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Influence of hydroxypropylation on retrogradation properties of native, defatted and heat-moisture treated potato starches

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Abstract

Recent studies have shown that defatting and heat-moisture treatment cause structural changes within the amorphous and crystalline regions of potato starch. Furthermore, the alkaline reagents (NaOH and Na_2SO_4) used during hydroxypropylation has been shown to cause structural changes within the amorphous and crystalline regions of native, defatted and heat-moisture treated starches. In this study, we have compared (using different techniques) the retrogradation properties of potato starch before and after physical (defatting and heat-moisture treatment), and chemical (alkaline treatment and hydroxypropylation) modification. Turbidity measurements showed that changes in turbidity during storage (4°C for 24 h and then at 40°C for 29 days) of native, defatted and heat-moisture treated gelatinized starch pastes were influenced by the interplay of two factors: (1) interaction between leached starch components (amylose–amylose, amylose–amylopectin, amylose–amylopectin), and (2) interaction between granule remnants and leached amylose and amylopectin. In alkali treated gelatinized native, defatted and heat-moisture treated starch pastes, turbidity changes on storage was influenced by aggregation of granule remnants. Hydroxypropylation decreased the rate and extent of increase in turbidity during storage of native, defatted and heat-moisture treated starches. The change in turbidity during storage of hydroxypropylated starch pastes was influenced by the interplay between: (1) steric effects imposed by hydroxypropyl groups on chain aggregation, (2) aggregation between small granule remnants, and (3) settling of large granule remnants beneath the path of the spectrophotometer beam. Stored gelatinized pastes of native, defatted and heat-moisture treated starches gave a 'B' type X-ray pattern. A similar pattern was also observed after alkaline treatment, and hydroxypropylation. However, the X-ray intensity of the strong reflection at 5.2 Å decreased after alkaline treatment and hydroxypropylation. The retrogradation endotherm (monitored by differential scanning calorimetry) occurred after 2 days storage in native, defatted and heat-moisture treated starches. A similar trend was also observed after alkaline treatment. However, the retrogradation endotherm appeared only after 7 days in hydroxypropylated starches. The enthalpy of retrogradation in all starches decreased on alkaline treatment and hydroxypropylation.

1. Introduction

Starch granules heated in excess water undergo an order–disorder phase transition called gelatinization over a temperature range characteristic of the starch source. This phase transition is a non-equilibrium process associated with the diffusion of water into the granule, hydration and swelling of the starch granules, uptake of heat, loss of birefringence, loss of double helical structure, loss of crystallinity and amylose leaching. On cooling, the starch chains (amylose and amylopectin) in the gelatinized paste associate, leading to the formation of a more ordered structure. These molecular interactions are termed collectively 'retrogradation' and have important textural and dietary implications. The influence of starch source (Hoover and Sosulski, 1985; Orford et al., 1987), concentration (Longton and LeGrys, 1981; Orford et al., 1987; Gudmundsson and Eliasson, 1990; Biliaderis and Tonogai, 1991), storage temperature (Colwell et al., 1969; Slade and Levine, 1987), salts (Ward et al., 1994), sugars (Biliaderis and Prokopowich, 1994; Seow et al., 1996), lipids (Biliaderis and Tonogai, 1991; Ward et al., 1994) and chemical modification (Hoover and Sosulski, 1986; Hoover et al., 1988; Wu and Seib, 1990) on starch retrogradation has been well documented. However, there is a dearth of information on the influence of physical modification techniques such as defatting

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and hydrothermal treatment on starch gelatinization and crystallization. Recently, Perera et al. (1997) have shown that defatting and heat-moisture treatment cause profound changes in the physical arrangement of the starch chains within the amorphous and crystalline domains of the potato starch granule. These changes in turn were shown to alter granule crystallinity, swelling power, thermal properties and amylose leaching. Thus, a comparative study of the retrogradation mechanism of starch gels from native, defatted and heat-moisture treated potato starches, may reveal how the molecular structure of the starch granule prior to gelatinization influences the rate and extent of chain aggregation and crystallization during retrogradation.

Hydroxypropylation has been shown to reduce starch retrogradation (Butler et al., 1986; Hoover et al., 1988; Yeh and Yeh, 1993). This has been ascribed solely to steric effects imposed by the bulky hydroxypropyl groups, (which prevent proper alignment of starch chains during chain aggregation and crystallization). However, to the author's knowledge, no research work has been published on the influence of the alkaline conditions (NaOH and Na_2SO_4) used during hydroxypropylation on starch retrogradation. Our recent study (Perera et al., 1997) showed that the above alkaline reagents disrupt double helices within the amorphous regions of ungelatinized potato starches (native > defatted > heat-moisture treated), and also alter crystallise orientation (native > defatted > heat-moisture treated). Thus, retrogradation studies on alkali treated native, defatted and heat-moisture treated potato starches before and after hydroxypropylation (at different levels of molar substitution) will further enhance our understanding on the influence of the molecular structure of the ungelatinized granule on the events that occur during retrogradation.

The influence of hydroxypropylation on starch retrogradation has been obtained mainly by measurements of the amount of water exuded (synerisis) during freezing and thawing (Butler et al., 1986; Hoover et al., 1988; Takahashi et al., 1989; Yeh and Yeh, 1993). However, it is difficult to compare the data obtained from synerisis measurements, since the extent of synerisis is influenced by the centrifugal forces applied, and in published works different centrifugal forces have been used.

The objectives of this study were to determine how the rate and extent of potato starch retrogradation is influenced by: (1) the structural changes that occur on defatting and heat-moisture treatment, (2) the alkaline reagents used during hydroxypropylation, and (3) the level (molar substitution) of hydroxypropylation. In this study, retrogradation has been examined using turbidity, X-ray diffraction, scanning electron microscopy and differential scanning calorimetry. Such a multidisciplinary approach allows a complete picture of starch retrogradation at both macroscopic and microscopic levels.

2. Materials and methods

2.1. Materials

Potato tubers (*Solanum tuberosum* cv Russett Burbank) were purchased from the local market. Crystalline porcine pancreatic α-amylase (EC 3211) type 1A was obtained from Sigma Chemical Co (St. Louis, MO, USA). Other chemicals and solvents were analytical grade. Solvents were distilled from glass before use.

2.2. Methods

2.2.1. Starch isolation

Potato tubers were divided into two lots representing whole sample. Each lot was further subdivided into two parts and starch was isolated according to the procedure of Hoover and Hadziyev (1981).

2.2.2. Preparation of defatted potato starch

Defatted starch was prepared by soxhlet extraction with 75% aqueous *n*-propanol for 7 h. The solvent was removed by vacuum evaporation and the starch was air dried to a moisture content of ~10%.

2.2.3. Preparation of heat moisture treated potato starch

The heat-moisture treatment was essentially that of Sair (1964). Starch (15 g dry basis) were weighed into glass containers. Starch moisture content was brought to 30%. The sealed samples (in glass jars) were heated in an air oven at 100°C for 16 h. After cooling the jars were opened and the starch samples were air-dried to a moisture content ~10%.

2.2.4. Preparation of hydroxypropyl potato starches

Native, defatted and heat-moisture treated potato starch samples were converted into a range of hydroxypropyl derivatives according to the procedure of Leegwater and Luten (1971). Samples (200 g, dry basis) from each of the above starches were weighed into 600 ml screw cap jars. Into each jar, a solution of NaOH (2.6 g) and Na_2SO_4 (30 g) in distilled water (240 ml) was added at room temperature. The jars with samples were placed in a water bath at 40°C and propylene oxide (0, 4, 10, 20, 30 and 50 ml) was added and the suspensions thoroughly mixed and the jars closed. The reaction was continued at 40°C for 24 h with shaking. The starch suspensions were then neutralized to pH 5.5 with dilute H_2SO_4 (1 M). The starch cakes were washed with distilled water until negative to sulfate ions when tested with $BaCl_2$. All hydroxypropylated suspensions were freeze dried until the moisture content was reduced to 10–12%. Control potato starch was prepared by treatment of native, defatted and heat-moisture treated starches (200 g, dry basis) with distilled water containing NaOH (2.6 g) and Na_2SO_4 (30 g) but without addition of

propylene oxide, according to the procedure for preparation of hydroxypropyl potato starch as described above.

2.2.5. Determination of molar substitution

The hydroxypropyl content was determined by the spectrophotometric method of Johnson (1969) and expressed in terms of molar substitution (MS). MS is defined as moles of substituent per mole of anhydroglucose unit. The results were means of 3 determinations.

2.2.6. Turbidity measurements

A 2% aqueous suspension of potato starch (native, defatted and heat-moisture treated) near neutral pH was heated in a boiling water bath for 1 h under constant stirring (mild shear forces). After the suspension was cooled for 1 h at 25°C, the turbidity was determined by measuring absorbance at 640 nm against a water blank with a Shimadzu UV-visible spectrophotometer (UV-260, Shimudzu Corporation, Kyoto, Japan). The development of turbidity was followed by storing samples for 1 day at 4°C followed by 2–35 days at 40°C. This sequential incubation at 4°C and 40°C was applied to obtain extensive retrogradation in a short time by favoring nucleation (formation of crystal nuclei) at 4°C and propagation (growth of crystallites from the nuclei formed) of starch crystallises at 40°C (Wunderlich, 1976).

2.2.7. Gel preparation for X-ray diffraction

Gels were prepared (with minor modifications) as described by Krusi and Neukom (1984). A 3% (w/v) potato starch gel was prepared by heating the suspension under gentle stirring for 15 min on a boiling water bath. After cooling to 30°C, sufficient starch was added to obtain a suspension with 40% (w/v) dry matter. These suspensions were then homogenized for 2 min at 8000 rpm and then heated in an air oven at 110°C for 2 h. After cooling, the gels formed were stored at 4°C for 1 day followed by 29 days at 40°C.

2.2.8. Gel powder preparation for X-ray diffraction

The procedure (with minor modifications) of Roulet et al. (1988) was used to convert freshly gelatinized and stored gels to a powder prior to examination by X-ray diffraction. The gels were rinsed with water, cut into small pieces and mixed with 100 ml acetone. After homogenization using a polytron, the mixture was left to decant for 5 min. The liquid was discarded and the rest was transferred to screw cap tubes. Acetone was again added, the mixture centrifuged (3000***g***) and the supernatant discarded. The procedure was repeated three times and the remaining mass was then freeze dried.

2.2.9. X-ray diffraction

X-ray diffractograms of gel powders were obtained with a Rigaku RU 200R X-ray diffractometer (Rigaku-Denki Co., Tokyo, Japan). The operating conditions were as described elsewhere (Hoover and Vasanthan, 1994). All experiments were replicated thrice.

2.2.10. Scanning electron microscopy (SEM)

The specimen preparation of freshly gelatinized and stored (1 day at 4°C) potato starch gels for SEM was carried out as follows: The starches (2%, w/v) were gelatinized under the conditions described for turbidity measurements, and then stored for 1 day at 4°C. The gels were then freeze dried and the samples were examined and photographed in a Hitachi (S570) scanning electron microscope. The SEM operating conditions were carried out by procedures outlined in an earlier publication (Perera et al., 1997).

2.2.11. Differential scanning calorimetry (DSC)

Thermal transitions of retrograded starches were investigated using a Perkin–Elmer DSC-2 (Norwalk, CT) differential scanning calorimeter equipped with a thermal analysis data station. Water (3 μl) was added, with a microsyringe to starch (3 mg) in DSC pans, which were then sealed, reweighed and kept for 1 h at room temperature. The scanning temperature range and the heating rate were 20–120°C and 10°C min^{-1}, respectively. The heated pans were then cooled to room temperature and stored for 1 day at 4°C followed by 2–7 days at 40°C. After this time period, the pans were left to equilibrate for 1 h at room temperature and then scanned under the same previous conditions. In all measurements an empty pan was used as reference. The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c) of the retrogradation endotherm. Indium was used for calibration. The enthalpy of retrogradation (ΔH_R) was estimated by integrating the area between the thermogram and the base line under the peak and expressed as joules per unit weight of dry starch (Jg^{-1}). All experiments were repeated thrice.

2.2.12. Statistical analysis

Analysis of variance was performed using Minitab statistical package (Minitab Inc., 1991). Duncan's new multiple range test was utilized for comparison among means.

3. Results and discussion

3.1. Turbidimetric and microstructural analysis

3.1.1. Initial turbidity and scanning electron microscopy of fresh pastes of native, defatted and heat-moisture treated potato starch pastes

The initial turbidity followed the order: heat-moisture treated > defatted > native (Fig. 1(A), (C) and (E)). The pellet of freshly gelatinized native potato starch paste was devoid of granule remnants and consisted of large sheets of leached amylose and amylopectin

(Jacobson et al., 1997) (Fig. 2(A)). However, fresh pastes of defatted (Fig. 2(B)) and heat-moisture treated (Fig. 2(C)) starches consisted of granule remnants (heat-moisture treated > defatted) connected by a matrix of leached amylose and amylopectin.

Turbidity effects have their origin in refractive index fluctuation over a distance scale comparable to the wavelength of observation. In a polymer-solvent system this is caused by density fluctuations over the same distance scale and is most likely due to extensive polymer–polymer aggregation (Gidley and Bulpin, 1989). Gidley and Bulpin (1989) have shown on the basis of their studies of amylose aggregation in aqueous systems, that even at the onset of detectable turbidity, highly aggregated polymer structures are present. Craig et al. (1989) have classified starch pastes into three categories depending on the behavior in light: (1) high clarity and almost no whiteness (due to little or no refraction of light because of a lack of swollen granular remnants, and little reflection of light (due to limited association of starch chains)); (2) moderate clarity and high whiteness (due to little refraction (few granular remnants) and high reflection of light (due to interchain association)); (3) low clarity and low whiteness (due to high refraction of light by swollen granular remnants, but little reflection of light by collapsed or associated starch granules).

The lower initial turbidity of native potato starch paste (Fig. 1(A)) can be attributed to its higher swelling factor (Perera et al., 1997), the absence of granule fragments (Fig. 2(A)), and to repulsion between the negatively charged phosphate groups (which are covalently bound to amylopectin). Differences in initial turbidity between defatted and heat-moisture treated starches (heat-moisture treated (Fig. 1(E)) > defatted (Fig. 1(C)) are

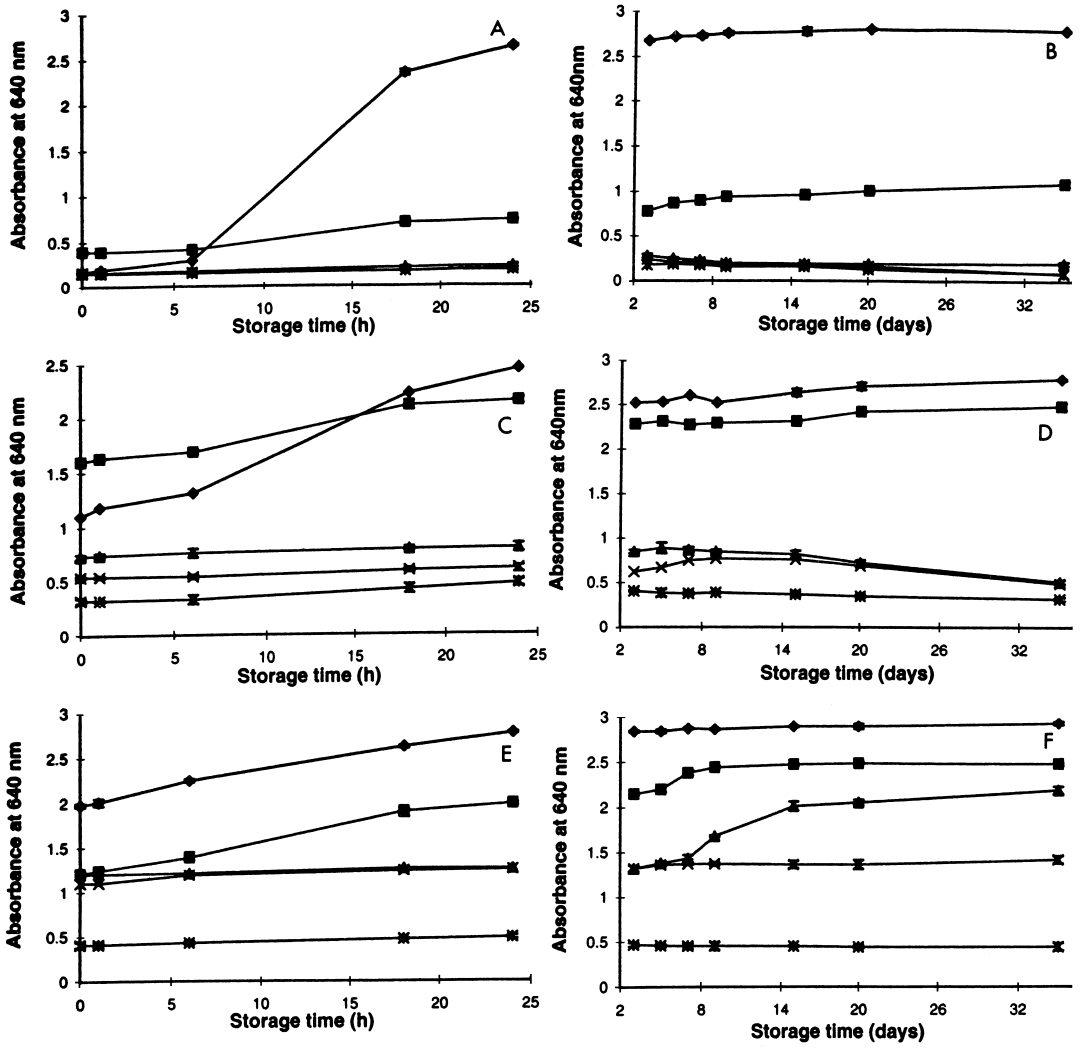

Fig. 1. Time course of turbidity development at 4°C (A,C,F) and 40°C (B,D,F). (A & B) ◆ native; ■ alkali treated native (MS 0.00); ▲ hydroxypropylated native (MS 0.11); **X** hydroxypropylated native (MS 0.18); * hydroxypropylated native (MS 0.25). (C & D) ◆ defatted; ■ alkali treated defatted (MS 0.00); ▲ hydroxypropylated defatted (MS 0.10), **X** hydroxypropylated defatted (MS 0.17), * hydroxypropylated defatted (MS 0.20). (E & F) ◆ heat-moisture treated; ■ alkali treated heat-moisture treated (MS 0.00); ▲ hydroxypropylated heat-moisture treated (MS 0.11); **X** hydroxypropylated heat-moisture treated (MS 0.20); * hydroxypropylated heat-moisture treated (MS 0.28).

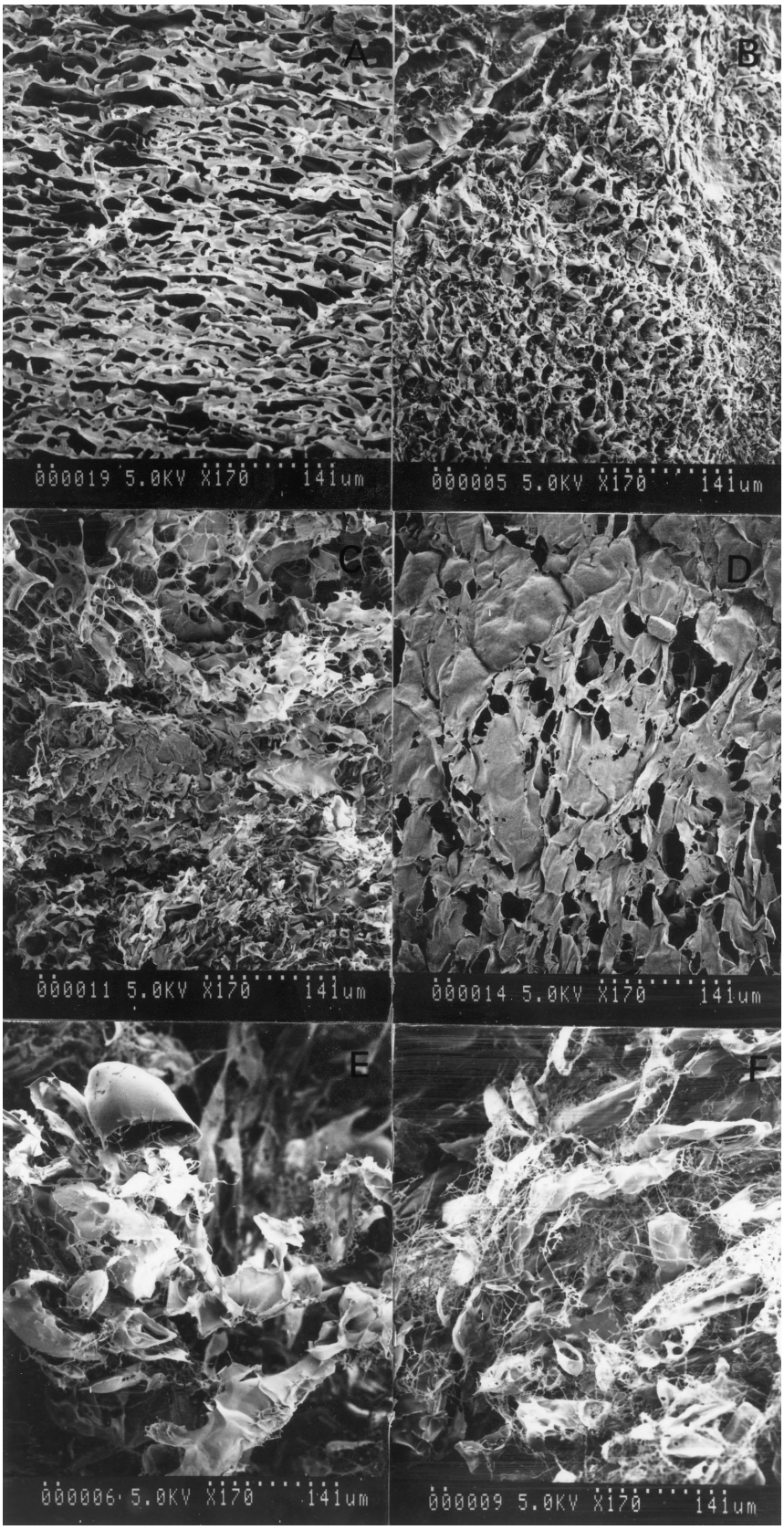

Fig. 2. Scanning electron micrographs of freshly gelatinized and stored (24 h at 4°C) potato starch pastes (A) fresh native potato stash, (B) stored native potato starch, (C) fresh defatted potato starch, (D) stored defatted potato starch, (E) fresh heat-moisture treated potato starch, (F) stored heat-moisture treated potato starch.

probably due to larger granule remnants (compare Fig. 2(C) (defatted) and Fig. 2(E) (heat-moisture treated)) and to lower granule swelling (Perera et al., 1997) in the latter.

3.1.2. Initial turbidity and scanning electron microscopy of alkali treated native, defatted and heat-moisture treated starch pastes

Alkaline treatment (NaOH and Na_2SO_4) increased the initial absorption of native and defatted starches (defatted > native), but decreased that of heat-moisture treated starch (Fig. 1(A), (C) and (E)). Few granule remnants were present in fresh pastes of alkali treated native starch (Fig. 3(A) and (B)), but more after alkali treatment of defatted (Fig. 4(A) and (B)) and heat-moisture treated (Fig. 5(A) and (B)) starches. Vasanthan and Hoover (1992) have shown that granule stability of potato starch increases on defatting (due to increase in crystallinity and interaction between amylose

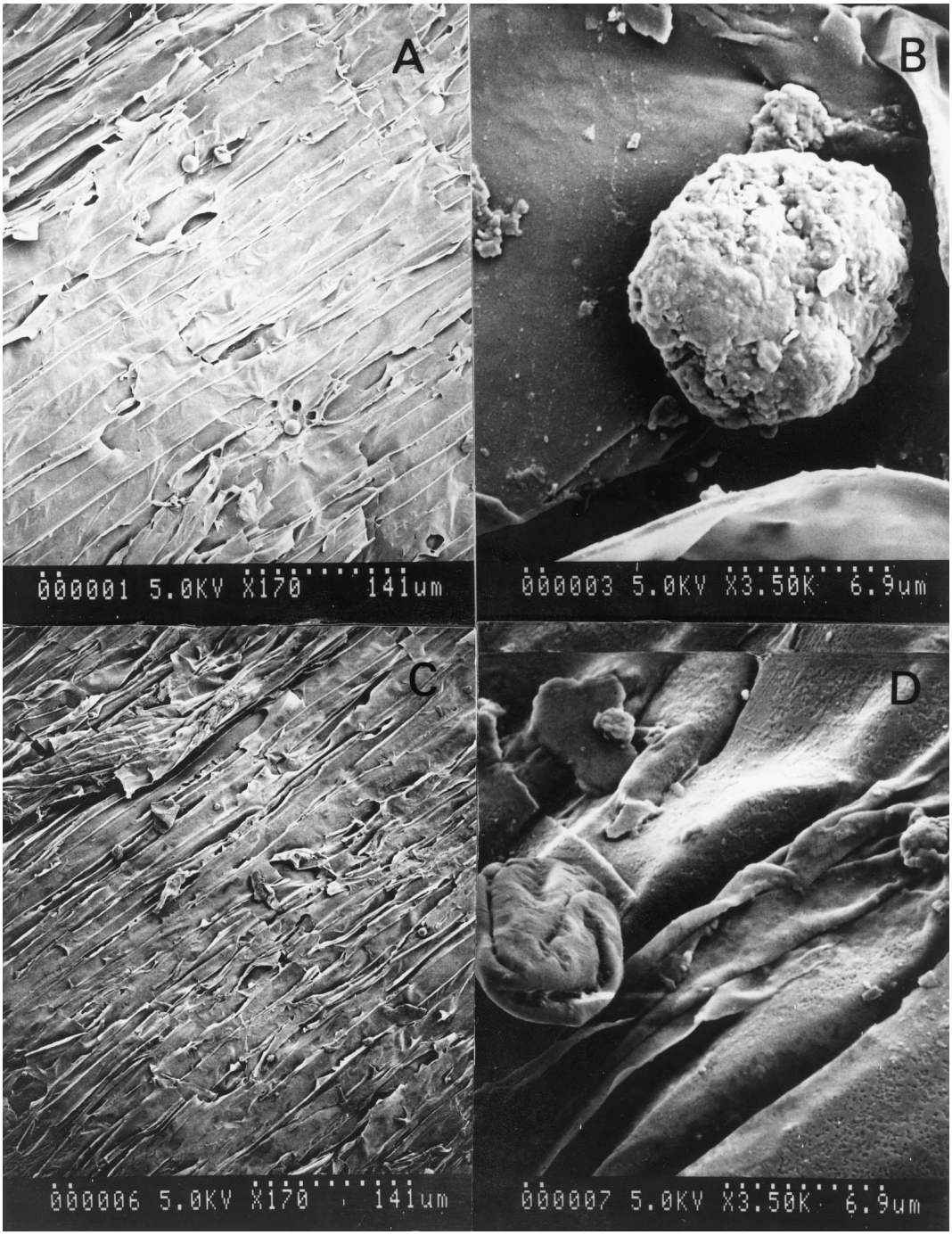

Fig. 3. Scanning electron micrographs of gelatinized and stored (24 h at 4°C) alkali treated native potato starch pastes. (A) and (B) fresh paste; (C) and (D) stored paste.

chains) and heat-moisture treatment (due to interaction between amylose chains). Perera et al. (1997) have shown that addition of NaOH and Na_2SO_4 (under the conditions used for hydroxypropylation) increases granule stability (native > defatted > heat-moisture treated) by changing crystallise orientation. This would then account for the presence of granule remnants after alkaline treatment of native starch (Fig. 3(A) and (B)) and to the increase in the number of granule remnants after alkaline treatment of defatted (Fig. 4(A) and (B)) and heat-moisture treated (Fig. 5(A) and (B)) starches. The results indicate that the initial turbidity of alkaline treated starches is influenced by the interplay of two factors: (1) an increase in granular swelling which tends to decrease light absorption (Perera et al. (1997) have shown that addition of NaOH and Na_2SO_4 increases

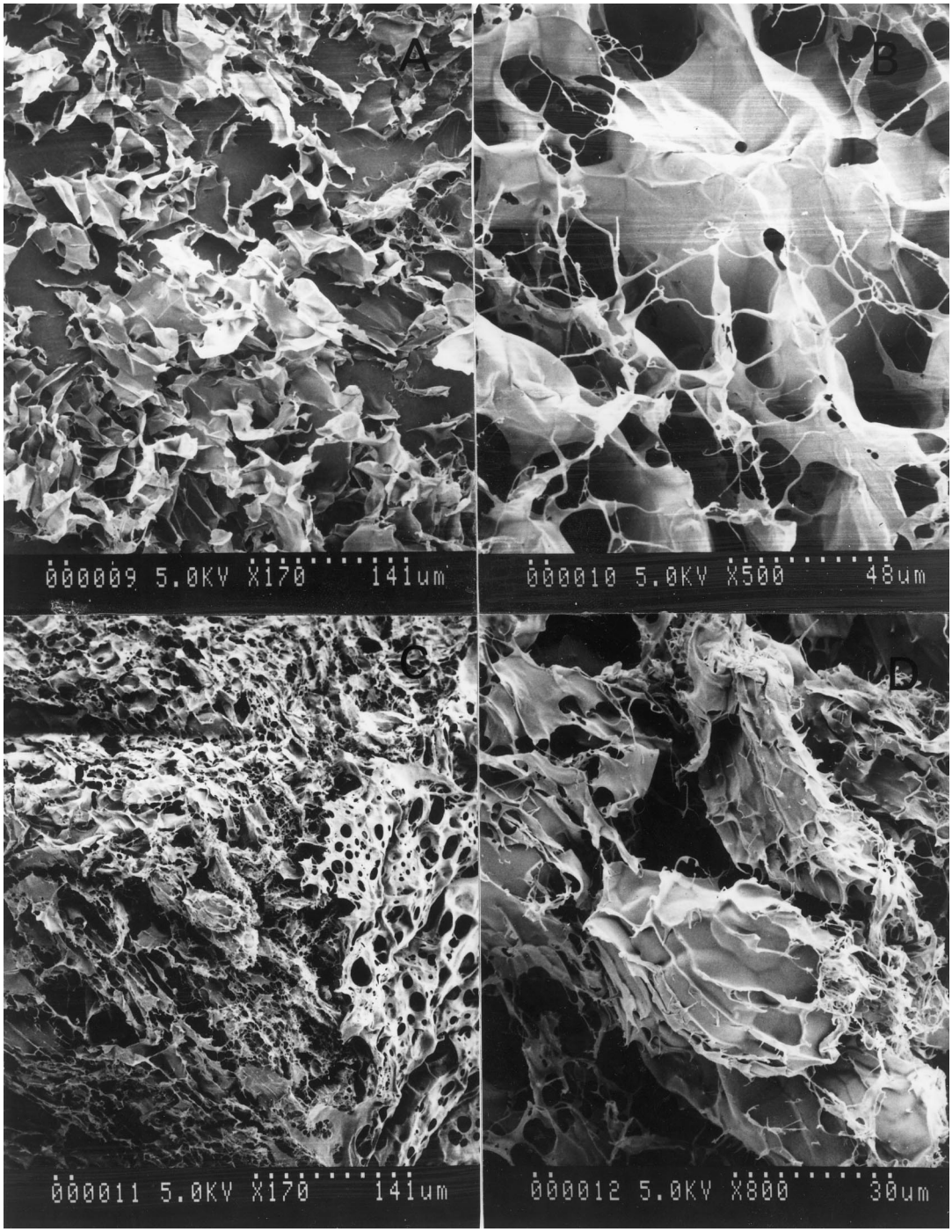

Fig. 4. Scanning electron micrographs of freshly gelatinized and stored (24 h at 40°C) pastes of alkali treated defatted potato starch (A) & (B) freshly gelatinized defatted starch, (C) & (D) stored defatted starch.

granular swelling (native > defatted > heat-moisture treated)); and (2) presence of granule remnants (increases light absorption). This absorption increase in alkali treated native (Fig. 1(A)) and defatted (Fig. 1(C)) starches suggests that the presence of granule remnants in these starch pastes probably negate the influence of increased granule swelling on light absorption. Hoover and Vasanthan (1994) have shown that heat-moisture treatment destroys crystalline regions in potato starch. This would increase flexibility of starch chains within the amorphous regions of the granule. Thus, the decrease in light absorption (in spite of an increase in the amount of granule remnants) on alkaline treatment of heat-moisture treated starch (Fig. 5(A) and (B)), is probably due to decreased interaction between leached amylose and/or amylopectin chains chains (compare Figs. 2(E) and 5(A) and (B).

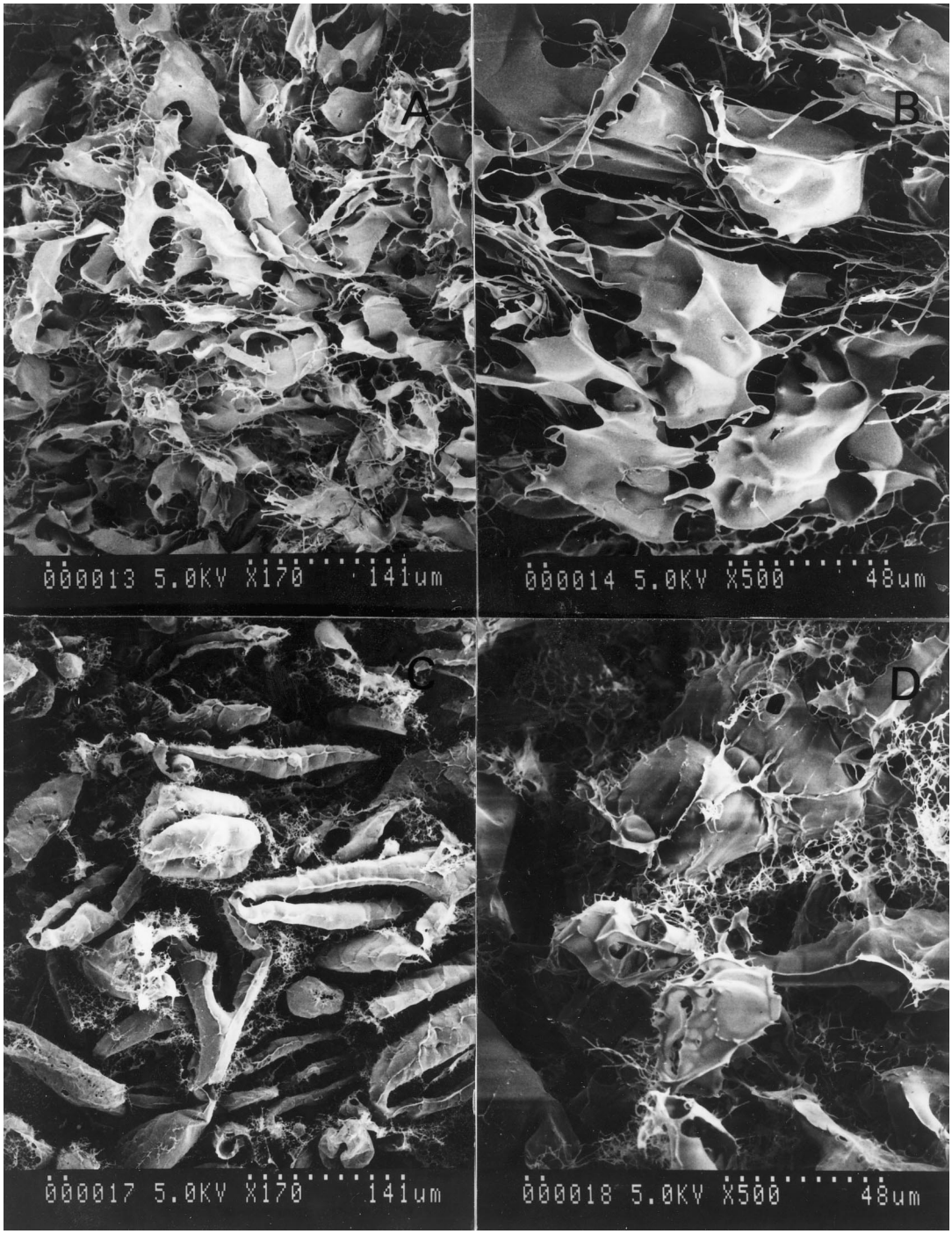

Fig. 5. Scanning electron micrographs of freshly gelatinized and stored (24 h at 4°C) starch pastes of alkali treated heat-moisture treated potato starch: (A) & (B) freshly gelatinized heat-moisture treated starch, (C) & (D) stored heat-moisture treated starch.

3.1.3. Initial turbidity and scanning electron micrographs of hydroxypropylated native, defatted and heat-moisture treated starches

The initial absorption of alkali treated starches decreased on hydroxypropylation (Fig. 1(A), (C) and (E)). In alkali treated native starch, absorption decreased by 58.9% at MS levels of 0.11 and 0.18. Whereas, at MS 0.25 the corresponding value was 61.2% (Fig. 1(A)). In alkali treated defatted starch, absorption decreased by 54.3, 66.2 and 80%, respectively, at MS 0.10, 0.17 and 0.20 (Fig. 1(C)). Whereas, in alkali treated heat-moisture treated starch, absorption decreased by 2.4, 10.0, 66.6%, respectively, at MS 0.11, 0.20 with 0.28 (Fig. 1(E)).

Scanning electron micrographs of freshly gelatinized pastes of native, defatted and heat-moisture treated starches after hydroxypropylation (under alkaline conditions) are presented in Fig. 6. Granules of all starches were disrupted on hydroxypropylation. The disrupted granules of hydroxypropylated (MS 0.11) native starch (Fig. 6(A)) and hydroxypropylated (MS 0.10) defatted starch (Fig. 6(C)) were in the form of elongated sheets connected by a network of amylose and amylopectin exudate. The morphology of the granule remnants of both freshly gelatinized native (Fig. 6(A)) and defatted (Fig. 6(C)) starches after hydroxypropylation were different from their unmodified counterparts (Fig. 2(A) (native), Fig. 2(C) (defatted)). In comparison with hydroxypropylated native and defatted starches, the extent of granule disruption was less marked in hydroxypropylated (MS 0.11) heat-moisture treated starch (Fig. 6(E) and (F)). Furthermore, there were many small intact granules (Fig. 6(E)) which seemed to have escaped disruption during hydroxypropylation.

The hydroxypropyl groups introduced into the starch chains are said to be capable of destroying inter and intra molecular hydrogen bonds, thereby weakening the granular structure (Wootton and Manatsathit, 1984). Kim et al. (1992) have shown by light microscopy that the central region of the hydroxypropylated potato starch granules become more and more disrupted with an increase in MS.

The results on hydroxypropylated native starch (Figs. 1(A) and 6(A)) suggests that although granule stability increases after alkaline treatment (Fig. 3(A) and (B)), the extent of this increase is not large enough to prevent granule breakdown (Fig. 6(A)) when hydroxypropylated native starch (MS 0.11) is heated under the conditions used for turbidity measurements. Thus, the decrease in initial absorption at MS 0.11 is

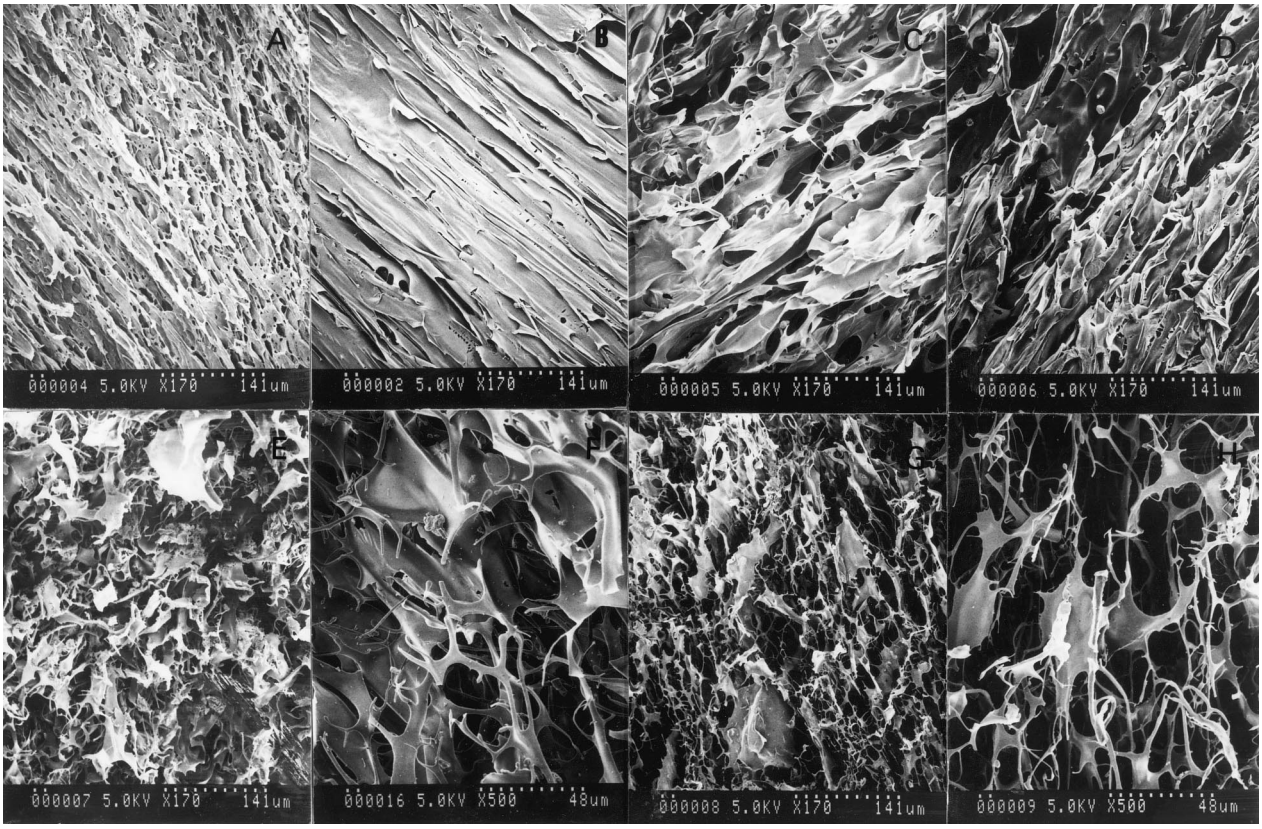

Fig. 6. Scanning electron micrographs of freshly gelatinized and stored (24 h at 4°C) pastes of hydroxypropylated potato starches (A) fresh hydroxypropylated (MS 0.11) native starch; (B) stored hydroxypropylated (MS 0.11) native starch; (C) fresh hydroxypropylated (MS 0.10) defatted starch; (D) stored hydroxypropylated (MS 0.10) defatted starch; (E) & (F) fresh hydroxypropylated (MS 0.11) heat-moisture treated starch; (G) & (H) stored hydroxypropylated (MS 0.11) heat-moisture treated starch.

due to a sharp decrease in the amount of granule structure present in the starch paste. This would also explain the lack of influence of increasing MS (0.11–0.25) on light absorption (Fig. 1(A)).

The progressive decrease in absorption with increase in MS for defatted (Fig. 1(C)) and heat-moisture treated (Fig. 1(E)) starches suggests that granule remnants of these starches are better able to resist (heat-moisture treated > defatted) the disruptive action of hydroxypropyl groups on granule structure than those of native starch. This can be attributed to the interactions that occur between starch chains on defatting (Vasanthan and Hoover, 1992) and heat-moisture treatment (Hoover and Vasanthan, 1994). At similar MS levels (0.20), the absorption decrease is higher in defatted (66%) (Fig. 1(C)) than in heat-moisture treated (10%) starch (Fig. 1(E)) due to greater stability of heat-moisture treated granules. The steep decrease in absorbance (66.2%) at MS 0.28 for heat-moisture treated starch (Fig. 1(E)), suggests that at this MS level granules undergo extensive disruption into small fragments.

3.1.4. Turbidity development and scanning electron micrographs of native, defatted and heat-moisture treated starch pastes during storage

The development of turbidity during storage of the above starches at 4°C (1 day) followed by storage at 40°C for 34 days are presented in Fig. 1(A)–(F). In all starches absorbance increased with storage time. The extent of this increase was more pronounced (native > defatted > heat-moisture treated) during the first day of storage (Fig. 1(A), (C) and (E)). The absorbance increase between day 1 and 35 of storage followed the order: defatted > native > heat-moisture treated (Fig. 1(B), (D) and (F)).

Scanning electron micrographs of the starch pastes after storage for 24 h (at 4°C) are presented in Fig. 2(B), (D) and (F). The microstructure of the stored pastes of native starch consisted of a dense network formed by leached amylose and amylopectin chains (Fig. 2(B)). Whereas, that of defatted starch (Fig. 4(D)) consisted of swollen granule remnants enmeshed and connected together by thick strands of the leached exudate. Heat-moisture treated granule remnants were enmeshed and held together by a network formed by thin strands of the leached exudate (Fig. 4(F)). Many researchers (Craig et al., 1989; Bello-Perez and Paredes-López, 1996; Jacobson et al., 1997) have shown that turbidity development during storage is influenced by factors such as granule swelling, granule remnants, leached amylose and amylopectin, amylose and amylopectin chain lengths, intra or intermolecular bonding, lipids, sucrose, cross-linking and substitution.

The first stage of retrogradation has been shown to involve mainly amylose aggregation and crystallization which is completed within the first few hours of storage. Whereas, the second stage that occurs over longer periods (days) involves mainly amylopectin aggregation and crystallization (Miles et al., 1985, Goodfellow and Wilson, 1990; Silverio et al., 1996).

The rapid increase in turbidity during the first 24 h of storage is thus, mainly due to continued interaction (via hydrogen bonding) between leached amylose and amylopectin chains and to a lesser extent to short range ordering of amylopectin side chains. These interactions would lead to the development of junction zones, which would reflect or scatter a significant amount of light.

Perera et al. (1997) have shown that the extent of amylose leaching during gelatinization is more pronounced in native than in defatted or heat-moisture treated starches (defatted > heat-moisture treated). Thus, the magnitude of interaction between leached amytose chains during storage would follow the above trend. This would then partially explain the extent of increase in absorption (native > defatted > heat-moisture treated) during the first 24 h of storage (Fig. 1(A), (C) and (E)). It is likely, that the magnitude of the increase in absorption during the 24 h storage period is also influenced by the presence of granule remnants in starch pastes of defatted (Fig. 2(C)) and heat-moisture treated (Fig. 2(E)) starches (heat-moisture treated > defatted). These remnants would hinder chain aggregation during storage. The slow increase in turbidity beyond 24 h storage (Fig. 1(B), (D) and (F)) can be attributed to slow interaction between the outer branches of amylopectin chains (Ring, 1987) of native, defatted and heat-moisture treated starches. Vasanthan and Hoover (1992) have shown that defatting increases crystallinity, whereas heat-moisture treatment disrupts crystallinity in potato starch. Consequently, the degree of separation (after gelatinization) between the outer branches of amylopectin chains would be much greater in heat-moisture treated than in defatted starch. Thus, during storage, the formation and lateral association of double helices involving amylopectin chains would be easier and much stronger in defatted than in heat-moisture treated or native starch (native > heat-moisture treated). This would then explain the observed order of increase in absorbance (defatted > native > heat-moisture treated) between the day 1 and 35 of storage (Fig. 1(B), (D) and (F)).

3.1.5. Turbidity development and scanning electron micrographs of alkaline treated native, defatted and heat-moisture treated starches during storage

In all starches, the extent of absorption increase during the first 24 h of storage was less pronounced after alkaline treatment (Fig. 1(A), (C) and (E)). For instance, during the above time period absorption increased by only 0.34, 0.55 and 0.77, respectively, in alkali treated native, defatted and heat-moisture treated starches (Fig. 1(A), (C) and (E)). The corresponding values for untreated starches were 2.48 (native), 1.35 (defatted) and 0.81 (heat-moisture treated), respectively. However, the extent of absorption increase between day

1 and 35 of storage was more pronounced after alkaline treatment (Fig. 1). Scanning electron micrographs of fresh (Fig. 3(A)) and stored (24 h at 4°C (Fig. 3(C)) pastes of alkali treated native starches were nearly similar. In fresh pastes of alkali treated defatted (Fig. 4(A) and heat-moisture treated (Fig. 5(A) starches, granule remnants were scattered throughout the matrix. The size of these remnants were larger in heat-moisture treated starch (Fig. 5(A)). However, in stored (24 h at 4°C) pastes, granule remnants of both alkali treated defatted (Fig. 4 (C) and (D)) and heat-moisture treated (Fig. 5(C) and (D)) starches were enmeshed in the amylose exudate. The above results have shown that the alkaline conditions used during hydroxypropylation influence starch chain aggregation during storage. In native starch, the magnitude of the increase in absorbance during storage (24 h at 4°C) decreases drastically (Fig. 1(A)), after alkaline treatment. Whereas, in stored pastes of alkali treated defatted and heat-moisture treated starches, starch chain aggregation is hindered by granule remnants (larger in heat-moisture treated) [Fig. 4(C) and (D) (defatted), Fig. 5(C) and (D) (heat-moisture treated]. This would then explain, differences in absorption increase (during the 24 h storage period at 4°C) between untreated and alkali treated starches (native (Fig. 1(A)) > defatted (Fig. 1(C)) > heatmoisture treated (Fig. 1(E))).

3.1.6. Turbidity development and scanning electron microscopy of hydroxypropylated native, defatted and heat-moisture treated starch pastes during storage

In hydroxypropylated native starches, absorbance increased with storage time, reaching a maximum value (after 3 days) of 0.28 and 0.24, respectively, at MS 0.11 and 0.18. Thereafter, absorption decreased gradually reaching a value of 0.20 and 0.16, respectively (after 35 days), at MS 0.11 and 0.18 (Fig. 1(B)). However, at MS 0.25, absorption remained constant (~0.15) until day 20 (Fig. 1(B)). Thereafter, absorption decreased gradually reaching a value of 0.09 at the end of the storage period (day 35). In hydroxypropylated defatted starch, absorption continued to increase with storage time reaching a maximum value of 0.89 (MS 0.10), 0.77 (MS 0.17) and 0.47 (MS 0.20), respectively, after 5 days (Fig. 1(D)), 9 days (Fig. 1(D)) and 1 day (Fig. 1(D)). Thereafter, absorption remained constant until day 15 at MS 0.10 and 0.17, and then decreased steeply reaching a value of 0.51 and 0.49, respectively (after 35 days) at MS 0.10 and 0.17 (Fig. 1(D)). However, at MS 0.20, absorption continued to decrease gradually beyond day 1, reaching a value of 0.32 after 35 days (Fig. 1(D)).

In hydroxypropylated heat-moisture treated starch, absorption continued to increase throughout the storage period at MS 0.11 and 0.20 (Fig. 1(E) and (F)). The extent of this increase was more pronounced at MS 0.11. However, at MS 0.28, absorption increased only until the hour 18 of storage (Fig. 1(E)). Thereafter, it remained unchanged throughout the storage period (Fig. 1(E) and (F)).

The scanning electron micrographs of stored (1 day) hydroxypropylated native (MS 0.11), defatted (MS 0.10) and heat-moisture treated (MS (0.11) starches are presented in Fig. 6. In hydroxypropylated stored native starch paste (MS 0.11), the leached amylose chains and granule remnants were fused together to form a compactly packed structure (Fig. 6(B)). The morphology was nearly similar to that of stored alkali treated native starch (Fig. 3(C)). Granule remnants and leached amylose and arnylopectin chains of stored hydroxypropylated defatted starch (MS 0.10) were loosely packed in the matrix (Fig. 6(D)). Furthermore, the morphology of this paste was totally different from that of stored alkali treated defatted starch (Fig. 5(D)). In stored hydroxypropylated heat-moisture treated starch (MS 0.11), granule remnants and leached starch chains were more loosely packed (Fig. 6(G) and (h)) than in stored pastes of hydroxypropylated (MS 0.10) defatted starch (Fig. 6(D)).

The results on hydroxypropylation indicate that changes in absorption during the entire storage period (35 days) is influenced by the interplay of 3 factors: (1) steric effects imposed by bulky hydroxypropyl groups (this decreases the magnitude and rate of increase in absorption by hindering starch chain alignment during retrogradation); (2) presence of small granule remnants which gradually aggregate during storage (this would increase the intensity of scattered light), and (3) settling of large aggregated granule remnants below the path of the spectrophotometric beam (this would increase light transmittance).

In hydroxypropylated (at MS 0.11 and 0.18) native starch, the gradual increase in absorbance (Fig. 1(A) and (B)) during the first 3 days of storage is mainly due to slow aggregation of starch chains. The gradual decrease in absorption beyond day 3 (Fig. 1(B)) at these MS levels, is mainly influenced by factor 2. At MS 0.25, absorption remained unchanged until day 20 due to factor 1, (which prevents chain aggregation). The decrease in absorption beyond day 20 at MS 0.25 (Fig. 1(B)), is mainly influenced by factor 2.

In defatted starch, the steep decrease in absorption (after day 15) at MS 0.10 and 0.17 is mainly due to factor 3. This decrease does not occur at MS 0.20, since granule remnants are probably disrupted (due to the large increase in granular swelling that occur at this MS level (Perera et al., 1997)). Consequently, at MS 0.20, the changes in absorption during storage are mainly influenced by starch chain aggregation.

As described earlier, granule crystallinity is destroyed on heat-moisture treatment. Consequently, heat-moisture treated granules would be more susceptible than defatted granules to disruption by the bulky hydroxypropyl groups. This would then explain the absence of large granule remnants (Fig. 6(E) and (F))

after hydroxypropylation (MS 0.11). The extent of increase in absorption during storage (35 days) of heat-moisture treated starches at the different MS levels (MS 0.11 > MS 0.20 > MS 0.28) is due to the interplay of factors 1 and 2. The results indicate that all granule remnants are not disrupted by hydroxypropyl groups at MS 0.11 (Figs. 6(E) and (F)). The undisrupted remnants probably aggregate slowly during storage. This would then explain the steep increase in absorption after day 7 of storage at MS 0.11. However, at MS 0.20 and 0.25, the extent of absorption increase is mainly influenced by factor 1.

3.2. X-ray diffraction

3.2.1. X-ray diffraction of fresh and stored gels of native, defatted and heat-moisture treated starches

The X-ray diffraction data of freshly gelatinized and stored (1 day at 4°C and 40°C for 29 days) pastes of native, defatted and heat-moisture treated starches are presented in Table 1 and Fig. 7. No significant peak could be distinguished in freshly gelatinized native (Fig. 7(A)), defatted (not shown) and heat-moisture treated (not shown) starches. The 'B' X-ray pattern which is typical of retrograded starch (d spacings at 16, 5.2, 3.9 and 3.7 Å) was evident (in all three starches) at the end of the storage period (Fig. 7(B)). The intensity of the spacing centered at 5.2 Å followed the order: defatted > native > heat-moisture treated (Table 1). As described earlier, the distance separating the outer branches of amylopectin chains in gelatinized starches followed the order: heat-moisture treated > native > defatted. Thus, due to closer proximity of the outer branches in gelatinized defatted starch, double helical formation and packing of double helices during storage would be much easier and more ordered in defatted than in the other starches. This would then account for the stronger X-ray diffraction intensites of retrograded defatted starch (Table 1). It has been shown that the 'B' pattern originates from hexagonal packing of double helices during retrogradation of both amylose (Gidley, 1989) and amylopectin (Zobel, 1988). As shown in Fig. 2, granule remnants are present in gelatinized pastes of defatted (Fig. 2(C)) and heat-moisture treated starches (Fig. 2(E)). The size of these remnants being greater in the latter. Presence of granule remnants could hinder aggregation and packing of double helices of both amylose and amylopectin. Thus, the recorded intensifies are probably influenced by the interplay of two factors: (1) degree of proximity of the outer branches of amylopectin chains to each other in gelatinized pastes; and (2) size of the granule remnants in the gelatinized paste.

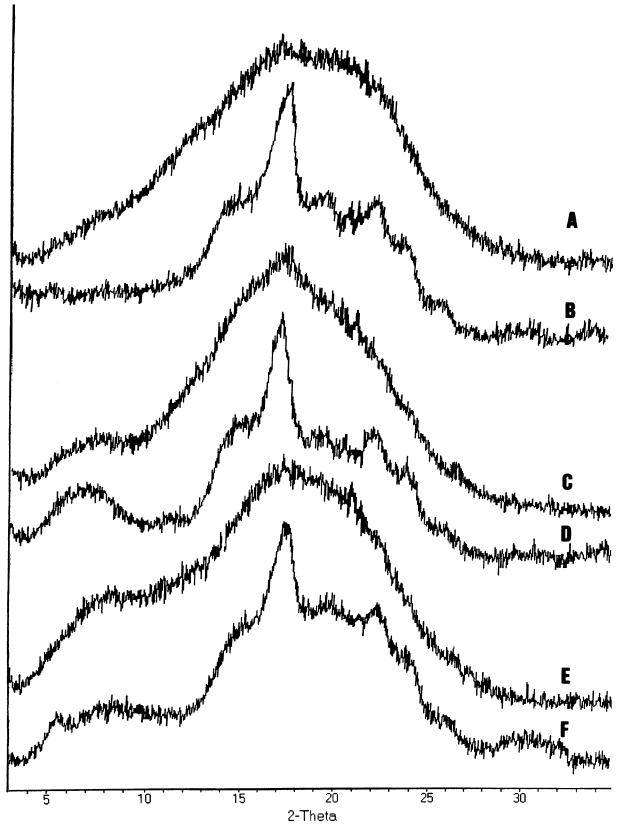

Fig. 7. X-ray diffraction patterns of freshly gelatinized and stored pastes (24 h at 4°C and then at 40°C for 29 days) of untreated, alkali treated and hydroxypropylated (MS 0.18) native potato starches (A) freshly gelatinized untreated native starch, (B) untreated native starch after 30 days storage, (C) freshly gelatinized alkali treated native starch, (D) alkali treated native starch after 30 days storage, (E) freshly gelatinized hydroxypropylated (MS 0.18) native starch, (F) hydroxypropylated (MS 0.18) native starch after 30 days storage.

3.2.2. X-ray diffraction of fresh and stored gels of alkali treated native, defatted and heat-moisture treated starches

No significant peak could be distinguished in freshly gelatinized pastes of native (Fig. 7(C)), defatted (not shown) and heat-moisture treated (not shown) starches. In all alkali treated starches, the X-ray diffraction pattern and the intensity of the 5.2 Å peak (Table 1) after 30 days storage was weaker than their untreated counterparts (Table 1 and Fig. 7(B)). The extent of this intensity

Table 1
X-ray diffraction intensities of stored[a] potato starch gels at 5.2 Å

Starch source and treatment	Moisture %	Intensity (cps[b]) at 5.2 Å
Native	9.6	1535
Native (alkali treated)	9.5	1496
Native (MS[c] 0.18)	9.6	990
Defatted	9.4	1895
Defatted (alkali treated)	9.5	1383
Defatted (MS[c] 0.17)	9.5	715
Heat-moisture treated	9.5	1225
Heat-moisture treated (alkali treated)	9.6	232
Heat-moisture treated (MS[c] 0.20)	9.5	194

[a] One day at 4°C and then at 40°C for 29 days.
[b] Counts per second.
[c] Molar substitution.

reduction followed the order heat-moisture treated > defatted > native (Table 1). This decrease can be attributed to the size of granule remnants (heat-moisture treated > defatted > native) in the alkali treated pastes, which hinder the formation, aggregation and packing of double helices in a crystalline array.

3.2.3. X-ray diffraction of fresh and stored gels of hydroxypropyl native, defatted and heat-moisture treated starches

No significant peaks could be distinguished in freshly gelatinized pastes of hydroxypropylated native (Fig. 7(E)), defatted (not shown) and heat-moisture treated (not shown) starches. The intensities at 5.2 Å decreased in stored hydroxypropylated starch gels (Table 1). The extent of this decrease in native (MS 0.18), defatted (MS 0.17) and heat-moisture treated (MS 0.20) starches were 35.5, 62.2 and 84.2%, respectively. This decrease is due to a decrease in chain aggregation resulting from the interplay of two factors: (1) steric effects imposed by hydroxypropyl groups on adjacent starch chains; and (2) aggregation of large granule remnants of hydroxypropylated defatted (MS 0.17) (Fig. 6(D)) and heat-moisture treated (MS 0.20) (Fig. 6(G)) starches.

3.3. Differential scanning calorimetry

3.3.1. Retrogradation enthalples of native, defatted and heat-moisture treated starches after gelatinization and storage

In all starches, the retrogradation endotherm occurred only after 2 days storage (Table 2). Retrogradation enthalpy (ΔH_R) at the end of the storage period (7 days) was more pronounced in native (6.7 J g^{-1}) than in defatted (4.5 J g^{-1}) or heat-moisture treated (4.8 J g^{-1}) starches. The transition temperatures T_o (onset), T_p (mid-point) and T_c (conclusion) of the retrogradation endotherm of native starch was higher than those of defatted and heat-moisture treated starches (not shown). However, differences between T_o, T_p and T_c of defatted and heat-moisture treated starches was only marginal (not shown in Table 2). In all starches, T_o, T_p and T_c remained practically unchanged during the time course of retrogradation (not shown).

Recrystallization of starch molecules occurs during gel storage. Reheating of an aged starch gel in a DSC produces an endothermic, transition which is not present in the DSC scan of freshly gelatinized samples. Such a transition is generally attributed to the melting of crystallized amylopectin. The enthalpy of retrogradation is generally considered to correspond to order–disorder transitions of crystallites (i.e. double helices present in extended ordered arrays) and regions of lesser crystalline order. Numerous researchers (Ring, 1987; Russel, 1987; Kalichevsky et al., 1990; Ward et al., 1994) have observed ΔH_R at similar temperatures well below the temperature range for gelatinization. Recrystallization of the amylopectin branch chains in a less ordered manner than what existed for the native starch is an explanation for the observed amylopectin retrogradation endotherm at a temperature range below that for gelatinization (Ward et al., 1994).

Hoover and Vasanthan (1994) have shown that during heat-moisture treatment crystallinity is disrupted within

Table 2
DSC[a] retrogradation enthalpy of untreated, alkali treated and hydroxypropylated starches (native, defatted and heat-moisture treated) after gelatinization and storage[b]

Treatment	Storage time (days)	Enthalpy of retrogradation (J g^{-1})		
		Starch source		
		Native	Defatted	Heat-moisture treated
Untreated	0	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	2	3.8 ± 0.1c	2.9 ± 0.3cd	3.3 ± 0.0c
	5	4.9 ± 0.1d	3.2 ± 0.1de	4.2 ± 0.2d
	7	6.7 ± 0.0f	4.5 ± 0.1f	4.8 ± 0.0f
Alkali treated	0	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	2	3.6 ± 0.2c	2.2 ± 0.1b	3.0 ± 0.1b
	5	4.6 ± 0.0d	2.7 ± 0.1c	4.0 ± 0.1d
	7	6.3 ± 0.0e	3.5 ± 0.1e	4.5 ± 0.1e
Hydroxypropylated[c,d]	0	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	2	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	5	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	7	2.7 ± 0.0b	2.1 ± 0.3b	3.4 ± 0.2c

[a] Values are means of 3 analysis.
[b] Starches were stored at 4°C for 24 h and then at 40°C. Storage beyond 7 days gave endotherms that were too broad for accurate determination of DSC parameters.
[c] Molar substitution: native (0.11), defatted (0.10) and heat-moisture treated (0.11).
[d] In all starches, a detectable endotherm was not observed at high molar substitution levels (> 0.11).
Means within a column followed by different letters are significantly different ($p \leq 0.05$).

granules of potato starch. Thus, after gelatinization, the degree of separation between the outer branches of adjacent amylopectin chain clusters would be more pronounced in heat-moisture treated than in native starch. Consequently, double helical formation (during storag(E)) between adjacent amylopectin chains of gelatinized heat-moisture starch would be much slower and reduced in number than in native starch. This would explain the slower increase in ΔH_R (during storage) and its decreased magnitude after heat-moisture treatment (Table 2). Vasanthan and Hoover (1992) have also shown that defatting increases granule crystallinity in potato starch. Thus, the number of ordered double helices that disrupt on gelatinization would be much lower in defatted than in native starch. Consequently, the number of new double helices that form during retrogradation would be much decreased after defatting. This would then explain the slower increase in ΔH_R (during storage) and its decreased magnitude after defatting (Table 2).

3.3.2. Retrogradation enthalpies of alkali treated native, defatted and heat-moisture treated starches after gelatinization and storage

In all starches, the magnitude and the rate of increase in ΔH_R (during storage decreased slightly after alkali treatment. The difference in magnitude of ΔH_R between untreated and alkali treated starches was more pronounced in defatted than in native and heat-moisture treated starches (Table 2). The results indicate that the number of double helices that form on storage is reduced after alkaline treatment.

3.3.3. Retrogradation enthalpies of hydroxypropylated native (MS 0.11), defatted (MS 0.10) and heat-moisture treated (MS 0.11) starches after gelatinization and storage

In hydroxypropylated starches, the retrogradation endotherm occurred only after 7 days of the storage period. Hydroxypropylation decreased ΔH_R in all starches (Table 2). At the same MS (0.11), the extent of decrease in ΔH_R (Day 7) was more pronounced in native than in heat-moisture treated starch (Table 2). The above decrease in ΔH_R suggests that hydroxypropyl groups within the bulk amorphous and intercrystalline amorphous regions hinder double helical formation by preventing proper alignment of the outer branches of the amylopectin chains during storage. The reduction in ΔH_R on hydroxypropylation is more pronounced in native than in defatted starch (Table 2) due to two reasons (1) the lower MS level of defatted starch (MS 0.10); and (2) the presence of higher amounts of crystallites in the gelatinized defatted starch paste (defatting increases crystallinity), which partially negates the influence of hydroxypropyl groups on chain alignment.

4. Conclusion

The results of this study have shown that the retrogradation properties of native, defatted and heat-moisture treated starches are indirectly influenced by the structural arrangement of starch chains within the amorphous and crystalline regions of the ungelatinized granule. Which in turn, influence the extent of granule breakdown during gelatinization, and the interactions that occur between starch chains during gel storage. Alkali treatment and hydroxypropylation, influence retrogradation by decreasing chain aggregation, and by their influence on granule stability.

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