A NOTE ON THE SEMIAUTOMATED DETERMINATION OF CATALASE IN WHEAT[1]

Cereal Chemistry 53(5): 796-801

J. E. KRUGER, Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Manitoba, Canada R3C 3G9

Catalase from wheat has been implicated in the oxidative reactions occurring in the breadmaking process (1,2) and in the bleaching of pigments (3). The mechanism of such involvements, however, is poorly understood and studies are hindered by the time-consuming and cumbersome nature of most catalase assays (4–10). In 1972, Sinha (11) devised a colorimetric method for determining the catalase from yeast and animal sources. It was based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of hydrogen peroxide. Catalase was incubated with hydrogen peroxide for different periods of time. The remaining hydrogen peroxide was determined by adding dichromate in acetic acid, heating, and measuring the resulting chromic acetate colorimetrically at 570 nm. The method compared well with the permanganate titration method (5). The present note describes the semiautomation of this method on the Technicon AutoAnalyzer and illustrates the suitability of the method for measuring the catalase activity in wheat.

MATERIALS AND METHODS

Wheat

Mature wheat samples were from 1972 plant breeders' varieties grown in

[1]Paper No. 357 of the Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Manitoba, Canada, R3C 3G9.

western Canada. Immature wheat kernels (var. Manitou) were collected at 24 days after flowering from a field plot grown at the University of Manitoba, Winnipeg. A sample of Neepawa wheat was malted in this laboratory. Two-gram or 40-kernel samples were finely ground with a Moulinex electric coffee mill and extracted with 8 ml of 12.5% sucrose for 2 hr. The suspension was centrifuged at 21,000 × *g* for 20 min and the clear extract used for assay of catalase. catalase.

Reagents

Dichromate/acetic acid was prepared according to Sinha (11) by mixing 1 vol of a 5% solution of potassium dichromate with 3 vol of glacial acetic acid. A stock solution of hydrogen peroxide was prepared by diluting 23 ml of 30% hydrogen peroxide to 100 ml with water (2.02*M*). One- to five-milliliter aliquots of stock hydrogen peroxide were diluted to 100 ml with 0.01*M* phosphate buffer, pH 7.0, and were run as controls. Substrate solution for the enzyme assay consisted of 3 ml of stock hydrogen peroxide diluted to 100 ml with 0.01*M* phosphate, pH 7.0 (0.06*M*).

Operational Procedure

The main modifications necessary for semiautomation of the method of Sinha (11) were the lowering of the temperature from 100° to 72° C for the reaction of dichromate in acetic acid with hydrogen peroxide and the increasing of the concentration of hydrogen peroxide in the assay.

The basic Technicon AutoAnalyzer (Technicon Corp., Chauncey, N.Y.) was used for semiautomation of the catalase assay. The system consisted of a 25° C heating bath; a Proportioning Pump II; a 72° C constant-temperature bath with a 20-ft glass coil; a colorimeter with a 570 nm filter; and a recorder, using absorbance paper. The flow diagram for the assay is shown in Fig. 1. The tubing inserted into the reaction vessel was of 0.86 mm inside diameter and its length was minimized as much as possible. Acidflex tubing was used in all lines except for manifold tubing of the air and sample lines.

The reaction was initiated by adding 0.5 ml of enzyme or diluted enzyme extract to 4.5 ml of hydrogen peroxide in a test tube in the 25° C constant-temperature bath. Immediately, the reaction mixture was assayed continuously

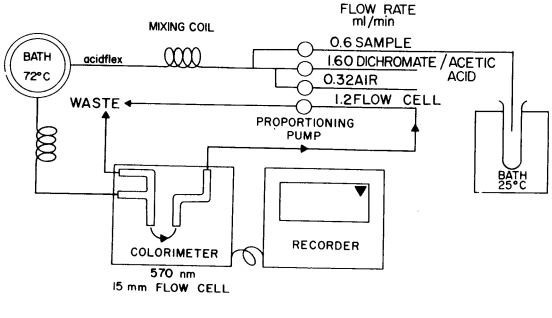

Fig. 1. Technicon AutoAnalyzer flow sheet for the semiautomated determination of catalase in wheat.

for breakdown of hydrogen peroxide with the AutoAnalyzer. The sample stream flowing at 0.6 ml/min met an air-segmented stream of dichromate in acetic acid flowing at 1.60 ml/min. After mixing, the stream passed into a 72° C constant-temperature bath for reduction of the dichromate in acetic acid by hydrogen peroxide to chromic acetate. The stream next passed through a colorimeter

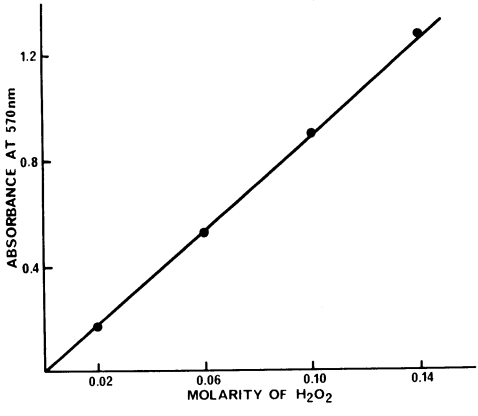

Fig. 2. Effect of hydrogen peroxide concentration on the absorbance at 570 nm using the AutoAnalyzer flow scheme shown in Fig. 1.

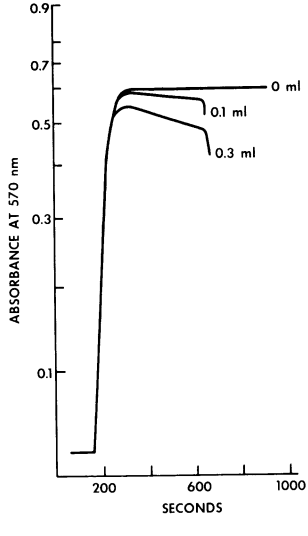

Fig. 3. AutoAnalyzer tracing showing the breakdown of hydrogen peroxide at enzyme concentrations of 0, 0.1, and 0.3 ml extract as measured by decreases in absorbance at 570 nm. The sharp decreases at the end of the 0.1- and 0.3-ml extract tracings indicate that sampling has been discontinued.

equipped with a recorder and the chromic acetate concentration was measured colorimetrically at 570 nm. Breakdown of dichromate in acetic acid was linear with increase in concentration of hydrogen peroxide up to 0.14*M* as shown in Fig. 2.

Typical traces on the AutoAnalyzer recorder that were obtained in the absence of enzyme, and with levels of 0.1 and 0.3 ml of enzyme extract in the reaction mixture are shown in Fig. 3. Decreases in absorbance at 570 nm were related to decreases in concentration of hydrogen peroxide by running controls with varying amounts of hydrogen peroxide (Fig. 2).

Calculation of Catalase Activity

Irvine *et al.* (1) have found that the decomposition of hydrogen peroxide by wheat catalase proceeds according to first-order kinetics. The first-order rate constant was found to be directly proportional to enzyme concentration and could be used as a convenient means of expressing the catalase activity of wheat and wheat products directly in terms of the constant. In the present study, the first-order constant was also used as a measure of enzyme activity and was calculated as follows:

$$k = \frac{2.303}{t} \log \frac{a}{a-x}$$

Where k is the observable rate constant, t is the time in seconds, a is the initial concentration of hydrogen peroxide, and a−x is the concentration of hydrogen peroxide after t. It was found that the initial concentration of hydrogen peroxide usually decreased with increasing concentration of enzyme (Fig. 3). In practice, therefore, the initial concentration was taken at the point where the absorbance was at its maximum and the final concentration as the absorbance recorded 200 sec later. The substrate concentration was normally kept at around 0.055*M* hydrogen peroxide to facilitate reading of absorbance changes on the recorder.

RESULTS

The semiautomated catalase assay described in this note was tested with extracts from ungerminated, malted, and immature wheat. In all cases, the rate constant k was found to be proportional to enzyme concentration (Fig. 4). This confirmed the findings of Irvine *et al.* (1) and indicated that the rate constant can be used as an index of wheat catalase activity. It was found convenient, therefore, to define a unit of catalase as having a k of 1×10^{-4}, sec^{-1} at 25° C. Eleven samples of hard red spring (HRS) wheat from Canadian plant breeders' material grown in 1972 were found to have catalase activities ranging from 40 to 80 units/g, with a mean of 56 units/g. Nine durum wheats that were tested also had substantial variation in catalase activity (36–88 units/g) with a mean (58 units/g) very similar to the HRS wheats. Irvine *et al.* (12) found that semolina of durum wheats contained less catalase than flour from HRS wheat, but indicated that the lower activity might be accounted for by the lower extraction used in semolina.

It should be possible to automate completely the present method by introducing a heating bath with the appropriate time coil and a sampler into the AutoAnalyzer train. A blank value would have to be determined, therefore, for

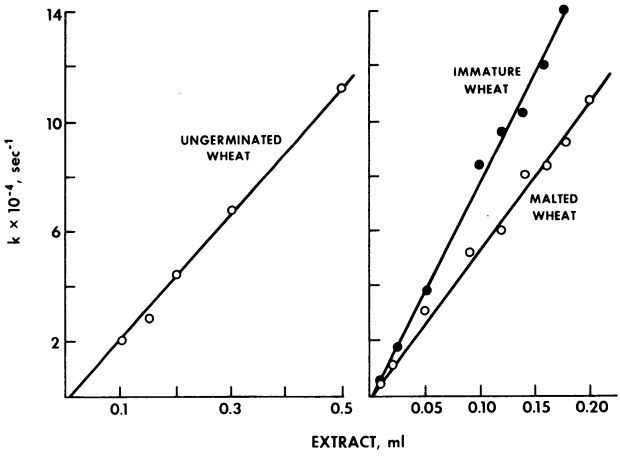

Fig. 4. Catalase observable rate constant as determined by the semiautomated method *vs.* amount of catalase extract from ungerminated, immature, and germinated wheats.

each sample that was run because of the varying initial uptake of hydrogen peroxide by the different wheat extracts. Even semiautomated, however, the method is more rapid than the commonly used manometric method (1,3,12) for determining catalase in wheat and wheat products. This makes the method particularly useful where a large number of samples have to be assayed. For example, it would facilitate the assay of samples from ion-exchange chromatography, isoelectric focusing, etc., by cereal researchers involved in the isolation and physical characterization of wheat catalase. Rust-susceptible varieties of wheats have been shown to have higher catalase activities than resistant varieties (13), and the method should be useful to plant breeders in predicting rust susceptibility. The method has been used in this laboratory to study changes in the catalase activity of HRS and amber durum wheat kernels during growth, maturation, and germination, and the results will be reported in a subsequent paper.

Literature Cited

1. IRVINE, G. N., BUSHUK, W., and ANDERSON, J. A. Kinetic studies of the catalase system of wheat. Cereal Chem. 31: 256 (1954).
2. HONOLD, G. R., and STAHMANN, M. A. The oxidation-reduction enzymes of wheat. IV. Qualitative and quantitative investigations of the oxidases. Cereal Chem. 45: 99 (1968).
3. HAWTHORN, J., and TODD, J. P. Catalase in relation to the unsaturated-fat oxidase activity of wheat flour. Chem. Ind. (London) 1955: 446.
4. CHANCE, B., and HERBERT, D. The enzyme-substrate compounds of bacterial catalase and peroxidases. Biochem. J. 46: 402 (1950).
5. SUMNER, J. B., and DOUNCE, A. L. Liver catalase. In: Methods in enzymology, ed. by S. P. Colowick and N. O. Kaplan. Vol. 2, p. 775. Academic Press: New York (1955).
6. PERLMANN, G. E., and LIPMANN, F. Catalase in serum and plasma. Arch. Biochem. Biophys. 7: 159 (1945).
7. WALKER, B. S. Direct measurement of catalase activity with the dropping mercury electrode (abstr.). Fed. Proc. 1: 140 (1942).
8. LACOPPE, J., and HOFINGER, M. Colorimetric method for studying catalase activity. Bull. Soc. Roy. Sci. Liege 37: 605 (1968).

9. BEERS, R. F., Jr., and SIZER, I. W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195: 133 (1952).
10. JOLLES, A. Beitrage zur Kenntnis der Blutfermente. Muenchen. Med. Wochenschr. 51: 2083 (1904).
11. SINHA, A. K. Colorimetric assay of catalase. Anal. Biochem. 47: 389 (1972).
12. IRVINE, G. N., BUSHUK, W., and ANDERSON, J. A. A note on the catalase activity of North American wheats. Cereal Chem. 31: 267 (1954).
13. NILOVA, V. P., and EGOROVA, G. N. The activity of catalase and peroxidase and the immunity of wheat to *Puccinia triticina*. Dokl. Vses. Akad. Sel'skokhoz. Nauk. V. I. Lenina 13: 34 (1948). [Chem. Abstr. 42: 6417i.]

[Received March 11, 1976. Accepted June 8, 1976]