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Recrystallisation behaviour of native and processed waxy maize starch in relation to the molecular characteristics

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Abstract

Molecular characteristics were determined for native waxy maize starch and maize starch modified in different way (by mechanical treatment or/and acid hydrolysis). Recrystallisation behaviour was studied. Methods used in this study were MALLS, HPAEC-PAD, NMR, DSC, SEM, light microscopy.

Five starch materials were subjected to storage under the same conditions in the presence of water (70 w/w%). Molecular weight, radius of gyration, initial crystallinity, and degree of polymerisation, degree of branching, chain length distribution profiles, were related to nucleation rate during the recrystallisation process, rate of recrystallisation, thermal stability and amount of obtained crystallinity. This allowed the following connections between the molecular characteristics and kinetic of recrystallisation to be proposed: Amylopectin molecular weight appeared to affect the number of starch crystallites formed and amount of crystallinity but not the stability of the rebuilt crystallites. The stability of rebuilt crystallites can be controlled by degree of polymerization, degree of branching and unit chain length distribution, characteristics which were similar for the starches.

A mixture of two starches, with and without crystalline structure in initial state but with molecular weight in same range, were stored and scanned in order to understand possible cocrystallisation effects.

Keywords: Waxy maize starch; Molecular weight; Degree of branching; Acid hydrolysis; Recrystallisation; DSC; SEM; Microspheres; Drug formulation

1. Introduction

Starch, the primary component of most plant storage organs, a component in many essential food products and common excipient with long tradition in drug formulation, has recently emerged as a candidate for new uses as a biodegradable polymer in microspheres within the pharmaceutical industry. Microspheres can be produced using waxy maize starch, which consists mainly of amylopectin. The starch is used in the production of the microspheres utilising an aqueous two-phase system with gelatinised starch and polyethylene glycol as the two separate phases. In addition, these starch particles may be coated with poly (lactic/glycolic) acid (PLGA) to obtain controlled release formulations (Reslow, Gustafsson, Jönsson, & Laakso, 2002).

PLGA-coated starch microspheres can be used for subcutaneous injections where the microspheres have a mean particle size range between 40 and 100 μm (Reslow, Jönsson, & Laakso, 2002). Pure PLGA microspheres, which have commonly been used for this purpose, have a major disadvantage in that an acidic micro-environment is formed during the hydrolysis of their ester bonds and such an environment can be detrimental to protein molecules (Fu, Pack, Klibanov, & Langer, 2000). Starch possesses advantages that make it more suitable for utilising in the microspheres: it contains no acidic groups, is biodegradable, biocompatible, cheap, available in large quantities and renewable and has unique colloidal properties.

The injected microspheres will form a depot allowing for controlled release of active substances for periods of weeks

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up to several months (Okada, Doken, Ogawa, & Toguchi, 1994, Putney & Burke, 1998). The advantages of such controlled release formulations include prolonged pharmacological activity and lower drug peak concentrations, which, in turn, results in decreased adverse effects. An interesting application of microspheres can be their use in delivery of vaccines for targeting of antigen to, for example, mucosal surfaces after oral administration or to antigen-presenting cells after parenteral inoculations (Singh & O'Hagan, 1999). Starch microspheres can also be used for intra-nasal drug delivery (Pereswetoff-Morath, 1998).

Starch structure can be modified in different ways prior to utilization in industrial applications of which enzymatic and chemical modifications are most common within industry. Acid hydrolysis of starch, one of the methods used in the present study, proceeds by breaking both (1 → 4) and (1 → 6)-α-D-glycosidic linkages. All starch granules independently of botanical source are cold-water insoluble and have a partially crystalline structure. Hydration, swelling, disruption of granule structures and ultimately solubilisation of starch molecules can be caused by heating of starch in the presence of water and this process is collectively described by the term gelatinisation. Physically, gelatinisation results in reduced crystallinity and loss of birefringence. On cooling, dissolved starch, being in a disordered state becomes less soluble, and some amylopectin/amylose molecules partially reassociate in an ordered conformation (double helices). It is known that crystallites begin to form eventually and that this process is accompanied by a gradual increase in rigidity and phase separation between polymer and solvent. However, a more accurate understanding of the factors, which control this reordering process, is needed.

The reordering of amylopectin after previous gelatinisation, i.e. the recrystallisation process, occurs at temperatures between the glass transition temperature (T_g) and melting temperature (T_m) and can be measured by DSC (differential scanning calorimetry), and the melting endotherm gives information on stability, heterogeneity, and degree and perfection of ordered structure (which is strongly linked to crystallinity). Generally, during thermal scanning of starch/water dispersions a broad distribution in the endothermic transition indicates crystalline heterogeneity, where the least stable crystallites melt at a low temperature and the remaining crystallites of increasing quality melt at higher temperatures (Biliaderis, 1992). It has also been indicated that there is a dependency between the length of outer chains and transition temperatures and melting enthalpy. Longer outer chains of amylopectin molecules give higher melting enthalpy values (Lundqvist, Nilsson, Eliasson, & Gorton, 2002; Würsch & Gumy, 1992).

Durrani & Donald (1995) performed a study on amylopectin gel evaluating different methods to determine its crystallinity and concluded that DSC gel melting endotherm provided a good indicator of the degree and perfection of the crystallinity in the gel. Thus, the DSC technique seems to be a good method for evaluating the crystallinity of starch and the results can be related to the molecular characteristics of the starch.

There are four modifications of the crystalline structure of starch granules. The two polymorphs that are commonly observed by X-ray diffraction of native starches are of A- and B-type. The A-type had been found in cereal starches, the B-type in tuber starches. A- and B-type polymorphs differ in packing arrangement. The A-type is dense with less room for water molecules, while the unit cell of the B-type can hold four times more water molecules (Imberty, Chanzy, Perez, Buleon, & Tran 1988a, Imberty and Perez 1988b). Gelatinisation of starch in the presence of water and following storage at lower temperature induces recrystallisation process which means development of crystallinity. Polymorphic packing arrangements can be effected by numerous of factors, e.g., storage temperature, concentration, additives, outer chain length. Waxy maize starch used in this study has a characteristic so-called A-type pattern while the X-ray pattern of an amylopectin gel has been seen to have diffraction peaks at the same positions as those of starch with a B-type pattern (Durrani & Donald, 1995). Therefore an amylopectin gel has a network which is highly crystalline, although it lacks the long range lamellar order which is present in a starch granule. A-type polymorph is the thermodynamic product, i.e. the most stable polymorph, and B-type is the kinetic product of amylopectin crystallisation (Gidley, 1987).

The recrystallisation process has great impact on the pharmaceutical applications described above since it will affect the integrity and encapsulation efficiency of the formulation during microsphere production. In this study, molecular properties of the native and processed waxy maize starch are characterized. Relation between molecular properties and the recrystallisation behaviours of the starch is suggested. Structural characteristics of acid hydrolysed and mechanically treated waxy maize starch in relation to the recrystallisation process during storage, mixing of components and different storage temperatures are discussed.

2. Experimental

2.1. Materials

Waxy maize starch (Cerestar SF 04201), S1n, and acid hydrolyzed waxy maize starch (Cerestar C* Pharm 06090), S2a, were obtained from Cerestar Inc. (Vilvoorde, Belgium). The mechanically processed forms, S3m and S4m, and the acid hydrolyzed S5a, were obtained from SkyePharma AB (Malmö, Sweden).The origin of the starch samples and their relations are illustrated in Fig. 1.

According to SkyePharma AB, for the starches S3m and S4m, in the following text annotated as mechanically treated starches, the processing was briefly as follows: the acid hydrolysed granules of S2a were washed with a solution of 0.1 M NaOH/76% (w/w) ethanol. The starch granules were thereafter solubilised by heating. The starch solutions were passed through a high-pressure homogeniser (Rannie Homogeniser type Rannie 12.56 H. APV, Denmark). After ion-exchange chromatography with Q-Sepharose FF (Amersham, Uppsala, Sweden) and filtration (5 μm and 0.5 μm) the starch solutions were spray-dried (Niro Atomiser Spray dryer Production Minor, Soeborg, Denmark). The acid hydrolysed S5a was produced by incubating 33% of S1n in 1% HCl during stirring for 24 h at 50 °C.

2.2. Methods

2.2.1. Static light scattering measurements

Light scattering measurements were performed essentially according to Frigård, Andersson and Åman (2002), with a DAWN DSP multiangle laser light scattering detector (MALLS) operating in chromatographic mode using a He–Ne laser light source (632.8 nm) (Wyatt Technology Corp., Santa Barbara, CA, USA). The mobile phase was 0.1 M sodium nitrate with 0.02% sodium azide, and three size exclusion columns in series Shodex OH-Pak 803-HQ, 804-HQ and 806-M-HQ (Shoko CO., Ltd, Tokyo, Japan) in a column oven set at a temperature of 30 °C were used. Samples were dissolved by heating in the mobile phase at 120 °C for 15 min during gentle shaking. All calculations in Astra were carried out on first degree fitted data using the Debye model. The dn/dc value used for amylopectin in water solutions (0.146 ml/g) was obtained from literature (Chanliaud, Roger, Saulnier, & Thibault, 1996; You, Fiedorowicz, & Lim, 1999).

2.2.2. Debranching and high performance anion exchange chromatography

Debranching of native starches as well as the partially treated amylopectins (~1.5 mg/ml) was performed essentially according to Lloyd, Hedley, Bull, & Ring (1996). The dissolved samples were incubated with isoamylase (29.5 U) for 16 h at 38 °C, after which the enzyme was inactivated by boiling for 10 min. Isoamylase (EC 3.2.1.68) from *Pseudomonase amyloderamosa*, with an activity of 71000 U/mg protein, was obtained from Hyashibara Biochemical Labs. (Okayama, Japan). Under the conditions described by Koch, Andersson, & Åman (1998), the debranched amylopectin was analysed by high performance anion exchange chromatography (HPAEC) system equipped with a pulsed amperometric detector.

2.2.3. Nuclear magnetic resonance spectroscopy (NMR)

Spectra were recorded on a Bruker DRX 400 spectrometer operating at 400 MHz for ^{1}H with the temperature regulated at 30 °C and using D_2O as solvent. Chemical shifts were calibrated using acetone sodium 3-(trimethylsilyl) propionate-2,2,3,3-d_4 (0 ppm, ^{1}H-NMR) as an internal reference. The degree of branching (DB; %) was calculated from the areas of the integrated peaks as the proportion of the branch point signal (4.94 ppm) relative to the total anomeric protons (4.94 and 5.36 ppm).

2.2.4. Microscopy

2.2.4.1. Light microscopy. The dry starch samples, as well as starches that had been dispersed in cold water (wet starch) and their heated dispersions, prepared as described in 2.2.5, were examined under light microscope Olympus DP 10 (Olympus Corp., Tokyo, Japan) in polarised light. Light microscopy photographs were taken with Olympus BH 2 camera (Olympus Corp., Tokyo, Japan) at a magnification of 156 × .

2.2.4.2. Scanning electron microscopy (SEM). Microscopy was performed on a JEOL JSM-5600 scanning electron microscope. The samples were attached to circular stubs with double-sided tape and coated with gold-palladium using Sputter Coater Polaron SC7640 (Thermo VGMicrotech). One of the samples, S2a (Fig. 4G), was visualized by a Philips XL30 scanning electron microscope controlled with the XL Microscope Control Software. Gold sputtering in this case was performed using Sputter Coater Cressington 108 Auto (Watford, UK). Samples were viewed by scanning the total specimen and a representative area was photographed at magnifications in ranges up to 1200 × .

2.2.5. Differential scanning calorimetry (DSC)

The crystallisation process was investigated using DSC on starch samples prepared by different methods as described below. The samples were characterized by the endothermic heat (ΔH) required to melt the crystalline structure of amylopectin of starch samples and the three temperatures, onset temperature (T_o), peak temperature of the dominating peak (T_m) and completion temperature (T_c), characterizing the melting.

Two alternative sample preparation methods were used. For initial scans and long-term recrystallisation experiments dry starch samples (3 mg) were weighed into coated aluminium pans, after which water was added to give a final concentration of 30% (w/w) starch material. The samples were allowed to rest for 3–5 h before heating in the DSC allowing for an initial scan of the sample at a rate of 5 °C/min. After initial scanning, the samples were stored at 6 °C for 14 days before DSC scanning at a rate of 5 °C/min for long-term recrystallisation.

Preparation of samples for short-term recrystallisation and two-step temperature experiments was performed outside the DSC pans by dispersing a 30% (w/w) starch/water mixture and heating it in a microwave oven (LG Electronics Inc., MS-194A). After this preparation samples of 10 mg were weighed into DSC pans, within 1 h, prior to further storage in the pans. The samples for short-term recrystallisation experiments were stored at 20 or 6 °C for 20 h before thermal scanning at a rate of 10 °C/min. The samples for two-step temperature experiment were stored at 6 °C for 29 h, followed by 14 h at 37 °C, and thereafter scanned by DSC at a rate of 5 °C/min. Two-step temperature treatment was performed in order to mimic the production of starch microspheres.

In addition to the individual starch samples, mixtures of two starch materials, S3m and S5a, were also investigated for short-term recrystallisation. The first set of the samples was prepared by mixing the dry starch materials before dispersing in water and heating. The second set of the samples for mixing experiment was prepared by addition of dry S5a to a heated dispersion of S3m. The prepared samples contained these starches in proportions of 50:50% (w/w) and had a final starch concentration of 30% (w/w). The samples were weighed and stored under the same condition as the individual starches in short-term recrystallisation experiment.

In all experiments the aluminium pans (TA Instruments, New Castle, USA, ref no. 900790.901 and 900796.901) were hermetically sealed and analysed in a DSC 6200 (Seiko Instruments Inc., Shizouka, Japan) with an empty pan as reference. The melting enthalpy was determined with a temperature sweep starting at 5–30 °C up to 130 °C and expressed on amylopectin dry weight basis. All measurements were made in at least duplicates. The true amylopectin/water ratio was determined by drying punctured pans at 105 °C overnight.

Two DSC scanning rates (5 and 10 °C/min) were used during the experiments in this study. Initially, a scan rate of 10 °C/min was chosen to use throughout the study. Unfortunately, in some experiments, resolution of the resulting endotherms at this scanning rate was worse than expected making it difficult to evaluate transition parameters. The scan rate was thus changed and all the following experiments were performed at 5 °C/min. However, the results of the 5 °C/min rates-experiments were never compared in the discussion with 10 °C/min rates-experiments. The comparisons of results were done only within same set of experiments or between experiments performed under the same experimental conditions.

3. Results and discussion

3.1. Characterisation of the starch materials

Some characteristics of the starch materials used in this study are shown in Table 1.

3.1.1. Properties on the molecular level

Average molecular weight ($\bar{M}_w$) and radius of gyration ($\langle \bar{s} \rangle_w$) for the starch materials were determined by HPSEC-MALLS. The $\langle \bar{s} \rangle_w$ was obtained from the light scattering data.

The highest $\bar{M}_w$ was detected for S1n, native waxy maize starch, whereas S5a had the lowest $\bar{M}_w$. The radius of gyration of S5a was on average at the same level or tended to be higher than $\langle \bar{s} \rangle_w$ of the mechanically treated starches, S3m and S4m. This indicated that the mechanically treated starches and acid hydrolysed starch S5a had different molecular superstructure of amylopectin molecules, and this distinction could probably be linked to differences in the processes of starch manufacture (Fig. 1). During mechanical treatment of previously acid hydrolysed and gelatinised starch, amorphous and crystalline material from the granular rings seemed to be displaced in a different way from what was obtained by the acid hydrolysis treatment alone. As a result, amylopectin molecules of the mechanical treated starches took up smaller space by gyration compared to the acid hydrolysed S5a although their $\bar{M}_w$ were in the same range.

The unit chain length distribution profiles for the starch samples as determined by HPAEC-PAD are shown in Fig. 2. Essentially no differences could be observed in the unit chain length profiles or the main peak maximum among the samples in spite of the differences obtained in $\bar{M}_w$. The average DP values (21–22) only showed minor differences due to the processes involved. The ^{1}H-NMR results reflect the results obtained from HPAEC-PAD, only minor differences could be observed in the spectra (Fig. 3). The broadening of the H-1(1 → 4) signal at 5.36 ppm was most likely due to an increase in viscosity as $\bar{M}_w$ increased. Because the broadening of NMR signals is just an observation that is expected as a consequence of increased viscosity it cannot be verified statistically. The only minor changes were observed in the H-1(t)

Table 1
Table illustrating varying initial characteristic differences by material, obtained during processing

Label	Processing description	Molecular weight ($\bar{M}_w$), g mol^{-1}	Radius of gyration $\langle \bar{s} \rangle_w$, nm	Presence of granules in dry powder
S1n	Native waxy maize starch (Unprocessed)	4.7×10^7	62	+
S2a	Acid hydrolysed starch (Commercial)	2.6×10^6	34	+
S3m	Mechanically treated starch	5.1×10^5	20	−
S4m	Mechanically treated starch	3.6×10^5	22	−
S5a	Acid hydrolysed starch	3.2×10^5	26	+

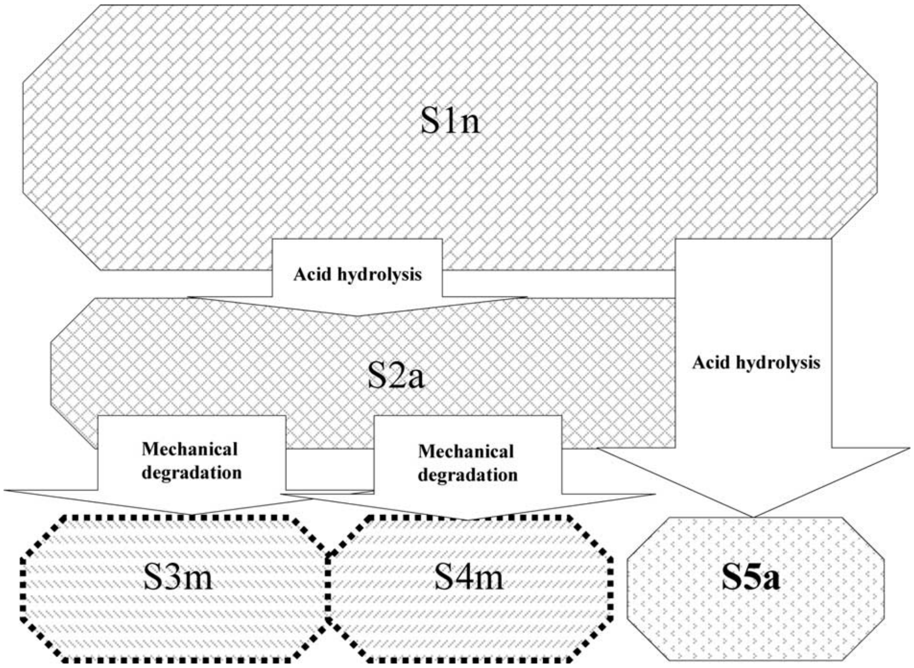

Fig. 1. Schematic picture over the starch materials used in the present study.

(the (t) means non-reducing terminal residue) or H-1(1 → 6) signals at 5.30 and 4.94 ppm, respectively, for the treated samples. The degree of branching was for the S1n-S5a starch samples, 3.8, 4.1, 4.5, 4.4, and 4.5%, respectively. The differences between the processed starch materials (average 4.4%) were very small and their fine structure should be considered to be similar.

3.1.2. Properties on the microscopic level

The particle appearance of the individual dry starch samples was investigated using SEM (Fig. 4). The appearance of native waxy maize starch was characteristic for this sort of starch, with distinct granules, having mixed shapes, with some being almost spherical, some angular, sometimes 'honeycomb' shaped with irregular surfaces, varying from small (3 μm) to intermediate (10–18 μm) in diameter. Relatively small differences were observed in appearance between native starch S1n and the two acid hydrolysed starches S2a and S5a (Fig. 4.A, B, E). These starch materials apparently contained distinct granules. Granules of S1n and S2a tended to be in clusters, but not granules of S5a. Moreover, the surface of S5a seemed to be less smooth compared to S1n and S2a. A rough outer surface layer could be clearly distinguished on the SEM-picture at magnification of 1200 × (Fig. 4J), although not at the lower magnifications. According to Atichokudomchai, Shobsngob and Varavinit (2000), the outer layer of the starch granule surface could be more or less perforated or completely destroyed due to erosion caused by acid hydrolysis and depth of the destruction depended on extent of the treatment, which also was confirmed by Jayakody and Hoover (2002).

In case of S3m and S4m, there were no typical waxy maize starch granules present in the samples (Fig. 4C and D). Instead, the structures observed were sometimes spherical or triangular shaped; varying from 7 to 40 μm in the diameter and sometimes they seemed to have a shell-like character. Obviously, granular surface and internal ordered structure of these samples were destroyed in processing, during the heating and shearing steps. This suggestion that the structures were non-granular constructions and seemed to be hollow, sometimes pinched, could be concluded from the SEM-micrographs at magnifications up to 1200 × (Fig. 4H and I).

Light microscopy observations confirmed the previous conclusions about the differences between the two groups of starches, where the native starch and the acid hydrolysed starches constituted one group (which contained intact granules) and mechanically treated starches constituted the other group (mainly without intact granules) (Fig. 5). Dry powders of native and acid hydrolysed starches showed the typical optical birefringence pattern (Fig. 6A and C). When these samples were wetted, the granules kept their general structure and integrity (Fig. 5A, B, E). In dry powder of mechanically treated starches some optical refraction at the outer part of the 'shells' could be observed (Fig. 6B and D), some kind of anisotropic structure which survived the processing or was built up during it. Upon wetting, the powders of S3m and S4m were almost completely dissolved and optical refraction which could

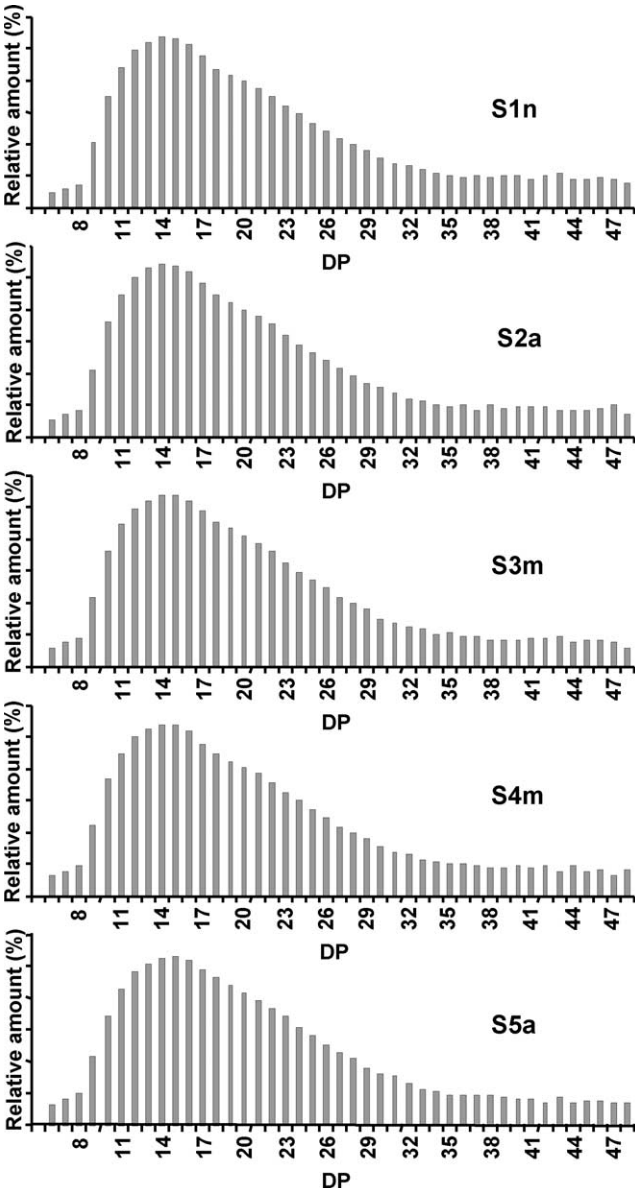

Fig. 2. The unit chain length distribution of native (S1n), acid hydrolysed (S2a and S5a) and mechanically treated (S3m and S4m) waxy maize starches.

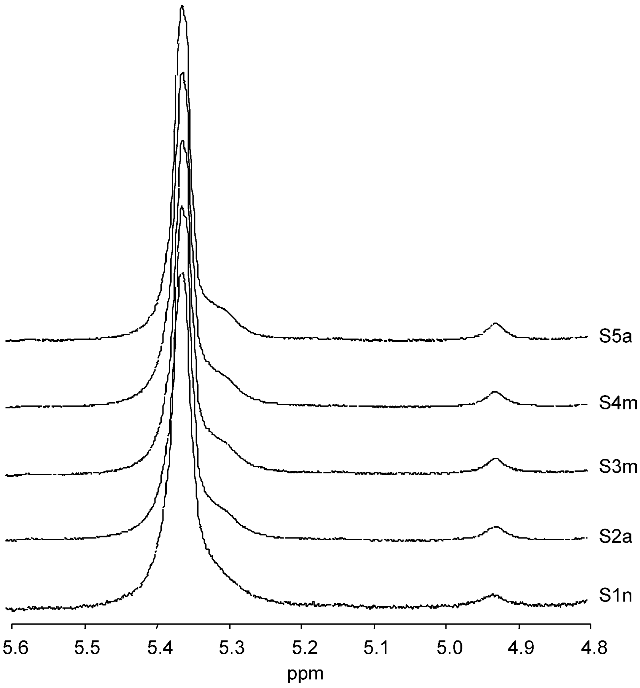

Fig. 3. 400-MHz ^{1}H-NMR spectra of native (S1n), acid hydrolysed (S2a and S5a) and mechanically treated (S3m and S4m) waxy maize starches recorded at 30 °C in D_2O.

point on the presence of some crystalline structure could not be seen anymore (Fig. 5C and D).

When these wetted starch dispersions were heated (in microwave oven as was described in 2.2.5), the main part of the granule structure in S1n and S2a samples disappeared but some particles possessing crystalline properties survived (Fig. 5F and G). In contrast, in the heated sample of S5a, which was the sample with the lowest molecular weight, native granules could not be found although micrographs of the wetted sample revealed presence of the crystalline and granular structure (Fig. 5J). Heated samples of S3m and S4m (Fig. 5H and J) hardly differed from wetted ones, which means that neither presence of intact granular or crystalline structure could be observed there. In the samples of S3m, S4m and S5a, after this final heating, only some fragments, like residues of the granules, could be observed.

3.1.3. DSC scan of non heated samples 0 days of storage

DSC scan performed on all samples prior to storage showed an endothermic transition for the native, S1n, and the acid hydrolysed, S2a and S5a, starches corresponding to the gelatinisation of the granules (Fig. 7). In contrast, initial DCS scans revealed an absence of an endotherm for the mechanically treated starches, S3m and S4m. Within the group of native and acid hydrolysed starches, only small differences were observed concerning peak melting temperatures or enthalpies (Table 2). However, the widths of the transition peaks were greater for S2a and S5a than for S1n when heated at 5 °C/min (Fig. 7). These observations were in line with Donovan and Mapes (1980) study, where the authors observed from DSC measurements of native and acid hydrolysed potato starch that, within experimental error, the specific enthalpy of the transition was constant (unchanged) for these materials, but the endotherm was significantly broadened for acid hydrolysed samples and their endotherm peak temperature were greater than that for native starch. The latter observation about peak temperature differed from our experimental results. This discrepancy might be related to differences in starch material, acid treatment extents, sample preparation methods and measurements conditions (like heating rate).

Two of five samples used in this study, S2a and S5a, were obtained by exposing of native starch S1n to acid hydrolysis

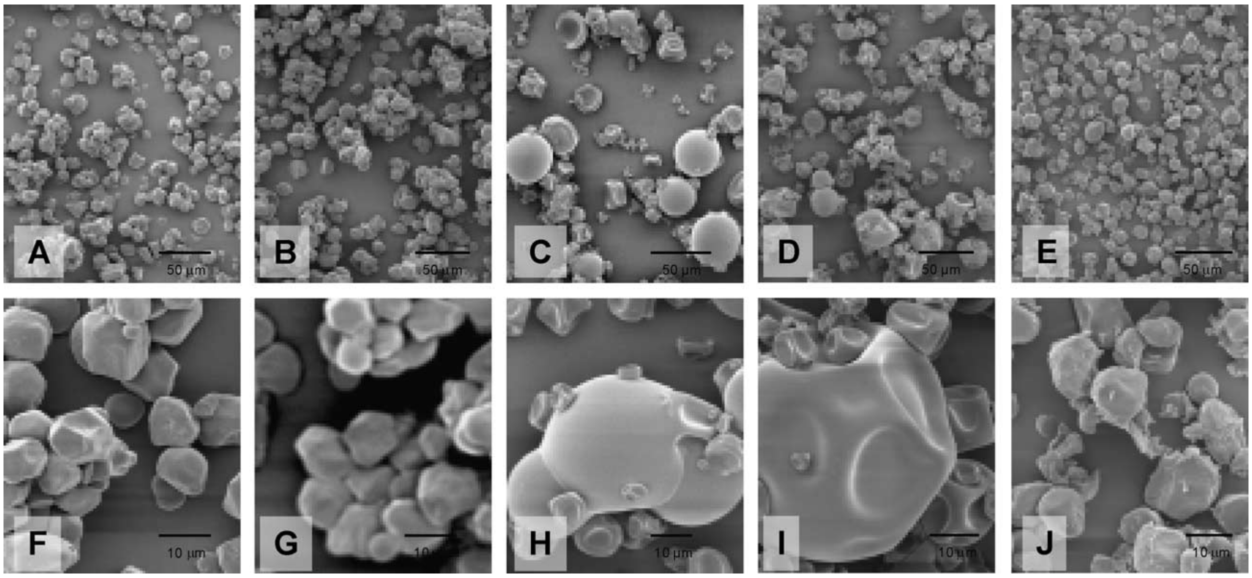

Fig. 4. Scanning electron microscopy micrographs of native S1n (A, F), acid hydrolysed S2a (B, G) and S5a (E, J), and mechanically treated S3m (C, H) and S4m (D, J) waxy maize starches. The micrographs A–E are taken at magnification of 300 ×. The micrographs F–J are taken at magnifications of 1000–1200 ×.

treatment in different extents (Fig. 1). Previously it has been suggested (Komiya & Nara, 1986, Komiya, Yamada, & Nara, 1987) that acid preferably attacks the amorphous regions of the starch granule and there the main part of degradation occurs. Even if the hydrolysis was not so extensive for our samples this effect has evidently been sufficient to reduce the molecular weight up to 100 times.

To judge from the enthalpy values, acid hydrolysis did not considerably alter the degree of crystallinity in the samples, but there were some effects on the granular structure and crystalline heterogeneity. According to Donovan and Mapes (1980), cleavage of starch chains in the amorphous regions of the granule due to acid hydrolysis reduces the destabilizing effect on the crystalline portions of the starch granule by weakening cooperativity between crystalline and amorphous parts of the granule, which could affect water transport within the granules. The broadening, observed in the DSC endotherms of S2a and S5a, indicated heterogeneity of the crystalline regions.

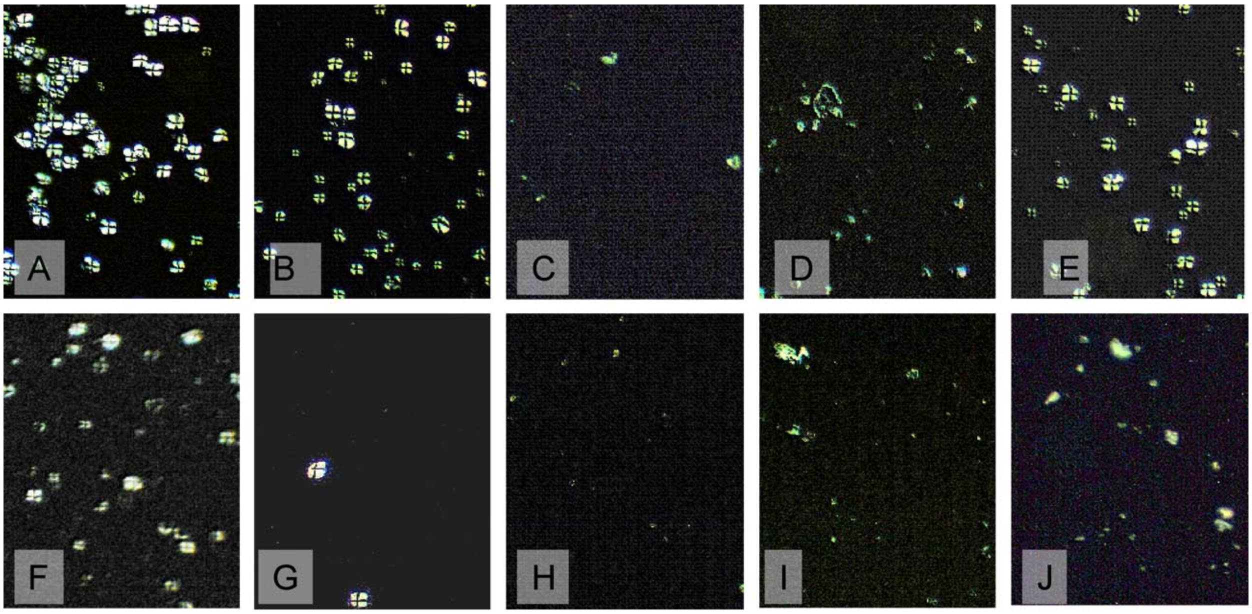

Fig. 5. Polarised light microscopy micrographs of wetted (A–E) starch dispersions and samples exposed to heating in presence of water (F–J). The samples A,F-of native (S1n), B,G-of acid hydrolysed S2a, G,H-of mechanically treated S3m, D,I-of mechanically treated S4m, and E,J-of acid hydrolysed S5a waxy maize starches.

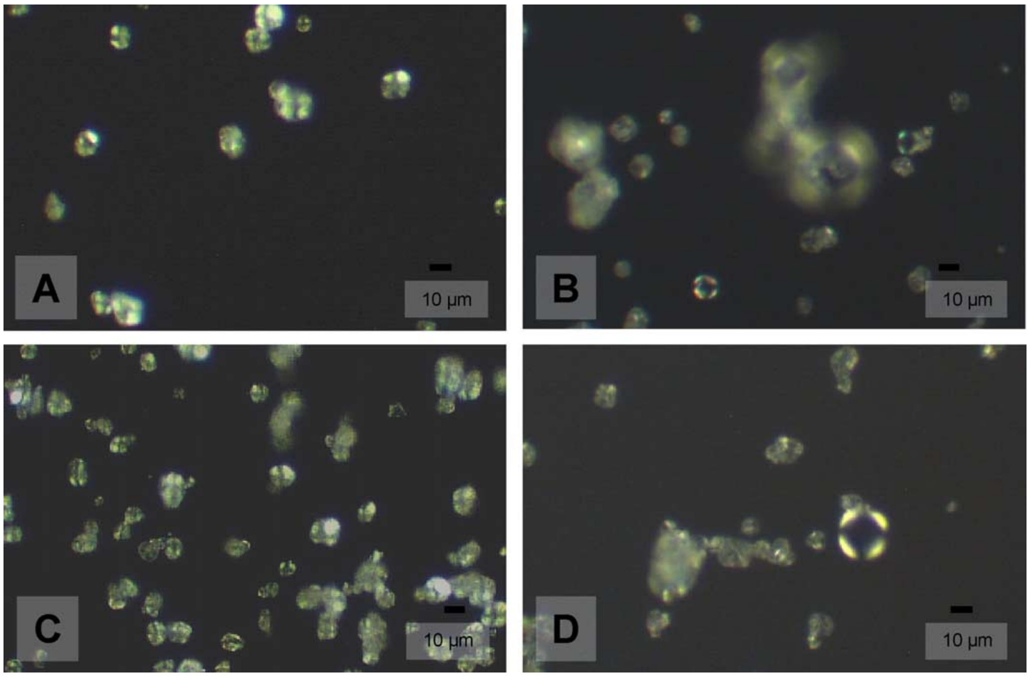

Fig. 6. Polarised light microscopy micrographs of dry powder of processed acid hydrolysed S2a (A) and S5a (C) and mechanically treated S3m (B) and S4m (D) waxy maize starches.

Absence of an endotherm in the initial DSC scan of the mechanically treated starches, S3m and S4m, could be expected, as light microscopy and SEM did not reveal any conventional granular structure in these samples. The lack of granules and crystallinity for this group of starches indicated that the mechanical treatment, which included

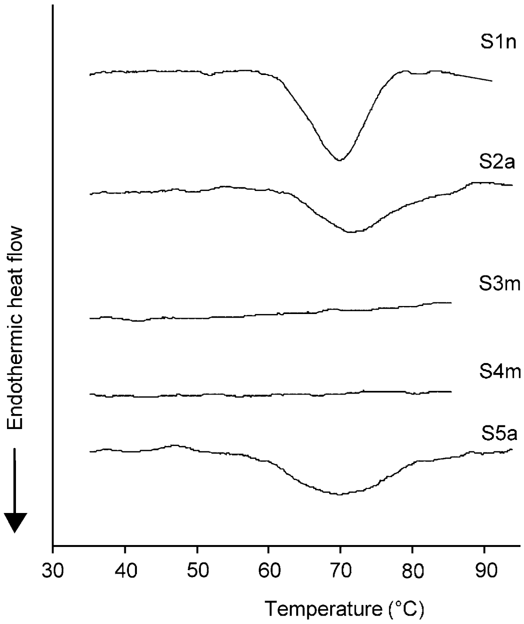

Fig. 7. Initial DSC ‘gelatinisation’ endotherms of native (S1n), acid hydrolysed (S2a and S5a) and mechanically treated (S3m and S4m) waxy maize starches (amylopectin/water ratio 3:7).

Table 2
The onset (T_o), peak (T_m) and final melting (T_c) temperature and transition enthalpy (ΔH) obtained from DSC analysis of processed and unprocessed waxy maize starch subjected to different storage times and temperature

Sample	Transition temperatures and enthalpy			
	T_o (°C)	T_m (°C)	T_c (°C)	ΔH (J/g)
Initial scan, 0 days of storage				
S1n	62.7(0)	69.9(0)	74.0(0)	15.8(0.5)
S2a	60.4(2.6)	71.9(0.6)	84.4(1.9)	15.7(1.6)
S3m	–	–	–	–
S4m	–	–	–	–
S5a	58.1(1.9)	70.0(2.1)	84.8(1.6)	14.5(0.8)
Long-term recrystallisation, 14 days of storage at 6 °C				
S1n	34.9(0.4)	47.7(0)	61.2(0.1)	10.6(1.6)
S2a	34.9(1.2)	48.9(1.9)	62.5(0.8)	11.9(1.1)
S3m	34.9(2.4)	49.0(1.2)	62.7(1.4)	6.9(0.3)
S4m	35.2(1.2)	48.1(1.1)	65.0(1.6)	7.7(0.9)
S5a	34.8(0.5)	48.0(1.3)	65.2(1.3)	13.7(0.9)
Short-term recrystallisation, 20 h of storage at 6 °C				
S1n	33.6(0.5)	56.7(1.2)	67.6(1.8)	2.9(0.9)
S2a	31.3(2.8)	54.0(1.1)	68.3(1.0)	4.2(0.4)
S3m	39.8(3.5)	54.4(3.5)	65.4(0.8)	3.1(0.3)
S4m	38.2(4.4)	56.7(2.5)	66.8(2.1)	2.6(0.6)
S5a	35.1(1.8)	55.1(0.3)	68.3(0.6)	5.6(0.4)
Short-term recrystallisation, 20 h of storage at 20 °C				
S1n	–	–	–	–
S2a	–	–	–	–
S3m	–	–	–	–
S4m	–	–	–	–
S5a	47.6(3.8)	62.0(2.2)	74.9(1.6)	1.3(0.2)

Standard deviation values are given within brackets; –, not observed.

gelatinisation prior to the mechanical degradation, was harsh enough to completely destroy the native starch structure. The processing and drying of the mechanically treated product was evidently such that no substantial recrystallisation of the starch material occurred.

3.2. Recrystallisation as investigated by DSC

3.2.1. One-step temperature treatment

3.2.1.1. Long-term recrystallisation. The thermograms, obtained during the melting of the stored individual starch dispersions, are presented in Fig. 8. The temperature range and peak temperature were nearly identical (Table 2). Recrystallisation at this period of storage (14 days) was assumed to be sufficient to achieve full recrystallisation and, thus, maximum value for transition enthalpy as reported by Roulet, Raemi and Wuersch (1987). Neither the acid hydrolysis nor the mechanical steps in the starch processing were able to influence the endothermic transition temperatures to a significant degree, which indicated that there was no significant influence from the processes on the type of crystals or the perfection of the crystals formed.

Some differences were revealed in the magnitude of the transition enthalpies. This variation could probably be explained by differences in the total amount of crystallites formed during the storage period, and the crystallisation rate. The enthalpy differences could also be related to the variations in molecular weight as seen with S5a having the highest enthalpy recorded. However, as the mechanically treated starches had lower enthalpies than the native and acid hydrolysed starches, it seemed likely that the results were influenced both by the molecular weight and by the process for molecular weight reduction.

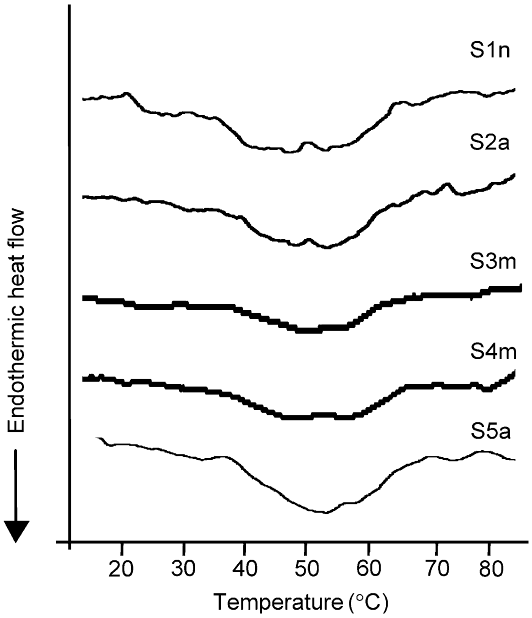

Fig. 8. Storage (14 days at 6 °C) DSC melting endotherms of native S1n, acid hydrolysed (S2a and S5a) and mechanically treated (S3m and S4m) waxy maize starches (amylopectin/water ratio: 3:7).

According to our observations, the main differences between mechanically treated and acid hydrolysed starches were that only the latter contained granules and crystalline material (seen from investigations by SEM and DSC), and that they seemed to have a less compact structure (seen from the radius of gyration). The presence of already crystalline material or granular remnants could function as nucleation points and thus favour recrystallisation as in the cases with the samples of S1n, S2a and S5a. Further, after acid treatment, which reduced the destabilizing effect of the amorphous part of granules in the starch samples, the remaining crystalline part of material might have a greater tendency to recrystallise. The effect of decreased molecular weight could be an increased mobility of the amylopectin and, thus, an increase in the rate of crystallisation.

The purpose of the 14-day storage time was to reach full recrystallisation. However, it is possible that the time chosen may only have been sufficient for some of the starches. Thus, the differences seen in the transition enthalpies could be due either to a difference in the number of crystallisation nuclei that can be formed in the melt and/or differences in rate of recrystallisation.

3.2.1.2. Short-term recrystallisation

3.2.1.2.1. Individual starch samples. Differences in endothermic transitions for the individual starch samples were observed already after the shorter storage time for 20 h at 6 °C (Table 2). This finding indicated that the rate of recrystallisation was affected by the type of starch sample, which were produced in the different modification way, by the action of acid hydrolysis alone, compared to that followed by mechanical treatment. The S5a again exhibited the largest melting enthalpy followed by S2a. The mechanically treated starches had the lowest enthalpy values and were approximately on the same level with the native starch.

After storage at 20 °C for 20 h, S5a was the only sample that showed any recrystallisation at all. Hydrolysis of amorphous material by acid treatment and the lower molecular weight probably aided the formation of double helices which act as crystal nuclei and thus, nucleation and growth of crystallites in S5a could be achieved under these storage conditions. Generally, it is well known that storage at a low temperature induces nucleation and thus the formation of crystallites. At temperatures just below T_m the crystallisation rate is extremely slow; as the temperature is decreased further the rate increases, passes through the maximum, and when the temperature is lowered sufficiently the rate seems to decrease again. The kinetics of an isothermal transformation can usually be explained by an adequate description of the nucleation and the growth mechanisms. To be effective in the transformation nuclei exceeding a thermodynamically defined critical size must be formed. However, foreign bodies, cavities, or other heterogeneities will facilitate the rate at which nuclei is formed (Mandelkern, 1956). In our case, it seemed that differences existing between the starches are such that during the storage at 20 °C nuclei could be formed only in sample S5a. Combination of lower molecular weight, and higher radius of gyration, presence of double helical structure and, maybe, higher mobility could be the reasons.

Increasing the storage temperature will favour the formation of more perfect crystals, which may be seen as an increase in the melting temperature of crystallites formed. This was also observed from the experiments with storage at 20 °C compared to 6 °C; T_m increased with 7 °C for the S5a sample.

3.2.1.2.2. Mixtures. Storage experiments of mixture of the starches S3m and S5a were performed in order to investigate which factors caused the differences in recrystallisation kinetics, seen between the group of native and acid hydrolysed and the group of mechanically treated starch materials. In the first mixing experiment, S3m and S5a were mixed prior to heating of the samples. The enthalpies obtained (Table 3) were close to what could be observed for 100% of S5a at the same storage conditions and higher than it could be observed for 100% of S3m, and thus higher than what would be expected for an additive effect from these two materials (i.e. if each starch is supposed to contribute 50% of the enthalpy of when it is present alone). The results did not conclusively show if the presence of S5a increased the amount of crystallites formed by S3m, or if the presence of S3m increased the amount of crystallites formed by S5a, or if it was a co-crystallisation effect on recrystallisation rate in this mixing experiment. During heating, the granules of S5a were ruptured and amylopectin molecules from both samples could interact and the gel network formation in this mixed sample seemed to develop in a co-operative manner. As reported by Smits, Kruiskamp, Soest, and Vliegenthart (2003), malto-oligosaccharides of DP > 6 might form small helices that co-crystallised with starch and thereby increased retrogradation. Obviously, such a co-crystallisation effect was present and enhanced the rate and, thereby, the process of nucleation and growth of crystallites in the mixture sample. However, with S3m apparently being a less crystalline material than S5a, it was likely that the presence of S5a was the critical factor for increased crystallinity.

Table 3
The onset (T_o), peak (T_m) and final melting (T_c) temperature and recrystallisation enthalpy (ΔH) obtained from DSC analysis and, also, the theoretical value of the minimal expected enthalpy of processed and unprocessed waxy maize starch subjected to mixing

Sample	Experimental transition temperature and enthalpy				Expected[a] ΔH (J/g)
	T_o (°C)	T_m (°C)	T_c (°C)	ΔH (J/g)	
Mixing, 20 h of storage at 6 °C					
Mix50bt[b]	37.4(2.5)	55.7(1.7)	65.8(1.2)	5.5(0.6)	4.4
Mix50bs[c]	62.2(0.1)	72.1(1.2)	87.5(3.7)	5.5(0.5)	8.8
Mixing, 20 h of storage at 20 °C					
Mix50bt[b]	49.5(4.3)	58.3(5.4)	65.4(6.4)	0.2(0.0)	0.7
Mix50bs[c]	58.9(2.3)	73.1(0.8)	90.9(2.1)	5.2(1.1)	7.3

Standard deviation values are given within brackets.

[a] Theoretical value of the minimal expected recrystallisation enthalpy of the individual starch samples prepared and stored at same conditions as in this experiment, in which they were mixed in equal proportions.

[b] Dry starches S3m and S5a were mixed followed by addition of water and heating (first mixing experiment).

[c] Initial preparation of S3m heated dispersion followed by the addition of dry S5a (second mixing experiment).

In the second experiment, dry sample of S5a was added to a pre-heated S3m dispersion after cooling to ambient temperature to see if the presence of non-dissolved granules as nucleation seeds would speed up the recrystallisation process. In this experiment S5a was not heated and gelatinised before mixing and storage and remained as cold water insoluble granules. Water uptake by the S5a was assumed to be negligible and the concentration of S3m in the dispersion was approximately 15%w/w (lower than 30%w/w as in all the other experiments). The DSC thermogram showed a transition peak which could correspond to the melting of dry granules of S5a and the values for the melting peak were in this case in the range of or slightly below what would be expected for the S5a alone (Table 3). To check the ability of S3m to recrystallise at 15%w/w starch concentration, the short-term recrystallisation experiments were performed which showed no recrystallisation at any of the experimental temperatures of storage. Thus, there was no increase in the recrystallisation enthalpy of the dissolved materials due to inoculation with dry high crystalline material.

One explanation for why acid hydrolysed starches recrystallised faster than the mechanically treated starches did, and also gave higher enthalpy values after shorter period of storage was that remnants of granules could work as nucleation points in starch dispersions. However, this explanation was disproved by the mixing experiments, which also was in line with results reported by Smits et al. (2003).

Thus, it was not the presence of remnants of granules that could explain the faster recrystallisation of the acid hydrolysed starch, but some other factors. One such factor could be that in the melted starch solution the amylopectin molecules that prior to melting formed the crystalline structure is still in proximity to each other in the non-stirred solution. This ‘memory’ of the former crystallinity could lead to fast reformation of small crystalline regions that, in turn, could function as nucleation sites. Gelatinisation at the final heating with water allowed penetration of water molecules into the granules, its swelling and rupture, and consequent unfolding of double helical structure of the crystallites. Being undamaged with regards to crystalline arrangement within granular structure, the crystallites of S5a could retrieve their ordered structure easier and recrystallise in shorter time then S3m did, which crystalline and granular structure, in contrast, was destroyed by mechanical treatment and, evidently, had not been able for restoration to an adequate level. On the other hand, variations in radius of gyration could also make a difference during recrystallisation process. Gelatinised amylopectin molecules of S5a had to take up more space in the heated dispersion because of higher value of radius of gyration compared to S3m, and thereby could be able to form more of nucleation sites because of contact between polymer branches in the dispersion facilitated. Finally, in the first mixing experiment, recrystallisation process was obviously speeded up by presence of gelatinised S5a. The latter was not observed in the second mixture experiment, in the absence of gelatinised S5a.

3.2.2. Two-step temperature treatment

As stated earlier, it is known that a temperature treatment affects both the rate of crystallite formation and the perfection of these crystallites. In the normal way of producing starch microspheres these aspects are considered by including two-step temperature treatment, at 6 and 37 °C, in the producing process. Therefore, such a treatment, with storage at these temperatures, was included in the present study. Treatment at 37 °C did increase the melting point, as could be expected due to crystal growth and perfection at higher temperatures, and also narrowed the melting interval (Table 4). We assumed that when crystallites were formed at low temperatures (4–6 °C) they were less perfect than crystallites formed at a higher storage temperature, and as an effect of the temperature value shift to a higher one, the least perfect crystallites melted and molecules would crystallise on the other, more perfect crystallites. Thus, the melting temperatures moved to higher values and the endothermic transition peak became narrower. These observations were also in line with reported by Durrani and Donald (1995).

As we have not investigated the X-ray patterns of our samples we do not know whether polymorphism is also a factor that contributes to differences in melting behaviours. As was reported by Durrani and Donald (1995), for amylopectin dispersions subjected to time/temperature treatment at 4 °C followed by 28–37 °C, crystalline structure of B-type could be registered by method of X-ray diffraction.

Similar to the results of long-term recrystallisation, a decrease in molecular weight seemed to increase the amount of crystallites formed as seen by an increase in enthalpy. The differences observed previously between the group of native and acid hydrolysed starches and the mechanically treated starches were not apparent in the measured enthalpy from the two-step temperature experiment. It is possible, that the amount of crystallites, formed during the low temperature phase in the mechanically treated starches, was enough to allow the subsequent formation and reformation of crystallites that occurred at 37 °C, to proceed with the same rate as for the acid hydrolysed samples.

Table 4
The onset (T_o), peak (T_m) and final melting (T_c) temperature and recrystallisation enthalpy (ΔH) obtained from DSC analysis of processed and unprocessed waxy maize starch subjected to two-step temperature treatment

Sample	Transition temperatures and enthalpy			
	T_o (°C)	T_m (°C)	T_c (°C)	ΔH (J/g AP)
Two-step temperature treatment: 29 h of storage at 6 °C followed by 14 h at 37 °C				
S1n	52.3(2.0)	61.2(0.6)	68.0(0.2)	2.5(0.3)
S2a	49.4(1.3)	61.1(0.3)	67.8(0.9)	3.9(0.5)
S3m	51.2(1.2)	60.5(0.6)	66.2(0.4)	4.7(0.6)
S4m	51.6(2.4)	61.8(0.0)	69.7(0.9)	3.2(0.4)
S5a	51.5(0.6)	61.9(0.3)	69.3(0.5)	5.0(0.2)

Standard deviation values are given within brackets.

4. Conclusions

Acid hydrolysis and mechanical treatment of waxy maize starch were performed in order to relate structural properties such as molecular weight, unit chain length distribution and degree of branching to endothermic transitions of recrystallisation after one-step and two-step temperature treatments, including mixing experiments. The results showed that a decrease in the molecular weight caused higher recrystallisation enthalpy or faster recrystallisation rate for given storage conditions. The recrystallisation enthalpy values were higher for the acid hydrolysed starch then for mechanically treated starches, whereas the endothermic transition temperatures, degree of branching and unit chain length distribution were retained unaffected by the different processes. Thus, stability of rebuilt crystallites was not influenced by processing conditions which was obvious from the melting range and peak temperature of the two-step temperature treated samples. Mixing of a highly crystalline and less crystalline material prior to heating revealed a higher effect of the inoculation on recrystallisation than what would be expected from an additive effect from these two samples. Mixing of dry material (more crystalline) to a heated dispersion (less crystalline) showed no effects on recrystallisation. Two-step temperature treatment increased the melting point of the starch materials and narrowed melting intervals.

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