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Hyperspectral imaging for non-destructive detection of process-related impact damage in whole Atlantic cod (*Gadus morhua* L.)

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Abstract

Mechanical impacts during on-board handling and processing challenge Atlantic cod (*Gadus morhua* L.) quality through muscle blood issues and structural damages. The present study investigated effects of controlled impacts on sensory quality and muscle hemoglobin (Hb) content, evaluated using hyperspectral imaging (HSI). A total of 120 farmed cod were assigned to a control group (n=30) or subjected to three impacts using 1, 2, or 4 kg plates dropped from 1.7 m (n=30 per group). Fish were assessed by sensory evaluation, Hb determination, and HSI-based estimation of Hb abundance. Increasing

impact severity caused skin damage, muscle fiber separation, blood spots, and vertebral fractures. Fractures occurred in 10%, 77%, and 100% of fish exposed to 1, 2, and 4 kg impacts, respectively, and were associated with internal blood staining and elevated muscle Hb. Chemical analysis showed significantly higher Hb content in the 2 and 4 kg groups than in the control and 1 kg group. HSI distinguished impacted fish from controls through higher Hb abundance but showed high within-group variability and no clear dose-response relationship. Thus, HSI shows potential for batch-level classification and defect detection, but optical effects from skin, tissue depth, scattering, and fish geometry limit accurate quantification and individual grading.

Introduction

Industrial seafood processing is undergoing increasing automation and digitalization, creating opportunities for data-driven process control and quality monitoring. Optical sensing technologies such as hyperspectral imaging have the potential to support these developments by enabling continuous quality assessment without interrupting production^{1,2}. A hyperspectral camera can measure many specific wavelengths across a spectrum that extends from the ultraviolet through the visible and into the near infrared. The spectral measurement is recorded for each pixel in the image, giving one spectral dimension and two spatial dimensions, making the full image data structure three-dimensional. The spectral data indicates how light has been scattered and absorbed within the sample and thus provides insight into the physicochemical composition. The spatial data helps visualize and quantify the distribution of these components across the sample surface³.

44 Research on the utilization of HSI for automated non-destructive quality
45 measurement of fish is advancing from the laboratory and into industrial
46 applications. Innovation such as interactance illumination by Wold, et al. ⁴ and
47 Sivertsen, et al. ⁵ gives deeper light penetration than diffuse reflectance, which
48 makes it possible to measure light that has passed through the skin and into the
49 fish's muscle, and can allow measurement of physicochemical components in whole
50 fish.

51 Robust chemometric models are necessary to translate spectral data into chemical
52 information. Traditional chemometric methods such as partial least squares (PLS)
53 are widely used because their latent-variable structure facilitates interpretation of
54 the relationship between spectral features and the response variable⁶. Machine-
55 learning approaches, including support vector machines and neural networks, can
56 accommodate more complex non-linear relationships within spectral data, but their
57 decision-making processes are often less transparent and may require additional
58 explainability methods for interpretation^{7,8}. These chemometric and machine
59 learning approaches have been successful at estimating various quality parameters
60 in seafood from hyperspectral image data, such as the prediction of freshness and
61 remaining shelf life of fish fillets⁹⁻¹¹, detection of parasites¹², and estimation of fatty
62 acids¹³.

63 Despite their success, chemometric and machine learning models are often sensitive
64 to variation in instruments, sample populations, and measurement conditions. As a
65 result, models developed under controlled laboratory conditions may not generalize

66 well to industrial environments, where differences in data distributions can reduce
67 predictive performance. This challenge, known as domain shift, remains a major
68 barrier to the robust industrial implementation of hyperspectral sensing
69 systems^{14,15}.

70 For industrial seafood production, it is therefore essential to develop models that
71 remain robust across hyperspectral cameras, production facilities, and operating
72 conditions. Skjelvareid, et al. ¹⁶ presented a solution to this issue by utilizing a priori
73 information in a constrained spectral unmixing model. The basis for this model is
74 the assumption that the spectrum is the sum of its components, and that by knowing
75 the spectral signatures of the components, their relative abundances can be
76 estimated from the sample's spectral signal. This gave a robust transferable model
77 for predicting blood concentrations in Atlantic cod fillets. Despite the promising
78 results for blood detection in fish fillets, the performance of HSI in whole fish
79 remains uncertain due to optical effects associated with skin, tissue heterogeneity
80 and sample geometry.

81 Blood-free Atlantic cod fillets are essential for producing high-value products.
82 Residual blood can reduce shelf life, cause discoloration, and negatively affect
83 texture^{17,18}. The effects of capture-related damages on fillet quality have been
84 extensively studied regarding fishing gear design and operation¹⁹⁻²³, while less
85 attention has been given to quality deterioration caused by on-board handling and
86 processing operations. Modern factories onboard larger fishing vessels are designed
87 for high-throughput production within confined spaces, increasing the risk of fish

colliding with equipment, hard surfaces, and processing infrastructure. Such impacts may cause bruising, localized hemorrhage, tissue damage, and skeletal fractures, resulting in products that fail to meet quality specifications²⁴.

Quality assessment is routinely performed both on board fishing vessels and at processing facilities through visual inspection of selected fish for residual blood, parasites, and other quality defects²⁵⁻²⁷. These inspections rely heavily on subjective assessments and are influenced by inspector experience and interpretation. Furthermore, only a small proportion of each catch is inspected, limiting the ability to accurately characterize batch quality. The absence of standardized and objective methods for quality assessment of headed and gutted fish may contribute to discrepancies in quality perception between fisheries and processors, potentially leading to production challenges and economic losses.

As seafood processing becomes increasingly automated, there is a growing need for objective, rapid, and non-destructive technologies capable of monitoring product quality throughout the production chain. Hyperspectral imaging (HSI) has emerged as a promising optical sensing technology for food quality assessment, offering the potential to detect internal and external defects without destructive sampling. However, its capability to detect and quantify process-induced impact damage in whole Atlantic cod remains largely unexplored. Therefore, the objectives of this study were (i) to establish a controlled experimental model for process-related impact damage in Atlantic cod and (ii) to evaluate the capability and limitations of

hyperspectral imaging as a non-destructive sensing technology for objective detection of such damage under industrially relevant conditions.

Results

Sensory quality

Across sensory attributes, increasing impact severity was associated with higher (worse) scores, indicating a dose-response relationship between impact and perceived quality degradation. The only exception was fillet gaping which indicated no relation to impact force and was excluded in further analysis. The full table summarizing mean values for all attributes of each group can be found in Supplementary Table S.1. No attributes were statistically distinguished from all adjacent groups, but the clearest separation was observed for fillet damage (muscle fiber separation). Where there were significant differences between the control and the first two impact groups (1 & 2 kg), and between the two latter groups and the 4 kg group as illustrated in Figure 2.

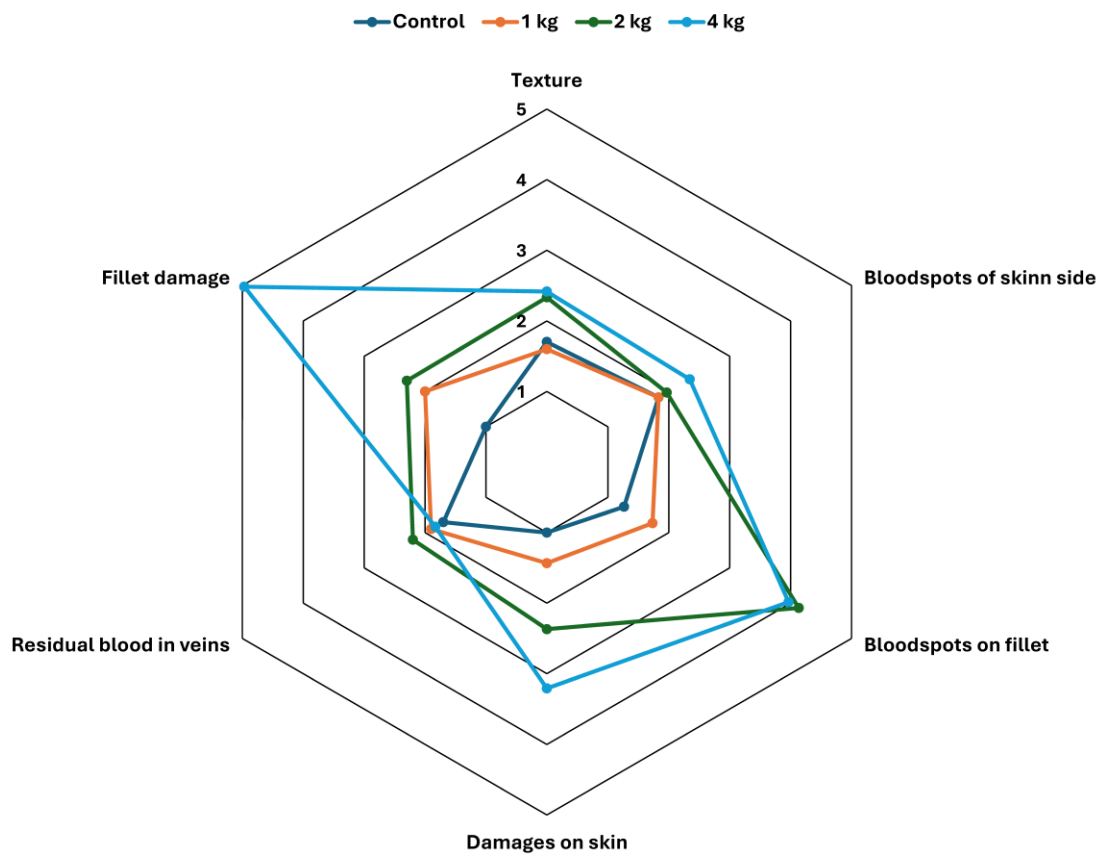


Figure 2. Spider plot of the mean sensory attribute scores by treatment group.

Principal component analysis (PCA) was conducted on the sensory data using two components, with PC1 explaining 60% of the variance and PC2 explaining 16%. The scoring plot (Figure 3) shows a more tightly separated grouping of the control and 4 kg sample groups along the PC1 axis. In between, the remaining 1 kg and 2 kg groups are more convoluted and spread along PC1. The correlation loading plot showed that blood spots in the fillet, skin damage, and fillet damage contributed strongly to PC1 and PC2. All parameters that pull to the right of the PC1 axis show a uniform correlation between higher sensory parameter scores and greater impact force. The second component

described the variation between the physical damage parameters (skin marks, muscle separation) and the blood-related parameters, suggesting some inconsistency between the two groups.

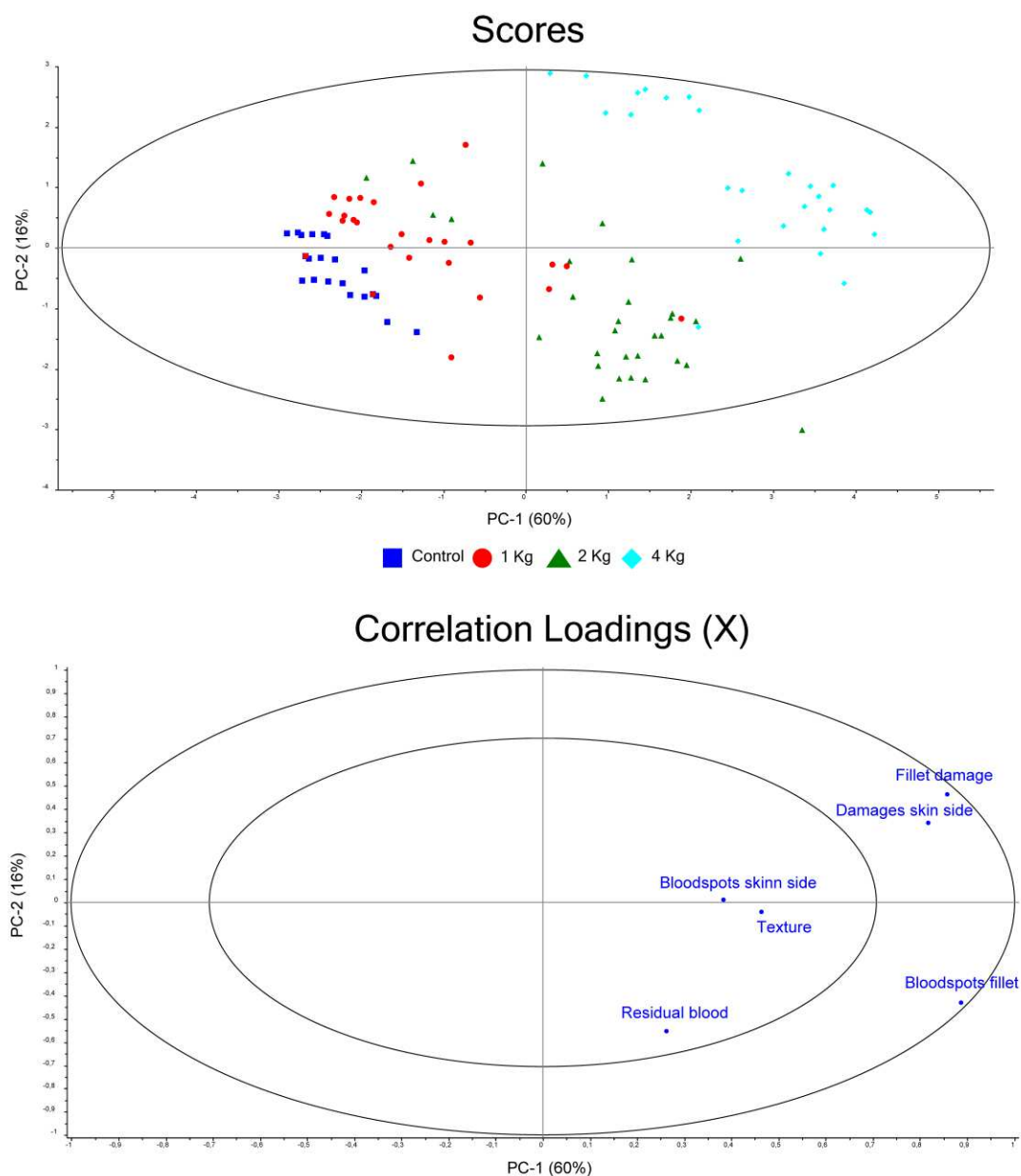


Figure 3. PCA score plot and loading plot for sensory attributes by treatment group.

Vertebral (spinal) fractures

Vertebral fractures were observed in all impacted groups, and frequency increased markedly with plate mass. Fractures occurred in 10% of fish in the 1 kg group (3/30), 77% in the 2 kg group (23/30), and 100% in the 4 kg group (30/30). No fractures were observed in the control group. In all cases, fractures occurred at the impact site and were associated with internal blood staining of fillets, consistent with rupture of the dorsal aorta.

Vertebral fractures were highly correlated with blood spots on the fillet (0.721, $p<0.001$), damages on skin and the fillet (0.687, $p<0.001$ and 0.742, $p<0.001$ respectively), moderate significant correlations to hemoglobin contents in the fillet (0.530, $p<0.001$), and lower correlation to the hemoglobin abundance estimates from HSI data (0.354, $p<0.001$). Correlations between all variables can be viewed in Supplementary Table S.2.

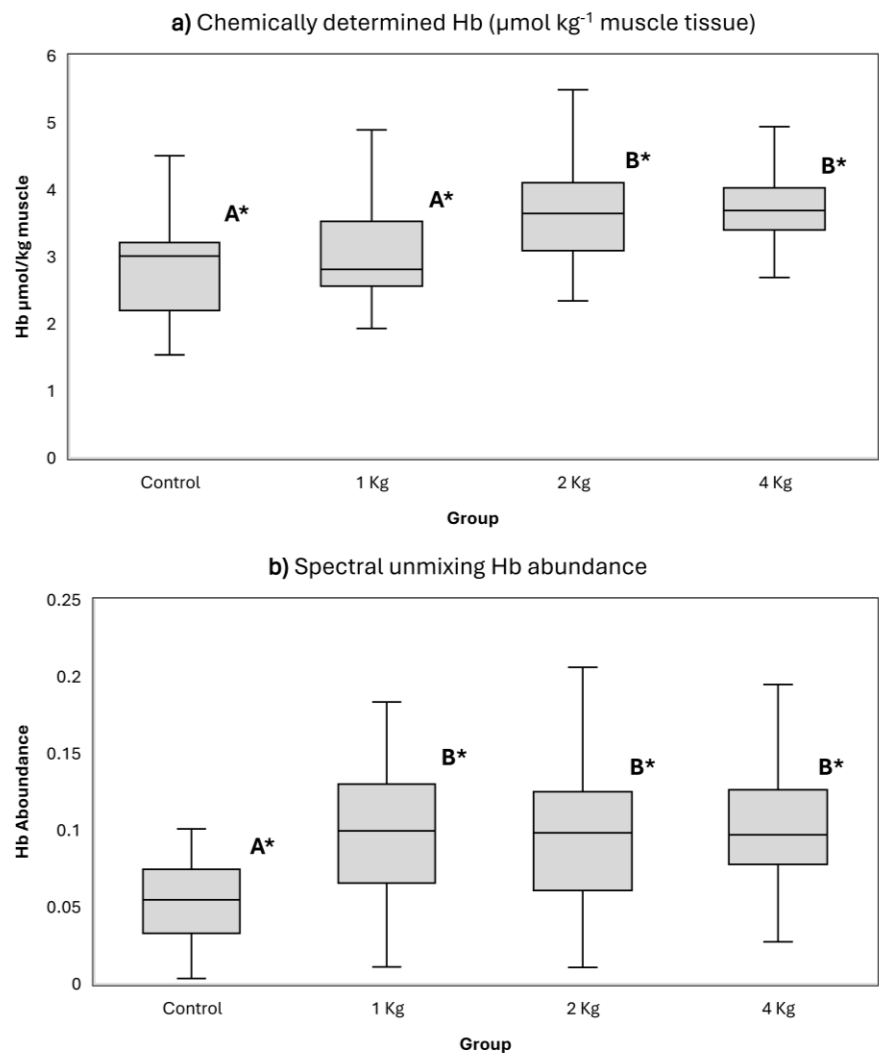
Muscle hemoglobin determination

Chemically determined hemoglobin (Hb) content increased with impact severity. The control group had the lowest mean Hb content ($2.77\pm0.69 \mu\text{mol Hb kg}^{-1}$). The 1 kg group showed a slightly higher mean ($3.11\pm0.82 \mu\text{mol Hb kg}^{-1}$), but it did not differ significantly from the control group ($p=0.256$) as seen in Figure 4a. The 2 kg and 4 kg groups exhibited higher Hb contents (3.62 ± 0.71 and $3.76\pm0.61 \mu\text{mol Hb kg}^{-1}$, respectively) and were significantly higher ($p<0.001$) than the control and 1 kg groups but not significantly different from each other.

Mean estimated hemoglobin abundances from HSI within the impact region were similar across the impacted groups (0.10 ± 0.04 , 0.10 ± 0.05 , and 0.09 ± 0.04 for the 1 kg, 2 kg, and

159 4 kg groups, respectively), whereas the control group showed a significantly ($p<0.001$)
 160 lower mean abundance (0.05 ± 0.03). The interquartile range of the impacted groups were
 161 above the control group, but some extreme cases are well below the control as illustrated
 162 in Figure 4b.

163



164

165 **Figure 4. Box and whiskers plot of a) Chemically determined muscle hemoglobin**
 166 **concentration ($\mu\text{mol Hb kg}^{-1}$), and b) average muscle Hb abundance from the**

spectral unmixing model by treatment group. *Annotated letters indicate statistically significant difference of mean values ($p < 0.05$) between subgroups.

There was a high degree of variation in Hb abundance within each group, as indicated by the high standard deviations of the mean values. Visual inspection of the heatmap images also revealed clear variation in Hb concentration and distribution, as shown in Figure 5. In the transformed images, high Hb concentrations were consistently observed along the dorsal side of the vertebra.

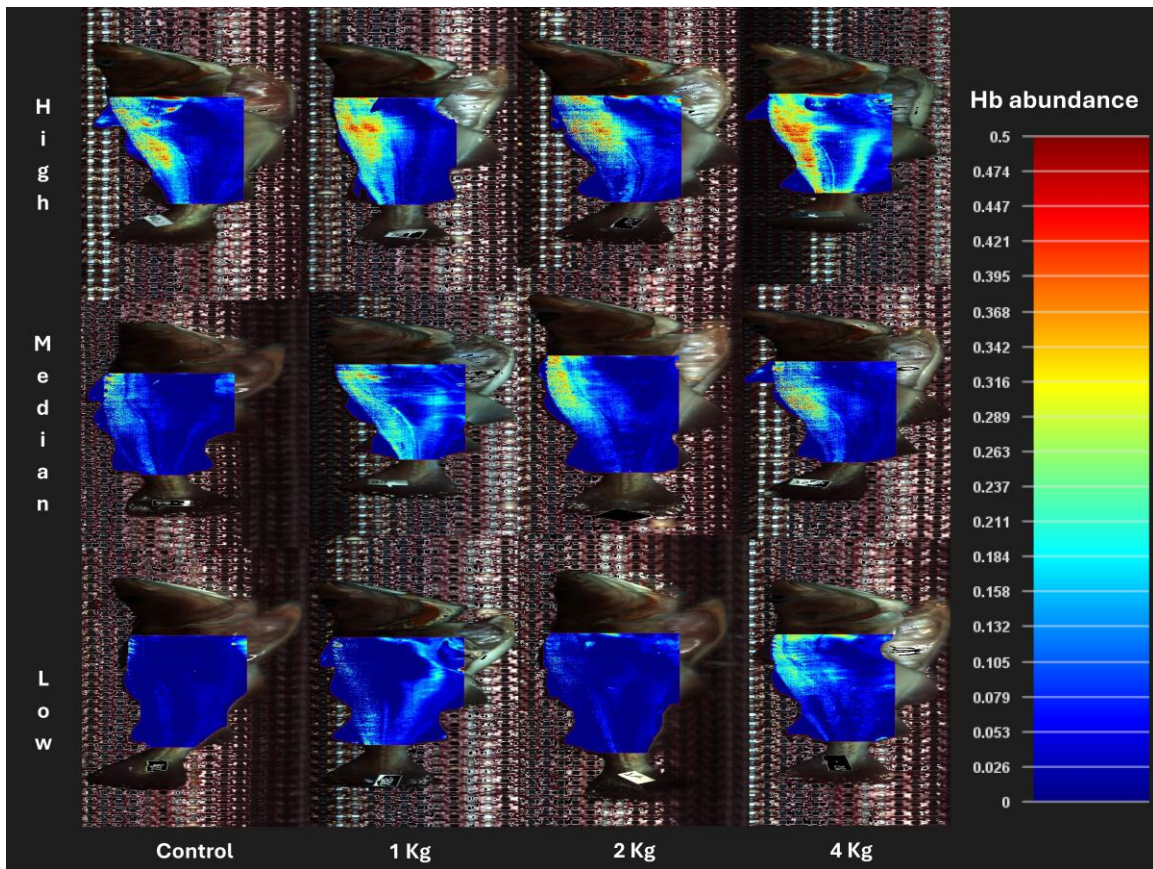


Figure 5. Representative unmixed abundance maps of Hb generated by constrained spectral unmixing of hyperspectral images acquired from whole Atlantic cod. Images illustrate within-group variability and show that elevated Hb-related

spectral responses were frequently localized near the vertebral column and dorsal musculature. Color scale has been adjusted to a range of 0.00 (dark blue) to 0.50 Hb abundance (dark red).

The effect of bleeding was also evaluated by subtracting the average Hb abundance from measurements after impact, measurements before impact, and measurements after bleeding. The standard deviation exceeded the mean in each group, and the distributions of the sample groups are shown in Figure 6. Negative numbers represent higher Hb abundance after impact, and positive numbers mean a reduction of Hb after impact. The variance in each sample group provides a basis for assessing the uncertainty in the accuracy of repeated measurements when monitoring changes in Hb content. The 2 kg group had the highest spread and, on average, (0.046 ± 0.078) significantly higher bleeding effect ($p=0.003$) than the 4 kg group, which had a mean Hb difference of 0.010 ± 0.046 . A clear trend of increased Hb retention with impact force is evident across the quartile ranges when excluding the 2 kg group.

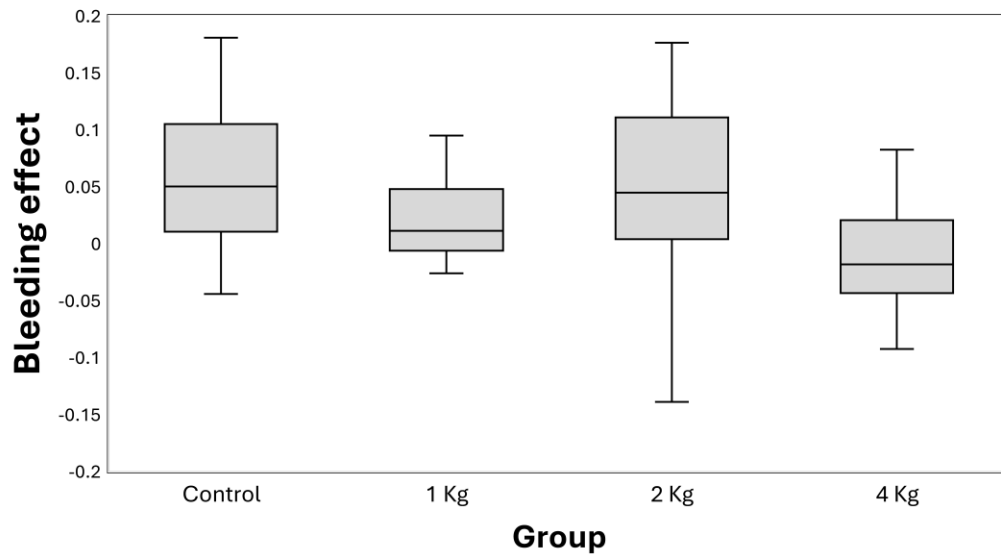


Figure 6. Box and Whisker plot of the bleeding effect measured by retracting the average Hb abundance after impact and out bleeding, from the measurement done before impact.

Discussion

The present study evaluated hyperspectral imaging (HSI) as a non-destructive sensing technology for detecting process-related impact damage in whole Atlantic cod under industrially relevant conditions. A controlled impact model was used to generate increasing levels of mechanical damage and to evaluate the relationship between HSI-derived hemoglobin (Hb) abundance, sensory quality attributes, and chemically determined Hb content. The results provide insight into both the capabilities and limitations of optical sensing for automated quality assessment in seafood processing.

Severe degradation in quality due to physical impacts was observed in groups exposed to impacts of 2 kg and above. Both sensory parameters and chemically determined Hb content differed significantly from the control group. In contrast, fish exposed to the 1 kg treatment were largely comparable to the control group, except for localized muscle fiber separation at the impact site. Notably, 10% of fish in the 1 kg group exhibited vertebral fractures. Similar fractures have previously been associated with electrical stunning in saithe ^{28,29}. However, the absence of fractures in the control group indicates that the stunning procedure did not contribute significantly to spinal damage under the present conditions.

Increasing the impact force to 2 kg resulted in vertebral fractures in 77% of the fish and produced extensive blood accumulation in the fillet, similar to that observed in the 4 kg group. The primary sensory differences between these two groups were related to the severity of skin damage and the depth of muscle separation, which were significantly greater in the 4 kg group. These findings suggest that once a threshold for structural damage (e.g., vertebral fracture) is exceeded, further increases in impact energy primarily affect the extent rather than the type of tissue damage. Together, these findings demonstrate that the experimental setup generated a reproducible gradient of process-related damage, providing a suitable framework for evaluating sensing technologies intended for quality monitoring in seafood processing.

The observed increase in Hb concentration in muscle tissue with increasing impact force is likely attributable to rupture of major blood vessels, particularly the ventral aorta, associated with vertebral fracture. Although visually blood-soaked tissue was avoided during sampling, diffusion of Hb into surrounding muscle tissue of the fillet, likely

contributed to the elevated Hb levels measured in the 2 kg and 4 kg groups. This interpretation is supported by the strong correlation (0.721, $p < 0.001$) between vertebral fractures and internal blood staining.

Although HSI successfully distinguished impacted fish from non-impacted controls, it did not provide a reliable quantitative measure of damage severity. One likely explanation is the limits of optical penetration depth of the interactance imaging configuration. While the interactance geometry increases sensitivity to subsurface features^{4,5}, the detected Hb signal is still weakened by the skin tissue due to absorption and scattering effects³⁰. Blood pooling located deeper within the musculature such as that associated with rupture of the ventral aorta may therefore contribute only weakly to the measured signal.

Additionally, the time difference between HSI acquisition (shortly after slaughter) and chemical analysis (after 6 days of storage) may have influenced the results. Over time, hemoglobin can diffuse within the muscle tissue, leading to a more homogeneous distribution and higher apparent Hb content in homogenized samples. In combination with increased muscle separation in higher-impact groups, this could facilitate deeper penetration of blood into the tissue, thereby strengthening the relationship between impact force and chemically measured Hb.

The constrained spectral unmixing approach used in this study assumes a linear combination of spectral end-members and applies a simplified polynomial correction for scattering effects. However, as demonstrated by Romeyn, et al.³⁰, this approach may be insufficient to account for the complex light transport processes in fish tissue, particularly

when imaging through skin. Their neural network-based model showed improved performance in estimating Hb beneath the skin, suggesting that more advanced modelling approaches may be required for accurate quantification under these conditions.

The high variability observed in HSI-derived Hb abundances can likely be attributed to several factors. First, blood distribution following mechanical impact is inherently heterogeneous, resulting in localized hemorrhages and uneven spatial patterns. Second, imaging through the skin reduces signal strength and contrast, as the skin introduces additional absorption and scattering. Third, the three-dimensional geometry of whole fish introduces variability in optical path length, illumination intensity, and surface reflections.

The measured hyperspectral signal represents a combined response from multiple tissue layers. Consequently, the spectral contribution of blood depends not only on its abundance, but also on its depth and the optical properties of the overlying tissue. A small superficial hemorrhage may therefore produce a similar spectral response to a larger but deeper blood accumulation. Likewise, variations in skin absorption and scattering can cause identical blood accumulations to appear different between fish. As a result, it is difficult to uniquely separate the effects of blood concentration, blood depth, and tissue optical properties. Importantly, this limitation is not solely related to penetration depth or signal strength but reflects an inherent ambiguity in the optical measurement itself. Improvements in instrumentation, illumination, and modelling may increase sensitivity and reduce measurement uncertainty, but they cannot fully eliminate this ambiguity. These challenges are particularly relevant for measurements performed on whole fish through the skin. In fillet applications, where skin effects and tissue depth are

reduced, the relationship between spectral response and hemoglobin content is likely to be more direct, potentially enabling more accurate quantification as demonstrated by Skjelvareid, et al.¹⁶.

Current quality assessment in the seafood industry relies primarily on visual inspection of a limited fraction of the catch. Such assessments are inherently subjective and may not accurately represent overall batch quality. The ability of HSI to identify systematic differences between damaged and undamaged fish suggests that optical sensing technologies may contribute to more objective quality monitoring. Although the present results indicate limitations for precise Hb quantification, HSI may still provide value through automated defect detection, quality screening, and batch-level assessment.

It should also be noted that the experimental fish were relatively uniform in size and geometry due to their farmed origin. In industrial settings, especially with wild-caught fish, greater variability in size, shape, and skin properties can be expected. Such variability may complicate interactance-based HSI measurements as the focal point cannot be optimally adjusted for every individual fish, reducing the measurement consistency. An additional challenge in an industrial setting is that only one side of the fish can typically be imaged, whereas in this experiment the fish were consistently oriented with the impact site facing the camera. Consequently, the measured spectral response was obtained directly from the affected area, a condition that cannot be guaranteed in commercial processing. These factors represent practical challenges that commonly arise when transferring machine-vision and optical sensing technologies from laboratory environments to industrial production lines.

Despite these limitations, HSI consistently identified higher average Hb abundance in impacted fish than in non-impacted controls, demonstrating sensitivity to process-related damage. However, high biological variability and optical uncertainty limited quantitative assessment of damage severity in individual fish. The results therefore indicate that HSI is better suited for classification, defect detection, and batch-level quality monitoring than for precise estimation of blood content in whole fish. From an industrial perspective, the technology shows promise as a non-destructive tool for automated quality assessment, although further advances in illumination, spectral data modelling, and sensor integration will be required to improve robustness under commercial processing conditions.

Conclusion

Physical signs of damage, including skin abrasion and muscle fiber separation, were clearly observed in all impacted groups, with severity increasing as impact force increased. Pronounced blood accumulation in the 2 kg and 4 kg groups was associated with rupture of major blood vessels and vertebral fractures. These findings indicate that, in current on-board production environments, collisions with hard surfaces or equipment edges following a fall of approximately 1.7 m can generate sufficient force to cause severe quality degradation in whole Atlantic cod. Lower impact forces, corresponding to approximately half the fish's own weight dropped from the same height, did not significantly increase Hb content in the fillet but still caused visible impact damage and resulted in vertebral fractures in 10% of the fish.

HSI using constrained spectral unmixing for Hb detected differences between impacted and non-impacted groups, but did not provide a clear quantitative relationship with

chemically determined Hb concentrations. These results indicate that HSI is better suited for classification and defect detection in whole fish than for precise quantification of hemoglobin abundance. The main limitations appear to be related to the optical properties of the skin, the geometry of whole gutted cod, and the inherent complexity of relating surface spectral measurements to blood abundance within deeper tissue layers. While the heuristic use of a polynomial to correct for light scattering and variable optical path lengths was insufficient, the higher average Hb abundance measured by HSI in impacted groups suggests that the method may have potential for batch-level detection of systematic process-related damage. In addition, comparing Hb abundance before and after impact and bleeding may provide objective information on blood retention during production.

Future research should focus on developing more accurate models for estimating Hb through the skin, particularly models capable of compensating for variation in absorption, scattering, and optical path length. Further work is also needed to address the effects of uneven fish geometry on light intensity and measurement accuracy. Such improvements may increase the sensitivity and robustness of HSI measurements and strengthen its use for classification, defect detection, and monitoring of process-related damage and bleeding efficiency in industrial whitefish production.

Materials and Methods

Sampling and experimental design

Samples of farmed Atlantic cod originated from a Norwegian Aquaculture company (Ode AS, Aalesund, Norway) and was slaughtered by a licensed commercial supplier (Hofseth

Aqua AS, Aalesund, Norway), in accordance with the Aquaculture act. The experimental fish were received after standard commercial euthanasia by electrical stunning (Stansas #1.1 110 V, SeaSide AS, Stranda, Norway) and gill cutting, performed by the commercial supplier during standard commercial operations. Euthanized experimental fish were then collected directly from the production line, manually gutted, and scanned using a Maritech Eye™ hyperspectral imaging system (Maritech AS, Molde, Norway) to measure the baseline characteristics.

After sampling, fish (gutted weight 2.0 ± 0.2 kg; fork length 58.9 ± 2.1 cm; $n=120$) were randomly assigned to four groups ($n = 30$ per group). Three groups were exposed within 5 min post-gutting to a guided falling plate released from 1.7 m to simulate process-related injuries (Figure 1). Plates of 1, 2, and 4 kg were used to create an impact severity gradient (Groups 1–3). The last group served as an unhandled control (Group 4). All impacts were delivered laterally just posterior to the pectoral fin to standardize impact location and imaging conditions.

Fish were then bled for 30 min in chilled seawater and subsequently rescanned using the HSI system to measure exposure-induced muscle damage. After rescanning, the fish were packaged in Styrofoam boxes and stored on ice in a cold room at 4°C for 6 days, resulting in a mean fish core temperature of -0.0 ± 0.3 °C. After storage, fish were filleted and evaluated by sensory assessment (Section 2.3.3). In addition, tissue from a standardized region was homogenized and stored at -28 °C for chemical analyses.

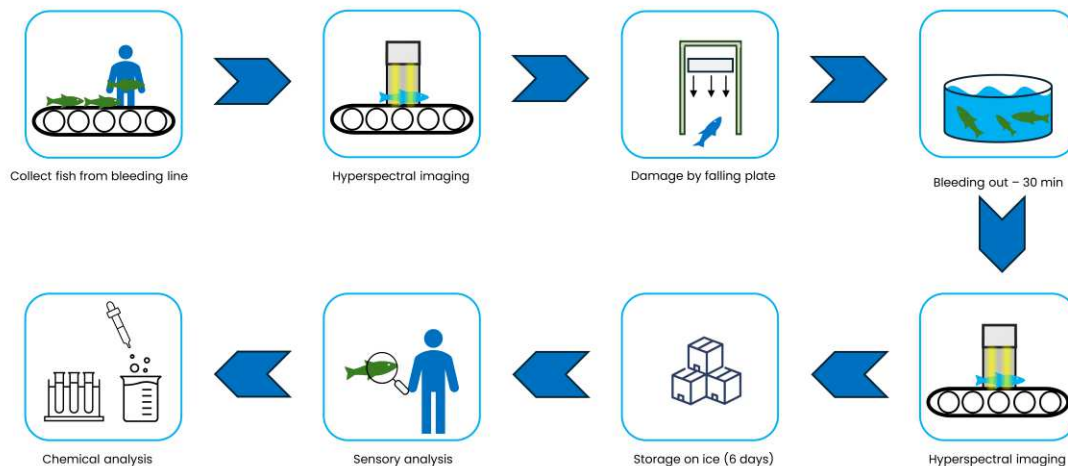


Figure 1. Experimental workflow conducted in this study

Impact damage simulation apparatus

To mimic impacts that may occur when fish collide with hard objects in on-board processing environments, a fixed drop apparatus was constructed to control drop height, force, and injury location. The apparatus comprised a steel frame with vertical guides that directed a rigid polytetrafluoroethylene (PTFE) plate onto the target region. The plate was 3 cm thick and had an estimated contact area with each fish of $\sim 30 \text{ cm}^2$.

The drop height was determined based on observations made aboard the Norwegian trawler M/Tr Kongsfjord (Finnmark Havfisk AS) during commercial operations in the Norwegian Sea. During the field experiment, a recurring vertical drop of approximately 1.7 m was documented in the bypass used for fish that were not successfully sorted in the grading tanks. Although higher drop heights were occasionally observed, these occurred

irregularly. Consequently, 1.7 m was selected as a representative and consistently occurring drop height for further analysis.

For the experiment, PTFE plate masses (1, 2, and 4 kg), which corresponded to approximately 50%, 100%, and 200% of the mean gutted weight of the sampled fish, were selected. This corresponded to nominal impact energies of 16.7 J (1 kg), 33.4 J (2 kg), and 66.7 J (4 kg).

Quality measurements

Hyperspectral image acquisition

Hyperspectral images were acquired using a commercially available Maritech Eye™ HSI system (Maritech AS, Molde, Norway). This system employs an interactance illumination geometry where two parallel light lines illuminate the sample, and the camera measures the diffusely scattered light emerging between the illumination lines. By adjusting the camera to sample distance, the focal point can be positioned within the fish musculature, enhancing sensitivity to subsurface features compared with conventional diffuse reflectance^{4,5}.

The Maritech Eye was fitted with a HySpex Baldur V-1024 N hyperspectral camera (Norsk Elektro Optikk AS, Oslo, Norway) providing spectral measurements from 493 to 945 nm across 88 bands at an approximate spectral resolution of 5.14 nm. Samples were transported on a conveyor at an industrially relevant speed of 50 cm s⁻¹, while the camera operated in push-broom mode, acquiring images line by line as the fish moved through the field of view.

Hyperspectral image processing

Hyperspectral image processing was conducted in the software Breeze (Prediktera AB, Umeå, Sweden). Prior to each sample set, a PTFE reference with high diffuse reflectance was measured. Mean reflectance from 100 lines of the PTFE reference was used to compute a reference frame, $Ra(\lambda, x)$. Sample absorbance was computed as $I(\lambda, x, y) = -\ln(Ri(\lambda, x, y)/Ra(\lambda, x))$, where $Ri(\lambda, x, y)$ is the raw sample image.

After calibration, images were segmented using the Segment Anything Model³¹ to remove the background. The head, caudal fin, and opened belly cavity were excluded by a fixed inset of the top, left, and bottom of the segmented images so that analyses were restricted to the ventral region containing the upper fillet musculature around the impact site. This also excluded exposed tissue in the belly cavity and neck area, preventing an artificially high hemoglobin response due to the absence of skin coverage. Hemoglobin abundance estimates were computed using constrained spectral unmixing following Skjelvareid, et al.¹⁶. The principle of linear unmixing models assumes that the measured spectrum is a sum of the component spectra of the different components within the sample. These component spectra are referred to as end-members, and their relative abundance in the sample can be estimated by finding the linear combination of their spectra which best fits the measured spectrum. For whole Atlantic cod the end-members used in the spectral unmixing model were hemoglobin (oxyHb, deoxyHb, and metHb), water, muscle tissue, and skin. The estimate of the relative abundance of each end-member was obtained using non-negative least squares regression, with a first-order polynomial included to account for scattering effects. It should be noted that the analysis

only finds the relative abundances between the end-members in the signal, which is not converted to the actual percentages of blood contained within the sample.

Since the samples were imaged shortly after being euthanized (>5 min), it was expected that hemoglobin would only exist in its deoxygenated and oxygenated form. A strong methemoglobin signal was, however, found running on the lateral side of the fish, which coincides with the position of the dark musculature of Atlantic cod. The dark musculature is located close to the skin and contains higher amounts of myoglobin than the white muscle tissue³². It was assumed that the signal of the methemoglobin end-member represented myoglobin from the dark muscle tissue and was therefore excluded in the final estimation of blood content in the fillet. Only the end-member signals from oxyHb and deoxyHb were used for blood content estimation.

Sensory evaluation

After 6 days of ice storage, fillets were evaluated by a panel of 5 trained assessors. Seven quality-related attributes were scored using a modified version of the Quality Index Method (QIM) for cod fillets³³. Attributes included texture, gaping, blood spots (skin side and internal side), skin damage, residual blood, and fillet damage. Each attribute was scored on a five-point scale, except for residual blood and blood spots on the fillet side, which were scored on a seven-point scale, as shown in Supplementary Table S.3. Instances of vertebral fractures were recorded for each fish.

Chemical determination of muscle hemoglobin

Tissue from the impact region was homogenized and total heme protein content was determined in triplicate as described by Chaijan and Undeland³⁴. The homogenate was

heated to 85 °C in a neutral phosphate buffer containing sodium dodecyl sulfate, centrifuged, and the absorbance of the supernatant was measured at 535 nm using a UV mini 1240 spectrophotometer (Shimadzu Corp., Kyoto, Japan). The total heme pigment amount was calculated using a standard curve made from bovine hemoglobin (Sigma-Aldrich, St. Louis, Missouri, USA).

Statistical analysis

All statistical analysis was conducted in the software SPSS 31.0.0.0 (IBM corp., New York, USA) except for Principal component analysis (PCA), where Aspen Unscrambler V12.1 (Aspen Technology Inc., Massachusetts, USA) was used. Sensory data were normalized to a scale of 1 to 5, and differences between groups were analyzed using non-parametric statistics (Kruskal-Wallis). Statistically significant variance between damaged groups and the control group for Hb-content and HSI predicted Hb-abundance was analyzed by one-way ANOVA with Tukey's post-hoc test. Equal variance was controlled for using Levene's test and normality of the residuals were checked by Shapiro-Wilk test for normality. Correlations between all variables were analyzed by the Pearson correlation coefficient. The alpha level for significance was set to $p < 0.05$ for all tests. All results are reported as mean $\pm$ standard deviation.

Sensory data was further analyzed by PCA on normalized data using the first two components.

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571 **Author Contributions**

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588 **Data availability**

589 Data used in this study is available upon reasonable request by contacting the author.

590 **Declarations**

591 The authors declare no competing interests.

592 **Ethical statement**

593 No live animal was involved in this study. All fish samples were provided by a
594 commercial supplier.

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