Near-Infrared Spectroscopy As Applied to Starch Analysis of Digestive Contents

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The digestibility of starch, which is an important and common source of energy for animals and humans, is widely studied in nutrition research. Starch content is determined either in intestinal or in fecal samples. However, the biochemical methods used to quantify starch in digesta are time consuming and destructive. A rapid and nondestructive method, based on near-infrared spectroscopy, was developed to measure the level of starch in the jejunal digesta of cannulated pigs. Samples were chemically analyzed to assess a prediction equation by applying a principal component regression. The equation was proved to be reliable and allowed the quantification of native and pregelatinized starch in the digestive residues. Near-infrared spectroscopy appears to be a simple and promising technique for nutritional studies, capable of providing a range of information of nutritional value.

Keywords: *Near-infrared spectroscopy; starch; nutrition*

INTRODUCTION

Starch is the principal storage component of higher plants such as cereals, pulses, and tubers and constitutes the main source of available carbohydrate in human and animal diets (Bornet *et al.*, 1990). Native starch exists in the form of crystalline granules, the size and form of which vary according to their origin. The digestibility of starch is affected by its source (Gallant *et al.*, 1992) as well as by the technological processing conditions applied to convert the native starch into food (Colonna *et al.*, 1992). The bioavailability of starch in the upper part of the intestine has been extensively studied during the past 20 years in order to find foodstuffs adapted to specific nutritional needs. As an example, athletes and patients suffering from diabetes would benefit from slowly digested and absorbed carbohydrates (Jenkins, 1982; Welch, 1991; Wolever and Miller, 1995). A common way to study *in vivo* digestion of starchy foods is to directly collect intestinal samples from humans using the intubation method (Flourié *et al.*, 1988; Faisant *et al.*, 1995a) or the ileostomy model (Andersson, 1992). It is also possible to use animal models such as pigs or rats by fitting them with cannulas in different parts of the small intestine (Sambrook, 1979). The pig, in particular, is similar to humans with respect to several aspects of its nutrition (Moughan *et al.*, 1994). Such an animal model allows complete studies of starch transit time, digestion, and absorption. Due to inherent variations between individuals, however, numerous samples need to be taken from a sufficiently large number of subjects to ensure representativity. As a consequence, a large number of samples are produced and analyzed. The methods commonly used to characterize starch in digesta are based on biochemistry (Thivend *et al.*, 1965; Karkalas, 1985; Englyst *et al.*, 1992; Faisant *et al.*, 1995b). Although these methods provide valid results, they are time consuming and destructive.

Near-infrared spectroscopy has been widely used to predict the chemical composition of raw materials and food products. Starch content, in particular, has been determined in wheat, barley, and peas (Williams and Norris, 1987), using absorption bands at about 2276 and 2100 nm and in the 1400−1600 nm region. The bands observed between 1400 and 1600 nm correspond to first overtones of hydroxyl groups. The precise position of these bands is very sensitive to hydrogen bonding in the starch molecule. While the wavelength at 2100 nm is assigned to a combination band that involves C−O stretch and O−H deformation vibrations, that at 2276 nm characterizes a combination band of O−H and C−C stretch vibrations. Few studies have attempted to characterize the chemical composition of digestive residues using near-infrared spectroscopy. Recently, however, Stein *et al.* (1996) proposed a method to quantify the faecal carbohydrates using the analysis of specific absorption bands in the 700−2500 nm near-infrared area. Although this method appears attractive, it is not specific to starch or starch digestion products.

In the present work, near-infrared spectroscopy and principle component analysis were used to develop a rapid method for limiting the number of chemical analyses required to predict the level of starch in pig digestive contents.

MATERIALS AND METHODS

Animals. Six female pigs, 40−45 kg weight at the time of operation, were used for the study. They were fitted with a simple Y cannula (8 mm internal diameter) in the upper jejunum, approximately 75 cm beyond the pylorus. The cannula was exteriorized through the body wall in the region of the right flank. Pigs were allowed 7 days recovery after the operation and were kept at room temperature in individual cages. They were fed twice a day (9:00 a.m. and 4:00 p.m.) with 500 g of food (dry matter basis). The experiment did not begin until the animals had returned to normal dietary intake. The day before the experiment, pigs received starch-free meals.

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Animal treatment was always in accordance with French legislation.

Experimental Diet. Two diets were composed of 225 g of meat flour (55% fat), 37.5 g of maize oil, 1.7 g of pea hulls, and 180 g of equivalent digestible maize starch. The starch (supplied by Roquette Industries, Lestrem, France) was either native (210 g, Amidon standard) or pregelatinized (190 g, PregefloM).

The meal contained 1447 kcal (31% from lipids, 28% from proteins, and 41% from carbohydrates).

Experimental Design. Animals ate each of the meals twice, making a total of four studies for each pig, except for two pigs where the study had to be interrupted before the fourth experiment. Before beginning the experiment, the cannula was rinsed with pure water heated at body temperature. Then, a rubber balloon inflated with 20 mL of air was placed through the cannula beyond the site of collection, to prevent any loss of digestive content. Animals were fed at 9:00 a.m. with the experimental diet and digesta were continuously collected in chilled receptacle at 0 °C during a period of 10 h. Every 30 min, the samples collected were weighed, frozen in liquid nitrogen, and stored at −70 °C. The samples were freeze-dried, ground and mixed with a spatula, and oven-dried (40 °C, 24 h) before further analysis. The total amount of studied samples was 462.

Chemical Analysis. Starch content in the digesta (100 mg of dry matter) was measured using the method developed by Faisant *et al.* (1995b), which is specific for starch and maltooligosaccharides produced by starch digestion. This method involves the gelatinization of starch at 100 °C and the dispersion of amylose and amylopectin in 2 N KOH. The hydrolysis of starch molecules is achieved by an amyloglucosidase (EC 3.2.1.3, 400 AGU, Novo Nordisk Bioindustries, U.K.), and the glucose released is quantified by a glucose-oxidase colorimetric method (Merckotest, catalog no. 14365, Merck, Darmstadt, Germany).

Spectral Data. The spectral data were recorded in the near-infrared region between 1100 and 2500 nm at 4 nm intervals using a Technicon 500 Infralyzer spectrometer. Two spectra were collected in the reflectance mode for each sample. The spectral data were centered and reduced in order to minimize variation of the specular reflection resulting from the heterogeneous particle size of the samples. The pretreatment of the spectral data was performed in the following way:

$$DC_{i,j} = (D_{i,j} - M_i)/S_i$$

where $D_{i,j}$ = absorbance at the wavelength j for the sample i, $DC_{i,j}$ = corrected spectral data at the wavelength j for the sample i, M_i = average absorbance value for the sample i, and S_i = standard deviation of the absorbance values for the sample i.

Mathematical Treatments. Principal component analysis is a multivariate statistical treatment commonly applied to near-infrared data in order to describe similarities between samples (Cowe and McNicol, 1985). This data reduction method allows the assessment of spectral wavelengths that exhibit the best discrimination between the samples.

In the present work, principal component analysis was first performed on all the recorded spectra (462) to select a set of 80 representative samples. The selected spectra were divided into calibration (40 samples) and validation (40 samples) subsets that were used to define a prediction equation for starch content in the digesta. The prediction equation was obtained by applying a principal component regression on to the calibration data, and its reliability was verified using the validation set. The principal components involved in the model were introduced according to their predicting ability and not to their corresponding eigenvalues. A spectral pattern associated to the equation of prediction was assessed by combining the eigenvectors of the selected principal components in the following way:

$$SP = -\sum \alpha_i V_i$$

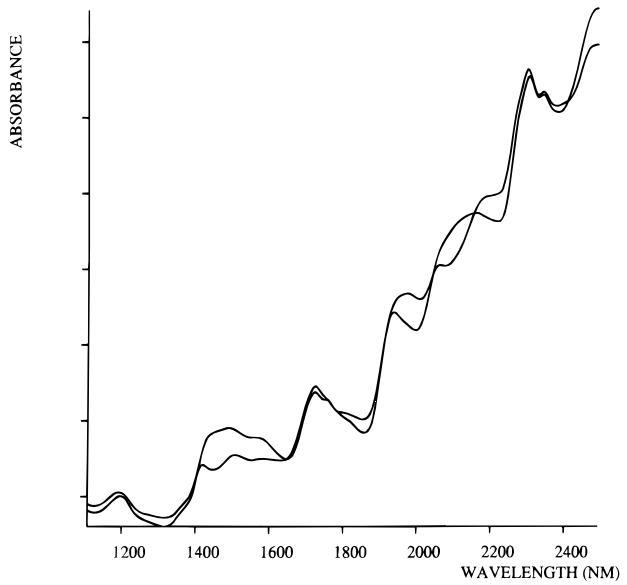

Figure 1. Near-infrared spectra of samples collected at two different times.

where SP = spectral pattern for the prediction equation, V_i = eigenvector for the selected principal component i, and α_i = regression coefficient for the selected principal component i.

RESULTS AND DISCUSSION

Near-infrared spectra recorded for two samples collected at different times from the same experiment are shown in Figure 1. Significant changes are observed between these two spectra in regions characteristic of starch molecules, i.e. between 1400 and 1600 nm, at about 2000 nm and in the wavelength range around 2100 nm. This result was indicative of the possibility of near-infrared spectroscopy for studying the digestion of starch in a complex meal.

The near-infrared spectra of all the samples were recorded and principal component analysis applied in order to select a set of 80 samples that was representative of the entire collection. The first two principle components that took 78.72% of the total variance into account defined the similarity map (Figure 2) that was used for the selection of the 80 samples. More precisely, the selection was achieved by regularly choosing the samples both along the principal components 1 and 2. In this way, the main variation of the spectral data for all the collection was described by only taking 17% of the samples into account. In addition, as the loading plot associated with the principal component 1 exhibited troughs at wavelengths characteristic of starch (see arrows in Figure 3), a regular selection of the samples along the first principal component was thought to be representative of starch content changes.

The starch content of the 80 chosen digesta were chemically analyzed. On the basis of the results, the calibration and validation samples were selected, such that the mean (M) and standard deviation (SD) values obtained for these two subsets were rather similar (calibration, M = 198.9 and SD = 133.3; validation, M = 201.8 and SD = 133.8). Moreover, regarding the calibration and validation data, particular attention was paid to ensure the presence in each subset of samples resulting from each *in vivo* experiment.

An equation of prediction for starch content in digesta was developed by applying principal component regression to the calibration samples. The first step of the

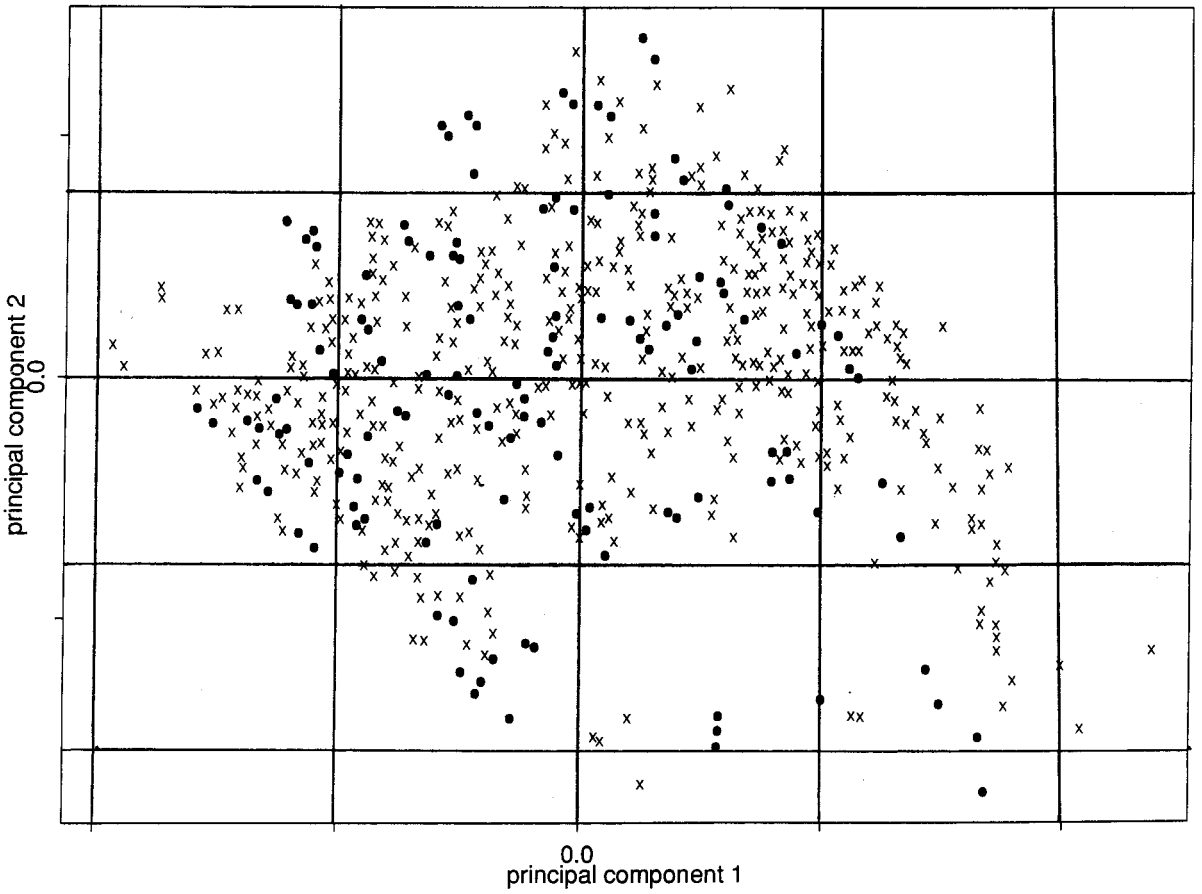

Figure 2. Principal component analysis applied to the complete collection. Similarity map is defined by principal components 1 and 2. (●) Selection of representative samples.

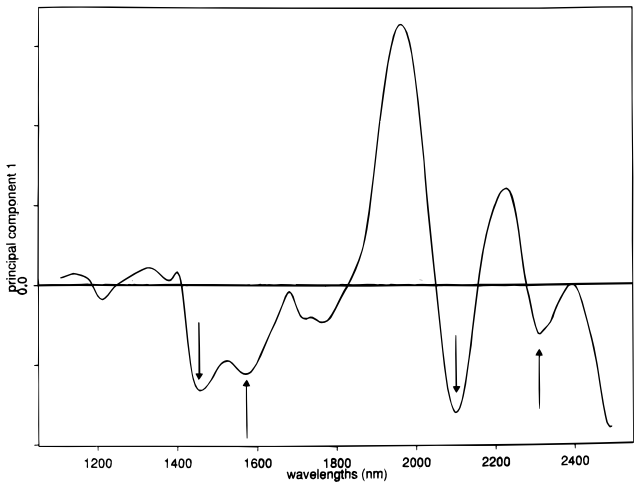

Figure 3. Principal component analysis applied to the complete collection. Loading plot is associated with principal component 1. Arrows = wavelengths characteristic of starch.

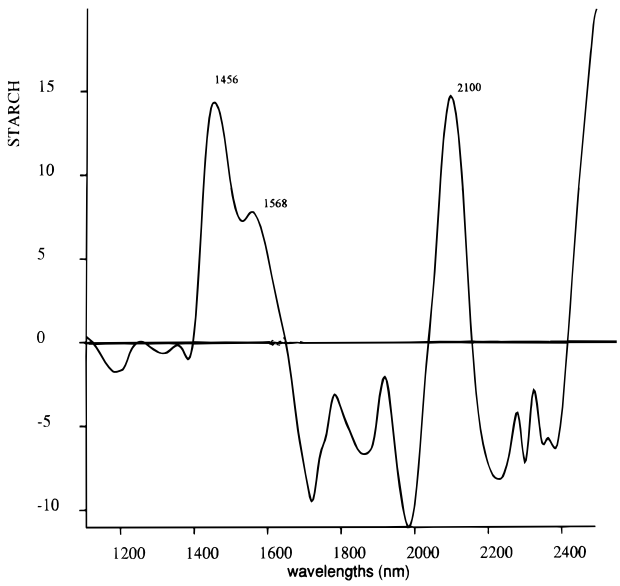

Figure 4. Spectral pattern associated to the prediction equation.

procedure (principal component analysis) indicated that only six principal components were required to describe 98.9% of the spectral variance. However, the equation, that only involved the first four principal components, was sufficient to give a good correlation to starch content. This prediction equation was characterized by a coefficient of determination and a standard error of calibration of 0.97 and 25.0, respectively. The spectral pattern associated to the equation (Figure 4) was assessed to verify the relevance of the model. The observation of characteristic wavelenghs at 1456, 1568, and 2100 nm, already assigned to overtones and combination bands of hydroxyl groups, confirmed that the equation efficiently described the digestion of starch. The significance of the equation was additionally tested by comparing the chemical and predicted starch content values for the samples in the validation set. The standard error obtained for the validation samples (26.7) was of the same order as the standard error for the calibration set (25.0). Moreover, the plot shown in Figure 5 revealed that the relationship between the predicted and chemical data was valid. The straight line fitted to the data was characterized by a constant

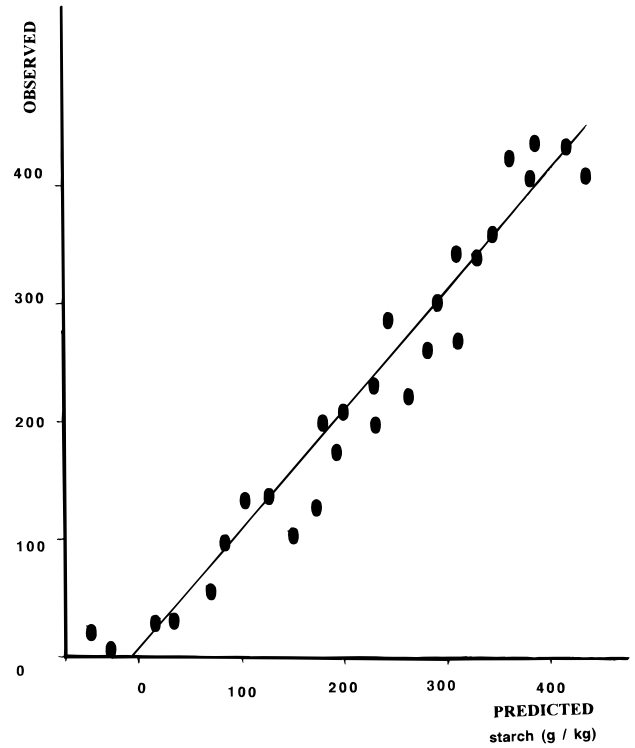

Figure 5. Prediction of starch content for the validation set, observed versus predicted values.

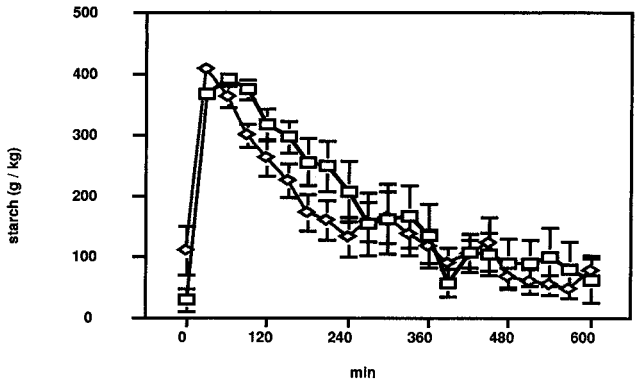

Figure 6. Starch concentration changes for (◊) native and (□) pregelatinized meals.

(3.9) and a slope (0.99) that were not significantly different from 0 (*t*-test, 0.90) and 1 (*t*-test, 0.46). The prediction equation assessed in the present work was determined from samples having starch contents lower than 400 g kg^{-1} dry matter. At the end of the small intestine, the starch level ranges between 0 and 300 g kg^{-1} (Faisant *et al.*, 1995a). The proposed method could therefore be applied to samples collected in different parts of the intestine.

The starch concentration of all the samples was determined using the prediction equation. The mean values obtained at each time point (Figure 6) revealed that the starch concentration changed in a similar way for the two meals. However, the concentration of starch in the digesta was slightly lower during the first 30 min following the meal based on pregelatinized starch and then higher between 60 and 240 min. Pregelatinized starch has a higher viscosity resulting from an increase of the water binding capacity (Colonna *et al.*, 1992). Thus, it may have been emptied more slowly from the stomach during the first 30 min, resulting in a lower concentration of starch in the digesta compared to native starch. Between 60 and 240 min, however, it is possible that the faster hydrolysis of pregelatinized starch led to a large production of maltooligosaccharides, emptied from the stomach and which could not be completely hydrolyzed and absorbed in the given time before the collection.

As the validity of near-infrared spectroscopy has been proved for the quantification of starch levels in digesta, new experiments could be performed by varying the nature of the meal (starch, proteins, ...) and the site for collecting the digesta (duodenum, ileum) in order to create a large data base. In this way, a robust equation of prediction could be determined and used for different experimental conditions.

Furthermore, near-infrared spectroscopy could be developed to study the kinetics of starch degradation. Chung and Arnold (1995) have shown that the acid-catalyzed hydrolysis of starch can be followed. They assessed a third-order polynomial function that fitted the spectral data to the concentration of oligosides in the liquid phase. The different oligosaccharides (glucose, maltose, maltotriose) were not identified using near-infrared spectroscopy; however, Safar (1995) has shown that glucose and maltose can be differentiated through the application of canonical correlation analysis to mid and near infrared spectra. It could then be expected a qualitative analysis of starch and maltooligosides resulting from the digestion.

Moreover, Millar *et al.* (1996) have shown that near-infrared spectroscopy allows the identification of physical changes during the extrusion cooking of wheat starch. Thus, infrared has the potential for the study of starch physical nature and could be employed to characterize the structural features of starch during the digestion process.

CONCLUSION

The near-infrared spectroscopic method developed in this paper can be used to analyze starch in digestive contents. The method allowed a considerable reduction of the number of biochemical analyzes required (i.e., time saving). It can be expected in the future to replace enzymatic methods, when a large data base is available.

Infrared spectroscopy is thus a simple and useful tool which can be adapted to nutritional studies. This promising technique could also be developed to describe the chemical nature and the physical structure of the digestive residues.

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