European Journal of Pharmaceutics and Biopharmaceutics 72 (2009) 574–586

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Starch-based coatings for colon-specific drug delivery. Part I: The influence of heat treatment on the physico-chemical properties of high amylose maize starches

A. Cristina Freire [a,c,*], Christiane C. Fertig [b], Fridrun Podczeck [a,1], Francisco Veiga [c], João Sousa [c]

[a] Inst. Pharmacy, Chemistry and Biomedical Sciences, University of Sunderland, Sunderland, UK
[b] Dept. Pharmaceutics, University of London, London, UK
[c] Dept. Pharmaceutics, University of Coimbra, Coimbra, Portugal

ARTICLE INFO

Article history:
Received 18 August 2008
Accepted in revised form 11 February 2009
Available online 20 February 2009

Keywords:
High amylose starches
Enzymatic digestion
Fourier Transform Infrared
Heat treatment
Modulated Differential Scanning Calorimetry
X-ray diffraction

ABSTRACT

In this study, the changes in the physico-chemical properties of different high amylose maize starches, i.e., Hylon® VII, Hylon® V and IM-DS acetate starch, were studied prior and after heat treatment used in the preparation of film coatings (WO 2008/012573 A1).

Characterisation of the unprocessed maize starches was carried out with regard to the outer particle morphology, particle size distribution, specific surface area, moisture content, apparent particle density, swelling, polarised light microscopy, Fourier Transform Infrared (FT-IR), X-ray powder diffraction and modulated Differential Scanning Calorimetry (mDSC). Pure amylopectin and low amylopectin samples (LAPS) were also used to aid the interpretation of the results. The effect of heat processing was evaluated in terms of degree of crystallinity, FT-IR and mDSC. Enzymatic digestibility of both processed and unprocessed maize starches was estimated qualitatively using various α-amylases resembling those present under *in vivo* conditions.

A significant decrease in the degree of crystallinity of the dried samples after processing was observed, in particular for amylopectin. Only LAPS and Hylon® VII samples showed differences in their thermal behaviour upon heat treatment, thus suggesting that a minimum amount of amylose is required for an effect to be detectable. High amylose starches maintained a well-ordered arrangement of their macromolecular chains, as was seen by X-ray and FT-IR studies. This effect could be explained by a formation of retrograded forms of the starches. The retrograded starches were found to be less digestible by various types of amylase, in particular those found in the upper intestines, indicating that the formation of a butanol complex as claimed elsewhere is not essential in the preparation of colon delivery devices.

1. Introduction

Starch is, after cellulose, the most abundantly distributed carbohydrate in the plant world. It occurs in cereals and vegetables in its native form and is stored in both granular and amorphous forms. Each starch granule consists of concentric growth rings of alternating amorphous and semi-crystalline structures composed of amylose and amylopectin [1]. Amylose is a long, essentially linear polymer and only sparsely branched, consisting of 1,4-linked-α-D-anhydroglucose units. Amylopectin is the main constituent of the starch granule (30–99%) and it appears as a highly branched polymer comprising of a large amount of short linear chains [2]. A great deal of attention has been devoted to starch and its derivatives mainly in the context of the Food, Plastics and Pharmaceutical industries. This is mainly due to the superior natural gelling ability of starches, their film forming properties and biodegradability. In the Pharmaceutical Industry, starch has been primarily used as a bulking agent, binder, disintegrant and thickening agent [3]. Additionally, native starches can undergo a number of chemical modifications such as acetylation, hydroxypropylation and cross linking, and physical alterations through application of heat and/or moisture to yield modified starches with distinctive properties. Such modified starches have been shown to be promising pharmaceutical excipients [4–6] and suitable additives for the food industries [7].

In the present study, high amylose starches with varying amylose contents were characterised before and after heat treatment.

Hylon® VII and Hylon® V starches are high amylose maize starches with amylose contents of 69% and 56%, respectively [8]

---

[*] Corresponding author. Dept. Pharmaceutics, Faculty of Pharmacy, University of Coimbra, Rua Do Norte, 3000-295 Coimbra, Portugal. Tel. +351 968208754; fax: +351 239855099.

E-mail address: anacfreire80@hotmail.com (A. Cristina Freire).

[1] Present address: Dept. Mechanical Engineering, University College London, Torrington Place, London WC1E 7JE, UK.

0939-6411/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2009.02.008

obtained from maize hybrid plants. The other high amylose starch, IM-DS acetate starch, is an acetylated form of the Hylon® VII with a degree of substitution of 1.5. Pure amylopectin and low amylopectin starch (LAPS) were used in a comparative way to ascertain the importance of the amylopectin-to-amylose ratio on the properties of the starches.

In a recent patent (WO 2008/012573 A1) it was reported that a heat treatment of these particular starches at 80 °C during an aqueous film coating process, using well-defined time spans of heating, dispersion, drying, curing and other coating conditions leads to films that do not release any drug in the stomach or upper intestines, but permit digestion of their starch portion in the colon, followed by drug release. Such behaviour was claimed to be impossible in patents on glassy amylose (e.g., EP 0343 993 and GB 0300 651); these earlier patents all claim that there is the need to extract the amylose into a butanol complex to make film coatings indigestible in the upper intestinal tract.

The aim of this work was to determine in which way the physico-chemical properties of the starches change during heat treatment used in the preparation of film coatings as described in patent WO 2008/012573 A1, and which of these changes are responsible for the observed digestibility of the starches by bacterial amylases.

2. Materials and methods

2.1. Materials

Hylon® VII (Batch No. FG 5514), Hylon® V (Batch No. BJ 9960), acetate maize starch with a degree of substitution (DS) of 1.5 (IM-DS acetate starch, Batch No. 78-0469) and low amylopectin maize starch (LAPS, Batch No. 374964) were donated by the National Starch and Chemical Company, Bridgewater, NJ, USA. The amylose content of these starches is listed in Table 1. Amylopectin (Batch No. 9561E) was supplied by ICN Biomedicals Inc., Aurora, OH, USA. Pancreatin (Batch No. 105K0689, EC No. 232-468-9) with an activity equivalent to at least the USP specifications was supplied by Sigma-Chemicals Co. (St. Louis, MO, USA). Hog pancreas α-amylase with an activity of 53.2 U/mg (Batch No. 10080, EC No. 232-565-6) was purchased from Fluka Biochemika GmbH (Buchs, Switzerland). *Bacillus licheniformis α-amylase* was supplied by Sigma-Chemicals Co. (St. Louis, MO, USA) as an aqueous suspension designed Type XII-A with 15% Sodium chloride and 25% sucrose (Batch No. 025K1132, EC No. 232-560-9). Its activity as determined by the Biuret-Method was 21 mg/ml (786 U/mg protein). For all enzymes used, one unit is defined as the amount of enzyme liberating 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.

2.2. Scanning Electron Microscopy

Maize powder samples were fixed onto specimen stubs by means of double-sided carbon conductive adhesive strips, followed by vacuum coating with a standard mixture of gold and platinum in a sputter coater (Polaron SC7620, Quorum Technologies, Newhaven, UK). An approximate coating thickness of 11.5–14.5 nm was used.

Images were taken with a Hitachi S-3000N scanning electron microscope (Polaron SC7620, Quorum Technologies, Newhaven, UK) with an emission of 20 kV and a magnification of 1500×.

2.3. Particle size analysis

Image analysis was used to determine the Feret diameter, which is the distance between pairs of parallel tangents to the projected outline of the particle [9] of 1000 particles of each maize starch sample. The system used comprised of a colour camera module (Axiocam MCR, Carl Zeiss, GmbH, Göttingen, Germany) attached to a microscope (Olympus BH-2, Olympus Optical Co., Japan) and PC, on which both data acquisition (Axiocam version 4.3, Carl Zeiss, GmbH, Göttingen, Germany) and image processing software (KS 400, version 3.0, Carl Zeiss, GmbH, Göttingen, Germany) had been installed. The pixel size was 0.52 μm in horizontal direction and 0.52 μm in vertical direction and an optical lens with a magnification of 10× (A10LP, Olympus, Japan) was used. The samples were prepared by dispersion of approximately 0.1 mg of powder in liquid paraffin on a glass slide, and a coverslip was added.

2.4. Specific surface area

The specific surface area of the starch samples was measured by gas adsorption using nitrogen (SA 3100 Surface Area Analyzer, Beckman Coulter UK Ltd., High Wycombe, UK). The experiment was run in triplicate. The samples were dried to mass constancy at 100 °C with an electronic moisture balance (Sartorius YTC01L, Sartorius GmbH, Göttingen, Germany). Five grams of the dried powder was filled into each tared measuring vessel of the surface area analyzer, and degassed over night at 90 °C under vacuum after flushing for 5 min with nitrogen gas. After reweighing of the closed vessels to establish the final powder weight, the specific surface area was determined at −70 °C using liquid nitrogen as a coolant.

2.5. Apparent particle density

The apparent particle density (Ph.Eur, Appendix XVII K, method 2.9.23) of the powders was evaluated using a helium pycnometer (Quanta Chrome Multi-pycnometer, MVP-1, Quanta Chrome Cor-

Table 1
Summary of physico-chemical properties of unprocessed maize starch samples.

Starch	% Amylose content (w/w)	Apparent particle density (g/cm^3)[c]	Moisture content (%)[b]		Specific surface area[d] (m^2/g)	IQR (μm)	Median particle size (μm)
			Initial	76% RH			
Hylon® VII	69[a]	1.44 ± 0.01	11.5 ± 0.2	17.3 ± 0.3	0.682 ± 0.005	10.2–17.2	13.2
Hylon® V	56[a]	1.43 ± 0.01	12.7 ± 0.2	16.9 ± 0.2	0.732 ± 0.044	7.9–14.4	10.7
IM–DS acetate	71[b]	1.30 ± 0.03	7.7 ± 0.0	9.7 ± 0.5	2.986 ± 0.008	10.7–24.8	15.5
Amylopectin	0[b]	1.45 ± 0.01	11.8 ± 0.1	16.7 ± 0.2	–	10.7–23.8	15.8
LAPS	95[b]	1.40 ± 0.03	11.8 ± 1.1	16.7 ± 0.5	0.960 ± 0.035	9.3–17.4	12.1

[a] As determined by near infrared spectroscopy [8].
[b] As provided by the supplier.
[c] Values are means ± standard deviation of five replicates.
[d] Values are means ± standard deviation of three replicates.

poration, New York, USA). Approximately 1–2 g of each powder was used and the results are the mean and standard deviation of five replicates.

2.6. Moisture uptake

The moisture content of the maize starch samples was determined using a Halogen Moisture Analyser (Model HG53, Mettler Toledo, Switzerland). Samples as received and after 3 days of storage in a desiccator containing a saturated solution of sodium chloride to attain 76% of relative humidity of the storage air were studied. The starch samples were dried at 200 °C and the sample weight recorded every 30 s until the mean weight loss did not exceed 1 mg during a 150 s interval. The results are the mean and standard deviation of three replicates, expressed as the % of moisture content.

2.7. Swelling index

The swelling behaviour of the different starches was determined in triplicate as follows: an exact amount of each starch powder was placed in a 25 ml graduated cylinder by tapping until no further changes in volume were observed. The target volume for the starch powder bulk was 5 ml. Distilled water maintained at 22 °C or pre-heated to 40 or 80 ± 2 °C was added until a final liquid volume of 25 ml was attained. The cylinder was placed in a water bath at 22, 40 or 80 °C and volume readings of the powder bulk were taken at pre-determined times. The final volume was recorded when no further changes were detected. The swelling index is the ratio between initial and final powder bulk volume in %.

2.8. Temperature processing

Approximately 3 g of maize starch powders was dispersed in 20 ml of distilled water and heated at 80 ± 5 °C in a glass vessel using a hot magnetic stirring plate for 30 min. After cooling down, the sediment was collected through a filter (cellulose nitrate membranes 0.45 μm, Whatman, International Ltd., Maidstone, UK) and was dried at room temperature for 24 h.

2.9. Polarised light microscopy

Samples of the various maize starches were analysed by polarised light microscopy with a Nixon eclipse E400 microscope instrumented with a Nixon Digital Slight DS-L1 Camera. Magnification lens of 40× was used (0.65P WD 0.65 Lens, Olympus, Japan).

The sample preparation was the same as described under Section 2.3. For the samples attained from the swelling studies at 40 °C and 80 °C, the starch dispersions were agitated manually to allow complete dispersion. A small aliquot was removed and placed on a glass slide. Before the addition of liquid paraffin the samples were allowed to dry at room temperature.

2.10. Fourier Transform Infrared analysis (FT-IR)

Infrared spectra of the maize starches were recorded in the wave number range of 4000 to 550 cm^{-1} with a Spectrum BX series spectrophotometer (Perkin Elmer, High Wycombe, UK). For each sample, a total of 16 scans were determined at a resolution of 4 cm^{-1} and velocity of 0.30 cm/s. The spectra were corrected for baseline shifts and deconvoluted automatically with the use of Spectrum BX series software version 2.19, which was also used to determine peak positions.

2.11. X-ray powder diffraction

X-ray powder diffraction analysis of the maize starches was performed with a Philips X′ Pert. Model PW 3040/00 equipped with a monochromatic Co-Kα radiation (1.78897 Å). Powder samples were tightly packed into rectangular aluminium cells and exposed to an X-ray beam with a voltage of 40 kV and a current of 35 mA. Other test conditions were: scanning range at 2θ of 5–60°; step size 0.025°, acquisition time 30 min, divergence slit 1°, receiving slit 0.25° and scattering slit 1°.

2.12. Determination of the degree of crystallinity

The degree of crystallinity of the maize starches was estimated quantitatively following the method of Nara and Komiya [10]. A baseline was plotted which connected the base of the main diffraction peaks. The areas above and below the curve were considered as the crystalline and amorphous portions, respectively. Both areas were integrated using Origin version Pro 6.1 software. The degree of crystallinity of the starch samples was calculated using the following equation:

$$\mathit{Crystallinity}(\%) = \frac{\mathrm{Ac}}{\mathrm{Ac} + \mathrm{Aa}} \times 100 \tag{1}$$

where Ac = crystalline area; Aa = amorphous area, both attained from the X-ray diffractograms.

2.13. Modulated Differential Scanning Calorimetry (mDSC)

Thermal behaviour of starch powder samples was studied by means of mDSC using a Differential Scanning Calorimeter (Q1000, TA Instruments, Waters LCC, DE, USA). Unprocessed and heat-processed maize starch samples ($n = 3$) were accurately weighed (4–6 mg) into aluminium pans (TA Instruments, DE, USA), hermetically sealed and scanned between 10.0 °C and 250.0 °C with an optimized modulation temperature amplitude of ±2.0 °C, modulation time of 40 s and a ramp rate of 3.0 °C/min. These conditions were shown to offer an adequate number of modulation cycles (more than four modulation cycles) over the temperature range of the transition. Also, larger modulation times have been shown [11] to offer better resolved weak glass transitions due to superior heat capacity measurements. A nitrogen gas supply with a flow rate of 50 ml/min and a refrigerating cooling system (RCS 90, TA Instruments, DE, USA) with a temperature range from −90 to 550 °C were used. The DSC cell was conditioned before calibration by heating it at 75 °C and holding isothermal for a period of 120 min. Two separate calibrations, i.e., cell resistance/capacitance, and cell constant and temperature were performed. The former involves a baseline run done without any samples or references, and the second run performed with two large sapphires (reference and sample sapphire). The cell constant and temperature calibrations were performed in the same way based on the melting peak of an indium sample.

Relevant endothermic or exothermic transitions and enthalpy of transition were analysed with the use of the TA universal analysis® 2000 software. Glass transition temperatures were determined on the reversible signal as the inflection point, i.e., the portion of the curve between the first and the third tangents with the steepest slope.

2.14. Enzymatic digestion

The enzymatic digestion was determined for both temperature processed and unprocessed starch samples, in order to establish the effect of temperature processing and/or enzyme affinity for distinct types of substrates. Amylopectin was used in its unprocessed form only, because treatment of this material at high temperatures caused the majority of the granules to fragment.

Powder samples (0.1 g) were dispersed in 100 ml of phosphate buffer with a pH of 7.2 comprising either pancreatin, hog pancreas α-amylase or *B. licheniformis* α-amylase (250 U/ml), and maintained at 37 ± 1 °C, for a period of 22 h. All solutions containing enzymes were freshly prepared every day.

2.15. Statistical analysis

The results were analysed by one-way or, where appropriate, two-way Analysis of Variance (ANOVA) and linear regression analysis using SPSS 14.0 for Windows (SPSS Inc., Woking, UK). A post hoc Scheffé test was employed when the overall *F*-test (ANOVA) indicated significant differences between samples.

3. Results and discussion

3.1. Physico-chemical characterisation of the unprocessed maize starches

3.1.1. Outer granule morphology

The Scanning Electron Microscopy (SEM) was used to assess the size and shape of the starch particles of the various starch samples used in this study.

The unmodified starches, owing to a common botanical source present similar outer particle morphology, i.e., spherical- and/or polygonal-shaped granules accompanied by a non-porous and smooth surface (see Figs. 1A, B, D and E). In the Hylon® VII and Hylon® V samples irregular tube-shaped particles, which are representative of the high amylose content, are seen in small proportions.

The intermediate-DS acetate starch particles present a very different morphology (see Fig. 1C). Particles appear to be larger comprising a very irregular surface and porous inner structure. Modification of the starch particles morphology through acetylation has been previously described by Singh et al. [7]. Starch acetylation involves the treatment with high concentrations of organic acid anhydride and alkaline reagent in a one-step process [12]. Such treatment causes fusion and deformation of the individual particles to form coherent multi-particulate agglomerates with a high intra-granular porosity.

3.1.2. Particle size, surface area and apparent particle density

Size, specific surface area and porosity of starch particles have been correlated to their propensity to undergo amylolysis [13]. Planchot et al. [14] used starches of different origins presenting a wide range of particle sizes to identify a clear relationship between granule size and degree of enzymatic digestibility of the starch granules. It was found that starch particles of a smaller size possessed a larger specific surface area resulting in a faster and more pronounced amylase hydrolysis. In addition, a higher surface porosity was found to facilitate the progression of the enzyme molecules towards the granule centre, which is known to have a high propensity to enzymatic hydrolysis. Hence, in this work the particle size, specific surface area and apparent particle density were determined.

Median particle size and interquartile range (IQR), specific surface area and apparent particle densities for each of the maize starch samples are listed in Table 1. Median particle size values were very similar and therefore not discriminating. However, the interquartile ranges identified the IM-DS acetate as the starch comprising a higher number of particles in the larger size classes, followed by amylopectin, Hylon® VII, LAPS and finally Hylon® V. Hylon® V starch is composed of a higher number of finer particles with 75% of the starch granules having a particle size smaller or equal to 14 μm.

The specific surface area of the IM-DS acetate starch is three times larger than that of LAPS and four times larger than the values obtained for all other starch samples studied. The statistically significantly higher specific surface area ($F \approx \infty$; $p < 0.001$) found for the acetylated starch is in agreement with its visibly high surface porosity (see Fig. 1C). No statistically significant differences were found between Hylon® VII and Hylon® V, but a difference between these starches and LAPS was identified. As LAPS and Hylon® VII samples have similar particle size distributions (see Table 1), the differences in specific surface area indicate an increase in the number of open surface pores in LAPS grains. In contrast to Hylon® VII, which contains approximately 70% of high molecular weight amylose, LAPS is composed of 75% of high molecular weight amylose and 20% low molecular weight amylose, and Hylon® V contains approximately 55% of high molecular weight amylose [15]. The difference in total amylose concentration and the presence of low molecular weight amylose might explain the differences in specific surface area between Hylon® VII and LAPS despite similar particle size distributions, while the considerably smaller particle size range observed for Hylon® V explains its slightly larger specific surface area when compared to Hylon® VII.

The IM-DS acetate starch sample has a considerable smaller apparent particle density (see Table 1). One-way ANOVA confirmed statistically significant differences in apparent particle density ($F = 40.513$; $p < 0.001$) between the IM-DS acetate starch and the remaining starches, and also between LAPS and amylopectin. The differences can be attributed to the chemical modification of the acetylated starch and to the different nature of the LAPS and amylopectin samples, respectively. The density of acetylated starches has been shown to be greatly affected by their degree of substitution. Korhonen et al. [5] reported a decrease in the apparent particle density values with increasing degree of substitution. Acetylation results in the formation of pores and crevices at the surface of the starch granules. However, the determination of the apparent particle density by helium pycnometry cannot account for closed pores and thus the density values reported for all starch samples will underestimate the true material density values to some degree.

3.1.3. Moisture content

Starches are hygroscopic materials which take up atmospheric moisture rapidly, the degree of which is intrinsically related to the starch species [16].

The initial moisture content of the various maize starch samples under handling conditions (room temperature and 50–55% RH) and after storage at 76% RH is summarised in Table 1. Initial moisture content for the chemically unmodified starch samples ranged from 11.5% to 12.7%, which is in agreement with values between 10% and 17% reported in the literature [17]. IM-DS acetate starch comprised much lower initial moisture content (7.7%), which might be due to its comparatively hydrophobic character [18].

The moisture content of the various starch samples increased on average by 4–6% when stored at a relative humidity of 76%. A smaller increase of approximately 2% was observed for the IM-DS acetate starch. The water sorption ability of starch materials is related to the presence of hydrophilic groups. An increased degree of substitution of the hydroxyl groups in acetylated starches results in a decreased affinity to water [18].

3.1.4. Swelling index

Due to a certain degree of elasticity of the intermicellar network of the starch granules, an entirely reversible phenomenon often termed as “real swelling” arises in the presence of cold water [17,19]. At higher temperatures, starch granules undergo gelatini-

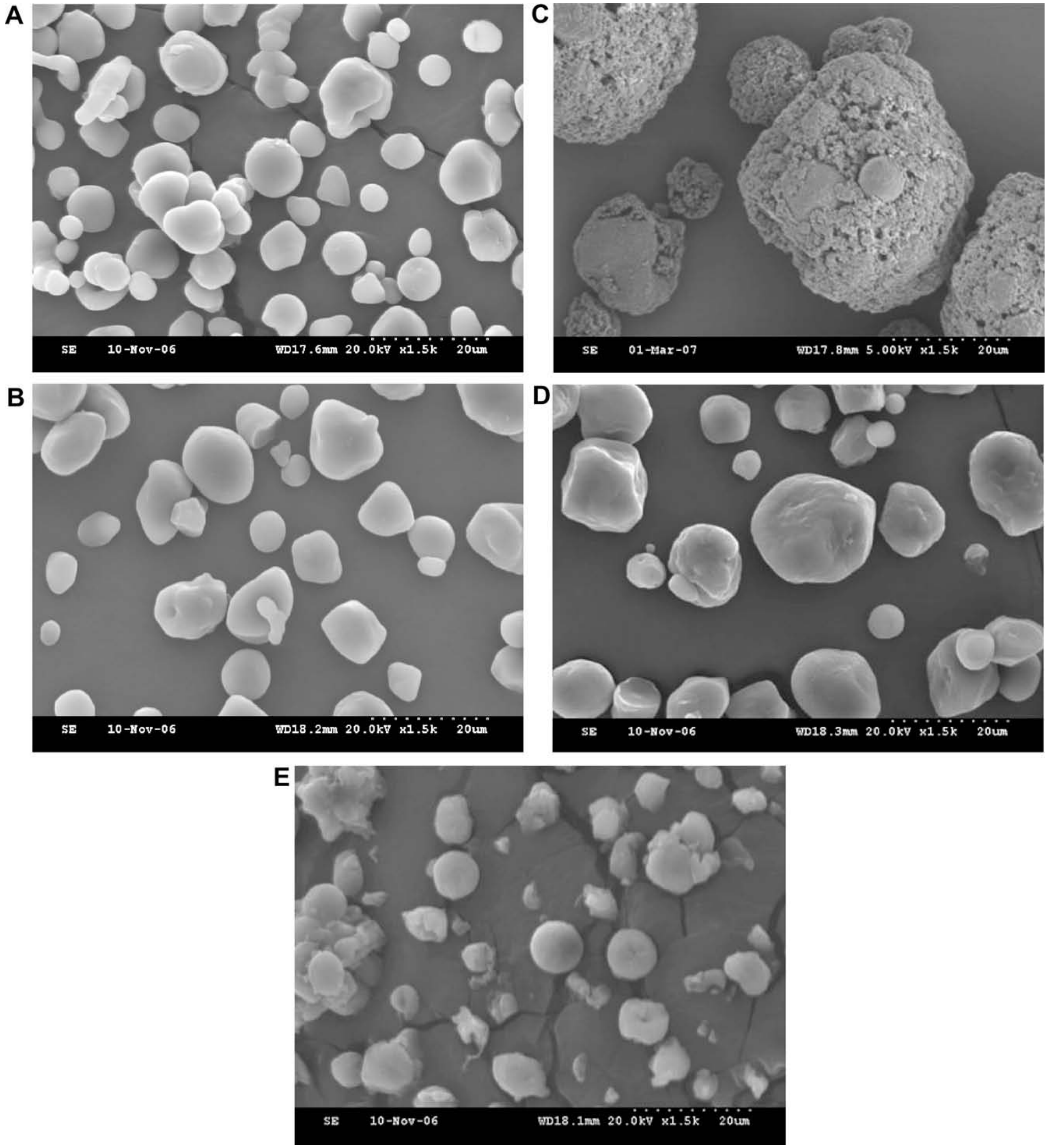

Fig. 1. Scanning Electron Microscopy images of maize starch powder samples (magnification 1500×). (A) Hylon® VII, (B) Hylon® V, (C) IM-DS acetate starch, (D) amylopectin, and (E) LAPS.

sation. This is an irreversible process described as the alteration of the granular starch into a turbid, viscoelastic paste or, at sufficient amylose concentrations, into an opaque elastic gel [19,20]. During gelatinisation, the starch granules swell to many times their original size, amylose is leached out, starch solubilisation increases, granule birefringence is lost and eventually the entire starch granule collapses [19].

The swelling propensity of the starch samples was determined at three different temperatures, i.e., 22, 40 and 80 °C as an indirect way of evaluating their gelatinisation behaviour and its temperature dependence. Values of the % swelling index (SI) are shown in Fig. 2. The sequence of polarised light microscopy images attained during the swelling studies is illustrated in Fig. 3 for the various starches tested.

Statistical analysis of these results by two-way ANOVA showed that both the temperature used and the type of starch sample are interacting factors ($F = 84.666$; $p < 0.001$), yet temperature ($F = 905.171$; $p < 0.001$) seemed to be statistically more important than the type of starch ($F = 109.861$; $p < 0.001$). In fact, a marked increase in the% SI with increased temperature was seen for all

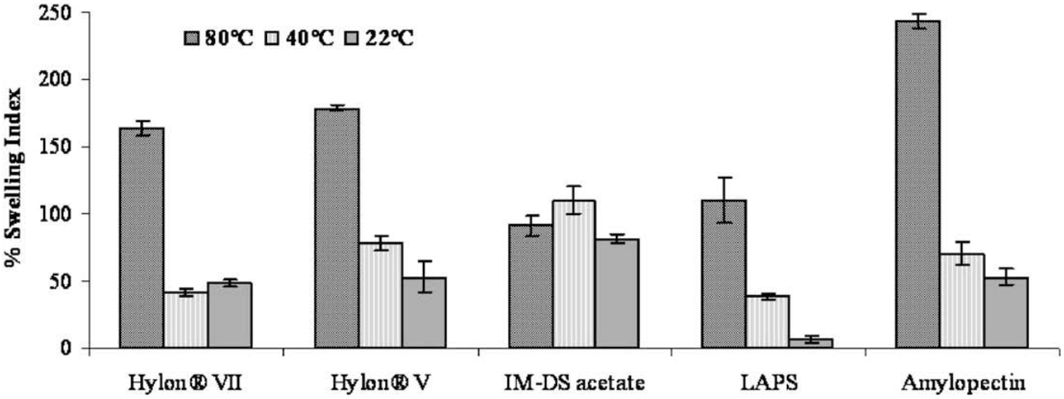

Fig. 2. Swelling index (%) as a function of the type of starch and the temperature of the aqueous medium ($n = 3$).

unmodified maize starch samples, whereas the IM-DS acetate starch presented fairly constant SI values over the temperatures studied. At a molecular level, acetylation results in the weakening of the hydrogen bonding between the starch polymers within the starch granule. As a consequence, the entire structure is opened up and the temperature for the onset of granule swelling is lower [21]. This is supported by the polarised light microscopy images attained at 40 °C, which show a decrease in the birefringence of the IM-DS acetate starch granules.

Looking at the remaining starch samples, at 80 °C, amylopectin showed the highest swelling index whereas LAPS had the lowest % SI. Hylon® VII, Hylon® V and LAPS exhibited clear granule swelling accompanied by a reduced granule birefringence (see Fig. 2). The characteristic Maltese cross-pattern disappeared and in its place

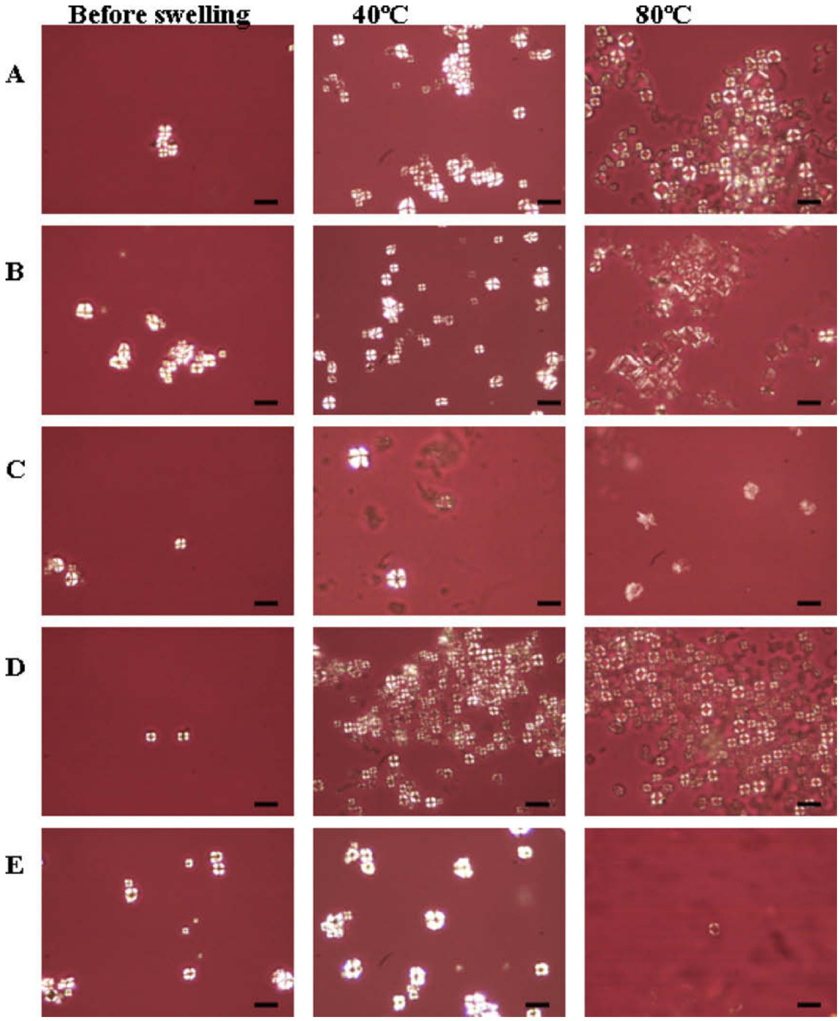

Fig. 3. Polarised light microscopy images of maize starch samples taken before and after swelling in aqueous medium at 40 °C and 80 °C (magnification 400×; scale bars correspond to 20 μm). (A) Hylon® VII, (B) Hylon® V, (C) IM-DS acetate starch, (D) LAPS, and (E) amylopectin.

a swollen halo was seen. In the case of amylopectin, gelatinisation was completed, with all granules collapsing, and therefore no granular structure or birefringence was detected.

These findings agree with those of Tester and Morrison [22] who showed that the amylopectin fraction in a starch grain controls the swelling and gelatinisation of the starch, whereas amylose acts as a dilutent. For this reason, Hylon® V due to its higher amylopectin content consistently presented higher % SI values than Hylon® VII. This relationship between amylose content x (in %) and % SI can be expressed statistically as ($R^2_{\text{adjusted}} = 0.943$; Root Mean Square = 6.6%):

$$SI(\%) = -1.350x + 248.238 \tag{2}$$

3.2. Effect of heat treatment on the physico-chemical properties of high amylose maize starches

3.2.1. Fourier Transform Infrared analysis (FT-IR)

The FT-IR spectra of the maize starches are shown in Fig. 4. The characteristic regions in these spectra can be summarised as follows: peaks in the region of 1450–1940 cm^{-1} are attributed to water absorption; peaks at approximately 1700 cm^{-1} denote the presence of lipid impurities, whereas those appearing between 2000 and 2200 cm^{-1} are ascribed to the presence of protein impurities. In addition, spectra of acetylated starches present three very characteristics bands, as indicated in Fig. 4, which allow their differentiation from unprocessed starch samples. These bands are [23] the C=O stretching band at 1750 cm^{-1}, a C—CH_3 deformation band at 1375 cm^{-1} and a C—O stretching band at 1240 cm^{-1}.

Further comparisons between the spectra of the unprocessed and heat-processed maize starches were undertaken using the spectral region of 1300 to 800 cm^{-1}. Amylopectin and all starches apart from IM-DS acetate starch and LAPS showed differences in their FT-IR spectra after heat treatment (see Fig. 5 and Table 2). Amylopectin FT-IR spectra changed in that the band intensity at 1000 cm^{-1} was clearly reduced, accompanied by the disappearance of the peak at 1042 cm^{-1}. For wheat starch, the intensity of the band situated at approximately 1047 cm^{-1} was shown to increase with an increasing degree of crystallinity [24]. The disappearance of the peak at 1042 cm^{-1} could hence be interpreted as a sign that amylopectin has become amorphous during heat treatment. In contrast, in the spectra of the processed LAPS, Hylon® VII and Hylon® V, this band was clearly maintained. In the case of the Hylon® VII and Hylon® V the spectra now show two peaks with distinct intensities, which are positioned at approximately 995 cm^{-1} and 1012–1016 cm^{-1}. LAPS, however, does not show any significant change in its FT-IR spectrum after heat treatment. This could be due to the fact that LAPS granules have the lowest amylopectin content (see Table 1), but also a highly ordered crystalline structure of amylose–lipid complexes [15], which cannot be destroyed by thermal treatment alone.

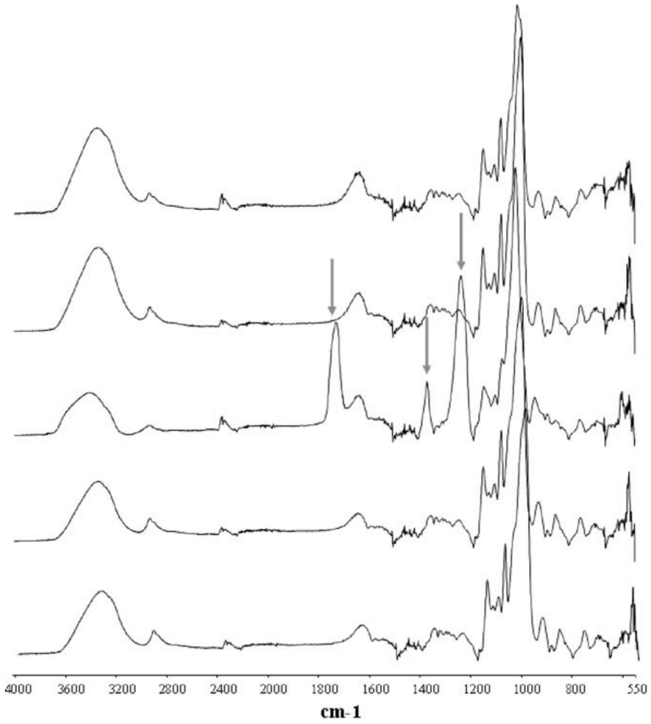

Fig. 4. FT-IR spectra of the maize starch samples in the region of 4000 to 550 cm^{-1}. From top to bottom: amylopectin, LAPS, IM-DS acetate starch, Hylon® V, and Hylon® VII. Arrows indicate the main differences between the acetylated starch and the unmodified starch samples.

3.2.2. X-ray powder diffraction

The X-ray diffractograms of the unprocessed maize starch samples are compared in Fig. 6A. A summary of the positions of the main peaks and their intensity as well as their crystal pattern classification and degree of crystallinity (%) is provided in Table 3. Diffraction peaks characteristic of the type B polymorphic form were seen for Hylon® VII, Hylon® V and LAPS with diffraction peaks at 2θ of approximately 6°, 17°, 19° and 23° and 25°. The very high intensity found at 2θ of 19° suggests a highly ordered crystalline structure of the amylose–lipid complexes in the starch granules [15].

The diffractogram observed for amylopectin identified a typical A-type polymorphic form [25] characterised by diffraction peaks at 2θ for 11°, 13°, 17° and 23°, plus an unresolved doublet between 19° and 20°. The diffractogram attained with the IM-DS acetate starch preserved some characteristic peaks of a B-type polymorph despite the modifications due to acetylation, seen in Fig. 6A and Table 3 from the peaks at 2θ of 6°, 17°, 19° and 22°. However, these peaks had a considerably lower intensity, which explains the lower degree of crystallinity determined. A completely amorphous starch can be obtained at higher degrees of acetylation; and when the degree of substitution was as high as 2.0 or 2.5, diffraction peaks could no longer be detected [18].

The degree of crystallinity decreased with an increase in amylose content. As pointed out by Shi et al. [15], a lower degree of crystallinity observed in X-ray diffraction is not necessarily similar to a predominantly amorphous state of the molecules in the granules. It could be the result of small-size crystallites, which would be in agreement with the much higher swelling indices of amylopectin and Hylon® V at 40 and 80 °C (see Fig. 2). Cheetham and Tao [26] using six commercially available maize starches with apparent amylose contents between 0% and 84%, found that the decrease in the relative degree of crystallinity with increased amylose content obeyed a two-stage relationship. Between 0% and 40% of amylose, the degree of crystallinity decreased in a linear fashion (stage I), while above 40% of amylose the degree of crystallinity decreased in a logarithmic manner (stage II), and therefore differences in crystallinity were less obvious and more difficult to determine. This is in agreement with the results of the present study where Hylon® V, Hylon® VII and LAPS, with amylose contents of 56%, 69% and 95%, respectively, presented very similar crystallinity values (see Table 3).

After heat treatment at 80 °C (see Section 2.8) the X-ray patterns of the studied starches indicated important changes, with all starches being converted into more amorphous entities (see Fig. 6B and Table 4). The degree of crystallinity of the processed starches now increased slightly with an increase in amylose content. IM-DS acetate starch was found to be fully amorphous after the heat treatment, which can be contributed to its lower gelatin-

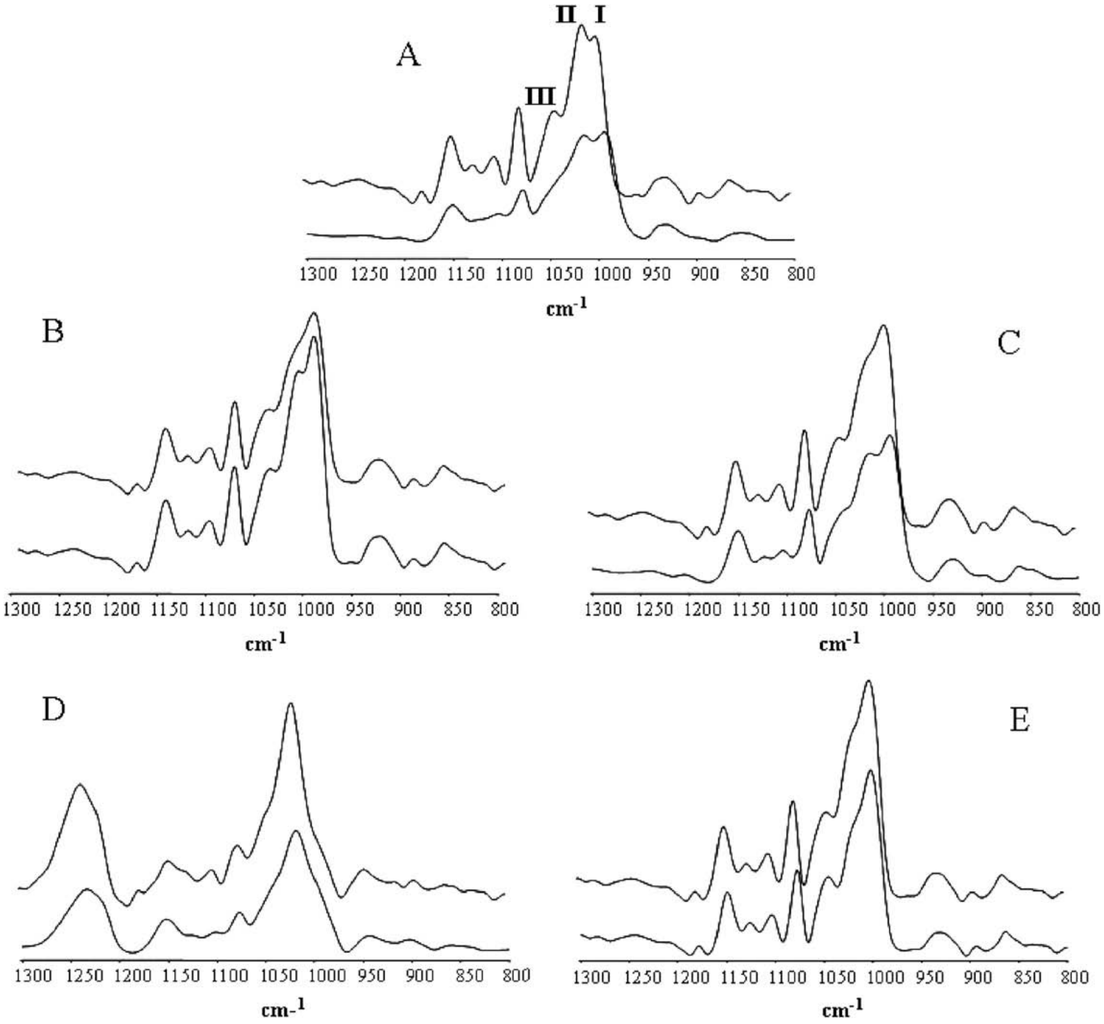

Fig. 5. FT-IR spectra comparing the heat-processed maize starch samples with the unprocessed maize starch samples in the region 1300 to 800 cm^{-1}. The peaks labelled in (A) with I, II and III as examples, were used to identify the differences between the spectra of the unprocessed (upper spectrum) and heat-processed (lower spectrum) maize starch samples. (A) amylopectin, (B) Hylon® VII, (C) Hylon® V, (D) IM-DS acetate starch, and (E) LAPS.

isation temperature. LAPS starch was able to retain part of its crystallinity. This is clearly seen in Fig. 6B, where LAPS was the only starch preserving the peak at 2θ of 6°, 19° and 22°, whereas Hylon® VII and Hylon® V presented clear diffraction peaks only at 2θ of 19° and 22°. LAPS requires higher temperatures for gelatinisation and consequently for the formation of an amorphous gel-like structure. X-ray investigations by Gidley et al. [27] on amylomaize V and VII resulted in similar X-ray patterns. The presence of some diffraction peaks resembling a B-type polymorph upon treatment of the granular starches at high temperatures was attributed to the formation of a retrograded arrangement of these starches.

Table 2
Comparison of the main peak positions (at approximately 1000 cm^{-1}) of unprocessed and heat-processed maize starches in the infrared spectra. For I, II and III see Fig. 7.

Starches	Peak position (cm^{-1})		
	I	II	III
Unprocessed			
Hylon® VII	996.2	–	1043.0
Hylon® V	996.5	–	1043.0
IM-DS acetate	1020.2	–	–
LAPS	1000.0	–	1043.5
Amylopectin	1000.3	1014.3	1042.3
Processed			
Hylon® VII	996.2	1011.8	1042.2
Hylon® V	994.3	1016.0	1044.7
IM-DS acetate	1019.0	–	–
LAPS	1002.2	–	1045.5
Amylopectin	994.9	1015.7	–

3.2.3. Modulated Differential Scanning Calorimetry (mDSC)

Numerous studies have previously reported the use of conventional DSC to study starch gelatinisation and/or glass transition and melting temperatures of starch materials [28–32]. Despite the extensive, and in many instances successful use of this technique, the investigation of the thermal behaviour of highly crystalline polymers such as starch is extremely difficult. For instance, glass transition temperatures (Tg) of starch materials appear as very weak transitions, as the more crystalline regions act as physical cross-links that restrain the mobility of the more amorphous regions [32]. It has been suggested that mDSC offers the advantage of an improved resolution of weak transitions such as glass transitions [11,28] over conventional DSC. Modulated DSC differs from conventional DSC in that it applies two simultaneous heating rates (linear and sinusoidal) to the sample. Whereas the linear or average heating rate provides the same information (total heat flow

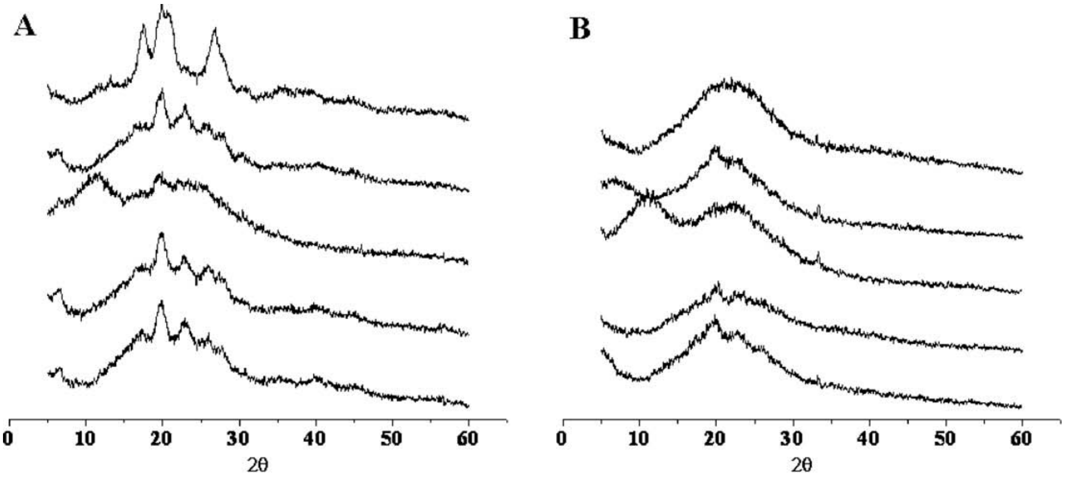

Fig. 6. X-ray diffractograms of the unprocessed (A) and heat-processed (B) maize starch samples. From top to bottom: amylopectin, LAPS, IM-DS acetate starch, Hylon® V, and Hylon® VII.

Table 3
Summary of the degree of crystallinity (%), crystal pattern and position/intensity of the main diffraction peaks of unprocessed maize starch samples.

Starch	Crystallinity degree (%)	Crystal pattern	Main diffraction peaks (and intensity) 2θ values									
			6°	11°	13°	17°	19°	23°	25°	27°	30°	35°
Hylon® VII	15.6	B-type	6.56 (33.71)	–	–	17.27 (39.5)	19.84 (105.4)	22.83 (63.28)	25.99 (39.22)	27.69 (29.56)	30.76 (19.66)	35.16 (24.86)
Hylon® V	17.1	B-type	6.71 (47.98)	–	–	16.49 (33.75)	19.94 (108.55)	22.91 (56.69)	25.81 (39.90)	28.12 (29.05)	31.24 (22.75)	36.79 (25.73)
IM-DS acetate	9.0	–	6.46 (31.33)	–	–	17.04 (26.18)	19.91 (55.66)	23.40 (23.20)	25.64 (21.51)	–	–	–
Amylopectin	32.7	A-type	–	11.71 (23.73)	13.21 (37.90)	17.51 (112.65)	19.81/20.70 (155.20)	23.00 (19.42)	–	–	30.83 (27.41)	35.11 (37.97)
LAPS	14.8	B-type	6.81 (29.77)	–	–	17.06 (33.37)	19.94 (104.94)	22.99 (67.72)	25.75 (32.88)	27.62 (42.92)	30.54 (26.53)	36.14 (16.48)

Table 4
Summary of the degree of crystallinity (%) and position /intensity of the main diffraction peaks of heat-processed maize starch samples.

Starch	Crystallinity degree (%)	Main diffraction peaks (and intensity) 2θ			
		6°	19°	22°	25°
Hylon® VII	6.81	–	19.79 (83.70)	22.69 (36.99)	26.21 (25.56)
Hylon® V	4.59	–	20.31 (32.36)	22.39 (28.45)	26.26 (24.92)
IM-DS acetate	0	–	–	–	–
Amylopectin	0	–	–	–	–
LAPS	7.15	6.86 (15.07)	19.74 (63.79)	23.36 (58.37)	–

rate) as conventional DSC, the sinusoidal (modulated) heating rate is used to determine the fraction of the total heat flow rate that responds to a changing heating rate. This fraction is referred to as reversing heat flow signal. A third signal can be found in typical mDSC thermograms which is the non-reversing heat flow and accounts for the difference between the total heat flow and the reversing heat flow [33].

The aim of this part of the study was to determine whether mDSC can detect differences between unprocessed and heat-processed starch samples with limited moisture content. Maize starches were used in the dry state as done before with X-ray diffraction and FT-IR analysis. This implies an important deviation from common analysis of starch materials and processed starch samples where starch/water mixtures are used.

Table 5 shows relevant thermal transitions over the temperature range of 10–250 °C of the unprocessed and processed maize starch samples. A very broad endotherm between 125 and 160 °C was identified on the non-reversible signal for all unprocessed starches, which is associated with the loss of residual water. The enthalpy (ΔH) related to these transitions was calculated and can also be found in Table 5. One-way ANOVA found statistical differences in relation to the enthalpy values of these transitions between the IM-DS acetate and the other starches, and between the LAPS and amylopectin samples. IM-DS acetate has the lowest enthalpy whereas the Hylon® V and amylopectin samples showed the highest enthalpy values. The endothermic peak is related to water loss from the starch granule structure and a higher enthalpy can be related to higher water losses. These results correlate well with the moisture content results described above, i.e., the IM-DS acetate starch had the lowest moisture content and lower water uptake ability.

The reversing signal obtained with the various starches can be seen in Fig. 7. Zeleznak and Hoseney [32] studied the glass transition of a wheat starch sample by means of conventional DSC under various moisture levels. The glass transition of the wheat starch sample was only identified within a narrow moisture range of

Table 5
Summary of the relevant thermal transitions detected on both the reversing and non-reversing signals of unprocessed and heat-processed maize starches determined by mDSC.

Starch	Unprocessed starch			Heat-processed starch		
	Reversing signal Tg (°C)	Non-reversing signal		Reversing signal Tg (°C)	Non-reversing signal	
		Endotherm (°C)	ΔH (J/g)		Endotherm (°C)	ΔH (J/g)
Hylon® VII	ND	129.2 ± 1.5	209.3 ± 1.5	ND	135.1 ± 1.4	201.0 ± 27.0
Hylon® V	ND	130.6 ± 6.9	230.0 ± 8.5	ND	128.1 ± 2.1	177.6 ± 35.0
IM-DS acetate	181.1 ± 0.9	126.1 ± 0.6	137.5 ± 3.8	179.0 ± 0.7	131.7 ± 4.0	90.9 ± 20.1
Amylopectin	ND	123.9 ± 1.8	219.4 ± 5.1	ND	129.7 ± 8.4	182.7 ± 50.0
LAPS	ND	143.3 ± 5.5	197.9 ± 12.9	ND	160.6 ± 5.0	127.8 ± 15.8

Values are means ± standard deviation of three replicates; ND = not detected, Tg = glass transition temperature, ΔH = enthalpy of transition.

13–22%. Below 13% moisture content the change in heat capacity was very gradual and small giving rise to a broad and ill-defined peak in the DSC thermogram. The starches used in this study had moisture contents between 7.7% and 12.7% (see Table 1) which might explain the inability to detect the glass transition. The unprocessed IM-DS acetate starch, however, showed a clear glass transition at 181.1 ± 0.9 °C (see Fig. 7). This is in line with the work of Zeleznak and Hoseney [32] who found that a pre-gelatinised starch showed a persistent well-defined glass transition even at lower moisture contents as a result of its amorphous organization.

Heat-processed starch samples presented a very similar thermal behaviour to that of the unprocessed samples. A broad endotherm was detected between 130 °C and 160 °C with enthalpies ranging from 90 to 210 J/g which in most cases was associated with a very large variation as seen by the high standard deviations (see Table 5). This might be explained by the inhomogeneous physical state of the dried processed starch samples. Whereas the unprocessed maize starches were present as individual particles, the heat-processed starches tended to form strong agglomerates upon drying.

Statistical differences between the various heat-processed maize starch samples were identified ($F = 30.584$, $p < 0.001$). Once more, LAPS had a higher endothermic peak value and values for the remaining starches were similar.

Comparing the endothermic peak values of the processed and unprocessed starch samples, statistical differences were only detected in the case of the Hylon® VII ($F = 31.067$; $p = 0.005$) and LAPS ($F = 16.625$; $p = 0.015$). In these two samples the endothermic peak was shifted to higher temperature values when the starches experienced heat treatment. Considering that these two starches have the higher amount of amylose, such results suggest that the thermal behaviour is dependent on the amylose content.

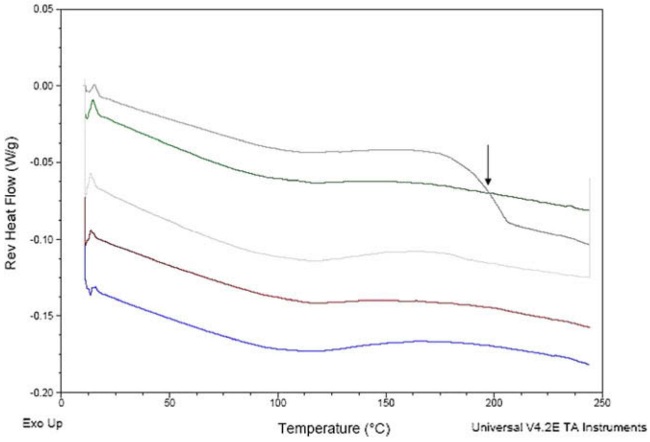

Fig. 7. Reversing signal of the mDSC thermograms of the unprocessed maize starch samples. The arrow identifies the Tg of the IM-DS acetate starch at around 181 °C. From bottom to top: Hylon® V, Hylon® VII, LAPS, amylopectin, and IM-DS acetate starch.

The heat-processed IM-DS starch showed a well-defined glass transition at a temperature of 179 °C. A statistical significant ($F = 13.042$; $p = 0.022$) decrease in the Tg value for this type of starch was observed. This can be attributed to the overall increased amorphous character of this starch after treatment, supported by the decrease in degree of crystallinity (%) (see Table 4).

3.2.4. Enzymatic digestibility of temperature-treated starch samples

A qualitative assessment of the efficacy of three different types of amylases (pancreatin, hog pancreas α-amylase and *B. licheniformis* α-amylase) to digest high amylose maize starches was made. The enzymes used were selected to mimic *in vivo* digestion of starch materials, whereby *B. licheniformis* α-amylase is often used in the assessment of starch films used for drug delivery to the colon, as it resembles the properties of colonic amylases very closely [34]. To represent the digestion occurring within the small intestine both pancreatin and hog pancreas α-amylase were used. Pancreatin is commonly used to represent digestion taking part in this portion of GI tract [35]. However, pancreatin is a complex enzyme mixture comprising not only amylases but also lipases, proteases and other minor impurities. Hence, its activity towards starch materials is lower than that of a pure hog pancreas α-amylase.

Planchot et al. [14] also employed techniques such as SEM to gain a qualitative insight into the digestion pattern of starch granules of different natures using *aspergillus fumigatus* α-amylase.

All maize starches were tested prior and after thermal treatment, while amylopectin was only studied in its unprocessed form. Unprocessed Hylon® VII, Hylon® V and LAPS granules revealed a characteristic pattern of digestion (see Figs. 8A, B and D), described earlier by Helbert et al. [36] as the appearance of pores on the surface of the starch granules. Pore formation is considered to be the result of radial hydrolysis of the amorphous regions within the starch granules, which results in channels directed towards the nucleus of the granules. The higher resistance of the high amylose starches to enzymatic digestion compared to that of the pure amylopectin sample is evident, with hardly any amylopectin granule remaining undigested.

Heat-treated starch granules of Hylon® VII, Hylon® V and LAPS revealed a less clear amylase digestion pattern, with only some of the smaller particles portraying a certain degree of amylolysis (see Figs. 9A, B and D). It is generally accepted that cooking of starch granules at high temperatures makes them more readily digested by the amylases. However, the exposure of starch to high temperatures followed by cooling down can trigger the linear amylose molecules to re-associate and ultimately form a crystalline structure through a phenomenon called starch retrogradation [20,37,38]. Retrograded starch is more resistant to enzymatic attacks due to a lower affinity shown by some α-amylases, such as pancreatic α-amylases, to the retrograded starch [39]. The α-amylases present in the colon are still able to digest the retrograded

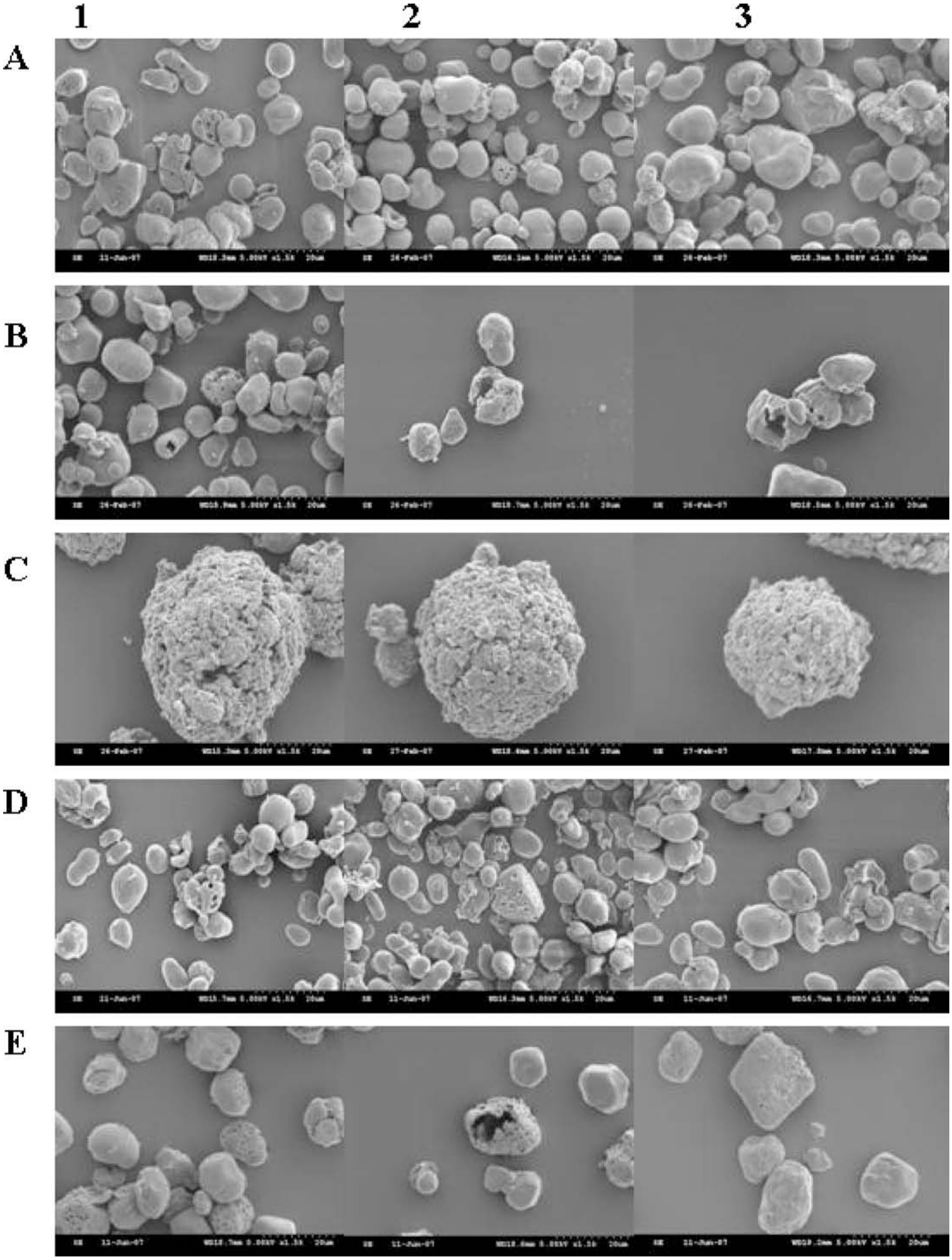

Fig. 8. SEM pictures of unprocessed maize starch samples (magnification 1500×) after exposure to enzymatic digestion using different enzymes, resembling *in vivo* conditions. (A) Hylon® VII, (B) Hylon® V, (C) IM-DS acetate starch, (D) LAPS, (E) amylopectin, (1) pancreatin, (2) hog pancreas α-amylase, (3) *Bacillus licheniformis* α-amylase.

starch [39], which makes heat-treated starch samples interesting as coating materials for drug delivery to the colon.

4. Conclusions

The observation that film coatings made from heat-treated high amylose starches could be used for colon-specific delivery leads to the investigation of the effect of heat treatment on the physicochemical properties of these starches. A heat treatment similar to that often received by starches during film coating preparation leads to a decrease in the digestibility of the starches by various α-amylases resembling the upper intestines conditions. As was seen by X-ray and FT-IR studies, upon heat treatment, these starches maintained a well-ordered arrangement of their macromolecular chains, which can be explained by a formation of retrograded forms of the starches. The present study has shown that high amylose starches have the potential to be used in film coating for colon drug delivery devices. This would obviate the need to ex-

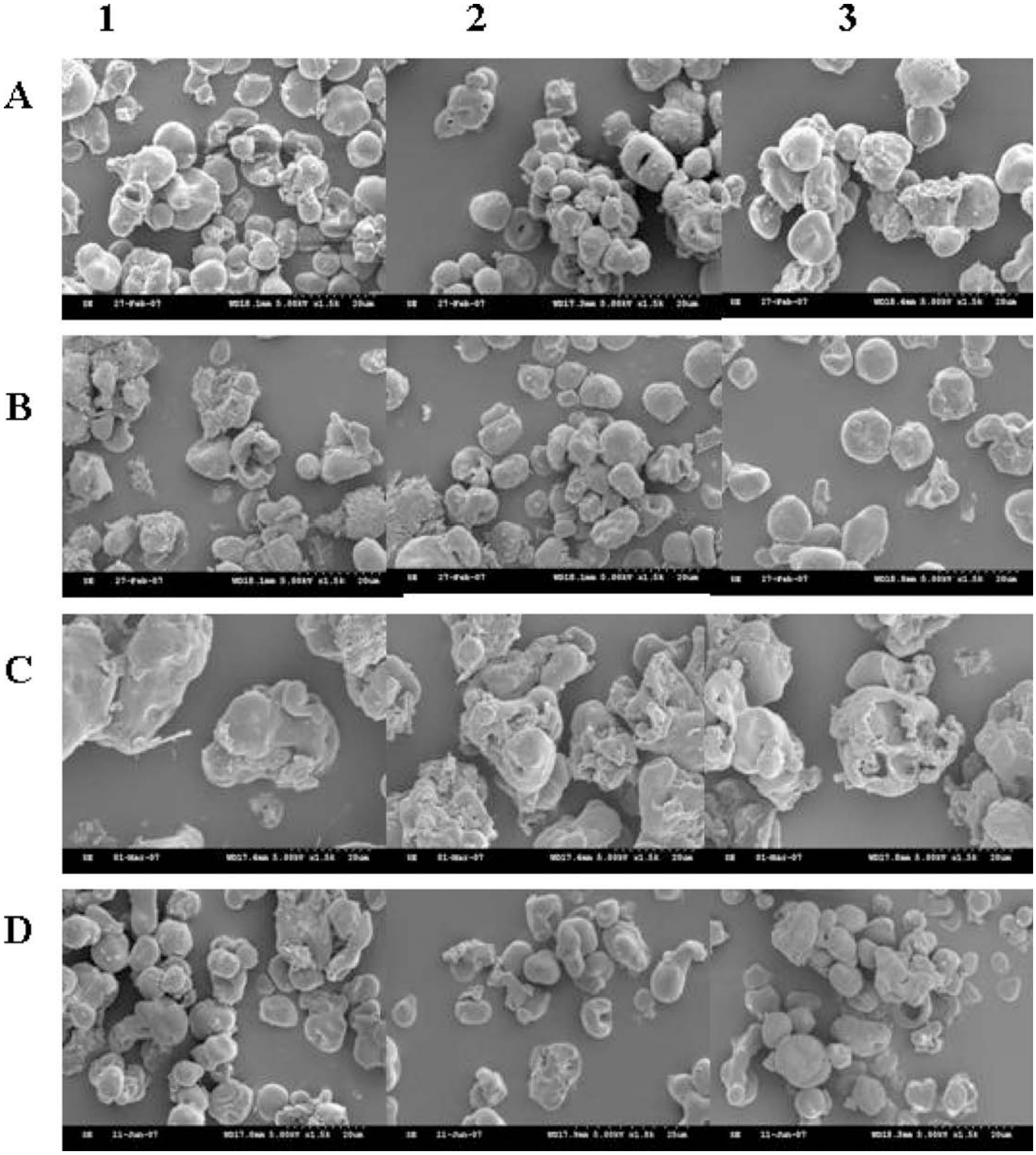

Fig. 9. SEM pictures of heat-processed maize starch samples (magnification 1500×) after exposure to enzymatic digestion using different enzymes, resembling *in vivo* conditions. (A) Hylon® VII, (B) Hylon® V, (C) IM-DS acetate starch, (D) LAPS, (1) pancreatin, (2) hog pancreas α-amylase, (3) *Bacillus licheniformis* α-amylase.

tract the amylose into a butanol complex, as claimed previously, hence cutting down expenses and the time required for the preparation of the film coating.

Acknowledgements

The authors would like to thank the Fundação para a Ciência e Tecnologia, Portugal, for their financial support (SFR/BD/18330/2004). We also like to thank Dr. Maria Teresa Vieira (Instituto Pedro Nunes) for the help with the X-ray and Dr. Lídia Gil for the assistance with polarised light microscopy.

References

[1] D.J. Gallant, B. Bouchet, A. Buléon, S. Pérez, Physical characteristics of starch granules and susceptibility to enzymatic degradation, Eur. J. Clin. Nutr. 46 (1992) 3–16.
[2] D.J. Gallant, B. Bouchet, P.M. Baldwin, Microscopy of starch: evidence of a new level of granule organization, Carbohydr. Polym. 32 (1997) 177–191.
[3] H. Röper, Applications of starch and its derivatives, Carbohydr. Eur. 15 (1996) 22–30.
[4] C. Chebli, I. Moussa, S. Buczkowski, L. Cartilier, Substituted amylose as a matrix for sustained drug release, Pharm. Res. 16 (1999) 1436–1440.
[5] O. Korhonen, P. Raatikainen, P. Harjunen, J. Nakari, E. Suihko, S. Peltonen, M. Vidgren, P. Paronen, Starch acetates—multifunctional direct compression excipients, Pharm. Res. 17 (2000) 1138–1143.
[6] M. Rahmouni, F. Chouinard, F. Nekka, V. Lenaerts, J.C. Leroux, Enzymatic degradation of cross-linked high amylose starch tablets and its effect on in vitro release of sodium diclofenac, Eur. J. Pharm. Biopharm. 51 (2001) 191–198.
[7] J. Singh, L. Kaur, O.J. McCarthy, Factors influencing the physico-chemical morphological thermal and rheological properties of some chemically modified starches for food applications – a review, Food Hydrocolloid 21 (2007) 1–22.
[8] C.C. Fertig, F. Podczeck, R.D. Jee, M.R. Smith, Feasibility study for the rapid determination of the amylose content in starch by near-infrared spectroscopy, Eur. J. Pharm. Sci. 21 (2004) 155–159.
[9] T. Allen, Particle size shape and distribution, in: T. Allen (Ed.), Particle Size Measurement, Chapman and Hall, London, 1990. pp. 126.
[10] S. Nara, T. Komiya, Studies in the relationship between water saturated state and crystallinity by the diffraction method for moistened potato starch, Starch/Stärke 35 (1983) 407–410.
[11] V.L. Hill, D.Q.M. Craig, L.C. Feely, Characterisation of spray-dried lactose using modulated differential scanning calorimetry, Int. J. Pharm. 161 (1998) 95–107.

[12] R.L. Billmers, M. Tessler, U.S. Patent 996322(5321132), 1994.

[13] E.A. Macgregor, α-Amylase structure and activity, J. Protein Chem. 7 (1988) 399–414.

[14] V. Planchot, P. Colonna, D.J. Gallant, B. Bouchet, Extensive degradation of native starch granules by alpha-amylase from *Aspergillus fumigatus*, J. Cereal Sci. 21 (1995) 163–171.

[15] Y.-C. Shi, T. Capitani, P. Trzasko, R. Jeffcoat, Molecular structure of a low-amylopectin starch and other high-amylose maize starches, J. Cereal Sci. 27 (1998) 289–299.

[16] G. Rowley, Starch, in: R.C. Rowe, P.J. Sheskey, P.J. Weller (Eds.), Handbook of Pharmaceutical Excipients, Pharmaceutical Press, London, 2003, pp. 603–608.

[17] H. Leach, Gelatinization of starch, in: R.L. Whistler, E.F. Paschall (Eds.), Starch: Chemistry and Technology, Academic Press, New York, 1965, pp. 289–308.

[18] R.L. Shogren, Preparation thermal properties and extrusion of high-amylose starch acetates, Carbohydr. Polym. 29 (1996) 57–62.

[19] J.A. Radley, The swelling and gelatinisation of starch, in: J.A. Radley (Ed.), Starch and Its Derivatives, Chapman and Hall, London, 1953, pp. 81–122.

[20] V.J. Morris, Starch gelation and retrogradation, Trends Food Sci. Technol. 1 (1990) 2–6.

[21] O.S. Lawal, Succinyl and acetyl starch derivatives of a hybrid maize: physicochemical characteristics and retrogradation properties monitored by differential scanning calorimetry, Carbohydr. Res. 339 (2004) 2673–2682.

[22] R.F. Tester, W.R. Morrison, Swelling and gelatinization of cereal starches. I – effects of amylopectin amylose and lipids, Cereal Chem. 67 (1990) 551–557.

[23] C. Fringant, J. Desbrieres, M. Rinaudo, Physical properties of acetylated starch-based materials: relation with their molecular characteristics, Polymer 37 (1996) 2663–2673.

[24] O. Sevenou, S.E. Hill, I.A. Farhat, J.R. Mitchell, Organisation of the external region of the starch granule as determined by infrared spectroscopy, Int. J. Biol. Macromol. 31 (2002) 79–85.

[25] A. Buléon, H. Bizot, M.M. Delage, B. Pontoire, Comparison of X-ray diffraction patterns and sorption properties of the hydrolyzed starches of potato, wrinkled and smooth pea, broad bean and wheat, Carbohydr. Polym. 7 (1987) 461–482.

[26] N.W.H. Cheetham, L. Tao, Variation in crystalline type with amylose content in maize starch granules: an X-ray powder diffraction study, Carbohydr. Polym. 36 (1998) 277–284.

[27] M.J. Gidley, D. Cooke, A.H. Darke, R.A. Hoffmann, A.L. Russell, P. Greenwell, Molecular order and structure in enzyme-resistant retrograded starch, Carbohydr. Polym. 28 (1995) 23–31.

[28] P. De Meuter, H. Rahier, B. Van Mele, The use of modulated temperature differential scanning calorimetry for the characterisation of food systems, Int. J. Pharm. 192 (1999) 77–84.

[29] R.A. Graaf, A.P. Karman, L.P.B. Janssen, Material properties and glass transition temperature of different thermoplastic starches after extrusion processing, Starch/Stärke 55 (2003) 80–86.

[30] A. Mizuno, M. Mitsuiki, M. Motoki, Effect of crystallinity on the glass transition temperature of starch, J. Agric. Food Chem. 46 (1998) 98–103.

[31] I. Tan, C.C. Wee, P.A. Sopade, P.J. Halley, Investigation of the starch gelatinisation phenomena in water-glycerol systems: application of modulated temperature differential scanning calorimetry, Carbohydr. Polym. 58 (2004) 191–204.

[32] K.J. Zeleznak, R.C. Hoseney, The glass transition in starch, Cereal Chem. 64 (1987) 121–124.

[33] L.C. Thomas, Modulated DSC® Basics; Calculation and Calibration of MDSC® Signals, T A Instruments Manual, New Castle, DE, USA, 2005.

[34] L.F. Siew, S.-M. Man, J.M. Newton, A.W. Basit, Amylose formulations for drug delivery to the colon: a comparison of two fermentation models to assess colonic targeting performance in vitro, Int. J. Pharm. 273 (2004) 129–134.

[35] S. Milojevic, J.M. Newton, J.H. Cummings, G.R. Gibson, R. Louise Botham, S.G. Ring, M. Stockham, M.C. Allwood, Amylose as a coating for drug delivery to the colon: preparation and in vitro evaluation using 5-aminosalicylic acid pellets, J. Controlled Release 38 (1996) 75–84.

[36] W. Helbert, M. Schulein, B. Henrissat, Electron microscopic investigation of the diffusion of *Bacillus licheniformis* [alpha]-amylase into corn starch granules, Int. J. Biol. Macromol. 19 (1996) 165–169.

[37] W.A. Atwell, P.L. Hood, R.D. Lineback, E. Varriano-Marston, F.H. Zobel, The terminology associated with basic starch phenomena, Cereal Foods World 33 (1988) 306–311.

[38] S.G. Ring, J.M. Gee, M. Whittam, P. Orford, I.T. Johnson, Resistant starch: its chemical form in foodstuffs and effect on digestibility in vitro, Food Chem. 28 (1988) 97–109.

[39] H.N. Englyst, G.T. Macfarlane, Breakdown of resistant and readily digestibible starch by human gut bacteria, J. Sci. Food Agric. 37 (1986) 699–706.