SASTA OLIPHANT SCIENCE AWARDS

Prize Winner

Scientific Inquiry

Year 11-12

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Which Fabaceae beans are the most effective α-amylase inhibitors?

Background Information:

Homeostasis is the maintenance of a stable internal environment in the human body within physiological tolerance limits (Oliveira & Malva, 2022), operating via feedback loops involving the nervous system and/or endocrine system (Cornell, 2023). The maintenance of a stable blood glucose concentration is an example of a homeostatic physiological process. It is controlled by the negative feedback loop between antagonistic hormones, insulin and glucagon, which are released from the Islets of Langerhans (LibreTexts, 2020).

All body cells require glucose during cellular respiration to synthesise ATP, a molecule which acts as an immediate power source for cells (BYJUS, 2019). Glucose is a monosaccharide that enters the body either eating foods containing glucose or carbohydrates that are chemically digested to glucose. This glucose is then absorbed into the bloodstream through capillaries of the small intestinal villi (Damon, 2014), increasing the blood glucose concentration. Simultaneously, body cells are constantly undergoing cellular respiration by breaking down glucose in the presence of oxygen to produce ATP (as well as by-products of carbon dioxide and water vapour) to meet bodily energy requirements. This process constantly lowers the concentration of glucose in the blood (Damon, 2014). Hence, the amount of glucose required in the blood to undergo cellular respiration will fluctuate according to demand, however because high levels of glucose in the blood creates hypertonicity, which can cause damage to cells, glucose levels must be regulated (Cornell, 2023).

Diabetes mellitus (DM) is a chronic disease that occurs when the body is unable to maintain a homeostatic, regulated blood glucose concentration (around 70-100mg/dL) (Riley). Characterised by hyperglycaemia, or a high blood glucose concentration, DM currently affects 1 in 10 adults worldwide (Diabetes Australia, 2023), and is caused by either β cells of the pancreas not producing enough insulin (Type I Diabetes) or when insulin produced cannot be effectively used by body cell receptors (Type II Diabetes) (WHO, 2023). Uncontrolled DM of either type may lead to diabetic nephropathy, diabetic neuropathy, cardiovascular disease etc.

Insulin is secreted from the β cells of the pancreas into the bloodstream when there is a high blood glucose concentration to reduce blood glucose levels by causing cells in the body to open protein channels in their plasma membranes. This allows the facilitated diffusion of glucose into cells along the concentration gradient, thus increasing the rate at which glucose is broken down by increasing the rate of cellular respiration. Insulin is also involved in stimulating the hepatocyte cells in the liver to absorb glucose from the hepatic portal vein and converting them into glycogen, a polysaccharide, which is then stored as granules in the cytoplasm of the hepatocytes, decreasing blood glucose levels. A similar process occurs in skeletal muscle cells as well, which also functions to reduce blood glucose levels via increased glucose uptake and glycogenesis.

When blood glucose levels fall below the threshold concentration (hypoglycaemia), e.g., after extensive exercise or limited food consumption, the hormone glucagon is secreted from α cells of the pancreas into the bloodstream. Glucagon stimulates hydrolysis of glycogen stored in liver hepatocytes, breaking the polysaccharide back down to glucose monosaccharides, thus increasing blood glucose concentration.

Starch is a common polysaccharide composed of glucose monomers, accounting for nearly 60% of all carbohydrates consumed by humans (Robertson, 2018), making it the most prevalent source of glucose in our diets (LibreTexts, 2020). It is an organic product of photosynthesis typically stored in plants as starch granules in chloroplasts or roots and exists as either linear chains (amylose) or branched chains (amylopectin), an commonly found in beans from the Fabaceae family.

Chemical digestion of starch begins in the mouth with salivary amylase and is continued by pancreatic amylase in the duodenum of the small intestine. Amylase is an enzyme, or a globular protein that acts as a biological catalyst to provide alternate pathways for metabolic reactions to occur with a lower activation energy, thus increasing reaction rate (NIH, 2023). It is specific to the digestion of starches (amylose and amylopectin), and catalyses the hydrolysis of these molecules (Gong, 2020).

Many beans from the Fabaceae family have evolved to contain α-amylase inhibitors which serve as a defence mechanism against the amylase in insects, preventing/reducing insect growth (Yamane, 2010), and acting as insecticides, while having no side-effects on humans (Kusaba-Nakayama, 2000). These α-amylase inhibitors block active sites of the α-amylase enzyme (Westermann, 2010), preventing amylose and amylopectin substrates from binding to the active sites of α-amylase. This decreases the hydrolysis of the α-1,4-glycosidic linkages of these starch molecules (Carlsen et al., 1983), thus limiting the number of molecules broken down into glucose and entering the bloodstream (disaccharides and polysaccharides cannot be absorbed into the bloodstream due to their large size) (Gong et al., 2020). This effectively decreases impacts

of starch consumption on blood glucose levels, and reduces the reliance of diabetics on insulin production, secretion and absorption to lower blood glucose concentration. Making dietary alterations to increase consumption of foods containing α-amylase inhibitors is emerging as a viable prophylactic treatment for Type II diabetes, as blood glucose concentrations can be lowered through α-amylase inhibitor consumption without the need of insulin being used by body cell receptors. A reduced digestion and absorption of carbohydrates due to α-amylase inhibitor consumption is also associated with decreased glycogenesis within the human body due to a decreased reliance on insulin, which may also have weight loss benefits (Peddio et al., 2022).

Personal Engagement:
Growing up, my dad would always encourage us to incorporate all kinds of beans into each of our meals, due to their wide variety of health benefits according to Traditional Chinese Medicine. When he developed kidney stones a couple of years ago, he was recommended red kidney beans for their diuretic properties, and I was really fascinated by how such tiny beans could contain chemicals that had such significant impacts. This led me to read about all the other different benefits packed into Fabaceae beans, and I was particularly intrigued by the weight loss benefits associated with eating beans and their potential as a more natural diabetes treatment. Thus, I decided to investigate which types of beans are the most effective α-amylase inhibitors and hence which are the best to consume for blood glucose regulation and diabetes treatment.

Research Question: Which cultivars of beans from the Fabaceae family are the most effective α-amylase inhibitors, as measured by the change in spectrophotometric absorption at 285nm of a bean powder suspension before and after adding α-amylase?

Aim: To determine which cultivars of beans from the Fabaceae family are the most effective α-amylase inhibitors and thus is most effective for the treatment of diabetes

Hypothesis:
Table 1: Table of Hypotheses

Null Hypothesis (H0):	There is no significant difference in the α-amylase inhibiting effect of different beans.
Alternative Hypothesis (H1):	There is a significant difference in the α-amylase inhibiting effect of different beans.

Materials:
40g dark red kidney beans
40g red beans
40g great northern white beans
40g black-eyed peas (*Vigna unguiculata)*
40g black turtle beans
40g borlotti beans
40g broad beans (*Vicia faba*)
7 x ceramic bowls
1 x oven
14 x sieves
1 x electronic digital balance
1 x 37°C water bath
7 x mortar and pestles
7 x glass jars
1L 1% α-amylase solution
10 x beakers
10 x 10mL test tubes
6 x rubber stoppers
2 x test tube racks
2 x micropipettes
3 x measuring cylinders
1L distilled water
25mL 0.1M hydrochloric acid
25mL 0.1M sodium hydroxide
119.424mg Sodium Phosphate Dibasic Heptahydrate
76.52mg Sodium Phosphate Monobasic Monohydrate
1 x Pasco wireless pH sensor
1 x retort stand and clamp
2 x 125mL beaker
7 x 50mL beakers
1 x magnetic stirrer
14 x 100mL volumetric flasks
1 x UV-340 Spectrophotometer
7 x cotton pipette filters

Methodology:
Preparation of Beans
1. 40g of each of the 7 bean cultivars were placed in a labelled ceramic bowl and soaked in 500mL of distilled water at room temperature for 24 hours
2. After 24 hours, the water from each bowl was drained and the beans were pealed to remove the hard outer coating (testa) to make them easier to crush (Figure 3).
3. All beans were patted dry using paper towels and placed in a tray to dry under the sun for 8 hours before being roasted in an oven at 70°C for a further 3 hours.


Figure 3: Each of the 7 cultivars of pealed beans after soaking

4. Each of the 7 different cultivars of Fabaceae beans were separately crushed in a mortar and pestle and ground to a fine powder before being sieved into a 50mL beaker, and again sieved a second time from the 50mL beaker into a labelled glass jar for storage (see Figure 4).

Figure 4: Second round of sieving bean powder into jar

Figure 5: pH meter setup for preparation of phosphate buffer solution

pH Buffer Solution:

1. 80mL distilled water was added to a beaker.
2. 119.424mg of Sodium Phosphate Dibasic Heptahydrate and 76.52mg Sodium Phosphate Monobasic Monohydrate were then measured out and added to the beaker before being mixed for 2 minutes at 600rpm using a magnetic stirrer.
3. A retort stand and clamp was set up above the beaker to hold a Pasco wireless pH sensor (see Figure 5) and the pH was adjusted to 6.7 by adding 0.1M hydrochloric acid and 0.1M sodium hydroxide until the suitable pH was reached to match the pH of the human mouth (where the majority of starch hydrolysis occurs in the human body)

Preparation of α-Amylase Solution:

1. A 125mL beaker was filled with 100mL distilled water.
2. 1g of α-amylase (diastase) powder was measured out using a digital scale and added to the beaker.
3. The beaker was placed on a magnetic stirrer and stirred at 200rpm until the α-amylase was dissolved.
4. The beaker was then placed into a water bath left to heat to 37°C.

Preparation of Bean Powder Filtrate*:*

1. 0.5g of each bean powder was extracted from a jar and measured out using a digital scale before being added to a separate labelled 10mL test tube
2. 5mL of the pH buffer solution was added to each test tube to create a 1:10 dilution of bean powder.
3. All 7 test tubes were sealed with a rubber stopper before being shaken vigorously to disperse the bean powder throughout the bean powder suspension.
4. 1mL of each bean powder suspension was extracted and added to a 100mL volumetric flask.
5. 100mL distilled water was then added to each volumetric flask to make up a 1:1000 dilution of the bean powder suspension.
6. Prior to taking absorption readings in the UV-340 Spectrophotometer, each bean powder suspension was filtered through a cotton pipette filter (see Figure 6) into a new labelled volumetric flask to make a bean powder filtrate and remove any bean powder sedimentation which may obstruct absorption measurements.

Figure 6: Cotton pipette filter used to remove undissolved bean powder particles from suspension

Spectrophotometry:

1. The UV-340 Spectrophotometer was turned on and left to warm up and calibrate for 15 minutes
2. While the spectrophotometer was warming up, the cuvettes were labelled and prepared by adding 0.75mL of the 1:1000 dilution of the red bean filtrate to 3 cuvettes using a micropipette.
3. Step 2 was repeated for the other 6 bean filtrate, and a blank cuvette was also made by adding 1.5mL of distilled water to a cuvette using a micropipette.
4. In 3 separate labelled cuvettes, 0.75mL of the 1% α-amylase solution was extracted from the water bath using a micropipette and added to each cuvette. This was repeated a further 6 times.
5. The 3 cuvettes with red bean filtrate were placed into the spectrophotometer, which was blanked before 3 absorbance (AU) readings at 285nm were taken for each sample.
6. Step 5 was repeated but with three cuvettes containing 0.75mL of 1% α-amylase solution
7. 0.75mL of the α-amylase solution was extracted from each of the 3 cuvettes using a micropipette and added to each of the 3 cuvettes with the dark red kidney bean filtrate, ensuring to expel the α-amylase into the cuvettes with some pressure to mix them together in a 1:1 ratio
8. The 3 cuvettes were left to react for 60 seconds before being placed into the spectrophotometer, along with the blank cuvette.
9. The spectrophotometer was blanked and 3 absorbance (AU) readings at 285nm were taken for each of the 3 cuvettes.
10. Steps 5-9 were repeated for the other 6 bean filtrates, ensuring to extract new samples of α-amylase from the water bath for each type of bean.

Variables:

Independent

Table 2: Independent variables in the experiment

What	How
The cultivar of bean from the Fabaceae family used	40g of 7 different Fabaceae bean cultivars were made into a bean powder filtrate: red, dark red kidney, borlotti, broad, great northern white, black-eyed and black turtle beans. This was to investigate which had the greatest α-amylase inhibiting effect, thus were most effective for lowering blood glucose concentrations.

Dependent

Table 3: Dependent variables in the experiment

What	How
Change in absorbance (AU) at 285nm before and after adding α-amylase to each bean filtrate	This was measured by scanning the absorption (AU) at 285nm of each bean filtrate, amylase solution, and bean + amylase filtrate using a UV-340 Spectrophotometer. This was because a change in absorption (AU) of the bean filtrate at 285nm signifies a change in protein content of the filtrate. If there is a decrease in absorption after adding α-amylase to the bean filtrate, this means protein content, and thus α-amylase content, has decreased and been inhibited. The greater the percentage decrease in absorption, the more effective of an α-amylase inhibitor the bean is.

Controlled

Table 4: Controlled variables in the experiment

What	How	Why
Volume (mL) and concentration (mol dm^{-3}) of α-amylase solution added to each bean powder filtrate in each trial	0.75mL of 1% α-amylase solution was added to each cuvette containing amylase only and the bean + amylase filtrate	Different volumes of varying concentrations of α-amylase solutions would impact the extent of starch hydrolysis that occurs within the given time period and thus hinder the effectiveness of the α-amylase inhibitors, as a greater volume or concentration of enzymes will increase the rate of reaction.
The absorption (AU) at 285nm of the α-amylase solution	The same α-amylase powder from the same source was used each trial, and the absorption (AU) at 285nm of each sample used was measured to ensure each α-amylase solution contained a consistent protein content	Significant differences in the absorbance (AU) of the α-amylase samples at 285nm suggests there is varying protein content in each sample. Thus, using α-amylase from the same source with a consistent absorbance ensures any changes in the absorbance of the bean powder filtrate after the addition of α-amylase are due solely because of the α-amylase inhibitory effects of the beans
Mass (g) of Fabaceae bean powder added	0.5g of bean powder from each cultivar was added to each bean powder filtrate before further dilutions were made.	A differing mass of Fabaceae bean powder added would change the amount of α-amylase inhibitors in the solution for a given bean powder filtrate and thus impact both the accuracy and precision of results
Same temperature (°C) of filtrate	The α-amylase solution was stored in a 37°C water bath immediately before the absorbance (AU) at 285nm was measured and the α-amylase was mixed with the bean powder filtrate.	To simulate the average temperature of the human mouth (Sund-Levander et al., 2002), where most starch hydrolysis occurs. An increase in temperature would increase the average kinetic energy of particles and thus lead to a greater rate of reaction. Extreme temperatures could also potentially denature proteins; therefore, it was crucial to keep the temperature of the samples consistent throughout the experiment
Same pH of filtrate	A phosphate buffer was made to pH 6.7 and added to each bean powder filtrate, as according to literature it was most	To simulate the average pH environment of the human mouth (Baliga et al., 2013) where most starch hydrolysis occurs. A differing pH could either speed up or slow down rate of reaction. Extreme pH could denature the proteins, thus it was crucial to

	suitable pH environment (Peddio et al., 2023)	keep the pH of samples consistent throughout the experiment.
The surface area of the individual bean powder particles in the bean powder	The bean powder for each Fabaceae bean cultivar was sieved twice to eliminate any larger chunks of bean powder particles	The greater the surface area, the more bean powder particles exposed to α-amylase, leading to a greater frequency of collisions and thus faster rate of reaction. Therefore, ensuring all bean particles were a similar size and surface area meant all α-amylase inhibiting bean particles were exposed to α-amylase equally
The time given (s) for the 1% α-amylase solutions and bean powder filtrates to react	Following addition of α-amylase, each cuvette of bean powder filtrate was given 60 seconds to react before 3 subsequent absorption (AU) readings at 285nm for each sample in the spectrophotometer.	If reactants are left to react for varying times, each bean filtrate sample will react to a varying extent, thus time given to react must be controlled to determine the relative α-amylase inhibiting effect of each bean cultivar. 60 seconds was chosen due to data from initial trials suggesting sufficient starch hydrolysis by α-amylase of the same concentration will occur taking into account time restraints.
Wavelength (nm) of light used in spectrophotometer to measure absorbance (AU)	The spectrophotometer was set to take 3 readings of the absorbance (AU) of each bean powder filtrate at 285nm	During initial trials, a full wavelength scan of each bean powder filtrate determined peak absorption (AU) occurs at 285nm. This corroborated with literature stating peak absorption of proteins occurs at similar wavelengths (Hayes, 2020). Thus, 285nm was chosen to measure the α-amylase inhibition of each bean.

Uncontrolled Variable:

Table 5: Uncontrolled variables in the experiment

What	Why
Final concentration of the bean powder filtrates	For each bean cultivar, a varying amount of powder dissolved, with some cultivars sedimenting more over the course of the investigation than others. However, during initial tests, it was found that using bean powder filtrates of lower concentrations in which little to no sedimentation developed did not produce α-amylase inhibiting effects that were significant enough to be measured, particularly in more easily soluble bean powder mixtures. Thus, the selected mass of bean powder added was deemed most appropriate to maximise the α-amylase inhibitory effects while attempting to minimise sedimentation.

Risk Assessment:

Table 6: Table of safety concerns

What	Hazard	Minimising Risk
Use of water baths	High water temperatures have risks of causing burns and scalding	Safety equipment such as gloves, glasses, enclosed shoes and a lab coat were worn to minimise impacts of hot water splashes
The presence of glassware	Risk of laceration if equipment breaks	Careful handling of instruments, storing them securely when not in use. Safety equipment (gloves, glasses, lab coat) were worn during the experiment.
Presence of Fabaceae beans	Individuals could potentially have an allergic reaction to the dried beans and particularly bean powder, with symptoms such as hives, skin swelling and vomiting	Hands were washed following handling of beans to reduce spread of allergens and experiment was conducted in a single laboratory to limit exposure to those with a potential bean allergy
Brightness of spectrophotometer	Intense light source may cause blinding if looked at directly	Only operated spectrophotometer when sample compartment was closed
Use of hydrochloric acid	Is corrosive to eyes and skin and toxic to inhale and ingest.	Hands were washed immediately after hydrochloric acid use, safety equipment was worn at all times, and first aid equipment was nearby if needed.

There were no environmental or ethical concerns throughout this investigation

Raw Data:

Qualitative Results

Table 7: Table of qualitative observations

Observation	Evidence
The processed and refined bean powder from all Fabaescus beans was unable to fully dissolve in the phosphate buffer solution	Sedimentation developed at the bottom of the test tube (see Figure 7).
Despite all the bean powders being a similar white powder, when dissolved, each type of bean formed a slightly different coloured suspension.	Broad beans, red beans and dark red kidney beans formed the darkest coloured suspensions, and great northern white beans and black-eye beans forming the lightest coloured suspensions (see Figure 7).
Foaming of bean powder suspensions occurred when shaking the suspensions to create dilutions.	This was most notable with broad beans, red beans, and dark red kidney beans (see Figures 8 and 10). However, some bean powder suspensions, including borlotti beans, had no foaming up (Figure 9).

Figure 7: Evidence of both foaming up of bean powder suspension and sedimentation of bean powder within the suspension

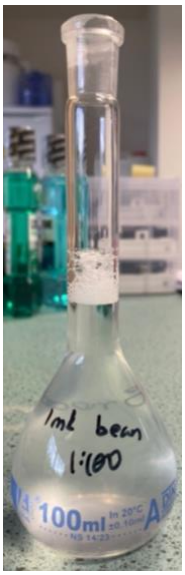


Figure 8: Significant foaming up in dark red kidney bean suspension

Figure 9: No foaming up in borlotti bean suspension

Figure 10: Some foaming up in red bean suspension

Quantitative Results

Table 8: Absorbance (AU) of each Red Bean filtrate, amylase, and the Red Bean + amylase filtrate at 285nm

		Absorbance (AU) ($\pm 0.003 AU$) at 285nm ($\pm 0.5 nm$)		
		Trial 1	Trial 2	Trial 3
Sample 1	Beans	0.521	0.517	0.516
	Amylase	0.450	0.448	0.450
	Beans + Amylase	0.481	0.482	0.485
Sample 2	Beans	0.593	0.590	0.586
	Amylase	0.463	0.462	0.464
	Beans + Amylase	0.701	0.649	0.644
Sample 3	Beans	0.556	0.558	0.558
	Amylase	0.452	0.455	0.455
	Beans + Amylase	0.481	0.478	0.481

Table 9: Absorbance (AU) of each Dark Red Kidney Bean filtrate, amylase, and Dark Red Kidney Bean + amylase filtrate at 285nm

		Absorbance (AU) ($\pm 0.003 AU$) at 285nm ($\pm 0.5 nm$)		
		Trial 1	Trial 2	Trial 3
Sample 1	Beans	0.264	0.262	0.264
	Amylase	0.470	0.470	0.473
	Beans + Amylase	0.852	0.847	0.853
Sample 2	Beans	0.245	0.245	0.247
	Amylase	0.473	0.467	0.247
	Beans + Amylase	0.952	0.955	0.958
Sample 3	Beans	0.255	0.254	0.253
	Amylase	0.476	0.472	0.473
	Beans + Amylase	0.926	0.926	0.927

Table 10: Absorbance (AU) of each Borlotti Bean filtrate, amylase, and the Borlotti Bean + amylase filtrate at 285nm

		Absorbance (AU) ($\pm 0.003AU$) at 285nm ($\pm 0.5nm$)		
		Trial 1	Trial 2	Trial 3
Sample 1	Beans	0.359	0.358	0.356
	Amylase	0.465	0.462	0.460
	Beans + Amylase	0.410	0.410	0.408
Sample 2	Beans	0.369	0.371	0.367
	Amylase	0.479	0.480	0.481
	Beans + Amylase	0.386	0.385	0.387
Sample 3	Beans	0.376	0.377	0.376
	Amylase	0.471	0.473	0.474
	Beans + Amylase	0.406	0.406	0.407

Table 11: Absorbance (AU) of each Broad Bean filtrate, amylase, and the Broad Bean + amylase filtrate at 285nm

		Absorbance (AU) ($\pm 0.003AU$) at 285nm ($\pm 0.5nm$)		
		Trial 1	Trial 2	Trial 3
Sample 1	Beans	0.474	0.469	0.473
	Amylase	0.474	0.469	0.469
	Beans + Amylase	0.519	0.521	0.515
Sample 2	Beans	0.479	0.481	0.479
	Amylase	0.482	0.479	0.478
	Beans + Amylase	0.534	0.531	0.536
Sample 3	Beans	0.462	0.462	0.461
	Amylase	0.474	0.474	0.475
	Beans + Amylase	0.527	0.527	0.528

Table 12: Absorbance (AU) of each Black-Eye Bean filtrate, amylase, and the Black-Eye Bean + amylase filtrate at 285nm

		Absorbance (AU) ($\pm 0.003AU$) at 285nm ($\pm 0.5nm$)		
		Trial 1	Trial 2	Trial 3
Sample 1	Beans	0.520	0.523	0.521
	Amylase	0.475	0.476	0.475
	Beans + Amylase	0.469	0.468	0.466
Sample 2	Beans	0.432	0.429	0.433
	Amylase	0.485	0.483	0.485
	Beans + Amylase	0.454	0.455	0.454
Sample 3	Beans	0.436	0.436	0.434
	Amylase	0.473	0.472	0.471
	Beans + Amylase	0.464	0.464	0.463

Table 13: Absorbance (AU) of each Black Turtle Bean filtrate, amylase, and the Black Turtle Bean + amylase filtrate at 285nm

		Absorbance (AU) ($\pm 0.003AU$) at 285nm ($\pm 0.5nm$)		
		Trial 1	Trial 2	Trial 3
Sample 1	Beans	0.446	0.445	0.446
	Amylase	0.466	0.468	0.466
	Beans + Amylase	0.450	0.451	0.455
Sample 2	Beans	0.463	0.461	0.462
	Amylase	0.464	0.463	0.466
	Beans + Amylase	0.430	0.431	0.434
Sample 3	Beans	0.430	0.427	0.426
	Amylase	0.468	0.469	0.466
	Beans + Amylase	0.467	0.466	0.464

Table 14: Absorbance (AU) of each Great Northern White Bean filtrate, amylase, and the Great Northern White Bean + amylase filtrate at 285nm

		Absorbance (AU) ($\pm 0.003AU$) at 285nm ($\pm 0.5nm$)		
		Trial 1	Trial 2	Trial 3
Sample 1	Beans	0.520	0.523	0.521
	Amylase	0.475	0.476	0.475
	Beans + Amylase	0.469	0.468	0.466
Sample 2	Beans	0.432	0.429	0.433
	Amylase	0.485	0.483	0.485
	Beans + Amylase	0.454	0.455	0.454
Sample 3	Beans	0.436	0.436	0.434
	Amylase	0.473	0.472	0.471
	Beans + Amylase	0.464	0.464	0.463

Due to the complex nature of the data collected, this was deemed the most suitable way of presenting the raw data

See Appendix A for full raw data table

Processed Data:

Table 15: Mean absorption (AU) at 285nm of beans, amylase, and beans + amylase, and the average %change in absorption and standard deviation for each cultivar of Fabaceae bean

		Mean Absorption (AU) ($\pm 0.003 AU$) of Beans at 285nm ($\pm 0.5 nm$)	Mean Absorption (AU) ($\pm 0.003 AU$) of Amylase at 285nm ($\pm 0.5 nm$)	Mean Absorption (AU) ($\pm 0.003 AU$) of Bean + Amylase at 285nm ($\pm 0.5 nm$)	Mean Change in Absorption (AU) ($\pm 0.003 AU$) at 285nm ($\pm 0.5 nm$)	Mean %Change in Absorption (AU) ($\pm 0.003 AU$) at 285nm ($\pm 0.5 nm$)	Standard Deviation
Cultivar of Fabaceae Bean	Red	0.555	0.455	0.542	0.468	-15.677	14.572
	Dark Red Kidney	0.254	0.447	0.911	-0.210	-182.394	44.732
	Borlotti	0.368	0.472	0.401	0.439	19.341	4.792
	Broad	0.471	0.475	0.526	0.420	-10.943	0.855
	Black-Eye	0.463	0.477	0.462	0.478	3.314	2.209
	Black Turtle	0.445	0.466	0.450	0.462	3.694	2.649
	Great Northern White	0.512	0.489	0.487	0.514	0.369	2.896

Calculation 1: Sample calculation for mean using absorption (AU) at 285nm using values for red beans

$$\bar{x} = \frac{\sum fx}{n}$$

$$mean = \frac{0.521 + 0.517 + 0.516 + 0.593 + 0.590 + 0.586 + 0.556 + 0.558 + 0.558}{9}$$

$$= 0.555\ AU$$

Calculation 2: Sample calculation for mean change in absorption (AU) at 285nm for red beans

$$Change = mean\ absorption\ of\ beans + mean\ absorption\ of\ amylase - mean\ absorption\ of\ beans + amylase$$

$$= 0.555 + 0.455 - 0.542$$

$$= 0.468\ AU$$

Calculation 3: Sample calculation for mean %Change in absorption (AU) at 285nm using values for red beans

$$\%Change = \frac{final - initial}{initial}$$

$$\%Change = \frac{Absorption\ Change - mean\ absorption\ of\ beans}{mean\ absorption\ of\ beans}$$

$$= \frac{0.468 - 0.555}{0.555}$$

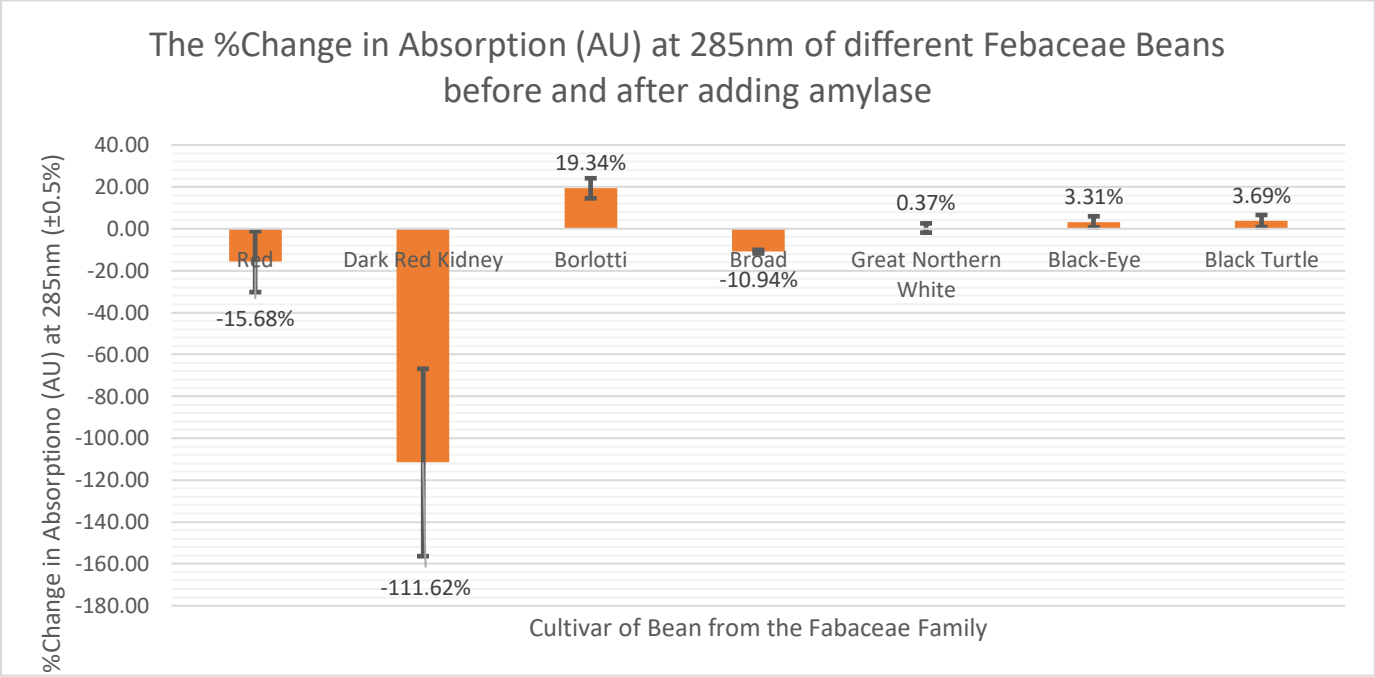
$$= -15.677\ AU$$

Calculation 4: Sample calculation for standard deviation using values for red beans

$$\sigma = \frac{\sqrt{\sum (x - \bar{x})^2}}{n}$$

$$= 14.572$$

See Appendix B for full calculation and data used to calculate standard deviation values



Graph 1: Bar chart of the %change in absorption (AU) of each cultivar of Fabaceae bean at 285nm after the addition of α-amylase

Explanation of Graph 1:
This graph shows the average percentage change in absorption (AU) at 285nm of different cultivars of Fabaceae beans using data from Table 8, displaying the mean percentage change in absorption (AU) at 285nm (±0.5%) for each of the 7 Fabaceae bean cultivars tested, with the error bars representing the standard deviation for each percentage value.

Statistically Tested Data:
From the graph alone, due to the high uncertainty of results it could not be fully ascertained whether there was actually a statistically significant difference in the α-amylase inhibiting effect of different cultivars of beans or if this was due to variances in the data. To determine whether differences between mean percentage change in α-amylase content of all Fabaceae bean cultivars tested was statistically significant, a one-way analysis of variance (ANOVA test) was conducted using the ANOVA: Single Factor Analysis tool from the Analysis ToolPak add-on in data analysis mode on Microsoft Excel following these instructions: https://www.statology.org/one-way-anova-excel/ (see Table 16).

Table 16: Single Factor ANOVA for the %Change in absorption (AU) at 285nm of different Fabaceae beans before and after adding α-amylase using and alpha level of 0.05 (See Appendix C for full summary of ANOVA Test)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	266203.323	6	44367.221	137.586	1.165E-31	2.266
Within Groups	18058.254	56	322.469			
Total	284261.577	62				

According to Table 16, since the F test statistic (137.586) > F critical value (2.266), there is sufficient evidence to reject the null hypothesis (H0).

This is supported by the P-value of 1.165E-31, which is significantly less than the alpha level used of 0.05. Therefore, there is a statistically significant difference between the α-amylase inhibiting effect of the different beans tested, and so the alternative hypothesis (H1) was accepted. Because the P-value < 0.05, a further Tukey-Kramer post hoc test was then conducted according to https://www.statology.org/tukey-kramer-post-hoc-test-excel/ to determine which specific results for the α-amylase inhibiting effect of each bean cultivar are statistically significant (see Table 17).

Table 17: Tukey-Kramer post hoc test to determine which results are statistically significant

Comparison	Absolute Mean Difference	Q Critical Value	Significant?
Red vs Dark Red Kidney	168.209	25.89	Yes
Red vs Borlotti	34.401	25.89	Yes
Dark Red Kidney vs Borlotti	202.610	25.89	Yes
Dark Red Kidney vs Broad	172.357	25.89	Yes
Dark Red Kidney vs Black-Eye	183.605	25.89	Yes
Dark Red Kidney vs Black Turtle	186.750	25.89	Yes
Dark Red Kidney vs Great Northern White	186.914	25.89	Yes
Borlotti vs Broad	30.253	25.89	Yes

See Appendix D for full Tukey-Kramer post hoc table.

Calculation 5: Sample calculation for Q Critical Value

$$Q\ Critical\ Value = Q\sqrt{\frac{S^2_{pooled}}{n}}$$

$$= 4.325\sqrt{\frac{322.469}{9}}$$

$$= 25.89$$

If the Absolute Mean Difference > Q Critical Value, the data is statistically significant. Thus, some of the differences seen in Table 8 and Graph 1 are not due to variances/uncertainties in the data, but due to the differing α-amylase inhibiting effect of the different beans

Analysis of Results:
According to Graph 1, the dark red kidney bean filtrate had the greatest α-amylase inhibiting effect, causing a -182.39% average percentage change in α-amylase content. This indicates it is the most effective α-amylase inhibitor. Because there is now less active α-amylase in the bean powder filtrate, this means less starch would be hydrolysed, and at a slower rate. Thus, less would be converted into glucose that may be absorbed into the bloodstream. Therefore, dark red kidney beans would be most effective for decreasing blood glucose concentration, and hence are best to be consumed for α-amylase inhibiting properties.

In contrast, Borlotti beans were the least effective α-amylase inhibitors, with an increase in α-amylase content of 19.34% following addition of α-amylase. This suggests they would be least effective at regulating blood glucose concentration. However, it was evident that there was still some degree of interaction between borlotti bean samples and α-amylase, as according to Table 15, the mean absorbance (AU) of the borlotti bean + amylase filtrate at 285nm (0.401AU) was less than the absorbance of only the borlotti beans (0.368AU) added to the absorbance of the amylase solution used (0.472). This is because if there was no interaction, the absorbance of the bean + amylase filtrate would have had an absorbance equal to the absorbance of just the beans (0.368) + absorbance of just amylase (0.472), ie 0.368 + 0.472 = 0.840AU, or a percentage change in absorption of 128%.

Standard deviation varied significantly for each bean cultivar. Broad beans had a standard deviation value of 0.855, indicating the most precise results. Dark red kidney beans had the largest standard deviation of 44.732.

The data obtained in this investigation supported the alternative hypothesis that there are statistically significant difference in the α-amylase inhibiting effect of different beans (see Table 16). More specifically, all measured values for dark red kidney beans, which according to Graph 1 caused the greatest percentage decrease in absorption (AU) at 285nm, were statistically significant (see Table 17), indicating data was due to the α-amylase inhibiting effects of dark red kidney beans, and not chance/uncertainties.

Comparison to Literature:
Specific literature values could not be obtained for the specific method, but values were found for the relative α-amylase inhibitor content of each bean cultivar tested, which indirectly supported the conclusion. According to various studies, *Phaseolus vulgaris* cultivars, including dark red kidney beans, red beans, and great northern white beans, exhibit moderate to high levels of α-amylase inhibitory activity, whereas black-eyed beans displayed lower for α-amylase inhibition (Abdelaziz et al., 2020; Ghorbani et al., 2018). According to another study, kidney beans contained high α-amylase inhibitor contents of 2-4g kg^{-1}, compared to the 0.1-0.2g inhibitor equivalent kg^{-1} in black-eyed beans (Grant et al., 1995). This largely supported data obtained in this investigation, with the most effective α-amylase inhibiting beans being in the order of dark red kidney beans > red beans > broad beans > great northern white beans > black-eye beans > black turtle beans > borlotti beans. Another study found that broad beans, a cultivar of the *Vicia faba* species, contain α-amylase inhibitor contents similar to that of dark red kidney beans of 2.94g kg^{-1} (Choudhary & Mishra, 2016), which contradicted data obtained, as dark red kidney beans were found to be significantly more effective α-amylase inhibitors than broad beans. Some differences between recorded data and literature values may be attributed to phylogenetic differences amonst cultivars, varying climatic conditions, location, soil type depending on where the beans were grown and sourced, and the crop year (Shi et al., 2017). Differences may also be due to various weaknesses and limitations in the methodology, which will be discussed in the evaluation.

Evaluation:
Strengths
Table 8: Strengths in the experiment

What	Why
Prior to being extracted from the volumetric flasks into the cuvettes for taking absorption measurements, each of the 7 bean powder filtrates were filtered using a cotton pipette filter to remove any solid undissolved bean powder particles.	This decreased chances of any solid particles obstructing and potentially interfering with absorbance readings, thus increasing accuracy of results.

Weaknesses/Improvements

Table 9: Weaknesses of the methodology

Error	Evidence	Impact on Results	Improvements
Sedimentation of bean powder in suspension	See Figure 7.	This systematic error meant the mixture was heterogenous, so not all bean powder was in solution. Thus, concentration would have differed slightly, as bean powder particles would likely not have been dispersed equally throughout the suspension when extracted for dilution or absorbance measurement. This had a relatively significant impact on accuracy of conclusions, as only a small amount of bean powder (0.5g) was added initially, so any sedimentation of bean powder when creating dilutions would have caused a significantly decreased concentration.	Place each volumetric flask in an oscillating water bath before extraction, leading to a more consistent concentration of bean powder throughout the filtrate that is dispersed more evenly in suspension.
Some volumetric flasks containing bean powder suspension developed foam after being mixed. Each suspension foamed to a different extent.	See Figures 7, 8, 9 and 10.	This obscured some of the graduation markings on the volumetric flasks, making it difficult to measure volume of water added. Furthermore, although the volumetric flasks were filled to the meniscus with water, foaming up of suspensions meant the volume of the volumetric flask was often greater than it should have been (100mL). This would have decreased the overall concentration of bean powder in filtrates where foaming occurred, while volumetric flasks with no foaming were unaffected. This random error had a minor impact on the conclusion, as the volume of water added to each dilution of the bean powder suspensions would have differed slightly, but only by a small amount relative to the total volume of the suspension.	Leave test tubes to allow foaming/frothing to settle, then once the meniscus becomes visible again, add distilled water to the volumetric flask accordingly. However, time restraints on data collection meant this was not possible.
After extraction from the 37°C water bath and while being added to each cuvette, the α-amylase solution likely would have cooled down from its initial temperature	Cuvettes felt noticeably cooler after measuring data collection.	This meant the rate of reaction in each sample would have been slightly slower than expected, but by different amounts each time, as a lower temperature means particles have a decreased average kinetic energy and thus lower frequency of successful collisions. Therefore, the α-amylase inhibiting effect of the beans tested would likely have been greater than recorded. This systematic error had a moderate impact on the results, as although the optimum temperature of the α-amylase solution used was 37°C, data recorded using the spectrophotometer was relatively efficient, and so the α-amylase solutions would have only cooled down slightly.	Use temperature-controlled cuvettes to ensure temperature of all filtrates remains constant throughout the experiment when taking absorption measurements to better simulate the environment of the human mouth. However, this is unrealistic for a school laboratory.
Not all bean powder was fully suspended in solution.	After shaking the 1:10 dilution of bean powder suspensions, some powder trapped between the rubber stopper and test tube was unable to mix.	This systematic error meant some bean powder was unable to be dissolved, so concentration of bean powder would have been lower than expected, but by different amounts each time. However, this only had a minor impact on the results, as only a small amount of bean powder was stuck between the rubber stopper and the test tube	Use an oscillating water bath to suspend and disperse bean powder instead of manually shaking the test tubes by hand. This would remove the need for a rubber stopper, ensuring all the bean powder remains within the mixture.

Limitations

Table 10: Limitations of the chosen methodology

What	Why
This method was a relatively indirect way of measuring the α-amylase inhibiting effects of different beans	Investigated changes in protein content in the bean powder filtrate before and after adding α-amylase, but did not directly investigate how this change in protein content impacted the rate and extent of starch hydrolysis. This limits the conclusions drawn from the results, as it is unknown whether a decrease in protein content and thus α-amylase content is necessarily correlated with a decrease in starch hydrolysis.
It is assumed that the only cause of changes in protein content of the bean powder filtrate following the addition of α-amylase was due to interactions between only the α-amylase and the α-amylase inhibitors, however it is unknown whether other proteins could potentially have interacted with the α-amylase as well.	This impacts validity of results, as changes in protein content may not necessarily reflect the inhibitory effects of α-amylase inhibitors in the beans on α-amylase, as changes in protein content could also be caused by other proteins. A potential improvement could be to use a starch-iodine test on each of the different bean cultivars to measure their respective changes in starch content over time, with more effective α-amylase inhibitors causing a slower rate of starch hydrolysis.

Extension:

Through this investigation I have become increasingly intrigued by the potential viability of dark red kidney bean consumption as a form of prophylactic treatment for decreasing blood sugar concentration and the regulation of Type II diabetes. In this investigation, all beans tested were dry, uncooked beans. I am curious how cooking may affect the α-amylase inhibiting effects of these various beans, they are often only consumed after being cooked. This is because α-amylase inhibitors are a protein (Svensson et al., 2004), meaning they may become denatured at extreme temperatures and/or pH. Because protein shape determines function, this could impact effectiveness of α-amylase inhibiting proteins. However, there is little literature available on specific conditions in which α-amylase inhibitors may be denatured. This could be measured using starch-iodine at regular time intervals and measuring changes in absorbance (AU) (Yap et al., 2020).

Word Count: 2191

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Appendices:

Appendix A:

Full Raw Data Results Table

		Red Beans (AU)						
		Trial 1	Trial 2	Trial 3	Average	Change in Absorption	%Change in Absorption	Standard Deviation
Sample 1	Beans	0.521	0.517	0.516	0.518	0.490	-5.950	14.572
	Amylase	0.450	0.448	0.450	0.449	0.483	-6.576	
	Beans + Amylase	0.481	0.482	0.485	0.483	0.481	-6.783	
Sample 2	Beans	0.593	0.590	0.586	0.590	0.355	-40.135	
	Amylase	0.463	0.462	0.464	0.463	0.403	-31.695	
	Beans + Amylase	0.701	0.649	0.644	0.665	0.406	-30.717	
Sample 3	Beans	0.556	0.558	0.558	0.557	0.527	-5.216	
	Amylase	0.452	0.455	0.455	0.454	0.535	-4.122	
	Beans + Amylase	0.481	0.478	0.481	0.480	0.532	-4.659	

		Dark Red Kidney Beans						
		Trial 1	Trial 2	Trial 3	Average	Change in Absorption	%Change in Absorption	Standard Deviation
Sample 1	Beans	0.264	0.262	0.264	0.263	-0.118	-144.697	44.732
	Amylase	0.470	0.470	0.473	0.471	-0.115	-143.893	
	Beans + Amylase	0.852	0.847	0.853	0.851	-0.116	-143.939	
Sample 2	Beans	0.245	0.245	0.247	0.246	-0.234	-195.510	
	Amylase	0.473	0.467	0.247	0.396	-0.243	-199.184	
	Beans + Amylase	0.952	0.955	0.958	0.955	-0.464	-287.854	
Sample 3	Beans	0.255	0.254	0.253	0.254	-0.195	-176.471	
	Amylase	0.476	0.472	0.473	0.474	-0.200	-178.740	
	Beans + Amylase	0.926	0.926	0.927	0.926	-0.201	-179.447	

		Borlotti Beans						
		Trial 1	Trial 2	Trial 3	Average	Change in Absorption	%Change in Absorption	Standard Deviation
Sample 1	Beans	0.359	0.358	0.356	0.358	0.414	15.320	4.792
	Amylase	0.465	0.462	0.460	0.462	0.410	14.525	
	Beans + Amylase	0.410	0.410	0.408	0.409	0.408	14.607	
Sample 2	Beans	0.369	0.371	0.367	0.369	0.462	25.203	
	Amylase	0.479	0.480	0.481	0.480	0.466	25.606	
	Beans + Amylase	0.386	0.385	0.387	0.386	0.461	25.613	
Sample 3	Beans	0.376	0.377	0.376	0.376	0.441	17.287	
	Amylase	0.471	0.473	0.474	0.473	0.444	17.772	
	Beans + Amylase	0.406	0.406	0.407	0.406	0.443	17.819	

		Broad Beans						
		Trial 1	Trial 2	Trial 3	Average	Change in Absorption	%Change in Absorption	Standard Deviation
Sample 1	Beans	0.474	0.469	0.473	0.472	0.429	-9.494	0.855
	Amylase	0.474	0.469	0.469	0.471	0.417	-11.087	
	Beans + Amylase	0.519	0.521	0.515	0.518	0.427	-9.725	
Sample 2	Beans	0.479	0.481	0.479	0.480	0.427	-10.856	
	Amylase	0.482	0.479	0.478	0.480	0.429	-10.811	
	Beans + Amylase	0.534	0.531	0.536	0.534	0.421	-12.109	
Sample 3	Beans	0.462	0.462	0.461	0.462	0.409	-11.472	
	Amylase	0.474	0.474	0.475	0.474	0.409	-11.472	
	Beans + Amylase	0.527	0.527	0.528	0.527	0.408	-11.497	

		Great Northern White Beans						
		Trial 1	Trial 2	Trial 3	Average	Change in Absorption	%Change in Absorption	Standard Deviation
Sample 1	Beans	0.478	0.475	0.482	0.478	0.467	-2.301	2.209
	Amylase	0.476	0.474	0.478	0.476	0.463	-2.526	
	Beans + Amylase	0.487	0.486	0.490	0.488	0.470	-2.490	
Sample 2	Beans	0.509	0.505	0.511	0.508	0.522	2.554	
	Amylase	0.494	0.491	0.493	0.493	0.518	2.574	
	Beans + Amylase	0.481	0.478	0.481	0.480	0.523	2.348	
Sample 3	Beans	0.551	0.548	0.551	0.550	0.559	1.452	
	Amylase	0.502	0.499	0.495	0.499	0.554	1.095	
	Beans + Amylase	0.494	0.493	0.495	0.494	0.551	0.000	

		Black-Eye Beans						
		Trial 1	Trial 2	Trial 3	Average	Change in Absorption	%Change in Absorption	Standard Deviation
Sample 1	Beans	0.520	0.523	0.521	0.521	0.526	1.154	2.649
	Amylase	0.475	0.476	0.475	0.475	0.531	1.530	
	Beans + Amylase	0.469	0.468	0.466	0.468	0.530	1.727	
Sample 2	Beans	0.432	0.429	0.433	0.431	0.463	7.176	
	Amylase	0.485	0.483	0.485	0.484	0.457	6.527	
	Beans + Amylase	0.454	0.455	0.454	0.454	0.464	7.159	
Sample 3	Beans	0.436	0.436	0.434	0.435	0.445	2.064	
	Amylase	0.473	0.472	0.471	0.472	0.444	1.835	
	Beans + Amylase	0.464	0.464	0.463	0.464	0.442	1.843	

		Black Turtle Beans						
		Trial 1	Trial 2	Trial 3	Average	Change in Absorption	%Change in Absorption	Standard Deviation
Sample 1	Beans	0.446	0.445	0.446	0.446	0.462	3.587	2.896
	Amylase	0.466	0.468	0.466	0.467	0.462	3.820	
	Beans + Amylase	0.450	0.451	0.455	0.452	0.457	2.466	
Sample 2	Beans	0.463	0.461	0.462	0.462	0.497	7.343	
	Amylase	0.464	0.463	0.466	0.464	0.493	6.941	
	Beans + Amylase	0.430	0.431	0.434	0.432	0.494	6.926	
Sample 3	Beans	0.430	0.427	0.426	0.428	0.431	0.233	
	Amylase	0.468	0.469	0.466	0.468	0.430	0.703	
	Beans + Amylase	0.467	0.466	0.464	0.466	0.428	0.469	

Appendix B:

Sample calculation for standard deviation using values for red beans

$$\sigma = \frac{\sqrt{\sum(x-\overline{x})^2}}{n}$$

$$= \frac{\sqrt{(-15.095--5.950)^2+(-15.095--6.576)^2+(-15.095--7.683)^2+(-15.095--40.135)^2+(-15.095--31.695)^2+(-15.095--30.717)^2+(-15.095--5.216)^2+(-15.095--4.122)^2+(-15.095--4.659)^2}}{9}$$

$$= 14.572$$

Full standard deviation results are above.

Appendix C:

Full summary of Single Factor ANOVA

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Pooled Variance*
Column 1	9	-135.853	-15.095	212.348	322.469
Column 2	9	-1649.735	-183.304	2000.951	
Column 3	9	173.753	19.306	22.965	
Column 4	9	-98.522	-10.947	0.732	
Column 5	9	2.706	0.301	4.879	
Column 6	9	31.015	3.446	7.018	
Column 7	9	32.490	3.610	8.389	

Appendix D:
Full Tukey-Kramer post hoc table

Comparison	Absolute Mean Difference	Q Critical Value	Significant?
Red vs Dark Red Kidney	168.209	25.89	Yes
Red vs Borlotti	34.401	25.89	Yes
Red vs Broad	4.148	25.89	No
Red vs Black-Eye	15.395	25.89	No
Red vs Black Turtle	18.541	25.89	No
Red vs Great Northern White	18.705	25.89	No
Dark Red Kidney vs Borlotti	202.610	25.89	Yes
Dark Red Kidney vs Broad	172.357	25.89	Yes
Dark Red Kidney vs Black-Eye	183.605	25.89	Yes
Dark Red Kidney vs Black Turtle	186.750	25.89	Yes
Dark Red Kidney vs Great Northern White	186.914	25.89	Yes
Borlotti vs Broad	30.253	25.89	Yes
Borlotti vs Black-Eye	19.005	25.89	No
Borlotti vs Black Turtle	15.860	25.89	No
Borlotti vs Great Northern White	15.696	25.89	No
Broad vs Black-Eye	11.248	25.89	No
Broad vs Black Turtle	14.393	25.89	No
Broad vs Great Northern White	14.557	25.89	No
Black-Eye vs Black Turtle	3.145	25.89	No
Black-Eye vs Great Northern White	3.309	25.89	No
Black Turtle vs Great Northern White	0.164	25.89	No

Bio IA Logbook

4/12/23

Now that exams at school are over I want to start working on my biology internal assessment. After compiling a list of potential ideas, I think I want to try something original that I don't think has been investigated much before, especially in a school laboratory. I also want to try out the new spectrophotometer that my school recently bought.

5/12/23

The laboratory technicians have acquired a variety of different beans for me to begin preliminary trials. These beans were purchased from a local wholefoods store, and today I began preparing them for use in my experiment. I am planning on turning them into a powder and dissolving them in solution to react with amylase solution, however even from trying this with such a small sample today I have found that it is incredibly difficult to crush these beans using just a mortar and pestle.

6/12/23

I tried using a food processor to crush these beans today, to no avail. I think the trick is to remove the hard outer testa of the beans by peeling them, as this is what is causing the majority of the hardness of the beans. However, I am not sure whether the outer coating of the beans may contain any nutritional value in terms of alpha-amylase inhibiting effect.

8/12/23

Rather than handpeeling beans without any prior preparation, I have found from watching YouTube videos today that it is much easier to soak the beans first before peeling. I have wasted so much time trying to peel these beans manually. However, I must ensure not to soak my beans for too long, otherwise they will sprout, as I unfortunately found out the hard way today.

10/12/23

Beans have been soaked, and peeled. However, crushing the beans when in this state leads to a mush. I think it would be better for me to dry the beans out in the sun first, and that way I will be able to create more of a powder

Turns out soaking in the sun for a day was not long enough. I will roast these in an oven at school over tomorrow to see if that is more effective

11/12/23

Beans are still slightly mushy on the inside, despite being very dry on the outside. I broke them into smaller fragments to increase the surface area exposed to the oven today, and also ensured to flip them to completely dehydrate the beans

12/12/23

Beans have finally been prepared, and I have spent all morning crushing them, sieving them, and packing them away into containers. Very satisfying to see such a large mess in the laboratory all be stored away in some glass vials. Began collecting data today

15/12/23
Apparently it turns out the wavelength that I have been operating the spectrophotometer at was the wrong wavelength. Fortunately, this new machine is so easy and convenient to use. The only annoying part is having to blank it each time I change the cuvettes being measured.

4/2/24
Practical has been completed, but I have no idea how I am going to cut down all of my words.

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☑ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: Caleb Tang ID: 0526-007

SCHOOL: Prince Alfred College

Activity: Give a brief outline of what you are planning to do.

Investigate which beans from the Fabaceae family are the most effective at inhibiting the action of the digestive enzyme alpha-amylase. This will be measured by their extent of breakdown of starch after a given time period according to absorbance at 285 nm

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

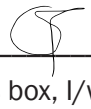
Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Risks	How I will control/manage the risk
Use of water baths. High water temperatures have risks of causing burns and scalding	Safety equipment such as gloves, glasses, enclosed shoes and a lab coat were worn to minimise impacts of hot water splashes
Use of glassware. Risk of laceration if equipment breaks	All apparatus was handled carefully and stored securely when not in use. Safety equipment such as gloves, glasses and a lab coat were worn.
Presence of Fabaceae beans could induce allergic reactions, with symptoms such as hives, skin swelling and vomiting	Hands were washed following handling of beans to reduce spread of allergens and experiment was conducted in a single laboratory to limit exposure to those with a potential bean allergy
Brightness of spectrophotometer may cause blinding if looked at directly	Only operated spectrophotometer when sample compartment was closed

(Attach another sheet if needed.)

Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)): Caleb Tang

SIGNATURE(S): 

☑ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: Fidias Andari

SIGNATURE:  DATE: 30/6/24