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GELATINIZATION AND MELTING OF MAIZE AND PEA STARCHES WITH NORMAL AND HIGH-AMYLOSE GENOTYPES*

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Key Word Index—*Pisum sativum*; Leguminosae; pea; *Zea mays* Gramineae; maize; starch; melting; gelatinization; amylose.

Abstract—Normal and high-amylose maize and pea starches (gene *ae* and *ra* respectively) were studied during gelatinization by their swelling and solubility patterns, as well as by differential scanning calorimetry. High-amylose (> 60%) starches showed restricted swelling and solubility, compared to normal maize (25%) and pea (35%) genotypes. At low water volume fractions ($v_1 < 0.75$), gelatinization occurred by two (pea and high-amylose maize) or three (normal maize) melting steps of crystallites, following the Flory equation. At high water volume fractions, melting of the crystallites and swelling are cooperative processes. On the basis of these experiments, explanations for the differences in behaviour between normal and high-amylose genotypes are discussed.

INTRODUCTION

Genetic variants, affecting the composition of the seeds, are known in several crop plants. Many mutants of maize show differences in starch characteristics, accumulated sugars, as well as in amino acid composition [1, 2]. For starch, several genes have been identified, among which the most important are waxy (*wx*), sugary-1 (*su1*), sugary-2 (*su2*), dull (*du*) and amylose extender (*ae*). In pea, the two major gene variants, affecting pulse composition, are those involving mutations in the *ra* (chromosome 7) and *rb* (chromosome 3) loci [3]. Both influence proteins [4], lipids [5] and storage carbohydrates [6, 7]. In addition, they lead to wrinkled seeds, whereas the normal genotype has smooth seeds. When considering starch properties, both the *ae* and *ra* genes appear equivalent. In both species, the major expression of these genes is to increase amylose content of the starch. Besides a slight reduction in average M_rs of amylose and amylopectin still with the same primary structure, a third component appears: the intermediate material. Whereas in high amylose maize starch, Baba *et al.* [8] claimed that this fraction was a short linear amylose, Colonna and Mercier [9] have demonstrated the relationship of intermediate material with amylopectin in the case of wrinkled pea starch. Comparatively, the gelatinization behaviour of such high-amylose starches has received relatively little attention up to now [10].

The purpose of this investigation was to determine the effects of increasing amylose content on gelatinization characteristics of pea and maize starches. The thermal transition was studied by the classical techniques of swelling, solubility and the Brabender viscoamylograph. In addition, differential scanning calorimetry (DSC), a thermoanalytical technique for monitoring changes in physical or chemical properties of materials, was used to investigate the heat changes associated with such transitions [11, 12].

RESULTS

The four starches studied were pure since their anhydroglucosyl content was greater than 99%. The iodine binding capacities ranged from 4.9–6.9 mg iodine bound/100 mg polysaccharide for normal genotypes to 12.1–14.9 for high amylose genotypes (Table 1), this corresponded to amylose contents of 25.0, 61.7, 35.2 and 76.0% for normal and high-amylose maize, smooth and wrinkled pea starches respectively. Their specific gravities were similar: 1.4757–1.4911 g/cm^3.

Gelatinization

Microscopic observation, using polarized light, was adequate for following gelatinization of normal genotypes, by observing the loss of birefringence through the polarization cross. The entire transition occurred over a large temperature range, of 49 (5% gelatinized granules)–60 (50%)–67° (95%) for normal maize and 55–65–70° for smooth pea starches. However for each granule, the transition occurs over a small temperature interval of 0.5–1.5°. In contrast, this method is unsuccessful for studying the gelatinization of high-amylose starches, since the absence of birefringence in native granules is worsened by the slight swelling of granules occuring during gelatinization.

Smooth pea and normal maize starches exhibited a double stage swelling (Fig. 1): a first step occurred around 60–75° for normal maize and 55–75° for smooth pea, followed in both samples by a final step at 90–95°. At 96°, normal maize starch absorbs 35 g water/g dry sediment instead of 17 for smooth pea starch. Wrinkled pea and

*Part 6 in the series "*Pisum sativum* and *Vicia faba* Carbohydrates". For Part 5, see ref. [9].

Table 1. Physicochemical characteristics of starches

		Maize		Pea	
		Normal	High amylose	Normal	High amylose
Amylose (% total starch)		25.0	61.7	35.2	76.0
Specific gravity (g/cm³)		1.4801	1.4757	1.4953	1.4912
Solubility					
65° 200 rpm	a	3.8	0.5	13.0	2.3
	b	72.9	41.3	88.3	81.6
	c	11.0	0.3	32.0	2.5
65° 750 rpm	a	4.8	0.6	13 5	3.5
	b	70.4	44.9	88.3	19.9
	c	13.5	0.4	33.0	3.9
96° 200 rpm	a	25.0	4.2	23 0	8.5
	b	64.2	73.5	85.2	98.5
	c	64.0	4.9	55.7	11.0
96° 750 rpm	a	39.4	5.9	45.0	8.8
	b	38.3	91.8	59.7	97.9
	c	60.0	8.7	76.3	11.4

a, % total starch; b, amylose content (%) relative to soluble material; c, amylose content (%) relative to total amylose.

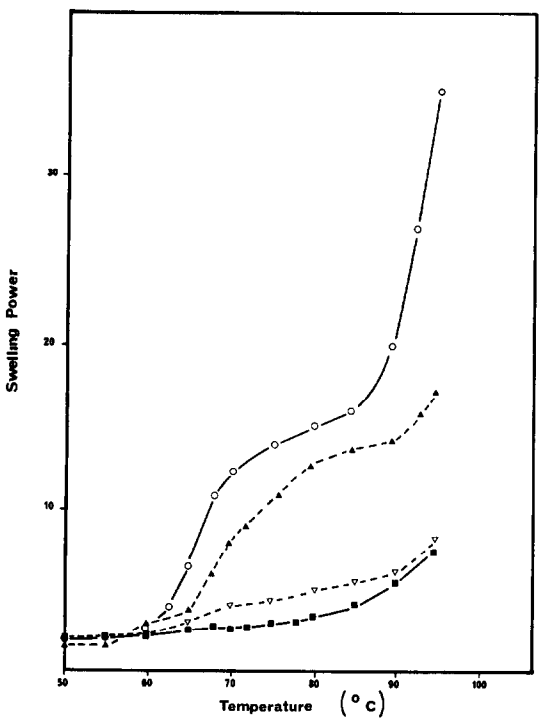

Fig. 1. Swelling patterns of smooth pea (▲), wrinkled pea (■), normal maize (○) and high amylose maize (▽) starches (expressed as g of water per g of dry starch in the sediment).

high-amylose maize starches have only a very low swelling, without any obvious transition temperature. At 96°, their swelling powers are rather low (~ 7 g water per g dry sediment).

The solubility behaviour (Fig. 2) as a function of

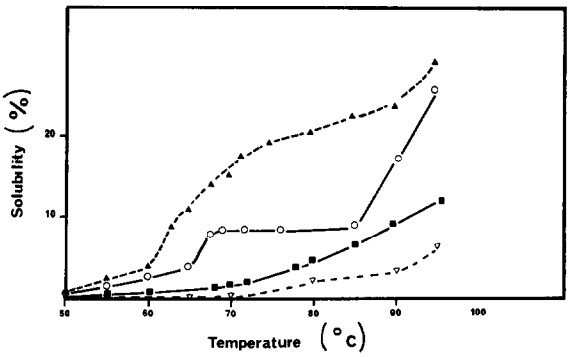

Fig. 2. Solubility patterns of smooth pea (▲), wrinkled pea (■), normal maize (○), and high amylose maize (▽) starches (expressed as ratio of soluble starch to total starch).

temperature displayed the same pattern as the swelling profile, indicating a relationship between these two processes. Starches from normal genotypes have solubility patterns with two important zones: the first one at 55–80° (smooth pea) or 65–85° (normal maize) and the second one at 90–95°. For high-amylose starches, solubility, which increases linearly with temperature, is still very low (5–10%).

In order to quantitatively study the leached material at 65 and 96°, soluble fractions were prepared in a heated round bottom vessel, with stirring by an anchor shaped blade at two different speeds of (200 and 750 rpm) (Table 1). At 65°, amylose was preferentially leached, except for high-amylose maize. By increasing the speed from 200 to 750 rpm, solubility increased slightly: at 750 rpm stirring, 13.5% of the total amylose was leached from normal maize, 33% from smooth pea, but only 0.4% and 3.9% from high-amylose maize and wrinkled pea

respectively. At 96°, total solubilities were increased for all starches. Normal maize and smooth pea starches displayed high rates of total amylose solubilization: 60 and 76.3% respectively at 750 rpm. High-amylose genotypes still have low solubilities, with mainly amylose being solubilized. The stirring rate slightly affects the solubilities of these starches; normal genotypes were rendered more soluble by operating at 750 rpm, than at 200 rpm. Under these conditions, amylopectin was only gradually leached.

The Brabender viscoamylograms of the four starches (Fig. 3) were determined at concentrations of 7.5% for normal genotypes and 10% for high-amylose genotypes. The higher concentration was required for the latter starches due to their very low paste viscosity. Temperatures of initial pasting were 77° and 70° for normal maize and smooth pea starches respectively. Smooth pea starch gives a Brabender curve of the C-type, according to the classification of Schoch and Maywald [13]; this corresponded to the absence of a pasting peak and a rather stable viscosity during cooking at 95°. Normal maize displays a pasting peak at 87° and a higher paste viscosity than smooth pea, increasing greatly during cooling to 55°: this is typical of B-type behaviour [13]. Both normal genotype starches exhibit considerable retrogradation during cooling. High-amylose starches have very low paste viscosities and no gel is formed (type D viscosity). Temperatures of initial pasting could not be determined for high amylose starches.

Differential scanning calorimetry (DSC)

With a large excess of water ($v_1 > 0.90$), the four starches exhibited only the endothermic peak P1 (Fig. 4). For normal genotypes, the temperature range, defined by t_1 and t_2, corresponded roughly to the gelatinization range, as determined by microscopy. Thermograms of gelatinized starches, obtained by a fast cooling (160°/min) and reheating under the conditions described previously, showed no transition, but demonstrated the existence of a modification of heat capacity (Cp) during this transition. Enthalpies of gelatinization (ΔH) are 3.0 and 3.2 cal/g dry starch for normal maize and smooth pea respectively. High-amylose starches give broad peaks, extending up to 125°. ΔH values are 0.8 and 1.1 cal/g dry starch for high amylose maize and wrinkled pea respectively.

When the volume fraction of water (v_1) decreased below 0.60, DSC thermograms of the four starches displayed several endothermic peaks: four for normal maize and three for the others. When v_1 decreased from 0.55 to 0.20, these peaks, which are overlapping, became small and broad in shape. No estimation of transition enthalpies was therefore due to the difficulty in drawing appropriate baselines (Fig. 5).

For all starches, the temperature of the first endothermic transition (P1) did not vary significantly with water content, but the transition enthalpy decreased when v_1 was below 0.70 and this peak disappeared completely when $v_1 < 0.45$. In contrast, the temperature of the other transitions was shifted towards higher temperatures as v_1 decreased. Furthermore these transitions disappeared completely at a high water volume fraction ($v_1 > 0.65$). Above 200°, cells generally leak or thermograms present downturn in heat flow, probably due to exothermic decomposition of starch molecules.

As starch is a semi crystalline material, transitions from an ordered state to solution can be treated as first order phase transition and fitted to the thermodynamic equation for crystallites melting in the presence of a diluent [11]. According to the Flory equation [14]:

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \frac{R}{\Delta H_u} \cdot \frac{V_u}{V_1} [v_1 - \chi_1 v_1^2]$$

where R is the gas constant, ΔH_u the fusion enthalpy per repeating unit (anhydroglucose), V_u/V_1 the ratio of the molar volume of the repeating unit to the molar volume of the diluent (water), T_m (°K) the melting point of the crystalline polymer + diluent, T_m^0 (°K) the true melting point of undiluted polymer crystallites, v_1 the volume

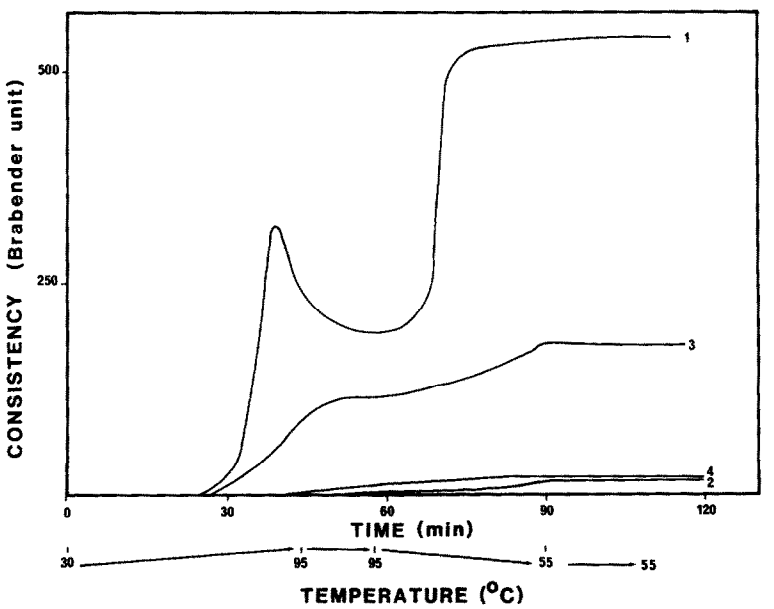

Fig. 3. Brabender viscoamylograms of peas and maizes starches: 1, normal maize; 2, high amylose maize; 3, smooth pea; 4, wrinkled pea.

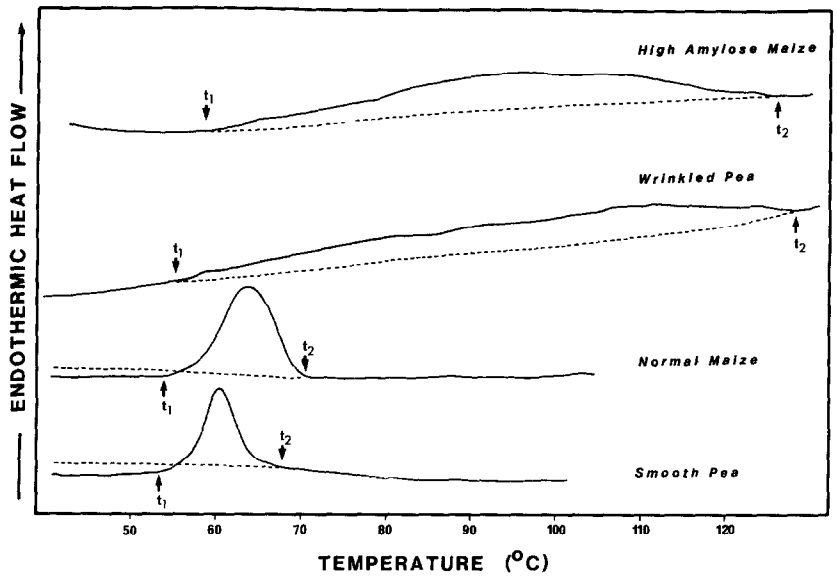

Fig. 4. Typical DSC curves of smooth (v_1 0.92) and wrinkled (v_1 0.96) peas, normal (v_1 0.93) and high amylose maize (v_1 0.89) starches; dotted curves represent second heating after fast cooling: t_1 and t_2 are the onset and concluding temperature respectively.

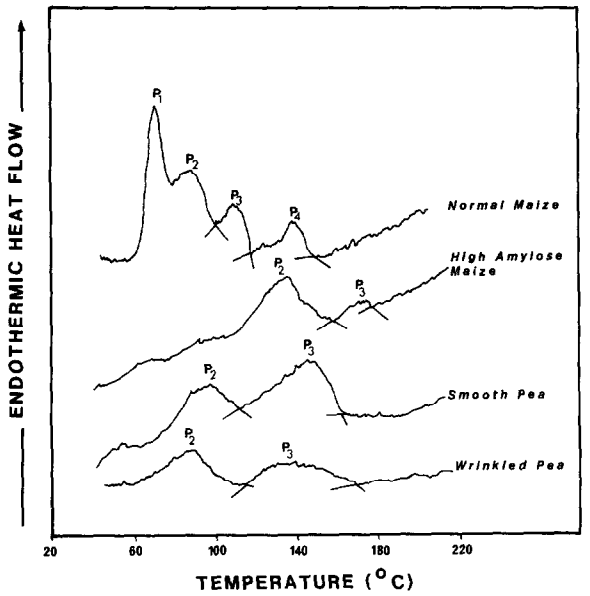

Fig. 5. Typical DSC curves for pea and maize starches, having different water volume fractions: wrinkled pea, 0.35; smooth pea, 0.29; high amylose maize, 0.20 and normal maize, 0.55

fraction of the diluent and χ_1 the Flory–Huggins interaction parameter (a dimensionless quantity characterizing the solvent–polymer interaction energy). Under our experimental conditions, v_1 is less than one, while χ_1 should be in the range of 0–0.5 [11]. Therefore $\chi_1 v_1^2$ can be neglected in front of v_1 and a linear relationship exists between $1/T_m$ and v_1 In this investigation, the concluding temperatures of the thermograms would represent the melting points of the most perfect crystallites for each peak (T_m). For smooth and wrinkled pea starches, plots of the reciprocal of the transition temperature T_m as a function of v_1 are shown for the two last transitions P2 and P3 (Fig. 6) and show good agreement for $v_1 < 0.55$. Each transition was then characterized by its extrapolated melting temperature T_m and the entropy of transition ΔS_u (on a molar monomer basis), calculated according to the relation $\Delta S_u = \Delta H_u/T_m$ (Table 2). On each thermogram, all the transitions do not present the same importance.

DISCUSSION

The amylose contents of pea starches (smooth: 35.2%; wrinkled: 76.0%) are higher than those of maize starches (normal: 25.0%; high-amylose maize: 61.7%). The high-amylose genotypes in both cases lead to some modification of the native granular structure of the starches, with a lower crystallinity, a B-type X-ray spectrum and a lower acid susceptibility than for their normal genotypes [15, 16]. However, the consistency in their specific gravities indicates that the packing of chains is not greatly modified.

All starches exhibit the gelatinization phenomenon, which is the disorganization of the semi-crystalline structure of the starch granules during heating in the presence of a sufficient amount of water ($v_1 > 0.9$). For normal genotypes, gelatinization occurs in two stages. The first step around 60–70° corresponds mainly to a swelling of the granules, with limited leaching. Loss of birefringence, which demonstrates that macromolecules are no longer oriented, occurs prior to any appreciable increase in viscosity. Compared to these techniques, DSC allows one to determine the gelatinization temperature more easily and more precisely than microscopy, and additionally the measured energy input needed to disorganize the crystalline structure of the granule. The second step, above 90°, is the complete disappearance of granular integrity by excessive swelling and solubilization. Nevertheless, this last transition is not detectable by DSC. Swollen granules

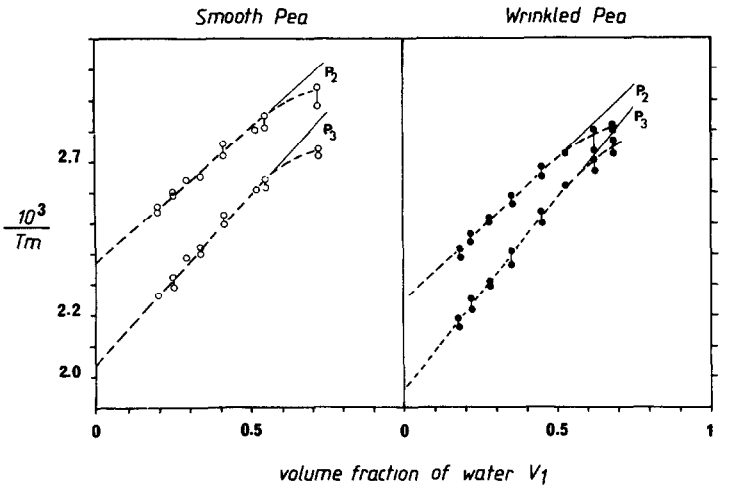

Fig. 6. Reciprocal melting point $1/T_m$ versus the water volume fraction (v_1) for smooth and wrinkled pea starches.

Table 2. Thermodynamic characteristics of melting transitions

	Transition								
	P2			P3			P4		
Starch	T_m° (°C)	ΔH^*	$\Delta S\dagger$	T_m° (°C)	ΔH^*	$\Delta S\dagger$	T_m° (°C)	ΔH^*	$\Delta S\dagger$
Smooth pea	157±7	12.3	28.6	244±8	8.6	16.6	—	—	—
Wrinkled pea	171±7	13.8	31.1	253±8	9.6	18.2	—	—	—
Normal maize	152±7	29.5	69.4	160±5	31.0	71.6	205±8	21.5	45.0
High amylose maize	152±7	38.5	90.6	240±8	17.8	34.7	—	—	—

*Expressed as cal/monomer unit.
†Expressed as cal/°K. monomer unit.

become fragile towards shear. Normal maize starch, with the highest swelling, has a pasting peak, due to granules breaking. In contrast, the limited swelling of smooth pea starch explains the absence of pasting peak and the type of hot-paste viscosity (Fig. 3). In both starches, high agitation during leaching leads to a breakdown of the swollen granules only at 96°, with an increase in solubility. Even after 30 min at 96° amylose leaching was never quantitative (Table 1) [17]. Therefore the swelling capability is not linked to the total amylose leaching, whereas in wheat starch, the incomplete leaching limits the swelling, whatever the agitation [18].

Normal maize starch, which has a more viscous paste than smooth pea starch, is less stable during cooking than pea starch. However the behaviour of pea starch, like those of others legume starches [13, 19–21], cannot be compared to the behaviour of cross-linked starch [22] as claimed by Vose [23], even if pea starch presents a stable-hot paste viscosity. Both high amylose genotypes have strongly reduced swelling and solubility, demonstrated by their resistance to mechanical fragmentation during amylose leaching experiments and their failure to give viscous pastes (Fig. 3). Brabender viscoamylograms of all starches are consistent with the observed swelling behaviour. Leached macromolecules are not essential to explain the rheological behaviour of gelatinized starch suspensions, confirming the work of Evans and Haisman [24]. It appears that the increase in amylose content in starch granules is responsible for the decrease in their swelling.

It must be noticed that these results give a description of the events during gelatinization but do not provide a physical explanation of the irreversible transition. DSC, when carried out with reduced water volume fractions, is a better approach in this respect. Under our experimental conditions, all the starches exhibit several endotherms. For normal genotypes, as the amount of water is reduced, the first transition (P1) at the lowest temperature changes only slightly with temperature (Fig. 5), as already reported for potato [11, 25, 26], wheat [27, 28] and legume [29] starches. P1 is considered to be the normal gelatinization transition, whose importance decreases with the increase of v_1. The other endotherms (P2, P3 and P4) correspond to the true melting of starch crystallites, following the Flory law. Transformation from helix (crystalline) to coil (amorphous in solution) represents an elementary manifestation of polymer melting. All determinations have been carried out on a large range of v_1 (0.15–0.65), which allows more precise extrapolation than the conditions used by Donovan [11]. As water is a part of the crystalline

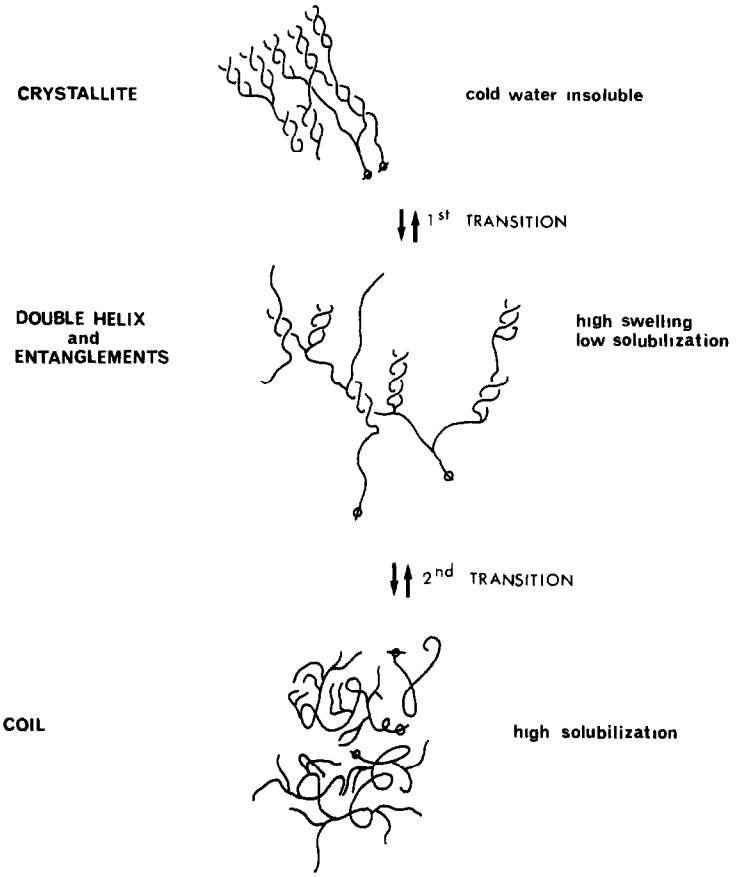

Fig. 7. Model of molecular transitions during starch gelatinization.

structure [30], the 'effective' water volume fraction is smaller, but any correction needs knowledge of the crystalline fraction, which is up to now, unknown. Each transition has its own thermodynamic characteristics (Table 2) with the following interpretations: T_m° represents the melting point of the pure undiluted polymer: ΔH_u is a property of the crystallizing chain repeating unit and is independent of the nature of the diluent used (water); ΔS_u is a measure of the entropy difference for a chain repeating unit between the crystalline and solution states, mainly the conformational entropy. In the case of a polymer–diluent system, the depression of the melting temperature is determined, to a first approximation, by the contribution of the entropy of mixing of the diluent with the polymer chains. The numerical values of the thermodynamic characteristics are of the same order as those measured for synthetic polymers containing ring structures (polyesters, polyanhydrides) [31].

The observed transitions can be compared to results published on other native starches [11, 26]. The transition P2, for normal maize starch, is clearly that of the usual amylose–lipid complex [32]. This transition is not observed with pea and high amylose maize starches, since their lipid content [7] is too low (< 0.1 %). The transition, called P3 for normal maize and P2 for all others starches, should correspond to the melting of ordered regions at reduced water content, by intermolecular transformation, as already observed on wheat [28, 33], potato [11, 25, 26] and legume [29] starches.

The last transition named P4 for normal maize and P3 for others starches has the highest extrapolated transition temperature, but enthalpy and entropy changes are lower than for the first transition P2. Donovan *et al.* [26] observed a similar endotherm on heat-moisture treated starches, which was attributed to another amylose–lipid complex. However, the explanation is not valid in our case, since the starches have low lipid contents. An intramolecular transformation from double helix to coil, over small portions of linear chains would be better hypothesis. At least it can explain the reversibility, due to easy conformational transitions on cooling and heating, and the lower values of H_u and S_u, compared to P2. It should correspond to the amylose transition from double helix [34, 35] to coil, observed in solution at 60° [36].

Except for the lipid–amylose complex endotherm, these two melting transitions cannot be related to the present crystallographic structures: high amylose starches are of B-type whereas maize starch is of A-type and pea starch an intermediate type between A and B [3]. The P2/P3 transition is higher with normal genotypes than with high amylose ones, due to their higher amylopectin content, which is less able to adopt a coil conformation as it is branched. All these determinations are based upon a nil value for χ_1. However, when v_1 is higher than 0.55, linearity between $1/T_m$ and v_1 no longer exists, proving that χ_1 is positive; our experimental results do not permit a precise determination of χ_1. When the water content is limited ($v_1 < 0.75$), it is clear that thermal disorganization

of starch cohesion is explained by the distinct melting of crystallites. At high water content, the rise in temperature increases translational and rotational diffusions of portions of chains within the amorphous parts of the granules. This leads to a reversible swelling, restricted by the crystallites that constitute cross links between the chains. When the temperature further increases, the melting of crystallites is the determinant event, independent of swelling, and allows increase in chain movement upon heating. The destabilizing action exerted by the amorphous parts of the granule is sufficient only because crystallites are already molten [25]. Swelling and melting are then cooperative processes; the entire gelatinization therefore appears as a semi-cooperative process [37]. The fact that when this transition occurs, the alpha-glucans appear as swollen gel particles rather than release into solution, indicates the presence of some structures within the gelatinized granules acting as cross links, like polymer entanglements or hydrogen bonds [14]. When the number of cross links in the gel particles increases, swelling decreases. In the starch–water system, ordered regions remain after gelatinization; the entropy of fusion and the entropy of mixing with water are therefore decreased. This phenomenon results in a rise in gelatinization temperature and a decrease in ΔH values. Therefore a possible explanation for the differences between normal and high amylose genotypes should involve two different physical characters: 1. The high order of native high amylose starches; the more ordered the initial state of the starch granule, the more order remains after gelatinization and so the less swelling occurs. This fact is consistent with gelatinization data and heterogeneous acid kinetics. However, the low crystallinity of high-amylose starches, when studied by X-ray diffraction, is not related to a lower organization, but is due to the small size of the crystallites [16]. 2. The interaction parameter χ_1 must be smaller for branched macromolecules (amylopectin) than for linear chains (amylose) [38] (i.e. amylose is more highly associated in solution than amylopectin). Therefore normal genotypes should be better hydrated than high amylose starches, leading to better swelling and solubilization.

All these physicochemical changes, taking place at the molecular level, explain the differences in gelatinization characteristics, between normal and high amylose genotypes of maize and pea starches. Yet now the unresolved problem is the arrangement of amylose and amylopectin macromolecules within the granule and their degree of entanglement.

EXPERIMENTAL

Material. Normal and high amylose maize (*Zea mays* L.) starches were provided by Roquette Frères (F-62136 Lestrem, France). Smooth and wrinkled pea (*Pisum sativum* L.) starches were prepared from their seeds as described previously [7].

Gelatinization methodology. The microscopic observation of gelatinization of native starches was followed under a polarizing microscope, fitted with a hot stage. Starch swelling and solubility were determined by the method of ref. [39] modified as follows: 0.25 g of starch was suspended in 25 ml H_2O and heated in a water bath adjusted to the desired temperature, with a slow magnetic stirring for 30 min. After centrifuging at 1500 *g* for 15 min, the amount of leached material was determined by the orcinol–sulphuric acid method; the sediment was weighed before and after desiccation to determine swelling and expressed as the total wt of H_2O in the sediment per/g of anhydrous sediment.

Pasting properties of native starches were determined on aqueous dispersion (7.5% for normal genotypes and 10% for high amylose genotypes, included starch moisture) using the Brabender Viscoamylograph (model E), fitted with a 250 cm/g head; the viscometer bowl was rotated at 75 rpm. For differential scanning calorimetry (DSC), a Perkin–Elmer DSC-2 was used with aluminium as inert thermal reference [25]. DSC scans were made at a heating rate of 10° per min from 20° to 190°; the instrumental sensitivity was 2 mcal/sec × inch. Samples (1–8 mg of starch on dry basis and 0–20 μl H_2O added by microsyringe) were hermetically sealed, allowed to equilibrate for at least 24 hr at 25° and then analysed by DSC. For each peak, two temps were measured: the onset temp (t_1) and the concluding temp (t_2). Enthalpy changes when determined, were evaluated by measuring the area under the gelatinization endotherm and measured as cal per g of starch on dry basis. Results were expressed as a function of vol. fraction of H_2O [11], calculated with specific value of density for each starch, according to the relation

$$v_1 = \frac{\text{water weight}}{\text{water weight} + \left(\dfrac{\text{starch weight db}}{\text{density}}\right)}$$

Specific gravity (g/cm^3) was determined by the pycnometer method (volume 23.449, 53 ml) using toluene at 25°. Samples were previously dried for 7 days in a vacuum oven at 45°. Each measurement, carried out on 2.5 g was triplicated. Starch purity was checked by the dual enzymatic amyloglucosidase–glucose oxidase method [7]. Iodine affinities of the starches and of their leached polysaccharides fractions were determined by amperometric titration [40] at 2.5°, Potato amylose (Avebe, Netherlands) complexing 19.6 mg iodine per 100 mg polysaccharide. Polysaccharide concentration was measured by the orcinol–H_2SO_4 method [41].

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