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# Structure and properties of waxy-rice (IR29) starch during development of the grain

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ABSTRACT

The structure and properties of a waxy-rice (IR29) starch were analysed at 7, 11, 15, and 30 days after flowering (DAF). The iodine affinity, blue value, and $\lambda_{max}$ of the iodine-stained solutions of the starches and their isoamylolysates, the beta-amylolysis limits, and the limiting viscosity number of the starches were largely constant during development of the grain. The number-average chain length and chain-length distribution were not significantly different. Starch granules, both spherical and polygonal, were smaller at 7 DAF than in the later stages, as revealed by scanning electron microscopy. These data suggest that only complete molecules are packed into the granules.

## INTRODUCTION

The changes in physical, chemical, and structural properties of rice starch during development of the grain have been investigated by several workers[1–4], and granular and molecular changes in various species have been reviewed[5–7]. Two types of α-D-glucan, linear or slightly branched (amylose) and branched (amylopectin), with widely different properties are assembled and placed in the same granule. However, waxy starches contain only branched amylopectin molecules. We now report on the structure and properties of rice starches isolated from grains of the waxy variety, IR29 (indica), harvested at 7, 11, 15, and 30 days after flowering (DAF).

## EXPERIMENTAL

*Materials.* — Developing IR29 rice grains were harvested at 7, 11, 15, and 30 days after flowering (DAF) and stored at −20°. Starch was isolated from these samples by the sodium dodecyl benzenesulfonate method[8]. The average wet weights (mg) of brown

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rice from the above samples were 7, 15, 18, and 17, and the starch contents (mg/grain) were 4.8, 9.7, 11.5, and 11.0, respectively. Isolated starches were stored at 4°, and then defatted by twice dissolving in dimethyl sulfoxide and precipitating with alcohol[9]. The sources of both beta-amylase and *Pseudomonas* isoamylase were as reported[10].

*Methods.* — Iodine affinities at 30° were determined by modified amperometric titration[11]. Blue values and $\lambda_{max}$ of iodine-stained solutions[11] and the limiting viscosity numbers[12] $[\eta]$ in M KOH at 22.5° were determined as described[10]. The number-average chain length $(\overline{c.l.}_n)$ of each amylopectin was determined by the rapid Smith-degradation method[13] and by hydrolysis with isoamylase[12]. Reducing sugar was determined by the methods of Somogyi[14] and Nelson[15] with extension of the time of heating to 30 min. Total carbohydrate was determined by the phenol–sulphuric acid method[16]. Beta-amylolysis was carried out as described[17].

The c.l. distributions (Fig. 1) of amylopectin were determined by h.p.l.c. combined with low-angle laser-light-scattering photometry as described[18,19], with three columns connected in the sequence Asahipak GS-320 (7.6 × 500 mm) × 2 and TSK-gel G3000PW (7.6 × 600 mm). The d.p. was calculated using a pullulan (P-10, 10,200 Da; Hayashibara Biochemical Laboratory).

High-performance anion-exchange chromatography involved a Dionex BioLC Model 4000i system and a Model PAD II pulsed amperometric detector consisting of an amperometric flow-through cell with a gold working electrode, a silver–silver reference electrode, and a potentiostat. The following pulse potentials and durations were used at range 2 (sampling period, 200 ms): $E_1$ 0.10 ($t_1$ 300), $E_2$ 0.60 ($t_2$ 120), $E_3$ 0.80 V ($t_3$ 300 ms).

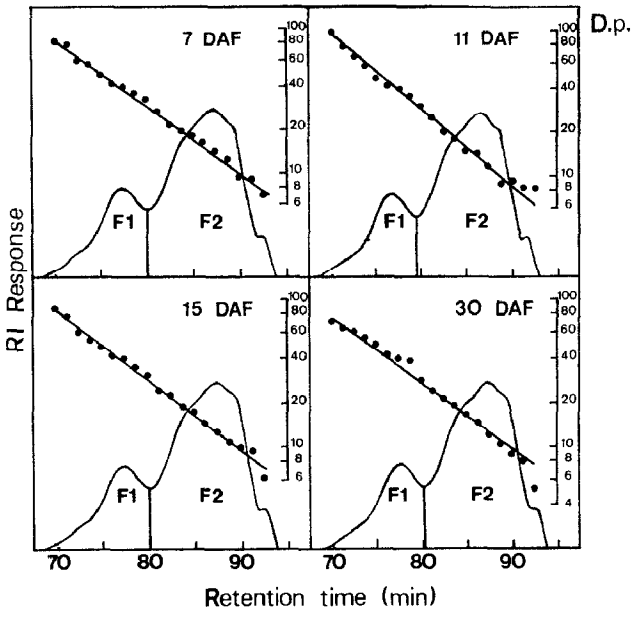


Fig. 1. H.p.l.c. (See Experimental) of isoamylolysates of waxy-rice (IR29) starches from various stages of grain maturity (DAF = days after flowering); F1 and F2 indicate the first and second fractions, respectively.

The response time of the detector was set to 1.0 s. A Dionex HPIC-AS6 column (250 × 4 mm i.d.) and an AG6 guard column (50 × 4 mm i.d.) were used. The eluent *A* was 150mM sodium hydroxide prepared from carbonate-free aqueous 50% sodium hydroxide in 18 MΩ cm deionised water. The eluent *B* was 150mM sodium hydroxide containing 500mM sodium acetate. The gradient programme was: % of eluent *B* = 40 at 0 min, 50 at 2 min, 60 at 10 min, and 80 at 40 min. A solution of debranched amylopectin (3 mg) in M sodium hydroxide (0.2 mL) was made up to 1 mL with deionised water, and aliquots (20–30 μL) were analysed.

Scanning electron micrographs were obtained with a Hitachi Model X-650 electron microscope at an accelerating voltage of 15 kV.

## RESULTS AND DISCUSSION

*Molecular properties.* — Some of the characteristics of the starches are summarised in Table I. The iodine affinities, blue values, $\lambda_{max}$, and beta-amylolysis limits were similar for the samples harvested at different stages of development of the grain. However, the limiting viscosity number increased slightly and then decreased, although the blue values and $\lambda_{max}$ of the isoamylolysates (debranched amylopectin) were similar (Table I). In related studies with both waxy[2–4] and non-waxy[1–4] varieties, the X-ray diffraction patterns[1–4] and gelatinisation characteristics on differential scanning calorimetry[4] were the same at all stages of development of the grain. These results suggest that there is no variation in the molecular structure of the amylopectin.

*Structural properties.* — The structural properties of the debranched amylopectin are given in Table II. Fig. 1 shows the h.p.l.c. results for samples harvested at various stages of maturity. The $\overline{c.l.}_n$ of the specimens, estimated by isoamylolysis and Smith degradation, were similar as were the c.l. distributions.

The individual components of debranched amylopectins can be separated by high-performance anion-exchange chromatography[20,21], and the elution profiles for debranched amylopectins, prepared from starches isolated 7 and 30 DAF, are shown in

TABLE I

Molecular properties of waxy-rice (IR29) starch

| *Properties* | *Days after flowering* | | | |
|---|---|---|---|---|
| | *7* | *11* | *15* | *30* |
| Iodine affinity (g/100 g) | 0.02 | 0.02 | 0.02 | 0.02 |
| Blue value | 0.05 | 0.05 | 0.04 | 0.04 |
| $\lambda_{max}$ (nm) | 528 | 528 | 527 | 527 |
| Beta-amylolysis limit (%) | 60 | 61 | 59 | 58 |
| $[\eta]$ (mL/g) | 125 | 132 | 131 | 122 |
| Isoamylolysate | | | | |
| Blue value | 0.17 | 0.17 | 0.17 | 0.17 |
| $\lambda_{max}$ (nm) | 554 | 554 | 553 | 552 |

TABLE II

Structural properties of waxy-rice (IR29) starch

| | *Days after flowering* | | | |
|---|---|---|---|---|
| | *7* | *11* | *15* | *30* |
| $\overline{C.l.}_n$ | | | | |
| Isoamylolysis | 18 | 19 | 18 | 18 |
| Smith degradation | 19 | 19 | 18 | 17 |
| $\overline{C.l.}_w$ | | | | |
| Whole | 22 | 23 | 22 | 22 |
| F1 | 46 | 47 | 47 | 46 |
| F2 | 14 | 15 | 14 | 14 |
| Peak-point c.l. | | | | |
| F1 | 40 | 40 | 40 | 39 |
| F2 | 14 | 14 | 13 | 12 |
| Weight ratio (F2/F1) | 3.0 | 3.1 | 3.1 | 3.0 |

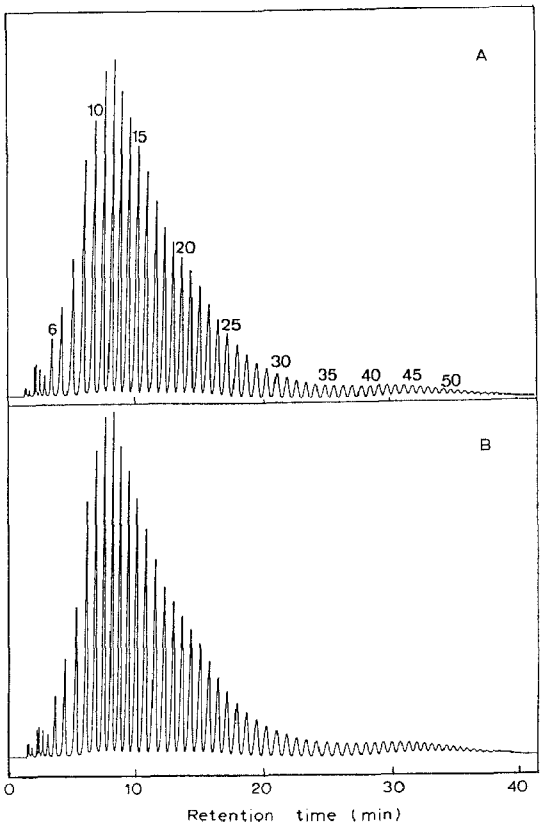


Fig. 2. High-performance anion-exchange chromatograms of isoamylolysates of developing waxy-rice (IR29) starches: *A*, 7 days; *B*, 30 days after flowering.

TABLE III

High-performance anion-exchange chromatography data for debranched amylopectins from developing waxy-rice (IR29) grains

| *Chain length (Glc units)* | *Retention time (min)* | *Peak area (%)* | | | |
|---|---|---|---|---|---|
| | | *7 DAF*[a] | *11 DAF* | *15 DAF* | *30 DAF* |
| 6 | 3.6 | 0.9 | 1.0 | 0.9 | 1.0 |
| 7–9 | 4.3–5.9 | 9.2 | 9.6 | 9.2 | 9.5 |
| 10 | 6.7 | 5.9 | 5.9 | 5.9 | 5.9 |
| 11–14 | 7.5–9.3 | 24.4 | 24.9 | 25.1 | 24.5 |
| 15 | 10.0 | 5.8 | 5.9 | 5.8 | 5.8 |
| 16–19 | 10.7–12.9 | 18.8 | 18.9 | 18.9 | 18.7 |
| 20 | 13.6 | 3.8 | 3.7 | 3.8 | 3.7 |
| 21–24 | 14.3–16.4 | 11.8 | 11.6 | 11.7 | 11.7 |
| 25 | 17.2 | 2.1 | 2.0 | 2.0 | 2.0 |
| 26–29 | 17.9–20.3 | 5.5 | 5.4 | 5.3 | 5.4 |
| 30 | 21.0 | 0.9 | 0.9 | 0.9 | 0.9 |
| 31–34 | 21.8–24.2 | 2.6 | 2.5 | 2.5 | 2.5 |
| 35 | 24.9 | 0.5 | 0.5 | 0.5 | 0.5 |
| 36–39 | 25.7–27.8 | 2.0 | 1.9 | 1.9 | 1.9 |
| 40 | 28.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 41–44 | 29.2–31.1 | 2.0 | 1.9 | 2.0 | 2.0 |
| 45 | 31.7 | 0.5 | 0.4 | 0.4 | 0.5 |
| 46–49 | 32.3–34.1 | 1.5 | 1.4 | 1.4 | 1.6 |
| 50 | 34.7 | 0.3 | 0.2 | 0.3 | 0.3 |
| >50 | up to 38.7 | 1.0 | 0.9 | 0.9 | 1.1 |
| Total | | 100.0 | 100.0 | 100.0 | 100.0 |

[a] Days after flowering.

TABLE IV

Relative molar distribution of chains with c.l. 6–17

| *C.l.* | *Relative detector response*[a] | *Relative molar distribution (%)*[b] | | | | |
|---|---|---|---|---|---|---|
| | | *7 DAF*[c] | *11 DAF* | *15 DAF* | *30 DAF* | *Average* |
| 6 | 0.74 | 2.8 | 3.0 | 2.6 | 2.8 | 2.8 |
| 7 | 0.82 | 4.8 | 5.0 | 4.6 | 4.9 | 4.8 |
| 8 | 0.89 | 7.0 | 7.2 | 6.9 | 7.2 | 7.1 |
| 9 | 1.00 | 10.3 | 10.3 | 10.3 | 10.4 | 10.3 |
| 10 | 1.10 | 11.7 | 11.7 | 11.8 | 11.7 | 11.7 |
| 11 | 1.20 | 11.3 | 11.3 | 11.4 | 11.3 | 11.3 |
| 12 | 1.31 | 10.6 | 10.6 | 10.8 | 10.6 | 10.7 |
| 13 | 1.38 | 9.8 | 9.7 | 9.9 | 9.7 | 9.8 |
| 14 | 1.46 | 9.1 | 9.0 | 9.2 | 9.1 | 9.1 |
| 15 | 1.55 | 8.3 | 8.2 | 8.3 | 8.2 | 8.3 |
| 16 | 1.59 | 7.6 | 7.5 | 7.6 | 7.5 | 7.6 |
| 17 | 1.65 | 6.6 | 6.5 | 6.6 | 6.5 | 6.5 |

[a] Data from ref. 22; also see Experimental. [b] The sum of relative molar distribution of chains with c.l. 6–17 was taken as 100%. [c] Days after flowering.

Fig. 2. The number on each peak indicates the c.l. of the corresponding component. The smallest c.l. for each preparation appeared to be 6, which was common to amylopectins, although negligible proportions of chains with c.l. $<5$ were observed, and the c.l. up to 50 were reflected as individual peaks. The chromatograms are clearly similar and the c.l. distribution data are summarised in Table III. Recently, a method for determining the relative responses in pulsed amperometric detection on a molar basis for malto-oligosaccharides with c.l. 6–17 has been described[22]. In this manner, the relative molar distributions (shown in Table IV) for c.l. 6–17 were calculated, but the results were not significantly different. Previous reports also showed that the amylopectins from waxy and non-waxy isogenic lines of japonica[4] and indica[23] varieties had uniformity in the chain length during development of the grain and were suggested to be synthesised in a similar way[4].

The environmental temperature during anthesis has a profound effect on the amylose content, and on the fine structure of both waxy and non-waxy amylopectins in the isogenic lines of japonica cultivars[2–4,24]. As the temperature increased from 25° to 30°, the amylose content of non-waxy starch decreased, the proportions of long B chains of both waxy and non-waxy amylopectins increased, and the proportions of short B and A chains decreased. These changes were prominent during the crucial period of 5–15 DAF when starch biosynthesis is active[2–4]. It seems that the synthesis and the fine structure are influenced greatly by the environment, especially during the early stages of development, rather than by genetic factors.

*Granular properties.* — By using thin sections for light microscopy, it was shown that the starch granules increased in size and the spherical granules became polygonal as the grain attained maturity. However, scanning electron microscopy of the isolated starch revealed that, even in the earlier stage (7 DAF) of development of the grain, substantial proportions of polygonal starch granules were present, in addition to spherical granules, although they were smaller. At later stages, the granules became larger and almost polygonal (Fig. 3). The differences may reflect the methodology, with loss of small granules formed in the initial stages during the isolation process in the later stages. It was presumed[1], from the increase in starch content per grain during development, that the number of granules were the same, but only their size increased and most of the granules were formed as early as 4 DAF. This prediction seems plausible, considering the results presented above.

Thus, it appears, as suggested[1], that the number of starch granules per cell may be determined in the very early stage of development of the grain. The granules are filled with fully synthesised molecules having the natural variation of c.l., and hence the structure and properties are similar from the onset of starch biosynthesis. The increase in the number of molecules per granule increases the size of granule and causes other morphological changes as the grain attains maturity.

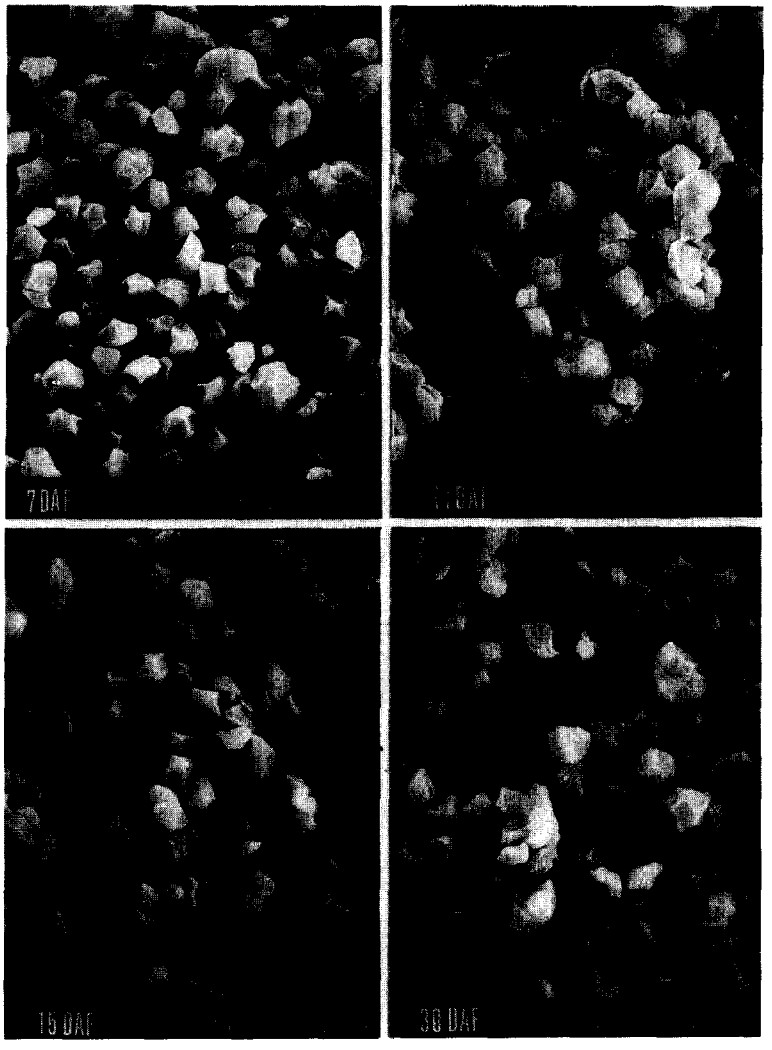


Fig. 3. Scanning electron micrographs of waxy-rice (IR29) starch granules.

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