AUTHENTICATION OF DEPURATED SALMON FILLETS BY NEAR INFRARED SPECTROSCOPY

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Abstract –This study examined the potential of near infrared spectroscopy (NIRS) to discriminate depurated from non-depurated salmon fillets. Sixty two Atlantic salmon were cultured within a commercial scale land-based recirculating aquaculture system (RAS). When salmon reached food-size, twelve salmon were removed from the RAS (non-depurated salmon) and fifty were transferred to partial water reuse systems to be depurated of existing off-flavours for 10 days (depurated salmon). Skin-off fillets were collected from all salmon, vacuum packed and frozen at -20°C. After thawing, the right-side, anterior third of the fillet was scanned over the visible and NIR range (350-2500 nm) on the flesh of the dorsal muscle. Partial least squares discriminant analysis based on NIR spectra correctly classified 100% of non-depurated and depurated salmon fillets; probably as a consequence of differences in the content of fat, water and off-flavour compounds between both samples, which could have been detected by NIR spectra. Thus, NIRS is a fast, inexpensive, solvent-free and non-destructive technology that can be used for the authentication of salmon with enhanced quality for marketing purposes.

I. INTRODUCTION

Certain species of actinomycetes, fungi and blue-green algae produce semi-volatile off-flavour compounds that are the main cause of earthy-musty odorants in water from aquaculture facilities (1, 2, 3). Those off-flavour compounds tend to bio-accumulate within fish flesh dependent on the concentration of the compound in the water supply, water temperature, fat content and mass of fish, and other abiotic and biotic factors (4). Although off-flavour compounds are harmless to human health, high levels within fish tissue lead to an undesirable taste which is typically regarded as being of unmarketable quality for consumption (5, 6). Pre-harvest processes such as depuration can be used to eliminate most of those off-flavour compounds and ultimately enhance salmon quality (5).

To assure quality control and guarantee to consumers that they are getting exactly what they paid for and not an inferior quality fish, methods to distinguish depurated salmon from those that have not been subjected to any pre-harvest process are required. Different extraction methods coupled with gas chromatography–mass spectrometry have been used to quantify off-flavour compounds in salmon (5, 7). However, those techniques require reactive chemicals and sophisticated and expensive analytical equipments, and they are time-consuming and technically demanding. Near infrared spectroscopy (NIRS) is a sensitive, fast, low cost and non-destructive technology, with minimum or no sample preparation, neither requiring reagents nor producing waste (8, 9). Another advantage of NIRS is its ability to record the response of the molecular bonds of chemical constituents (e.g., O-H, N-H, and C-H bonds) to the near infrared irradiation and, thereby, build a characteristic spectrum that behaves as a "fingerprint" of the sample (10).

Therefore, the aim of this study was to test the potential of NIRS technology to distinguish depurated from non-depurated salmon fillets.

II. MATERIALS AND METHODS

A. Samples and depuration process

Atlantic salmon were cultured to food-size (3–5 kg) at the Conservation Fund's Freshwater Institute (Shepherdstown, WV, USA) within a commercial scale (150 m^3) land-based recirculating aquaculture system (RAS). Twelve salmon were removed from the 150 m^3 RAS (non-depurated salmon) and fifty were transferred to identical partial water reuse systems (0.5 m^3) that were used to depurate/purge the fish of existing off-flavours that might be present in the flesh (depurated salmon). Namely, those 50 salmon were taken off-feed one-day prior to transfer to the depuration system and then remained off-feed during 10 days of depuration process, as is the common practice for food-fish produced in RAS. The depuration system design was relatively simple, consisting of a circular culture tank with a bottom, center drain and a PVC water aeration column (1.52-m tall × 0.23-m diameter). Water aeration columns containing media were packed with 1.37-m of individual 5-cm NSW Nor-Pac rings (Jaeger Environmental, Eldorado, KS, USA). A 1/8-hp magnetic pump (Model MD-55RLT, Iwaki Co. Ltd, Tokyo, Japan) was used to pump approximately 90 L/min of water from mid-depth of the culture tank and lift it to the top of the corresponding aeration column. Depuration system was operated with an average makeup water flow rate of 3.8 ± 0.1 L/min (approximately 1 gpm) and thus a 95% recycle rate on a flow basis. Skin-off fillets were collected from all salmon, vacuum sealed in individual plastic bags, and immediately frozen at -20°C until spectra collection.

B. Spectra collection

The salmon fillets were thawed overnight at 2°C. The right-side, anterior third of the fillet was collected from each fish and randomly scanned on the intact flesh of the dorsal muscle using a portable LabSpec®4 Standard-Res spectrometer (Analytical Spectral Device-ASD Inc., Boulder, CO, USA) equipped with an ASD fibre-optic high intensity contact probe (21 mm window diameter) (Figure 1). The spectrometer scanned 50 times per reading (~5 s) over the visible and NIR range (350-2500 nm) in reflectance mode, and spectra were averaged by the equipment software. The data were interpolated to produce measurements in 1 nm steps, resulting in a diffuse reflectance spectrum of 2151 data points. Absorbance data were stored as log (1/*R*), where *R* was the energy reflected. Two spectra per salmon fillet were collected in different locations to increase the area of muscle scanned and reduce the sampling error (10), visually examined for consistency and then averaged. Instrument control and initial spectral manipulation were performed with the Indico™ Pro software package (Analytical Spectral Device-ASD Inc., Boulder, CO, USA).

C. Statistical analysis

Principal component analysis (PCA) was performed to decompose and compress the data matrix in order to examine the possible grouping of samples. Partial least squares discriminant analysis (PLS2-DA; 11) was applied to classify salmon fillets into non-depurated and depurated. This model seeks to correlate spectral variations (X) with defined classes (Y), attempting to maximize the covariance between the two types of variables for group differences and ignoring variance within a class. In this type of approach, Y is a dummy matrix with arbitrary numbers assigned to the different classes to be distinguished (non-depurated = 1, depurated = 2). According to this equation, a sample was classified as salmon belonging to a specific category (non-depurated or depurated) if the predicted value was within ±0.5 of the dummy value. The accuracy of the models obtained was evaluated using the percentage of correctly classified samples. Cross-validation (leave one-out) was performed to validate calibrations and to restrict the number of PLS terms incorporated in the regression, to prevent over-fitting. Spectral data management, PCA and PLS2-DA were performed by means of The Unscrambler® software (version 10.2, Camo, Trondheim, Norway).

III. RESULTS AND DISCUSSION

A. Spectral information

As observed in Figure 1, the mean spectra of non-depurated and depurated salmon fillets showed the same pattern although clear differences were observed in the near infrared region. The main differences were found at wavelengths of 1450 (O–H stretch first overtone) and 1940 nm (O–H

bend second overtone), and at 1215 (C–H stretch second overtone), 1720-1760 (C–H stretch first overtone) and 2310 nm (C–H bend second overtone). Since those O–H and C–H molecular bonds are related to water and fat absorption, respectively (8, 12), differences in those chemical components could be used to discriminate depurated from non-depurated salmon fillets. On the contrary, no differences were observed in the visible region (400-700 nm) between the spectra of non-depurated and depurated salmon fillets.

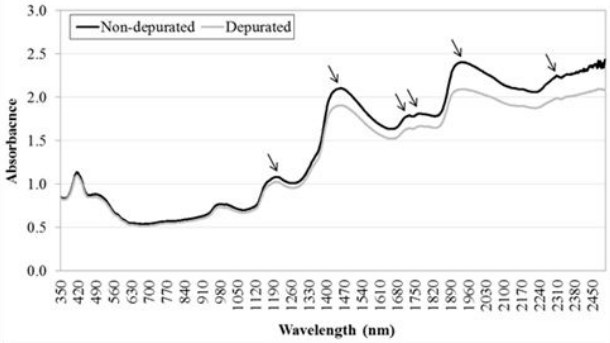

Fig. 1. Average visible and near infrared reflectance spectra of non-depurated (n = 12) and depurated (n = 50) salmon fillets.

B. Discrimination of salmon fillets

During PCA, the raw absorbance data matrix was reduced to a coordinate axis system, so each sample was defined by the corresponding scores for each PC. As a result, when the whole sample set was represented on an XY plane according to the scores for PC1 and PC2, these first two PCs accounted for 98% of the variation in the spectra of salmon fillets and two different clusters (1 = non-depurated, 2 = depurated) were observed (Figure 2).

The regression model developed using a PLS2-DA and including 3 PLS terms correctly classified 100% of both non-depurated and depurated salmon fillets; all predicted values for non-depurated samples were within the range from 0.5 to 1.5 and those for depurated samples were higher than 1.5 and lower than 2.5. Similar results were observed when the calibration model was cross-validated where again all depurated salmon fillets were segregated from those non-depurated with an overall accuracy of 100%.

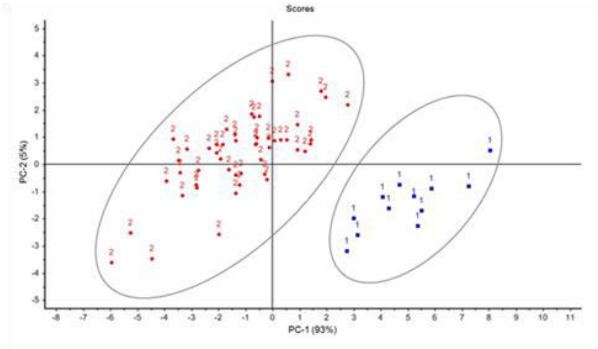

Fig. 2. Score plot for principal component 1 and 2 of salmon fillets based on near infrared spectra (1 = non-depurated, 2 = depurated).

Because salmon remained off-feed during the depuration period, possible differences in the fat content between non-depurated and depurated salmon fillets might have been responsible for the different absorption peaks of the C–H and O–H bonds in both samples (Figure 1); the latter as a consequence of the inverse relationship between fat and water content in fish muscle (13). In this study, the fat content of the samples was not analysed. Nevertheless, a lipid content decrease in depurated salmon samples has been previously reported by Burr et al. (5). Hence, the different fat and water content could be one reason for NIRS to successfully discriminate depurated salmon fillets. As fat content decreases during the depuration process, the off-flavour compounds accumulated in this tissue are also reduced. Indeed, significant differences in the content of some off-flavours compounds between the non-depurated and depurated salmon fillets from this study were previously described (7). Because off-flavour compounds are organic substances, they may have absorbance in the near infrared region. Therefore, the different content of those compounds in both salmon fillets could have been detected by NIR spectra; hence providing the excellent segregation of depurated from non-depurated salmon fillets found in this study.

IV. CONCLUSION

NIRS technology discriminated depurated from non-depurated Atlantic salmon fillets with accuracy up to 100% in the population used in this study. The work reported here constitutes a feasibility study and requires further development

with considerably more salmon fillets of different species before its potential may be implemented by the salmon industry. Further studies are needed to test NIRS technology for on-line applications in the salmon industry, where portable equipment applied directly on the whole salmon through the skin could be used to authenticate salmon with enhanced quality for marketing purposes.

ACKNOWLEDGEMENTS

Financial support from the NRC-CHRC Industrial Research Assistance Program is gratefully acknowledged. Dr. N. Prieto thanks the Alberta Crop Industry Development Fund (ACIDF) for funding support. The Conservation Fund Freshwater Institute's research on the kinetics of off-flavour depuration in Atlantic salmon was supported by the Atlantic Salmon Federation (St. Andrews, New Brunswick, Canada), the Gordon and Betty Moore Foundation (Palo Alto, California), and the USDA Agricultural Research Service under Agreement No.59-1930-5-510. All experimental protocols and methods were in compliance with the Animal Welfare Act (9CFR) requirements and were approved by the Freshwater Institute's Institutional Animal Care and Use Committee.

REFERENCES

1. Auffret, M., Pilote, A., Proulx, É., Proulx, D., Vandenberg, G. & Villemur, R. (2011). Establishment of a real-time PCR method for quantification of geosmin-producing Streptomyces spp. in recirculating aquaculture systems. Water Research, 45(20): 6753-6762.

2. Tucker, C. S. (2000). Off-Flavor Problems in Aquaculture. Reviews in Fisheries Science, 8(1): 45-88.

3. Smith, J. L., Boyer, G. L. & Zimba, P. V. (2008). A review of cyanobacterial odorous and bioactive metabolites: Impacts and management alternatives in aquaculture. Aquaculture, 280(1): 5-20.

4. Kankaanpää, H.T., Holliday, J., Schröder, H., Goddard, T. J., von Fister, R. & Carmichael, W. W. (2005). Cyanobacteria and prawn farming in northern New South Wales, Australia—A case study on cyanobacteria diversity and hepatotoxin bioaccumulation. Toxicology and Applied Pharmacology, 203(3): 243-256.

5. Burr, G. S., Wolters, W. R., Schrader, K. K. & Summerfelt, S.T. (2012). Impact of depuration of earthy-musty off-flavors on fillet quality of Atlantic salmon, Salmo salar, cultured in a recirculating aquaculture system. Aquacultural Engineering, 50: 28-36.

6. Robertson, R. F., Jauncey, K., Beveridge, M. C. M. & Lawton, L. A. (2005). Depuration rates and the sensory threshold concentration of geosmin responsible for earthy-musty taint in rainbow trout, Onchorhynchus mykiss. Aquaculture, 245(1): 89-99.

7. Ruan, E. D., Aalhus, J. L., Summerfelt, S. T., Davidson, J., Swift, B. & Juárez, M. (2013). Determination of off-flavor compounds, 2-methylisoborneol and geosmin, in salmon fillets using stir bar sorptive extraction–thermal desorption coupled with gas chromatography–mass spectrometry. Journal of Chromatography A, 1321: 133-136.

8. Osborne, B. G., Fearn, T. & Hindle, P. H. (1993). Near infrared spectroscopy in food analysis. Harlow: Longman Scientific and Technical.

9. Prieto, N., Roehe, R., Lavín, P., Batten, G. & Andrés, S. (2009). Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. Meat Science, 83: 175-186.

10. Downey, G. & Hildrum, K. I. (2004). Analysis of Meats. In L. Al-Amoodi, R. Craig, J. Workman, & J. Reeves III, Near-infrared spectroscopy in agriculture (pp 599-632). Winconsin: American Society of Agronomy Inc., Crop Science Society of America Inc., Soil Science Society of America Inc.

11. Naes, T., Isaksson, T., Fearn, T. & Davies, T. (2002). A user-friendly guide to multivariate calibration and classification. Chichester: NIR Publications, 420 pp.

12. Williams, P. C. & Norris, K. H. (eds.) (1987). Near-infrared technology in the agricultural and food industries. St. Paul: American Association of Cereal Chemists.

13. Sargent, J., Henderson, R. J. & Tocher, D. R. (1989). Lipids. In J. E. Halver, Fish Nutrition (2nd ed., pp 153-218). London: Academic Press.