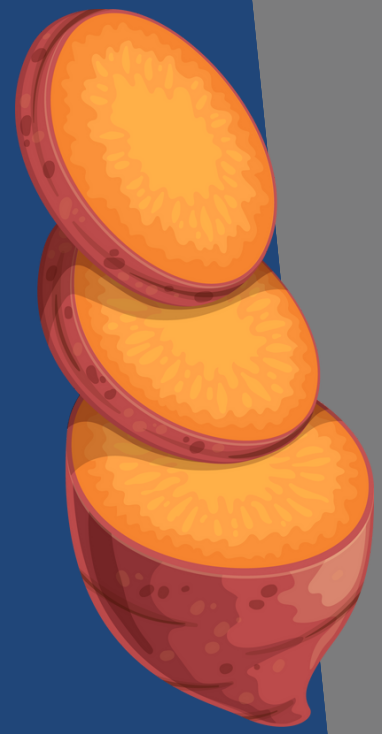
PROTOCOL

# INVESTIGATING BETA AMYLASE ACTIVITY IN SWEET POTATOES

*Extract beta amylase from sweet potatoes and investigate the breakdown of amylose*

sserc



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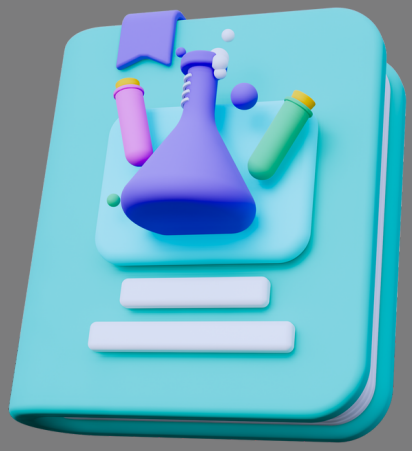


## Background

Starch is an important source of energy for many organisms. In plants, it is often found in seeds and tubers, serving as a storage form of carbohydrate. It is a mixture of two polymers called amylose (approximately 10-30%) and amylopectin. Amylose is a linear polysaccharide made of glucose units joined together with α-1,4-glycosidic bonds. Amylose chains spiral into coils and iodine snugly fits into the coils forming an amylose-iodine complex which results in iodine turning from a yellow to a blue/black colour.

The enzyme β-amylase, found in yeasts, bacteria and plants, can break every second bond to leave 2 glucose units joined together - this molecule is called maltose. When iodine is added to a solution of maltose, it does not change colour (remains yellow). This allows the breakdown of amylose into maltose to be monitored by colorimetry using iodine.

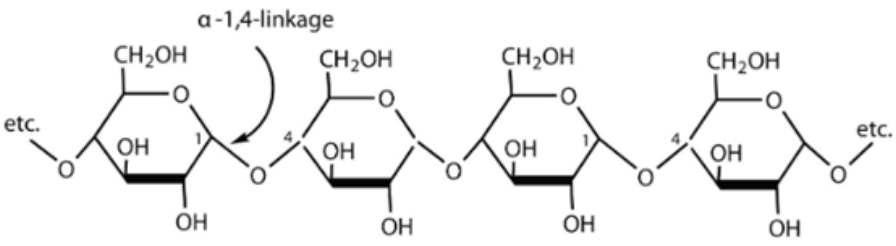



**Figure 1**: Amylose is a linear molecule formed from glucose units joined by α-1-,4-glycosidic bonds. Beta-amylase can break every second α-1,4-bond, resulting in 2 glucose units joined together - this product is called maltose.

Amylopectin is a branched-chain polysaccharide, made from glucose units joined with α-1-,4-glycosidic bonds and α-1-,6-bonds (Figure 2). However, β-amylase cannot break down the α-1-,6-glycosidic bonds and so the complex carbohydrate does not fully break down to maltose units. This means that, when reacted with iodine, the blue-black complex remains and never fully turns yellow.

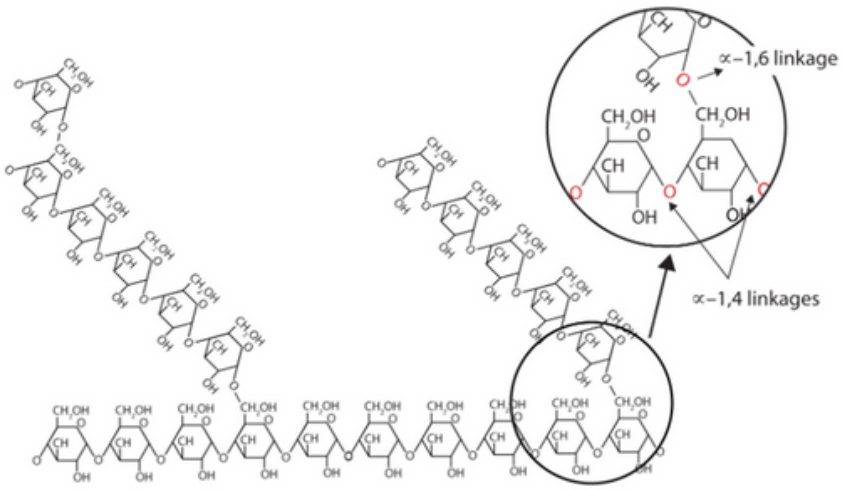



**Figure 2:** Amylopectin is a branched carbohydrate molecule formed from glucose units joined by α-1-,4-glycosidic and α-1-,6-glycosidic bonds. β-amylase cannot break down the branched linkages.

Potato starch provides the source of the substrate in this investigation. It contains both amylose and amylopectin. The amylopectin must be removed first so the effect of β-amylase on amylose can be monitored using its reaction with iodine by colorimetry. This removal might be carried out by a science technician depending on the nature of the investigation.

## AIM

**Aim: To investigate the effect of temperature on β-amylase activity in sweet potatoes.**

This protocol can be adapted to investigate:

- effect of pH on β-amylase activity
- effect of end-product inhibition on β-amylase (using maltose)
- effect of enzyme concentration on β-amylase activity.

## RISK ASSESSMENT

A risk assessment for this activity can be downloaded from the SSERC website. Click here. This should be adapted for your centre, where appropriate.

There are several hazards outlined in this model risk assessment, which should be carefully read prior to commencing this activity. Some of these hazards are pertinent to technicians, while others are more relevants to learners and/ or teachers.

## PART 1: EXTRACTION OF AMYLOSE FROM POTATO STARCH

**Materials:** These quantities below will yield approximately 70 $cm^3$ of amylose. Each investigation will require a minimum of 24 $cm^3$ amylose (allowing for accuracy / precision errors).

| | |
|---|---|
| 0.5 g Potato starch | 55 $cm^3$ 0.16 M NaOH |
| 5 $cm^3$ slightly cooled boiled water | 15 $cm^3$ 0.6 M HCl containing 0.75 g NaCl |
| Centrifuge (6000 rpm) | 6x Centrifuge tubes |
| 3 $cm^3$ Plastic pipette | Dimple tile |
| Iodine solution | 100 $cm^3$ beaker |
| Weigh boat | Spatula |
| Balance | Suitable container for prepared amylose substrate |
| Magnetic stirrer | Magnetic flea |

**Method:**

1. To the beaker, add 0.5 g potato starch with 5 $cm^3$ boiling water. Add a magnetic flea to the beaker and place on the magnetic stirrer.
2. Add 55 $cm^3$ 0.16 M NaOH to the beaker, adjusting the magnetic stirrer to the slowest setting. After 1 minute, turn off the stirrer.
3. Allow to stand with occasional swirling for 5 minutes. During this time, the suspension will separate into a clear layer (amylose) and a cloudy precipitate (amylopectin).
4. Return the beaker to the magnetic stirrer using the slowest setting. Neutralise by stirring in 15 $cm^3$ 0.6 M HCl containing 0.75 g NaCl.
5. Divide the mixture between 6 centrifuge tubes and centrifuge at 6000 rpm for 5 minutes. Amylopectin will sediment as a pellet and the supernatant will contain a solution of amylose which can be used as the substrate for β-amylase. Use a plastic pipette to transfer the supernatant to a suitable container and label this as "amylose".
6. Add a couple of drops of the prepared amylose to a dimple tile with 1 drop of iodine. Iodine should turn an intense blue-black colour.

## PART 2: EXTRACTION OF BETA AMYLASE FROM SWEET POTATOES

**Materials per pair (although this will produce enough extract for a class of 20 learners)**

| | |
|---|---|
| 30 g sweet potato | 30 $cm^3$ distilled water |
| Blender | Muslin |
| 100 $cm^3$ beaker for extract | 50 $cm^3$ measuring cylinder |
| Marker pen | Balance |
| Weigh boat | Chopping board / white tile |
| Knife | Spoon |

**Method:**

1. Blend 30 g sweet potato with 30 $cm^3$ water.
2. Filter the extract through a piece of muslin. This will produce about 25 $cm^3$ of enzyme.
3. Store the filtrate in a labelled container, e.g. beaker or universal, until required. The extract will keep in the fridge for a few days without significant loss of activity.

**Method overview**

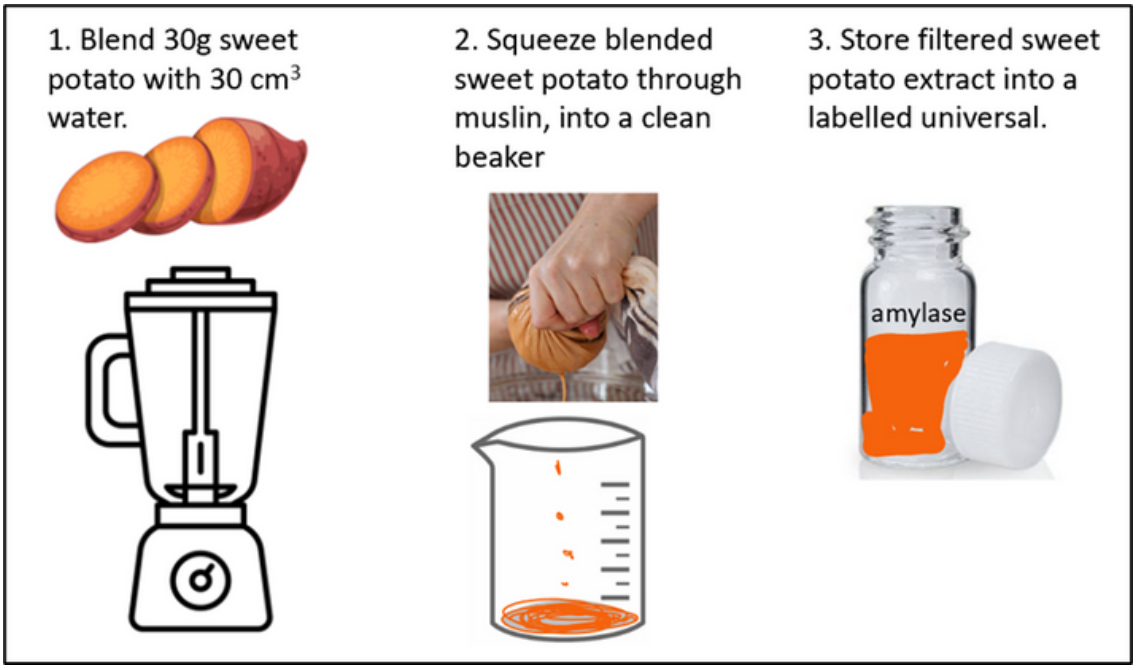



**Figure 3:** Integrated diagram of Part 2 of the investigation.




**Figure 4:** This procedure allows the extraction of beta-amylase (enzyme) from sweet potato. A 30 g sample of sweet potato is cut, blended and then passed through muslin. The collected liquid contains the enzyme and can be stored.

Although research suggests this remains activity for a few days, it is best to prepare fresh for a lesson.

These quantities should yield enough for a full class but if learners are working in pairs or groups, quantities could be reduced proportionally.

## PART 3: THE BETA-AMYLASE ASSAY

| | |
|---|---|
| 24 $cm^3$ amylose (substrate) – from Part 1 | 56 $cm^3$ 1% iodine solution made up in 0.1M HCl |
| 1200 µl β-amylase (enzyme) – from Part 2 | 7x cuvettes |
| 24 $cm^3$ citrate-phosphate buffer at pH 6.0 | Colorimeter (red diode or 635 nm) |
| 4 test tubes and rack | Thermotube block set to 60 ºC |
| thermometer | Kettle of boiling water & polystyrene cup |
| 3 $cm^3$ plastic pipettes | 100 µl automatic pipette and tips |
| Stopwatch | 10 cm x 10 cm bubble wrap (dimension to allow it to cover the top of the polystyrene cup) |
| 100 µl distilled water | Access to a Bunsen burner and tripod with a 250 $cm^3$ beaker of water |

**Iodine solution:** This is required to stop the reaction. Standard bench iodine will not stop the reaction - this must be an acidified preparation. This can be prepared as follows:

1. Combine 1 g potassium iodide with 1 g iodine in a mortar. Grind, using a pestle, to a fine powder.
2. Add a very small volume of 0.1 M HCl and mix.
3. Make up to 200 $cm^3$ with 0.1 M HCl.
4. Store in a suitable container that restricts light transmission.

**Method overview**

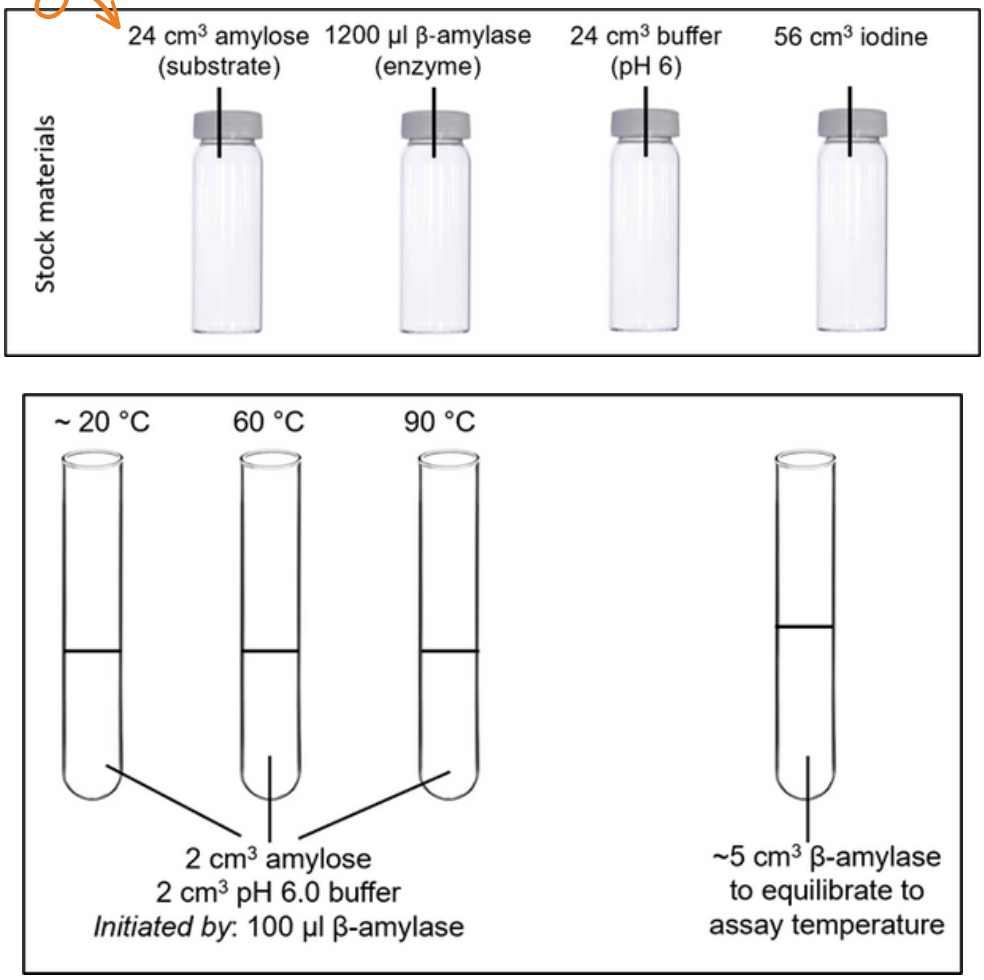



**Figure 5:** Integrated diagram of the beta-amylase assay. Preparation of 3 test tubes for assays at different temperatures. Substrate and buffer added first, equilibrated to the assay temperature, and reactions initiated by the addition of temperature-equilibrated enzyme. The investigation looks at the effect of three temperatures on beta-amylase activity, which will each be performed in triplicate.

1 To three test tubes, add 2 cm³ amylose (substrate) and 2 cm³ pH 6 buffer.

2 Set up 7 cuvettes in the cuvette rack.
Cuvette 1 (colorimetric blank): Add 2 $cm^3$ iodine solution + 100 µl distilled water. This will be used to zero the colorimeter using the red diode (635 nm).

3 To Cuvette 2-7: Add 2 $cm^3$ iodine solution.

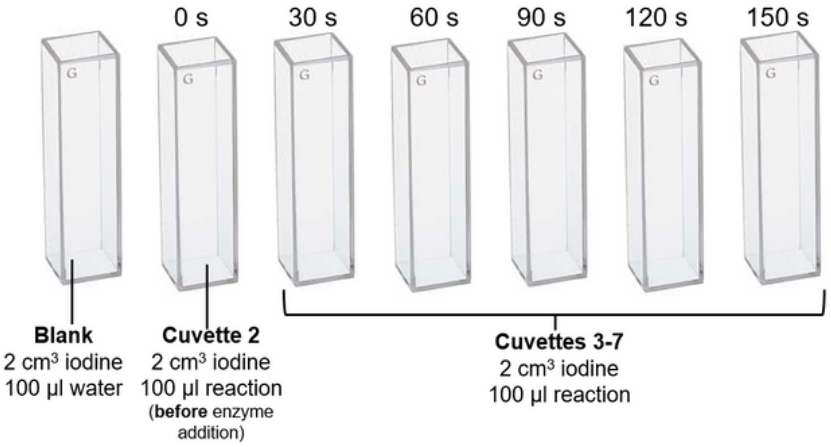



**Figure 6:** For the assay, 7 cuvettes will be required for each temperature. Cuvette 1 represents the colorimetric blank. Cuvette 2 will provide the absorbance of the solution prior to any enzyme activity. For cuvettes 3-7, iodine will be added followed by 100 µl assay reaction mixture every 30 s after enzyme addition.

4 Start with room temperature: Stand the first test tube in a test tube rack at room temperature (note the room temperature displayed on the thermometer in the results table).

5 Using the automatic pipette, remove 100 µl from the test tube and add to cuvette 2 (this will represent the 0 s time point).

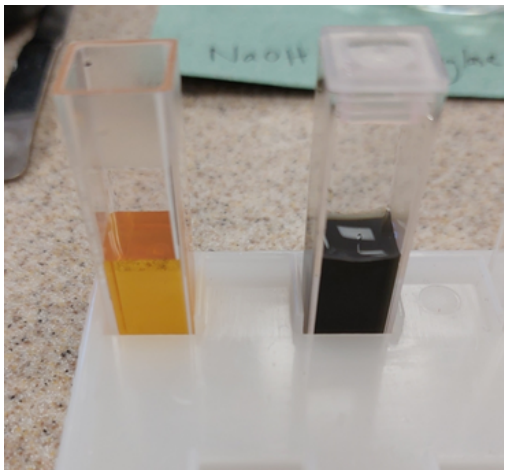
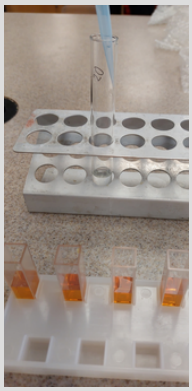


**Figure 7:** For cuvette 1 - this provides the "background colour" that exists in all the tubes due to presence of iodine. This is used to blank the colorimeter.

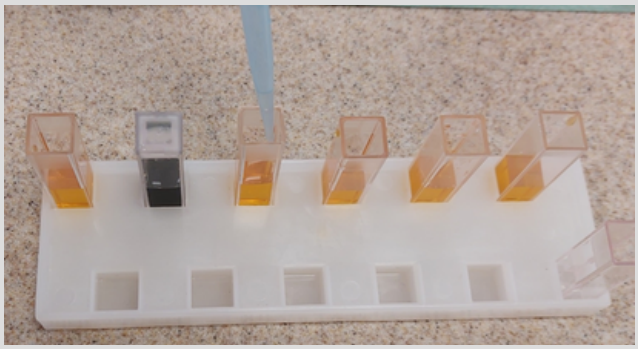
Cuvette 2: 0s time point. This is the colour that iodine will turn in the presence of amylose, prior to any enzyme activity. The final absorbance value collected at the end of the time course will be compared to this sample.

6 **To initiate the reaction**, use the automatic pipette to add 100 µl β-amylase (enzyme) to the substrate / buffer mix. Start the stopwatch immediately.

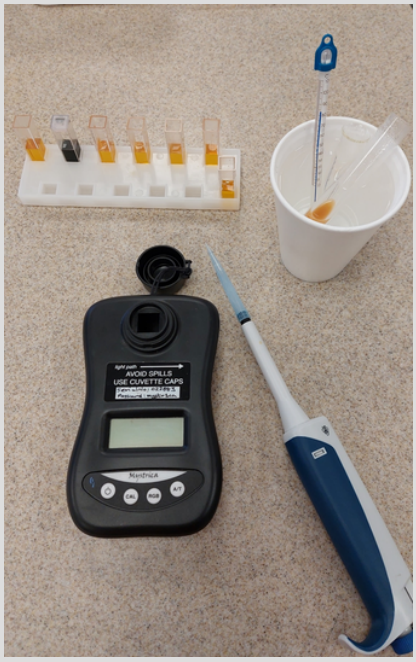


**7** After 30 s, remove 100 µl of the reaction mixture and add to cuvette 3. Repeat every 30 s* until 150 s have elapsed.

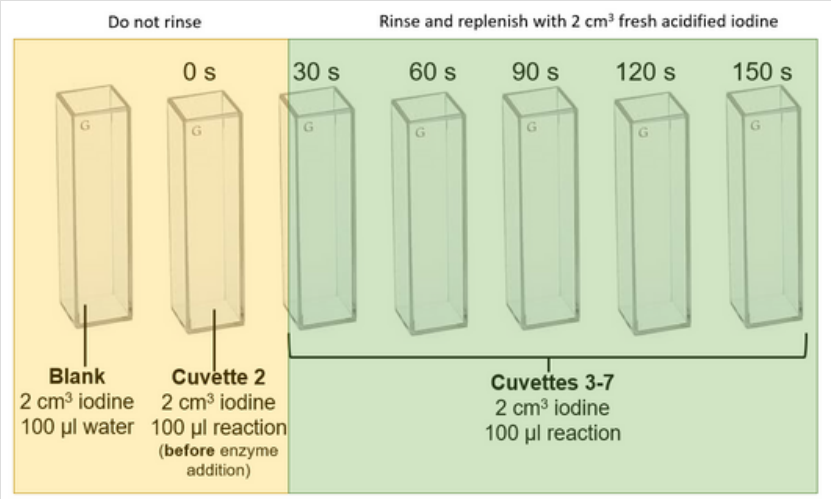
*Note: Every sweet potato is different so you might find that 30 s time intervals are too low and the reaction does not proceed quickly enough. This might even be affected by season or size of sweet potato (larger sweet potatoes tend to work quicker!). If this is the case, increase to 60 s sampling intervals.



**8** Use **cuvette 1** to **zero** the colorimeter (set to the red diode, or 635 nm). Read each of the remaining solutions in the cuvettes.



**9** Retain cuvettes 1 and 2 for the remaining temperatures. Wash cuvettes 3-7 and blot dry. Replenish cuvettes 3-7 with 2 $cm^3$ iodine.

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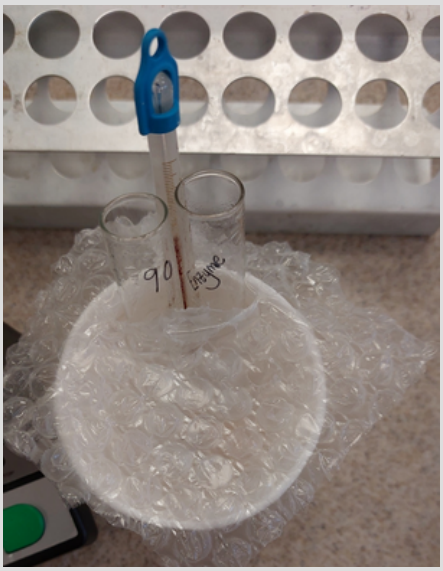
Repeat the experiment at 60 °C and 90 °C.

- For 60 °C, place approximately 5 $cm^3$ enzyme into a test tube and incubate this in the thermotube block at 60 °C. Place the test tube containing the substrate and buffer in the thermotube block at the same time. Allow 5 minutes for temperature equilibration before initiating the reaction with the addition of 100 µl enzyme. You do not need to repeat the 0s time point as you have retained this cuvette from the room temperature assay.

- For 90 °C, boil a beaker of water over a Bunsen burner and immerse a test tube of enzyme into the boiling water for 5 minutes to denature the enzyme. Boil the kettle and transfer boiling water to a polystyrene cup, covered with the piece of bubble wrap. Make a hole in the bubble wrap to allow two test tube to pass through. Immerse the test tube of denatured enzyme and the remaining test tube from step 1 in the polystyrene cup of boiling water. Allow 3 minutes for temperature equilibration before initiating the reaction with the addition of 100 µl enzyme. Keep an eye on the temperature of the water using the thermometer - replace if necessary to retain temperatures > 80 °C. You do not need to repeat the 0s time point as you have retained this cuvette from the room temperature assay.

- A wider range of temperatures can be explored. The published optimum temperature for this enzyme is approximately 70 °C so higher temperatures will be required to observed loss of activity due to denaturation.


## RESULTS

Complete the results table below, including the temperature indicated on the thermometer for the "room temperature" assay. Include the sampling intervals in the table, e.g. these might be every 30 s or every 60 s. In the final column, "%decrease over time", the comparison should be made between the final sample and the 0 s sample.

| Temperature (°C) | Absorbance at sample time points | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 s | | | | | | %decrease over time |
| | | | | | | | |
| 60 | | | | | | | |
| 90 | | | | | | | |

## SAMPLE RESULTS

The table below indicates typical results that might be obtained using this protocol.

| Temperature (°C) | Absorbance at sample time points | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 s | 30 s | 60 s | 90 s | 120 s | 150 s | % decrease over time |
| 2 | 1.438 | 1.403 | 1.361 | 1.284 | 1.211 | 1.150 | 20 |
| 20 | 1.438 | 0.790 | 0.511 | 0.503 | 0.336 | 0.264 | 81 |
| 60 | 1.438 | 0.532 | 0.421 | 0.348 | 0.219 | 0.138 | 90 |
| 90 | 1.438 | 1.431 | 1.449 | 1.419 | 1.398 | 1.422 | 1 |

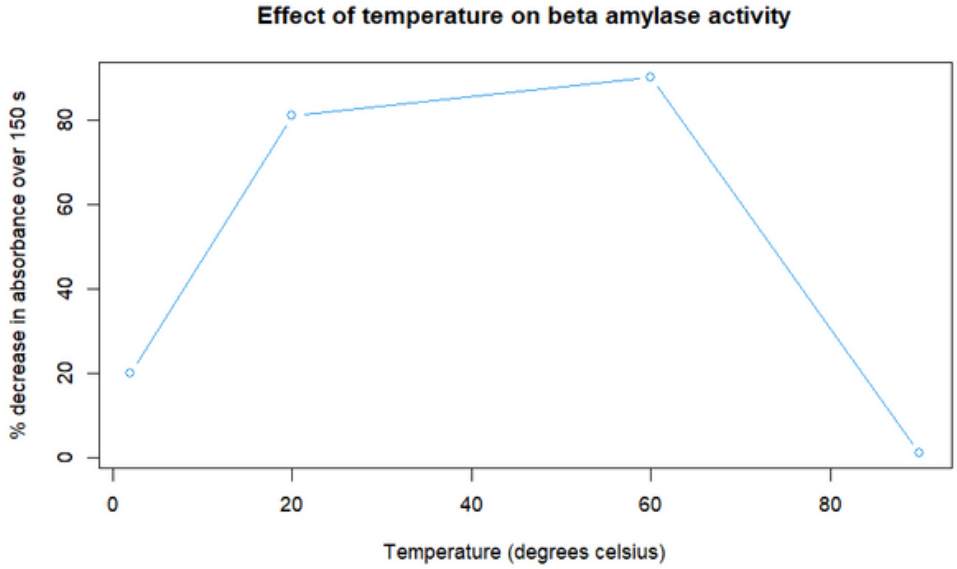
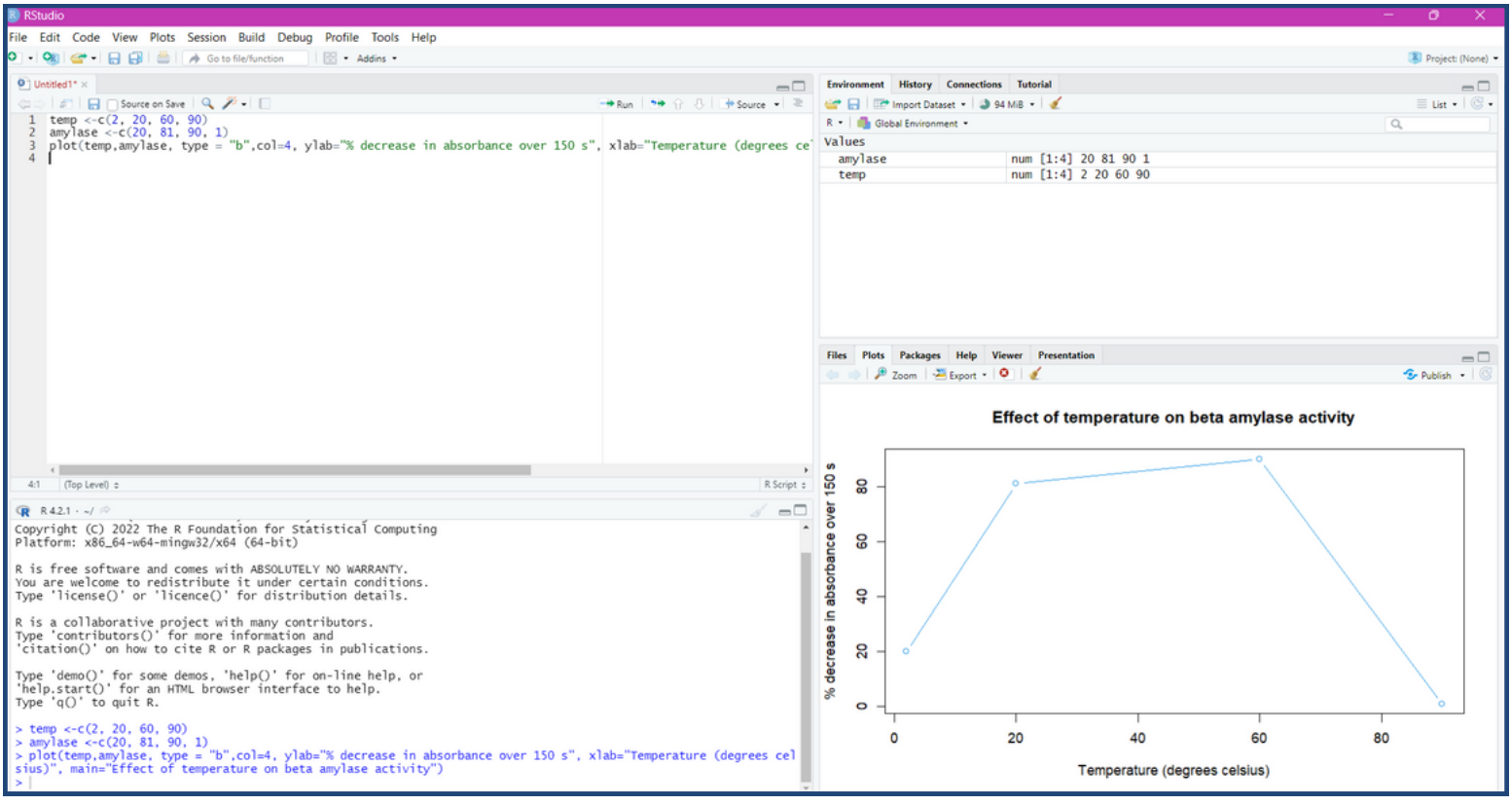



**Figure 8:** R Studio was used to produce a summary plot of the data from the table above. This shows that the optimum temperature, based on the temperatures investigated, was 60 °C. The R Studio code used to produce this plot is shown below. A screenshot of the R Studio environment has also been included.

```
temp <-c(2, 20, 60, 90)

amylase <-c(20, 81, 90, 1)

plot(temp,amylase, type = "b",col=4, ylab="% decrease in absorbance over 150 s", xlab="Temperature
(degrees celsius)", main="Effect of temperature on beta amylase activity")
```



## SAMPLE RESULTS WITH ALTERNATIVE INDEPENDENT VARIABLES

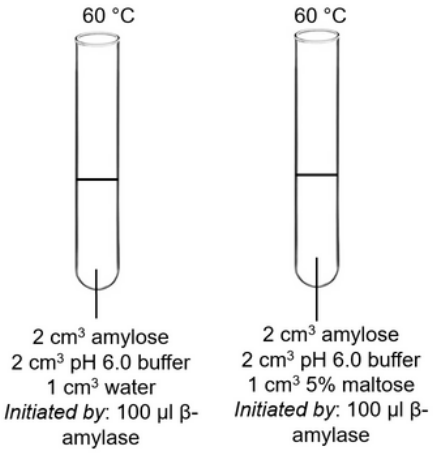
### pH

The experiment was repeated at 60 °C in the presence of a pH 5, pH 6 or pH 7 buffer. The level of beta amylase activity rapidly declines as the pH moves away from pH 6.

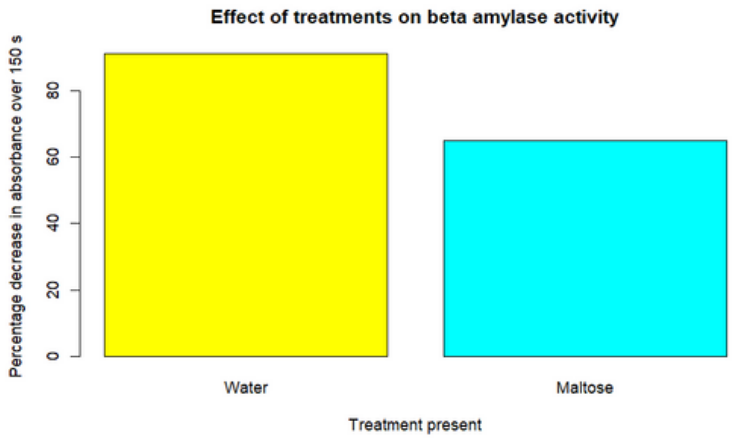
| pH | Absorbance at sample time points | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 s | 30 s | 60 s | 90 s | 120 s | 150 s | % change over time |
| 5 | 1.510 | 1.307 | 1.226 | 1.217 | 1.211 | 1.239 | -18 |
| 6 | 1.438 | 0.790 | 0.511 | 0.503 | 0.336 | 0.264 | -81 |
| 7 | 1.449 | 1.440 | 1.433 | 1.531 | 1.498 | 1.502 | +4 |

### Enzyme inhibition by maltose

The experiment was repeated at 60 °C, in the presence of a pH 6 buffer, and maltose. The assay was set up with 2 $cm^3$ pH 6 buffer, 2 $cm^3$ amylose, and 1 $cm^3$ 5% maltose (or water for comparison). Maltose inhibited the activity of beta amylase.




| Treatment present | Absorbance at sample time points | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 s | 30 s | 60 s | 90 s | 120 s | 150 s | % decrease over time |
| Water | 1.470 | 0.674 | 0.425 | 0.301 | 0.141 | 0.128 | 91 |
| Maltose | 1.470 | 0.644 | 0.567 | 0.537 | 0.489 | 0.514 | 65 |




## ACKNOWLEDGEMENTS

This protocol was sourced, with minor adaptation for classroom use, from www.mystrica.com. Thank you to Douglas Macdonald for publishing this protocol on his open-access website.

Much thanks to Neal Gwynne (Head of Biology) and his S3 pupils at Morrison's Academy for trialling the protocol and highlighting the challenges they faced. This led to important adaptations in the final protocol.