Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(5): 239-243
Received: 01-07-2016
Accepted: 02-0-2016

Tanmai Vachhani
The Galaxy School, University Road, Opposite Akashwani Quarter, Rajkot, Gujarat, India

Correspondence
Tanmai Vachhani
The Galaxy School, University Road, Opposite Akashwani Quarter, Rajkot, Gujarat, India

Comparing *α* amylase kinetics using differential calculus

Tanmai Vachhani

Abstract
The paper's aim is to prove that there is a significant difference in kinetics of *α* amylase from three different sources. To prove this, the paper devises a new procedure that employee's spectrophotometer to measure the optical density of starch solutions and make a starch standard curve. Using the obtained information, the paper derives a mathematical derivative function for each type of amylase. The derivative function is than used to calculate the kinetics of *α* amylase.

Keywords: Kinetics, *α* amylase, starch standard curve

1. Research Question
Will there be a significant difference in kinetics of *α* amylase from three different sources - *Homo sapiens*, *Escherichia coli* and *Fungi?*

2. Hypothesis
Yes, there will be a significant difference in kinetics of all three types of enzyme sources. Even though all the three types of amylase, considered in the research paper, are *α* amylase there will be a difference in their kinetics. The primary reason for this is amylase from different sources constitute of different things, for instance, salivary amylase from *Homo sapiens* has 99.5% of water and 0.5% of electrolyte, mucus, glycoprotein, antibacterial, lysozyme and enzymes and amylase from *Escherichia coli* is composed of ribosomes, cytoplasm, DNA and many more organelles. Hence, the source that has the maximum amount of amylase would have the fastest kinetics.

3. Introduction
Amylase is an enzyme which catalysis the hydrolysis of starch. In *Homo sapiens*, salivary gland and pancreas produce amylase that breaks down incoming starch from food into glucose. Apart from *Homo sapiens*, plants, bacteria and fungus also produce amylase. Amylase is divided into three groups depending on the properties:

- Alpha amylase (*α*) - this type of amylase cannot function in absence of calcium. It breaks bonds in the long chain of starch randomly and thus converting starch into glucose. It has a optimum pH of 6.7 to 7.0 and optimum temperature of 37c. It is found in humans, plants, fungi and bacteria.
- Beta amylase (*β*) - It is synthesised by bacteria, fungi and plants but not by *Homo sapiens*. This amylase breaks two maltose molecules at a time and its optimum pH is 4.0 to 5.0.
- Gamma amylase (*γ*) - It splits the glycosidic linkages and has the most acidic optimum pH of 3.

The research paper only compares *α* amylase from three different sources. *α* amylase has many applications when it comes to industrial use. It is used in food, fermentation, pharmaceutical, textile, detergent and paper industries. It is so widely used that it constitutes for approximately 25% of enzyme market. Hence, answering the research question might help these industries to cut the production cost. Assume a hypothetical situation where a industry is using an amylase which can be replaced with another amylase with faster kinetics. The faster amylase would cut the production time and; therefore, reducing the costs.

4. Variables

Table 1: Independent Variables

Independent Variables		Measuring It
Fungal	α	
Amylase Salivary Amylase Bacterial Amylase	α α	The optical densities (±0.01 OD) of all the three variables were measured using spectrophotometer and then were plotted on standard curve of starch.

Table 2: Dependent Variables

Dependent Variables	Measuring It
Color change in solution	The rate of hydrolysis was measured by amount of starch present at different time intervals (determined by colour change).

Table 3: Control Variables

Control Variables	Method of Controlling	Rational for Controlling
The pH level	Sodium Phosphate buffer (Na_2HPO_4) was added to amylase to ensure an optimum pH of 7.	At optimum pH enzymes can hydrolyse the fastest, if it is too low or high then they might get denatured.
Temperature of reaction	All the reactions that include hydrolysis of starch were carried out at room temperature (30^oc)	Controlling temperature would ensure that the enzymes work near the optimum temperature.
Starch solution	Starch solution was prepared - 1g/L with Sodium Phosphate buffer (Na_2HPO_4) and then 0.1ml of it was used in each trial	Specific amount of starch would ensure substrate to enzyme ratio is constant
Amylase	0.1ml of amylase from each of the three sources was used in each trial	Specific amount of amylase would ensure substrate to enzyme ratio is constant
Time intervals	There were equal time intervals setup 1,2,3,4 and 5 seconds for measuring the hydrolysis of starch	Keeping time intervals constant would ensure that the results can be compared.
Starch standard curve	A standard curve of starch was prepared using dilutions and optical density	This ensures that the concentration of starch we get is relative to one standard reading.

Table 4: List of apparatus

Apparatus	Uncertainty	Quantity Required
Test Tube	-	66
Micropipette 100μ	-	1
Micropipette 50μ	-	1
Syringe (5ml)	(±0.1 ml)	5
Spectrophotometer	(±0.01 OD)	1
Spatula	-	2
Stop Watch	(±0.001 sec)	1
Measuring Cylinder (10ml)	(±0.25 ml)	1
Measuring Cylinder (50ml)	(±0.05 ml)	1
Weighing Machine	(±0.001 grams)	1
Test Tube rack	-	3-5

Table 5: Chemicals Used

Name of Chemical	Quantity used
Starch	1 gram
Sodium Phosphate Buffer (Na_2HPO_4)	2 grams
Distilled Water	800 ml
Potassium Iodide (Na_2HPO_4)	0.28 grams
Iodine (I_2)	0.03 grams
Concentrated Hydrochloric Acid (HCL)	6 ml

5. Procedure

5.1 Starch Standard Curve

Step 1: Prepare 500ml of 0.02 molar concentration of Sodium Phosphate buffer (Na_2HPO_4)

Step 2: Weigh 0.25 grams of starch on weighing machine; then add 25 ml of Sodium Phosphate buffer (Na_2HPO_4) to it and than heat it to its boiling temperature.

Step 3: Make the following dilutions using Micropipette and Syringe

Table 6: Starch Dilutions

Dilution	Starch Stock Solution (ml)	Volume of Buffer (ml)
1/1	1	0
1/2	0.5	0.5
1/3	0.4	0.6
1/4	0.3	0.7
1/5	0.1	0.9

Step 3: Measure the absorbance of each dilution at 590 absorbance using the spectrophotometer and than plot a standard curve.

5.2 Acidic Iodine Solution (Stop Solution)

Step 1: Weigh 0.28 grams of Potassium Iodide (KI) on weighing machine.

Step 2: Weigh 0.03 grams of Iodine (I) and add it to 500ml of distilled water with previously prepared Potassium Iodide (KI)

Step 3: Add 6 ml of Concentrated Hydrochloric Acid (HCL) to the above solution and preserve it in a dark space.

5.3 Measuring Kinetics of Fungal Amylase.

Step 1: Prepare 5% starch solution by adding 1.25 grams of starch in 25ml of Sodium Phosphate buffer (Na_2HPO_4). After adding starch, heat the solution to its boiling point.

Step 2: Add 0.5 grams of fungal amylase to 249.5 ml of Sodium Phosphate buffer (Na_2HPO_4). This will dilute the amylase by 500 folds

Step 3: Now take a test tube rack, place three test tubes in it. Add 0.1 ml of starch solution in each test tube using Micropipette and then add 0.1 ml of amylase. Immediately start the stopwatch after doing this.

Step 4: After 1 minute add Acidic Iodine Solution (stop solution) to all the three test tubes.

Step 5: Determine the absorbance of all the three solution present in test tube using spectrophotometer at 590 absorbance.

Step 6: Repeat the steps 3 to 5 for 2, 3, 4 and 5 minutes time interval.

5.4 Measuring Kinetics of Homo sapiens salivary amylase.

Step 1: Prepare 5% starch solution by adding 1.25 grams of starch in 25ml of Sodium Phosphate buffer (Na_2HPO_4). After adding starch, heat the solution to its boiling point.

Step 2: Add 0.5 ml of saliva and add it to 49.5 ml of Sodium Phosphate buffer (Na_2HPO_4)

Step 3: Now take a test tube rack, place three test tubes in it. Add 0.1 ml of starch solution in each test tube using Micropipette and than add 0.1 ml of amylase. Immediately start the stopwatch after doing this.

Step 4: After 1 minute add Acidic Iodine Solution (stop solution) to all the three test tubes.

Step 5: Determine the absorbance of all the three solution present in test tube using spectrophotometer at 590 absorbance.

Step 6: Repeat the steps 3 to 5 for 2, 3, 4 and 5 minutes time interval.

5.5 Measuring Kinetics of Bacterial amylase

Step 1: Prepare 5% starch solution by adding 1.25 grams of starch in 25ml of Sodium Phosphate buffer (Na_2HPO_4). After adding starch, heat the solution to its boiling point.

Step 2: Take a part from *E.coli* culture and add it to 10 ml of distilled water with a pinch of *Trypticase* enzyme.

Step 3: Now take a test tube rack, place three test tubes in it. Add 0.1 ml of starch solution in each test tube using Micropipette and than add 0.1 ml of amylase. Immediately start the stopwatch after doing this.

Step 4: After 1 minute add Acidic Iodine Solution (stop solution) to all the three test tubes.

Step 5: Determine the absorbance of all the three solution present in test tube using spectrophotometer at 590 absorbance.

Step 6: Repeat the steps 3 to 5 for 2, 3, 4 and 5 minutes time interval.

6. Data Collection and Processing

Table 7: Raw data table of starch standard curve

Dilution	Trial 1/±0.01 OD	Trial 2/±0.01 OD	Trial 3/±0.01 OD
1/1	1.3	1.2	1.2
1/2	0.58	0.57	0.57
1/3	0.39	0.38	0.38
1/4	0.23	0.24	0.24
1/5	0.4	0.3	0.3

Table 8: Raw data table of fungal amylase

Time/minutes (±0.001 sec)	Trial 1/±0.01 OD	Trial 2/±0.01 OD	Trial 3/±0.01 OD
1	1.7	1.72	1.69
2	1.42	1.48	1.49
3	1.15	1.15	1.18
4	0.87	0.85	0.89
5	0.71	0.7	0.76

Table 9: Raw data table of salivary amylase

Time/minutes (±0.001 sec)	Trial 1/±0.01 OD	Trial 2/±0.01 OD	Trial 3/±0.01 OD
1	1.84	1.74	1.74
2	1.63	1.62	1.6
3	1.54	1.53	1.53
4	1.5	1.51	1.51
5	1.41	1.43	1.49

Table 10: Raw data table of bacterial amylase

Time/minutes (±0.001 sec)	Trial 1/±0.01 OD	Trial 2/±0.01 OD	Trial 3/±0.01 OD
1	1.84	1.81	1.69
2	1.51	1.5	1.52
3	1.4	1.42	1.46
4	1.4	1.41	1.42
5	1.22	1.2	1.25

7. Processed Data

7.1 Starch Standard Curve

The average of each trial and dilution of starch from Table 7 was calculated. It was than multiplied by 5 to get an approximate value of 5% starch solution. Using these calculations one can make a starch standard curve. The following formula was used for the calculations:

$$\frac{Trial1 + Trail2 + Trail3}{3} \times 5 \quad (1)$$

Table 11: Average optical density of starch

Dilution/ml	Average OD (±0.01 OD)
1/1	6.16
1/2	2.87
1/3	1.92
1/4	1.18
1/5	0.17

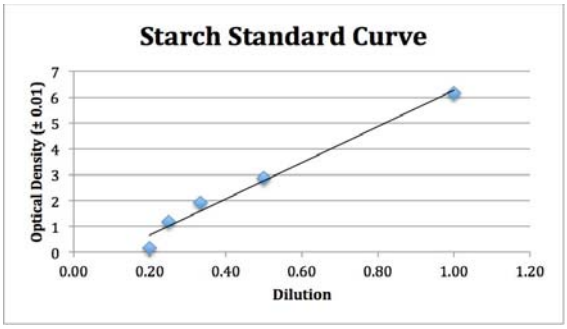

Fig 1: Starch Standard Curve

It can be deduced from Figure 1 that there is an inverse relationship between dilution and optical density. Hence, a starch solution with high optical density will have lower concentration of starch.

7.2 Processed data for Amylase.

The average of each trial and dilution of all three types of amylase was calculated from Table 8, 9 and 10. The following formula was used for the calculation:

$$Average = \frac{Trial1 + Trail2 + Trail3}{3} \quad (2)$$

$$StandardDeviation = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2} \quad (3)$$

Table 12: Average optical density of fungal amylase

Time/minutes (±0.001 sec)	Average OD (±0.01 OD)	Standard Deviation
1	1.7	0.02
2	1.46	0.04
3	1.16	0.02
4	0.87	0.02
5	0.72	0.03

Table 13: Average optical density of salivary amylase

Time/minutes (±0.001 sec)	Average OD (±0.01 OD)	Standard Deviation
1	1.77	0.06
2	1.62	0.02
3	1.53	0.01
4	1.51	0.01
5	1.44	0.04

Table 14: Average optical density of bacterial amylase

Time/minutes (±0.001 sec)	Average OD (±0.01 OD)	Standard Deviation
1	1.78	0.08
2	1.51	0.01
3	1.43	0.03
4	1.41	0.01
5	1.22	0.03

After calculating average optical density of all three amylase, *=trend* function from Microsoft Excel was employed to determine the starch content. The *trend* function plots the optical density of amylase against the starch standard curve to determine starch content.

Once the starch content was determined, starch concentration - time graph was created for all the three amylase. The graphs were used to determine the equation of the trend line, which can be used to calculate the rate of hydrolysis (kinetics).

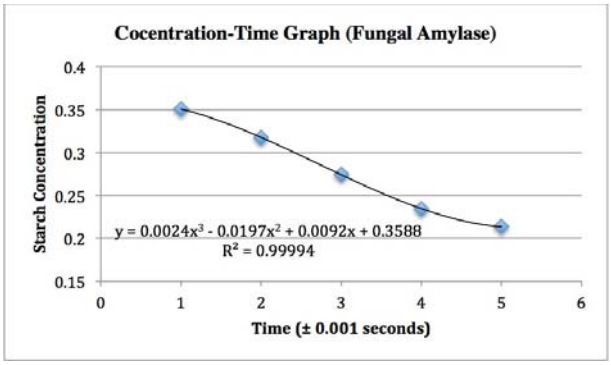

Fig 2: Starch concentration time graph - fungal amylase

$$F = 0.0024t^3 - 0.0197t^2 + 0.0092t + 0.3588 \quad (4)$$

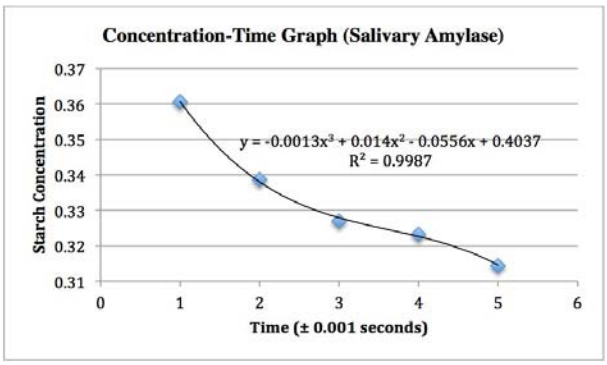

Fig 3: Starch concentration time graph - salivary amylase

$$S = -0.0013t^3 + 0.014t^2 - 0.0556t + 0.4037 \quad (5)$$

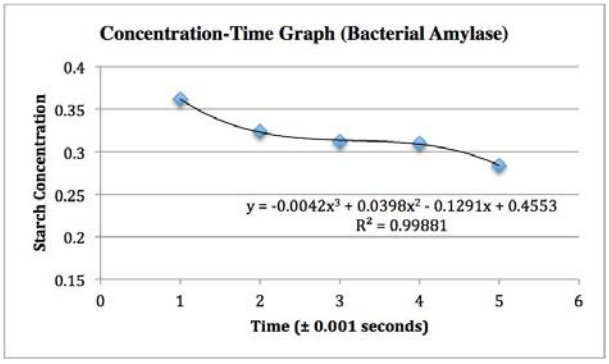

Fig 4: Starch concentration time graph - bacterial amylase

$$B = -0.0042t^3 + 0.0398t^2 - 0.1291t + 0.4553 \quad (6)$$

Equations derived from Figure 2, 3 and 4 can be used to calculate the differential equation of each amylase. The following are the differential equations:

$$\frac{dF}{dt} = 0.0072t^2 - 0.0394t + 0.0092 \quad (7)$$

$$\frac{dS}{dt} = -0.0039t^2 + 0.028t - 0.0556 \quad (8)$$

$$\frac{dB}{dt} = -0.0126t^2 + 0.0796t - 0.1291 \quad (9)$$

Using the differential equations 7, 8 and 9 the rate of hydrolysis (kinetics) of each amylase per minute can be calculated.

Table 15: Kinetics of amylase

Time/minutes (±0.001 sec)	Fungal amylase	Salivary amylase	Bacterial amylase
1	-0.02	-0.03	-0.06
2	-0.04	-0.02	-0.02
3	-0.04	-0.01	-0.01
4	-0.03	-0.01	-0.01
5	0.00	-0.01	-0.05
Average rate of hydrolysis/minute	-0.0298	-0.0145	-0.0289

8. Conclusion

The experiment was trying to determine that" Will there be a significant difference in kinetics of *α* amylase from three different sources - *Homo sapiens*, *Escherichia coli* and *Fungi?"*

It was found that there is a significant difference in kinetics of Fungal, Salivary and Bacterial amylase and; hence, the hypothesis is correct. All three types of *α* amylase under consideration have a different *average rate of hydrolysis per minute* as shown in Table 15. The fungal *α* amylase has the fastest kinetics. It hydrolyses 0.0298/ml of starch every minute. The fast kinetics of fungal amylase suggests that it has lesser quantity of biological materials such as cell organelles and more quantity of amylase compared to other sources. Thus, it would be beneficial for an industry, using amylase, to use fungal amylase as it has the fastest kinetics.

It is quite peculiar that the final answer of average rate of hydrolysis is a negative number. However, mathematically it is correct because the amount of starch is supposed to be subtracted from the total solution containing starch, when an enzyme such as amylase is added.

9. Evaluation

Table 16: Evaluation and error analysis

Error	Type of error	Significance	Improvement
The experiment should have concentration as a control variable; however, this was not possible in the experiment as lab was not well equipped.	Random	High	Use accurate equipments to achieve the desired results
There were less number of time intervals for measuring the optical density	Human	Low	Increase the number of time intervals.
Number of Trials	Human	Medium	Increase the number trials from 3 to 5, this will lead to reliable results

10. Acknowledgements

It is a genuine pleasure to express my thanking and gratitude to my biology teachers Madhuri Parmari, Deepthi Uthaman and Anila Patel. Their mentoring and guiding helped me immensely in writing this paper.

I am also extremely thankful to Kapil Savjani for guiding me through the mathematics portion of the paper.

11. References

1. Wikipedia. Saliva, 2015. Retrieved, fromWikipedia: https://en.wikipedia.org/wiki/Saliva
2. Singh S, Singh S, Bali V, Sharma L, Mangla J. Production of Fungal Amylases Using Cheap, Readily Available Agriresidues, for Potential Application in Textile Industry, 2014. Retrieved from BioMed Research International
3. Souza DPM, Magalhaes O. Application of microbial alpha amylase in industry. Retrieved from Brazilian Journal of Microbiology. 2010.
4. Worthington Biochemical Corporation Amylase, 2015. Retrieve, from Worthington: http://www.worthington-biochem.com/BA/default.html
5. Alariya SS, Sethi S, Gupta S, Gupta B. Amylase activity of a starch degrading bacteria isolated from soil. Retrieved, from Scholars, 2015.
6. Research Library, 2015. Retrieved, from http://scholarsresearchlibrary.com/aasrvol5-iss1/AASR-2013-5-1- 15-24.pdf