Swelling and Gelatinization of Cereal Starches. III. Some Properties of Waxy and Normal Nonwaxy Barley Starches[1]

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ABSTRACT

Starches were isolated from 12 waxy and six nonwaxy genotypes of barley grown in adjacent field plots to minimize edaphic variation. Amylopectin (AP) in all of the starches appeared to be very similar in structure. For the colorimetric determination of amylose (AM), a revised calibration curve was necessary because barley AP gave a lower λ_{max} (529 nm) and absorbance with I_2/KI reagent than AP from other cereal starches used previously. The waxy starches contained AM (<7.4%) and lipids (120–630 mg/100 g), giving an AM-lipid relationship ($r = 0.990$) different from that in the nonwaxy starches; the B-granules in the waxy starches had about half of the AM and lipids found in the A-granules. The fatty acid composition of the lipids in the waxy starches was much more saturated than in the nonwaxy starches, and it was negatively correlated with total lipid content. Starch granule swelling at 70 and 80°C, after gelatinization (disordering) of AP is complete, is a property of the whole AP molecule. Swelling factors for the AP fraction, over the range of 4–35 for all starches from the present and previous studies, were inversely correlated with lipid content ($r = -0.918$, $n = 30$).

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Early observations showed that waxy cereal starches have little or no lipids compared with the corresponding normal starches, whereas high-amylose (AM) starches have elevated levels (Morrison 1988). In starches from the Triticeae, the lipids are almost entirely lysophospholipids (LPL) and account for nearly all of the phosphorus in the starch. In starches from other cereals, the lipids consist of free fatty acids (FFA) and LPL. In the nonwaxy starches of rice and maize, about 5–10% of the total phosphorus is in hexose-phosphate (Morrison 1988, Morrison and Karkalas 1990).

High correlations between AM and lipid contents in these early studies were not truly significant because the waxy, normal, and high-AM starches were in discrete clusters, and there was no clear relationship within any cluster. Better evidence was obtained in subsequent studies where other variables were closely controlled. In starch granules isolated from developing wheats, AM contents increased from about 20 to 30% and LPL contents from about 0.4 to 1.0% over the period 20–60 days after anthesis, and in three cultivars there was a different AM-LPL relationship in the A- and B-type granules (Morrison and Gadan 1987). A similar pattern of results was obtained with starches from developing waxy, normal, and high-AM barleys (McDonald et al 1991). Waxy Oderbrucker starch was particularly interesting because AM increased from 2 to 6% and LPL from 0.08 to 0.39% in the A-granules, whereas the B-granules consisted entirely of amylopectin (AP) at all stages of development (lipids were not measured in the latter). Further evidence of a direct AM-lipid relationship was obtained from analysis of starch from single kernels of F_2 generation crosses of barleys exhibiting zero to three doses of the high-AM gene (Morrison 1987).

The most complex situation studied so far was in maize, where anomalous types of AP gave rise to misleading values for AM when assayed colorimetrically or by gel permeation chromatography of native starches (South et al 1991). When long-chain α-1,4-glucan was used as a measure of true AM content, an excellent AM-lipid relationship was obtained for a waxy gene dosage series and for a collection of single and double mutants affecting starch composition. In 15 nonwaxy maize starches, true AM content (23–51%) was highly correlated ($r = 0.906$) with lipid content (0.49–1.15%), and the linear regression extrapolated through the cluster of waxy starches near the origin (0–1% AM, 0–0.07% lipid) so that the overall correlation ($r = 0.959$, $n = 20$) was also very significant (South et al 1991).

An unsatisfactory aspect of the AM-LPL relationship in barley is that waxy barley starches seem to have much more lipid than waxy rice or waxy maize starches, and regressions extrapolate to 0.15–0.25% LPL at zero AM (Morrison et al 1984, 1986; Tester et al 1991). For this reason we decided to examine starches from a collection of waxy and normal barleys grown together at one site to minimize edaphic variation. Since lipids strongly inhibit starch granule swelling during and after gelatinization (Tester and Morrison 1990a, Tester et al 1991), these properties were also studied. The results are presented here. Other aspects of AM and LPL organization within the starch granule and their effects on some properties of the starches will be described later (Morrison et al, in press).

MATERIALS AND METHODS

Starches

Twelve waxy and six nonwaxy (normal) genotypes of barley were grown in adjacent field plots at the Scottish Crop Research Institute, Dundee. Seven of the 12 waxy barleys were of Japanese origin, Chalbori was Korean, and Wapana, Wanupana, and Washonupana were developed originally in the United States (from Compana). Nine barleys were naked or nude types (Summire Mochi 3724, Dango Mugi 7982, Masan Naked 1 OUJ043, Tokushima Mochimugi OUJ783 and 8028, Iyatomi Mochi 8026, Bozu Mochi 8024, Wanupana, and Washonupana).

Starches were isolated from cracked grain by steeping and washing, and they were then purified by centrifuging through cesium chloride (South and Morrison 1990, Tester and Morrison 1990a). In previous studies (Morrison et al 1984, 1986), proteases were used to assist in removal of protein and mercuric chloride, or brief treatment at pH 2 was used to prevent amylase activity. None of these treatments was used here.

Physical Measurements

Starch granule dimensions were measured using a Coulter Multisizer (Hialeah, FL) recording 256 channels, with a 70-μm aperture counting tube. Starch granule numbers per endosperm were calculated from Coulter data and the starch content of the grains (Table I), assuming that hydrated granules contained 43% moisture and had a density of 1.35. Swelling factors (SF) of starches heated in excess water at 80°C for 30 min (SF_{80}) were determined using a blue dextran dye exclusion method (Tester and Morrison 1990a). SF_{80} divided by the fraction of AP in the starch gave $SF(AP)_{80}$, the notional SF of the undiluted AP in the starch (Tester et al 1991).

Chemical Analyses

Moisture content was taken as weight loss after heating at 130 ± 3°C for 1 hr, and all results are corrected to a dry weight basis. Apparent and total AM contents (with native lipids present

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or removed, respectively) were determined colorimetrically with I_2/KI reagent (Morrison and Laignelet 1983), and λ_{max} was recorded under assay conditions for total AM. The original calibration, which was developed for all cereal starches, included a correction for the absorbance of AP that was too high for barley AP. Consequently, AM contents determined for waxy starches were too low. A new calibration curve was prepared, using the absorbance obtained for pure barley AP isolated by preparative gel permeation chromatography on Sepharose CL-2B (South et al 1991), giving the following equation: Percent AM = (28.00 × blue value) − 4.65, where analytical conditions and blue value are as before (Morrison and Laignelet 1983).

Lipids were extracted with propanol-water (3:1, v/v) at 100°C (Morrison and Coventry 1985) for methanolysis and determination of total lipids as fatty acid methyl esters (FAME) by gas chromatography (Morrison et al 1975, 1980). Phosphorus was determined (Morrison 1964) on dry starch and on aliquots of extracted lipids. After correction for nonlipid P (see results), total starch P was converted to weight of LPL using the factor (P − 2) × 16.16. Starch content was determined on replicate batches of five kernels (crushed with pliers and steeped in α-amylase solution at 80°C overnight) by the method of Karkalas (1985). Damaged starch was determined on 50- to 100-mg samples using a small-scale modification of the standard AACC procedure (Karkalas et al 1992). Starches were debranched with isoamylase, and the mean chain length (CL) of the linear α-1,4-glucan chains from AP was determined by high-performance liquid chromatography (Tester and Morrison 1990b).

Analytical Errors

Errors of determinations (in brackets) were well within acceptable limits for 1,000-kernel weight (0.1 g), starch content (2.5 mg per kernel), percent starch in kernel (1.9%), A-granule diameter (0.02 μm) and volume (4 μm^3), B-granule diameter (0.003 μm) and volume (0.06 μm^3), damaged starch content (0.1%), apparent and total AM contents (0.08%), phosphorus content (0.6 mg/100 g), FAME content (3.8 mg/100 g), and SF (0.1).

RESULTS AND DISCUSSION

Starch Contents and Granule Dimensions

The waxy barleys had smaller kernels and less starch per endosperm than the nonwaxy genotypes (Table I). Wapana (waxy), Wanupana (waxy, nude), and Washonupana (waxy, short awn, nude) lines, developed from Compana, had larger kernels and more starch per endosperm than the other waxy barleys, but less than in their parent Compana or Shopana (short awn Compana).

The waxy endosperms were extremely hard, especially in the Japanese barleys. Therefore, extraction of starch was difficult and recoveries of waxy starches (36.3 ± 4.1% of grain weight, wet basis) were lower than for nonwaxy kernels (43.8 ± 4.6%). Levels of damage to granules were acceptable, considering the wet grinding of steeped grain that was necessary to release starch, and would have had no significant effects on SF and other properties discussed below.

Although starch yields (70–85%) were a little low, this had no evident effect on the results reported here. A- and B-granule numbers and dimensions were essentially the same when measured on bulk preparations of starch (Table I) and on starch isolated from single kernels using our cesium chloride procedure (South and Morrison 1990).

The waxy starches had similar proportions of A- and B-granules (by number and by weight) to the nonwaxy starches (Table I), in agreement with previous results (Morrison et al 1986), although the values were about 10% less by number and 1.6% less by weight on average than before. These results indicate that mutations affecting the waxy protein gene caused a reduction in the synthesis of AM that was not compensated by increased synthesis of AP, so that generally starch granule dimensions, endosperm content, and kernel size were reduced. However, we have also found, in experiments with grain grown at 10 and 15°C, that Waxy Oderbrucker kernel size and endosperm starch content can be as large as that in the nonwaxy cultivars Triumph and Golden Promise, although the waxy starch granule dimensions were smaller (Tester et al 1991).

The numbers of A-granules per endosperm can be taken as the number of amyloplasts if it is accepted that there is one A-granule per amyloplast (Parker 1985). The nonwaxy starches had about 50% fewer A-granules (Table I) than found previously in field-grown Triumph (Morrison et al 1986), Glacier, and Oderbrucker barleys (McDonald et al 1991). B-granule numbers were also much less than reported previously and greatly variable between genotypes. The waxy starches had 50% more amyloplasts and A-granules than the nonwaxy starches, as noted before when comparing Waxy Oderbrucker with Oderbrucker (McDonald et al 1991), whereas B-granule numbers were much less than previously recorded. To some extent this may have been due to the difficulty in extracting starches from the very hard endosperms, which would have caused selective losses of very small B-granules. In barleys grown in controlled-environment chambers (Tester et al 1991), A- and B-granule numbers and dimensions were much reduced in grain grown at higher temperatures, but even in grain grown at 10°C the values were less than those found in this study; Waxy Oderbrucker had much more A- and B-granules with smaller dimensions than the nonwaxy starches.

Composition of Starches

All starches were of high purity (96.1 ± 0.9% α-glucan), and traces of protein and other nonlipid materials were considered to have no effect on the properties discussed below. Nonstarch lipid impurities (Morrison 1981) were generally insignificant.

The AM and lipid contents of the starches are given in Table II. Lipid interferes with binding of polyiodide ions by AM so that apparent AM of native starches is always less than total AM of lipid-free starches (Morrison and Laignelet 1983). The difference, ΔAM, will be discussed further in a future article (Morrison et al, in press).

The debranched starches gave three peaks of CL 45.7, 22.7 and 14.6 in the high-performance liquid chromatograms from the AP component, as reported previously (MacGregor and Morgan 1984, Tester et al 1991). The chromatograms were all very similar and showed no features that could be related to variation in any chemical or physical property. However, Kang et al (1985a,b) did find small quantitative differences in the ratio of their fractions III and II (estimated CL <35 and >35, respectively) from Bozu, Iyatomi Mochi, and four other varieties. Nevertheless, we conclude that all of the starches used here had essentially identical types of AP. In previous work there was no evidence for anomalous types of AP with enhanced iodine-binding capacity even in high-AM barley starches (Tester et al 1991); therefore, the total AM contents of barley starches determined colorimetrically should be reliable. This is not the case with high-AM types of maize (South et al 1991).

TABLE I
Comparison of Kernel Weights and Starch Granule Dimensions in 12 Waxy and Six Nonwaxy Barley Genotypes

Property	Waxy Genotypes	Nonwaxy Genotypes
1,000-kernel weight, g	28.8 ± 7.9	35.2 ± 6.2
Starch per endosperm, mg	18.6 ± 3.8	22.6 ± 3.0
Starch in endosperm at 10.6% moisture, %	51.1 ± 2.5	52.2 ± 1.7
Damaged starch, % by wt	3.1 ± 1.1	3.3 ± 1.5
A-granules		
Mean diameter, μm	11.1 ± 0.6	13.4 ± 0.8
Mean volume, μm^3	864.6 ± 138.6	1,526.8 ± 283.1
Number (10^6) per endosperm	26.4 ± 6.0	17.6 ± 2.5
B-granules		
Mean diameter, μm	2.6 ± 0.15	3.2 ± 0.3
Mean volume, μm^3	13.4 ± 2.4	28.2 ± 6.2
Number (10^6) per endosperm	94.7 ± 41.0	54.1 ± 36.4
% by number	73.9 ± 15.4	76.0 ± 16.3
% by volume or wt	6.1 ± 4.9	7.2 ± 3.6

TABLE II
Composition and Properties of Starches from 12 Waxy and Six Nonwaxy Barley Genotypes

Genotype	Amylose,[a] %		λ_{max}[b] (nm)	Phosphorus,[c] mg/100 g		Lipid,[d] mg/100 g		SF[e]	
	Apparent	Total		Lipid	Total	ex. Total P	ex. FAME	SF_{80}	$SF(AP)_{80}$
Waxy									
Summire Mochi	0.8	1.7	532	7.9	9.5	120	174	33.8	34.4
Dango Mugi	0.9	2.1	534	10.1	12.5	169	267	33.3	34.0
Masan Naked	1.1	2.4	530	13.2	15.0	209	272	28.7	29.4
Tokushima Mochimugi OUJ783	1.8	3.1	537	15.7	17.3	246	331	25.5	26.3
Chalbori	1.9	3.4	539	13.4	16.5	233	300	25.7	26.6
Tokushima Mochimugi 8028	2.1	3.6	534	18.2	20.6	300	312	30.9	32.1
Iyatomi Mochi	1.8	3.9	536	18.1	20.4	296	304	26.2	27.3
Waxy Oderbrucker	2.7	5.2	550	23.8	26.6	397	412	21.0	22.2
Bozu Mochi	2.4	5.4	546	24.4	30.5	460	528	21.2	22.4
Wapana	3.4	6.5	559	30.1	31.7	480	505	19.5	20.9
Wanupana	3.2	6.5	560	30.2	32.5	493	536	25.2	27.0
Washonupana	3.8	7.4	578	35.1	37.2	569	630	19.1	20.6
Nonwaxy									
Chalky Glen	22.8	29.2	636	47.7	56.0	873	874	10.2	14.4
Midas	23.8	30.2	636	54.4	56.5	881	810	12.5	17.9
Hector	24.3	30.4	639	50.6	49.9	774	757	12.2	17.5
Shopana	23.1	30.5	635	61.2	63.8	1,000	1,012	11.5	16.6
Compana	22.2	30.5	634	60.3	65.8	1,032	1,021	11.0	15.8
Glen	25.8	32.7	645	55.9	61.9	969	1,025	13.7	20.4

[a] Apparent amylose measured using native starch; total amylose measured using lipid-free starch.
[b] λ_{max} measured under conditions for colorimetric determination of total amylose.
[c] Phosphorus from lysophospholipid extracted with hot propanol-water from starch, and total phosphorus in dry starch, respectively.
[d] Lipid, calculated as lysophospholipid from (total starch P − 2) × 16.16, and from total extractable fatty acid methyl esters (FAME) × 1.63.
[e] Swelling factor (SF) (blue dextran method) at 80°C of native starch, and value calculated on basis of amylopectin content (AP), respectively.

The total AM contents of the waxy starches (Table II) were in good agreement with previous reports in which lipid-free starches were used (Kang et al 1985a,b; Morrison et al 1986; MacGregor, in press). There is always the danger that a few nonwaxy kernels could have been present as contaminants in the waxy grain samples, giving elevated levels of AM (South and Morrison 1990). However, the kernels were checked visually for impurities before use, and analysis of starch from single kernels gave essentially identical results as those from bulk-extracted samples. The AM-lipid relationship (below) would also have been quite different if nonwaxy kernels had been the source of the AM (see discussion below). Thus, the AM contents of the waxy starches were real and reached much higher levels than in waxy rice and waxy maize (Morrison et al 1984, Shannon and Garwood 1984, Juliano 1985, Morrison and Azudin 1987, South and Morrison 1990). AM contents were correlated with A-granule volumes and to some extent may reflect the duration of starch synthesis or maturity of the granules (Kang et al 1985b, McDonald et al 1991). There was a strong curvilinear relationship between λ_{max} and AM content, which gave an extrapolated value of 527 ± 2 nm (at zero AM content) for pure AP, whereas the AP isolated for calibration of the colorimetric method gave 529 nm.

The total and apparent AM contents and λ_{max} of the nonwaxy starches were of the expected order, except for Glen, which had slightly high AM values compared with previous results for other normal types of barley starches (Morrison et al 1984, 1986; MacGregor, in press).

The starch lipids were almost exclusively LPL, as in all Triticeae starches isolated under conditions that avoid adsorption of nonstarch lipid FFA as a type of artifact (Morrison 1981, 1988). Total phosphorus content was closely correlated with lipid phosphorus (Table III, line 1). The intercept of the regression line gave a value of 1.86 mg of nonlipid phosphorus per 100 g of starch, which was probably inorganic phosphate with traces of hexose phosphate, and the slope indicates that only 97% of the LPL was recovered in the lipid extract. Since total starch P can be determined more easily and accurately than lipid P, a correction of 2 mg of nonlipid P per 100 g of starch was used before converting P to LPL. Hence, LPL = (total starch P − 2) × 16.16 in this study.

The fatty acids (FA) in the Japanese waxy starches were mostly saturated, particularly in Iyatomi Mochi. Waxy Oderbrucker and the three waxy lines derived from Compana were intermediate compared with the other nonwaxy starches (Table IV). These results agree with previous reports on the FA composition of lipids in barley starches (Acker and Becker 1971, Becker and Acker 1972, Tester et al 1991), but they do not agree with the results of Goering et al (1975), who appear to have analyzed mostly nonstarch lipid contaminants (Morrison 1978). Percent saturated FA and LPL content were inversely related (Table III, line 2). Lipid in waxy starches from other cereals also tends to be more saturated (Morrison 1988, South et al 1991), but the significance of this is not known.

TABLE III
Linear Regression Equations (form $y = A + Bx$) for Data in Table II

Starches	n	Equation[a]	r[b]
All	18	Total P = 1.86 + 1.036 lipid P	0.995
All	16[c]	Saturated FA = 79.5 − 0.0293 LPL	−0.974
Waxy	12	LPL (ex. total P) = 7.7 + 75.8 total AM	0.990
Waxy	12	Lipid (ex. total FAME) = 79.6 + 70.6 total AM	0.964
Waxy	12	SF_{80} = 35.7 − 2.319 total AM	−0.864
Waxy	12	SF_{80} = 33.8 − 0.030 LPL (ex. total P)	−0.857
All	18	SF_{80} = 33.8 − 0.024 LPL (ex. total P)	−0.944
Waxy	12	$SF(AP)_{80}$ = 36.1 − 0.0278 LPL (ex. total P)	−0.829
All	18	$SF(AP)_{80}$ = 33.0 − 0.0177 LPL (ex. total P)	−0.887
All	30[d]	$SF(AP)_{80}$ = 31.5 − 0.0176 LPL (ex. total P)	−0.907

[a] FA, fatty acids; LPL lysophosopholipids; AM, amylose; FAME, fatty acid methyl esters; SF, swelling factor; AP, amylopectin; 80, at 80°C. Units: P = mg/100 g starch, saturated FA = %, total AM = %, LPL and lipid (ex. FAME) = mg/100 g starch, SF_{80} and $SF(AP)_{80}$ are dimensionless.
[b] $P < 0.001$ for all values.
[c] Omitting Iyatomi Mochimugi and Bozu Mochi where the lipids appeared to have been slightly oxidized, overall correlation including these starches was $r = -0.926$ ($n = 18$).
[d] Including data for 12 starches given by Tester et al (1991), AM and AP values from original calibration of colorimetric method.

AM-Lipid Relationship

In previous studies (Morrison et al 1984, 1986), barleys were obtained from several sources, and variation in the composition of their starches undoubtedly included edaphic effects. In particular, it has been found that ambient temperature during the

period of grain filling and starch synthesis affects lipid content with little or no effect on AM content (Morrison et al 1986, Tester et al 1991). In the present study edaphic effects were minimized by growing all samples at the same site in the same year, although some varieties did ripen earlier than others and therefore experienced slightly different solar radiation and weather.

In the waxy starches, there were highly significant correlations between total AM and lipid contents (Table III, lines 3 and 4). The high intercept for lipid (calculated from total FAME) indicates that the starches may have had about 80 mg of nonstarch lipids (FFA artifacts) per 100 g, which is comparable with previous results (Morrison et al 1986). The intercept of the regression of LPL on AM (Table III, line 3) was very low, and this regression was probably a more accurate description of the AM-lipid relationship in the waxy barley starches. The high intercept values of LPL reported previously (Morrison et al 1986, McDonald et al 1991) were caused by low values for AM determined with the original colorimetric calibration discussed above.

In practice, the AM-lipid relationship described only the A-type granules, which were about 93% by weight of the starch (Table I). No AM has been reported in Waxy Oderbrucker B-granules (McDonald et al 1991). However, that observation may have been incorrect because AM was determined using the original colorimetric calibration curve rather than the recalibrated method used here. In the present study, pure B-granules had 2.4 and 4.3% AM and 92 and 262 mg of LPL/100 g from Summire Mochi and Washonupana, respectively. Kang et al (1985a) also reported small amounts of AM in the B-granules of waxy starches.

Further aspects of the AM-lipid relationship in these studies will be discussed in detail in a future article (Morrison et al, in press). For the purposes of this article, it is sufficient to recognize that lipid content will be closely correlated with AM content in starches from waxy barleys grown under the same conditions, but that lipid content will be particularly susceptible to change as a result of edaphic variation or of changes in ambient temperature during the grain-filling period (Morrison et al 1986, Tester et al 1991).

Starch Granule Swelling

Granule swelling begins at the onset of gelatinization or disordering of AP (measured by differential scanning calorimetry) and continues to temperatures well above the conclusion temperature, T_c (Tester and Morrison 1990a,b). In a previous study, SF was measured at 70°C (Morrison et al 1986), but measurements at 80°C are preferable since T_c is often >70°C but nearly always <80°C (Tester et al 1991). Swelling is primarily a property of AP and AM appears to act as a diluent, whereas lipids strongly inhibit swelling through inclusion complexes with some of the AM (Tester and Morrison 1990a). This effect of lipid has been demonstrated in Triumph and Golden Promise starches of constant AM content, but it can also be shown by recalculating SF_{80} on the basis of AP content to obtain $SF(AP)_{80}$ values (Tester et al 1991) (Table II). Since measurements were made above T_c, SF were not affected by gelatinization in the restricted meaning of disordering AP. It should be noted that it was reported previously that lipids did not affect the swelling of Oderbrucker starch at temperatures above 70°C (Tester and Morrison 1990a), but in all other studies (Morrison et al 1986, Tester et al 1991), including the present one, they did have a strong effect. The Oderbrucker result was clearly anomalous.

In the waxy starches, SF was correlated with AM content, but it was equally well correlated with LPL (Table III, lines 5 and 6) and with lipid calculated from FAME (not shown). Since lipid is the active inhibitor of swelling (Tester et al 1991) and AM and lipid contents are closely correlated, the correlation of SF with AM was consequential. This is confirmed by the fact that $SF(AP)_{80}$ was correlated with LPL content (Table III, lines 8–10) and with lipid (calculated from FAME). Within the nonwaxy group, SF_{80} and $SF(AP)_{80}$ showed limited ranges of variation that were not correlated with either lipid or AM contents.

The SF/AM regression line (Table III, line 5) for the waxy starches predicted zero swelling at 14.2% AM and was thus useless for predicting SF of nonwaxy starches that contained 29–33% AM and had measurable SF. However, SF and SF(AP) regressions against LPL (Table III, lines 6 and 8) or lipid calculated from FAME (not shown) gave better predictions of SF for the nonwaxy starches, and the overall correlation coefficients for all 18 starches (Table III, lines 7 and 9) were high.

In a previous study where barleys were grown in controlled-environment chambers at various temperatures and with constant illumination (Tester et al 1991), SF(AP) was highly correlated with LPL for waxy, normal, and high-AM starches ($r = -0.987$, $n = 12$). However, lipid contents were generally much higher and, consequently, $SF(AP)_{80}$ was much lower than values for the corresponding starches in the present study. Nevertheless, the combined data ($n = 30$) could be described by a single regression line with a highly significant correlation coefficient (Table III, line 10).

SFs measured at 70°C (SF_{70}) are about 70% of SF_{80} (Tester et al 1991) but otherwise follow the same trends. Using data (Morrison et al 1986) for starches comparable with those used

TABLE IV
Fatty Acid Composition of Lipids in Barley Starches

Genotype	Fatty Acid, %					Total Saturated Fatty Acids (%)
	16:0	18:0	18:1	18:2	18:3	
Waxy						
Summire Mochi	71.8	4.0	5.2	19.1	0	75.8
Dango Mugi	73.3	2.1	5.5	18.9	1.3	75.4
Masan Naked	71.8	1.4	5.2	20.7	1.0	73.2
Tokushima Mochimugi OUJ783	70.1	2.0	5.5	21.4	1.0	72.1
Chalbori	74.0	1.3	4.4	19.2	1.1	75.3
Tokushima Mochimugi 8028	71.0	0	5.9	23.1	0	71.0
Iyatomi Mochi[a]	80.6	3.2	2.4	13.8	0	83.8
Waxy Oderbrucker	63.0	5.0	3.3	28.6	0	68.0
Bozu Mochi[a]	73.5	1.2	3.3	21.0	1.0	74.7
Wapana	59.7	5.6	3.9	30.8	0	65.3
Wanupana	58.6	1.7	3.6	34.5	1.6	60.3
Washonupana	61.9	1.7	3.3	31.8	1.5	63.6
Nonwaxy						
Chalky Glen	49.0	0.4	3.0	44.4	3.2	49.4
Midas	51.5	1.2	2.5	40.8	4.0	52.7
Hector	56.3	1.0	3.3	36.5	2.9	57.3
Shopana	50.8	1.7	3.8	40.6	3.1	52.5
Compana	47.5	5.6	3.3	40.9	2.7	53.1
Glen	49.2	0.7	3.6	43.2	3.3	49.9

[a] Low levels of 18:2 and 18:3 with corresponding elevated levels of other fatty acids indicate losses of polyunsaturates by autoxidation.

in the present study (i.e., omitting starches with high FFA values) and recalculating SF_{70} as $SF(AP)_{70}$, seven waxy, five normal, and two high-AM starches gave the equation $SF(AP)_{70} = 24.8 - 0.0148$ LPL ($r = -0.903$, $n = 14$). Adding data from our later study (Tester et al 1991), the equation $SF(AP)_{70} = 21.8 - 0.0113$ LPL was obtained ($r = -0.902$, $n = 30$). If the constants are scaled up (divided by 0.7) to estimate $SF(AP)_{80}$, the equations are remarkably close to those in Table III (lines 8–10).

Although the simple linear regressions described above have very significant correlation coefficients ($P < 0.001$), they are not entirely satisfactory because the data are not strictly linear. Consequently, the equations predict low values for the swelling of very low-AM and high-AM starches. This problem will be addressed in a later article (Morrison et al, in press).

CONCLUSIONS

AM contents of barley starches calculated using the revised calibration for the colorimetric method were about 1–2% higher than those calculated by the original method. The AM-LPL regression for the waxy starches passed close to the origin, whereas previously it gave a value of 120–160 mg of LPL/100 g of starch at zero AM content, which was unsatisfactory for a simple direct relationship. Since the regression for the waxy starches did not describe the nonwaxy starches, it implies that there is a fundamental difference. This is explained in a later article (Morrison et al, in press). The waxy barley starches had much more AM and lipids than waxy rice (which has none) or waxy maize (which has very low levels) starch. This suggests that expression of the waxy gene, which controls AM synthesis, is less affected in barley than in rice and maize; expression also changes as barley grain development and starch accumulation proceed (McDonald et al 1991).

The combined data from the present and previous studies (Morrison et al 1986, Tester et al 1991) show that SF(AP) at 70 and 80°C are negatively correlated with the lipid content of the barley starches. Hence, swelling behavior depends on AP (or AM) content, which is primarily a heritable character, and on lipid content, which is also heritable but much more susceptible to edaphic variation. This, and several other properties of the starches, will be examined in greater depth in a future article (Morrison et al, in press).

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