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Composition, molecular structure, and physicochemical properties of starches from four field pea (*Pisum sativum* L.) cultivars

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Abstract

Starch from four cultivars (Carneval, Carrera, Grande and Keoma) of field pea (*Pisum sativum* L.) was isolated and its physicochemical properties were compared with those of other legume starches. The yield of starch was in the range 32.7–33.5% on a whole seed basis. The starch granules were round to elliptical with smooth surfaces. The free lipid was 0.05% in all starches. However, bound and total lipids ranged from 0.24 to 0.29% and from 0.28 to 0.34%, respectively. The total amylose content ranged from 48.8–49.6%, of which 10.9–12.3% was complexed by native lipid. The degree of polymerization (DP) of amyloses ranged from 1300 to 1350. The chain length distributions of debranched amylopectins of the starches were analyzed using high performance anion-exchange chromatography equipped with a post-column amyloglucosidase reactor and a pulsed amperometric detector. The proportion of short branch chains, of chain length DP 6-12, ranged from 16.2 to 18.6%. Keoma displayed a larger portion (19.4%) of long branch chains (DP > 37) than the other three starches (16.2–16.9%). The average amylopectin branch chain length ranged from 22.9 to 24.2. The maximum detectable DP was higher in Keoma (71) than in the other three starches (64–65). The X-ray pattern was of the 'C' type. The relative crystallinity was in the range 20.8–25.1%. The proportion of 'B' polymorphic form was higher in Keoma (25.6%) than in the other three starches (22.1–24.1%). There were no significant differences in swelling factor. The extent of amylose leaching at 95°C ranged from 25.20 to 26.85. All four starches exhibited nearly identical gelatinization transition temperatures and enthalpies. However, the gelatinization temperature range (T_c–T_o) followed the order: Grande∼Keoma > Carneval∼Carrera. The four starches showed identical pasting temperatures and exhibited only marginal differences with respect to 95°C viscosity and to the increase in consistency during the holding period at 95°C. However, the set-back viscosity for Carneval was lower than that of the other starches. There were no significant differences in the extent of acid hydrolysis. However, susceptibility towards hydrolysis by α-amylase followed the order: Carneval∼Carrera∼Grande > Keoma. The extent of retrogradation (monitored by changes in enthalpy) during storage at 40°C/24 h followed the order: Carneval > Carrera > Grande > Keoma. However, differences in the extent of retrogradation among starches were not discernable by freeze-thaw stability measurements.

Keywords: Composition; Molecular Structure; Physicochemical properties; Starch; Field pea

1. Introduction

Legumes are dicotyledonous seeds of plants that belong to the family *Leguminosae* (16,000–19,000 species in ∼750 genera; Allen & Allen, 1981). The grain legumes, collectively, are ranked fifth in terms of annual world grain production (171 million metric tons). The pea comprises two species, viz; *Pisum sativum* and *Pisum fulvum*. Field pea (*Pisum sativum* L.) — which is also known as common pea, dry pea, green pea (green seeded cultivars), yellow pea (yellow seeded cultivars), and garden pea — is a cool season crop, grown in the sub-tropics and at higher altitudes in the tropics. It is one of the four important cultivated legumes (others include soy-beans, groundnuts and dry-beans) in the world. Pea is a predominant export crop in world trade and represents about 35–40% of the total trade in pulses. In 1999, Canada contributed 19% to the total world production of 11,699,171 Mt (FAO, 1999), and Canadian pea production increased by 30% in the 1998/1999

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season (Agriculture and Agrifood Canada, 2000). Canada's largest export market for field pea is Western Europe. Field pea is utilized in Canada as follows: (1) whole or split in soups and stews; (2) hulls in high fibre breads; (3) pea protein in human food and in hog rations as an alternative protein source to soy and canola meal, and (4) pea starch in production of adhesives and carbonless paper. The air-classification and wet-milling processes of field peas (Tyler, Youngs, & Sosulski, 1981) have permitted the fractionation of field pea flour into protein and starch concentrates (60–80% of starch). However, due to its poor functional properties, the starch concentrate is not used in food formulations. Therefore, it is economically important to explore possible avenues for improving the functional properties of field pea starch for it to be successfully utilized in the food industry.

Total carbohydrates of food legumes vary from 24% (winged beans) to 68% (cowpeas). Starch is the most abundant carbohydrate in the seed (22–45%; Hoover & Sosulski, 1991). Starches from different cultivars of wheat (Wootton & Mahdar, 1993), maize (Yun & Matheson, 1993), proso millet (Yanez, Walker, & Nelson, 1991), rye (Gudmundsson & Eliasson, 1991) and cassava (Asaoka, Blanshard, & Rickard, 1991) have been shown to vary in composition and properties. However, such variations in starch composition and properties among field pea cultivars have not been the subject of a detailed study. Most of the studies on field pea starch have been on a single cultivar. Therefore, it is difficult to ascertain whether the data reported are truly representative of field pea starch. Furthermore, there is a dearth of information on the retrogradation properties of field pea starch. Therefore, it was considered worthwhile to investigate the chemical composition, granule crystallinity, polymorphic composition, thermal properties, rheological properties and retrogradation characteristics in starches isolated from four cultivars of field pea. At the present time, there is increased emphasis on developing value-added products from Canadian legume products. Therefore, the results of this study would form the basis of further investigations on physical and chemical modification to improve the functionality of field pea starches.

2. Materials and methods

2.1. Materials

Field pea (*Pisum sativum* L.) cultivars (Carneval, Carrera, Grande and Keoma) were grown on experimental plots (under identical environmental conditions) at the Morden Research Center, Agriculture and Agri-Food Canada in Morden, M.B. Crystalline porcine pancreatic α-amylase (EC 3.2.1.1, type 1A), α-amylase from sweet potato (EC 3.2.1.2) and amyloglucosidase from *Rhizopus* mold (EC 3.2.1.3) were purchased from Sigma Chemical Co., (St. Louis, MO, USA). Isoamylase (EC 3.2.1.68), from *Pseudomonas amyloderamosa*, and maltopentaose were purchased from Hayashibara Biochemical Laboratories Inc., (Okayama, Japan). Maltotriose, maltotetraose, maltohexaose and maltoheptaose were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Nucleosil 300-10 silica gels were purchased from Altech (Deerfield, IL, USA). Potato starch and waxy corn starch were donated by National Starch and Chemical Co., Bridgewater, NJ, USA. All other chemicals and solvents were of ACS certified grade. Solvents were distilled from glass before use.

2.2. Starch isolation

Three lots of field pea seeds were taken, representing whole samples from the experimental plots of each cultivar. Starch was extracted from each lot using the procedure of Hoover and Sosulski (1985). Sub-sub samples from each sub sample of starch from each cultivar were taken for the experiments.

2.3. Granule morphology

Granule morphology of native starches was studied by scanning electron microscopy. Starch samples were mounted on circular aluminium stubs with double sticky tape and then coated with 20 nm of gold and examined and photographed in a Hitachi-S 570 scanning electron microscope (Nissei Sangyo Inc., Rexdale, ON, Canada) at an accelerating potential of 20 kV.

2.4. Chemical composition of starch

Quantitative estimation 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

Apparent and total amylose content was determined by a modification (Hoover & Ratnayake, 2000) of the method of McGrance, Cornell, and Rix (1998).

2.5.1. Apparent amylose content

Starch (20 mg, db) was dissolved in 90% dimethylsulfoxide (8 ml) in 10 ml screw-cap reaction vials. The contents of the vials were vigorously mixed for 2 min and then heated in a water bath (with intermittent shaking) at 85°C for 15 min. The vials were then cooled to ambient temperature, and the contents diluted with water to 25 ml in a volumetric flask. 1.0 ml of the diluted

solution was mixed with water (40 ml) and 5 ml I_2/KI solution (0.0025M I_2 and 0.0065M KI) and then adjusted to a final volume of 50 ml. The contents were allowed to stand for 15 min at ambient temperature, before absorbance measurements at 600 nm.

2.5.2. Total amylose content

The total amylose contents of starch samples were determined by the above procedure, but with prior defatting with hot *n*-propanol-water (3:1 v/v) for 7 h. In order to correct for over estimation of apparent and total amylose content (due to complex formation between I_2 and the long outer branches of amylopectin), amylose content was calculated from a standard curve prepared using mixtures of pure potato amylose and amylopectin (over the range 0–100% amylose).

2.6. Starch fractionation

Amylose and amylopectin were extracted from the field pea starches utilizing the aqueous leaching procedure described by Montgomery and Senti (1958).

2.7. Determination of purity of isolated amylose and amylopectin using gel permeation chromatography

Purity of isolated amylose and amylopectin were determined by the method of Jane and Chen (1992) using columns (1.5 i.d.×100 cm) packed with Sepharose CL-2B gel. The columns were run in the ascending mode. A sample solution (5 ml) containing amylose (~15 mg) and glucose (0.5 mg as a marker) was injected into the column. The eluent was a NaCl aqueous solution (0.02%) with a flow rate of 30 ml/h. Fractions of 4.8 ml were collected and were subjected to total carbohydrate (Dubios, Gilles, Hamilton, Rebers, & Smith, 1956) and amylose content (Hoover & Ratnayake, 2000) measurements. Percent purity of amylose was calculated by dividing the total carbohydrate peak area of the amylose peak by the sum of the area of the amylose and amylopectin peaks.

2.7.1. Degree of polymerization (DP) of amyloses

Isolated amylose (0.10 g) was completely dissolved in 10 ml of dimethylsulfoxide by heating at 60°C in a water bath. The resulting solution was divided into two equal volumes and the DP was calculated using the following equation (Jane & Robyt, 1984).

$$\mathrm{DP} = \frac{\text{Total carbohydrate (μg)}}{\text{Reducing sugar (as μg of maltose)}} \times 2$$

Total carbohydrates and total reducing power were calculated according to the procedures outlined by Dubois et al. (1956) and Bruner (1964), respectively.

2.8. Branch chain length distribution of amylopectin

Isolated amylopectins were debranched using isoamylase according to the procedure of Jane and Chen (1992). Branch chain-lengths were obtained by using a high performance anion-exchange chromatograph with a post column amyloglucosidase reactor and a pulsed amperometric detector (Wong & Jane, 1997). The separation of debranched samples was carried out using a PA-100 anion exchange analytical column, a PA-100 guard column (Dionex, Sunnyvale, CA, USA) and an AS 40 automated sampler. The mobile phase used for separation consisted of eluent A (100 mM NaOH) and eluent B (100 mM NaOH with 300 mM $NaNO_3$) with a flow rate of 0.5 ml/min. The separation gradient was programmed as follows: 0–5 min, 99% A and 1% B; 5–30 min, linear gradient to 8% B; 30–150 min, linear gradient to 30% B; 150–200 min, linear gradient to 45% B. The eluent degas module (Dionex, Sunnyvale, CA, USA) was set at a system pressure of 7 psi (never exceeding 10 psi). Pump A (Dionex DX-300 standard bore gradient pump), which delivered the gradient for sample separation, was operated at 600 psi pressure (never exceeding 5000 psi). Pump B (Dionex DX-300 micro bore gradient pump), which delivered 0.5 N acetate buffer for pH adjustment, was operated at a minimum pressure 600 psi (not exceeding 5000 psi). Pump C (Dionex pneumatic pump), which delivered 750 mM NaOH solution for pH adjustment, was operated at 47 psi. The entire system was operated using an AI-450 software interface with an IBM compatible computer.

2.9. X-ray diffraction

2.9.1. X-ray pattern and relative crystallinity

X-ray diffractograms were obtained with a Rigaku RU 200R X-ray diffractometer (Rigaku-Denki Co., Tokyo, Japan) with operating conditions as: target voltage 40 kV, target 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 calculated using the method of Nara, Mori, and Komiya (1978) using peak-fitting software (Origin — Version 6.0, Microcal Inc., Northampton, MA, USA). Amorphous starch was prepared by heating a 10% starch solution at 95°C for 30 min with continuous agitation and then drying it at 100°C for 24 h. The dried sample was ground into a free-flowing powder using a RP 202 Pulaerit comminutator (Geoscience Instruments Corp., New York, NY, USA) with denatured alcohol as the solvent. The ground sample was air-dried for 24 h and passed through a 250-μm sieve. Powdered quartz was used as the 100% crystalline reference.

2.9.2. Determination of 'A' and 'B' polymorphic composition by X-ray diffraction

The proportion of "A" and "B" polymorphic composition of the starches was calculated using the method outlined by Davydova, Lent'ev, Genin, Sasov, and Bogracheva (1995). The peak at 5.45° 2θ (see Fig. 3 below) is characteristic of 'B' type starches. The 'B' polymorph content was calculated by determining the ratio of the area under the diffraction peak at 5.45° 2θ to the summed areas of all the peaks of the diffractogram, together with a calibration curve derived from mixtures of pure 'B' type (0–100% potato starch) and pure 'A' type (100–0% waxy corn starch). The moisture contents of the field pea starches and the starch mixtures used for calibration were adjusted to 16%(w/w), and allowed to equilibriate in sealed containers for one week prior to analysis.

2.10. Swelling factor (SF)

The SF of the starches when heated to 50–95°C in excess water was measured according to the method of Tester and Morrison (1990). This method measures only intragranular water, and hence, the true SF at a given

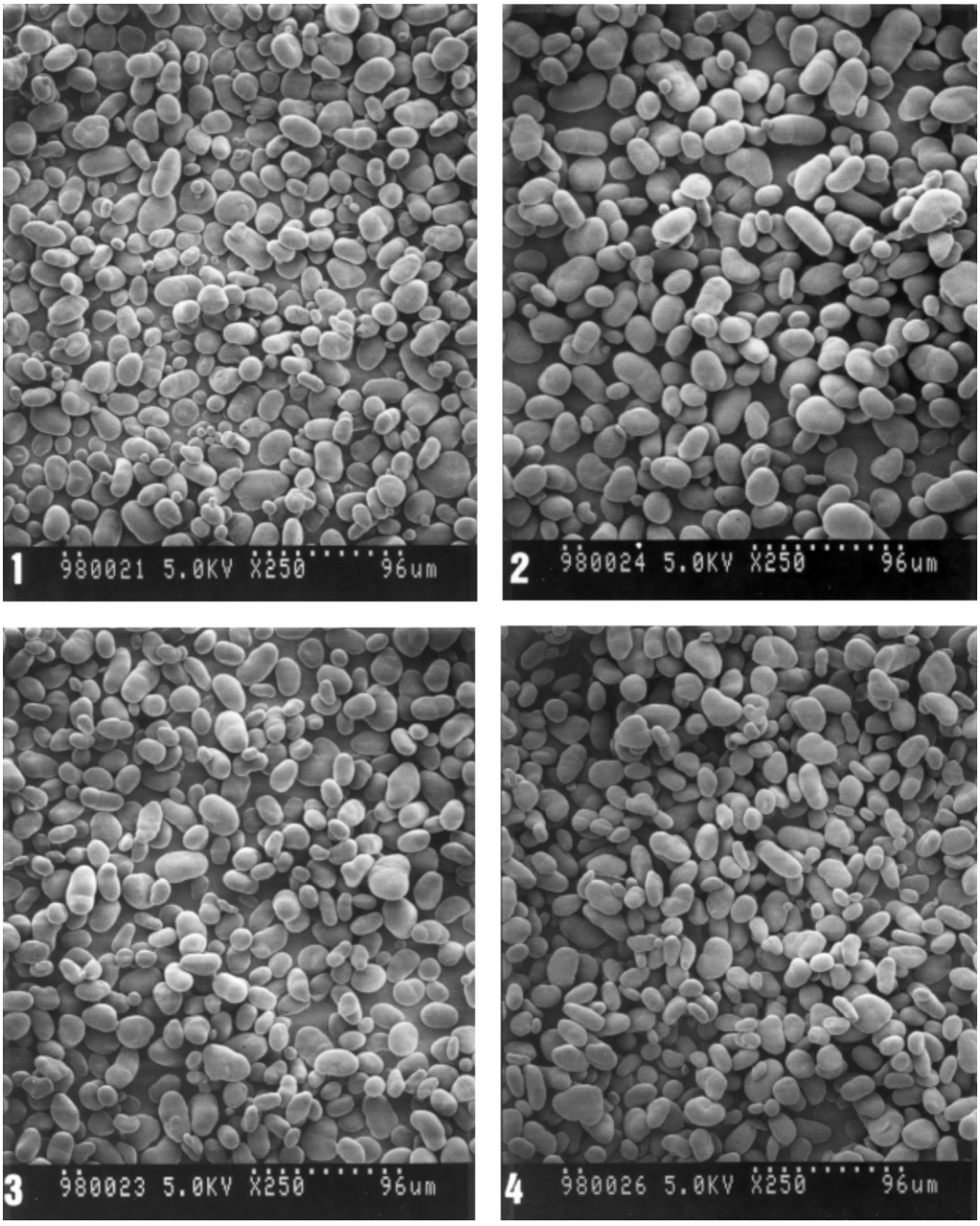

Fig. 1. Scanning electron micrographs of field pea starches: (1) Carneval, (2) Carrera, (3) Grande, and (4) Keoma.

temperature. The SF is reported as a ratio of the volume of swollen starch granules to the volume of the dry starch.

2.11. Amylose leaching (AML)

Starches (20 mg, db) in water were heated (50–95°C) in volume-calibrated sealed tubes for 30 min. The tubes were then cooled at ambient temperature (25–27°C) and centrifuged at 2000 *g* for 10 min. The supernatant liquid (1 ml) was withdrawn and its amylose content was determined as described by Hoover and Ratnayake (2000). Percentage amylose leaching was expressed as mg of amylose leached per 100 g of dry starch.

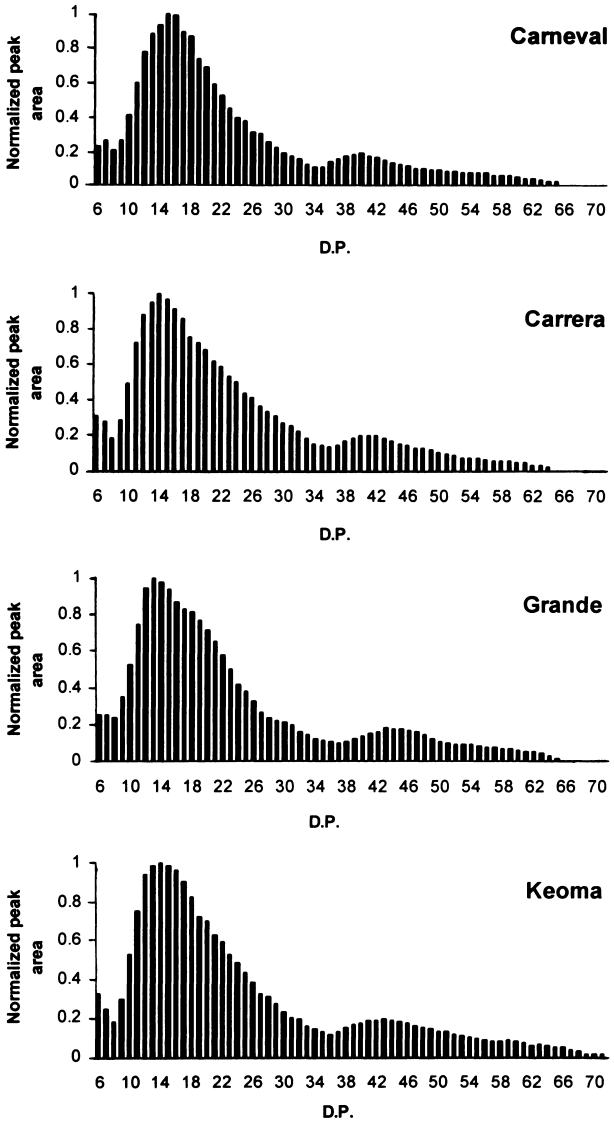

Fig. 2. Normalized peak area chromatograms of isoamylase debranched amylopectins of field pea starches by the use of high performance anion exchange chromatography equipped with an on-line amyloglucosidase reactor and a pulsed amperometric detector.

2.12. Differential scanning calorimetry (DSC)

Gelatinization parameters of native and retrograded starches were measured using a Seiko DSC 210 (Seiko Instruments Inc., Chiba, Japan) differential scanning calorimeter equipped with a thermal analysis data station and data recording software.

2.12.1. Gelatinization parameters of native starch

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 2 h at room temperature before DSC analysis to attain an even distribution of water. The scanning temperature range and the heating rates were 20–120°C and 10°C/min, respectively. In all measurements, the thermogram was recorded with an empty aluminium pan as the reference. The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c). The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram and a base line under the peak and was expressed in terms of Joules per gram of dry starch.

2.12.2. DSC parameters of retrograded starch

Water (3 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 6 h at room temperature for moisture equilibration. The sealed pans were then heated (20–120°C at 10°C/min) to gelatinize the starch. The gelatinized samples were stored at 40°C for 24 h to enhance the propagation of crystallites. Subsequently, the samples were equilibrated at room temperature for 2 h, and then rescanned in the calorimeter from 20 to 120°C at 10°C/min to measure retrogradation transition temperatures and enthalpy.

2.13. Pasting properties

A Brabender viscoamylograph (Model VA-V), equipped with a 700 cm cartridge (C.W. Brabender Instruments

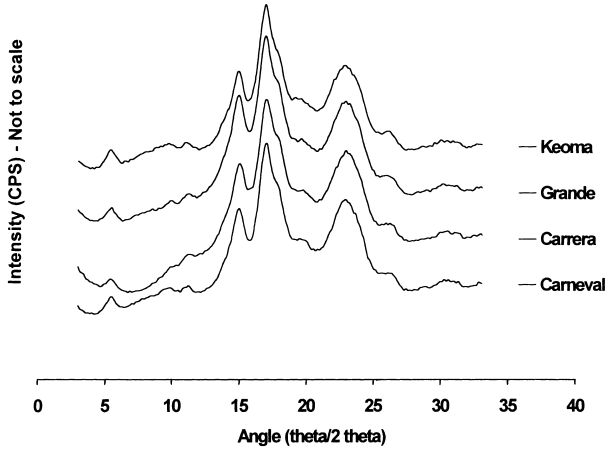

Fig. 3. X-ray diffraction patterns of native field pea starches (moisture content adjusted to 16%).

Inc., South Hackensack, NJ, USA), was used to study pasting properties at a concentration of 9% (w/v) and pH 5.5. Duplicate measurements were used for this determination.

2.14. Acid hydrolysis

Starches were hydrolyzed with 2.2 M HCl at 35°C (1 g starch/40 ml acid) for periods ranging from 0 to 20 days. The extent of hydrolysis was estimated by the procedure described elsewhere (Hoover & Vasanthan, 1994).

2.15. Enzymatic digestibility

Enzymatic digestibility (0–72 h) studies on field pea starches were carried out using crystalline porcine pancreatic α-amylase (Sigma Chemical Co., St. Louis, MO, USA) in 2.9M NaCl containing 3 mM $CaCl_2$ (in which the concentration of α-amylase was 30 mg/ml and the specific activity was 790 units per milligram of protein (one unit was defined as the α-amylase activity which liberates 1 mg maltose in 3 min at 20°C at pH 6.9). The details of the procedure have been outlined in an earlier publication (Hoover & Vasanthan, 1994).

2.16. Freeze-thaw stability

Aqueous suspensions of starches (6%, w/v) were rapidly heated to 95°C under constant agitation. These suspensions were then kept at 96°C for 30 min before being cooled to 25°C. The gels thus obtained were subjected to cold storage at 4°C for 24 h (to increase nucleation) and then frozen at −16°C (for 24 h). The frozen gels were then thawed at 25°C for 6 h and then refrozen at −16°C. Six freeze-thaw cycles were performed. The exuded water, at the end of each cycle, was gravimetrically determined by vortexing the thawed gels for 15 s, followed by centrifugation at 1000 *g* for 20 min.

2.17. Statistical analysis

All determinations were replicated three times and mean values and standard deviations reported. Analyses of variance were performed and the mean separations were performed by Tukey's HSD test ($P < 0.05$) using SigmaStat® Version 2.0 (Jandel Scientific/SPSS Science, Chicago, IL, USA).

3. Results and discussion

3.1. Morphological characteristics of granules

Scanning electron microscopy showed that field pea starch granules from the four cultivars had irregular shapes, which varied from round (5–7 μm) to elliptical (shorter diameter, 10 μm; longer diameter, 25 μm; Fig. 1). These values were lower than those reported for other legume starches (Czuchajowska, Otto, Paszczynska, & Baik, 1998; Hoover & Sosulski, 1991). The surfaces of the above starches appeared to be smooth and showed no evidence of fissures (Fig. 1).

3.2. Chemical composition of the starches

The data on yield and composition are presented in Table 1. The purity of the starches was judged on the basis of composition (low nitrogen and low ash content) and microscopic observation (absence of any adhering protein). The yields (on a total seed basis) of pure starch (32.7–33.7%; Table 1) were in the range reported (18–45%) for other legume seeds (Hoover & Sosulski, 1991). The nitrogen content was in the range of 0.04–0.07%. The low nitrogen indicated the absence of non-starch lipids (lipids associated with endosperm proteins). Therefore, the total lipids (0.28–0.34%; Table 1) obtained by acid hydrolysis mainly represent free and bound starch lipids (Vasanthan & Hoover, 1992). The total lipid content of the field pea starches was within the range reported for mung bean (0.32%; Hoover, Li, Hynes, & Senanayake, 1997), and lentil (0.27–0.38%; Hoover & Manuel, 1995) starches, but was lower than that reported for beach pea (0.16%), green pea (0.19%) and grass pea (0.12%) starches (Chavan, Shahidi, Hoover, & Perera, 1999). Most of the data on the total lipid content of other legume starches have been obtained by the use of solvent systems that have proved to be ineffective in removing internal starch lipids. Therefore, a comparison of our results with published data cannot be made. The free lipid (obtained by extraction with chloroform-methanol 2:1 v/v at 25°C) constituted 0.05% of the total weight of lipid in all four field pea starches. The bound lipid content (obtained by extraction of the choloform-methanol residue with 1-propanol-water 3:1 v/v for 7 h) was in the range 0.24–0.29% (Table 1). The bound lipid content was higher than the values reported for beach pea (0.10%), grass pea (0.07%), green pea (0.12%; Chavan et al., 1999), pigeon pea (0.10%; Hoover, Swamidas, & Vasanthan, 1993), lima bean (0.22%; Hoover, Rorke, & Martin, 1991), but was comparable to that reported for mung bean (0.27%; Hoover et al., 1997) starch.

The total amylose content of the field pea starches was in the range 48.8–49.6% (Table 1). These values were much higher than those reported by Chavan et al. (1999) for beach pea (29.0%), green pea (36.7%), grass pea (36.4%) starches, and lower than those of smooth pea (52.6–57.0%) and wrinkled pea (94.0%; Czuchajowska et al., 1998), but was comparable to that of mung bean starch (45.3%; Hoover et al., 1997). The apparent amylose (determined by I_2-binding, before

removal of bound lipids) and total amylose (determined by I_2-binding, after removal of bound lipids) contents were in the range 42.9–43.7, and 48.8–49.6%, respectively, in the four field pea starches. A comparison of the apparent and total amylose content (Table 1) showed that 10.9, 12.3, 12.1, and 12.0% of the total amylose was complexed by native lipids in Carneval, Carrera, Grande, and Keoma starches, respectively (Table 1).

3.3. Molecular structure

3.3.1. Degree of polymerization of amylose

The DP (Table 2) of the isolated amylose followed the order: Keoma (1350) > Grande (1322)~Carrera (1320) > Carneval (1300). These values were close to that reported for smooth pea starch (1400; Biliaderis, Grant, & Vose, 1981).

3.3.2. Amylopectin branch chain length (CL) distribution

The normalized branch CL distributions of debranched amylopectins of the four starches are presented in Fig. 2 and Table 3. The first peak in the bimodal peak distribution was at a peak CL of 14 for Carrera and Keoma, and at 13 and 15 for Carneval and Grande, respectively, whereas the second peak was at 43 for Grande and Keoma and at 42 and 40, for Carneval and Carrera, respectively. Among the four starches, Keoma had the longest average CL, of DP 24.2, while those of Carneval, Carrera, and Grande were 22.9, 23.1, and 23.0, respectively. The proportion of short-branch chains (DP 6-12) followed the order: Grande (18.6%) > Carrera (17.2%) > Keoma (17.0%) > Carneval (16.2%). The proportion of long branch chains (DP > 37) followed the order: Keoma (19.4%) > Grande (16.9%) > Carneval (16.4%) > Carrera (16.2%). The maximum detectable CL was higher in Keoma (DP 71) than in the other three starches (DP 64-65; Table 3). CL of each of the four starches (Table 3) was comparable to those reported for other legume starches (CL 20–26; Biliaderis et al., 1981).

3.3.3. Wide angle X-ray diffraction

The field pea starches showed the characteristic 'C' type pattern of legume starches (Colonna, Buleon, Lemaguer, & Marcier, 1982; Davydova et al., 1995; Gernat, Radosta, Damaschun, & Shierbaum, 1990; Hoover & Sosulski, 1985). The X-ray pattern (Fig. 3) was characterized by strong intensity peaks at 5.9, 5.2, 5.0 and 3.8°A, and a weak intensity peak at 15.7°A ($2\theta = 5.54$). The peak at 15.7°A is generally characteristic of tuber starches. Gernat et al. (1990) have shown that the 'C' crystalline polymorph is a mixture of 'A'

Table 1
Chemical composition (%) and some of the properties of field pea starches

Characteristic	Composition (%)[a]			
	Carneval	Carrera	Grande	Keoma
Yield (% initial material)	33.7±1.5p	33.2±1.6p	32.7±1.5p	33.5±1.3p
Moisture	13.3±0.11	9.2±0.08	12.3±0.07	11.2±0.09
Ash	0.03±0.01p	0.04±0.01p	0.03±0.01p	0.14±0.01q
Nitrogen	0.04±0.01p	0.06±0.00p,q	0.04±0.01p	0.07±0.01q
Lipid				
Solvent extracted				
Chloroform-methanol[b]	0.05±0.00p	0.05±0.00p	0.05±0.01p	0.05±0.00p
n-Propanol-water[c]	0.27±0.04p	0.25±0.04p	0.24±0.03p	0.29±0.03p
Acid hydrolyzed[d]	0.31±0.03p	0.30±0.04p	0.28±0.02p	0.34±0.04p
Amylose content				
Apparent[e]	43.7±0.03s	43.5±0.03r	42.9±0.03p	43.2±0.07q
Total[f]	49.1±0.14q	49.6±0.02r	48.8±0.06p	49.0±0.09p,q
Amylose complexed with native lipid[g]	10.9±0.04p	12.3±0.06s	12.1±0.05r	12.0±0.07q
Starch damage	1.73±0.08p	2.3±0.04q	2.50±0.04r	2.55±0.08r
Granule size (μm)				
Round	5–7	5–7	5–7	5–7
Elliptical				
Shorter diameter	10	10	10	10
Longer diameter	25	25	25	25

[a] All data reported on dry basis and the values followed by the same letter in each row are not significantly different ($P < 0.05$) by Tukey's HSD test.
[b] Lipid obtained from native starch by chloroform-methanol 2:1 (v/v) at 25°C (mainly unbound lipids).
[c] Lipid extracted by hot *n*-propanol-water 3:1 (v/v) from the residue left after chloroform-methanol extraction (mainly bound lipids).
[d] Lipids obtained by acid hydrolysis (24% HCl) of native starch (total lipids).
[e] Apparent amylose determined by iodine binding without removal of free and bound lipids.
[f] Total amylose determined by I_2-binding after removal of free and bound lipids.
[g] $\frac{\text{Total amylose} - \text{apparent amylose}}{\text{Total amylose}} \times 100$.

and 'B' unit cells, and that legume starches contain pure 'A' and 'B' polymorphs in varying proportions. Both '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, 1988; Imberty, Chanzy, Ring, & Hedley, 1988). Bogracheva, Morris, Ring and Hedley (1998) have concluded from DSC and X-ray studies of pea starch gelatinized in 0.6M KCl solution, that the 'A' and 'B' polymorphs in pea starch are present in the same granule and that the 'B' polymorph is situated in the centre of all granules and is surrounded by the 'A' polymorph. Hizukuri (1986) and Hizukuri, Kaneko, and Takeda (1983) have shown that starches with amylopectins of short chain length (< 20 residues) exhibit 'A' type crystallinity, whereas those with amylopectins of longer average CL show the 'B' pattern. The results showed that the amount of 'B' polymorph followed the order; Keoma (25.5%) > Carneval (24.1%) > Grande (23.3%) > Carrera (22.1%) (Table 4). The higher 'B' polymorph content of Keoma can be attributed to its longer amylopectin chain length (Table 3). The values shown above were lower than that reported (Cairns, Bogracheva, Ring, Hedley, & Morris, 1997; Davydova et al., 1995) for smooth pea starches (26–49%).

The relative crystallinity followed the order: Carneval (25.06%)~Keoma (24.71%) > Grande (21.96%) > Carrera (20.38%; Table 4). These values were lower than those reported (Davydova et al., 1995) for five varieties of smooth pea starches (26–32%). X-ray diffraction studies on maize starches of different amylose content (Cheetham & Tao, 1997) have shown that starch crystallinity is influenced by amylose content, average CL of amylopectin, and the mole percentage of short chain fractions of amylopectin (DP 10-13). The differences in total amylose content (Table 1), CL (Table 3), and the amount of short chains (DP 6-12; Table 3) between the four starches were marginal and furthermore, differences in moisture content of the starches used for X-ray diffraction were also marginal. Therefore, the observed differences in crystallinity probably represent differences in crystallite size and/or different orientations of the double helices within the crystallite.

Table 2
Degree of polymerization of field pea amylose

Cultivar	Degree of polymerization[a]
Carneval	1300±11p
Carrera	1320±12p,q
Grande	1322±15p,q
Keoma	1350±10q

[a] The values followed by the same superscript are not significantly different by Tukey's HSD test at $P < 0.05$ level.

3.4. Swelling factor (SF) and amylose leaching (AML)

The swelling factor (SF) and amylose leaching (AML) were investigated over the temperature range 50–95°C (Table 5). There were no significant differences in SF among the starches. The SF (at 95°C) of the field pea starches were lower than those reported for beach pea (30.72), green pea (34.1; Chavan et al., 1999), mung bean (43.6), CC gold lentil (31.0) but were comparable to that of laird lentil (26.0; Hoover & Manuel, 1995) starch. The extent of AML followed the order: Keoma~Carneval~Grande > Carrera (Table 5). AML at 95°C was much higher than those reported (Chavan et al., 1999) for beach pea (12.94), green pea (17.08), grass pea (19.07), but lower than those reported for CC gold (35.5) and laird lentil (38.5) starches (Hoover & Manuel, 1995). In all four starches, SF and AML increased dramatically between 60 and 85°C (Table 5), thereafter the increases were gradual. A similar trend has also been observed for other legume starches (Chavan et al., 1999; Hoover & Manuel, 1995; Hoover & Sosulski, 1985; Schoch & Maywald, 1968; Tolmasquim Correa, & Tolmasquim, 1971). The SF has been shown to be influenced by amylose-lipid complexes (Hoover & Manuel, 1996; Maningat & Juliano, 1980; Tester & Morrison, 1990; Tester, Morrison, & Schuimann, 1993) and amylopectin molecular structure (Tester et al., 1993). The similar SF values shown by the four starches (Table 5)

Table 3
Branch chain length distributions of debranched field pea amylopectins

Starch source	First peak	Second peak	Distribution (%)[a]					
			DP 6-12	DP 13-24	DP 25-36	DP ≥ 37	Average chain length (CL)	Maximum detectable DP
Carneval	15	40	16.2±1.9a	52.9±0.4c	14.6±1.7a	16.4±1.7a	22.9±0.5a	65
Carrera	14	42	17.2±0.3a	48.2±0.5a	17.5±0.8b	16.2±0.6a	23.1±0.6a,b	64
Grande	13	43	19.6±1.4a	50.8±0.9b,c	13.9±0.7a	16.9±0.1a,b	23.0±0.3a,b	65
Keoma	14	43	17.0±0.1a	48.5±1.6a,b	15.1±0.5a,b	19.4±1.1b	24.2±0.4a,b	71

[a] The total relative peak area was used to calculate percent distribution. Values followed by the same letter in the same column are not significantly different ($P < 0.05$) by Tukey's HSD test.

suggests that differences in% bound lipid (Table 1), lipid content (Table 1) and amylopectin chain length (Table 3) are too small to make any significant impact on SF. The lower extent of AML in Carrera (Table 5) reflects its higher bound lipid content (Table 1). The rapid increase in SF and AML, between 60 and 85°C (Table 5), may be due to an increase in molecular mobility of the amorphous region, which causes unravelling, and melting of the double helices present within the amorphous (double helices formed between amylose chains and between amylose and the branched chains of amylopectin) and crystalline domains (double helices formed between the outer branches of amylopectin).

Table 4
Relative crystallinity and polymorphic composition of field pea starches[a]

Starch source	Relative crystallinity[b] (%)	'B' polymorphic composition[c] (%)
Carneval	25.1±0.52s	24.1±0.31r
Carrera	20.4±0.47p	22.1±0.32p
Grande	22.0±0.45q	23.3±0.30q
Keoma	24.7±0.50r,s	25.6±0.32s

[a] Values followed by the same letter, in the same column are not significantly different ($P<0.05$) by Tukey's HSD test. The moisture content of the starches were 16% (w/w).
[b] % crystallinity = $\Sigma|I_s-I_a|/|I_c-I_a|\times100$, where I_s-I_a = difference between the sample and amorphous intensities and I_c-I_a = difference between the 100% crystalline (quartz) and amorphous intensities.
[c] Proportion of 'B' polymorph α [area of the peak at 5.54 (2θ)/total peak area].

3.5. Gelatinization parameters

The gelatinization transition temperatures [T_o (onset); T_p (midpoint); T_c (conclusion)] and the enthalpies of gelatinization (ΔH) of the four starches are presented in Table 6. T_o, T_p, T_c and ΔH/AP (enthalpy calculated on the basis of amylopectin content) did not vary

Table 5
Swelling factor (SF) and amylose leaching (AML) of field pea starches at different temperatures[a]

Starch source	Temperature (°C)						
	50	60	70	80	85	90	95
Carneval							
SF	4.2±0.21	8.5±0.25	13.7±0.16	19.4±0.11	24.3±0.04	26.5±0.03	26.7±0.21
AML	0.0±0.00	10.5±0.23q	16.3±0.17q,r	19.6±0.12q	25.1±0.03	26.3±0.22q	26.6±0.16q,r
Carrera							
SF	4.2±0.22	8.6±0.21	13.8±0.23	19.4±0.05	24.2±0.05	26.4±0.21	26.7±0.24
AML	0.0±0.00	101±0.22p	15.1±0.12p	18.1±0.25p	24.8±0.24p	25.1±0.19p	25.2±0.10P
Grande							
SF	4.1±0.21	8.4±0.22	13.8±0.11	19.4±0.10	24.2±0.05	26.5±0.16	26.7±0.23
AML	0.0±0.00	10.5±0.23q	16.0±0.10q	20.2±0.08r	25.7±0.10r	26.0±0.12q	26.2±0.09q
Keoma							
SF	4.1±0.18	8.4±0.22	13.3±0.11	19.2±0.20	24.1±0.20	26.4±0.20	26.5±0.05
AML	0.0±0.00	10.7±0.15q	16.6±0.20r	20.3±0.06r	25.5±0.16r	26.6±0.20r	26.8±0.02r

[a] The values of AML followed by the same letter, in the same column are not significantly different ($P<0.05$) by Tukey's HSD test. No significant differences ($P<0.05$) were observed among the values for SF within the same column by Tukey's HSD test.

Table 6
Gelatinization[a] characteristics of field pea starches

Starch source	Transition temperature[b](°C)			T_c-T_o[c] (°C)	ΔH[d] J/g	ΔH/(AP)[e] J/g
	T_o	T_p	T_c			
Carneval	61.4±0.20q	67.0±0.22p	76.0±0.23q	14.6±0.11q	11.5±0.02q	22.6±0.12q
Carrera	61.0±0.22p	66.8±0.15p	75.0±0.11p	14.0±0.05p	11.4±0.8p,q	22.6±0.10q
Grande	61.0±0.31p	67.5±0.16q	76.0±0.13q	15.0±0.08r	11.2±0.10p	21.9±0.14p
Keoma	61.0±0.15p	67.0±0.19p	76.0±0.17q	15.0±0.10r	11.2±0.08p	22.0±0.16p

[a] Starch:water ratio = 1:3 (w/w dry basis).
[b] T_o, T_p and T_c indicate the temperatures of the onset, midpoint and end of gelatinization respectively.
[c] T_c-T_o indicates the gelatinization temperature range.
[d] Enthalpy of gelatinization.
[e] Enthalpy of gelatinization (ΔH) expressed on the basis of amylopectin content (AP). Values followed by the same letter, in the same column are not significantly different ($P<0.05$) by Tukey's HSD test.

significantly among the starches. However, the gelatinization temperature range (T_c–T_o) followed the order: Grande∼Keoma > Carneval > Carrera. The results indicate that the numbers of double helices (in the amorphous and crystalline domains) that unravel and melt during gelatinization are nearly similar in all four starches. However, the differences in T_c–T_o, suggest that the degree of heterogeneity of the starch crystallites within granules of Keoma and Grande are greater than those in Carneval and Carrera. The T_o, T_p, T_c and ΔH of the field pea starches were within the range reported for other legume starches (Hoover & Sosulski, 1991).

3.6. Pasting properties

The pasting properties of field pea starches are presented in Table 7. The four starches showed identical pasting temperatures, and exhibited only minor differences with respect to 95°C viscosity and to the increase in consistency during the holding period at 95°C. However, Carneval differed from the other starches with respect to the extent of increase in viscosity (85 BU) on cooling to 50°C, and to the final viscosity (230 BU) at 50°C. The corresponding values for the other three starches were in the range 150–200 BU and 300–350 BU, respectively. The starch pasting properties have been shown to be influenced by granule swelling, friction between swollen granules, amylose leaching, starch crystallinity, and CL of the starch components (Hoover 1996; Rasper, 1982). The difference in the gel-forming tendency (during the cooling cycle) between Carneval and other starches reflects the smaller amylose chain length (Table 2) and the smaller proportion of DP 6-12 chains (16.2%; Table 2) of Carneval.

3.7. Acid hydrolysis

The hydrolysis of field pea starches by 2.2M HCl during a 20-day period is presented in Table 8. All starches exhibited a two-stage solubilization pattern. A relatively higher rate was observed during the first 8 days (corresponding to the degradation of the amorphous region of the granule), followed by a slower rate (corresponding to degradation of the crystalline region) between 8 and 20 days. There were no significant differences in the extent of hydrolysis among starches during the first 8 days of hydrolysis. At the end of the 8th day of hydrolysis the starches were hydrolyzed to the extent of ∼26%. This suggests that the degrees of packing and orientation of the starch chains in the amorphous regions are probably identical in all four starches. The extent of hydrolysis during the first 8 days was comparable to that reported for other legume starches (Chavan et al., 1999; Hoover & Manuel, 1995; Hoover et al., 1993).

There were also no significant differences among the starches with respect to the extent of increase in hydrolysis beyond the 8th day. At the end of 20 days, the starches were hydrolyzed to the extent of ∼37%. This was within the range reported for other legume starches (Chavan et al., 1999; Hoover & Manuel, 1995; Hoover et al., 1993). The results suggest that the amount of double helices

Table 7
Pasting characteristics of field pea starches[a]

Starch source	Pasting temperature[b] (°C)	Viscosity[c] at 95°C (BU)d	Viscosity[c] after 30 min at 95°C (BU)[d]	Viscosity[c] at 50°C (BU)[d]
Carneval	79.5±1.5	70.0±2.5q	145±4.5q,r	230±5.5p
Carrera	79.5±2.0	60.0±5.5p,q	150±5.0r	300±6.0q
Grande	79.5±2.5	55.0±4.8p	150±4.5r	350±6.5r
Keoma	79.0±2.0	55.0±4.5p	130±5.0p	300±5.8q

[a] Starch concentration 9% (w/v) and pH 5.5.
[b] No significant differences ($P < 0.05$) were observed among the values by Tukey's HSD test.
[c] The values followed by the same letter in the same column are not significantly different ($P < 0.05$) from each other by Tukey's HSD test.
[d] Brabender units.

Table 8
Extent (%) of acid hydrolysis of native field pea starches[a]

Starch source	Number of days										
	0	1	2	3	4	5	8	12	15	18	20
Carneval	0.0±0.0	4.8±0.1	10.3±0.1	13.5±0.1	16.8±0.4	20.0±0.2	26.1±0.2	31.7±0.2	34.6±0.2	36.8±0.2	37.8±0.3
Carrera	0.0±0.0	5.1±0.1	10.3±0.1	13.8±0.1	16.8±0.1	19.7±0.1	26.8±0.2	31.9±0.2	35.1±0.2	26.2±0.2	26.5±0.3
Grande	0.0±0.0	5.5±0.1	10.8±0.1	14.5±0.1	17.8±0.2	20.4±0.2	26.3±0.2	31.8±0.2	35.7±0.3	37.5±0.3	38.3±0.3
Keoma	0.0±0.0	5.1±0.1	10.8±0.1	14.6±0.2	17.9±0.2	20.0±0.2	26.4±0.2	31.8±0.2	34.4±0.3	37.0±0.3	38.8±0.3

[a] No significant differences ($P < 0.05$) were observed among the values in the same column by Tukey's HSD test.

within the crystalline region and crystallite size are probably similar in all four starches. Thus, differences that were observed in X-ray intensities (Fig. 3) could reflect differences in crystallite orientation.

3.8. In vitro digestibility by porcine pancreatic α-amylase

The extent of hydrolysis of native field pea starches followed the order Carneval > Carrera > Grande > Keoma (Fig. 4). For instance, after 24 h, Carneval, Carrera, Grande and Keoma were hydrolyzed to the extent of 22.2, 21.5, 20.7 and 18.2%, respectively. In comparison, beach pea, grass pea, green pea, laird lentil, CC gold lentil and mung bean starches are hydrolyzed (in 24 h) to the extent of 35, 22, 16, 14.5, 35 and 71%, respectively (Chavan et al., 1999; Hoover & Manuel, 1995; Hoover et al., 1997). Differences in the in vitro digestibility of native starches, among and within species, have been attributed to the interplay of many factors, such as starch source (Ring, Gee, Whittam, Orford, & Jhonson, 1988), granule size (Snow & O'Dea, 1981), amylose/amylopectin ratio (Hoover & Sosulski, 1985), extent of molecular association between starch components (Dreher, Berry, & Dreher, 1984), degree of crystallinity (Hoover & Sosulski, 1985) and amylose lipid complexes (Holm et al., 1983; Hoover & Manuel, 1995). Furthermore, it has been reported (Franco, Preto, Ciacco, & Tavares, 1988; Marsden & Gray, 1986) that hydrolysis by α-amylase predominantly occurs in the amorphous regions of the granule. Planchot, Colonna, Buleon, and Gallant (1997) have shown that there is a clear relationship between the hydrolysis rate of lintnerized starches and their crystalline type. Regardless of morphology, particles with 'A' type crystallinity were found to be more susceptible to amylolysis than those with the 'B' type. 'A' type lintners (waxy maize) showed the highest rates, whereas the rates for 'C' type lintners (mixtures of 'A' and 'B' type structures) was dependent on the 'A' type ratio. 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 derived from branch linkages located inside the crystalline regions, which produces an inferior crystalline structure. This inferior crystalline structure containing α (1-6) linked branch points and the short double helices are more susceptible to enzyme 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 'A' type starches, and hence more resistant to α-amylolysis. Thus, the difference in the extent of α-amylase hydrolysis (Fig. 4) between Keoma and the other starches could only be attributed to the higher 'B' polymorph content in Keoma (Table 4), since the starches differ only marginally with respect to granular size, crystallinity, amylose content,% amylose complexed lipids, and extent of molecular association between starch components.

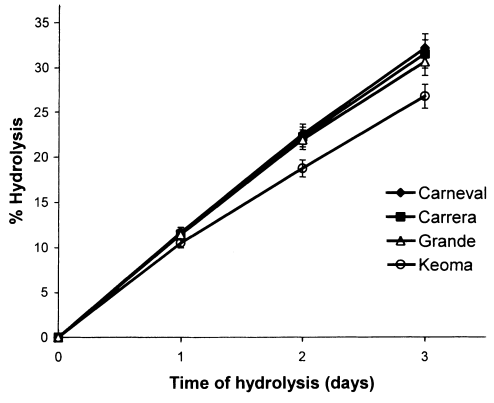

Fig. 4. Time course of hydrolysis (%) of native field pea starches by porcine pancreatic α-amylase.

3.9. Retrogradation of starch gels

The extent of retrogradation during gel storage was monitored by determining changes in retrogradation enthalpy and changes in freeze-thaw stability.

3.9.1. Differential scanning calorimetry

The retrogradation endotherms of the field pea starches (40°C/24 h) are presented in Table 9. In all four starches, T_o, T_p and T_c of retrograded gels were lower than those for the gelatinization endotherm (Table 6), and $T_c - T_o$ for retrogradation was broader than for the gelatinization endotherm (Table 6). The magnitude of ΔH_R (enthalpy of retrogradation) followed the order; Carneval > Carrera > Grande > Keoma whereas $T_c - T_o$ followed the order: Keoma > Grande > Carneval > Carrera (Table 9). The melting temperature range ($T_c - T_o$) gives an indication of the quality and heterogeneity of the recrystallized amylopectin. Thus, a wide melting range might imply crystals with a large variation in stability, whereas a narrow range could suggest crystals of a more homogeneous quality and similar stability. The results indicate that, at 40°C, variations in stability among crystallites (formed during retrogradation) are greater in Keoma than in the other starches. The ΔH_R reflects the unravelling and melting of double helices formed during gel storage. Differences in ΔH_R among starches have been explained on the basis of amylopectin unit CL distribution (Fredriksson, Silverio, Andersson, Eliasson, & Aman, 1998; Kalichevsky, Orford, & Ring, 1990; Lai, Lu, & Lii 2000; Lu, Chen, &

Table 9
Thermal characteristics of retrograded field pea starch gels

Parameter measured (40°C for 24 h)[a]	Cultivar			
	Carneval	Carrera	Grande	Keoma
ΔH_R/AP	12.2±0.08d	10.8±0.21c	10.1±0.20b	8.1±0.15a
T_o	60.2±0.21a	61.6±0.14a	60.1±0.28a	60.1±0.25a
T_p	69.5±0.27a	70.1±0.31a	70.1±0.14a	70.7±0.25b
T_c	78.3±0.23a	79.0±0.21b	78.4±0.28a	78.6±0.24a,b
T_c-T_o	18.1±0.21b	17.4±0.34a	18.3±0.24c	18.5±0.21d

[a] Samples of 3 mg (db) of starch with 3 μl of water, in hermetically sealed DSC pans were gelatinized and stored at 40°C for 24 h. Values followed by the same letter, in the same row (within the same treatment) are not significantly different by Tukey's HSD test at $P < 0.05$. ΔH_R = retrogradation enthalpy (J/g of starch, db); ΔH_R/AP = retrogradation enthalpy (J/g) /%Amylopectin;. T_o = Onset temperature (°C) T_p = peak temperature (°C); T_c = conclusion temperature (°C).

Table 10
Freeze-thaw stability of field pea starches[a]

Starch source	Syneresis (%) at different freeze-thaw cycles.					
	1	2	3	4	5	6
Carneval	18.6±1.2	23.0±1.4	24.0±0.5	24.0±1.4	25.5±0.8	27.0±2.1
Carrera	19.0±1.5	25.0±1.3	25.0±0.7	26.0±1.2	27.5±1.8	28.0±2.0
Grande	22.0±1.1	22.0±0.8	26.0±1.9	26.0±0.9	26.5±1.9	28.0±2.0
Keoma	22.0±1.6	23.0±2.0	26.5±1.4	27.0±2.0	28.0±1.9	30.0±2.1

[a] A 6% starch (db) solution. No significant differences ($P < 0.05$) were observed among the values in the same column by Tukey's HSD test.

Lii, 1997; Shi & Seib, 1992; Ward, Hoseney, & Seib, 1994). Liu and Thompson (1998) have shown by DSC studies that dull waxy maize starch retrogrades faster than waxy maize starch. They have attributed this difference to the presence of a larger number of branch points, in close proximity, in the amylopectin clusters of dull waxy starch, which hinder large scale motion of the outer A chains during retrogradation. This would then favour formation, proper alignment and organization of double helices. In this study, differences in amylopectin unit CL distribution were marginal. Therefore, differences in ΔH_R between Keoma and the other three starches could only be attributed to differences in the number of branch points (Carneval∼Carrera∼Grande > Keoma), in close proximity, in the disordered amylopectin clusters of these starches. The reduced number of branch points in Keoma would impart greater flexibility to the outer 'A' chains, resulting in poorly formed and/or improperly aligned doubl- helices during gel storage. Since DSC monitors double helical dissociation (Cooke & Gidley, 1992), the energy required to dissociate double helices in retrograded Keoma would be much less (accounting for the low ΔH_R value) than in the other starches.

3.9.2. Freeze-thaw stability

The freeze-thaw stability of a starch gel is evaluated by the amount (%) of water released (syneresis) when starch chains retrograde (reassociate) during the freeze-thaw cycles. The degree of syneresis of the starch gels is presented in Table 10. There were no significant differences in the extents of syneresis among these starches. In non-waxy starches, both amylose and amylopectin crystallization influence the degree of syneresis. Gidley and Bulpin (1987) showed that the kinetics of aggregation of amylose chains and the variation of gel strength with amylose concentration show a dependence on amylose chain length. These authors showed that precipitation and gelation occur for amylose CL of 250–660 residues whereas, for longer chains (>1100 residues), gelation predominates over precipitation. The results (Table 10) suggest that the differences in amylose CL (Table 2), amylopectin unit CL distribution (Table 3), and the number of branch points (in close proximity to disordered clusters of amylopectin) among the starches were not large enough to cause significant differences in the extents of syneresis.

4. Summary and conclusions

This study showed that differences in starch structure and properties could occur among the cultivars of the same species, even under identical environmental conditions. For instance, there were minor differences in structure and physicochemical properties among Car-

neval, Carrera and Grande. However, differences were much greater between Keoma and the above starches with respect to amylopectin branching, 'B' polymorph content, susceptibility towards α-amylase, and extent of retrogradation.

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