

Flatulence and Chemistry: An Introductory Enzyme Experiment

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Abstract

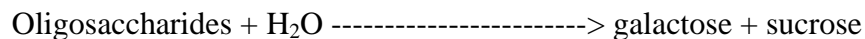
An inexpensive introductory level enzyme experiment was developed using raffinose family sugars extracted from split green peas as a substrate and the enzymes alpha-galactosidase and sucrase found in Beano^R. The reaction studied was the hydrolysis of raffinose family sugars to galactose, glucose, and fructose with the reaction rate determined using a retail glucometer to measure the concentration of glucose. Results are given on the effect of substrate concentration, enzyme concentration, temperature, and heavy metals on enzyme activity.

Introduction

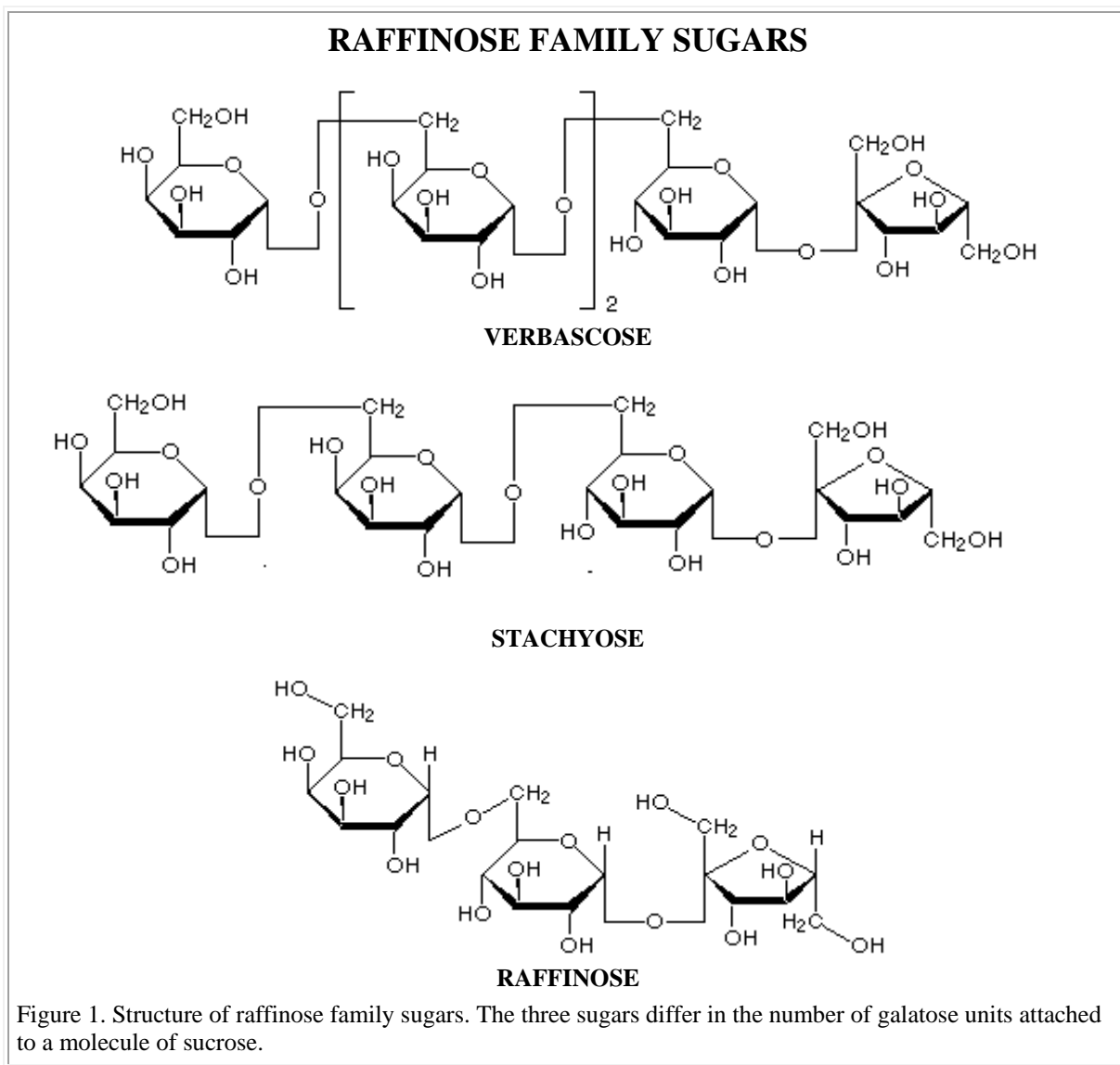
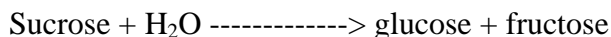
Enzyme activity experiments are traditional in biochemistry and health science chemistry laboratories (1). Depending on the level of the laboratory, these experiments may focus on enzyme kinetics and the effects of enzyme concentration, temperature, pH, and inhibitors upon enzyme activity. This inexpensive experiment introduces students to enzymes by using a substrate readily available from green peas, an enzyme available in the commercial product, Beano^R, and analytical equipment that can be purchased from any local pharmacy.

Certain foods such as peas and beans contain appreciable levels of complex sugars (raffinose, stachyose, and verbascose) known as oligosaccharides (Figure 1). Alpha-galactosidase and sucrase are the two enzymes required to completely hydrolyze the oligosaccharides into monosaccharides which can be readily absorbed into the bloodstream. However, the human gastrointestinal tract does not possess alpha-galactosidase (2,3); thus, the hydrolysis of ingested oligosaccharides is incomplete. The unhydrolyzed oligosaccharides are eventually fermented by anaerobic microorganisms in the colon to produce flatulent gases such as carbon dioxide, hydrogen, and methane. The commercial product Beano^R (advertised as a social and scientific breakthrough) supplies alpha-galactosidase and sucrase and purportedly helps prevent intestinal gas by catalyzing the hydrolysis of these complex sugars. In this work Beano was used as a source of enzymes for an introductory experiment on enzymes based on the reactions

alpha-galactosidase



sucrase



Experimental

The substrate was obtained by soaking 50.0 g of raw split green peas in 100 mL of deionized water for 12 hours. Solutions were kept in a constant temperature bath for 30 minutes before 10 microliters of Beano^R were added. The rate of hydrolysis was observed by following the rate of appearance of glucose. One drop of solution was periodically removed from the solutions and analyzed with a Glucometer Elite (distributed by the Bayer Corporation). Calibration curves of

meter readings versus glucose concentration were made for both the Glucometer Elite^R and the Profile One Step^R from Johnson and Johnson, but only the Glucometer Elite^R was used for enzyme activity experiments. The Glucometer Elite^R provided glucose readings in 30 seconds. Since the glucometers measure directly the concentration of the beta anomer of glucose, all glucose standard solutions were prepared 12 hours in advance of use to allow equilibrium to be reached between the alpha and beta anomers.

Results and Discussion

Calibration curves for the two types of glucometers are shown in Figure 2. A calibration curve is necessary because glucometers are calibrated at the factory for glucose readings in blood samples but viscosity and other matrix differences cause inaccurate readings in aqueous solutions (4).

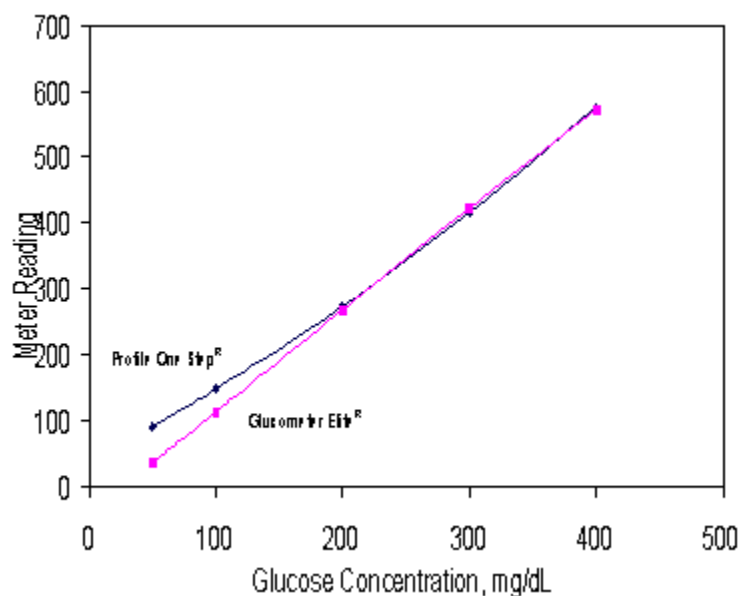


Figure 2. Calibration Curves for Glucometers

Since the Profile One Step^R showed slight deviations from linearity at low glucose concentrations the Glucometer Elite^R was used for all analyses. Because the glucometer is sensitive to only the beta anomer, this has led us to another experiment where glucose solutions are prepared and students immediately begin testing for glucose. As mutarotation occurs the observed glucose concentration will increase with time and students can "observe" mutarotation taking place. A plot of glucometer reading versus time allows students to observe mutarotation and the approach of the system to equilibrium. The substrate was obtained by soaking split green peas in water. Other beans or peas could be used, but split green peas were chosen because they contain relatively high levels of all three raffinose family sugars (5). A practical application of aqueous extraction of raffinose sugars is the soaking of beans or peas in water prior to cooking. The soaking extracts raffinose family sugars which are discarded and thereby lessens flatulence from the fermentation of these sugars. Figure 3 shows the effect of substrate concentration on

enzyme activity. Since one mole of glucose is formed for every one hydrolyzed molecule of raffinose, verbascose, stachyose, and sucrose, the initial slope of each line in figure 3 is equal to the initial reaction rate.

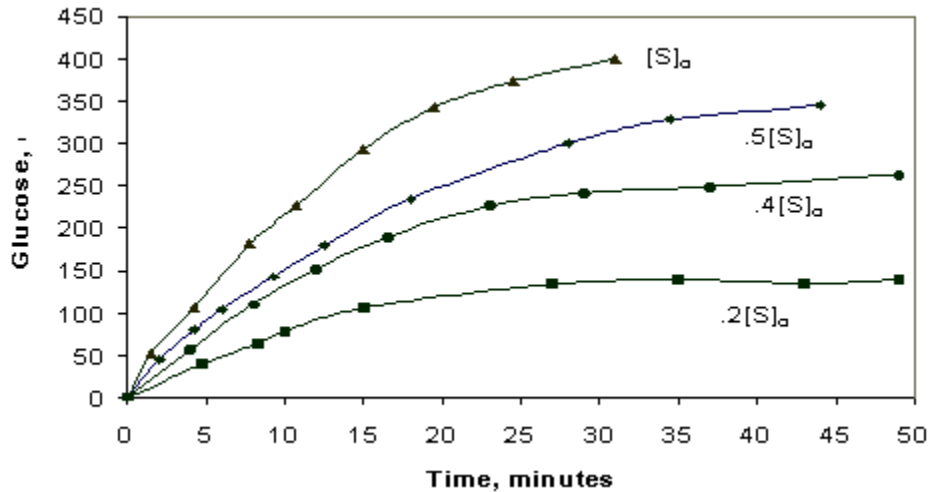


Figure 3. Effect of Substrate Concentration on Enzyme Activity

Figure 3, therefore, shows a typical increase in enzyme activity with an increase in substrate concentration. Health science majors will simply observe more glucose formed in a given period of time for the higher the substrate concentrations.

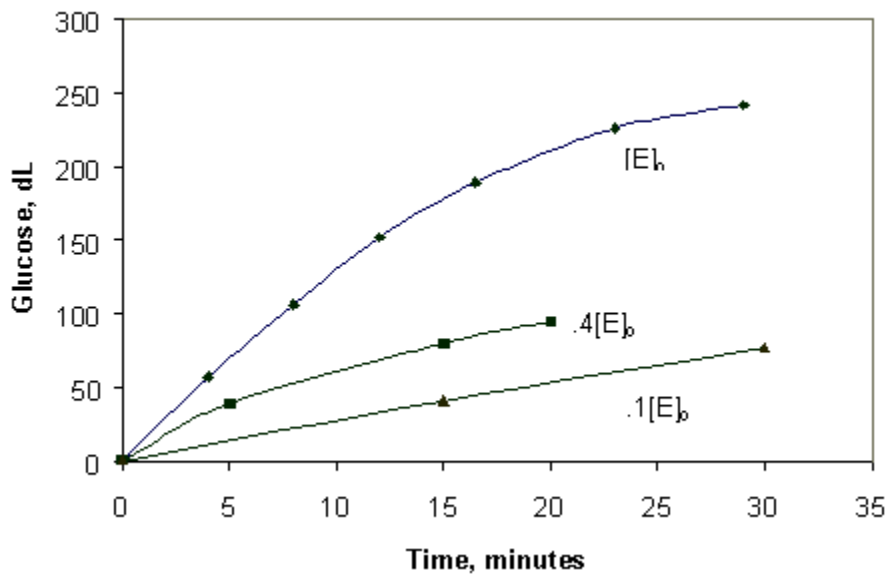


Figure 4. Effect of Enzyme Concentration on Reaction Rate

Figure 4 shows that the reaction rate increases with an increase in enzyme concentration at a constant substrate concentration. Figures 5 and 6 show the inhibitory effect of high temperature and five drops of 0.1 M mercury (II) chloride. Solvents, other heavy metals, acids, and bases can also be used to show similar effects. It must be kept in mind, however, that experiments designed for the observation of inhibition of enzyme activity are complicated by the fact that the glucometer uses the enzyme glucose oxidase. Such experiments do not distinguish between inhibition of alpha- galactosidase and inhibition of glucose oxidase.

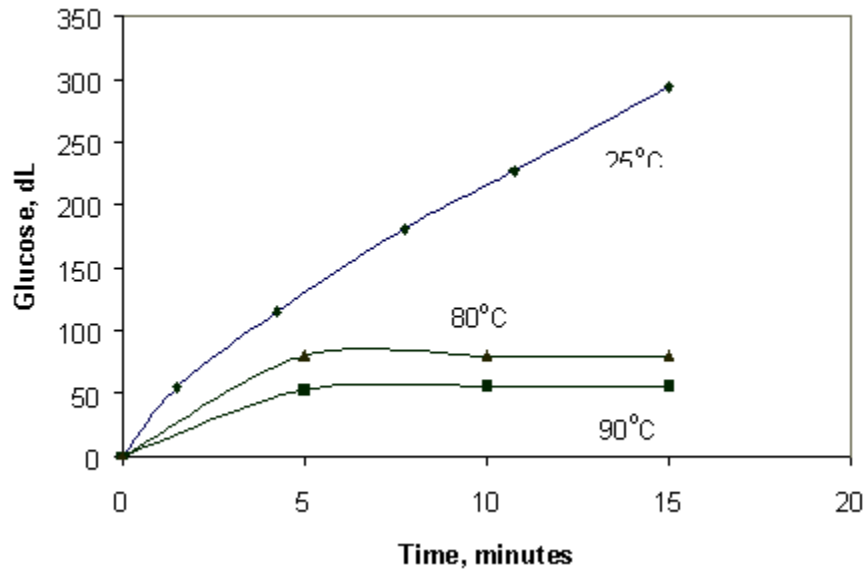


Figure 5. Effect of Temperature on Enzyme Activity

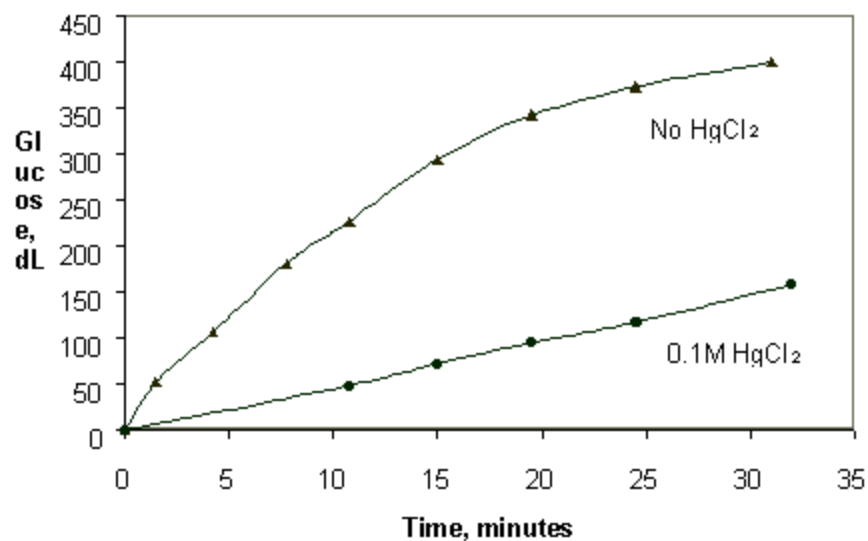


Figure 6. Effect of Mercury on Enzyme Activity

Glucometers can be purchased from local pharmacies for about \$40.00 and test strips run about \$35.00 for a pack of 50. In addition to being relatively inexpensive and convenient, this experiment has the advantage of introducing health science majors to the idea of a calibration curve and to the use of glucometers which we find especially instructive for nursing majors. In addition to introducing enzymes and the factors effecting enzyme activity, this experiment allows an instructor to introduce to students a new group of sugars and leads to discussions of nutrition, blood sugar levels, and the use of glucometers by diabetics.

Acknowledgements

One of the authors, T.M.M., thanks the Arkansas Silo Advisory Committee for her Student Undergraduate Research Fellowship. We also are grateful to the Henderson State University Undergraduate Research Committee for its support.

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Biographies

John R. Hardee is the Jack and Ida Byrne Kennedy Professor of Premedical Studies in the chemistry department at Henderson State University. In May of 1998, he completed his 12th year as a faculty member at HSU. He presented this paper at the national meeting of the American Chemical Society at Dallas in April, 1998.

Tina M. Montgomery is a senior biology major at Henderson State University. In the 1997-98 academic year, Tina received a Student Undergraduate Research Fellowship from the Arkansas SILO Advisory Council for this work.

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