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# Effect of defatting on starch structure and physicochemical properties

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Wheat, corn, lentil, potato and cassava starches were defatted with hot *n*-propanol/water (3:1, v/v), resulting in almost complete removal of lipids. The relative crystallinity of potato and lentil starches increased by 21 and 7·8%, respectively, on defatting, while that of the other starches remained virtually unchanged. Defatting eliminated the pasting peak of cereal starches and increased the thermal stability and reduced the hot paste consistency of all starches. However, these changes were larger in potato and lentil. The swelling factor of all starches decreased on defatting, with the decrease being more pronounced in potato and lentil. In comparison with their native counterparts, the extent of amylose leaching at different temperatures was higher in wheat and corn starches. The extent of acid hydrolysis of native and defatted starches of wheat, corn and cassava were similar throughout a common interval. During the first stage of hydrolysis, defatted granules of lentil and potato were hydrolyzed (4–7 days, respectively) to a greater extent than were their native counterparts. Thereafter, the native and defatted starches were hydrolyzed to approximately the same extent. Defatted granules of all starches were hydrolyzed by porcine $\alpha$-amylase to a greater extent than were native starches. The gelatinization temperature of native and defatted starches of wheat, corn and cassava were similar. Defatted granules of potato and lentil starch gelatinized over broader and higher temperature ranges than did native starches. The results suggest that amylose and amylopectin chains in native granules are more associated with each other in potato and lentil than in the other starches.

## INTRODUCTION

Lipids associated with cereal, tuber, root and legume starch granules have been found to occur on the surface as well as inside the granule (Morrison, 1981). The surface lipids are mainly triglycerides, followed by free fatty acids, glycolipids and phospholipids (Morrison, 1981; Galliard & Bowler, 1987; Vasanthan & Hoover, 1992). The internal lipids are predominantly monoacyl lipids, with the major components being lysophospholipids and free fatty acids (Hargin & Morrison, 1980; Morrison, 1981; Vasanthan & Hoover, 1992). The amount of total starch lipids (surface and internal) has been generally found to be in the range 0·7–1·2% in cereals (Morrison & Milligan, 1982; Takahashi & Seib, 1988; Vasanthan & Hoover, 1992), 0·01–0·87% in legumes (Hoover & Sosulski, 1991) and 0·08–0·19% in tubers and roots (Emiola & Delarosa, 1981; Goshima *et al.*, 1985; Vasanthan & Hoover, 1992). It is likely that starch lipids may be present in the free state as well as bound to starch components, either linked *via* ionic or hydrogen bonding to hydroxyl groups of the starch components or in the form of amylose-inclusion complexes in which the ligand resides within the central hydrophobic core of the helix (Mikus *et al.*, 1946; Morrison, 1981). Controversy still exists with regard to the lipid binding ability of the short linear (15–20 glucose units) portions of the outer branches of amylopectin (Lagendijk & Pennings, 1970; Krog, 1971; Goering *et al.*, 1975; DeStefanis *et al.*, 1977; Evans, 1986; Biliaderis & Vaughan, 1987; Hahn & Hood, 1987; Eliasson & Ljunger, 1988; Gidley & Bociek, 1988). A 'V' X-ray diffraction pattern is seen when lipid containing starches are subjected to extrusion cooking (Mercier *et al.*, 1980), and after addition of monoacyl lipids to starch under appropriate conditions (Hoover & Hadziyev, 1981; Biliaderis *et al.*, 1986). Native (untreated) cereal starches do not exhibit the 'V'

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pattern (Galliard & Bowler, 1987). This means that either the complexes do not exist in native starch, and are formed only on heating, or more probably, that they do exist but only in partially helical or crystalline conformation (Galliard & Bowler, 1987). Many researchers (Medcalf *et al.*, 1968; Goering *et al.*, 1975; Lorenz, 1976, 1983; Melvin, 1979; Maningat & Juliano, 1980; Goshima *et al.*, 1985; Kawano *et al.*, 1989) have used different lipid extractants, many of which are now known to differ in their ability to extract firmly bound lipids (Morrison, 1981; Morrison & Coventry, 1985; Vasanthan & Hoover, 1992). Therefore, it is difficult to interpret coherently the effect of defatting on starch functionallity. Leach *et al.* (1959) postulated that the bonding forces within the starch granule influence the extent of swelling. Thus, highly associated starch granules should be relatively resistant to swelling and amylose leaching. Tester and Morrison (1990*a*) reported that the swelling behavior of cereal starches was primarily a property of their amylopectin content; amylose acts both as a dilutent and as an inhibitor of swelling, especially in the presence of lipids (natural components of non-waxy cereal starch granules). Tester & Morrison (1990*b*) showed by study on waxy rice starches (no native lipids) that crystallites within the amylopectin molecule determine the onset of swelling and gelatinization. There are conflicting reports in the literature (Lorenz, 1983; Goshima *et al.*, 1985; Tester & Morrison, 1990*a*) with regard to the effect of defatting on swelling and amylose leaching. Lorenz (1983) reported that defatting with 80% methanol increased the swelling power and solubility of wheat starch, whereas in potato starch the swelling power remained unchanged while the solubility decreased. Goshima *et al.* (1985) reported that the swelling power and solubility of potato starch increased on defatting with 99% methanol. Tester and Morrison (1990*a*) reported that partial extraction of lipids from wheat starch with anhydrous methanol at 100°C increased the swelling factor by 30%.

The literature is replete with conflicting information with regard to the effect of defatting on pasting properties (Lorenz, 1976, 1983; Melvin, 1979; Goshima *et al.*, 1985; Takahashi & Seib, 1988). Lorenz (1976) reported that lipid removal (0·54–0·61%) from wheat starch with 80% methanol did not affect the peak viscosity at 92°C. Melvin (1979) reported that lipid removal (0·4–0·5%) from corn and wheat starches by slurrying at 70°C with water-saturated *n*-butanol (5 h), or by Soxhlet extraction using 85% aqueous methanol (72 h ), reduced the pasting temperature but increased the pasting peak and paste consistencies. Takahashi and Seib (1988), however, showed that lipid removal from wheat (1·00%) and corn starches (0·82%) with boiling 75% ethanol eliminated the pasting peak, reduced consistency and setback and decreased the pasting temperature. Biliaderis and Tonogai (1991) observed increased gel firmness and viscosity when lipids were extracted from rice (0·82%) and wheat (0·64%) starches using 85% methanol (4 h). They monitored viscosity and gel texture changes using the Bohlin VOR rheometer. The above researchers attributed the discrepancy between their results and those of Takahashi and Seib (1988) to differences in starch concentration (20–30% versus 6·5–7·5%). Lorenz (1983) showed that lipid removal from potato starch with 80% methanol (48 h) did not significantly alter the amylograph consistencies. Goshima *et al.* (1985), however, reported that lipid removal (0·064%) from potato starch with 99% methanol (15 h) in a Soxhlet extractor reduced the pasting temperature, increased paste consistency at 67·8°C and caused no change in thermal stability during the holding period.

There is very little information in the literature with respect to the effect of defatting on starch crystallinity. Lorenz (1983) observed that wheat and potato starches showed no changes in their X-ray pattern on defatting with 80% methanol for 48 h. Furthermore, defatting was shown to cause a decrease in relative crystallinity (RC), which amounted to 1·7% and 6·8% in potato and wheat starch, respectively.

It is necessary at this stage to give a brief description of the structure of amylose and amylopectin chains in some of these starches, which will allow a subsequent discussion of the changes in crystallinity during defatting.

Bender *et al.* (1982) showed that the cyclodextrin glycosyl-transferase (CGT) from *Klebsiella pneumoniae* catalyzes the simultaneous cyclization (exo-attack) and chain shortening (endo-attack) of amylopectin with formation of a mixture of cyclic and non-cyclic degradation products. Analysis of maize and potato using CGT revealed that 57% of the maize amylopectin and 64% of the potato amylopectin was recovered as non-cyclic products which had β-amylolysis limits ranging from 24% to 34%, respectively. This indicated that the clusters were not equally susceptible to attack by CGT. The fragments of potato amylopectin still contained marked amounts of longer B chains. This seemed to imply that the associated clusters of A chains (which are primarily responsible for crystallinity) are not so tightly packed, and the long B chains of the basic structure carry interchain branches throughout their lengths. In contrast, maize amylopectin is composed of tightly packed clusters connected by more or less unbranched, long B chains of the basic structure. It thus seems plausible, that the observed differences in RC of native potato and maize starches may be attributed to differences in the extent of A chain clustering. Gidley (1987) showed that in the A-type starch structure there is a close-packed arrangement of double helices, whereas in the B-type the structure is more open, with a greater amount of interhelical water. Hizukuri (1985) showed by means of gel permeation HPLC with monitoring with a low angle light scattering photometer and

differential refractometer that the amylopectin molecules of A-type starches have shorter average chain length (CL) than the B-type starches. These values were 25, 26, 28 and 34 in wheat, corn, cassava and potato, respectively, while the corresponding value for lentil was reported to be 20 (Biliaderis *et al.*, 1981*a*). Swinkels (1985) reported that the degree of polymerization (DP) of amylose in wheat and corn was 800, while that of potato and cassava was 3000. However, Takeda *et al.* (1984) reported DP values of 570, 2260 and 4920 for amylose in wheat, tapioca and potato, respectively. The above authors also showed that the amylose from potato starch (7·5 chains per molecule) and tapioca (7·8 chains per molecule) were more highly branched than that of wheat starch (1·9 chains per molecule). A DP of 1600 has been reported for amylose from lentil starch (Biliaderis *et al.*, 1981*a*).

Zobel (1988) presented several arguments to support the concept that amylose is more closely associated with amylopectin in potato than in cereal starches. Zobel (1988) postulated that lipids in starches may be responsible for effecting an amylose separation within the granules. This would imply that the starch components of low lipid containing starches (potato, lentil, cassava) may be more associated with each other in the native granule than those of high lipid-containing starches (wheat, corn). Although this may be partly true, it is the authors' opinion that the extent to which the starch components are associated with each other within the native granule may also depend on their respective CL. Long amylose CL may facilitate easier association with the short DP (20–25) glucopyranose residue chains of amylopectin. It is therefore likely that in potato starch the degree of association between starch components may be higher than in other starches, due to its low lipid content and long amylose CL.

The purpose of this investigation was to study the effect of lipid removal, using hot *n*-propanol/water (3:1, v/v) (PW), on the structure and physicochemical properties of starches from various plant sources.

## MATERIALS AND METHODS

Corn and potato starch was obtained from Sigma Chemical Co., St Louis, MO, USA. Cassava starch was obtained from A. E. Staley Manufacturing Company, Decatur, IL, USA. Dimodan PM, a commercially distilled monoglyceride derived from fully hydrogenated lard (*c.* 30% palmitic acid and 65% stearic acid) containing 92% 1-monoglycerides was obtained from Grinsted Products, Brabrand, Denmark. Alpha-amylase from hog pancreas was also purchased from Sigma Chemical Co. Other chemicals and solvents were analytical grade. Solvents were distilled from glass before use.

### Proximate analysis and lipid extraction

Proximate analysis of moisture and nitrogen were performed using the standard AACC (1984) procedures. Amylose determinations were by the method of Chrastil (1987). Starch lipids (free and complexed) were Soxhlet extracted with PW at 90–100°C for 7 h. Starch granules were hydrolyzed with 24% HCl 70–80°C for 30 min, and the hydrolyzate then extracted three times with *n*-hexane (Goshima *et al.*, 1985).

### Preparation of starch/dimodan complex

Dimodan powder (1 part by weight) was slowly added to water (9 parts by weight) and the mixture was preheated to 70°C, with agitation, until a translucent dispersion was obtained. This dispersion was cooled to ambient temperature (25–27°C) and then added to a starch/water suspension (10%, w/w) preheated to 50°C in the starch to dispersion weight ratio of 5:1. The combined volume was heated at 50°C with agitation for 6 h. The suspension was then cooled to ambient temperatures, and the insoluble fraction was recovered by filtration through a Buchner funnel. Any non-interacting dimodan, was removed from the insoluble fraction by extraction at ambient temperature with chloroform/methanol (2:1, v/v) and agitation for 10 min. The solid fraction remaining was dried under vacuum at 40°C. Complex formation was monitored using differential scanning calorimetry and X-ray diffraction.

### Relative crystallinity by X-ray diffraction

X-ray diffractograms were obtained with a Rigaku RU 200R X-ray diffractometer with a chart speed of 20 mm $min^{-1}$. The starch powder was scanned through the $2\theta$ range of 3–35°C. Traces were obtained using Cu–K$\alpha$ radiation detector with a nickel filter and a scintillation counter operating under the following conditions: 40 kV, 50 mA, 1°/1° divergence slit/scattering slit, 0·30 mm receiving slit, 1 s time constant, and 3° per minute scanning rate. Relative crystallinity was measured by the method of Hermans and Weidinger (1948). Quartz was used as the 100% reference crystal.

### Acid hydrolysis

The starches were hydrolyzed with 2·2N HCl at 35°C (1·0 g starch per 40 ml acid) for 25 days. The starch slurries were shaken by hand daily to resuspend the deposited granules. At 24 h intervals, aliquots of the reaction mixtures were neutralized and centrifuged (3500 rpm) and the supernatant liquid was assayed for total carbohydrate (Bruner, 1964). The extent of hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial dry starch.

### Determination of the swelling factor

The swelling factor (SF) of the starches when heated to 50–95°C in excess water was measured according to the method of Tester and Morrison (1990*a*), This method measures only intragranular water and hence the true SF at a given temperature. The SF is reported as a ratio of the volume of swollen starch granules to the volume of the dry starch.

### Extent of amylose leaching

Various concentrations of native and defatted starch (10–15 mg) in water were heated in volume calibrated sealed tubes (50–95°C) for 30 min. The tubes were then cooled to ambient temperature and centrifuged at 3 500 rpm for 10 min. One milliliter of the supernatant liquid was withdrawn and its amylose content was determined by the method of Chrastil (1987).

### Pasting behavior

A Brabender viscoamylograph, Model VA-V equipped with a 700 cmg cartridge was used to study pasting properties at a concentration of 6% (w/v) and pH 5·5.

### Differential scanning calorimetry

Gelatinization temperatures were measured and recorded on a Perkin-Elmer DSC-2 differential scanning calorimeter, with a heating rate of 10°C $min^{-1}$, a sensitivity of 0·5 m cal $s^{-1}$, and a chart speed of 20 mm $min^{-1}$. Water (8·0 $\mu$l) was added with a microsyringe to starch (2·5 mg) in the DSC pans, which were then sealed, reweighed and allowed to stand overnight at room temperature. The scanning temperature range was 20–120°C. The thermogram was recorded with water as reference. The transition temperatures reported are the onset ($T_0$), peak ($T_p$) and conclusion ($T_c$) of the gelatinization endotherm. Indium was used for calibration.

### Enzymatic digestion

Enzymatic digestion studies on native and defatted corn, potato and cassava starches were done using crystalline porcine pancreatic $\alpha$-amylase in 0·5 M saturated sodium chloride containing 3 mM calcium chloride (Sigma Chemical Co.), in which the concentration of $\alpha$-amylase was 23·9 mg $ml^{-1}$, and the specific activity was 1240 units per milligram of protein. A unit of activity was defined as that which liberates 1 mg maltose in 3 min at 20°C and pH 6·9.

The procedure used was that of Knutson *et al.* (1982). However, a higher concentration of enzyme was used in this study. Starch granules (100 mg) were suspended in distilled water (25 ml) and 5 ml aliquots were placed in a constant temperature water bath (37°C). Then 4·0 ml of 0·1 M phospate buffer (6·9), containing 0·006 M sodium chloride were added to the slurry. The mixture was gently stirred before adding 4 $\mu$l $\alpha$-amylase suspension. Then 1·0 ml aliquots were removed at specified time intervals, pipetted into 0·2 ml of 95% ethanol, and centrifuged. Aliquots of the supernatant were analyzed for soluble carbohydrate by the 3,5-dinitrosalicylic acid method of Bruner (1964), and reported as maltose. Controls without enzyme but subjected to the above experimental conditions were run concurrently.

## RESULTS AND DISCUSSION

### Proximate analysis

Proximate analyses of the starches are presented in Table 1. The nitrogen contents represent the contributions from endosperm storage proteins, lysophospholipids and proteins located inside starch granules (Morrison, 1981). The nitrogen contents of the purified starches were in the range 0·01–0·04% (dry basis) indicating the absence of endosperm proteins and by implication, most of the non-starch lipids (Morrison, 1981).

### Acid hydrolysis

The total starch lipids extracted after acid hydrolysis ranged from 0·11% (potato) to 0·76% (corn). Defatting with hot PW resulted in removal of >98·6% of the lipids from most of the starches, the exception being wheat, in which there was a 96·4% removal (Table 1).

### Amylose content

The total amylose content and the apparent amylose content of native, and dimodan complexed starches are presented in Table 2. A comparison of the apparent and total amylose gives an indication of the proportion of

**Table 1. Lipid content of some cereal, legume, tuber and root starches[a]**

| Starch source | Lipids[b] (mg per 100 g dry starch) | |
|---|---|---|
| | Acid hydrolyzed[c] | Solvent extracted[d] |
| Wheat | 704 | 678 |
| Corn | 760 | 759 |
| Lentil | 136 | 136 |
| Potato | 107 | 107 |
| Cassava | 188 | 188 |

[a] Values are average of three determinations.
[b] Includes both surface and internal starch lipids.
[c] Lipids obtained by extraction after acid hydrolysis (24% HCl) of native starches at 70–80°C for 30 min.
[d] Lipids obtained by *n*-propanol/water (3:1 v/v) extraction at 90–100°C for 7 h.

**Table 2. Amylose content of native and dimodan complexed starches[a]**

| Starch source | Treatment | Amylose content (% of total starch) | |
|---|---|---|---|
| | | Apparent[b] | Total[c] |
| Wheat | Native | 21·1 | 27·3 |
| | Native complexed[d] | 18·7 | |
| | Defatted[e] complexed | 18·2 | |
| Corn | Native | 22·1 | 27·2 |
| | Native complexed | 18·5 | |
| | Defatted complexed | 18·6 | |
| Lentil | Native | 36·7 | 38·9 |
| | Native complexed | 25·3 | |
| | Defatted complexed | 20·5 | |
| Potato | Native | 21·9 | 24·7 |
| | Native complexed | 10·7 | |
| | Defatted complexed | 7·6 | |
| Cassava | Native | 18·5 | 21·5 |
| | Native complexed | 8·3 | |
| | Defatted complexed | 8·6 | |

[a] Values are means of three replicates.
[b] Apparent amylose was determined by iodine binding without removal of total native starch lipids and/or dimodan.
[c] Total amylose was determined by iodine binding after removal of total starch lipids with hot *n*-propanol/water (3:1, v/v).
[d] Complexed with 5% dimodan (starch dry weight basis). Non-interacting dimodan was removed with chloroform/methanol) (2:1, v/v) at 25°C.
[e] Soxhlet extraction with hot *n*-propanol/water (3:1, v/v) for 7 h.

amylose complexed with lipid. In the case of native starches, the proportion of amylose complexed with naturally occurring lipids amounted to 23, 19, 5·6, 11·3 and 14% in wheat, corn, lentil, potato and cassava, respectively.

The amount of amylose complexed by native lipids and dimodan was 31·5% (wheat), 32% (corn), 35% (lentil), 56·6% (potato) and 61·4% (cassava). The above values did not change significantly on the addition of dimodan to lipid-free wheat (33·3%), corn (31·6%) and cassava (60%) starches (Table 2). However, additional amylose complexation was evident in defatted lentil (47·3%) and potato (69·2%) starches.

### X-Ray diffraction

The X-ray diffraction patterns and the relative crystallinity (RC) of native and defatted starches are shown in Fig. 1. Native potato starch exhibited the typical 'B' pattern of tuber starches, with peaks at 16·7, 5·9, 5·2, 4·0 and 3·7 Å. Defatting resulted in an increase in RC (21%), and the disappearance of the peaks at 16·7 and 5·9 Å. The X-ray pattern changed to a combination of one half 'A' and one half 'B' pattern, indicating that a clustering of the outer A chains of amylopectin may have occurred, resulting in the formation of

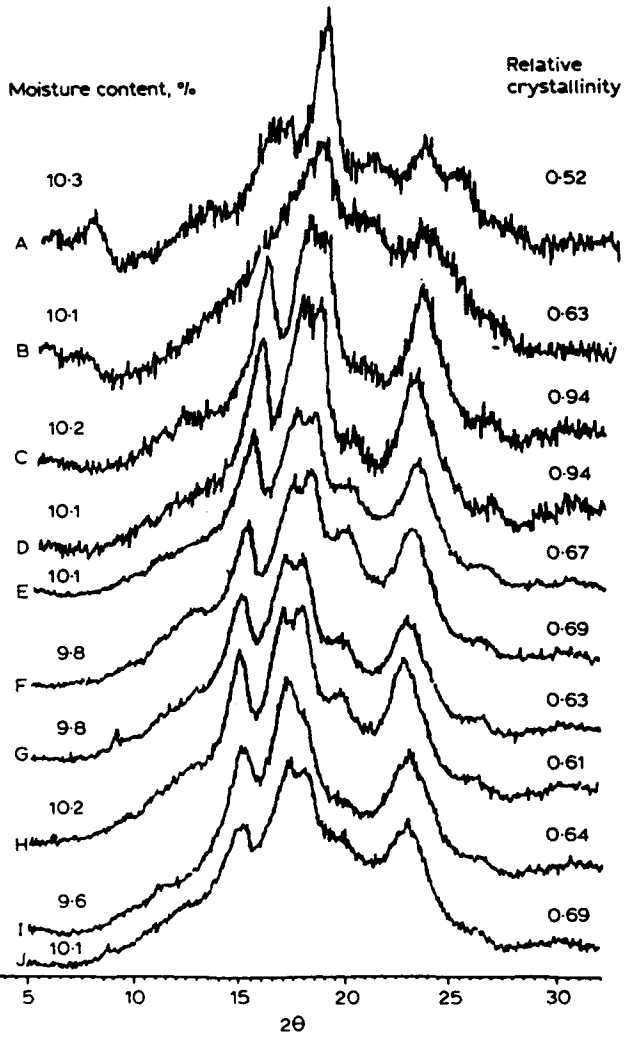


**Fig. 1.** X-Ray diffraction patterns of native and defatted starches. (A) Native potato starch; (B) defatted potato starch; (C) native cassava starch; (D) defatted cassava starch; (E) native wheat starch; (F) defatted wheat starch; (G) native corn starch; (H) defatted corn starch; (I) native lentil starch; (J) defatted lentil starch.

a close-packed arrangement of double helices (Gidley, 1987). A similar transformation has been observed in heat-moisture treated potato starch (Donovan *et al.*, 1983).

The X-ray patterns of native and defatted wheat and corn starches were similar (A-type) showing spacings at 5·9, 5·2 and 3·8 Å. Both these starches showed no significant changes in their RC on defatting (Fig. 1). The C-type X-ray pattern was seen in both native and defatted cassava and lentil starches (Fig. 1). The RC of cassava starch remained unchanged, while that of lentil increased by 7·8% on defatting.

Long range molecular ordering (crystallinity) in starch granules has been attributed to regular packing of double helices formed from adjacent clusters of the short DP chains of amylopectin (French, 1984). It is therefore likely that starches containing amylose chains entrapped between adjacent chain clusters of amylopectin would exhibit a lower degree of long range molecular

order (by preventing close association of adjacent amylopectin chains) than those in which amylose and amylopectin chains are well separated within the granule. The unchanged X-ray pattern and the very low increase in relative crystallinity seen in defatted starches of wheat, corn, cassava and lentil (Fig. 1) shows the degree of separation of amylose and amylopectin in the native granules to be in decreasing order: corn ~ wheat ~ cassava > lentil > potato.

The additional amylose complexing (Table 2) and the increases in relative crystallinity (Fig. 1) observed in defatted lentil and potato starches seem to suggest that the moisture and thermal energy increases the mobility of entrapped amylose chains, resulting in their release into the amorphous regions of the granule. The discrepancy between the authors' observation and that of Lorenz (1983) with regard to changes in potato starch crystallinity on defatting, is possibly due to differences in the composition of the extracting solvent systems. The authors' results showed that both PW and 80% methanol extracted the same quantity of lipid from potato starch. However, the maximum temperature experienced by potato starch granules during refluxing with PW and 80% methanol for 7 h was 82 and 67°C, respectively. This would then explain the low degree of structural change observed by Lorenz (1983), who defatted potato starch by refluxing with 80% methanol for 48 h. It is likely that at 67°C the thermal energy may have been insufficient to cause the release of entrapped amylose chains into the amorphous regions.

**Pasting curves**

Removal of lipids decreased the pasting temperatures of wheat and corn by 3 and 7°C, respectively (Fig. 2(1)), and increased those of potato and lentil by 26 and 21°C, respectively (Figs 2(2) and 2(4)). That of cassava starch was unaffected (Fig. 2(3)). Defatting also eliminated the pasting peak of corn and wheat starches (Fig. 2(1)), increased the thermal stability and reduced the hot paste consistencies of all starches (Figs 2(1)–2(4)). The magnitude of the changes in thermal stabilities and hot paste consistencies decreased in the order: potato > lentil > corn > cassava > wheat. The viscosity profile of defatted potato and lentil starches during the holding cycle at 95°C (Figs 2(2) and 2(4)) resembled that of cross-linked starches. The results suggest that in

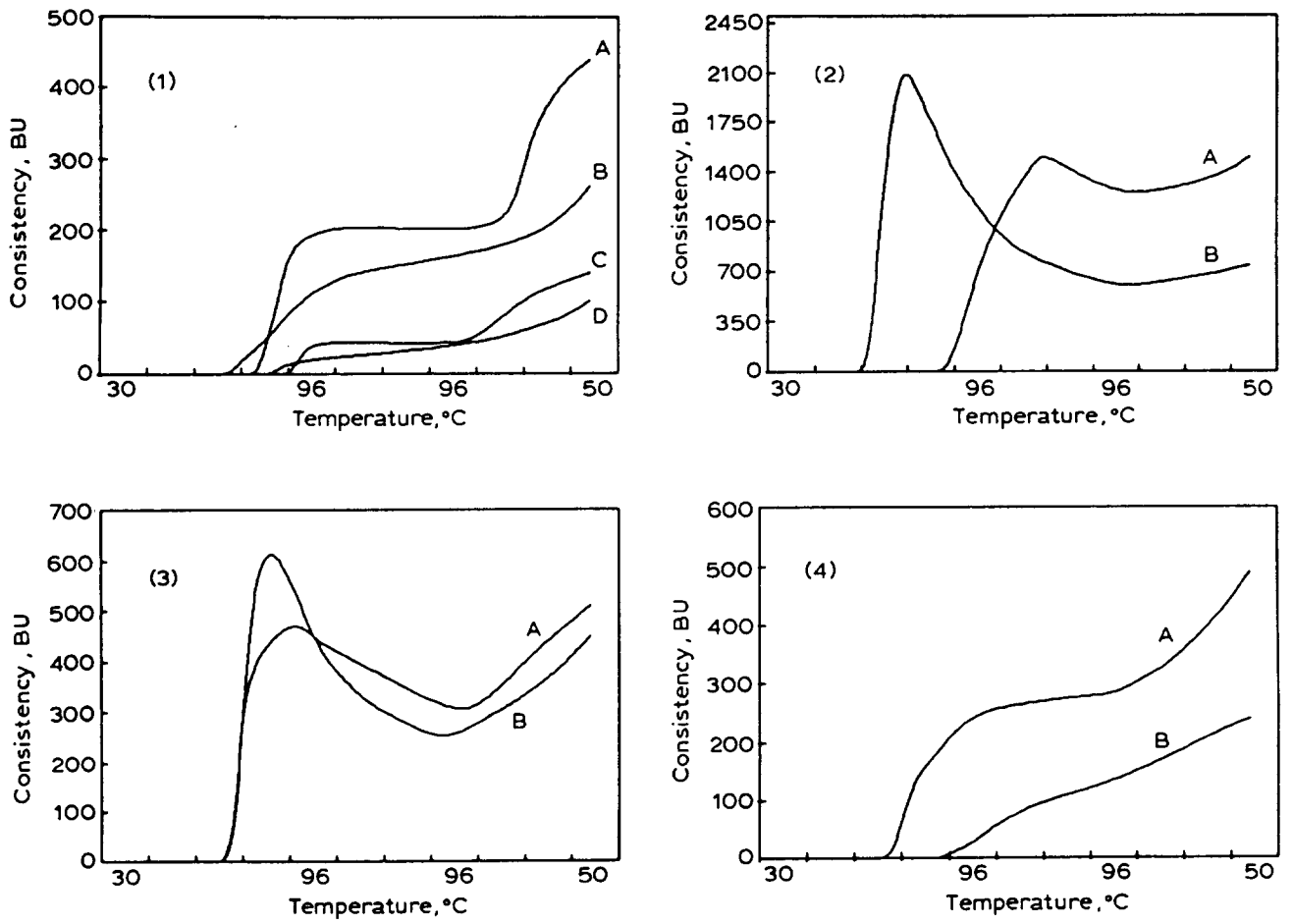


Fig. 2. Brabender amylograms of native and defatted starches. (1): (A) Native and (B) defatted corn starch; (C) native and (D) defatted wheat starch. (2): (B) Native and (A) defatted potato starch. (3): (B) Native and (A) defatted cassava starch. (4): (A) Native and (B) defatted lentil starch.

potato and lentil starches, the interaction between amylopectin chain clusters during defatting may have been the main causative factor responsible for the large decrease in pasting temperature, increased thermal stability and reduced hot paste consistency. It is likely that in wheat, corn and cassava, the observed changes in pasting curves on defatting may reflect mainly the amount of bound lipids removed. This seems plausible, since the changes in pasting curves were greater in defatted corn than in wheat, probably due to the presence of solvent unextractable bound lipids still remaining within the granules of the latter (Table 1). The differences in the pasting curves of native and defatted cassava starches (Fig. 2(3)) were not as marked as those of other starches (Fig. 2(1,2,4)). This is not surprising since this starch is known to contain only traces of bound lipids (Vasanthan & Hoover, 1992).

The amylograms of defatted wheat and corn starches are in general agreement with those of Takahashi and Seib (1988), but differ from those of Lorenz (1976) and Melvin (1979). The amylograms of defatted potato starch differed from those reported by Goshima *et al.* (1985) and Lorenz (1983). The present authors' results seem to indicate that discrepancies in the literature with respect to changes in rheological properties on lipid removal are due to differences in the maximum temperatures experienced by starch granules during lipid removal, and in the nature and composition of the extracting solvent system.

**Swelling factor and amylose leaching**

Swelling factor (SF) and amylose leaching (AML) at different temperatures are presented in Figs 3 & 4, respectively. The SF and AML of native and defatted starches increased with a rise in temperature (Figs 3 & 4). The SF of all starches decreased on defatting, the decreases being highest with potato and lentil (Figs 3(2) and 3(4)). Defatting also decreased the extent of AML in potato cassava and lentil starches (Figs 4(2)–4(4)) and increased those of wheat corn (Fig. 4(1)). The results seem to suggest that the decrease in SF on defatting is indicative of increased granular stability arising from interaction between amylopectin chain clusters, the magnitude of this interaction being strongest in potato starch and weakest in cereal starches. The increase in granular stability is also responsible for the decreased AML seen in defatted potato, lentil and

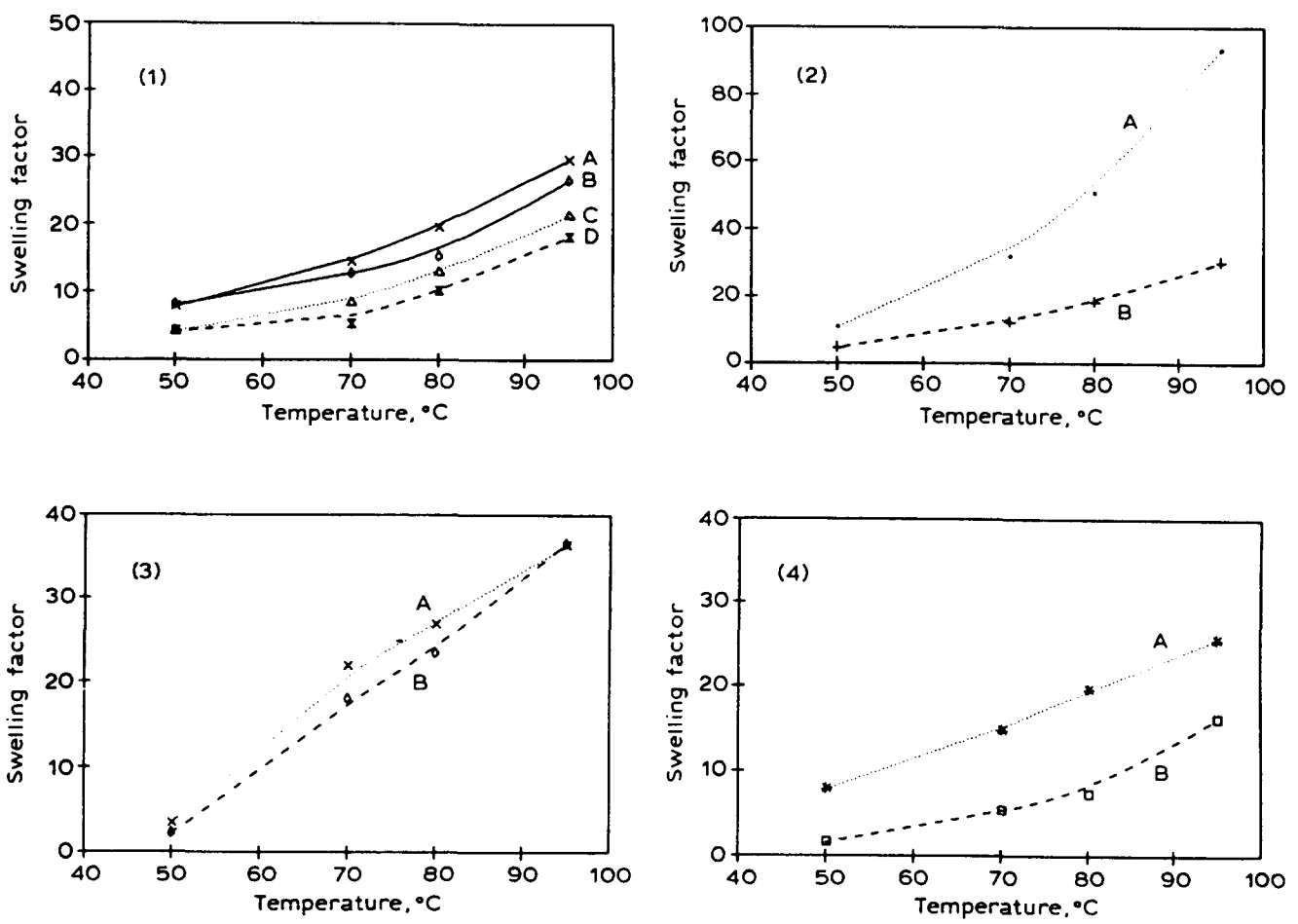


**Fig. 3.** Swelling factor of native and defatted starches. (1): (A) Native and (B) defatted wheat starch; (C) native and (D) defatted corn starch. (2): (A) Native and (B) defatted potato starch. (3): (A) Native and (B) defatted cassava starch. (4): (A) Native and (B) defatted lentil starch.

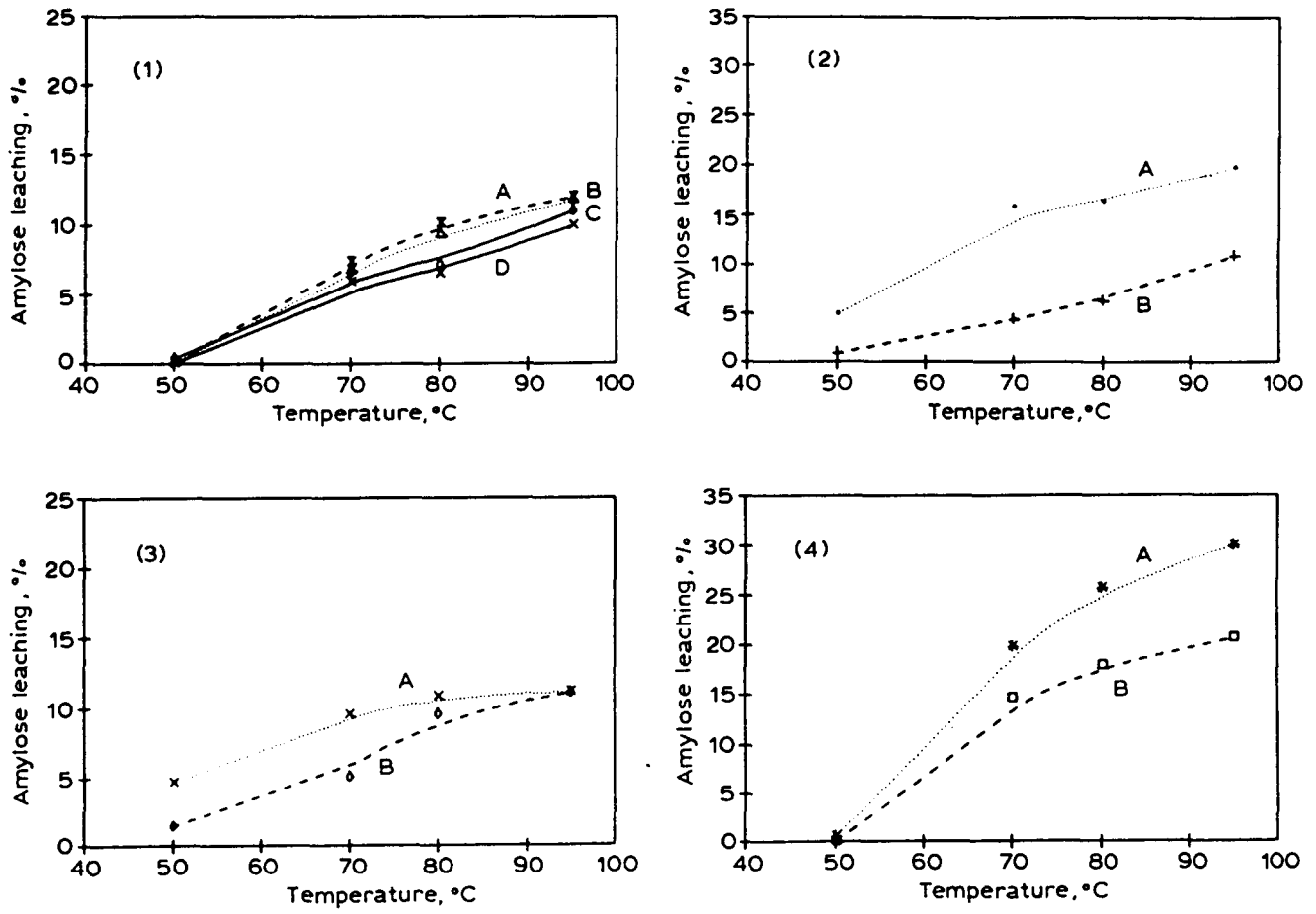


**Fig. 4.** Amylose leaching in native and defatted starches. (1): (A) Defatted and (B) native corn starch; (C) defatted and (D) native wheat starch. (2): (A) Native and (B) defatted potato starch. (3): (A) Native and (B) defatted cassava starch. (4): (A) Native and (B) defatted lentil starch.

cassava starches. However, in cereal starches , the increased AML probably represents to a larger extent the removal of bound lipids. These discrepancies between the authors' findings and those of Lorenz (1983), Goshima *et al.* (1985) and Tester and Morrison (1990*a*) may have been due to the same differences as outlined in the previous section and to a lesser extent to differences in the methodology employed for measuring the extent of swelling. Lorenz (1983) and Goshima *et al.* (1985) used the method adopted by Leach *et al.* (1959) for measurement of swelling power, which does not distinguish between intragranular water and intergranular or interstitial water. However, in the method (Tester & Morrison, 1990*a*) used in this study only the intragranular water, and hence the true swelling factor at a given temperature, is measured.

### Acid hydrolysis

The solubilization patterns of native and defatted starches are presented in Fig. 5. All starches exhibited a two-stage hydrolysis pattern. A relatively faster rate of hydrolysis was observed during the first nine days of hydrolysis followed by a slower rate between days 9 and 12. Similar hydrolysis patterns have been reported for native corn, waxy corn, wheat, potato, cassava, rice, lima bean, mung bean, lentil and wrinkled pea starches (Robin *et al.*, 1974; Biliaderis *et al.*, 1981*b*; Nara *et al.*, 1983; Komiya *et al.*, 1987; Hoover *et al.*, 1991). The faster hydrolysis rate during the initial eight days has been shown to correspond to the destruction of the amorphous regions of the starch granule. During the second stage the crystalline region is slowly degraded (Robin *et al.*, 1974; French, 1984). Lipid removal from wheat, corn and cassava starches did not cause any significant changes in the extent of hydrolysis. This seems to indicate that native lipids complexed with amylose does not render the amorphous regions of the starch granule resistant to degradation by cold aqueous acid. Potato and lentil starches, however, showed increased rates of hydrolysis on defatting (Figs 5(2) and 5(4)). This increase was higher in potato. The higher rate of hydrolysis was evident for the first four days in defatted lentil starch and the first seven days in defatted potato starch (Figs 5(2) and 5(4)). Thereafter, the rate of hydrolysis was lower than those of their native counterparts (Figs 5(2) and 5(4)). The higher extent of degradation seen in defatted starches of lentil and potato during

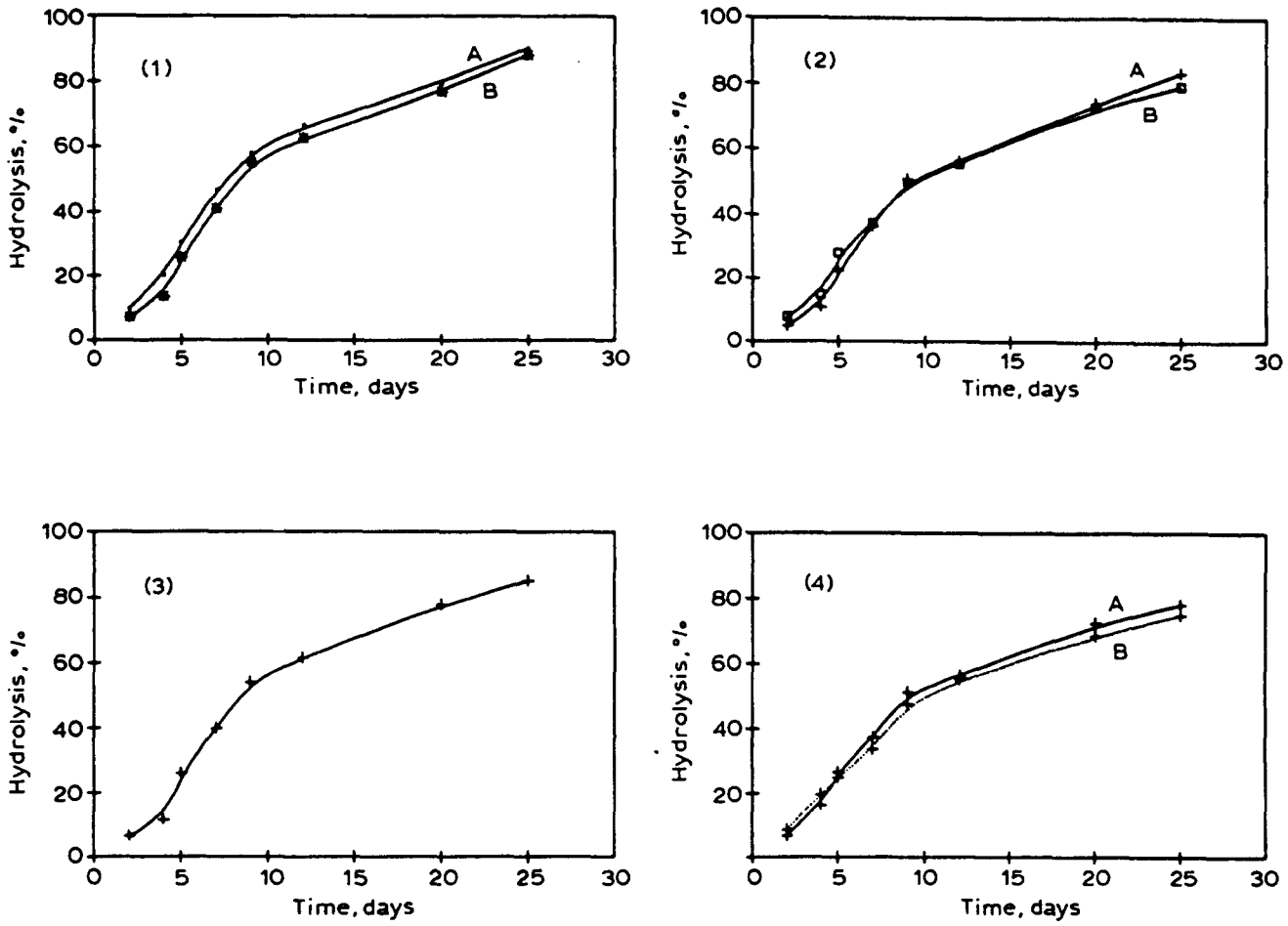


**Fig. 5.** Heterogenous hydrolysis of native and defatted starches in 2·2 N HCl at 35°C. (1): (A) Native wheat starch; (B) native corn starch. (2): (A) Native potato starch; (B) defatted potato starch. (3): Native cassava starch. (4): (A) Native lentil starch; (B) defatted lentil starch.

the first four and seven days of hydrolysis, respectively, is indicative of hydrolysis of released amylose chains, that were originally a part of the amylopectin structure (in the native granule). Once the released amylose chains are hydrolyzed the effect of increased crystallinity on acid hydrolysis becomes evident.

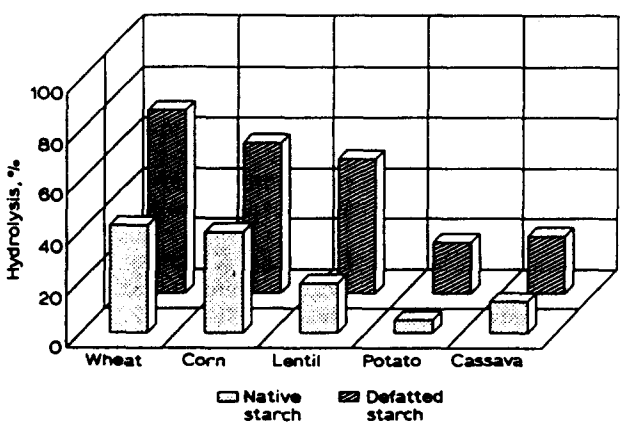


**Fig. 6.** Hydrolysis of native and defatted starches by porcine pancreatic α-amylase.

**In-vitro digestibility of native and defatted starches by porcine pancreatic α-amylase**

The extent of α-amylase hydrolysis of native and defatted starches is presented in Fig. 6. Defatting increased the extent of hydrolysis in all starches. The extent of increase followed the decreasing order: potato > lentil > cassava > wheat > corn. This seems to suggest that defatting increases the accessibility of amylose chains to α-amylase. In wheat, corn and cassava this could be attributed to a change in amylose conformation (V-helix → random coil) on lipid removal, with the result that a larger surface area becomes available for enzyme action. Previous studies have shown that amylose/lipid complexes show reduced susceptibility to α-amylase digestion (Larrson & Meizis, 1979; Holm *et al.*, 1983; Seneviratne & Biliaderis, 1991). However, in potato and lentil starches, a change in amylose conformation is less likely (since these starches have been shown to contain only trace quantities of complexed lipids (Vasanthan & Hoover, 1992)). Therefore, it seems plausible that in potato and lentil the increase reflects the action of α-amylase on the released amylose chains.

**Table 3. Gelatinization temperatures of native, defatted and lipid complexed starches[a]**

| Starch source | Treatment | $T_0$[b] (°C) | $T_p$ (°C) | $T_c$ (°C) |
|---|---|---|---|---|
| Wheat | Native | 57 | 62 | 67 |
| | Defatted | 56 | 61 | 65 |
| | Native complexed | 64 | 66 | 69 |
| | Defatted complexed | 65 | 67 | 70 |
| Corn | Native | 60 | 68 | 73 |
| | Defatted | 61 | 67 | 74 |
| | Native complexed | 64 | 71 | 74 |
| | Defatted complexed | 84 | 73 | 77 |
| Lentil | Native | 56 | 61 | 66 |
| | Defatted | 60 | 66 | 73 |
| | Native complexed | 63 | 67 | 71 |
| | Defatted complexed | 68 | 73 | 79 |
| Potato | Native | 55 | 59 | 65 |
| | Defatted | 59 | 65 | 71 |
| | Native complexed | 58 | 62 | 67 |
| | Defatted complexed | 60 | 67 | 75 |
| Cassava | Native | 60 | 67 | 72 |
| | Defatted | 61 | 66 | 72 |
| | Native complexed | 64 | 70 | 74 |
| | Defatted complexed | 67 | 73 | 77 |

[a] $T_0$, $T_p$ and $T_c$ indicate the temperatures of the onset, midpoint and end of gelatinization, respectively.
[b] Average standard deviation = 0·1 ($n$ = 3).

### Gelatinization temperatures

The gelatinization parameters of native and defatted starches are presented in Table 3. Defatting did not significantly affect the gelatinization temperatures of wheat, corn and cassava starches. However, defatted granules of potato and lentil gelatinized over a broader and higher temperature range than did the corresponding untreated control starches (Table 3). The final gelatinization temperature of lentil starch increased on defatting from 66 to 73°C, while that of potato increased from 65 to 71°C. The range over which the starch granules gelatinized increased from 10 to 13°C in lentil and from 10 to 12°C in potato starch. These increases are an indication of increased order within the defatted starch granules of potato and lentil. Takahashi and Seib (1988) showed that extraction of wheat and corn starches with 75% ethanol did not cause any significant change in their gelatinization temperatures. Similar observations were reported by Lorenz (1983) (80% methanol) and Goshima *et al.* (1985) (99% methanol) on potato starch and by Lorenz (1983) on wheat starch. Biliaderis and Tonogai (1991) reported that the gelatinization temperature of rice starch decreased on extraction with 85% methanol. Differences in extracting solvent systems are mainly responsible for these discrepancies.

The gelatinization temperatures of dimodan complexed native and defatted starches of wheat, corn and cassava were fairly close (Table 3). However, those of potato and lentil were widely different (Table 3), with defatted complexed starches exhibiting higher gelatinization temperatures than native complexed starches. This is indicative of increased amylose complexing (due to release of amylose chains) in defatted starches of potato and lentil.

This work has demonstrated that the changes in starch granule structure and physicochemical properties on defatting depends on the type of crystalline structure (A, B or C), nature and composition of the extracting solvent system, maximum temperature experienced by the starch granules, extent of association between amylose and amylopectin chains in the native granule, and on the lipid content.

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