The Enzyme Bromelin

SUSAN HIGGINS

R. M. SAYRE

THE STUDY OF ENZYMES by high-school students is quite inadequate, considering the role enzymes play in many biochemical reactions. For this reason we would like to add to the repertoire of projects, suitable to the high-school laboratory, in the field of enzymology. The project correlates the simple breakdown of a protein with the physical change resulting from the addition of pineapple juice to gelatin.

Intentions and Scope of the Project

Because enzymes mediate a multitude of specific chemical reactions occurring in simple and complex living organisms, biology students should be aware of their contribution in animal and plant biochemistry. Many enzymatic reactions are too subtle for a high-school laboratory and are not readily visualized. The laboratory exercises suggested here should help to overcome some of these difficulties by allowing the student to observe a physical change and to separate and examine some of the end products of an enzymatic reaction.

Because it deals with enzymes, the project pertains to several sciences, not just biology. The project might be equally beneficial to students of home economics or chemistry. Virtually all the necessary laboratory supplies and equipment probably would be available in the combined laboratories. In home economics, one of the exercises will demonstrate why fresh pineapple should not be used in gelatin salads. The chemistry student is introduced to molar calculations, buffer solutions, and the identification of organic compounds by paper chromatography. The biology student will learn how to extract enzymes from a plant source and initiate a study to characterize enzymes.

Specifically, the student will be following the hydrolysis of an animal protein, gelatin, by a plant enzyme, bromelin. The four separate experiments make use of a crude preparation of bromelin from fresh pineapple. (i) The activity of the enzyme upon the gelatin is observed. (ii) The temperature at which the bromelin is destroyed is determined. (iii) Enzyme activity is compared at various pHs. (iv) For more advanced students, chromatographic techniques for the separation and identification of end products of protein hydrolysis are taught.

Materials and Methods

An extract of crude bromelin is made from a medium-sized peeled pineapple. The fleshy parts of the pineapple are cut into small pieces, reduced to a fine pulp in a food blender, and strained through several layers of cheesecloth. The final liquid, which can be readily pipetted, is stored in a deepfreeze until needed. (Fig. 1.)

The protein substrate is made from commercial unflavored gelatin. One tablespoon (7 g) of powdered gelatin is mixed in half a cup (118 ml) of cold water and added to one cup (236 ml) of boiling water. The gelatin is then allowed to set in a refrigerator for 4 hours.

A viscometric method is used to measure the activity of bromelin. A 250-ml graduated cylinder is filled with 220 ml of gelatin solution. (Graduated cylinders of other sizes would serve equally well.) The time it takes a marble, placed on the surface of the gelatin, to sink to the bottom of the cylinder is recorded (fig. 2); this serves as a measure of the initial viscosity of the gelatin.

1 ml of pineapple juice is added to the gelatin in the column and allowed to set in the refrigerator. After the gelatin has set, the time required for the marble to sink through the gelatin is again noted; the change in viscosity is the visible measure of the amount of protein degradation. The same procedure is followed with each new addition of diluted pineapple juice. To obtain a curve it may be necessary to dilute the initial crude enzyme preparation, because hydrolysis may proceed too rapidly.

The effects of temperature of the enzyme also are measured. 1-ml samples of pineapple juice are heated to 35 C, 50 C, and 85 C and added to 50 ml

Susan L. Higgins is a senior at Virginia Polytechnic Institute and State University, Blacksburg, Va. 24060. The present paper is based on her high-school science-fair project. She served during the summer of 1969 as an aide to Richard M. Sayre, who is a nematologist in the Plant Protection Institute of the USDA's National Agricultural Research Center, Beltsville, Md. 20705. A 1951 graduate of Oregon State University, he holds a master's degree from that institution and has his Ph.D., in plant pathology, from the University of Nebraska. His 30 publications include "Microfauna of Moss Habitats" (1971: *ABT* 33: 100–102, 105) and "Prey–Predator Interaction of Nematodes and Turbellarians" (1970: *ABT* 32: 487–490). Sayre is a member of the board of governors of the American Institute of Biological Sciences.

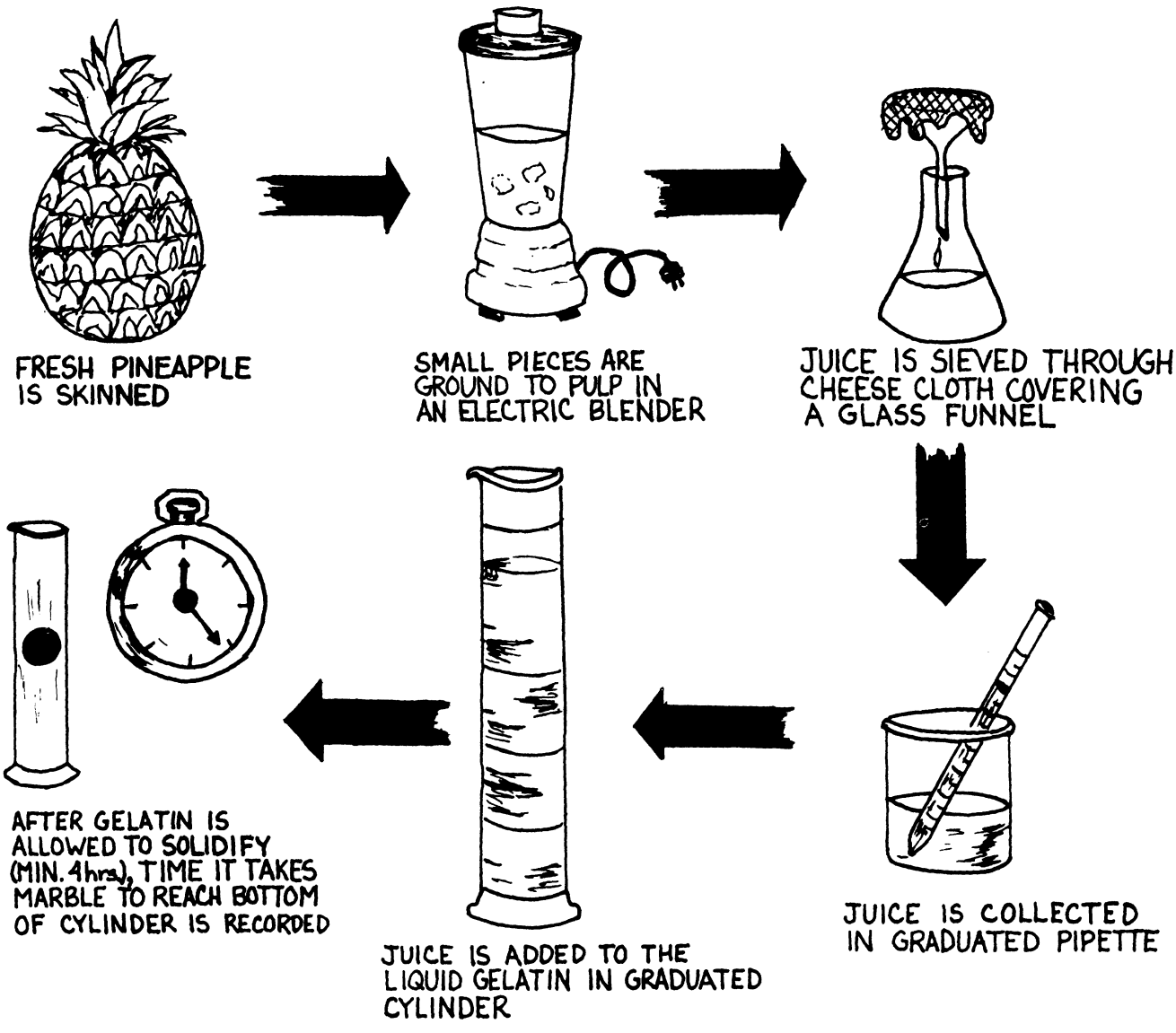

Fig. 1. Flow diagram showing the preparation of the enzyme bromelin in crude form and a method for determining its enzymatic activity on the protein gelatin.

of gelatin in test-tubes. After refrigerating for 4 hours, gelatin consistency is again measured.

pH optima for the enzyme are determined. Dibasic sodium phosphate (23.38 g/L) and citric acid (21.01 g/L) are combined in different amounts (table 1) to prepare a series of buffers having pH values of 4 to 8 (Barka, 1965). Each of five test-tubes is filled with 40 ml of gelatin, 25 ml of a buffer, and 0.5 ml of pineapple juice. The resulting pH of each tube is recorded. After the gelatin is refrigerated overnight, the changes in gelatin consistency are measured. Because of the variability of crude-enzyme preparation it may be necessary to reduce the amount of added pineapple juice until the concentration is found that does not obscure the influence of the buffers.

Paper chromatographs are prepared in an attempt to identify some of the products of protein digestion. A simple ascending method of paper chromatography is outlined in the student laboratory guide of the BSCS Yellow Version (Biological Sciences Curriculum Study, 1963). We use a descending method. 10 μl, 20 μl, and 30 μl (microliters, or lambdas) of the partially digested gelatin are spotted along the origin line of the chromatograph. Along with these are 10-μl, 20-μl, and 30-μl spots of the plain gelatin solution. The solvent used to develop the chromatograph is a 4:1:5 mixture of *n*-butanol, glacial acetic acid, and water. The reagents in the solvent are combined by shaking in a separatory funnel, and the lower layer is removed before the solvent is added to the tray in the chamber. After it has hung overnight in the chamber, the chromatograph is removed and dried, the solvent front is marked, and then the chromatograph is sprayed with a color reagent, ninhydrin. (The reagent is available in convenient spray can [Ninspray, a product of Nutritional Biochemicals, Cleveland], but it may be prepared in the laboratory from triketohydrindene hydrate in 95% ethanol.) After drying in an oven or under a hair

dryer at about 60 C, the amino acids, peptides, and proteins appear as purple spots on the chromatograph. Spots are circled, R_f (retardation factor) values for each are calculated, and their color is described. The calculated R_f values are then compared with the values found by others for amino acids.

Observations and Results

The change in the viscosity of the gelatin solution is used as a measure of the activity of the enzyme. The increasing concentration of the pineapple juice added is plotted against the time taken for the marble to reach the bottom of the gelatin (fig. 2). Initially, hydrolysis proceeds rapidly with small amounts of the crude enzyme; however, a plateau is reached at which increasing concentrations of enzyme fail to result in decreased viscosity. The hydrolysis of the protein proceeds at a slower rate once this plateau has been passed.

Freezing temperatures are not harmful to the enzyme: throughout the project the juice was stored in a deepfreeze. However, temperatures of 75 C and above denatures the protein–enzyme molecule.

Protein hydrolysis proceeds most rapidly at pH 4. Enzyme activity is also very much in evidence at

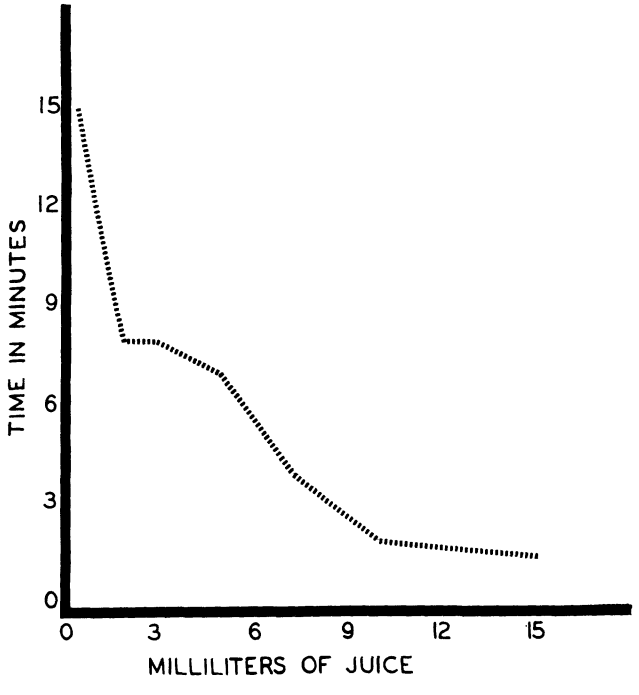

Fig. 2. The effect of increasing concentrations of crude bromelin on the rate at which a marble falls in a gelatin column.

pH 5 and pH 6.5, but it is only in the decidedly alkaline range that activity is suppressed.

Two clear spots show up on the chromatograph: a dark-purple spot of R_f 0.23 and a lavender spot of R_f 0.036. The purple spot has the same horizontal range as the glycine sample when it is run concurrently on the same chromatograph. Because glycine constitutes 25.5% of the amino acids found in gelatin, it is not surprising that this amino acid is readily found in the partly digested protein.

Preparation of buffer solutions. From Barka, 1965.

pH	*0.2M Na_2HPO_4 (ml)*	*0.1M citric acid (ml)*
4	77.1	122.9
5	103.0	97.0
6	126.3	73.7
7	164.7	35.3
8	194.5	5.5

Discussion

These experiments introduce the student to bromelin, one of a number of protein-digesting enzymes that have been found in plant cells. The enzyme has been studied extensively, and some information is available about its abilities to hydrolize proteins and peptides to amino acids and products of protein hydrolysis (Balls, Thompson, and Kies, 1941; Murachi and Neurath, 1959). The experiments should lead the student to an understanding of the action of the enzyme on its substrate and its effects on the peptide bonds linking the amino acids of a protein. Clearly, the student should be able to observe that something has happened to the protein, and he can follow the action visibly through the changes in the viscosity of the gelatin. Also, he will see the products of hydrolysis as they are resolved on the paper chromatograph.

REFERENCES

BALLS, A. K., R. R. THOMPSON, and M. W. KIES. 1941. Bromelin: properties and commercial production. *Industrial and Engineering Chemistry* 33 (7): 950-953.

BARKA, T. 1965. *Histochemistry.* Harper & Row, Inc., New York.

BIOLOGICAL SCIENCES CURRICULUM STUDY. 1963. Student laboratory guide for *Biological science, an inquiry into life.* Harcourt, Brace & World, Inc., New York. P. 55-57.

HAIS, I. M., and K. MACEK. 1963. *Paper chromatography.* Academic Press, Inc., New York. P. 435-478.

MURACHI, T., and H. NEURATH. 1959. Fractionation and specificity studies of stem bromelin. *Journal of Biological Chemistry* 235 (1): 99-107.

PERLMAN, G., and L. LORAND. 1970. Proteolytic enzymes. In *Methods in enzymology,* vol. 19. Academic Press, New York. P. 273-284.

REED, G. 1966. *Enzymes in food processing.* Academic Press, New York. P. 128-130.

Bike Paths Along Highways

The Oregon Department of Highways is building—with gasoline tax money—a number of bicycle paths where youngsters ride bikes to and from schools, along highways. The asphalt paths are painted yellow and are separated by a buffer strip.