Park, J., Difford, G., Ahmad, A., Jacob, N., Ødegård, J., Dvergedal, H., Klemetsdal, G., Wold, J., Berg, P., Lintvedt, T., Afseth, N. (2026) Raman-based prediction of fillet fatty acid composition and genetic architecture in Atlantic salmon (*Salmo salar L.*), *Proceedings 13th World Congress on Genetics Applied to Livestock Production* 23705: https://doi.org/10.31274/wcgalp.23705 ©2026 Park, et al. CC BY-NC-ND

Raman-based prediction of fillet fatty acid composition and genetic architecture in Atlantic salmon (*Salmo salar L.*)

J. Park[1*], G.F. Difford[1], A. Ahmad[1], N. Jacob[1], J. Ødegård[3], H. Dvergedal[3], G. Klemetsdal[1], J.P. Wold[2], P. Berg[1], T.A. Lintvedt[2], and N.K. Afseth[2]

[1]*Faculty of Biosciences, Norwegian University of Life Sciences (NMBU), Ås, Norway*; [2]*The Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima), Ås, Norway*; [3]*AquaGen, Trondheim, Norway;* [*]*Corresponding author: jisoo.park@nmbu.no*

Introduction

Atlantic salmon (*Salmo salar L.*) is one of the most valuable aquaculture species in Norway and an important dietary source of omega-3 long-chain polyunsaturated fatty acids (n-3 LC PUFA), particularly eicosapentaenoic (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3). These fatty acids (FAs) provide well-documented health benefits for human consumers and play essential roles in fish physiology. Fillet lipid content and FA composition are heritable traits that influence flesh quality, nutritional value, and marketability. As the aquaculture industry moves toward precision and sustainable breeding, the availability of reliable and scalable phenotyping methods for fillet quality traits is becoming critical. Gas chromatography (GC) is the gold-standard method for quantifying FA composition. Although it is precise, it's expensive, time-consuming, and sample-destructive, limiting its use in large-scale measurements. Raman spectroscopy, on the other hand, can offer an alternative method to measure FA composition, with the advantages of being rapid, cost-effective, and non-destructive, for high-throughput phenotyping. Although Raman spectroscopy has been studied for general lipid profiling (Afseth *et al*., 2022) and its application to intact salmon fillets (Lintvedt *et al*., 2023), the estimation of genetic parameters based on Raman spectroscopy is not yet fully explored. The objective of this study was to evaluate the feasibility of using Raman spectroscopy to predict omega-3 FA contents of Atlantic salmon fillets. Secondly, we estimated the heritability, genetic correlations, and genomic architecture of Raman-predicted FA traits to assess their potential for integration into selective breeding programs.

Materials & Methods

Fish sample, Phenotypes, and Raman spectra. All 834 Atlantic salmon from 30 full-sib families from AquaGen AS (Trondheim, Norway) were reared under common environmental conditions from hatching until tagging, smoltification, and saltwater rearing. During this time, they were fed the same diets until they reached an average body weight of 1.3 kg. After slaughter, Raman spectra were acquired from all fillet samples using a MarqMetrix All-in-One Raman system (MarqMetrix Inc., Seattle, WA, USA) equipped with a 785nm laser. For preprocessing, a Raman shift range of 500-1800 cm^{-1} was selected, and Savitzky-Golay was applied for spectral smoothing. Asymmetric Least Squares and Extended Multiplicative Signal Correction were applied for baseline correction and normalization, respectively. A total of 51 samples were selected by using the Kennard-Stone method and analyzed using gas chromatography fatty acid methyl ester (GC-FAME) to quantify the FA composition. Five traits related to omega-3 FAs (expressed as % of total FAs) were selected and evaluated in terms of structural relationships: PUFA, the sum of EPA and DHA (EPA+DHA), EPA, DHA, α-linolenic acid (ALA; C18:3n-3).

Statistical model for Phenotypes and Genotypes. A Partial Least Squared Regression (PLSR) model was developed using statistical software R (R Core Team, 2024) and built for FA prediction using Raman as predictor and GC measurements as a reference in the training data set; Raman-predicted FA traits for all individuals were subsequently used as phenotypes for genetic analyses. The optimal number of components was selected based on the minimum value of the predicted residual error sum of squares using leave-one-out (LOO) cross-validation (CV). The prediction ability was evaluated using the coefficient of determination (R^2), root-mean-squared-error (RMSE) and mean-absolute-percentage-error (MAPE). All fish samples were genotyped using the axiom Affymetrix SNP Genotyping Array. Quality control (QC) was performed to retain SNPs with a call rate > 0.9, minor allele frequencies > 0.01, Hardy-Weinberg equilibrium p-value > 10^{-7} from an initial 62,557 SNPs for all individuals. After QC, 43,644 SNPs and 807 individuals were available to construct a genomic relationship matrix. Heritability and genetic parameters were estimated using DMU software (Madsen *et al*., 2014) and the following linear mixed model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \quad (1)$$

where y is a vector of the FA traits, **X** is the incidence matrix for fixed effects and **b** is the vector of the fixed effects including the effects of sex (2 levels; Female, Male), Tank information (8 levels), **Z** is the incidence matrix for random additive genetic effects and **u** is the vector of random additive genetic effects, and **e** is the vector of random residuals. The assumption of the random effects were: $\mathbf{u} \sim N(0, \mathbf{G}\sigma_g^2)$, $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$, where **I** is the identity matrix, **G** is the GRM, σ_g^2 is the additive genetic variance, and σ_e^2 is the residual variance. Genetic and phenotypic correlations (R_G and R_P, respectively) between FA traits were estimated using bivariate linear mixed models implemented in DMU following the same model as Eq (1). Genome-wide association studies (GWAS) for all FA traits were performed using the Leave One Chromosome Out function implemented in GCTA software (Yang *et al*., 2011). P-values were adjusted by Bonferroni correction, with significant at the genome level if -log10(P-value) was greater than -log10(0.05/n), where n represents the number of markers across the genome.

Results

Among the FAs, PUFA had the highest abundance (32.4%), of which EPA had the least (2.1%). The coefficient of variation (CoV) indicated overall low phenotypic variability across FA traits, below 10%, with PUFA showing the lowest variability and DHA showing the highest (CoV = 1.83 and 7.26, respectively). Across all FAs, the MAPE was less than 3%, and the R^2 was greater than 0.72 for all traits except ALA.

Table 1. Summary statistics and Raman calibration results on the fatty acid composition (proportional level; %) and Raman calibration performance of 51 selected fillet samples in Atlantic salmon

Trait	N	Min - Max	Mean	SD[1]	CoV (%)[2]	R^2 [3]	$RMSE_{cv}$[4]	MAPE[5]
PUFA	51	31.4 – 34.2	32.4	0.59	1.83	0.75	0.29	0.76
EPA + DHA	51	6.9 – 8.5	7.5	0.39	5.21	0.81	0.17	1.87
EPA	51	1.8 – 2.5	2.1	0.15	7.15	0.72	0.08	2.99
DHA	51	4.6 – 6.5	5.4	0.39	7.26	0.77	0.19	2.91
ALA	51	6.3 – 7.8	7.1	0.29	4.06	0.55	0.19	2.21

[1]SD: Standard Deviation, [2]CoV: Coefficient of Variation (SD/Mean × 100), [3]R^2: Coefficient of determination, [4]RMSEcv: Cross-validation of Root Mean Square Errors, [5]MAPE: Mean Absolute Percentage Error

Heritability estimates from Raman-predicted phenotypes varied among the traits, ranging from 0.07 ± 0.03 for EPA to 0.44 ± 0.06 for PUFA. Among major omega-3 individual FAs, DHA had the highest heritability (0.42 ± 0.07), followed by ALA (0.17 ± 0.04), and EPA (0.07 ± 0.03). Genetic correlations (R_G) between traits were generally high and positive, with significant correlations observed between EPA+DHA and DHA (R_G = 1.00) and EPA and DHA (R_G = 0.97). Phenotypic correlations (R_P) showed similar patterns but were lower in magnitude than the corresponding genetic correlations.

Table 2. Genetic parameter estimates of Raman-predicted fatty acid traits in Atlantic salmon fillet

Trait	PUFA	EPA + DHA	EPA	DHA	ALA
PUFA	**0.44 (0.06)**	0.88 (0.03)	0.77 (0.10)	0.91 (0.03)	0.50 (0.13)
EPA + DHA	0.80 (0.02)	**0.43 (0.07)**	0.98 (0.05)	**1.00 (0.00)**	0.03 (0.18)
EPA	0.64 (0.02)	0.56 (0.02)	**0.07 (0.03)**	0.97 (0.07)	-0.05 (0.28)
DHA	0.74 (0.02)	0.97 (0.01)	0.38 (0.03)	**0.42 (0.07)**	0.10 (0.18)
ALA	0.47 (0.03)	-0.12 (0.04)	0.32 (0.03)	-0.22 (0.04)	**0.17 (0.04)**

Note. heritability estimates (h^2) on diagonal, genetic correlations (R_G) in the upper diagonal, phenotypic correlations (R_P) in the lower diagonal, with standard errors (SE) in parentheses.

Discussion

This study demonstrates that Raman spectra acquired directly from Atlantic salmon fillets gain meaningful information related to omega-3 FA composition. Consistently low $RMSE_{CV}$ and MAPE values across the selected traits indicate that Raman-predicted estimates provide stable and informative phenotypic measures, supporting its feasibility for genetic analysis (Park *et al*., 2026). Heritability estimates derived from Raman-predicted phenotypes for EPA+DHA and DHA are moderate (0.44 and 0.42), suggesting the potential for genetic selection in breeding programs. In contrast, EPA showed a low heritability (0.07), similar to previous studies using GC (Horn *et al*., 2018). The strong genetic correlations among proportional omega-3 traits, especially between EPA+DHA and DHA (R_G = 1.00) and EPA and DHA (R_G = 0.97) highlights substantial shared genetic control of DHA proportions and may suggest that selection on DHA proportion is likely to influence overall EPA+DHA composition. In this study, no significant SNPs were detected in GWAS across all given traits, which may suggest that all given traits have too small-effect variants across genome. However, given the limited reference sample size and relatively less precision of Raman-based predicted phenotypes (e.g., ALA), this could be one possible reason for attenuating the GWAS signals.

Overall, our results suggest that Raman spectroscopy has powerful potential as a rapid and large-scale non-contact phenotyping tool within slaughter facilities and breeding programs, complementing GC-FAME analysis by reducing costs and increasing throughput. The combination of adequate prediction accuracy for omega-3 FAs and moderate heritability estimates further supports the feasibility of integrating Raman-based phenotyping into genetic improvement strategies targeting Atlantic salmon fillet quality. However, FA composition is highly affected by dietary composition and, in the case of Atlantic salmon, may vary depending on the two different life cycle stages: freshwater and saltwater. Further research should validate these results by using larger populations and different environments to evaluate the robustness and generalizability of Raman-derived predictions.

Conclusions

This study demonstrates that Raman spectroscopy provides a promising approach for phenotyping omega-3 FAs in Atlantic salmon fillet. The moderate but significant heritability estimates, and strong genetic correlations of the Raman-derived traits indicate that their potential value for integration into selective breeding programs for improving omega-3 content. Although no significant SNPs were identified in GWAS, these results show a polygenic genetic architecture of studied traits and suggest the need for larger cohorts to improve precision.

Acknowledgements

This work was supported by Norwegian Research Council with grant number [334821 (DigiFishent)], Foods of Norway, Center for Research-based Innovation (Research Council of Norway with grant number [23784/O30].

References

Afseth, N. K., Dankel, K., Andersen, P. V., *et al*. (2022). Foods, 11. https://doi.org/10.3390/foods11070962

Horn, S. S., Ruyter, B., Meuwissen, T. H. E., *et al*. (2018). Genet. Sel. Evol. 50. https://doi.org/10.1186/s12711-018-0394-x

Lintvedt, T. A., Andersen, P. V., Afseth, N. K., *et al*. (2023). Talanta, 254. https://doi.org/10.1016/j.talanta.2022.124113

Madsen, P., Jensen, J., and Sahana, G. (2014). https://www.researchgate.net/publication/268109868

Park, J., Difford, G. F., Horn, S. S., *et al*. (2026). Aquaculture, 614. https://doi.org/10.1016/j.aquaculture.2025.743578

R Core Team. (2024). https://www.R-project.org/

Yang, J., Lee, S. H., Goddard, M. E., *et al*. (2011). Am. J. Hum. Genet. 88, https://doi.org/10.1016/j.ajhg.2010.11.011