The structure and function of barley amylases.

Aim:
To determine if the there is a relationship between the primary structure of barley (*Hordeum vulgare*) amylases and the effect of pH buffers (ranging from pH 3 – pH 11) on them.

Ex: Focussed research question

Introduction:
Enzymes are large proteins that catalyze chemical reactions. They achieve this by lowering the activation energy of a chemical reaction – the initial input of energy required to start the fabrication of the products of the reaction between the enzyme and its substrate. Enzymes have a very complex three-dimensional shape called the active site in which the enzyme-substrate complex forms during the reaction; this part of the enzyme is structurally and biochemically opposite to its substrate, thereby making each enzyme specific to a range of substrates that share a similar bio-molecular and chemical structure. Enzymes are also not consumed by the reaction.

Ex: Scientific context relevant

However, enzymatic reactions can also be affected by certain factors such as the concentration ratio between the enzyme and its substrate, the temperature, as well as the pH of the environment the reaction takes place in. This experiment will investigate the effect of pH on the enzymatic reaction between plant amylase and starch, and therefore further information on the effect of pH on enzyme activity is needed.

The active site of an enzyme has a specific biochemical surface that corresponds to that of its substrate. The amino acids on the active site can be either positively charged (H^+) or negatively charged (OH^-); therefore, increasing the pH of the environment around the enzymatic reaction causes more OH^- ions to bond with the H^+ ions on the surface of the active site, thereby causing the enzyme to become less efficient, or even completely inactive sometimes. If an enzyme is placed in a medium with a lower pH, a similar scenario will occur, only this time with the H^+ ions of the solution it is in bonding with the OH^- ions of the protein's active site. Extreme pH levels can also cause denaturation – a permanent deformation of the enzyme's active site that henceforth inhibits any reaction to take place between it and its substrate.

There are three major kinds of amylase: alpha, beta, and gamma. The alpha-amylases function with calcium to break down long carbohydrates into maltose, and have an optimum pH of 5.5-6.8[1]. It is produced by plants, such as barley, animals, fungi and bacteria. The beta-amylases are synthesized by bacteria, fungi and plants, and break down starch into maltose during the ripening of fruits. They have an optimum pH of 3.5-6.2[2]. The gamma-amylases show the lowest pH optimum at a level of pH 3 [3]. Germinating barley seeds, the source of the enzyme preparation used here synthesise alpha- and beta-amylases so we shall concentrate on these.

The optimum pH of an enzyme is reflected in its primary structure. Those enzymes that work best at a high pH have a lot of basic amino acids in their structure and those that work at a low pH tend to have a lot of acidic amino acids in their structure. Of the 20 amino acids that are used to make proteins two are acidic (aspartic acid and glutamic acid). Based up on this we can predict that alpha-amylases should have slightly less acidic amino acids in their structure than beta-amylases.

Determining the proportion acidic amino acids in the structure of alpha and beta amylase from Barley

Ex: Aim stated

The primary structures of these enzymes was obtained from UniProt a protein structure data base associated with the European Bioinformatics Institute (EBI).

A Basic Local Alignment Search Tool (BLAST) was used to search for proteins with similar structures and those from Barley (Hordeum) were selected. These were then aligned to see how much similarity there is between their structures.

Ex: Method stated
Why barley is selected could also be be included

Though there are several types of alpha- and beta-amylase enzymes found in barley, the most common type of alpha- and beta-amylase were selected. The primary structures where the amino acids are represented by single letters were placed in an MSExcel spread sheet. Using the data filtering tool the acidic (D and E), basic (R, H and K) and polar (C, N, Q, S, T and Y) amino acids were identified by their letters (see key page 3). The proportions of these amino acids was calculated for each of the enzymes.

Ex: Method appropriate

Comm: Could be more clearly expressed

[1] http://www.brenda-enzymes.info/php/result_flat.php4?ecno=3.2.1.1&Suchword=&organism%5B%5D=Hordeum+vulgare&show_tm=0
[2] http://www.brenda-enzymes.org/php/result_flat.php4?ecno=3.2.1.2&Suchword=&organism%5B%5D=Hordeum+vulgare&show_tm=0
[3] http://en.wikipedia.org/wiki/Amylase

Results:

An: Raw data presented clearly

The amino acids in red are part of the active sites.

Alpha amylase Type A primary structure[4]

```
                  10         20         30         40         50         60
MGKNGSLCCF SLLLLLLAG LASGHQVLFQ GFNWESWKQS GGWYNMMMGK VDDIAAAGVT
                  70         80         90        100        110        120
HVWLPPPSHS VSNEGYMPGR LYDIDASKYG NAAELKSLIG ALHGKGVQAI ADIVINHRCA
                 130        140        150        160        170        180
DYKDSRGIYC IFEGGTSDGR LDWGPHMICR DDTKYSDGTA NLDTGADFAA APDIDHLNDR
                 190        200        210        220        230        240
VQRELKEWLL WLKSDLGFDA WRLDFARGYS PEMAKVYIDG TSPSLAVAEV WDNMATGGDG
                 250        260        270        280        290        300
KPNYDQDAHR QNLVNWVDKV GGAASAGMVF DFTTKGILNA AVEGELWRLI DPQGKAPGVM
                 310        320        330        340        350        360
GWWPAKAATF VDNHDTGSTQ AMWPFPSDKV MQGYAYILTH PGIPCIFYDH FFNWGFKDQI
                 370        380        390        400        410        420
AALVAIRKRN GITATSALKI LMHEGDAYVA EIDGKVVVKI GSRYDVGAVI PAGFVTSAHG
                 430
NDYAVWEKNG AAATLQRS
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Beta amylase primary structure[5]

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                  10         20         30         40         50         60
MEVNVKGNYV QVYVMLPLDA VSVNNRFEKG DELRAQLRKL VEAGVDGVMV DVWWGLVEGK
                  70         80         90        100        110        120
GPKAYDWSAY KQLFELVQKA GLKLQAIMSF HQCGGNVGDA VNIPIPQWVR DVGTRDPDIF
                 130        140        150        160        170        180
YTDGHGTRNI EYLTLGVDNQ PLFHGRSAVQ MYADYMTSFR ENMKDFLDAG VIVDIEVGLG
                 190        200        210        220        230        240
PAGEMRYPSY PQSHGWSFPG IGEFICYDKY LQADFKAAAA AVGHPEWEFP NDVGQYNDTP
                 250        260        270        280        290        300
ERTQFFRDNG TYLSEKGRFF LAWYSNNLIK HGDRILDEAN KVFLGYKVQL AIKISGIHWW
                 310        320        330        340        350        360
YKVPSHAAEL TAGYYNLHDR DGYRTIARML KRHRASINFT CAEMRDLEQS SQAMSAPEEL
                 370        380        390        400        410        420
VQQVLSAGWR EGLNVACENA LPRYDPTAYN TILRNARPHG INQSGPPEHK LFGFTYLRLS
                 430        440        450        460        470        480
NQLVEGQNYV NFKTFVDRMH ANLPRDPYVD PMAPLPRSGP EISIEMILQA AQPKLQPFPF
                 490        500        510        520        530
QEHTDLPVGP TGGMGGQAEG PTCGMGGQVK GPTGGMGGQA EDPTSGIGGE LPATM
```

Using BLAST analysis, 26 other alpha-amylases were found in varieties of barley, of which the similarity between the molecules were all over 97%. For beta-amylase 17 matches were found. They showed more variation from 82-100% similarity. However the regions of beta-amylase where the amino acids are involved in the active site (#184 and #378) are found are quite conservative.

An: Some analysis of data

Segments involving amino acids #184 and #378 (in pink) showing the alignment of the 17 matches found for beta amylase[6]

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181  PAGEMRYPSYPQSQGWVFPGIGEFICYDKYLEADFKAAAAKAGHPEWELPDDAGEYNGTP  240  Q4VM10   Q4VM10_HORVD
181  PAGEMRYPSYPQSQGWVFPGIGEFICYDKYLEADFKAAAAKAGHPEWELPDDAGEYNDTP  240  D6BU17   D6BU17_HORVS
181  PAGEMRYPSYPQSQGWVFPGIGEFICYDKYLEADFKAAAAKAGHPEWELPDDAGEYNDTP  240  Q4VM11   Q4VM11_HORVD
169  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  228  Q6SNP7   Q6SNP7_HORVU
179  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  238  Q9FUK6   Q9FUK6_HORVU
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  240  C1IIM6   C1IIM6_HORVU
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  240  Q9FSI3   Q9FSI3_HORVU
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  240  Q84T20   Q84T20_HORVD
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  240  Q9AVJ8   Q9AVJ8_HORVU
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDAGQYNDTP  240  P82993   AMYB_HORVS
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDAGQYNDTP  240  E0W6Z7   E0W6Z7_HORVS
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDAGQYNDTP  240  D6BU16   D6BU16_HORVS
179  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  238  Q9FUK7   Q9FUK7_HORVU
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  240  A8CFR3   A8CFR3_HORVU
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  240  Q84T19   Q84T19_HORVD
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  240  Q9SBH7   Q9SBH7_HORVU
181  PAGEMRYPSYPQSHGWSFPGIGEFICYDKYLQADFKAAAAAVGHPEWEFPNDVGQYNDTP  240  P16098   AMYB_HORVU
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[4] http://www.uniprot.org/uniprot/P00693
[5] http://www.uniprot.org/uniprot/P16098
[6] http://www.uniprot.org/align/2013071640L0KRY3A7

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361  VRQVLSAGWREGLHVACENALSRYDATAYNTILRNARPKGINENGPPEHKLFGFTYLRLS  420  Q4VM10   Q4VM10_HORVD
361  VQQVLSAGWREGLHVACENALSRYDATAYNTILRNARPKGINENGPPEHKLFGFTYLRLS  420  D6BU17   D6BU17_HORVS
361  VQQVLSAGWREGLHVACENALSRYDATAYNTILRNARPKGINENGPPEHKLFGFTYLRLS  420  Q4VM11   Q4VM11_HORVD
349  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  408  Q6SNP7   Q6SNP7_HORVU
359  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  418  Q9FUK6   Q9FUK6_HORVU
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  C1IIM6   C1IIM6_HORVU
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  Q9FSI3   Q9FSI3_HORVU
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  Q84T20   Q84T20_HORVD
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  Q9AVJ8   Q9AVJ8_HORVU
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  P82993   AMYB_HORVS
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  E0W6Z7   E0W6Z7_HORVS
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  D6BU16   D6BU16_HORVS
359  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  418  Q9FUK7   Q9FUK7_HORVU
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  A8CFR3   A8CFR3_HORVU
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  Q84T19   Q84T19_HORVD
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  Q9SBH7   Q9SBH7_HORVU
361  VQQVLSAGWREGLNVACENALPRYDPTAYNTILRNARPHGINQSGPPEHKLFGFTYLRLS  420  P16098   AMYB_HORVU
```

Amino acid abbreviations[7]

Comm: Useful information but this key could have been presented earlier

Amino acid	Three letter code	One letter code
alanine	ala	A
arginine	arg	R
asparagine	asn	N
aspartic acid	asp	D
cysteine	cys	C
glutamic acid	glu	E
glutamine	gln	Q
glycine	gly	G
histidine	his	H
isoleucine	ile	I
leucine	leu	L
lysine	lys	K
methionine	met	M
phenylalanine	phe	F
proline	pro	P
serine	ser	S
threonine	thr	T
tryptophan	trp	W
tyrosine	tyr	Y
valine	val	V

Acidic in pink
Basic in blue
Polar non-charged in green[8]

[7] http://www.uiowa.edu/~ghemical/doc/aa_table.html
[8] http://www.sigmaaldrich.com/life-science/metabolomics/learning-center/amino-acid-reference-chart.html#neu

Results the analysis of the amino acid properties – amino acids involved in the active site are framed in bold.

Alpha amylase

1	MGKNGSLCCF	SLLLLLLLAG	LASGHQVLFQ	GFNWESWKQS	GGWYNMMMGK
51	VDDIAAAGVT	HVWLPPPSHS	VSNEGYMPGR	LYDIDASKYG	NAAELKSLIG
101	ALHGKGVQAI	ADIVINHRCA	DYKDSRGIYC	IFEGGTSDGR	LDWGPHMICR
151	DDTKYSDGTA	NLDTGADFAA	APDIDHLNDR	VQRELKEWLL	WLKSDLGFDA
201	WRL**D**FARGYS	PEMAKVYIDG	TSPSLAVA**E**V	WDNMATGGDG	KPNYDQDAHR
251	QNLVNWVDKV	GGAASAGMVF	DFTTKGILNA	AVEGELWRLI	DPQGKAPGVM
301	GWWPAKAATF	VDNHDTGSTQ	AMWPFPSDKV	MQGYAYILTH	PGIPCIFYDH
351	FFNWGFKDQI	AALVAIRKRN	GITATSALKI	LMHEGDAYVA	EIDGKVVVKI
401	GSRYDVGAVI	PAGFVTSAHG	NDYAVWEKNG	AAATLQRS	

Amino acids: Acidic: 11.6% Basic: 11.9% Neutral polar: 21.4%

Beta amylase

1	MEVNVKGNYV	QVYVMLPLDA	VSVNNRFEKG	DELRAQLRKL	VEAGVDGVMV
51	DVWWGLVEGK	GPKAYDWSAY	KQLFELVQKA	GLKLQAIMSF	HQCGGNVGDA
101	VNIPIPQWVR	DVGTRDPDIF	YTDGHGTRNI	EYLTLGVDNQ	PLFHGRSAVQ
151	MYADYMTSFR	ENMKDFLDAG	VIVDIEVGLG	PAG**E**MRYPSY	PQSHGWSFPG
201	IGEFICYDKY	LQADFKAAAA	AVGHPEWEFP	NDVGQYNDTP	ERTQFFRDNG
251	TYLSEKGRFF	LAWYSNNLIK	HGDRILDEAN	KVFLGYKVQL	AIKISGIHWW
301	YKVPSHAAEL	TAGYYNLHDR	DGYRTIARML	KRHRASINFT	CAEMRDLEQS
351	SQAMSAPEEL	VQQVLSAGWR	EGLNVAC**E**NA	LPRYDPTAYN	TILRNARPHG
401	INQSGPPEHK	LFGFTYLRLS	NQLVEGQNYV	NFKTFVDRMH	ANLPRDPYVD
451	PMAPLPRSGP	EISIEMILQA	AQPKLQPFPF	QEHTDLPVGP	TGGMGGQAEG
501	PTCGMGGQVK	GPTGGMGGQA	EDPTSGIGGE	LPATM	

Amino acids: Acidic: 11.5% Basic: 11.8% Neutral polar: 23.7%

Conclusion:

Though there are proportionally more polar amino acids in beta-amylase, there is not a great difference between the proportions of the different types of amino acids in the two enzymes. If anything, alpha amylase has slightly more acidic amino acids in proportion to its size. It is also worth noting that both the enzymes have acidic amino acids in their active sites. Alpha amylase also uses calcium in its active site and it binds to the starch molecules using special binding sites as well as the active site.[9] These may also be influenced by pH.

An: Processed data presented
An: Processing appropriate and effective

Comm: Could be expressed more clearly

Ev: Conclusion drawn which is supported by the data

Ev: Supporting scientific context given

[9] http://www.chem.uwec.edu/Webpapers2005/leee/barley.html

4

Determining the optimum pH for barley amylase used in school.

Ex: Aim given

Variables:
Independent: The pH buffer was the factor of the experiment that was changed at intervals of 1 pH level, ranging from pH 3 to 11.

Dependent: The ways in which the initial rate of the chemical reaction concerning the digestion of starch was affected by the different pH buffers.

Controlled: The concentrations and volumes of the pH buffers, amylase, and starch solutions were kept at constant levels inside each cuvette. The volume of iodine solution placed into each cuvette was kept constant at 3 drops. The temperature could not be controlled whilst the reaction took place in the colorimeter but all the solutions had been kept at room temperature and this did not vary during the experiment. The time for which the data collection took place was limited to 150 seconds. The colorimeter was also recalibrated between each try.

Ex: The ambient temperature should have been monitored.

Materials:

LabQuest	3 x 2ml syringe
Colorimeter probe	Amylase solution (4%)
Colorimeter cuvettes and caps	Starch solution (2%)
4 x 50ml beakers	pH buffer solutions (3, 4, 5, 6, 7, 8, 9, 10, and 11)
Marker pen	Iodine solution
	Dropping pipette

Ex: Sufficient range and intervals for the independent variable

Method:
Colorimeter cuvettes were labeled from 3 to 11 but not in the pathway of the colorimeter light – showing which pH buffer was in each – and a solution of 1 ml of the pH buffer and 1ml of 2% starch solution were placed in each cuvette and mixed. Three drops of iodine solution were then placed into each cuvette, and the contents were shaken in order for the entire starch in each cuvette to become colored. The colorimeter was set at a wavelength of 635 nm, as this proved to be the setting that gave appropriate results to the outcomes of the reaction; previous pilot experiments were performed in order to determine this. The cuvettes were then, each in turn, placed into the colorimeter, and the 1 ml of 4% amylase solution was squirted into the contents of the particular cuvette using a 2ml syringe. The lid of the colorimeter was then immediately closed and the data collection was started every 0.5s for a total of 150s. A Labquest device was used for this data collection. A blank cuvette (containing distilled water) was placed in the colorimeter between each trial to recalibrate the device.

Ex: Adequate control of volumes, concentrations and apparatus.

Results:
Qualitative data:

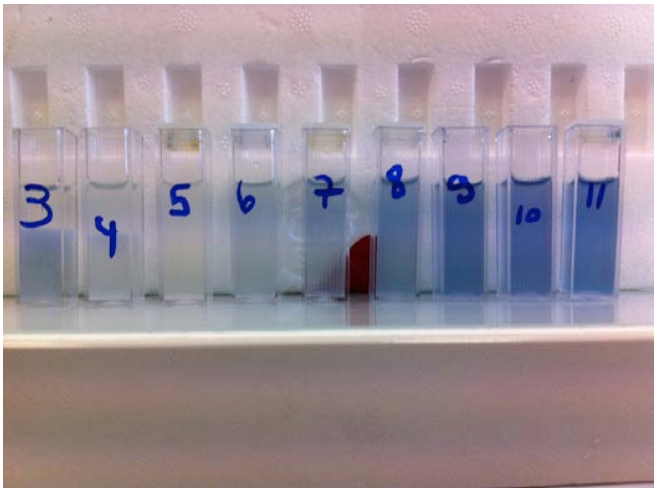


Figure 1: Image showing the colorations of the solutions with different pH levels after data collection process with colorimeter.

This image allows for key qualitative observations to be made. Firstly, one can observe that there appears to be a clear visible relationship between the pH level and the efficiency of the hydrolysis of the starch molecules. pH 5

An: Useful qualitative observations.

seems to be the optimum pH level for 4% amylase to digest 1% starch, as it is the cuvette containing the clearest solution. A symmetrical coloration of the solutions in the adjacent cuvettes is also visible, in that the ones at an equal distance from the optimum share the same coloration. This is seen by observing the identical colorations in cuvettes of pH 4 and 6, as well as those with pH 3 and 7. Lastly, one can see that, as the pH levels reach the extremes, the colorations become denser, signifying that less starch was digested.

Quantitative data:

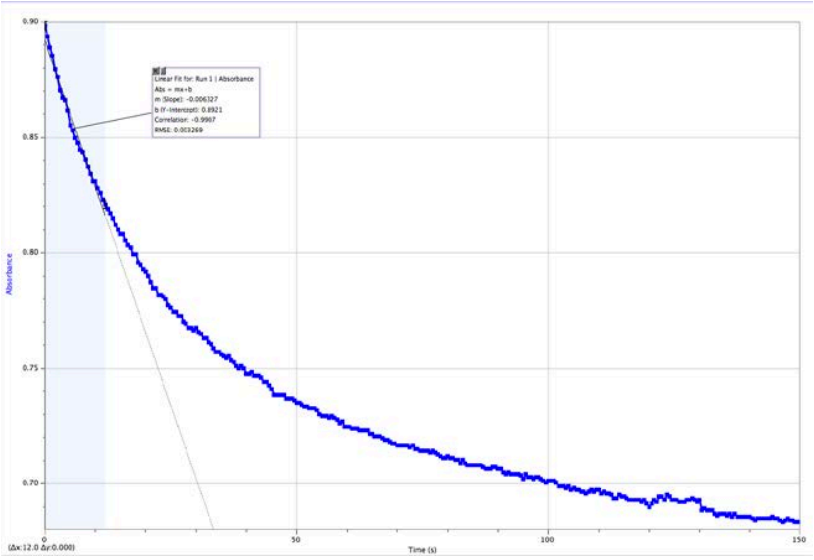


Figure 2: Absorbance levels for starch hydrolysis at pH 3

Figure 2 shows how the raw data was collected for the results at pH3. A linear trend fit was drawn in Logger Pro for the first 12.5s (25 results). Logger Pro calculates the slope of this line to give the initial reaction rate. The other 8 graphs of this sort can be found in the appendix section at the end of this report. A similar trend was observed in each graph. It is important to note that, when determining the initial rate of decrease in starch levels, the first 25 results starting from the peaking value of absorbance were considered – this can be seen by the highlighted section of the curve above.

An: Data logger trace can be considered as raw data. Linear fit and calculation represents processing.

An: Processing appropriate

The overall percentage change in absorption was also calculated from the peak absorbance value. The positive values for the initial rates were taken to draw the graph that determined the optimum pH as well as the percentage change in in absorption levels after 150s.

Comm: Clear but could be more concise.

Could be more explicit here.

Results extracted from the graphs of the raw data

pH Level	Rate of Starch Digestion (positive values) / Abs s^{-1}	Percentage change in Absorption Levels after 150 s / %
3	0.006327	23.9
4	0.007154	37.1
5	0.011500	55.5
6	0.011150	44.4
7	0.009020	44.4
8	0.004084	24.3
9	0.007163	34.8
10	0.006837	33.3
11	0.006978	33.3

Table 1: Rates of Starch digestion at the different pH values.

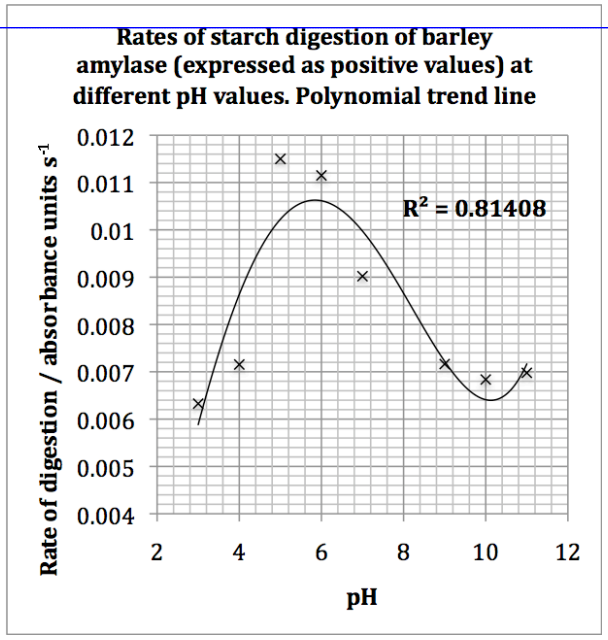
An: Processing successful

Comm: Conventions mostly respected. That the given pH buffers and the absorbance values have no uncertainties is acceptable but uncertainties should be given for % absorption levels.

Comm: It would have been clearer if the candidate had stated that column 2 is the initial reaction rate.

The result for pH 8 appears to be an outlier and it is excluded from the following graphs.

An: Outlier identified though at this stage its exclusion appears arbitrary.



An: Processed data graphed appropriately.

Comm: Conventions respected

An: Uncertainties expressed as scatter about trend line and R^2 value

Figure 3: Graphing of results shown in first column of *Table 1.*

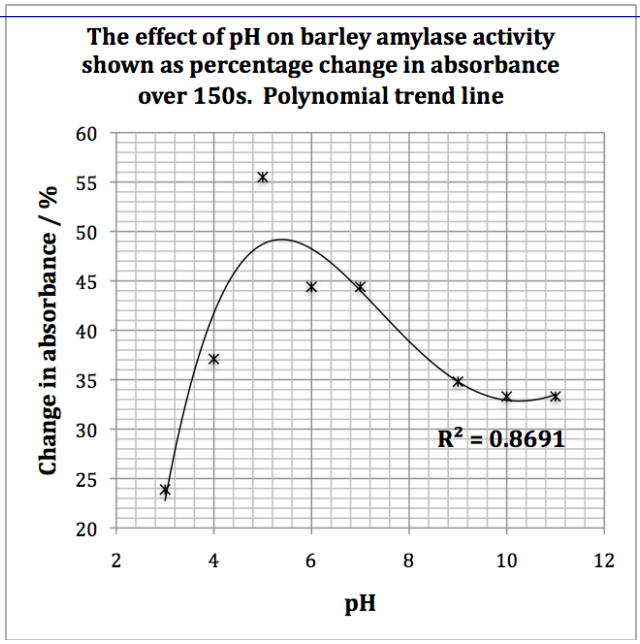
Observations on *Figure3*:
One can notice that the maximum value of the rate of starch digestion is between pH 5 and 6. The data points also appear to stabilize as they tend towards the extremes. Moreover, when observing the overall trend given by the data points of *figure 3*, one can see that a plateau of the minimum rate of change of starch levels forms at approximately 0.007abs s^{-1}.

An: Data interpreted appropriately.

The processed figure above shows that the live reading obtained for pH 8 does not correspond with the overall trend of starch digestion. It is therefore judged an as an outlier. This is supported by the fact that the raw data of the starch digestion at pH 8 (see appendix, *figure 9*) shows some fluctuation in the digestion of starch during the initial period compared to the other figures. This could suggest that pH 8 is the limit by which the enzyme can undertake the reaction efficiently. It also undermines the validity of this datum, as its trend differs from the other figures except that of *figure 10* (see appendix), which shows a similar fluctuation. However, this theory can be rejected, as *figures 3 & 4* show otherwise – higher pH values still cause for a reaction to occur in line with the general trend. A potential source of error for this part of the experiment could be due to the strength at which the amylase was squirted into the cuvette. If done so with force, the perturbation caused in the cuvette could have affected the readings of the colorimeter, thereby causing them to fluctuate.

Ev: Outlier identified and justifies removing it from the graphical presentation. This decision is a bit arbitrary. A better approach would have been to plot the graph twice once with all data and one with the outlier removed to show a possible interpretation.

Ev: Errors in the protocol used are considered.



An: Processed data presented by appropriate graph.

Figure 4: Graphing of results shown in second column of *Table 1*.

Observations on *Figure 4*:
Figure 4 shows a similar trend to that seen in *figure 3* with a slightly better fit to the trend line. The greatest percentage change is that of 55.5% at a pH of 5. Also, the percentage change of starch is identical for pH levels of 6 and 7. As seen in *figure 3*, the data points at lower pH levels than the optimum (3 & 4) show a steeper increase to this maximum than those after pH 5 (pH 6 & 7). Less of an equal plateau between the acidic pH values and the basic ones can be seen on this graph, although a stabilization does appear towards the higher pH levels. The value obtained for pH 8 is treated as an outlier again, as it does not correspond to the overall trend.

Conclusion from pH optimum:
It appears the optimum pH for the barley amylase is pH 5-6. This would imply that there is a mixture of amylases both alpha-amylase, which has an optimum of pH 5.5-6.8, and beta amylase, which has an optimum of pH 3.5-6.2. Unfortunately the analysis of the primary structures of these enzymes does not indicate clearly why there should be this difference in the optima.

Ev: Overall conclusion made that is supported by the data.

The measured values for the rate of activity of amylase, *figure 3*, do not exactly correspond to the observed colours at the end of the experiment, *figure 1*. pH 6 shows a relatively low rate of digestion, but is still significantly greater than the value shown at pH 4. This contradicts the observations made from the colour of the cuvettes (*figure 1)*: it appears as though the measured 'distance' from the optimum of pH 5 does not bring the contents of the cuvettes the same state of digestion as is suggested by the qualitative observations from *figure 1*. This statement is justified by making a similar observation regarding the rates of starch decomposition for pH values of 3 and 7; *figure 3* shows that the value at pH 7 has a faster rate of of starch digestion than that of pH 3.

Therefore, the rise towards the maximum value at pH 5 from the more acidic pH levels (3 & 4) is steeper than that from the basic pH levels (6 & 7). Such a contrast may suggest that the plant amylase enzyme used can handle a greater range of pH levels that tend more towards neutral values than those that have more acidic ones. This would correspond to a greater proportion of alpha-amylase (optimum pH given to be 5.5-6.8[10]) in the mixture than beta amylase (optimum pH of 3.5-6.2[11]). Nonetheless, *figure 3* shows the 'cut-off' points of the enzymes optimum as being pH values of 4 and 7, which is within the range of both types of amylase.

These conclusions are sustained by the observations made for *figure 4*. This graph corresponds more to the results from the initial rate of reaction than the observed colours of the cuvettes at the end of the investigation.

Ev: Not surprising the percentage change is calculated from the reading at 150s and not the initial reaction rate.

[10] http://www.brenda-enzymes.info/php/result_flat.php4?ecno=3.2.1.1&Suchword=&organism%5B%5D=Hordeum+vulgare&show_tm=0
[11] http://www.brenda-enzymes.org/php/result_flat.php4?ecno=3.2.1.2&Suchword=&organism%5B%5D=Hordeum+vulgare&show_tm=0

Evaluation and Improvements:

The evident source of error in this experiment can be seen when observing the results obtained for pH 8. As it was discussed previously, this error may have arisen due to the strength of the squirting of the amylase into the cuvette. A more controlled mixing of cuvettes is needed. However, shaking the cuvette would have delayed the collection of results in the colorimeter and there is a greater risk of putting finger prints on the cuvettes.

Ev: Appropriate suggested improvement.

The though a large range of pHs was tested the investigation on each pH was only carried out once. This no doubt led to the poor fit, $R^2 = 0.81408$, between the trend line and the data. A greater number of repeats (at least five) could be carried out to see if the results are consistent.

Ev: Weakness identified and reasonable suggested improvement made.

There were only three pHs tested in the region of the optimum pHs for these enzyme. A more focussed analysis of the pHs around pH 5 would be helpful (e.g. every half pH unit from pH 4 to 7).

Ev: Weakness identified and reasonable suggested improvement made.

A more thorough analysis of the protein data bases to determine why the optima for alpha- and beta-amylase should be different. The variety of barley used to produce the school's supply of amylase was not known, yet it was seen that different varieties of barley produce different alpha- and beta-amylases. If this information could be known it would help to focus the investigation further.

Ev: Weakness identified and reasonable suggested improvement made.

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Appendix: Raw data

An: Raw data presented
Comm: Reasonably clear presentation

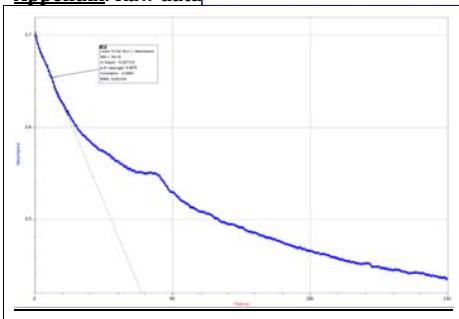

Figure 5: Absorbance levels for starch hydrolysis at pH 4

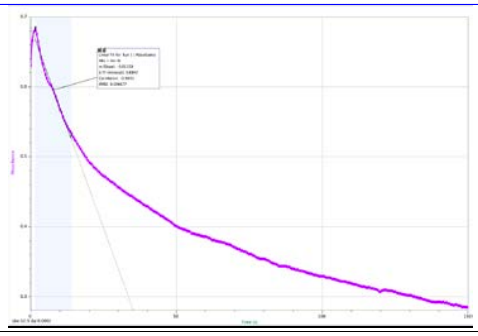

Figure 6: Absorbance levels for starch hydrolysis at pH 5

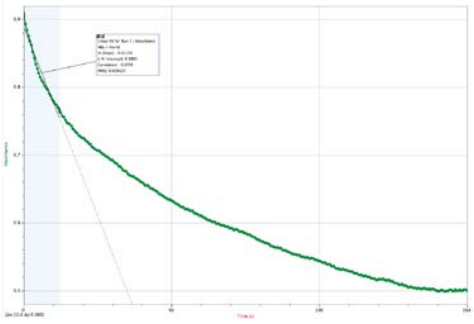

Figure 7: Absorbance levels for starch hydrolysis at pH 6

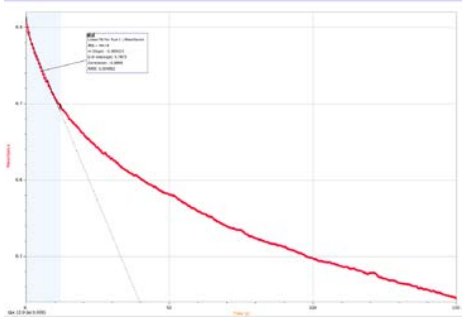

Figure 8: Absorbance levels for starch hydrolysis at pH 7

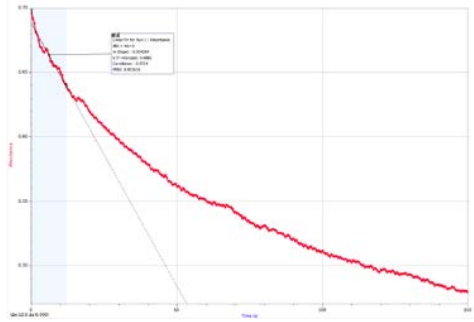

Figure 9: Absorbance levels for starch hydrolysis at pH 8

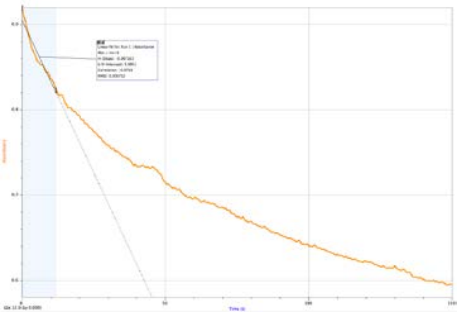

Figure 10: Absorbance levels for starch hydrolysis at pH 9

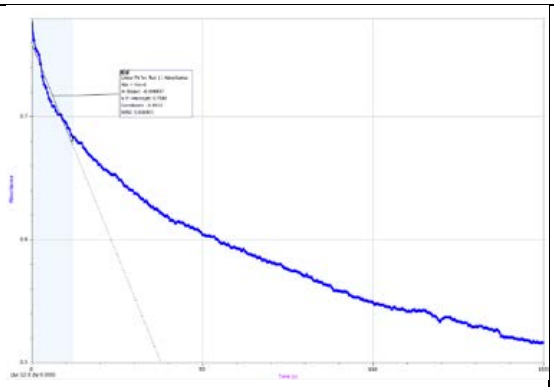

Figure 11: Absorbance levels for starch hydrolysis at pH 10

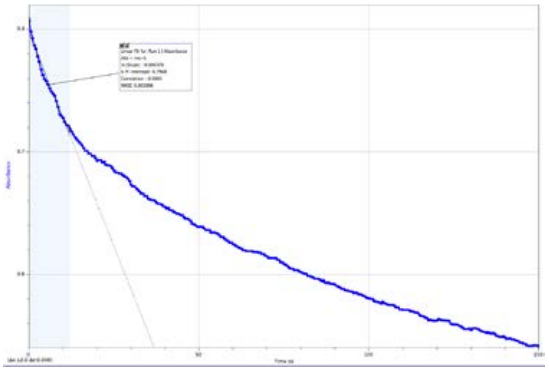

Figure 12: Absorbance levels for starch hydrolysis at pH 11