

An investigation to
find the effect of bile
salts of on the
digestion of lipids e...



**ASSIGN
BUSTER**

AN INVESTIGATION TO FIND THE EFFECT OF BILE SALTS OF ON THE

DIGESTION OF LIPIDS I have investigated that if there is an effect of bile salts on the digestion of lipids in the milk. When lipids are broken down in to fatty acids and glycerol (see below), the acid lowers the pH of the mixture. To help me determine and measure the digestion of lipids, a pH indicator phenolphthalein has been used to measure the pH of the mixtures. The pH indicator will change the colour from pink to either white (if bile is not contained) or brown (if bile is contained), this will indicate that the solution is changing from alkaline to acidic.

So if I want to use phenolphthalein, I will have to firstly make the mixtures alkaline. This will be achieved by adding sodium carbonate to the milk until it is a strong alkaline which is just above pH 10. I used a universal indicator paper to find out if the pH is 10. Lipids? Fatty Acids + Glycerol My null hypothesis is that bile salts have no effect on the digestion of lipid and lipase will still breakdown the fat, because the fat in the milk itself is probably already partly emulsified, the rate of reaction will be the same. I drew a table, which showed different reagents in each tube and the time that took for the lipase to digest the lipids. The first 4 mixtures I created were controlled and were also not timed.

They were used as colour standards which were maintained throughout the investigation. This was done to make it easier to determine the end point of the digestion of lipids. In tube 1 and 2, bile was added to the mixture but no lipase was added. Phenolphthalein was not added to tube 2. These two tubes were produced to become colour standards when tube 5 and 7 are experimented.

<https://assignbuster.com/an-investigation-to-find-the-effect-of-bile-salts-of-on-the-digestion-of-lipids-essay/>

Tube 1 was produced as a colour standard to see how the solution should look like before the lipase is added. However, tube 2 was used to help me to judge the end point of the digestion of lipids. On the other hand, tube 3 and 4 were added with lipase but no bile was added.. Phenolphthalein was not added in tube 4. Also in tube 3, the lipase was heated up to make it inactive.

This when energy from heat break the bonds of the active site which changes the shape of the active site so the substrate is unable to bind with enzyme. As the lipase is inactive, it will not have any effect on the digestion so there will be no production of fatty acids to lower the pH of the solution as there is no change in the pH of the solution, there will be no change in the colour so the solution would stay pink due to the addition of phenolphthalein . Tube 4 does not contain phenolphthalein so it can act as the colour standard to determine the end point of tube 8 and 10. The time taken for the colour of the solution to change to the same colour as tube 4 will be measured.

Bile was added to tube 5, 6 and 7 but bile was not added to tube 8, 9 and 10. The effect of the addition of bile on the time taken for the digestion of lipid in milk

Treatment tube no.	Presence of bile	Time taken/ s
5	Yes	12
6	Yes	14
7	Yes	16
8	No	101
9	No	98
10	No	103

All the times are measured to 1/100th of a second but I have rounded up the numbers to the nearest second. This will make it easier for me when I calculate and if I kept it to 1/100th of a second, it would make my results more precise but I do not need to be that precise results in my experiment.

While experimenting, when I noticed that the colour of the tube that I am experimenting on had become the same colour as the standard I produced earlier, I quickly stopped the timer and recorded the time. But the time between judging the colour and stopping the timer had changed my results to some extent, so experiment is not accurate. The tubes with bile are x and the tubes without bile is y $x = ? x/n1 = 302/3 = 100.67$ seconds $y = ? y/n2 = 42/3 = 14$ seconds I will now work out the standard deviation of both the 'x' and 'y' treatments to see whether my data is very spread out and unreliable or close together and pretty reliable. $s_x = \sqrt{\frac{\sum x^2 - (\sum x)^2}{n-1}}$ I will draw a table in which I will work out ' $\sum x^2$ ' and ' $(\sum x)^2$ '.

Treatment tube number Time/seconds (x) $\sum x^2$

8	101	10201
9	99	9801
6	604	364816

1010310609 $\sum x = 302$ $\sum x^2 = 30414$ $(\sum x)^2 = 91204$ The standard deviation for the y: $s_x = \sqrt{\frac{\sum x^2 - (\sum x)^2}{n-1}} = \sqrt{\frac{30414 - (91204/3)}{3-1}} = \sqrt{\frac{30414 - 30401.33}{2}} = \sqrt{6.33} = 2.51$ The standard deviation for the y: $s_y = \sqrt{\frac{\sum y^2 - (\sum y)^2}{n-1}}$ I will draw a table in which I will work out ' $\sum y^2$ ' and ' $(\sum y)^2$ '.

Treatment tube number Time/seconds (y) $\sum y^2$

5	144	20736
6	196	38416
7	256	65536

$\sum y = 42$ $\sum y^2 = 596$ $(\sum y)^2 = 1764$ $s_y = \sqrt{\frac{\sum y^2 - (\sum y)^2}{n-1}} = \sqrt{\frac{596 - (1764/3)}{3-1}} = \sqrt{\frac{596 - 588}{2}} = \sqrt{4} = 2$ My null hypothesis is that bile salts have no effect on the digestion of lipid and lipase will still breakdown the fat, the reaction times will be the same. The critical value of probability is 0.05. The critical value at '4' degrees of freedom with probability '0.

05' is 2.776. $t_{calc} = \frac{x - y}{\sqrt{\frac{s_x^2}{n_1} + \frac{s_y^2}{n_2}}} = \frac{100.67 - 14}{\sqrt{\frac{6.35}{3} + \frac{4}{3}}} = \frac{86.67}{\sqrt{3.45}} = 147.3$

$6.35 + 4.00 = 10.35$ $\frac{10.35}{3} = 3.45$ $\sqrt{3.45} = 1.86$ $\frac{86.67}{1.86} = 46.6$

<https://assignbuster.com/an-investigation-to-find-the-effect-of-bile-salts-of-on-the-digestion-of-lipids-essay/>

416 = 25.35 As t_{calc} value is much greater than the t_{critical} value, I have rejected the null hypothesis and so there is a 95% confidence and that there is a change in the time taken for the digestion of lipids in the presence and absence of bile salts. After calculating, I can see that there is a difference in the reaction times. This is because bile salts are alkaline providing alkaline conditions for the lipase to work in (optimum conditions) so the more bile added, the more alkaline the conditions, therefore the optimum pH is reached. The pH in which an enzyme works is very important, and even slight changes in pH can affect the rate at which an enzyme works dramatically.

So bile has a big effect for this reason. When bile salts are added into the mixture, the digestion of lipid would happen much more quickly than without bile. Also Bile salts have detergent action on particles of fat, which causes them to break down or be emulsified into minute, microscopic droplets called micelles, which are only 0.5- 1.

0.5 μm in diameter. These, however, are still not small enough to pass through the membranes, and need to be broken down further. This emulsification creates a greater surface area of fat on which the lipase can work. Effectively there is more substrate, and so all the lipase enzymes are working to their full capacity. Bile is an important part of the digestion of fats. It is made in the liver, and is stored in the gall bladder.

Bile contains bile salts, electrolytes, bilirubin, cholesterol, and phospholipids. The bile salts are mainly derived from Sodium glycocholate and sodium taurocholate. The Bile salts we are using in this investigation are synthesized

sodium taurocholate. They do not work in exactly the same way as bile in our bodies, and so the effects on the digestion of fats in this investigation may be slightly slower than in real life. In the human stomach, the warmth and churning action turn solid fat into liquid.

However, because it is insoluble and does not dissolve in the watery contents of the stomach, the fats form a suspension of large droplets. Bile secreted from the liver, and stored in the gall bladder, is secreted via the bile duct into the duodenum. Although it does not contain enzymes; however bile salts such as sodium taurocholate and glycocholate emulsifies large droplets of fat into tiny globules. There are several other salts derived from cholesterol present within bile as well. This emulsification enables a larger surface area for the enzyme lipase to act upon.

However, at the high concentrations, the bile salts may inhibit the breakdown of fats, because the enzymes do not collide with the substrate molecules due to "traffic" within the mixture. As the t_{calc} value was much larger than the $t_{critical}$ value at a probability level of 0.05, I decided to find out if my results more accurate by finding out if it is greater than the $t_{critical}$ value at a probability level of 0.01, which is '4.604'.

So my t_{calc} is larger than the $t_{critical}$ so now I am able to say that I have 99% confidence, that there is a change in the reaction times for the digestion of lipids in the presence and absence of bile salts. Evaluation: The procedure I have used for this experiment was suitable because I have fair and reasonable results. The results I produced followed a trend which was predicted. This showed me that my results were reliable.

. Techniques followed during the experiment allowed that the results were quite reliable. ' Treatment tubes 5-7' had the same volume of different reagents and therefore were the same. This is also true for ' treatment tubes 8-10'.

Hence, three readings were taken for both; with and without bile. This ensured that if there were any anomalies, then they would show up. After this the mean of the results were taken. This procedure and method did make the experiment more reliable as it was an average of 3 different results instead of using just one result.

Also, the total volumes of the reagents were kept constant throughout the experiment. This made the experiment fair as if this step was not practised, then the times would be different. If one test tube had 10cm³ and the other had 5cm³, then although both have or do not have bile, the 10cm³ treatment tube will take much longer than the 5cm³ tube. Also, the same type of milk was used throughout the experiment.

This made the experiment fair as if different types of milk was used in the experiment, inaccuracies would be caused. If in one treatment, normal milk was used, in another semi-skimmed milk was used and in another fat-rich milk was used, then all the different types of milks would have different concentrations of fat. Also, using the same species' milk was important as different species have different concentrations of fat in their milk. A change in concentration would affect the rate of enzyme activity. In high-fat milk, the rate will increase as there is a high concentration of fat. Furthermore, throughout the experiment, I did not change the temperature.

This is because if the temperature was changed then the test would not have been fair as temperature is a factor affecting the rate of enzyme activity. If the temperature was increased, the rate of enzyme activity would also increase and vice versa. This is because particles would gain more energy and move faster and there would be more chance of a substrate molecule interacting with the active site of the enzyme. This would happen until the temperature was raised to a certain optimum after which if the temperature was increased, the rate would rapidly decline as the enzyme begins to unravel. Also, during the experiment, I used syringes to place my reagents into the test tubes. I also mixed the solution thoroughly after placing in any new reagent to ensure that the reagents had mixed properly.

I also made sure that the initial pHs of all the different treatments were the same. This was done by first making the solution, checking the pH, and then placing them into the different treatment tubes. This was necessary as if one treatment had a higher pH and one had a lower one, then the one with the higher pH would take much longer to reach the end-point. Control tubes were also made to act as colour standards so that the colour of the treatment tubes can be compared to them to determine the end-point. As all these precautions were taken and adhered to, I can say that the result were pretty accurate, fair and reliable. If these techniques were not followed, the rate of enzyme activity would be changed and a fair result on the effect of bile in the rate of digestion of milk fat would not be made.

However, there were quite a few errors in the procedures followed which could have caused inaccuracies in the results. Firstly, although the

temperature was not changed, however it was not monitored either to see <https://assignbuster.com/an-investigation-to-find-the-effect-of-bile-salts-of-on-the-digestion-of-lipids-essay/>

whether the temperature changed on its own accord. If the temperature changed halfway through the experiment, there would be a change in the rate of enzyme activity. If the temperature increased the rate of lipase activity would increase and vice versa. This is because as the temperature increases, the particles gain more energy and move faster. Hence, there is a higher chance in the interaction of a particle with an active site of an enzyme.

If the temperature was increase past a certain optimum, the rate of lipase activity would start to decline as the atoms with the enzyme would start vibrating and the weak bonds holding together the tertiary structure of the enzyme would break. This would make the specific shape of the active site unravel and the particle would no longer fit into it. This source of error could be eliminated by controlling the temperature. An electronic water bath could have been used to maintain a temperature. The thermostat could have been set and the reagents placed into the water bath.

This would make sure that there would be no effect of temperature on the rate of enzyme activity and a fair result for the effect of bile could be obtained. This would greatly improve the reliability of the experiment. I had used a syringe to measure out the reagents. However, the syringes, although quite accurate, have a much higher percentage error than burettes and pipettes. This is because burettes and pipettes are much thinner.

In a syringe, if you erred slightly from a marked quantity, then it would be quite a lot compared to if you erred by the same in a pipette or burette. This could have caused inaccuracies in my experiment as if one reagent was

added slightly more or less than the relative amounts of each reagent would change. Hence, by using a pipette or burette, if there was an error there would not be as much a change in the relative concentrations and therefore would be more accurate than syringes. The more accurate the measurements taken, the more reliable the experiment will be. Also, determining the end-point was difficult.

This was because by fixing your eye on the treatment tube, it would seem to be a bit pinkish, although in reality they were not. The colour standards did help to a certain extent to remove this doubt, however some still remained. This could be avoided by doing the treatments in special test tubes which can be inserted into a colorimeter. The control tubes can be placed in the colorimeter so that it can be used as a standard and then when the treatment tube is placed and when the value on the colorimeter is according to the standard, the time should be stopped and noted.

The stop clocks used were only accurate up to 1/100th of a second. To further improve the accuracy, I could have used a stopwatch that was accurate to the nearest millisecond. However, I think that this would not be too ideal as this would be implying precision that was not upheld. The reaction time that it would have taken me to stop the timer would have been much greater than a millisecond.

Also, whilst starting the timer, it was hard to pour the lipase into the solution, mix it thoroughly with a glass rod and start the timer simultaneously. Ideally, the timer should be started when the first drop of lipase mixes with the solution. However, this was very hard to determine with the naked eye. Data

loggers could have been used so that they would start a timer as soon as the first drop of lipase dropped into the solution and stop it when the reaction ended. This would make the recorded times more accurate and therefore the experiment more reliable. Also, I might not have mixed all the solutions in the same manner.

It is highly possible that I mixed one more than another which would affect their rates. In this experiment there were no obvious anomalous results. This emphasises on the accuracy of the experiment. However, as I have mentioned the places of improvement above, these improvements are useful to make the experiment more accurate.

These improvements may show that there might have been a trend or pattern of mistakes in the experiment, hence not resulting in an obvious anomaly. I never chose to collate any group data and work out the average of the total lot as this would not be accurate and reliable. This is because different people will have had different initial pHs as they would have added different amounts of sodium carbonate. A solution with a higher pH would take longer to end and one with a lower pH would take less time. Due to this, the standard deviation might also be very large and make it seem as though the data is not reliable.

This would also have an effect on the t_{calc} value.