Hong Kong Student Science Project Competition 2023

Team Number: SBBC178

Project Title: Study of α-amylase Inhibitor in Plants

Project Type: Investigation

To our best knowledge, there *are* / ~~*are no*~~ * *similar works in the field*; (if there are,) related research links are as below:

https://www.sciencedirect.com/science/article/abs/pii/S096399699800074X

The enhancement our project made / the difference with related research are:

We try to simplify the experiment to see if the tested plants can inhibit a-amylase to lower the blood glucose level of diabetic patients

I. Background

According to the World Health Organisation (WHO), among the world population, about 422 million people are diagnosed with diabetes, the majority living in low-and middle-income countries, and 1.5 million deaths are directly attributed to diabetes each year. A research found that the average economic cost per person was projected to be HK$105,920 for diagnosed diabetes, HK$34,000 for undiagnosed diabetes, HK$4000 for prediabetes.The tremendous economic cost of diabetes is conservatively projected to make up 2.2% of the global GDP by 2030. No matter globally or individually, diabetes has a serious economic impact on the world.

Diabetes is a common chronic health condition. There is an increasing number of young people diagnosed with diabetes in recent years. Apart from medication, patients also need to be aware of their eating habits and food choices. Through literature review, some Chinese herbal medicines are also helpful in lowering the blood glucose level. However, Chinese herbal medicine requires a doctor's prescription, which may not be very convenient, effective and accessible to the general public. Consequently, we would like to find out if there is any plant food, which is available in the market, that can help regulate the blood glucose level. As it is not ethical to involve human subjects in our research projects, therefore we are going to carry out the experiments in the laboratory with different chemicals. In junior form science lessons, we learnt that digestive enzymes are involved in the digestion process. For example, amylase can help the conversion of starch (polysaccharide) into maltose (disaccharide) and different carbohydrates. In this research project, we are going to find out the enzyme (e.g. amylase and different carbohydrates) inhibitors that are present in different food plants. We have tested at least 30 food plants, including okra, kimchi, Chinese broccoli, celery, kimchi, white kidney bean, bitter melon flesh, etc.) We are going to conduct an iodine test, Benedict’s test and glucose test, pH value test to test for the presence of substrates and products in the enzymatic reaction. The more the substrates (polysaccharide) remained or the less the products (monosaccharides) formed indicate the better the enzyme inhibitory effect in a certain part of food. This suggests the possibility of a certain food substance in lowering the blood glucose level in humans. Our research is to investigate which type of food plant is most effective in inhibiting the a-amylase.

II. Objective

The aim of this research is to assess the relationship between type 1 & 2 diabetes and white kidney bean and bitter melon among the patients. Using quantitative methods, we measure the rate of inhibition of the residue of white kidney beans and juice of flesh of bitter melon by testing the presence of starch and reducing sugar after mixing the juice with a certain concentration of amylase solution and also starch solution.

III. Hypothesis

For the result, we predict that there are amylase inhibitors in the residue of white kidney beans and juice of flesh of bitter melon which can reduce the rate of breakdown of starch into simpler carbohydrates. We believe that this can help people control their blood glucose level and thus relieve diabetes. The effectiveness to inhibit a-amylase can be tested by iodine test by comparing the colour intensity of the food samples to the control

Also, we believe that the longer time the white kidney beans and the bitter melon are boiled, the less effective in reducing the rate of breakdown of starch they are, since a number of amylase inhibitors in the white kidney beans and bitter melon will become denatured. Iodine test is carried out at a two-minute interval until all the food samples, which originally are mixed with starch solution and amylase and are boiled in a water bath at 90 degrees Celsius, mixed with the iodine solution turn brown. By comparing the time of the food samples needed to turn brown can help us to know the time the food samples can be boiled.

II. Methodology

- <u>Materials:</u>

- White kidney bean
- Cowpea
- Adzuki bean
- Kimchi
- Broccoli
- Kale
- Okra
- Raspberry
- Blackberry
- Celery
- Red kidney bean
- Red grape
- Chia seed
- Peanut
- Ginger
- Bitter melon
- Aloe Vera
- Distilled water

- Blending of food substances

Apparatus used:

- Blender
- Centrifuge
- 22 mL Test tube
- Food Substance
- Water

In order to find out different statistics of the white kidney bean (and also other food substances), we first blended the beans, which is easier for us to test for starch if it is in liquid form. The diluted solution was then transferred to the centrifuge for about 8 minutes at 5000 rotates per minute, so that the measurement of the liquid and the residue can be done effortlessly by separating the content and for easier comparison to draw conclusions.

- Benedict's Test for testing the presence of reducing sugar

Apparatus used:

- Water bath
- Test tube rack
- 22 mL Test tube
- 0.10% amylase solution
- 1.00% starch solution
- Food solution
- Benedict's solution

To test the presence of reducing sugar in the white kidney bean, we use Benedict's solution to find out the exact amount of reducing sugar in a certain volume of the solution. First, we put the mixture in boiling water of 90 degrees celsius. After 15 minutes, we transferred the mixture of Benedict's solution and the juice to the centrifuge, so that we can observe the quantity of red precipitate of each juice by separating the precipitate and the liquid inside the test tube. Eventually, we draw the conclusion based on the results carried out by the experiment.

- Starch test after the addition of food solution into a mixture of amylase and starch

Apparatus used:

- 22 mL Test tube
- Food solution
- 0.10% Amylase solution
- 1.00% Starch solution
- Iodine solution
- Spotting tiles

To test the presence of starch after mixing the amylase solution and the starch solution with the juice, we carried out the iodine test. First, we incubated the amylase solution for 15 minutes at 37.5℃. Then, we added an equal volume of juice of 2 mL into the amylase solution and waited for 15 minutes since we have to ensure that there is enough time for the juice to inhibit the amylase. After that, we

mixed amylase, starch and different vegetable juices to start off the reaction. We transferred a drop of the reacting mixture to an iodine drop on the spotting tile at 2-minute intervals. Finally we stop when the iodine drop remains brown because this is the time at which all the starch in the reacting mixture is broken down.

- pH value test

Apparatus used:

- pH metre with data logger
- 100 mL Beaker
- Food solution

We also test the food solution's pH value since pH value is a factor that affects the catalysing rate of α-amylase. First, a certain amount of the food solution is poured into the beaker. Then, the pH value of the food solution is tested with a pH metre connected to a data logger.

III. Results

Starch intensity of all tested food samples (after addition of food solution to the amylase)

adzuki bean	+++++	ginger	+++
aloe vera	-	kale	-
bitter melon	++++	kimchi	++
blackberry	-	okra	-
broccoli	-	peanut	-
celery	-	raspberry	++
chia seed	+	Red grape	+
cowpea	+	Red kidney bean	-
distilled water (control)	-	White kidney bean	+++++

- Result

Based on the data collected from the iodine test, we concluded that the juice of flesh of bitter melon is slightly better than the juice of skin of bitter melon in terms of the effectiveness of inhibiting the α-amylase activity, since there is correspondingly more starch present in the solution.

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I. If your team will compete the Social Innovation Award, please list the target group or social issue the project focuses on, and provide justification for competing for this award.

Diabetes is a disease that affects many people all over the world with more people being diagnosed with diabetes. It is one of the most common diseases in Hong Kong. However, diabetes patients can only be cured by injecting insulin up to date, which is costly to the general public. Regardless of the low death rate of about 0.6% in our society, its long-term consequences should not be overlooked. More people are diagnosed with diabetes at a young age in recent years and the trend is alarming. Without proper treatments, it can lead to devastating complications, such as heart disease, nerve damage, blindness, kidney failure and even amputations. So for patients who suffer from diabetes, it is essential to receive medications such as metformin. However, there could be side effects including diarrhoea. Therefore, through this experiment, we hope that we can find some cheaper and edible plants which can relieve diabetic patients. It is hoped that all diabetes patients can all receive suitable treatment, no matter what their background and financial status is. Therefore, we are dedicated to promoting other alternative ways of inhibiting a-amylase temporarily to ensure everyone in our society not only has a gauge of knowledge about diabetes but also knows how to relieve it.

IV. Conclusion

To conclude, the hypothesis was supported by our results. We can conclude that boiled flesh of bitter melon and unboiled residue of white kidney beans are the most effective in inhibiting α-amylase inhibitors in plants.

For the boiled flesh of bitter melon, it can inhibit the reaction which can effectively lower the intake of blood glucose level by inhibiting α-amylase. Comparatively, boiled flesh of bitter melon has a more significant impact to inhibit α-amylase than boiled skin of bitter melon.

For the residue of the white kidney bean, it can inhibit the reaction which can effectively lower the intake of blood glucose level by inhibiting α-amylase so the starch would not be digested.

It can also reach the conclusion that unboiled residue of white kidney beans is the most effective compared with all the food samples.

Also, we believe that the longer the white kidney beans and the bitter melon are boiled, the less effective on reducing the rate of breakdown of starch they are, since a number of amylase inhibitors in the white kidney beans and bitter melon will become denatured.