Determination of the degree of gelatinisation of starch by near infrared spectroscopy

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Introduction

Starch is widely distributed in various plant storage organs in the form of tiny white granules: in cereal grains (corn, wheat, barley, oat, Sorghum), in roots (sweet potatoes, cassava, arrowroots, yam), in tubers (potatoes), in stems (sago palm) and in legume seeds (beans, peas). It is used as an ingredient in many foods and is the most important source of carbohydrate in human nutrition.

The properties of starch prepared from different sources vary considerably and the chemical composition and the physical characteristics are essentially related to the biological origin of the starch. Starch, in the form of granules of different plant species, differs in shape, size, size distribution, composition and crystallinity of granules.

Native starch molecules are classified as either linear (amylose) or branched (amylopectin). **Amylose** is essentially a linear polymer of (1 →4) linked α-D-glucopyranosyl units, while **amylopectin** is a group of large and highly branched molecules with branch-points of (1 →4) and (1 →6) linked α-D-glucopyranosyl units.[1]

To increase the digestibility and storage stability of many starch-based foods, starch is modified during industrial processing, by undergoing heat treatments. Native starch granules are, essentially, insoluble in cold water. Little happens, even at room temperature: they can absorb water and swell slightly but the swelling is reversible. When starch granules are treated with boiling water, the viscosity of the suspension rises steeply as a part of the amylose diffuses out of the granules and goes into solution. The granules swell until they collapse and then the starch crystallites melt to form a polymer network.

This process of swelling is referred to as gelatinisation. But the most comprehensive definition was generated by Atwell[2] to clarify terms for better communication among researchers: "*Starch gelatinisation is the collapse of molecular orders with the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence and starch solubilisation. The point of initial gelatinisation and the range over which it occurs is governed by starch concentration, method of observation, granule type and heterogeneities within the granule population under observation.*" Gelatinisation depends not only on the botanical origin of the starch and the gelatinisation temperature but also on the water level content of the suspension. Any dried starch with less than 5% water will undergo slight changes up to 190°C, whereas starch granules with 60% moisture will change greatly.

Food processors generally prefer starches with better characteristics, digestibility and storage stability than are provided by native starches. The role of starch is principally either to take up water or to produce gels with various viscosities in order to provide desired textural qualities. The extent of starch

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gelatinisation in baked goods strongly effects the properties of the products, their storage behaviour and digestibility, but not metabolisability.[3]

Several analytical methods for gelatinisation determination have been proposed: birefringence determination,[4] differential scanning calorimetry,[5] enzymatic susceptibility,[6,7] nuclear resonance spectroscopy,[8] viscosity[9] and X-ray diffraction.[10] The degree of gelatinisation is determined by two techniques; loss of birefringence by gelatinised starch measurement and its high sensitivity to enzyme attack were selected among others to determine the degree of gelatinisation.

The enzymatic method is the simple one that is based on the hydrolysis of the gelatinised starch into glucose. Several enzymes, such as amyloglucosidase, ^{b}-amylase and diastase, are used to cleave the gelatinised starch. However, the use of amyloglucosidase is a more suitable method for quantification of glucose content, because it easily converts gelatinised starch into glucose, whilst other enzymes such as ^{b}-amylase release maltose. A large number of carbohydrate molecules are produced during the conversion with amyloglucosidase. These are released when ^{a}-amylase is utilised.

The degree of gelatinisation (DG) is calculated in two steps, first, by calculating glucose produced by the action of amyloglucosidase alone (*gelatinised starch*) and second, by calculating the amount of glucose produced by the combined action of ^{a}-amylase and amyloglucosidase (*total starch*).

Materials and methods

Samples used were from commercially available corn flours, extruded flours, oat flours, rice semolina, tapioca and wheat bran supplied by different European and American millers and flour trades. Throughout this experimentation, the reference method used was the enzymatic method as it is described in Starch: Chemistry and Technology.[1]

Reference method—enzymatic method

Amyloglucosidase from *Aspergillus niger* (Merck 1332) and thermostable α-amylase (Termamyl 120 L) from Nova; Buffer solutions: 1 mM calcium acetate adjusted at pH 6.5 and 0.05 mM sodium acetate adjusted at pH 4.6 and a glucose oxidase–peroxidase from BioMérieux (Glucose enzymatic PAP for glucose determination.

Step A (gelatinised starch content)

Into the sample (flask A), 50 mL calcium acetate buffer pH 4.6, hydrolyse with 50 mg of amyloglucosidase from *Aspergillus niger* (Merck 1332) were added, incubated for 30 min at 37°C under agitation and the reaction stopped by increasing the pH to 7.6 and dipping the flask in cold water.

Step B (total starch content)

Into the sample (flask B) 25 mL calcium acetate buffer pH 6.5, hydrolyse with α-amylase with 1 µL (Termamyl 120 L) was added, incubated for 30 min in a boiling water bath under agitation,the sample was allowed to cool and add 25 mL calcium acetate buffer pH 4.6, hydrolyse with 50 mg amyloglucosidase, incubate 30 min at 37°C under agitation and the reaction stopped.

The determination of the gelatinised starch content (*method A*) and total starch content (*method B*) were applied to all the commercial flour samples used in this study. The degree of gelatinisation was then calculated from the two methods according to the following equation:

$$DG\% = \frac{A}{B} \times 100$$

DG; degree of gelatinisation, **A**; glucose resulting from method **A**, **B**; glucose resulting from method **B**.

NIR measurement

All spectroscopic measurements were done using an NIRSystems model 6500 spectrophotometer (Foss-NIRSystems, Silver Spring, MD, USA) in reflectance mode. Spectra of the flour samples were measured using a small ring quartz cup (55 mm in diameter) and transport mechanism. All reflectance data were recorded as log (1/*R*) from 400 nm to 2498 at 2 nm intervals; and 24 co-added scans for each sample were referenced to 12 co-added scans of a ceramic reference material and saved as the average to the hard disk of the computer of (Table 1). A four-point Fourier smoothing was applied to the data prior to further processing.

Data treatment and analysis

All data analyses were carried out using Windows Infrasoft International (WinISI II) software v. 1.02A. (NIRSystems, Silver Spring, MD, USA). The raw optical data were collected for the effects of scatter standard normal variate (*SNV*)[11] and detrend and transformed into second derivatives, (Figures 1, 2 and 3) using a 16 nm gap and a height-point (16 nm) smoothing function. Modified partial least squares (MPLS) regression analysis was performed to establish the relationship between the degree of gelatinisation from its enzymatic method and NIR absorbance values (log 1/*R*).

Results and discussion

The results show that a rapid quantitative determination of the degree of gelatinisation in commercial flours is possible with an acceptable standard error of performance (*SEP*). An MPLS regression

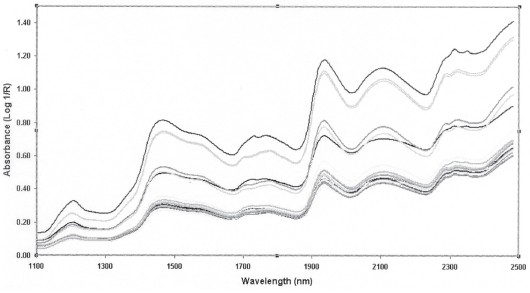

Figure 1. The NIR raw spectra of gelatinised starch flours with different degrees of gelatinisation

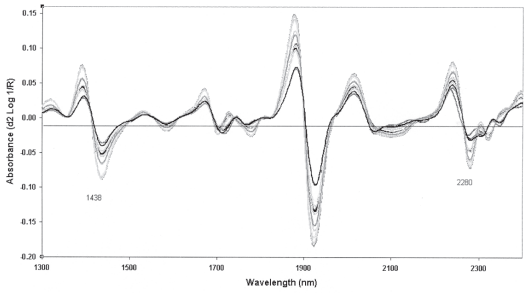

Figure 2. Second derivatives of NIR spectra of gelatinised starch flours with different degree of gelatinisation.

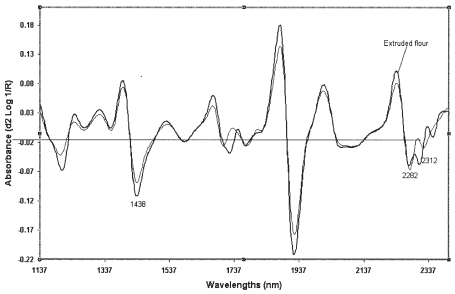

Figure 3. Comparison of a spectrum of gelatinised rice flour and a spectrum of extruded rice flour with a high degree of gelatinisation.

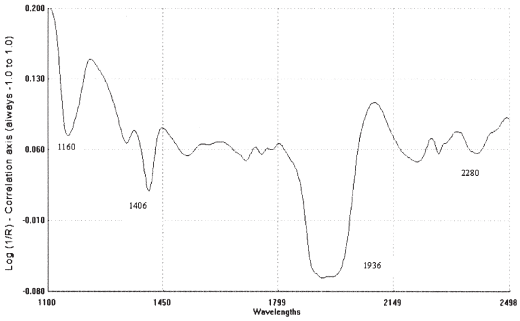

Figure 4. Correlation plot of degree of gelatinisation of gelatinised starch in flour.

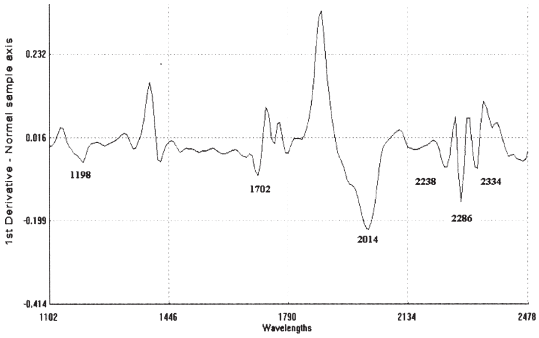

Figure 5. Loading plot PCA factor of degree of gelatinisation for gelatinised starch in flour.

analysis performed to establish the relationship between the degree of gelatinisation from enzymatic method and NIR (using the ISI Windows NIR software (WinISI II v. 1.02A).

The correlation plot exhibits minima at 1150, 1406 and at 1936 nm the 2nd overtone region moisture band corresponds to areas of high correlation with a degree of gelatinisation (Figure 4). The load-

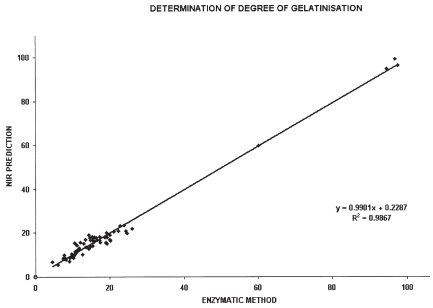

Figure 6. Scatter plot of NIR predicted values v. enzymatic method of degree of gelatinisation.

ing plot v. wavelengths, (Figure 5) with negative correlation at 1198, 1702, 2014, 2238, 2286 and 2334 nm gives more correlative information between spectral data and reference values of DG of gelatinised starch.

Table 1 summarises the degree of gelatinisation of flour samples in the calibration and validation sets determined by the enzymatic digestibility method. Samples are similar in the calibration set and the validation set. A good correlation was obtained between the degree of gelatinisation determined by

Table 1. Constituent statistics of NIR calibration equation of degree of gelatinisation for gelatinised flours.

DG			
Calibration	Statistics	Validation	Statistics
Mean	20.00	Mean-Ref.	19.99
SD	20.40	Mean-NIR	20.00
SEC	2.27	SD-Ref.	20.11
R^2	0.988	SD-NIR	19.96
SECV	2.89	*SEP*	2.90
1 – *VR*	0.980	R^2	0.987
N (samples)	90	N (samples)	50
T	11	Bias	–0.039
Range	(4.5 – 97.5)	Slope	1.001

DG**:** Degree of gelatinisation
SD: Standard deviation
SEC: Standard error of calibration
R^2: coefficient of determination in calibration
SECV: standard error of cross-validation
1 – *VR*: coefficient of determination on cross-validation
n: number of samples
T: number of PLS terms in model
SEP: Standard error of performance
RSQ: coefficient of determination in validation

the enzymatic method and NIR with a coefficient of determination (R^2) of 0.988 and a standard error of calibration (*SEC*) of 2.27, which are comparable to the standard error of performance or prediction (*SEP*) of 0.990 obtained when the calibration equation was applied to the validation set samples.

Conclusion

The enzymatic method is accurate, however it needs time and chemicals. The results show that the NIR as a straightforward and accurate method, might be used as an alternative, rapid method for the determination of the degree of gelatinisation in commercial flours and in food processing.

References

1. R.L. Whistler, J.N. BeMiller and E.F. Paschall, (Eds) Starch: Chemisty and Technology, 2nd Edition. Academic Press, NY, USA (1984).
2. W.A. Atwell, L.F. Hood, D.R. Lineback, E. Varriona-Marston and H.F. Zobel, *Cereal Foods World* **33,** 306 (1988).
3. R.D. James and R.L. Whistler, in *Chemical Changes in Food During Processing,* Vol. 4, Ed by T. Richardson and J.W. Finley. AVI Publishing, Westport, CT, USA (1985).
4. H.W. Leach, in *Starch-Chemistry and Technology.* Academic Press, New York, USA, p. 289 (1965).
5. D.J. Stevens and G.A. Elton, *Starch* **23,** 8 (1971).
6. R.M. Shetty, D.R. Lineback and P.A. Seib, *Cereal Chem.* **51,** 364 (1974).
7. B.-Y. Chiang and J.A. Hohnson*, Cereal Chem.* **54(3),** 429 (1977).
8. E. Jaska, *Cereal Chem.* **48,** 437 (1971).
9. R.M. Snadstedt, W. Kempf and R.C. Abbott, *Starch* **12,** 333 (1960).
10. R. Collison, in *Starch and its Derivative.* Chapman and Hall, London, UK, p. 168 (1968).
11. R.J. Barnes, M.S. Dhanoa and S.J. Lister, *Appl. Spectrosc*. **43,** 772 (1989).