Portable visible/near infrared spectroscopy for assessing flesh quality of aquaculture produce

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Aquaculture can deliver seafood of high and consistent quality in response to market demand. As quality is a key determinant of processing returns and market pricing, the ability to assess flesh properties rapidly and objectively would provide a great benefit to the industry. This is not only important at point of harvest but during the entire process chain, to understand and be able to monitor the effects of harvesting and processing treatments on flesh characteristics. Efficient methods for measuring quality traits are also essential if improving or monitoring product quality traits is to be included within the objectives of selective breeding programmes.

This study assesses the potential for portable visible/near infrared (VIS/NIR) reflectance spectroscopy to measure quality traits of two aquacultured species. First, it was used to measure the pigment (astaxanthin) and fat levels in Atlantic salmon (*Salmo salar*) from the Salmon Enterprises of Tasmania (SALTAS) selective breeding programme. These components are considered important contributors to the quality and marketability of salmon. Second, NIR spectroscopy was tested on meat from cultured abalone (hybrids from crossing *Haliotis laevigata* and *H. rubra*) to assess whether it was capable of discriminating product according to freezing protocol.

Materials and methods

VIS/NIR spectroscopy was performed with a LabSpec 5000 (350–2500 nm) fitted with an A122300 contact probe (ASD Inc., Boulder, CO, USA) and data were analysed using Grams/AI software (Thermo Fisher Scientific, Waltham, MA, USA). Partial least squares (PLS-1) regression was used for calibration and validation in salmon samples, and cross-validation was used for

evaluation of models. One-fifth of the samples were set aside for validation. Principal component analysis (PCA) was used to discriminate abalone samples, based on NIR spectra.

For salmon, ≈300 fish (1–6 kg) from a Tasmanian marine farm were sampled on six occasions during August and October 2007, May and July 2008, and, March and April 2009. Fish were killed using rested harvest techniques, either by anaesthesia with 17 ppm Aqui-S (Aqui_S NZ Ltd, Lower Hutt, New Zealand) or following standard commercial harvest using the SI5 flowthrough system (Seafood Innovations, Sheldon, QLD, Australia). Once stunned, fish were bled by cutting the isthmus (ventral aorta), then stored in ice for 1–3 days. A standard fillet, i.e. "Norwegian Quality Cut" or NQC,[1] the region from immediately behind the dorsal fin to the anus, was removed from the right side of each fish. NIR spectroscopy scans were undertaken by placing the probe on the flesh of the NQC from six regions (Figure 1) and the reflectance spectra were averaged.

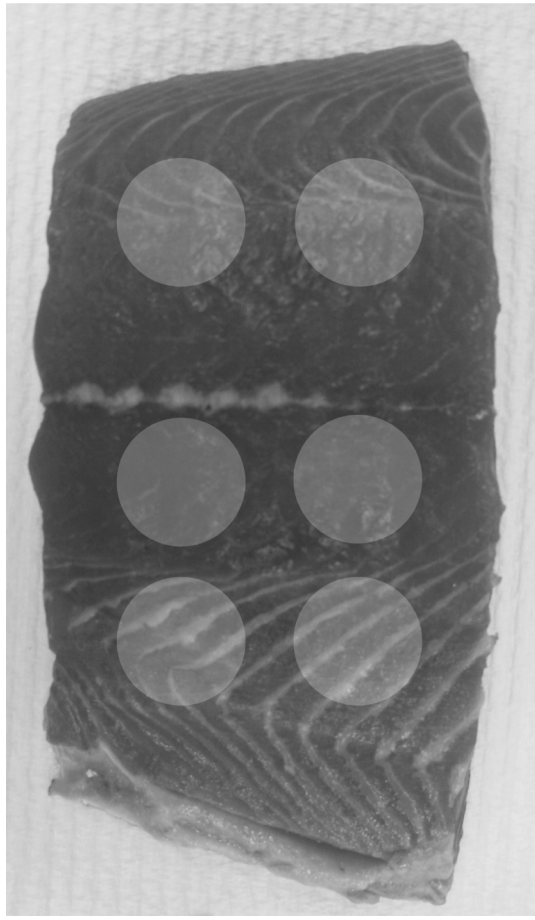

Figure 1. Positions scanned on the salmon NQCs.

The NQCs were then stored at –20°C for 1–4 weeks. Samples were thawed at 4°C, and the flesh was minced with a food processor. Subsamples (two per fish; spectra averaged) were spread into 4 cm diameter × 1.5 cm deep wells of a silicone tray, then scanned. Subsamples (5 g) were also stored frozen prior to chemical analysis (≤ 4 weeks). However, as these took up significantly less space than the NQCs, it was practical to store the subsamples at –80°C to minimise the likelihood of any sample deterioration. Astaxanthin was estimated spectrophotometrically, after extraction with acetone, and measurement of absorbance at 475 nm.[2] Fat was estimated gravimetrically after sample extraction.[3] Reproducibility (co-efficient of variation) of the chemical assays were ± 1.7% and ± 1.5%, respectively.

For abalone, 36 hybrid animals (*Haliotis laevigata* × *H. rubra;* 101 ± 14 g weight) from a Tasmanian abalone farm were placed within a sealed plastic bag containing 100% oxygen and chill-packs, and then within a styrofoam box. They were air-transported to Brisbane, and analysed 6 h after initial harvesting. Twelve individuals were shucked immediately, the foot of each was scanned by NIR spectroscopy in two positions (0.5 cm from the head and rear) and spectra averaged. The remaining abalone samples were frozen by one of two methods. One group of 12 abalone were frozen by immersion for 20 mins in a brine (23% salt)/ice mixture (–21.5°C), then transferred to a –21°C freezer. The other group of 12 abalone was air-frozen (–21°C freezer). Samples were stored for one week, thawed overnight at 4°C, then scanned. Half of the thawed abalone were cooked by steaming (80°C; 30 min), then after cooling, rescanned. All meat samples were between 10°C and 12°C when scanned.

Results and discussion

VIS/NIR spectroscopy models were developed for fat and astaxanthin prediction in salmon (Table 1). Samples were collected over two years to provide as much natural temporal variation in the data; thus providing models enabling prediction throughout the season. Because of the processing time

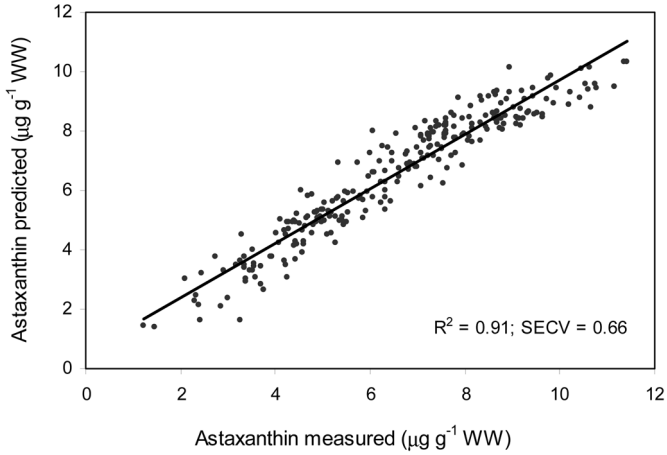

Figure 2. Measured versus VIS/NIR spectroscopy-predicted concentrations of astaxanthin in salmon NQCs from calibration sample set.

mincing samples was not amenable for rapid throughput, and scans were made on six positions of intact NQCs (Figure 1). Preliminary modelling of spectra from NQCs compared models based on individual scan regions, and various combinations of spectra averaged from all regions (Figure 1); however averaging scans from all regions gave the best results (Figure 2 and Table 1).

Data pretreatment using either multiplicative scatter correction (MSC) or standard normal variate with detrending (SNVd) significantly improved the calibration models based on r^2 and *SECV* values. The astaxanthin model had better predictability than the fat model; presumably because astaxanthin is more evenly distributed than fat, which is in higher concentrations at the ventral portion (i.e. near belly-flap) of the NQC. The current focus of our studies is to assess whether we can apply our portable system to develop similar models from non-invasive scanning of live-anaesthetised fish (enabling individual selection of quality trait measured fish); such prediction in salmon has recently been achieved from another study, albeit using a prototype commercial system.[1]

Table 1. Models (PLS-1) developed for salmon. Laboratory data from the reference samples are in parentheses. Spectra were mean centred (700–1700 nm for fat; 350–2500 nm for astaxanthin).

Component/sample type	Calibration					Validation	
	n	Data pretreatment	PC	R^2	*SECV*	R^2	*RMSEP*
% Fat (9.5 ± 2.7 % WW; range 2.3 to 15.3% WW)							
—minced	191 (3)	SNVd; 2nd deriv. (SG 31)	8	0.95	0.58	0.92	0.72
—intact NQC	216 (1)	SNVd; 1st deriv. (SG 25)	7	0.86	0.98	0.86	1.0
Astaxanthin (7.2 ± 2.1 μg g^{-1} WW; range 1.2 to 11.7 μg g^{-1} WW)							
—minced	170 (1)	MSC; 2nd deriv. (Gap 15)	8	0.95	0.53	0.94	0.56
—intact NQC	260 (1)	MSC; 1st deriv. (Gap 25)	6	0.91	0.66	0.88	0.70

n = no. of samples (outliers removed). PC = principal components; R^2 = coefficient of determination; *SECV* = standard error of cross-validation; SNVd = standard normal variate and detrending correction; MSC = multiplicative scatter correction; *RMSEP* = root means square error of prediction; SG = Savitzky–Golay algorithm.

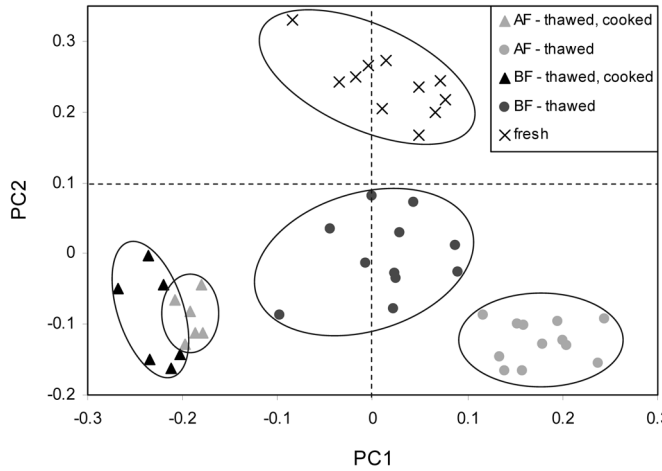

Figure 3. PCA scores for PC1 and PC2 for NIR spectroscopy of abalone meat. Data treatment: Mean centring, multiplicative scatter correction, 1st derivative (SG 15), 900–1800 nm. Explained variance: PC1 49%; PC2 41%; PC3–4 8%. AF = air-frozen; BF = brine/ice frozen.

The two freezing methods tested here on abalone are among several used by the aquaculture industry; another (not tested here) is using liquid nitrogen vapour. Anecdotal information is that there are sensory differences of product according to the freezing protocol, though this has not been rigorously tested. PCA demonstrated that NIR spectroscopy could discriminate between fresh abalone meat, frozen-thawed abalone and the latter product after cooking (Figure 3).

Although the sample populations were small, there was clear discrimination between frozen-thawed product according to freezing method.

Acknowledgments

We thank SALTAS, Tassal Operations Pty Ltd and Huon Aquaculture Group Pty Ltd for support of this study. Peter Mansour, Anna Do and Grant West also provided technical assistance.

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