Pergamon 0031-9422(95)00390-8 Phytochemistry, Vol. 40, No. 4, pp. 1057–1064, 1995
Elsevier Science Ltd
Printed in Great Britain
0031-9422/95 $9.50 + 0.00

PROPERTIES OF POTATO STARCH: EFFECTS OF GENOTYPE AND GROWING CONDITIONS

JOAN E. COTTRELL,* CAROL M. DUFFUS, LINDA PATERSON and GEORGE R. MACKAY†

Crop Science and Technology Department, Scottish Agricultural College, West Mains Road, Edinburgh, U.K.;
†Scottish Crop Research Institute, Invergowrie, Dundee, U.K.

(*Received in revised form 7 April 1995*)

Key Word Index—*Solanum tuberosum*; Solanaceae; potato tubers; low-temperature sweetening; gelatinization temperature; differential scanning calorimetry; amylose; phosphorus; α-amylase.

Abstract—The aim of this work was to determine whether genotype and growing conditions influence the composition and physical properties of potato tuber starch granules. Starch is thought to be the source of the reducing sugars produced during low-temperature sweetening. Genotype influenced the properties of starch granules but there was no consistent difference between sweetening and non-sweetening cultivars in the properties of their starch granules. Growing conditions affected starch granule characteristics with starch from tubers grown in the heated glasshouse conditions having higher gelatinization temperatures and amylose contents as well as greater resistance to degradation by bacterial α-amylase.

INTRODUCTION

Crisp colour depends mainly on the concentration of the reducing sugars, glucose and fructose in the potato tuber prior to processing [1]. Concentrations of reducing sugars in excess of 0.2% fresh weight cause the crisps to become unacceptably dark when fried. The dark-brown colour is the result of the Maillard reaction which involves the conjugation, during the frying process, of reducing sugars and amino acids [2]. Greater amounts of reducing sugars accumulate when tubers are stored at temperatures below 5° and this phenomenon is known as low-temperature sweetening [3]. The source of the reducing sugars is believed to be starch and many of the studies into the biochemical basis of low-temperature sweetening have concentrated on measuring the activities of phosphorylytic and amylolytic enzymes involved in starch breakdown [4–8]. The cold labile glycolytic enzymes involved in sugar breakdown have also been studied extensively [9–11].

Cultivars exist which are relatively resistant to low-temperature sweetening [12–14] and a number of these have been shown to have lower activities of starch-degrading enzymes than cultivars which sweeten in the cold [7, 8]. However, sensitivity to low-temperature sweetening could also be a function of some property of the starch granules themselves which may influence their susceptibility to degradation by endogenous enzymes. For example, a hybrid of *Solanum tuberosum* and *S. phureja* which does not sweeten at low temperatures has starch granules which have higher gelatinization temperatures, crystallinity and amylose contents, as well as greater resistance to degradation by bacterial α-amylase than starch granules produced by *S. tubserosum* cv. Norchip, which sweetens in the cold [13]. Differences in the properties of the starch granules of commercial, sweetening and non-sweetening cultivars within *S. tuberosum* have not been investigated. Additionally, it may be that susceptibility of tubers to low-temperature sweetening is influenced via effects of growing conditions on the physical properties of the tuber starch granules which, in turn, affect their susceptibility to hydrolysis during storage at low temperature.

This paper reports the effect of genotype and growing conditions on the following properties of starch granules; gelatinization temperature, crystallinity, phosphorus and amylose content, susceptibility to bacterial α-amylase and granule size. Four genotypes were used in the study, Record and Pentland Dell, which are susceptible to low-temperature sweetening, and Brodick and Eden, which are resistant [3].

RESULTS AND DISCUSSION

Gelatinization temperature and crystallinity

The temperature at which 50% of the granules lost their birefringence coincided with the onset of the endothermic transition and was generally *ca* 3° lower than the temperature at which the peak endotherm (T_{p1}) occurred

*Author to whom correspondence should be addressed at Tree Improvement Branch, Forestry Commission, Northern Research Station, Roslin, Midlothian, U.K.

using differential scanning calorimetry (DSC) (Table 1a). In starch granules extracted from field grown tubers, T_{p1} occurred between 60.6 and 62.9 . This is significantly lower than the estimates obtained by Barichello *et al.* [13, 14] who found that T_{p1} occurred between 71 and 76 . Most other workers quote values of 62 66 for potato starch granules [15 17].

Where water was present in excess ($v_1 = 0.83$) all samples exhibited a single endotherm, T_1 (Table 1a). Starch granules are composed of crystalline and amorphous regions. The single endotherm which is exhibited when water is present in excess is thought to be due to hydration, followed by swelling and increased motion of the amorphous regions. As the crystalline regions are closely coupled to the amorphous regions, the swelling and movement in the amorphous regions induce strain in the crystallites which tears molecular chains away from the crystallites so that they melt cooperatively at a lower temperature than they would if isolated from the amorphous region. Swelling of the amorphous region and melting of the crystallites therefore occur synchronously and appear as a single endotherm.

Analysis of variance indicated that there was a significant ($P < 0.05$) effect of genotype on the temperature at which T_{p1} occurred (Table 1a). This was greatest in cv. Record and lowest in the cvs Eden and Pentland Dell (Table 1a). In contrast to the results of Barichello *et al.* [13, 14], there was no correlation between the temperature at which T_{p1} occurred and the susceptibility of a cultivar to low-temperature sweetening.

Analysis of variance also indicated that growing conditions had a significant ($P < 0.001$) effect on the temperature at which T_{p1} occurred (Table 1a). This was highest in starch granules extracted from tubers grown in the heated glasshouse; it was lowest in starch granules extracted from field grown tubers (Table 1a). High

Table. 1. (a) Effect of genotype and growing conditions on the DSC characteristics of extracted tuber starch. Volume fraction of water (v_1) was 0.83 throughout. The temperature at which 50% of the granules lost their birefringence is also included. (b) Effect of volume fraction of water (v_1) on the DSC characteristics of extracted tuber starch granules of cv. Eden

(a)

		Endothermic transition				
Cultivar	Growing conditions	Onset T_o (°)	Peak T_{p1} (°)	End T_m (°)	ΔH (J g^{-1})	50% loss of birefringence (°)
Pentland Dell	Field	57.8	61.2	66.5	15.4	57.5
	Unheated glasshouse	60.8	63.8	69.4	14.0	60.5
	Heated glassshouse	68.3	71.8	77.5	14.7	69.0
Eden	Field	57.0	60.6	66.9	14.3	57.5
	Unheated glasshouse	60.8	63.6	69.7	14.8	61.0
	Heated glasshouse	68.3	72.4	78.0	15.5	68.0
Brodick	Field	59.2	62.2	68.2	15.3	58.5
	Unheated glasshouse	61.6	64.0	68.9	14.6	61.5
Record	Field	59.4	62.9	68.3	15.6	60.0
	Unheated glasshouse	63.2	66.4	71.8	13.0	63.0

(b)

		Endothermic transition (°)			
Growing conditions	Volume fraction of water v_1	Onset T_o	Peak T_{p1}	Peak T_{p2}	End T_m
Field	0.83	57.0	60.6	—	66.9
	0.50	55.7	60.1	74.5	80.1
	0.33	71.0		85.3	100.2
	0.10	89.6		108.4	118.2
Unheated glasshouse	0.83	60.8	63.6	—	69.7
	0.50	58.2	63.5	78.0	84.3
	0.33	75.5		89.5	102.8
	0.10	95.3		113.6	126.0
Heated glasshouse	0.83	68.3	72.4	—	78.0
	0.50	66.1	71.4	82.3	92.2
	0.33	84.6		98.4	112.0
	0.10	102.3		116.0	126.8

temperatures for T_{p1} are thought to be indicative of more stable amorphous regions, a more ordered crystalline structure and/or a higher ratio of crystalline to amorphous regions [13].

More detailed DSC measurements were conducted on the starch granules from cv. Eden in which the volume fraction of water in each sample was reduced. At restricted water contents ($v = 0.5$), two endotherms were observed (T_1 and T_2) (Table 1b). The temperature at which T_{p1} occurred was independent of the water content of the sample. A further reduction in the water content caused T_1 to disappear and T_2 to be shifted to a higher temperature (Table 1b). T_1 is thought to represent gelatinization, whereas T_2 is due to the melting of crystallites. The upward shift of T_2 at progressively lower water contents is due to a reduced effect of the swelling of the amorphous regions. At extremely low water contents, T_1 disappears and gelatinization occurs entirely by melting of the crystallites [15, 18, 19]. The fact that the temperature at which T_{p2} occurred was highest in granules extracted from tubers grown in the heated glasshouse may indicate that these granules had greater stability in their crystalline regions [13].

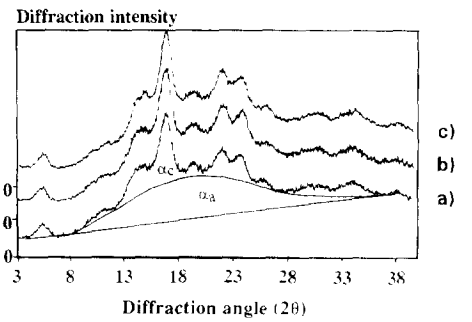

Fig. 1. Effect of growing conditions on X-ray diffraction patterns produced by starch extracted from tubers of cv. Eden (a, field; b, unheated glasshouse; c, heated glasshouse). To separate crystalline and amorphous regions, a smooth line was drawn which connected each part of minimum intensity; the region above this curve (α_c) was taken to represent the crystalline region. A straight line was drawn to connect the two points of intensity at 4° and 37° 2θ; the area between this straight line and the crystalline region (α_a) was considered to represent the amorphous region. Relative crystallinity was calculated as $\alpha_c/\alpha_c + \alpha_a$.

As a high degree of crystallinity has previously been associated with resistance to low-temperature sweetening [13], X-ray crystallography was carried out on all samples. The X-ray diffraction patterns obtained for the samples examined were characteristic of B-type starch from tubers [20] and the position of the peaks was similar to that described for potato starch by earlier workers [21]. The major peak occurred at 17.2° 2θ, corresponding to 5.15 Å, and there were smaller peaks at 14.8, 19.6, 22.0 and 23.8° 2θ. Estimates of relative crystallinity were virtually identical for all samples (Fig. 1). In contrast to the results of Barichello *et al.* [13], resistance to low-temperature sweetening was not associated with high relative crystallinity. It should be borne in mind when considering these results that interpretation of X-ray data is known to be difficult for starch due to the small size of the crystallites and the imperfection of the crystallites [18]. Estimates of crystallanity can be affected by sample preparation, with the method of drying the starch [22] and the water content of the starch [23] having major effects. As crystallinity is dependent on the chain-length of the amylopectin and its degree of branching, more information would possibly have been gained by measuring these two parameters independently. A highly branched amylopectin may lead to a reduction in the degree of intra- and intermolecular hydrogen bonding required for stability [24, 25].

Phosphorus and amylose content of starch granules

Phosphorus contents of starch granules ranged between 0.07 and 0.10% (Table 2). Analysis of variance indicated that there was no significant effect ($P > 0.05$) of genotype on the phosphorus content of granules and phosphorus content was unrelated to the susceptibility of a genotype to low-temperature sweetening. Analysis of

Table 2. Effect of genotype and growing conditions on the phosphorus and amylose content of starch granules

Cultivar	Growing conditions	Percentage phosphorus	Percentage amylose (± 95% confidence intervals)
Pentland Dell	Field	0.07	25.4 ± 0.1
	Unheated glasshouse	0.08	25.7 ± 0.8
	Heated glasshouse	0.07	28.5 ± 1.2
Eden	Field	0.07	24.4 ± 2.4
	Unheated glasshouse	0.10	24.7 ± 3.0
	Heated glasshouse	0.07	27.6 ± 1.7
Brodick	Field	0.09	26.2 ± 0.4
	Unheated glasshouse	0.10	27.6 ± 0.8
Record	Field	0.07	27.3 ± 2.1
	Unheated glasshouse	0.09	30.9 ± 0.7

variance also indicated that there was a significant effect ($P < 0.05$) of growing conditions on the phosphorus contents of the starch granules, with starch granules from tubers grown in the unheated glasshouse having the highest phosphorus contents and those extracted from tubers grown in the heated glasshouse having the lowest (Table 2).

The amylose content of starch granules ranged between 24 and 31% (Table 2). Analysis of variance indicated a significant effect ($P < 0.001$) of genotype with cv. Record producing granules with the highest amylose contents. Barichello *et al.* [13] found resistance to low-temperature sweetening was correlated with high amylose content but current results do not support this finding. Amylose content was significantly ($P < 0.001$) influenced by growing conditions, being highest in granules extracted from tubers grown in the heated glasshouse. High amylose contents are thought to confer a more ordered crystallinity to the granule [14] and this may account for the fact that high amylose contents were generally accompanied by high gelatinization temperatures.

Susceptibility of α-amylase and granule size

The Somogyi–Nelson test, which measures μmol of reducing sugar released, provides a measure of the number of times the glycosidic linkages in the starch are hydrolysed by α-amylase to produce sugar molecules with reducing ends. As this is a more sensitive test than that used to measure total soluble carbohydrate released, it enabled the hydrolysis of starch to be measured throughout the incubation period. Hydrolysis occurred at a steady rate throughout the incubation period (Fig. 2). The amount of total soluble carbohydrate released was extremely low and could only be measured accurately at the end of the incubation period using the phenol–sulphuric acid test. At the end of the incubation period with α-amylase, it was found that percentage degradation of granules never exceeded 3% (Table 3). Scanning electron micrographs (not presented) showed virtually no evidence of erosion on the surface of the granules. These results confirm that starch granules extracted from potato tubers are relatively resistant to breakdown by bacterial α-amylase [26–29]. The results are difficult to reconcile with those of Barichello *et al.* [13] who reported up to 35% hydrolysis of granules after only 3 hr of incubation. Their estimation would appear to be correct as their photomicrographs demonstrate a considerable amount of breakdown, with a number of granules showing bore holes and roughened surfaces.

Genotype had a significant effect ($P < 0.01$) on the susceptibility of starch granules to degradation by α-amylase. Resistance to α-amylase has previously been correlated with inability to sweeten in the cold but this is not supported by current results, because cv. Record, which sweetens in the cold, produced starch granules which were most resistant to attack by α-amylase

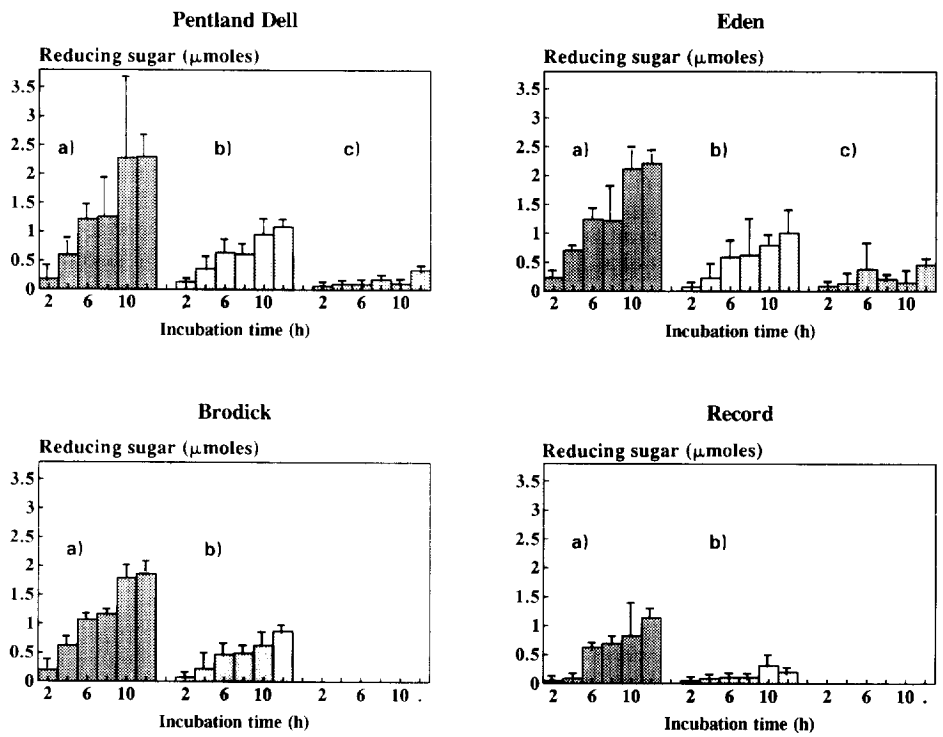

Fig. 2. Effect of cultivar and growing conditions on the susceptibility of extracted starch to applied α-amylase. Susceptibility to α-amylase expressed as μmoles of reducing sugar released (a, field; b, unheated glasshouse; c, heated glasshouse).

Table 3. Effect of genotype and growing conditions on the susceptibility of extracted starch to α-amylase. Susceptibility to α-amylase is expressed as percentage hydrolysis of granules after 12 hr of incubation with α-amylase

Cultivar	Growing conditions	Percentage degradation (± 95% confidence intervals)
Pentland Dell	Field	1.74 ± 1.04
	Unheated glasshouse	0.96 ± 0.36
	Heated glasshouse	0.61 ± 0.49
Eden	Field	2.21 ± 1.15
	Unheated glasshouse	0.67 ± 0.14
	Heated glasshouse	0.11 ± 0.47
Brodick	Field	0.78 ± 0.49
	Unheated glasshouse	0.75 ± 0.08
Record	Field	0.87 ± 0.14
	Unheated glasshouse	0.61 ± 0.18

(Table 3, Fig. 2). This finding must not be taken to indicate that cv. Record produces granules which are most resistant to digestion by endogenous enzymes present in the potato tuber. It remains uncertain which enzymes are responsible for breakdown of starch during low-temperature sweetening and, even if α-amylase is one of the enzymes responsible, it is unlikely that the isoforms of this enzyme present in the tuber have identical properties to those of the bacterial α-amylase used for incubations in the present work.

The reason for the differences in susceptibilities between cultivars may lie to some extent in the relative sizes of their granules. There was a higher proportion of large granules in tubers of cv. Record, the cultivar which produced starch which was least susceptible to degradation by α-amylase (Fig. 3). It is only the surface of the granule which is available for initial hydrolysis, so that the effective starch concentration is relatively lower when the population is composed of a high proportion of large granules. The idea that the surface area to weight ratio may be an important factor in determining susceptibility to α-amylase is supported by our finding that when large and small granules from a population are separated, it is the latter which show the greatest susceptibility to hydrolysis by α-amylase (Fig. 4). There were only minor differences in the other properties of the large and small granules (Fig. 4).

However, granule size cannot account for the significant ($P < 0.001$) effect of growing conditions on susceptibility to α-amylase. Tubers grown in the heated glasshouse produced starch granules which were most resistant to degradation by α-amylase but this was not accompanied by any increase in the average size of granules (Fig. 3). Some inherent property of the starch, independent of granule size, must have been influenced by the warm growing conditions which enabled these granules to have greater resistance to α-amylase.

Resistance to α-amylase has previously correlated with high gelatinization temperatures in beans [30] and potatoes [13]. In addition, it has also been correlated with high amylose contents in barley [31], maize [32, 33],

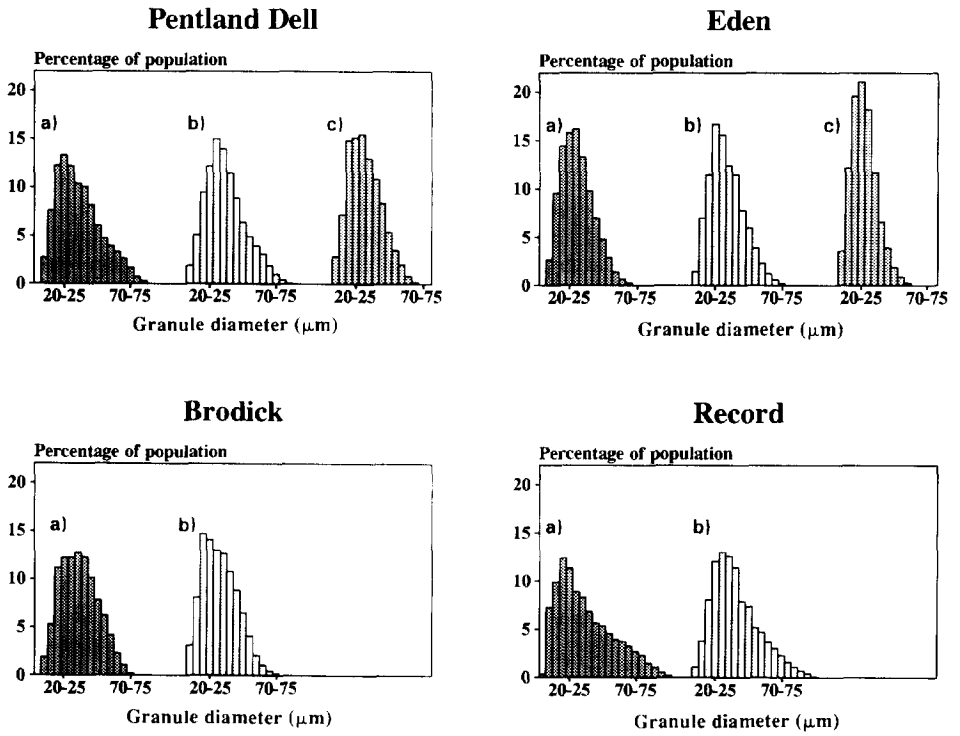

Fig. 3. Effect of cultivar and growing conditions on size distribution of starch granules extracted from tubers (a, field; b, unheated glasshouse; c, heated glasshouse).

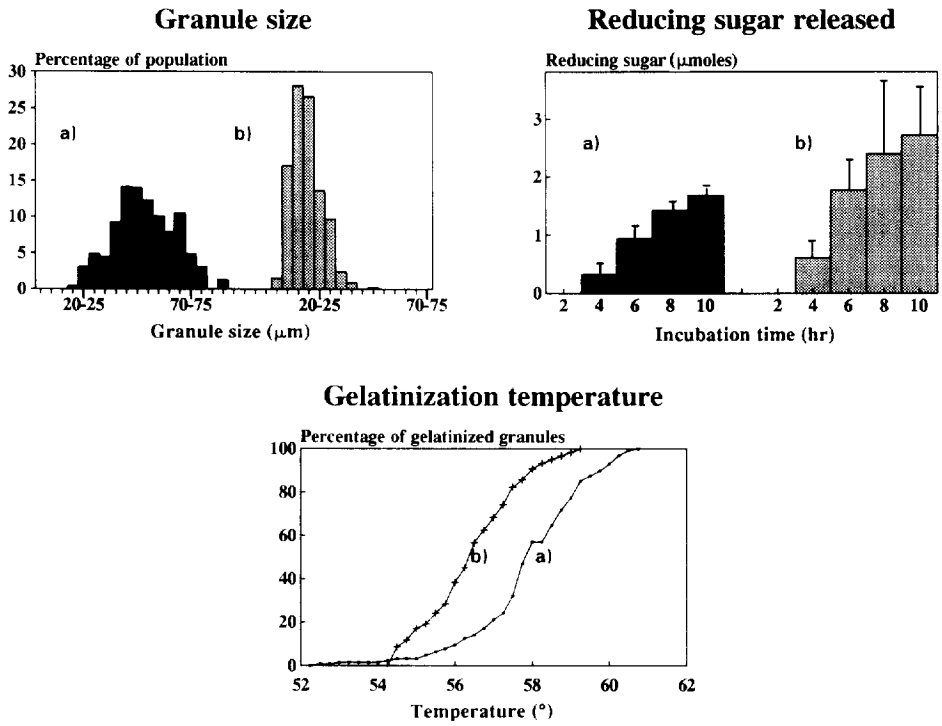

Fig. 4. Size profile, susceptibility to α-amylase and gelatinization temperatures of large and small granules extracted from cv. Eden grown in the field (a, large granules; b, small granules).

beans [30] and potatoes [13] but the reason for this correlation remains unclear. More hydrolysis occurred when sugar beet α-amylase was presented with amylopectin rather than amylose as a substrate [34] but substrate preference is unlikely to be the basis of the correlation. It is more likely that the arrangement of amylose and amylopectin within the granule is the important determinant of susceptibility to α-amylase. The exterior of the granule is known to be very resistant to degradation and the composition of the outer layer is probably more important than that of the granule as a whole. The outer layer of starch granules from cereal grains is composed of individual chains of amylose and amylopectin protruding from the surface of the granule [35, 36] and the arrangement of the chains may affect how accessible the α-(1,4) glycosidic bonds are to α-amylase.

In summary, no correlation was found to exist between the composition or physical properties of tuber starch granules of a cultivar and its tendency to sweeten during low-temperature storage. Warm growing conditions resulted in high gelatinization temperatures, high amylose contents and greater resistance to hydrolysis by bacterial α-amylase. It remains unknown whether growing conditions influence the susceptibility of a cultivar to low-temperature sweetening and this question requires to be investigated.

EXPERIMENTAL

Plant material and growing conditions. Seed tubers of cvs Record and Pentland Dell (low-temp sweetening cvs), and Brodick and Eden (low-temp sweetening resistant cvs), obtained from the Scottish Crop Research Institute (SCRI), were planted in field plots at SCRI and in an unheated glasshouse at the Scottish Agricultural College (SAC), Edinburgh, U.K.). Tubers of cvs Pentland Dell and Eden were also grown in a heated glasshouse at SAC. Temps gradually increased throughout the growing season and the mean daily temps from planting until foliage removal were 13.1 19.3 and 23.1° in the field, unheated glasshouse and heated glasshouse, respectively. Field plots were planted on 16 April 1992 and harvested on 14 September 1992. In both glasshouses, tubers were planted on 19 March 1992 and harvested on 5 August 1992. In the glasshouses, single tubers were planted in 12″ plastic pots two-thirds filled with John Innes No. 1 compost. Emerging shoots were 'earthed up' by filling the remaining space in the pot with compost. Foliage was removed 2 weeks prior to tuber harvest when the leaves were beginning to senesce. Tubers obtained from the heated glasshouse were much smaller than those from the other two growing conditions. Similar effects of high temps on tuber yields have been reported previously [37, 38].

Starch extraction. Starch extraction, using the methods of Refs [13] and [39], was performed within 24 hr of tuber harvest. Tubers were peeled, roughly diced and placed in a beaker containing ice-cold infiltration medium (0.3 M K_2HPO_4 containing 10 mM $MgCl_2$, 10 mM EDTA, 0.2% (w/v) BSA (fatty acid free) and 12% (w/w) sucrose). The beaker was placed in a desiccator containing ice and vacuum-infiltrated for 30 min. In

order to isolate starch granules in a cold room, a 50 ml conical flask was attached to the base of a vertical glass tube (length 32.5 cm, diameter 2.5 cm). A glass funnel containing a nylon net was attached to the top of the glass tube. The flask and the lower half of the glass tube were then filled with a pre-cooled medium (0.3 M K_2HPO_4 containing 10 mM $MgCl_2$, 10 mM EDTA and 15% (w/w) sucrose). An equivalent vol. of pre-cooled infiltration medium was layered on top so that the nylon net and glass funnel were partially filled with liquid. Diced tuber tissue was removed from the desiccator and thinly sliced into the nylon net. When the net was full, tuber slices were discarded and more were added. Granules were left to settle into the conical flask for 1 hr, after which the flask was detached. Granules were rinsed $\times 5$ in ice cold dist. H_2O, taking care to ensure that all granules had settled to the bottom before the H_2O was removed. Washed granules were transferred to plastic weighing boats and dried at 30°. When there was no further loss of wt granules were placed in stoppered bottles and stored in a desiccator. The surface of the granules, when viewed with the scanning electron microscope was smooth and there was no evidence of damage during extraction.

Gelatinization temperature. Two methods, loss of birefringence and DSC were used to estimate gelatinization temps. For loss of birefringence measurements, a 0.15% starch suspension in H_2O was placed on a microscope slide and covered with a coverslip [40]. The slide was placed in a Mettler FP82 Hot Stage which was linked to a Mettler FP90 central processor programmed to produce a starting temp. of 30° followed by a steady increase in temp. at 2° min^{-1} up to 90°. Granules were viewed using a polarizing microscope and the proportion of granules which had lost their birefringence was recorded with every 0.25° increase in temp. Gelatinization curves were constructed for each treatment from the average of 5 runs. Gelatinization temp. is defined as the temp. at which 50% of the granule population had lost their birefringence.

DSC measurements were conducted using a Mettler 12E differential scanning calorimeter and a Mettler data analysis programme. Deionized H_2O (10 μl) was added to 3 mg of dry, accurately weighed starch. Samples were hermetically sealed in aluminium DSC pans and heated at a rate of 10° min^{-1} with a starting temp. of 20°. The onset (T_o) peak (T_p) and end (T_m) endotherms, as well as the heat enthalpy (ΔH) were obtained using the Mettler computer software.

To determine the effect of the volume fraction of water in the starch, samples of starch from cv. Eden grown in the three conditions were subjected to DSC after addition of varying amount of H_2O. The vol. fr. of H_2O was calculated as described in Ref. [13].

X-ray crystallography. Dry, powdered samples of starch were scanned using a Philips PW1730 X-ray diffractometer from 3 to 53° 2θ. A radiation source (Kα) from a copper target was used. Relative crystallinity was considered to be the ratio of the crystalline portion to the sum of the crystalline and amorphous portions [41].

Phosphorus content. Dry starch granules (0.5 g) were placed in a graduated digestion tube and digested for 2 hr at 150° with 5 ml of HNO_3 (70%)–perchloric acid (60%) (4:1). The temp. was then raised to 180° to boil off the excess HNO_3 and to reduce the vol. to 2 ml. After cooling, the vol. was made up to 25 ml with hot ultra-pure H_2O and the P-content measured using inductively-coupled plasma atomic emission spectrometry. Because this assay required a relatively large quantity of starch it was only possible to conduct one analysis per starch sample.

Amylose content. Dry starch (50 mg) in a test tube was wetted with 300 μl DMSO after which 10 ml 0.1 M NaOH were added with constant stirring [42]. Tubes were then heated at 100° for 30 min until all the starch had dissolved. After cooling, solns were neutralized with *ca* 10 ml 0.1 M HCl and made up to 50 ml with 0.1 M NaCl. To measure the amylose content of these solns, 80 ml of H_2O were added to 10 ml of starch soln. I_2–KI soln (2 mg I_2 and 20 mg KI ml^{-1}) (2 ml) were added and the total made up to 100 ml with H_2O. A was read at 635 nm at 20° after 15 min against an I_2–KI blank. Percentage amylose was calculated from the equation given in Ref. [43]. Analyses were conducted in triplicate.

Treatment with α-amylase. Potato α-amylase has not yet been purified to homogeneity so bacterial α-amylase Type II-A from *Bacillus* species (Sigma) with an activity of 2100 units mg^{-1} solid was used. This source of α-amylase was used because it was found to be free from contamination with β-amylase when tested with the Betamyl kit (Megazyme). 0.02 M NaPi buffer (2 ml) containing 6.7 ml NaCl and 0.02% sodium azide were added to 50 mg of dry starch granules. The stoppered tubes were placed in a shaking waterbath overnight at 37° to rehydrate the granules. The following morning, a 100 μl suspension containing 0.72 mg, i.e. 1500 units of bacterial α-amylase, were added to each tube. Three tubes with α-amylase and three control tubes without α-amylase were set up for each starch sample at each sampling time. Tubes were incubated for 2, 4, 6, 8, 10 and 12 hr at 37°, after which the starch granules were centrifuged down at 10000 g and the supernatants removed and frozen. They were then analysed for total sol. carbohydrate using the method of Ref. [44] and for reducing sugars using the Somogyi–Nelson test [45].

Granule size. Starch granule diameter was measured using a Coulter multisizer recording 256 channels and 0.9% saline as suspending electrolyte [46]. Results are expressed as the percentage of granules which fall into each 5 μm size category.

Separation of large and small granules. A modified version of the method of Ref. [47] was used. Dried granules of cv. Eden grown in the field were rehydrated overnight at 37° in 0.02 M Pi buffer (pH 6.9) containing 6.7 mM NaCl [48]. Ludox, a colloidal suspension of silica, was obtained as a gift from DuPont (U.K.) Ltd. It was found that the viscosity of Ludox varied between batches, so the set of conditions used to separate the starch granules had to be tailored for each bottle of Ludox. In the expt reported here, a discontinuous

gradient was set up by layering 2 ml of 75, 70 and 65% Ludox in a 15 ml centrifuge tube. Tubes were spun at 5 *g* for 10 min on a centrifuge with a swing-out head. The fr. contained in the 70% Ludox was mostly small granules (size range 1–50 μm) and that in the pellet in the 75% Ludox was mostly large granules (size range 15–90 μm). Granule dimensions were obtained by microscopic measurement of 5 fields of view of granules suspended on a microscope slide. Separated granules were rinsed × 3 in dist. H_2O and dried at 30°.

Statistical analysis. Minitab was used to perform ANOVA GLM analysis on data. Where indicated, levels of significance are quoted as $P < 0.05$, 0.01 or 0.001. Bars on figures represent 95% confidence limits.

Acknowledgements—The authors are grateful to, J. Taylor (cultivation of plants), R. Tester (granule measurements), R. Bottom and L. Troughton (DSC measurements), J. Paterson (P measurements) and A. Duncan (X-ray crystallography). The work was funded by the BBSRC.

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