Biology Coursework

Is To Investigate The Effect Of Bile Salts On The Digestion Of Fat

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Variables

Controlled variables are the variables, which should be kept constant to ensure a fair test.

Controlled variables are: - **The same temperature (37oC)**
The same amount of time taken (10mins)
The same volume of lipase
The same volume of milk
The same volume of sodium carbonate
The same variety of milk
The same equipment used

From my preliminary experiment I found that these controlled variables worked well and feel that I should use the same controlled variables in this experiment.

Independent variable is the variable you change in the experiment.

Independent variable is: - The concentration of bile salts. The concentrations of bile salts I will use will be 0%, 1%, 2%, 3%, 4% and 5%.

I decided to pick these as I wanted a wide range of concentrations of bile salts to see what effect the concentrations will have on the rate of reaction.

Dependant variable is the variable you measure in the experiment.

Dependant variable is: - The pH of the mixture

Prediction

I predict that as the concentration of bile salts increases, also the rate of reaction will increase the activity of lipase in the solution. This will in turn make the amount of fats emulsified into smaller droplets increase. Therefore a larger surface area for the enzyme lipase to break down the triglycerides to fatty acids and glycerol. This is because the more surface area the more active sites for the substrate concentration to get to for the enzyme-substrate complex to take place. Therefore the reaction will take place quicker as there is more enzyme –substrate complex taking place.

Preliminary Experiment

I carried out a preliminary experiment, firstly to see what variables I will use and change and also to get to grips with the equipment that I will use in the actual experiment.

In the preliminary experiment the method was:-

- **Collect apparatus and set up apparatus.**
- **Wash some apparatus used if appropriate with water and then with distilled water.**
- **Put the following into four different beakers, mark each one clearly :**

  1. Milk with bile
  20cm^3 of milk
  10cm^3 of sodium carbonate
  5cm^3 of bile salts

  2.Milk without bile
  20cm^3 of milk
  10cm^3 of sodium carbonate
  5cm^3 of distilled water

  3. Cream with bile
  20cm^3 of cream
  10cm^3 of sodium carbonate
  5cm^3 of bile salts

  4. Cream without bile
  20cm^3 of cream
  10cm^3 of sodium carbonate
  5cm^3 of distilled water
- **Place the four beakers, in the water bath, which is set at 37oC. Allow five minutes for temperature equilibration.**
- **Start recording the pH by using pH probes linked to the la ptop via analogue to digital converter and immediately add 5cm^3 of lipase solution, into each beaker. Record the pH change by printing off the data.**

From carrying out my preliminary experiment I have gained appropriate knowledge and skill of how to use the apparatus correctly. I am going to use milk in my actual experiment instead of cream because milk is a lot cheaper. I am going to stir each solution every 30 seconds in the actual experiment to mix it up more so that it doesn't settle. Also I will take the measurements of the solutions in the syringes to the bottom of the meniscs level. Overall this will make my results more precise.

Results

The results will be recorded initially by using a tabular format. (see below)

Using the information obtained from above, the results will also be displayed in graphical format. (see below)

pH

Time (mins)

Apparatus

- **Water Bath (set at 37oC)**
- **100cm^3 Beaker ***
- **20cm^3 plastic syringe ***
- **10cm^3 plastic syringe ***
- **5cm^3 plastic syringe ***
- **full fat milk**
- **Lipase solution 5%**
- **Sodium carbonate solution 0.1mol dm**
- **Stock solution of 5% bile salts**
- **Distilled water**
- **pH probe ***
- **Datalogger**
- **Laptop**

*** means wash with water and then distilled water.**

Drawing of apparatus

Safety

During the course of the experiment it is imperative that the following safety checks are carried out.

1.
2. **Standard laboratory safety rules must be observed.**
3. **Take care when working with mains voltage – equipment (laptops etc..) near to water and other aqueous solutions.**

METHOD

1. **Collect all apparatus listed. Set up apparatus as shown in diagram.**

2. **Wash all apparatus stared (*) with water and then distilled water.**

3. **Put 20cm^3 of full milk into two beakers, followed by 10cm^3 of sodium carbonate solution in each beaker.**

4. **Then for the first concentration of bile salts which is 0% put 5cm^3 of distilled water (see page for the other concentrations measurements)**

5. **Place the two beakers in the water bath, which is set at 37oC. Allow 5 minutes for temperature equilibration.**

6. **Start recording the pH by using pH probes linked to the laptop via analogue to digital converter and immediately add 5cm^3 of lipase solution, into each beaker. Stir the solution every 30 seconds. Record the pH change by printing off the data.**

7. **Repeat the process for 1%, 2%, 3%, 4% and 5% of bile salts, using fresh mixtures of milk, sodium carbonate (as detailed for the experiment of 0% of bile salts.) To each repeat, add the enzyme lipase as you immediately start recording the pH.**

Concentration of bile salts

Concentration (%)	Distilled water (cm^3)	Stock solution of bile salts (cm^3)
0	5	0
1	4	1
2	3	2
3	2	3
4	1	4
5	0	5

Each beaker will contain for the different concentrations:-

For 0% of Bile = 20 cm^3 of milk
= 10cm^3 of sodium carbonate solution
= 5 cm^3 of distilled water
= 5 cm^3 of lipase

For 1% of Bile = 20 cm^3 of milk
= 10cm^3 of sodium carbonate solution
= 4 cm^3 of distilled water
= 1 cm^3 of bile salts
= 5 cm^3 of lipase

For 2% of Bile = 20 cm^3 of milk
= 10cm^3 of sodium carbonate solution
= 3 cm^3 of distilled water
= 2 cm^3 of bile salts
= 5 cm^3 of lipase

For 3% of Bile = 20 cm^3 of milk
= 10cm^3 of sodium carbonate solution
= 2 cm^3 of distilled water
= 3 cm^3 of bile salts
= 5 cm^3 of lipase

For 4% of Bile = 20 cm^3 of milk
= 10cm^3 of sodium carbonate solution
= 1 cm^3 of distilled water
= 4 cm^3 of bile salts
= 5 cm^3 of lipase

For 5% of Bile = 20 cm^3 of milk
= 10cm^3 of sodium carbonate solution
= 5 cm^3 of bile salts
= 5 cm^3 of lipase

Method for connecting pH probes to the datalogging box.

1. **Collect tray with the dataloggers and pH sensors.**
2. **Plug in the power to the box.**
3. **Plug in the pH adaptor into the short connecting lead.**
4. **Plug this into port 1 of the box.**
5. **Plug in pH probe to the pH adaptor (push and screw)**
6. **Keep the pH probe in distilled water when not in use.**
7. **You can use another probe by connecting it into the box.**

Using the datalogger.

1. **Push 'enter' using the scroll down keys.**
2. **Go to 'system' and push 'enter'.**
3. **Scroll down to 'set time' and push 'enter'.**
4. **'Set date' and push 'enter'**
5. **Press 'stop'.**
6. **Scroll to 'remote' and press 'enter'.**
7. **Set duration to whatever length of time you want your results to be recorded for eg 10 minutes. This time will set the trigger.**
8. **Press 'enter' to start logging.**
9. **At the end of logging press 'enter'.**

Transferring the data from the box to the laptop

1. **Log on: Science**
2. **User name: pupil**
3. **Click on 'graph' icon**
4. **Click on 'collect remote'**
5. **Retrieve data from appropriate time.**
6. **Drag boundary on LHS of screen to reveal the table.**
7. **Go to 'File' and then 'print', 'table'. It will give you the option to print the graph or the table.**

Introduction

Bile :- ***Bile is a bitter yellow liquid produced by the liver which helps the digestion of fat.*** **(Collins Dictionary)**

Bile :- ***A greenish liquid containing bile salts, bile pigments and cholesterol, produced in the liver and stored in the gall bladder before being released into the duodenum.*** **(Text book)**

Statistical Test

Firstly I am going to find out the standard deviation of my results. The standard deviation is a measure of the extent of which the individual measurements vary around the mean. I can calculate the standard deviation using the following formula:

$$Sx = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}$$

After we have found the Standard deviation of all the sample results we will then use the Students T test.
The main reason for caring out a test of significance is to see whether the results support the hypothesis.
The hypothesis requires a comparison between two or more samples. The difference between the samples may go from very small to very big.

Null Hypothesis = There is no significant difference between the results. Any difference has occurred only by chance.

$$\text{Now } t = \frac{\text{difference in mean}}{\text{standard error of mean}} \qquad \frac{x_x \; - \; x_y}{\frac{Sx^2}{n_x} + \frac{Sy^2}{n_y}}$$

I can then determine exact level of significance for $n_x + n_y - 2$ degrees of freedom. To see if the means of X and Y are significantly different or not.

I will now carry out the statistical tests on 0% and 5% bile at 1 minute.

0% Bile at 1 min

X	X^2
9.07	82.26
8.98	80.64
9.02	81.36
9.08	82.45
9.40	88.36
9.27	85.93
8.94	79.92
9.22	85.01
9.39	88.17
9.52	90.63
$\Sigma x = 91.89$	**$\Sigma x^2 = 844.73$**

Mean of x = 91.89 ÷ 10
= 9.189

$$Sx = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}$$

$$Sx = \frac{844.73 - \frac{(91.89)^2}{10}}{10-1}$$

$$Sx = \frac{844.73 - 844.38}{9}$$

Sx = 0.20

5% Bile at 1 min

Mean of y = 87.13 ÷ 10
= 8.713

$$Sy = \frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{n-1}$$

$$Sy = \frac{760.77 - \frac{(87.13)^2}{10}}{10-1}$$

$$Sy = \frac{760.77 - 759.16}{9}$$

Sy = 0.42

$$\text{Now } t = \frac{\text{difference in mean}}{\text{standard error of mean}} = \frac{x_x - x_y}{\frac{Sx^2}{n_x} + \frac{Sy^2}{n_y}}$$

$$t = \frac{9.189 - 8.713}{\frac{(0.20)^2}{10} + \frac{(0.42)^2}{10}}$$

$$t = \frac{0.476}{0.147}$$

t = 3.24

Is this significant?

Degrees of freedom $= n_x + n_y - 2$

$= 10 + 10 - 2$

$= 18$

The value 3.24 falls between p= 0.01 and p = 0.02 (see t table)

The level of rejection in biological investigations is normally taken at p =0.05. Therefore there is a significant difference between the two sets of results so the null hypothesis is rejected because p = 0.01.

Now t = difference in mean / standard error of mean

$$t = \frac{\text{difference in mean}}{\text{standard error of mean}} = \frac{x_x - x_y}{\frac{Sx^2}{n_x} + \frac{Sy^2}{n_y}}$$

$$t = \frac{8.602 - 7.361}{\frac{(0.44)^2}{10} + \frac{(0.15)^2}{10}}$$

$$t = \frac{1.241}{0.147}$$

t = 8.44

Is this significant?

Degrees of freedom $= n_x + n_y - 2$
$= 10 + 10 - 2$
$= 18$

The value 8.44

The level of rejection in biological investigations is normally taken at p =0.05. Therefore there is a significant difference between the two sets of results so the null hypothesis is rejected because it falls after this.

Now t = $\frac{\text{difference in mean}}{\text{standard error of mean}}$ = $\frac{x_x - x_y}{\sqrt{\frac{Sx^2}{n_x} + \frac{Sy^2}{n_y}}}$

t = $\frac{7.542 - 7.134}{\sqrt{\frac{(0.25)^2}{10} + \frac{(0.26)^2}{10}}}$

t = $\frac{0.408}{0.114}$

t = 3.58

Is this significant?

Degrees of freedom = $n_x + n_y - 2$
= 10 +10 – 2
= 18

The value 3.58 falls between p= 0.01 and p = 0.02 (see t table)

The level of rejection in biological investigations is normally taken at p =0.05. Therefore there is a significant difference between the two sets of results so the null hypothesis is rejected because p = 0.01.

Now t = $\dfrac{\text{difference in mean}}{\text{standard error of mean}}$ $\dfrac{x_x - x_y}{\dfrac{Sx^2}{n_x} + \dfrac{Sy^2}{n_y}}$

t = $\dfrac{7.270 - 7.034}{\dfrac{(0.14)^2}{10} + \dfrac{(0.12)^2}{10}}$

t = $\dfrac{0.236}{0.058}$

t = 4.07

Is this significant?

Degrees of freedom = $n_x + n_y$ -2
= 10 +10 – 2
= 18

The value 4.07

The level of rejection in biological investigations is normally taken at p =0.05. Therefore there is a significant difference between the two sets of results so the null hypothesis is rejected because it falls after this.

$$\text{Now } t = \frac{\text{difference in mean}}{\text{standard error of mean}} \quad \frac{x_x \; - \; x_y}{\frac{Sx^2}{n_x} + \frac{Sy^2}{n_y}}$$

$$t = \frac{6.903 - 6.857}{\frac{(0.12)^2}{10} + \frac{(0.86)^2}{10}}$$

$$t = \frac{0.046}{0.275}$$

$t = 0.17$

Is this significant?

$$\text{Degrees of freedom} = n_x + n_y - 2$$
$$= 10 + 10 - 2$$
$$= 18$$

The value 0.17

I will now carry out the statistical tests on 0% and 5% bile at 2 minute.

0% Bile at 2 min

X	X^2
8.40	70.56
8.20	67.24
8.11	65.77
8.17	66.75
8.31	69.06
8.51	72.42
9.19	84.46
9.26	85.74
8.99	80.82
8.88	78.85
Σx= 86.02	**Σx^2= 741.67**

Mean of x = 86.02 ÷ 10
= 8.602

$$Sx = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}$$

$$Sx = \frac{741.67 - \frac{(86.02)^2}{10}}{10-1}$$

$$Sx = \frac{741.67 - 739.94}{9}$$

Sx = 0.44

5% Bile at 2 min

Mean of y = 73.61 ÷ 10
= 7.361

$$Sy = \frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{n-1}$$

$$Sy = \frac{542.04 - \frac{(73.61)^2}{10}}{10 - 1}$$

$$Sy = \frac{542.04 - 541.84}{9}$$

Sy = 0.15

I will now carry out the statistical tests on 0% and 5% bile at 3 minute.

0% Bile at 3 min

x	X^2
7.52	56.55
7.35	54.02
7.48	55.95
7.46	55.65
8.03	64.48
7.97	63.52
7.42	55.06
7.43	55.20
7.32	53.58
7.44	55.35
Σx= 75.42	**Σx^2= 569.36**

Mean of x = 75.42 ÷ 10
= 7.542

$$Sx = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}$$

$$Sx = \frac{569.36 - \frac{(75.42)^2}{10}}{10 - 1}$$

$$Sx = \frac{569.36 - 568.81}{9}$$

Sx = 0.25

5% Bile at 3 min

Mean of y = 71.34 ÷ 10
= 7.134

$$Sy = \frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{n-1}$$

$$Sy = \frac{509.56 - \frac{(71.34)^2}{10}}{10 - 1}$$

$$Sy = \frac{509.56 - 508.94}{9}$$

Sy = 0.26

I will now carry out the statistical tests on 0% and 5% bile at 4 minute.

0% Bile at 4 min

5% Bile at 4 min

x	X^2
7.24	52.42
7.18	51.55
7.28	53.00
7.30	53.29
7.58	57.46
7.38	54.46
7.14	50.98
7.25	52.56
7.09	50.27
7.26	52.71
Σx= 72.70	**Σx^2= 528.70**

Mean of x = 72.70 ÷ 10
= 7.270

Mean of y = 70.34 ÷ 10
= 7.034

$$Sx = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}$$

$$Sy = \frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{n-1}$$

$$Sx = \frac{528.70 - \frac{(72.70)^2}{10}}{10 - 1}$$

$$Sy = \frac{494.89 - \frac{(70.34)^2}{10}}{10 - 1}$$

$$Sx = \frac{528.70 - 528.53}{9}$$

$$Sy = \frac{494.89 - 494.77}{9}$$

Sx = 0.14

Sy = 0.12

I will now carry out the statistical tests on 0% and 5% bile at 5 minute.

0% Bile at 5 min

x	X^2
7.12	50.69
6.99	48.86
7.03	49.42
7.16	51.27
7.02	49.28
7.13	50.84
7.40	54.76
7.18	51.55
7.14	50.98
7.14	50.98
$\Sigma x = 71.31$	**$\Sigma x^2 = 508.63$**

Mean of x = 71.31 ÷ 10 = 7.131

$$S_x = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}$$

$$S_x = \frac{508.63 - \frac{(71.31)^2}{10}}{10 - 1}$$

$$S_x = \frac{508.63 - 508.51}{9}$$

Sx = 0.12

5% Bile at 5 min

Mean of y = 69.64 ÷ 10 = 6.964

$$S_y = \frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{n-1}$$

$$S_y = \frac{485.08 - \frac{(69.64)^2}{10}}{10 - 1}$$

$$S_y = \frac{485.08 - 484.97}{9}$$

Sy = 0.11

I will now carry out the statistical tests on 0% and 5% bile at 10 minute.

0% Bile at 10 min

x	X^2
6.96	48.44
6.84	46.79
6.90	47.61
6.93	48.02
6.70	44.89
6.83	46.65
6.98	48.72
7.16	51.27
6.80	46.24
6.96	48.44
$\Sigma x = 69.06$	**$\Sigma x^2 = 477.07$**

Mean of x = 69.06 ÷ 10
= 6.9026

$$Sx = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}$$

$$Sx = \frac{477.07 - \frac{(69.06)^2}{10}}{10-1}$$

$$Sx = \frac{477.07 - 476.93}{9}$$

Sx = 0.12

5% Bile at 10 min

Mean of y = 68.57 ÷ 10
= 6.857

$$Sy = \frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{n-1}$$

$$Sy = \frac{470.27 - \frac{(68.57)^2}{10}}{10-1}$$

$$Sy = \frac{470.27 - 476.93}{9}$$

Sy = 0.86

y	y^2
9.09	82.63
8.48	71.91
8.80	77.44
8.77	76.91
9.14	83.54
9.23	85.19
8.65	74.82
8.90	79.21
8.05	64.80
8.02	64.32
Σy= 87.13	**Σy^2= 760.77**

y	y^2
7.51	56.40
7.20	51.48
7.26	52.71
7.27	52.85
7.40	54.76
7.58	57.46
7.39	54.61
7.56	57.15
7.20	51.84
7.24	52.42
Σy= 73.61	**Σy^2= 542.04**

y	y^2
7.17	51.41
6.84	46.79
7.35	54.02
7.27	52.85
7.05	49.70
7.07	49.98
7.15	51.12
7.31	53.44
7.06	49.84
7.10	50.41
Σy= 71.34	**Σy^2= 509.56**

y	y^2
7.02	49.28
6.82	46.51
7.21	51.98
7.13	50.84
6.96	48.44
6.98	48.72
7.04	49.56
7.19	51.70
6.99	48.86
7.00	49.00
Σy= 70.34	**Σy^2= 494.89**

y	y^2
6.97	48.58
6.75	45.56
7.13	50.84
7.05	49.70
6.93	48.02
6.91	47.75
6.96	48.44
7.12	50.69
6.87	47.20
6.95	48.30
Σy= 69.64	**Σy^2= 485.08**

y	y^2
6.85	46.93
6.71	45.02
6.96	48.44
6.88	47.33
6.80	46.24
6.74	45.43
6.86	47.06
6.97	48.58
6.81	46.38
6.99	48.86
Σy= 68.57	**Σy^2= 470.27**

$$s = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n_x} + \Sigma y^2 - \frac{(\Sigma y)^2}{n_y}}{n_x + n_y - 2}$$

$$s = \frac{741.67 - \frac{(86.02)^2}{10} + 542.04 - \frac{(73.61)^2}{10}}{10 + 10 - 2}$$

$$s = \frac{741.67 - 739.94 + 542.04 - 541.84}{18}$$

$$s = \frac{1.73 + 0.2}{18}$$

$$s = 0.11$$

$$\text{Now } t = \frac{\text{mean of X} - \text{mean of Y}}{\text{standard error of mean}} \qquad \frac{\text{mean of X} - \text{mean of Y}}{\frac{1}{n_x} \quad \frac{1}{n_y}}$$

$$s = \frac{\Sigma x^2 - \dfrac{(\Sigma x)^2}{n_x} + \Sigma y^2 - \dfrac{(\Sigma y)^2}{n_y}}{n_x + n_y - 2}$$

$$s = \frac{569.36 - \dfrac{(75.42)^2}{10} + 509.56 - \dfrac{(71.34)^2}{10}}{10 + 10 - 2}$$

$$s = \frac{569.36 - 568.82 + 509.56 - 508.94}{18}$$

$$s = \frac{0.54 + 0.62}{18}$$

$$s = 0.06$$

$$\text{Now } t = \frac{\text{mean of X} - \text{mean of Y}}{\text{standard error of mean}} \qquad \frac{\text{mean of X} - \text{mean of Y}}{\dfrac{1}{n_x} \quad \dfrac{1}{n_y}}$$

$$s = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n_x} + \Sigma y^2 - \frac{(\Sigma y)^2}{n_y}}{n_x + n_y - 2}$$

$$s = \frac{528.70 - \frac{(72.70)^2}{10} + 494.89 - \frac{(70.34)^2}{10}}{10 + 10 - 2}$$

$$s = \frac{528.70 - 528.53 + 494.77 - 494.77}{18}$$

$$s = \frac{0.17 + 0.12}{18}$$

$$s = 0.16$$

$$\text{Now } t = \frac{\text{mean of X} - \text{mean of Y}}{\text{standard error of mean}} \qquad \frac{\text{mean of X} - \text{mean of Y}}{\frac{1}{n_x} \quad \frac{1}{n_y}}$$

$$s = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n_x} + \Sigma y^2 - \frac{(\Sigma y)^2}{n_y}}{n_x + n_y - 2}$$

$$s = \frac{508.63 - \frac{(71.31)^2}{10} + 485.08 - \frac{(69.64)^2}{10}}{10 + 10 - 2}$$

$$s = \frac{508.63 - 508.51 + 485.08 - 484.97}{18}$$

$$s = \frac{0.12 + 0.11}{18}$$

$$s = 0.013$$

$$\text{Now } t = \frac{\text{mean of X} - \text{mean of Y}}{\text{standard error of mean}} \qquad \frac{\text{mean of X} - \text{mean of Y}}{\frac{1}{n_x} \quad \frac{1}{n_y}}$$

$$s = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n_x} + \Sigma y^2 - \frac{(\Sigma y)^2}{n_y}}{n_x + n_y - 2}$$

$$s = \frac{477.07 - \frac{(69.06)^2}{10} + 470.27 - \frac{(68.57)^2}{10}}{10 + 10 - 2}$$

$$s = \frac{477.07 - 476.93 + 470.27 - 476.93}{18}$$

$$s = \frac{0.14 + 6.66}{18}$$

$$s = 0.38$$

$$\text{Now } t = \frac{\text{mean of X} - \text{mean of Y}}{\text{standard error of mean}} \qquad \frac{\text{mean of X} - \text{mean of Y}}{\frac{1}{n_x} \quad \frac{1}{n_y}}$$

Student's t values exceeded with probability p

d.f.	P = 0.1	0.05	0.02	0.01	0.02	0.001
1	6.314					
2	2.920					
3	2.353					
4	2.132					
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
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26						
27						
28						
29						
30						
40						
60						
120						
∞						