Use of near infrared transmittance in quality analysis of fish

Christel Solberg
Department of Fisheries and Natural Sciences, Bodø College, N-8002 Bodø, Norway.

Introduction

Fish and fish products turns out to be one of Norway's most important export products, with a total value of 19.4 billion Norwegian crowns in 1994,[1] where cod and farmed Atlantic salmon are the most important species. To be able to optimise farming conditions and carry out quality control, we need rapid, simple to use and accurate analytical methods: from that aspect use of near infrared (NIR) methods is very promising.

Fish differ from land living animals because they can survive starving for a long time due to limited access to food, too low temperature or maturity and spawning.[2] They do not need muscles for standing up, only for swimming, and can use a large part of their own white muscle tissue as a food reserve. During spawning, a fish usually starves and in a lean fish, as for example cod, the protein in the muscle will be replaced with water and result in a seasonal variation.

Some of the water in the fish muscle can be looked on as free water and is easily removed from the tissue when the fibre is cut. By gentle centrifugation, according to the net test, the unbound liquid or the water holding capacity in the muscle sample can be measured.[3] The liquid loss varies with the temperature of the sample, caused by changes in the connective tissue and in the muscle proteins.[4] In our cold-water fishes, a substantial part of the fat is polyunsaturated and has a melting point close to 0°C. In salmon, the fat is distributed both within the muscle fibre and in the myosepta between different muscle segments.[4] After death this fat is easily released and after a simple cut with an ordinary knife, one can see an oil-film on the cut surface. The risk for liquid loss at room temperature must be considered during NIR measurements. The free liquid can easily separate from the rest of the sample and create a water- or fat-mirror. Measurements in the transmission mode are generally less sensitive for inhomogenities in the sample than measurements in the reflectance mode. The objective of this study was to find out if near infrared transmittance is a feasible method for analysis of high moisture samples such as fish.

Material and methods

Cod (*Gadus morhua*) collected from different typical fishing grounds, throughout the year, were gutted and stored on ice for three days before filleting and mincing in a food processor.

Samples of capeline (*Mallotus villosus*) were taken directly from the boat during the winter season. Three buckets of raw material from each catch were taken from the top, the middle and the bottom part of the boat. After mixing, a subsample of 5 kg was minced. A 1/2 L plastic box was filled to 3/4 and frozen at –20°C. Analysis of the sample was made within a month. In the laboratory the samples were thawed to room temperature and mixed on an ESGE Bamix for about 15 sec.

Atlantic salmon (*Salmo salar*), weighing from 300 g to 3000 g, were randomly sampled from sea cages at the Aqua culture station, Tromsø. The fish were bled at the farm and kept on ice until

further processing on the same day. Prior to analysis the fish were filleted and minced in a food processor.

Water was estimated as weight loss after drying at 105°C overnight. For capelin the crude fat content was measured by extraction with ethyl acetate. A 10 g sample was mixed with 20 g water free sodium sulphate. 50 g ethyl acetate was added and the mixture was shaken for 10 min. After about 20 hr it was shaken for another 10 min. After filtering, 20 mL filtrate was evaporated at 105°C until constant weight. For salmon, the crude fat content was measured by extracting with petroleum ether for 1 hr (AOAC, No. 24.005 and No. 7.045). Protein was analysed according to Kjeldahl with the Kjeltec equipment (Tecator AB, Höganäs, Sweden). pH was measured in a 1 : 1 mixture of minced fish and 0.15 M KCl with a combined glass electrode.

The liquid loss was measured as the percent of weight released per 15 gram of coarsely chopped sample. The samples were either centrifuged directly at 5°C or heated (in a metal tube with a rubber stopper at each end) to the required temperature, held at that temperature for 10 minutes, and then cooled to room temperature by keeping the metal tube in an ice bath. The samples were transferred to centrifugation tubes and thereafter centrifuged at $210 \times g$ for 15 min at 20°C. Mean values for the liquid loss in the salmon samples were calculated from 6 trials of 2 replicates heated to 10, 20, 30, 40, 50 and 60°C respectively. The liquid from four replicates heated to 25 or 45°C was pooled into a measuring cylinder and the separation of the liquid to fat and water were observed.

NIR spectral data were collected by using an Infratec 1225 Food and Feed Analyzer (Tecator AB, Höganäs, Sweden). Spectral transmission readings were obtained at 2 nm intervals over a region 850–1050 nm. The sample thickness was 6 mm for capelin and 23 mm for cod and salmon.

Principal component analysis (PCA) and partial least square (PLS) regression analysis were used for analysis of the spectral data, with the aid of the "Unscrambler" program (CAMO AS, Trondheim, Norway).

Results and discussion

Cod

Figure 1 shows the score plot for the first and second principal component from the NIR transmittance scan of capelin, cod and salmon. In spite of the very small differences between the samples in the cod group, compared with those of the capelin and salmon group, a further analysis of the cod samples alone shows a clear seasonal variation.[5] The results have not only given us new information about seasonal variation in the raw material, but also strongly indicate the necessity of collecting samples for further calibration over at least a whole year to cover the expected variation in future analysis.

The unbound water at 5°C increases from 12% to about 36% after only three months storage at –20°C. This fact makes it more difficult to analyse frozen cod samples, because water very easily separates and can result in wrong data in the NIR transmittance measurement and the wet chemical analysis.

Table 1 shows us that calibration of cod against NIR transmittance gives us good prediction values, making it possible to replace the usual time consuming chemical analysis with rapid NIR transmittance measurements.

The variation in protein and water is relatively small and is associated with long time starving or feeding periods. pH on the other hand varies much more. Well fed cod deposits glycogen in the muscle and results in *post mortem* low pH. Fish muscle with a low pH has an increased tendency for gaping because of decreased breaking strength in the connective tissue,[6] and is less suitable for frozen storage because it develops an unacceptable tough texture after freezing.[7]

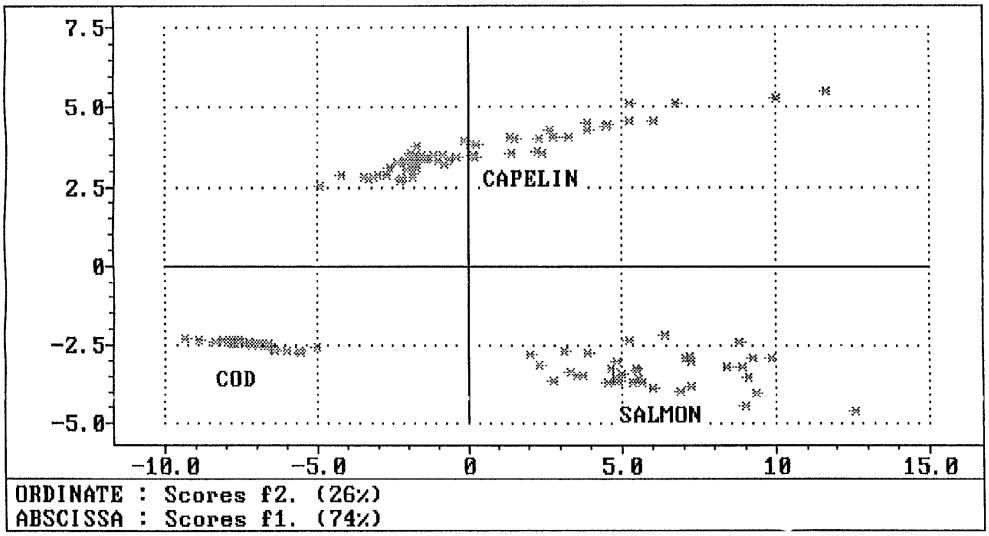

Figure 1. PCA score plot of the NIR transmittance scan of different cod, capelin and salmon samples.

Table 1. Characteristics of calibration of minced cod (fillet).

Component	*r*	*SEP*	Min.	Max.
Water %	0.98	0.18	80.3	83.6
Protein %	0.96	0.22	15.6	18.4
pH	0.96	0.06	6.2	7.2

So by using NIR transmittance, we can easily collect "all" the chemical information during the season and from year to year and in that way make it possible to monitor the quality changes in one of our most important species.

Capelin

Figure 2 shows the calibration of whole minced capelin against fat content. The precision is not as good as desired in the low and high concentration areas and the results are the same for analysis of dry matter. When the fishing season starts in January, the fat content is 18–19%. It decreases from week to week and is about 7% at the beginning of March. The gonads are then fully developed, and constitute about one fifth of the fish weight. The capelin will now start to spawn. During all this time the capelin starves continuously and does so until the bitter end when it dies after finishing the spawning.[8]

We have found that we can improve the calibration by separating it into two parts,[9] one covering the time before spawning and one covering the time after the start of spawning, as shown in Table 2.

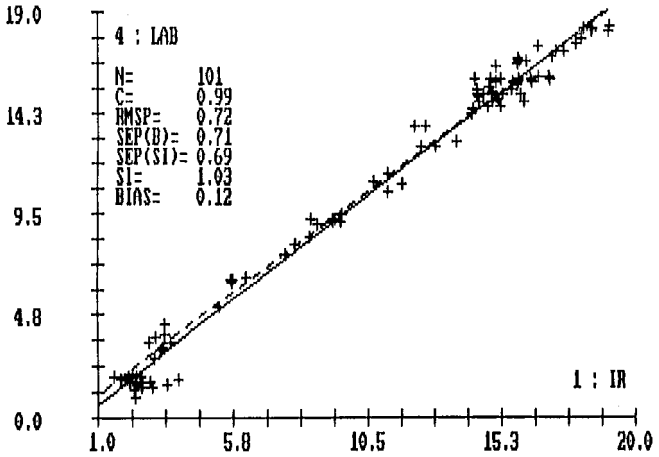

Figure 2. Analysis of % fat in capelin by NIR transmittance measurement (abscissa) and wet chemical data (ordinate).

Table 2. Characteristics of calibration of whole minced capelin.

	Component	*r*	*SEP*	Min.	Max.
Before spawning	Fat %	0.99	0.34	9.0	19.5
After spawning	Fat %	0.99	0.32	1.2	9.4

Salmon

Farmed salmon, on the other hand, will have no limitation in the food supply. The growth rate and the chemical composition of the muscle will, among several things, be dependent of the kind of feed it gets and the temperature of the water.

The liquid loss after centrifugation of minced salmon is less than that found in cod. However, at room temperature, which is the common for NIR measurements, the liquid loss increases (Figure 3). Analysis of the liquid released after heating the mince to 25°C, showed that up to 50% of the liquid is fat. This fat will rapidly concentrate on the surface and create a fat mirror. The high fat content in the released liquid is much higher than reported by others.[4] The difference is caused by the difference in the centrifugation temperature 20°C versus 10°C. At 10°C the fat in the liquid will have a high viscosity and will be entrapped as "free liquid", visible to the naked eye, in the mince. Measurements in the transmission mode in a horizontally oriented cup, as is used in the "Infratec", are less sensitive for the development of a water or fat mirror on the surface than measurements in the reflectance mode. In any way, after mincing a fish sample we have to be certain that the subsample we take for the NIR measurements and the chemical–physical analysis really is representative. Table 3 shows that analysis with NIR is acceptable.

These calibrations are made on minced, whole fillets. New calibration will be done by using the new standardised "Norwegian quality cut"; that is the muscle tissue from the back fin to the gut.[10] The calibration will also be extended to cover higher fat content up to 15–20 % fat and lower water and protein concentrations. NIR transmittance measurements turn out to be very useful in

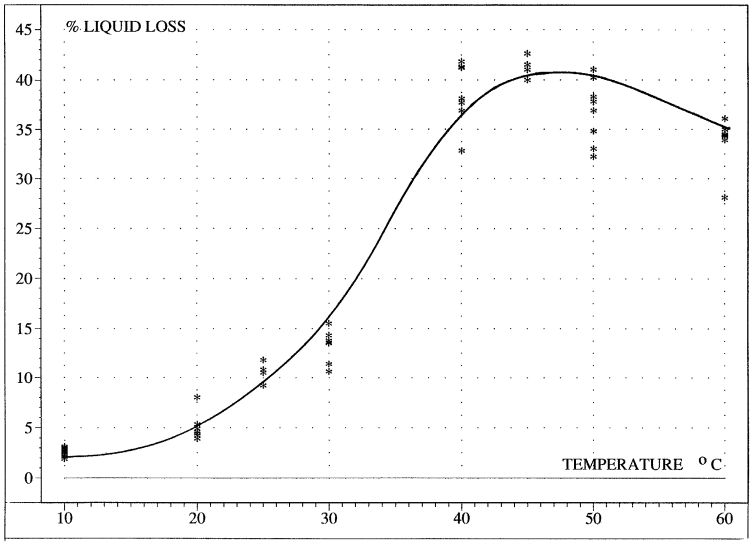

Figure 3. The % liquid loss after centrifugation of heat treated minced salmon.

Table 3. Characteristics of calibration of minced salmon (fillet).

Component	*r*	SEP	Min.	Max.
Fat %	0.99	0.33	0.5	10.0
Water %	0.99	0.21	69	78
Protein %	0.99	0.20	19	21

feeding experiments. In this way large numbers of samples can be analysed rapidly at a reasonable cost.

Reference

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