On-line monitoring of enzymatic degradation of wheat starch by near infrared

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Introduction

Near infrared (NIR) reflectance spectroscopy is well known as far as the analysis of ground agricultural material is concerned and many calibration results are given in the literature.[1,2] The development of new measuring cells allows the measurement of entire seeds or fresh material.[3,4]

More and more NIR reflectance spectroscopy is investigated for the real-time monitoring of chemical reactions[5] and of biotransformation reactions.[6] Continuous flow cells were first investigated by the latter authors for this kind of application. The recent development of fibre optic probes enables the *in situ* analysis of the broth during the reaction.[7]

In our study, a continuous flow cell (CFC) and a bundle fibre optic probe (FO) were compared for the real-time monitoring of the enzymatic amylolysis of ground wheat by NIR reflectance spectroscopy.

Material and methods

- NIRSystems 6500 scanning instrument with a transport system and a continuous flow cell alimented by a peristaltic pump (Chemap, 60 mL min^{-1});
- NIRSystems 6500 scanning instrument with a bundle fibre optic probe (NIRSystems, anhydroguide interactance immersion probe NR6640-AN035);
- Infrasoft International ISI V3.0 NIR software;
- Chemap bio-reactor CMF 100 (5 L) and a CBC 5 control unit;
- α-amylase of *Bacillus lichenifornis* (Termamyl 120 L, Novo Nordisk) for the liquefaction
- Mixture of amyloglucosidase and pullulanase obtained from selected strains of *Aspergillus niger* and *Bacillus acidopullulyticus* (Dextrozyme 225/75 L, Novo Nordisk).
- HPLC Hewlett-Packard 1090 equipped with a C18 reversed phase column (BioRad Aminex HPX87H) and refractive index detector.

The experimental set-up for the monitoring of the enzymatic hydrolysis of wheat by CFC and FO devices is illustrated in Figure 1. The experimental protocol is summarised in Figure 2.

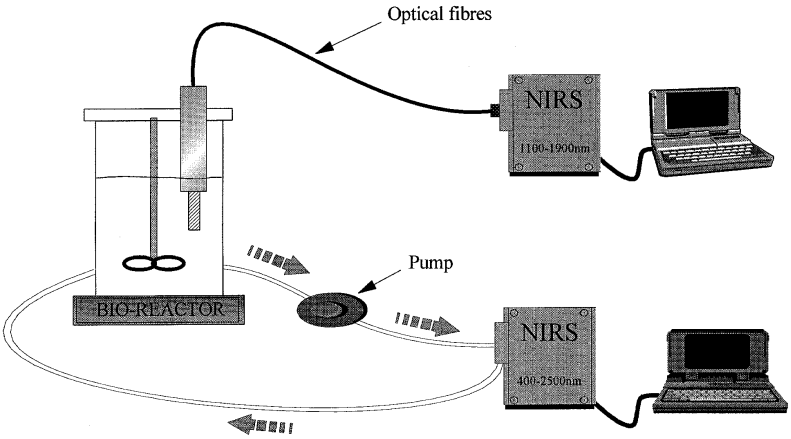

Figure 1. Experimental set-up for reaction control by a continuous flow cell and a fibre optic probe.

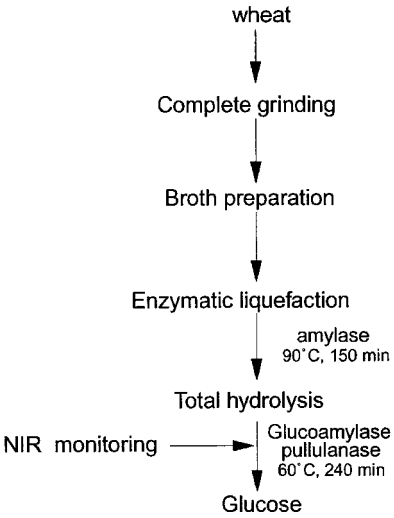

Figure 2. Experimental protocol for the monitoring of the enzymatic degradation of starch.

Results and discussion

Qualitative aspects

As the spectral bands for glucose and starch are in the same regions of the NIR spectrum, it was difficult to pinpoint the spectral changes during the reaction. Principal components analysis (PCA) was very sensitive to the global changes of the matrix during the reaction.

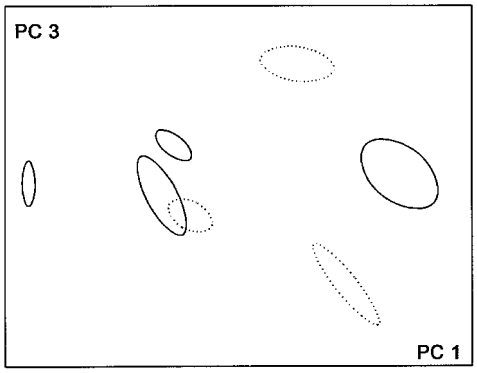

Figure 3. Variation between batches in axes 1 and 3 of the principal components analysis (CFC).

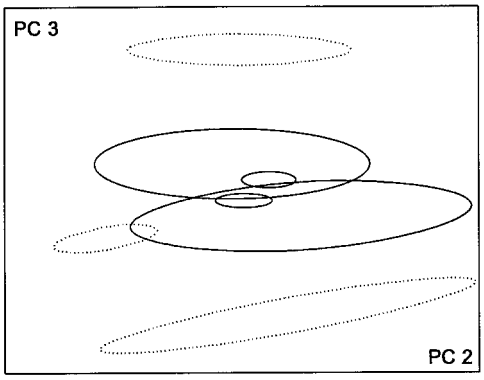

Figure 4. Variation between batches in axes 2 and 3 of the principal components analysis (CFC).

A given technological process can be traced in the firsts principal components axes. This has been recorded for a desiccation process. The same technique was used for the qualitative monitoring of the catalytic reduction of 4-nitro-*m*-xylene.[5] From our experiment, based on a more complex matrix (entire wheat, aqueous medium, particles...) we noticed a clear trace of one given reaction in the plan formed by PC2 and PC3. However, it was impossible to superimpose another reaction (same conditions) on the same track. PCA and mainly PC1 is largely affected by the batch to batch variations. These variations disable the use of PCA for the qualitative monitoring of the enzymatic liquefaction of wheat. This is illustrated for the CFC (Figures 3–5). Similar observations were made for the FO.

Quantitative aspects

One hundred and forty samples from seven different batches were analysed by HPLC for the glucose content (g/100g). The NIR calibrations were obtained by partial least squares (PLS) procedures. Due to the optical characteristics of the fibre optic probe, the wavelength range was

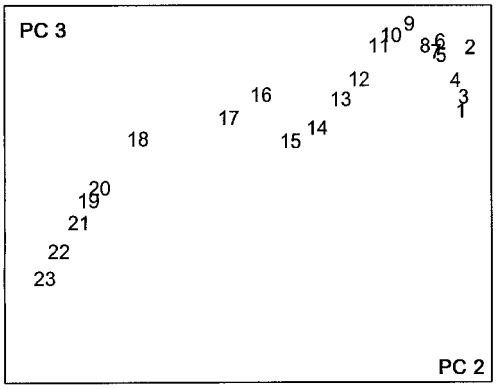

Figure 5. Spectral changes during a given reaction (CFC).

Table 1. Calibration results for the monitoring of enzymatic degradation of wheat starch by NIR using a continuous flow cell (CFC) and a fibre optic probe (FO).

Spectral pretreatment		CFC (700-2500 nm)		CFC (1100–1900 nm)		FO (1100–2500 nm)	
(1)	(2)	*SECV*	R^2	*SECV*	R^2	*SECV*	R^2
0,0,1	NONE	0.40	0.97	0.37	0.97	0.82	0.86
	DET	0.43	0.96	0.39	0.97	0.90	0.85
	SNVD	0.46	0.96	0.40	0.97	1.07	0.79
0,0,5	NONE	0.41	0.97	0.38	0.97	0.83	0.86
	DET	0.41	0.97	0.38	0.97	0.93	0.85
	SNVD	0.48	0.95	0.40	0.97	1.11	0.78

(1) Derivative, gap, smooth.
(2) Scatter correction : None, Detrend, Standard Normal Variate.

limited to 1100–1900 nm. A trial with a larger spectral range was performed for the CFC. From the results given in Table 1, the best accuracy levels were obtained for the CFC using the 1100–1900 nm range. The results are very similar when the range is extended to 700–2500 nm. The CFC device ensures an accurate determination of the glucose content which is compatible with the real time monitoring of the enzymatic process. The standard errors of cross-validation are much higher with the FO. In the latter case, the accuracy should be improved to allow a good estimate of the glucose content in the broth.

Conclusions and further prospects

The enzymatic reaction under study shows a clear pattern in the three firsts PCA axes due to the spectral changes during the reaction (PC2 and 3). PC1 represents the variations between the batches. Due to these batch to batch variations, it seems difficult to have a reliable, qualitative monitoring of the process only by the principal scores. Therefore, quantitative models must be developed from several batches.

The calibrations obtained for the CFC device allow the real-time monitoring of the reaction by a continuous prediction of the glucose content in the broth.

The accuracy obtained with the FO device needs improvements to have a good estimate of the glucose concentration. The lack of accuracy could be due to :

- the limitations of the fibre optic material itself (noisy spectra above 1900 nm)
- the way of taking the reference which is common to all the spectra; a double beam instrument should overcome this drawback.
- the broth under study is rather complex (aqueous medium, particle size effects, turbulence in the broth...)

Acknowledgements

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