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Short communication

Predicting glycogen concentration in the foot muscle of abalone using near infrared reflectance spectroscopy (NIRS)

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ABSTRACT

Near infrared reflectance spectroscopy (NIRS) was used to predict glycogen concentrations in the foot muscle of cultured abalone. NIR spectra of live, shucked and freeze-dried abalones were modelled against chemically measured glycogen data (range: 0.77–40.9% of dry weight (DW)) using partial least squares (PLS) regression. The calibration models were then used to predict glycogen concentrations of test abalone samples and model robustness was assessed from coefficient of determination of the validation (R_2^{val}) and standard error of prediction (SEP) values. The model for freeze-dried abalone gave the best prediction (R_2^{val} 0.97, SEP = 1.71), making it suitable for quantifying glycogen. Models for live and shucked abalones had R_2^{val} of 0.86 and 0.90, and SEP of 3.46 and 3.07 respectively, making them suitable for producing estimations of glycogen concentration. As glycogen is a taste-active component associated with palatability in abalone, this study demonstrated the potential of NIRS as a rapid method to monitor the factors associated with abalone quality.

1. Introduction

The natural variability in abalone meat quality means that the abalone industry, like other seafood and meat industries, struggles to market a consistent and controlled product to the consumer. There have been a number of studies conducted, predominantly in south east Asia, indicating seasonal variation of taste-active components and textural properties in the foot of abalones (Webber, 1970; Watanabe, Yamanaka, & Yamakawa, 1992; Hatae et al., 1995; Chiou, Lai, & Shiau, 2001). However, the harvesting of these marine gastropods continues year round with little attention given to variability in meat quality (Fleming, Van Barneveld, & Hone, 1996). Having a better understanding of the underlying factors that influence variability in meat quality could provide the industry with the knowledge required to make improved harvesting and processing decisions, particularly if equipped with a fast, instantaneous and non-destructive technique that assesses quality.

Traditionally, measuring food quality parameters is complex, time consuming and costly, relying on laborious chemical analysis and subjective sensory panel assessments (Cozzolino, Murray, & Scaife, 2002). The developing technology of near infrared reflectance spectroscopy (NIRS) has attracted significant attention over the past 30 years as an analytical tool in the agricultural and food industries (Prieto, Roehe, Lavin, Batten, & Andres, 2009). NIRS has shown potential as a rapid, non-destructive method for measuring food quality parameters (Givens, De Boever, & Deaville, 1997). For example, in the seafood industry, the Norwegians have led the way in using NIRS to measure quality traits in Atlantic salmon. Their studies initially began with the determination of fat and moisture in salmon fillets (Isaksson, Togersen, Iversen, & Hildrum, 1995), and have since successfully progressed to the non-invasive measurement of meat quality traits in live salmon (Folkestad et al., 2008).

For abalone, glycogen in the foot muscle is a major energy store that is depleted during the metabolically expensive period of gonad growth, and also during chronic stress, such as starvation and disease (Webber & Giese, 1969; Webber, 1970; Hayashi, 1983; Braid et al., 2005). Glycogen concentration varies significantly and can account for up to 40% of the dry weight of the abalone foot (Hayashi, 1983; Watanabe et al., 1992; Braid et al., 2005). Critical to the issue of quality, glycogen is a taste-active component in abalone meat. Abalones with little glycogen in the muscle tissue during spawning season are reportedly watery and unpalatable (Watanabe et al., 1992). These findings are supported by sensory studies conducted on scallop muscle which found that glycogen, although tasteless on its own, enhanced the meat's overall fullness, complexity and palatability (Konosu, Watanabe, Koriyama, Shirai, & Yamaguchi, 1987). Few examples of glycogen concentration determination by NIRS exist in the literature, with research limited in earlier years to the brewing industry, but more recently also extending into beef meat quality studies (Mochaba, Torline, &

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Axcell, 1994; Rosenvold et al., 2009; Lomiwes, Reis, Wiklund, Young, & North, 2010). The objective of this study was to evaluate NIRS as a potential tool for measuring meat quality parameters in abalone, comparing both destructive and non-invasive methods. To our knowledge this is the first study investigating the application of NIRS for measuring glycogen as a parameter of quality in abalone meat.

2. Materials and methods

2.1. Samples and sample preparation

Cultured hybrid abalones (*Haliotis laevigata* × *H. rubra*) with an average weight and shell length of 99 ± 16.2 g and 90.5 ± 3.9 mm, respectively, were sampled monthly (n = 15) from April to December 2009, giving a total sample size of 135 abalones. Abalones were collected live from a farm 60 km southeast of Hobart (Tasmania, Australia), transported in plastic lined polystyrene boxes to the CSIRO Laboratories in Hobart and processed within 4 h. Excess moisture on the live abalone was removed using a paper towel, and NIRS scans were taken by placing the contact probe in the middle of the foot. Abalones were then shucked, the viscera removed, a spatula was used to gently scrape the biofilm from the surface of the foot and the foot was blotted to remove excess moisture. A second NIRS scan was then taken from the middle of the foot. The abalone foot was cut in half longitudinally, snap frozen using liquid nitrogen, transferred to a freeze drier (Labconco, FreeZone 6, Kansas City, MO, USA) and dried under vacuum over 72 h. Dried samples were ground to a fine powder using a domestic grater prior to further NIRS analysis and chemical glycogen analysis.

2.2. Near infrared reflectance spectroscopy (NIRS) measurement

The reflectance spectra of the abalones were collected using an Analytical Spectral Device (ASD Inc., Boulder, CO, USA) LabSpec 5000 analyser. For the measurements of the live and shucked abalones the analyser was fitted with a 20 mm ASD High Intensity Contact Probe and configured with Indico Pro Data Software. For the scanning of the compacted freeze-dried powder the analyser was fitted with a smaller 3 mm ASD Pro Reflectance Probe. Approximately 150 mg of each powdered sample was packed into a 15 mm wide partition of a 24 well polystyrene microplate. The probe was positioned in direct contact with the compacted powder and scanned three times over a wavelength range of 350–2500 nm at 2 nm intervals. Raw spectral files were imported into GRAMS/AI Spectroscopy Software Suite (Thermo Fisher Scientific, Walthan, MA, USA) for further analysis and calibration. The spectra of 107 samples were used to develop the live and shucked calibration models. The freeze-dried calibration model was developed using the spectra of 78 samples. To test the calibrations, 27 randomly selected samples that had not been included in the original models were used as a validation set.

2.3. Data analysis

Partial Least Squares (PLS) calibration models with full cross validation were developed for each form of abalone (live, shucked and freeze-dried). To obtain the most robust calibrations, various mathematical and spectral pre-treatments (e.g. Standard Normal Variate (SNV), Multiplicative Signal Correction (MSC) and Savitzky Golay Smoothing (SG)) offered by GRAMS/AI were tested.

The statistics calculated for assessing the robustness of the calibration models included the coefficient of determination of calibration (R^2_{cal}) and the standard error of cross validation (SECV). SECV is the standard deviation of differences of the residuals between the NIRS and chemically determined glycogen concentrations (Williams, 2008), and has been reported to be the best estimate for the prediction capability of calibrations (Shenk & Westerhaus, 1996). The best calibrations were selected based on the lowest SECV and by the highest R^2_{cal}. For validation of the coefficient of determination of validation (R^2_{val}), the standard error of prediction (SEP) and the residual predictive deviation (RPD) were used to test the prediction accuracy of the models (Williams, 2001). The RPD is defined as the standard deviation (SD) of the chemical method divided by the SEP.

2.4. Glycogen concentration

Glycogen concentration of the abalone samples was determined using a protocol adapted from Burton, MacKenzie, Davidson, and MacNair (1997) and Braid et al. (2005), whereby amyloglucosidase is used to convert glycogen to glucose for measurement by colorimetry. Powdered tissue (50 mg) from each of the 135 abalones was analysed.

3. Results and discussion

3.1. Glycogen concentration

The glycogen concentration of the abalone muscle tissue was highly variable, accounting for 0.77–40.9% of the dry weight, with a mean ± SD of 23.3 ± 8.8%. The standard error of the chemical analysis was 0.76%. Such a large variation in the calibration set is considered useful when developing robust NIRS models, as the calibration curve is more likely to encompass future samples, allowing the interpolation instead of the extrapolation of unknown samples (Shenk & Westerhaus, 1996; Tsuchikawa, 2007).

3.2. NIR calibration of glycogen prediction model

The glycogen concentrations, determined through chemical analysis, were modelled against the associated spectra to develop calibration models for predicting glycogen. Best models were obtained utilising the wavelength range 800–2000 nm, where spectral pre-processing included mean centering, first derivative conversion (SG, 25 points) and SNV transformation either with detrending (live and shucked abalones), or without (freeze-dried abalone). Freeze-dried abalone gave the best calibration model for glycogen concentration with an R^2_{cal} of 0.98 and a SECV of 1.16% (Table 1). The live and shucked abalones had R^2_{cal} of 0.77 and 0.82, and SECV of 4.24% and 3.69%, respectively. As the freeze-dried samples were more homogenous than the live or shucked abalone, and the spectra were not overshadowed by peaks associated with water, it was not unexpected that the freeze-dried calibration model was more robust.

3.3. NIR validation of glycogen prediction model

The calibration models were validated by predicting the glycogen concentration of a subset of samples (n = 27) not used to build the original calibration models (Fig. 1). The results of the validations support the strength of the freeze-dried calibration model with an R^2_{val} value of 0.97, SEP of 1.71 and RPD of 5.43 (Table 1). As Shenk and Weterhaus (1996) state, calibration models with R^2 values greater than 0.90 are highly suitable for providing quantitative information about unknown samples. According to Williams (2008), a RPD value between 5 and 6.4 is indicative of efficient NIR reflectance predictions and is considered excellent in the analysis and quality control of feeds. The results of the validation for live and shucked samples had R^2_{val} values of 0.86 and 0.90, SEP of

Table 1
Data pre-treatment and statistics of the calibration and prediction models developed for predicting glycogen concentration in live, shucked and freeze dried abalone.

	Calibration				Validation		
	No. PLS factors	Outliers	SECV (%) [a]	R^2_{cal} [b]	SEP [c]	R^2_{val} [d]	RPD (%) [e]
Live	10	3	4.24	0.77	3.46	0.86	2.69
Shucked	10	2	3.69	0.82	3.07	0.90	3.03
Freeze-dried	4	3	1.16	0.98	1.71	0.97	5.43

[a] Standard error of cross validation.
[b] Coefficient of determination of calibration.
[c] Standard error of prediction.
[d] Coefficient of determination of prediction.
[e] Ratio of standard error of prediction validation to standard deviation.

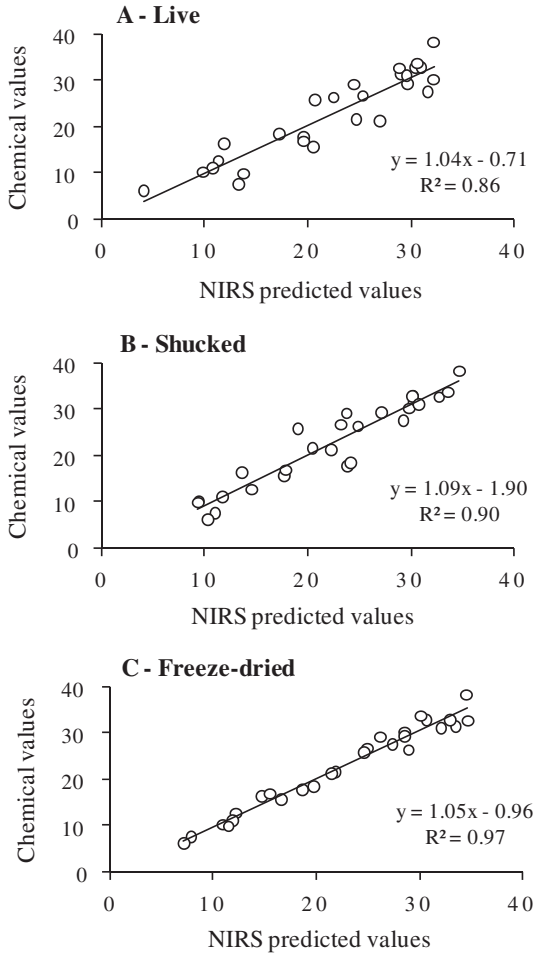

Fig. 1. Validation of NIRS predicted versus chemically determined values of glycogen concentration (% DW) in (A) Live, (B) Shucked and (C) Freeze-dried abalone (R^2= coefficient of validation).

3.46 and 3.07 and RPD values of 2.68 and 3.03, respectively. Based on these statistics, these models may prove useful in estimating glycogen concentrations and be applied for screening purposes (Shenk & Westerhaus, 1996; Williams, 2008).

3.4. Potential application of the NIRS models

The NIRS model for freeze-dried abalone may be used for accurate quantitative applications. Getting the abalones to this form still requires some effort and involves the destructive sampling of the abalone; however, being able to predict glycogen concentration through NIRS as opposed to the described chemical method has considerable benefits in terms of laboratory time and expense saved. Scanning a sample with the NIRS probe takes less than a minute, whilst the chemical analysis of glycogen requires multiple steps and the overnight incubation of samples.

NIRS and the associated models developed for live and shucked abalones could be applied as non-destructive screening tools, for estimating the glycogen concentration. As glycogen concentrations are linked to sensory properties and the physiological status of abalones, NIRS may, thereby assist in breeding, harvesting and processing decisions, for both the farmed and wild fishery industries. For example, NIRS could be used to determine which abalones are best suited to live transport, freezing, canning or drying based on estimated glycogen content in the foot muscle. As an extension of this study, CSIRO's abalone breeding programme is currently applying these models for predicting glycogen, to gain an insight into the physiological condition and quality, and hence to inform breeding decisions for selecting broodstock. Given the promising results reported herein further work should be carried out to develop similar NIRS calibration models for predicting additional components in the abalone foot, such as protein, fat and collagen.

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