Changes in Barley Kernels During Growth and Maturation[1]

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ABSTRACT

Samples of Conquest barley were collected at regular intervals, from flowering to maturity, during the 1969 crop-growing season. These were analyzed for moisture, kernel weight, protein, ash, fat, crude fiber, sugars, starch, and alpha- and beta-amylase activities. The major components, protein and starch, reached their maximum values 32 to 36 days after ear emergence, but the sugars rose to a maximum 16 days after ear emergence and then declined. Alpha-amylase activity increased and then declined rapidly between 5 and 11 days after ear emergence. "Free" beta-amylase activity increased from 9 days after ear emergence to a maximum level after 35 days and then declined, whereas "bound" beta-amylase was first detected 16 days after ear emergence and its activity rose to a maximum at maturity.

The main starch-degrading enzymes of malted barley, alpha- and beta-amylases, have received much attention for many years, as they are important in industrial processing and, more recently, as factors to be considered in plant breeding programs for the development of improved malting varieties.

It has long been considered that sound barley contains only small amounts of alpha-amylase (1), and recent studies (2,3) suggest that malt alpha-amylase develops in the aleurone layer of barley during malting. However, there is evidence that an alpha-amylase is present in barley kernels during early stages of growth (4,5), but there is very little information available on the properties of this enzyme, and its relation to malt alpha-amylase is not known.

It is generally accepted that there are two forms of beta-amylase present in mature barley kernels: the "free" form which is readily extracted by water or salt

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solutions, and the "bound" form which is extracted only by the proteolytic enzyme papain or by reducing agents such as thiol groups. During malting, saccharifying activity increases, and it is thought that the "bound" beta-amylase component is converted into the "free" component, but the mechanism of this conversion is not known. Further characterization of the beta-amylase system in barley is a necessary prerequisite for the elucidation of this complex process.

To correlate changes of amylase activity with changes in other barley constituents during kernel growth, a detailed study of the development of barley kernels from ear emergence to maturity was undertaken in 1969. In addition to alpha- and beta-amylases, the starch, sugar, protein, fat, fiber, and ash contents of developing barley grain were studied. Preliminary results of this investigation are presented in this paper. They provide a detailed picture of the changes which occur in the developing grain of a typical Canadian barley during the relatively short growing season of Western Canada.

MATERIALS

Barley Samples

The Conquest barley used in this study was grown by the Plant Science Department of the University of Manitoba. The barley was sown on April 24, 1969. Eighteen samples were collected at regular intervals between June 30 (date of ear emergence) and August 14 (barley had reached maturity). The temperature, sunshine, and precipitation record for Winnipeg during this period is shown in Table I, along with normal values. Apart from lower temperatures during June, the weather conditions were close to normal for the rest of the growing season, and were excellent for growing high-quality barley.

The barley samples were harvested in the morning and brought directly into the laboratory. About 20 kg. of fresh material was collected randomly throughout the plot at each harvest. In the laboratory the heads were removed from the stems (when necessary), and the kernels from ten heads were bulked for moisture and alpha-amylase determinations. The heads were then deep-frozen and, except for a few which were stored in the frozen state, freeze-dried to a moisture content of about 4%.

TABLE I. TEMPERATURE, PRECIPITATION, AND SUNSHINE RECORD FOR THE 1969 CROP GROWING SEASON[a]

	Temperature (°C.)			Precipitation	Sunshine
	High	Low	Average	in.	hr.
April	54.3	30.8	42.6	1.0	273
	(47.5)	(28.5)	(38.0)	(1.2)	(206)
May	63.0	38.7	50.9	3.0	288
	(64.6)	(40.6)	(52.6)	(2.0)	(244)
June	65.7	43.7	54.7	4.1	264
	(72.6)	(50.8)	(61.7)	(3.2)	(248)
July	75.5	55.0	65.3	4.0	298
	(79.7)	(56.8)	(68.3)	(3.7)	(310)
August	81.2	57.9	69.6	2.3	304
	(77.5)	(54.5)	(66.0)	(2.8)	(269)

[a]Normal values are given in parentheses.

The dried heads were threshed by placing them in small cotton sacks and rubbing them together by hand until all the kernels had been liberated. The awns and other extraneous material were removed from the early samples (0 to 11 days) by hand sieving and from the other samples by a commercial grain cleaner. Samples of dried, cleaned kernels from each harvest were ground in a Wiley mill through a 1-mm. sieve to give a set of uniform grists.

Date of Anthesis

Twenty heads from the 4-, 7-, 9-, and 11-day samples of barley which had been stored at −15°C. were analyzed for anthesis by examining the anthers in the middle spikelet on the middle floret on one side of the ears. The 4-day sample showed 70% anthesis, the 7-day 90%, and the 9- and 11-day samples 100%. Therefore, the 9-day sample was the earliest to completely anthese, and the date of anthesis was taken to be July 9.

Enzymes

Glucoamylase from *Rhizopus* genus mold (130,000 units of activity per g.) was obtained from Sigma Chemical Co., St. Louis, Mo. Analytical grade glucose oxidase from *Aspergillus niger* (130,000 units of activity per g.) and horseradish peroxidase (250 units of activity per g.) were obtained from Mann Research Laboratories, Orangeburg, N.Y.

METHODS

Many of the procedures used in this study are recommended methods of the American Association of Cereal Chemists (6), and only brief accounts of these are given in this paper. Other procedures, such as the sugar and starch determinations and the extraction and determination of the amylases, were developed during the study, and these are presented in detail.

Moisture

Three 1-g. samples of freshly harvested barley kernels were heated in a vacuum oven for 18 hr. at 100°C., and their loss in weight was measured.

Kernel Weight

The number of kernels in 5-g. samples of the freeze-dried material was determined. The moisture content of this material was determined, and the kernel counts were corrected to give number of kernels per unit weight of dry matter.

Protein, Ash, and Fat

These were determined according to standard AACC methods (6).

Crude Fiber

This was determined by a modified AACC method (7).

Sugars

Duplicate 125-mg. samples of barley grist from each harvest were extracted three times with 25-ml. portions of 50% ethanol at 70°C. for 15 min. (8). The suspensions were centrifuged (12,500 × g) between each extraction, and the

supernatant solutions were combined. A small amount of barium carbonate solution was added to each extract before it was evaporated to small volume on a flash evaporator (40°C.). The solutions were then diluted to 25 ml. with water. Ten-milliliter portions of these solutions were acidified (1.1 ml. of 2.5M sulfuric acid), heated on a boiling-water bath for 10 min., cooled to room temperature, neutralized with sodium hydroxide, and diluted to 25 ml. with water. These hydrolyzed extracts were assayed for reducing sugars by the automated ferricyanide-cyanide method as modified by LaBerge and Meredith (9).

Extraction of Starch

Ground samples of freeze-dried barley (100 g.) were made into a thick paste with water. The paste was rolled into a ball and placed in a very fine-meshed piece of silk. Starch was extracted by gently kneading the silk-encased samples under a slow stream of water.

The extracted material was allowed to settle for one hr., and the supernatant solution was decanted. The impure starch granules were suspended in water and centrifuged at 800 X g for 10 min. The supernatant solution was decanted, and the top tailings on the pellet were removed with a spatula and discarded. The remainder of the pellet was resuspended in water and centrifuged three additional times with removal of tailings each time to give a sample of prime starch.

Determination of Amylose

The amylose content of the prime starches was determined on 30-mg. oven-dried samples by an amperometric technique (10,11). The amylopectin content was determined by difference.

Starch Granule Size Distribution

Endosperm tissue was dissected from five barley kernels from each harvested sample. This material had been stored in a freezer. The tissue was ground in a mortar and a small sample was placed on a microscope slide with a drop of 0.2% iodine in 2% potassium iodide. The diameter of at least 300 granules for each harvested sample was determined by microscopy using a calibrated ocular eyepiece and a haemocytometer chamber to grade the granules into various particle size ranges (12).

Starch Determination—Preliminary Experiments

Eight 125-mg. oven-dried samples of the 45-day Conquest barley grist were weighed into centrifuge tubes and extracted three times each for 15 min. with 25-ml. portions of 80% ethanol at 70°C. (13). The suspensions were centrifuged (15,000 X g; 15 min.), and the supernatant solutions discarded. Twenty-five milliliters of distilled water was added, and the residues were stirred thoroughly and placed in a boiling-water bath. Two samples were removed at hourly intervals and cooled to room temperature. One milliliter of glucoamylase solution (20 mg. glucoamylase in 10 ml. of 0.2M acetate buffer, pH 4.6) was added to each with thorough mixing, and the stoppered digests were incubated at 45°C.

After 24 hr., one digest from each set of duplicates was heated at 100°C. for 5 min. to destroy glucoamylase activity and then cooled to room temperature. The

remaining tubes were treated in a similar manner after 40 hr. All samples were diluted to 100 ml. with water and filtered.

Two 4-ml. aliquots from each solution were diluted to 50 ml. and assayed with glucose oxidase (14) and ferricyanide reagents (9). To a further 1-ml. portion of each solution was added 10 ml. of water, 2 ml. of 0.3N barium hydroxide, and 2 ml. of 5% zinc sulfate. The latter two solutions must neutralize one another (15). The suspensions were shaken thoroughly and filtered to yield clear solutions that were assayed with glucose oxidase and ferricyanide reagents.

Determination of Starch in Plant Tissues

The standard method for determining starch in plant tissues requires that the starch be completely extracted from the tissues by perchloric acid, purified by iodine precipitation, liberated from the iodine complex with alkali, and then determined directly or in solution by any one of a number of methods (13). This technique, because of the extreme care required for its successful operation, does not readily lend itself to the analysis of large numbers of samples.

An extremely simple procedure for determining alpha-linked glucose polymers in plant tissues, developed by Macrae and Armstrong (16), did not require prior extraction of the starch. The plant tissues were heated in boiling water to gelatinize the starch, which was then hydrolyzed to glucose with the enzyme glucoamylase. The glucose was determined with the glucose oxidase technique. Since these workers were interested in determining the total glucose content of the tissues and not just that portion produced from the starch, they did not remove small sugars from the plant tissue prior to analysis. This method was modified in the present study so that the starch content of plant tissues could be specifically and readily determined.

The soluble sugars in the barley grist were quantitatively extracted with three portions of hot 80% ethanol. Further extraction did not yield additional soluble material. Some of these extracts were evaporated to a small volume and tested with iodine, but no iodine-staining material was detected. This alcohol treatment also inactivated the barley amylases.

Because glucoamylase has little or no action on raw starch granules, it is important to ensure that the starch is completely gelatinized before it is incubated with the enzyme. The results in Table II indicate that the sample to be analyzed must be maintained at a temperature of 100°C. for 3 to 4 hr. to completely gelatinize the starch. These results confirm the findings of Macrae and Armstrong (16).

Although the glucoamylase hydrolyzed most of the starch within 24 hr. at an incubation temperature of 45°C., a small increase in free glucose was detected after a further period of 16 hr. In practice, therefore, an incubation time of 40 hr. was used.

Glucose is essentially the only product of starch hydrolysis by glucoamylase. Two methods for its quantitative determination were compared: the glucose oxidase procedure (14) which is specific for glucose and the automated alkaline ferricyanide method for determining reducing sugars (9). The results shown in Table II indicate that the methods give similar results, but since the automated procedure offers advantages in both time and precision, it is the method of choice.

TABLE II. EFFECT OF GELATINIZATION PERIOD, TIME OF INCUBATION WITH ALPHA-AMYLOGLUCOSIDASE, AND METHOD OF ANALYSIS ON THE DETERMINATION OF STARCH IN A SAMPLE OF BARLEY

Gelatinization Period hr.	Incubation with Alpha-Amyloglucosidase hr.	Percentage Starch[a]			
		Glucose Oxidase Method		Alkaline Ferricyanide Method	
		Untreated	Protein removed	Untreated	Protein removed
1	24	52.0	51.2	53.1	53.1
1	40	53.0	52.7	54.1	54.1
2	24	50.8	50.1	52.3	52.2
2	40	51.7	51.0	52.3	52.9
3	24	50.2	50.1	51.9	52.0
3	40	50.8	50.5	52.4	52.1
4	24	54.0	53.8	55.0	55.7
4	40	54.2	54.0	54.6	55.0

[a]A value of 54.2% starch was found for this sample of barley by the method of Hassid and Neufled (13).

Because the automated alkaline ferricyanide procedure is nonspecific (i.e., any reducing agent will interfere with the determination), protein in the starch digests could give erroneous results. The effect of removing protein from the digests, by the method of Somogyi as described by Nelson (15), prior to the determination of glucose was studied. The results are shown in Table II. This treatment had no appreciable effect on the starch determinations, indicating that the samples were free from protein. Therefore, it was eliminated from the adopted method.

The results obtained with this procedure (54.6% starch in the barley sample) were in good agreement with those obtained with the perchloric acid extraction method of Hassid and Neufeld (13) (54.2% starch).

The method finally used was as follows: The ethanol-extracted barley grists were heated with water for 4 hr. at 100°C., digested with glucoamylase for 40 hr. at 45°C., diluted to 100 ml. with water, filtered, and, after appropriate dilution, assayed on the AutoAnalyzer.

Alpha-Amylase Activity

Extraction of Fresh Material. Duplicate 20-kernel samples from each harvest were homogenized in a mortar with 5-ml. portions of 0.2M acetate buffer, pH 5.5, containing 0.001M calcium chloride. The extracts were centrifuged (15,000 X g; 10 min.) to yield clear solutions that were analyzed in triplicate for alpha-amylase activity.

Extraction of Freeze-Dried Material. Two 200-mg. portions of each ground sample were shaken gently with 50-ml. portions of acetate buffer (0.2M; pH 5.5; 0.001M calcium chloride) for 18 hr. at 5°C. The extracts were filtered to yield clear solutions that were analyzed in triplicate. This whole procedure was repeated three times so that the results shown in Fig. 6 are average values obtained from six separate determinations.

Determination of Activity. The method proposed by Briggs (17) was used with the following modifications. The amylopectin beta-limit dextrin was replaced by waxy maize starch beta-limit dextrin. This substrate was prepared directly from the

parent starch without prior fractionation. Two milliliters of substrate solution (0.5 mg. of substrate per ml. of 0.2M acetate buffer, pH 5.5, containing 0.001M calcium chloride) and 2 ml. of suitably diluted enzyme extract were used for each determination. The enzyme-substrate digests were incubated at 35°C., and the reaction was terminated by the addition of 10 ml. of iodine solution (50 mg. of potassium iodide and 5 mg. of iodine per 100 ml. of 0.05M hydrochloric acid). These solutions and appropriate standards and blanks were allowed to stand at room temperature for at least 30 min. before their absorbances were measured at 540 nm. on a Beckman DU spectrophotometer. The results were expressed in I.D.C. units. One I.D.C. unit is the amount of enzyme required to lower the absorbance of a standard digest from 0.6 to 0.4 in 100 min.

Beta-Amylase Activity

Extraction. Free beta-amylase was extracted from 500 mg. of barley grist with 50 ml. of 0.1M citrate buffer, pH 6.0, containing 0.001M thioglycerol, by shaking gently overnight at 5°C. Total beta-amylase was extracted as described above, but the citrate buffer contained both 1% papain and 0.001M thioglycerol. These extracts were filtered to give clear solutions.

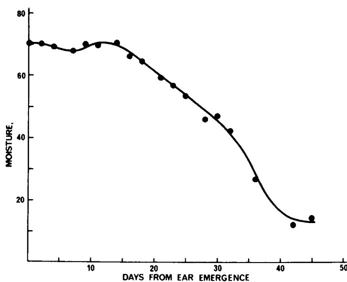
Determination of Activity. Aliquots (0.5 ml.) of the filtered solutions were incubated for 5 min. at 30°C. with 1-ml. portions of 1% starch in 1M acetate buffer, pH 4.5. The reaction was stopped by addition of dinitrosalicylic acid reagent (18). The digests were heated for 5 min. in a boiling-water bath, cooled rapidly to room temperature, and diluted with 20 ml. of water; the absorbances were determined on a spectrophotometer at 525 nm.

The value for bound beta-amylase was calculated from the difference between the activity of the papain extract and that obtained without papain in the buffer. A unit of beta-amylase is defined as the number of mg. of maltose produced by the enzyme during 5 min. of incubation with 1% starch at 35°C. and pH 4.5.

The results shown in Fig. 7 are average values obtained from a number of separate determinations.

RESULTS AND DISCUSSION

To obtain sufficient material for detailed analysis of the various barley components and especially for the isolation and characterization of the amylases,



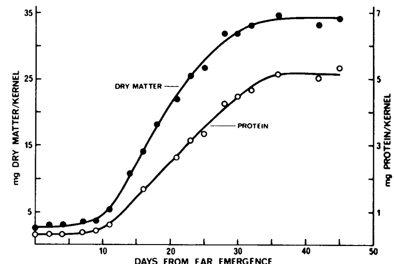


Fig. 1 (left). Moisture content of barley samples.

Fig. 2 (right). Development of dry matter and protein in barley kernels.

large samples of barley were collected at each sampling date. It was impossible, therefore, to determine the exact stage of growth of each sample, but an attempt was made to obtain an approximate date of anthesis for the crop as a whole by examining several heads from the 4-, 7-, 9-, and 11-day samples. The kernels in the 9-day sample had anthesed completely, so this date was taken as the approximate date of completion of anthesis, and thus the beginning of true kernel formation.

The results obtained in this study are expressed on a per kernel basis because this is a more informative way of presenting such data. The results can be converted to a dry weight basis by using the dry matter graph in Fig. 2.

Moisture

The moisture content of the barley kernels during growth and maturation is shown in Fig. 1. During the first 14 days after ear emergence, the moisture level remained between 67 and 70%, but thereafter it fell steadily to a level of 42% after 32 days and then very rapidly to 13% after 45 days. It is surprising that the moisture content of the immature kernels remained almost constant during the initial stages of growth, but this may have resulted from the cloudy, wet weather experienced during this period.

Kernel Weight

The dry matter curve in Fig. 2 outlines the growth pattern of the individual kernels. Over 90% of the kernel dry weight was synthesized during the 25-day period between the 9th and 36th day after ear emergence. This is in contrast to the much longer periods of synthesis (5 to 7 weeks) required by barley varieties grown in Europe (16,18).

Synthesis of kernel dry matter ceased after 36 days when the kernels had reached a moisture level of 27%. At this stage the kernels had reached their maximum size, and further desiccation to 13% moisture led to a reduction in kernel size which was readily detected by visual examination. Mature kernels were smaller and more wrinkled than those at the 36-day stage of growth.

Protein

No attempt was made in this study to separate the nitrogenous constituents into different groups before determining their concentration, although it was realized that the early samples in particular would contain high levels of nonprotein nitrogen (19). The total nitrogen figures determined by the Kjeldahl method were multiplied by 6.25 to give the protein values shown in Fig. 2. Therefore, it is not possible to determine detailed changes in the nitrogen metabolism of the maturing kernels from these figures. However, it is apparent that the total nitrogen content of the barley kernels increased at the same rate as the dry matter content and reached a maximum at 36 days after ear emergence. Although the protein content remained constant at a value of 5.2 mg. per kernel until maturity, there is evidence that nitrogen metabolism does continue during this period (19).

Fiber

The fiber content of the samples (Fig. 3) increased regularly from 0 to 30 days after ear emergence, and thereafter remained essentially constant at 1.7 mg. per

kernel. This was the only kernel constituent examined that increased during the initial stages of growth (0 to 5 days), but the increase was so small (0.3 mg. per kernel) that it was not detected in the determinations of dry matter per kernel (Fig. 2). However, these results do indicate that the outer layers of the grain are among the first portions to be synthesized.

Ash

The increase in the ash content of the barley samples (Fig. 3) followed the pattern of dry matter deposition, reaching a maximum at 32 days and then remaining constant at 1.1 mg. per kernel until maturity.

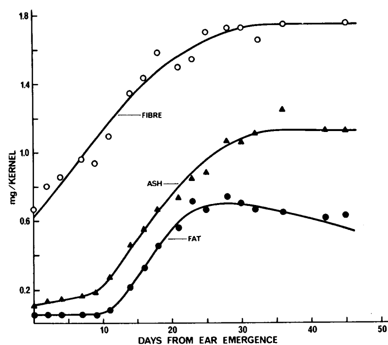
Fat

The fat (Fig. 3) remained at a low level for 9 days, increased to a maximum of 0.7 mg. per kernel after 28 days, and then declined to a value of 0.6 mg. per kernel after 45 days. This decline in the fat content during the final stages of maturation suggests that it is involved in the metabolic processes of ripening.

Sugars

This group comprises those sugars that are soluble in 50% ethanol and includes not only the simple sugars, glucose, fructose, sucrose, maltose, and raffinose, but also the low-molecular-weight fructosans (8). High-molecular-weight polysaccharides such as the gums are not included.

Detailed analysis of the individual sugars in this group will be the subject of a future publication, but preliminary results indicate that the predominant sugars are sucrose and fructosans. This finding is in agreement with the results of other workers (19,20). Since these carbohydrates are readily hydrolyzed to their constituent monosaccharides by dilute acid, the ethanolic extracts of the barley kernels, after removal of the ethanol, were subjected to mild acid hydrolysis (experimental section). Under these mild conditions no appreciable destruction of the simple sugars already present occurred, but raffinose was hydrolyzed to fructose and melibiose. Because fructose predominated in the resulting



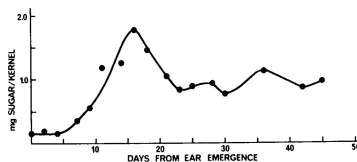


Fig. 3 (left). Development of fiber, ash, and fat in barley kernels.

Fig. 4 (right). Development of sugars in barley kernels.

hydrolysates, the results (Fig. 4) were expressed in terms of fructose rather than the more usual glucose.

The rapid increase in the sugar content of the barley kernels between the 7th and 16th days after ear emergence was due mainly to the synthesis of fructosan.

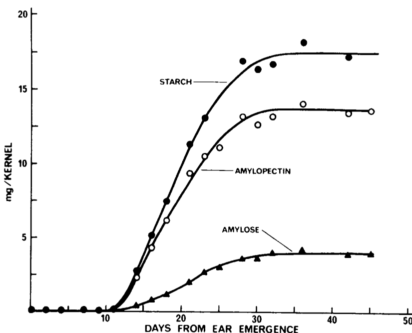
If the sugar composition of the samples is expressed on a percentage basis, then the sugar content appears to reach a maximum at 11 days and declines thereafter. This decline coincides with the initiation of starch synthesis (Fig. 5), suggesting that this polysaccharide was produced at the expense of the simple sugars. Reference to Fig. 4, however, indicates that within the individual kernels, sugar accumulation continued rapidly until the 16th day before starting to decline. These findings emphasize the importance (not always appreciated by earlier workers) of reporting analytical observations on maturing grain not only as a percentage of the dry matter but also on a per kernel basis.

Starch

Since starch is a major component of barley, it is not surprising that its deposition in the barley kernel mirrors the increase in dry matter of the grain (Fig. 5). Starch synthesis started about 11 days after ear emergence, continued rapidly for 14 days, and then more slowly until day 32 when it stopped.

Ninety-five percent of the starch was synthesized in the remarkably short period of 17 days between the 11th and 28th days after ear emergence. Reports from Britain (21,22), where the growing season is much longer than in Western Canada, indicate that a period of 35 to 40 days is required for a similar accumulation of starch in barley kernels.

The curves showing the synthesis of the individual starch components, amylose and amylopectin, are similar to those of other workers (19,21). It is apparent that the ratio of the two polysaccharides is not constant throughout the growing season. The amylose content of the starches increased from a value of 13.8% after 14 days to 22.5% after 30 days and remained constant at this value to maturity. These results indicate that during the early stages of growth, the amylopectin fraction was synthesized at a relatively faster rate than the amylose. This appears to be a general



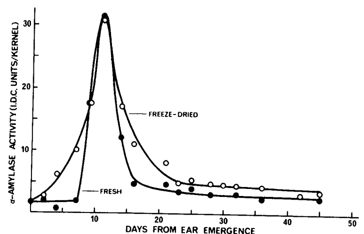


Fig. 5 (left). Development of starch in barley kernels.

Fig. 6 (right). Development of alpha-amylase in barley kernels.

TABLE III. PARTICLE SIZE DISTRIBUTION OF STARCH GRANULES (%)

Days from Ear Emergence	Mean Particle Diameter	0– 2.7 μ	2.8– 5.3 μ	5.4– 8.0 μ	8.1– 10.7 μ	10.8– 13.5 μ	13.6– 16.0 μ	16.1– 18.7 μ	18.8– 21.4 μ	21.5– 24.0 μ	24.1– 26.7 μ	26.8– 29.4 μ
11	3.7	46.5	40.1	12.7	0.3	0	0.3	0	0	0	0	0
14	8.0	4.9	20.0	29.6	22.5	14.9	6.1	2.0	0	0	0	0
16	10.5	4.2	12.1	14.7	16.6	24.6	18.8	6.7	1.9	0.3	0	0
18	10.2	13.2	9.9	9.1	19.3	20.6	12.3	11.1	2.5	1.2	0.8	0
21	5.2	67.1	7.5	3.4	2.2	3.4	7.5	4.1	2.8	0.6	1.9	0.3
23	4.4	75.9	7.6	1.3	1.0	2.9	4.1	4.8	1.3	1.6	1.6	0
25	3.3	64.4	28.2	0.9	0.6	0.6	0.9	0.3	2.5	1.2	0.3	0
28	4.6	57.0	27.2	0.5	2.5	2.5	6.0	2.5	2.7	0.8	0.3	0
30	4.7	59.9	21.3	2.5	1.3	2.5	3.1	3.1	2.2	2.5	1.3	0.3
36	4.6	65.9	17.7	1.9	0.8	1.1	2.4	2.9	2.6	3.2	0.8	1.1
42	3.6	64.9	24.7	1.0	0.7	1.0	1.0	2.4	2.4	0.7	1.0	0
45	3.6	73.7	15.0	0.3	0.3	1.0	1.6	3.5	1.0	2.2	1.0	0

phenomenon associated with starch biosynthesis because similar patterns of development have been found in other cereals as well as in maturing potato tubers (23).

Many multi-component pathways have been proposed for the mechanism of starch biosynthesis (24,25,26,27). In most of these, attempts were made to explain this complex process in terms of the enzyme systems present in mature, starch-containing tissues. It is possible that such tissues no longer contain all the enzymes required for starch biosynthesis in a fully active form, with the result that material in which starch is still being actively synthesized would be a much better source of the requisite enzyme systems. Therefore, the barley samples harvested between the 14th and 20th days after ear emergence should be ideal material for a study on the biosynthesis of starch in barley.

Recent developments in starch biosynthesis suggest that sugar nucleotides and ADP-glucose in particular are important intermediates during the synthesis of starch from glucose (28). It is interesting that Moore and Turner (29), working with wheat kernels, were able to detect only small amounts of ADP-glucose pyrophosphorylase—one of the important enzymes in such a system—in mature samples, but that large amounts of the enzyme were found in immature kernels, which were still synthesizing starch.

Table III provides information on the size distribution of the starch granules at various stages of grain development. The mean granule size increased sharply from 3.7 μ at 11 days to 10.5 μ at 16 days and then declined just as sharply from 10.2 μ at 18 days to 3.3 μ after 25 days. From this stage until maturity there was a slight increase in the average granule size.

There was a dramatic change in the small granule (2.7 μ) content of the starch between the 16-day (4.2% small granules) and 21-day samples (67.1% small granules). Succeeding samples also contained a high percentage of small granules. The influx of small granules at this stage explains why the mean granule size fell rapidly after the 18th day. These results are similar to those reported by other workers who also found that the percentage of small granules increased as the barley kernels matured (12,21).

Because the over-all rate of starch synthesis did not change appreciably during this period (Fig. 5), the rapid accumulation of small granules suggests that a change took place in the mechanism of starch deposition after the 18th day from ear emergence. During the early stages of growth (days 11 to 18), the small granules initially produced increased in size by apposition (30). In the later stages granule growth was suppressed, and to maintain a high rate of starch synthesis, the rate of granule formation was increased.

Alpha-Amylase

Development and subsequent decline of alpha-amylase within barley kernels during growth and maturation are shown in Fig. 6. The samples were analyzed for alpha-amylase within 6 hr. of being harvested and again 3 months later after they had been deep-frozen, freeze-dried, and stored at 5°C. There is good agreement between the two sets of data indicating that very little activity, if any, had been lost during the drying process.

The alpha-amylase activity increased very rapidly from ear emergence to 11 days and then declined sharply to one-tenth of its maximum level after 28 days. Thereafter, it remained almost constant until maturity. These results are similar to those reported by Duffus (5), who analyzed a two-rowed barley variety and showed that the alpha-amylase activity was maximal 30 days after anthesis and declined to one-fifth of this value 15 days later. As early as 1936, Chrzaszcz and Janicki (4) noted that immature barley kernels contained some alpha-amylase.

There are isolated reports of similar findings in wheat. In 1946, Sandstedt and Beckord (31) showed that the alpha-amylase content of wheat increased sharply from flowering until 6 days afterwards and then decreased to 60% of the maximum value after 12 days. Unfortunately, no further determinations were recorded. More recently, Olered (32) reported high values for alpha-amylase in three wheat varieties 86 days after seeding. These activities had decreased to very low levels by harvest time.

The results from this study confirm earlier reports of the presence of alpha-amylase in mature barley (33). They show unequivocally that the small amounts of alpha-amylase found in sound barley are produced during maturation and are not necessarily the result of limited germination caused by unfavorable harvest conditions.

Because Grabar and Daussant (34) were unable to detect protein material in barley that reacted immunologically with antibodies corresponding to malt alpha-amylase, they concluded that barley does not contain alpha-amylase. However, the barley and malt alpha-amylases may have different immunological properties. Also, they did not test immature barley kernels, and it is possible that their technique is not sufficiently sensitive to detect the small amounts of amylase present in mature kernels.

Beta-Amylase

Although several workers (35,36,37) have investigated the properties of barley beta-amylase, there is very little information available on the development of the "free" and "bound" forms of the enzyme in maturing barley kernels. Chrzaszcz and Janicki (4) analyzed barley, wheat, and oat samples collected over a very short

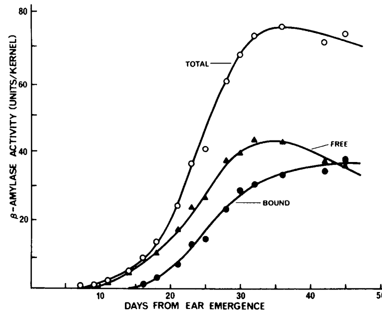


Fig. 7. Development of total, free, and bound beta-amylase in barley kernels.

period of time and concluded that the free beta-amylase content of these cereal grains increased initially and then decreased as the kernels reached maturity. Schwimmer (38) analyzed the water, salt, and papain-soluble beta-amylase components in developing winter and spring wheats and showed that during early stages of growth all the beta-amylase present was soluble in water. As the kernels matured, all three components increased in amount, but the water-soluble beta-amylase represented a diminishing proportion of the total enzyme. At maturity only half of the "total" enzyme was extracted with water.

Sandstedt and Beckord (31) analyzed wheat kernels from flowering to 12 days afterwards and showed that the water-soluble beta-amylase content of the grain increased during this period. However, they did not determine total beta-amylase.

The results of the present study are shown in Fig. 7.

During the initial period of plant growth (7 to 16 days), the small amount of beta-amylase synthesized was in the "free" form. This was followed by a period of rapid beta-amylase synthesis in which both the "free" and "bound" forms accumulated at almost equal rates. The increase in beta-amylase paralleled the dry matter increase, and differed from the general pattern for cereal grains suggested by other workers in that most of the beta-amylase was formed prior to the rapid increase in dry weight of the kernels (39). This discrepancy could be caused by a number of factors such as cereal variety, environment, and weather conditions.

The total beta-amylase reached a maximum level after 35 days and then decreased slowly. The "free" component behaved similarly, but it decreased more rapidly during the later stages of maturation while the bound beta-amylase continued to increase slowly during this period. At maturity, equal amounts of free and bound beta-amylase were present in the barley kernels.

CONCLUSIONS

The appearance and fertilization of barley flowers take place over a period of days. In this study it was found that fertilization was completed and kernel development had begun, on the average, on the ninth day after ear emergence. The results shown in Figs. 1-7 show that the peak of synthetic activity occurred shortly after fertilization; structural components were laid down early, and the reserve materials were synthesized over a longer period. The enzyme systems developed at

different rates and at different times, but the relation between the amylase enzymes and starch in the immature kernels is not clear. Additional studies are under way to clarify some of the problems associated with the nature of starch biosynthesis, the presence of alpha-amylase in immature barley, and the biosynthesis of barley beta-amylase.

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