Monitoring of the de-esterification and depolymerisation of pectins by Near Infrared Reflectance spectroscopy

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Introduction

Pectin is a natural polymer made of α 1-4 galacturonic acid units that is largely found in plant cell walls. The monomers can be methylatyed or acetylated leading to pectins with various degrees of esterification (DE). Pectic substances can be demethylated by pectin-methyl esterases or can be also depolymerised by polygalacturonases[1,2].

The aim of the study is to evaluate the potential and the limitations of NIR for the monitoring of bio-reactions. The work is focussed on the enzymatic hydrolysis of pectic substances, mainly their de-esterification (or demethylation) by pectin methyl esterases (PME) and their depolymerisation by polygalacturonases (PG). The results obtained on the liquid samples (LS) were compared to those obtained on the corresponding freeze-dried samples (FDS).

Materials and methods

Depolymerisation of galacturonic acid

To prevent any side reaction of de-esterification, pure polygalacturonic acid (PGA) is used as substrate for the sole monitoring of the depolymerisation. Calibrations were derived from the anion-exchange HPLC determinations of individual oligogalacturonides[3,4].

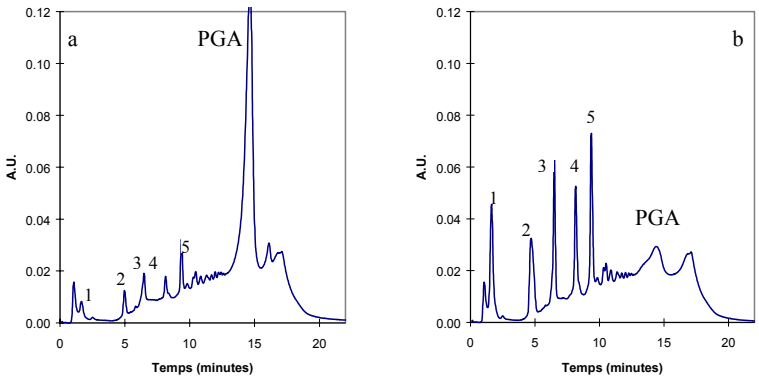

Figure 1. HPLC chromatograms showing individual oligogalacturonides (1-5) and the polygalacturonic acid used as substrate.

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De-esterification of a high methoxyl pectin

A high methoxyl pectin (DE=71) (1% aq solution) is de-esterified by a pure PME (Sigma P-0764). The degree of esterification (DE) is obtained by the HPLC analysis of the released methanol.

Simultaneous de-esterification and depolymerisation of pectin

The same pectin is hydrolysed by an enzymatic preparation containing both pectin methyl esterases (PME) and polygalacturonases (PG). The increase of reducing ends (RED) due to the PG activity is measured by UV at 280 nm. The DE is determined by the HPLC determination of the released methanol.

NIR measurements and data treatments

In the course of their hydrolysis, the liquid pectins LS (1% aq. solution) were measured using a transmittance continuous flow-cell. Some samples were collected, freeze-dried (FDS) for reference and NIR measurements (Foss-NIRSystems 6500 with small ring cups). The data were treated using the Foss-Infrasoft Winisi software v1.5. Calibrations are developed on n batch reactions and are validated on the n+1 reaction. For the LS trial, the number of samples with a reference value could be enlarged by interpolation[3].

Results and discussion

Depolymerisation of galacturonic acid

For the sole monitoring of the depolymerisation of the pure polygalacturonic acid, provided a bias correction is applied, LS measurements allow a global monitoring of the reaction on the basis of the sum of the oligogalacturonides (SD/SEPc= 10.9) (figure 2). The SD/SEP values are too low to allow the quantitative determination of individual oligogalacturonides (figure 2).

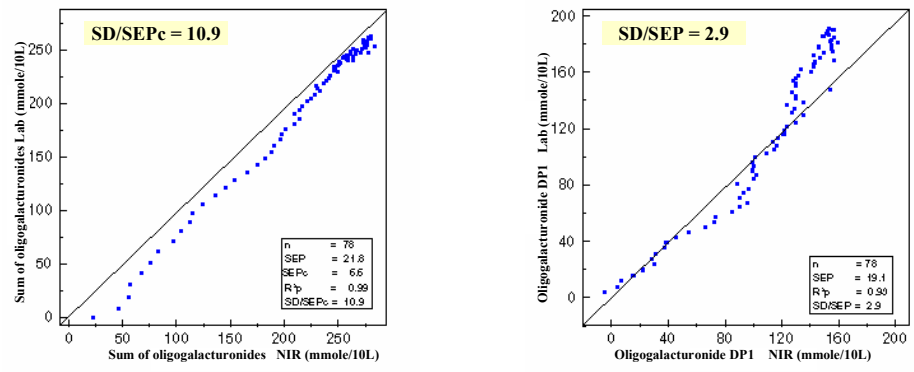

Figure 2. Calibration developed on the liquid samples (1% aq. solution) for the sum of the oligogalacturonides and for the monomer (DP1) freed during the depolymerisation of polygalacturonic acid.

For a better understanding of the mechanisms involved, a freeze drying preparation step is required. The NIR measurements achieved on the freeze dried samples (FDS) lead to useful calibration results for the major individual oligogalacturonides. For DP1, DP2, DP3 and DP4, the SD/SEP ratios are respectively 7.4, 5.8, 3.5 and 2.0. NIR on the freeze dried samples as well as the HPLC allow the monitoring of individual oligogalacturonides for a better understanding of the mechanism involved (figure 3).

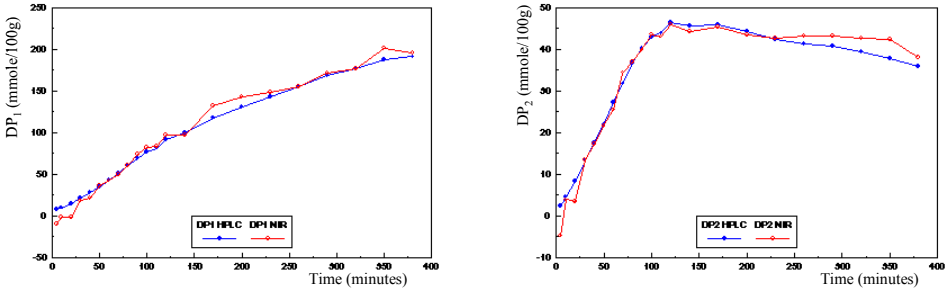

Figure 3. HPLC and NIR monitoring of individual oligogalacturonides (DP1 and DP2) freed during the depolymerisation of polygalacturonic acid (freeze dried samples).

Depolymerisation of galacturonic acid

Major spectral changes due to the de-esterification of a high methoxyl pectin can be noticed between 2000 and 2400 nm, especially on the freeze dried samples (figure 4). These spectral bands were also observed in previous work[5]. A clear correlation (R^2=0.983) can be observed between the DE and the first axis of the PCA (figure 5). With SD/SEP of respectively 9.4 and 8.8, the DE can be predicted either on the liquid or on the freeze dried samples.

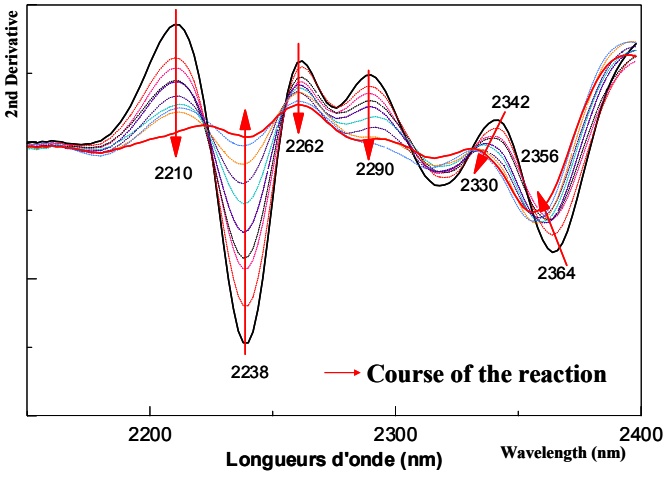

Figure 4. Spectral changes (second derivative) observed between 2000 and 2400 nm during the course of the de-esterification of a high methoxyl pectin.

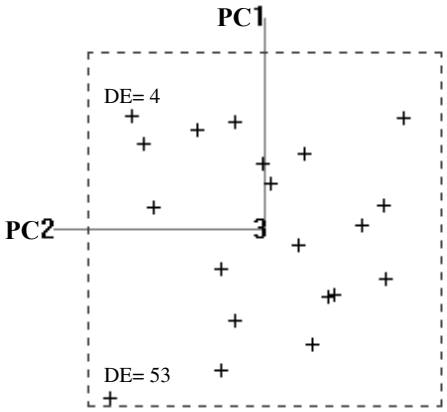

Figure 5. Principal component analysis (PC1 – PC2 projection) showing the correlation between the degree of esterification and PC1.

De-esterification and depolymerisation of a high methoxyl pectin

Enzymatic complexes usually contain both PME and PG enzymes so that the de-esterification and depolymerisation are taking place at the same time and are influencing each other. These reactions were monitored using the degree of esterification (DE) and reducing ends (RED) determinations. Validation remains satisfactory; however linearity problems negatively affect the validation results (table 1).

Table 1. Performances of the calibration equations developed for the determination of the degree of esterification and the reducing ends during the course of the de-esterification and the depolymerisation of high methoxyl pectin.

		Calibration				Validation			
		n	**SD**	**SEC**	**SD/SEC**	**n**	**SD**	**SEP**	**SD/SEP**
DE	**FDS**	158	10.1	1.31	7.8	44	7.5	4.19	1.8
	LS	224	9.3	0.98	9.5	64	7.2	1.43	5.0
RED	**FDS**	158	3165	223	14.3	44	2749	652	4.2
	LS	224	3103	220	14.8	64	2656	325	8.2

FDS = freeze dried samples, LS = liquid samples

n = number of samples, SD = standard deviation, SEC = standard error of calibration

SEP = standard error of prediction.

Conclusions and further prospects

About pectins and pectic substances

The main difficulty of pectins solutions lies in the low concentration levels. Due to their gelling properties, above 1% pectins can rapidly forms strong gels.

Depolymerisation of galacturonic acid

NIR measurements of liquid samples allow a global monitoring of the reaction by predicting the sum of the oligogalacturonides. For their individual prediction, a freeze drying step is required prior to the NIR measurements.

De-esterification of a high methoxyl pectin

The de-esterification of a HM pectin can be monitored by predicting the degree of esterification either on the liquid or on the freeze-dried samples.

Simultaneous de-esterification and depolymerisation of a high methoxyl pectin

The simultaneous monitoring of both the de-esterification and the depolymerisation of a HM pectin can be achieved either on the liquid or on the freeze-dried samples. In the case of liquid measurements, the interpolation on the basis of reference determinations allows an increase of the number of samples available for calibration and by the way improves SEP values.

References

1. A. Baron and J.-F. Thibault, Les enzymes pectinolytyiques. In Collection Biochimie appliqué. Bordas-Paris, 143-163 (1985).
2. D. Campos Guttierez, PHD thesis, Faculté Universitaire des sciences agronomiques, Gembloux-Belgium, 262 p (1993).
3. G. Sinnaeve, PHD thesis, Faculté Universitaire des sciences agronomiques, Gembloux-Belgium, 204 p (2001).
4. H. Endress, H. Omran and K. Gierschner, Lebensm. Wiss. U Technol., 24, 80-85 (1991).
5. H. Haas and M. Jäger, J. Food sc., 1087-1089 (1986). http://fsmail.freeserve.com/webmail/graphics/Smiley/animes/sy_eclat.gif