Near infrared reflectance assessment of the degree of retting of flax stems

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Introduction

Fibre makes up a small fraction of the mass of freshly harvested flax stems; for example, the cultivar used in this study was 27% fibre. Retting is the chemical, enzymatic or microbial process that releases this fibre from the waxy cuticle and the lignified core so that it can be separated mechanically. Enzymatic retting is a promising technology because it is rapid and produces good fibre characteristics if the product is not under- or over-retted. Conventional methods for determining the end-point of retting produce crude estimates and often require subjective judgement. As such, we have investigated spectral methods for monitoring the enzyme retting process with an aim toward applications in laboratory studies as well as for trade and industry.[1] Because near infrared (NIR) reflectance analysis can measure the composition of intact plant tissue, an NIR method to monitor flax retting may be feasible, provided that the calibration models can be made insensitive to the flax colour, stem packing geometry and moisture content, all of which are likely to vary considerably among flax specimens.

Experimental

As described previously,[1] stems from the flax cultivar Ariane were retted for various times in test tubes with mild agitation at 50°C in a buffered solution containing a chelator and an enzyme mixture known as Flaxzyme™ (Novo Nordisk, Franklinton, NC, USA). At the various time points, stems were removed from the incubation bath, rinsed and dried in air. Intact retted stems were aligned and loaded into 38 mm i.d. windowless round clamps. Reflectance spectra were measured over the range 400 to 2500 nm with an NIRSystems Model 6500 spectrometer, which produces $-\log[R_{\text{sample}} \cdot R_{\text{ceramic}}^{-1}]$, where R's are in units of measured reflectance power. Spectral data were acquired with two sampling accessories, the "spinning cup" (three repeat scans) and the stationary 'transport device' (six repeat scans at various angular orientations). The spectral measurements were collected after equilibration of the samples in laboratory air ranging from 40 to 68% relative humidity over a data acquisition period spanning three weeks. All spectral and reference measurements were done with measurement order randomised with respect to the retting time sequence. The degree of retting was monitored by several techniques,[1] but most important was the Fried's test,[2] which measures the degree of stem disintegration in boiling hot water after the sample is given a controlled amount of agitation. Fried's test requires an observer to classify the appearance of the treated stems into one of four levels (0, 1, 2 and 3). One observer evaluated a randomly coded set of subsamples (Figure 1). Because of the large uncertainty in the Fried's test data v. retting time, a smooth function was fit to the data and the smoothed Fried's test scores (SFTS) were used as reference data for the NIR partial least squares regression (PLSR) calibra-

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tions. Moreover, since the enzyme retting is believed to be complete after six to eight hours, over-retted samples beyond 14 hours were not included in the calibration set. For the purpose of quantifying the ability of PLSR models to distinguish under- and over-retted samples, the endpoint was defined as an SFTS value of 1.4, which is equivalent to 6.5 hours of enzyme retting. All SFTS predictions were produced by a modification of full cross-validation on each spectral sample, where, in the cross-validation prediction segment of a certain specimen, all the spectral samples of treatments of that specimen were omitted from the calibration set of that segment. This ensures that the prediction model of each spectral sample is calculated from spectra of an independent set of specimens.

Results and discussion

A technique we call 'spectral window preprocessing' was used to locate the spectral regions that are most effective in predicting SFTS regardless of the sample conditioning or sample presentation treatments. Model statistics were computed for a grid of spectral window widths (10, 20, …, 1000 nm) and spectral window positions (beginning at 800, 810, …, 2490 nm). In this study each tested window was background corrected prior to calculation of the PLSR model and generation of cross-validated predictions. Background correction for each spectrum of a spectral window was accomplished with the following algorithm: (1) subtraction of the line between the beginning and end of the spectral window; (2) normalisation of the spectrum vector by subtracting its mean and dividing by the standard deviation; and (3) projection of the spectral segment back onto the same region of the median spectrum. Step three does not greatly affect the PLSR result, but makes the corrected data appear more like typical –log[*R*] data. By evaluating the most effective PLSR spectral window model for each number of factors, it was concluded that three factors are sufficient to predict the SFTS data and that variations in the root mean squared error of cross-validation (*RMSECV*) are fairly representative of the other statistics (Figure 2). The spec-

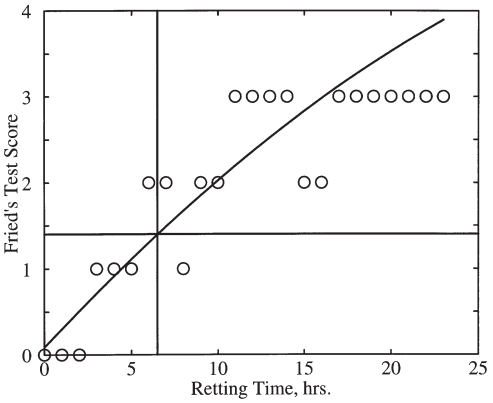

Figure 1. Estimation of the degree of fibre release of a sequence of flax stems retted with enzymes for times ranging from 0 to 23 hours. The circles are single-blind determinations of Fried's test visual scores. The horizontal and vertical lines divide the under- and over-retted samples, while the curve is a fit line to generate the smoothed Fried's test score (SFTS) reference data used in the NIR calibration study.

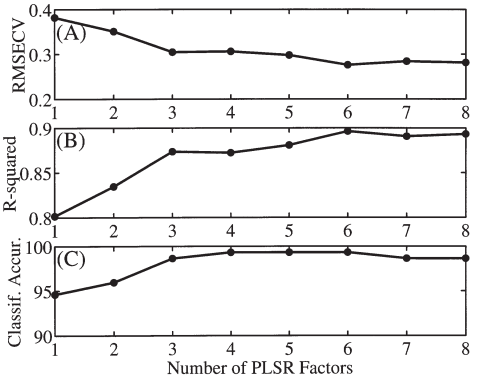

Figure 2. Best values among all individual tested NIR spectral windows for PLSR performance statistics v. number of factors, as generated by a cross-validation procedure that preserves the independence of each specimen: (a) *RMS* error in predicting the known SFTS; (b) correlation between predicted and known SFTS; and (c) percent of samples correctly identified as either under- or over-retted. For each statistic the sample retting times ranged from 0 to 14 hours.

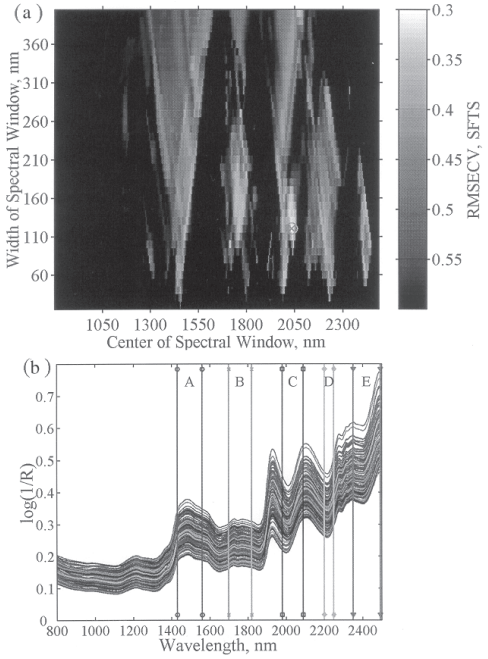

Figure 3. Determination of five spectral windows with good performance for prediction of SFTS: (a) three-factor spectral window preprocessing image with the best individual model noted with the symbol ; and (b) unprocessed –log[*R*] NIR spectra with indication of the five locally optimal spectral windows A–E. Both plots have the same range of abscissa values.

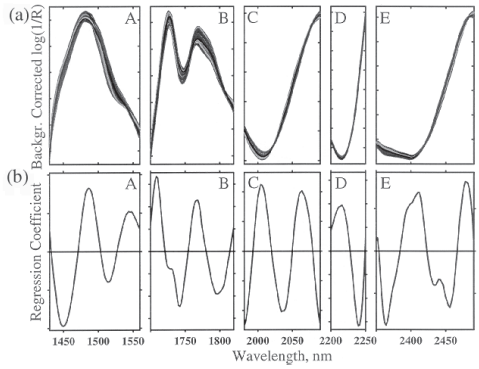

Figure 4. Spectral basis of PLSR calibration models for determining SFTS from NIR reflectance of flax stems: (a) background-corrected locally optimal spectral windows A–E; and (b) corresponding regression vectors. The expansion per wavelength is the same for all of abscissa scales. See Table 1 for statistics.

tral window image of *RMSECV* for three factor models was used to identify the best window limits for five effective spectral regions A–E (see Figure 3 and Table 1). Three-factor models were optimal for every window except (d), in which the 1-factor model was nearly as good as the 3-factor model (data not shown). Moderate spectral band changes in the background-corrected spectra are used by the five models, including: for (a) O–H 1st overtones and 1st overtones of C–H combinations; for (b) C–H 1st overtones; for (c), O–H combinations; for (d), O–H and C–H + C–H combinations and for (e) C–H + C–C combinations. Variation in these vibrational bands would be expected for the

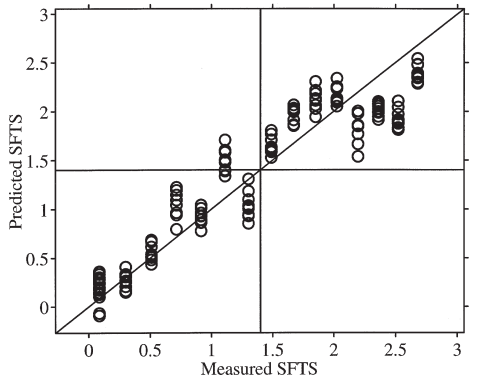

Figure 5. Predicted v. known SFTS values with predictions generated by weighted combination of the PLSR predictions for five NIR spectral windows A–E. The predicted values were estimated by leave-one-out cross-validation with specimen independence preserved by omitting the multiple treatments for a particular sample from that cross-validation segment. The predicted values from the different models were combined after weighting by the inverse of the mean squared error of cross-validation for the model. The horizontal and vertical lines divide the under- and over-retted specimens. See Table 1 for statistics.

Table 1. Summary of the performance of NIR reflectance models for predicting smoothed Fried's test scores of intact flax stems retted with enzymes for times ranging from 0 to 14 hours.

Model name	Wavelength Range, nm	# PLSR Factors	*RMSECV*, SFTS[a]	*R*-squared[b]	*RMS* Repeat.[c]	Classfication Accuracy, %[d]
A	1430 –1560	3	0.368	0.816	0.169	92.6
B	1700 – 1820	3	0.350	0.833	0.161	94.6
C	1980 – 2090	3	0.305	0.874	0.181	96.6
D	2200 – 2250	1	0.382	0.802	0.181	90.5
E	2350–2490	3	0.361	0.825	0.158	95.3
(A–E)[e]	—	—	0.296	0.884	0.108	95.3

[a]Root of the mean-squared error of cross-validation for independent prediction of the SFTS for each sample and treatment.
[b]correlation between predicted and measured SFTS for all samples.
[c]estimate of the root of the mean-squared deviation in repeated prediction of SFTS of a single specimen as obtained by pooling treatment variance across all specimens.
[d]accuracy in classifying stem specimens as either under-retted (SFTS < 1.4) or over-retted (SFTS ≥ 1.4): [(number of true positives) + (number of true negatives)]•[total number of samples]$^{-1}$•100%.
[e]combined predictive ability of all five windows by weighted averaging of the predictions of models A to E.

compositional changes known to occur during the retting process (loss of wax, pectins and lignin relative to cellulose). Spectral window C performs substantially better than the other windows (see Table 1) and most of this improvement is for the over-retted specimens (data not shown). This performance difference is consistent with the interpretation that this region is measuring the relative amount of structural polysaccharides that are exposed by the retting process. The single-factor model (d) is effective for the under-retted samples, perhaps by using C–H + C–H combination bands to track the loss of wax from the flax stems. A weighted combination of cross-validation predictions generated from the five regions is superior to any individual model, particularly in terms of the repeatability of determining SFTS for a single specimen under varying conditions of hydration and sample orientation during optical measurement (Figure 5 and Table 1). However, the model uncertainty is still limited by either error in selection of the spectroscopic subsamples, or variation in the retting rate induced in different subsamples. Note though, that even the poorest model was able to classify under- and over-retted samples with an accuracy of better than 90% (Table 1).

References

1. D.D. Archibald, G. Henrikssen, D.E. Akin and F.E. Barton, II, in *Fourier Transform Spectroscopy: Eleventh International Conference: Athens, GA August 1997*, Ed by J.A. de Haseth. American Institute of Physics, p. 653 (1998).
2. H. Fried, *Faserforschung* **12,** 197 (1939).