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A comparative study of the physicochemical properties of starches from two lentil cultivars

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Starches from two lentil (*Lens culinaris* Medik) cultivars (Laird and CC Gold) were isolated and some of the important characteristics determined. The yield of starch was 22% for Laird and 25% for CC Gold on a whole-seed basis. The shape of the starch granule was oval to elliptical to irregular, with granules 2·5–25 μm in diameter. Scanning electron micrographs revealed the presence of smooth surfaces. The total amylose content of Laird and CC Gold were 33·0 and 34·5%, respectively. The two starches differed with respect to the amount of amylose complexed by native lipids, granule crystallinity, the extent of granular swelling and amylose leaching, the degree of hydrolysis by porcine pancreatic α-amylase and 2·2 M HCl, pasting properties, gelatinization parameters and the extent of retrogradation.

INTRODUCTION

Legumes are the dicotyledonous seeds of plants that belong to the family Leguminosae, which contains about 600 genera and 13000 species. The lentil (*Lens culinaris* Medik) is an important legume crop in developing countries and is exclusively used in human foods. Canada has become the largest lentil producer in North America and the largest lentil exporter in the world. Canadian lentil production in 1992 was 350×10^3 tons, grown on 300×10^3 hectares (Bhatty, 1993). Saskatchewan, is the major lentil producing area in Canada. Lentil cultivars grown in Saskatchewan are Laird, commercial Chilean, Eston and CDC Richlea (yellow cotyledons), CDC Rose (red cotyledons) and CC Gold, (zero tannin, yellow cotyledon lentil). Recently, processes for the air-classification of finely ground lentils have permitted the fractionation of the flour into protein and starch concentrates, the latter product containing 75–80% of starch. However, due to the paucity of information about its physicochemical properties, the starch concentrate is not used in food formulations. Starches form different cultivars of wheat (Wootton & Mahdar, 1993), Proso millet (Yañez *et al.*, 1991), oat (Paton, 1977), rye (Gudmundsson & Eliasson, 1991) and cassava (Asaoka *et al.*, 1991) have been shown to vary in protein, ash, lipid, amylose content, crystallinity and gelatinization properties. However, such variations in starch composition and properties between legume cultivars have not been the subject of a detailed study. Differences in starch physicochemical properties between cultivars will affect the functional properties of the starch and its suitability for specific end use. Furthermore, the rate and extent of retrogradation in legume starches has received only scant attention. Therefore, as a part of studies in improving the functionality of legume starches, it was considered worthwhile to investigate the chemical composition, thermal properties, rheological behavior and the gelation and crystallization mechanism in starches from two lentil cultivars grown at the same location and in the same year in Saskatchewan. Such a study would form the basis for further investigations on physical and chemical modification to improve the functionality of lentil starches.

MATERIALS AND METHODS

Materials

Lentil (*Lens culinaris* Medik) cultivars (Laird, CC Gold) were grown on experimental plots at the University of Saskatchewan. Crystalline porcine pancreatic α-amylase (EC 3211) type 1A was obtained from Sigma Chemical Co. (St Louis, MO, USA). Other chemicals and solvents were analytical grade. Solvents were distilled from glass before use.

Methods

Starch isolation

Starch was isolated from lentil seeds by the procedure outlined in an earlier publication (Sosulski *et al.*, 1989).

Chemical composition of starch
Quantitative estimations of moisture, ash and nitrogen were performed by the standard AACC (1984) procedures. Starch lipids were determined by procedures outlined in an earlier publication (Vasanthan & Hoover, 1992). Apparent and total amylose content were determined by the method of Chrastil (1987).

Swelling factor
The swelling factor 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 swelling factor at a given temperature. The swelling factor is reported as a ratio of the volume of swollen starch granules to the volume of the dry starch. Results used for calculation were means of triplicate measurements.

Extent of amylose leaching
Various concentrations of the starches (15–20 mg) in water were heated in volume calibrated sealed tubes (50–95°C) for 30 min. The tubes were then cooled to ambient temperature and centrifuged at 2000 *g* for 10 min. The supernatant liquid (1 ml) was withdrawn and its amylose content was determined by the method of Chrastil (1987). Results used for calculation were means of triplicate measurements.

X-ray diffraction
X-ray diffractograms were obtained with a Rigaku RU 200R X-ray diffractometer with a chart speed of 20 mm/min. The operation conditions were as described elsewhere (Hoover & Vasanthan, 1994).

Pasting behaviour
A Brabender viscoamylograph (Model VA-V) equipped with a 700-cm cartridge was used to study pasting properties at a concentration of 6% (w/v). Two replicates were used for this determination.

Differential scanning calorimetry
Gelatinization temperatures were measured and recorded on a Perkin–Elmer DSC-2 (Norwalk, CT, USA) differential scanning calorimeter (DSC), equipped with a thermal analysis data station, as reported previously (Hoover & Vasanthan, 1994). All DSC experiments were replicated three times.

Enzymatic digestibility
Enzymatic digestibility studies on lentil starches were done using a crystalline suspension of porcine pancreatic α-amylase in 0·5 M saturated sodium chloride containing 3 mM calcium chloride (in which the concentration of α-amylase was 23·9 mg/ml and the specific activity was 1240 units per milligram of protein. The details of the procedure have been outlined in an earlier publication (Hoover & Vasanthan, 1994). The above experiment was replicated twice.

Scanning electron microscopy
Granule morphology and the mode of action of α-amylase were studied by scanning electron microscopy (SEM). Specimen preparation and SEM were carried out by procedures outlined in an earlier publication (Hoover & Sosulski, 1985).

Acid hydrolysis
Lentil starches were hydrolyzed with 2·2 M HCl at 35°C (1·0 g starch/40 ml acid) for periods ranging from 2 to 15 days. The extent of hydrolysis was estimated by the procedure described elsewhere (Hoover & Vasanthan 1994). Results used for calculation were means of triplicate measurements.

Gel preparation
Gels (40%, w/v) were prepared as described by Krüsi and Neukom (1984). Lentil starches (4 g dry basis) were carefully weighed into circular aluminum molds (diameter 3·0 cm, height 3·0 cm) with removable tops and bases and then mixed with 10 ml of distilled water containing 0·02% $Na_2S_2O_5$ as preservative. The molds were then heated in a water bath at 95°C for 30 min. The resulting gels were allowed to cool within the molds for 30 min at 4°C prior to storage at 25°C for periods ranging from 1 to 15 days.

Gel powder preparation
The extent of retrogradation of stored lentil starch gels (at 25°C) was followed by X-ray diffraction and DSC. The procedure (with minor modifications) of Roulet *et al.* (1988) was used to convert the stored gels to a powder prior to examination by DSC and X-ray diffraction. The gels were rinsed with water, cut into small pieces and mixed with 100 ml of acetone. After homogenization using a polytron, the mixture was left to decant for 5 min. The liquid was discarded and the rest was transferred to screw-cap tubes. Acetone was again added, the mix centrifuged (3000*g*) and the supernatant discarded. This procedure was repeated three times and the remaining mass was dried in an air-oven for 6 h at 30°C.

Gel strength
The resistance to penetration of the gels during storage at 25°C was determined with a model 6000 R Lloyd texture testing machine (Omnitronix Instruments Ltd, Mississauga, Ontario, Canada) equipped with a data acquisition and processing station (Lloyds Instruments Inc.). The 5 and 50 N load cells were used. The gels within the aluminum molds were placed on the compression table. The load cell was fitted to the cross-beam, and driven down so as just to touch the gel surface. The cylindrical probe (5 mm diameter) was then driven at a constant speed (0.5 mm/min) into the gel for a distance of 6 mm. The load at 1 mm compression was termed firmness. The resulting readings were in units of load grams. Values are the means of three replicates.

Freeze–thaw stability
The gels (6% db) were subjected to cold storage at 4°C for 16 h (to increase nucleation) and then frozen at

–16°C. To measure freeze–thaw stability, the gels frozen at –16°C for 24 h were thawed at 25°C for 6 h and then refrozen at –16°C. Five cycles of freeze–thaw were performed. The excluded water was determined by centrifuging the tubes (30 diameter × 100 mm) at 1000 *g* for 20 min after thawing. Values are the means of three replicates.

RESULTS AND DISCUSSION

Morphological granular characteristics of the starches

The granules of the lentil cultivars were morphologically similar to other legume starch granules (Naivikul & D'Appolonia, 1979; Hoover & Sosulski, 1991). The starch granules appeared to be round, oval and elliptical, with characteristic dimensions in the range 2·5–25 μm. The surfaces appeared to be smooth and showed no evidence of fissures when viewed under the scanning electron microscope (Fig. 1).

Chemical composition of the starch

The data on composition and yield are presented in Table 1. The purity of the starch was judged on the basis of composition and microscopic observations. The yield of starch from Laird and CC Gold was 22 and 25%, respectively, on total-seed basis. This was within the range reported for other legume starches (Hoover & Sosulski, 1991). The nitrogen content was 0·15 and 0·20% in Laird and CC Gold, respectively. These low values indicated the absence of non-starch lipids (lipids associated with endosperm proteins). Therefore, total lipids (obtained by acid hydrolysis) in Laird (0·38%) and CC Gold (0·27%) mainly represent the free and bound starch lipids.

The total lipid content (Table 1) was beyond the range reported for other legume starches (Hoover & Sosulski, 1991). The amount of free lipids (obtained by extraction with chloroform–methanol) were almost identical in both starches (Table 1). However, the bound lipids (obtained by extraction of chloroform–methanol residues with *n*-propanol–water) (Table 1) was greater in Laird (0·35%) than in CC Gold (0·13%). The bound lipid content of Laird starch was beyond the range (0·10–0·23) reported for other legume starches (Hoover & Sosulski, 1991; Hoover *et al.*, 1991; Vasanthan & Hoover, 1992).

The apparent and total amylose content of Laird starch was 34·5 and 39·4%, respectively, while the corresponding values for CC Gold were 33·0 and 35·4%. These were within the range (21·2–65%) reported for other legume starches (Hoover & Sosulski, 1991). A comparison of the apparent and total amylose contents (Table 1) showed that 5·6 and 12·4% of the total amylose was complexed by native starch lipids in CC Gold and Laird, respectively. The above values were higher

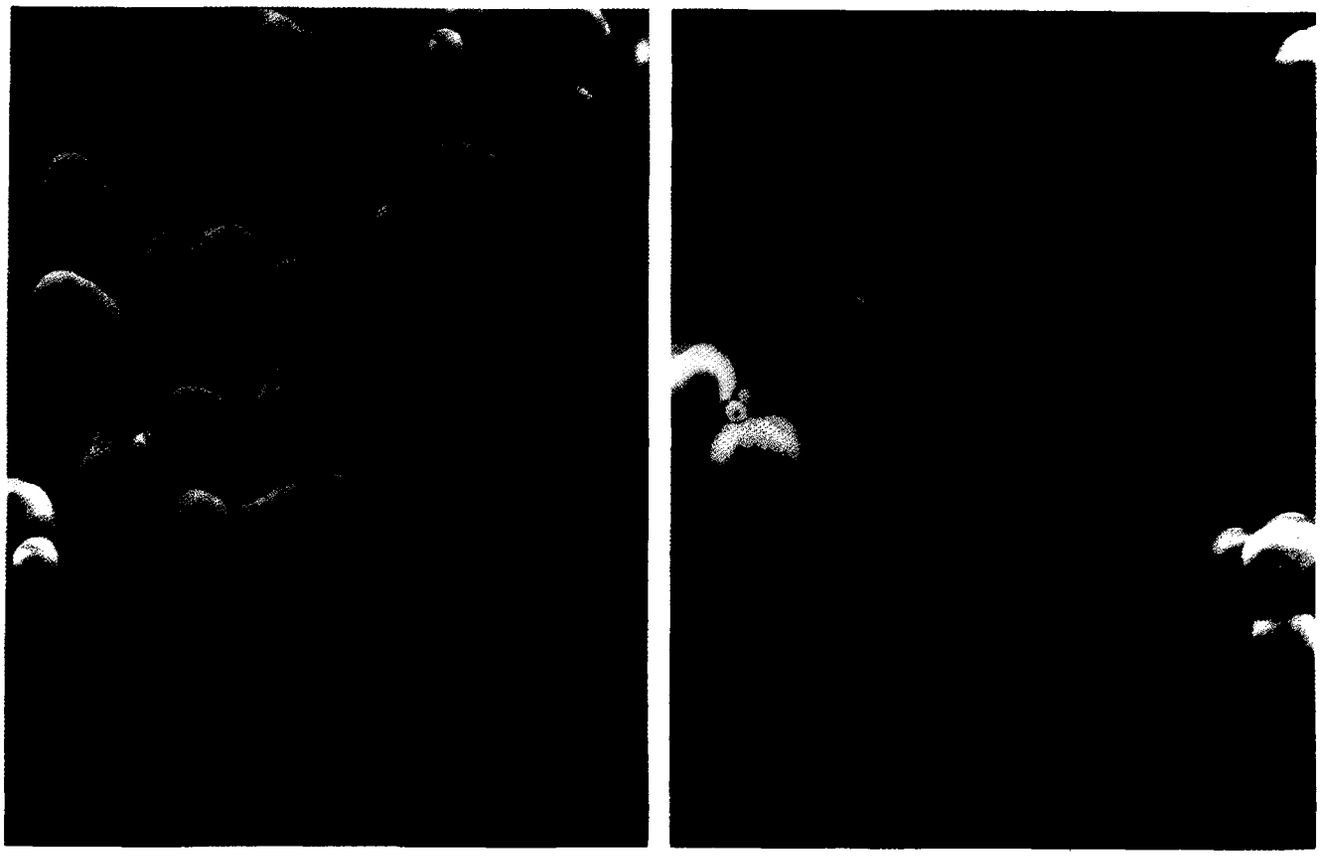

Fig. 1. Scanning electron micrographs of native lentil starches: (A) Laird; (B) CC Gold.

Table 1. Chemical composition (%) of lentil starches[a]

Characteristic	Starch source	
	Laird	CC Gold
Yield (% initial material)	22·0	25·0
Moisture	9·6 ± 0·1	9·6 ± 0·1
Ash	0·10 ± 0·01	0·10 ± 0·01
Nitrogen	0·15 ± 0·01	0·20 ± 0·02
Lipid		
Acid-hydrolyzed[b]	0·38 ± 0·04	0·27 ± 0·03
Solvent-extracted		
Chloroform–methanol[c]	0·03 ± 0·01	0·04 ± 0·01
n-Propanol–water[d]	0·35 ± 0·02	0·13 ± 0·01
Amylose content (% of total starch)		
Apparent[e]	34·5 ± 0·3	33·0 ± 0·4
Total[f]	39·4 ± 0·2	35·4 ± 0·2
Amylose complexed with native lipid[g]	12·4	5·6
Starch granule characteristics		
Granular shape	Oval to elliptical to round	Oval to elliptical to round
Granular size (μm)	2·5–25	2·5–25

[a]All data reported on dry basis and represent the mean of three determinations.
[b]Lipids obtained by acid hydrolysis (24% HCl) of the native starch (total lipids).
[c]Lipids extracted from native starch by chloroform–methanol 2:1 (v/v) at 25°C (mainly unbound lipids).
[d]Lipids extracted by hot *n*-propanol–water 3:1 (v/v) from the residue left after chloroform–methanol extraction (mainly bound lipids).
[e]Apparent amylose was determined by iodine binding without removal of free and bound lipids.
[f]Total amylose was determined by iodine binding after removal of free and bound lipids.
[g]$\frac{\text{Total amylose} - \text{Apparent amylose} \times 100}{\text{Total amylose}}$

than that reported for pigeon pea (2·7%) starch (Hoover *et al.*, 1993).

X-ray diffraction

The lentil starches showed the characteristic C-pattern of legume starches (Lai & Varriano-Marston, 1979; Colonna *et al.*, 1981; Hoover & Sosulski, 1985; Gernat *et al.*, 1990). In Laird the X-ray pattern was characterized by two strong intensity lines at 5·17 and 5·11 Å and three medium lines at 5·93, 5·33 and 5·26 Å; in CC Gold the strong intensity lines occurred at 5·21 and 5·14 Å and the medium intensity lines at 5·88, 5·26 and 3·80 Å. The intensity of the above peaks was higher in Laird than in CC Gold. This indicated that crystallites within granules of Laird starch are probably more compactly packed and/or are better oriented (to diffract X-rays) than those of CC Gold starch.

Swelling factor and amylose leaching

The swelling factor (SF) and amylose leaching (AML) were investigated over the temperature range 50–95°C. The results are presented in Figs 2 and 3, respectively. The SF and AML of both starches were within the range reported for other legume starches (Vasanthan and Hoover, 1992; Hoover *et al.*, 1993). The SF of CC Gold was generally higher than that of Laird (Fig. 2), whereas AML occurred to a greater extent in Laird than in CC Gold (Fig. 3). In both starches, SF and AML increased dramatically between 70 and 80°C. Similar rapid increases have also been observed to occur within temperature ranges of 60–90°C in other legume starches (Schoch & Maywald 1968; Tolmasquim *et al.*, 1971; Wankhede & Ramteke, 1982; El Faki *et al.*, 1983; Hoover & Sosulski 1985). Hoover and Sosulski (1985) have postulated that this may be due to melting of the crystallites, which may involve a solvation assisted helix → coil transition of their chains

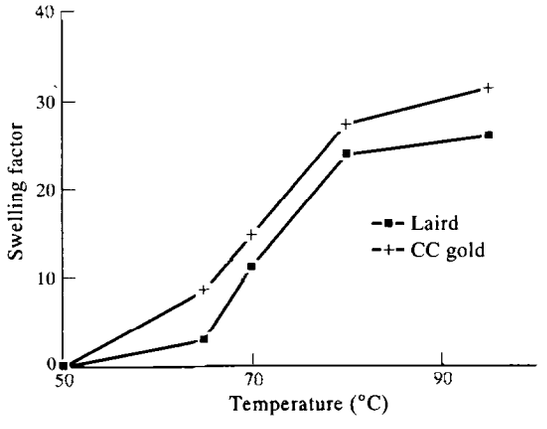

Fig. 2. Swelling factor of native lentil starches: (–■–) Laird; (–+–) CC Gold.

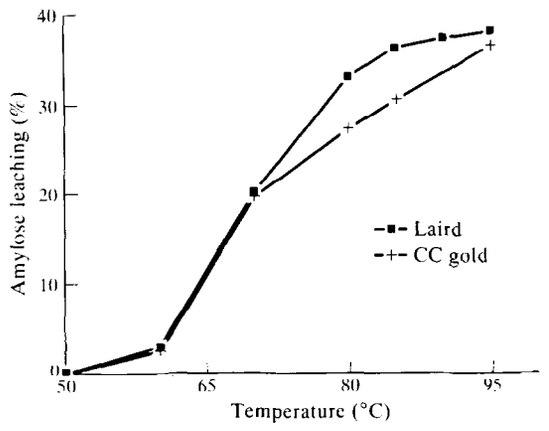

Fig. 3. Amylose leaching in native lentil starches: (–■–) Laird; (–+–) CC Gold.

(Biliaderis *et al.*, 1980). This in turn would result in a gain in entropy that would offset the hydrogen bonding occurring in the crystalline regions, leading to increased swelling and solubility. The differences in SF (Fig. 2) suggest that the degree of crystallite packing within starch granules of CC Gold are probably of a lower order of magnitude than in Laird.

Pasting characteristics

The pasting characteristics of the starches at a concentration of 6% (w/v) and pH 5·5 are presented in Table 2. At this pH and concentration most legume starches (Schoch & Maywald, 1968; Hoover *et al.*, 1993) exhibit pasting temperatures in the region 65–87°C, 95°C viscosities greater than 80 Brabender units (BU) and a gradual increase in viscosity (40–140 BU) during the holding period at 95°C. The pasting curves of both starches were typical of legume starches. In comparison to Laird, CC Gold exhibited a higher pasting temperature, a lower viscosity during heating to 95°C and lower viscosities both at 95 and 50°C. As seen in Fig.2, the SF of CC Gold is higher than that of Laird. This suggests that granules of CC Gold would be fragile and hence more susceptible to the shearing forces encountered during the pasting process than those of Laird. This would then explain the differences in the pasting parameters of these starches.

Gelatinization parameters

The gelatinization transition temperatures T_o (onset), T_p (mid-point), T_c (conclusion) — and the enthalpy of gelatinization (ΔH) of Laird starch were 55·0, 62·0, 73·0°C and 3·2 cal/g, respectively. The corresponding values for CC Gold were 52·2, 61·2, 69·0°C and 2·1 cal/g. T_o, T_p, T_c, T_c–T_o (gelatinization temperature range) and the ΔH of Laird and CC Gold starches were comparable to those of other legume starches (Hoover & Sosulski, 1991). However, the gelatinization temperatures of CC Gold were lower than those of Laird. This suggests that the two starches may differ with respect to crystallite size and/or the degree of crystallite association within the granule. Recently, Cooke and Gidley (1992) showed by X-ray spectroscopy and ^{13}C solid state NMR that ΔH values reflect mainly the loss of double-helical order than crystalline register. Therefore, the differences in ΔH between these two starches probably reflect differences in the number of double helices (within amorphous and crystalline regions of the granule) unravelling and melting during gelatinization.

***In-vitro* digestibility**

The extent of α-amylase hydrolysis of the starches is presented in Table 3 and Fig. 4. CC Gold was hydrolyzed to a much greater extent than Laird (Table 3). However, the amount of hydrolysis was lower than that reported for other legume starches (Hoover & Sosulski, 1985). For instance, after 6 h of hydrolysis, Laird and CC Gold were hydrolyzed to the extent of 5·8 and 11·9%, respectively. However, during the same

Table 2. Pasting characteristics of lentil starches[a]

Starch source	Pasting temperature (°C)	Highest viscosity reached during heating to 95°C (BU)[b]	Viscosity at 95°C (BU)	Viscosity after 30 min at 95°C (BU)	Viscosity at 50°C (BU)
Laird	75	250	200	250	410
CC Gold	83	180	140	180	280

[a]Values obtained using 6% starch and averaged over two determinations.
[b]Brabender units.

Table 3. The course of hydrolysis (%) of native lentil starches by porcine pancreatic α-amylase[a]

	Hydrolysis time (h)					
Starch source	0·5	1·0	6·0	24	48	72
Laird	0·5 ± 0·3	2·0 ± 0·2	5·8 ± 0·1	16·5 ± 1·5	23·8 ± 1·5	22·3 ± 2·0
CC Gold	1·3 ± 0·3	3·1 ± 0·1	11·9 ± 0·2	35·1 ± 1·8	43·1 ± 1·0	50·3 ± 2·5

[a]The data represents the mean of three determinations.

time interval, granules of black bean, navy bean, northern bean, pinto bean and kidney bean have been reported (Hoover & Sosulski, 1985) to be hydrolyzed to the extent of 34·8, 32, 29, 25·2 and 31·4%, respectively,

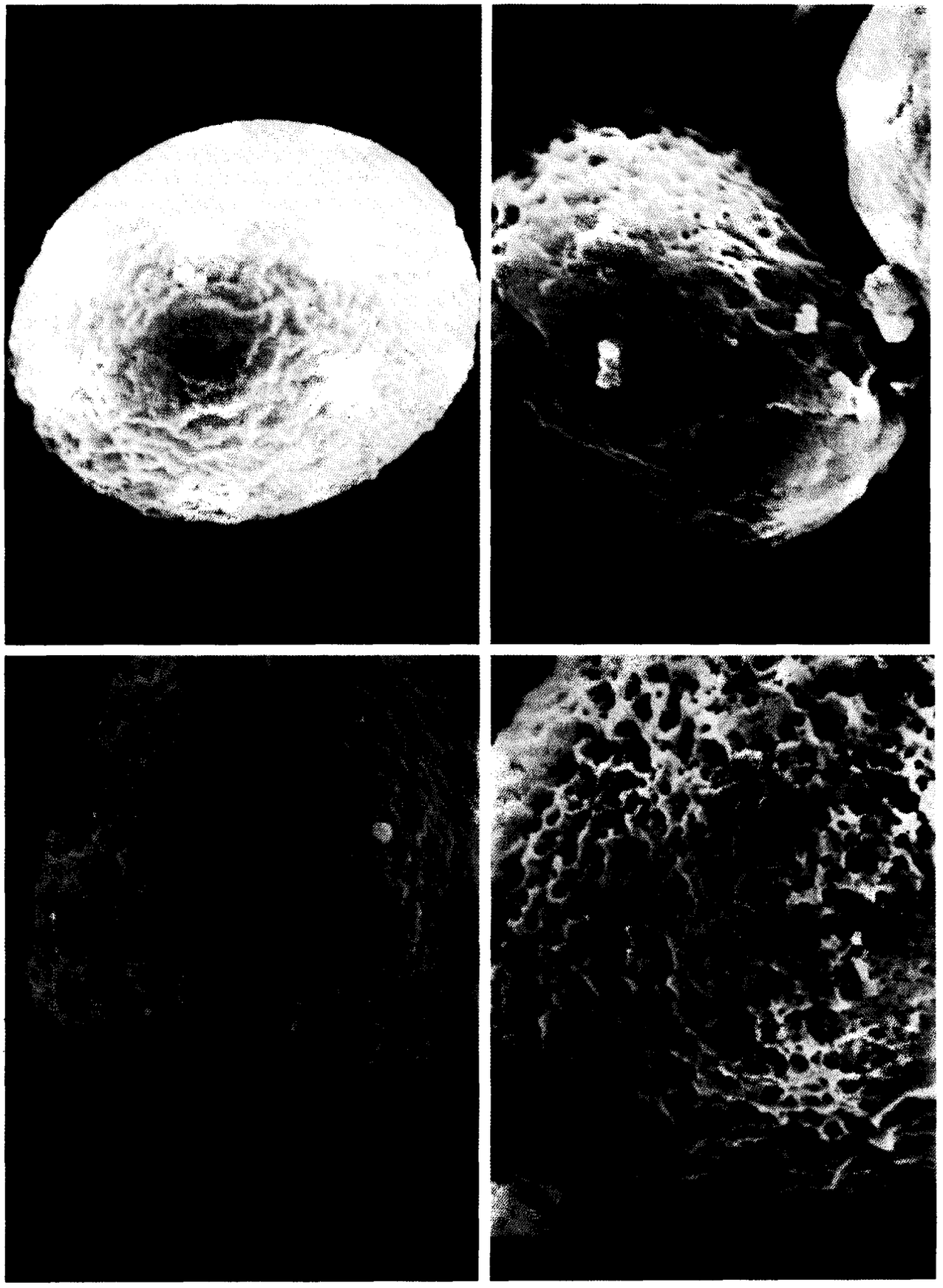

Fig. 4. Scanning electron micrographs of α-amylase hydrolyzed native starch granules: (A) Laird after 24 h hydrolysis; (B) CC Gold after 24 h hydrolysis; (C) Laird after 72 h hydrolysis; (D) CC Gold after 72 h hydrolysis.

by porcine pancreatic α-amylase (concentration of enzyme used was the same as in this study).

The 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 *et al.*, 1988), granule size (Snow & O'Dea, 1981; Ring *et al.*, 1988), amylose–lipid complexes (Holm *et al.*, 1983), starch–protein interaction (Würsch *et al.*, 1986) and amylose/amylopectin ratio (Dreher *et al.*, 1984; Hoover & Sosulski, 1985; Holm & Björck, 1988; Ring *et al.*, 1988). It is likely that, since differences between the two starches with respect to granule size, protein content and amylose/amylopectin ratio are only marginal (Table 1), the major factor influencing the degree of susceptibility of Laird and CC Gold towards hydrolysis by α-amylase (Table 3) is probably the higher proportion of amylose-complexed lipids in the former (Table 1). Thoma (1968) postulated that the enzyme-catalyzed hydrolysis of the α-D-(1→4)-glucosidic bonds of the starch molecule involves enzyme-induced ring distortion of one of the D-glucosyl residues from the $^4C_1$ chair conformation to a 'half chair' conformation. This ring distortion decreases the enthalpy of activation and increases the susceptibility of the glucosyl residues to nucleophilic attack by functional groups on the enzyme and water. László *et al.* (1978) have shown that ring distortion or a 'half chair' conformation is involved in the transition state of α-amylase. It is therefore plausible that conformational changes (chair → half chair) during α-amylase hydrolysis may be difficult for those amylose chains that are complexed by native lipids (due to decreased chain flexibility). This would then explain the differences in the degree of susceptibility of Laird (12·4% of amylose complexed by lipid) and CC Gold (5·6% of amylose complexed by lipid) starches towards hydrolysis by α-amylase.

The mode of attack by α-amylase on Laird and CC Gold starches (Fig. 4) was investigated by SEM. After 24 and 72 h hydrolysis, the surface of Laird starch (Figs 4A, C) exhibited highly roughened surfaces which were covered with numerous fissures. However, during the same time interval, the surface of CC Gold (Figs 4B, D) were covered not only with fissures but with numerous craters of varying size and depth, as if the α-amylase had entered the granule and preferentially hydrolyzed the interior portion.

The thermal characteristics of the residues left after attack (24 and 72 h) by α-amylase are presented in Table 4. In both starches, the thermal transition temperatures increased after enzyme hydrolysis. These increases were more pronounced in CC Gold. After 72 h hydrolysis, the increase in T_o, T_p and T_c were 11·3, 5·8 and 12°C, respectively, in CC Gold, whereas the corresponding values for Laird were 3·2, 3·5 and 4·2°C. In both starches, ΔH was only marginally affected by enzyme hydrolysis (Table 4). These results also suggests that amylose–lipid complexes are resistant to attack by α-amylase.

Acid hydrolysis

The solubilization pattern of the starches are presented in Table 5. Both starches exhibited a two-stage solubilization pattern. A relatively higher rate was observed during the first 6 days, followed by a lower rate thereafter (Table 5). The percentage of solubilization of CC Gold was slightly higher than that of Laird throughout the time-course of hydrolysis. At the end of the 8th day of hydrolysis (corresponding to the degradation of the amorphous regions of the granule), CC Gold and Laird were hydrolyzed to the extent of 27·7 and 24·4%, respectively. These values are comparable to that reported for pigeon pea (Hoover *et al.*, 1993) and mung bean (Biliaderis *et al.*, 1981) starches.

Table 4. Differential scanning calorimetry characteristics of enzyme-treated granular residues following hydrolysis with porcine pancreatic α-amylase

Starch source	Time of hydrolysis (h)	Transition temperatures (°C)[a] T_o	T_p	T_c	ΔH (cal/g)[b]
Laird	24	56·2 ± 0·1	64·0 ± 0·2	78·0 ± 0·1	3·4 ± 0·1
	72	57·5 ± 0·1	67·0 ± 0·2	80·0 ± 0·2	3·1 ± 0·7
CC Gold	24	58·2 ± 0·2	65·5 ± 0·1	77·2 ± 0·2	2·1 ± 0·1
	72	63·5 ± 0·2	67·0 ± 0·1	81·0 ± 0·3	2·0 ± 0·1

[a]Transition temperatures: T_o (onset); T_p (mid-point); T_c (conclusion).
[b]Enthalpy of gelatinization.

Table 5. Heterogenous acid hydrolysis (%) of lentil starches[a]

Starch source	Hydrolysis time (days)						
	2	4	6	8	10	12	15
Laird	3·8 ± 0·1	11·6 ± 0·2	18·4 ± 0·1	24·4 ± 0·1	28·9 ± 0·2	32·7 ± 0·1	34·8 ± 0·7
CC Gold	4·3 ± 0·1	13·3 ± 0·1	20·1 ± 0·2	27·7 ± 0·1	31·5 ± 0·1	35·3 ± 0·1	38·7 ± 0·7

[a]The data represents the mean of three determinations.

During treatment of starch granules with mineral acids at temperatures below the gelatinization temperature, the amorphous regions are more rapidly attacked than are the crystalline regions (Kainuma & French 1971). Therefore, the observed extent of hydrolysis during the first 8 days (CC Gold > Laird) seems to suggest that the amorphous regions of CC Gold are more accessible than those of Laird to the entry of H_3O^+.

The X-ray diffraction intensities of the residues resulting from acid hydrolysis (2 and 10 days) of Laird and CC Gold starches are presented in Table 6. In both starches, the d-spacings of the major peaks showed only marginal changes on acid hydrolysis. However, the intensities of the peaks increased with hydrolysis time (Table 6). The extent of this increase (CC Gold > Laird) after the 10th day of hydrolysis was almost double that of the native starch. The increase in intensity could be attributed to the removal of amorphous material. The higher increase in intensity for CC gold starch suggests that its amorphous regions are less compactly packed than those of Laird.

It is therefore plausible that the greater susceptibility of CC Gold to α-amylase hydrolysis (Table 3) may have also been due to the greater accessibility of the amorphous region to penetration by α-amylase.

The thermal characteristics of acid-treated (2 days) granular residues of lentil starches are presented in Table 7. T_o, T_p and T_c increased (CC Gold > Laird) on acid hydrolysis (2 days). However, in both starches the gelatinization temperature range (T_c–T_o) remained unaffected by hydrolysis. The ΔH decreased (Table 7) substantially on hydrolysis. The extent of this decrease was approximately 19 and 43% in Laird and CC Gold, respectively.

The large increase in T_o, T_p and T_c may have been due to the action of H_3O^+ on starch chains in the amorphous regions. Scission of these chains would remove destabilizing constraints on the crystalline areas of the

Table 6. X-ray diffraction spacings and intensities of the major peaks of the residues resulting from acid hydrolysis of lentil starches[a]

Starch source	Hydrolysis time (days)	Moisture content (%)	Interplanar spacing (d) in Å with intensities (CPS)[b]		
Laird	0	9·6	5·93 (630)	5·17 (998)	3·90 (762)
	2	9·5	5·93 (680)	5·16 (1021)	3·88 (834)
	10	9·7	5·84 (1386)	5·16 (1969)	3·89 (1434)
CC Gold	0	9·6	5·88 (481)	5·21 (861)	3·80 (699)
	2	9·6	5·84 (587)	5·18 (931)	3·83 (801)
	10	9·6	5·86 (1260)	5·20 (2048)	3·80 (1425)

[a]X-ray diffraction intensities were recorded on the residues obtained after hydrolysis with 2·2 M HCl at 35°C.
[b]Counts per second.

Table 7. Differential scanning calorimetry of acid-treated granular residues of lentil starches[a]

Starch source	Transition temperatures (°C)[b]			
	T_o	T_p	T_c	ΔH (cal/g)[c]
Laird				
Native	56·0 ± 0·2	62·0 ± 0·1	73·0 ± 0·3	3·2 ± 0·1
Acid-hydrolyzed	65·2 ± 0·3	74·0 ± 0·4	83·0 ± 0·2	2·6 ± 0·1
CC Gold				
Native	52·2 ± 0·2	61·2 ± 0·2	69·0 ± 0·2	2·1 ± 0·1
Acid-hydrolyzed	69·0 ± 0·4	76·0 ± 0·2	86·0 ± 0·4	1·2 ± 0·1

[a]The starches were hydrolyzed with 2·2 M HCl and 35°C for 2 days.
[b]Transition temperatures: T_o (onset); T_p (mid-point); T_c (conclusion).
[c]Enthalpy of gelatinization.

Table 8. X-ray diffraction spacings and intensities of the major peaks of stored lentil starch gels[a]

Starch source	Days of storage at 25°C	Moisture content (%)	Interplanar spacings (d) in Å with intensities (CPS)[b]			
Laird	4	9·6	5·20 (529)	4·44 (383)	4·05 (395)	3·80 (310)
	7	9·5	5·20 (761)	4·40 (506)	4·07 (492)	3·85 (390)
CC Gold	4	9·5	5·22 (496)	4·40 (384)	4·02 (440)	3·79 (359)
	7	9·6	5·22 (608)	4·43 (439)	4·03 (496)	3·86 (439)

[a]The gels (40%) stored at 25°C for 4 and 7 days were converted to powders prior to X-ray examination.
[b]Counts per second.

granules. Consequently, a higher input of thermal energy would be required to melt the starch crystallites. The above results are in agreement with those of Donovan and Mapes (1980), who reported that on acid hydrolysis (3 days), T_p of potato starch increased by 4·6°C, while T_c–T_o remained practically unchanged. However, Morrison *et al.* (1993) showed that transition temperatures of normal and waxy barley starches are unaffected by acid hydrolysis (140 h), whereas increases occur in T_c–T_o.

The decrease in ΔH (Table 7) is probably due to the action of H_3O^+ on the double helices within the amorphous regions of the starch granules. It is highly unlikely that the decrease in ΔH is due to disruption of double helices by H_3O^+ within crystalline lattices, since X-ray diffraction intensities increase on acid hydrolysis (Table 6). These results suggest that amylose–lipid complexes (single V_6 helices) within amorphous regions of the granule influence the extent to which T_o, T_p, T_c and ΔH change on acid hydrolysis. The above changes are more marked in CC Gold than in Laird, due to the presence of higher quantities of amylose–lipid complexes in the latter (Table 1). These results are in agreement with those of Morrison *et al.* (1993), who showed that lipid-complexed segments of single-chain V_6-amylose helices are resistant to acid hydrolysis.

Retrogradation of starch gels

The extent of retrogradation during gel storage was monitored by determining changes in gel strength, X-ray intensities and freeze–thaw stability.

Gel strength

The gel strength of Laird starches after storage (at 25°C) for 24 and 48 h was 136·4 and 149·7 g, respectively. The corresponding values for CC Gold were 89·2 and 89·8 g. The extent of increase in gel strength on storage was higher in Laird.

Since the short-term development of the structure and crystallinity of starch gels is dominated by irreversible (T < 100°C) gelation and crystallization of amylose within the gel matrix, an increase in AML or a decrease in SF (highly swollen granule, occurring between adjacent amylose chains would hinder their association during retrogradation) would theoretically be expected to increase gel firmness. As seen in Figs 2 and 3, the SF and extent of AML in Laird was lower and higher, respectively, than that of CC Gold. Thus, the differences between the two starches with respect to gel strength and its increase on storage is explained.

X-ray intensity

The X-ray diffraction spacing and intensities of the major peaks of Laird and CC Gold starch gels stored at 25°C for 4 and 7 days are presented in Table 8. The 'B' pattern, which is typical of retrograded starch, was evident in the X-ray spectra of Laird and CC Gold starch gels. The intensity of the 'B' pattern increased on storage in both starches (Table 8). However, this was more pronounced in Laird (Table 8).

The 'B' pattern has been shown to result from the retrogradation of both amylose (Gidley, 1989) and amylopectin (Zobel, 1988). Since the storage period was only 7 days (Table 8), the intensity increase probably represents mainly the extent of retrogradation of amylose chains both within and outside the gelatinized granule. The intensity increase is higher in Laird due to its higher amylose content (Table 1) and the presence of more leached amylose in the continuous matrix (Fig. 3).

Freeze–thaw stability

The freeze–thaw stability of starches are generally determined by estimation of the amount of water exuded from the starch gel system stored at low temperatures. The amount of water exuded would be the result of increased inter-molecular and intra-molecular hydrogen bonding due to aggregation of starch chains (retrogradation) during frozen storage. The percentage exudate (syneresis) from Laird and CC Gold starch gels stored at −16°C (after two freeze–thaw cycles) was 74 and 56%, respectively. The degree of syneresis was higher than that reported for pigeon pea starch (Hoover *et al.*, 1993) but comparable to that of lima bean starch (Hoover *et al.*, 1991). It is evident from the above results that both Laird and CC Gold starches, showing poor resistance to freeze–thaw stability, must be chemically modified if they are to be incorporated into food systems that must undergo freezing and thawing before consumer use.

CONCLUSIONS

The effect of cultivar on physicochemical properties of lentil starch was studied. The starches differed significantly with respect to the amount of amylose complexed by native lipids, the extent of crystallinity and the degree of accessibility of the amorphous regions of the granule to the entry of H_3O^+, water molecules and α-amylase. These differences influence the observed variations in swelling factor, amylose leaching, pasting properties, enzyme digestibility, acid hydrolysis, gelatinization parameters and the extent of retrogradation.

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