Research Travel Grant:

To Attend Workshops and NIR 2009 Conference

Malcolm Brown,

CSIRO Marine and Atmospheric Research, and

Food Futures Flagship, Hobart, Tasmania, Australia

Email: malcolm.brown@csiro.au

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PRINCIPAL INVESTIGATOR: Malcolm Brown

ADDRESS: CSIRO Marine and Atmospheric Research, and Food Futures Flagship,
GPO Box 1538 Hobart, Tas 7001, Australia

OBJECTIVES OF RESEARCH TRAVEL GRANT:

- To develop expertise in Visible-Near InfraRed Spectroscopy (VNIRS) to apply in current and future Seafood CRC projects assessing and discriminating seafood quality.
- To establish scientific networks with VNIRS expertise, as potential collaborators for current or future Seafood CRC projects.

NON TECHNICAL SUMMARY:

Visible-near infrared reflectance spectroscopy (VNIRS) is a rapid, objective technique that has been used within CSIRO Food Futures (FF) Flagships projects over the past few years to assess flesh quality of animals within breeding programs. More recently, our group has applied VNIRS as part of a Seafood CRC Project ("Understanding Abalone Quality", No: 2008/701; Miriam Fluckiger, Ph.D student) and will use this in a new Seafood CRC/FRDC Project "Incorporation of selection for reproductive condition marketability and survival into a breeding strategy for Sydney rock oysters and Pacific oysters." (Applicant: Australian Seafood Industries and NSW Select Oyster Company).

This project aimed to enhance my (Malcolm Brown) capability in VNIRS, and apply these skills to current CRC projects. Another objective was to establish scientific networks with VNIRS expertise, as potential collaborators for current or future Seafood CRC projects. To this end, a range of activities were incorporated into the travel schedule, i.e conferences, training workshops and site visits. As the benefits from these activities were to flow to other non-CRC CSIRO projects (eg. salmon selective breeding program) CSIRO contributed the major funding to this travel grant, with the CRC providing supplementary funds to help extend the range of activities.

Two conferences were attended, i.e. Asia-Pacific Aquaculture 2009 (Nov 3-6; Kuala Lumpur) and NIR 2009 (Nov 9-13; Bangkok) where oral presentations were given. NIR 2009, which brought together 500 delegates with expertise in VNIRS was especially valuable, covering many technical aspects across broad application areas, including seafood and meat quality. I met with Christel Solberg – a pioneer of VNIRS for salmon quality assessment – and she provided useful discussion and positive feedback.

Two VNIRS training workshops were attended; one prior to the NIR 2009 conference, and the other a 2 day, one-on-one training with ASD Inc. – the manufacturer of our instrument. The workshops gave valuable instruction on the approaches to examine spectral data, identify outliers, and build and test appropriate VNIRS models. As well as applying this new knowledge to my current projects, I have passed on the key points to Miriam Fluckiger, for her to apply in her CRC project on abalone quality.

Site visits were undertaken to three institutions using VNIRS and other spectral methods for assessing meat quality. They were NOFIMA, (Oslo, Norway; hosted by Dr Jens Petter Wold), the Food Science & Technology Dept., (University of Nebraska, USA; hosted by Prof. Jeyam Subbiah) and Dr Steven Shackelford and his team from the US Dept of Agriculture, Meat and Animal Research Center (Clay Center, Nebraska). Collectively, the visits gave me a good insight into different methodology and instrumentation, and valuable information exchange on many VNIRS aspects. Relevant to our CRC project on oysters, Dr Wold has successfully used VNIRS to scan live crabs to predict roe and liver yield, and is testing his system with other shellfish. Dr Wold's view was that the non-invasive scanning of oysters might prove difficult because of the irregular nature of the shell (and associated reflection). Nevertheless, Dr Wold offered to provide further advice on this and other projects, and for potential collaboration. I gave seminars to staff and students at both NOFIMA and the University of Nebraska on our VNIRS research, including that undertaken within CRC projects.

In summary, the activities have significantly enhanced my understanding and skills in VNIRS, and these are being applied to benefit current CRC projects. The site visits and conference activities provided important linkages to key experts in VNIRS, and opportunities for collaboration will be investigated into the future during the evolution of existing and new CRC projects.

OUTCOMES ACHIEVED TO DATE:

The key outcomes arising from activities supported by the travel grant are:

- Enhancement of my capability and understanding of VNIRS, and transfer of some of this knowledge to Miriam Fluckiger, (CRC Ph.D student) to apply in her project on understanding abalone quality. While this is difficult to quantify in the short term, during the course of current CRC projects these enhanced skills will give rise to more robust models, to assess quality parameters in oysters and abalone.
- Linkages were made with international experts in applying VNIRS for assessing seafood and meat quality. Opportunities for collaboration within current and future CRC will be investigated in the future.

OUTPUTS DEVELOPED AS RESULT OF TRAVEL GRANT:

Conference Presentation:

Brown, M.R., Fluckiger, M., Tume, R., Sikes, A., Kube, P., Taylor, R., Brock, M. and Elliott, N. (2009). Portable VIS/ NIRS for Assessing Flesh Quality of Aquaculture Produce. – presented at NIR 2009, Nov 2009, Bangkok.

Publications:

Brown M. Fluckiger, M., Tume, R., Sikes, A., Kube, P., Taylor, R., Brock, M. and Elliott, N.et al. (2010). Portable VIS/ NIRS for Assessing Flesh Quality of Aquaculture Produce. Proceedings of the 14th International Conference on Near Infrared Sprectroscopy (NIR 2009). In press.

Reports:

Brown, M.R. (2010). "Visible-Near Infrared Reflectance Spectroscopy (VNIRS): A tool for rapidly assessing seafood quality. A report of activity and training undertaken between November 2-25 as part of the CMAR Capability Development Fund – Senior Scientists Program", Internal CSIRO Report, 28 pp.

Seminars:

Brown, M.R. "Portable VIS/NIR reflectance spectroscopy for assessing flesh quality of aquaculture produce" – to staff at NOFIMA, Norway, Nov 18, 2009.

Brown, M.R. "Portable VIS/NIR reflectance spectroscopy for assessing flesh quality of aquaculture produce" – to staff and students, Dept of Food Science, University of Nebraska, USA. Nov 23, 2009

Brown, M.R. "Visible-Near Infrared Reflectance Spectroscopy (VNIRS): A tool for rapidly assessing seafood quality" CSIRO Divisional seminar, Hobart, Mar 12, 2010.

BACKGROUND AND NEED:

In 2006, during the initial phases of CSIRO breeding program research, a need was identified for appropriate methods to monitor product quality to ensure selection decisions were not compromising quality. Also, associated research on abalone quality also required methods to examine the effects of environmental conditions and processing on quality. A requisite was the method be objective, accurate, inexpensive – and capable of rapid sample throughput. Of the methods that were considered, visible-near infrared reflectance spectroscopy (VNIRS) appeared the most suitable. This technology, which combines spectral examination of samples in the visible (350-700 nm) and near-infrared (NIR; 700-2500 nm) regions, has been successfully used to discriminate quality and to estimate chemical constituents associated with quality (eg. fat, protein, moisture, pigment and many others) in a variety of meat and seafood products.

Approximately two years ago our group acquired a portable VNIRS spectrometer, and in the intervening period good progress has been in developing VNIRS capability. The technology is currently being applied by a CRC Ph.D student, Miriam Fluckiger to examine aspects of abalone quality. Also, in a new CRC project "Incorporation of selection for reproductive condition marketability and survival into a breeding strategy for Sydney rock oysters and Pacific oysters" we will be applying VNIRS to estimate protein, fat, glycogen and moisture, as indicators of oyster condition.

Despite our positive start with VNIRS, it was clear that we still had much to learn. With that background, in mid-2009 I submitted an application to CSIRO's "CMAR Capability Development Fund" to undertake activities aimed to significantly advance my (and hence the team's) capability. The application was approved, and the Seafood CRC (through this travel grant) provided supplementary funds to assist in broadening the scope of activities, in particular to include site visits relevant to our CRC research.

RESULTS:

The travel itinerary, and scheduling of activities, is shown below.

Date	Travel	Description of Activity
Mon 2 Nov	Hobart → Melb – Kuala Lumpur (KL)	Travelled to Kuala Lumpur (KL).
Tues 3 to Fri 6 Nov		**Asia-Pacific Aquaculture (WAS) 2009 Conference, KL** Oral Presentation: "A Comparison of VNIRS with Other Methods for Measuring Quality Traits of Cultured Atlantic Salmon"
Sat 7 Nov	KL → Bangkok	Travelled to Bangkok
Sun 8 Nov	Bangkok	**VNIRS training course: "Advanced Chemometrics"**
Mon 9 to Fri 13 Nov	Bangkok	**NIR 2009 Conference** Oral presentation: "Portable VNIRS for Assessing Flesh Quality of Aquaculture Produce". Networking with experts in VNIRS; insight into other applications of VNIRS for new project opportunities at CSIRO.

Sun 15 Nov	Bangkok → Oslo	Travel
Mon 16 Nov	Ås, Oslo region, Norway	**Visit to Norwegian Institute of Fisheries and Aquaculture Ltd. (NOFIMA - MAT)** – hosted by Dr Jens Petter Wold. The group has developed VNIRS systems for estimating the liver and roe yield on live crabs, and doing research on other shellfish. The invitation included viewing the prototype systems and discussion of their results. I gave a seminar on our research to their group.
Wed 18 Nov	Oslo → Denver	Travelled to Denver, then Boulder, Colorado, USA
Thurs/Fri 19, 20 Nov	Boulder, CO, USA	**ASD Intermediate/Advanced Chemometric and VNIRS Training** One-on-one training by ASD Inc. Our instrument and data files were used in training and model development. The course included instruction on maintenance and optimisation of our instrument.
Sun 22 Nov	Boulder, CO → Lincoln, NE	Travelled (8 h by car) to Lincoln, Nebraska.
Mon 23 Nov	Lincoln, NE	**Visit to Food Science and Technology, University of Nebraska** Met with Dr Jeham Subbiah. The group has expertise in using VNIRS to predict characteristic components in food and other imaging for food quality inspection. I gave a seminar on our research to their group.
Tues 24 Nov	Clay Center, NE	**Visit to USDA Meat Animal Research Center** Dr Steven Shackelford. The group has expertise in using VNIRS and other methodology to evaluate and discriminate meat quality in beef.
Tues 24 Nov	Clay Center, NE → Denver, CO	Mid–afternoon; return to Denver, prior to return flight
Wed 25 Nov	Denver → Hobart	Return flight to Hobart

Details of these activities and how they might relate to current or future VNIRS work within the CRC are summarised below.

Conference: Asia-Pacific Aquaculture 2009, 3-6 Nov 2009, Kuala Lumpur

Though this activity wasn't formally identified in my CRC Travel Grant application (fully funded by CSIRO) there were some CRC-related interactions that are relevant to this report. I gave a presentation on the application of VNIRS for assessing quality of salmon as part of our selective breeding program, and afterwards had discussions with Dr Graham Mair (Pgm Manager, Product Innovation) about the application of VNIRS for assessing seafood quality. Graham had some previous discussions with Dr Jens Petter Wold of NOFIMA (one of my subsequent site visits – see below) about the potential of VNIRS for non-invasive scanning of shellfish, and he briefed me on those discussions. During the conference I also met up with Wayne O'Connor a co-investigator on the new Seafood CRC "Incorporation of selection for reproductive condition marketability and survival into a breeding strategy for Sydney rock oysters and Pacific oysters", so we had the opportunity to discuss strategies for VNIRS analysis of oysters within the project.

Conference: The 14th International Conference on Near Infrared Spectroscopy (NIR 2009), 9-13 Nov 2009, Bangkok, Thailand

This international conference, held every 2 years, attracted ≈ 500 participants from 36 countries, including 20 from Australia. It covered many applications of VNIRS and NIRS, including food and agriculture, pharmaceutical and medical, polymer chemistry, petrochemical, biology and the environment.
My talk "Portable NIR Reflectance Spectroscopy for Assessing Flesh Quality of Aquaculture Produce" was within the Food Session and covered our work on salmon, and our abalone quality work done within the CRC (see abstract; Appendix 1).

Interesting talks or poster presentations, relevant to seafood quality applications included:

- detect blood spotting on salmon fillets (K. Heia)
- detect nematodes in cod fillets (using VNIRS imaging) (A. Silvertsen)
- validate authenticity of fresh and frozen thawed fish products (L. Fasalota)
- predict shelf-life extension of pork (A. Soldado), and to detect meat spoilage (J.M. Carstensen).
- measure free amino acids (FAA) in cheese (L. Marinoni) (*application to our current research of FAA in abalone*)
- assess oil (fat) quality and source of origin (several talks; Y. Le Dreau, V. Venkataraman)
- assess beef quality, including glycogen measure (*application to our current research of glycogen in abalone*) (M. Challies).

A full list of abstracts of all presentations is available (hard copy) upon request.

The conference provided an excellent overview of the wide-ranging applications of VNIRS and NIRS. There were many talks on application of "hyperspectral imaging". Essentially, this is a high-resolution version of VNIRS. In our current VNIRS research we take one spectrum of any entire sample for component prediction; but with hyperspectral imaging a large number of spectra are taken from different regions of the sample simultaneously. An analogy to this is the composition of a digital photo, which is a composite of R-G-B value of individual pixels. Hence, through hyperspectral imaging one can build up a detailed "NIR image" of a sample, eg. one could precisely map the fat distribution across a salmon fillet. Hyperspectral imaging was seen as an area for continued development and application, and a trend towards systems with greater portability for field applications.

Training Workshops:

Two VNIRS training workshops were undertaken. The first, a one-day workshop on "chemometric modelling" attended by 30 delegates was given by Dr David Hopkins as part of NIR 2009. This course covered the use of principal components analysis (PCA) and score plots in the initial examination of spectral data, and also factor loading plots. These approaches provide a good insight into the integrity of data, and help to build robust models. I learned some valuable information from this course. A two-day workshop at ASD Inc (Boulder, Colorado, USA), i.e. the manufacturer of our VNIRS instrument, provided one-on-one training with Dr Dan Shiley. Through prior consultation, the training was targeted specifically to our project needs. Dr Shiley briefly reinforced some of the information covered by Dr Hopkins, but the main value was using our own spectral data and models, and using ASD's chemometric software,

as part of the training process. Other informative components of the training included spectral data management, various software “tips and short-cuts” and operational protocols for ensuring optimal instrument performance.

Site Visit: NOFIMA (Mat) in Ås, Norway, 18th Nov 2009

NOFIMA is a business oriented research group working in R&D for the aquaculture, fisheries and food industry in Norway. NOFIMA (Mat) is situated in Ås (outside of Oslo), Norway, and focuses on food quality, raw materials processing and nutrition.

My visit was hosted by Dr Jens Petter Wold. Dr Wold and his NOFIMA group are highly regarded for their expertise in VNIRS, including imaging, for seafood quality assessment. The visit included a tour of the laboratories, discussions with Dr Wold and colleagues, and I gave a seminar to NOFIMA staff and the associated University.

One system demonstrated to me was a conveyor belt VNIRS for on-line scanning, With rapid sensors and approximately 20 wavelength channels the unit is being used for VNIRS imaging. Recently, Dr Wold has mapped the meat content (specifically, liver and roe) within live crabs using this system, enabling on-line discrimination on the process line for further process methods (work to be published shortly). Dr Wold was also investigating this system for use with other shellfish. I had some discussion with Dr Wold about the feasibility of this approach to non-destructively scan live oysters (i.e. to link to new current CRC project work with oysters), to assess meat yield or physiological status. While conceding it might be possible, Dr Wold suggested it could prove difficult because of the calcareous nature and irregularity of the shell. The team has also used this system to examine the fat and colour distribution within salmon fillets.

The NOFIMA team has also done much research on the quality assessment of Atlantic salmon using VNIRS, and developed a system for non-invasive scanning of the fish. This area was also of significant interest and aligns with CSIRO quality work within our selective breeding program.

Overall, the visit to NOFIMA was very valuable and provided a co-operative and open exchange of information. Dr Wold and I agreed to stay in touch, and examine opportunities to collaborate in the future, especially with ongoing salmon work, and the new oyster project.

Site Visit: Food Science and Technology, Uni of Nebraska, Lincoln, NE, USA, 23 Nov 2009

A day visit was hosted by Jeyam Subbiah, Assistant Professor of Biological Systems Engineering and Food Science & Technology.

Prof. Subbiah’s applications with VNIRS and spectral imaging include prediction of beef texture and tenderness. He gave a detailed tour of his laboratory and explained his various VNIRS and spectral imaging systems, most of which were developed in-house by Prof. Subbiah and his group. I consulted with Prof. Subbiah on the potential of VNIRS to predict meat yield in oysters; he (like Dr Wold) thought this unlikely because of the shell thickness and irregularity.

Some advice was provided by Prof. Subbiah to get more consistent spectra for CSIRO’s salmon work, i.e. by using a turntable system with associated mug light. As

was the case at NOFIMA, I provided a 30 min seminar giving an overview of our seafood quality work, focusing on our progress with VNIRS. The talk was attended by University staff, technicians and undergraduate students.

Prof. Subbiah expressed interest in collaborating to assist us to set up imaging systems for seafood assessment. Possible mechanism to follow up on this (eg. exchange fellowships; DEST, Flagship or Industry funding) will be investigated.

Site visit: US Dept of Agriculture – Meat and Animal Research Center, Clay Center, Nebraska, USA, 24 Nov

The Meat and Animal Research Centre (MARC) are developing and evaluating non-invasive instrumentation (especially VNIRS) to predict value-determining characteristics of meat. I was interested in visiting this group because they utilised similar instrumentation to our ASD system, and the meat (product) discrimination process was also relevant to our CRC research on abalone and oysters.

Dr Steven Shackelford, who leads this research, invited me to join a morning field trip to a large abattoir/ packing plant in Schuyler, Nebraska, where his team were undertaking quality assessments using VNIRS on beef carcasses. During the transit of carcasses through the process line, a ≈ 30 cm cut was first made in the midregion to expose the rib-eye steak region. The section was scanned using an ASD LabSpec Pro unit with a hand held probe – similar to our system. After the VNIRS measurement, steaks were cut from the same region, placed on ice, then transported back to the laboratory for assessment by the laboratory methods, and calibration again the VNIRS data.

Although this visit was brief, the discussions I had with project staff, and viewing of the process, were useful. For example, it was interesting to see a commercial operation using VNIRS imaging for quality analysis, and to see alternative probe options in use with the ASD instrument. I also picked up a few small tips in using the VNIRS spectra-capturing software that will help streamline operations in future CSIRO work.

EXTENSION ACTIVITIES:

The following extension activities have been (or will be) undertaken:

- Written reports on trip activities, both to the Seafood CRC (this report) and an internal CSIRO report (submitted).
- Seminar on activities undertaken as part of my VNIRS capability development - at CSIRO Marine and Atmospheric Research, Hobart, March 12, 2010.
- Transfer of knowledge and know-how to other team members using VNIRS in our CRC oyster and abalone projects. Application of enhanced capability to these CRC projects.
- Feedback on talks presented at the NIR 2009 conference, and discussion with Prof. Subbiah (regarding VNIRS and microbiological/ food safety applications) to Judith Fernandez Piquer, Seafood CRC Ph.D student (Project: “Protecting the Safety and Quality of Australian Oysters Using Predictive Models Integrated with “Intelligent” Cold Chain Technologies”)
- Conference presentation: An abstract has been submitted to Australasian Aquaculture 2010 “Visible-near infrared reflectance spectroscopy (VIS/NIRS): A

rapid method for assessing quality of aquaculture produce". The presentation will give an update to industry of our VNIRS research, including research within the CRC abalone and oyster projects.

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY):

As discussed in a previous section, the immediate project outcomes are an enhancement of my (Malcolm Brown's) capability in VNIRS and establishment of international contacts with high-level expertise in this area. As this enhanced capability is employed, i.e. in current (oyster and abalone) or future CRC projects, more tangible industry-linked outcomes should follow. For example, in the case of the oyster reproductive condition project, if we can demonstrate that VNIRS can act as an effective tool for quality-associated discrimination, this would greatly assist selective breeding undertaken within that project, and more generally could be adopted by industry as a tool to assist in quality discrimination.

SUMMARY OF CHANGE IN INDUSTRY (WHAT IMMEDIATE CHANGES ARE EXPECTED):

No immediate change – longer term changes expected; covered in section above.

WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED:

Covered in above section "Project Outcomes".

FURTHER ACTION REQUIRED IN REGARDS TO COMMUNICATION:

An oral presentation on the application of VNIRS, including some of the contacts and activities reported here, will be given at the Australasian Aquaculture 2010 Conference, in Hobart, May 2010.

I will maintain contact with Drs Petter Wold and Subiah, in regards to ongoing VNIRS research and collaboration opportunities, including that within the CRC.

FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION? (IP PROTECTION, LICENSING, SALES, REVENUE ETC):

Not applicable.

LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS:

The funds provided from this CRC travel grant provided a valuable supplementation to funds also provided by CSIRO for my capability development in VNIRS. Specifically, the CRC funds enable me to extend my site visit activities. In this regard, the visit to NOFIMA (with which the CRC already has a research agreement) and Dr Jens Petter Wold was especially rewarding.

ACKNOWLEDGEMENTS:

Activities described in this report were jointly funded by CSIRO through the CMAR Capability Development Fund and Food Futures, and through a Travel Grant from the Seafood CRC (Project 2009/754).

Appendix 1 Abstract of Paper given at NIR 2009

Portable NIR Reflectance Spectroscopy for Assessing Flesh Quality of Aquaculture Produce

M. Brown,[1,4]* M. Fluckiger,[1,2,5] R. Tume,[3,4] A. Sikes,[3,4] M. Brock[1,4] and N. Elliott[1,4]

[1]CSIRO Marine and Atmospheric Research, Hobart 7000 Australia, E-mail: malcolm.brown@csiro.au;
[2]University of Tasmania, Launceston Australia; [3]Food Science Australia, Cannon Hill 4170 Australia;
[4]CSIRO Food Futures Flagship; [5]Australian Seafood Co-Operative Research Centre

[Introduction] The ability to rapidly and objectively measure or discriminate flesh quality would provide a great benefit to the aquaculture industry. In this study we report on the potential of portable near infrared reflectance spectroscopy (NIRS) to fulfill this need in two specific examples: 1) quantitatively, to measure the colour (astaxanthin) and fat levels in flesh of Atlantic salmon (*Salmo salar*) - two important traits contributing to flesh quality, and 2) qualitatively, to discriminate cultured abalone (hybrids from crossing *Haliotis laevigata* and *H. rubra*) subjected to different post-harvest processing treatments.

[Materials and Methods] NIRS was performed with a LabSpec 5000 (350-2500nm) instrument with a high-intensity contact probe (ASD Inc., Boulder, CO, USA). Data was analysed using Grams/AI software (Thermo Fisher Scientific, Waltham, MA, USA). For salmon, approx. 200 fish (1 to 6 kg) were tested. A standard fillet, i.e. "Norwegian Quality Cut" or NQC was removed from each fish. Scans were undertaken on the flesh of the NQC (6 regions; spectra averaged), and also the NQC after mincing. Minced samples were chemically analysed for fat and astaxanthin to model the data. For abalone, 36 individuals were used. The foot was scanned (900-1800 nm) of freshly shucked abalone, or abalone either air-frozen (-20°C) or frozen in a brine/ice mixture (-21.5°C), then thawed. Half of the thawed samples were cooked (steamed, 7 min), and rescanned. PCA analysis with cross-validation was performed to assess if product could be discriminated by NIRS.

[Results and Discussion] Robust models were developed for the prediction of components in salmon NQCs and mince (Table 1). Models using salmon mince have been validated and used to predict astaxanthin and fat in several thousand fish within a selective breeding program, thus informing breeding decisions. Future work will validate NQC models, and investigate models based on non-invasive scanning (i.e. scanning on skin surface). PCA analysis demonstrated that NIRS could discriminate between fresh abalone meat, frozen-thawed abalone, and the latter product after cooking (Fig. 1). Further, there was clear discrimination between frozen-thawed product according to freezing method. Though these data must be considered preliminary because of a small sample size, the experiment demonstrated the potential of NIRS for discriminating abalone produce. Further experiments will examine other process treatments, and explore whether NIRS can predict glycogen and moisture in abalone.

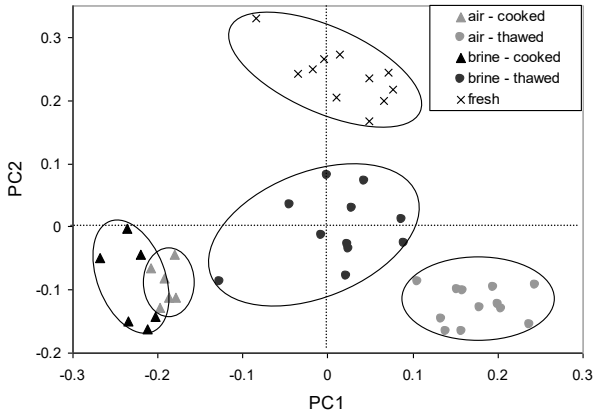

Figure 1. Scores of PC1 and PC2 used to discriminate abalone products. Data treatment: mean centering, MSC and 1D (SG 15)

Table 1. Models developed for salmon by cross validation. Mean ± SD of components 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=20)	
	n	Data Pretreatment	PC	R^2	SECV	R^2	RMSEP
% Fat (10.2 ± 2.7 % wet wt)							
- minced	154 (2)	SNVd	8	0.95	0.58	0.86	0.79
- intact NQC	148 (2)	SNVd; 1st deriv. (SG 25)	4	0.84	1.05		
Astaxanthin (7.2 ± 2.1 mg/kg)							
- minced	150 (0)	SNVd; 1st deriv. (SG 15)	8	0.94	0.53	0.92	0.52
- intact NQC	147(0)	SNVd; 1st deriv. (Gap 25)	4	0.90	0.66		

n = no. of samples in model (outliers removed in parentheses). PC = principal components; R^2 = Coefficient of determination; SECV = standard error of cross-validation; SNVd = standard normal variate and detrending correction; RMSEP = root means square error of prediction; SG = Savitzky-Golay algorithm.