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Composition and properties of starches extracted from tubers of different potato varieties grown under the same environmental conditions

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Abstract

Twelve commercially relevant potato cultivars were grown in a field plot in Perthshire (UK) in 1996. Starches were extracted from these tubers and their physico-chemical properties were investigated with a particular focus on aspects of granule order and corresponding gelatinisation characteristics. There was little variation in (average) granule diameter (21.4±1.6 μm), amylose content (28.7±1.9%) or phosphorus (0.54±0.16 μg mg^{-1}) content. Structural elements of amylopectin were essentially constant in terms of unit distributions and chain lengths (average DP 28±2) with long (F_1) and short (F_2) chains with average DP of 56±2 and 19±1, respectively. The structures of the (debranched) β-limit dextrins of the amylopectin molecules were also very similar with the average DP of the two major fractions ($F_{1\beta}$ and $F_{2\beta}$) being 48±3 and 37±2, respectively. Peak gelatinisation temperatures (T_p) and enthalpy (ΔH) were also correspondingly similar (63.2 °C±1.2 and 18.6 °C±0.8, respectively). The constancy of physico-chemical properties for these starches is in marked contrast to variation in different parameters induced by environmental factors, especially growth temperature.
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1. Introduction

Potato starch has been a subject of pure and applied research for many centuries. It has been utilised extensively in food systems because of its unique gel forming properties and the clarity of the gels produced. Different reviews (e.g. Hoover, 2001; Morrison & Karkalas, 1990; Tester & Karkalas, 2002) have discussed the unique physico-chemical properties of potato starch, especially in terms of the extensive phosphorylation of the amylopectin molecules. There is a tendency to believe that 'starch is starch' and, if a potato starch of any origin is used in a product, it will provide the required characteristics. An associated common belief in starch research is that, if a particular variety of any given species is used the properties and quality of the starch will be retained, regardless of environmental conditions experienced during starch deposition. However, this is not true. Environmental conditions (especially temperature) have a very significant effect on the synthesis and properties of starches (Tester & Karkalas, 2001). These (varietal and environmental) effects have been discussed and evaluated in relation to potato tuber (Haase & Plate, 1996; Jansen, Flamme, Schuler, & Vandrey, 2001; Morrison et al., 2001; Peshin, 2001; Protserov et al., 2002; Singh & Singh, 2001; Svegmark et al., 2002; Vasanthan, Bergthaller, Driedger, Yeung, & Sporns, 1999; Zrust, 1988) and potato microtuber starch (Debon, Tester, Millam, & Davies, 1998) although the interplay between different varieties and environmental conditions is not clear from these studies. In particular, the relationship(s) between varietal versus environmental control of amylopectin crystallisation and associated gelatinisation temperature control is not understood but is highly significant with respect to the utilisation of potato starches in food (and non-food

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industrial) products. In view of this paucity of knowledge, the following study was conducted where twelve varieties of potato were grown under identical conditions in field plots (same site and same season) to ensure that differences in starch properties reflected varietal rather than environmental change. Tubers were chosen to reflect common potatoes used in the UK for a variety of different purposes.

2. Materials and methods

2.1. Materials

Twelve potato cultivars (Desiree, Duke of York, Edward King, Edzell Blue, Golden Wonder, Kara, Kerrs Pink, Maris Piper, Nicola, Pink Fir Apple, Premiere, Vanessa) were grown in the same field plots in Perth, Scotland in 1996. Two commercial potato starches (S-4251, Sigma Chemical Company and BDH product 30262) were used as references for analysis of these starches.

2.2. Starch extraction

Potato tubers were washed in cold water, liquidised in cold sodium thiosulphate:sodium chloride (1% with respect to both salts). The material was filtered through muslin cloth, concentrated by centrifugation (2000 rpm, 1500 *g* for 5 min) before purifying by centrifuging through 80% (w/v) CsCl (30,000 *g* for 20 min at 15 °C as described by Tester & Morrison, 1990a). The purified starch was extensively washed (six times) with excess volumes of cold water with intermittent centrifugation (2000 rpm, 1500 *g* for 5 min) to concentrate the starch. Finally, the washed starches were rinsed twice with acetone and allowed to air dry to obtain a flowing dry powder.

2.3. Analytical methods

The moisture content was obtained by weight loss of oven-dried starches (100 mg) for 1 h at 130 °C. Nitrogen was determined using a standard Kjeldahl-type methodology where $P = N \times 6.25$. The α-glucan content was determined as glucose (×0.9) by the α-amylase hydrolysis method of Karkalas (1985). Amylose content was determined according to the iodine-binding method of Morrison and Laignelet (1983) while phosphorus was determined as phosphomolybdic blue complexes according to Morrison (1964). Damaged starch, as a function of α-amylase hydrolysis, was determined according to Karkalas, Tester, and Morrison (1992).

The extent of β-amylase hydrolysis (β-amylolysis limit) of the starches was determined as follows: samples (50 mg, triplicate) were accurately weighed into clean 10ml screw-cap tubes to which 5 ml 0.2 M acetate buffer, pH 5.6, was added by pipette and the sealed tubes were boiled to dissolve the starches. Next, 24 μl β-amylase (500 units, Sigma A-7005 from *Ipomoea batatas*) were added by pipette and the mixed and sealed tubes were stored at 37 °C for 24 h. The tubes were then shaken and centrifuged (2000 *g*, 10 min), whereupon 1 ml aliquots were transferred by pipette to clean screw cap-tubes, which were then diluted by addition of 9 ml distilled water. These were re-mixed thoroughly. Into clean 10 ml tubes, 0.1 ml of the diluted solution was transferred by pipette and to these tubes 0.82 ml of water and 80 μl maltase (Sigma M-3145 from bakers yeast EC: 3.2.1.20; 15.5 units) was added. After sealing, they were again mixed and incubated overnight at 37 °C. after this period, the samples were cooled to room temperature and 5 ml of glucose oxidase peroxidase (GOP, Karkalas et al., 1992) were added and the tubes were incubated at 37 °C for a further 40 min. Finally, the absorbance was measured at 505 nm against reference (1 ml distilled water plus GOP) and the amount of glucose was calculated, based on a 0.1 mg/ml glucose standard as for the α-glucan method of Karkalas et al. (1992).

2.4. Chromatography

Native starches were debranched with iso-amylase and the linear fractions were separated by gel permeation chromatography (GPC) as follows. Starch samples (5–10 mg) were weighed into 10 ml Pyrex screw-cap tubes. Into these tubes, 0.4 ml of dimethyl sulphoxide (DMSO) was added by pipette and the starches were gelatinised upon heating in boiling water for 5–10 min. After cooling, 1.6 ml of acetate buffer (0.2 M, pH 3.7) and 10 μl (590 Units) iso-amylase (Hayashibara Inc., Okayama, Japan) were added by pipette. The tubes were mixed, sealed and then incubated at 37 °C for 24 h, whereupon they were boiled for 10 min to denature the enzyme. To this boiled digest, 2.5 ml of 0.05 N KOH and internal standard (0.5 ml maltoheptaose, 1 mg/ml) was added, whereupon the solutions were mixed then centrifuged (2000 *g*, 5 min). Finally, 1 ml aliquots were separately applied to a Sephacryl S-200 HR column (1.6 cm id × 100 cm) to fractionate, as described in elsewhere (Karkalas & Tester, 1992).

2.5. Physical properties

Granule dimensions and distributions were determined using a Coulter Counter Multisizer (Coulter Electronics, Luton, Bedfordshire) with a pre-calibrated (PDVP latex particles) 100 or 120 μm aperture tube (Tester, Morrison, Gidley, Kirkland, & Karkalas, 1994).

Native starch gelatinisation temperatures and gelatinisation enthalpy were determined with a Mettler DSC

30 Low Temperature Cell linked to a TC10A processor, driven by a 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 were added by microsyringe and the contents were stirred with a needle. The pans were 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 three appropriate metal (temperature) and indium (enthalpy) standards. All measurements were performed at least in triplicate. Starch swelling factors (hydrated volume expansion of starch at a given temperature) were determined by blue dextrin dye exclusion where the starch samples were swollen in 10 ml water at 40, 60 or 80 °C for 30 min according to Tester and Morrison (1990a, 1990b).

A Brucker DSZ 200 NMR operating at 200MHz 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 adamantine, respectively. The proportion of (relative) double helices was determined manually with reference to extensively ball-milled amorphous (48 h ball-milled 2 g samples of starch) and acid-etched (40 days in 2M HCl at room temperature) crystalline controls. For the C-6 peak, the ratio of peak height to width at half height was determined. For C-1, the height of the peak at 93ppm (dominant in the amorphous maize but virtually absent in the crystalline maize) was determined. In both cases, the amount of double helices in each sample (M) was calculated as $M = Cx + A(1-x)$, where C and A refer to the '100%' crystalline potato starch and '0%' crystalline potato starch respectively.

X-ray diffraction data were 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 & Tao, 1998).

2.6. Analytical variation

Analytical variation was typically with a coefficient of variation (cv) of 1% or better, although the determination of gelatinisation enthalpy, crystallinity (X-ray diffraction) and proportion of double helices (NMR) was $<5\%$.

3. Results and discussion

3.1. Granule dimensions and composition

Potato starch granules are typically reported to be oval and ellipsoidal with a mean diameter ranging from 5 to 110 μm (Hoover, 2001; Tester & Karkalas, 2002) but more typically 20–40 μm (Peshin, 2001). In this study (Table 1), the average diameter ranged from 17.99 to 23.06 μm with a mean of 21.4 ± 1.6, indicating that, although different cultivars were investigated, the granule size was surprisingly constant, reflecting the identical growing environment. The starches were very pure with a protein content of $<0.45\%$ (Table 2). Amylose ranged from 25.8 to 31.2% with an average of 29.0% and, although comparable to data reported by some authors (Tester, Debon, Davies, & Gidley, 1999), this was greater than figures of 23–25% reported elsewhere (Galliard & Bowler, 1987; Hoover, 2001). The amylose to amylopectin ratio (Table 2) ranged from 1:2.20 to 1:2.87 and this reflects the spread of amylose contents. The amylose content by iodine binding and hence determination of blue-values (Table 2) is greater than figures generated from chromatograms of debranched starches (Table 3).

Phosphorus is relatively high in potato starch (amylopectin) compared to other starches which corresponds 0.37–0.75 μg mg^{-1} starch or 0.53–1.06 μg mg^{-1} amylopectin in this study (Table 2) and again is comparable to data reported elsewhere (Galliard & Bowler, 1987; Hoover, 2001; Morrison & Karkalas, 1990; Tester et al., 1999). The starches were prepared carefully and the amount of damaged starch was minimised ($0.33 \pm 0.16\%$), as shown in Table 2. Hence, any variation in physical properties (below) were not significantly influenced by the damaged starch content.

3.2. Amylopectin structure

It has been reported (Hizukuri, 1985; Tester et al., 1999) that debranched potato amylopectin comprises two major fractions with a modal degree of polymerisation by weight (DPw) of 52-57 (Fraction 1, F1) and 19-21 (F2). Comparable figures were obtained in this study where the average chain length (CL) for F1 and F2 was 57 and 19, respectively (Fig. 1 and Table 3). These data indicate the constancy of the amylopectin unit chain distribution in these starches (from the twelve cultivars). The β-amylolysis limits of the potato starches were very similar with an average of 55.7 ± 0.9 (Table 4). In terms of the internal structure of the β-limit dextrins, the DP of the two major fractions ($F_{1\beta}$ and $F_{2\beta}$) averaged

Table 1
Potato starch granule dimensions

Cultivar	Modal diameter (μm)	Mean diameter (μm)	Mean surface area (μm^2)	Mean volume (μm^3)	Specific surface area (m^2/g)
Desiree	11.66	20.96	1746	9385	0.132
Duke of York	10.02	19.93	1632	8676	0.133
Edzell Blue	12.32	22.92	2094	12,140	0.122
Golden Wonder	11.66	22.22	2080	12,830	0.115
Kara	11.66	19.36	1510	7539	0.142
Kerrs Pink	14.62	22.44	2050	11,950	0.122
King Edward	16.26	21.92	1905	10,600	0.128
Maris Piper	13.63	23.05	1992	10,440	0.136
Nicola	11.33	21.73	1959	11,640	0.119
Pink Fir Apple	12.65	17.99	1303	6071	0.152
Premiere	15.93	23.06	2059	11,350	0.129
Vanessa	14.29	21.19	1724	8791	0.139
Average	13.0±1.9	21.4±1.6	1838±256	10,118±2053	0.131±0.011

Table 2
Composition of potato starch granules

Cultivar	Protein[a] (%) (N×6.25)	Amylose[b] (%)	AM/AP[c]	Phosphorus[a] (μg/mg)	Phosphorus (μg/mg) AP basis	Damaged starch[b] (%)
Desiree	0.44	31.2	1 : 2.20	0.39	0.57	0.55
Duke of York	0.41	29.5	1 : 2.40	0.37	0.53	0.58
Edzell Blue	0.31	26.4	1 : 2.79	0.46	0.63	0.23
Golden Wonder	0.37	27.7	1 : 2.61	0.72	1.00	0.43
Kara	0.36	31.2	1 : 2.20	0.73	1.06	0.45
Kerrs Pink	0.38	28.1	1 : 2.56	0.75	1.04	0.11
King Edward	0.30	29.7	1 : 2.37	0.43	0.61	0.14
Maris Piper	0.31	26.0	1 : 2.84	0.48	0.65	0.24
Nicola	0.39	29.2	1 : 2.43	0.60	0.85	0.42
Pink Fir Apple	0.32	30.1	1 : 2.32	0.74	1.06	0.18
Premiere	0.33	25.8	1 : 2.87	0.41	0.55	0.34
Vanessa	0.42	29.3	1 : 2.42	0.39	0.55	0.27
Average	0.36±0.05	28.7±1.9	1 : 2.42	0.54±0.16	0.76±0.22	0.33±0.16
BDH Prod 30262	0.16	26.3	1 : 2.80	0.61	0.82	0.70

[a] Dry basis.
[b] α-Glucan basis.
[c] AM = amylose and AP = amylopectin.

Table 3
Chain length distribution of isoamylase debranched potato starch using a Sephacryl S 200 HR column

Cultivar	Amylose (%)	DP[a]	F_1[b]		F_2[c]		F_2:F_1	
			CL	Weight, %	CL	Weight, %	Weight	Mole[d]
Desiree	24	32	58	38	20	51	1.3	3.8
Duke of York	26	25	58	27	19	49	1.8	5.4
Edzell Blue	18	27	54	32	18	51	1.6	4.7
Golden Wonder	26	27	58	31	19	50	1.6	5.0
Kara	27	29	58	36	19	41	1.1	3.4
Kerrs Pink	24	27	54	33	18	47	1.4	4.2
King Edward	19	28	54	32	20	50	1.5	4.2
Maris Piper	21	28	54	38	18	42	1.1	3.3
Nicola	23	26	56	29	19	50	1.7	5.0
Pink Fir Apple	22	30	56	37	20	46	1.2	3.5
Premiere	25	26	57	29	18	49	1.7	5.4
Vanessa	33	28	56	30	20	54	1.8	5.0
Average	24±4	28±2	56±2	33±4	19±1	48±4	1.5±0.3	4.4±0.8
BDH Prod 30262	23	29	58	33	20	48	1.5	4.2

[a] Average DP calculated according to Hizukuri (1985).
[b] F_1 = B2–B4 chains.
[c] F_2 = A + B_1 chains.
[d] $F_1(CL)/F_2(CL) \times F_2/F_1$ (by weight).

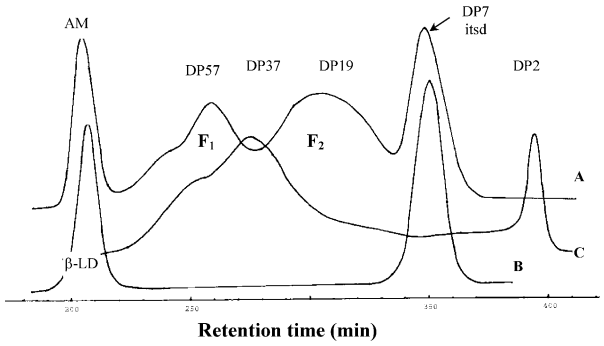

Fig. 1. Representative gel permeation chromatography profile [Sephacryl (HR) S-200 column] of debranched potato starch/amylopectin (A) and β-limit dextrin before (B) and after (C) debranching. itsd: Internal standard maltoheptaose (G7).

48±3 and 37±2, respectively. A broad tail on these major peaks was also present with an average DP range of 7–12. In addition (but outside the resolution of this column), a low molecular weight peak was also present, representing maltotriose, maltose and glucose generated from stubs of hydrolysed exterior chains. The similarity of the amylopectin molecules is further reinforced by the similarity of the average exterior (ECL) and interior chain lengths (ICL), representing 17±1.2 and 9±0.9, respectively (Table 4).

3.3. Physical properties

The swelling factors at 40, 60 and 80 °C are presented in Table 5 and show a broad genotypic variation in this respect. Although there is unity with respect to swelling for all the starches at 40 °C, at 60 °C swelling factors ranged from 36 to 88 while, at 80 °C, these ranged from 94 to 146. Comparable figures have been reported by Tester (1997) and reflect both compositional differences and crystalline characteristics within the starch granules (Tester & Morrison, 1990a, 1990b). The amount of limited acid hydrolysis is typically correlated with starch amorphous material and, consequently, the positive correlation ($r=0.9969$, $P=0.001$) between this parameter (3 days in 2M HCl at 35 °C, Table 5) and the amount of amylose (Table 2). Upon more extensive (9 days) erosion (where amorphous material is extensively hydrolysed but crystalline material hydrolysis is initiated), this parameter becomes inversely correlated with amylose ($r=-0.6310$, $P=0.02$) where the crystallinity of the amylopectin fraction becomes a more important parameter with respect to the extent of hydrolysis (discussed below).

Tester et al. (1999) have shown that, when potatoes (Maris Piper) are grown between 10 and 25 °C, for

Table 5
Swelling factor (SF) of potato starches incubated in excess water at 40, 60 and 80 °C and acid solubles generated by hydrolysis in 2 M HCl

Cultivar	Swelling factor[a]			Acid solubles[b] (%, db)	
	40 °C	60 °C	80 °C	3 Days	9 Days
Desiree	1.0	37	99	23.5	70.1
Duke of York	1.0	48	94	25.8	74.6
Edzell Blue	1.0	43	114	18.4	77.6
Golden Wonder	1.0	55	138	25.8	74.6
Kara	1.0	83	146	27.2	69.2
Kerrs Pink	1.0	88	137	23.5	74.8
King Edward	1.0	57	114	19.0	76.1
Maris Piper	1.0	45	109	20.5	76.7
Nicola	1.0	36	118	23.3	72.0
Pink Fir Apple	1.0	47	126	21.7	72.5
Premiere	1.0	43	108	25.0	71.2
Vanessa	1.0	63	113	33.1	71.2
Average	1.0	54±17	118±16	23.9±3.8	73.4±0.6

[a] α-Glucan basis.
[b] Starches hydrolysed in 2 M HCl at 35 °C.

Table 4
β-Amylolysis limit and unit chain distribution of debranched potato starch β-limit dextrins (LD) using a Sephacryl S 200 HR column

Cultivar	β-Amylolysis limit (%)	Debranched chain lengths (CL)[a]			Exterior CL (AP[b])	Interior CL (AP)
		$F_{1\beta}$	$F_{2\beta}$	Minor tail		
Desiree	55.6	47	37	10–22	20	11
Duke of York	54.6	47	36	6–14	16	8
Edzell Blue	54.9	49	37	5–18	17	9
Golden Wonder	55.0	49	34	9–16	17	9
Kara	56.5	52	40	6–18	18	10
Kerrs Pink	56.1	45	34	8–17	17	9
King Edward	57.6	44	37	5–11	18	9
Maris Piper	56.1	50	37	8–19	18	9
Nicola	55.0	46	34	9–18	16	9
Pink Fir Apple	54.6	53	39	8–15	18	11
Premiere	55.1	48	37	7–16	16	9
Vanessa	56.0	51	36	7–16	17	9
Average	55.6±0.90	48±3	37±2	7–17	17±1	9±0.9

[a] Excludes G1–G3 from unit chain stubs.
[b] AP: amylopectin.

Table 6
Gelatinisation properties (DSC), crystallinity (X-ray diffraction) and number of double helices (NMR) of potato starches

Cultivar	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J g^{-1}, db)	ΔH (J g^{-1}, AP[a])	Crystallinity (X-ray)	Double helices (NMR)
Desiree	59.2	63.4	70.4	11.2	17.0	24.7	28	nd
Duke of York	58.1	61.7	67.8	9.7	19.1	27.0	24	65
Edzell Blue	58.5	62.7	70.2	11.7	17.6	23.9	25	65
Golden Wonder	58.9	62.5	69.1	10.2	18.4	25.4	25	nd
Kara	58.8	63.7	70.9	12.1	19.3	26.7	32	64
Kerrs Pink	59.3	63.5	70.3	11.1	19.1	26.1	24	64
King Edward	59.7	63.8	71.0	11.4	19.3	26.9	24	64
Maris Piper	58.7	62.0	68.6	9.9	17.6	23.8	25	nd
Nicola	58.9	64.1	71.5	11.5	19.2	27.1	24	nd
Pink Fir Apple	61.0	66.0	72.7	11.8	19.0	27.2	24	nd
Premiere	57.9	61.8	69.1	11.2	19.0	25.6	28	nd
Vanessa	59.9	62.9	71.4	11.5	18.3	25.8	27	nd
Average	59.1±0.8	63.2±1.2	70.3±1.3	11.1±0.7	18.6±0.8	25.9±1.2	26±2	64±1
BDH Prod 30262	59.3	65.6	74.74	9.1	18.0	24.5		

[a] AP: amylopectin.

every 1 °C increase in growth temperature, the starch gelatinisation temperature increases by at least 0.5 °C. In this work—the primary reason for conducting this study—it is apparent that there is little variation in the onset (T_o, from 58.1 to 61.0 °C), peak (T_p, from 61.7 to 66.0 °C) or conclusion (T_c, from 67.8 to 72.7 °C) temperatures by DSC or the gelatinisation enthalpy (ΔH, from 17.0 to 19.3 J g^{-1}), as shown in Table 6. Similar data, focussing on the crystalline structure of potato starch grown at different temperatures, has also been published (Protserov et al., 2002) Clearly, environmental temperature has much more influence on the gelatinisation temperatures than cultivar specific variation—at least for these cultivars. This constancy of gelatinisation temperatures is also reflected in the constancy and percentage (26±2%) of starch crystallinity (X-ray diffraction) and the proportion of double helices by NMR (64±1%).

The implications of this work are very important both academically and commercially. In terms of the proportion of amylopectin double helices, starch crystallinity and gelatinisation temperatures, normal potato cultivars (specifically these) do not show much variation in these characteristics. Compositional differences would be expected to influence swelling characteristics more overtly and, with a contribution form the respective amorphous and crystalline compartments, the extent of variation is apparent. With respect to the gelatinisation characteristics of the starches, the influence of variation between the cultivars (grown under the same environmental conditions) is much less apparant than the influence of environmental temperature (reported elsewhere). This is explored in more detail at the molecular level in a subsequent publication.

4. Conclusions

This work was primarily, conducted to quantify variations in potato starch physico-chemical properties when grown at the same site. Clearly potatoes are not uniform in size and, unlike grain, manageable sample numbers are small. Within these samples, the amylopectin structure showed little or no variation in terms of unit chain distribution profiles or lengths. In terms of order within the granules, there is almost constancy with respect the amount of crystallinity (26±2) and double helices (64±1%). Additionally, the amount of variation regarding gelatinisation parameters is relatively small. This contrasts with the influence of growth temperature on gelatinisation parameters (discussed elsewhere) which has a very profound effect on starch gelatinisation temperatures.

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