

Cereal Chemistry

Vol. 49

July-August 1972

No. 4

Changes in the Amylases of Hard Red Spring Wheat during Growth and Maturation¹

J. E. KRUGER, Canadian Grain Commission, Grain Research Laboratory, Winnipeg 2, Manitoba

ABSTRACT

The α - and β -amylases of hard red spring wheat kernels were studied during growth and maturation of the wheat, by polyacrylamide-slab electrophoresis at pH 9.0. Three α -amylase isozymes are formed during early growth and persist until near full maturation, at which time their quantity decreases greatly. Two main β -amylase components are formed in early growth and increase in quantity during growth. In addition, one minor β -amylase is formed during early growth which later disappears, and four minor β -amylase components are formed during final maturation. The electrophoretic mobilities of the major β -amylase components for the varieties analyzed were not identical, but fell into either of two patterns. The α -amylase was found largely in the pericarp, whereas the β -amylase was found mainly in the endosperm, except for the early-formed minor β -amylase component which was present only in the pericarp. Free and latent β -amylases had identical electrophoretic mobilities.

The number of different forms of α - and β -amylases found in wheat, and changes that may occur in these forms during growth, maturation, and germination, are still uncertain. For example, Tipples and Tkachuk (1) found that saline extracts of wheat flour contained three major and two minor β -amylase components, as shown by DEAE ion-exchange chromatography. There was, however, a lack of reproducibility between runs. The writer has found two main β -amylase components present in wheat flour, as shown by DEAE- and CM-cellulose ion-exchange chromatography (2). The components found in malted wheat were quite different. Nummi et al. (3) have also found two main β -amylase components in extracts of wheat by exclusion chromatography on Sephadex, although upon storage there was a transformation of one component into the other. Alexandrescu and Mihailescu (4) found, on using agar-gel electrophoresis, that only one main β -amylase component was present in wheat extracts, which did not alter in electrophoretic behavior during germination.

¹Paper No. 312 of the Canadian Grain Commission, Grain Research Laboratory, Winnipeg 2, Manitoba, Canada.

A similar confusion also exists regarding the number of α -amylase isozymes and what changes can occur in these forms throughout growth, maturation, and germination. In this laboratory (5), wheat α -amylase has been isolated from malted wheat, and four components were found by DEAE-cellulose ion-exchange chromatography. Daussant and Corvazier (6) find that two α -amylase isozymes are formed during germination, and that both are formed *de novo* and not due to release of pre-existing α -amylases. Olered and Jonsson (7,8) have also detected the formation of two α -amylase forms during germination, by agar-gel electrophoresis, with one form being electrophoretically similar to and presumably identical with a form present in immature kernels of wheat. This "green" α -amylase diminished with decreasing moisture at near maturity, but could be regenerated by increasing the moisture level of the kernel. Alexandrescu and Mihailescu (4), also using agar-gel electrophoresis, found two to three electrophoretically different α -amylase components present in both ungerminated and germinated wheat. Germination altered the electrophoretic mobility of one of the components and changed the intensity of the bands. Studies on other cereal grains indicate that many multiple forms of both α - and β -amylases are present. In germinated barley, Frydenberg and Nielsen (9) found five α -amylases, two β -amylases, and two unknown forms. Similarly, Tanaka and Akazawa (10) treated embryoless barley half seeds with gibberellic acid and detected two major α -amylase fractions, each containing two to three isozymes. These workers have also found four major and nine to ten minor α -amylase-isozyme bands in germinating rice seeds (11).

The importance of wheat amylases, and in particular α -amylase, in affecting breadmaking quality, is well known; and it is essential, therefore, that the number of components present be identified, and that the alteration of these components with changes in the seed be well established. This paper describes a sensitive method that has been developed using polyacrylamide-gel electrophoresis to separate and detect α - and β -amylases, and illustrates the changes that occur in hard red spring wheat during growth and maturation. A later paper will deal with the changes in amylase isozymes that occur during germination of hard red spring wheat.

MATERIALS

The hard red spring wheat varieties Marquis; Manitou; Thatcher; a variety not licensed but referred to commonly as "Prairie Pride"; and a soft wheat, Pitic 62, were grown in the Canada Department of Agriculture experimental plots at Glenlea, Manitoba, during the summer of 1970, and sampled at various growing stages. The intact heads of wheat were excised and stored in a deep freeze until analysis.

EXPERIMENTAL

Extraction of Amylases

Kernels of wheat with low moisture content were finely ground, by either a Micro-Wiley mill equipped with a 40-mm.-mesh sieve, or an electric coffee mill. Kernels with very high moisture levels, such as very immature growth samples, were ground directly in small centrifuge tubes using a glass stirring rod and with the extracting solution present. Fifty kernels were extracted with 1 ml. of cold 0.01M calcium chloride solution, overnight at 4°C. In some cases, extraction was for 3 hr. at room temperature. The extract was then centrifuged at 54,000 \times g at 4°C. for 20 min. and the filtrate saved.

Polyacrylamide-Slab Electrophoresis

The technique for electrophoresis was similar to that described in the Ortec model 4200 electrophoresis-system manual (12), and employed a pulsed constant power supply and a continuous pH in the gel system. An Ortec model 4200 slab-electrophoresis system was used with an Ortec model 4100 pulsed constant power supply (Ortec Inc., Oak Ridge, Tenn.). An 8% polyacrylamide separating gel with 0.375M tris-sulfate, pH 9.0, was first prepared in the $9 \times 10 \times 0.3$ -cm. I.D. casting cell to a depth of 7.7 cm. Four milliliters of 8% polyacrylamide containing 0.075M tris-sulfate, pH 9.0, was then overlaid on the separating gel, followed by the insertion of a 12-sample Teflon well former. Following polymerization and removal of the well former, 12 sample wells 1.3 cm. in height were formed. To each well was added 0.05 ml. of a sample solution consisting of equal parts of the amylase extract and 80% sucrose dissolved in 0.075M tris-sulfate, pH 9.0. An 8% polyacrylamide sealing cup containing 0.075M tris-sulfate, pH 9.0, was finally polymerized onto the top of each sample layer. The cell was placed in the tank assembly and the upper (cathode) and lower (anode) chambers filled with 0.065M tris-borate buffer, pH 9.0, cooled to 4°C.; 0.2 ml. of 0.1% aqueous bromophenol blue was added as a tracking dye to the upper-tank buffer. Electrophoresis was carried out at 325 v., with a pulsed constant power, and with samples moving towards the anode. The pulse rate was started at 75 p.p.s., increased at 5 min. to 150 p.p.s., at 10 min. to 225 p.p.s., and finally kept at 300 p.p.s. after 15 min. The electrophoresis run was complete after 1 hr. The polyacrylamide slab was removed from the cell by first reaming the edges of the cell with a 21-gauge 3-in. cannula attached to a water-filled syringe and then gently forcing the gel out of one end with a plate inserted in the other end.

Detection of Amylases

Starch or β -limit dextrin in agar slabs was first prepared on 4-in.-square glass plates. Starch (0.5%) in 1% agar slabs was prepared by adding 0.5 g. of soluble starch (Lintnerized) in a small amount of water and 1.0 g. agar in a small amount of water to approximately 75 ml. of boiling water containing 20 ml. of 0.5M acetate buffer, pH 4.6, and bringing the final volume to 100 ml. The glass plates were placed in a group of eight on a levelled table, and 10 ml. of the above hot solution placed on each slab and allowed to cool. β -Limit dextrin (0.1%) in 1% agar slabs was prepared in the same way except that 0.1 g. β -limit dextrin was substituted for the starch. Waxy-maize starch (American Maize Products Co., Roby, Ind.) is composed virtually entirely of amylopectin, and was, therefore, chosen for the preparation of the β -limit dextrin. Twenty grams of waxy-maize starch was suspended in 1 liter of 90% dimethyl sulfoxide and stirred overnight. The solution was diluted to 5 liters with water containing 100 ml. of 0.2M acetate buffer, pH 4.6, and 0.2 ml. of sweet potato β -amylase suspension added. The solution was stirred for 24 hr., followed by addition of 0.2 ml. of sweet potato β -amylase and a further 24 hr. stirring. The resulting solution was exhaustively dialyzed against distilled water to remove maltose, and freeze-dried. Although the β -limit dextrin prepared in this way contained β -amylase, this enzyme was inactivated in the boiling required to prepare the agar slabs. The prepared slabs were used within 3 hr. of preparation.

Incubation was carried out by sandwiching the polyacrylamide gel between a starch slab and a β -limit-dextrin slab and placing the "sandwich" in a tray

containing enough water at 35°C. to cover it. On top of the "sandwich" was placed a 4-in.-square metal plate weighing 261.8 g., and the tray then placed in an incubation bath at 35°C. Incubation time was usually 45 min. for growth samples. Following incubation, the starch slab was placed in a solution of 0.04% iodine and 0.4% potassium iodide, and the β -limit-dextrin slab in a solution of 0.02% iodine and 0.2% potassium iodide. Following this, the gels were rinsed with distilled water and placed in 7% acetic acid if they were to be stored moist. Pink or colorless bands against a blue background on the starch slab indicated the location of the α - and β -amylases, whereas colorless bands against a pink background on the β -limit-dextrin gels indicated the location of the α -amylase components. By comparison of the two slabs, differentiation of the α - and β -amylases can be made on the starch slab. The resolution of the bands on both slabs was sharpest just after staining. The patterns of the bands on the starch gel do remain reasonably constant however, and on drying, provide a permanent record of the zymogram. With β -limit-dextrin slabs, however, the bands become blurry with time, presumably due to the greater mobility of the lower molecular weight β -limit-dextrin products. It is sometimes advantageous to also stain the polyacrylamide gel following incubation, as some of the starch and β -limit dextrin moves into the gel, and amylases are detected as colorless bands against a blue background.

RESULTS

Amylase Enzymes in the Growing Kernel

Varieties of hard red spring wheat were planted on June 17, 1970, and were sampled at appropriate times ranging from very early stages of kernel development to full maturity. The development of amylases for Manitou wheat at six stages in the development of the kernel is shown in Figs. 1 and 2. The β -limit-dextrin gel (Fig. 1) indicates that three forms of α -amylase are present. The three forms appear very early in the growth, and remain at a reasonably constant level during the ripening period. As full maturity approaches, the level of the three amylases falls drastically, and with some varieties no α -amylase activity could be found at full maturity, by the present experimental conditions. The intensity of the α -amylases, relative to each other, did not change for any particular growth stage, nor was there any change in their electrophoresis behavior. No new α -amylases are formed during the entire growth and maturation period. On the starch gel (Fig. 2), the three α -amylases — components α -1, α -2, and α -3 — can again be detected and identified by comparison with the β -limit-dextrin gel. The remaining bands on the starch gel can, therefore, be attributed to the β -amylase isozymes. Two major components, β -2 and β -3, are formed during the growth of Manitou wheat. In contrast to the α -amylases, these components are present in very small amounts in early growth samples, and increase in quantity as the ripening process continues. In the later stages of the ripening process, a number of electrophoretically slower β -amylase components, β -5, β -6, β -7, and β -8, comprising only a small portion of the apparent total β -amylase activity, are also formed. Another minor β -amylase component, β -4, is formed early in the growth of the wheat and disappears during the later ripening stages. α -Amylase can be differentiated from β -amylase by its ability to withstand heating at 70°C. for 15 min., and extracts heat-treated in this manner were subjected to electrophoresis. On both β -limit-dextrin and starch gels, only

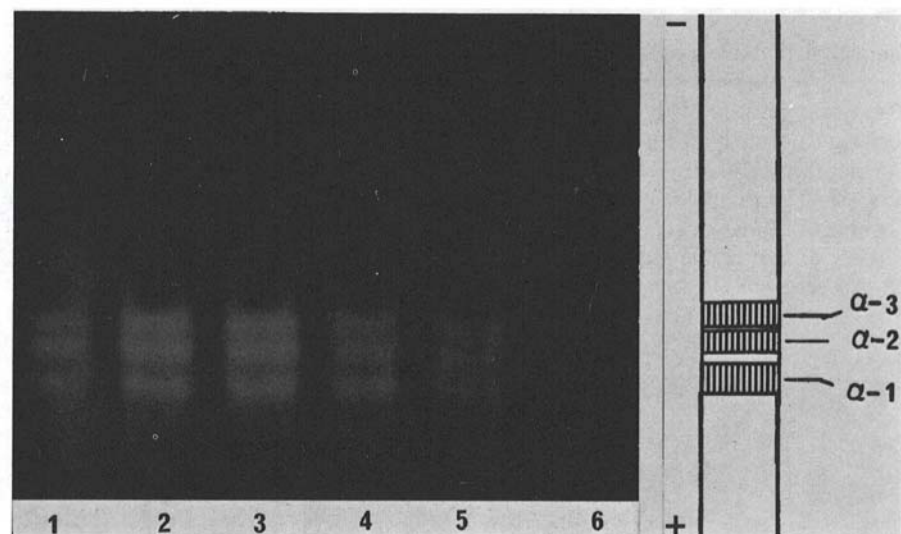


Fig. 1. Zymograms on β -limit-dextrin gels of α -amylase from Manitou wheat during 1970 growth. Wheat was planted on June 17 and harvested at: 1) Aug. 13; 2) Aug. 19; 3) Aug. 26; 4) Sept. 2; 5) Sept. 9; 6) Sept. 30.

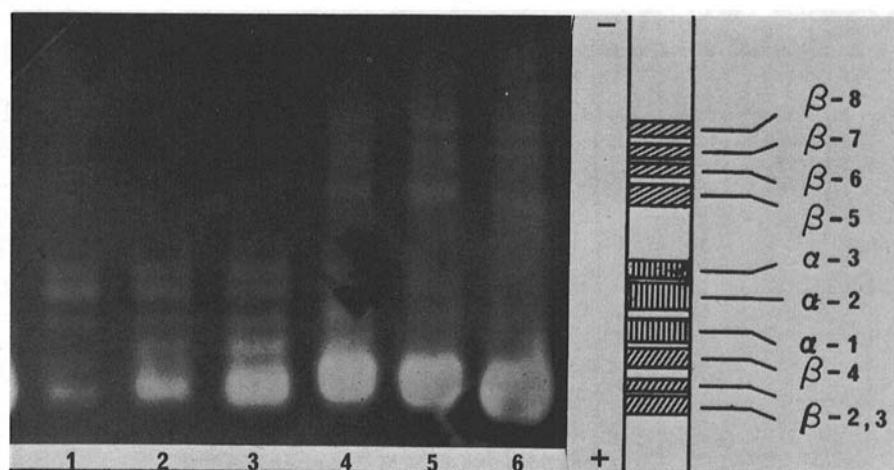


Fig. 2. Zymograms on starch gel of amylases from Manitou wheat during 1970 growth. Planted and harvested as in Fig. 1.

components $\alpha-1$, $\alpha-2$, and $\alpha-3$ remained after this treatment. Hard red spring varieties that have been analyzed during growth and found to behave in a way similar to Manitou, were Thatcher, Marquis, and "Prairie Pride". In all cases, three α -amylase isozymes, two major β -amylase isozymes, and five minor β -amylase isozymes were formed. The intensity of the isozymes at the various harvest dates does vary somewhat; but this should be expected, as kernel maturities for the

different varieties may be different. Analysis of the soft wheat variety Pitic 62 indicated that, although the α -amylase isozymes were similar in nature and behavior (Fig. 3) to those of the other varieties, there were some striking differences in the β -amylase patterns (Fig. 4). In the very early stages of development, the variety behaved normally in that the two major components, β -2 and β -3, were synthesized. As development progressed, however, a new faster-moving β -amylase component, β -1, was formed and component β -3 disappeared. (The pattern of the minor β -amylase components, β -5, β -6, β -7, and β -8, in Fig. 4 was blurred, but appeared similar to that of the Manitou wheat.)

To see whether this electrophoretic variation in β -amylase activity was peculiar to Pitic 62 alone, the mature kernels from 21 Canadian plant breeders' varieties of

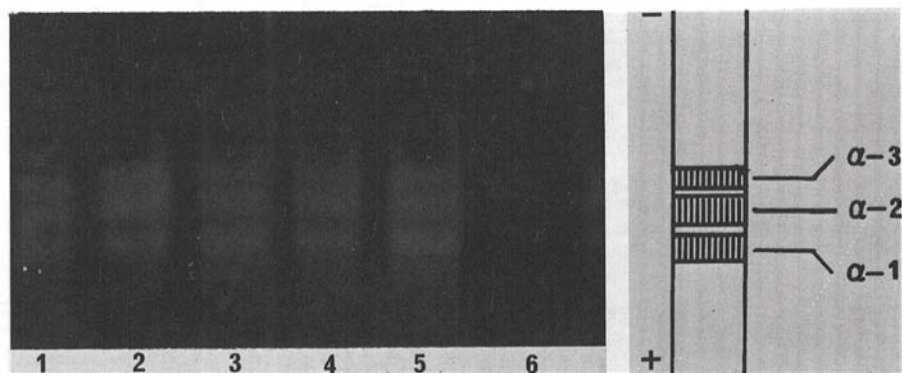


Fig. 3. Zymograms on β -limit-dextrin gel of α -amylase from Pitic 62 wheat during 1970 growth. Wheat planted on June 17 and harvested at: 1) Aug. 19; 2) Aug. 26; 3) Sept. 2; 4) Sept. 9; 5) Sept. 25; 6) Sept. 30.

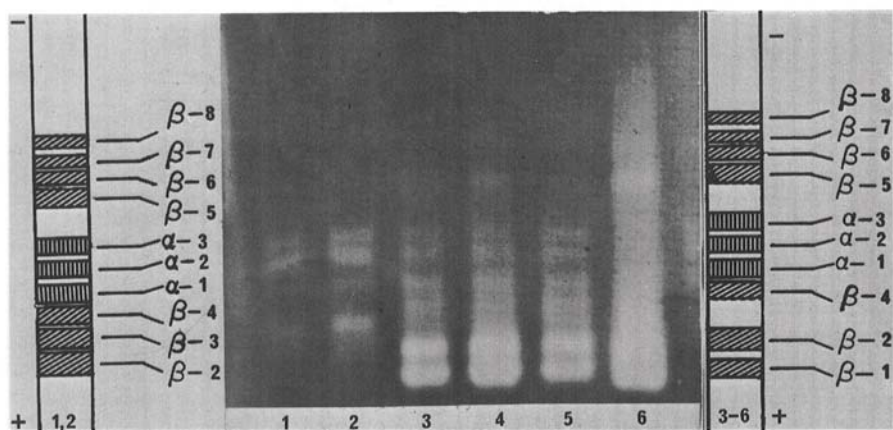


Fig. 4. Zymograms on starch gel of amylases from Pitic 62 wheat during 1970 growth. Wheat planted and harvested as in Fig. 3.

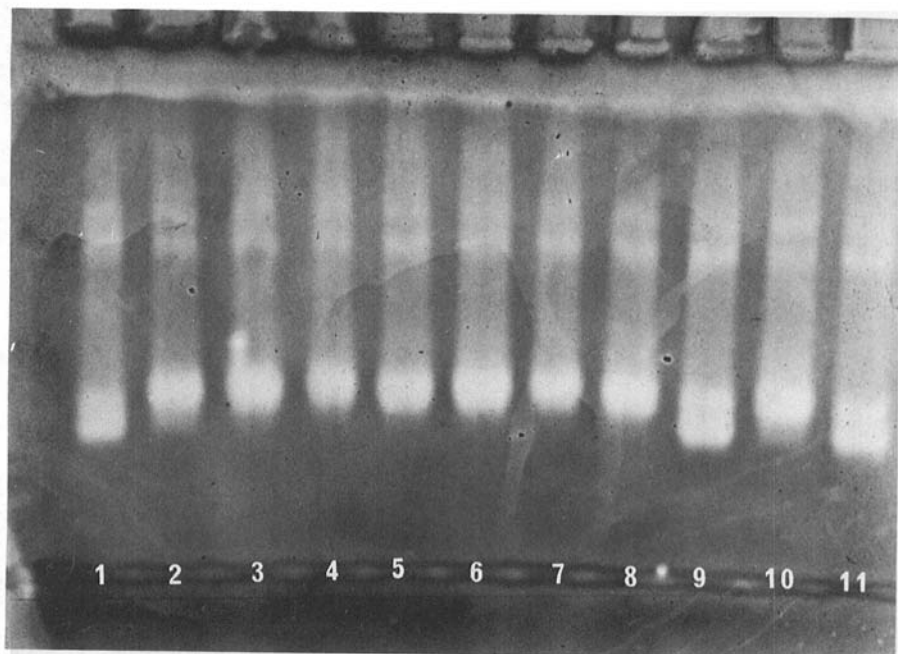


Fig. 5. Zymograms on starch gel of amylases from 11 different varieties of hard red spring wheat.

HRS wheat from the 1969 crop year were analyzed with starch gels. The results for 11 varieties are shown in Fig. 5. Zymogram variations in which component β -3 is absent and component β -1 is present do appear to exist in a number of varieties, although the normal zymogram, with components β -2 and β -3 as the major β -amylase forms, certainly predominates. The polyacrylamide gels on which the electrophoresis was carried out were also stained for protein with Amido Schwarz (Fig. 6). Quite evident is the difference in protein pattern of extracts from varieties in which component β -3 has been replaced by component β -1, with proteins of greater electrophoretic mobility being found. For varieties containing β -2 and β -3, the large amount of β -amylase activity and their close electrophoretic mobility make it difficult to be certain that two components are present in zymograms, although they are readily differentiated in early-growth samples. Examination of the protein components in the region of these enzymes, however, does indicate that two closely spaced proteins are present.

The above 21 varieties were also incubated with β -limit-dextrin gels under the same experimental conditions. Very little or no evidence of α -amylase activity was found, indicating that, for analysis of α -amylase from most sound varieties, this technique will have to be modified either by extending the incubation time, increasing sample concentration, or both.

Amylase Distribution in Green Kernels

Kernels of Marquis and "Prairie Pride", planted on June 17, 1970, and harvested

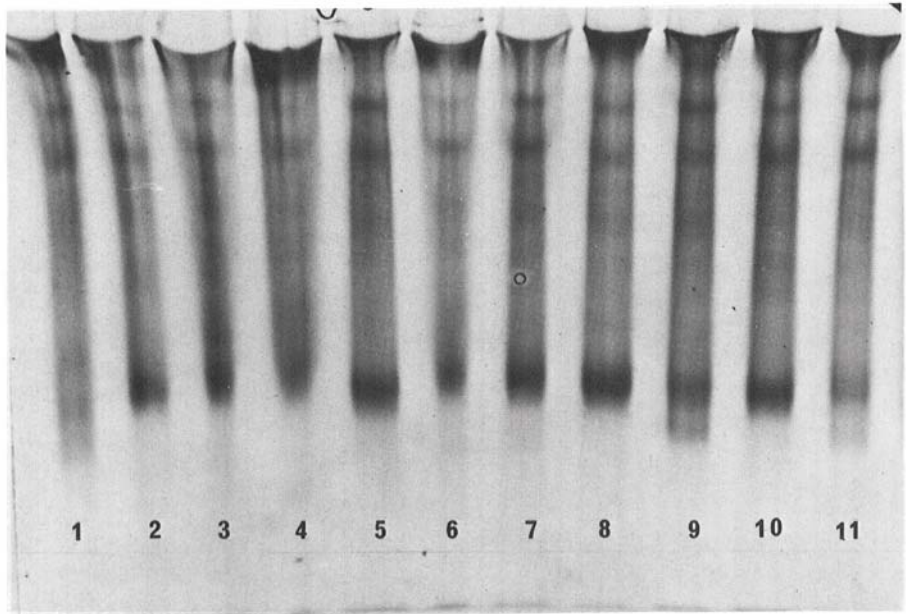


Fig. 6. Electrophoretic pattern of the proteins present in the varieties analyzed for amylases in Fig. 5.

on August 26, 1970, were dissected into pericarp, seed coat and aleurone layer, endosperm, and germ, in order to determine the morphological distribution of the amylases. The dissected portions were finely ground, extracted (25 kernels per ml. buffer), and submitted to electrophoresis. The resulting zymograms on a β -limit-dextrin gel are shown in Fig. 7, and on starch gel in Fig. 8. The bulk of the α -amylase activity (Fig. 7) was found in the pericarp and very little in the endosperm. Some α -activity was also found in the seed coat and aleurone, but virtually none was found in the embryo. Both Marquis and "Prairie Pride" exhibited a similar morphological distribution of their α -amylases. The major β -amylase components β -2 and β -3, by contrast, were found largely in the endosperm, with only a small amount present in the pericarp. Quite a large amount of β -amylase activity was also found in the seed coat and aleurone layer, but none in the embryo. The β -amylase activity of the "Prairie Pride" samples is lower than that of the Marquis, because the former is at an earlier stage of kernel development. Quite interesting is the fact that the minor β -amylase component β -4, which normally is present in early growth samples but disappears as maturity approaches, resides entirely in the pericarp. The electrophoretically slower β -amylases, β -5, to β -8, can only be detected in the Marquis samples, and appear to be present in the endosperm.

The three α -amylase isozymes are not preferentially separated from each other, and all are present in the same relative proportions in the particular anatomical portion of the kernel in which they are found.

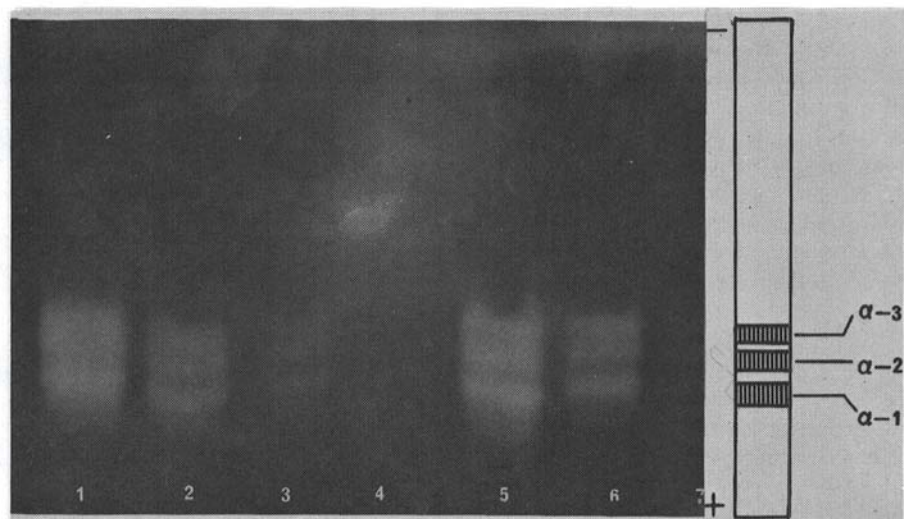


Fig. 7. Zymograms on β -limit-dextrin gel of α -amylase isozymes from different anatomical parts of immature Marquis (1,2,3,4) and "Prairie Pride" (5,6,7) wheats: 1, 5, pericarp; 2, 6, seed coat and aleurone layer; 3, 7, endosperm; 4, embryo. "Prairie Pride" embryo is not shown, but no amylases were present.

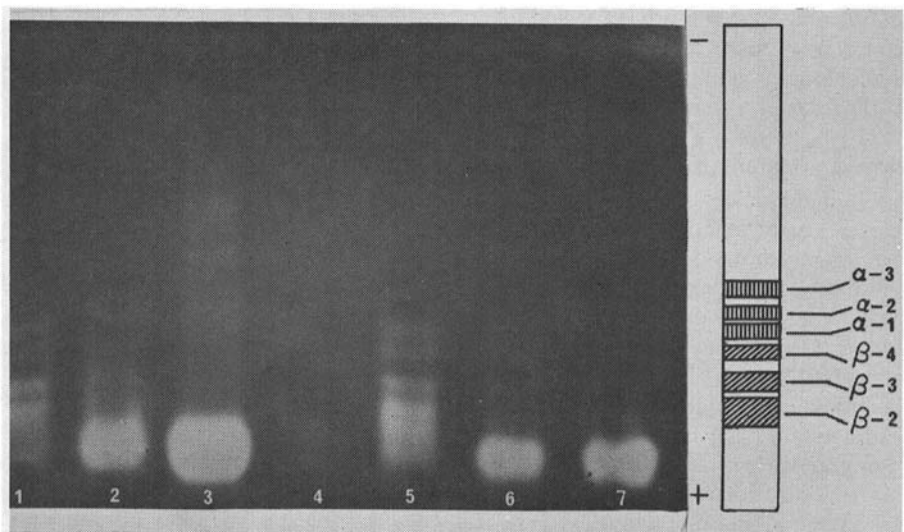


Fig. 8. Zymograms on starch gel of amylase isozymes from different anatomical parts of immature Marquis (1,2,3,4) and "Prairie Pride" (5,6,7) wheats: 1, 5, pericarp; 2, 6, seed coat and aleurone layer; 3, 7, endosperm; 4, embryo. "Prairie Pride" embryo is not shown, but no amylases were present.

Nature of the Latent β -Amylases in Wheat

Fully mature kernels from Marquis (components β -2 and β -3 isozyme types), and Pitic 62 (components β -1 and β -2 isozyme types), were extracted in buffer with and without the addition of 0.05 ml. thioglycerol. No difference in the electrophoretic mobility of the two extracts was found, nor were any new components present. Re-extraction, with buffer containing 0.1 ml. thioglycerol, of the insoluble residue from kernels extracted without thiol, released a substantial additional amount of β -amylase activity. The electrophoretic mobility of these latent β -amylase components were again identical with the thiol-free extracts, and no additional components appeared.

DISCUSSION

To study the multiple forms of amylases found in HRS wheat, it was first necessary to devise a sensitive method for their separation and detection. Electrophoresis on various supporting media, including agar, cellulose acetate, and polyacrylamide gels, has been used by many investigators to separate multiple forms of enzymes. Polyacrylamide, because of its sharp resolution, was chosen as the supporting medium for electrophoresis in this study. Conventional disc electrophoresis was not used, as the circular shape of the disc gel made a poor contact with slabs of starch or dextrin, and consequently hindered detection of the isozymes. Boettcher and de la Lande (13) have tried the direct incorporation of 1% starch into the gels prior to electrophoresis. They found, however, that at any pH used for electrophoresis, amylase complexed with the starch in the gel. More successful were Van Onckelen and Verbeck (14), who found that, by allowing a β -limit dextrin to diffuse into the gel and then staining with potassium iodide, they were able to detect six multiple forms of α -amylase. The use of a polyacrylamide slab in the present study provided a very good contact between gel and slab, with consequent sharp resolution of amylase bands. In addition, 12 different samples could be run simultaneously, facilitating comparisons between and among samples. Utilization of a pulsed constant power supply reduced the time of electrophoresis from the normal 2 hr. to approximately 1 hr., with the advantage that protein denaturation or artifact formation by heat buildup was minimized.

In preliminary studies, a variety of chemicals were tested for their ability to preferentially inactivate α - or β -amylases after polyacrylamide electrophoresis. On agar gels, cupric sulfate and mercuric chloride have been found effective in inhibiting β -amylase, while sodium hexametaphosphate preferentially inhibited α -amylase (9). None of these chemicals was found completely satisfactory on polyacrylamide gels for, although there was a preferential weakening of the expected bands, all amylase bands showed some weakening. The "sandwich" technique, employing a β -limit-dextrin plate on one side of the polyacrylamide slab and a starch plate on the other side, was very satisfactory for distinguishing α - and β -amylases. Heat-treated extracts also served as confirmation of α -amylase zones. It must be mentioned that the β -limit dextrin was much more sensitive than the starch gel for detecting α -amylases and, in many cases, indicated location of α -amylase bands even though no evidence of the enzyme was seen on starch gels.

The electrophoretic conditions described in this paper have been found satisfactory for resolving both the α - and β -amylases of wheat into their multiple forms. Three α -amylase forms of apparently similar activity are formed very early in

the growth of the wheat kernel. As the final ripening occurs, the three forms decrease greatly and, in some cases, disappear. All HRS wheat varieties analyzed had the three forms, and all with identical electrophoretic mobility. The α -amylase was found largely in the pericarp and, presumably, is partially responsible for breakdown of starch, which is also located in these tissues in very immature wheat kernels. The finding by Olered and Jonsson (7) that α -amylase in "green" wheat is responsible in some cases for variations in falling-number values, suggests that levels of such "green" α -amylase may be an important quality factor in wheat.

Two main forms of β -amylase appear during growth of HRS wheat kernels. The two forms are very similar electrophoretically, and consequently blur into one zone of activity in the final growing stages. The finding that there are two main forms of β -amylase in the fully mature kernel, and that these forms are similar to the "latent" forms, confirms previous results with DEAE- and CM-cellulose ion-exchange chromatography (2).

A number of electrophoretically slow β -amylase components, comprising only a small portion of the apparent total β -amylase activity, are found in kernels approaching full maturity. Although it may be that they are newly synthesized at this stage of kernel development, other explanations are also possible. It may be that part of the two main forms binds into larger-molecular-weight gluten proteins during the period in which these storage proteins are being laid down. It is, in fact, known that "latent" β -amylase is found in the glutenin (15,16) fraction of wheat. Another possibility is that the minor β -amylases are synthesized in early kernel development along with the major β -amylase components, but because of the small amounts present, would not be detected by the present technique.

β -Amylase component β -4, is particularly interesting in that it is found exclusively in the pericarp and disappears during later kernel growth, in contrast to other β -amylase components. This is suggestive that its purpose is to be part of the operative machinery, along with the α -amylase, for breakdown of starch in the pericarp. Once this has been accomplished, it disappears during final kernel development, along with the α -amylase.

Certain varieties, such as Pitic 62, differed in the electrophoretic mobility of their major β -amylase components, with a faster moving β -amylase component being present. This stresses the fact that caution should be used in generalizing about the multiple forms of enzymes found in wheat or other cereals when only a few varieties are selected for examination. The protein patterns of these varieties also reflected the presence of an electrophoretically faster component. The pattern cannot be used as an indicator of poor protein quality, however, for although Pitic 62 has poor milling and baking qualities, such is not the case for some other electrophoretically similar varieties, such as Ciano 67.

The finding that the bulk of the β -amylase in immature wheat kernels resides in the endosperm, whereas the bulk of the α -amylase is in the pericarp, confirms the previous results of Sandstedt and Beckord (17). Using photomicrographs and activity determinations, they found that α -amylase was principally in the pericarp, with only traces in the endosperm. β -Amylase was found in the endosperm, although the pericarp also contained considerable amounts of β -amylase.

Acknowledgments

The author wishes to thank Miss N. Turriff for competent technical assistance and P. C. Williams for assistance in connection with the planting and harvesting of wheat samples.

Literature Cited

1. TIPPLES, K. H., and TKACHUK, R. Wheat beta-amylases. I. Isolation. *Cereal Chem.* 42: 111 (1965).
2. KRUGER, J. E. On the relationship between free and latent beta-amylase in wheat. *Cereal Chem.* 47: 79 (1970).
3. NUMMI, M., DAUSSANT, J., MIKU-PAAVOLA, M. L., and ENARI, T. M. Comparative immunological and chromatographic properties of some plant beta-amylases. *J. Sci. Food Agr.* 21: 258 (1970).
4. ALEXANDRESCU, V., and MIHAILESCU, F. Electrophoretic studies of wheat amylases during the first growing stages. *Rev. Roum. Biochim.* 7: 3 (1970).
5. KRUGER, J. E., and TKACHUK, R. Wheat alpha-amylases. I. Isolation. *Cereal Chem.* 46: 219 (1969).
6. DAUSSANT, J., and CORVAZIER, P. Biosynthesis and modifications of alpha- and beta-amylases in germinating wheat seeds. *FEBS Letters* 7: 191 (1970).
7. OLERED, R., and JONSSON, G. Elektrophoretische Studien von alpha-amylase in Weizen. *Getreide Mehl* 18: 95 (1968).
8. OLERED, R., and JONSSON, G. Electrophoretic studies of alpha-amylase in wheat. II. *J. Sci. Food Agr.* 21: 385 (1970).
9. FRYDENBERG, O., and NIELSEN, G. Amylase isozymes in germinating barley seeds. *Hereditas* 54: 123 (1966).
10. TANAKA, V., and AKAZAWA, T. Alpha-amylase isozymes in gibberellic acid-treated barley half-seeds. *Plant Physiol.* 46: 586 (1970).
11. TANAKA, V., ITO, T., and AKAZAWA, T. Enzymic mechanism of starch breakdown in germinating rice seeds. III. Alpha-amylase isozymes. *Plant Physiol.* 46: 650 (1970).
12. ANONYMOUS. Instruction manual for model 4200 electrophoresis system. Ortec Incorporated, 100 Midland Road, Oak Ridge, Tenn.
13. BOETTCHER, B., and De La LANDE, F. A. On the electrophoresis of amylase in gels containing starch. *Anal. Biochem.* 34: 1 (1970).
14. Van ONCKELEN, H. A., and VERBECK, R. La formation des isozymes de l'alpha-amylase durant la germination de l'orge. *Planta* 88: 255 (1960).
15. ROWSELL, E. V., and GOAD, L. J. The constituent of wheat binding latent beta-amylase. *Biochem. J.* 84: 73p (1962).
16. ROWSELL, E. V., and GOAD, L. J. Latent beta-amylase of wheat: Its mode of attachment to glutenin and its release. *Biochem. J.* 84: 73p (1962).
17. SANDSTEDT, R. M., and BECKORD, O. C. Photomicrographic studies of wheat starch. II. Amylolytic enzymes and the amylase inhibitor of the developing wheat kernel. *Cereal Chem.* 23: 548 (1946).

[Received August 3, 1971. Accepted January 11, 1972]