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# Relationship between α-amylase degradation and the structure and physicochemical properties of legume starches

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**Abstract**

Starch from cultivars of black bean, pinto bean, smooth pea, lentil and wrinkled pea starches were isolated and their composition, physicochemical properties and susceptibility towards porcine pancreatic α-amylase determined. The yield of starch ranged from 16.4 to 34.1% on a whole seed basis. The shape of the granules varied from round to oval to irregular. Bound and total lipids ranged from 0.26 to 0.80% and 0.35 to 0.84%, respectively. The total amylose content ranged from 30.5 to 78.4%, of which 10.3 to 12.2% was complexed by native starch lipids. The X-ray diffraction pattern was of the 'B' type in wrinkled pea starch and of the 'C' type in other starches. The relative crystallinity and the 'B' polymorphic content ranged from 17.7 to 33.4% and 27.1 to 92.2%, respectively. The starches differed in the degree of swelling, extent of amylose leaching, gelatinization temperatures and gelatinization enthalpy. The above differences were more pronounced between cultivars of black bean and lentil. The results showed that starch chain interactions within the amorphous domains were more pronounced in wrinkled pea starch. All starches exhibited a biphasic hydrolysis pattern, a relatively rapid rate at the initial stage followed by a progressively decreased rate thereafter. Wrinkled pea starch exhibited a much higher initial velocity than the other starches. Cultivars of black bean and lentil showed significant differences in their initial velocities. However, differences in initial velocity between cultivars of smooth pea and pinto bean were not significant. Black bean, lentil and wrinkled pea starches showed a plateau at 93, 85 and 65% hydrolysis, respectively. The time taken for the appearance of the plateau was identical for black bean cultivars, but was different for cultivars of lentil. Pinto bean and smooth pea cultivars showed no plateau. At the end of the assay period (120 h), cultivars of each legume species were hydrolyzed to the same extent, and the extent of hydrolysis among the legume species followed the order: black bean > lentil > smooth pea > pinto bean > wrinkled pea. The X-ray pattern and the 'B' polymorphic content of all starches remained unchanged on hydrolysis. However, the relative crystallinity increased in wrinkled pea, but remained unchanged in the other starches. On hydrolysis, the apparent amylose content decreased in all starches. The extent of this decrease was most pronounced in wrinkled pea. In all starches, the enthalpy of gelatinization decreased, and the gelatinization transition temperatures increased slightly on hydrolysis. This study showed that the rate and extent of hydrolysis was influenced by structural organization of the starch chains within the native granule, and by the extent of interaction between hydrolyzed amylose chains.


*Keywords:* Legume starches; Physicochemical properties; α-Amylase hydrolysis

## 1. Introduction

The susceptibility of starches with 'A' and 'B' type crystalline polymorphic forms towards porcine pancreatic α-amylase in terms of enzyme adsorption, action pattern, extent of hydrolysis, hydrolysis products, and structure and properties of the enzyme resistant residues has been reported. However, the susceptibility of legume starches, which are known to contain a mixture of 'A' and 'B' type crystalline polymorphs in varying proportion (Cairns, Bogracheva, Ring, Hedley & Morris, 1997; Davydova, Leont'ev, Genin, Sasov, & Bogracheva, 1995; Gernat, Radosta, Damaschun, & Schierbaum, 1990; Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001) towards α-amylase have not been investigated to as great an extent. Furthermore, most of the previous work on legume starch susceptibility to α-amylase was based on studies on a single cultivar. This approach makes the results difficult to

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interpret, since it is not known, whether the data truly represents the species in general.

'A' and 'B' type starches are based on parallel stranded double helices in which the helices are closely packed in the 'A' type starch, but loosely packed in the 'B' type starch. Furthermore, they also differ in content of intrahelical water (B > A, Imberty Chanzy, Pérez, Buléon, & Tran, 1988). Bogracheva, Morris, Ring, and Hedley (1998) have shown that in pea starch the 'B' polymorph is situated in the center of all granules and is surrounded by the 'A' polymorph.

Jane, Wong, and McPherson (1997) have explained the susceptibility differences between 'A' and 'B' type starches towards α-amylase in the following way: in 'A' type starches, the branch points are scattered in both amorphous and crystalline regions. Consequently, there are many short A chains ('A' and 'B' chains of amylopectin do not signify 'A' or 'B' crystalline types) derived from branch linkages located inside the crystalline regions, which produces an inferior crystalline structure. This inferior crystalline structure containing α(1 → 6) linked branched points and the short double helices are more susceptible to α-amylase hydrolysis, leading to 'weak points' in the 'A' type starches. These weak points are readily attacked by α-amylase. However, in 'B' type starches more branch points are clustered in the amorphous region, and furthermore there are fewer short branch chains. Consequently, the crystalline structure is superior to that of the 'A' type starches, and hence more resistant to α-amylolysis. Ratnayake et al. (2001) have shown by studies on starches from different cultivars of field peas that resistance to α-amylase increases with increase in 'B' polymorph content. Gerard, Colonna, Buleon, and Planchot (2001) have postulated that orientational distribution and packing of 'B' type crystallites within the granule could be a factor influencing resistance towards α-amylase. In addition to differences in type and proportion of polymorphic forms, other factors such as granule size (Snow & O'Dea, 1981), surface porosity (Huber & BeMiller, 1997; Kong, Kim, Kim, & Kim, 2003), extent of molecular association between starch components (Dreher, Berry, & Dreher, 1984), amylose/amylopectin ratio (Hoover & Sosulski, 1985), degree of crystallinity (Hoover & Sosulski, 1985), amylose-lipid complexes (Guraya, Kadan, & Champagne, 1997; Holm et al., 1983; Hoover & Manuel, 1995; Nebensy, Rosicka, & Tkaczyk, 2002; Seneviratne & Biliaderis, 1991; Tufvesson, Skrabanja, Björch, Liljeberg-Eelmstahl, & Eliasson, 2001) and antinutrients (Thompson & Gabon, 1987) have been shown to influence the susceptibility of starches towards α-amylase.

The reduced bioavailability of legume starches has been attributed to the presence of intact tissue/cell structures enclosing starch granules, high levels of amylose (30–65%), high content of viscous soluble dietary fiber components, presence of a large number of antinutrients, 'B' type crystallites and strong interactions between amylose chains (Deshpande & Cheryan, 1984; Hoover & Sosulski, 1985; Siddhuraju & Becker, 2001; Tovar, de Francisco, Björch, & Asp, 1991; Wursch, Del Vedovo, & Koellreuter, 1986).

Legume starches make better substrates than cereal and tuber starches for gaining a deeper insight into the structural factors that influence α-amylolysis due to the following reasons: (1) absence of pores on the granule surface (Hoover & Sosulski, 1985); (2) trace quantities (0.002–0.011%) of phosphate monoesters (Lim, Kasemsuwan, & Jane, 1994); (3) presence of only trace quantities of bound lipids (Hoover & Sosulski, 1991); and (4) uniformity in granule size (Hoover & Sosulski, 1991).

Thus, a comparative study of the susceptibility of legume starches belonging to different cultivars towards α-amylase may lead to the identification of the structural factors that limit α-amylolysis. This in turn may help us understand as to why legume starches exhibit a lower glycemic index than cereal or tuber starches (Annison & Topping, 1994; Foster-Powell & Miller, 1995; Jenkins et al., 1987; Tovar, Granfeldt, & Björch, 1992; Truswell, 1992; Urooj & Puttaraj, 1994).

The objective of this study was four fold: (1) to determine the composition and physicochemical properties of starches from cultivars of black bean, pinto bean, lentil, smooth pea and wrinkled pea; (2) to determine the susceptibility of the above starches towards α-amylase hydrolysis; (3) to determine the morphology, X-ray pattern, relative crystallinity, thermal characteristics and apparent amylose content of the residues remaining after different time periods of hydrolysis; and (4) to relate differences in the rate and extent of α-amylase hydrolysis to differences in legume starch structure and properties.

## 2. Materials and methods

### 2.1. Materials

Black bean (*Phaseolus vulgaris*) cultivars (*CDC nighthawk*, *black jack*); pinto bean (*P. vulgaris*) cultivars (*othello*, *sierra)*; lentil (*Lens culinaris*) cultivars (*CDC robin*, *CDC redwing*); smooth pea (*Pisum sativum* L.) cultivars (CDC mozart, CDC sonata) and wrinkled pea (*P. sativum* L.) were obtained from the Crop Development Center, University of Saskatchewan, Canada. Crystalline porcine pancreatic α-amylase (E.C. 3.2.2.1, type 1A) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Chemicals and solvents were of ACS-certified grade.

### 2.2. Starch isolation

Starch was extracted from legume seeds using the procedure of Hoover and Sosulski (1985).

### 2.3. Granule morphology

Granule surface was studied by scanning electron microscopy. Starch samples were mounted on circular

aluminum stubs with double sticky tape and then coated with 20 nm of gold and examined and photographed in a Hitachi scanning electron microscope (S570, Nissei Sangyo, Inc., Rexdale, ON, Canada) at an accelerating potential of 5 kV. The size and shape of native starches were examined by a Leica Gallen III microscope. The range of granule size was determined by measuring the length and width of 40 granules from a 1.0% starch suspension at 100 × measured with an eye-piece micrometer.

### 2.4. Chemical composition of starch

Quantitative estimations of moisture, ash, nitrogen and starch damage were performed by the standard AACC methods (1984). Starch lipids were determined by procedures outlined in an earlier publication (Vasanthan & Hoover, 1992).

### 2.5. Amylose content

Amylose content of native and enzyme treated residues was determined by a modification (Hoover & Ratnayake, 2001) of the method of McGrance, Cornell, and Rix (1998).

### 2.6. X-ray diffraction

#### 2.6.1. X-ray pattern and relative crystallinity

X-ray diffractograms were obtained by a Rigaku RU 200R X-ray diffractometer (Rigaku—Denki Co., Tokyo, Japan) with operating conditions as: target voltage, 40 KV; current, 100 mA; aging time, 5 min; scanning range, 3–35°; scan speed, 2.000°/min; step time, 4.5 s; divergence slit width, 1.00; scatter slit width, 1.00 and receiving slit width, 0.60.

Relative crystallinity of the starches was quantitatively estimated following the method of Nara and Komiya (1983) by using a software (Origin—version 6.0, Microcal Inc., Northampton, MA, USA). A smooth curve, which connected peak baselines was computer-plotted on the diffractogram. The area above the smooth curve was considered as the crystalline portion, and the lower area between the smooth curve and a linear baseline was taken as the amorphous portion. The ratio of the upper area to the total diffraction area was calculated as the relative crystallinity.

The moisture contents of all starch samples for X-ray diffraction were adjusted to ~19% by being kept in a desiccator over saturated $BaCl_2$ solution (25 °C, $a_w = 0.9$) for one week (Barron, Buléon, Colonna, & Valle, 2000).

#### 2.6.2. Determination of 'A' and 'B' polymorphic component

The proportion of 'A' and 'B' polymorphic component of the native and enzyme treated residues was calculated using the method outlined by Davydova et al. (1995). The 'B' polymorph content was calculated by determining the ratio of the area under the diffraction peak at 5.6° 2θ to the total crystalline area (as described above) together with a calibration curve derived from mixtures of pure 'B' type (0–100% potato) and pure 'A' type (100–0% waxy corn) starch.

### 2.7. Swelling factor (SF)

The SF of native starches when heated at 80 °C in excess water was measured according the method of Tester and Morrison (1990).

### 2.8. Amylose leaching

Native starches (20 mg, db) in water were heated at 80 °C in volume-calibrated sealed tubes for 30 min. The tubes were then cooled to ambient temperature (25–27 °C) and centrifuged at 2000 rpm for 10 min. The supernatant liquid (1 ml) was withdrawn and its amylose content determined as described by Hoover and Ratnayake (2001). Amylose leaching was expressed as percentage of amylose leached per 100 g of starch.

### 2.9. Differential scanning calorimetry (DSC)

Gelatinization parameters of native and enzyme treated residues were measured and recorded on a Seiko DSC 210 (Seiko Instruments Inc., Chiba, Japan) differential scanning calorimeter equipped with a thermal analysis data station and data recording software. Water (11 μl) was added with a microsyringe to starch (3.0 mg) in the DSC pans, which were then sealed, reweighed, and allowed to stand for 24 h before DSC analysis. The scanning temperature range and the heating rate were 20–120 °C and 10 °C/min, respectively. In all measurements, the thermogram was recorded with an empty aluminum pan as the reference. The transition temperatures reported are the onset ($T_o$), peak ($T_p$), and conclusion ($T_c$) of the gelatinization endotherm. The enthalpy of the gelatinization ($\Delta H$) was estimated by integrating the area between the thermogram and a base line connecting the points of onset and conclusion temperature, and was expressed in terms of mJ/mg starch. All DSC experiments were replicated thrice.

### 2.10. Enzymatic digestibility

Enzymatic digestibility studies on native starches were conducted using a crystalline suspension of porcine pancreatic α-amylase in 2.9 M saturated sodium chloride containing 3 mM calcium chloride (Sigma Chemical Co., St Louis, MO, USA), in which the concentration of α-amylase was 32 mg/ml and the specific activity was 1370 units/mg protein. The procedure was essentially that of Knutson, Khoo, Cluskey, and Inglett (1982). However, a higher concentration of enzyme was used in this study (12 units/mg starch). Starch granules (0.2 g, db) were suspended in distilled water (11 ml) and then 9 ml of 0.1 M phosphate

buffer (pH6.9) containing 0.006 M NaCl were added. The slurry was pre-warmed for 30 min at 37 °C and gently stirred before adding 54.7 μl α-amylase suspension. The reaction mixtures were shaken manually on a daily basis to resuspend the deposited granules. Then 1.0 ml aliquots were withdrawn at specific time intervals, pipetted into 0.2 ml of 95% ethanol, and centrifuged (2000 rpm). Aliquots of the supernatant were analyzed for reducing sugar content (Bruner, 1964). The extent of hydrolysis was calculated as the amount of maltose (mg) released per 100 mg of dry starch. Residues obtained at various time intervals of hydrolysis were washed three times with distilled water, centrifuged (2000 rpm) and freeze-dried. Controls without enzyme but subjected to the above experimental conditions were run concurrently. The experiment was replicated at least thrice.

### 2.11. Statistical analysis

All determinations were replicated three times, mean values and standard deviations were reported. Analysis of variance (ANOVA) was performed by Turkey's HSD test ($P < 0.05$) using statistical software SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

## 3. Results and discussion

### 3.1. Chemical composition of the starches

Data on the composition of the legume starches are presented in Table 1 The purity of the starches was judged on the basis of composition and microscopic examination. The ash content ranged from 0.01 to 0.04%. This low value indicated that the starches were relatively free of hydrated fine fibers, which are derived from the cell wall enclosing the starch granules. The nitrogen content (Table 1) was low in all starches (0.02–0.07%), indicating the absence of non-starch lipids (lipids associated with endosperm proteins). Therefore, the total lipid (obtained by acid hydrolysis—Table 1) in the legume starches (0.35–0.84%) mainly represent the free and bound lipids. In all starches, the bound lipid content (0.26–0.81%) was higher than the surface lipid (0.01–0.10%, Table 1). Significant differences in bound lipid content between cultivars were evident only in black bean [*black jack* (0.43%) > *CDC nighthawk* (0.26%)] and pinto bean [*othello* (0.57%) > *sierra* (0.43%)] starches. The amounts of bound lipids in wrinkled pea (0.80%) and lentil (0.72–0.81%) were higher than those in the other legume (0.26–0.48%) starches (Table 1). In all starches, there was no significant difference between the amount of lipids extracted by acid hydrolysis and that extracted by solvent extraction. Most of the data on the total lipid contents of legume starches reported in the literature have been obtained by the use of solvent systems that have been proven to be ineffective in removing bound lipids (Hoover & Sosulski, 1991). Therefore, a meaningful comparison cannot be made.

The total amylose content of legume starches has generally been reported (Hoover & Sosulski, 1991) to be in the range of 24–65%. The total amylose content of the legume starches (Table 1) ranged from 30.5% (lentil, *CDC redwing*) to 78.4% (wrinkled pea). There was no significant difference in total amylose content between cultivars of the same species (Table 1). The extent of granule damage in wrinkled pea starch (3.54%) was higher than that in other legume starches (0.22–0.43%) (Table 1). Significant differences in starch damage were observed only between cultivars of lentil [*CDC robin* (0.30) > *CDC redwing* (0.15)].

### 3.2. X-ray diffraction

The X-ray diffraction pattern, relative crystallinity and the 'B' polymorphic content of the legume starches are presented in Table 2 and Fig. 1a and b (the X-ray spectrum of smooth pea, lentil and pinto bean starches (not shown) were similar to that of black bean starch (Fig. 1a)). With the exception of wrinkled pea starch, all other starches showed the characteristic 'C' pattern of legume starches (Cheetham & Tao, 1998; Gernat et al., 1990; Hoover & Sosulski, 1985). The 'C' X-ray pattern was characterized by peaks at diffraction angles $2\theta$ of 5.6, 15, 17, 20, and 23°. The X-ray spectrum of wrinkled pea (Fig. 1b) starch was of the 'B' type, representative of high amylose maize and tuber starches, with prominent peaks at diffraction angles $2\theta$ of 5.6, 15, 17, 20, 22, and 23°. The intensity of the peak at $2\theta = 5.6°$ (characteristic of the 'B' polymorphic form) in wrinkled pea starch was higher than that in the other legume starches. However, the overall intensity of the peaks in wrinkled pea starch was much lower than those of the other legume starches (Fig. 1a). The relative crystallinity (RC) of wrinkled pea starch (17.7%) was much lower than that of other legume starches (29.9–33.4%) (Table 2). There was no significant difference in RC either among or between cultivars of black bean, pinto bean, smooth pea and lentil starches (Table 2). The lower RC of wrinkled pea starch could be attributed to its low amylopectin content (Table 1) and/or to a poorly organized crystalline structure. This explanation seems plausible, since potato starch having a higher amylopectin content (75%) than wrinkled pea starch (21.7%) also exhibits a low RC (25%) (Zobel, 1988). The 'B' polymorphic content of wrinkled pea starch (92.2%) was much higher than those of the other legume starches (27.1–33.5%) (Table 2). There was no significant difference in the 'B' polymorphic content (Table 2) between cultivars of black bean, pinto bean and smooth pea starches. However, the 'B' polymorphic content of lentil cultivars differed significantly [*CDC redwing* (36.1%) > *CDC robin* (28.1%)].

Table 1
Chemical composition (%)[1] and some of the properties of legume starches

| Characteristics | Black bean | | Pinto bean | | Lentil | | Smooth pea | | Wrinkled pea |
|---|---|---|---|---|---|---|---|---|---|
| | CDC nighthawk | Black jack | Othello | Sierra | CDC robin | CDC redwing | CDC mozart | CDC sonata | |
| Yield (% of initial seeds) | 16.37 ± 0.82$^{e}$ | 21.80 ± 1.06$^{c,d}$ | 28.25 ± 1.25$^{b}$ | 25.01 ± 1.50$^{b,c}$ | 27.44 ± 1.62$^{b}$ | 34.07 ± 2.04$^{a}$ | 19.40 ± 1.02$^{d,e}$ | 28.90 ± 2.20$^{b}$ | 21.60 ± 1.08$^{c,d}$ |
| Moisture | 10.99 ± 0.16$^{b,c}$ | 10.82 ± 0.10$^{b,c}$ | 11.38 ± 0.12$^{b}$ | 12.22 ± 0.20$^{a}$ | 8.98 ± 0.32$^{e}$ | 9.87 ± 0.18$^{d}$ | 9.47 ± 0.15$^{d,e}$ | 10.47 ± 0.25$^{c}$ | 11.76 ± 0.23$^{a,b}$ |
| Ash | 0.04 ± 0.01$^{a}$ | 0.03 ± 0.00$^{a,b}$ | 0.03 ± 0.01$^{a,b}$ | 0.02 ± 0.01$^{a,b}$ | 0.03 ± 0.01$^{a,b}$ | 0.03 ± 0.01$^{a,b}$ | 0.02 ± 0.01$^{a,b}$ | 0.02 ± 0.00$^{a,b}$ | 0.01 ± 0.00$^{b}$ |
| Nitrogen | 0.03 ± 0.01$^{b,c}$ | 0.05 ± 0.01$^{a,b,c}$ | 0.07 ± 0.02$^{a,b}$ | 0.08 ± 0.03$^{a}$ | 0.04 ± 0.02$^{a,b,c}$ | 0.05 ± 0.01$^{a,b,c}$ | 0.08 ± 0.02$^{a}$ | 0.02 ± 0.01$^{c}$ | 0.03 ± 0.01$^{b,c}$ |
| Lipid | | | | | | | | | |
| *Solvent extracted* | | | | | | | | | |
| Surface lipid[2] | 0.10 ± 0.01$^{a}$ | 0.08 ± 0.01$^{a,b}$ | 0.06 ± 0.02$^{b,c}$ | 0.04 ± 0.01$^{c,d,e}$ | 0.01 ± 0.01$^{e}$ | 0.01 ± 0.01$^{e}$ | 0.02 ± 0.01$^{d,e}$ | 0.03 ± 0.01$^{c,d,e}$ | 0.05 ± 0.01$^{b,c,d}$ |
| Bound lipid[3] | 0.26 ± 0.02$^{d}$ | 0.43 ± 0.03$^{c}$ | 0.57 ± 0.03$^{b}$ | 0.43 ± 0.02$^{c}$ | 0.81 ± 0.03$^{a}$ | 0.72 ± 0.05$^{a}$ | 0.47 ± 0.04$^{b,c}$ | 0.48 ± 0.03$^{b,c}$ | 0.80 ± 0.05$^{a}$ |
| *Acid hydrolyzed*[4] | 0.35 ± 0.02$^{e}$ | 0.52 ± 0.03$^{d}$ | 0.62 ± 0.04$^{c}$ | 0.48 ± 0.01$^{d}$ | 0.83 ± 0.02$^{a}$ | 0.71 ± 0.03$^{b}$ | 0.48 ± 0.03$^{d}$ | 0.52 ± 0.03$^{d}$ | 0.84 ± 0.02$^{a}$ |
| Amylose content | | | | | | | | | |
| Apparent amylose[5] | 35.21 ± 0.68$^{b}$ | 33.07 ± 1.19$^{b,c}$ | 28.36 ± 1.62$^{d,e}$ | 27.83 ± 0.81$^{d,e}$ | 28.78 ± 1.29$^{d,e}$ | 27.35 ± 1.70$^{e}$ | 31.04 ± 0.21$^{c,d}$ | 30.63 ± 0.31$^{c,d,e}$ | 68.84 ± 1.71$^{a}$ |
| Total amylose[6] | 39.32 ± 1.70$^{b}$ | 37.17 ± 0.68$^{b,c}$ | 31.93 ± 2.60$^{d,e}$ | 31.34 ± 0.36$^{d,e}$ | 32.29 ± 1.05$^{d,e}$ | 30.51 ± 0.63$^{e}$ | 35.09 ± 0.64$^{c,d}$ | 34.73 ± 1.09$^{c,d}$ | 78.42 ± 1.52$^{a}$ |
| Lipid-complexed amylose[7] | 10.37 ± 2.82$^{a}$ | 11.04 ± 3.14$^{a}$ | 11.18 ± 1.92$^{a}$ | 11.21 ± 1.06$^{a}$ | 10.88 ± 0.90$^{a}$ | 10.34 ± 1.74$^{a}$ | 11.54 ± 1.29$^{a}$ | 11.83 ± 1.76$^{a}$ | 12.22 ± 1.96$^{a}$ |
| Starch damage | 0.28 ± 0.03$^{c,d}$ | 0.27 ± 0.04$^{c,d}$ | 0.22 ± 0.03$^{d,e}$ | 0.24 ± 0.02$^{c,d}$ | 0.30 ± 0.01$^{c}$ | 0.15 ± 0.02$^{e}$ | 0.40 ± 0.03$^{b}$ | 0.43 ± 0.03$^{b}$ | 3.54 ± 0.02$^{a}$ |
| Granule morphology | | | | | | | | | |
| Size range (mm) | | | | | | | | | |
| Width | 10.0–32.0 | 10.0–37.5 | 10.0–29.0 | 10.0–32.0 | 8.0–28.0 | 6.0–27.0 | 8.0–32.0 | 9.0–34.0 | 5.0–34.0 |
| Length | 10.0–40.0 | 10.0–41.0 | 10.0–40.0 | 10.0–42.0 | 8.0–36.0 | 6.0–37.0 | 8.0–50.0 | 10.0–50.0 | 5.0–37.0 |
| Shape | Round to oval | Round to oval | Round to oval | Round to oval | Round to oval to irregular | Round to oval to irregular | Round to oval to irregular | Oval to irregular | Irregular to compound/rounded rosette |

[1] Data with the same superscript in the same row are not significantly different ($P < 0.05$) by Tukey's HSD test. All data reported on dry basis and represent mean ± SD of three determinations.
[2] Lipids extracted by chloroform-methanol 2:1(v/v) at 25 °C (mainly unbound lipids).
[3] Lipids extracted by hot *n*-propanol–water 3:1 (v/v) from the residue left after chloroform–methanol extraction (mainly bound lipids).
[4] Lipids obtained by acid hydrolysis (24% HCl) of the native starch (total lipids).
[5] Apparent amylose determined by iodine binding without removal of free and bound lipids.
[6] Total amylose determined by iodine binding after removal of free and bound lipids.
[7] ((Total amylose − apparent amylose)/total amylose)100.

Table 2
X-ray diffraction pattern, relative crystallinity and 'B' polymorphic content of legume starches[1]

| Starch source and cultivar | Crystalline pattern | Relative crystallinity (%)[2] | 'B' polymorphic content (%)[2] |
|---|---|---|---|
| Black bean | | | |
| CDC nighthawk | C | 32.1 ± 1.0[a] | 32.1 ± 2.4[b,c] |
| Black jack | C | 32.7 ± 2.2[a] | 33.1 ± 2.7[b,c] |
| Pinto bean | C | | |
| Othello | C | 33.4 ± 3.0[a] | 32.1 ± 2.0[b,c] |
| Sierra | C | 33.0 ± 0.6[a] | 37.5 ± 2.1[b] |
| Lentil | C | | |
| CDC robin | C | 31.7 ± 2.5[a] | 28.1 ± 1.8[c] |
| CDC redwing | C | 32.3 ± 2.2[a] | 36.1 ± 3.2[b] |
| Smooth pea | C | | |
| CDC Mozart | C | 30.0 ± 2.0[a] | 27.1 ± 2.7[c] |
| CDC sonata | C | 30.3 ± 2.4[a] | 28.8 ± 2.1[c] |
| Wrinkled pea | B | 17.7 ± 2.3[b] | 92.2 ± 3.0[a] |

[1] Moisture content of all starches ~19.0%.
[2] Mean ± SD of three determinations. Data with the same superscript within the same column are not significantly different ($P < 0.05$).

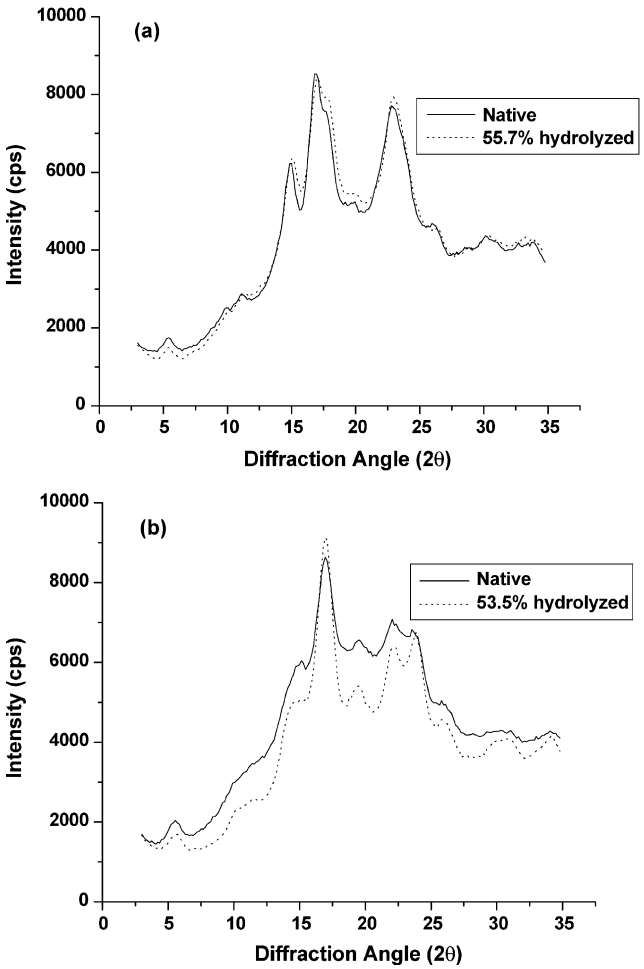


Fig. 1. X-ray diffraction patterns of native and hydrolyzed black bean (*black jack*) (Fig. 1a) and wrinkled pea starch (Fig. 1b). The X-ray pattern (native and hydrolyzed) of all other starches used in this study were similar to that of Fig. 1a.

### 3.3. Swelling factor (SF) and amylose leaching (AML) at 80 °C

The SF and AML of native legume starches are presented in Table 3 The SF ranged from 3.4 (wrinkled pea) to 18.4 (lentil—*CDC robin*). No significant difference ($P < 0.05$) in SF was observed between cultivars of pinto bean, lentil, and smooth pea. However, cultivars of black bean differed in their SF [*black jack* (17.7) > *CDC nighthawk* (8.2)]. The SF of black bean, lentil, pinto bean and smooth pea starches were generally lower than those reported for green pea (21.1), field pea (19.4), mung bean (31.9), but comparable to those of beach pea (18.4) and grass pea (13.0) (Chavan, Shahidi, Hoover, & Perera, 1999; Ratnayake et al., 2001). The extent of AML at 80 °C, ranged from 11.0 (pinto bean—*othello*) to 17.8% (smooth pea—*CDC sonata*). There was a significant difference in AML between cultivars of black bean (*black jack* > *CDC nighthawk*), pinto bean (*sierra* > *othello*) and lentil (*CDC robin* > *CDC redwing*). However, cultivars of smooth pea showed no significant difference ($P < 0.05$) in AML. The extent of AML exhibited by the legume starches (Table 3) was comparable to that reported for beach pea (9.5%), grass pea (15.1%), green pea (14.3%) but was lower than that reported for mung bean starch (Chavan et al., 1999; Hoover, Li, Hynes, & Senanayake, 1997).

SF has been shown to be influenced by: (1) amylose-lipid complexes (Manningat & Juliano, 1980; Tester & Morrison, 1990; Tester, Morrison, & Schulman, 1993); (2) amylose content (Sasaki & Matsuki, 1998); (3) extent of interaction between starch chains within the amorphous and crystalline domains of the granule (Hoover & Manuel, 1996); and (4) amylopectin molecular structure (Sasaki & Matsuki, 1998; Shi & Seib, 1992; Tester et al., 1993). The differences in SF among legume starches and between cultivars of the same species (Table 3) could be attributed to the interplay of

Table 3
Swelling factor (SF) and amylose leaching (AML) of native legume starches at 80 °C

| Starch source and cultivar | SF[1] | AML (%)[1] |
|---|---|---|
| Black bean | | |
| CDC nighthawk | 8.2 ± 1.9[b] | 13.6 ± 0.5[b] |
| Black jack | 17.7 ± 0.4[a] | 16.5 ± 0.6[a] |
| Pinto bean | | |
| Othello | 10.4 ± 0.9[b] | 11.0 ± 0.4[b] |
| Sierra | 9.9 ± 0.8[b] | 13.0 ± 0.6[b] |
| Lentil | | |
| CDC robin | 18.4 ± 0.9[a] | 17.7 ± 0.9[a] |
| CDC redwing | 16.0 ± 1.0[a] | 13.6 ± 0.3[b] |
| Smooth pea | | |
| CDC mozart | 16.2 ± 1.3[a] | 17.6 ± 0.5[a] |
| CDC sonata | 16.6 ± 0.5[a] | 17.8 ± 0.2[a] |
| Wrinkled pea | 3.4 ± 0.5[c] | 11.1 ± 0.5[c] |

[1] Mean ± SD of three determinations. Data with the same superscript within the same column are not significantly different ($P < 0.05$).

factors 2–4, since there was no significant difference in the amount of lipid-complexed amylose chains (Table 1). The lower SF (3.4) of wrinkled pea starch (Table 3) could be attributed to its lower amylopectin (17.1%) content (Table 1) and/or to strong interactions between amylose chains.

The extent of AML has been shown to be influenced by: (1) the extent of interaction between amylose chains (AM–AM) and/or between amylose and the outer branches of amylopectin (AM–AMP); and (2) the amount of lipid-complexed amylose chains (Hoover & Ratnayake, 2001; Ratnayake et al., 2001). In this study, the extent of AML is mainly influenced by starch chain (AM–AM, AM–AMP) interactions within the native granule, since differences in the amount of lipid-complexed amylose chains between and among legume cultivars were not significant (Table 1). The results (Table 3) indicate that the extent of AM–AM and AM–AMP interactions between cultivars follows the trend: *CDC nighthawk* > *black jack*; *othello* > *sierra*; *CDC redwing* > *CDC robin*; *CDC mozart* ~ *CDC sonata*. The results also indicate that the total amylose content per se does not influence AML, since wrinkled pea starch with its much higher amylose content (78.4%) exhibited nearly the same degree of AML as did pinto bean starch (32.0% amylose) (Table 1).

### 3.4. Gelatinization parameters

The gelatinization transition temperatures ($T_o$ (onset), $T_p$ (peak), $T_c$ (conclusion) and $\Delta H$ (gelatinization enthalpy)) of native starches are presented in Table 4. $T_o$, $T_p$, $T_c$ and $T_c - T_o$ of both cultivars of pinto bean were significantly ($P < 0.05$) higher than those of the other legume starches. There were significant ($P < 0.05$) differences in $T_o$, $T_p$ and $T_c$ between cultivars of black bean (*CDC nighthawk* > *black jack*), pinto bean (*othello* > *sierra*) and lentil (*CDC redwing* > *CDC robin*). Wrinkled pea starch showed no endotherm (Table 4). Significant differences in $\Delta H$ were evident only between cultivars of black bean (*CDC nighthawk* > *black jack*) and lentil (*CDC redwing* > *CDC robin*). Noda et al. (1998) demonstrated that gelatinization temperatures are influenced by the molecular architecture of the crystalline region which corresponds to the distribution of amylopectin short chains (DP6-11) and not by the proportion of crystalline region, which corresponds to the amylose/amylopectin ratio. The above authors showed by studies on 51 cultivars of sweet potato and 27 cultivars of buckwheat starches that a low $T_o$, $T_p$ and $T_c$ reflects the presence of abundant short amylopectin chains. Shi and Seib (1995) have also shown by studies on ae wx, ae du wx, wx and du wx maize starches that ae wx starch having the lowest proportion of short chains (DP6-11) exhibited the highest gelatinization temperature and enthalpy. This suggests that the higher $T_o$, $T_p$ and $T_c$ shown by black bean and pinto bean starches indicate the presence of longer amylopectin chains (Table 4). The wider $T_c - T_o$ exhibited by pinto and black bean starches (Table 4) suggests the presence of crystallites of varying stability.

Waigh et al. (2000) have postulated that two stages are involved during starch gelatinization in excess water. The first stage involves a slow side by side dissociation of helices and the second stage involves a rapid helix ⟶ coil transition. Cooke and Gidley (1992) have claimed that $\Delta H$ reflects primarily the loss of double helical order rather than loss of crystalline register. The larger $\Delta H$ values for starches of black bean and pinto bean cultivars (Table 4), suggest that interactions (via hydrogen bonding) between double helices (that are packed in clusters) forming the crystalline region of the above starches are probably more extensive (due to longer chains in amylopectin) than in smooth pea and lentil starches. Consequently, the $\Delta H$ associated with dissociation and unraveling (hydrogen bonds are broken during both stages of gelatinization) and melting of

Table 4
Gelatinization characteristics of native legume starches[1]

| Starch source and cultivar | $T_o$[2] (°C) | $T_p$ (°C) | $T_c$ (°C) | $T_c - T_o$ (°C) | H/AP[3] (mJ/mg) |
|---|---|---|---|---|---|
| Black bean | | | | | |
| Black jack | 61.0 ± 0.2$^{d}$ | 70.9 ± 0.3$^{c}$ | 81.2 ± 0.3$^{d}$ | 20.3 ± 0.5$^{b}$ | 17.8 ± 0.6$^{b,c,d}$ |
| CDC nighthawk | 65.7 ± 0.3$^{a}$ | 74.9 ± 0.4$^{b}$ | 86.7 ± 0.2$^{b}$ | 21.0 ± 0.1$^{b}$ | 20.1 ± 1.0$^{a}$ |
| Pinto bean | | | | | |
| Othello | 64.5 ± 0.2$^{b}$ | 76.5 ± 0.6$^{a}$ | 88.8 ± 0.3$^{a}$ | 24.3 ± 0.4$^{a}$ | 17.9 ± 0.3$^{b,c}$ |
| Sierra | 63.3 ± 0.2$^{c}$ | 70.9 ± 0.2$^{c}$ | 85.1 ± 0.7$^{c}$ | 21.8 ± 0.5$^{b}$ | 18.8 ± 0.1$^{a,b}$ |
| Smooth pea | | | | | |
| CDC sonata | 60.1 ± 0.2$^{e}$ | 66.0 ± 0.2$^{e}$ | 76.4 ± 0.2$^{e}$ | 16.3 ± 0.4$^{c}$ | 15.5 ± 0.5$^{e,f}$ |
| CDC Mozart | 60.0 ± 0.4$^{e}$ | 66.6 ± 0.1$^{e}$ | 77.5 ± 0.4$^{e}$ | 17.5 ± 0.7$^{c}$ | 16.6 ± 0.8$^{c,d,e}$ |
| Lentil | | | | | |
| CDC redwing | 63.9 ± 0.1$^{b,c}$ | 70.6 ± 0.1$^{c}$ | 80.1 ± 0.9$^{d}$ | 16.2 ± 1.0$^{c}$ | 16.3 ± 0.4$^{d,e}$ |
| CDC robin | 61.1 ± 0.2$^{d}$ | 67.7 ± 0.1$^{d}$ | 77.3 ± 0.3$^{e}$ | 16.2 ± 0.2$^{c}$ | 14.6 ± 0.1$^{f}$ |
| Wrinkled pea | –[4] | – | – | – | – |

[1] Mean ± SD of three determinations. Data with the same superscript in the same column are not significantly different ($P < 0.05$).
[2] $T_o$, $T_p$, $T_c$ indicate the onset, peak and conclusion temperature of gelatinization, respectively.
[3] Gelatinization enthalpy (mJ/mg)/Amylopectin content (%).
[4] Not detected within the temperature range 25–145 °C.

the double helices would be of a higher order of magnitude in pinto bean and black bean starches.

Jenkins (1994) has postulated that in excess water, gelatinization is primarily a swelling driven process. Water uptake by the amorphous background regions is accompanied by swelling within these regions. Swelling acts to destabilize the amylopectin crystallites within the crystalline lamellae, which are ripped apart. Thus, the DSC endotherm represents solvation assisted melting of amylopectin crystallites. This suggests that the absence of an endotherm for wrinkled pea starch (within the temperature range 20–145 °C) is probably due to a reduced intake of water by the amorphorus background regions of the granule (due to strong interaction between hydroxyl groups of adjacent amylose chains) and consequently, a higher thermal input (>145 °C) would be required for crystallite melting.

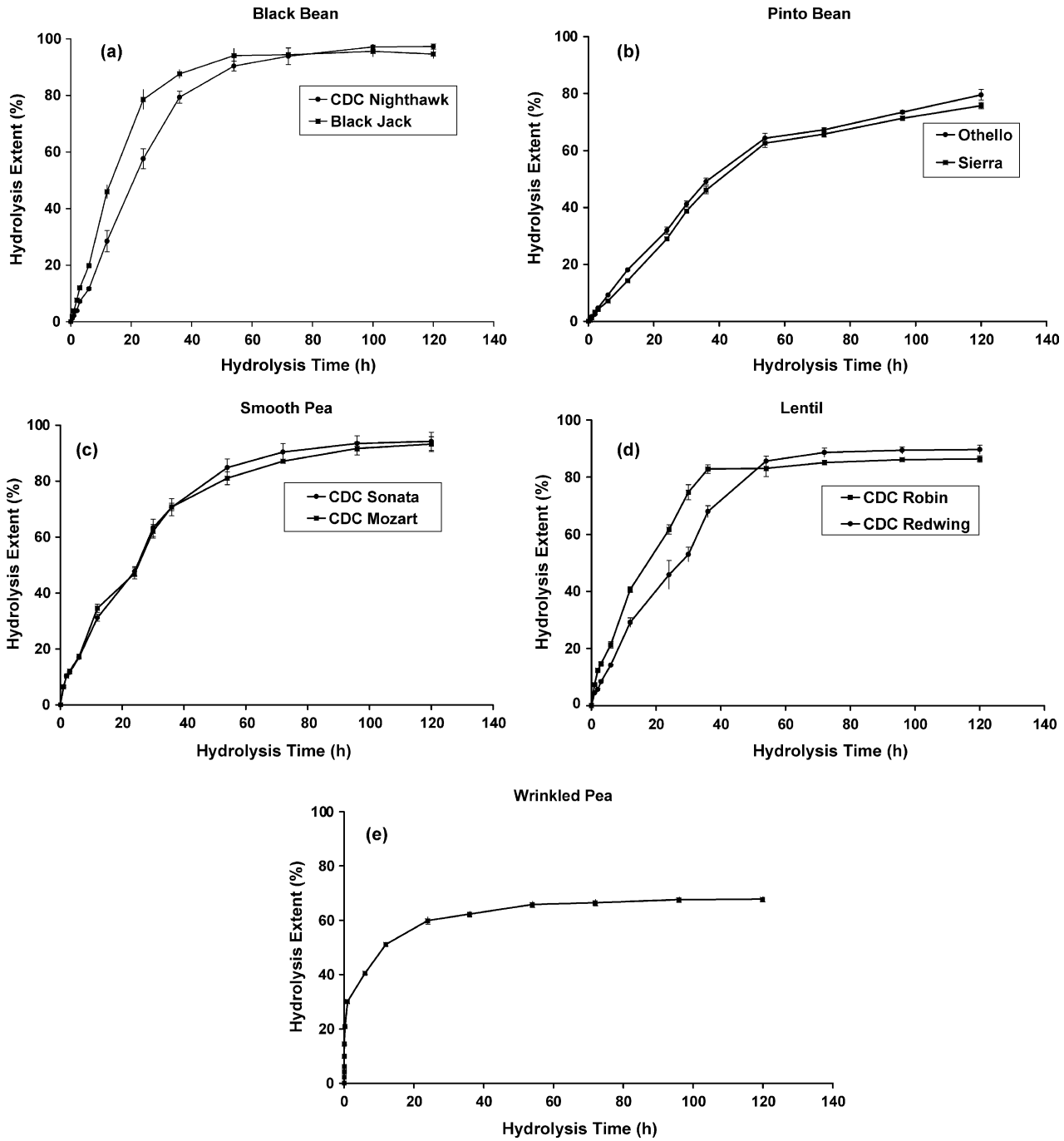


Fig. 2. Hydrolysis kinetics (37 °C) by porcine pancreatic α-amylase of legume starches: (a) black bean; (b) pinto bean; (c) smooth pea; (d) lentil; and (e) wrinkled pea.

Table 5
Initial velocity of α-amylase hydrolysis of legume starches[1]

| Starch source and cultivar | Initial velocity[2] (%/h) |
|---|---|
| Black bean | |
| Black jack | 3.9 ± 0.3[c] |
| CDC nighthawk | 2.3 ± 0.4[d,e] |
| Pinto bean | |
| Othello | 1.5 ± 0.3[e] |
| Sierra | 1.5 ± 0.2[e] |
| Smooth pea | |
| CDC sonata | 5.5 ± 0.4[b] |
| CDC mozart | 5.4 ± 0.5[b] |
| Lentil | |
| CDC robin | 5.4 ± 0.2[b] |
| CDC redwing | 2.9 ± 0.3[d] |
| Wrinkled pea | 241.6 ± 5.6[a] |

[1] Initial velocity calculation for wrinkled pea and the other legume starches are based on the data within the first 20 min and 4 h, respectively. Data represent mean ± SD of three determinations.
[2] Data with the same superscript in the column are not significantly different ($P < 0.05$).

### 3.5. Hydrolysis kinetics

The hydrolysis by porcine pancreatic α-amylase in black bean (Fig. 2a), lentil (Fig. 2d) and wrinkled pea (Fig. 2e) starches was biphasic, a relatively rapid rate at the initial stage followed by a progressively decreased rate thereafter (Fig. 2a, d and e). However, in pinto bean (Fig. 2b) and smooth pea (Fig. 2c) starches, the decrease in the rate of hydrolysis, following the initial rapid increase was much less than in the other starches (Fig. 2a, d and e). The hydrolysis curves of black bean cultivars (Fig. 2a), smooth pea (Fig. 2c), lentil cultivars (Fig. 2d), and wrinkled pea (Fig. 2e) showed a plateau at hydrolysis levels of 93 (Fig. 2a), 91 (Fig. 2c), 85 (Fig. 2d) and 65% (Fig. 2e), respectively. The time for the appearance of the above plateau was identical for black bean (55 h) and smooth pea (100 h) cultivars, but was different for cultivars of lentil [*CDC robin* (35 h), *CDC redwing* (55 h)]. Cultivars of pinto bean (Fig. 2b) starches did not exhibit a plateau during the time course of hydrolysis. Wrinkled pea

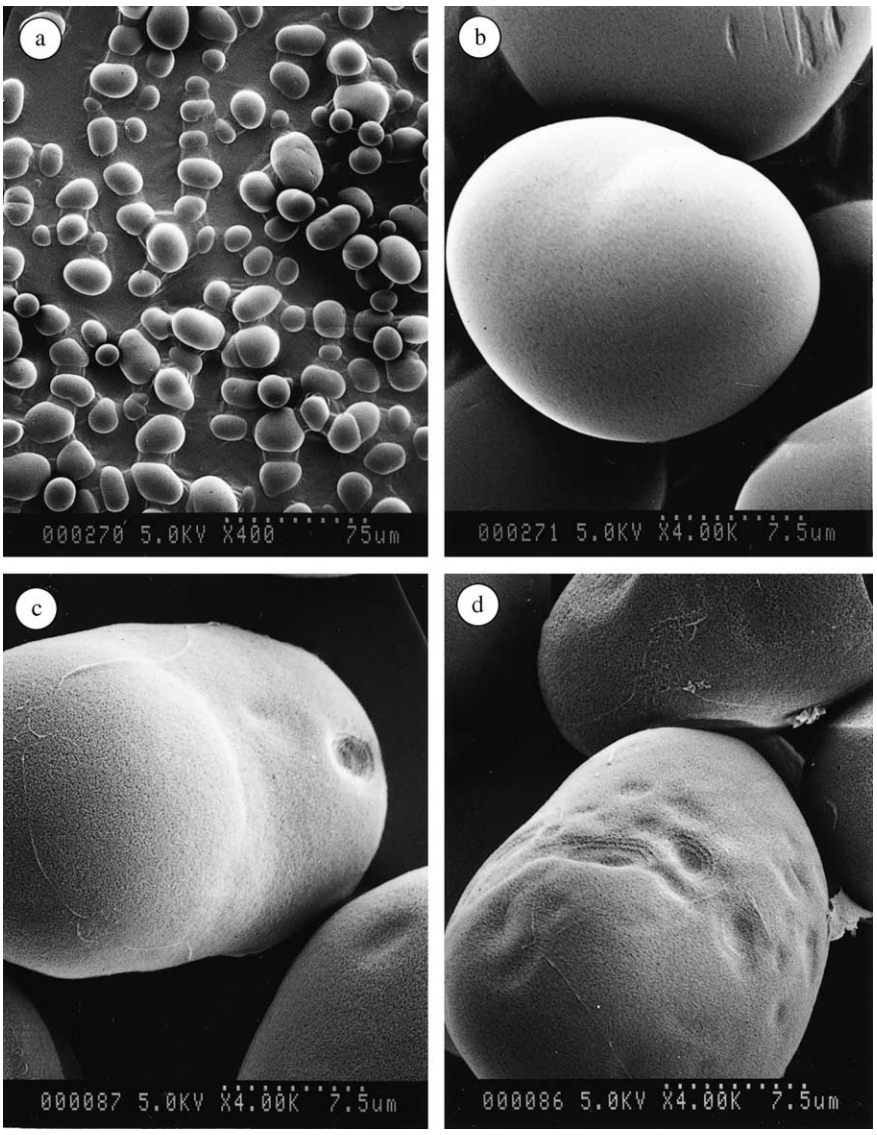


Fig. 3. Scanning electron micrographs of native black bean (*black jack*) granules (a and b) and hydrolyzed (15.4%) granules (c and d).

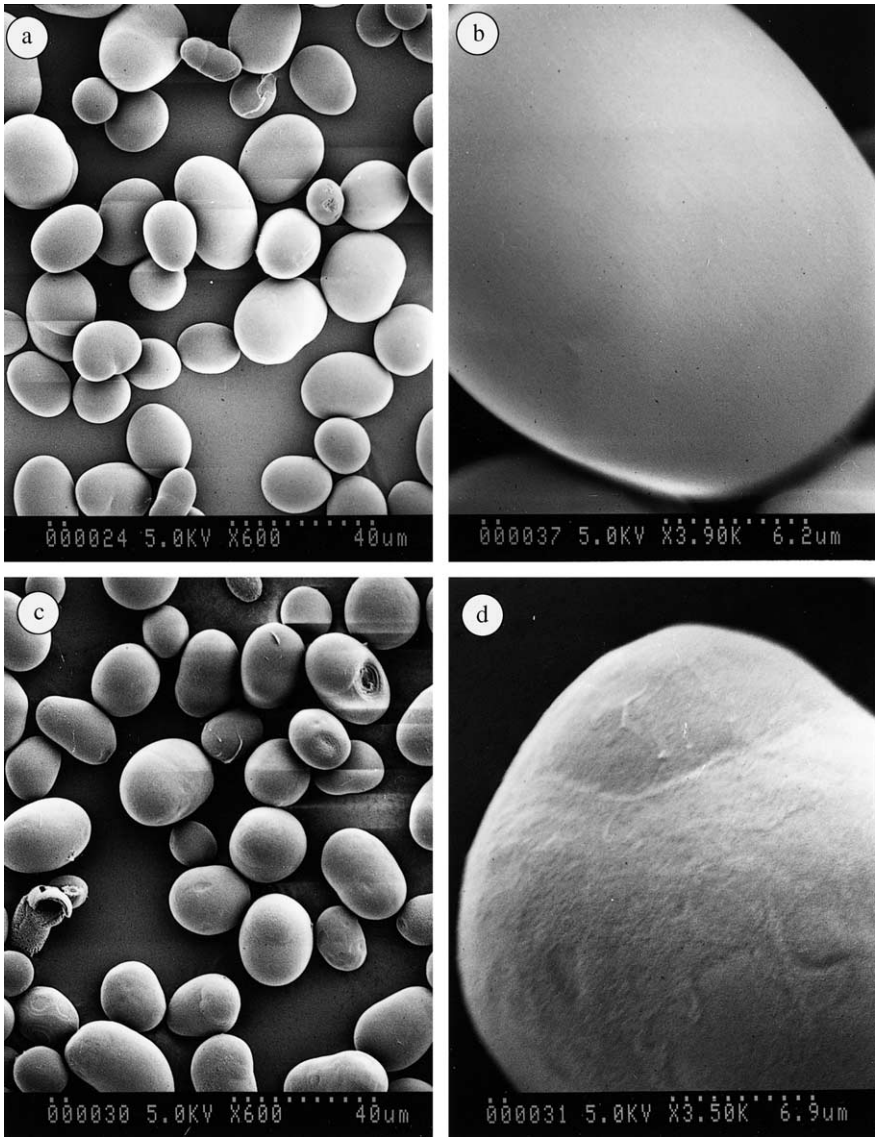


Fig. 4. Scanning electron micrographs of native pinto bean (*othello*) granules (a and b) and hydrolyzed (18.1%) granules (c and d).

starch exhibited a higher initial velocity (241.6%/h) than the other legume starches (1.4–5.5%/h) (Table 5). Difference in initial velocity between cultivars was evident only in black bean [*black jack* (3.9%/h) > *CDC nighthawk* (2.3%/h)] and lentil [*CDC robin* (5.4%/h) > *CDC redwing* (2.9%/h)]. During the initial rapid phase of hydrolysis, cultivars of black bean (Fig. 2a) and lentil (Fig. 2d) were hydrolyzed to different extents. This difference was most marked between the 5th and 40th hour of hydrolysis in both black bean (*black jack* > *CDC nighthawk*) and lentil (*CDC robin* > *CDC redwing*) cultivars. However, during the above time period, there was no significant difference ($P < 0.05$) in the extent of hydrolysis between cultivars of pinto bean (Fig. 2b) and smooth pea (Fig. 2c) starches. After 120 h, there was no difference in the extent of hydrolysis between cultivars of each legume species (Fig. 2). At the end of this time period, the extent of hydrolysis among the legume starches followed the order: black bean > lentil > smooth pea > pinto bean > wrinkled pea (Fig. 2).

It is appropriate at this stage to give a brief description of the mechanism of α-amylase action, which would then enable a subsequent discussion of the hydrolysis kinetics of the legume starches.

Porcine pancreatic α-amylase (PPA) has been shown to have five binding sites with the catalytic site located between subsites 2 and 3, with two subsites to the right and three subsites to the left of the catalytic site (Robyt & French, 1970). These authors have shown that only the chain to the right diffuses away after the initial cleavage and the remaining chain to the left diffuses to fill the open binding subsites to give maltose (G2), maltotriose (G3) and maltotetraose (G4) as products in a multiple attack mechanism. The products of hydrolysis particularly G2 and G3 are known to have an inhibitory effect on the action

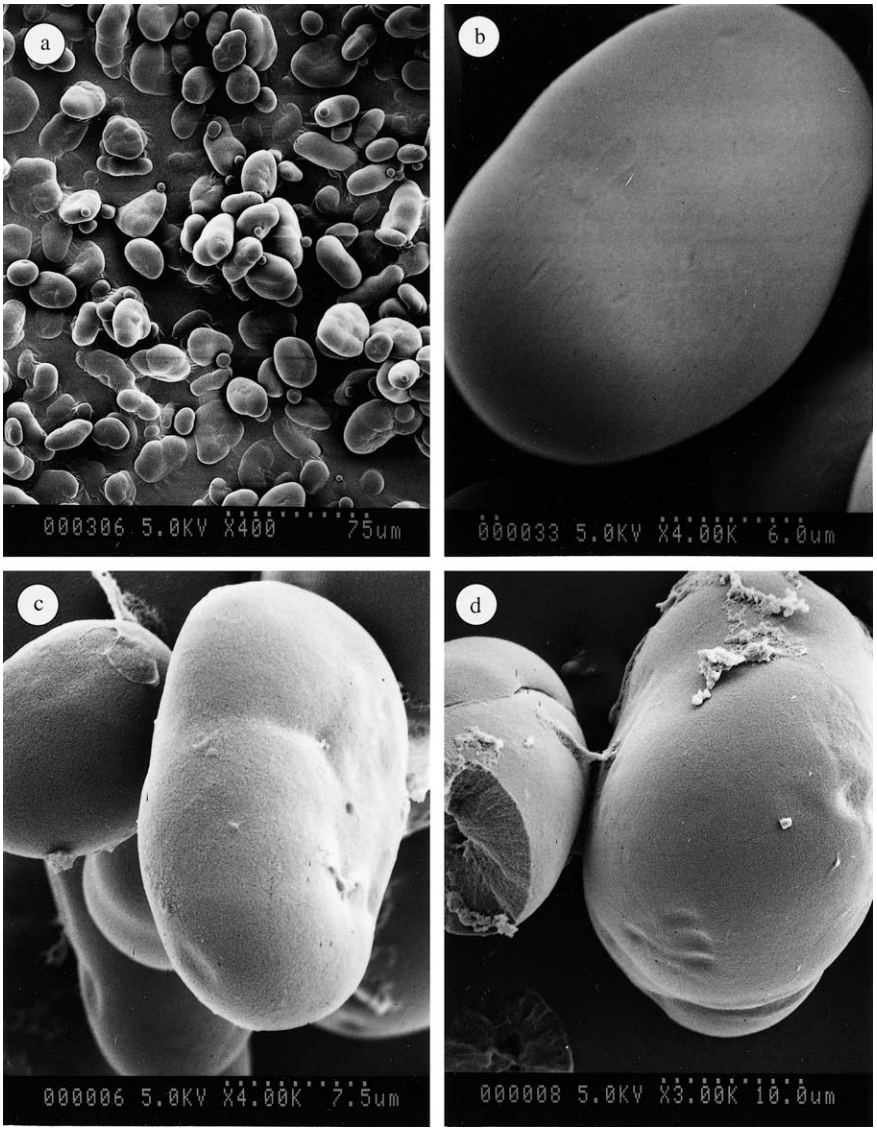


Fig. 5. Scanning electron micrographs of native smooth pea (*CDC sonata*) granules (a and b) and hydrolyzed (17.0%) granules (c and d).

of α-amylase in vitro (Elodi, Mora, & Krysteva, 1972; Leloup, Colonna, & Ring, 1991; Robyt & French, 1970). G2 and G3 have been shown to bind strongly to PPA, thereby impeding their adsorption onto crystalline spherulites of short chain amylose (Leloup et al., 1991).

The appearance of a plateau during hydrolysis of black bean (Fig. 2a), smooth pea (Fig. 2c), lentil (Fig. 2d), and wrinkled pea (Fig. 2e) starches reflects the interplay of the following factors: (1) inhibition of α-amylase activity by G2 and G3 (the occupation of the subsites to the left of the catalytic center by G2 and G3 would prevent further hydrolysis of starch chains) and (2) formation of crystalline regions during hydrolysis (hydrolyzed amylose chains may retrograde forming crystalline regions which could hinder the accessibility of α-amylase to the glucosidic bond). The absence of a plateau in pinto bean starch (Fig. 2b), even after 120 h of hydrolysis, suggests strong interactions between starch chains within the amorphous and crystalline domains of the native granule. These interactions probably reduce the degree of accessibility of the glucosidic bonds to α-amylase, thereby decreasing the rate of release of G2 and G3 during hydrolysis. Thus, the time taken for α-amylase inhibition by G2 and G3 would be much longer in pinto bean than in other legume starches.

Jenkins and Donald (1995) have postulated that co-crystallization of amylose with amylopectin disrupts amylopectin crystallites. Their postulation was based on the observation that the electron density difference between the crystalline and amorphous lamella decreased with increase in amylose content. Cheetham and Tao (1998) have shown by X-ray diffraction studies on native maize starches of varying amylose content (0–84%) that crystallinity decreases with increased amylose content in both 'A' and 'C' type starches. This suggests that the low RC (17.7%) of wrinkled pea starch (Table 2) is probably due to its lower amylopectin content and/or to disrupted

amylopectin crystallites. The extent of this disruption is likely to be higher in wrinkled pea starch than in the other legume starches, due to its higher amylose content (78.1%) (Table 1) and longer amylopectin chain length (CL 32–45 vs. CL 24–27 in the other legume starches (Biliaderis, Grant, & Vose, 1981; Colonna, Buléon, LeMaguer, & Mercier, 1982; Colonna & Mercier, 1984; Hoover & Sosulski, 1991; Ratnayake, Hoover, & Warkentin, 2002)). Thus, the higher initial velocity (Table 5) exhibited by wrinkled pea starch could be due to the interplay of the following factors: (1) lower amylopectin content; (2) disrupted crystalline structure; and (3) higher extent of starch damage during starch isolation (Table 1).

Tester and Sommerville (2000) have postulated that granular swelling is controlled by granule order which controls α-amylolysis. The differences between black bean cultivars with respect to SF (*black jack* > *CDC nighthawk*) and AML (*black jack* > *CDC nighthawk*) at 80 °C (Table 3) suggest that starch chain interactions (amylose–amylose, amylopectin–amylopectin, amylose–amylopecin) within native starch granules are of a higher order of magnitude in *CDC nighthawk*. Strong interactions between starch chains would reduce granular swelling at 37 °C (assay temperature). This would then explain the initial velocity difference between the black bean cultivars (*black jack* > *CDC nighthawk*) (Table 5). The difference in initial velocity between cultivars of lentil (*CDC robin* > *CDC redwing*) can be attributed to a lower degree of interaction between starch chains in *CDC robin* (indicated by a higher SF and a higher degree of AML (Table 3)). The marginal difference in SF and AML between cultivars of pinto bean and smooth pea (Table 3) may explain their nearly identical initial velocities (Table 5).

The above explanation based on SF and AML seems plausible, since differences between cultivars with respect to granule size (Table 1), amylose content (Table 1), starch

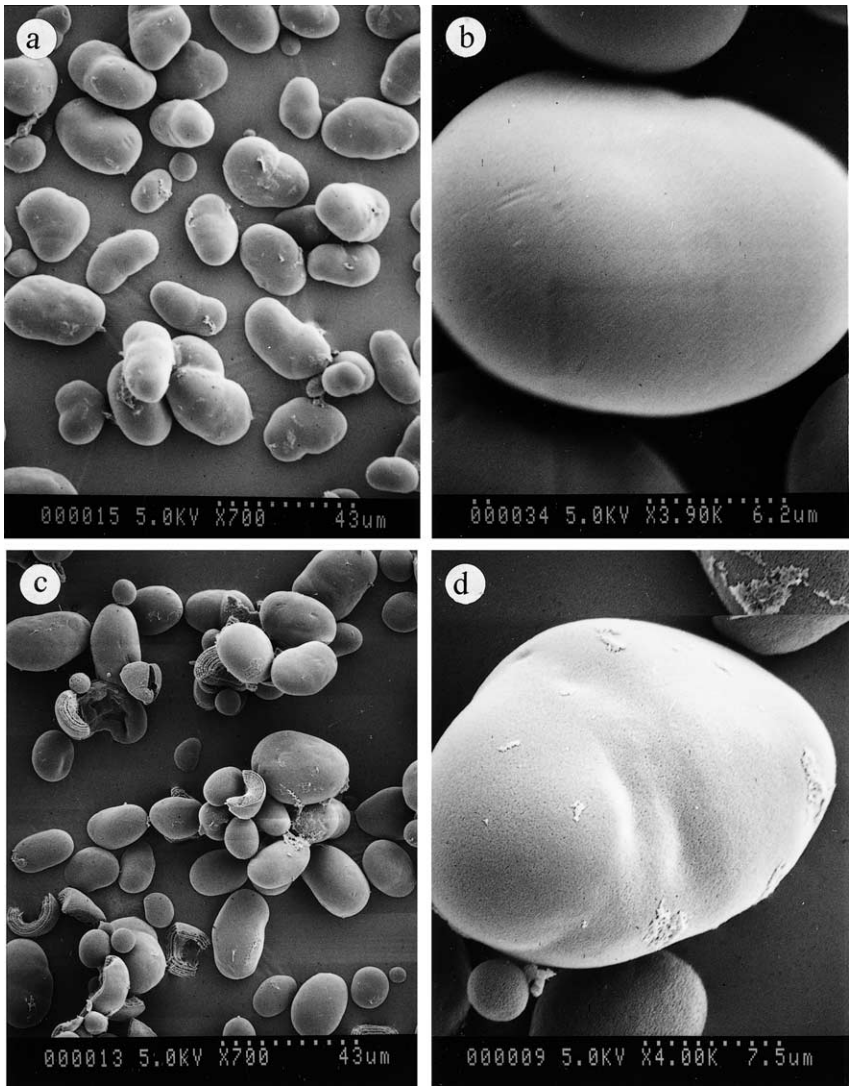


Fig. 6. Scanning electron micrographs of native lentil (*CDC redwing*) granules (a and b) and hydrolyzed (14.2%) granules (c and d).

damage (Table 1), lipid-complexed amylose chains (Table 1), relative crystallinity (Table 2) and 'B' polymorph content (Table 2) are too small to account for the large difference in initial velocity between cultivars of black bean and lentil.

### 3.6. Morphology of native and hydrolyzed starch residues

The morphologies of native legume starches and their hydrolyzed residues (at nearly equivalent levels of hydrolysis) are presented in Figs. 3–7. The granules of native black bean (Fig. 3a and b), pinto bean (Fig. 4a and b), smooth pea (Fig. 5a and b) and lentil (Fig. 6a and b) ranged from oval to irregular in shape. The width and length of the granules were within the range of 5.0–37.5 and 5.0–50 μm, respectively (Table 1). Wrinkled pea starch appeared to be a mixture of simple and compound granules (Fig. 7a–c). Many of the compound granules contained clusters (3–5) of individual granules. Many of the simple granules (mainly small granules) were round in shape; whereas large granules (forming the cluster) were irregular in shape. The width and length of small and large granules ranged from 5.0 to 34.0 and 5.0 to 37.0 μm, respectively (Table 1). In native wrinkled pea starch, some of the larger granules showed extensive damage resulting in splitting and exposure of the internal layering (Fig. 7b). A similar observation was reported by Bertoft, Manelius, and Quin (1993). The granule surfaces of native pinto bean (Fig. 4a and b), smooth pea (Fig. 5a and b) and lentil (Fig. 6a and b) starches were smooth and showed no evidence of pores, fissures or indentations. However, in black bean (Fig. 3a and b) and wrinkled pea (Fig. 7a–c) starches, indentations were present on the surface of some granules, whereas others were smooth

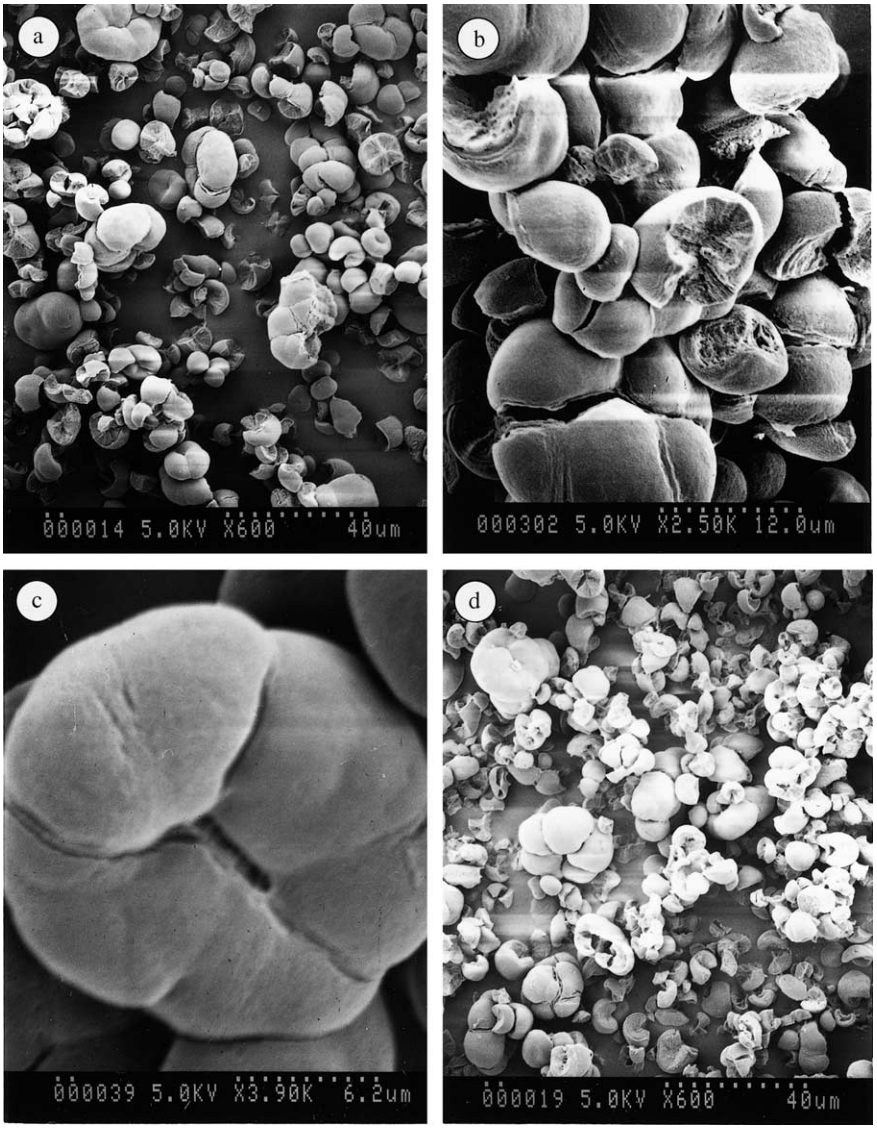


Fig. 7. Scanning electron micrographs of native (a–c) wrinkled pea starch and hydrolyzed (16.2%) granules (d).

and free of pores, fissures and indentations. There was no difference in granule morphology between cultivars of the same legume species.

The mode of α-amylase attack was examined by SEM during the early stages of hydrolysis (<20%), and at nearly equivalent levels of hydrolysis. Hydrolyzed (15.4%) black bean starch (*black jack*) showed slightly roughened surfaces and disc like depressions and the number of depressions varied from granule to granule (Fig. 3c and d). Roughened surfaces and disc like depressions were also visible on the surfaces of hydrolyzed (18.1%) pinto bean (*othello*) starch. However, the depth of these depressions (Fig. 4d) were much lower than in black bean (Fig. 3d) starch. In hydrolyzed (17%) smooth pea starch (*CDC sonata*), some granules (~ < 1% of the total population) had fragmented so that their interior parts were exposed (Fig. 5d), whereas the major population of the granules was intact and exhibited only roughened surfaces and disc like depressions (Fig. 5c and d). Lentil starch (*CDC robin*) at 14.2% hydrolysis behaved similarly to smooth pea starch with respect to roughened surfaces and granule fragmentation (<1% of the granule population). However, none of the hydrolyzed granules showed disc like depressions on their surfaces. In hydrolyzed (16.2%) wrinkled pea starch (Fig. 7d), several of the large granules had fragmented, exposing their interior structure. However, some granules were still intact with no evidence of α-amylase attack. Furthermore, the extent of fragmentation in wrinkled pea starch was much higher than that in smooth pea and lentil starches. A similar pattern of hydrolysis was observed by Bertoft et al. (1993) on wrinkled pea starch hydrolyzed by *Bacillus amyloliquefaciens*.

### 3.7. X-ray analysis of hydrolyzed residues

The X-ray patterns of native and control (treated without α-amylase) and the hydrolyzed residues of the legume starches are presented in Fig. 1a and b and Table 6. There was no significant difference in the X-ray pattern, relative crystallinity or 'B' polymorphic content between native and control starches (Table 6). Hydrolysis did not change the X-ray pattern, relative crystallinity or the 'B' polymorphic content of black bean (*black jack*), pinto bean (*othello*), smooth pea (*CDC sonata*), and lentil (*CDC robin*) starches (Table 6). This was also true for the other cultivars of the above starches (data and figure not shown). The hydrolyzed residues of wrinkled pea starch also showed an unchanged X-ray pattern (Fig. 1b) and 'B' polymorphic content (Table 6). However, relative crystallinity increased (17.8–33.4%) substantially on hydrolysis (Table 6).

Several researchers have shown that α-amylases can simultaneously solubilize both amorphous and crystalline regions of starch granules (Colonna, Buléon, & Lemarié, 1988; Lauro, Forssell, Suortti, Hulleman, & Poutanen, 1999; Leach & Schoch, 1961). This was based on the observation that α-amylolysis did not produce an increase in crystallinity. However, crystallinity and gelatinization enthalpy of barley starches have been shown to decrease during the later stages of α-amylolysis (Lauro et al., 1999). This suggests that extensive hydrolysis effectively destroys and solubilizes the crystalline areas of the granule. However, the exact mechanism by which starch crystallites are degraded by α-amylase remains controversial. Comparison of our X-ray data with that observed for barley starches (Lauro et al., 1999), suggests that crystallites of legume starches are more resistant to α-amylolysis than the 'A' type crystallites of barley starch. This is based on the observation that even at 55% hydrolysis, the relative crystallinity of black bean starch remained unchanged (Table 6). The increase in relative crystallinity on hydrolysis of wrinkled pea starch could be attributed to extensive degradation of the amorphous regions of the granule.

Table 6
X-ray diffraction parameters of α-amylase hydrolyzed legume starch residues

| Starch source and cultivar | Crystalline pattern | Relative crystallinity (%)[1] | 'B' Polymorphic content (%)[1] |
|---|---|---|---|
| *Black bean* | | | |
| Black jack | | | |
| Native | C | 32.7 ± 2.2[a] | 33.1 ± 2.7[f] |
| Control[2] | C | 32.3 ± 2.2[a] | 33.8 ± 2.8[f] |
| Hydrolyzed (55.7%) | C | 28.7 ± 2.5[a] | 31.7 ± 2.0[f] |
| *Pinto bean* | | | |
| Othello | | | |
| Native | C | 33.4 ± 3.0[b] | 32.1 ± 2.0[g] |
| Control[2] | C | 32.9 ± 2.6[b] | 32.4 ± 1.2[g] |
| Hydrolyzed (45.6%) | C | 29.4 ± 2.0[b] | 33.4 ± 1.9[g] |
| *Smooth pea* | | | |
| CDC sonata | | | |
| Native | C | 30.3 ± 2.4[c] | 28.8 ± 2.1[h] |
| Control[2] | C | 30.0 ± 1.7[c] | 28.0 ± 1.7[h] |
| Hydrolyzed (41.6%) | C | 29.0 ± 1.7[c] | 27.1 ± 2.6 + |
| *Lentil* | | | |
| CDC robin | | | |
| Native | C | 31.7 ± 2.5[d] | 28.1 ± 1.8[i] |
| Control[2] | C | 31.0 ± 2.0[d] | 28.1 ± 2.1[i] |
| Hydrolyzed (48.9%) | C | 28.2 ± 2.1[d] | 27.8 ± 2.8[i] |
| *Wrinkled pea* | | | |
| Native | B | 17.7 ± 2.3[e] | 92.2 ± 3.0[j] |
| Control[2] | B | 17.8 ± 1.7[e] | 91.3 ± 2.3[j] |
| Hydrolyzed (53.5%) | B | 33.4 ± 3.1[e] | 89.8 ± 2.2[j] |

[1] Mean ± SD. For each cultivar, data with the same superscript in the same column are not significantly different ($P < 0.05$).
[2] Treated without α-amylase but sujected to the same experimental conditions.

### 3.8. Apparent amylose content of hydrolyzed starches

The changes in apparent amylose content (AAC) at different time periods of hydrolysis are presented in Fig. 8. In all starches, AAC decreased with hydrolysis time,

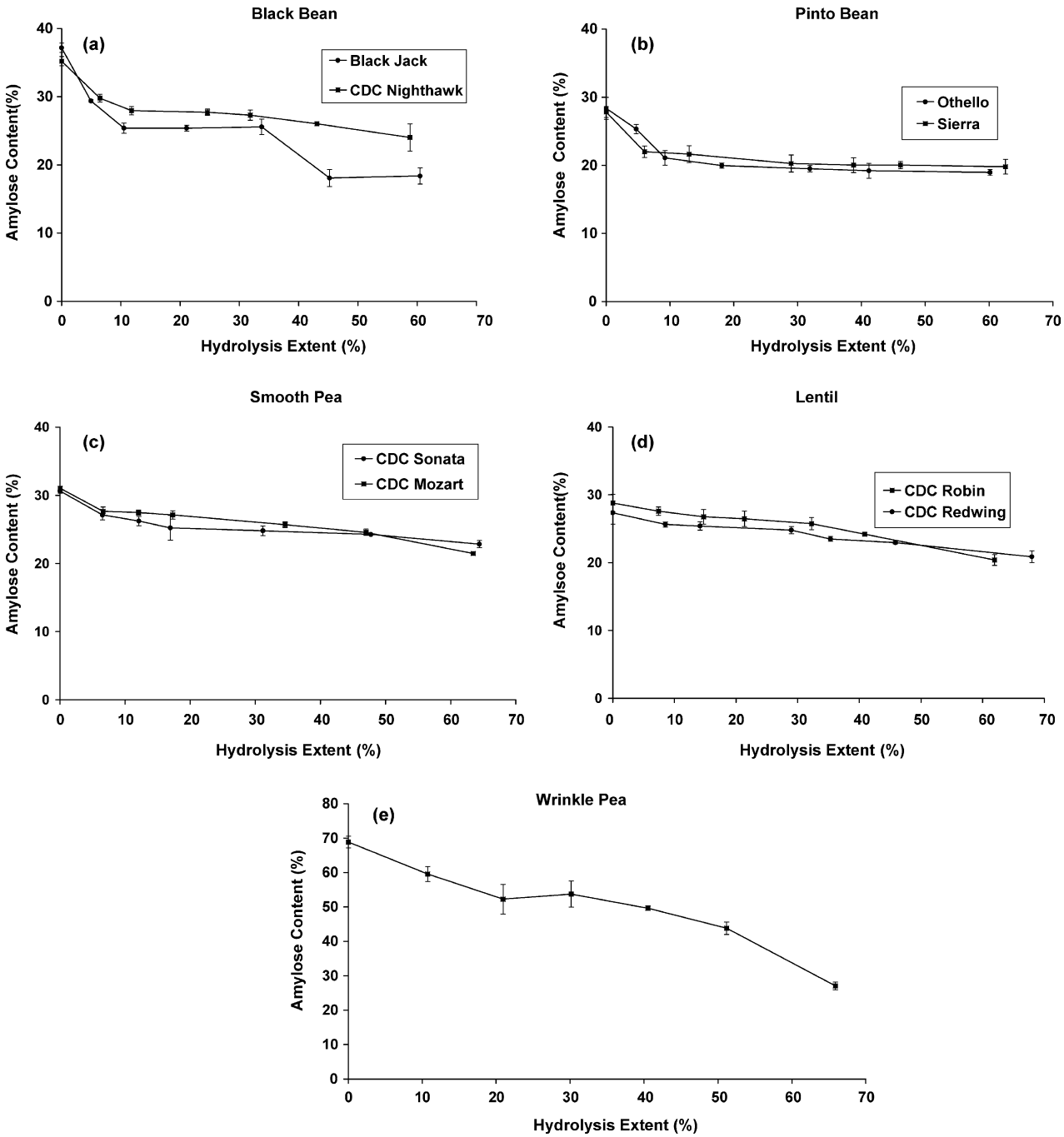


Fig. 8. Apparent amylose content of legume starches at different time periods of α-amylase hydrolysis.

the extent of this decrease was most pronounced in wrinkled pea starch. At 60% hydrolysis, AAC decreased from 68.5 to 28.0% for wrinkled pea starch. For the other legume starches, the decrease in AAC at 60% hydrolysis ranged from 9 to 21% (Fig. 8). There was no significant difference in the extent of decrease in AAC between cultivars of pinto bean, smooth pea and lentil starches. However, in black bean starches, the decrease in AAC was higher in *black jack* (21%) than in *CDC nighthawk* (12.0%).

As discussed earlier, disruption of amylopectin crystallites by amylose was most pronounced in wrinkled pea starch. Consequently, the degree of accessibility of α-amylase to amylose chains within the amorphous domains of the granule would be of a very high order of magnitude in wrinkled pea starch. This would explain the rapid and large decrease in AAC content upon hydrolysis (Fig. 8e). Amylose leaching measurements (Table 3) showed that among legume starches, differences in the magnitude of starch chain interactions (AM–AM and/or AM–AMP) were more pronounced between cultivars of black bean (*CDC nighthawk* > *black jack*). This suggests that the differences in the extent of decrease in AAC

Table 7
DSC parameters[1] of α-amylase hydrolyzed legume starch residues

| Starch source and cultivar | | $T_o$[2] (°C) | $T_p$ (°C) | $T_c$ (°C) | $T_c - T_o$ (°C) | $H$[3] (mJ/mg) |
|---|---|---|---|---|---|---|
| *Black bean* | | | | | | |
| Black jack | | | | | | |
| Native | | 61.0 ± 0.2$^{b}$ | 70.9 ± 0.3$^{a}$ | 81.2 ± 0.3$^{b}$ | 20.3 ± 0.5$^{a,b}$ | 11.2 ± 0.1$^{a}$ |
| Control[4] | | 60.7 ± 0.3$^{b}$ | 71.3 ± 0.3$^{a}$ | 81.4 ± 0.4$^{b}$ | 20.8 ± 0.5$^{a}$ | 11.4 ± 0.2$^{a}$ |
| Hydrolysed | 28.3% | 62.6 ± 0.2$^{a}$ | 70.8 ± 0.2$^{a,b}$ | 82.5 ± 0.4$^{a}$ | 19.9 ± 0.4$^{a,b}$ | 10.2 ± 0.2$^{b}$ |
| | 55.7% | 63.5 ± 0.6$^{a}$ | 70.2 ± 0.1$^{b}$ | 82.5 ± 0.3$^{a}$ | 19.0 ± 0.7$^{b}$ | 10.0 ± 0.3$^{b}$ |
| CDC nighthawk | | | | | | |
| Native | | 65.7 ± 0.3$^{e}$ | 74.9 ± 0.4$^{e}$ | 86.7 ± 0.2$^{e}$ | 21.0 ± 0.1$^{e}$ | 12.2 ± 0.6$^{e}$ |
| Control[4] | | 65.6 ± 0.5$^{e}$ | 75.0 ± 0.3$^{e}$ | 86.3 ± 0.4$^{e}$ | 20.6 ± 0.4$^{e}$ | 12.1 ± 0.3$^{e}$ |
| Hydrolyzed | 24.6% | 66.5 ± 0.4$^{e,f}$ | 75.6 ± 0.1$^{e}$ | 86.7 ± 0.1$^{e}$ | 20.1 ± 0.5$^{e}$ | 10.3 ± 0.7$^{f}$ |
| | 58.8% | 67.2 ± 0.6$^{f}$ | 75.4 ± 0.5$^{e}$ | 88.2 ± 0.4$^{f}$ | 21.0 ± 0.6$^{e}$ | 11.0 ± 0.5$^{e,f}$ |
| *Pinto bean* | | | | | | |
| Othello | | | | | | |
| Native | | 64.5 ± 0.2$^{g}$ | 76.5 ± 0.6$^{g}$ | 88.8 ± 0.3$^{g}$ | 24.3 ± 0.4$^{g}$ | 12.2 ± 0.2$^{g}$ |
| Control[4] | | 64.5 ± 0.4$^{g}$ | 76.5 ± 0.3$^{g}$ | 88.8 ± 0.4$^{g}$ | 24.3 ± 0.6$^{g}$ | 12.4 ± 0.2$^{g}$ |
| Hydrolyzed | 18.1% | 66.5 ± 0.3$^{h}$ | 77.1 ± 0.2$^{g}$ | 89.9 ± 0.2$^{h}$ | 23.4 ± 0.3$^{g}$ | 10.7 ± 0.3$^{h}$ |
| | 41.2% | 66.9 ± 0.3$^{h}$ | 77.0 ± 0.2$^{g}$ | 90.2 ± 0.2$^{h}$ | 23.4 ± 0.2$^{g}$ | 10.2 ± 0.4$^{h}$ |
| Sierra | | | | | | |
| Native | | 63.3 ± 0.2$^{l}$ | 70.9 ± 0.2$^{l}$ | 85.1 ± 0.7$^{l}$ | 21.8 ± 0.5$^{l}$ | 12.9 ± 0.1$^{l}$ |
| Control[4] | | 63.5 ± 0.4$^{l}$ | 70.8 ± 0.4$^{l}$ | 85.1 ± 0.2$^{l}$ | 21.6 ± 0.4$^{l,m}$ | 12.9 ± 0.1$^{l}$ |
| Hydrolyzed | 29.0% | 64.3 ± 0.2$^{m}$ | 70.7 ± 0.1$^{l}$ | 85.7 ± 0.6$^{l}$ | 21.5 ± 0.8$^{l,m}$ | 10.6 ± 0.2$^{m}$ |
| | 46.1% | 65.6 ± 0.2$^{n}$ | 71.1 ± 0.2$^{l}$ | 86.0 ± 0.3$^{l}$ | 20.4 ± 0.3$^{m}$ | 10.9 ± 0.4$^{m}$ |
| *Smooth pea* | | | | | | |
| CDC sonata | | | | | | |
| Native | | 60.1 ± 0.2$^{q}$ | 66.0 ± 0.2$^{q}$ | 76.4 ± 0.2$^{q}$ | 16.3 ± 0.4$^{q}$ | 10.1 ± 0.3$^{q}$ |
| Control[4] | | 60.2 ± 0.4$^{q}$ | 66.0 ± 0.2$^{q}$ | 76.3 ± 0.3$^{q}$ | 16.2 ± 0.6$^{q}$ | 10.1 ± 0.3$^{q}$ |
| Hydrolyzed | 17.0% | 58.2 ± 0.4$^{r}$ | 65.8 ± 0.2$^{q}$ | 76.7 ± 0.1$^{q}$ | 18.5 ± 0.4$^{r}$ | 8.5 ± 0.8$^{r}$ |
| | 47.8% | 60.2 ± 0.5$^{q}$ | 67.1 ± 0.6$^{r}$ | 78.4 ± 0.2$^{r}$ | 18.2 ± 0.6$^{r}$ | 8.8 ± 0.2$^{r}$ |
| CDC Mozart | | | | | | |
| Native | | 60.0 ± 0.4$^{s}$ | 66.6 ± 0.1$^{s}$ | 77.5 ± 0.4$^{s}$ | 17.5 ± 0.7$^{s}$ | 10.8 ± 0.5$^{s}$ |
| Control[4] | | 60.2 ± 0.5$^{s}$ | 66.7 ± 0.2$^{s}$ | 77.3 ± 0.3$^{s}$ | 17.2 ± 0.6$^{s}$ | 11.0 ± 0.3$^{s}$ |
| Hydrolyzed | 17.3% | 59.4 ± 0.3$^{s}$ | 67.2 ± 0.3$^{s}$ | 77.6 ± 0.3$^{s}$ | 18.2 ± 0.5$^{s}$ | 9.2 ± 0.3$^{t}$ |
| | 47.0% | 61.2 ± 0.1$^{t}$ | 68.5 ± 0.3$^{t}$ | 80.9 ± 0.4$^{t}$ | 19.7 ± 0.3$^{t}$ | 10.1 ± 0.4$^{s,t}$ |
| *Lentil* | | | | | | |
| CDC redwing | | | | | | |
| Native | | 63.9 ± 0.1$^{u}$ | 70.6 ± 0.1$^{u}$ | 80.1 ± 0.9$^{u}$ | 16.2 ± 1.0$^{u}$ | 11.3 ± 0.3$^{u}$ |
| Control[4] | | 63.9 ± 0.2$^{u}$ | 70.5 ± 0.3$^{u}$ | 80.6 ± 0.5$^{u}$ | 16.7 ± 0.5$^{u}$ | 11.2 ± 0.2$^{u}$ |
| Hydrolyzed | 29.0% | 64.2 ± 0.4$^{u}$ | 71.3 ± 0.2$^{v}$ | 79.8 ± 0.3$^{u}$ | 15.6 ± 0.1$^{u}$ | 9.6 ± 0.1$^{v}$ |
| | 52.6% | 64.3 ± 0.3$^{u}$ | 71.8 ± 0.1$^{v}$ | 81.1 ± 0.3$^{u}$ | 16.9 ± 0.1$^{u}$ | 9.6 ± 0.5$^{v}$ |
| CDC robin | | | | | | |
| Native | | 61.1 ± 0.2$^{w,x}$ | 67.7 ± 0.1$^{w}$ | 77.3 ± 0.3$^{w}$ | 16.2 ± 0.2$^{w}$ | 9.9 ± 0.1$^{w}$ |
| Control[4] | | 60.9 ± 0.4$^{w}$ | 67.6 ± 0.3$^{w}$ | 77.1 ± 0.4$^{w}$ | 16.2 ± 0.5$^{w}$ | 9.9 ± 0.2$^{w}$ |
| Hydrolyzed | 21.4% | 61.7 ± 0.3$^{x}$ | 68.3 ± 0.2$^{x}$ | 78.0 ± 0.3$^{w}$ | 16.2 ± 0.2$^{w}$ | 9.1 ± 0.3$^{x}$ |
| | 61.9% | 63.9 ± 0.2$^{y}$ | 70.7 ± 0.1$^{y}$ | 81.1 ± 0.4$^{x}$ | 17.3 ± 0.5$^{x}$ | 8.8 ± 0.2$^{x}$ |
| *Wrinkled pea* | | | | | | |
| Native | | –[5] | – | – | – | – |
| Control[4] | | – | – | – | – | – |
| Hydrolyzed | 22.1% | – | – | – | – | – |
| | 53.5% | – | – | – | – | – |

[1] For each cultivar, data with the same superscript in the same column are not significantly different ($P < 0.05$). The data represent the mean ± SD of three determinations.
[2] $T_o$, $T_p$, $T_c$ indicate the onset, peak and conclusion temperature of gelatinization, respectively. $T_c - T_o$ indicates the temperature range of gelatinization.
[3] $\Delta H$, gelatinization enthalpy.
[4] Treated without α-amylase but sujected to the same experimental conditions.
[5] Not detected within the temperature range 25–145 °C.

between black bean cultivars (*black jack* > *CDC nighthawk*) (Fig. 8a) reflect differences in the degree of accessibility (*black jack* > *CDC nighthawk*) of α-amylase to amylose chains within the amorphous domains of the granule.

### 3.9. Thermal properties of hydrolyzed starches

The gelatinization parameters of native, control and hydrolyzed residues are presented in Table 7 In general, gelatinization transition temperatures ($T_o$, $T_p$, $T_c$) increased slightly on hydrolysis. The extent of this increase was nearly of the same order of magnitude in all starches. However, increases in $T_o$, $T_p$ and $T_c$ were evident only for *CDC mozart* and *CDC robin* (Table 7). *Sierra*, *CDC redwing* and *CDC sonata* showed increases only in $T_o$, $T_p$ and $T_p$ and $T_c$, respectively (Table 7). Whereas, *black jack*, *CDC nighthawk* and *othello* showed increases only in $T_o$ and $T_c$ (Table 7). In all starches, $\Delta H$ decreased on hydrolysis and the extent of this decrease was nearly the same for all starches (Table 7). The decrease in $\Delta H$ on hydrolysis suggests that α-amylase is able to hydrolyze crystallites from amylopectin side chains and retrograded amylose (formed by hydrolyzed amylose chains). The slight increase in $T_o$, $T_p$ and $T_c$ on hydrolysis suggests that some retrograded amylose chains, which had longer chain length than the crystallites in the native starch, may have been present in the hydrolyzed residues.

We postulate that the crystallites formed by amylose retrogradation probably differ in number and size among the hydrolyzed legume starches. This would explain why increases in $T_o$, $T_p$ and $T_c$ on hydrolysis occur only in some starches, whereas in others only one or two of the above parameters increase on hydrolysis (Table 7). Furthermore, the absence of an endotherm in hydrolyzed wrinkled pea starch (Table 7) also suggests a large extent of amylose retrogradation during hydrolysis. This seems plausible, since the swelling factor of 58.5% for hydrolyzed wrinkled pea starch residue (1.8) was lower than that of its native counterpart (3.4).

## 4. Summary and conclusions

This study has shown that the granular morphology, relative crystallinity, X-ray pattern ('C' type), extent of starch damage, 'B' polymorphic content and composition differed only marginally among black bean, pinto bean, smooth pea and lentil starches. However, swelling power, amylose leaching and differential scanning calorimetry measurements showed that the extent of starch chain interactions within the amorphous and crystalline domains of the native granule were more pronounced in pinto bean than in black bean, lentil and smooth pea starches. Wrinkled pea differed from the other legume starches in exhibiting a higher extent of starch damage, a higher content of bound lipids, a different X-ray pattern ('B' type), lower relative crystallinity, different granule shapes and sizes, a highly disrupted crystalline structure and strong interaction between amylose chains.

The rate and extent of α-amylolysis of black bean, pinto bean, smooth pea and lentil starches were mainly influenced by the interplay of: (1) the magnitude of interaction between starch chains within the amorphous domains of the native granule, and (2) extent to which hydrolyzed amylose chains interact with each other during the time course of hydrolysis. However, in wrinkled pea starch, in addition to the above two factors, a disrupted crystalline structure (influenced by its higher amylose content and longer amylopectin chain length), a higher 'B' polymorphic content and a higher extent of starch damage were also causative factors influencing the rate and extent of hydrolysis. Differences in hydrolysis between cultivars were only evident in black bean and lentil starches.

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