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Exponential Fit to Food Degradation Experiment

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Abstract

Students in the Principles of Biology I course use a lot of course time preparing solutions each week in their course based research experience. This experiment was designed to determine the degradation rates of prepared solutions of various plant-based foods (i.e., Napa cabbage, rice, peanuts, and apple) under refrigeration as a means of reducing the amount of time for solution preparation. Scaling from the original lab procedures, approximately sixty milliliters of filtrate were prepared from the listed foods and testing was conducted on a thrice-weekly schedule. The concentration of proteins and carbohydrates were measured using the Bradford's assay and Benedict's test, respectively. The data revealed a noticeable trend in degradation in protein for all the samples. The rice sample proved to be an outlier with a marked increase in reducing sugars, while the other samples gradually decreased.

Keywords: Exponential fit, Bradford's assay, Benedict's test, best fit, coefficient of determination

Mathematics Subject Classification:92-10

Introduction

In a course-based undergraduate research experience (CURE) in the Principles of Biology I lab at Savannah State University, students examined the concentration of proteins and carbohydrates in various plant-based foods (i.e., Napa cabbage, rice, peanuts, and apple). In previous semesters, students would have to prepare new samples each week, taking up valuable instructional time. Determining the length of time a single sample can be used benefits students and instructors. Students would be able to engage in data analysis and interpretation of data more while faculty could spend more time emphasizing techniques, understanding data, and developing scientific communication skills.

Organic molecules such as proteins and carbohydrates are essential to the health and function of living organisms. The stability of organic (i.e., carbon-based) molecules is an interesting and challenging topic as there are many different types of functional groups, molecular configurations, and molecular collisions to consider.

The study was done by applying the same methods that lab participants would use in the Principles of Biology I lab class. And further an exponential fit using statistical regression analysis is performed on the data and we measured coefficient of determination to see how good the fits are.

Materials and Methods

Preparing the Filtrates

We prepared two samples each of twelve grams of each food item (Napa cabbage, apple, rice, and peanut) that were ground with a mortar and pestle then mixed with 60 mL of distilled water. We filtered the mixture with funnels lined with filter paper naturally into two 30 mL test tubes. We stored the samples in a Frigidaire Electrolux refrigerator

(model no. FRT18S6AWC, manufactured 03-04) set to 5.3 on a 6-point scale on the temperature gauge, which produced temperatures less than 19°C internally. From one testing period to the next, the samples would partially or completely freeze.

Testing for Proteins and Carbohydrates and Measuring Optical Densities (OD)

We used 2.5mL of each filtrate with 2.5mL of the testing reagent (Coomassie Brilliant Blue dye for Bradford's Assay for proteins and Benedict's Reagent for testing for reducing sugars) in a cuvette for testing with a Spectronic 20D spectrophotometer; an additional tube of 2.5 mL distilled water plus the reagent were used for a blank to calibrate the spectrophotometer. For the Benedict's test, filtrates and Benedict's reagent were first warmed in a 12 mL test tube using a 100 mL beaker was filled half-way with water and gradually heated to 100°C for a water bath. One at a time, each test tube was placed within the prepared hot water bath for exactly fifty seconds and removed. This process was intended to activate the reagent's binding to the carbohydrate molecules; a water bath is not needed for the Bradford's Assay. Thereafter, the contents were transferred to cuvettes and permitted to cool for five minutes before optical densities (OD) were measured using a spectrophotometer.

After the cooling period, we recorded the optical densities (OD) at 450nm for Benedict's Test and 595nm for Bradford's Assay. With the potential of reducing sugars exceeding absorbance range on the spectrophotometer, the mixtures were serially diluted with distilled water until values were in the range of the standard curve. We avoided reheating the dilutions to avoid exacerbated carbohydrate breakdown. For first days testing, the reducing sugar content for the apple and cabbage samples expectantly exceeded expectation and necessitated three half dilutions (1:8 dilution) to compatibly fit within the spectrophotometer's absorbance range. For the future Benedict's tests, the apple and cabbage samples would undergo the same 1:8 dilution to maintain consistency. A test model for the Benedict's test revealed an inverse relation between the temperature of the treated mixtures and the given optical density.

From initial to ambient temperatures, the readings for the same cuvette would vary drastically. A five-minute cooldown period, along with avoiding reheating the dilutions, was implemented as a new control variable. After each OD was measured, we returned the samples to the refrigerator.

Experimental Data Summary

The average OD values for each sample using the two methods mentioned above are presented in the following tables.

Table 1: Average Optical Density (OD) Values for Benedict's (Reducing Sugars) Test Results

	Day 0	**Day 3**	**Day 5**	**Day 8**	**Day 10**	**Day 12**	**Day 15**
Rice	0.071	0.064	0.095	0.191	0.121	0.307	0.385
Apple	0.264	0.171	0.170	0.186	0.161	0.089	0.075
Cabbage	0.355	0.224	0.261	0.225	0.219	0.136	0.111
Peanut	0.485	0.313	0.296	0.300	0.295	0.148	0.157

Data Fitting

We used regression analysis to fit exponential functions of the form

$$\hat{y} = ae^{bx} \tag{1}$$

Table 2: Average Optical Density (OD) Values for Bradford Assay (Proteins) Test Results

	Day 0	**Day 3**	**Day 5**	**Day 8**	**Day 10**	**Day 12**	**Day 15**
Rice	0.089	0.070	0.102	0.051	0.041	0.041	0.025
Apple	0.048	0.033	0.067	0.022	0.012	0.023	0.017
Cabbage	0.085	0.07	0.099	0.022	0.016	0.010	0.005
Peanut	0.778	0.535	0.690	0.358	0.215	0.290	0.284

where $x = 0, 3, 5, 8, 10, 12, 15$ and y represents the data from the experiment, using the normal equations

$$\sum \ln y = n \ln a + b \sum x \tag{2}$$

$$\sum x \ln y = \ln a \sum x + b \sum x^2 \tag{3}$$

to obtain the values a and b. And further we used the formula

$$R^2 = 1 - \frac{\text{the sum of squares of residuals}}{\text{the total sum of squares}} = 1 - \frac{SSR}{SST} \tag{4}$$

where

$$SSR = \sum_{i=1}^{7} (y_i - \hat{y}_i)^2 \tag{5}$$

and

$$SST = \sum_{i=1}^{7} (y_i - \bar{y})^2, \quad \bar{y} = \frac{\sum y_i}{7} \tag{6}$$

to measure how good the fit is for each kind of food considered. Solving the normal equations we get the values listed in the following tables:

Table 3: Model Variables and R^2 Values

	Bradford Assay			**Benedict**		
	a	b	R^2	a	b	R^2
Rice	0.1037	-0.0859	0.7105	0.0558	0.1236	0.8759
Apple	0.0511	-0.0834	0.4305	0.2603	-0.0748	0.7659
Cabbage	0.1314	-0.2092	0.6499	0.3452	-0.0685	0.8030
Peanut	0.7453	-0.0802	0.7711	0.4543	-0.0711	0.7864

Analysis and Results

The Bradford assay results showed a predominantly negative slope, with a very good R2 values for rice and peanut but not so good for apple and cabbage, which suggests either more data is needed to get a better exponential fit or need to use a different fit. On day 3 of data collection each variable experienced a stark increase in optical density of proteins. We believe this may be due to reversing the order of testing; in this instance, the Benedict's test was completed first instead of the Bradford assay, which led us to hypothesize that larger displacement of filtrate created with this test affected the Bradford assay results, either because of variance in the filtrates' concentrations at different volume levels or by agitating the precipitate via pipetting. In either situation, the change of procedural execution was a liable contributor, particularly for the apple sample. Reviewing the Benedict's test data, the volume of reducing sugars for the apple, cabbage, and peanut were markedly declining and shows very good R^2 values (Table III). The

rice data was unanticipated with the increasing reducing sugars throughout the weeks. The prospective explanation was the process of starch gelatinization. With 90% of rice being starch, with between 1% and 37% being amylose (Techawipharat et al., 2008), the extended interaction of the hydroxyl bonds of the starch and the water promoted retrograding into basal mono- and disaccharides – into simple sugars or reducing sugars. Holger Fleischer does explore the efficacy of the iodine test, which does not require heating to show results, in lieu of the Benedict's test which was worth consideration in prospective research (Fleischer, 2019). Based on these data, there is variation in each of the samples.

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