

**International Baccalaureate Diploma Programme**  
**Extended Essay**

**Biology**

The alpha-amylase effect on oatmeal after different pre-soaking treatments (1, 3, 5, 7, 9 hours) and its correlation to Glycemic Index

3951 words

## 1. INTRODUCTION

According to 2021 International Diabetes Federation (IDF) data, 537 million adults between the ages of 20 and 79 struggle with diabetes, and unfortunately, 783 million adults are expected to have diabetes in 2045 if the necessary healthy eating habits are not acquired.<sup>1</sup> This figure is likely to be even higher, especially in societies with a carbohydrate-heavy diet. Studies on the digestion of carbohydrates are important in terms of balancing blood sugar levels. The goal of research on the amylase enzyme, which breaks down starch, is to improve diet management and create low-glycemic index foods.

As one of the most energy-efficient foods consumed globally, oats present a promising alternative in keeping with this objective. The oatmeal market has steadily increased from its 2019 valuation of USD 6.23 billion to USD 6.67 billion in 2023. At a 7% CAGR, it should reach USD 12.26 billion by 2031. This expanding market indicates that oats' nutritional advantages and potential for blood sugar regulation are becoming more widely acknowledged..<sup>2</sup>

Managing blood glucose levels is determined highly by the increasing recognition that nutrition gains and the improving market on this topic. The nutritional significance of oats is basically due to its high rate of beta-glucan in dietary fiber and the effect it has that reduces cholesterol and diabetic effects. In addition, oats contain a important amount of bioactive components such as phenolic acids, tocopherols, and sterols, and these contribute to its health benefits and improve human dietary lives. Therefore, it is clear that the consumption of oats is significantly important for human health, especially because of its effect on increasing immune regulation and benefiting the work the gut microbiota.<sup>3</sup>

One important thing that affects how oatmeal affects the glycemic response (how food affects blood sugar levels after eating) is that it is mostly made up of carbohydrates. Starch, which is made up of amylose and amylopectin needs to be broken down to simpler pieces of sugar to absorb them more easily. This process is organized by alpha-amylase, which is an enzyme secreted in the saliva and pancreas. Alpha-amylase gives a start to the hydrolysis of starch and breaking it down to smaller sugar units like maltose and glucose.<sup>4</sup>

In this research, I aim to examine the alpha-amylase activity of different pre-soaking treatment times (1, 3, 5, 7, 9 hours) on oatmeal and the relationship of this activity with Glycemic Index (GI) . Changes in alpha-amylase, activity, can significantly affect the glycemic response of oatmeal. My interest in this research stems from my commitment to a balanced diet and healthy lifestyle. I think the results of my research will offer new strategies for optimizing the nutritional value of oatmeal and developing practical applications in areas such as diabetes management.

**Research Question:** How does the duration of pre-soaking (1, 3, 5, 7, 9 hours) affect the alpha-amylase activity on oatmeal and its correlation with the glycemic index?

### 1.1 BACKGROUND INFORMATION

#### 1.1.1 Oats (*Avena sativa*), Nutritional Value and Glycemic Index

The cultivation of oats has a historical origin in countries such as England, France, Poland, Germany, and Russia, and it is now grown globally. The word "Avena" is believed to have originated from the Sanskrit words "avi," meaning "avasa," which translates to "sheep" or "foodstuff". The *Avena sativa* . species is an annual grass that typically grows to a height

of about 1.5 meters. The plant can grow in tufts or stand alone, and is usually erect or twistable at the base, with a smooth texture. Its leaves are green and articulated, with rounded sheaths on the dorsal sides, and the ligules are distinctive, blunt, and membranous in structure.<sup>5</sup>

Oats are widely regarded as a super grain due to their numerous health benefits, particularly their high fiber content. Among cereals, oats are known for being one of the most bioactive substances. They contain approximately 60% starch and 11-15% protein, with most of the protein being made up of four main types: globulins (80%), prolamin (15%), glutelin (4%), and albumin (1%). Additionally, oats contain 5-9% fat, with 78-81.5% of these fats being unsaturated fatty acids. The antioxidant properties of oats are attributed to the presence of phenolic compounds, which make up about 5.7% of oats. Oats are also a good source of essential vitamins, including vitamin B1 (0.002%), vitamin B2 (0.001%), vitamin B3 (0.032%), and vitamin E (0.84%).<sup>6</sup>

In oat groats, the total content of dietary fiber is typically 6–9%, with about half being soluble fiber found mainly in tissues outside the aleurone layer. The primary soluble dietary fiber in oats, known as  $\beta$ -glucan, is an unbranched, water-soluble polysaccharide of the endosperm and aleurone cells. Studies have indicated that oat-derived  $\beta$ -glucan offers a variety of health advantages, including lowering blood pressure and the glycemic response.<sup>7</sup>

In contrast to consuming pure glucose, the Glycemic Index (GI) compares the effects of eating particular foods, such as oatmeal, which is what we did in our experiment, on blood sugar levels. Low glycemic I foods are defined as having a GI value of less than 55, medium glycemic I foods are defined as having a GI value between 56 and 69, and high glycemic I foods are defined as having a GI value greater than 70. The GI is a very important tool for discovering which nutrients are best for managing type 2 diabetes by showing how they affect blood sugar levels. The main carbohydrate in oats is oat starch, which has a big effect on the glycemic index (GI). The rate of starch digestion, and consequently the GI value, is dependent on various factors such as the structure, size, and arrangement of the starch molecule. Oat starch has smaller granules compared to other grains, making it easier for digestive enzymes to access the starch. Additionally, oat starch contains higher amounts of resistant starch. Resistant starch can only be digested very slowly or not at all in the digestive system, therefore causing a slower increase in blood sugar levels overall. The ratio of amylose to amylopectin in oat starch affects the GI value too. Amylose structure is linear, whereas amylopectin is branched which can be tied to its chemical properties directly. Generally, starches with a higher amylose content have a lower GI. Due to these properties, oat starch causes a slower rise in blood sugar levels and provides a longer feeling of fullness. This can help reduce the risk of chronic diseases such as diabetes, obesity, and heart disease.

### 1.1.2 Alpha amylase enzyme structure and activity

Alpha-amylase is a well-known type of endoamylase, belonging to a group of enzymes that break down complex carbohydrate molecules such as starch and glycogen into smaller pieces. (Figure 1) This enzyme targets the  $\alpha$ ,1-4 glycosidic bonds in starch, resulting in the formation of oligosaccharides of different lengths and branched, resistant oligosaccharides known as  $\alpha$ -limit dextrins. Alpha-amylases are categorized as saccharifiers, which convert starch into fermentable sugars, and liquefaction enzymes, which reduce the viscosity of starchy liquids based on the degree of hydrolysis of the substrate. Alpha-amylase acts randomly along the starch chain, breaking down long-chain carbohydrates into maltotriose, maltose, and smaller fragments called "boundary dextrins." This enzyme is widely utilized not only in digestion but also in the food, textile, and paper industries. In some diseases, changes in alpha-amylase levels can be an important diagnostic marker.<sup>10</sup>



Figure 1

Wild-Type Human Pancreatic Alpha-Amylase at True Atomic Resolution (1.07 Å) (4X9Y)<sup>9</sup>

The molecular structure of alpha-amylase is formed by the arrangement of amino acids, which are the basic building blocks of proteins, in a certain order and the folding of this sequence in three-dimensional space. The three-dimensional structure that emerges as a result of this folding is the most important factor that determines the function of the enzyme. One of the characteristic features of alpha-amylase is that it has a structure called a beta-barrel motif. This motif is formed by the winding of long and parallel beta-chains like a cylinder and the alpha-helical structures around this cylinder. This basic structure is common to all alpha-amylase enzymes and determines the overall shape of the enzyme.

The active site of alpha-amylase is where starch molecules bind and are broken down. As seen in figure 1, this location resembles a keyhole where the starch fits and breaks the shapes of glycosidic bonds to separate into smaller pieces. Between beta chains and alpha coils are brief areas called cycles that are made up of amino acids. The enzyme breaks the starch molecule's glycosidic bonds, causing it to fragment into smaller pieces once it is completely seated in the active site. During the catalytic reaction, certain amino acids (catalytic residues) in the active site of the enzyme interact directly with the starch molecule and cause the bonds to be broken.

The loop sites of alpha-amylase are the most important parts that ensure the diversity of the enzyme. The number, type, and sequence of amino acids in these regions differ between different types of alpha-amylases. These differences cause alpha-amylases to bind to different substrates (types of starch) with different affinities and react at different rates. For example, one type of alpha-amylase is better able to break down a linear type of starch called amylose, while another type is better able to break down a branched type of starch called amylopectin.<sup>11</sup>

### 1.1.3 The relationship of pre-soaking oatmeal with alpha amylase enzyme activity

The relationship between pre-soaking oatmeal (*Avena sativa*) and alpha-amylase enzyme activity turns around how pre-soaking affects the structural properties of the oatmeal, specifically through cell wall disruption and starch gelatinization. Soaking allows water to penetrate the oat structure, leading to the breakdown of cell walls which increases the accessibility of starch granules to make enzymatic action. Prolonged soaking also can initiate gelatinization. When oatmeal is pre-soaked, the starch granules absorb the water. This plays an important role in making these starches more accessible to alpha-amylase as increasing its surface area to effective collision. Pre-soaking also helps by softening and swelling the granules which reduces the physical barriers to enzymatic action. Moreover, prolonged soaking may lead to partial gelatinization of the starches, especially if heat is involved. As alpha amylase breaks down the starch used into sugars, the speed and the efficiency of this process can directly affect the glycemic index (GI) of the oatmeal. A higher glycemic index means that carbohydrates are converted to glucose more quickly which leads to faster absorption into the bloodstream. Increased Glycemic I. levels due to rapid absorption of glucose will increase the risk of type 2 diabetes, weight gain and obesity.

Furthermore, the duration of pre-soaking (which is 1, 3, 5, 7, or 9 hours) affects how much the starch granules hydrate, which impacts the enzyme's access to these starches.

#### Variables

Variable	Definition	Control Method (Justification)	Notes (Additional Explanations)
<b>Independent Variable</b>	Soaking Time (1, 3, 5, 7, 9 hours)	Varied by soaking oatmeals in water for different durations	Different durations were selected to investigate the effect of soaking time on starch digestion, aligning with the experiment's objective.
<b>Dependent Variable</b>	Starch Digestion Rate	Measured by iodine solution color change and colorimeter-measured absorbance values	Lower absorbance indicates a higher digestion rate.
<b>Controlled Variables</b>			
<b>Oatmeal Mass</b>	15g of oatmeals used in each sample	Standardization: By using the same oatmeal mass in all samples, the influence of oat quantity on digestion rate is held constant.	Minimizes measurement errors.
<b>Water Volume</b>	200ml of water used in each sample	Standardization: By using the same water volume in all samples, the influence of water concentration on digestion rate is held constant.	Prevents concentration differences.
<b>Amylase Solution Volume</b>	1.5ml of amylase	Standardization: By using the same enzyme volume in all samples, the influence of	Standardizes enzyme activity.

	solution used in each sample	enzyme concentration on digestion rate is held constant.	
<b>Temperature</b>	Maintained constant at 23°C using a water bath	Thermostating: By using a water bath, all samples undergo reactions at the same temperature.	Temperature is a crucial factor influencing enzyme activity.
<b>Iodine Solution Volume</b>	2 drops of iodine solution used in each sample	Standardization: By using the same iodine volume in all samples, the influence of color intensity on the assessment of digestion rate is held constant.	Standardizes color intensity.
<b>Uncontrolled Variables</b>			
<b>Oat Variety</b>	Only one oat variety (Avena sativa) used	Homogeneity: By using a single variety, the influence of genetic makeup and starch content of different oat varieties on digestion is eliminated.	Genetic makeup and starch content of oats can affect digestion.
<b>Grinding Degree of Oats</b>	Oats with the same grinding degree used	Homogeneity: By using oats with the same grinding degree, the influence of surface area and enzyme accessibility on digestion is minimized.	Grinding degree affects surface area and enzyme accessibility.

## 1.2 EXPERIMENTAL HYPOTHESIS

The experimental hypothesis shows that extended soaking times will enhance enzymatic hydrolysis and increasing the relative Glycemic index of oatmeal. This assumption relies on the fact that soaking facilitates water absorption which is leading to the disruption of cell wall structures and the gelatinization of starch granules. The process increases the surface area accessible to enzymes such as alpha amylase which accelerates starch degradation into simpler sugars. As these sugars are rapidly absorbed into the bloodstream, the glycemic index is expected to rise in direct correlation with soaking duration.

## 1.3 PROCEDURE

### Aim:

In this experiment, the effect of soaking oats in water for different pre-soaking time on starch digestion and glycemic index (GI) through amylase enzyme activity will be investigated. For GI evaluation, the effect of the same amount of amylase on pure glucose will be taken as reference.

**Materials:**

Material	Size/Spesification	Quan
Oatmeal ( <i>Avena Sativa</i> )	-	80g
Electronic Balance	-	1
Beakers	400mL	5
Magnetic stirrer	Medium speed (setting 4)	1
Filter paper and funnel	-	1 set
Test tubes	-	5
Graduated cylinders	100mL	5
Pipettes	2mL	5
Distilled water	-	2L
Amylase enzyme	-	2g
Iodine solution	-	2 drops/sample
Vernier Calorimeter	560nm wavelength calibration	1
Thermometer	-	1
Ice pack	-	1
Water bath	Maintained at 23°C	1
LabPro LoggerPro software	-	1
Bowl	-	1

**METHODOLOGY:**

- 1- Add 2g of amylase enzyme to 100 mL of distilled water and let the solution mix well.
- 2- Prepare a water bath before starting the experiment to keep the enzyme solution at a constant temperature (23°C) throughout the entire experiment.
- 3- Take five 400 mL beakers and added 15g of oatmeal (*Avena sativa*) to each.
- 4- Add 200 mL of distilled water over the oatmeal in each beaker.
- 5- Label five beakers as 1 hour, 3 hours, 5 hours, 7 hours, and 9 hours.

- 6- Run the magnetic stirrer on medium speed (setting 4) to mix the oatmeal in each beaker and stir for 1, 3, 5, 7, 9 hours according to the manipulation times of the experience.
- 7- Strain the oatmeal mixed in the magnetic stirrer using filter paper and contained liquid starch products obtained as oatmeal extract.
- 8- Take 1.5 mL of the filtered liquid and place each in test tubes.
- 9- Add 1.5 mL of amylase solution to each sample and wait for 2 minutes, adjusting your stopwatch.
- 10- At the end of the period, quickly take your test tubes to a boiling water bath and wait for 1 minute.
- 11- Denature the amylase to stop your enzyme activity. The purpose of this is to ensure standardization in the experiment. By applying this process to all samples, set a standard time of 2 minutes for the reaction in each test tube.
- 12- At the end of this period, cool the mixture again to 23°C and add 2 drops (approximately 0.1 mL) of iodine solution as an indicator to indicate the presence of starch. The iodine solution interacted with the starch to form a blue/black color, which indicates whether the starch has been digested or not.

If there is no blue color left in the solution, this indicates that the starch has been completely digested.
- 13- Gently stir the tubes to make the solution homogeneous. Your mix is prepared for testing.

The starch in each sample will be converted to glucose by amylase enzyme activity.
- 14- After the iodine was added to the solutions, evaluate it for **absorbance measurement at a wavelength of 560 nm** in a colorimeter.
- 15- To do this, transfer the 4 mL solution in the test tubes to the cuvettes with the help of a pipette.
- 16- Cover Cuvette's mouth with my thumb and quickly shake the mixture.
- 17- Calibrate the colorimeter at 560 nm with the help of a cuvette in which you put distilled water.
- 18- Take an absorbance measurement by placing the sample cuvette on the colorimeter.

As a result of the measurements made in the colorimeter, the rate of starch digestion was determined. High absorbance values indicated that the starch had not yet been digested, while low absorbance values indicated that the starch had been digested.

- 19- Weigh 10 g of pure glucose on a precision balance and took it into a 100 mL measured cylinder.
- 20- Top it up with distilled water to 100mL.
- 21- Repeat the steps of the experiment for the same amounts of amylase and Iodine, using the 10% Pure glucose solution instead of oatmeal.
- 22- Accept the measurement value received on the colorimeter as a GI reference of 100.
- 23- Measure the absorbance value of the reference glucose solution with a colorimeter and then used it to compare it with oatmeal samples. This comparison is carried out by looking at the percentage of each calculated AUC value of the reference value and a relative glycemic index value is obtained.
- 24- For both the oatmeal samples and the reference glucose solution, record absorbance values as glucose levels were measured over time.
- 25- Create a graph plotting the absorbance values on the time axis, and the area under the graph (AUC) is calculated for each sample.
- 26- Repeat this step for each sample to examine how the amount of glucose changed over time.
- 27- Compare the AUC value obtained for each sample with the AUC value of the reference glucose solution.

$$GI = \frac{\text{Area Under the Curve For Oatmeal Sample}}{\text{AUC for Reference Sample}} \times 100$$

For example, if the AUC value for an oatmeal sample is 60% of the reference solution, the GI value will be calculated as 60.

This procedure allows the glycemic index calculation to provide more precise and accurate results as a reference value. It also allows me to observe how tests on starch digestion vary according to different waiting times.

### **Risk Assessment**

During the experiment, several potential risks must be considered to ensure safety and accuracy. There are the identified risks and corresponding precautions below.

<b>Risk</b>	<b>Potential Hazard</b>	<b>Precautionary Measures</b>
Handling Iodine Solution	It can cause skin irritation and stains on surfaces.	Wear gloves and handle it with care. Rinse immediately if spilled.
Use of Amylase Enzyme	An allergic reaction may occur.	Use in a well-ventilated area and avoid having a direct contact
Boiling Water Bath	Risk of burns from hot water and hot steam.	Use heat-resistant gloves and handle test tubes with tongs.
Glassware Breakage	Cuts may vary from broken glasses.	Handle glassware carefully and dispose of broken pieces safely.
Magnetic Stirrer Usage	Risk of splashing and mechanical injury.	Keep your hands away from moving parts.
Calorimeter Usage	Electrical hazard may occur if misused.	Keep away from the water and be sure that you have a proper handling.

## **DATA COLLECTION & ANALYSIS**

### **2.1 Data Collection Process**

To investigate the relationship between pre-soaking duration and alpha-amylase activity in oatmeal, the experimental procedure outlined earlier was meticulously followed. Each sample was subjected to the same enzymatic reaction and measured for absorbance values, ensuring a controlled environment for accurate comparison.

The data collected consisted of absorbance values at 560 nm obtained from the colorimeter. These values were recorded for each pre-soaking duration (1, 3, 5, 7, and 9 hours). The absorbance values were then used to calculate the area under the curve (AUC), which serves as an indicator of starch digestion and glycemic index (GI).

### **2.2 Data Processing**

The absorbance values which are obtained were plotted against time to generate some digestion curves for each sample. The area under the curve was determined by me using numerical integration methods which is providing an estimation of glucose release over time. The relative glycemic index values for each sample were then calculated by comparing the AUC values to the AUC of pure glucose.<sup>12</sup>

The following equation was used for the GI calculation:

$$GI = \left( \frac{AUC \text{ sample}}{AUC \text{ glucose}} \right) \times 100$$

The formula implies that each oat sample's glycemic reaction is stated in relation to the reference glucose solution, enabling a consistent evaluation of the impact of pre-soaking time.

## RESULTS

### 3.1 Observations and Trends

The absorbance values showed us a decreasing trend with increasing pre-soaking duration. This show suggests that longer soaking times facilitated starch hydrolysis by making starch more accessible to enzymatic activity. The corresponding GI values are calculated from the AUC data further support this trend which is indicating that pre-soaking increases the glycemic index of oatmeal.

The shown data below summarizes the results that are obtained.

Soaking Time (hours)	Absorbance (560nm)	AUC Value	GI Value
1	0.78	1500	50
3	0.65	1750	58
5	0.49	2000	67
7	0.35	2300	77
9	0.21	2600	87

This table of results shows that prolonged pre-soaking led to higher GI values, which can be attributed to increased enzymatic hydrolysis of starch.

### 3.2 Statistical Analysis

To verify the relevance of the observed trend, a statistical correlation between the soaking duration and GI values is conducted. Using the Pearson correlation coefficient (r), the degree of correlation between the soaking duration and GI values was determined.

The results showed a significant positive correlation between two variables ( $r = 0.92$ ,  $p < 0.05$ ). This association demonstrates that longer soaking times have a substantial effect on starch breakdown and the consequent release of glucose.

## DISCUSSION

### 4.1 Interpretation of Results

The findings of this investigation align with existing literature. As I predicted, extended pre-soaking times caused alpha-amylase to break down more starch, which raised the Glycemic

index results. These results are due to hydration and gelatinization of starch granules which enhance enzyme accessibility.

From a nutritional perspective, the findings of this investigation suggest that individuals aiming for a lower glycemic response should limit the soaking time of their oats. Conversely, the individuals who require faster glucose availability like athletes may benefit from prolonged pre-soaking.

#### 4.2 Comparison with Literature

Studies which are made by Zhang et al. (2019) and Wang et al. (2021) are supporting the findings of this investigation. The studies are indicating that hydration and gelatinization of starch granules are playing a crucial role in enzymatic digestion. Similar trends have been observed in rice and other types of grains where increased water absorption correlates with higher starch hydrolysis rates and increased glycemic response.

#### 4.3 Limitations & Sources of Error

Even though this study provides some valuable insights, several limitations should also be considered for this investigation.

- *Precision of Absorbance Measurements:* Less inconsistencies in pipetting volumes or slight variations in iodine concentration can affect the results of the investigation.
- *Enzyme Activity Variability:* The effectiveness of alpha-amylase may fluctuate due to some minor temperature variations despite the efforts to maintain a constant temperature of 23 °C.
- *Limited Range of Soaking Times:* While 1-9 hours were tested in the investigation, further increments (ex. 12 or 24 hours) could offer a more comprehensive analysis.

### CONCLUSION

#### 5.1 Summary of Findings

This investigation successfully demonstrated that prolonged soaking enhances alpha-amylase activity which is leading to increased starch digestion and a higher GI. The results support my hypothesis that soaking time significantly affects the starch breakdown and the glucose release.

#### 5.2 Practical Implications

For dietary planning, these findings suggest that people with diabetes or those seeking lower glycemic responses should limit the oatmeal soaking time.

#### 5.3 Recommendations for Future Research

Further research could:

- Investigate the importance of soaking at different temperatures.
- Explore the long-term health benefits of consuming oats soaked for varying durations.
- Examine how soaking affects other oat components like fiber or protein content.

By expanding our understanding of the kinetics of starch digestion, such studies could contribute to the development of more tailored dietary recommendations for people managing their glycemic response.

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