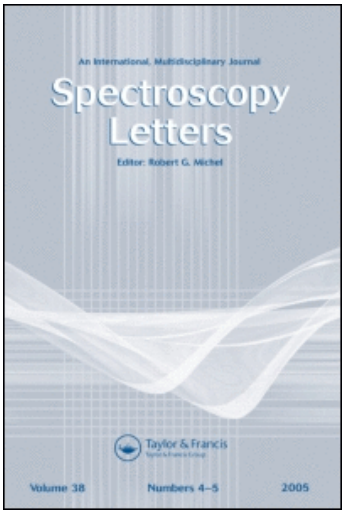

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α-NH_2 Determination from Biological Infrared Spectra

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α–NH_2 DETERMINATION FROM BIOLOGICAL INFRARED SPECTRA

Key words: α–NH_2, biological samples, Mid-FTIR, multivariate analysis.

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Abstract.

The use of Mid-infrared spectroscopy for the analysis of food products has only recently developped with the advent of Fourier Transformed spectroscopy and other techniques like Attenuated Total Reflectance, diffused reflection combined to the use of powerful micro-computers. We have recently reported to use a combination of multidimensional statistical analysis and Mid-FTIR spectroscopy for the quantitative determination of sugars in a biological sample. In this paper we have evaluated the use of such a method for the quantitative measurement of α–amino groups from amino-acids, peptides and proteins. The spectral

region where the characteristic absorption bands of such groups are located, ranges between 1200 cm-1 and 1900 cm-1. Water features a major absorption band in this region (1500-1700 cm-1). This superimpose with amide I and II bands.

The standard deviation for each and every wavelength, calculated for all the spectra of the calibration set, show the existence of two absorption bands in the 1500-1900 cm-1 region which means that the observed variations in this zone are not only due to water but are also due to two peaks centered at 1650 cm-1 and 1540 cm-1 (with a hollow at 1600 cm-1) that are characteristic of protids.

The contribution of the first four axes of the PCA, axes 5, 1, 4 and 2, to the total inertia percentage are 2.37%, 53.36%, 3.92% and 28.82% respectively. The correlation coefficient between the major axis, axis 5, and the chemical values of

α–NH_2 is 0.311 and the second axis, axis 1, increases this value to 0.541. The first 10 axes were used to establish the prediction equation; the correlation coefficient value is very high : 0.978.

Good predictions were obtained; mean and standard deviation associated to the predicted concentrations of α–NH_2 content, valued 0 g/ml and 0.12 g/ml respectively. Hence, we have established the possibility of determining, from a MIR spectra, the α–NH_2 content.

INTRODUCTION

Mid-infrared spectroscopy is widely used for the determination of polypeptides and proteins secondary

structures (Elliott, 1950). Miyazawa et al., (1960) carried out normal coordinate calculations on model compounds which showed three strong infrared bands of proteins, called amide I, II and III. The most representative spectral region is that between 1600 and 1700 cm-1 which is characteristic of amide I.

Near Infrared Reflectance spectroscopy is the most widely used method for the quantitative analysis of major biochemical constituants in food industry. More and more constituants among which, water, proteins, lipids and sugars, are being analysed by this method (Osborne et Fearn, 1986; Williams & Norris, 1987).

With the advent of Fourier Transformed Infrared spectroscopy and of new techniques (Coates et al., 1987a-b) such as Attenuated Total Reflectance (ATR), photoacoustic detection and diffused reflection, combined with the use of powerful micro-computers, considerable progress have been made in the field of Mid-infrared spectroscopy. ATR is an analytical technique that has important potentialities with regard to food products analysis (Fuller and Griffiths 1978, Depecker & al., 1985; Van de Voort & Ismail, 1991; Cadet & al, 1991). Few cases of applications of MIR spectroscopy to food analysis has been reported among which cereals and other cereal products (Renard et al., 1987) and the quantitative dosage in milk and milk products (Crocombe & al., 1987)

The price of the sugar cane is based on the sucrose content measurements. It is also important to be able to measure and forecast, during the industrial process, the quality of the sugar cane juices, the amount of sugar than can cristallize and the lost of sugars in molasso. For the determination of these different parameters, in addition to the sucrose content

measurement, other constituants should be quantified, notably glucose, reducing sugars, potassium and sodium ions and alpha-amino acids. We have recently shown that it is possible to measure by MIR spectroscopy, to quantify sugars in sugar cane juices (Cadet & Offmann, 1995; Cadet & Offmann, 1996).

The aim of the present work is to evaluate the interest of using principal component analysis and Principal Component Regression on the mid infrared spectra, to predict the concentrations of the α–NH_2 (amino-acids, peptids, proteins) in sugar cane juices.

MATERIAL AND METHODS

Biological samples.

After pulverization in a desintegrator, 1000 g of sugarcane is pressed for two and a half minutes at 250 bars by an hydraulic press. The raw juices that are obtained and that contains impurities and fibres are filtered. Via a highly porous plastic filter, this filtration procedure is carried out instantenueously when ATR cells are filled.

The reference α–amino group content is measured colorimetrically according to the nynhydrine method from the International Commission for Uniform Methods of Sugar Analysis (Schneider, 1982).

The calibratrion set is constituted of 20 biological samples while the verification set is composed of 15 samples.

Mathematical treatment

The softwares used for the mathematical treatments were developped written in "C" language in our laboratory.

Principal Component Analysis (PCA), Principal Component Regression (PCR) and the "spectra pattern" notion used in this paper were extensively described previously (Cadet & al, 1991, Cadet & al., 1995).

Mid FTIR spectra.

Mid-Fourier Transform Infrared (Mid-FTIR) spectra were collected on a Michelson-100 Fourier transform spectrophotometer. Attenuated total reflectance spectra were obtained with a Specac Overhead ATR system. The crystal of the reflectance element is made from zinc selenide, a material that is quite inert to water; it is quite rapidly cleaned between samples by being sprayed with water and then dried with filter paper.

The data were recorded from 1180 to 1900 cm^{-1} in 4 cm^{-1} increments at log(1/R), in which R is the ratio of the reflected intensity for the background to that of the sample. Although the ATR experiment does involve the reflection of the radiation within a crystal, the interaction of the radiation with the sample is the transmittance of radiation through the sample; this depth of penetration is wavelength dependent, but it is passing through a finite layer of the sample. For this reason, plots can read according to absorbance (or transmittance). The combination of four scans resulted in an average spectrum. The intensity the spectra was low; the highest peaks had log(1/R) values less than 0.6 on baseline spectra.

RESULTS AND DISCUSSION

Reference values and spectra.

The calibration set is constituted of a collection of 20 biological samples. The α–NH_2 content of the calibration set ranged

from 0.34 % to 1.12 % (g/100 ml) with a mean and standard deviation (SD) values of 0.58 % and 0.22 % respectively. The verification set is constituted of 15 samples. The $\alpha-NH_2$ content ranged from 0.33 % to 1.05 % (g/100 ml) with a mean and standard deviation (SD) values of 0.58 and 0.21%.

The major bands that are characteristic of proteins and that are of interest in MIR, are located between 1200 and 1700 cm-1 (Susi, 1972).

These bands are :
- amide I, C=O stretch, 1710-1580 cm-1,
- amide II, N-H bending, 1580-1500 cm-1,
- amide III, C-N stretch and bending, 1300-1200 cm-1.

The absorption wavelengths differ in the case where a peptide is isolated to that when it is included in a protein. This is due to intra and inter-chains coupling effects. The nature and the secondary structure of the protein considerably influence the position of these absorption bands.

The analysis protids faces a major obstacle : water is a strong IR absorber between 1500 and 1800 cm-1. This band is superimposed to the bands characteristic of amides I and II. One way in which this problem may be solved is to dissolve the proteins in heavy water (D_2O) (Susi et al, 1967, Timasheff et al., 1967). However, this cannot be readily applied to biological samples.

The Mid-FTIR spectrum of a sample raw sugar cane juice is given in figure 1. The spectrum was recorded between 1180 and 1900 cm-1. The spectra obtained with such complex biological samples are the result of the different absorption bands of the major constituants: water and protids. The absorption band of water (1500-1700 cm-1) is more

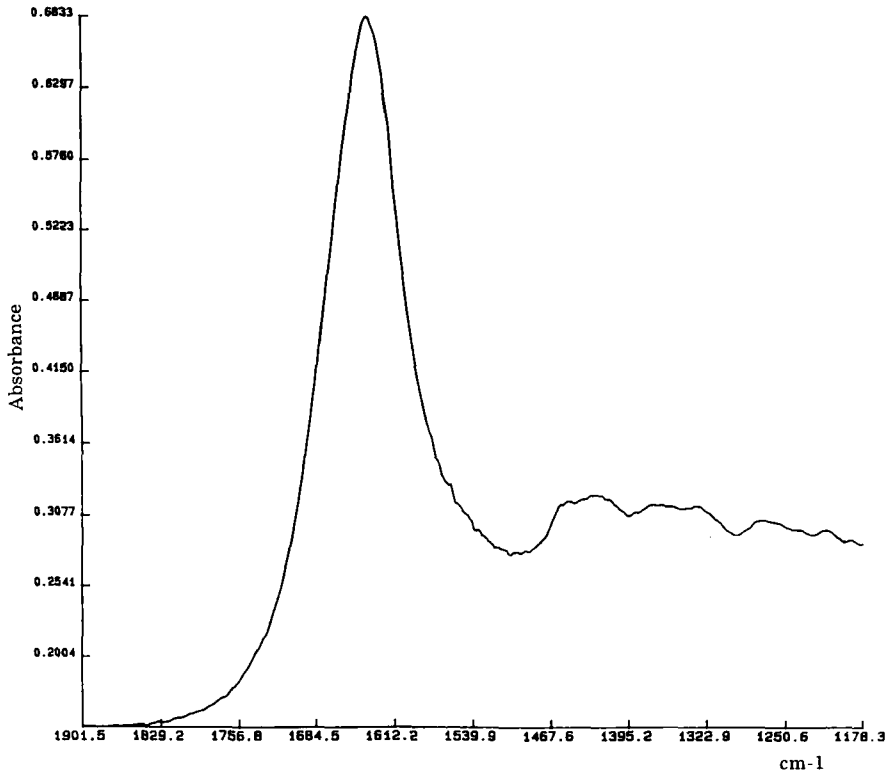

Figure 1. Mid-FTIR of a Biological sample.

important than that of the protids between 1500 and 1180 cm-1. The peak found between 1500-1700 cm-1 completely masks the protids amide I and II bands. The deconvolution of a pure water spectrum and of a biological sample spectrum where several shoulders are observed, shows that in the latter the peak is a result of several phenomenons (Figure 2).

The mean absorbance and the standard deviation for each and every point (wavelength) have been calculated for all the spectra of the calibration set. The standard deviation (SD)

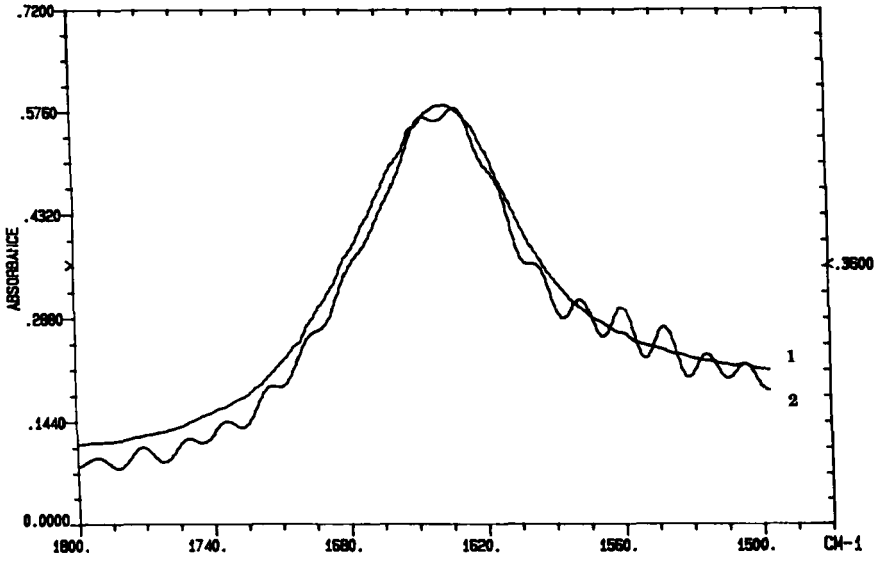

Figure 2. Mid-FTIR spectra after deconvolution: (1) water, (2) biological sample.

values are featured in figure 3. This figure shows : (i) firstly, that the SD variation is associated with the constituants content variation and that therefore, a quantification in the zones where such variation exists is possible ; (ii) secondly, that the existence of two absorption bands in the 1500-1900 cm-1 region, (water features only a single large band in this region), means that the observed variations in this zone are not only due to water but are also due to protids characterised here by two peak centered at 1650 cm-1 and 1540 cm-1 and by a hollow at 1600 cm-1 (Yang, 1985).

PCA, PCR and predictions.

Multidimensional statistical analyses give a simple way to have a global description of a set of variables. Performing

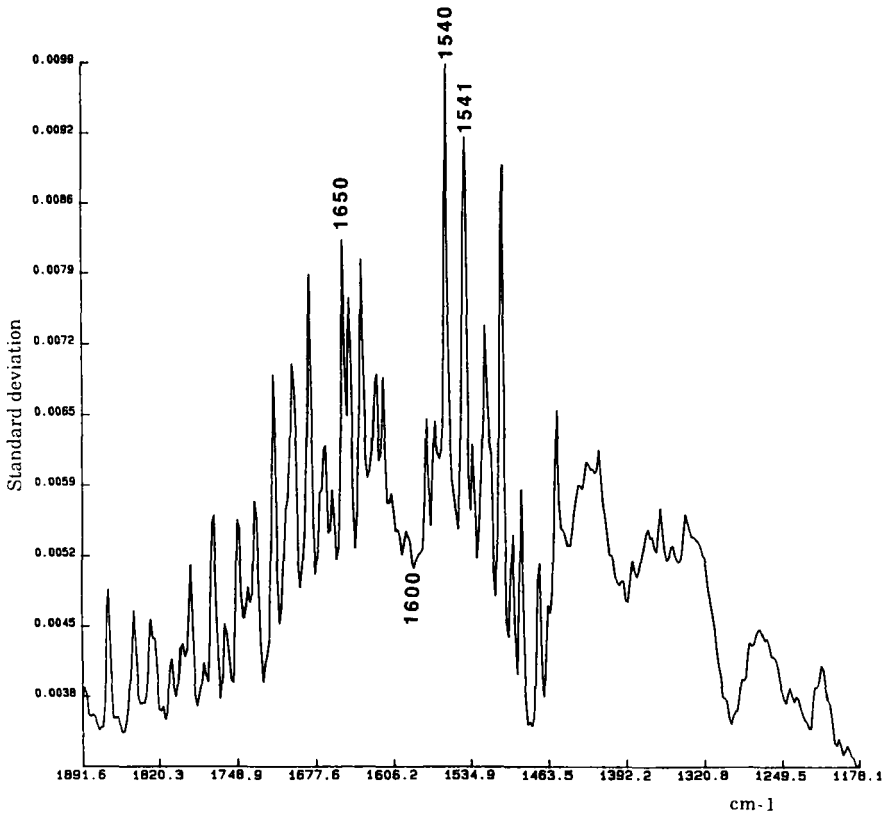

Figure 3. Standard deviation of the absorbance values at each wavelength for the calibration set.

an analysis is simply an attempt to find new system of axes better adapted to the descrition of data than the old one. This creation of axes can be done with no assumption of the nature of the differences between samples. Principal Component Analysis (PCA) allows the creation of such a system of axes not correlated with each other (the principal component) which are a linear combination of the original ones. Hence, in order to extract the spectral information that correspond to

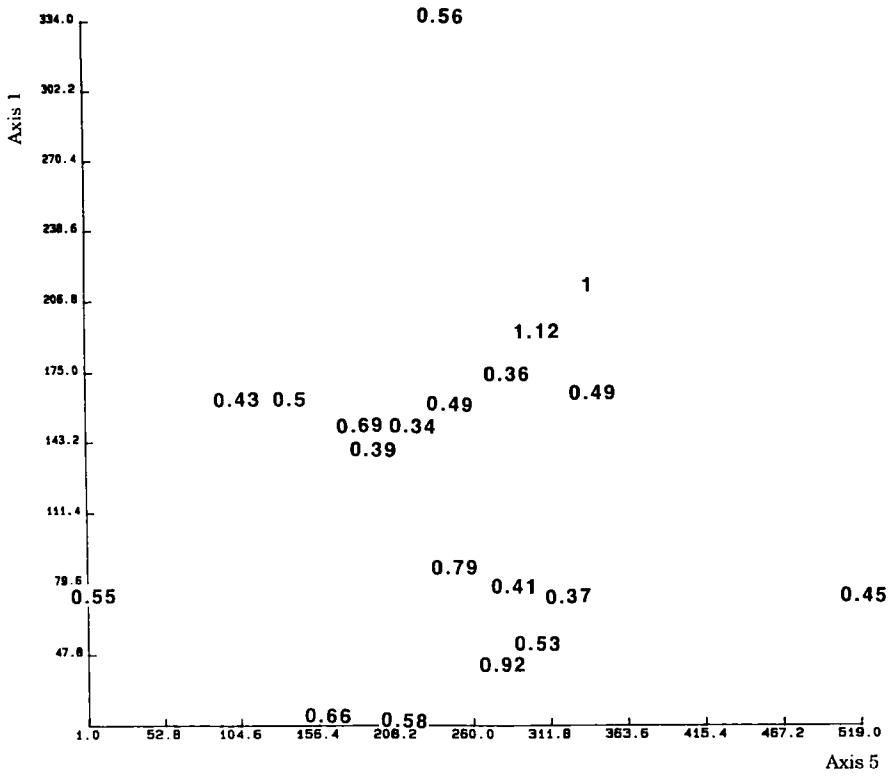

Figure 4. Factorial map associated with α–NH_2 as described by axis 5 and axis 1.

protids, the collected spectra of all the biological samples from the calibration set were entered into a PCA.

The contribution of the first four axes of the PCA (axes 5, 1, 4 and 2) to the total inertia percentage are 2.37%, 53.36%, 3.92% and 28.82% respectively.

The projection of the different samples in the plane formed by axis 5 and axis 1 (figure 4) does not clearly classify the samples according to the concentrations of α–NH_2.

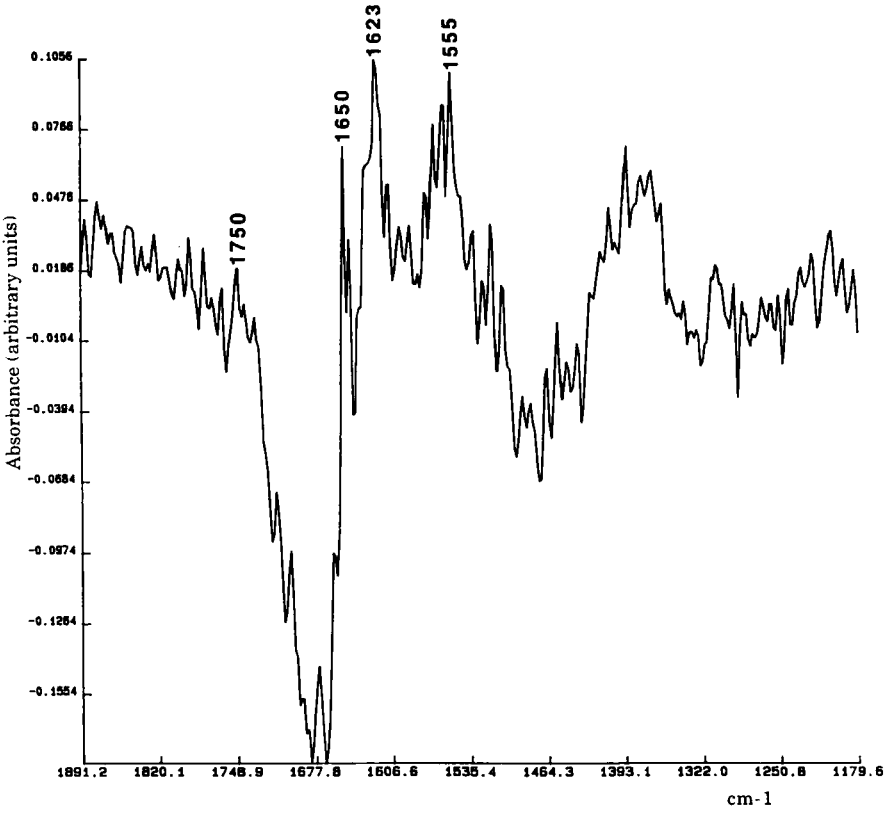

Figure 5. Spectral pattern associated with axis 5.

The collection of spectra is modelised by PCA into a sum of characteristic signals which form a spectral pattern (Le Nouvel., 1981, Robert *et al.*, 1987, Devaux et al, 1988). This spectral representation of the principal component of PCA features characteristic absorption bands of biochemical constituants in a sample.

The spectral representations of the two major axis which are essentially associated with α–NH_2 content are

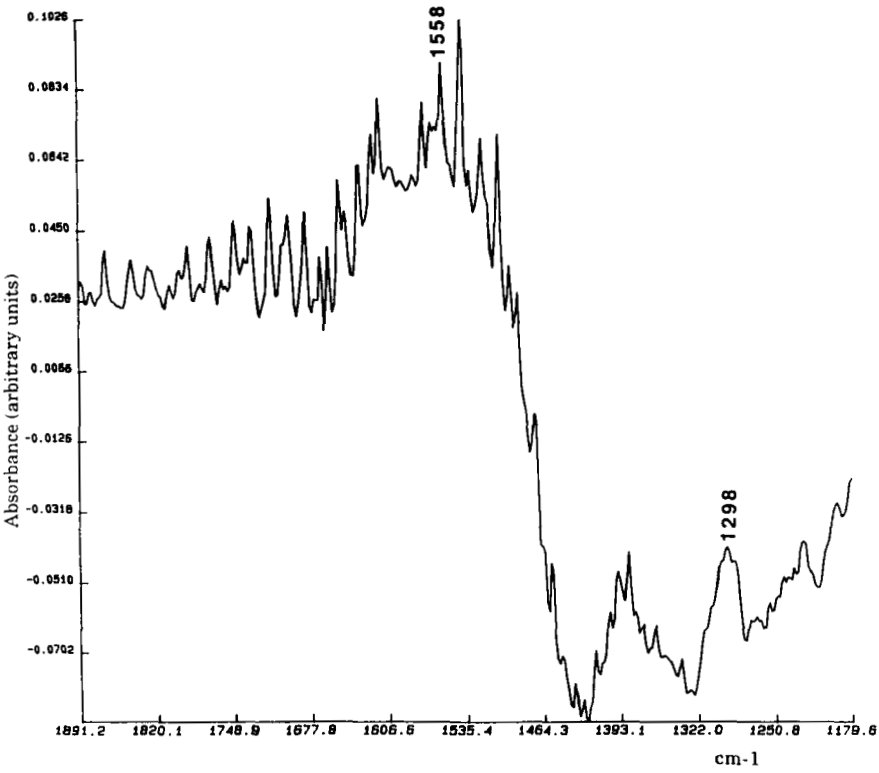

Figure 6. Spectral patterns associated with axis 1.

given in Figure 5 and 6. The spectral pattern that describes the principal component (axis 5), as shown in figure 5, features absorption bands centered at 1555 cm-1 and 1623 cm-1 that can be associated are with protids (amide II and amide I). These zones are opposed to the 1650-1750 cm-1 region which is essentially representative of H_2O. The correlation coefficient between axis 5 and the chemical values of fructose is 0.311 and axis 1 increases this value to 0.541. Figure 6 (axis 1) show absorption bands centered at 1298 cm-1 and 1558 cm-1 that can be associated with the amide III, C-N stretch and bending and the amide II, N-H bending.

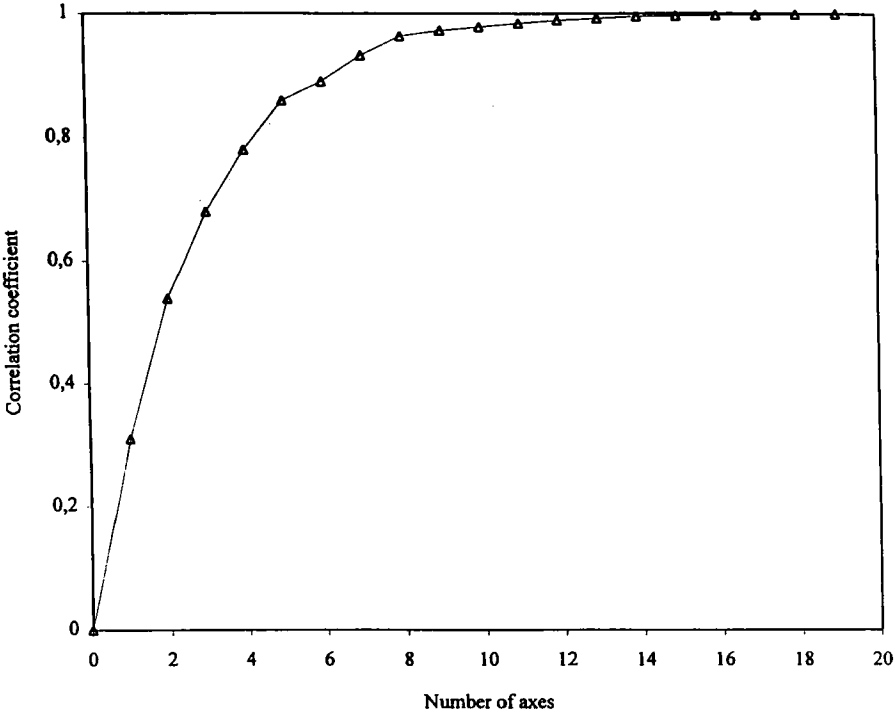

Figure 7. Percentage of inertia as a function of the number of axes as assessed by PCA applied to the calibration set.

Principal Component Regression on the calibration set scores as assessed by PCA were carried out in order to establish prediction equations that linked spectral data to α–NH_2 content.

Figure 7 gives the correlation patterns between the axes and the α–NH_2 content. The first 10 axes were used to establish the prediction equation. The correlation coefficient value is still very high (0.978).

Table 1. Difference between the reference and the predicted α–NH_2 values.

Sample number	Reference (a)	Predicted (b)	b-a
1	0.82	0.80	-0.02
2	0.47	0.41	-0.06
3	0.37	0.61	0.24
4	1.05	0.80	-0.25
5	0.72	0.66	-0.06
6	0.4	0.41	0.01
7	0.8	0.70	-0.10
8	0.33	0.33	0.00
9	0.74	0.59	-0.15
10	0.42	0.44	0.02
11	0.59	0.58	-0.01
12	0.51	0.63	0.12
13	0.56	0.67	0.11
14	0.56	0.70	0.14
15	0.38	0.35	-0.04
		mean	0.00
		Standard deviation	0.12

Table 1 gives the predicted concentrations of α–NH_2 content, mean and standard deviation valued 0 g/ml and 0.12 g/ml; good predictions were obtained.

CONCLUSION

We have recently shown that it is possible to identify and to measure with a good precision the concentrations of all sugars from a biological sample constituted of a ternary mixture of oses (sucrose, fructose and glucose). In this paper we have established the possibility of determining, from the same MIR spectra but in a different spectral region, α–NH_2 content.

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