

The structure and function of barley amylases.

Aim:

To determine if 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: Focused 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 5.5-6.8¹. 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 3.5-6.2². 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 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:

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

An: Raw data presented clearly

Alpha amylase Type A primary structure⁴

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10      20      30      40      50      60
MGKNGSLCCF SLLLLLLLAG LASGHQVLFQ GFNWESWKQS GGWYNMMGK VDDIAAAGVT
70      80      90     100     110     120
HWLPPPSHS VSNEGYPGR LYDIDASKYG NAAELKSLIG ALHGKGVQAI ADIVINHRCA
130     140     150     160     170     180
DYKDSRGIYC IFEGGTS DGR LDWGPHEMICR DDTKYSDGTA NLDTGADFAA APDIDLHNDR
190     200     210     220     230     240
VQRELKEWLL WLKSDLGFDA WRLDFARGYS PEMAKVYIDG TSPSLAVAEV WDNMATGGDG
250     260     270     280     290     300
KPNYDQDAHR QNLVNWVDKV GGAASAGMVF DFTTKGILNA AVEGELWRLI DPQKAPGVM
310     320     330     340     350     360
GWWPAKAATF VDNHDTGSTQ AMWPFPSDKV MQGYAYILTH PGIPCIFYDH FFWNGFKDQI
370     380     390     400     410     420
AALVAIRKRN GITATSALKI LMHEGDAYVA EIDGKVVVKI GSRYDVGAVI PAGEVTSAHG
430
NDYAVWEKNG AAATLQRS

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Beta amylase primary structure⁵

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10      20      30      40      50      60
MEVNVKGNV VQVYVMLPLDA VSVNRRFEKG DELRAQLRKL VEAGVDGMV DVWVGLVEGK
70      80      90     100     110     120
GPKAYDWSAY KQFLFELVQKA GLKLQAIMSF HQCGGNVGDA VNIPIQWVR DVGTRDPDIF
130     140     150     160     170     180
YTDGHGRINI EYLTGLVDNQ PLFHGRSAVQ MYADYMTSFR ENMKDFLDAG VIVDIEVGLG
190     200     210     220     230     240
PAGEMRYPSY PQSHGWSFPF IGEFICYDKY LQADFKAAAA AVGHPEWEPF NDVQYNDTP
250     260     270     280     290     300
ERTQFFRDNG TYLSEKGRFF LAWYSNNLIK HGDRILDEAN KVFLGYKVQL AIKISGIHWW
310     320     330     340     350     360
YKVPSHAAIL TAGYYNLHNR DGYRTIARML KRHRASINFT CAEMRDLEQS SQAMSAPPEL
370     380     390     400     410     420
VQQVLSAGWR EGLNVACENA LPRYDPTAYN TILRNARPHG INQSGPPEHK LFGFTYLRLS
430     440     450     460     470     480
NQLVEGQNVV NFKTFVDRMH ANLPRDPYVD PMAPLPRSGP EISIEMLQA AQPQLQPFPP
490     500     510     520     530
QEHTDLVPGF TGGMGQAEG PTCGMGGQVK GPTGMGGQA EDPTSGIGGE LPATM

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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 amylase⁶

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181 PAGEMRYPSYPQSQGWVFPFGIGEFICYDKYLEADFKAAAAKAGHPPEWLPDDAGEYNGTP 240 Q4VM10 Q4VM10_HORVD
181 PAGEMRYPSYPQSQGWVFPFGIGEFICYDKYLEADFKAAAAKAGHPPEWLPDDAGEYNDTP 240 D6BU17 D6BU17_HORVS
181 PAGEMRYPSYPQSQGWVFPFGIGEFICYDKYLEADFKAAAAKAGHPPEWLPDDAGEYNDTP 240 Q4VM11 Q4VM11_HORVD
169 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 228 Q6SNP7 Q6SNP7_HORVU
179 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 238 Q9FUK6 Q9FUK6_HORVU
181 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 240 C1IIM6 C1IIM6_HORVU
181 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 240 Q9FSI3 Q9FSI3_HORVU
181 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 240 Q84T20 Q84T20_HORVD
181 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 240 Q9AVJ8 Q9AVJ8_HORVU
181 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 240 P82993 AMYB_HORVS
181 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 240 E0W6Z7 E0W6Z7_HORVS
179 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 240 D6BU16 D6BU16_HORVS
179 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 238 Q9FUK7 Q9FUK7_HORVU
181 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 240 A8CFR3 A8CFR3_HORVU
181 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 240 Q84T19 Q84T19_HORVD
181 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 240 Q9SBH7 Q9SBH7_HORVU
181 PAGEMRYPSYPQSHGWSFPFGIGEFICYDKYLQADFKAAAAAVGHPEWEPFNDVGQYNDTP 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 VRQVLSAGWREGLHVACENALSRYDATAAYNTILRNARPKGINENGGPEHKLFGFTYLRLS 420 Q4VM10 Q4VM10_HORVD
361 VQQVLSAGWREGLHVACENALSRYDATAAYNTILRNARPKGINENGGPEHKLFGFTYLRLS 420 D6BU17 D6BU17_HORVS
361 VQQVLSAGWREGLHVACENALSRYDATAAYNTILRNARPKGINENGGPEHKLFGFTYLRLS 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

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Amino acid abbreviations⁷

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 green⁸

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

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	M	G	K	N	G	S	L	C	C	F	S	L	L	L	L	L	L	A	G	L	A	S	G	H	Q	V	L	F	Q	G	F	N	W	E	S	W	K	Q	S	G	G	W	Y	N	M	M	M	G	K	
51	V	D	I	A	A	A	G	V	T	H	V	W	L	P	P	P	S	H	S	V	S	N	E	G	Y	M	P	G	R	L	Y	D	I	D	A	S	K	Y	G	N	A	A	E	L	K	S	L	I	G	
101	A	L	H	G	K	G	V	Q	A	I	A	D	I	V	I	N	H	R	C	A	D	Y	K	D	S	R	G	I	Y	C	I	F	E	G	G	T	S	D	G	R	L	D	W	G	P	H	M	I	C	R
151	D	D	T	K	Y	S	D	G	T	A	N	L	D	T	G	A	D	F	A	A	P	D	I	D	H	L	N	D	R	V	Q	R	E	L	K	E	W	L	L	W	L	K	S	D	L	G	F	D	A	
201	W	R	L	D	F	A	R	G	Y	S	P	E	M	A	K	V	Y	I	D	G	T	S	P	S	L	A	V	A	E	V	W	D	N	M	A	T	G	G	D	G	W	D	N	M	A	T	G	G	D	G
251	Q	N	L	V	N	W	V	D	K	V	G	G	A	A	S	A	G	M	V	F	D	F	T	T	K	G	I	L	N	A	A	V	E	G	E	L	W	R	L	I	A	V	E	G	E	L	W	R	L	I
301	G	W	W	P	A	K	A	A	T	F	V	D	N	H	D	T	G	S	T	Q	A	M	W	P	F	P	S	D	K	V	M	Q	G	Y	A	Y	I	L	T	H	M	Q	G	Y	A	Y	I	L	T	H
351	F	F	N	W	G	F	K	D	Q	I	A	A	L	V	A	I	R	K	R	N	G	I	T	A	T	S	A	L	K	I	L	M	H	E	G	D	A	Y	V	A	L	M	H	E	G	D	A	Y	V	A
401	G	S	R	Y	D	V	G	A	V	I	P	A	G	F	V	T	S	A	H	G	N	D	Y	A	V	W	E	K	N	G	A	A	A	T	L	Q	R	S	A	A	A	T	L	Q	R	S				

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

An: Processed data presented
An: Processing appropriate and effective

Beta amylase

1	M	E	V	N	V	K	G	N	Y	V	Q	V	Y	V	M	L	P	L	D	A	V	S	V	N	R	F	E	K	G	D	E	L	R	A	Q	L	R	K	L	V	E	A	G	V	D	G	V	M	V	
51	D	V	W	W	G	L	V	E	G	K	G	P	K	A	Y	D	W	S	A	Y	K	Q	L	F	E	L	V	Q	K	A	G	L	K	L	Q	A	I	M	S	F	H	Q	C	G	G	N	V	G	D	A
101	V	N	I	P	I	P	Q	W	V	R	D	V	G	T	R	D	P	D	I	F	Y	T	D	G	H	G	T	R	N	I	E	Y	L	T	L	G	V	D	N	Q	P	L	F	H	G	R	S	A	V	Q
151	M	Y	A	D	Y	M	T	S	F	R	E	N	M	K	D	F	L	D	A	G	V	I	V	D	I	E	V	G	L	G	P	A	G	E	M	R	Y	P	S	Y	P	Q	S	H	G	W	S	F	P	G
201	I	G	E	F	I	C	Y	D	K	Y	L	Q	A	D	F	K	A	A	A	A	A	V	G	H	P	E	W	E	F	P	N	D	V	G	Q	Y	N	D	T	P	E	R	T	Q	F	F	R	D	N	G
251	T	Y	L	S	E	K	G	R	F	F	L	A	W	Y	S	N	L	I	K	T	H	G	D	R	I	L	D	E	A	N	K	V	F	L	G	Y	K	V	Q	L	A	I	K	I	S	G	I	H	W	W
301	Y	K	V	P	S	H	A	A	E	L	T	A	G	Y	N	L	H	D	R	D	G	Y	R	T	I	A	R	M	L	K	R	H	R	A	S	I	N	F	T	C	A	E	M	R	D	L	E	Q	S	
351	S	Q	A	M	S	A	P	E	E	L	V	Q	Q	V	L	S	A	G	W	R	E	G	L	N	V	A	C	E	N	A	L	P	R	Y	D	P	T	A	Y	N	T	I	L	R	N	A	R	P	H	G
401	I	N	Q	S	G	P	P	E	H	K	L	F	G	F	T	Y	L	R	L	S	N	Q	L	V	E	G	Q	N	Y	V	N	F	K	T	F	V	D	R	M	H	A	N	L	P	R	D	P	Y	V	D
451	P	M	A	P	L	P	R	S	G	P	E	I	S	I	E	M	I	L	Q	A	A	Q	P	K	L	Q	P	F	P	F	Q	E	H	T	D	L	P	V	G	P	T	G	G	M	G	Q	A	E	G	
501	P	T	C	G	M	G	G	Q	V	K	G	P	T	G	G	M	G	G	Q	A	E	D	P	T	S	G	I	G	G	E	L	P	A	T	M															

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.

Ev: Conclusion drawn which is supported by the data
Ev: Supporting scientific context given

⁹ <http://www.chem.uwec.edu/Webpapers2005/lee/barley.html>

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
Colorimeter probe
Colorimeter cuvettes and caps
4 x 50ml beakers
Marker pen

3 x 2ml syringe
Amylase solution (4%)
Starch solution (2%)
pH buffer solutions (3, 4, 5, 6, 7, 8, 9, 10, and 11)
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.

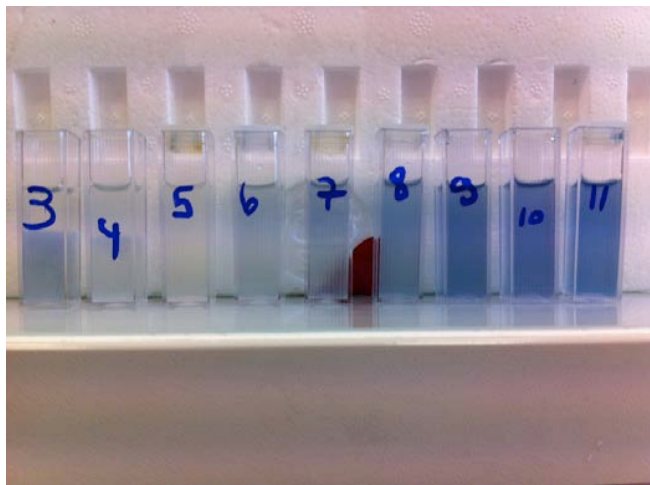
Results:**Qualitative data:**

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:

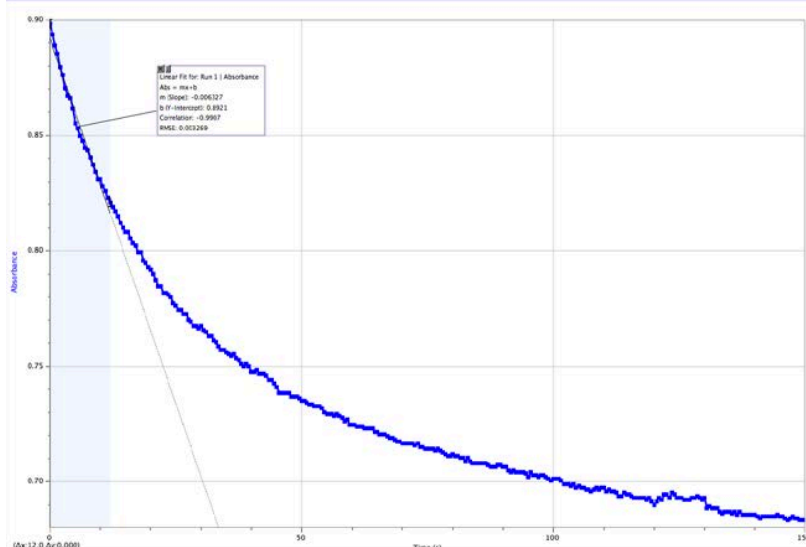


Figure 2: Absorbance levels for starch hydrolysis at pH 3

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 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

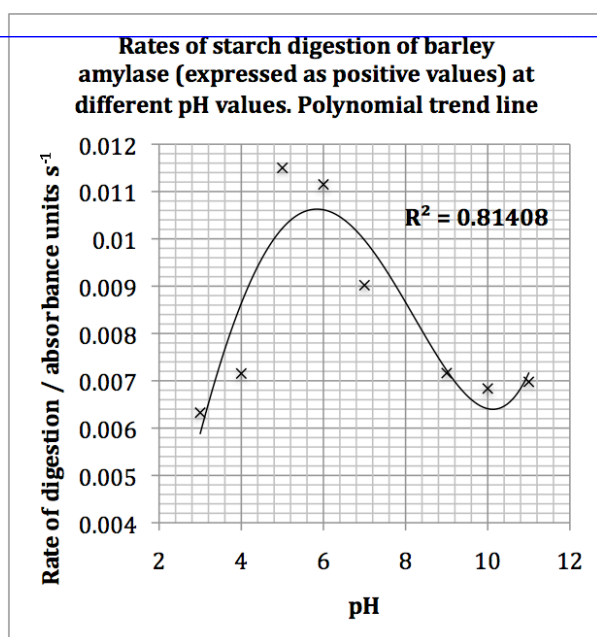
Table 1: Rates of Starch digestion at the different pH values.

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 R² value

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

Observations on Figure 3:

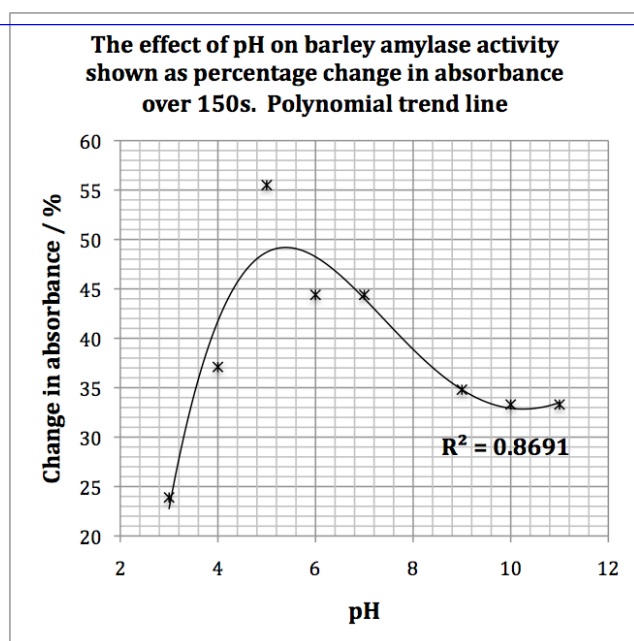
One can notice that the maximum value of the rate of starch digestion is between pH 5 and 6. The data points also appear to stabilize as they tend towards the extremes. Moreover, when observing the overall trend given by the data points of figure 3, one can see that a plateau of the minimum rate of change of starch levels forms at approximately 0.007 abs 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 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 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 5.5-6.8¹⁰) in the mixture than beta amylase (optimum pH of 3.5-6.2¹¹). 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.

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

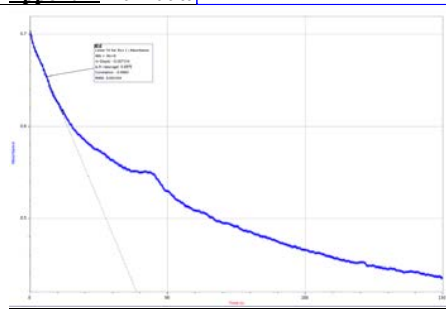


Figure 5: Absorbance levels for starch hydrolysis at pH 4

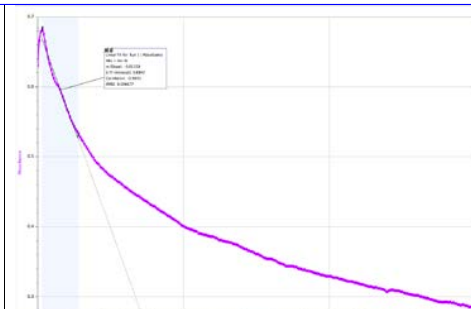


Figure 6: Absorbance levels for starch hydrolysis at pH 5

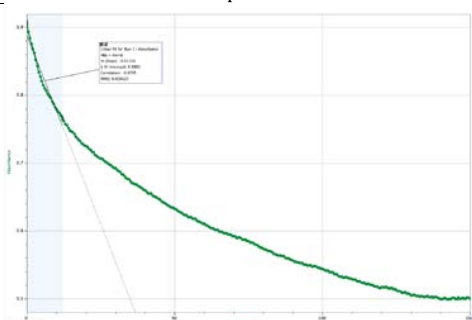


Figure 7: Absorbance levels for starch hydrolysis at pH 6

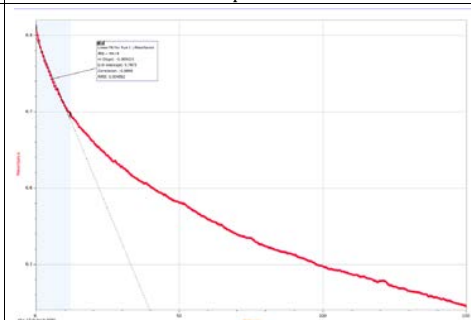


Figure 8: Absorbance levels for starch hydrolysis at pH 7

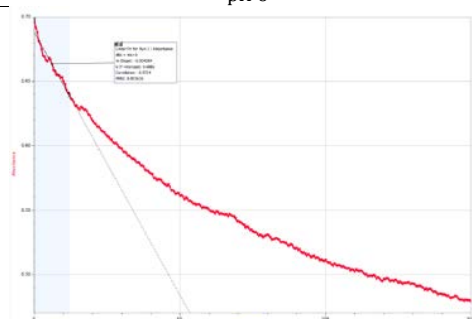


Figure 9: Absorbance levels for starch hydrolysis at pH 8

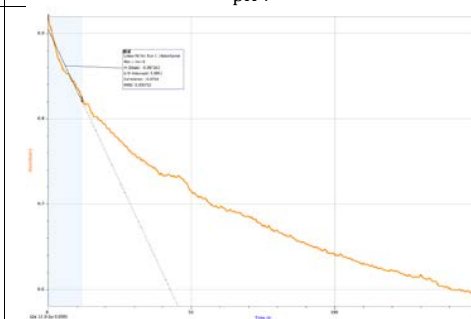


Figure 10: Absorbance levels for starch hydrolysis at pH 9

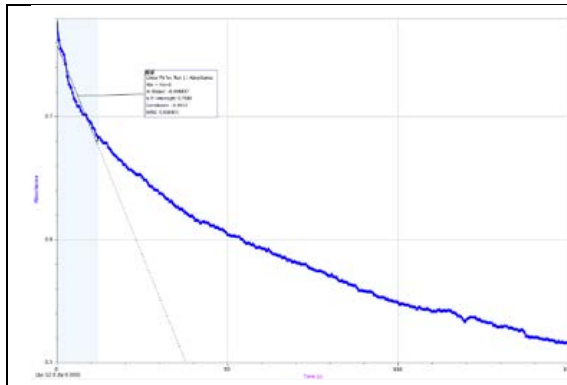


Figure 11: Absorbance levels for starch hydrolysis at pH 10

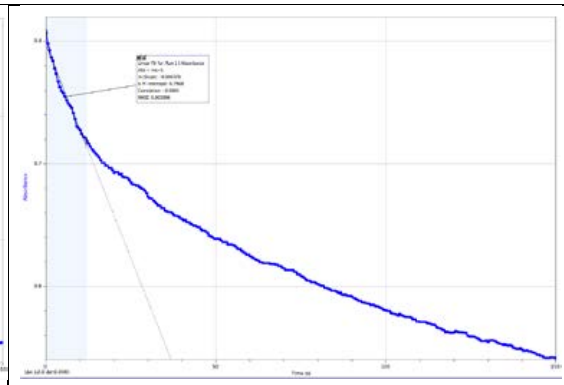


Figure 12: Absorbance levels for starch hydrolysis at pH 11