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Starch Hydrolysis by Amylase and Detergents

The effect of amylase concentration on the breakdown of starch, and the comparison of hydrolysis of starch by different brands of laundry detergents at different temperatures.

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STARCH HYDROLYSIS BY AMYLASE AND DETERGENTS

OLIPHANT SCIENCE AWARDS | SIENNA HILL | OUR LADY OF THE SACRED HEART

ABSTRACT

Amylase is an enzyme that is commonly added to laundry detergents to remove stains containing starch. The purpose of this investigation was to determine the optimum concentration of amylase that best breaks down starch, and to then compare different brands of laundry detergent for the hydrolysis of starch at different temperatures.

In Part A, the three concentrations of alpha-amylase that were tested were 1%, 5% and 10%. The results demonstrated that the 10% amylase was the most effective at breaking down starch. In Part B, three brands of laundry detergent – Almat, Dynamo and Radiant, were assessed for their effectiveness in digesting starch at both 20°C and 37°C. The results showed that the Almat detergent performed best at 37°C, however, the Dynamo and Radiant detergents were more effective at hydrolysing starch at 20°C. This investigation revealed that the best performing detergent in eliminating starch stains was Almat (37°C) and the worst performing detergent was Dynamo (37°C). This result suggests that Almat had the highest concentration of Amylase and that Dynamo had the lowest concentration of amylase.

Interestingly, the results also showed that at 37°C, the Almat laundry detergent was more effective at digesting starch compared to the 10% amylase at 37°C. This result implied that the Almat detergent had a concentration of amylase that was higher than 10%. At 37°C both the Dynamo and Radiant was less effective at breaking down starch compared to the 10% amylase at 37°C but was more effective than the 5% amylase at 37°C. These results implied that both detergents had an amylase concentration between 5-10%. It is concluded that to increase detergent performance in eliminating starch, both Dynamo and Radiant should increase their concentration levels of amylase.

In Part A, the optimum concentration of amylase was discovered through heating starch and amylase to 37°C and then mixing them. The presence of starch was detected using iodine. To assess the degree of starch digestion, the mixed starch/amylase solution was added to the iodine at 2-minute intervals. In Part B, a similar method was followed for finding the best laundry detergent, except the reaction was compared at two different temperatures (20°C and 37°C). From the results obtained, it was concluded that the first hypothesis in Part A was supported whereas the second hypothesis in Part B was not supported.

INTRODUCTION

Enzymes are proteins that are produced by any living organism, and they act as biological catalysts, meaning that they speed up the rate of a chemical reaction. A chemical reaction is a process that converts one or more substances to another type of substance, and these catalysts can speed this up, without being affected themselves. 'Enzymes are made up of amino acids, in the form of a chain'[11]. They have a special area that is shaped in a certain way, called the active site.

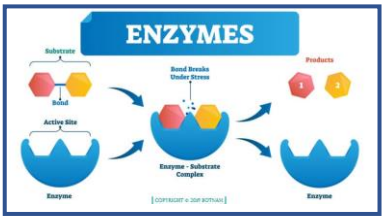


Figure 1 - Botnam. 2019. Enzymes Are Proteins: A Definitive Guide Of 4000+ Words (Updated). [picture]] Available at: <https://botnam.com/enzymes/> [Accessed 2 July 2020].

Substrate(s) are complementary in shape to their enzyme's active site and this enables the substrate(s) to bind to the active site, much like how a key can fit into a lock (*Figure 1*). At this stage, an enzyme-substrate complex has formed (*Figure 1*). 'Usually, the substrate is held in place by weak bonds, and then an 'induced fit' occurs, where the enzyme can alter its shape so that the substrate fits perfectly' [10]. The active site is where the chemical reaction occurs. Depending on the reaction, the enzyme can either break down the substrate into two products or join substrates together to form a larger product. If an enzyme becomes overheated, it may denature. This means that the shape of the active site has changed, so consequently the substrate can no longer bind to the active site.

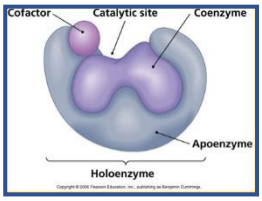


Figure 2 - Biovision.com. Coenzymes & Cofactors | Metabolism Assays | <https://www.biovision.com/products/metabolism-assays/coenzymes-cofactors.html> [Accessed 2 July 2020].

Enzymes do not work alone, as they sometimes require the help of cofactors and coenzymes (*Figure 2*). Cofactors are typically metal ions (e.g. Iron), and coenzymes are organic molecules (e.g. Vitamins). They can be found next to the substrate, or on the active site (*Figure 2*). 'The cofactors and coenzymes help enzymes build up or break down substrates into products' [11]. The difference between the two is the fact that coenzymes are organic substances (meaning they contain carbon), and cofactors are inorganic substances (meaning they lack carbon-hydrogen bonds).

Enzymes can be found throughout our bodies and are an integral part of the digestive system. 'Digestive enzymes are secreted in the pancreas, stomach, and small intestine' [2]. These enzymes include protease which breaks down proteins, pancreatic lipase that breaks down fats, and amylase, which can be found in our saliva. Amylase acts as a catalyst for digestion, and it breaks down large starch molecules into smaller sugar molecules (*Figure 3*). Starch is broken down into maltose, which then is converted into glucose which is used for energy (*Figure 4*).

'Starch is a carbohydrate that is present many foods and is very important for the human body' [5]. It can be found in potatoes, bread, pasta, and beans. Starch is stored in plants and is used as an energy source. It is kept in the chloroplasts and amyloplasts (both organelles in the plant cell). Starch is made up of two specific polymers (chains of monomers) and two examples include amylopectin and amylose. Amylase is the enzyme that breaks down amylose, which is found in starch. This type of digestion occurs in our mouth and in the small intestine.

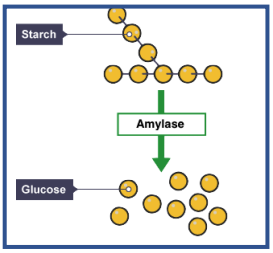


Figure 3 - Igcse-biology-2017.blogspot.com. 2020. 2.29: Understand The Role Of Digestive Enzymes. [online] Available at: <http://igcse-biology-2017.blogspot.com/2017/06/229-understand-role-of-digestive.html> [Accessed 2 July 2020].

'In some detergents, proteases, lipases, and amylases can be found to improve detergency' [1]. Most detergents include amylase enzymes to aid in the hydrolysis of starch. These enzymes help remove tough starch containing stains, and the process is environmentally friendly because they reduce the use of toxic detergent ingredients.

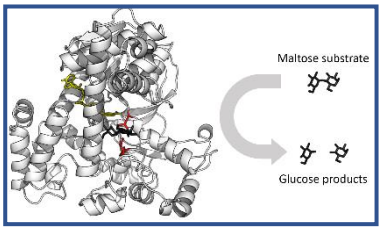


Figure 4 - En.wikipedia.org. 2020. Enzyme. [online] Available at: <https://en.wikipedia.org/wiki/Enzyme> [Accessed 2 July 2020].

'While proteases are the most used enzyme in detergent, amylase is not far behind' [6]. Amylase removes a variety of common food stains that contain starch. Amylase in detergent degrades starch to short-chain sugars, and common stains include sauces, gravy, and ice-cream. 'Protease acts on stains that contain proteins, and the use of protease helps break down proteins into peptides' [9]. Typical stains include blood stains, grass and soil stains. Lipases are used to break down lipids, and common stains include oil and grease. Cellulases act on dust and mud stains.

The best solution to getting rid of stains may be perceived as washing clothes at high temperatures, with vigorous mixing for a long period of time. Unfortunately, this method of washing is very expensive because of the cost of heating the water. Plus, the lengthy mixing of the clothes will shorten the life of the material. The use of enzymes allows for lower temperatures to be employed. Once the enzymes have done their job, the broken-down particles can easily be washed away with warm water in a washing machine.

Originally, the enzymes that were used in cleaning came from animal glands, but now they are produced through fermentation of fungi and bacteria (*Figure 5*). 'The three steps involved in enzyme production are fermentation, recovery, and standardisation' [13]. The fermentation in which industrial enzymes are produced begins with a vial of either dried or frozen microorganisms. The microorganism is kept at optimal pH, temperature and nutrient conditions during fermentation. After this, the next step is recovery. During this stage, the enzyme solution is separated from the biomass. Then the enzyme is concentrated through the removal of water and other impurities. Finally, standardisation occurs, which involves preparing the final cleaning product.

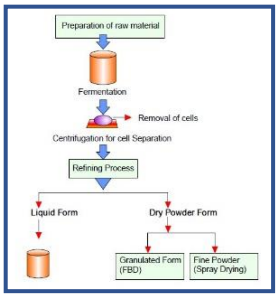


Figure 5 - Raj Process Equipments And Systems Custom Equipments Rajprocessequipments.com. Available at: <https://www.rajprocessequipments.com/detergent-powder-plant.html> [Accessed 2 July 2020].

PART A

AIM

To determine the optimum concentration of amylase that best breaks down starch. The presence of starch will be tested using dilute iodine.

HYPOTHESIS

It is hypothesised that if the 10% amylase concentration is mixed with 1% starch, then it will best eliminate starch. This is because the reaction rate will increase as the concentration of the catalyst is increased.

VARIABLES

Independent Variable

The independent variable of the practical was the amylase concentration. The concentrations of amylase that were tested were 1%, 5% and 10%.

Dependent Variable

The dependent variable of the experiment was the time taken for the enzyme/starch mixture to give a yellow colour with iodine.

Controls

The controls of the experiment were:

1. Iodine and 1% starch
2. Iodine and a-amylase (1%, 5% and 10%).

Controlled Variables

Factors held constant include:

1. Amount of amylase (10mL)
2. Amount of starch (10mL)
3. Amount of iodine added to detect the presence of starch. (1 drop)
4. Size of spotting tile dimples
5. The temperature at which the hydrolysis occurred in (37°C)
6. The pH of the amylase (pH = 7)
7. The time intervals at which the colour of the iodine was recorded (every 2 minutes)
8. The type of amylase (alpha-amylase)
9. The amount of starch/amylase solution that was added to iodine (0.5mL)

MATERIALS

1. 1 spotting tile
2. 1% Starch
3. Dilute iodine solution
4. 2 x pipettes
5. Stopwatch
6. 2 x 10mL measuring cylinders
7. Marker
8. Alpha-amylase (1%, 5%, 10%)
9. 2 x thermometers
10. 3 x 50mL beakers
11. 2 x 250mL beakers
12. Stirring rod
13. Heating block

METHOD

1. The equipment was collected and set out on the bench, and the heating block was set to 50°C.
2. The spotting tile was prepared, with a drop of iodine in each dimple.
3. Two water baths (one for the starch solution and one for the 10% amylase) were prepared by filling up two 250mL beakers with 50mL of water. Both beakers were placed on the heating block.
4. A control was prepared for the 10% amylase. To prepare this control, 10mL of 10% amylase solution was measured with a 10mL measuring cylinder which was then added to a 50mL beaker. This solution was then warmed to 37°C in a water bath as described in Step 3. 0.5mL of this 10% amylase solution was then added to a dimple in the spotting tile that contained 1 drop of iodine. The colour of the iodine solution was recorded. This control served as a guide for how the final colour should look when starch had completely digested.
5. For the second control, 10 mL of 1% starch solution was measured with a 10mL measuring cylinder which was then added to a 50mL beaker. This solution was warmed to 37°C in a water bath as described in Step 3. 0.5mL of this 1% starch solution was added to a dimple in the spotting tile that contained 1 drop of iodine with a pipette. The colour of the iodine solution was recorded. This control served as a guide for how the initial colour should look when starch was not digested by amylase.
6. For the experimental samples, 10mL of starch and 10mL of 10% amylase were then measured out using two different 10mL measuring cylinders and then placed in 50mL beakers.
7. The starch and 10% amylase were both placed in a water bath on the heating block, and a thermometer was inserted into both beakers. They were heated to 37°C - the optimum temperature for enzyme activity.
8. The starch and amylase were mixed into a third 50mL beaker, and straight away a pipette was used to place 0.5mL of the starch/amylase solution into the first dimple on the spotting tile. The colour of the iodine solution was recorded.
9. Every 2 minutes, a drop of 0.5mL of the starch/amylase solution was added to the iodine on the spotting tile, and the colour of the iodine was observed.
10. Step 9 was repeated until the iodine turned to the colour of the 10% amylase/iodine control.
11. Steps 1-10 were repeated with the 1% amylase and 5% amylase.

WHY WAS THIS METHOD CHOSEN?

This method was chosen because after completing some research, it was founded that a few different practical's involving amylase and starch followed a very similar method. The method used in this investigation was based on past experiments, such as the amylase and starch experiment method from 'Writeonline.ca. n.d. *Annotated Lab Report - Enzymes*. [online] Available at: <http://writeonline.ca/media/documents/LabReport-AnnotatedFull.pdf>'

RISK ASSESSMENT

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☑ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

NAME: Sienna Hill ID: 0462

SCHOOL: Our Lady of the Sacred Heart College

Activity: Give a brief outline of what you are planning to do.

The purpose of this investigation is to determine the optimum concentration of amylase that best breaks down starch and to compare different brands of laundry detergent for the hydrolysis of starch. Through the comparison of laundry detergents, the most effective detergent at eliminating starch will be established, and the ideal temperature for this reaction. The optimum concentration of amylase will be discovered through heating starch and amylase to 37 degrees Celsius and then mixing them.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Type of Risk	What is the risk?	How will I manage/control the risk?
Chemical risks: α-amylase solution (1%, 5% 10%), starch solution (1%), iodine solution (potassium triiodide)	Amylase: low toxicity. Liquid droplets may cause allergy or asthma symptoms or breathing difficulties, if inhaled. Lung-irritant vapour of iodine evolved from the concentrated solution. Toxic. May cause an allergic reaction on skin. Solution of iodine in potassium iodide solution, containing mostly triiodide ions. Toxicity depends on the concentrations of iodine and potassium iodide.	Avoid inhalation of aerosol droplets while handling solutions and a well-ventilated area will be used to conduct the experiment. When placing drops of iodine in the spotting tile, extra precautions will be taken to protect skin from coming in contact with iodine.

Type of Risk	What is the risk?	How will I manage/control the risk?
Thermal Risks: Electric hotplate	Possibility of burns during heating and even after hotplate is turned off because the hotplate retains heat. Electric cord may be damaged by heat and cause electric shock.	Inspect regularly for signs of damage to cord, cord loose in plug, cord loose at entry to hotplate, or any signs of corrosion or other damage. Test and tag at regular intervals. I will ensure that the hotplate has a heatproof cord.
Sharps risks: Glassware (250 mL beaker, 50 mL beaker & 10 mL measuring cylinder, spotting tile, alcohol thermometer)	Breakage of beaker, cuts from chipped rims. Breakage of thermometer, glass cylinder may break, possibility of cuts from broken glass. Tile can break to form sharp fragments, which may cause injury.	Sweep up broken glass with brush and dustpan; do not use fingers. Inspect and discard any chipped or cracked beakers, no matter how small the damage. Discard any cracked or broken measuring cylinders. Do not heat any liquid in a measuring cylinder, since not designed for heating. Inspect and discard any chipped or cracked tiles. Sweep up ceramic fragments from a broken tile with brush and dustpan; do not use fingers.
Electrical Risks: Electric hotplate	Possibility of burns during heating and even after hotplate is turned off because the hotplate retains heat. Electric cord may be damaged by heat and cause electric shock.	Inspect regularly for signs of damage to cord, cord loose in plug, cord loose at entry to hotplate, or any signs of corrosion or other damage. Test and tag at regular intervals. I will ensure that the hotplate has a heatproof cord.
Other hazards: Marker pen	Inhaling the contents may be harmful, due to toxic volatile solvents. May cause severe irritation, if used on skin as a cosmetic. An allergic reaction is possible. Pen liquid may be flammable.	Marker will be recapped tightly after use. Extra precautions will be taken to ensure I do not inhale the fumes. I will consult the safety data sheet from the manufacturer before use.

RESULTS

Colour Intensity Chart that I Developed and Used

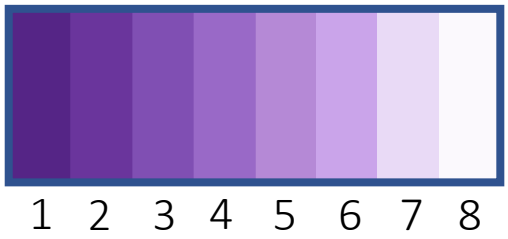


Table 1 – Results

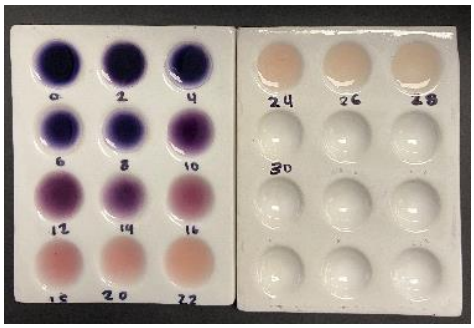
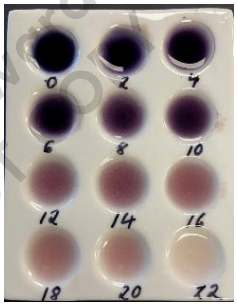
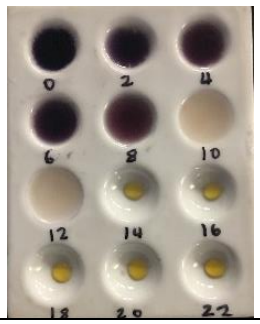
Concentration of Amylase (%)	Time (Minutes)	Colour (according to colour intensity chart)
1	0	1
1	2	1
1	4	1
1	6	1
1	8	2
1	10	2
1	12	3
1	14	3
1	16	3
1	18	4
1	20	5
1	22	6
1	24	7
1	26	8

Concentration of Amylase (%)	Time (Minutes)	Colour (according to colour intensity chart)
5	0	1
5	2	1
5	4	2
5	6	3
5	8	4
5	10	4
5	12	5
5	14	5
5	16	5
5	18	6
5	20	7
5	22	8

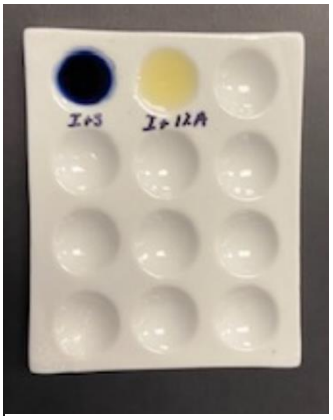
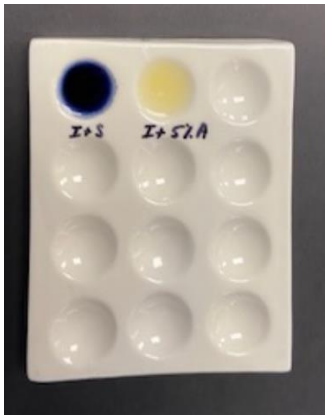
Concentration of Amylase (%)	Time (Minutes)	Colour (according to colour intensity chart)
10	0	1
10	2	1
10	4	2
10	6	3
10	8	5
10	10	7
10	12	8

Concentration of Amylase (%)	Time Taken to Completely Digest Starch (Minutes)
1	26
5	22
10	12

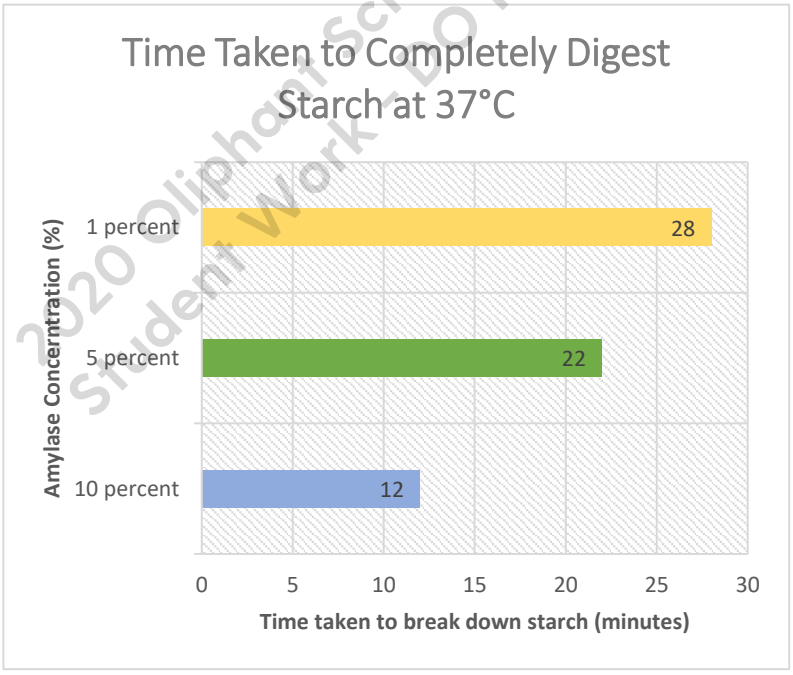
Table 2 – Images of results

Concentration of Amylase	Image of Results
1%	
5%	
10%	

Controls

Control Image (1% amylase)	Control Image (5% amylase)	Control Image (10% amylase)
 *Control for 1% Amylase.* *I + S = Iodine and starch* *I + 1%A = Iodine and 1% amylase.*	 *Control for 5% amylase.* *I + S = Iodine and starch* *I + 5% A = Iodine and 5% amylase.*	 *Control for 10% amylase.* *I + S = Iodine and starch* *I + 10% A = Iodine and 10% amylase.*

Graph of results



DISCUSSION

The practical conducted observed how the concentration of amylase affected the hydrolysis of starch. Iodine solution was used to detect the presence of starch due to the reaction that occurs between iodine and starch. The results showed that the 10% amylase was the most effective enzyme at breaking down starch. For the 1% amylase solution, the starch was completely digested at the 26-minute time point (colour intensity = 8). For the 5% amylase solution, the colour intensity was 8 at the 22-minute mark which indicated that the starch had been fully broken down. For the 10% amylase solution, the hydrolysis was completed at 12 minutes (colour intensity = 8). The general trend was that as the concentration of amylase solution increased, the time that was required for complete starch digestion decreased.

The reason for the 10% amylase solution being the most effective would be because as the concentration of the amylase increased, so did the number of active sites. This allowed the substrate (starch) to be broken down at a more efficient rate. In other words, there would be more enzymes present to collide with the substrate molecules. Increasing the concentration allows for more of the substrate to bind to the enzyme, as the amount of enzyme is higher.

The reason that the 1% amylase did not perform as well as the 10% was because, if all the enzymes bind to the substrate, the remaining substrate must wait until the enzymes become available. The enzyme would become available after the reaction, meaning that because the 1% amylase had a lower concentration, more starch was leftover. This caused the rate of reaction to be slower. Increasing the enzyme concentration (which is the limiting factor, as it determines whether the reaction can be completed at a faster rate) would speed up the hydrolysis, as it allows for another factor to become the limiting factor. It was discovered that the amylase worked best at 37°C.

The purpose of the amylase/iodine control for this experiment (*refer to Method, Step 4*) was to serve as a benchmark for colour when starch was completely digested in experimental samples. This control proved the experiment's validity, as it is the sample to which no treatment is administered (in this case, no starch was added).

The objective of the 1% starch/iodine control (refer to Method, Step 5) was to show that amylase was essential for starch breakdown. This control also served as a guide for how the initial colour should look when starch was not digested by amylase. This control that was used in this experiment proved the experiment's validity, as it is the samples to which no treatment is administered (in this case, no amylase was added).

This experiment was a fair test because the pH of the amylase concentrations was consistent, and so was the temperature at which the reactions occurred in. The reason why the pH was the same for all the amylase concentrations was because changes in the pH of the enzyme would affect the bonds that hold the enzyme together. Therefore, this would change the shape of amylases' active site and affect the rate of enzyme activity, causing the experiment to be an unfair test. This experiment was also fair because the type of amylase (alpha-amylase) and the concentration of substrate (1% starch) used were both consistent. If the concentration of substrate differed across samples, this experiment would not be a fair test because there would be either greater or fewer substrate molecules present to bind to amylase's active site. This would have affected the amount of starch hydrolysis. For this reason, it was very important to keep the substrate concentration consistent throughout the experiment.

Random errors are caused by unknown variations within the experiment that are unpredictable and difficult to avoid. These errors cannot be controlled and are found to result in slight result discrepancies. The changes affect the precision of results and are defined as natural variation within the data. It is quite difficult to minimise the effect of these errors, as they are hard to avoid. Random errors made in this investigation include the amount of iodine added to each dimple of the spotting tile, the amount of starch/amylase solution added to each iodine sample and the slight fluctuations of temperature. To reduce the effect of random errors, it is very important to repeat the experiment numerous times, increase the sample size and to calculate an average result.

Systematic errors are potential errors in the method, or possible human errors, which can be controlled by the experimenter. These errors can affect the accuracy of the results obtained within the experiment. To highlight systematic errors, it is important to conduct the experiment multiple times. A systematic error in this investigation was the minimum temperature on the temperature dial of the hot plate. The minimum temperature was 50°C, which was higher than the optimum temperature for enzyme activity. The amylase/starch solution was constantly taken on and off the hotplate numerous times throughout the experiment so that the temperature could remain as close as possible to 37°C. Unfortunately, it was very difficult to maintain a constant temperature at 37°C, which caused anomalous results at certain timepoints. If the water bath was left on the hot plate for too long, the enzyme was at risk of being denatured, which would have slowed down the rate of starch hydrolysis because the starch (substrate) could not complementary bind to amylases' (enzyme) active site as effectively. If this experiment were to be repeated next time, it would be better to use a hotplate that has a minimum temperature setting of 37°C. This would ensure that the results were accurate, and valid.

Another systematic error that occurred was that the sample readings were taken every 2 minutes. This was an error because only an approximate time for the reaction would have been recorded. Unknowingly to the experimenter, the hydrolysis of starch could have been completed between the set sample reading times. To obtain more accurate results, this error could have been improved by recording the colour of the iodine every minute.

A limitation experienced during this investigation was that only 1%, 5% and 10% concentrations of amylase were assessed due to a lack of time. The results would have been more comprehensive if more concentrations of amylase were tested (e.g. 6%, 7%, 8%, 9%, 11%, 12%, 14% and 15%). This is important because the results from Part A of this investigation were compared to Part B. An improvement for next time would be to test more amylase concentrations between 1-15%.

Another limitation would have been that currently, there was no available colour chart of starch digestion by alpha-amylase, so there was no technique to quantitatively describe the results. This meant there would have been no tool to fairly compare each reaction for each concentration of alpha-amylase. This limitation was resolved by developing and using a colour intensity chart to accurately assess the digestion of starch by the enzyme, alpha amylase.

CONCLUSION

The aim of this investigation was to determine the optimum concentration of amylase that best breaks down starch. This was achieved through trials in which different concentrations of amylase (1%, 5% and 10%) were mixed with starch and the hydrolysis of starch was observed. The hypothesis was supported because the results showed that for the 10% amylase the level 8 colour intensity was achieved at a time point of 12 minutes, whereas the 5% took 22 minutes and the 1% took 26 minutes. The reason that the 10% amylase was the most efficient concentration was because there were more alpha-amylase enzymes present and therefore more active sites available to bind with the complementary shaped starch. This caused more product to be produced because the hydrolysis of starch occurred at a faster rate.

PART B

AIM

To compare several brands of laundry detergent to determine the best detergent for breaking down starch, and to find the optimum temperature for this hydrolysis. The effectiveness of the solution would be tested using iodine solution to detect the presence of starch and the laundry detergents will be tested at 20°C and 37°C.

HYPOTHESIS

It is hypothesised that when the different types of detergents are compared, the Dynamo detergent in the hot temperature will be the most effective detergent to break down starch stains because, based on recent Australian review by a reputable organisation, it has been stated that the Dynamo Superior Stain Removal Front Loader removed chocolate ice cream stains (that contain starch) with 95% efficiency, compared to 69% (Radiant) and 71% (Almat). CHOICE. n.d. *Laundry Detergent Reviews | CHOICE*. [online] Available at: <https://www.choice.com.au/home-and-living/laundry-and-cleaning/laundry-detergents/review-and-compare/laundry-detergents> [Accessed 28 June 2020].

VARIABLES

Independent Variables

The second part of this investigation had two independent variables. The first was the detergent used to break down starch (Dynamo, Radiant, and Almat). The second was the temperature at which the experiment occurred in (20°C and 37°C).

Dependent Variable

The dependent variable of the experiment was the time taken for the starch/detergent solution to best break down starch.

Controlled Variables

The factors held constant include:

1. Amount of starch (10mL)

2. Amount of laundry detergent (10mL)
3. Amount of iodine added to detect the presence of starch (1 drop)
4. Size of spotting tile dimples
5. The time intervals at which the colour of the iodine was recorded (every 2 minutes)
6. The temperature at which the hydrolysis occurred in (20°C and 37°C)
7. The amount of starch/detergent solution that was added to iodine (0.5mL)

The Controls

The controls for this experiment include:

1. Iodine and starch (20°C and 37°C)
2. Iodine and 10% amylase (20°C and 37°C)
3. Iodine and Almat (20°C and 37°C)
4. Iodine and Dynamo (20°C and 37°C)
5. Iodine and Radiant (20°C and 37°C).

MATERIALS:

1. 1 spotting tile
2. 1% Starch
3. Dilute iodine solution
4. 2 x pipettes
5. Stopwatch
6. 2 x 10mL measuring cylinders
7. Marker
8. Detergents (Radiant, Dynamo and Almat)
9. 2 x thermometers
10. 3 x 50mL beakers
11. 2 x 250mL beakers
12. Stirring rod
13. Heating block

METHOD:

Hydrolysis of Starch at 37°C

1. The equipment was collected and set out on the bench, and the heating block was set to 50°C.
2. The spotting tile was prepared, with a drop of iodine in each dimple.
3. Two water baths (one for the starch solution and one for the detergent) were prepared by filling up two 250mL beakers with 50mL of water and were placed on the heating block.
4. A control was prepared for the Almat detergent. To prepare this control, 10mL of Almat was measured with a 10mL measuring cylinder which was then added to a 50mL beaker. This solution was then warmed to 37°C in a water bath as described in Step 3. 0.5mL of this Almat was then added to a dimple in the spotting tile that contained 1 drop of iodine with a pipette. The colour of the iodine solution was recorded. This control served as a guide for how the final colour should look when starch had completely digested.

5. For the second control, 10 mL of 1% starch solution was measured with a 10mL measuring cylinder which was then added to a 50mL beaker. This solution was then warmed to 37°C in a water bath as described in Step 3. 0.5mL of this 1% starch solution was then added to a dimple in the spotting tile that contained 1 drop of iodine with a pipette. The colour of the iodine solution was recorded. This control served as a guide for how the initial colour should look when starch was not digested by amylase.
6. For the experimental samples, 10mL of starch and 10mL of Almat detergent were then measured out using two different 10mL measuring cylinders and then placed in 50mL beakers.
7. The starch and Almat detergent were both placed in a water bath on the heating block, and a thermometer was inserted into both beakers. They were heated to 37°C - the optimum temperature for enzyme activity.
8. The starch and Almat detergent were mixed into a third 50mL beaker, and straight away a pipette was used to place 0.5mL of the starch/Almat solution into the first dimple on the spotting tile. The colour of the iodine solution was recorded.
9. Every 2 minutes, a drop of 0.5mL of the starch/Almat solution was added to the iodine on the spotting tile, and the colour of the iodine was observed.
10. Step 9 was repeated until the iodine turned to the colour of the Almat/iodine control.
11. Steps 1-10 were repeated with the Dynamo and Radiant detergents.

This method was chosen based on the method for Part A, to try to make this experiment a fair test.

Hydrolysis of Starch at 20°C

1. The equipment was collected and set out on the bench
2. The spotting tile was prepared, with a drop of iodine in each dimple.
3. A control was prepared for the Almat detergent. 0.5mL of Almat detergent was then added to a dimple in the spotting tile that contained 1 drop of iodine with a pipette. The colour of the iodine solution was recorded. This control served as a guide for how the final colour should look when starch had completely digested.
4. For the second control, 0.5mL of 1% starch solution was added to a dimple in the spotting tile that contained 1 drop of iodine with a pipette. The colour of the iodine solution was recorded. This control served as a guide for how the initial colour should look when starch was not digested by amylase.
5. For the experimental samples, 10mL of starch and 10mL of Almat detergent were then measured out using two different 10mL measuring cylinders and then placed in 50mL beakers.
6. The starch and Almat detergent were mixed into a third 50mL beaker, and straight away a pipette was used to place 0.5mL of the starch/Almat solution into the first dimple on the spotting tile. The colour of the iodine solution was recorded.
7. Every 2 minutes, a drop of 0.5mL of the starch/Almat solution was added to the iodine on the spotting tile, and the colour of the iodine was observed.
8. Step 7 was repeated until the iodine turned to the colour of the Almat/iodine control.
9. Steps 1-8 were repeated with the Dynamo and Radiant detergents.

RISK ASSESSMENT

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☑ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

NAME: Sienna Hill ID: 0462

SCHOOL: Our Lady of the Sacred Heart College

Activity: Give a brief outline of what you are planning to do.

The purpose of this investigation is to determine the optimum concentration of amylase that best breaks down starch and to compare different brands of laundry detergent for the hydrolysis of starch. Through the comparison of laundry detergents, the most effective detergent at eliminating starch will be established, and the ideal temperature for this reaction. The optimum concentration of amylase will be discovered through heating starch and amylase to 37 degrees and then mixing them.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Type of Risk	What is the risk?	How will I manage/control the risk?
Chemical risks: Laundry detergents (Almat, Dynamo, Radiant), starch solution (1%), iodine solution (potassium triiodide)	Amylase: low toxicity. Liquid droplets may cause allergy or asthma symptoms or breathing difficulties, if inhaled. Lung-irritant vapour of iodine evolved from the concentrated solution. Toxic. May cause an allergic reaction on skin. Solution of iodine in potassium iodide solution, containing mostly triiodide ions. Toxicity depends on the concentrations of iodine and potassium iodide. Starch has low toxicity, as has very low risks.	Avoid inhalation of aerosol droplets while handling solutions and a well-ventilated area will be used to conduct the experiment. When placing drops of iodine in the spotting tile, extra precautions will be taken to protect skin from coming in contact with iodine.

Type of Risk	What is the risk?	How will I manage/control the risk?
Thermal Risks: Electric hotplate	Possibility of burns during heating and even after hotplate is turned off because the hotplate retains heat. Electric cord may be damaged by heat and cause electric shock.	Inspect regularly for signs of damage to cord, cord loose in plug, cord loose at entry to hotplate, or any signs of corrosion or other damage. Test and tag at regular intervals. I will ensure that the hotplate has a heatproof cord.
Sharps risks: Glassware (250 mL beaker, 50 mL beaker & 10 mL measuring cylinder, spotting tile, alcohol thermometer)	Breakage of beaker, cuts from chipped rims. Breakage of thermometer, glass cylinder may break, possibility of cuts from broken glass. Tile can break to form sharp fragments, which may cause injury.	Sweep up broken glass with brush and dustpan; do not use fingers. Inspect and discard any chipped or cracked beakers, no matter how small the damage. Discard any cracked or broken measuring cylinders. Do not heat any liquid in a measuring cylinder, since not designed for heating. Inspect and discard any chipped or cracked tiles. Sweep up ceramic fragments from a broken tile with brush and dustpan; do not use fingers.
Electrical Risks: Electric hotplate	Possibility of burns during heating and even after hotplate is turned off because the hotplate retains heat. Electric cord may be damaged by heat and cause electric shock.	Inspect regularly for signs of damage to cord, cord loose in plug, cord loose at entry to hotplate, or any signs of corrosion or other damage. Test and tag at regular intervals. I will ensure that the hotplate has a heatproof cord.
Other hazards: Marker pen	Inhaling the contents may be harmful, due to toxic volatile solvents. May cause severe irritation, if used on skin as a cosmetic. An allergic reaction is possible. Pen liquid may be flammable.	Marker will be recapped tightly after use. Extra precautions will be taken to ensure I do not inhale the fumes. I will consult the safety data sheet from the manufacturer before use.

RESULTS

Colour Intensity Chart that I Developed and Used

Summary of Results

Type of Laundry Detergent	Time Taken to Completely Digest Starch at 20°C (Minutes)	Time Taken to Completely Digest Starch at 37°C (Minutes)
Almat	8	4
Dynamo	16	18
Radiant	8	14

Almat Detergent – 20°C

Type of Laundry Detergent	Time (Minutes)	Colour (according to colour intensity chart)
Almat	0	1
Almat	2	4
Almat	4	6
Almat	6	7
Almat	8	8

Almat Detergent – 37°C

Type of Laundry Detergent	Time (Minutes)	Colour (according to colour intensity chart)
Almat	0	2
Almat	2	5
Almat	4	8

Dynamo Detergent – 20°C

Type of Laundry Detergent	Time (Minutes)	Colour (according to colour intensity chart)
Dynamo	0	1
Dynamo	2	1
Dynamo	4	1
Dynamo	6	2
Dynamo	8	3
Dynamo	10	5
Dynamo	12	6
Dynamo	14	7
Dynamo	16	8
Dynamo	18	8
Dynamo	20	7
Dynamo	22	8

Dynamo Detergent – 37°C

Type of Laundry Detergent	Time (Minutes)	Colour (according to colour intensity chart)
Dynamo	0	1
Dynamo	2	2
Dynamo	4	3
Dynamo	6	3
Dynamo	8	5
Dynamo	10	5
Dynamo	12	6
Dynamo	14	6
Dynamo	16	7
Dynamo	18	8

Radiant Detergent – 20°C

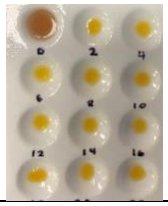
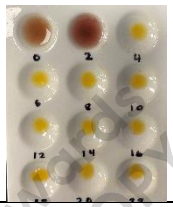
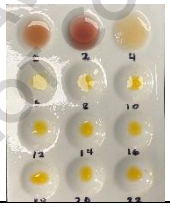
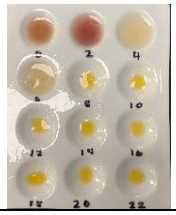
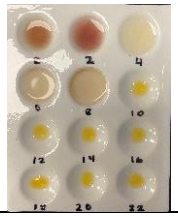
Type of Laundry Detergent	Time (Minutes)	Colour (according to colour intensity chart)
Radiant	0	1
Radiant	2	3
Radiant	4	4
Radiant	6	7
Radiant	8	8
Radiant	10	8
Radiant	12	7
Radiant	14	7
Radiant	16	8
Radiant	18	7
Radiant	20	8

Radiant Detergent – 37°C

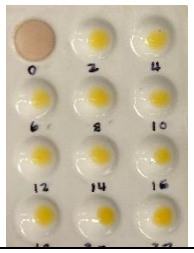
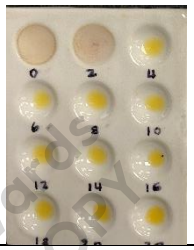
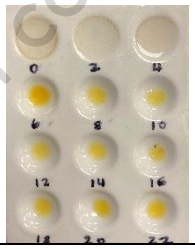
Type of Laundry Detergent	Time (Minutes)	Colour (according to colour intensity chart)
Radiant	0	2
Radiant	2	2
Radiant	4	3
Radiant	6	5
Radiant	8	6
Radiant	10	6
Radiant	12	7
Radiant	14	8

Table with images of results

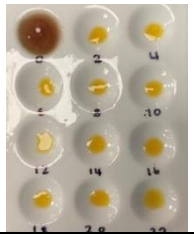
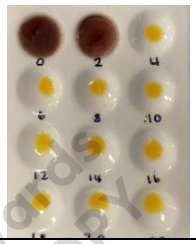
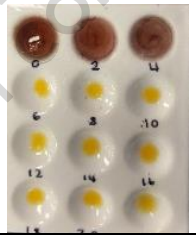
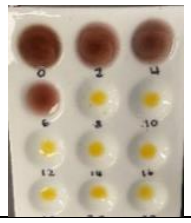
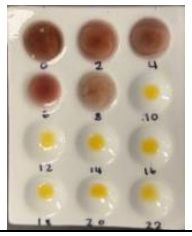
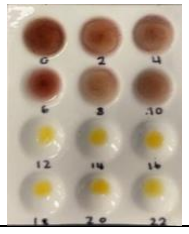
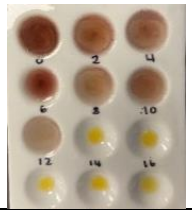
Almat Results – 20°C

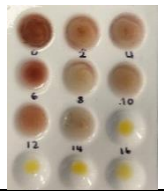
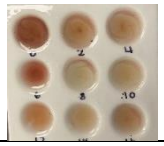
Type of Laundry Detergent	Time (Minutes)	Image of results (20°C)
Almat	0	
Almat	2	
Almat	4	
Almat	6	
Almat	8	

Almat Results – 37°C

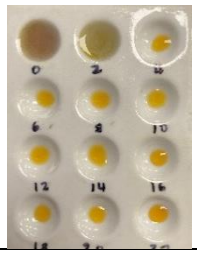
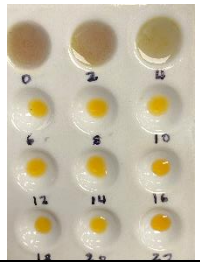
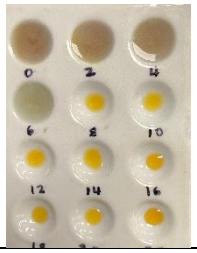
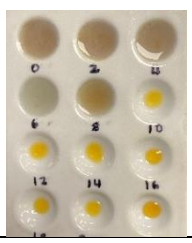
Type of Laundry Detergent	Time (Minutes)	Image of results (37°C)
Almat	0	
Almat	2	
Almat	4	

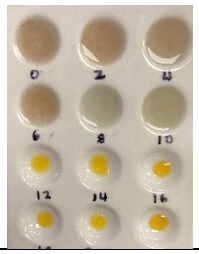
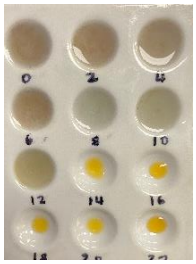
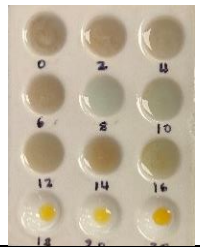
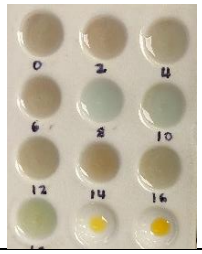
Dynamo Results – 20°C

Type of Laundry Detergent	Time (Minutes)	Image of results (20°C)
Dynamo	0	
Dynamo	2	
Dynamo	4	
Dynamo	6	
Dynamo	8	
Dynamo	10	
Dynamo	12	

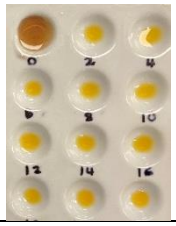
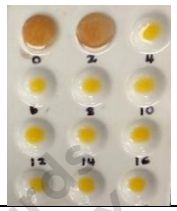
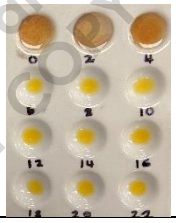
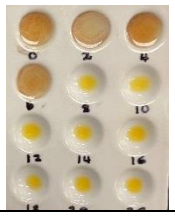
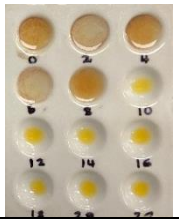
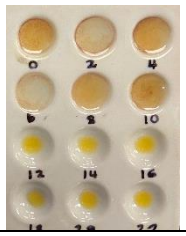
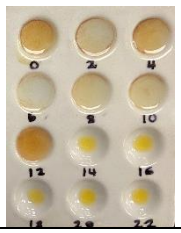
Dynamo	14	
Dynamo	16	

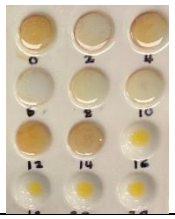
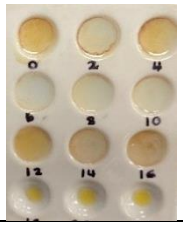
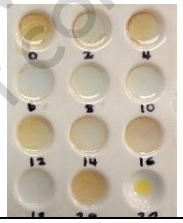
Dynamo Results – 37°C

Type of Laundry Detergent	Time (Minutes)	Image of results (37°C)
Dynamo	0	
Dynamo	2	
Dynamo	4	
Dynamo	6	
Dynamo	8	

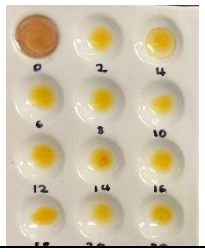
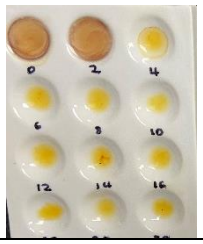
Dynamo	10	
Dynamo	12	
Dynamo	14	
Dynamo	16	
Dynamo	18	

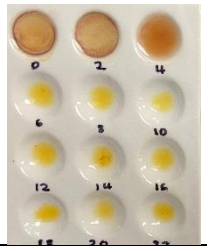
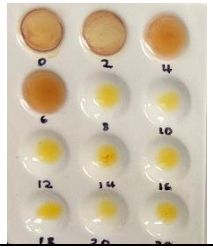
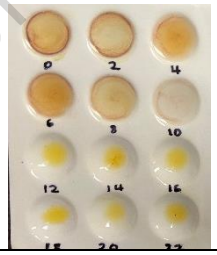
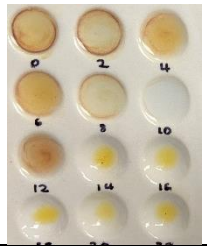
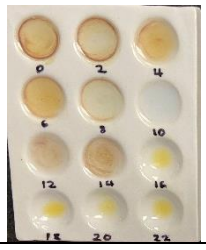
Radiant Results – 20°C

Type of Laundry Detergent	Time (Minutes)	Image of results (20°C)
Radiant	0	
Radiant	2	
Radiant	4	
Radiant	6	
Radiant	8	
Radiant	10	
Radiant	12	

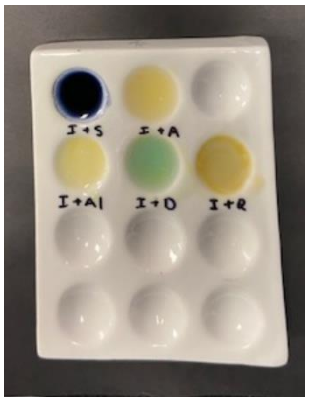
Radiant	14	
Radiant	16	
Radiant	18	
Radiant	20	

Radiant Results – 37°C

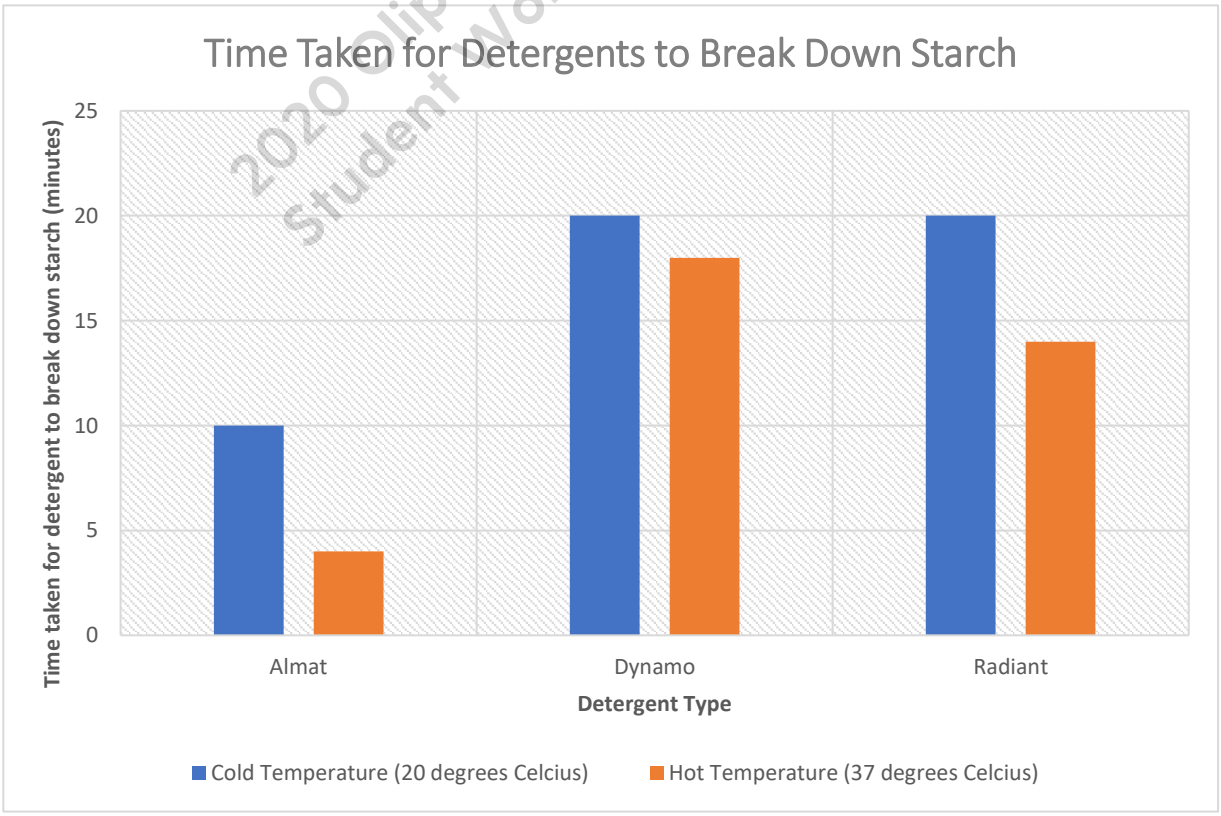
Type of Laundry Detergent	Time (Minutes)	Image of results (37°C)
Radiant	0	
Radiant	2	

Radiant	4	
Radiant	6	
Radiant	8	
Radiant	10	
Radiant	12	
Radiant	14	

Controls

Control Image (20°C)	Control Image (37°C)
	
Control for each detergent at 20°C (Almat, Radiant and Dynamo). *I + S = iodine and starch* *I + A = iodine and 10% amylase* *I +Al = iodine and Almat* *I + D = iodine and Dynamo* *I + R = iodine and Radiant*	*Control for each detergent at 37°C (Almat, Radiant and Dynamo).* *I + S = iodine and starch* *I + A = iodine and 10% amylase* *I +Al = iodine and Almat* *I + D = iodine and Dynamo* *I + R = iodine and Radiant*

Graph of result



DISCUSSION

The results showed that for the Almat detergent at 37°C, the starch was fully digested at the 4-minute time point (colour intensity = 8) and at 20°C, it took 8 minutes (colour intensity = 8). The Dynamo detergent and starch solution at 37°C took 18 minutes to reach the colour intensity of 8, and at 20°C, it took 16 minutes. For the Radiant detergent, the amylase in the detergent took 14 minutes to break down the starch in a 37°C environment, whereas it took 8 minutes in the 20°C environment.

The results showed that the Almat detergent performed best at 37°C, however, the Dynamo and Radiant detergents were more effective at hydrolysing starch at 20°C. This investigation revealed that the best performing detergent in eliminating starch stains was Almat (37°C) and the worst performing detergent was Dynamo (37°C). These results suggest that Almat had the highest concentration of amylase and that Dynamo had the lowest concentration of amylase. The reason that Almat was the most effective detergent would be because it had the most amylase enzyme present to collide with the starch substrates. This allowed the substrate (starch) to complementary bind to the active sites more readily so that it could be broken down at a more efficient rate.

A possible explanation for why the Almat digested starch better at 37°C rather than 20°C would be because at the higher temperature of 37°C, the movement of both starch and amylase was faster compared to a lower temperature of 20°C (as stated in the particle theory of matter). At the higher temperature of 37°C, more kinetic energy was gained which ended in a faster collision rate between the substrate (starch) and enzyme (alpha-amylase). At the lower temperature of 20°C, the substrate and enzyme moved slower and therefore the rate of complementary binding was less efficient. This theory suggests that an anomalous result occurred for Dynamo (37°C) and Radiant (37°C) because these detergents hydrolysed starch best at 20°C. These anomalous errors are likely to be due to systematic errors that are discussed later in the report.

Interestingly, the results also showed that at 37°C, the Almat laundry detergent was more effective at digesting starch compared to the 10% amylase at 37°C (Part A). This result implied that the Almat detergent had a concentration of amylase that was higher than 10%. At 37°C, both the Dynamo and Radiant were less effective at breaking down starch compared to the 10% amylase at 37°C (Part A) but was more effective than the 5% amylase at 37°C (Part A). These results implied that both detergents had an amylase concentration between 5-10%. The results of this experiment suggest that to increase detergent performance in eliminating starch, both Dynamo and Radiant should increase their concentration levels of amylase.

The purpose of the detergent/iodine control for this experiment (*refer to Method, Step 4*) was to serve as a benchmark for colour when starch was completely digested in experimental samples. This was especially important because some of the detergents contained a colour dye. For example, the Dynamo contained a blue colour dye, so it was important to fairly assess its benchmark colour when mixed with iodine. This control proved the experiment's validity, as it is the sample to which no treatment is administered (in this case, no starch was added).

The objective of the 1% starch/iodine control (*refer to Method, Step 5*) was to show that amylase was essential for starch breakdown. This control also served as a guide for how the initial colour should look when starch was not digested by amylase. This control that were used in this experiment proved the experiment's validity, as it is the samples to which no treatment is administered (in this case, no amylase (detergent) was added).

This experiment was a fair test because a colour intensity scale was developed and used to fairly assess the final colour of the detergent/iodine samples when the starch was fully digested. Before this investigation, there was no technique available to quantitatively describe the results of starch digestion by detergent. A colour intensity scale was integral in the results for this investigation because otherwise, the comparisons would not be fair. Part B of this investigation used a very similar method that was followed in Part A. It would not be a fair comparison if the method of Part B were different from Part A, because the results from both experiments were compared in this investigation. Part B was a fair test because there was an attempt to keep the controlled variables consistent. The controlled variables included the amount of starch (10mL), amount of laundry detergent (10mL), amount of iodine added to detect the presence of starch (1 drop), the time intervals at which the colour of the iodine is recorded (every 2 minutes) and temperature (either 20°C or 37°C). If the controlled variables were not maintained, then the results could not be fairly compared because it would be impossible to determine if the results were due to the independent variable or due to changes in the controlled variables.

Random errors cannot be controlled by the experimenter and can cause slight variations in the results. Random errors made in this investigation include the amount of iodine added to each dimple of the spotting tile, the amount of starch/detergent solution added to each iodine sample and the slight fluctuations of temperature at both 20°C and 37°C. To reduce the effect of random errors, it is very important to repeat the experiment numerous times, increase the sample size and to calculate an average result.

Systematic errors are potential errors in the method, or possible human errors, which can be controlled by the experimenter. To highlight systematic errors, it is important to conduct the experiment multiple times. A systematic error that occurred was that the samples were assessed only every 2 minutes. This was an error because only an approximate time for the reaction would have been recorded. Unknowingly to the experimenter, the hydrolysis of starch could have been completed between the set sample reading times. To obtain more accurate results, this error could have been improved by recording the colour of the iodine every minute.

Another systematic result was that the minimum temperature on the hotplate was 50°C. Consequently, the detergent/starch solution was constantly taken on and off the hotplate numerous times throughout the experiment so that the temperature could remain as close as possible to 37°C. Unfortunately, it was very difficult to maintain a constant temperature at 37°C, which at certain time points led to anomalous results. When the temperature exceeded 37°C, the amylase enzyme might have begun to change shape. This would have slowed down the rate of starch hydrolysis because the starch (substrate) could not complementary bind to amylases' (enzyme) active site as effectively. This might explain why Dynamo and Radiant digested starch more effectively at 20°C than at 37°C. If this experiment were to be repeated next time, it would be better to use a hotplate that has a minimum temperature setting of 37°C.

A limitation that occurred was that due to a lack of time, only three laundry detergents were tested for their effectiveness in starch removal. If more laundry detergents were tested, this would give the results more scope and help buyers to make better-informed decisions when buying detergents. This investigation can help consumers make better decisions when purchasing detergents, and provides information on the best temperature to wash clothes in. The results showed that for the fastest starch removal, clothes should soak in Almat detergent at 37°C for 4 minutes. This information would be very useful to people who lead a busy lifestyle. However, if people are energy and environmentally conscious, the next best detergent to soak clothes in would be the Almat detergent at 20°C for 8 minutes or in Radiant at 20°C for 8 minutes.

Another limitation would have been that currently, there was no available colour chart of starch digestion by alpha-amylase, so there was no technique to quantitatively describe the results. This meant there would have been no tool to fairly compare each reaction for each concentration of alpha-amylase. This limitation was resolved by developing and using a colour intensity chart to accurately assess the digestion of starch by the enzyme, alpha-amylase.

CONCLUSION

The aim of this investigation was to compare a few brands of laundry detergent to determine the best detergent for breaking down starch, and to find the optimum temperature for this hydrolysis. This was achieved through trials in which different detergents (Almat, Dynamo, and Radiant) were mixed with starch and the hydrolysis was observed. The results showed that the Almat detergent performed best at 37°C, however, the Dynamo and Radiant detergents were more effective at hydrolysing starch at 20°C.

The hypothesis was not supported because this investigation revealed that the best performing detergent in eliminating starch stains was Almat (37°C) and the worst performing detergent was Dynamo (37°C). These results suggest that Almat had the highest concentration of amylase and that Dynamo had the lowest concentration of amylase. The reason that Almat was the most effective detergent would be because it had the most amylase enzyme present to collide with the starch substrates. This allowed the substrate (starch) to complementary bind to the active sites more readily so that it could be broken down at a more efficient rate.

A possible explanation for why the Almat digested starch better at 37°C rather than 20°C would be because at the higher temperature of 37°C, the movement of both starch and amylase was faster compared to a lower temperature of 20°C. At the higher temperature of 37°C, more kinetic energy was gained which ended in a faster collision rate between the substrate (starch) and enzyme (alpha-amylase). This theory suggests that an anomalous result occurred for Dynamo (37°C) and Radiant (37°C) because these detergents hydrolysed starch best at 20°C. These anomalous errors are likely to be due to systematic errors which were discussed in the Discussion section in Part B of this report.

The results also showed that at 37°C, the Almat laundry detergent was more effective at digesting starch compared to the 10% amylase at 37°C (Part A). This result implied that the Almat detergent had a concentration of amylase that was higher than 10%. At 37°C, both the Dynamo and Radiant was less effective at breaking down starch compared to the 10% amylase at 37°C (Part A) but was more effective than the 5% amylase at 37°C (Part A). These results implied that both detergents had an amylase concentration between 5-10%. The results of this experiment suggest that to increase detergent performance in eliminating starch, both Dynamo and Radiant should increase their concentration levels of amylase.

APPENDIX

Equipment used

Part A

Figure 6 - Equipment used in Part A of investigation

Part B

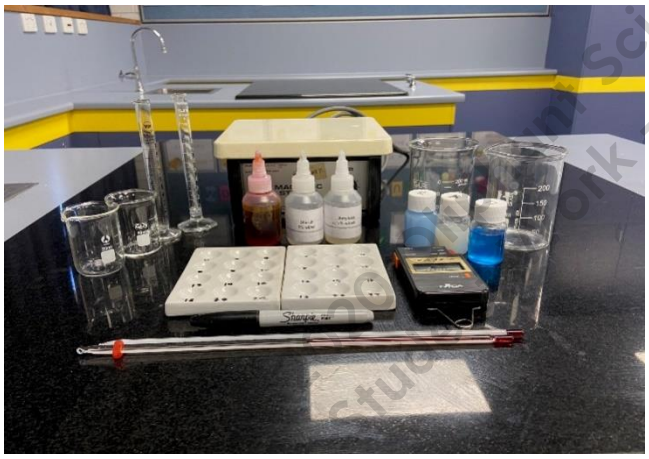


Figure 7 - Equipment used in Part B of investigation.

Figure 8 - Detergents (left to right): Almat, Dynamo, Radiant and 10% alpha-amylase.

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