STRUCTURAL CHARACTERIZATION OF ACYLATED STARCHES WITH INCREASED DELIVERY OF SHORT-CHAIN FATTY ACIDS

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INTRODUCTION

Short-chain fatty acids (SCFA), mainly acetic, propionic and butyric acids, are produced during fermentation of carbohydrates in the human colon and are critical for the maintenance of bowel health and colonic function (Topping and Clifton, 2001). However, an increased delivery of specific SCFA to the large bowel can also be obtained by acylation of carbohydrates, using a CSIRO proprietary technology.

Understanding the effects of structure on functionality is essential for the design and manufacture of acylated starches with specific health and therapeutic effects. The aim of the present study is to determine the effects of the level of acylation (degree of substitution), the molecular size of the esterified acid and the composition of the base maize starch on the structure of the acylated starch. Low and high amylose maize starches acylated with acetic, propionic and butyric acids at three degrees of substitution were compared with the structure of the unmodified maize starches.

MATERIALS AND METHODS

Materials

Two maize starches were studied before and after modification with acetic, propionic and butyric anhydrides—a standard maize starch containing 30% amylose (3401C base starch) and a high amylose maize starch (HAMS, Hi-maize^{TM}; 85% amylose). Both starches were obtained from Penford Australia (Lane Cove Road, North Ryde, NSW).

The procedure used for production of these acylated starches is described elsewhere (Annison et al., 2003). Three different degrees of substitution (low, medium and high) were investigated (see Table 1 for more detailed information). The degree of substitution (DS) indicates the average number of substitutions per anhydroglucose unit.

Table 1. Degree of substitution (DS) of the different acylated maize starches

	Normal maize starch (3401C)			High-amylose maize starch		
	Acetylated	Propionylated	Butyrylated	Acetylated	Propionylated	Butyrylated
Low DS	0.13	-	-	0.10	0.15	0.15
Medium DS	0.21	0.214	0.23	0.23	0.24	0.25
High DS	-	-	0.35	0.32	0.39	0.50

Small angle X-ray scattering (SAXS)

Small angle X-ray scattering measurements were performed on a Bruker Nanostar SAXS camera, with pin-hole collimation for point focus geometry. The instrument source is a copper rotating anode (0.3mm filament) operating at 45kV and 110mA, fitted with cross coupled Göbel mirrors, resulting in Cu Kα radiation wavelength 1.54 Å. The SAXS camera is fitted with a Hi-star 2D detector (effective pixel size 100 μm). The sample to detector distance was chosen to be 650 mm which provided a q-range from 0.02 to 0.3 $Å^{-1}$. Samples were presented in 2mm glass capillaries. The optics and sample chamber were under vacuum to minimise air scatter. Scattering files were normalised to sample transmission, background subtracted and then radially averaged using macros written in the Igor software package (Wavemetrics, Lake Oswego, Oregon, USA).

X-ray Diffraction (XRD)

X-ray diffraction was carried out on a Panalytical X'Pert Pro diffractometer. The instrument was equipped with a Cu long fine focus tube, programmable incident beam divergence slit and diffracted beam scatter slit (both fixed at 0.125°) and an X'celerator high speed detector. The samples were examined over the angular range of 2 to 40 degrees with a step size of 0.0332° and a count time of 800 seconds per point. Crystallinity determination was carried out using the X'Pert software. This programme automatically determines the amorphous background of the diffraction pattern and the crystallinity can be easily calculated from the intensity ratio of the diffraction peaks (I_{net}) and of the sum of all intensity measured (I_{total}):

$$\text{Crystallinity (\%)} = 100 \times (I_{net} / I_{total})$$

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was conducted on a JEOL (JSM-6400) microscope (JEOL, LTD, Tokyo, Japan) at an accelerating voltage of 15 KV and a working distance down to 14 mm. The dried starch materials before and after processing and digestion were sprayed on circular metal stubs previously covered with double-sided adhesive, and coated with carbon. After examination of the samples, different regions depicting interesting morphological features were selected and photographed.

FTIR spectroscopy

Diffuse reflectance infrared spectra were recorded with a Nicolet Nexus 8700 FTIR spectrometer equipped with a liquid nitrogen-cooled HgCdTe detector using dispersions of the starch samples in potassium bromide (KBr) (4%w/w). Spectra were scanned in the range 4000 – 650 cm^{-1} with an effective resolution of 4 cm^{-1}, co-adding 256 scans. A spectrum of KBr was recorded as background.

RESULTS AND DISCUSSION

Microstructural characterisation

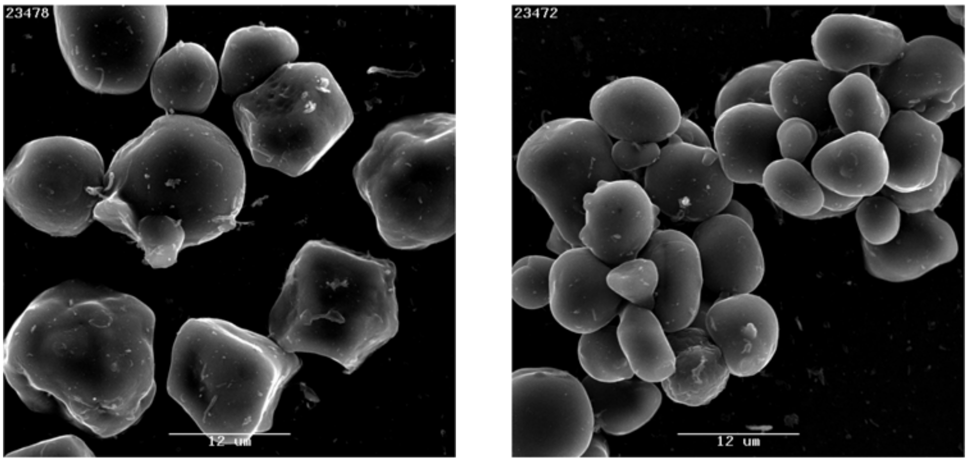

Figure 1. Scanning electron micrographs of acetylated 3401C (left) and acetylated high-amylose (right) maize starches.

Acylation at the different degrees of substitution was confirmed by FT-IR, using the band at 1740 cm^{-1} which indicates the presence of an ester group (results not shown). SEM showed that, in general, acylation does not alter significantly the microstructure of starches. The normal maize starch (3401C) showed more angular granules, which remained intact upon acylation with the different fatty acids and at the various degrees of substitution. On the other hand, high-amylose starch is characterized by more rounded and even elongated granules. Neither addition of propionic nor butyric acid caused any modification in the microstructure. However, upon acetylation some granule aggregation was observed (see Figure 1) which has been previously ascribed to disruptions in the surface as a consequence of the chemical modification (Singh et al., 2004).

Nanostructural characterisation

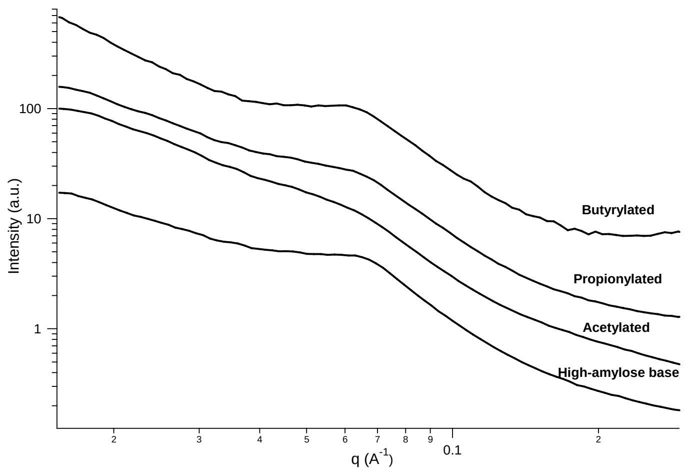

Figure 2. SAXS curves of high-amylose maize base starch and acylated high-amylose starches (high degree of substitution). Data have been offset for clarity.

Chemical modification of both starches with short-chain fatty acids leads to a decrease in the electron density contrast between the amorphous and crystalline lamellae, with this decrease being more pronounced for the starches substituted with the lower molecular weight fatty acids. At the highest decrease of substitution (see Figure 2), the typical 9 nm lamellar repeat of starches is practically unchanged for the butyrylated starch, decreases when propionic groups are added and, in the case of acetylated starches it is relatively unchanged.

Crystallinity of the chemical modified starches

Table 2. Crystallinity content (%) of the base, acetylated (A), propionylated (P) and butyrylated (B) starch samples.

	Normal maize starch (3401C)				High-amylose maize starch			
	Base	A	P	B	Base	A	P	B
Low DS	26.4	25.9	-	-	18.9	18.9	18.6	17.6
Medium DS	26.4	25.9	24.8	24.8	18.9	18.7	17.9	17.7
High DS	26.4	-	-	25.3	18.9	13.6	17.1	18.9

In Table 2 the crystallinity values of the different samples are displayed. In general, chemical modification leads to some disruption of the crystallinity. However different trends are observed depending on the molecular weight of the fatty acid. At low degrees of substitution (DS), acetylated starches are scarcely modified which, in agreement with previous studies

(Chen et al., 2004), could indicate that this type of chemical modification, at low DS, mostly takes place in the amorphous regions of the starch. At medium DS a slight decrease in crystallinity is observed and high DS results in the greatest decrease in crystalline content. In the case of propionylated starch, increasing the degree of substitution from low to medium and from medium to high, leads to similar decreases in crystallinity. On the other hand, while low and medium degrees of substitution with butyric acid cause a decrease in the crystallinity, at the higher degree of substitution the crystalline content is higher. The results suggest that the shorter chain lengths are acting as defects in the crystalline structure of starch, while butyrate chains are probably folding parallel to the amylopectin branch chains to minimize crystalline strain resulting, therefore, in improved lamellar order and recovery of electron density contrast (as observed by SAXS).

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