Rapid on-line non-destructive NIR measurement on alive and filleted Atlantic salmon

Christel Solberg

Department of Fisheries and Natural Science, Bodø Regional University, N-8049 Bodø, Norway

Introduction

One of the reasons for the success of Norwegian salmon farming is the result of a 30-year continuous breeding program and the concomitant improvement in the chemical composition of the feed. Since 1972 the fully grown weight of farmed Atlantic salmon (*Salmo salar*) has increased by 10-12% on average in every generation and is now almost 80% greater than that of the original wild salmon. During the 1970s the upper limit for dietary fat in salmon feed was 17-18% in the feed.[1] Today, new production technology using extrusion and coating has made it possible to increase the fat content in the feed,[2] which is now usually around 40%. This increased fat content, however, has resulted in a higher fat content in the fillet of the salmon.[2-5] Different markets have different preferences for fat content and this has raised the need for non-destructive measurement of fat in salmon. Measurements on live salmon would make it possible to monitor the feeding regime and also to determine the fat content before slaughter for future product tailoring for different markets. Non-destructive measurement of the fat content in live salmon would also make it possible to select salmon for breeding from the fat content in the muscle tissue, besides other quality factors. It has been shown that it is possible to measure fat in live salmon by near infrared (NIR) diffuse spectroscopy.[6] In this paper the influence of different temperature in the fish and the mortem status is investigated.

Half of the cost for the feed is related to the added pigment astaxanthin. The pigment is necessary to get the red colour in the flesh, another important quality parameter. It has been shown that it is possible to measure the astaxanthin content if the salmon is filleted and the measurement is performed from the flesh side[7] and this experiment has now been repeated.

Material and methods

100 Atlantic salmons, ranging from 1 to 11 kg were analysed in June 2001, when the temperature in the water was 14° C. Another 40 salmons, ranging from 1 to 6 kg were analysed in February 2002, when the temperature in the water was 4° C. The salmons were taken one by one from a sea pen, and transferred to a vessel containing 200 mg kg^{-1} Metacainum® (MS 222, m-aminobenzoic acid etyl ester metanesulphonate; Tamro, Copenhagen, Denmark). The salmon was anaesthetized within 2 min and then analysed on an diode array near infrared (DA-NIR) instrument (Perten 7000 Flexi-mode, Perten Instrument, Huddinge, Sweden). The instrument was used in a "up-view" mode. Excess of water on the surface of the salmon was wiped off with a piece of tissue paper, and then the salmon was simply placed directly on a circular glass plate (diameter 125 mm) on the instrument, so the area from the backfin to the gut was illuminated in 3 seconds. The sample was illuminated with full spectrum light, and the diffuse reflectance (log(1/R)) of the light was measured from 400 nm to 1700 nm in 5 nm steps. On the samples from February the measurement was repeated after the fish was killed and bled. In this experiment all the fish was killed and a sample corresponding to the flesh between backfin and the gut, the so-called "Norwegian quality

Near Infrared Spectroscopy: Proceedings of the 11th International Conference.

cut" (NQC) was cut off, and analysed for fat content by extraction with ethyl acetate, according to Norwegian standards[8]. In another experiment measurements were performed with the same instrument on post rigor filleted salmon at the flesh side. The NQC was cut of and samples were analysed for fat content and astaxanthin. Astaxanthin was measured after separation of the pigment from the ethyl acetate extract by absorption chromatography on a silicic acid column. After chromatography the solution was evaporated the pigment residue was dissolved in 10 mL hexane and the concentration of astaxanthin was measured in a spectrophotometer at 472 nm.

Partial least squares (PLS) regression was used for calibration and validation[9]. To evaluate the calibration models, full cross-validation was applied. Regression analysis was performed using UNSCRAMBLER ver.7.8 (Camo Process AS, Oslo, Norway).

Results and discussion

Figure 1 shows the diode array spectrum of five live salmon. The wavelength region 400 to 900 nm simply reflects the uneven colour of the salmon skin and this region of the spectrum must be omitted before the PLS calibration.

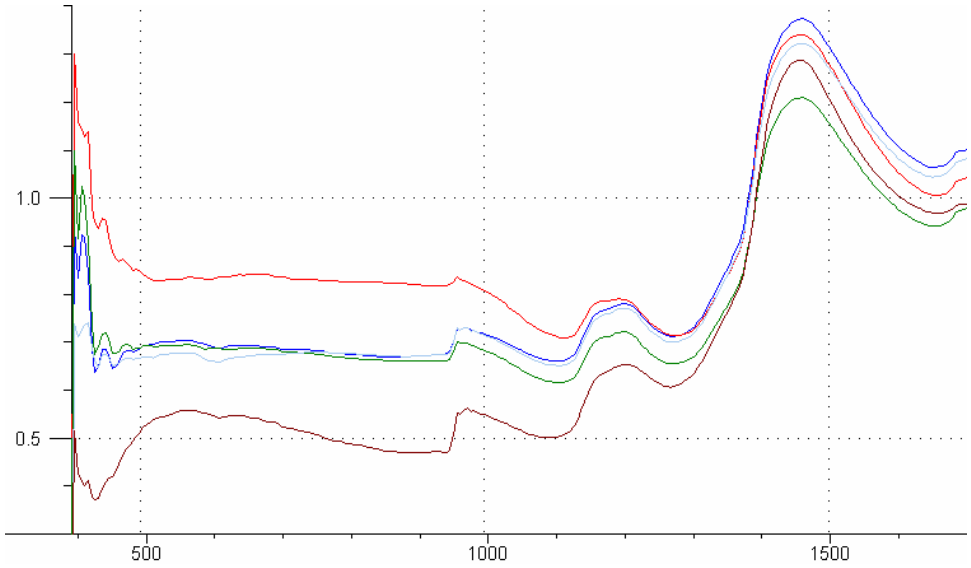

Figure 1. Diode array spectra of live salmons from 400 to 1700 nm.

An earlier calibration of the summer samples has been done.[10] This calibration, made of samples at higher temperature, results in a large deviation and a 2% bias for the predicted winter samples. However, when a combined calibration model consisting of both the summer and the winter samples was made, there was no bias and the calibration could be used to predict the fat values for the salmon independently for the temperature in the sample. Earlier it was found that a calibration made on pre rigor salmon could not be used for prediction of post rigor samples.[10] To analyse if any difference could be measured whether the fish was alive or dead, NIR measurements were repeated after killing the fish but before the fish enters rigor. The mortem status shows only a minor effect on the prediction performance. The correlation coefficient remained the same whether dead samples

were included or not. So, it is possible to make a calibration that could be used to predict the fat value in whole salmon and this could be made on the living fish or just after killing the fish. This means that this instrument could be installed in the slaughter house and the salmon could be sorted according to the fat content on the production line.

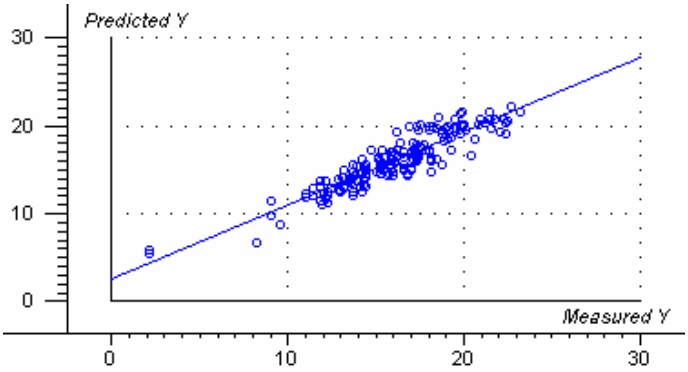

Figure 2. NIR-predicted versus reference method % fat of 100 live summer and 40 live and dead salmon. Correlation 0.91, RMSEP 1.3 %, 9PC.

The predictive performance for fat in whole salmon is shown in figure 2. The wavelength range 900-1700 nm was used for prediction with 9 PC, resulting in a correlation of 0.91 and a RMSEP of 1.3 %.

Measurement from the fillet side made it possible to extend the wavelength range into the visible area and in that way enhancing the performance for prediction of fat content by using the wavelength range 475-1700 nm, figure 3 and also made it possible to measure the astaxanthin content in the ppm level by using the wavelength range 520-1700nm, figure 4.

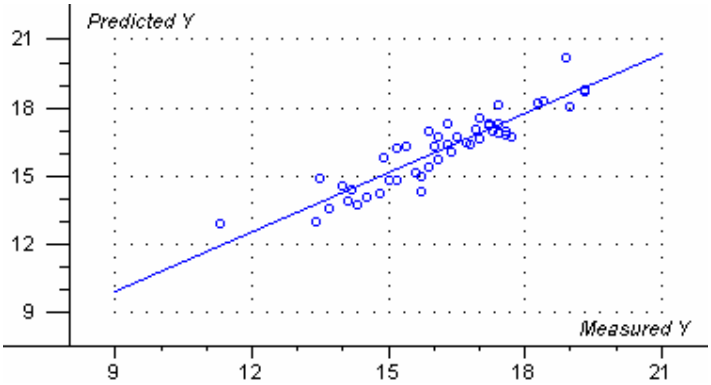

Figure 3. Prediction of the fat content in the fillet from the flesh side. Corr. 0.91, RMSEP 0.69% fat, 8 PC.

The optimal wavelength ranges were different for the different applications. Further selection of the wavelength by "Jack knifing" had little influence on the calibration performance for these two applications.

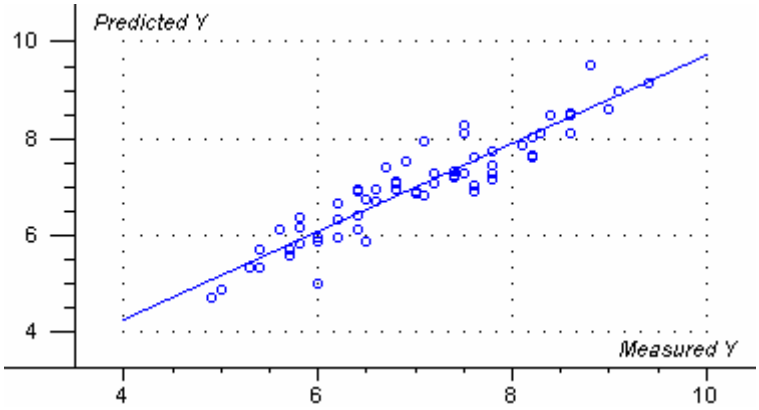

Figure 4. Prediction of the astaxanthin content in the fillet from the flesh side. Corr. 0.91, RMSEP 0.40 mg astaxanthin/kg muscle, 11 PC.

Conclusion

Diode-array NIR measurement, could be used as a screening method to determine the crude fat content in live farmed Atlantic salmon. It is necessary to avoid the visible range of the spectrum. It is also necessary to include samples representative for the future expected temperature range.

By making the measurement on a fillet from the flesh side, the visible region of the spectrum should be used. In that way the calibration performance for the fat analysis were strongly enhanced and it was also possible to measure the astaxanthin content in the fillet.

The short analysis time with a diode-array makes it suitable for on-line analysis.

Acknowledgement

The Research Council of Norway is acknowledge for financial support, grant no 136440/420, and Aquagen AS for excellent cooperation.

References

1. E. Austreng, *Sci. Rep Agric Univ Norway*. **55**, 16 (1976).
2. F. Johnsen, A. Wandsvik, in *Nutritional Strategies and Aquaculture waste*, Ed by C.B. Cowey and C.Y. Cho. University of Guelph, Guelph, p 51 (1991).
3. M. Hillestad, F. Johnsen , *Aquaculture*. **124**, 109 (1994).
4. E. Watne, *Dr scient Thesis*. Agricultural University of Norway, Ås, N. (1995).
5. G.I. Hemre, K. Sandnes, *Aquaculture Nutr.* **5**, 9 (1999).
6. C. Solberg, E. Saugen, L.P. Swenson, L. Bruun, T. Isaksson, *J Sci Food Agric.* **83**, 692 (1993).
7. C. Solberg, in *Near Infrared Spectroscopy: Proceedings of the 9th International Conference,* Ed by A.M.C. Davies and R. Giangiacomo. NIR publication, UK, p889 (2000).
8. Norwegian Standard Association, NS 9401/9402 (1994).
9. H. Martens, T. Næs, *Multivariate Calibration.* Wiley, Chichester (1989).