Carbohydrate Polymers 27 (1995) 109–115
Elsevier Science Limited.
Printed in Great Britain.
0144-8617/95/$09.50+.00

ELSEVIER

0144-8617(95)00052-6

Some physico-chemical properties of smooth pea starches

N.I. Davydova,[a] S.P. Leont'ev,[a] Ya.V. Genin,[b] A.Yu. Sasov[c] & T.Ya. Bogracheva[a]*

[a]The Institute of Food Substances RAS, Russia
[b]The Institute of Elementoorganic Compounds, Russia
[c]Moscow State University, Moscow, Russia

(Received 26 August 1994; revised version received 20 October 1994; accepted 31 March 1995)

The physico-chemical characteristics of the starches of five smooth pea varieties have been investigated. X-ray diffraction analysis has shown that all of the starches have 'C' polymorphs with different contents of 'A' and 'B' polymorphs. DSC analysis has demonstrated a correlation between the thermodynamic parameters of gelatinization and the content of 'B' polymorphs in the starches. The influence of KCl concentration on the gelatinization process was investigated. The single peak of gelatinization in excess water doubled in salt solutions. A model is proposed which suggests that 'A' and 'B' polymorphs are in different independent co-operative structures which results in bifurcation of the peaks in KCl solutions.

INTRODUCTION

Two pea seed phenotypes exist, smooth and wrinkled, that are genetically different and produce unique starches. The starch granules of smooth peas are generally larger in size than those found in wrinkled peas and may be round, oval or irregular in shape (Bertorft *et al.*, 1993; Stute, 1990). In addition, the starch granules have a semi-crystalline nature with 'C' polymorphs, which are a mixture of 'A' and 'B' polymorphs, with intermediate behaviour and properties (Blanshard, 1987; Gernat *et al.*, 1990; Sarco & Wu, 1978). The properties of starches from different round pea genotypes could vary and it is possible that these distinctions are related to the content of 'A' and 'B' polymorphs in the starches.

When heated, an aqueous suspension of starch in excess water undergoes co-operative endothermic transition known as gelatinization. The molecular events responsible for this transition are uncertain but entail the melting of crystallites (Biliaderis *et al.*, 1980; Blanshard, 1987; Noel & Ring, 1992; Donovan, 1979). The solute acts as a plasticizer in this process and influences to a high degree the thermodynamic parameters of this transition. In general, low concentrations of inorganic salts have been found to raise the gelatinization temperatures of both 'A' and 'B' type starches (Evans & Haisman, 1982; Jay-Lin & Ames, 1993).

The present study investigates the structure and properties of starches using five varieties of round seeded peas which have different contents of 'A' and 'B' polymorphs.

MATERIALS AND METHODS

Starch isolation procedure

Samples of starches were isolated from five smooth seeded pea varieties, the seeds of which were harvested in the same year and locality (Central Russia). Seeds were firstly dehulled, then pin-milled, and the meal slurried at a solid/liquid ratio of 1:5 at pH 8·5. The mixture was centrifuged at 2500*g*, the supernatant removed and the precipitate resuspended in distilled water, screened and recentrifuged. The resulting precipitate was reslurried in water, rescreened and recentrifuged a further three times. The starch thus obtained was then air-dried.

General methods

Protein content was determined from total nitrogen analysis using the Kjeldahl method (Perrin, 1953). Lipid content was determined following extraction with CCi_4 in a Soxslet apparatus (Richter *et al.*, 1969). Starch contents in pea flour were determined by measuring optical rotation using HCl (Richter *et al.*, 1969). The specific optical rotation for 5% suspension of pea starches was found to equal 180·0. Amylose contents were

---

*Author to whom correspondence should be addressed.

determined by complexing starch with iodine (Kozimina, 1971). 100 milligrams of starch were suspended in distilled water and 1 ml of C_2H_5OH was added. The mixture was made up to a total volume of 100 ml with distilled water and heated in a water bath for 3 min at 100°C under stirring. Five millilitres of this solution was mixed with 40 ml of distilled water, neutralized with 0·05 M HCl and a solution of iodine and potassium iodide was added to give a final concentration of 0·2% iodine and 2% potassium iodide. The solution was made up to 100 ml with distilled water, stirred for 20 min and the absorption at 590 nm measured. Amylose concentrations were determined using a calibration curve obtained using potato amylose (AVEBE, Veendam, The Netherlands).

Starch grain size

Starch grain size distribution was determined using automatic quantitative analysis of scanning electron microscope (SEM) images (Sergeev *et al.*, 1984*a*, 1984*b*). The electron microscope was a JSM-U3 SEM linked to an Electronika D3-28 microcomputer. The calculations were based on measurements from approximately 100 starch grains, which had been shown previously to be sufficient for reproducible results.

Starch crystallinity measurements

A DRON-3 diffractometer (Russian) with CuK_α radiation was used for wide-angle X-ray diffraction analysis of the crystalline structure of the starches. The crystallinity of the starches was calculated using the method of Hermans and Weidinger (1961). An amorphous sample of starch was prepared by roll-drying a 30% suspension of starch (Smaract variety) under 180°C on the rolls. The X-ray diffractogram of amorphous starch is shown in Fig. 1. Its crystallinity was postulated to be 0%.

DSC measurements

A DASM-4 (Biopribor, Russia) differential adiabatic scanning calorimeter with Pt DSC cell was used. The temperature range was 20–110°C and the scanning rate was 1°C per min with an excess pressure of 1·5 atm. In each experiment the heat capacity scale of the microcalorimeter was calibrated by Joule–Lentz effect. The specific heat capacity increment (ΔC_p), the peak temperature (T^p) and the width of the peak (ΔT) were determined (Fig. 4). The specific enthalpy of cooperative transition (ΔH^{cal}) was determined by measuring the peak area by planimetry and calculated as described by Pfeil & Privalov (1979). Starch concentration was 0·4–1·5% (w/w).

Swelling and solubility

Swelling and solubility behaviour of starches were measured using standard methods described in detail elsewhere (Richter *et al.*, 1969). Centrifuge tubes with 1·4% aqueous suspensions of starch were heated at a rate of 1·5°C per min up to temperatures in the range 50–90°C and held at any fixed temperature for 30 min. During the procedure the starch was kept in suspension by gentle stirring. The tubes were then cooled rapidly and centrifuged at 2500 *g*.

Viscograph measurement

The viscograph profiles were determined using the Brabender Viscograph (Richter *et al.*, 1969). The conditions of measurement were: starch concentration, 5·6% (w/w); temperature rate rise, 1·5°C/min; the highest temperature was 96°C which was maintained for 30 min.

RESULTS

Chemical composition

The chemical composition of the starches is presented in Table 1. The high protein concentration of some of the starches (Orlovchanin and Smaract varieties) reflects the difficulty in extracting starches from seeds; the highest protein contents characterizing starches from varieties with higher protein content in flour. Varietal differences were also found for lipid content extracted from the starch. The variety with both the lowest protein and the lowest lipid content in the starch was Sproot. The amylose content in the starches ranged from 30 to 43% between varieties.

Table 1. Chemical composition of pea flour and starches

Variety of pea	Flour, % dry weight				Starch, % dry weight			
	Starch	Protein	Lipids	Ash	Protein	Lipids	Ash	Amylose
Sproot	50	22	1·2	2·3	0·04	0·2	0·01	43
Vyatich	52	22	1·7	3·1	0·04	0·4	0·01	43
Orpella	51	23	1·4	2·6	0·04	1·1	—	42
Orlovchanin	58	27	2·1	3·1	0·36	1·0	—	33
Smaract	—	26	1·7	—	0·24	0·5	—	30

Analysis of starch grain size and shape

Previously, SEM studies have shown that native starch grains vary in size and shape. The granules may be spherical, oval or irregular in shape (Bertorft *et al.*, 1993; Stute, 1990) which makes comparative studies of sizes between different pea varieties difficult. This may explain why most authors do not define precisely what is meant when they measure the diameter of starch grains.

In the present study, the size of the granules (D) was determined as the diameter of a sphere with the same area as the starch granule image. The average size of granules was determined as:

$$\bar{D} = \frac{1}{n}\sum_{n} D_{\mathrm{n}}, \qquad (1)$$

where $\bar{D}$ = the average size of granules, D_{n} = the diameter of a circle with the same area as the area of the granule number n, where $n = 1, \ldots, 100$.

The size distributions of starch granules are represented in Table 2. The size of granules ranged from 1·45 to 90·00 μm but the majority of the granules were represented within the range 22·6–45·00 μm. In addition, there was some variation between varieties for the $\bar{D}$ of the granules, which ranged from 22 to 30 μm.

In order to compare the shapes of the different starch granules we have used the relationships:

$$F = \frac{4\pi S}{P^2} \qquad (2)$$

$$\bar{F} = \frac{1}{n}\Sigma F_{\mathrm{n}}, \qquad (3)$$

where F = form factor; $\bar{F}$ = average form factor; S = the area of the granule image; P = perimeter of the granule image.

The analysis of the distributions for F and $\bar{F}$ for starch grains of the different varieties indicated that most of the granules were either oval or almost spherical (Table 3).

Starch crystallinity

X-ray diffraction revealed that all the starches are represented by polymorph type 'C' and have a similar degree of crystallinity (Fig. 1; Table 4). On the other hand, there were relatively large differences in the X-ray intensity at $2\theta^\circ = 5{\cdot}75$, which expresses the different proportions of 'B' modifications in the starches (Sarco & Wu, 1978).

It was apparent that intensity of the peak at $2\theta^\circ = 5{\cdot}75$ could be related to the extent of the 'B' polymorph in the 'C' type starch. This was substantiated by our experiments with 'A' type (corn) and 'B' type (potato) starch mixtures. The relative intensity of the above reflection to the overall intensity of crystalline reflections of 'B' type starch was found to be 13·4%. The relationship between the proportion of the relative intensity of the peak at $2\theta^\circ = 5{\cdot}75$ and the intensity of this peak for the potato starch and for different mixtures of potato and corn starch is given in Fig. 2. This curve was used to estimate the proportion of 'B' polymorphs in the starches from the smooth pea seeds, which was found to be from 26 to 49% (Table 4).

DSC measurements

The DSC thermograms of the five pea starches during heating in excess water are given in Fig. 3 and the ther-

Table 2. Size distribution of starch granules

Pea variety	The quantity (n) of granules in each size class (μ)							Average size (μ)
	1·41	1·42–2·81	2·82–5·63	5·64–11·25	11·26–22·50	22·60–45·00	45·10–90·00	
Sproot	2	3	2	1	18	74	1	26
Vyatich	14	2	5	0	21	56	1	23
Orpella	10	0	3	3	19	65	0	23
Orlovchanin	6	4	0	9	31	51	0	22
Smaract	0	2	1	0	13	82	2	30

Table 3. Form factor distribution of starch granules

Pea variety	The quantity (n) of granules in each form factor class					Average form factor
	0·2	0·3–0·4	0·5–0·6	0·7–0·8	0·9–1·0	
Sproot	1	0	9	43	47	0·77
Vyatich	3	5	11	23	58	0·74
Orpella	2	0	14	20	63	0·78
Orlovchanin	0	2	16	43	40	0·75
Smaract	1	2	10	48	39	0·75

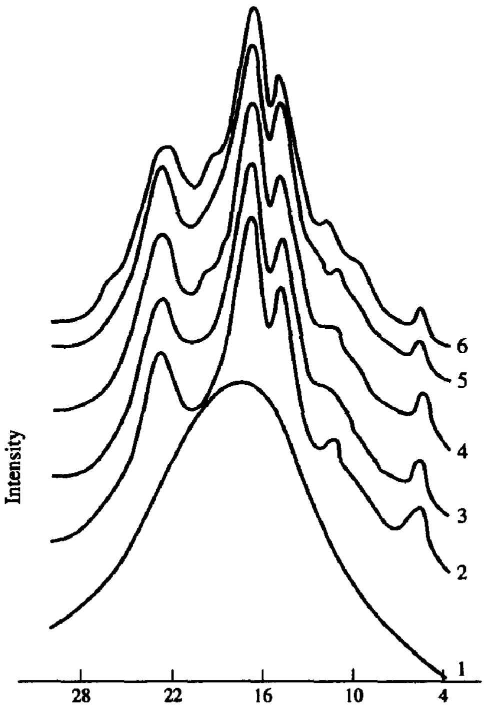

Fig. 1. X-ray patterns of pea starches. 1, amorphous sample; varieties 2, Sproot; 3, Orpella; 4, Vyatich; 5, Smaract; 6, Orlovchanin.

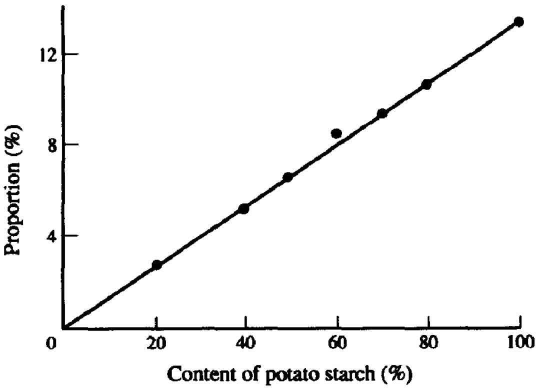

Fig. 2. The relationship between the proportion of the relative intensities of the peaks at $2\theta° = 5{\cdot}75$ for different mixtures of potato and corn starch and for pure potato starch.

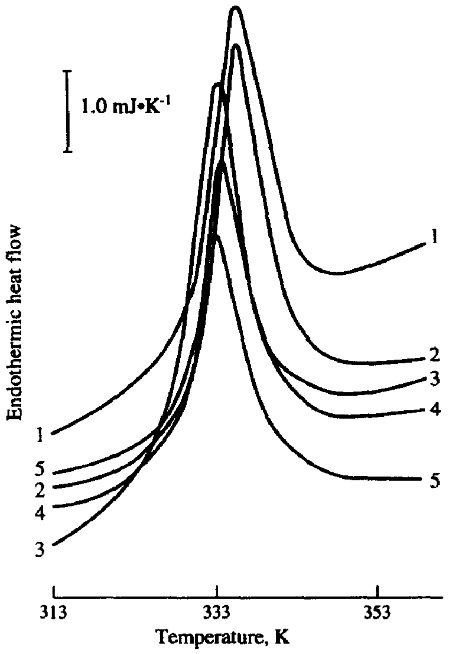

Fig. 3. The DSC thermograms of starches from various pea varieties in excess water. Heating rate = 1°C per min. Varieties and concentrations of the starch suspensions (w/w): 1, Smaract, 0·42%; 2, Orlovchanin, 0·50%; 3, Sproot, 0·44%; 4, Orpella, 0·42%; 5, Vyatich, 0·38%.

modynamic parameters of gelatinization are shown in Table 4. All the thermograms were obtained in quasi-equilibrium conditions; the concentration of aqueous suspension of starch was 0·4–1·5% and the heating rate 1°C/min (Shiotsubo & Takahashi, 1984). Under these conditions it is very unlikely that melting will occur in addition to the gelatinization process. In addition, there is no evidence that under these conditions KCl promotes such a phenomenon, although this may not be the case in more concentrated suspensions. All of the curves are represented by endothermal symmetrical peaks of gelatinization, with gelatinization temperatures (T^{p}) in the range 333·0–336·2 K, and the enthalpy of gelatinization (ΔH^{cal}) changing from 14·1 to 17·0 J/g. The higher gelatinization temperatures are characteristic of starches with a higher content of 'A' polymorphs. This agrees with a previous study which has shown a correlation between higher temperatures of gelatinization of type 'A'

Table 4. X-ray diffraction and DSC analysis of the starches

Variety of pea	% Crystallinity	% Content of 'B' pattern	$T^{\mathrm{p}a}$ (K)	ΔC_{p} (J/gK)	ΔH^{cal} (J/g)	ΔT^{b} (K)
Sproot	31	49	333·5	1·9	17·0	12·2
Vyatich	32	40	333·0	1·6	16·8	12·2
Orpella	26	44	333·7	1·7	16·8	12·1
Orlovchanin	27	26	335·7	1·7	15·0	12·7
Smaract	30	33	336·2	1·7	14·1	12·8

[a] T^{p}, Gelatinization temperature, determined as shown in Fig. 4.
[b] $\Delta T = T_2 - T_1 \cdot T_2$ and T_1, determined as shown in Fig. 4.

compared with the type 'B' starches (Cooke & Gidley, 1992). The molecular events responsible for DSC transition are uncertain but they include melting of crystallites. It is known that the packing energy for 'B' polymorphs is higher then for 'A' polymorphs (Sarco & Wu, 1978) and that the enthalpy changes for the gelatinization transitions for type 'B' starches is higher then for type 'A' (Noel & King, 1992; Kugimiya *et al.*, 1980). It is not surprising, therefore, that starches with a higher content of 'B' polymorphs have higher ΔH^{cal}. During the gelatinization process water acts as a plasticizer, so that the changes in its quality should result in changes in the thermodynamic parameters of gelatinization.

Figure 4 shows the thermograms for starch gelatinization in the presence of KCl. When the concentration of salt is greater than 0·1–0·2 M the single peak doubles. Under these conditions, therefore, there are two temperatures of gelatinization. The higher the concentration of KCl, the higher the temperature of gelatinization for each of the peaks (Figs 5, 6). It appears that the presence of salt in the solution exposes the existence of two different types of independent co-operative structures in the starches. These structures seem to have the same gelatinization temperatures in water, but different gelatinization temperatures in salt solutions.

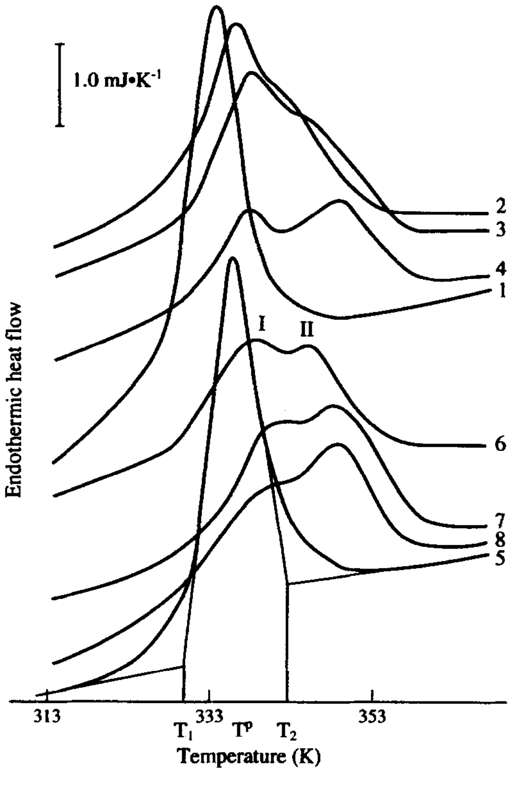

Fig. 4. The DSC thermograms of the starches of Sproot and Orlovchanin varieties at different concentrations of KCl. Heating rate, 1°C/min. Sproot variety: 1, distilled water; 2, 0·3 M KCl; 3, 0·5 M KCl; 4, 1·5 M KCl. Orlovchanin variety: 5, distilled water; 6, 0·3 M KCl; 7, 0·5 M KCl; 8, 1·5 M KCl. Concentration of starch suspensions (w/w): 0·44–0·50%.

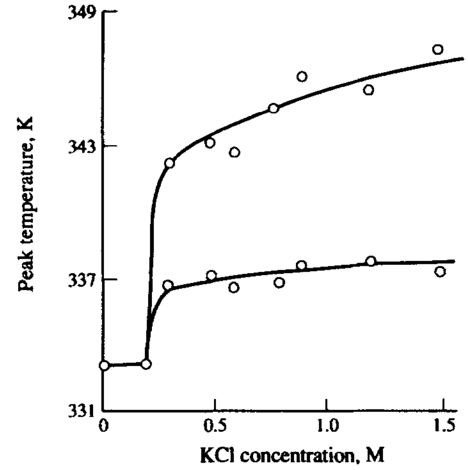

Fig. 5. The effect of KCl concentration on the gelatinization temperatures of starch from Sproot variety. Concentration of starch suspensions (w/w): 0·4–1·5%.

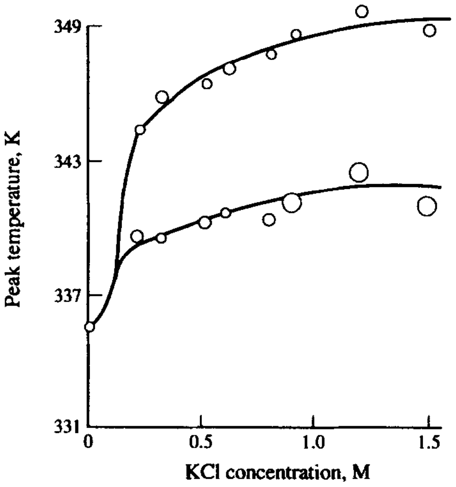

Fig. 6. The effect of KCl concentration on the gelatinization temperatures of starch from Orlovchanin variety. Concentration of starch suspension (w/w): 0·4–1·5%.

Splitting into two peaks has been observed in water alone for a few pea varieties, but not those used in the present study. In these cases the process was further accentuated in aqueous salt solutions (pers. observ.). It has been proposed that 'A' and 'B' type crystallites are placed in different independent co-operative structures. If so, the peaks of 'A' and 'B' type starches should behave in a similar way with increasing salt concentrations. It is known that the gelatinization temperature of these starches (namely, corn and potato) increases under higher salt concentrations in a different manner (Evans & Haisman, 1982; Jay-Lin & Ames, 1993). Comparing our experimental data with the information from these papers allows us to equate low temperature peak I with type 'B' crystallites and to equate high temperature peak II with type 'A' crystallites of the type 'C' starches.

The relationship between peak I and peak II appears to be influenced by the content of 'B' polymorphs in the starch. This is illustrated by the thermograms in KCl solutions of the starches with different contents of 'B' polymorphs (Fig. 4).

Swelling and solubility of the starches

The solubility and swelling ability of starch is dependent on the temperature and the source of the starch. There were no differences between three of the varieties for these parameters (Fig. 7). As expected, both the solubility and swelling increase during heating. The slope of the solubility with temperature increases slowly until approximately 333 K (the gelatinization temperature of these starches) and then dramatically changes above this temperature. The curve reflecting the swelling characteristics also has a bending point just after 333 K, but the slope of the curve after this point is similar to that of the first part of the curve. It is likely, therefore, that the increase in solubility and swelling takes place as a result of gelatinization, which is in good agreement with the literature (Doublier, 1987).

Rheological behaviour

The consistency of a starch suspension during heating is a very important characteristic of starch. It is often measured by stirring a starch suspension during a standardised heating and cooling cycle. The behaviour of starch from the Smaract pea variety was studied using this technique (Fig. 8). The curve obtained for this variety is similar to those obtained and reported in the literature for other smooth pea starches (Doublier, 1987; Stute, 1990). The starch was characterized by a plateau for the viscosity while maintaining the temperature at 96°C, which is higher than that reported in the above papers. The differences reduce after cooling. The viscosity seems to be influenced not only by the pea variety, but also by the extraction procedure. The value of viscosity after cooling seems not to be so critical to these parameters.

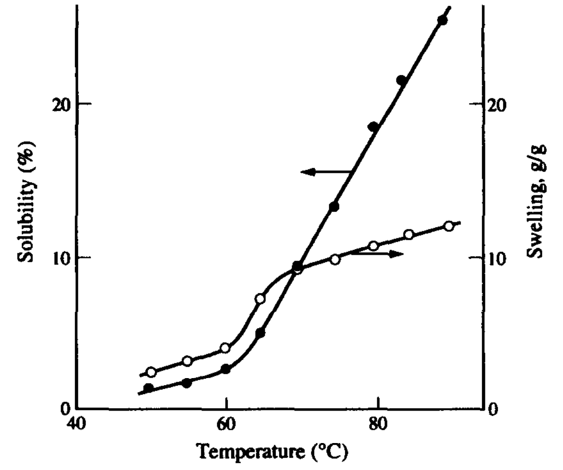

Fig. 7. Swelling–solubility curves of smooth pea starches (Sproot, Vyatich and Orpella varieties). Concentration of starch suspension (w/w): 1·4%. Heating rate, 1·5°C/min. The samples were held at fixed temperatures for 30 min.

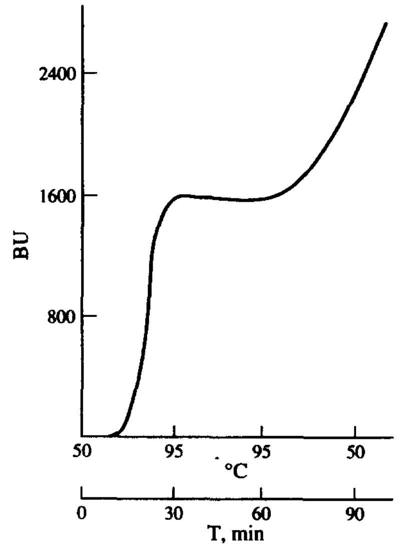

Fig. 8. Brabender viscogram of pea starch (Smaract variety).

DISCUSSION

The proposed model of type 'C' starch gelatinization suggests the existence of two types of independent co-operative structures, containing either 'A' or 'B' polymorphs. The total enthalpy change as a result of the transition can be expressed, therefore, by the equation:

$$\Delta H^{\mathrm{cal}} = a\Delta H_a^{\mathrm{cal}} + b\Delta H_b^{\mathrm{cal}}, \qquad (4)$$

where ΔH^{cal} = the total enthalpy change of the independent co-operative structures; $\Delta H_a^{\mathrm{cal}}$ and $\Delta H_b^{\mathrm{cal}}$ = the enthalpy changes of the independent co-operative structures containing either 'A' or 'B' polymorphs; a and b = content of 'A' and 'B' polymorphs in the starch.

According to theoretical representations the ratio of the packing energies for 'B' to 'A' polymorphs is equal to 1·7 (Sarco & Wu, 1978). The experimental data for the starches which contained either 'A' or 'B' patterns in excess water gave approximately the same ratio: 1·5–1·7 (Kugimiya *et al.*, 1980; Noel & Ring, 1992). Since half of the crystallites of the starch of the Sproot variety are represented by 'B' polymorphs, it is apparent, from this model that peak I should be larger then peak II (Fig. 4). The comparative study using starches with different 'B' polymorph content (Fig. 4) is in agreement with the proposed model.

With the increasing concentration of KCl, peak II became higher then peak I. This is because both peaks widen under these conditions. From Table 4, Figs 3, 7 and 8 it can be seen that during heating, the critical increase in solubility, swelling and viscosity begins just after the peak temperature of gelatinization. This supports the theory that it is necessary to achieve an initial level of swelling prior to the beginning of gelatinization (Biliaderis *et al.*, 1980; Donovan, 1979). Thus high solubility, swelling and viscosity are consequences of the gelatinization process.

ACKNOWLEDGEMENTS

The authors wish to thank the Joint Scientific & Production Enterprise KORUND for financial support. They also express their thanks to Dr. F. Schierbaum (Germany) for his kind help in viscosity measurements and A.N. Danilenko (Russia) for useful discussions about the manuscript.

REFERENCES

Bertorft, E., Manelin, S.R. & Qin, Z. (1993). *Starch/Starke*, **45,** 215–220.

Biliaderis, C.G., Maurice, T.J. & Vose, J.R. (1980). *J. Food Sci.*, **45,** 1669.

Blanshard, J.M.V. (1987). In *Starch: Properties and Potential*, ed. T. Galliard. Published for Society of Chemical Industry by John Wiley, Chichester, pp. 16–54.

Cooke, D. & Gidley, M.J. (1992). *Carbohydr. Res.*, **227,** 103–112.

Donovan, J. (1979). *Biopolymers*, **18,** 263.

Doublier, J.L. (1987). *J. Cereal Sci.*, **5,** 247–262.

Evans, I.D. & Haisman, D.R. (1982). *Starch/Starke*, **34,** 224–231.

Gernat, Ch., Radosta, S. & Damashun, G. (1990). *Starch/Starke*, **42,** 175–178.

Hermans, P.H. & Weidinger, A. (1961). *Macromol. Chem.*, **24,** 44–46.

Jay-Lin, J. & Ames, I.A. (1993). *Starch/Starke*, **45,** 161–166.

Kozimina, N.I. (1971). *Sugar Industry*, **11,** 59 (in Russian).

Kugimiya, M., Donovan, J.W. & Wong, R.Y. (1980). *Starch/Starke*, **32,** 265–270.

Noel, T.R. & Ring, S.G. (1992). *Carbohydr. Res.*, **227,** 203–213.

Perrin, C.N. (1953). *Analyt. Chem.*, **25,** 928.

Pfeil, W. & Privalov, P.L. (1979). In *Biochemical Thermodynmics*, ed. M.N. Jones. Elsevier, Amsterdam, pp. 75–115.

Richter, M., Augustat, S. & Schierbaum, F. (1969). *Auscgewahlte Methoden der Starkechemie*, VEF fachbuchferlag.

Sarco, A. & Wu, H.-C.H. (1978). *Starch/Starke*, **30,** 73–78.

Sergeev, Y.M., Spivak, G.V., Sasov, A.Yu, Osipov, V.I., Sokolov, V.N. & Rau, E.I. (1984*a*). *J. Microscopy*, **135,** 1–12.

Sergeev, Y.M., Spivak, G.V., Sasov, A.Yu., Osipov, V.I., Sokolov, V.N. & Rau, E.I. (1984*b*). *J. Microscopy*, **153,** 13–24.

Shiotsubo, T. & Takahashi, K. (1984). *Agric. Biol. Chem.*, **48,** 9–17.

Stute, R. (1990). *Starch/Starke*, **42,** 178–184.