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PISUM SATIVUM AND *VICIA FABA* CARBOHYDRATES: PART IV – GRANULAR STRUCTURE OF WRINKLED PEA STARCH

P. COLONNA,* A. BULEON,* M. LEMAGUER† & C. MERCIER*

**Institut National de la Recherche Agronomique, Centre de Recherches Agro-Alimentaires, Laboratoire de Biochimie des Aliments, Chemin de la Géraudière, 44072 Nantes, Cédex, France*

† *University of Alberta, Department of Food Science, Edmonton T6G 2N2, Canada*

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ABSTRACT

The granular structure of wrinkled pea starch, compared to two other B-type starches, potato and amylomaize, has been studied, using physical, chemical and enzymic methods both before and after lintnerisation (2·2 N HCl, 35°C). Wrinkled pea starch, which was composed mainly (90%) of compound granules, had an apparent amylose content of 75·4% when measured at +2°C. Native granules showed weak B-type crystallinity. The fraction (27·4%) which was easily degraded during lintnerisation and which corresponded to the amorphous phase, was smaller than for other starches. The degradation rate of the more organised phase was low (6% in 17 days). The residue remaining after exposure to acid for 42 days presented a very high, B-type crystallinity but with the same sorption properties as native starch, which indicates that water is part of the crystallites. The crystalline phase is composed of linear chains of $\overline{DP}$ *25, distributed asymmetrically. The native starch showed a single gelatinisation endotherm between 117 and 133°C and with a* ΔH *of 0·7 cal.* g^{-1} *dry starch, which is somewhat lower than other B-type starches.*

INTRODUCTION

The amylose content of most starches from tubers and seeds varies from between 20 to 30%. There are others, however, which deviate significantly from this norm. For example, glutinous or waxy cereal starches are composed almost entirely of amylopectin while some other starch genotypes are very rich in amylose, e.g. 60–70% for maize (Deatherage *et al.*, 1954) and pea (Nielsen & Gleason, 1945; Hilbert &

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McMasters, 1946; McCready *et al.*, 1950) and 45% for barley (Walker & Merritt, 1969). The chemical structures of their principal constituents, amylose and amylopectin, present little variation, the amylose to amylopectin ratio being the variable between the different starch types (Greenwood & Thomson, 1962; Banks *et al.*, 1974). However, 'intermediate' material, which has properties closer to amylose with a lower molecular weight, is present in a proportion from 30 to 35% of the parent starches of high-amylose content.

Starches with a high amylose content have unusual granular forms, weaker birefringence patterns and, when heated in water, swell to a much lesser extent than do their normal counterparts, indicating a very high degree of intermolecular association (Leach *et al.*, 1959; Greenwood & Thomson, 1962; Banks *et al.*, 1974). The granules must be treated at about 125°C before any extensive disruption becomes evident. The crystalline structure, observed by X-ray diffractometry, is characterised by a weak B-type pattern (Kainuma & French, 1971), indicating a low degree of crystallinity and yet these starches are water insoluble after prolonged acid treatment. Moreover, the extent of erosion of the starch granules during acid treatment is very much less than those of the 'normal' and low amylose starches (Kainuma & French, 1971). No satisfactory explanation has been advanced for their granular structure.

This paper reports an investigation into the structure of wrinkled pea starch using physical, chemical and enzymic methods before and after mild acid hydrolysis. Its behaviour compared with two other B-type starches, one with a normal amylose content (potato) and a second with a high amylose content (amylomaize), will be discussed.

EXPERIMENTAL

Materials

The starch used was isolated from air-classified flour of wrinkled seeded pea (*Pisum sativum* L., Frogel variety) according to the method of Colonna *et al.* (1980).

Lintnerisation of the native starch (2·2 N HCl, 35°C, 1/10 w/v) and measurement of the extent of starch hydrolysis were performed as described by Colonna *et al.* (1981). When x% of the initial material was removed, the acid resistant material was called 'x% lintnerised starch'. For subsequent analysis of fine structure by enzymes, lintnerised starch was solubilised in 95% (v/v) DMSO by magnetic stirring at room temperature for 48 h.

Pullulanase (EC 3.2.1.41) of *Enterobacter aerogenes* (*Aerobacter aerogenes*) (Hayashibara Biochemical Laboratories Inc., Okayama, Japan), barley β-amylase (EC 3.2.1.2) (Fluka AG, Buchs SG, Switzerland), *Aspergillus niger* glucose oxidase (EC 1.1.3.4) (grade II) and horse radish peroxidase (EC 1.11.1.7) (grade I) (Sigma Chemical Company, St Louis, Mo., USA), *A. niger* amyloglucosidase (EC 3.2.1.3) (E. Merck, Darmstadt, West Germany) and ABTS [2,2'-azino-di(3-ethyl-benzo-

thiazoline sulphonic acid)] (Boehringer Mannheim GmbH, Mannheim, West Germany) were used. Sephacryl S-200 Superfine was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Industrial amylose (AVEBE, Veendam, The Netherlands) was used as standard amylose.

All other reagents were of analytical grade.

Enzymic Methods

Debranching of the lintnerised starch was performed in 20% (v/v) DMSO as recommended by Mercier & Kainuma (1975). The digest (P_1) contained approximately 5 mg of substrate and 8·32 nKatals* of pullulanase per ml of 20% (v/v) DMSO.

β-Amylolysis of the debranched lintnerised starches ($P_1\beta_1$) was carried out after inactivation of pullulanase by heating the digest for 20 min at 100°C and addition of 10 nKatals of β-amylase per ml of P_1 digest.

The total concentration of starch-type polysaccharide was measured in 20% (v/v) DMSO by the successive action of amyloglucosidase (Lee & Whelan, 1966) and glucose oxidase-peroxidase (Lloyd & Whelan, 1969), as modified by Colonna *et al.* (1981).

All other determinations were performed as described previously (Colonna *et al.*, 1981).

Gel Permeation Chromatography

A column (diameter 2·5 cm; bed height 90 ± 1 cm), maintained at 40°C, was filled with Sephacryl S-200 and eluted with an upward flow of degassed, 40% (v/v) DMSO at a rate of 10 ± 1 ml h^{-1}. Carbohydrate samples (5–10 mg in 2–4 ml) were applied to the column and fractions of 10 ml were collected. The starch-type polysaccharide in each fraction was determined enzymically (amyloglucosidase, glucose oxidase-peroxidase). In all cases the recovery was 90–100%. The position of each fraction was characterised by its own partition coefficient $K_{av} = (V_e - V_0)/(V_t - V_0)$; V_e, V_0 and V_t being elution volume of the fraction, the void volume (as dispersed native starch elution volume) and total volume (as glucose elution volume), respectively. The elution patterns obtained represent the weight of polysaccharide expressed as μg of glucose per mg of recovered polysaccharide versus K_{av}.

Other Chemical Determinations

The apparent amylose content was determined by the amperometric method (Larson *et al.*, 1953) at 2 and 25°C, using amylose as a reference material.

Iodine-absorption spectra were obtained from mixtures containing 1 ml of 0·02% substrate solution (after solubilisation for 30 min in 1 N KOH), 1 ml of 1 N HCl, 2·1 ml of 0·33 M KCl and 0·208 ml of aqueous iodine solution (0·2% iodine + 2·0% potassium iodide) made up to 10 ml with distilled water.

The average chain length $\overline{DP}$ was determined using the periodate oxidation method (Greenwood & Thomson, 1962).

* 1 I.U. = 16·67 nKatals.

Thermal Analysis

A sample of pea starch of known water content was mixed with an appropriate amount of distilled water to give a mass ratio of 1 part starch: 3 parts water. Samples of about 10 mg were hermetically sealed in aluminium pans and heated in a Perkin-Elmer DSC-2. An empty pan was used as the reference. The experiment was performed using a heating rate of $5°C\ min^{-1}$ over the temperature range 20–150°C and with a sensitivity of $0{\cdot}5\ mcal.\ s^{-1}$. The endothermic transition enthalpy was determined by measuring the peak area.

X-Ray Diffraction Technique

Powder X-ray spectra were determined using a Compagnie Générale de Radiologie X-ray spectrometer Sigma 2080 equipped with Guinier monochromator and scintillation counter. The powder diagrams were recorded in the transmission mode, using the Debye-Scherrer procedure. The operation conditions were as follows: CuK_α radiation, 1·54105 Å; angular scanning velocity, $1°2\theta\ min^{-1}$; chart speed, $10\ mm\ min^{-1}$.

Sorption Isotherm Determinations

Sorption measurements were performed, using a classical desiccator-saturated salt solution method under vacuum. Samples of about 200 mg were predried to constant weight with P_2O_5 as desiccant at 50°C. The subsequent sorption process took about 10 days to reach constant weight (±0·1 mg at 25°C ± 0·2). Four saturated salt solutions were used with the following water activities (Leopold & Johnston, 1927; Stokes & Robinson, 1949): $LiCl,H_2O = 0{\cdot}1105$; $NaBr,2H_2O = 0{\cdot}5770$; $NaCl = 0{\cdot}7528$; $K_2SO_4 = 0{\cdot}9690$.

Results were plotted using a least square regression method on a H.P. 9825 + 9871 A mini computer system (R^2 is the value of the correlation coefficient). As shown by Bizot *et al.* (1981) using the Guggenheim-Anderson formula $W/W_m = (Ck_{a_w}/(1-k_{a_w}))[1+(c-1)k_{a_w}]$, only four sorption equilibria are necessary with starchy products to determine the full isotherm where W represents the amount of absorbed vapour per gram sorbant (dry basis) at equilibrium, W_m is the value of W when all active sites bear one molecule of water, a_W is the water activity (P_w/P'_w), c is a coefficient related to the heat of absorption and k a factor correcting for the different sorbant structure as compared to bulk liquid.

This absorption model equation has been widely used by Van Den Berg *et al.* (1975) who stressed its advantages as a preferred alternative to the classical BET (1938) equation, particularly in determining the primary adsorption sites (formerly named 'monolayer').

Light Microscopy Observations

Starch granules were observed in aqueous suspensions on an Olympus light microscope (model Vanox) equipped to work in the polarisation mode.

RESULTS

The apparent amylose content of native wrinkled pea starch was higher when determined at 2°C (75·4%) than at 25°C (62·8%).

Microscopic Observations

When observed by light microscopy, starch appeared to be a mixture of simple and compound granules, the latter being composed of 3–10 individual sub-units joined together (Fig. 1). The numerical ratio between simple and compound granules was about 40 : 60 but assuming that both types of granules had the same density, the mass ratio between simple and compound granules was approximately 10 : 90. It is possible that some splitting of the compound granules into their sub-units may have

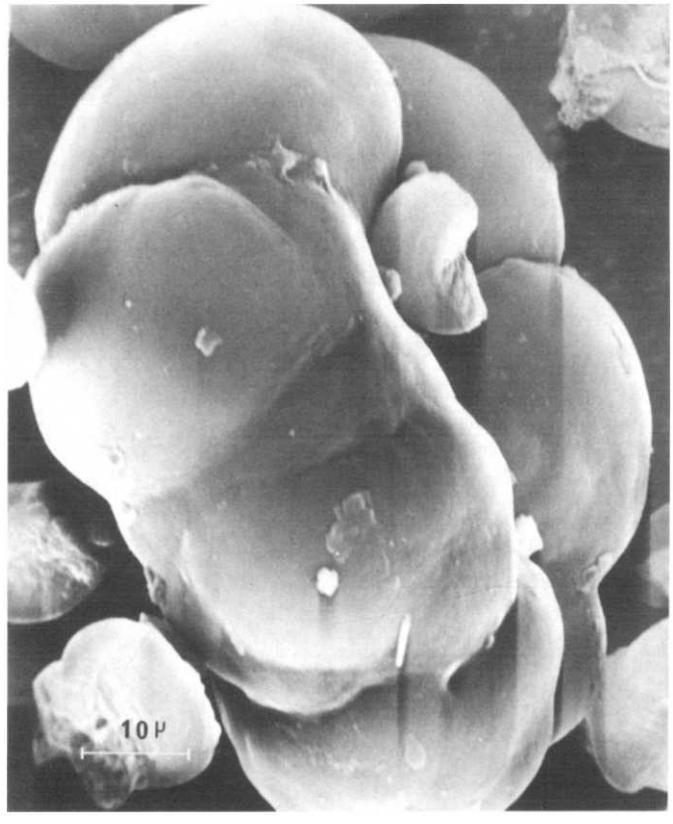


Fig. 1. Scanning electron micrographs of native wrinkled pea starch granules.

occurred during purification, thereby increasing the apparent numerical importance of simple granules. It is believed, therefore, that the starch sample studied can be considered to consist almost entirely of compound granules. The birefringence of the native granules was weak and no Maltese crosses were observed; the starch granules had only small bright sectors under polarised light.

Lintnerised starch did not show any cohesion between granules, but rather they were split into birefringent fragments when observed under polarised light.

Hydrolysis Kinetics

A plot of the extent of hydrolysis (x) versus time (t) presented two distinct stages (Fig. 2). The first one required 8-10 days and was characterised by 25% hydrolysis whereas the second, much slower one, yielded 43% lintnerised starch after 42 days. This distinction is emphasised by plotting $\log_{10}[(100)/(100-x)]$ versus time. The linear regression relationship between $\log_{10}[(100)/(100-x)]$ and time is:

first stage

$$\log_{10}[(100)/(100-x)] = 0{\cdot}0118 + 0{\cdot}023\,t$$

second stage

$$\log_{10}[(100)/(100-x)] = 0{\cdot}139 + 2{\cdot}46 \times 10^{-3}\,t$$

where t is expressed in days, x is the hydrolysed starch as a percentage of the initial starch, and r equals 0·973 with 5 points for the first stage and 0·996 with 8 points for the second stage.

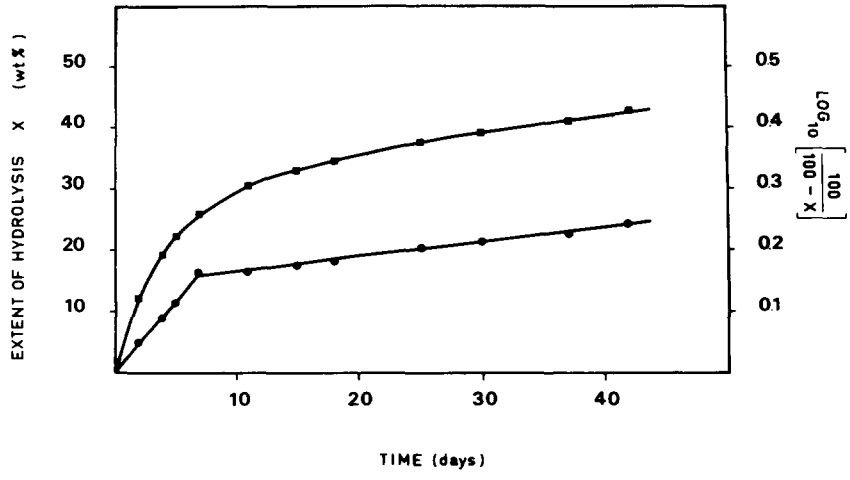


Fig. 2. Hydrolysis of wrinkled pea starch in 2·2 N HCl at 35°C. The percentage x (■) of hydrolysed starch and the $\log_{10}[(100)/(100-x)]$ (●) are plotted versus time of hydrolysis (days).

X-Ray Diffractometry

X-ray spectra of native and lintnerised wrinkled pea starches (Fig. 3) were of the B-type, representative of tuber starch, with two main reflections at 5·5° and 17° 2θ angles. The native starch revealed poor structural organisation with peaks which were both broad and weak, even after equilibration to a 39·5% moisture content. Lintnerisation increased the crystallinity of samples. The spectra of lintnerised starch with moisture contents of 15·6 and 37·0% were very sharp and contained more than seven important reflections, indicating that acid hydrolysis had removed the amorphous parts of the starch granules. No significant differences in the diffraction spectra of 37 and 43% lintnerised starches were observed. In every case, a high moisture content yielded a better crystalline organisation, particularly for the 5·5° reflection, as is generally observed with starches (Katz, 1930).

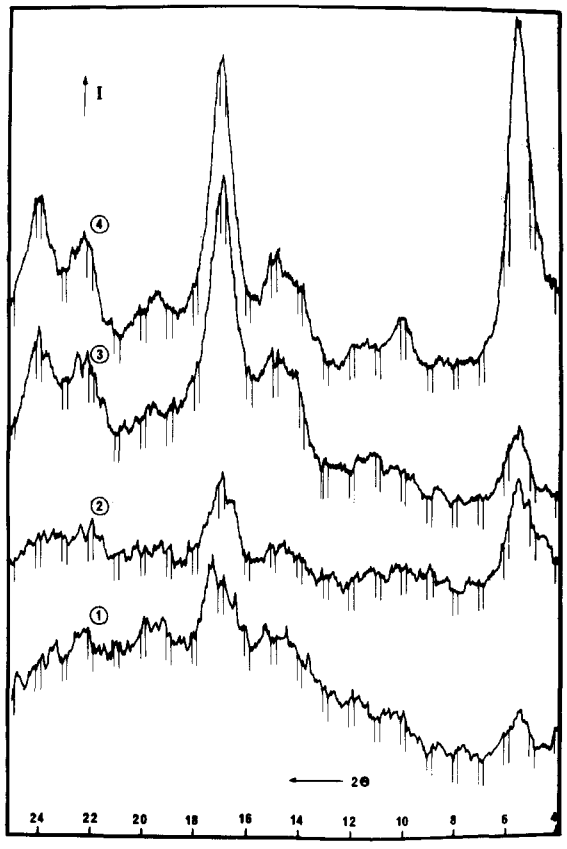
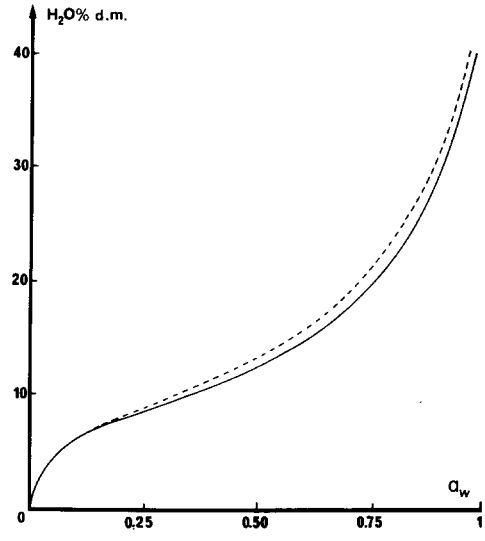


Fig. 3. X-ray diffraction diagrams of native (1 and 2) and lintnerised (3 and 4) wrinkled pea starches at different moisture contents: (1) 14·2%, (2) 39·5%, (3) 15·6%, (4) 37·0%.

Sorption Isotherms

The sorption isotherms for native and 43% lintnerised wrinkled pea starches (Fig. 4) were almost similar; the first parts of the curves up to 15% relative humidity were identical. Thereafter, the curve for native starch was higher than for 43% lintnerised starch, but the difference never exceeded a 2% moisture content.



	a_w				W_m	R^2
	0·111	*0·577*	*0·753*	*0·969*		
Lintnerised starch, H_2O% d.m.	6·25	14·17	19·26	37·09	7·674	0·9980
Native starch, H_2O% d.m.	6·09	15·40	20·18	39·50	8·432	0·9881

Fig. 4. Sorption isotherms of native (– – –) and 43% lintnerised (——) wrinkled pea starches at 25°C.

Characteristics of Lintnerised Starches

While the dispersed native starch was completely excluded from the Sephacryl S-200, the elution pattern of dispersed 37% lintnerised starch (25 days of treatment) showed two peaks. A small one (2·7% of total material) was located at K_{av} 0·26 and the major one at K_{av} 0·67. After 42 days of lintnerisation (43% lintnerised starch), the same profile was obtained (Fig. 5), the first peak representing 3·1%. Furthermore, the β-amylolysis limit of these two fractions were similar: 106·6% for the 37% lintnerised starch and 104·9% for the 43% lintnerised starch.

No apparent amylose could be detected by amperometry in the dispersed lintnerised starches. However, the peak absorption wavelengths of their iodine-polysaccharide

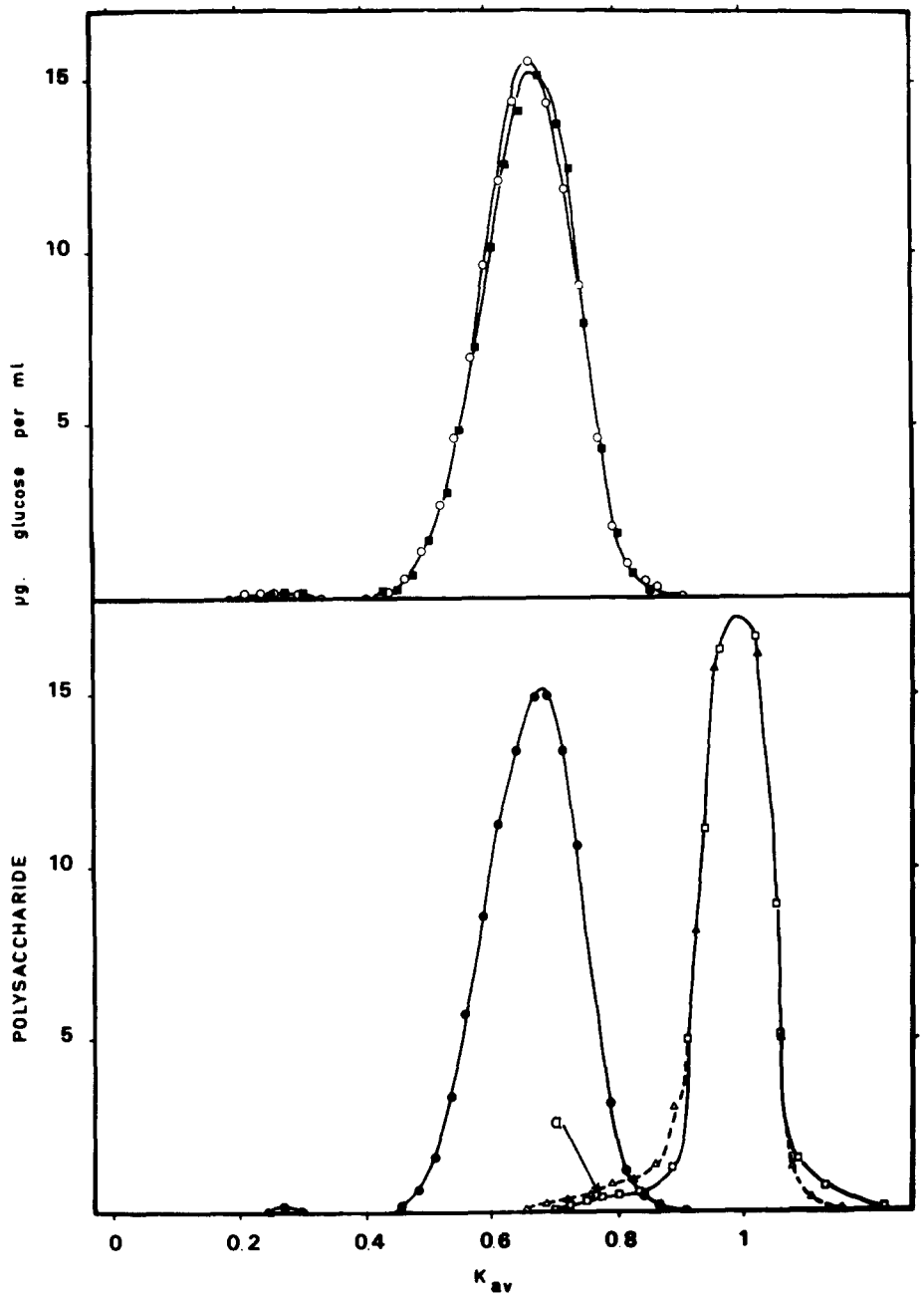


Fig. 5. Elution patterns from Sephacryl S-200 of dispersed lintnerised wrinkled pea starch. Untreated 37% lintnerised starch (■). 43% lintnerised starch: untreated (○), P_1 (●), $P_1\beta_1$ (□) and β_1 (△).

complexes (λ_{max}) were at 555-560 nm (a purple colour). The average chain lengths, determined from the reducing power (Nelson method; Nelson, 1944) were 24·5 for the 37% lintnerised starch and 23·4 for the 43% lintnerised starch. When using the periodate oxidation method, the average chain lengths were found to be 25·1 for the 37% lintnerised starch and 24·7 for the 43% lintnerised starch.

After pullulanase action, the elution pattern of debranched, lintnerised starch (P_1) showed no difference from the undebranched sample (Fig. 5). Further β-amylolysis ($P_1\beta_1$) (Fig. 5) transformed the material into maltose, maltotriose with an enzyme resistant fraction *a* (3% of total material) as a weak peak located at K_{av} 0·78.

The gel permeation chromatography of the 43% lintnerised starch, directly treated by β-amylase (Fig. 5) revealed a major peak, at the total volume, with a small enzyme resistant fraction (4·1% of total material), beginning at K_{av} 0·65. Further debranching β_1P_1 and a final β-amylolysis $\beta_1P_1\beta_2$ did not modify the β-amylase dextrin.

Calculation of Molecular Weight Distribution from the Gel Permeation Chromatography Pattern

Only the main peak (97%) of the 43% lintnerised starch elution pattern was considered in this study. The calibration of the Sephacryl S-200 column was carried out with debranched waxy-maize starch. By plotting log average chain length ($\overline{CL}$) versus K_{av} in the range of K_{av} 0·27-0·87 (Fig. 6), a linear regression between these two

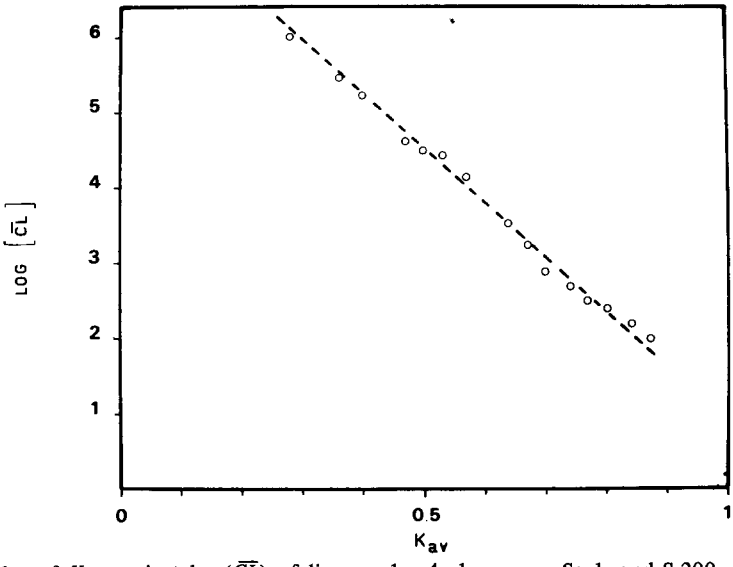


Fig. 6. Plot of K_{av} against log ($\overline{CL}$) of linear α-1→4 glucans on Sephacryl S-200, eluted with 40% (v/v) DMSO.

variables was obtained from the following equation:

$$\log \overline{CL} = -7{\cdot}3K_{av} + 8{\cdot}2$$

$r = -0{\cdot}995$ with 15 points.

The yields and average chain lengths of the different fractions of 43% lintnerised starch are shown in Table 1. The parameter $W(CL)$ is defined as the cumulative fractional amount to the mid-point of each fraction. The integral and differential chain length distribution curves are shown in Fig. 7. From these data, it is possible to calculate the different molecular weights:

number molecular weight $\bar{M}_n = 4000$

weight molecular weight $\bar{M}_w = 5304$

These values can be corrected for peak broadening due to axial dispersion (Yau *et al.*, 1979). The corrected values are $\bar{M}_n = 4390$ and $\bar{M}_w = 4858$ with a polydispersity $I = \bar{M}_w/\bar{M}_n = 1{\cdot}106$. The asymmetrical coefficient γ_1, calculated as the ratio of moment of order 3 divided by the moment of order 2 raised to the 3/2th power, is high ($\gamma_1 = 1{\cdot}51$).

TABLE 1
Fractionation data for 43% lintnerised wrinkled pea starch

Fraction	$\overline{CL}$	*Yield (% wt)*	W(CL)[a]
1	244·8	0·1	999·9
2	200·3	0·3	999·8
3	162·8	0·8	999·2
4	133·2	2·9	997·3
5	108·9	7·9	991·9
6	88·6	17·9	979·0
7	72·4	34·5	952·8
8	58·9	58·7	906·2
9	48·2	87·0	833·3
10	39·2	115·3	732·2
11	32·0	141·6	603·7
12	26·2	147·8	459·1
13	21·3	139·6	315·4
14	17·4	115·3	187·9
15	14·2	74·8	92·9
16	11·6	36·4	37·3
17	9·4	13·1	12·6
18	7·7	4·0	4·0
19	6·3	1·0	1·5
20	5·1	0·6	0·7
21	4·2	0·4	0·2

[a] Cumulative yield to the mid-point of each fraction.

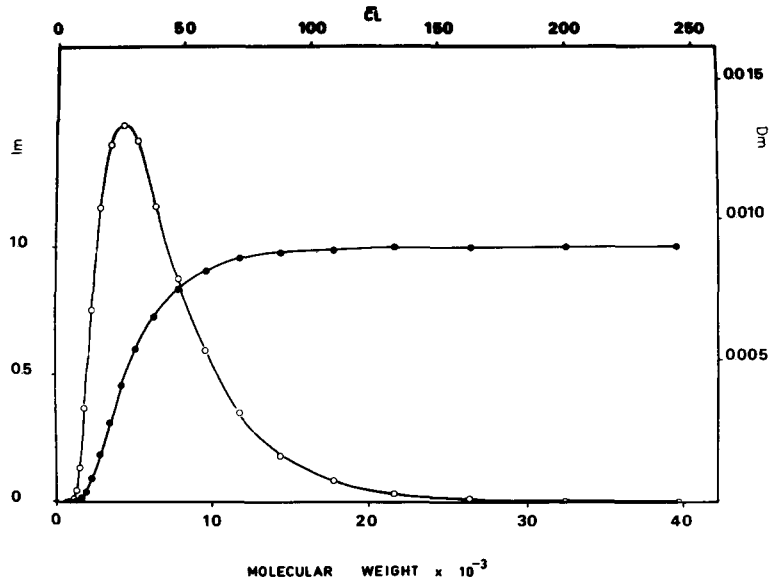


Fig. 7. The integral ($Im = W(M)$; •) and differential ($Dm = dW(M)/dM$; ○) molecular weight distribution curves for 43% lintnerised wrinkled pea starch.

Thermal Analysis

On heating the native wrinkled pea starch granules in excess water up to 150°C, an endothermic peak was observed. This commenced at 117°C, terminated at 138°C and possessed a peak temperature at 133°C. The enthalpy change calculated from the area of the endotherm was 0·7 cal. g^{-1} dry starch. No thermal transition was observed on repeating this heating process which suggested that the wrinkled peak starch had been completely gelatinised.

DISCUSSION

The apparent amylose content obtained at 2°C (75·4%) was higher (+ 20%) than the value obtained at 25°C (62·8%), already observed in a previous work (Colonna *et al.*, 1980). This difference is due to the difficulty in extrapolating to a zero concentration of free iodine as the iodine-binding titration curve is without an inflection at 25°C, whereas at 2°C extrapolation is easily accomplished. The same observations were made by Adkins & Greenwood (1966) and Mercier (1973) on amylomaize starch. The apparent amylose content of the present sample is very high and of the same order as that of the *perfection* variety of wrinkled pea starch (Hilbert & McMasters, 1946).

The starch sample studied was composed almost completely of compound granules. It is important to emphasise this feature because Banks & Greenwood (1975) suggested

that the compound granules were mainly composed of amylose whereas the simple ones had normal amylose content. The same distinction, based on granule size, was established on high-amylose maize (63·8% of apparent amylose) (Cluskey *et al.*, 1980) where filamentous granules were suspected to have a higher proportion of amylose than normal ones.

X-ray diffraction spectra of wrinkled pea starch were poor and the degree of crystallinity seemed to be very low in comparison with native potato starch, which has the same kind of B-type spectrum. This could be due either to an inherently low degree of order or to excessively small crystallites. The hydration increased, as usual, both the sharpness of patterns and the intensity of the 5·5° peak, but it was never possible, even with high moisture content to obtain very satisfactory patterns. The wrinkled pea starch granules did not show Maltese crosses, demonstrating that the crystallites, in the granules, were not organised in spherulites. On the contrary, the 43% lintnerised wrinkled pea starch pattern presented a very high degree of crystalline order, which was similar to the pattern of 85% lintnerised potato starch (Robin *et al.*, 1974). These results confirm the previous work of Kainuma & French (1971) on native wrinkled pea starch and its Nägeli type amylodextrin. At present, in the structural models proposed for β-amylose and B-type starch (Cleven *et al.*, 1978; Sarko & Wu, 1978; Bluhm *et al.*, 1980), the different authors suggest that water molecules play a major role in the crystallinity. The calculated unit cells may contain up to 33% (on a dry weight basis) which corresponds to equilibrium of the sample with very high relative humidities. So, in native starch granules, the water, during the sorption process, can be absorbed either in the amorphous regions containing a lot of free hydroxyl groups, or in those regions which can crystallise with water. The difference existing in water sorption properties of native and lintnerised samples is very small in comparison with the difference in their diffraction diagrams. This can be explained by a great amount of water present in the crystallites or by a low degree of crystallinity of lintnerised starch. Even if this sample were not absolutely crystalline, the quality of the diffraction diagrams argues for the first possibility, which is in good agreement with the claims of Cleven *et al.* (1978), Sarko & Wu (1978) and Bluhm *et al.* (1980).

The w_m values (i.e. water monolayer) given by the Guggenheim regression are 7·67 and 8·43 respectively for 43% lintnerised and native wrinkled pea starches. Those values, proportional to the number of active sites for the water sorption process, are similar for the two samples, showing that the accessibility to water is not very different for the native and 43% lintnerised starches. However, the smaller value for the lintnerised sample indicates that some sites, which might otherwise be available for binding water molecules, are already blocked through interchain hydrogen bonding either in crystalline or less specifically organised regions.

Lintnerisation releases crystalline parts, by hydrolysing preferentially the amorphous parts of the starch granules (Robin *et al.*, 1974; Robin *et al.*, 1975; Robin,

1976). However, annealing could take place either by cleavage of just a few molecular chains or recrystallisation of the newly released chains. The amorphous phase is estimated by determining the easily degradable starch fraction, accomplished by extrapolating to zero time the second phase process (10–42 days) in the graph of $\log_{10}[(100)/(100-x)]$ versus time. The proportion of amorphous phase is smaller for wrinkled pea starch (27·4%) than for potato (36·4%) (Robin *et al.*, 1974), wheat (51·5%), maize (50·8%), waxy-maize (40·2%) (Robin *et al.*, 1975) and amylomaize (36·9%) starches (Robin, 1976). This observation indicates that native wrinkled pea starch granules have a higher crystalline/amorphous phase ratio than other starches, which seems to be in contradiction with the low degree of crystallinity observed by X-ray diffractometry. The degradation rate of the more organised phase, calculated from the slope of the curve $\log_{10}[(100)/(100-x)]$ versus time for the second phase (10–42 days) is low and of the same order as that for amylomaize (Robin, 1976). As already observed for maize (Robin *et al.*, 1975) and rice (Maningat & Juliano, 1979) starches, there is an inverse relationship between the amylose content of the native starch and the resistance to acid hydrolysis during the second stage of lintnerisation. This should result either from the crystalline type or from the size and the shape of crystallites. The fact that wrinkled pea and potato starches, both showing B-type X-ray crystallinity, are different in their resistance to lintnerisation, suggests that the geometrical features of the crystalline phase are mainly responsible for the differences between the lintnerisation rates of starches. Since amylose appears to be an ordering factor in starch structure, it should contribute to the crystalline phase to a higher extent, explaining why leaching of amylose is so difficult with wrinkled pea starch (Greenwood & Thomson, 1962). Moreover, the similarity between the elution patterns of 37 and 43% lintnerised starches indicate that wrinkled pea starch contains a homogeneous crystalline phase.

The gel permeation chromatography of the acid-resistant residue shows a main peak (97% of total material), essentially linear, as demonstrated by no pullulanase debranching, a direct β-amylolysis limit of 104·9% and one reducing end (Nelson method; Nelson, 1944) per non-reducing end (periodate oxidation). The average chain length of 25 explains the considerable difficulty in solubilising the residue in water. However, the elution pattern, after β-amylolysis, reveals a small proportion of material, located at K_{av} 0·75–0·80, corresponding to short β-limit dextrins ($\overline{DP}$ 2–10), according to the calibration curve. The existence of single glucose stubs on the main chains seems to be the most likely explanation of these enzyme resistant structures which could be formed by acid cleavage of an α-1,4 linkage near the α-1,6 branching point, as claimed by Robin *et al.* (1974, 1975). The fine structure of the lintnerised wrinkled pea starch is quite different from that of lintnerised potato starch (Robin *et al.*, 1974), where two chain populations, a linear one of $\overline{DP}$ 15 and a singly branched one of $\overline{DP}$ 25, are present. Furthermore, with potato starch the proportion of residual linear chains increases with the rate of lintnerisation, whereas the second population is preferentially hydrolysed. Since the 43% lintnerised wrinkled pea starch is almost linear and

homogeneous on Sephacryl S-200, the molecular weight distribution (MWD) was determined by gel filtration. This MWD is asymmetrical with a $\bar{M}_n$ of 4390 and a $\bar{M}_w$ of 4860, giving a low polydispersity of 1·106. Those results explain why the acid resistant residue gives purple iodine complexes (Fales, 1980), as already observed for wrinkled pea Nägeli amylodextrins (Kainuma & French, 1971). The MWD can be compared to those calculated from previous studies on normal and waxy starches (Robin *et al.*, 1974, 1975; Robin, 1976). From the data of Robin (1976) a 48% lintnerised amylomaize starch (Fig. 8) is characterised by a number molecular weight M_n of 4200, a polydispersity I of 1·08 and an asymmetrical coefficient γ_1 of 1·33. With 85% lintnerised potato starch (Robin *et al.*, 1974), M_n, I and γ_1 are 2345, 1·03 and 0·41, respectively. It appears, therefore, that wrinkled pea and amylomaize lintnerised starches are different from potato lintnerised starch, with higher number molecular weights, polydispersities and asymmetries. The phenomenon of asymmetry is important because it governs the geometrical features of crystallites. A symmetrical MWD points to lamellar crystallites, as is the case for potato starch. With wrinkled pea and amylomaize starches, the asymmetry points to a different organisation, either to different sizes of the same crystallites or to crystallites with shapes other than lamellar. Moreover, the small fraction (about 3%) of higher molecular weight is part of the crystalline phase and any model of the crystalline phase must take into account this fraction. The resistance of crystallites towards lintnerisation is certainly a function of the crystallite size, due to thermodynamic stability.

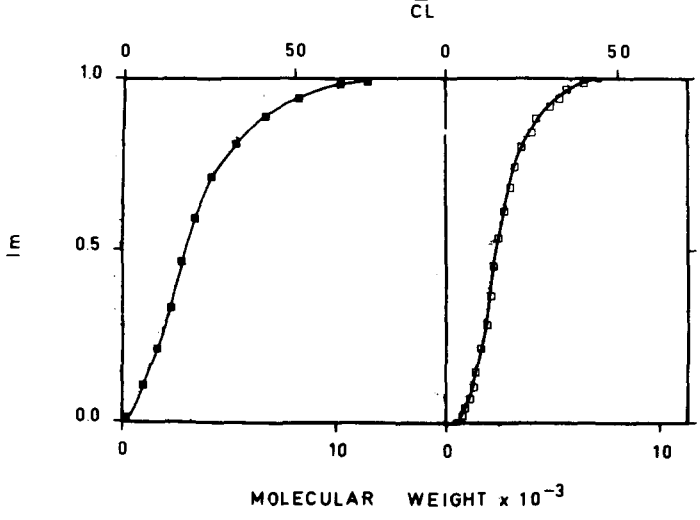


Fig. 8. The integral ($Im = W(M)$) of molecular weight distribution curves for 48% lintnerised amylomaize (■) and 85% lintnerised potato (□) starches.

When applied to the wrinkled pea starch : water system the differential scanning calorimetry technique allows us to observe the physical transitions of structure as a function of temperature.

The transition enthalpy (0·7 cal. g^{-1} dry starch) of wrinkled pea starch was found to be low, in contrast to those of potato (Donovan, 1979; Donovan & Mapes, 1980), cereals (Stevens & Elton, 1971; Wootton and Bamunuarachchi, 1979) and legume (Biliaderis *et al.*, 1980; Colonna *et al.*, 1981) starches. The higher temperature of the gelatinisation endotherm agrees with the fact that wrinkled pea starch is very difficult to gelatinise in excess water; for example, no birefringence end-point temperature could be detected with a polarising microscope. Amylomaize starch, which presents similar behaviour, showed a similar, rather low transition enthalpy when studied by Stevens & Elton (1971) (2 cal. g^{-1} dry starch at $v_1 = 0{\cdot}39$); however, the value obtained by Wootton & Bamunuarichchi (1979) (7·6 cal. g^{-1} dry starch at $v_1 = 0{\cdot}56$) was much higher. The differences between these amylomaize samples could be due to variations in raw materials, the starch samples possessing significant differences in structure.

The present results do not allow us to make any firm statements on the nature of the interactions between the crystalline and non-crystalline phases inside the starch granules. However, the acid-resistant residue material ($\overline{CL}$ 15) of normal and waxy starches (Robin *et al.*, 1974, 1975; Robin, 1976; Maningat & Juliano, 1979; Hall & Manners, 1980; Watanabe & French, 1980), which corresponds to the outer chains of amylopectin, has been thought to be mainly responsible for the crystallites of the granules. Therefore in the case of wrinkled pea starch, it is tempting to relate the particular molecular weight distribution of lintnerised starch ($\overline{CL}$ 25) to the amylose and/or amylopectin structures, since amylomaize amylopectin has been demonstrated to be composed of longer chains (Mercier, 1973; Ikawa *et al.*, 1981). Further work is now in progress on the fine structure of the wrinkled pea starch components in order to gain more precise insight into the granular structure.

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