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Journal of Cereal Science 39 (2004) 57–66

Journal of CEREAL SCIENCE

www.elsevier.com/locate/jnlabr/yjcrs

Molecular basis of the gelatinisation and swelling characteristics of waxy barley starches grown in the same location during the same season. Part II. Crystallinity and gelatinisation characteristics

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Received 24 February 2003; revised 26 June 2003; accepted 7 July 2003

Abstract

Nine waxy barley samples (grown at the same site during the same season) were investigated to identify those molecular aspects of amylopectin structure and architecture which define the order and gelatinisation characteristics. Using ^{13}C CP-MAS/NMR it was confirmed that the number of double helices within the starches were approximately constant although differences in crystallinity were identified by X-ray diffraction. These differences in terms of amount of crystalline order correlated well with gelatinisation temperatures. The onset (T_o), peak (T_p) and conclusion (T_c) gelatinisation temperatures were 53.4, 59.2 and 68.1 °C on average with the associated enthalpy (ΔH) of 11.0 and 13.5 J g^{-1} on a starch and amylopectin basis. Annealing of the starches below T_o elevated T_o, T_p and T_c by +11.9, +8.2 and +5.1 °C on average and sharpened the gelatinisation range ($T_c - T_o$). Acid hydrolysis after annealing increased T_o, T_p and T_c (especially T_c) by +2.3, +17.4 and +34.7 °C on average. Annealing in the presence of α-amylase elevated similarly the gelatinisation parameters by +10.2, +7.1 and +2.8 °C for T_o, T_p and T_c, respectively. Crystalline lamellae lengths were found to be 5.2 ± 0.7 and 6.2 ± 0.4 nm using high sensitivity differential scanning micro-calorimetry and differential scanning calorimetry, respectively.

Keywords: Barley; Structure; Amylose; Amylopectin; Gelatinisation; Crystallinity

1. Introduction

In a previous publication (Qi et al., 2003) the difficulties with respect to correlating those genetic aspects controlling composition, polysaccharide structure and granule architecture with the molecular events surrounding gelatinisation and swelling were discussed. It is apparent that the molecular events regulating starch architectural development during biosynthesis and, the impact in terms of the physical chemistry associated with the gelatinisation process, are still not fully understood.

The benefits of using waxy starches to study amylopectin crystallisation independently of amylose are obvious with waxy starches also being low in lipid content or lipid free (Qi et al., 2003). Waxy barley starches are unusual compared with other waxy starches in that they contain significant amounts of lipids although the amylose content is very low (Tester and Qi, 2003). These low levels of lipid, however, can influence the order within the waxy barley starch granules by associating with the amylose (Tester and Qi, 2003).

One objective of this work was to differentiate between the forms of molecular association within waxy barley

Abbreviations: ^{13}C CP-MAS/NMR, ^{13}C cross polarisation-magic angle spinning/nuclear magnetic resonance; CL, chain length; DP, degree of polymerisation; DSC, differential scanning calorimetry; DSM, differential scanning microcalorimetry; FAME, fatty acid methyl ester; T_o, T_p and T_c, onset, peak and conclusion gelatinisation temperatures, respectively; ΔH, gelatinisation enthalpy; XRD, X-ray diffraction.

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0733-5210/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0733-5210(03)00066-3

starch granules and use this to build a more coherent picture with respect to the crystalline order within the granules (with potential relevance to other starches). The second objective was to differentiate (if possible) between the different waxy starches in terms of crystallite dimensions (genetic variation independently of environmental factors). Thirdly, the work was structured to identify the key architectural aspects (α-glucan associations) of the starch granules that provide starch specific gelatinisation characteristics and model these accordingly.

Because the starches were extracted from grains grown in the same location at the same time, environmentally induced variations in starch deposition patterns were constant. This publication forms part II of a waxy barley starch investigation published in this journal edition.

2. Materials and methods

2.1. Reagents

All chemicals, reagents and solvents were of Merck Analar quality or better.

2.2. Samples

Nine samples of waxy barley grown under the same environmental conditions during the same season in field plots were obtained from the Scottish Crop Research Institute (SCRI), Dundee, UK as previously described (Tester and Qi, 2003).

2.3. Starch extraction

Starches were extracted as discussed previously (Tester and Qi, 2003). Compositional and α-glucan structural data for the starches are similarly described elsewhere (Tester and Qi, 2003).

2.4. Preparation of amorphous and crystalline starch

Amorphous cereal starch controls were prepared by ball milling commercial maize (Fisher, S/7880/69) or wheat (BDH, 30265) starches (10 g) for 24 h. The amorphous conversion was confirmed by differential scanning calorimetry (DSC) and both the α-glucan and damaged starch contents were quantified (Karkalas, 1985; Karkalas et al., 1992) Completely crystalline controls were prepared by steeping the same (native) starches in 9 volumes 2 M HCl at 37 °C for 40 days (with regular mixing). The residues were extensively washed with cold distilled water (with centrifugation in between washes) and then washed twice with acetone to promote drying. The residues were air equilibrated on a glass plate for 1 week.

2.5. Acid and α-amylase hydrolysis of starches

Acid hydrolysis was performed on 100 mg (±0.1 mg) samples of starch as described previously (Tester and Qi, 2003) where solubilised α-glucan was determined similarly (Karkalas, 1985).

The digestibility of native or annealed starches with fungal (*Aspergillus oryzae*) *alpha*-amylase was assayed according to the method of Karkalas et al. (1992) with the following modifications. To triplicate samples of starch (circa 50 mg, accurately weighed into 10 ml screw-cap Sovirel type tubes) 2.5 ml distilled water was added by pipette. The sealed tubes were placed horizontally (within a holder) in a gently shaking water bath at the required temperature for 30 min to swell the starch. Once cooled to 30 °C, 2.5 ml 'double strength' (2.5 mg ml^{-1}) according to the standard method (Karkalas et al., 1992) *alpha*-amylase solution was added to each tube by pipette. The tubes were vortex mixed immediately and then incubated at 30 °C for 15 min exactly. The solubilised (hydrolysed) starch was then quantified as per the standard protocol (Karkalas et al., 1992).

2.6. Physical properties

Crystalline (lamellae) dimensions (lengths) were determined by high sensitivity differential scanning microcalorimetry (DSM) and conventional DSC as described elsewhere (Kiseleva et al., 2003). Starch gelatinisation temperatures and gelatinisation enthalpy were determined with a Mettler DSC 30 Low Temperature Cell linked to a TC10A processor driven by computer running Mettler STARe software. Samples (accurately weighed circa 3.5 mg) of starch were weighed into standard 40 μl pans to which 15 μl (boiled and cooled) distilled water was added by micro-syringe and the contents were stirred with a needle. The pans were pressure sealed (ME 27330 press) then endotherms were obtained by heating from 5 to 100 °C at 10 °C min^{-1} against pierced empty reference pans. The instrument was pre-calibrated by appropriate three metal (temperature) and indium (enthalpy) standards. All measurements were performed in at least triplicate. Onset, peak and conclusion gelatinisation temperatures (T_o, T_p and T_c, respectively) together with gelatinisation enthalpy (ΔH, J g^{-1}) were determined.

2.7. Annealing

Starches were annealed in excess water (>96% by weight). Triplicate samples of starch (1 g) were accurately weighed into 100 ml screw-cap conical flasks to which 100 ml deionised water containing 0.02% sodium azide (as a bacteriostatic agent) was added. After sealing and equilibrating at room temperature for 30 min, the samples were placed in incubators at the required temperatures (below T_o by DSC) for the required times (see Table legends).

After this period the samples were recovered by centrifugation (2000*g* for 10 min), washed three times with cold deionised water (with centrifuging between washing) then washed twice with acetone before allowing to air dry and equilibrate.

Annealing was also done in the presence of *alpha*-amylase. Here, in triplicate, starch samples (100 mg) were accurately weighed into 10 ml screw-cap Sovirel tubes to which 5 ml deionised water, 5 ml acetate buffer (0.2 M, pH 4.7) containing 25 mg (370 U) fungal (*Aspergillus oryzae*) *alpha*-amylase (Sigma A-0273) and 10 μl toluene (bacteriostatic agent) were added by pipette. After sealing, mixing and equilibrating at room temperature for 30 min, the samples were placed in an incubator to anneal at the required temperature for the required time.

2.8. X-ray diffraction and NMR

A Brucker DSZ 200 NMR operating at 200 MHz with a standard Brucker 7 mm PH MASV probe head was used to obtain ^{13}C cross-polarisation magic angle spinning nuclear magnetic resonance (^{13}C CP-MAS/NMR) spectra. The instrument was operated at a spinning rate of 5–6 kHz with a recycle delay of 1.5 s where 35,000 scans were averaged for each spectrum. Internal and external standards used were TKS and adamantane, respectively.

X-ray diffraction data was generated with a Philips PW 1840 Bragg-Brentano type parafocusing diffractometer mounted on a PW 1066/11 sealed tube X-ray generator operating at the Cu K_α wavelength (1.5406 Å). Samples were packed into aluminium cells and were exposed to X-ray beams with the generator running at 40 KV and 40 mA. The total diffraction intensity was measured over the angular range 4–30° 2θ. The overall degree of crystallinity was quantified as the ratio of the area of crystalline reflections to the overall diffraction area as reported elsewhere (Cheetham and Tao, 1998).

3. Results and discussion

3.1. Granule order by X-ray diffraction and NMR

The (A-type) X-ray diffractograms generated from the native waxy barley starches are presented in Fig. 1 and indicate how different the proportion of crystallinity is for the group of starches–ranging from 36.4 to 44.8%. The proportion of absolute crystallinity is reported (calculated as the ratio of the area of crystalline reflections to the overall diffraction area) rather than relative (to the crystalline standard). These data are comparable to data generated for waxy rice starches (Qi et al., 2003) but are higher than (normal) wheat starch (Morrison et al., 1994) (~35.5%) because of the amylose dilution effect. Others have reported relative crystallinity (relative to amorphous and crystalline standards) figures of 33.0–37.1% for waxy barley starches (Tang et al., 2002) but 22.0–27.4 for normal barley starch (Tang et al., 2001). Typical A-type diffraction patterns for waxy barley starches are presented elsewhere (Tang et al., 2000). Some authors (Czuchajowska et al., 1998; Vasanthan and Bhatty, 1996) have reported, however, that certain X-ray diffraction peaks of waxy barley starches have different intensities compared to normal or high amylose barley starches.

The ^{13}C CP-MAS/NMR spectra of the waxy barley starches (eight) are presented in Fig. 2. They appear to be very similar regardless of genotype. Using the approach described to evaluate the proportion of amorphous to crystalline component of starch granules based on calculation and the NMR spectra (Qi et al., 2003), the amount of double helical material corresponds to ~65% in common with the waxy rice starches (Qi et al., 2003) previously investigated.

These two techniques, X-ray diffraction and NMR, determine the amount of crystallinity and double helices, respectively, and indicate that although within the group of waxy barley starches investigated the proportion of double helices is constant, there are some differences in the proportion of crystallinity. Some starches, therefore, have better ordered structures than others.

3.2. Gelatinisation and annealing

The gelatinisation temperatures of the waxy barley starches are presented in Table 1 where the average onset (T_o), peak (T_p) and conclusion (T_c) temperatures are 53.4 ± 1.5, 59.2 ± 1.4 and 68.1 ± 1.4 °C, respectively. Hence, there is a small amount of genetically induced variation which is independent of any growth related factors. The difference $T_c - T_o$ reflects the variation in gelatinisation parameters (14.7 ± 0.8) while the gelatinisation enthalpy (ΔH) similarly exhibits variation (11.0 ± 0.7 J g^{-1} on a starch basis and 13.5 ± 0.7 J g^{-1} on an amylopectin basis). These data are comparable to data published elsewhere (Tang et al., 2002; Czuchajowska et al., 1998; Yasui et al., 2002; Li et al., 2001; Zheng et al., 1998; Tester et al., 1991; Morrison et al., 1993a; Gudmundsson and Eliasson, 1992; Shi and Seib, 1992).

Annealing at 48 °C in excess water (Table 2) increases T_o (+11.9 °C on average), T_p (+8.2 °C on average) and T_c (+5.1 °C on average) where T_o is clearly increased by the greatest extent followed by T_p then T_c. The variation in T_o, T_p and T_c (65.3 ± 0.3, 67.5 ± 0.5 and 73.2 ± 1.1 °C, respectively) is much less than for the native starches with the difference between T_c and T_o (7.8 °C) similarly much less. Hence, all the starches have been annealed to a more similar state of order. The gelatinisation enthalpy is little affected by annealing (Table 2).

The number of double helices post annealing would be expected to be constant although the crystalline order (registration) would be enhanced (Tester et al., 1998). With the barley starches investigated here, clearly some had more

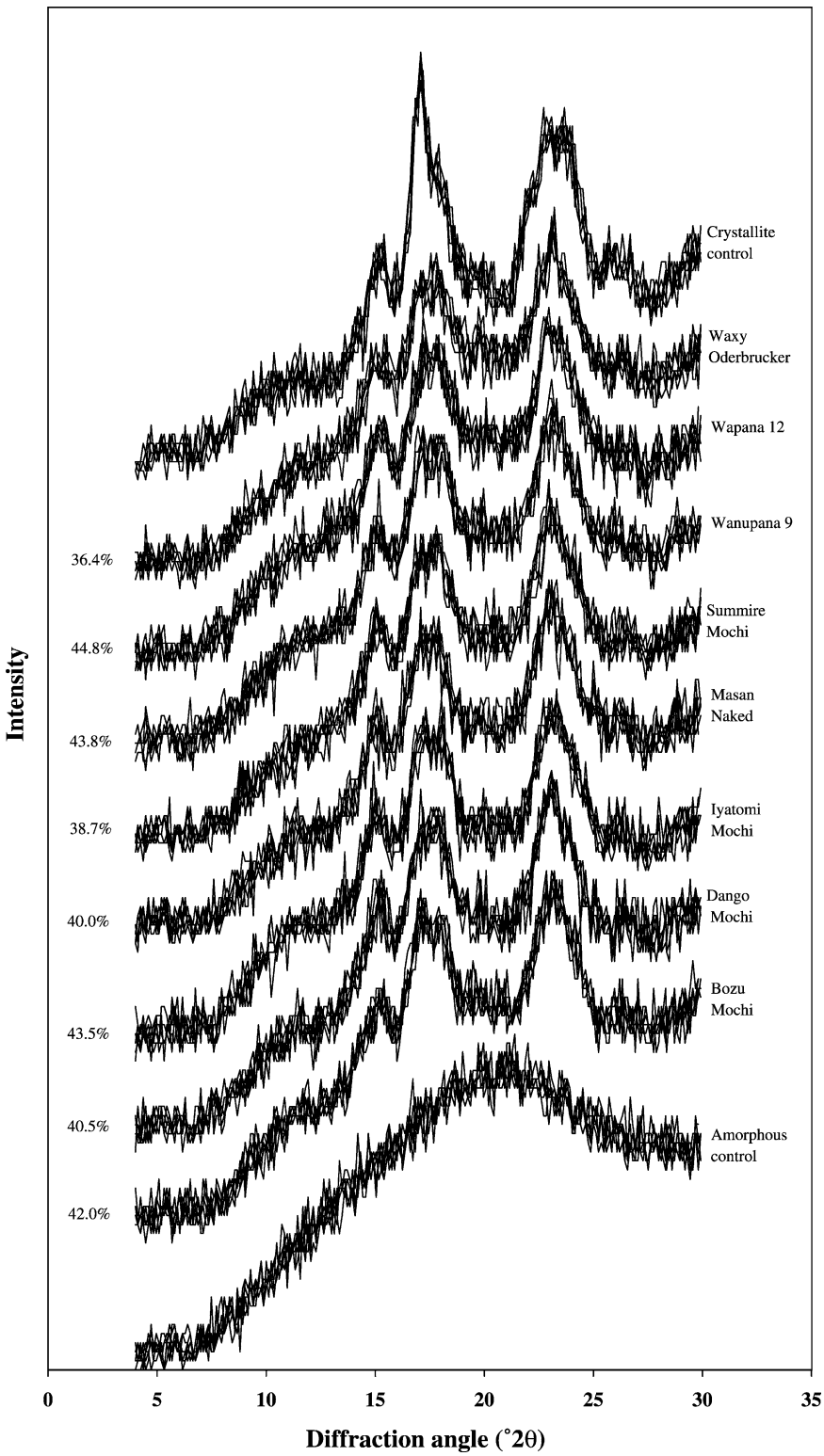

Fig. 1. X-ray diffractograms of waxy barley starches with amorphous and crystalline controls. Estimated proportion of crystallinity shown on the left.

perfect crystalline order (evident from X-ray data) than others but contained the same proportion of double helices. The annealing processes enhanced ordering of the less perfectly ordered double helices into an optimised crystalline registration.

Gelatinisation temperatures might be regarded as the thermal reflection of the ease in which water can penetrate starch granules and provide hydration for double helices to permit dissociation. The water will also facilitate the gelatinisation process by plasticising the amorphous glass

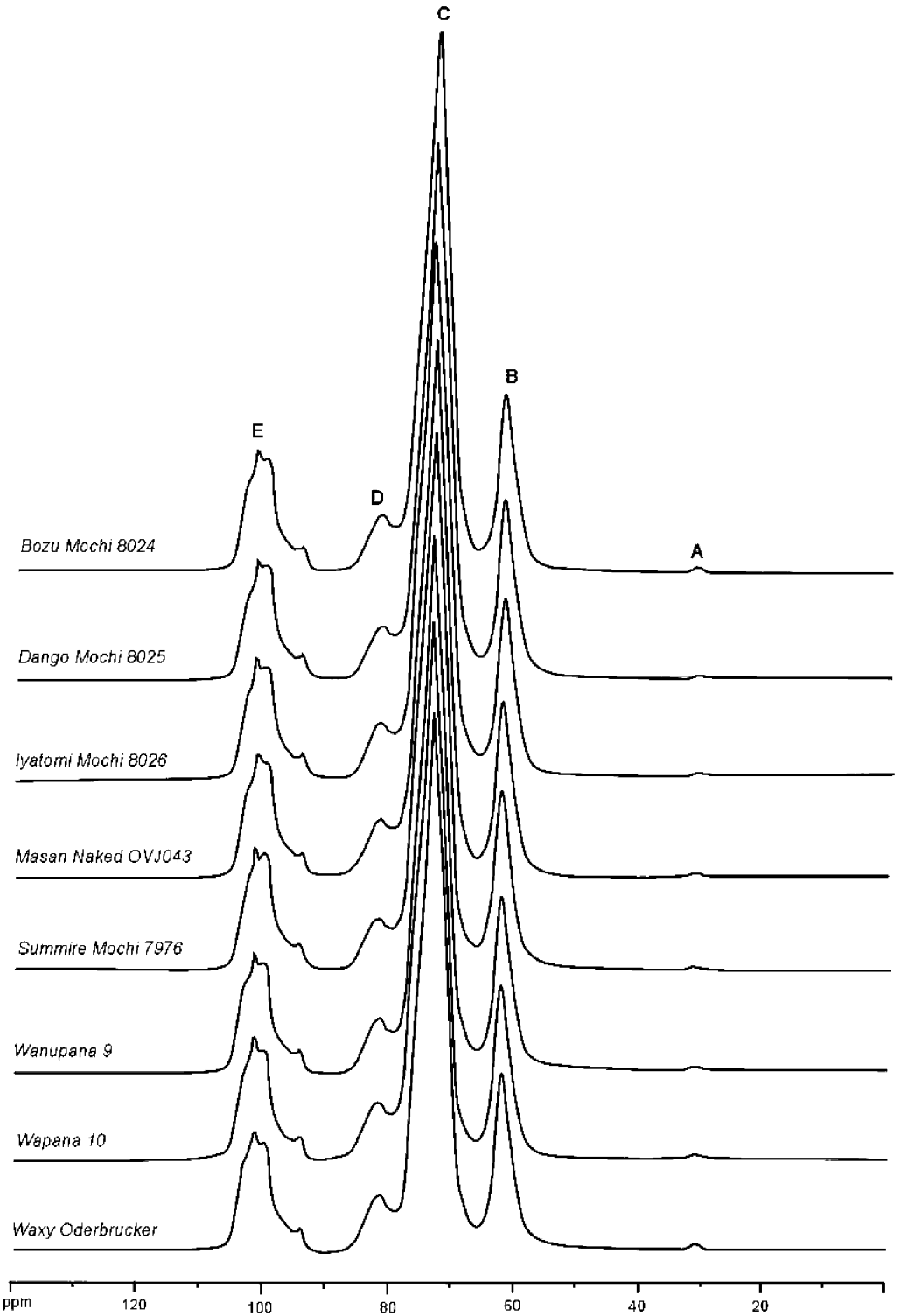

Fig. 2. The ^{13}C-CP/MAS NMR spectra (25 MHz) of waxy barley starches. Resonance lines represent: $A = 31.2$ ppm, mid-chain methylene carbons of lysophospholipid fatty acids; B = C-6; C = C-2, C-3 and C-5; D = C-4; E = C-1.

transition (T_g) preceding the gelatinisation event itself. With this in mind, if double helices were not ordered, one would expect a relatively low-temperature 'gelatinisation' event with double helices simply hydrating and uncoiling as they receive enough thermal energy to uncoil with double helix rupture—the basis of the gelatinisation endotherm (Cooke and Gidley, 1992). Depending on the ease of water access to different double helices within this hypothetical starch structure, this could happen over a relatively consistent short (uniformly hydrated at the same time) or

Table 1
Gelatinisation parameters of waxy barley starches by DSC

Cultivar	Gelatinisation temperatures			$T_c - T_o$	Enthalpy (J g^{-1})	
	T_o	T_p	T_c		Starch	Amylopectin
Bozu Mochi 8024	53.0 ± 0.1	59.0 ± 0.4	68.6 ± 0.5	15.6	10.8 ± 0.2	13.3 ± 0.3
Dango Mochi 8025	52.3 ± 0.2	58.1 ± 0.1	67.2 ± 0.7	14.9	10.1 ± 0.1	12.1 ± 0.1
Iyatomi Mochi 8026	53.4 ± 0.1	59.5 ± 0.2	69.4 ± 0.5	16.0	11.8 ± 0.5	14.4 ± 0.6
Masan Naked OVJ043	52.2 ± 0.3	57.9 ± 0.2	66.6 ± 0.7	14.4	11.8 ± 0.3	14.2 ± 0.3
Summire Mochi 7976	52.8 ± 0.1	58.6 ± 0.2	67.5 ± 0.7	14.7	11.8 ± 0.4	14.0 ± 0.4
Wanupana 9	55.2 ± 0.5	60.7 ± 0.3	70.0 ± 0.3	14.8	11.0 ± 0.5	13.7 ± 0.5
Wapana 12	55.1 ± 0.3	60.6 ± 0.3	69.0 ± 0.4	13.9	10.9 ± 0.4	13.5 ± 0.4
Washonupana 10	55.4 ± 0.2	61.2 ± 0.0	68.7 ± 0.5	13.3	10.6 ± 0.7	13.7 ± 0.9
Waxy Oderbrucker 7986	51.0 ± 0.2	57.4 ± 0.2	65.9 ± 0.2	14.9	9.8 ± 0.5	12.7 ± 0.5
Mean ($n = 9$)	53.4 ± 1.5	59.2 ± 1.4	68.1 ± 1.4	14.7 ± 0.8	11.0 ± 0.7	13.5 ± 0.7

Table 2
Gelatinisation parameters of waxy barley starches by DSC after annealing in excess water

Cultivar	Gelatinisation temperatures			$T_c - T_o$	Enthalpy (J g^{-1})	
	T_o	T_p	T_c		Starch	Amylopectin
Bozu Mochi 8024	65.4 ± 0.1 [+12.4][a]	67.4 ± 0.1 [+8.4]	73.3 ± 0.2 [+4.7]	7.9 [−7.7]	11.0 ± 0.5 [+0.2]	13.6 ± 0.6 [+0.3]
Dango Mochi 8025	65.1 ± 0.0 [+12.8]	67.0 ± 0.1 [+8.9]	71.7 ± 0.2 [+4.5]	6.6 [−8.3]	9.9 ± 0.5 [−0.2]	11.9 ± 0.6 [−0.2]
Iyatomi Mochi 8026	65.2 ± 0.0 [+11.8]	67.4 ± 0.2 [+7.9]	73.6 ± 0.1 [+4.2]	8.4 [−7.6]	12.4 ± 0.9 [+0.6]	15.1 ± 1.1 [+0.7]
Masan Naked OVJ043	65.4 ± 0.0 [+13.2]	67.3 ± 0.1 [+9.4]	72.5 ± 0.1 [+5.9]	7.1 [−7.3]	11.4 ± 0.2 [−0.4]	13.6 ± 0.2 [−0.6]
Summire Mochi 7976	65.2 ± 0.0 [+12.4]	67.3 ± 0.2 [+8.7]	73.8 ± 0.0 [+6.3]	8.6 [−6.1]	11.7 ± 0.1 [−0.1]	13.9 ± 0.2 [−0.1]
Wanupana 9	65.6 ± 0.0 [+10.4]	68.1 ± 0.0 [+7.4]	74.2 ± 0.0 [+4.2]	8.6 [−6.2]	12.8 ± 0.5 [+1.8]	15.9 ± 0.6 [+2.2]
Wapana 12	65.6 ± 0.1 [+10.5]	68.0 ± 0.3 [+7.4]	74.5 ± 0.1 [+5.5]	8.9 [−5.0]	12.4 ± 0.8 [+1.5]	15.4 ± 1.0 [+1.9]
Washonupana 10	65.7 ± 0.1 [+10.3]	68.1 ± 0.0 [+6.9]	73.3 ± 0.1 [+4.6]	7.6 [−5.7]	11.0 ± 0.4 [+0.4]	14.2 ± 0.5 [+0.5]
Waxy Oderbrucker 7986[b]	64.6 ± 0.1 [+13.6]	66.6 ± 0.1 [+9.2]	71.5 ± 0.1 [+5.6]	6.9 [−8.0]	8.2 ± 0.0 [−1.6]	10.6 ± 0.1 [−2.1]
Mean ($n = 9$)	65.3 ± 0.3 [+11.9]	67.5 ± 0.5 [+8.2]	73.2 ± 1.1 [+5.1]	7.8 [−6.9]	11.2 ± 1.4 [+0.2]	13.8 ± 1.7 [+0.3]

Annealing in excess water at 48 °C for 7 days.
[a] Difference between these data and un-annealed starches (Table 1).
[b] Slight gelatinisation possible.

Table 3
Gelatinisation parameters of acid hydrolysed waxy barley starches by DSC after annealing

Cultivar	Gelatinisation temperatures			$T_c - T_o$	Enthalpy (J g^{-1})	
	T_o	T_p	T_c		Starch	Amylopectin
Bozu Mochi 8024	53.6 ± 0.4 [+0.6][a]	72.3 ± 0.3 [+13.3]	102.2 ± 0.4 [+33.6]	48.6 [+33.0]	15.3 ± 0.3 [+4.5]	18.9 ± 0.3 [+5.6]
Dango Mochi 8025	53.4 ± 0.3 [+1.1]	73.2 ± 0.5 [+15.1]	102.3 ± 0.2 [+35.1]	48.9 [+34.0]	20.9 ± 0.1 [+10.8]	25.2 ± 0.1 [+13.1]
Iyatomi Mochi 8026	53.0 ± 0.2 [−0.4]	75.0 ± 0.5 [+15.5]	103.6 ± 0.4 [+34.2]	50.6 [+34.6]	18.7 ± 0.1 [+6.9]	22.9 ± 0.1 [+8.5]
Masan Naked OVJ043	53.1 ± 0.1 [+0.9]	72.7 ± 0.1 [+14.8]	102.2 ± 0.5 [+35.6]	49.1 [+34.7]	18.1 ± 0.2 [+6.3]	21.7 ± 0.2 [+7.5]
Summire Mochi 7976	53.4 ± 0.1 [+0.6]	76.7 ± 0.3 [+18.1]	103.6 ± 0.4 [+36.1]	50.2 [+35.5]	21.8 ± 0.3 [+10.0]	26.0 ± 0.4 [+12.0]
Wanupana 9	55.4 ± 0.2 [+0.2]	79.7 ± 0.2 [+19.0]	101.3 ± 0.4 [+31.3]	45.9 [+31.1]	18.3 ± 0.1 [+7.3]	22.9 ± 0.1 [+9.2]
Wapana 12	58.4 ± 0.2 [+3.3]	79.1 ± 0.3 [+18.5]	102.2 ± 0.1 [+33.2]	43.8 [+29.9]	18.9 ± 0.4 [+8.0]	23.5 ± 0.5 [+10.0]
Washonupana 10	57.0 ± 0.2 [+1.6]	79.1 ± 0.7 [+17.9]	102.4 ± 0.5 [+33.7]	45.4 [+32.1]	17.4 ± 0.4 [+6.8]	22.5 ± 0.5 [+8.8]
Waxy Oderbrucker 7986[b]	64.1 ± 0.9 [+13.1]	81.3 ± 0.4 [+23.9]	105.5 ± 0.3 [+39.6]	41.4 [+26.5]	17.8 ± 0.2 [+8.0]	23.1 ± 0.2 [+10.4]
Mean ($n = 9$)	55.7 ± 3.7 [+2.3]	76.6 ± 3.4 [+17.4]	102.8 ± 1.2 [+34.7]	47.1 [+32.4]	18.6 ± 1.9 [+7.6]	23.0 ± 2.0 [+9.5]

Acid hydrolysis in 2 M HCL at 35 °C for 6 days. Annealing in excess water at 48 °C for 7 days.
[a] Difference between these data and un-annealed starches (Table 1).
[b] Slight gelatinisation possible.

Table 4
Gelatinisation parameters of waxy barley starches by DSC after annealing in the presence of α-amylase

Cultivar	Gelatinisation temperatures			$T_c - T_o$	Enthalpy (J g^{-1})	
	T_o	T_p	T_c		Starch	Amylopectin
Bozu Mochi 8024	63.7 ± 0.2 [+10.7][a]	65.8 ± 0.1 [+6.8]	70.3 ± 0.2 [+1.7]	6.6 [−9.0]	11.3 ± 0.4 [+0.5]	14.0 ± 0.5 [+0.7]
Dango Mochi 8025	63.3 ± 0.1 [+11.0]	65.7 ± 0.2 [+7.6]	69.9 ± 0.1 [+2.7]	6.6 [−8.3]	10.1 ± 0.5 [±0.0]	12.1 ± 0.6 [±0.0]
Iyatomi Mochi 8026	63.8 ± 0.2 [+10.4]	66.1 ± 0.1 [+6.6]	71.0 ± 0.3 [+1.6]	7.2 [−8.8]	12.8 ± 0.3 [+1.0]	15.7 ± 0.3 [+1.3]
Masan Naked OVJ043	63.1 ± 0.4 [+10.9]	65.5 ± 0.1 [+7.6]	69.6 ± 0.4 [+3.0]	6.5 [−7.9]	11.3 ± 0.3 [−0.5]	13.5 ± 0.4 [−0.7]
Summire Mochi 7976	61.3 ± 0.3 [+8.5]	65.8 ± 0.2 [+7.2]	69.0 ± 0.3 [+1.5]	7.7 [−7.0]	8.2 ± 0.2 [−3.6]	9.8 ± 0.3 [−4.2]
Wanupana 9	64.1 ± 0.1 [+8.9]	67.1 ± 0.1 [+6.4]	72.1 ± 0.2 [+2.1]	8.0 [−6.8]	10.9 ± 0.3 [−0.1]	13.5 ± 0.4 [−0.2]
Wapana 12	65.0 ± 0.3 [+9.9]	67.6 ± 0.3 [+7.0]	73.2 ± 0.4 [+4.2]	8.2 [−5.7]	11.7 ± 0.4 [+0.8]	14.5 ± 0.4 [+1.0]
Washonupana 10	64.7 ± 0.2 [+9.3]	67.5 ± 0.2 [+6.3]	72.8 ± 0.3 [+4.1]	8.1 [−5.2]	11.3 ± 0.5 [+0.7]	14.6 ± 0.6 [+0.9]
Waxy Oerbrucker 7986[b]	63.1 ± 0.1 [+12.1]	65.5 ± 0.1 [+8.1]	70.6 ± 0.5 [+4.7]	7.5 [−7.4]	10.5 ± 0.2 [+0.7]	13.6 ± 0.3 [+0.9]
Mean ($n = 9$)	63.6 ± 1.1 [+10.2]	66.3 ± 0.9 [+7.1]	70.9 ± 1.5 [+2.8]	7.3 [−7.4]	10.9 ± 1.9 [−0.1]	13.5 ± 2.4 [±0.0]

Annealing in α-amylase at 48 °C for 6 days.
[a] Difference between these data and un-annealed starches (Table 1).
[b] Slight gelatinisation possible.

long (non-uniform hydration rate) temperature-time period. However, where double helices are packed into crystalline domains with more or less perfect order, variations in hydration rate will be created with the more ordered (registered) structures restricting hydration more than less ordered regions and hence requiring higher temperatures to facilitate the hydration with associated increases in gelatinisation temperatures.

With the above model in mind, annealing enhances order of double helices without creating more double helices. There may be a small improvement in double helix length if 'ends' were not optimally formed as double helices although this is not apparent from any significant overall change with respect to the gelatinisation enthalpy post annealing. This annealing event then restricts hydration and elevates the gelatinisation temperature (reflecting the higher temperatures required to 'drive' hydration and dissociation of the double helices). This would also be associated with a diminished capacity of water to hydrate amorphous regions and hence plasticise T_g preceding gelatinisation.

When the annealed starches are hydrolysed by acid to remove amorphous regions (Table 3) it is relevant to consider the outcome of the hydrolysis process. Firstly amorphous glass transition events plasticised by water would presumably be removed by the acid hydrolysis of these regions. Secondly, the crystalline regions would be 'concentrated' by this hydrolysis. Consequently the facilitation of gelatinisation by T_g (hydration of amorphous regions) and hydration of double helices themselves would be restricted. Elevated temperatures would then be required

Table 5
Thermal properties and crystalline laminate thickness of barley starches using differential scanning microcalorimetry (DSM)

Cultivar	T_{mcrl}[a] (°C)	ΔH_m[b] (J g^{-1})	ΔH^{vH}[c] (kJ mol^{-1})	$\Delta_g C_p$[d] (J $mol\ K^{-1}$)	T_{AML}[e] (°C)	ΔH_{AML}[f] (kJ mol^{-1})	ν^g	L_{crl}[h] (nm)
Bozu Mochi 8024	58.0	17.9 [13.3][i]	36.4	55.1	92	0.1	12.6 [16.9][i]	4.4 [5.9][i]
Dango Mochi 8025	57.0	14.8 [12.1]	37.6	32.9	–	–	15.7 [19.2]	5.5 [6.7]
Iyatomi Mochi 8026	57.6	19.1 [14.4]	37.8	27.5	–	–	12.2 [16.2]	4.3 [5.7]
Masan Naked OVJ043	56.0	13.0 [14.2]	36.7	–	–	–	17.4 [16.0]	6.1 [5.6]
Summire Mochi 7976	56.0	17.3 [14.0]	40.9	19.5	–	–	14.6 [18.0]	5.1 [6.3]
Wanupana 9	59.3	16.0 [13.7]	41.8	82.0	88	0.2	16.1 [18.8]	5.6 [6.6]
Wapana 12	58.8	15.4 [13.5]	40.2	26.0	94	0.3	16.1 [18.4]	5.6 [6.4]
Washonupana 10	nd	nd	nd	nd	nd	nd	nd	nd
Waxy Oderbrucker 7986	nd	nd	nd	nd	nd	nd	nd	nd
Mean ($n = 7$)	57.5 ± 1.3	16.2 ± 2.1 [13.6 ± 0.8]	38.8 ± 2.2	-	91 ± 3	0.2 ± 0.1	15.0 ± 1.9 [17.6 ± 1.3]	5.2 ± 0.7 [6.2 ± 0.4]

[a] T_{mcrl} represent gelatinisation peak temperature.
[b] ΔH_m represent enthalpy.
[c] ΔH^{vH} represents van't Hoff's enthalpy.
[d] $\Delta_g C_p$ is the difference in heat capacity between the native and molten states.
[e] T_{AML} represent amylose-lipid peak dissociation temperature.
[f] ΔH_{AML} represent enthalpy.
[g] Equals $\Delta H^{vH}/\Delta H_m$ which is gelatinisation of the co-operative (crystalline) unit.
[h] L_{crl} equals 0.35ν the thickness of crystalline lamellae.
[i] Values in brackets represent figures derived from conventional DSC.

Table 6
Correlations (r) and significance for physico-chemical properties of waxy barley starches

Parameter	Diameter A-granules	Diameter B-granules	Total amylose (TAM)	Free amylose (FAM)	Δ-amylose (ΔAM)	Lipid	T_o	T_p	T_c	Enthalpy (ΔH)	Swelling Factor (SF, °C)		Hydrolysis		XRD
											70	80	Acid	Enzyme	
Dia.	+0.79														
B-granules	$P < 0.01$														
TAM	+0.61	+0.54													
FAM	+0.48	+0.48	+0.96												
			$P < 0.001$												
ΔAM	+0.68	+0.55	+0.97	+0.87 P < 0.01											
			$P < 0.001$												
Lipid	+0.70	+0.59	+0.98	+0.91	+0.98 < 0.001										
			$P < 0.001$	$P < 0.001$											
T_o	+0.83	+0.83	+0.33	+0.15	+0.46	+0.42									
	$P < 0.01$	$P < 0.01$													
T_p	+0.84	+0.86	+0.44	+0.26	+0.55	+0.51	+0.99								
	$P < 0.01$	$P < 0.01$					$P < 0.001$								
T_c	+0.71	+0.82	+0.21	+0.12	+0.28	+0.25	+0.85	+0.86							
		$P < 0.01$					$P < 0.01$	$P < 0.01$							
Enthalpy	−0.05	−0.08	−0.54	−0.53	−0.52	−0.58	+0.17	+0.12	+0.29						
SF 70	−0.82	+0.67	−0.92	−0.85	−0.93	−0.97	−0.88	−0.88	−0.66	+0.36					
	$P < 0.01$		$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.01$							
SF 80	−0.78	−0.72	−0.92	−0.86	−0.91	−0.96	−0.85	−0.86	−0.70	+0.45	−0.98				
	$P < 0.01$	$P < 0.02$	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.01$			$P < 0.001$				
Hydrol. Acid	−0.08	−0.55	+0.13	+0.06	+0.18	+0.16	−0.26	−0.27	−0.57	−0.21	+0.10	+0.24			
Hydrol. Enzyme	−0.79	−0.89	−0.36	−0.23	−0.44	−0.43	− ‘0.92	−0.93	−0.88	−0.14	−0.83	−0.88	+0.44		
	$P < 0.01$	$P < 0.01$					$P < 0.001$	$P < 0.001$	$P < 0.01$		$P < 0.01$	$P < 0.01$			
XRD	+0.65	+0.88	0.02	−0.07	+0.10	+0.11	+0.90	+0.90	+0.91	+0.31	−0.27	−0.34	−0.62	−0.96	
		$P < 0.01$					$P < 0.01$	$P < 0.01$	$P < 0.01$					$P < 0.001$	
Crystal length	+0.65	+0.88	0.00	−0.08	+0.07	+0.09	+0.89	+0.89	+0.89	+0.33	−0.81	−0.88	−0.62	−0.96	−0.16
		$P < 0.01$					$P < 0.01$	$P < 0.01$	$P < 0.01$		$P < 0.01$	$P < 0.01$		$P < 0.001$	

NMR data constant (~65% double helices) and hence correlations are not calculated.

to cause the hydration assisted dissociation of these double helices. This is what happens (Table 3, where it is assumed that there is little hydrolysis of the double helices themselves) (Morrison et al., 1993b). Unlike unhydrolysed starches, non-helical material would be removed (hydrolysed) by the acid and the apparent enthalpy would increase due to this concentrating effect. Again, this is apparent (Table 3).

The primary role of annealing with respect to causing enhanced crystalline order can be proved further by annealing in the presence of *alpha*-amylase where one assumes that (accessible) amorphous regions may be predominantly initially hydrolysed under controlled conditions leaving ordered crystalline domains free to order into more optimally registered structures. In Table 4 this is apparent. Annealing occurs largely independently of the enzyme. It might be argued that the enzyme was ineffective in hydrolysing the starch. However it is apparent that acid and amylase hydrolysis occurs quite extensively (Tester and Qi, 2003).

3.3. Thickness of crystalline lamellae

According to Cameron and Donald (1992), starch (wheat) crystallite (double helical) lengths are 6.65 nm interspersed with amorphous lamellae of 2.2 nm. In respect of the data generated in this study (Table 5) using differential scanning micro-calorimetry (DSM) and differential scanning calorimetry (DSC), crystalline lamellae dimensions were 5.2 ± 0.7 and 6.2 ± 0.4 nm, respectively, and are consequently similar to the data reported by Cameron and Donald. Importantly, the lamellae dimensions within the waxy barley starches are similar and provide a significant contribution to the gelatinisation temperatures as shown in Table 6 (which reflects data from this paper and elsewhere (Tester and Qi, 2003)). These data represent a major contribution to the knowledge base with respect to the crystalline composition of starch granules and the variation within a particular botanical species.

As anticipated, based on the significant ($r = +0.89$, $P < 0.01$) positive correlation between gelatinisation parameters and crystallite lengths (Table 6), the crystallite lengths contribute to the origin of gelatinisation temperatures but do not provide the full underpinning architectural basis for these parameters. There is similarly a high positive correlation between the amount of crystallinity (XRD) and the gelatinisation temperatures ($r = +0.90$, $P < 0.01$) which reflects the three dimensional nature of the crystallite material and not just specifically length. In addition, non-crystalline double helices may also be significant with respect to the gelatinisation process where they may be construed as defects in starch crystalline structure (Kiseleva et al., 2003). In this context, gelatinisation of crystalline domains may initially be focused in these non-crystalline double helical regions ('defects' (Kiseleva et al., 2003)) and be inversely correlated with gelatinisation temperatures.

In contrast with the above, there is no correlation between the starch crystalline length and the amount of crystallinity by XRD. The gelatinisation enthalpy is independent of the indicators of crystallinity (crystalline length and XRD parameters)—also as anticipated—since the enthalpy reflects the number of double helices rather than crystallinity per se.

4. Conclusions

This work was conducted to understand more extensively how starch crystalline structure (order, crystallite dimensions and architecture within granules) controls gelatinisation parameters. It is apparent that crystalline lamellar regions control the gelatinisation parameters of the starch. It is concluded that although crystallite length contributes to the origin of the gelatinisation temperatures (but not enthalpy), it is the overall optimisation of registration of these double helices that control gelatinisation by in effect more or less restricting hydration of the granules when heated in water. The more exact role of amylose in this process will be explored.

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