis of wooden chips. A reduction of muscle pH from 6.2 to 5.8 in PCS treated samples do probably contribute to reduced water holding capacity (WHC) and thereby increased DL. Lowest WHC is observed at pH levels close to the isoelectric point (pI), where pI of myosin, one of the main muscle proteins is 5.4 (Rotabakk, Jørpeland, & J, 2018). Muscle pH close to pI will diminish protein-water interactions, and consequently led to muscle shrinkage and loss of water. This is earlier observed in pork meat where Qiao et al. (2007) observed pH and drip loss to be negatively correlated. The effect of added KL on the DL was insignificant both among PCS treated samples ($P > 0.166$), and those smoked traditionally ($P > 0.199$).

3.2. NaCl concentration and total phenolic content (TPC)

The NaCl content was on average slightly higher for traditionally processed samples as compared to those processed with PCS (on average: 2.9 ± 0.4 and 2.3 ± 0.3%, $P = 0.006$). On group level however, no effects were found (Table 2, $P > 0.168$). Since the salting protocol was performed equally for both batches, observed differences might be a result of differences in the raw material characteristic affecting the salt uptake, e.g. contents of lipids (Gallart-Jornet et al., 2007; Wang, Tang, & Correia, 2000) and or muscle temperature at point of salting (Telis, Romanelli, Gabas, & Telis-Romero, 2003). The muscle salt content was moreover not affected by added levels KL ($P > 0.748$). This observation agreed with Fulladosa et al. (2009) who did not, after replacing 36% of the salt with KL, observe any increases in salty taste of salt reduced dry-cured ham. By comparing salt content observed in the present study with those reported earlier by Espe, Kiessling, Lunestad, Torrissen, and Rørå (2004); Leroi et al. (2001); Løvdal (2015), our samples are regarded as lightly salted products. Bacterial growth in CSS is in general known to be affected by the level of salt where inhibition of growth can be seen as a function of increased salt content (Leroi & Joffraud, 2000).

Levels of phenols observed in the present study (Table 2), expressed as total phenolic content (TPC), was on average significantly higher in fillets smoked with atomized PCS (0.85 ± 0.06 mg/100 g fish) compared to those smoked traditionally (0.61 ± 0.09 mg/100 g fish, $P < 0.001$). Increased levels of KL did not show any significant effects on absorption of phenols neither in traditionally nor PCS treated products ($P > 0.820$ and $P > 0.104$, respectively). TPC levels observed in the present study were on average at similar levels or higher than required by the French Standard NF V45-065. In lightly salted products, such as those presented in this study, other barriers than sufficient levels of salt need to be strengthened to maintain microbiological stability and food safety (Tocmo et al., 2014). One such barrier could therefore be to increase the level of phenols in the product (Cornu et al., 2006; Løvdal, 2015). In the present study, 38% higher content of phenols was found in samples produced with atomized PSC as compared to those produced with traditionally smoking. High content of phenols is known to have antimicrobial potential (Giménez & Dalgaard, 2004; Løvdal, 2015; Porsby et al., 2008; Suñen et al., 2003; Tocmo et al., 2014) that might explain lower microbial counts observed for PCS treated samples in the presented study (Fig. 3). However, in traditional CSS, Leroi and Joffraud (2000) found that APC and total lactic acid bacteria were mainly inhibited by the salt concentration (5% w/w) in the meat and to a lesser extent by the content of phenols. Due to higher TPC of PCS treated fillets, a significant negative correlation between TPC and muscle water content was observed ($r = -0.505$, $P = 0.033$).

3.3. Muscle pH and water activity

Muscle pH in traditionally CSS was on average significantly higher than those processed with atomized PCS (on average: 6.14 ± 0.10 and 5.96 ± 0.19, $P < 0.001$, Fig. 2). Initially, just after processing,

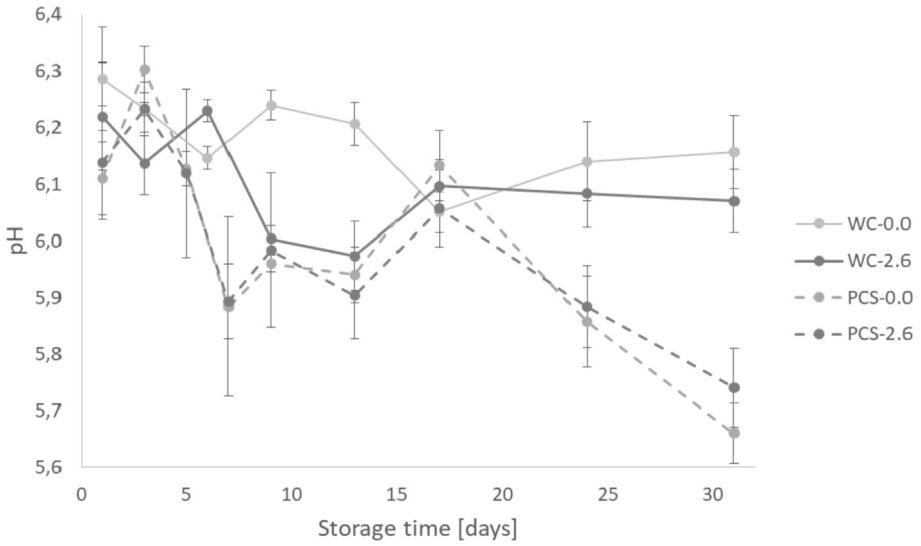

Fig. 2. Average muscle pH as a function of 31 days refrigerated storage (4 °C) for cold-smoked salmon with and without added potassium lactate (% KL) smoked either by traditional wooden chips (WC) or purified condensed smoked (PCS). Error bars indicate one standard deviation.

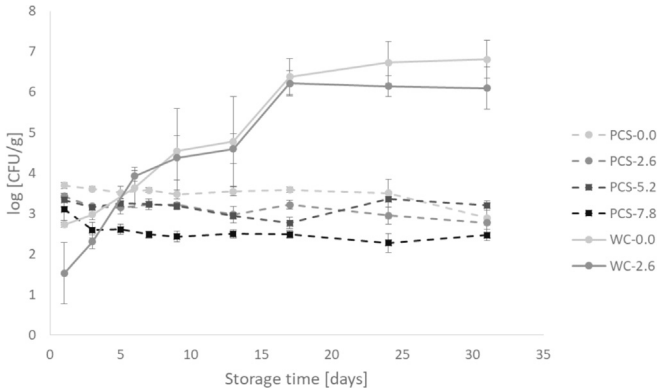

Fig. 3. Aerobic plate count (APC) of samples collected from both traditional wooden chips (WC) and purified condensed smoke (PCS) salmon with increasing potassium lactate (% KL) concentration.
H_2S-producing bacteria, representing specific spoilage bacteria in fish, appeared only sporadically.

traditionally CSS had a pH of 0.15 units higher than samples treated with atomized PCS. As a function of cold-storage this difference increased extensively from day 17 and throughout storage ending approximately 0.4 pH-units lower for PCS treated samples compared to those smoked traditionally (Fig. 2). Based on the experimental design, no major effects of added KL on muscle pH was observed ($P > 0.686$). This agreed with Kin et al. (2011) who reported unchanged pH of catfish fillets after adding of KL and potassium acetate. In a meat based fermented sausages however, Guàrdia et al. (2008) reported KL to significantly affect pH, probably due to the inhibitory effect of KL on growth of lactic acid bacteria (LAB). Reduced pH in PCS treated samples as a function of time presented in Fig. 2 is suggested to be a result of the acidic content of the PCS (9–12% acetic acid, pH = 2.0–3.2), which first acidify the product surface (not measured) followed by a diffusion of organic acids from the fillet surface to the sample core over time. Low pH-values are moreover known as an important hurdle to reduce bacterial growth (Rhee, Lee, & Lee, 2011; Theron & Lues, 2007).

The water activity (a_w) measured on the product surface and inside the muscle is presented in Table 2. The average muscle a_w was significantly higher in traditionally CSS compared to those treated with PCS ($P < 0.001$, $a_w = 0.95 \pm 0.009$ and 0.92 ± 0.011, respectively). The same trend was observed in the fillet surface, showing water activity of 0.94 ± 0.009 and 0.92 ± 0.015, respectively, for traditionally and PCS treated samples ($P = 0.013$). Lower a_w and pH of CSS produced by PCS protocols showing tougher processing conditions probably caused by more extensive drying in combination with lower pH of the atomized smoke compared to the smoke generated by pyrolysis of wooden beech chips. By lowering a_w and pH in presented lightly salted products, barriers against potential pathogens such as e.g. *Listeria monocytogenes* will be strengthened giving e.g. improved food safety. This was recently stated by Heir, Liland, Carlehög, and Holck (2019) who showed inhibitory effects of Verdad N6 (a vinegar-based food additive) on the growth of *L. monocytogenes* in CSS. Addition of KL in combination with PCS gave moreover reduced a_w as a function of increased amounts KL added ($P < 0.001$, Table 2). This was however not observed in traditionally CSS, probably due to the restrictive KL gradient presented in those samples (limited to two different concentrations).

3.4. Microbial growth

The APC was significant affected by the experimental design showing higher counts in traditionally smoked compared to CSS produced by atomized PCS (Fig. 3, $P < 0.001$). The APC levels of PCS groups varied between 2.5 and 3.2 log CFU $\times$ g^{-1} and no bacterial growth was observed throughout 31 days of cold storage. Thus, compared to traditionally CSS, smoked traditionally smoked an approximately 3.5–4.5 log reduction of APC was observed after 31 days of storage (Fig. 3). The growth inhibiting effects observed in PCS treated salmon is suggested to be related to the sum of improved barriers compared to those reached by traditional smoking, i.e. lower water content, a_w, pH, and higher DM and TPC levels (Løvdal, 2015; Tocmo et al., 2014). This was moreover supported by significant correlations between APC and a_w ($r = 0.880$, $P < 0.001$ and $r = 0.664$, $P = 0.003$, for muscle and surface a_w, respectively), and APC and pH ($r = 0.292$, $P = 0.003$). Obtained differences in TPC (Table 2), did however not affect APC significantly ($r = -0.337$, $P = 0.172$). The PCS used in the present study consisted of 9–12% acetic acid (pH 2.0–3.2) carrying a potential to reduce surface pH and to inhibit bacterial growth. This was moreover supported by Heir et al. (2019) where Verdad N6 was found to inhibit *L. moncytogenes* in CSS throughout storage. The linkage between APC and growth of *L. moncytogenes* is known to be highly correlated (Al-Zeyara, Jarvis, & Mackey, 2011), supporting observed bacterial inhibition in our study by PCS to also affect growth of *L. moncytogenes*.

The results indicate a minor effect of KL, showing lower APC counts as a function of increased KL added (P = 0.001). Lower inhibitory effect of KL shown in the present study compared to results obtained by Vogel, Ng, Hylding, Mohr, and Gram (2006) is probably related to the fillet content of KL that were lower in the present study.

This was probably due to the low occurrence of bacteria in PCS treated samples or, regarding both smoking technologies, to the sum of applied barriers (Tocmo et al., 2014). LAB was only sporadically observed in samples treated with PCS, whereas growth of LAB reached the same level as APC after approximately 17 days of cold-storage for CSS processed by smoke generated from wooden chips (data not shown). Gou, Guerrero, Gelabert, and Arnau (1996) reported that the growth of LAB decreased by increased addition of lactate due to a reduction in pH. Leroi and Joffraud (2000) stated that a 0.5 pH-unit reduction could contribute to an overall microbial inhibition in CSS. In the presented study, it was observed a total drop of 0.4 pH-units for PCS treated and approximately 0.1 for traditionally produced CSS. The correlation between pH and LAB was however low but significant (r = 0.24, P = 0.003). With only sporadically occurrence of LAB, the spoilage effect of those bacteria was suggested to be low in PCS treated samples.

3.5. Colorimetric properties and textural analyses

The fillet appearance in the present study was affected by applied smoking technology (Wilks' MANOVA, P < 0.001) whereas no effect of added KL was observed (P > 0.46). As far as we know, no literature is available describing KL-induced colorimetric changes of salmon fillets. In a study on catfish however, Kin et al. (2011) reported darker colour after addition of 1.5% KL as compared to controls. The effect of added KL on fillet appearance might therefore be dependent on amount added, type of species and the chemical composition of the muscle in general. Independent of added KL, fillets smoked with PCS were darker (lower L^*-value, GLM, P < 0.001), less reddish (lower a^*- value, GLM, P = 0.004) and less yellowish (lower b^*-value, GLM P = 0.001) as compared to those smoked traditionally (Table 3). Smoke-processing of salmon fillets are in general known to give darker, less reddish and more yellowish fillets compare to the raw material (Birkeland, 2004; Birkeland et al., 2004; Cardinal et al., 2001; Lerfall, Akse, Østerlie, & Birkeland, 2011; Lerfall & Rotabakk, 2015). This colour change is related to smoke-induced carbonyl-amino reactions of the Maillard type (Horner, 1997; Martins, Jongen, & van Boekel, 2000) and structural changes on the fillet surface caused by changes in pH, content of phenols, drying and oxidation of proteins and lipids (Cardinal et al., 2001; Hidalgo & Zamora, 2000; Lerfall et al., 2016). Colour changes is however known to be affected by the water activity where Maillard browning predominates at high a_w (a_w > 0.97) whereas protein-lipid browning is dominant in products with lower a_w (Sikorski, Haard, Motohiro, & Pan, 1998). Observed differences in water activity between samples of the different protocols (Table 2) support process-induced colorimetric changes in the present study to be related to the specific water activity of each sample.

As a function of time, cold-storage of CSS is known to give paler (higher L^*-values), more reddish (higher a^*-values) and yellowish (higher b^*-values) fillets as compared to those newly smoked (Jørgen Lerfall et al., 2016). As a result, the appearance of long-term cold-stored CSS often became more like what observed for the raw material (Lerfall et al., 2016; Lerfall & Rotabakk, 2015). In the present study, 31 days cold-storage of traditionally smoked fillets resulted in slightly darker but more reddish and yellowish fillets compared to those newly smoked (Table 4, P < 0.001, P < 0.001 and P = 0.024, respectively). PCS treated fillets did however behave differently, giving darker, less reddish and less yellowish/more blueish fillets (Table 4, P < 0.001). Colour is a key attribute of food items (Francis, 1995) and an important decision maker for consumers when purchasing raw (Anderson, 2000) and smoked salmon products (Gormley, 1992; Røra, Monfort, & Espe, 2004). The observed decrease in yellowness/increase in blueness in the present study as an effect of storage might therefore be a concern. There is however, a potential to optimize the dosage of PCS in the protocol to give a more stable colour development throughout storage (unpublished data).

The overall breakage force (B_f, N) needed to rupture the fillet surface and the fillet firmness (F80, N) is shown in Table 4 and was affected by applied smoking technology (Wilks' MANOVA, P < 0.001) whereas no effect of added KL was observed (P > 0.970). The B_f of traditionally CSS showed an average of 14.2 ± 4.7 N while those treated with PCS had an average of 23.2 ± 4.4 N. Textural properties of CSS is often related to the processing yield where water fluxes and salt uptake are the main discriminants (Indrasena, Hansen, & Gill, 2000). Atomization of PCS to generate smoke particles, will over time generate condensates in the smoking cabinet that has to be removed by drying. It is therefore important to optimize the drying process to avoid soft and pasty fillets that often is regarded as poor quality among consumers (Indrasena et al., 2000). Pasty fillets are often pink in colour, whereas firmer ones are more orange/brownish (Cardinal et al., 2004). This effect does moreover support the relationship between fillet firmness and colorimetric properties (CIE, 1994) observed in our study (r = −0.646 to −0.816, P < 0.001). In the present study, neither B_f (N) nor F80 (N) was affected by time of storage or by added amount of KL.

Table 3
Colorimetric properties (CIE, 1994) of experimental cold-smoked salmon (CSS) as affected by smoking technology.

Parameter		Traditionally CSS (WC)	PCS treated CSS	*P-value*
L^*	Raw	58.6 ± 0.6	58.8 ± 0.6	*> 0.281*
	Smoked	57.2 ± 0.3	55.5 ± 0.3	*< 0.001*
	P-value	*< 0.001*	*< 0.001*	
a^*	Raw	14.6 ± 0.7	14.4 ± 1.0	*> 0.355*
	Smoked	12.2 ± 0.6	10.1 ± 1.5	*< 0.001*
	P-value	*< 0.001*	*< 0.001*	
b^*	Raw	14.2 ± 0.6	13.1 ± 1.1	*= 0.001*
	Smoked	7.1 ± 1.8	0.9 ± 3.8	*< 0.001*
	P-value	*< 0.001*	*< 0.001*	

Abbreviates: WC = wooden chips and PCS = purified condensed smoke. Presented values are an average of 14 and 30 fillets for WC and PCS treated salmon, respectively. Statistical differences were calculated using a student t-test where α = 0.05.

4. Conclusion

It is concluded that smoke generated by atomization of PCS inhibit bacterial growth and thereby have a potential to enhance shelf life of lightly salted CSS products. Combination of barriers such as decreased water activity and pH is of highly importance to obtain a stabilized product. In the present study, the contribution of potassium lactate and TPC as barriers was found to be minor.

The quality of lightly salted CSS produced with atomized PCS was significantly different compared to what obtained of those smoked with pyrolysis of wooden chips (traditional protocol). It was concluded that the main discriminant was the fillet appearance (darker, less reddish and yellowish) and slightly firmer texture of PCS treated samples. Both factors are related to the muscle water activity, showing the importance of optimizing the ratio between drying and atomization to obtain a high-quality product when atomization of PCS is used to produce CSS.

CRediT authorship contribution statement

Torunn Valø: Writing - original draft, Data curation. **Anita Nordeng Jakobsen:** Supervision, Methodology, Writing - review & editing. **Jørgen Lerfall:** Supervision, Writing - original draft,

Table 4
Colorimetric- (CIE, 1994) and textural properties (breakage force (B_f) and fillet firmness at 80% compression (F80)) of experimental cold-smoked salmon (CSS) stored at 4 °C for 31 days.

Parameter	Day	Traditionally CSS		PCS treated CSS				*GLM*			
		WC-0.0	WC-2.6	PCS-0.0	PCS-2.6	PCS-5.2	PCS-7.8	P_M	P_P	P_{KL}	P_S
L^*	*0*	57.1 ± 0.3^{aA}	57.2 ± 0.3^{a}	55.4 ± 0.3^{bA}	55.4 ± 0.4^{bA}	55.4 ± 0.4^{bA}	55.7 ± 0.3^{bA}				
	17	57.6 ± 0.3^{aA}	57.3 ± 0.2^{ab}	55.0 ± 0.2^{bAB}	54.7 ± 0.3^{bAB}	54.4 ± 0.8^{bB}	54.2 ± 0.4^{bB}				
	31	55.8 ± 0.3^{aB}	56.5 ± 0.8^{a}	54.5 ± 0.4^{bB}	54.2 ± 0.5^{bB}	54.5 ± 0.5^{bB}	54.1 ± 0.6^{bB}				
	GLM							*< 0.001*	*< 0.001*	*> 0.81*	*< 0.001*
a^*	*0*	12.1 ± 0.8^{aB}	12.4 ± 0.8^{aB}	10.7 ± 1.4^{bcA}	11.0 ± 1.4^{bA}	9.1 ± 1.4^{cA}	10.0 ± 1.4^{bcA}				
	17	14.3 ± 0.9^{aA}	14.0 ± 0.8^{aA}	6.5 ± 0.3^{bB}	6.7 ± 1.0^{bB}	5.5 ± 0.8^{bB}	5.4 ± 0.7^{bB}				
	31	13.2 ± 0.5^{aAB}	14.4 ± 1.4^{aA}	5.8 ± 0.4^{bB}	5.6 ± 0.6^{bB}	5.3 ± 0.2^{bB}	5.3 ± 0.6^{bB}				
	GLM							*< 0.001*	*> 0.001*	*> 0.22*	*< 0.001*
b^*	*0*	7.2 ± 2.0^{aB}	6.9 ± 1.8^{a}	2.4 ± 3.6^{bcA}	3.1 ± 3.8^{bA}	-2.0 ± 3.1^{cA}	0.5 ± 3.3^{bcA}				
	17	10.3 ± 2.4^{aA}	8.7 ± 1.5^{a}	-11.4 ± 1.4^{bB}	-10.6 ± 3.6^{bB}	-15.0 ± 2.1^{bB}	-15.2 ± 2.1^{bB}				
	31	8.3 ± 0.6^{aAB}	9.5 ± 2.5^{a}	-15.0 ± 1.3^{bC}	-15.1 ± 1.7^{bB}	-16.5 ± 0.3^{bB}	-16.1 ± 1.0^{bB}				
	GLM							*< 0.001*	*> 0.001*	*> 0.16*	*< 0.001*
B_f, *N*	*3*	15.2 ± 5.9	17.9 ± 3.5	21.8 ± 3.9	21.3 ± 1.2	20.2 ± 9.4	22.0 ± 2.3				
	17	16.5 ± 3.6^{ab}	12.3 ± 1.8^{b}	22.3 ± 3.2^{ab}	24.7 ± 1.0^{ab}	23.3 ± 2.7^{ab}	26.9 ± 2.6^{a}				
	31	12.5 ± 1.9^{b}	12.9 ± 0.4^{b}	25.3 ± 5.4^{a}	22.5 ± 2.9^{a}	25.8 ± 6.8^{a}	18.4 ± 6.5^{ab}				
	GLM							*< 0.001*	*< 0.001*	*> 0.97*	*> 0.53*
F80, *N*	*3*	18.8 ± 5.9	21.7 ± 6.2	25.0 ± 5.1	28.8 ± 1.8	26.6 ± 11.3	25.1 ± 1.2				
	17	21.7 ± 5.9^{ab}	16.8 ± 0.3^{b}	28.2 ± 4.2^{a}	32.6 ± 6.1^{a}	32.3 ± 2.2^{a}	31.7 ± 6.6^{a}				
	31	17.9 ± 4.5^{b}	15.0 ± 1.5^{b}	$33.3 \pm .5.2^{a}$	27.6 ± 4.1^{a}	33.4 ± 9.7^{a}	31.8 ± 2.6^{a}				
	GLM							*< 0.001*	*< 0.003*	*> 0.918*	*> 0.287*

Colorimetric values are an average seven individuals per sampling point whereas textural measurements were performed in duplicates of three samples per group per day. General Linear Model (GLM) analyses of variance, where P_M, P_P, P_{KL}, and P_S are the significance levels for the effects of the model, smoking technology, potassium lactate added and storage time, respectively. Different superscripts (^{abc}) within each row and (^{ABC}) within each column indicate significant variation ($P < 0.05$) between groups by a one-way ANOVA and Tukey's comparison test.

Methodology, Data curation, Writing - review & editing.

Declaration of competing interest

We declare that there is no conflict of interest to disclose.

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