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Composition, molecular structure, and physicochemical properties of starches from two grass pea (*Lathyrus sativus* L.) cultivars grown in Canada

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Abstract

The morphology, molecular structure and physicochemical properties of starches from two cultivars (NC8A97, Lath 96) of grass pea (*Lathyrus sativus* L.) were examined. In both cultivars, starch granules were irregular to oval to round to elliptical shaped, with smooth surfaces. No significant differences was observed between the cultivars with respect to mean granule diameter (26.3–27.3 μm), mean granule length (32–34.5 μm), amylose content (37.95–38.30%), bound lipid content (0.08%), amylopectin chain length distribution, average chain length (19.2) and X-ray diffraction pattern ('C' type). However, NC8A97 and Lath 96 starches differed significantly with respect to the degree of crystallinity (Lath 96 > NC8A97), B-polymorphic content (Lath 96 > NC8A97), granular swelling (NC8A97 > Lath 96), extent of amylose leaching (NC8A97 > Lath 96), peak viscosity (Lath 96 > NC8A97), shear stability (Lath 96 > NC8A97), set-back (NC8A97 > Lath 96) and susceptibility towards enzyme and acid hydrolysis (NC8A97 > Lath 96). The results showed that physicochemical properties of the grass pea starches were influenced by the extent of interaction between starch chains (Lath 96 > NC897) in the amorphous regions, amount of crystallites (NC8A97 > Lath 96) and crystallite heterogeneity (NC8A97 > Lath 96).

Keywords: Grass pea starches; Structure; Physicochemical properties

1. Introduction

Legumes are the dicotyledonous seeds of plants that belong to the family Leguminosae, which contains about 600 genera and 13,000 species. Canada is the fifth largest legume producer (4.2 million tonnes; FAO, 2004), the total world production being 61 million tonnes (FAO, 2004). Legume seeds can be fractionated to obtain starch concentrates, protein concentrates and, as a by-product of the process, dietary fiber. Research investigations into all legume fractions are intensifying; particularly as food and non-food markets look towards plants for components with unique functionalities to meet consumer needs (Patterson, 2003). Starch is the most abundant carbohydrate in the legume seed (22–45%). Legume starches have been shown to have unique properties imparting resistance to hydrolysis by digestive enzymes (Hoover & Sosulski, 1991). The high resistant starch content of legume seeds may have a significant impact in eliciting a low glycemic response (Hoover & Sosulski, 1991). Grass pea (*Lathyrus sativus* L.) is a food, feed and fodder crop belonging to the family Leguminoseae, sub family papilinoideae, tribe viciae (Campbell, 1997). It is also known as kesari, chickling pea, chickling vetch, guaya and san lee dow. Grass pea is an important crop of economic significance in India, Bangladesh, Pakistan, Nepal and Ethiopia. The grass pea could become a useful rotation crop in the brown soil zone

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of the Canadian prairie, an area that lacks an adapted annual legume alternative. Much research has been devoted to understanding the structure–property relationships in pea and lentil starches. However, there is a dearth of information on the molecular structure and physicochemical properties of grass pea starches. Therefore, as part of our studies on legume starch functionality, it was considered worthwhile to investigate the molecular structure and physicochemical properties of starches isolated from two cultivars of grass pea grown under the same environmental conditions in Mordern, Manitoba, Canada.

2. Materials and methods

2.1. Materials

Two cultivars (Lath 96 and NC8A97) of grass pea seeds (*L. sativus*, L.) were obtained from Kade Research Limited (Morden, Manitoba). Crystalline porcine pancreatic α-amylase (type 1A, 790 units/mg protein), Isoamylase (68,000 units/mg protein) from *pseudomonas amylodermosa* were purchased from Hayashibara Biochemical Laboratories Ltd. (Okayama, Japan). Pure potato amylose (100% amylose, type III) and potato amylopectin (100% amylose free) were obtained from Sigma Chemical Co (St. Louis, MO, USA) and Fluka Biochemica, Buchs, Switzerland, respectively. All chemicals and solvents were of ACS certified grade.

2.2. Methods

2.2.1. Starch isolation

Grass pea seeds were steeped in deionized water (1:5 w/v) at 4 °C for 48 h. The softened seeds were wet milled in a Waring blender for 2 min and magma screened through a sieve of 200-mesh (W.S. Tyler, Mentor, OH), and centrifuged (4300*g*) for 20 min. The brownish grey sediment which accumulated on top of the white starch pellet during centrifugation was carefully scraped off and discarded. The pellet was then dispersed in deionized water (1:5) and centrifuged (4300*g*) for 20 min, with the brownish grey sediment scraped off after each wash. The process was repeated three times. The starch pellet was dried at 40 °C in a mechanical convection oven. The dried starch cake was ground and passed through an 80-mesh screen.

2.2.2. Granule morphology

The granule surface and shape was studied by scanning electron microscopy (SEM). 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 Philips 505 SEM (Philips, Eindhoven, NL) at an accelerating potential of 30 kV. The volume mean diameter and granule size distributions of the starches were determined by low angle light scattering using a Malvern Mastersizer (Model 2000 SM, Malvern Instruments Ltd., Malvern, UK). Deionized water was used as the dispersant at a starch concentration of 10% w/v.

2.2.3. Chemical composition of starch

Quantitative estimation of moisture, ash, and nitrogen were determined by the standard AACC (2000). Starch lipids were determined by procedures outlined in an earlier publication (Hoover, Swamidas, & Vasanthan, 1993).

2.2.4. Amylose content

Total amylose content was determined on defatted [hot-*n*-propanol–water (3:1 v/v) for 7 h] grass pea starches by the iodometric method of Chrastil (1987). Amylose content was calculated from a standard curve using mixtures of pure potato amylose and potato amylopectin (over the range 0–100% amylose).

2.2.5. Amylopectin branch chain length distribution

Isoamylase debranching of whole starch accompanied by high pressure anion exchange chromatography with pulsed ampermetric detection (HAPAEC-PAD) was used to determine the branch chain length distribution of the grass pea starches following the procedure of Jayakody, Hoover, Liu, and Weber (2005).

2.2.6. X-ray pattern and relative crystallinity

X-ray diffractograms were obtained with a Rigaku RPT 300 PC X-ray diffractometer (Rigaku–Denke Co., Tokyo, Japan). The hydrated samples (0.5 g dry basis) were packed tightly into an elliptical aluminum holder. The operating conditions were: target voltage 40 kV, target current 100 mA, aging time 5 min; scanning range 3–35°, step scan size 0.03°, scan speed 2.000°/min; step time 0.9 s, divergence slit width 1.0°; scatter slit width 1.0° and receiving slit width 0.6 mm. Starches for X-ray diffraction measurements were kept in a dessicator (at 25 °C) over saturated K_2SO_4 ($a_w = 0.98$) up to sorption equilibrium (20 days).

Crystallinity of the starches was quantitatively estimated following the method of Nara and Komiya (1983) by using a software package (Origin-version 6.0 Microcal Inc., Northampton, MA, USA). A line connecting 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 crystallinity. The moisture content of the samples was determined before and after scanning.

2.2.7. Gelatinization characteristics

Gelatinization parameters of native starches were measured using a Seiko differential scanning calorimeter (DSC 210; Seiko Instruments Inc., Chiba, Japan) equipped with a thermal analysis data station and data recording software. Water (11 μl) was added with a microsyringe to starch (3.0 mg db) in the DSC pans, which were then sealed, reweighed and allowed to stand overnight at room

temperature before DSC analysis. The scanning temperature range and the heating rates were 25–130 °C and 10 °C/min, respectively. In all measurements, the thermogram was recorded with an empty aluminum pan as a reference. During the scans, the space surrounding the sample chamber was flushed with dry nitrogen to avoid condensation. 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; four replicates per sample was analyzed.

2.2.8. Swelling factor

The swelling factor in the range 60–90 °C was determined by the method of Tester and Morrison (1990).

2.2.9. Amylose leaching

The extent of amylose leaching in the range 60–90 °C was determined by the method of Jayakody et al. (2005).

2.2.10. Pasting properties

A Rapid Visco Analyzer RVA-4 (Newport Scientific Pty. Ltd., Warmewood, NSW, Australia) was employed to measure the pasting properties of starches (7% db, 25 g total weight). Experiments were performed using AACC method 76-21 (AACC, 2000), in which the sample is equilibrated at 50 °C for 1 min, heated at 6 °C/min to 95 °C, held at 95 °C for 5 min, cooled at 6 °C/min to 50 °C, and held at 50 °C for 2 min. The speed was 960 rpm for the first 10 s, then 160 rpm for the remainder of the experiment. The reported values for pasting temperature, peak viscosity and final viscosity are the means of duplicate measurements.

2.2.11. Shear stability

Shear stability was determined for 5% (w/v) aqueous starch suspensions as described by Praznik, Mundigler, Kogler, Pelzl, and Huber (1999). Viscosity profiles were determined with a concentric cylinder geometry rheometer (Rheolyst AR 1000, TA instruments Inc., Newcastle, Delaware, USA) for shear rates of 100 S^{-1} (5 min), 1000 S^{-1} (5 min) and again at 100 S^{-1} (5 min). Shear stability was computed as the ratio of viscosity (η) at the end of the first period (η_{before}) and viscosity after the end of the second period (η_{after}) of shear stress in terms of shear stability percentage.

$$\text{Shear stability } (\%) = \frac{\eta_{after}}{\eta_{before}} \times 100$$

2.2.12. Acid hydrolysis

Starches were hydrolyzed with 2.2 N HCl at 35 °C (1 g db/40 ml acid) for periods ranging from 0 to 15 days. The extent of hydrolysis was estimated by the Somogyi–Nelson method (Nelson, 1944; Somogyi, 1952). Three replicates per sample were analyzed.

2.2.13. Enzymatic digestibility

Enzymatic digestibility studies on native starches were conducted using a crystalline suspension of porcine pancreatic α-amylase in 2.9 M sodium chloride containing 3 mM calcium chloride (Sigma Chemical Co., St. Louis, MO, USA), in which the concentration of α-amylase was 32 mg protein/ml and the specific activity was 1122 units/mg protein. Starch granules (20 mg db) were suspended in 10 ml of 0.02 M phosphate buffer (pH 6.9) containing 0.006 M NaCl. A 5.5 μl of α-amylase suspension was added, the mixture gently mixed and digested at 37 °C in a water bath (New Brunswick Scientific, G76D, Edison, NJ, USA) for 72 h. The reaction mixtures were vortexed on a daily basis to resuspend the deposited granules. The digestion reaction was terminated by adding 5 ml of absolute ethanol to the digestion mixture. The hydrolysate was recovered by centrifugation (at 2000 rpm/5 min) of the mixture. Aliquots of the supernatant were analyzed for reducing sugar content (Nelson, 1944; Somogyi, 1952). Controls without enzyme but subjected to the above experimental conditions were run concurrently. The reported values are the means of four replicates.

2.3. Statistical analysis

Analysis of variance (ANOVA) was performed by Tukey's HSD test ($P < 0.05$) using statistical software SPSS 14 for Windows (SSPS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Granule morphology

Microscopic examination showed that the starch granules of both NC8A97 and Lath 96 had irregular shapes, which varied from oval to round to elliptical. A large variability existed in starch granule length 12–60 μm [NC8A97], 10–55 μm [Lath 96] and diameter 18–32 μm [NC8A97], 17–30 μm [Lath 96]. The volume mean diameter and the surface area of Lath 96 and NC8A97 starch granules (determined using the Malvern Mastersizer) were 27.3 μm^3 and 0.161 m^2 and 26.3 μm^3 and 0.167 m^2, respectively. The granule surfaces of both cultivars appeared to be smooth and showed identations when viewed under the scanning electron microscope (Fig. 1a–d).

3.2. Chemical composition of the starches

The data on yield and composition are presented in Table 1. Starch yield from NC8A97 and Lath 96 were 21.1% and 25.5%, respectively. The purity of the starches was judged on the basis of composition (low nitrogen and low ash content) [Table 1] and microscopic examination. There was no significant difference between NC8A97 and Lath 96 starches with respect to total amylose (37.3–38.2%), surface lipid (0.05%) and bound lipid (0.08%) contents (Table 1). The yield and chemical compo-

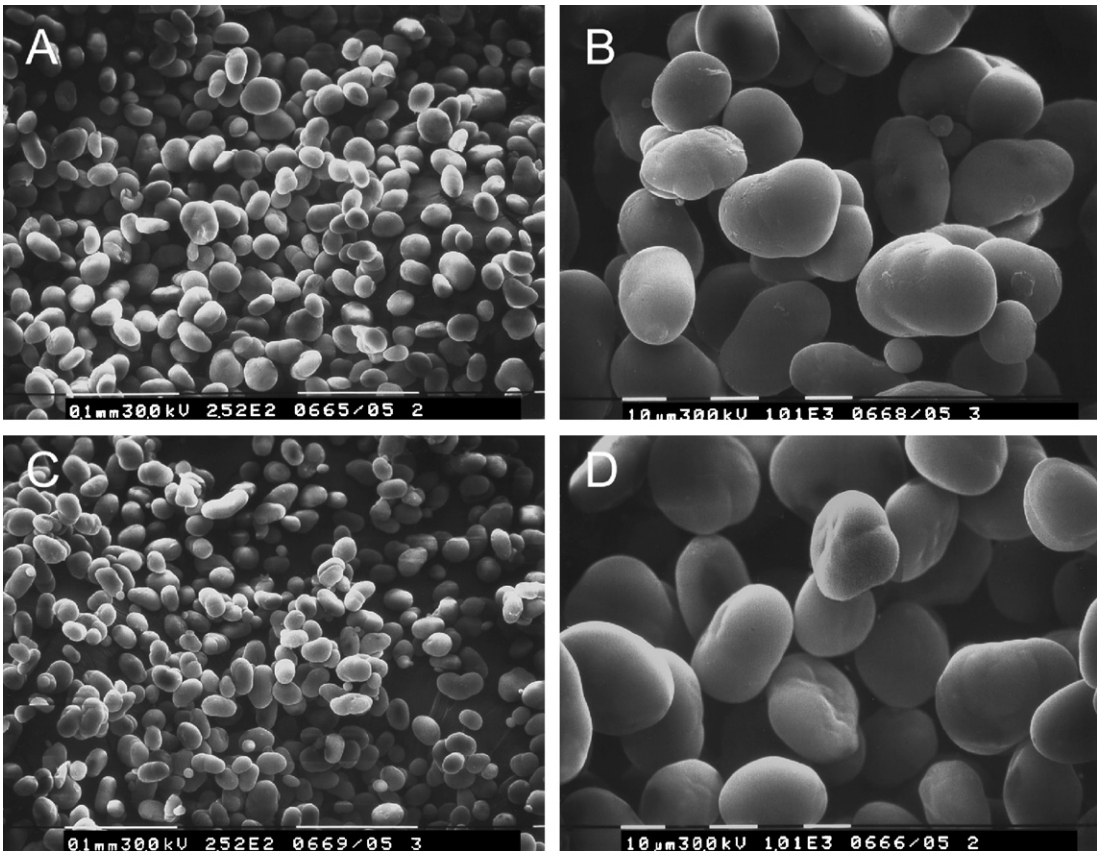

Fig. 1. Scanning electron micrographs of grass pea starches: A and B (NC8A97), C and D Lath 96, A and C (300×), B and D (1000×).

Table 1
Chemical composition (%), of native grass pea starches[A]

Characteristics	Cultivar	
	NC8A97	Lath 96
Starch yield (% initial material)	21.1	25.5
Nitrogen	0.11 ± 0.01^{a}	0.03 ± 0.01^{b}
Ash	0.15 ± 0.03^{a}	0.18 ± 0.04^{a}
Lipid (solvent extracted)		
Chloroform–methanol (2.1)[B]	0.05 ± 0.02^{a}	0.04 ± 0.01^{a}
1-Propanol–water (3:1)[C]	0.08 ± 0.01^{a}	0.08 ± 0.02^{a}
Total amylose content (% of total starch)[D]	38.30 ± 0.40^{a}	37.95 ± 0.50^{a}

[A] All data reported on dry basis and represent the means of three determinations. Means in each row with different superscripts are significantly different ($P < 0.05$).
[B] Lipids extracted from native starch by chloroform–methanol (2:1 v/v) at 25 °C (mainly unbound lipids).
[C] Lipids extracted by hot-1-propanol–water (3:1 v/v) from the residue left after chloroform–methanol extraction (mainly bound lipids).
[D] Total amylose was determined by iodine binding after removal of free and bound lipids.

sition of the grass pea starches was within the range reported for other legume starches (Chavan, Shahidi, Hoover, & Perera, 1999; Grelda, Farlas, Moreno-Valencia, del Refugio, & Barron-Hoyos, 1997; Hoover & Sosulski, 1985, 1991; Hoover & Ratnayake, 2002; Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001; Srisuma, Rueng-Sakulrach, & Ubersax, 1994).

3.3. Amylopectin branch chain length distribution

The chain length distribution and the average chain length (CL) of the debranched amylopectins of NC8A97 and Lath 96 starches were nearly similar (Table 2). The percentage distribution of chains with DP 6–12, DP 13–14, DP 25–36 and DP > 37 in NC8A97 and Lath 96 were within the range reported for other legume starches (Ratnayake et al., 2001; Yoshida et al., 2003). The CL in most legume starches have been shown to be in the range 20–25 (Biliaderis, Grant, & Vose, 1981; Ratnayake et al., 2001; Yoshida et al., 2003). However, the corresponding value for the grass pea starches (19.2–19.3) was lower (Table 2).

3.4. Gelatinization characteristics

The gelatinization transition temperatures [T_o (onset), T_p (mid point), T_c (conclusion)], gelatinization temperature range ($T_c - T_o$) and the enthalpy of gelatinization (ΔH) are presented in Table 3. The above parameters were significantly ($P < 0.05$) higher in NC8A97 starch (Table 3). T_o, T_p and T_c have been shown to be influenced by amylose content (Demenke, Huel, Abdel-Aal, Baga, & Chibbar, 1999; Inouchi et al., 1993; Stevenson, Domoto, & Jane, 2006; Visser, Suurs, Steeneken, & Jacobsen, 1997), distribution of amylopectin chains (Noda et al., 1998; Stevenson et al., 2006; Vandeputte, Vermeylen, Geerons, & Delcour,

Table 2
Branch chain length distribution and average chain length ($\overline{CL}$) of native grass pea starches[A]

Starch source	Distribution (%)[B]				
	DP 6–12[C]	DP 13–24[C]	DP 25–36[C]	DP 37–50[C]	(CL)[C]
NC8A97	18.8 ± 0.7[a]	59.2 ± 0.6[a]	17.5 ± 0.3[a]	4.5 ± 0.3[a]	19.3 ± 0.2[a]
Lath 96	19.1 ± 0.7[a]	59.2 ± 0.5[a]	16.8 ± 0.5[a]	4.8 ± 0.4[a]	19.2 ± 0.2[a]

[A] All data represent the mean of three replicates.
[B] Total relative area was used to calculate percent distribution.
[C] Values followed by the same superscript in each column are not significantly different ($p < 0.05$) by Tukey's HSD test.

2003) and lipid complexed amylose chains (Hoover & Ratnayake, 2002; Jayakody et al., 2005; Vandeputte et al., 2003). Cooke and Gidley (1992) have shown that ΔH reflects the loss of double helical order. Whereas, Tester and Morrison (1990) have postulated that ΔH reflects the overall crystallinity (quality and amount of crystallites) of amylopectin. As discussed earlier, the grass pea starches did not differ significantly with respect to amylose content, (Table 1) amylopectin branch chain length distribution (Table 2), and lipid complexed amylose chains (Table 1). This suggests, that the differences in T_o, T_p, T_c and ΔH between Lath 96 and NC8A97 (Table 3) starches, is likely due to a higher content of crystallites and/or to stronger interaction between the outer A chains of amylopectin in the latter. The difference in $T_c - T_o$ (NC8A97 > Lath 96) suggests a higher degree of heterogeneity in crystallites of NC8A97 starch. The T_o, T_p, T_c and ΔH of the grass pea starches were of a higher order of magnitude than those reported (T_o, 58–69 °C, T_p 64–71 °C, T_c 74–82 °C, ΔH 13.4–16.8 J/g) for other legume starches (Biliaderis et al., 1981; Chavan et al., 1999; Hoover & Sosulski, 1985; Hoover & Sosulski, 1991; Hoover, Li, Hynes, & Senanayake, 1997; Ratnayake et al., 2001; Tjahjadi & Breene, 1984).

3.5. X-ray diffraction

The X-ray pattern and crystallinity of the grass pea starches are presented in Fig. 2. Pure B-type starches such as potato and wrinkled pea have been shown to exhibit strong peaks at 2θ: 5.4°, 15.0°, 17°, 22° and 24° (Buléon, Bizot, Delage, & Pontoire, 1987; Hoover & Hadziyev, 1981; Wu & Sarko, 1978). Whereas, pure A-type starches such as wheat and maize have been shown to exhibit a shoulder at: (1) $2\theta = 18°$; (2) a unique peak around $2\theta = 23°$ instead of the doublet $2\theta = 22$–$24°$ and (3) an increase in the relative intensity of the band at $2\theta = 15°$. In this study both NC8A 97 and Lath 96 starches exhibited the characteristic C-type X-ray pattern of legume starches. (Chavan et al., 1999; Colonna, Buléon, & Mercier, 1981; Gernat, Radosta, Damaschun, & Schierbaum, 1990; Hoover & Sosulski, 1985). Gernat et al. (1990) have shown that the legume starch "C" crystalline polymorph is a mixture of 'A' and 'B' unit cells, and that these starches contain pure 'A' and 'B' polymorphs in varying proportions. In the grass pea starches, the 'C' pattern was characterized by a weak peak at $2\theta = 5.4°$ (characteristic of "B" polymorphs) and strong peaks at 17.5° and 23° 2θ (Fig. 2). The intensity of the peak at 5.4° 2θ was higher in Lath 96 (Fig. 2). This suggests that the proportion of 'B' unit cells in Lath 96 is higher than in NC8A97. The crystallinity of NC8A97, (33.0%) was significantly different ($P < 0.05$) from that of Lath 96 (34.0%). This was rather surprising, since the above starches did not differ in amylopectin structure (branch chain length distribution, $\overline{CL}$) [Table 2] amylose/amylopectin ratio (Table 1) or bound lipid content (Table 1). This suggests that the slightly higher crystallinity of Lath 96 could be due to better orientation of its crystallites to the X-ray beam. The crystallinity of the grass pea starches was within the range (17.0–34.0%) reported (Davydova, Leont'ev, Genin, Sasov, & Borgracheva, 1995; Hoover & Ratnayake, 2002; Ratnayake et al., 2001) for other legume starches.

3.6. Amylose leaching (AML) and swelling factor (SF)

The extent of AML and SF in the temperature range 60–90 °C are presented in Figs. 3 and 4, respectively. In both starches, AML increased dramatically between 60 and

Table 3
Gelatinization parameters[A] of native grass pea starches

Starch source	Gelatinization parameters[A]				
	T_o (°C)[B]	T_p[B] (°C)	T_c[B] (°C)	$T_c - T_o$ (°C)[C]	ΔH (J/g)[D]
NC8A 97	68.3 ± 0.23[a]	75.5 ± 0.07[a]	85.4 ± 0.35[a]	17.2 ± 0.63[a]	15.32 ± 0.29[a]
Lath 96	66.6 ± 0.00[b]	73.3 ± 0.14[b]	83.2 ± 0.00[b]	16.6 ± 0.00[b]	14.15 ± 0.10[b]

All data reported on dry basis and represent the mean of at least four replicates. Values followed by the same superscript in each column are not significantly different ($P < 0.05$) by Tukey's HSD test.
[A] Starch:water ratio = 1:3 (w/w dry basis).
[B] T_o, T_p, T_c, indicate the temperature of the onset, midpoint and end of gelatinization, respectively.
[C] $T_c - T_o$ indicates the gelatinization temperature range.
[D] Enthalpy of gelatinization ΔH.

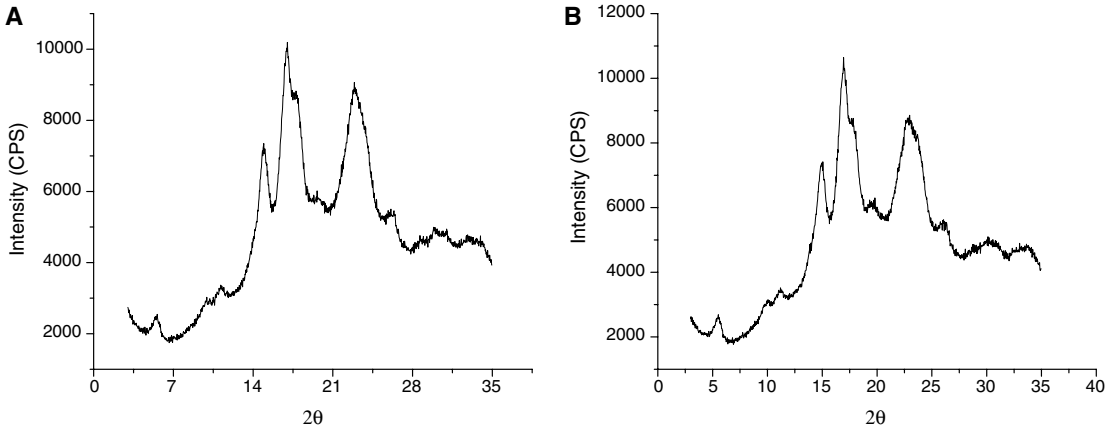

Fig. 2. X-ray diffraction patterns (C-type) of: (A) NC8A97 (moisture content 22.0%, crystallinity 33.0%) and (B) Lath 96 (moisture content 20.0%, crystallinity 34%) starches. The contribution of the peak centered at ~5.4° 2θ (characteristic of the B-polymorph) to total crystallinity was 2.3%, 2.7% in NC8A97, and Lath 96, respectively. The crystallinity values which were the means of four replicates were significantly ($p < 0.05$) different by Tukey's HSD test.

90 °C. A similar trend has also been observed for other legume starches (Chavan et al., 1999; Hoover & Manuel, 1995; Hoover & Sosulski, 1985; Ratnayake et al., 2001; Schoch & Maywald, 1968; Tolmasquim, Correa, & Tolmasquim, 1971). In the temperature range 70–90 °C, the extent of AML in NC8A97 was significantly ($P < 0.05$) higher than in Lath 96. (Fig. 3). This difference was most pronounced at temperatures beyond 80 °C. The data suggests, that interaction between amylase–amylose (AM–AM) and/or amylose–amylopectin (AM–AMP) chains are probably of a higher order of magnitude in Lath 96. This explanation seems plausible, since the starches did not differ significantly ($P < 0.05$) with respect to amylase–lipid complexes and amylose content (Table 1). The extent of AML at 90 °C was within the range (12.9–38.5%) reported for legume starches (Chavan et al., 1999; Hoover et al., 1997; Ratnayake et al., 2001). In both starches, significant granule swelling was evident only at temperatures beyond 60 °C (Fig. 4). At all temperatures, SF was significantly lower ($P < 0.05$) in Lath 96. This difference was more pronounced at temperatures exceeding 80 °C (Fig. 4). SF differences among starches has been shown to be influenced by: (1) lipid complexed amylose chains

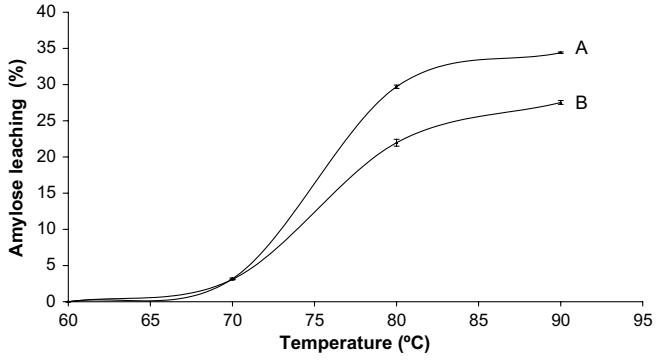

Fig. 3. Amylose leaching of grass pea starches at different temperatures: (A) NC8A97; (B) Lath 96 starches.

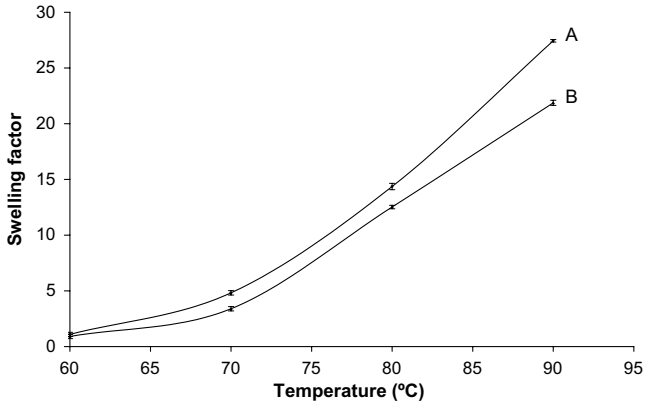

Fig. 4. Swelling factor of grass pea starches at different temperatures: (A) NC8A97; (B) Lath 96.

(Hoover & Manuel, 1995; Swinkels, 1985; Tester & Morrison, 1990); (2) amylose content (Morrison, Tester, Gidley, & Karkalas, 1993; Sasaki & Matsuki, 1998); (3) amylopectin structure (Sasaki & Matsuki, 1998; Shi & Seib, 1992; Srichuwong, Sunarti, Mishima, Isono, & Hismatsu, 2003; Tester, Morrison, & Schuiman, 1993), extent of interaction between starch chains in the native granule (Hoover & Manuel, 1996; Tester, Debon, & Sommerville, 2000) and (4) granule crystallinity (Jayakody et al., 2005). The results indicate that the higher degree of swelling exhibited by NC8A97 is likely influenced by the interplay of the following factors: (1) lower degree of crystallinity (Fig. 2); (2) lower degree of interaction between AM–AM and/or AM–AMP chains (Fig. 3), and (3) lower proportion of long branch chains (DP 37-50) [Table 2]. The SF data suggests that the differences in AML (NC8A97 > Lath96) between the two starches (Fig. 3) may also have been influenced by differences in granular swelling (NC8A 97 > Lath 96). The SF of NC8A97 and Lath 96 was within the range (12.2–43.0) reported (Hoover & Manuel, 1995; Ratnayake et al., 2001) for other legume starches.

3.7. Pasting properties

Pasting properties of the grass pea starches are presented in Fig. 5 and summarized in Table 4. NC8A97 exhibited lower peak viscosity, higher breakdown viscosity (peak viscosity–trough viscosity) and a higher set-back than Lath 96. Studies have shown that peak viscosities and shear stability are influenced by amylose content (Jane et al., 1999; Kuno, Kainuma, & Takahashi, 2000; Stevenson et al., 2006; Wang, White, & Pollak, 1993) and by the proportion of amylopectin branch chains of DP 13-24 and DP > 37 (Han & Hamaker, 2001; Li, Vasanthan, Rossnagel, & Hoover, 2001; Stevenson et al., 2006). In this study, Lath 96 and NC8A97 showed no significant difference ($P < 0.05$) in their amylopectin branch chain length distribution (Table 2) or amylose content (Table 1). However, these differences although small may have contributed to some extent to the observed differences in shear stability, peak viscosity and set-back in the grass pea starches. For instance, the slightly higher proportion of chains of DP 37–50 (Table 2) in Lath 96 could hold the integrity of the swollen granules for a longer period of time during the pasting process and thus may have contributed to its higher peak viscosity and lower extent of viscosity breakdown. Whereas, the higher amylose content of NC8A97 starch may have contributed to its higher set-back. We postulate, that the differences in shear stability and peak viscosity between the grass pea starches (Table 4) may have been also due to amylose chains in the amorphous regions being less compactly packed (weaker interactions between amylose chains) in NC8A97 (data from AML studies [Fig. 3]). Thus, when heated in water under shear, granule disintegration during swelling will be higher in NC8A97. This would then explain the lower peak viscosity and the high extent of viscosity breakdown during the heating cycle displayed by NC8A97 (Fig. 5). The higher set-back exhibited by NC8A97 (Fig. 5) could be attributed to its higher extent of amylose leaching (Fig. 3).

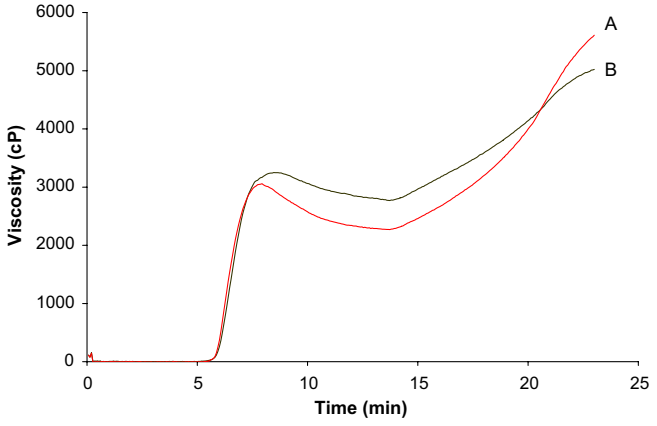

Fig. 5. RVA viscograms of grass pea starches: (A) NC8A97; (B) Lath 96.

3.8. Shear stability

The shear stability of grass pea starch suspensions determined using a Rheolyst AR1000 rheometer is presented in Table 5. The results showed that the drop in viscosity after the application of a shear stress of 1000 s^{-1} for 5 min was more pronounced in NC8A97. This data also suggests that interaction between amylose chains is weaker in NC8A97. This is in agreement with the RVA data (Table 4).

3.9. Acid hydrolysis

The extent of hydrolysis (2.2 N HCl at 35 °C) of the grass pea starches are presented in Table 6. Starches subjected to acid treatment have been shown to initially exhibit a faster rate of hydrolysis followed by a slower rate thereafter. The faster rate has been attributed to the hydrolysis of the amorphous domains (amorphous background and the thin amorphous lamella within the crystalline region)

Table 5
Shear stability[A] of native grass pea starch

	Cultivar	
	NC8A 97	Lath 96
$Viscosity_{before}$ (mPa)[B]	46.20 ± 0.60^{a}	55.42 ± 2.30^{b}
Shear stress		
$Viscosity_{after}$ (mPa)[C]	40.76 ± 1.20^{c}	51.53 ± 2.25^{d}
Shear stability (%)[D]	88.20	92.30

[A] 5% w/w starch suspension.
[B] Viscosity determined during the first 5 min under a shear stress of 100 S^{-1} (Shear rate of 100 S^{-1} was used to initiate rotation and to provide gentle mixing).
[C] Viscosity determined after the suspension was subjected to a shear rate of 100 S^{-1} (for 5 min) followed consecutively by shear rates of 1000 S^{-1} (for 5 min) and 100 S^{-1} (for 5 min).
[D] $\frac{\text{viscosity before}}{\text{viscosity after}} \times 100$.

Table 4
Pasting properties[A] of native grass pea starches

Starch Source	Peak time (min)	Pasting temperature (°C)	Peak viscosity during heating cycle (C_p)[D]	Trough viscosity (C_p)[D]	Breakdown in viscosity[B] (C_p)[D]	Final viscosity (C_p)[D]	Set-back[C] (C_p)[D]
NC 8A 97	7.9 ± 0.0^{a}	74.3 ± 0.7^{a}	3074 ± 22^{a}	2273 ± 6^{a}	806 ± 16	5622 ± 17^{a}	3349 ± 11
Lath 96	8.5 ± 0.1^{b}	74.1 ± 0.3^{b}	3226 ± 34^{b}	2735 ± 4.9^{b}	491 ± 16	5004 ± 26^{b}	2269 ± 23

[A] At 7% w/w starch suspension. Values followed by the same letter, in the same column are not significantly different ($p < 0.05$) by Tukey's HSD test.
[B] Peak viscosity during heating cycle–trough viscosity.
[C] Final viscosity–trough viscosity.
[D] Centipoise.

of the starch granule, whereas during the second stage, the crystalline regions are slowly degraded (Hoover, 2000; Jayakody & Hoover, 2002; Jayakody et al., 2005). The time taken for the degradation of the crystalline region by acid has been shown to vary widely (9–25 days) depending upon the starch source (Hoover, 2000; McPherson & Jane, 1999). Differences in the extent of acid hydrolysis among starches has been attributed to differences in: (1) granule size (Vasanthan & Bhatty, 1996); (2) the amount of lipid complexed amylose chains (Morrison et al., 1993; Waduge, Hoover, Vasanthan, Gao, & Li, 2006); (3) characteristics of the amorphous lamella (Srichuwong, Isono, Mishima, & Hisamatsu, 2005a); (4) proportion of amylopectin short chains with DP 6-12 relative to that with DP 6-24 (Srichuwong et al., 2005a), extent of interaction between starch chains (Chavan et al., 1999; Hoover & Manuel, 1996) and proportion of 'B' type unit cells (Gérard, Colonna, Buléon, & Planchot, 2002). In this study NC8A 97 was hydrolyzed to a greater extent than Lath 96 (Table 6). In both starches, the rate and extent of hydrolysis increased continuously during the time period of hydrolysis (15 days). However, the rate of increase was not uniform through the time course of hydrolysis. For instance, the rate of increase in hydrolysis beyond the 12th day was much higher than during the initial stages (Table 6). The continuous increase in hydrolysis suggests that the crystalline lamellae of the starch granules was not attacked during the time period of hydrolysis. If so, the rate of hydrolysis would have decreased. The results suggests that the increase in hydrolysis mainly reflects the action of H_3O^+ on the $\alpha(1 \rightarrow 4)$ glucosidic bonds of amylose chains in the bulk amorphous region and on the $\alpha(1 \rightarrow 6)$ glucosidic bonds present in the intercrystalline regions. We postulate, that near the granule surface, amylose chains may have been more compactly packed than amylose chains in the interior. Consequently, the rate of hydrolysis would not have been uniform. This would then explain the enhanced rate of hydrolysis beyond the 12th day, where the attack of H_3O^+ may have been on the $\alpha(1 \rightarrow 4)$ and $\alpha(1 \rightarrow 6)$ glucosidic bonds of loosely packed amylose chains and amylopectin, respectively. This hypothesis seems plausible, since, Lath 96 in which the amylose chains were more compactly packed was hydrolyzed to a lesser extent than NC8A97. The lower susceptibility of Lath 96 towards H_3O^+ could also be attributed to its higher proportion of longer amylopectin chains (DP > 37) and higher proportion of B-type unit cells (Fig. 2). Srichuwong et al. (2005a) and Vermeylen et al. (2004) have postulated that long amylopectin chains could form stable helices that are resistant to mild acid hydrolysis. Gérard et al. (2002) have shown by studies on maize mutant starches, that starch susceptibility to acid was lower and the number of residual structures greater as the amount of B-type crystallites increased. The resistance of B-type crystallites to acid hydrolysis was attributed to their intrinsic stability, three dimensional size or a greater degree of perfection as compared to A-type crystallites.

3.10. Enzyme hydrolysis

The susceptibility of the grass pea starches towards hydrolysis by porcine pancreatic α-amylase are presented in Table 7. After 72 h of hydrolysis, NC8A97 was hydrolyzed (80.5%) to a higher extent than Lath 96 (66.9%). Differences in vitro digestibility of starches among and within species has been attributed to differences in: (1) granule size (Snow & O'Dea, 1981); (2) amylose/amylopectin ratio (Hoover & Sosulski, 1985); (3) amount of amylose-lipid complexes (Holm et al., 1983; Hoover & Manuel, 1995); (4) amylose content (Behall, Scholfield, Yuhania, & Canary, 1989); (5) specific area and granule porosity (Gallant, Bouchet, Buléon, & Perez, 1992); (6) unit cell structure (Jane, Wong, & McPherson, 1997); (7) amylopectin chain length distribution (Srichuwong, Candra, Takashi, Naoto, & Makoto, 2005b) and (8) extent of interaction between starch chains (Cummings & Englyst, 1995; Dreher, Berry, & Dreher, 1984; Hoover & Sosulski, 1985). As shown in Tables 1 and 2 and Figs. 1 and 3, differences in the extent of α-amylase hydrolysis between NC8A97 and Lath 96 starches cannot be attributed to factors 1 to 7. Thus, it is likely that the main causative factor influencing the difference in susceptibility of Lath 96 and NC8A97 starches towards α-amylase hydrolysis is weaker interactions

Table 7
Hydrolysis of native grass pea starches by porcine pancreatic α-amylase at 37 °C[A]

Starch source	Hydrolysis (%)[B]
NC8A97	80.5 ± 2.5^{a}
Lath 96	67.0 ± 1.5^{b}

[A] All data reported on a dry basis.
[B] After 72 h. Means ± SD ($n = 3$). Hydrolysis values are significantly different ($p < 0.05$) by Tukey's HSD test.

Table 6
Acid hydrolysis of native grass pea starches

Starch source	Acid hydrolysis					
	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
NC8A 97	1.11 ± 0.18^{a}	12.91 ± 3.07^{c}	26.36 ± 1.07^{e}	45.31 ± 1.73^{g}	56.86 ± 0.31^{i}	79.12 ± 0.39^{k}
Lath 96	0.94 ± 0.01^{b}	9.18 ± 0.01^{d}	25.58 ± 0.09^{f}	43.58 ± 0.36^{h}	54.38 ± 0.22^{j}	71.34 ± 0.53^{l}

Data with the same superscript in the same column are not significantly different ($P < 0.05$) by Tukey's HSD test. All data reported on dry basis and represent the means ± SD of three determinations.

between starch chains within the amorphous domains of the latter.

4. Conclusion

The results showed that starches from the two cultivars (NC8A97, Lath 96) of grass pea showed no significant differences in chemical composition, granular morphology, amylopectin structure and X-ray pattern. However, in NC8A97 starch, degree of crystallite heterogeneity, gelatinization parameters, extent of viscosity breakdown during shear and heating, extent of set-back, granular swelling, amylose leaching and susceptibility towards acid and α-amylase were higher than in Lath 96. Whereas, crystallinity, proportion of 'B' unit cells, peak viscosity, shear stability and interaction between starch chains within the amorphous domains of the granule were lower than in Lath 96.

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