COMMUNICATION TO THE EDITOR

Amylase Activity of Sprout-Damaged, Malted, and Immature Wheat

DEAR SIR:

Experience in this Institute over the last 30 years has repeatedly led us to question that the effects of sprout damage (weathering, premature germination) seen in our commercial wheat crops can necessarily be reproduced by sprouting or germination in the laboratory, or that malted wheat is a satisfactory substitute in investigations of the effects.

Particular circumstantial evidence is that the many tests we use based on paste or gel strength, viscosity, crumb stickiness, saccharogenic and dextrinogenic activities do not rank all affected flours in the same order between themselves or relative to laboratory germination and malting. Such observations are most readily explained by the postulate that more than one major heat-stable, endo-starch-degrading enzyme is involved.

It has been commonly assumed that laboratory-germinated grain, or malt, can be used for work on sprout damage. References (1) and (2) are merely examples of many that could be quoted illustrating the assumption, and we trust that these authors will forgive us for using them as examples. We here present evidence, obtained incidentally to another investigation, that two commercial sprouted wheats contained a second important starch-thinning enzyme activity as well as that usually attributed to α-amylase.

Some recent work has focused attention on amylase activity associated with the pericarp of developing grain (3–5). We have compared this amylase with that of typical commercial wheat showing a moderate degree of sprout damage and with malted wheat prepared in a pilot plant from a similar kind of sound wheat. Fractionations have been made by gel-exclusion column chromatography. Assay of the column effluent has been made by a method that is sensitive only to heat-stable, starch-viscosity-lowering power.

The undeveloped wheat was of the cultivar Aotea of the 1971 harvest season, cut and frozen 18 days after ear-emergence, freeze-dried, and ground. Malted wheat was prepared from sound, ripe, commercial, bulked wheat cv. Aotea of the 1971 harvest, in a pilot plant[1] under the following conditions: steeped 60 hr. at 12.8°C. in 6-hr. alternating periods, germinated 6 days at 11.7°, and kilned 22 hr. at 49°. The sprouted wheat was a typical commercial damaged wheat from the Southland area of New Zealand, cv. Hilgendorf, of the 1972 harvest season. A second confirmatory sample of sprouted wheat was from a bulk of damaged samples from the Southland area of the 1968 harvest. All four samples had been stored in deep-freeze conditions before grinding for analysis. Their activities, determined by dilution in the amylograph, were 10.8, 25.7, 0.43, and 0.76 amylograph units, respectively. One amylograph unit is the activity of 600 mg. ground wheat or flour that, added to a slurry of 60 g. acid-inactivated (6) flour with 440 ml. liquid, lowers the amylogram peak reading to one half.

[1]We are indebted to J. Smart and the Canterbury (N.Z.) Malting Co. for the malting.

Copyright © 1973 American Association of Cereal Chemists, Inc., 3340 Pilot Knob Road, St. Paul, Minnesota 55121. All rights reserved.

Samples were prepared for gel-exclusion chromatography by extracting 1 to 5 g. ground wheat by shaking with 50 ml. 0.1M acetate buffer pH 4.7 containing 0.05% calcium acetate, for 20 min. at 27°C. After centrifugation, the supernatant was saturated with ammonium sulfate and the precipitate centrifuged down, the supernatant being discarded. The precipitate was dissolved in about 10 ml. of 0.02M acetate buffer, pH 4.7, containing 0.05% calcium acetate and 3M urea. The solution was dialyzed against 0.02M acetate buffer with 0.05% calcium acetate and recentrifuged before being introduced onto the chromatography column. The column, 2.5 X 75 cm., contained about 25 g. BioGel P 150 in 0.02M acetate buffer with 0.05% calcium acetate and was jacketed at about 10°. Elution was continued with the same buffer at 15 to 20 ml./hr., fractions about 1.6 ml. being collected. For analysis an aliquot of 0.5 ml. was taken from every third fraction. The void volume of the column was 120 ml.

Each sample aliquot was heated 20 min. at exactly 60°C. to inactivate labile amylases and cooled to 27°C. Ten milliliters soluble starch solution was added (5%,

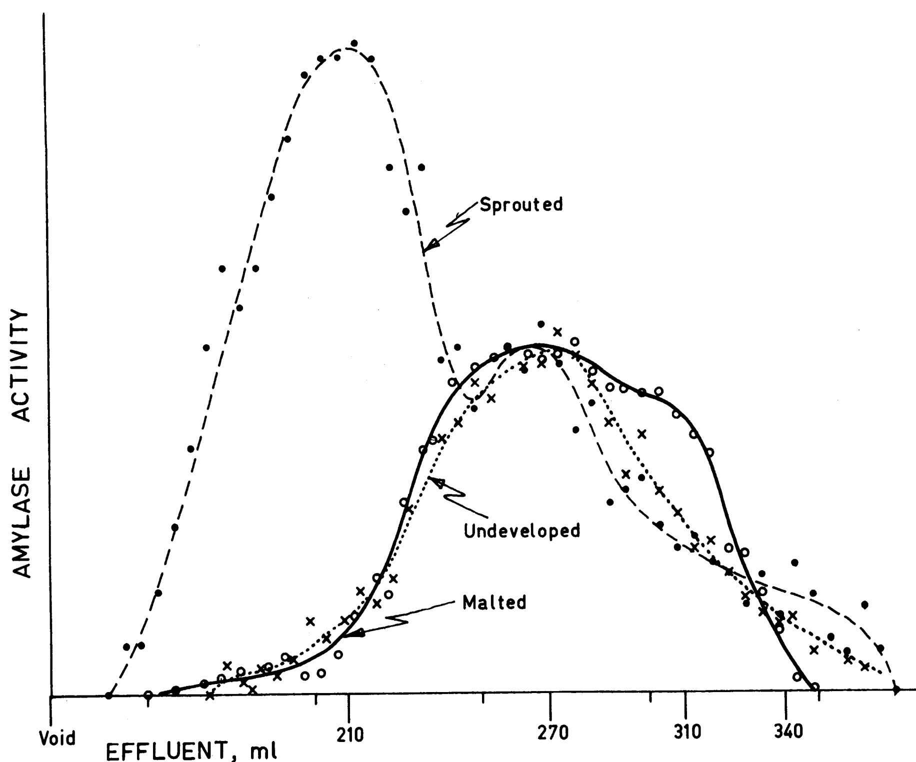

Fig. 1. Gel-exclusion column chromatograms of amylase activities extracted from malted, sprouted, and undeveloped wheats. Because of large differences in amounts of materials extracted and separated, the heights of the curves are not indicative of the original activities of the wheats, but are scaled to equal peak height at the 270 ml. effluent point to best show the elution pattern.

in 0.2M acetate buffer, pH 4.7) and the mixture incubated 2 hr. at 27°C. Without further treatments, the viscosity of the incubated mixture was determined in an Ostwald U-tube viscometer, also at 27°C., the grade of viscometer being such that water had a flow time of 70 sec. Units of activity were calculated as the ratio of flow time for no activity (base-line samples) to flow time for the active sample, minus one. The results have been scaled to equal peak height at the 270 ml. elution point.

Figure 1 shows one major peak in the elution patterns for the undeveloped wheat and for the malted wheat, at 270 ml. However, the sprouted wheat showed also a second, larger peak at 210 ml. corresponding to a component of higher molecular weight. This pattern was confirmed in the second sample of sprouted wheat of another season.

These patterns, and others of amylases determined by different analytical criteria, show reproducible fine detail suggesting several iso-enzymes. The malt probably has another component at about 310 ml., while both sprouted wheats showed a definite minor component at about 340 ml. elution volume.

The additional main peak for sprouted wheat clearly indicates an amylolytic component that could account for observed differences of behavior between malted and sprouted wheats.

PETER MEREDITH and LYNETTE D. JENKINS

Wheat Research Institute
Dept. of Scientific and Industrial Research
Christchurch, New Zealand

Literature Cited

1. KRUGER, J. E., and TKACHUK, R. Wheat alpha-amylases. I. Isolation. Cereal Chem. 46: 219 (1969).
2. DRONZEK, B. L., HWANG, P., and BUSHUK, W. Scanning electron microscopy of starch from sprouted wheat. Cereal Chem. 49: 232 (1972).
3. MEREDITH, P. Starch synthesis in the developing grain. Proc. 21st Conf. Royal Australian Chemical Institute, Cereal Chemistry Division (October 1971).
4. BANKS, W., EVERS, A. D., and MUIR, D. D. The location of alpha-amylase in developing cereal grains. Chem. Ind. (London) 1972: 573.
5. STODDART, J. L. Sequential changes in amylase isozymes during grain maturation in barley. Planta 97: 70 (1971).
6. MEREDITH, P. Inactivation of cereal alpha-amylase by brief acidification: The pasting strength of wheat flour. Cereal Chem. 47: 492 (1970).