An experiment to investigate the action of saliva on starch

Research question

Do enzymes in our saliva break up starch into simple sugars (mono- or disaccharides)?

Introduction

Enzymes are globular proteins, which act as biological catalysts. They speed up reactions by binding to a substrate, such as for example the enzyme amylase, which binds to starch (a polysaccharide) in order to break it down into di - and monosaccharides. They are present in all organisms in thousand of different forms, most enzymes specific to only one substrate. An enzyme has an active site, at which it binds to a substrate, which is specific to its shape. An example for this is the enzyme amylase which catalyses the breakdown of starch, a polysaccharide for example found in potatoes. When it is digested by amylase, it will be broken down into disaccharides, which are sugars such as maltose. Then another enzyme, maltase, will break the disaccharide down into glucose, which is the monomer starch is made of.
Starch is a carbohydrate consisting of a large number of glucose units joined together. It is produced by all green plants as an energy store. It is the most important carbohydrate in the human diet and is for example contained in potatoes, wheat, maize (corn) and rice. Human saliva is rich in amylase, and the pancreas also secretes the enzyme.
Iodine solution is used to test for starch; a dark blue colour indicates the presence of starch.

Hypothesis:

The longer starch and saliva are mixed, the more starch is broken down and the iodine will become brighter. As the solution is mixed with Benedict's solution and heated in a water bath, it will change it's colour, indicating that the starch in the solution has been broken down to reducing sugars by the amylase in the saliva.

- The longer the amylase is reacting with the starch solution, the less starch will be present
  Therefore towards the end, the iodine does not turn blue/black anymore, because there is no starch anymore.

Method

1) Test one drop of the starch suspension provided with iodine solution, on a white dish or spotting tile.

2) Rinse mouth with $20cm^3$ of distilled water: spit this back into the sink.
3) Rinse mouth a second time with $20cm^3$ of distilled water. This time keep the water in the mouth for 30 seconds, moving it around with the tongue. Collect this diluted saliva in a beaker.
4) Using a pipette place one drop of iodine solution in each of the depressions on the spotting tile.
5) Use a clean $5cm^3$ syringe to take up $2cm^2$ of the starch suspension. Rinse the outside of the syringe with distilled water.
6) Now take up $2cm^3$ of dilute saliva into same syringe. Draw in a little air and quickly and thoroughly mix the contents of the syringe by inverting it two or three times.
7) Hold the syringe vertically and immediat ely squeeze the plunger carefully to expel one drop of the mixture into the first drop of iodine solution on the tile. Do not let the tip of the syringe touch the iodine solution. Note the time.
8) Continue to test single drops of the mixture at intervals of 30 seconds until the iodine solution shows no further change in colour.
9) Now expel $1cm^3$ of the remaining mixture in the syringe into a test -tube. Test this for the presence if reducing sugars using Benedict's solution. Put it into a water bath and leave it for 10 minutes. Then take it out.
10) Ten expel another $1cm^3$ of the remaining mixture in the syringe into a test -tube. Test this for the presence of reducing sugars, using Benedict's solution. Put it into a water bath and leave it for 10 minutes. Then take it out again.
11) Place about $5cm^3$ of the remaining dilute saliva in a test -tube and boil it gently for about 10seconds.Leave it to cool.
12) Repeat steps 4 to 7, but this time mix boiled saliva with starch suspension in a clean syringe. Continue to test single drops of this mixture for as long as you did with the unboiled saliva and starch.

Variables

(i) Independent variable:

The time for the reaction of amylase upon the starch to take place: 30 seconds for each result.

Use a watch/stopwatch to check the tim e before dropping another drop of the mixture onto the spotting tile.

(ii) Dependent variable:

The colour changes when iodine solution is added to the mixture of saliva and starch

(iii) Controlled variable:

What stays the same	How it is controlled
Amount of starch solution	By using a syringe
Amount of saliva	By using a syringe
Amount of iodine dropped	Using a pipette and spotting tile
Amount of drinking water	Using a beaker to measure water

Results

Time/sec ($^{+/-}$0.5 sec)	Colour

Start	Black-blue
30sec	Dark blue
60sec	Navy blue
90sec	Dark purple
120sec	Dark brown
150sec	Brown
180sec	Light brown
210sec	Dark yellow
240sec	Orange

After heating the remaining solution (with the added Benedict's solution) in a water bath for 10 minutes, it turns brick red.
That means that the starch in the solution has been broken down to reducing sugars by the amylase in the saliva.

Questions-Answers

- 1) What appears to have happened to the starch during the course of this experiment?
  -The starch broke down the longer it was left

- 2) What do you think was the purpose if rinsing out your mouth the first time?
  -To stimulate the salivary glands to produce saliva, which is important for the experiment
  OR
  -To remove food residues which could influence the experiment's outcome.

- 3) What conclusion can you draw from the results of the tests in steps 9 and 10?

- The solution turns brick red.
  That means that the starch in the solution has been broken down to reducing sugars by the amylase in the saliva.

- 4) What test should you perform on the original starch suspension before you can be confident of your answer to question 3?
  -Iodine test to see if starch has completely been broken down. If it turns black blue, starch is still present!

- 5) How are the properties of saliva affected by boiling?
  - The enzyme amylase in saliva is denatured and so the starch in the saliva stays unchanged

- 6) Saliva normally acts on starch at body temperature (37°C). If you had carried out the whole experiment at this temperature, what difference would you expect this to have made to the results you obtained?
  -The results would have been much faster. The colour would change much faster for example from blue/black directly to navy blue or dark purple instead of

changing much slower. (as shown in the result table) The experiment would be much faster to do and to finish.