Screening of pectolytic microorganisms by near infrared reflectance spectroscopy

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Introduction

Pectinolytic enzyme preparations are widely used in the food industry, mainly for extraction, clarification and stabilisation of fruit juices;[1] The preparation of these pectinolytic extracts can be improved by strain selection, breeding and fermentation optimisation. The enzymatic preparations very often contain several types of pectinolytic activity.[1,2] There is a need for screening techniques to detect pectinolytic activities and to characterise the type of pectinase for optimal use.

Near infrared (NIR) reflectance spectroscopy is a well known analytical technique in the field of food and feed.[3] More recently, its uses have been investigated in the chemical and pharmaceutical industries.[4] It has also been used for monitoring starch hydrolysis[5,6] or for the on-line monitoring of enzymatic degradation of wheat starch.[7] Some references deal with the determination of the degree of esterification of pectins by NIR,[8,9] but none could be found for the screening of strains for their pectinolytic activities.

Material and methods

Mould strains and culture conditions

Twenty mould strains, with a presumed pectinolytic activity, were selected. Eighteen of them were provided by CWBI (Centre Wallon de Bio-industries, University of Liège, Belgium) and the two remaining ones were isolated from fruits of Burkina Faso. The strains were kept at 4°C on a Potato Dextrose Agar medium.

The medium is made up of a 2% solution of a high methoxyl pectin (Herbstreith DM = 71%); 0.75% $(NH_4)_2SO_4$; 0.5% KH_2PO_4; 0.03% $MgSO_4$ $3H_2O$ and 0.05% Chloramphenicol.[10]The pH is adjusted at 4.5 with NaOH after sterilisation at 121°C for 20 minutes.

All the fermentation trials were conducted in 1 L flasks containing 200 mL medium. The spores collected were suspended in a 0.9% NaCl solution at a concentration of 10^7 spores mL^{-1}. Each fermentation flask is inoculated with $4*10^7$ spores. After five days incubation at 30°C under agitation, each broth was filtered and the filtrates containing the enzymes were kept at 4°C.

Polygalacturonase activity

10 ml of the enzymatic preparation were added to 50 ml of a 1% (w/v, pH 5.0) solution of polygalacturonic acid (PGA, Sigma P1879) and incubated for 60 minutes at 30°C. The reaction was stopped by heating at 100°C for ten minutes. The use of PGA enables the sole reaction of polygalacturonase without any effect due to methyl esterase or pectin lyase. The hydrolysates were then freeze-dried before taking their NIR spectrum. For calibration and validation purposes, the samples were analysed for their polygalacturonase activity using a UV-visible method.[1] The reducing groups freed from the PGA by the esterases react with copper sulfate and arsenomolybdate to give a complex absorbtion at 520 nm.

Methyl esterase activity

The hydrolysis of methyl esters bounds by methyl esterase leads to free acid functions and to the production of methanol. Methyl esterase activity was determined by the titration of acid functions with NaOH 0.02N.

Acquisition of the spectra, data processing

The NIR spectra (1100–2500 nm by steps of 2 nm) of the freeze-dried hydrolysates were acquired on a NIRSystems 5000 scanning monochromator (Foss-NIRSystems) using minicups and rings of 1 cm. The spectral data were obtained and treated with the ISI software (Infrasoft International–Foss).[11] The calibrations were obtained by multiple linear regression (MLR) or partial least squares regression (PLS). The ISI software allow calibration on the basis of raw spectra, of their first or their second derivatives as well as baseline correction. Trial and error is the only way to get the best analytical performances.[11] The latter are given by the determination coefficient (R^2c), the standard error of calibration (*SEC*) in the calibration step and by the determination coefficient (R^2v), the standard error of validation without or with bias correction (*SEV* or *SEVc*). A ratio *SD*/*SEC* (*SD* = standard deviation of the population) of more than 3.0 is required for quantitative determination. The higher this value the more accurate the model.[12]

Results and discussion

Screening of moulds for polygalacturonase activity (PG)

Spectral changes between 2000 and 2400 nm, due to reducing groups freed by PG, can be observed (Figure 1). The enzymes extracted from a selection of eight moulds were used to hydrolyse sodium

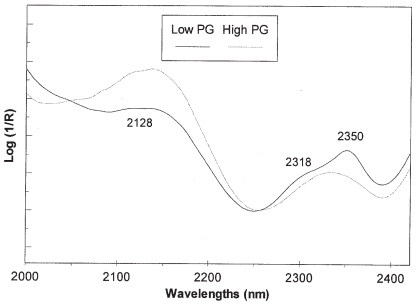

Figure 1. Comparison between hydrolysates obtained from enzymatic preparations with a low and a high polygalacturonase activity.

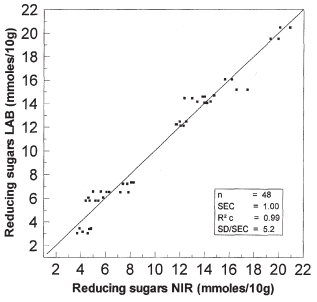

Figure 2. Relationship between actual and predicted reducing sugars (calibration set). The substrate was made of sodium pectate.

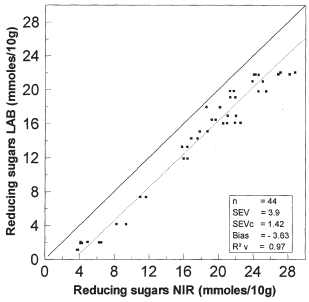

Figure 3. Relationship between actual and predicted reducing sugars (validation set). The substrate was made of sodium pectate.

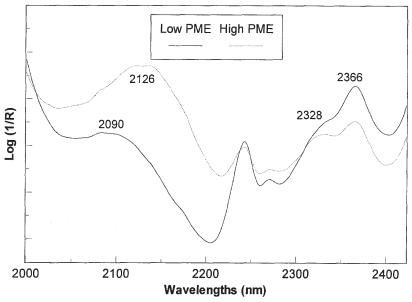

Figure 4. Comparison between hydrolysates obtained from enzymatic preparations with a low and a high pectin methyl esterase activity.

pectate. The NIR spectra of their hydrolysates were then acquired in duplicate. Sixteen other samples obtained from the hydrolysis of sodium pectate, using a purified depolymerase (Sigma P-1879), were added to build the calibration data base. The best analytical performances were obtained using the raw spectra with a "standard normal variate and detrend" baseline correction[13] (Figure 2). The hydrolysates obtained from another 20 moulds and two commercial enzymatic preparations were then used as an independent test set to validate the model (Figure 3). The validation shows a bias, probably due to variation either in the culture or in the hydrolysis step. Even without any bias correction, the model enables a classification of the moulds according to their ability to produce depolymerase. To get a quantitative estimation of free-reducing ends, it is necessary to include three enzyme preparations with known activities to cover the range and to correct the bias.

Screening of the moulds for pectin methyl esterase activity (PME)

Major spectral changes, due to esterification of a high methoxyl pectin by two enzymatic mixtures having a low and a high PME activity, can be observed between 2000 and 2400 nm (Figure 4).

The enzymes extracted from a selection of eight moulds were used to hydrolyse a high methoxyl pectin (Degree of esterification = 75). The NIR spectra of their hydrolysates were then acquired in du-

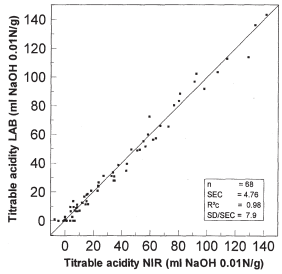

Figure 5. Relationship between actual and predicted titrable acidity (calibration set). The substrate was made of a high methoxyl pectin (DE = 75).

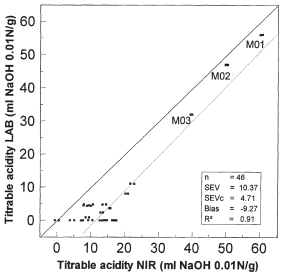

Figure 6. Relationshio between actual and predicted titrable acidity (validation set). The substrate was made of a high methoxyl pectin (DE = 75).

plicate. Twenty six other samples obtained from the hydrolysis of the same pectin using a commercial enzyme (Novo–Nordisk, Pectinex 100L) were added to build the calibration database. The best analytical performances were obtained using the raw spectra with a "standard normal variate and detrend" baseline correction (Figure 5). The hydrolysates obtained from another batch of 20 moulds and three commercial enzymatic preparations were then used as an independent test set to validate the model (Figure 6). Again, the validation shows a bias, probably due to variation either in the culture or in the hydrolysis step. Even without any bias correction, the model enables a classification of the moulds according to their ability to produce pectin methyl esterase. Only three mould strains showed interesting PME activities. To get a quantitative estimation of the titrable acidity linked to the PME activity, it is necessary to include three enzyme preparations with known values to cover the range and to be able to correct the bias.

Conclusions

NIR reflectance spectroscopy allows the screening of enzymatic preparations for their polygalacturonase activity by predicting the concentration in reducing groups freed by the hydrolysis, in standardised conditions, of sodium pectate. Quantitative results can be obtained if a bias correction is performed with three standards covering the range. NIR also enables a classification of enzymatic preparations according to their ability to hydrolyse methyl ester bounds of a high methoxyl pectin. The quantitative determination of pectin methyl esterase activity also requires a bias correction with three commercial enzymatic preparations covering the whole range of titrable acidity. Of the 20 mould strains under evaluation, only three showed a significant PME activity.

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