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Co-products from the Atlantic salmon filleting industry – Their properties, stability and potential as human food ingredients

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ABSTRACT

The present study impacts the growing knowledge about co-products obtained from the primary processing of Atlantic salmon (*Salmo Salar* L.) as food ingredients by highlighting their microbial and chemical composition and stability. The proximate composition varied between the co-products from the fatty belly-flap (34% lipid, 15% protein) to the leaner deboned meat (10% fat, 22% protein) ($p < 0.001$), whereas no differences were observed between the superior and production qualities ($p > 0.31$). However, the co-products from the processing of production quality salmon had a higher microbial load of psychotropic aerobic-, H₂S-producing- and *Pseudomonas* spp. bacteria than those of superior quality salmon origin (4.0, 5.2, and 4.3 log CFU/g units higher, respectively). Fatty co-products, such as the belly flap, were especially susceptible to ATP degradation, giving higher Hx concentrations after 7/10 days of ice-storage than leaner fractions ($p < 0.001$). Moreover, a positive correlation between the lipid level and degradation of ATP was observed ($r = 0.62$, $p = 0.006$). To conclude, co-products from superior and production quality salmon contain valuable nutrients suitable for human consumption. However, the co-products are vulnerable to microbial and chemical deterioration, presumably due to disruption of biological membranes during processing, and should be handled accordingly.

1. Introduction

Inadequate food resource utilization remains a considerable challenge in the face of a growing world population, the increased need for food, and the necessity to maintain food security while reducing the environmental impact of global food production. Meanwhile, the United Nations 12th Sustainable Development Goal calls for more responsible and sustainable utilization of available food resources. As the largest producer of Atlantic salmon in the world (FAO, 2022), the Norwegian aquaculture industry could contribute to a more sustainable production and stimulate economic growth by increasing the amount of rest raw materials used for human consumption (Asche et al., 2018; Stevens et al., 2018).

The filleting yield of Atlantic salmon varies from 45% to 62% of total wet weight depending on the trimming grade, resulting in as much as 55% residual raw material (Olsen & Tobiassen, 2004; Stevens et al., 2018). The terminology defining “residual raw materials” varies in the literature. The present paper will use the same terminology as Aspevik

et al. (2017) which was first defined by the European parliament: The main products are what the industry produces for direct sale, such as fillets or whole fish. Any remaining biomass is defined as rest raw material, or residual raw material. Rest raw materials suitable for human consumption are defined as co-products, while those unsuitable for human consumption are defined as by-products.

The Norwegian aquaculture industry sort all salmon as “Superior quality” (Sup) or “Production quality” (Prod) as described in the Norwegian industry standard NBS 10–01 (NISF, 1999). This classification is based on the salmon’s physical appearance as well as external and internal faults. Both Sup and Prod salmon are subject to the Norwegian food safety regulation FOR-2013-06-28-844 for fish products intended for human consumption (Lovdata, 2013). The Sup salmon is primarily exported as head-on-gutted fish (75–80%) and processed in other countries, thereby rendering the most significant part of the rest raw material unavailable for further processing in Norway. The Prod salmon, however, are sorted and processed domestically in line with Norwegian regulations and are therefore an important source of co-products for the

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Norwegian seafood industry. In Norway, Prod salmon represents 2–7.5% of the total salmon production (Furuset, 2020(Furuset, 2020), which corresponds to 31 000–108 000 tons of available raw material annually based on the total annual production (Directorate of Fisheries, 2022).

Myhre et al. (2022) estimate that 94% of the raw material from aquaculture that was available for the Norwegian processing industry were utilized in 2021. Most of these materials are used as by-products in the production of ensilage or marine protein and oils intended for feed or biogas production. However, rest raw materials, such as meat left on frames, Bits & Pieces, belly flaps, and heads has the potential to be used as co-products directly in foods (Aspevik et al., 2017), and the amount utilized for human consumption has been steadily increasing from 2017 to 2021 (Myhre et al., 2022). The current market trends further facilitate increased use of co-products for human consumption; Traditionally, fish have been sold whole from the suppliers and processed to varying degrees along the supply chain, including the consumer's home, which limits or prevents the potential for further processing of rest raw materials by the industry. While this practice still dominates in many parts of the world (FAO, 2022), the Norwegian salmon market has shifted towards trade of fillets and ready-to-eat products. This has introduced significant changes, as the domestically available rest raw materials are now wholly generated in the industry's processing facilities instead of distributed throughout the supply chain, and thus more readily available for further valorisation.

The utilization of salmon co-products, either as ingredients in existing foods or as novel products (e.g., minced fish products, fish soups, and other commercially pre-made dishes, including salmon co-products as a minor part of the dish), is contingent on a high and reliable product quality. Co-products require more processing than whole fish and fillets and are especially vulnerable to quality deterioration. Processing equipment, as well as seawater and fish inherent microbiota, have previously been identified as contamination routes of spoilage bacteria into salmon fillets (Møretro et al., 2016; Thomassen et al., 2022). Therefore, microbial control during production is essential to prevent contamination of salmon co-products to prevent spoilage and ensure high-quality products. Particular emphasis must be placed on halting processed meats' decomposition and lipid autoxidation during production, storage, and transportation (Aspevik et al., 2017). Simultaneously, the successful commercialization of foods based on salmon co-products will require a thorough understanding of their chemical composition and nutritional quality. Co-products from both Sup and Prod salmon have the potential to contribute to the global food production to a greater extent as the industry develops towards more sustainable production.

The aim of the present study was to investigate the chemical and microbial composition and stability of co-products from farmed Sup and Prod Atlantic salmon. Belly flaps, Bits & Pieces, deboned meat, and fillet cut-offs were collected and analysed to identify the proximate composition, including fatty acid profile, protein solubility, and ATP degradation. The microbial community and stability were studied in all fractions except the fillet cut-offs. However, the Bits and Pieces fraction could be regarded as a part of the fillet since it was a co-product obtained from trimmed fillets as part of the commercial production of consumer-friendly portions. By contributing to the growing knowledge of salmon co-products as food ingredients, the present study aims to increase the sustainability of salmon aquaculture and global food production.

2. Material & methods

2.1. Experimental design and sample preparation

The chemical and microbial quality and composition were assessed in Superior (Sup) and Production (Prod) graded Atlantic Salmon, respectively. The Sup salmon was collected freshly slaughtered directly from the slaughtering facility. The Prod salmon were first slaughtered at one location before it was collected from a processing facility. It should

be stressed that the Prod salmon used in the first experiment was collected 1 day post mortem (Fig. 2A), while the Prod salmon used in the second experiment was collected 3 days post mortem (Fig. 2B).

The first experiment measured the proximate composition (protein, water, ash and lipid content) fatty acid composition, protein solubility and ATP degradation in ten farmed Atlantic Salmon (Fig. 2A). Of these, five individuals had Sup quality and five Prod quality. After arriving at NTNU– Norwegian University of Science and Technology, the fish were filleted and sorted into the co-product fractions; Bits & Pieces (P), belly flaps (B), and deboned meat (S) from the backbone. In addition, samples from the fillet (F) were merged and used as a reference (Fig. 3).

With four fractions (P, B, S, and F) and two qualities (Sup and Prod) the process resulted in eight sample batches, where each batch contained a mixture of meat from five individual salmons to reduce the individual sample variability (Fig. 2A). Each batch was divided into two aliquots. One aliquot was minced and frozen (−80 °C) in vacuum-packed portions immediately, hereby referred to as the Day 0/1 post mortem samples. The second aliquot was stored on ice in a refrigerated room (0–4 °C) in open plastic bags for 7 days, hereby referred to as Day 7/8 post mortem samples. After 7 days, the samples were minced and frozen (−80 °C) in vacuum-packed portions. Samples were minced using a Blixer 6 mincer (Robot Coupe, France), and vacuum-packed using a SuperMax-C vacuum packaging machine (Webomatic, Germany).

In the second experiment (Fig. 2B), microbial quality and community of the raw material were measured in six individuals with Sup and Prod quality, respectively. All individuals were sorted into the co-product fractions; Bits & Pieces (P), belly flaps (B), and deboned meat (S) from the backbone (Fig. 3). Microbial plate counting parameters were analysed in the Bits & Pieces (P), belly flaps (B), and deboned meat (S) fractions at days 0 and 7 post mortem for the Sup quality. However, due to the commercial standard of processing Prod salmon post rigor, these samples were analysed at days 3 and 10 post mortem. All measurements were performed in triplicates. Microbial community analysis was performed on days 0 for Sup and 3 for Prod samples. A more detailed description of all salmon processing steps are provided in the OSF depository of the study (https://osf.io/jyatr/?view_only=64eb73c368ec4f1387b2cf70cbb81056).

2.2. Proximate composition

Proximate analysis of total protein determination, water and ash content, total lipid, WSP and SSP, and fatty acid profile was performed on Day 7/8 samples of all four fractions (P, B, S, and F) of both Sup and Prod salmon.

2.2.1. Protein determination by the Kjeldahl method

The protein content was determined according to method 981.10 of the AOAC International (AOAC, 2016). Triplicates of 1–2 g salmon mince were weighed on Kjeldahl papers and placed in 750 mL sample tubes together with 2 Kjeldahl catalyst tablets and 15 mL 97% sulfuric acid (H₂SO₄). The samples were digested by heating at 320 °C for 20 min and 420 °C for 90 min. After that, the fully digested samples were titrated with a KjelMaster K-375 (Büchi, Switzerland) to determine the total nitrogen in the samples. The protein content was calculated from total Nitrogen using a conversion factor of 6.25.

2.2.2. Water and ash determination

The water and ash content in the samples were analysed according to the ISO standard ISO 6496:1999 (ISO, 1999). The water content was determined gravimetrically after drying triplicates of 3–4 g sample in an oven at 105 °C for 22–24 h. The ash content was determined by heating the samples further in a muffle furnace for 22–24 h at 550 °C.

2.2.3. Lipid determination by Bligh and Dyer

The lipid content in the salmon mince was determined using a modification of the method described by Bligh and Dyer (1959).

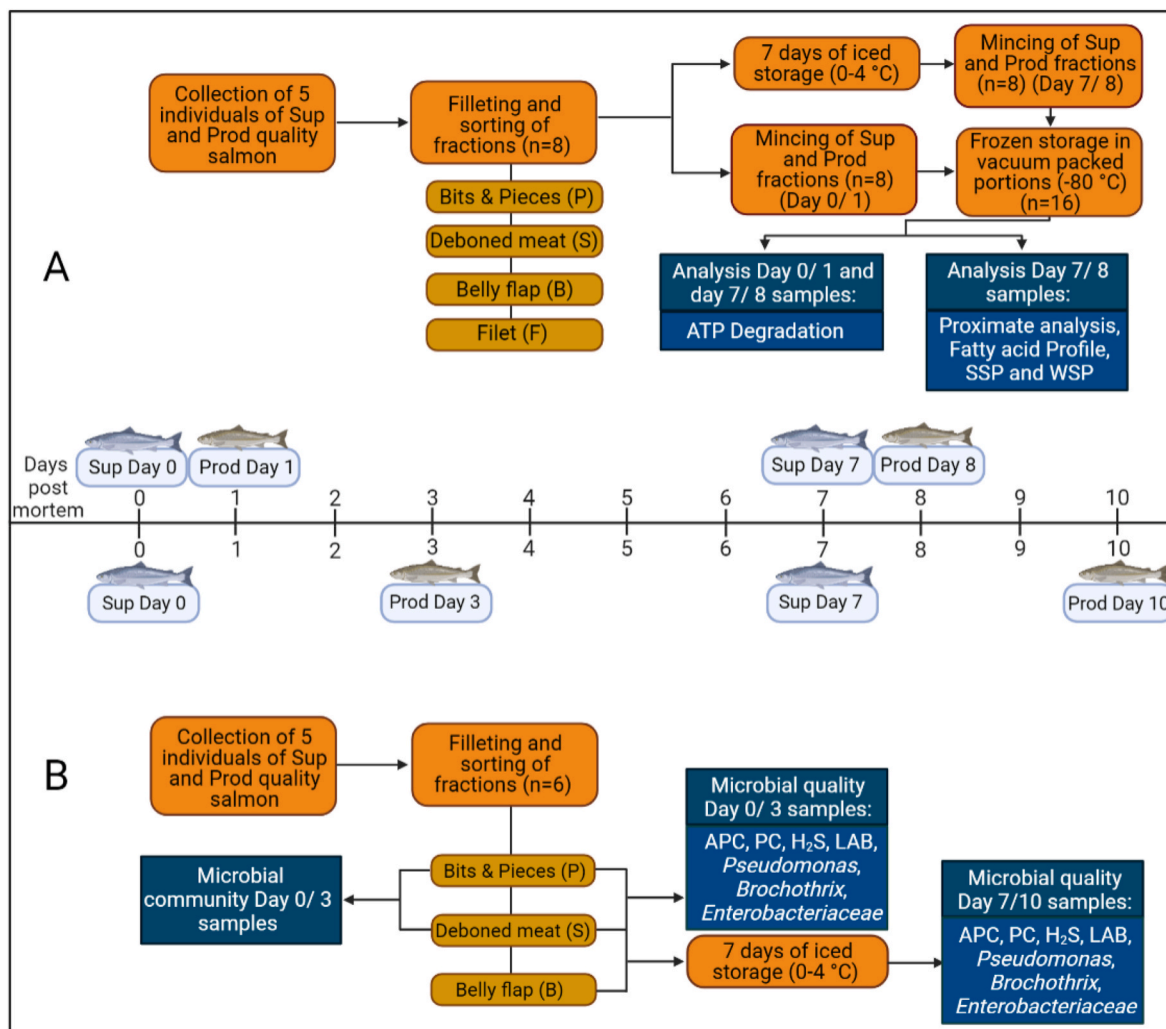


Fig. 2. Flowchart of experimental design and timeline for superior-grade (Sup) and production-grade (Prod) Atlantic Salmon in days post-mortem. A: Timeline and processing of salmon for chemical composition and ATP degradation analysis. B: Timeline and processing of salmon for microbial quality and community analysis. The figure includes denotations used in the study when referring to different salmon fractions (P, B, S, and F), storage groups (Day 0/1, Day 0/3, Day 7/8 and Day 7/ 10), and qualities (Sup and Prod). The process of preparing samples for analysis is illustrated in orange boxes, the salmon fractions in yellow boxes, and the performed analysis in blue boxes. GPA = General proteolytic activity; SSP= Salt-soluble protein; WSP = Water-soluble protein; APC = Aerobic plate count; PC= Pyschrotrophic aerobic plate count; H₂S= H₂S Producing bacteria plate count; LAB = Lactic acid bacteria. Figure created with BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

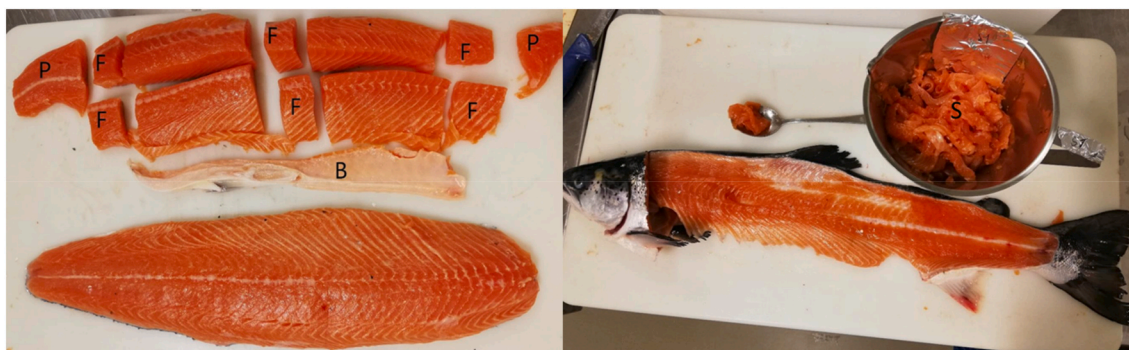


Fig. 3. Salmon meat fractions used in the present study; Bits & Pieces (P), belly flaps (B), Fillet (F) and deboned meat (S). All samples used in the study are mixed mince from five individual salmon. The pictures are taken during filleting and sorting of a superior quality salmon Day 0 post mortem used in experiment A., before mincing and storage.

Triplicates of 5–10 g of minced salmon, 16 mL distilled water (dH₂O), 40 mL methanol (CH₃OH) and 16 mL chloroform (CHCl₃) were added to 200 mL centrifugation tubes. The resulting samples were homogenized with an Ultra Turrax T-25 for 2 min before 20 mL CHCl₃ was added. The samples were then homogenized for 20 s before 20 mL dH₂O was added, and finally homogenized for another 20 s and centrifuged (3000 g, 15 min, 4 °C). The chloroform phase containing the lipids was then pipetted into a separate container. The lipid content was determined gravimetrically by evaporating two parallels of 2 mL of the organic phase in pre-weighed reagent glasses.

2.3. Water- and salt-soluble protein

The water-soluble protein (WSP) and salt-soluble protein (SSP) were extracted using a modification of the method described by Anderson and Ravesi (1968). The extracts were prepared by homogenizing 4 g salmon mince and 50 mL 0.05 M Monopotassium phosphate (KH₂PO₄), pH 7 using an Ultra-Turrax T25 for 10 s. The samples were then centrifuged (20 min, 3000g, 4 °C), and the supernatant was decanted into a separate tube through filter paper to obtain the WSP fraction. The precipitate was resuspended in 50 mL with 0.05 M KH₂PO₄, 0.6 M Potassium chloride (KCl), pH 7, and the process was repeated to obtain the SSP fraction.

The amount of nitrogen in the WSP and SSP extracts was determined using a modified Kjeldahl method. Briefly, duplicates of, 16–20 mL of the WSP and SSP extracts were pipetted into Kjeldahl tubes. Before digestion, the heating block was preheated to 150 °C. The samples were then heated at 170 °C for 15 min, 220 °C for 30 min, and 420 °C for 100 min. The protein extracts were produced in triplicates, while the protein content of each extract was measured in duplicates.

2.4. Fatty acid profile

Fatty acid (FA) profile was determined by Gas Chromatography (GC). FA methyl-esters were prepared as described by Metcalfe et al. (1966) and injected (1 µL) into an Agilent 6850 GC-system (Agilent Technologies, USA) equipped with a polyethylene glycol capillary column (HP-INNOWax) 30 m × 250 µm × 0.25 µm and a flame ionization detector (FID, 310 °C). Helium was used as the carrier gas, and the oven had an isothermal temperature of 210 °C. As a reference, the chromatogram was compared to a fatty acid methyl ester (FAME) standard (Supelco, CRM47885). The percentage distribution of each fatty acid was determined by calculating the intensity of each specific peak against the total intensity of FAMES in each specific sample (Gutnikov, 1995). The analysis was performed in one experimental parallel using pooled samples of five individual salmon.

2.5. ATP degradation

The Adenosine triphosphate (ATP) degradation products Inosine monophosphate (IMP), Inosine (Ino) and hypoxanthine (Hx) were determined by High Performance Liquid Chromatography (HPLC). First, extracts were prepared by homogenizing 1.2 g salmon mince in 7.5 mL TCA (7.5% w/v) with an Ultra Turrax T-25 for 60 s. The samples were then centrifuged (20 min, 3000 g, 4 °C) before the supernatant were filtered twice through a 0.2 µm nylon syringe filter and frozen at –80 °C until analysis. Extracts were prepared in triplicates from both Day 0/1 and Day 7/8 samples of Prod and Sup salmon.

The extracts were analysed using an Agilent 2190 HPLC system (Agilent Technologies, USA) equipped with an infinity diode array detector and a Poroshell 120 column (EC-C18 3,0 × 100 mm, pore size 2,7 µm). The mobile phase consisted of 0.215 M monopotassium phosphate (KH₂PO₄) and 0.0023 M Tetrabutylammonium hydrogen sulphate ([CH₃(CH₂)₃]₄N(HSO₄)) in 3.5% acetonitrile (CH₃CN) adjusted to pH 6.25. The column temperature was 20 °C. The flow rate was set to 0.2 mL/min for minutes 0–2, 0.8 mL/min for minutes 2–9 and 0.2 mL/min

for minutes 9–10. The resulting chromatogram was analysed using standard containing IMP (Sigma, ≥98%, CAS:352 195-40-5), Ino (Sigma, ≥99%, CAS:58-63-9), and Hx (Sigma, ≥99.0% CAS:68-94-0) as reference.

The Ki-value (Equation I) (Karube et al., 1984) and H-value (Equation II) (Luong et al., 1992) were calculated based on the measured concentrations of IMP, Ino and Hx using the following equations:

$$Ki - value = \frac{[(Ino + Hx)]}{[(IMP + Ino + Hx)]} * 100 \quad \text{Equation I}$$

$$H - value = \frac{[(Hx)]}{[(IMP + Ino + Hx)]} * 100 \quad \text{Equation II}$$

2.6. Microbiological analyses

A 10 g sample of fish muscle was aseptically transferred to a sterile stomacher bag and diluted 1/10 with sterile peptone water (1.0 g bacteriological peptone and 8.5 g/L NaCl) and homogenized vigorously for 60 s in a Stomacher 400 Lab Blender (Seward Medical Ltd., Gwent, UK). Appropriate serial dilutions were made in sterile peptone water and spread at their respective agar plates. Psychrotrophic aerobic plate count (PC) was quantified by spreading on Long and Hammer agar (L&H) with 10 g/L NaCl to support the growth of the salt requiring *P. phosphoreum* (NMKL, 2006). Plates were incubated at 15 °C for six days. Aerobic plate count (APC) and H₂S-producing bacteria were enumerated by pour plating on Lyngby's iron agar (IA) (Oxoid, Oslo, Norway) supplemented with 0.4 g/L l-cysteine (Sigma-Aldrich, Oslo, Norway) as total and black colonies, respectively. Plates were incubated at 22 °C for 72 h. *Pseudomonas* spp. was quantified on *Pseudomonas* agar base (CM0559), supplemented with *Pseudomonas* CFC selective supplement SR0103 (Oxoid, Oslo, Norway) by spread plating and incubated aerobically at 25 °C for 48 h. *Listeria* spp. were quantified on Brilliance™ *Listeria* agar (BLA) containing Brilliance™ *Listeria* selective supplement (Oxoid CM1080 and Oxoid SR0227, Oxoid, Oslo, Norway) prepared as described by the manufacturer and incubated aerobically at 37 °C. *Brochothrix* spp. were quantified on streptomycin-thallos acetate (STA) agar containing STA selective supplement (Oxoid CM0881 and Oxoid SR0162, Oxoid, Oslo, Norway) prepared as described by the manufacturer and incubated aerobically at 22 °C (22.0 ± 0.3 °C) for 48 ± 2 h. Lactic acid bacteria (LAB) were quantified by spreading on de Man, Rogosa and Sharp agar (MRS) (Oxoid) that was incubated under anaerobic conditions (BD GasPack EZ, Aerobe Container system with indicator) at 25 °C for five days (NMKL, 2007). *Enterobacteriaceae* was quantified using 3M™ Petrifilm™ *Enterobacteriaceae* Count Plates (Sigma-Aldrich) incubated at 37 °C for 24 ± 2 h. The pour plating methods (APC, H₂S and *Enterobacteriaceae*) have a detection limit of 1 log CFU/g, while a detection method of 1 log CFU/g is valid for the other microbiological methods applied.

2.6.1. Analysis of microbial community

Total microbial genomic DNA was extracted from pooled samples from the fractions Bits & Pieces (P) and deboned meat (S) at the initial sampling. Pooling was done by mixing 1 mL from 3 parallels of homogenized samples of the respective fractions. DNA was extracted from 1 mL of the pooled samples using the dNeasy Blood and Tissue Kit (Qiagen, Oslo, Norway), as described by the manufacturer in the protocol for Gram-positive bacteria. A nested polymerase chain reaction (PCR) strategy was applied to avoid a possible co-amplification of 18S rRNA from the fish (Bakke et al., 2011). The purity and the concentration of the DNA were analysed using spectrophotometry (PowerWaveXS, BioTek, Winooski, USA) to ensure an optical density (OD) 260/280 nm ≥ 1.8 and OD 260/230 nm ≥ 1.9. The band length and degradation were analysed using gel electrophoresis (agarose gel 1% m/v, 70 V) using 1 kb Plus ladder (Qiagen, Oslo, Norway). Eurofins Genomics amplified and Illumina MiSeq sequenced the V3–V4

hypervariable region of the 16S rRNA gene (~445 bp) to identify bacterial operational taxonomic units (OTUs). For the amplification of the V3–V4 region, the 16S_f with sequence 5'TACGGGAGGCAGCAG 3' and 16S_r with sequence 5'CCAGGTATCTAATCC 3' (Turner et al., 1999) were used. Microbiome analysis was performed by Eurofins Genomics using the company's standard procedure.

2.7. Statistics

All data and standard curves were processed in Microsoft excel (2017), while statistical analysis was performed in IBM SPSS Statistics v.27. Statistical analysis on microbial growth was done at log-transformed data. Samples with no detected bacterial counts were scored as 1 CFU/g before log-transformation. All results are presented as mean values \pm standard error. Only one instrumental parallel was used to determine the fatty acid profile since the extracts were made from pooled samples consisting of fractions of 5 individual salmon.

All statistical tests in this study use a significance level of $\alpha = 0.05$. A 2-tailed independent samples *t*-test with a Levene's Test for Equality of Variance was used for a main effect analysis testing for significant differences between Prod and Sup samples and their initial- and stored samples according to Fig. 2 (the microbial community and proximate composition were only analysed on initial- or stored samples, respectively). Prior to this main effect analysis, all results in the relevant categories were combined to generate two means that were compared using the *t*-test. For instance, the statistical difference in protein content between Sup and Prod salmon was determined by calculating the mean protein content of all Sup and Prod fractions before comparing the means of the datasets with the *t*-test. Significant differences between salmon fractions (P, B, S, and F) were tested with a one-way Analysis of Variance (ANOVA) using Tukey's multiple comparisons test for post-hoc. Correlations between measured parameters were analysed with a Pearson correlation test. All the raw data from each experiment are provided in the OSF repository for the study (https://osf.io/jyatr/?view_only=64eb73c368ec4f1387b2cf70cbb81056).

3. Results & discussion

3.1. Proximate composition and fatty acid profile

There was no significant difference in the percentage content of

water ($p \geq 0.92$), ash ($p \geq 0.39$), protein ($p \geq 0.60$), or lipid ($p \geq 0.31$) between combined fractions of Sup and Prod salmon (Fig. 4). These results indicate that Prod and Sup salmon share a similar proximate composition, which is to be expected from fish that are classed based on physical appearance and/or external and internal blemishes.

Similar to the manual deboned meat reported by Østvik et al. (2005), the manual deboned meat in the present study had a lipid content close to 10% (Fig. 4). Consequently, the deboned meat was significantly leaner ($p < 0.001$) than the fillet and had a significantly higher ($p < 0.001$) protein and water content. The lipid content of industrial produced deboned meat is expected to increase towards 23% as softer, fatty tissue is removed from the backbone (Østvik et al., 2005). The sensory tests performed by Østvik and Grimsmo (2012) showed that the deboned meat is of the highest quality when the red meat of the backbone is removed without the fatty, soft tissue. However, this would decrease the amount of meat extracted from the backbone. A solution could be to remove the red meat from the frames before removing the remaining meat, including the fatty, soft tissue. While this would be a more labour-intensive process, it would also allow all the meat to be extracted without compromising the high quality of the red meat left on the frames.

On average, the Bits & Pieces of Sup and Prod quality had a similar lipid and protein content to the fillet. However, the lipid content in the Bits & Pieces varied significantly ($p < 0.001$) between the Sup and Prod samples (Fig. 4). This result is unlikely to be caused by differences of Sup and Prod quality as there was no significant difference in the fat content in the other co-product and fillet fractions. The Bits & Pieces used in this study were sampled from the low-fat posterior end and the fattier anterior part of the salmon fillet (Fig. 2). The lipid content varies from 2 to 18% in these fillet parts (Katikou et al., 2001). The observed fat and water content difference is likely caused by including different cuts in the two batches. This difference is interesting since it illustrates a challenge with Bits & Pieces as a co-product; composition will likely vary between batches. The variation would be reduced in production lines that always remove the same pieces from the fillet.

The FA profiles reveal a similar FA distribution in Sup and Prod salmon (Table 1). Furthermore, all the co-products (P, B, and S) and the fillet (F) had a similar FA distribution, with 14–16% saturated FA, 52–56% monounsaturated FA (MUFA), and 29–31% polyunsaturated FA (PUFA). The amount of n-3 FA was 12–14% of total lipids, with an n-3/n-6 ratio of 0.8–1. The most abundant FA was the MUFA oleic acid

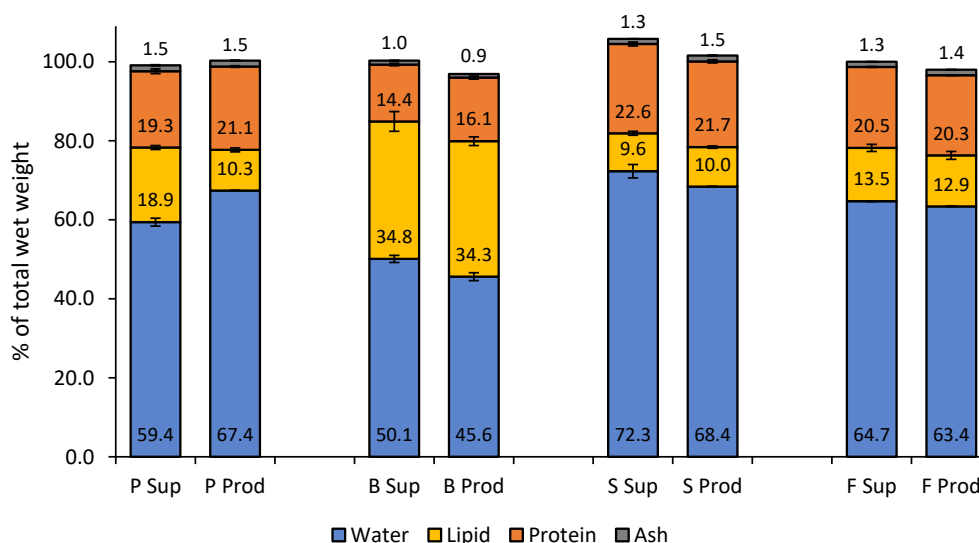


Fig. 4. Proximate composition of farmed Atlantic salmon. The results are presented as Superior (Sup) and Production (Prod) quality, and in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S) and Fillet (F). All values are presented as the weight fraction of total wet weight, expressed as mean with SE as error bars. Sample size: water ($n = 5-6$), ash ($n = 2-6$), protein ($n = 3$) and lipid ($n = 3-5$).

Table 1

Fatty acid profile as proportional content (% of total fatty acids w w⁻¹) of farmed Atlantic Salmon. The results are presented as Superior and Production quality, and in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S), and Fillet (F) (n = 1, based on pooled sample consisting of five individual salmon).

Fatty acid	Superior quality				Production quality			
	P	B	S	F	P	B	S	F
C 14:0	2.1	2.1	1.9	1.9	1.9	2.1	2.0	2.0
C 16:0	9.8	9.8	10.6	10.8	10.8	9.9	10.4	9.9
C 16:1n-7	2.6	2.6	2.4	2.4	2.4	2.7	2.5	2.6
C 18:0	2.9	2.9	3.1	2.9	2.9	2.8	2.8	2.7
C 18:1n-9	41.4	41.6	42.3	40.0	40.0	42.0	40.4	40.8
C 18:1n-7	2.7	2.8	3.1	2.7	2.7	2.9	2.8	2.6
C 18:2n-6	14.5	14.6	14.4	13.6	13.6	14.7	13.7	14.0
C 18:3n-3	7.3	7.4	7.0	5.7	5.7	6.0	5.9	6.1
C 18:4n-3	0.7	0.7	ND	0.8	0.8	ND	0.7	0.7
C 20:1n-9	3.7	3.7	3.8	4.3	4.3	4.4	4.4	4.3
C 20:4n-6	1.0	1.0	1.1	1.1	1.1	1.2	1.1	1.0
C 20:5n-3	2.8	2.6	2.8	2.9	2.9	2.7	3.0	2.8
C 22:1n-9	2.4	2.0	2.7	3.6	3.6	3.6	3.6	3.6
C 22:5n-3	1.2	1.2	1.2	1.2	1.2	1.2	1.3	1.2
C 22:6n-3	3.6	3.6	3.8	4.7	5.0	3.7	4.5	4.3
Σ SAF	14.7	14.7	15.5	14.9	15.6	14.7	15.1	14.6
Σ MUFA	52.8	52.7	54.2	52.8	53.1	56.1	53.7	53.8
Σ PUFA	31.0	31.1	30.2	30.8	30.3	29.1	30.2	30.1
Σ n-3 PUFA	14.3	14.3	13.6	14.1	14.4	12.5	14.2	13.9
Σ n-3/Σ n-6	0.9	0.9	0.9	0.9	1.0	0.8	1.0	0.9

(C18:1n9), which constituted 40–42% of fAs in all samples. The FA profile of the fillet is within the expected range specified in the FAO standard for farmed salmon (FAO, 2022). Therefore, the similar FA distribution in all fractions indicates that salmon co-products are an equally good source of n-3 PUFAS as the salmon fillet and that the total amount of nutritional lipids such as DHA and EPA in foods based on salmon co-products mainly depend on the amount of fat in the co-products. It is therefore of less importance wherever the lipid originates in the fillet, belly flap, or deboned meat.

The belly flap had significantly higher ($p < 0.001$) lipid content and significantly lower ($p < 0.001$) water and protein content compared to the other salmon co-products and fillets (Fig. 4). The high fat content, combined with a similar FA profile as the fillets, makes the belly flap a promising source of omega-3 FA in foods. When used in fish mince products, the belly flap's increased fat content could also enhance the mince's desirable functional properties, texture, and mouthfeel (Belton, 2000).

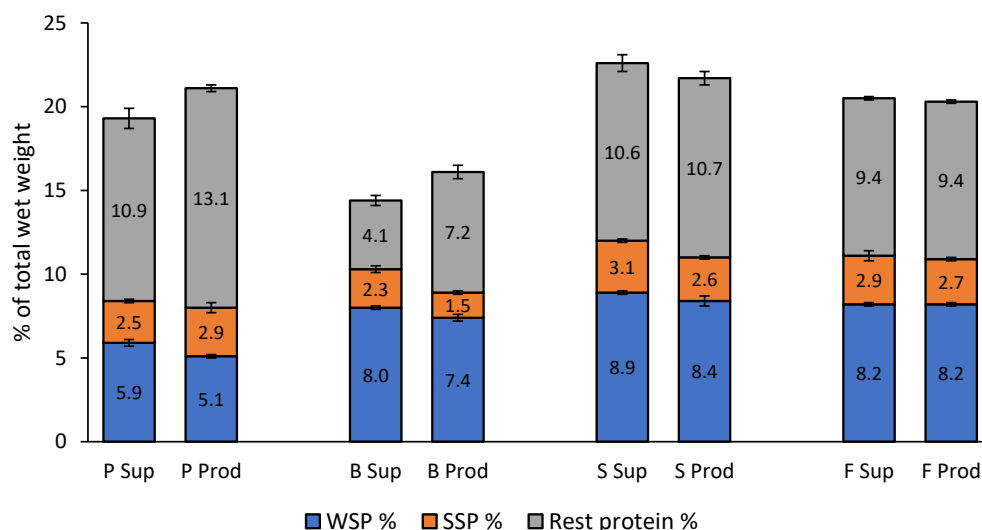


Fig. 5. Water-soluble protein (WSP), Salt-soluble protein (SSP), and rest protein of farmed Atlantic salmon. The results are presented as Superior (Sup) and Production (Prod) quality, and in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S), and Fillet (F). WSP and SSP are presented as the weight fraction of total wet weight, expressed as mean with SE as error bars (n = 3). Rest protein is calculated as Rest protein % = Total protein % - (WSP% + SSP%). SSP = Salt-soluble protein; WSP = Water-soluble protein.

3.2. Salt- and water-soluble protein

There was no significant difference in the content of WSP ($p \geq 0.40$) or SSP ($p \geq 0.30$) between Sup and Prod salmon (Fig. 5). The Bits & Pieces had a significantly lower ($p < 0.001$) amount of WSP than the other co-products, while the deboned meat had a significantly higher ($p < 0.001$) amount of WSP than other fractions. The belly flap is the only fraction with a significantly lower SSP ($p = 0.007$) compared to the others.

The WSP content of 8.2% found in the Sup and Prod quality fillets in this study agrees with previously reported results by Hultmann and Rustad (2004). Meanwhile, the fillet SSP content of 2.5–2.7% is lower than the 8% reported by Hultmann & Rustad in the same study. The lower amounts of SSP are likely due to freeze denaturation of the SSP during the eight weeks of storage at -80 °C in vacuum-sealed bags before protein extraction (Duun & Rustad, 2007; Mackie, 1993). SSP becomes insoluble upon freeze denaturation and is therefore measured as rest protein in this experiment. The freeze denaturation of SSP also explains the high measurements of insoluble rest protein, which should only be connective tissue (3–10% of total protein) in fresh salmon (Mackie, 1993). The freeze denaturation of myofibrillar proteins negatively affects (Mackie, 1993). The problem of freeze denaturation could be solved by processing the co-products while they are fresh. If the co-products must be frozen, Tolstorebrov et al. (2016) recommend that long termed stored fish are stored at -35 °C in air-tight conditions.

3.3. ATP degradation

The average Ki-value of all samples significantly ($p < 0.001$) increased during iced storage from Day 0/1 ($42.4 \pm 5.4\%$, n = 24) to Day 7/8 ($82.5 \pm 4.0\%$, n = 24) (Fig. 6). During a storage experiment of sliced salmon fillets stored at 1 °C, Sallam (2007) found that the K-value, which is highly correlated to the Ki-value (Hong et al., 2017), of fillets, was 18% at day 0, 55% at day 7, and 70% at day 15. The fillet fraction in the present study exhibits a somewhat faster increase in the Ki-value than reported by Sallam (2007). The rapid increase in Ki-value could be caused by the mincing of the salmon muscle, where biological membranes are disrupted and air is mixed into the samples. These results indicate that minced co-products are generally more susceptible to ATP degradation than whole fish or fillets.

The belly flap fractions had an exceptionally high Ki-value, with an initial value of around 80% and a Ki-value of 100% on Day 7/8 (Fig. 6). The high Ki-values suggest that the belly flap fraction is highly susceptible to ATP degradation and should be handled accordingly.

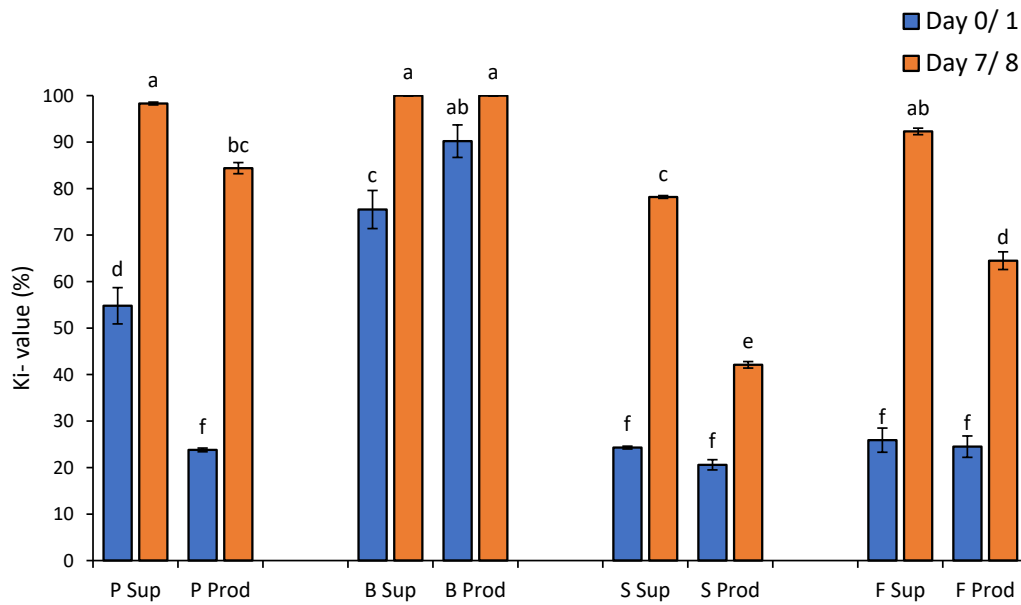


Fig. 6. Ki-value calculated from the Inosine monophosphate (IMP), Inosine (Ino), and Hypoxanthine (Hx) values presented in Table 2. The results are presented as Day 0/1 and Day 7/8, Superior (Sup) and Production (Prod) quality, and in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S) and Fillet (F). All values are expressed as means with SE as error bars (n = 3). Different letters (a–f) indicate a significant difference (p < 0.05) between fractions.

Meanwhile, the Ki-values of the deboned meat Sup and Prod samples are significantly lower (p < 0.001) than their fillet Sup and Prod counterparts on Day 7/8. The low Ki-value is surprising as deboned meat could be contaminated with blood from the frames that contain endogenous enzymes (Falch et al., 2007). It is essential to know the differences between manually deboned meat in this study (Fig. 3.2) and industrial deboned meat. The manual deboning of meat results in a mince containing less blood and soft, fatty tissue than the industrial deboned meat, depending on the type of machine used (Østvik et al., 2005). The low Ki-values found in the deboned meat in this study indicate that manually deboned meat is stable regarding ATP degradation Table 2.

There was no significant difference between Sup and Prod samples in a main effect analysis for the concentrations of IMP (p ≥ 0.230), Ino (p ≥ 0.393), and for Ki-value (p ≥ 0.164) (Table 2). The Sup quality average Hx concentration of 0.57 ± 0.07 μ mol/g (n = 24) was found to be significantly higher (p = 0.039) than the Prod quality average Hx concentration of 0.40 ± 0.04 μ mol/g (n = 24). This observation was unexpected since the Sup fractions showed less microbial growth than the Prod salmon (further described in chapter 3.3). Furthermore, the

Prod Day 1 salmon used in the present study was one day older than the Sup quality Day 0 salmon (Fig. 2A), which would usually result in higher Hx concentrations (Sallam, 2007). An inconsistent cold chain, contamination during processing, or individual variation between salmon could cause higher concentrations of Hx in the Sup salmon. Nonetheless, the difference in Hx concentrations was too small to make the H-value of the Sup quality (19.2 ± 3.0%, n = 24) significantly different (p ≥ 0.064) from the H-value of the Prod quality (12.5 ± 9.2%, n = 24). The total Hx concentrations are low compared to previously reported values in Atlantic salmon fillets at the end of shelf life (Jakobsen et al., 2022).

Interestingly, a correlation was found (r = 0.62, p = 0.006) between the lipid content and ATP degradation using a Pearson correlation test. The lean deboned meat and fillets exhibit the lowest Ki-values, while the fatty belly flap and Sup quality Bits & Pieces co-products have the highest Ki-values. Furthermore, the total concentration of ATP-related compounds (IMP, Ino, and Hx) is significantly higher (p < 0.001) in the fillet (4.0 ± 0.2 μ mol/g, n = 12) than in the fatty belly flap (2.5 ± 0.1 μ mol/g, n = 12). ATP is found in high concentrations in the mitochondria of muscle cells, which contain myofibrillar proteins (Mackie,

Table 2

Concentrations of IMP, Ino and Hx (μ mol/g) in all samples. The results are presented as Superior and Production quality, in the fractions of Bits & Pieces (P), Belly flap (B), Deboned meat (S) and Fillet (F), and as Day 0/1 and Day 7/8. All values are expressed as mean ± SE. The sample size (n) and p-value of an ANOVA analysis to test significant difference between fractions within each category are given. Different letters (a–f) within the same row indicate significant difference (p < 0.05) between fractions.

	Superior				Production				p	n
	P	B	S	F	P	B	S	F		
IMP (μ mol/g), Day 0/1	1.55 ± 0.16 ^b	0.59 ± 0.07 ^{a,b}	4.37 ± 0.52 ^c	3.48 ± 0.32 ^c	3.75 ± 0.14 ^c	0.26 ± 0.10 ^a	3.77 ± 0.07 ^c	3.52 ± 0.27 ^c	<0.001	3
IMP (μ mol/g), Day 7/8	0.04 ± 0.01 ^{a,b}	0.00 ± 0.00 ^a	0.81 ± 0.03 ^d	0.24 ± 0.02 ^b	0.49 ± 0.02 ^c	0.00 ± 0.00 ^a	2.47 ± 0.10 ^f	1.30 ± 0.08 ^e	<0.001	3
Ino (μ mol/g), Day 0/1	0.54 ± 0.09 ^{b,c}	1.36 ± 0.02 ^{a,b,c}	1.15 ± 0.19 ^{a,b,c}	1.00 ± 0.22 ^{a,b,c}	0.94 ± 0.03 ^{a,b}	1.61 ± 0.16 ^c	0.78 ± 0.03 ^a	0.96 ± 0.06 ^{a,b}	0.002	3
Ino (μ mol/g), Day 7/8	1.90 ± 0.45 ^{a,b,c}	1.29 ± 0.07 ^a	2.21 ± 0.10 ^{b,c}	2.33 ± 0.05 ^c	2.16 ± 0.09 ^{b,c}	1.89 ± 0.09 ^{a,b,c}	1.45 ± 0.05 ^{a,b}	1.96 ± 0.09 ^{a,b,c}	0.008	3
Hx (μ mol/g), Day 0/1	0.32 ± 0.02 ^{a,b}	0.51 ± 0.21 ^{a,b}	0.25 ± 0.00 ^a	0.23 ± 0.04 ^{a,b}	0.23 ± 0.02 ^{a,b}	0.59 ± 0.02 ^b	0.19 ± 0.03 ^a	0.17 ± 0.01 ^a	0.013	3
Hx (μ mol/g), Day 7/8	0.75 ± 0.06 ^c	1.22 ± 0.17 ^d	0.70 ± 0.01 ^{b,c}	0.59 ± 0.01 ^{a,b,c}	0.51 ± 0.02 ^{a,b,c}	0.74 ± 0.04 ^c	0.34 ± 0.02 ^a	0.41 ± 0.01 ^{a,b}	<0.001	3

IMP = Inosine monophosphate; Ino = Inosine; Hx = Hypoxanthine.

1993). Hence, the low concentrations of ATP-related compounds in the belly flap are likely caused by the significantly lower ($p = 0.007$) concentrations of myofibrillar SSP in the belly flap than in the other fractions (Fig. 5). To summarize, these findings suggest that salmon fractions high in fat or low in myofibrillar muscle tissue have low concentrations of ATP-related compounds but a high ratio of Hx compared to IMP. This results in the high Ki-value observed in fatty salmon co-products.

3.4. Microbial quality

Microorganisms are the most frequent cause of the deterioration of fish products, so exploring microbial diversity is essential in assessing the suitability of raw material for human consumption (Parlapani, 2021). In the present study, the microbial quality affected by the design factors has been investigated. By looking into the main effects of the design variables (Table 3), the fixed factor Quality (Sup versus Prod, $p < 0.001$) and Storage time (Day 0/3 and Day 7/10, $p = 0.008$) stand out as the main discriminants regarding all investigated microbial parameters (Table 3). The higher microbial counts in Prod samples are most likely caused by more extensive handling during processing and storage and hence contamination from the processing plants' in-house microbiota (Møretro et al., 2016). *Listeria* spp. was not detected in any fraction, regardless of quality or sampling time. Among the different fractions, Bits & Pieces stand out as the fraction with the lowest microbial quality, highlighted by significantly higher log CFU/g values for all measured parameters ($p = 0.004$ – 0.043), except from the counts of H₂S-producing bacteria ($p > 0.263$).

Significant differences in microbial quality between Sup and Prod qualities might mask some fundamental differences between the investigated fractions. The development of psychrotrophic aerobic plate count (PC), H₂S-producing bacteria, and *Pseudomonas* spp. has previously been identified as an essential parameter affecting salmon products' microbial quality and shelf-life (Møretro et al., 2016). A significantly higher microbial load was observed among Prod compared to the Sup quality fractions (Fig. 7). Accounting that the Prod samples were three days older (Fig. 2B), this difference was expected but maybe not so prominent. Looking into the specific development of these parameters within each fraction, the belly flap of the Sup quality fraction has the lowest storage stability regarding the development of PC and H₂S-producing bacteria (an increase of 4.5 and 3.6 log CFU/g, respectively) from day 3–10). Among the Prod quality fractions, the highest load of PC and H₂S-producing bacteria was found in the Bits & Pieces at day 3 (7.4 and 6.3 log CFU/g, respectively), whereas an increase of

approximately 2–3 log CFU/g was observed during the seven days storage of the fractions Belly flap and Deboned meat. Overall high initial counts in all fractions, compared to those previously observed in fresh ice stored post rigor filleted salmon (Chan et al., 2020), (Chan et al., 2020), indicate poor handling of the raw material. The bacterial growth within these fractions, as a function of storage time, gave a comparable log CFU/g value among all investigated fractions of the Prod quality at day 10 (PC: ranging from 7.5 to 8.2 log CFU/g, $p > 0.05$, and H₂S producing bacteria: 6.3 to 7.3 log CFU/g, $p > 0.05$). The development of *Pseudomonas* spp. showed a similar trend among the Prod fractions as observed for the PC and H₂S-producing bacteria. For the Sup fractions, significant development of PC and *Pseudomonas* spp. was observed during the seven days of storage, of which the Belly flap fraction increased the most (Fig. 7).

Møretro et al. (2016) detected higher concentrations of *Pseudomonas* spp. and *Shewanella* spp. in industrially filleted salmon (Prod quality) compared to salmon filleted under strictly controlled conditions (in the present study represented by the Sup quality). Some *Pseudomonas* strains can produce a sour smell that will be noticeable at concentrations log CFU/g > 7 in ice-stored salmon fillets (Langsrud, 2015). Moreover, it has been indicated that *Pseudomonas* concentrations above 7–8 log CFU/g significantly affect the mucus produced, showing deteriorated quality (Nychas et al., 2008) Several fractions originating from the Prod qualities had *Pseudomonas* counts higher than 7 log CFU/g, indicating the Prod quality fractions deteriorated at Day 10.

3.5. Microbial community

Illumina MiSeq sequencing generated a total of 181 334 raw sequence reads. 181 266 remaining sequences were obtained after quality control processing, filtering of raw reads, and chimera detection and filtering, of which 89.2% were assigned to OTUs. The total numbers of OTUs were 316. The microbiome profiling (Fig. 8) revealed that members of the genus *Photobacterium* were the most abundant in all samples. However, the relative abundance of *Photobacterium* was higher in Prod (80.2% and 93.5% in Bits & Pices and Deboned meat, respectively) than in Sup samples (18.8% and 25% Bits & Pices and Deboned meat, respectively). *Pseudomonas* and *Shewanella* were the only other genera detected in Prod samples, accounting for 1.6–5.0% and 1.1–1.3%, respectively. Møretro et al. (2016) have previously reported cross-contamination routes for *Pseudomonas* and *Shewanella* from processing equipment to salmon fillets, while *Photobacterium* most likely originates from fish and seawater. *Photobacterium*, *Pseudomonas*, and *Shewanella* are well-known spoilage bacteria in salmon (Gram & Huss,

Table 3

Effects of the fixed factors Quality (Superior and Production), Fraction (Bits & Pieces, Belly flap, and Deboned meat), and Storage (Day 0/3 and Day 7/10) on the microbial quality (log CFU/g) of the raw material.

Factor	Microbial quality (log CFU/g)						
	APC	PC	HSPB	<i>Pseudomonas</i>	<i>Brochotrix</i>	LAB	<i>Enterobacteriaceae</i>
Quality (n = 18)							
Superior	2.6 ± 1.3 ^b	1.7 ± 2.3 ^b	1.7 ± 1.6 ^b	2.0 ± 2.0 ^b	0.2 ± 1.0 ^b	1.7 ± 1.5 ^b	0.7 ± 0.8 ^b
Production	6.6 ± 1.6 ^a	6.9 ± 1.4 ^a	5.8 ± 1.4 ^a	6.3 ± 1.6 ^a	3.9 ± 2.4 ^a	4.5 ± 1.8 ^a	2.7 ± 1.0 ^a
P _Q -value ^a	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fraction (n = 12)							
Bits & Pieces	4.8 ± 2.7 ^a	3.9 ± 4.1 ^b	4.0 ± 2.8	4.6 ± 3.0 ^a	2.7 ± 2.9 ^a	3.7 ± 2.5 ^a	2.3 ± 1.4 ^a
Belly Flap	4.3 ± 2.5 ^b	4.9 ± 2.4 ^a	3.3 ± 2.4	3.8 ± 2.7 ^b	1.8 ± 2.1 ^{ab}	2.8 ± 1.9 ^b	1.3 ± 1.1 ^b
Deboned meat	4.8 ± 2.5 ^a	4.2 ± 3.2 ^{ab}	3.8 ± 2.6	4.0 ± 3.0 ^b	1.7 ± 2.6 ^b	2.7 ± 2.2 ^b	1.4 ± 1.5 ^b
P _F -value ^a	= 0.019	= 0.043	>0.263	= 0.006	= 0.024	= 0.010	= 0.004
Storage (n = 18)							
Day 0/3	3.4 ± 2.1 ^b	3.2 ± 2.9 ^b	2.8 ± 2.3 ^b	2.6 ± 2.7 ^b	1.1 ± 1.9 ^b	1.9 ± 1.9 ^b	1.4 ± 1.1 ^b
Day 7/10	5.8 ± 2.2 ^a	5.4 ± 3.3 ^a	4.7 ± 2.5 ^a	5.7 ± 2.1 ^a	3.1 ± 2.8 ^a	4.3 ± 1.7 ^a	2.0 ± 1.5 ^a
P _S -value ^a	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	= 0.008

^a General Linear Model (GLM) analyses of variance, where P_Q, P_F, and P_S are the significance levels of the fixed factors Quality (Superior and Production), Fraction (Bits & Pieces, Belly flap, and Deboned meat), and Storage (Day 0/3 and Day 7/10 (Superior/Production)). Different superscripts (^{ab}) within each column indicate significant variation ($P < 0.05$) between variables by Tukey's comparison test. APC = Aerobic plate count; PC= Psychrotrophic aerobic plate count; H₂S= H₂S Producing bacteria plate count; LAB = Lactic acid bacteria.

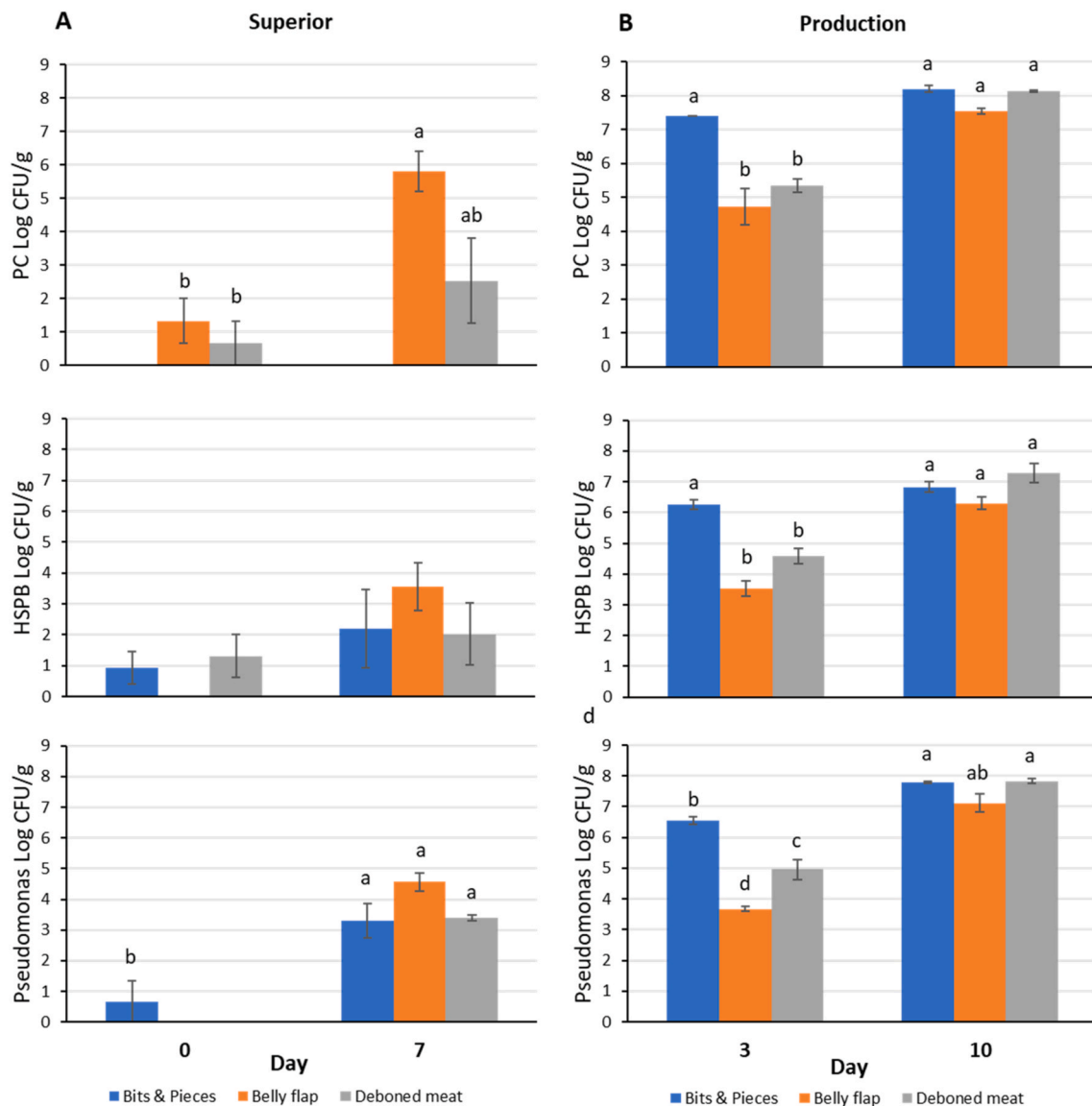


Fig. 7. The development of psychrotrophic aerobic plate count (PC), H₂S-producing bacteria (HSPB), and *Pseudomonas* spp. The different fractions (Bits & Pieces, Belly flap, and Deboned meat) are separated on the fixed factor quality (A: Superior and B: Production) for 7 days storage. *Superior/production* samples were analysed on Day 0/3 and Day 7/10, respectively. Error bars indicate one SE, and different lowercase letters (a–d) within each chart indicate significant variation ($P < 0.05$) between groups by Tukey's comparison test.

1996; Macé et al., 2012). *Photobacterium* species are generally very efficient producers of trimethylamine (TMA) under anaerobic conditions (Dalgaard, 1995) and tolerate high concentrations of CO₂ (Devlieghere & Debevere, 2000), e.g., in modified atmosphere packaging. *Shewanella* species also perform an anaerobic reduction of trimethylamine oxide (TMAO) to TMA, but less efficiently than *Photobacterium*, and their proliferation can more easily be prevented by, e.g., applying CO₂ in gas-packaging of the raw material (Devlieghere & Debevere, 2000). Growth of *Pseudomonas* is considerably reduced in O₂-limited conditions. However, *Pseudomonas* is associated with quality changes such as slime production and off-odor development of chilled air-stored seafood (Gram & Dalgaard, 2002). Furthermore, *Pseudomonas*, *Photobacterium*, and *Shewanella* species are regarded as biogenic amine-producing bacteria (Visciano et al., 2012). However, salmon's biogenic amines are not considered a safety hazard since only insignificant amounts of histamine are produced (Emborg et al., 2002). Nevertheless, biogenic amines such as cadaverine and putrescine contribute to salmon spoilage, and emphasis on prevention and control methods is highly important, as highlighted in a recent review by Gao et al. (2023).

The Sup samples had an overall higher microbial diversity and a high abundance of reads categorised as "others", many due to reads that were only assigned at higher taxonomy levels (e.g., phylum). Another abundant genus in Sup samples was *Vibrio*, accounting for 8.8% and 4.6% in Bits & Pieces and Deboned meat, respectively. *Vibrio* spp. are widespread in aquatic environments, and the genus includes potential human pathogenic species such as *V. cholera*, *V. parahaemolyticus*, and *V. vulnificus* (Baker-Austin et al., 2018). These pathogenic *Vibrio* have occasionally been detected in Norwegian marine environments on the Soth coast of Norway (Naseer et al., 2019). However, these species are more abundant in a marine environment with higher temperatures (>18 °C) and lower salinity levels (<25‰) (Vezzulli et al., 2013) than the coast of Mid-Norway. A recent comprehensive study of *Vibrio* spp. from the Norwegian marine environment (Håkonsholm et al., 2020) did not detect pathogenic *Vibrio* species on the west coast of Norway. Thus, *Vibrio* detected in our study are most likely non-pathogenic species. However, further studies should elaborate on these findings.

Pseudomonas and *Shewanella* were also detected in the Sup samples. A diverse psychrotrophic bacterial community, constituting several

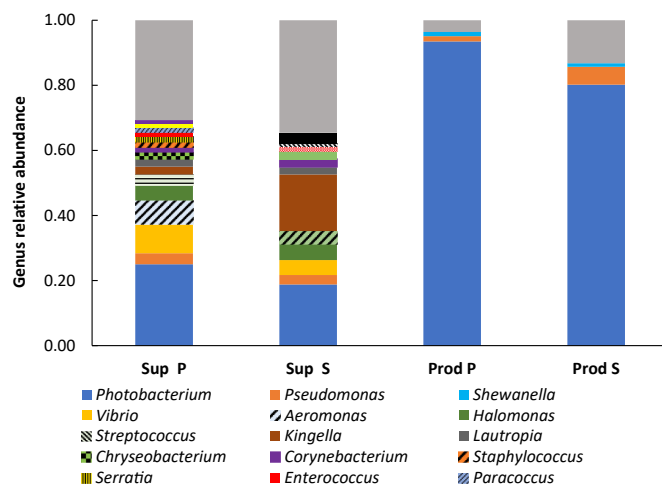


Fig. 8. Genus relative abundance in pooled samples ($n = 3$) at Day 3. The x-axis is sorted by sample type, i.e., Superior (Sup) and Production (Prod) quality, and in the fraction Bits & Pieces (P) and Deboned meat (S). All the genera with $>1.0\%$ relative abundance are shown. All taxonomic units with less than 1% of reads, or reads not assigned to the taxonomic level of genera are assembled in the category “others”.

bacteria with spoilage potential, e.g., lactic acid bacteria (Françoise, 2010) and *Aeromonas* (Jakobsen et al., 2020) were detected. The high diversity is following communities reported for salmon processing environment after cleaning and disinfection (Møretro et al., 2016; Thomassen et al., 2022).

4. Conclusion

The present study demonstrated that both Sup and Prod salmon co-products consist of valuable nutrients suitable for human consumption. The co-products' proximate composition varied among the fractions, while the FAs were equally distributed. Moreover, it is concluded that the belly flaps and Bits & Pieces are more susceptible to ATP degradation than deboned meat and the fillet.

The main barrier to producing foods based on Salmon co-products lies in retaining its microbial and chemical quality during processing and storage. Co-product processing breaks biological membranes and introduces oxygen into the product, which enhances microbial growth, lipid oxidation, ATP degradation, and freeze denaturation of salt-soluble proteins during storage. The presented microbial analysis highlights the importance of the processing environment and good hygienic routines during processing, i.e., indicating the Sup raw material to have the highest potential to be utilized directly into value-added products intended for human consumption. Further studies should assess the stability of lipids and protein during frozen storage. Industry standards should be developed to optimize the processing and storage of the salmon co-products before being used in products intended for human consumption. Simultaneously, food products based on salmon co-products should be developed and assessed with sensory analysis to test consumer acceptance.

CRediT authorship contribution statement

Didrik Ulleberg: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Ingrid Bøe Sletten:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Anita Nordeng Jakobsen:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Visualization, Supervision. **Jon Brage Svenning:** Formal analysis, Writing – review & editing, Visualization, Supervision. **Jørgen Lerfall:** Conceptualization,

Methodology, Formal analysis, Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data available at OSF depository through the link in chapter "2.9 Statistics" of the manuscript. OSF link: https://osf.io/jyatr/?view_only=64eb73c368ec4f1387b2cf70cbb81056

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