Investigating the Digestive Enzyme Inhibitors of Bean Extracts

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Summary

This project aims to study the digestive enzyme inhibitors found in beans that we eat everyday. Enzymes are biological catalysts that speed up chemical reactions such as digestion. In this project, our study focuses largely on amylase, an enzyme found in our body that is used to break down starch into smaller and simpler substances such as maltose. In addition to investigating whether the bean extracts contain inhibitors that are able to prevent amylase from functioning, this project also attempts to understand the nature of these inhibitors.

Abstract

Our project aims to investigate the digestive enzyme inhibitory properties of bean extracts. The experiment involves conducting alpha-amylase inhibition assays on 4 different beans, namely mung bean (*Vigna radiate*), kidney bean (*Phaseolus vulgaris*), black-eyed bean (*Vigna unguiculata unguiculata*), and rice bean (*Vigna umbellate*). The assays were also performed against an increasing starch concentration in order to determine whether the inhibitor found in the bean extracts are of competitive or non-competitive nature. Finally, different pre-treatment tests were also conducted to characterize the properties of the alpha- amylase inhibitors found in the bean extracts. Of the 4 beans tested, only kidney bean (*Phaseolus vulgaris*) has shown significant inhibition against alpha-amylase, and the inhibitors found in the kidney bean extract were determined to be non-competitive of nature. In addition, both soaking and boiling are capable of reducing the amount of alpha amylase inhibitory activity significantly, although dehulling seems to have no effect on that front. Future experiments could be done to identify the specific components responsible for the amylase-inhibitory properties via HPLC and to test the effect of the bean extracts *in vivo* against storage pests.

Introduction

Rationale

There has been growing concerns about the heavy use of chemical pesticides in farming which has led to adverse effects on both human health and the environment. For instance, *Rana Sphenocephala,* a species of frog, is known to suffer from deformities or even mortality when exposed to chemical pesticides. (Bridges, 2000) Humans, especially children, are also at risk, as pesticides are known to have a correlation with the causation of asthma and other developmental disabilities. (Philip, *et al*., 1999) Lastly, most chemical pesticides are resistant to decomposition, and their effects are able to last through a long period of time. Therefore, there is a need for natural sources that have pesticidal effects so that such adverse effects can be negated.

In addition, it is known that digestive enzyme inhibitors found in food can cause various negative health effects, such as the production of flatulence. By consuming food that contains digestive enzyme inhibitors, more undigested food substances such as undigested starch will enter the colon, which is in turn broken down by bacteria living in the colon, thus producing a greater amount of flatulence. (Rackis, 1981) However, there are positive health effects as well. It is possible to harness these digestive enzyme inhibitor to treat diabetes, since it can reduce the amount of glucose broken down and absorbed by the body . (Chris, *et al.,* 2008) In addition, this function can also be utilized as "starch blokcers" to combat weight gain.

Beans are known to be a rich source of such defensins. Defensins are small, cysteine rich proteins found in most plants. They are known to have anti-fungal, anti-bacterial and digestive enzyme inhibitory properties. (Lay & Anderson, 2005) For instance, soya beans are known to have defensins which have trypsin inhibitory activity (Kakade, *et al.*, 1973) while mung beans are known to have the defensin VrD1, which have alpha-amylase inhibitory properties. (Liu, *et al.*, 2006) Therefore, we wish to find out if there are digestive enzymes inhibitors found in other plants and, if the enzyme inhibitors are effective enough, utilise them to create products ranging from pesticides to medicines.

Objectives

The objectives of this project are:

1. To determine the alpha-amylase inhibitory activity of various bean extracts
2. To determine the type of enzyme inhibition performed by the bean extracts
3. To find out the effect of different treatment on the alpha-amylase inhibitors in bean extracts

Hypothesis

1. Different bean extracts have varying levels of amylase inhibitory activity.
2. The inhibitors found in the bean extracts are non-competitive of nature.
3. Certain pre-treatments before consumption would be able to decrease the amylase inhibitory activity of the bean extract.

Experimental Procedure

Methodology

The following is an overview of the methods used:

1. Maltose standard curve
2. Preparation of crude bean extract
3. Amylase inhibition assay
4. Starch concentration test
5. Treatment test

Maltose standard curve

Maltose was used as standard solution in the concentration as 2mg/ml.Varying concentrations of maltose solution was then prepared by pipetting 1ml, 0.8ml, 0.6ml, 0.4ml and 0.2ml of maltose standard solution into respective containers and making up their contents to 2ml by adding deionized water. 1 ml of DNS solution was then added to each container before they were placed in a boiling water bath for 10 minutes to allow the DNS solution to bind to the maltose to form a colouring reagent. (Figure 1) After cooling, 8ml of deionized water was added to each container before mixing the solution by inversion. The absorbance value of each solution was then recorded using the spectrometer at 540nm.

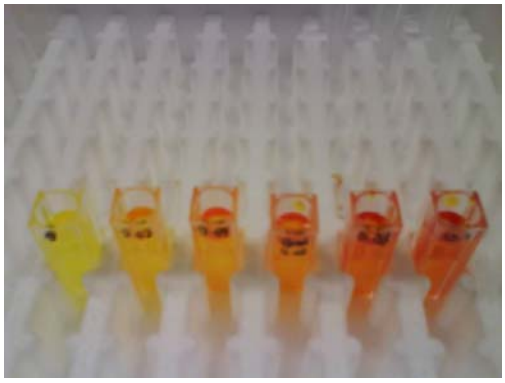

Figure 1: Colouring reagent formed by the DNS-maltose complex upon boiling

Preparation of crude bean extract

The following four beans were used in this project:

- Mung Bean (*Vigna radiate*)
- Kidney Bean (*Phaseolus vulgaris*)
- Rice Bean (*Vigna umbellata*)

- Black-eyed Bean (*Vigna unguiculata unguiculata*)

25g of beans were blended and homogenized in 50ml of 20mM sodium phosphate buffer, pH 6.9, containing 300mM sodium chloride. (Figure 2)The crude extract was then centrifuged at 8000rpm for 10 minutes. The supernatant was collected and centrifuged again at 12,000 rpm for 10 minutes to obtain the crude bean extract. (Figure 3)

Figure 2: Homogenizing the bean extract in a blender

Figure 3: Supernatant obtained after centrifuging

Amylase Inhibition Assay

For the amylase inhibition assay, four different solutions were prepared: blank, control, bean-only and test sample. 1ml of 0.1% bovine serum albumin buffer was added to each solution in order to stabilise the amylase and prevent adhesion of the amylase to the reaction tubes and tip surfaces. For the blank solution, 60µl of deioinized water was added. For the control solution, 10µl of amylase solution and 50µl of deionized water was added which represents the uninhibited solution. For the bean-only solution, 50µl of crude bean extract and 10µl of deionized water was added to measure the endogenous amylolytic activity of the bean extract itself. For the test solution, 50µl of crude bean extract and 10µl of amylase solution was added which represents the inhibited solution. The reaction mixtures were then incubated at 25°C for 15 minutes to allow the amylase inhibitor to bind to the amylase molecule. 250µl of the reaction mixture were then added to 250µl of deionized water and 500µl of 1% starch solution before further incubating at 37°C for 10 minutes in order to allow the enzyme-substrate complex to form. (Figure 4) 500ml of 3,5-Dinitrosalicylic acid solution was then added to each solution before heating them in a boiling water bath. (Figure 5)After cooling, the volume in each container was made up to 5 ml by adding de-ionized water and the contents were mixed by inversion before the absorbance of each solution were taken at 540nm. Triplicates were also done for each bean extract used.

Figure 4: Incubating the reaction mixtures at 37°C for 10 minutes

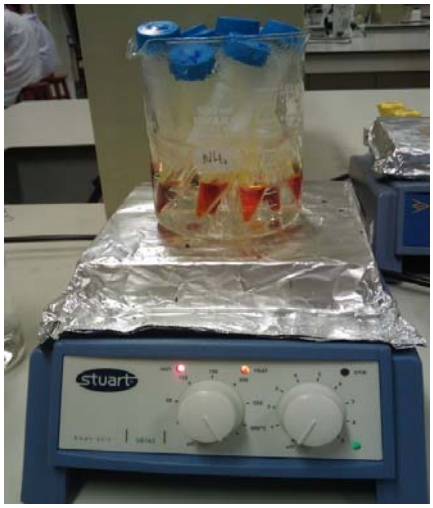

Figure 5: Reaction mixtures placed in boiling water bath to develop colouring reagent

Starch concentration Test

For the starch concentration test, the same amylase inhibition assay was performed on the bean extracts, but with the various starch concentrations: 1%, 2%, 3%, 4% , 5% and 6% This test assumes that the amylase concentration is not limiting and that the enzyme-inhibitor interaction is reversible.

Treatment Test

Three types of pre-treatment tests were conducted: pre-soaking, boiling and dehulling. For the pre-soaking treatment, the beans were soaked in water at a 1:5 (weight/volume) bean to water ratio overnight and then dried at room temperature for 12 hours. For the boiling treatment, the beans were heated in boiling water bath for 30 minutes before preparing the bean extract. For the dehulling treatment, the beans were manually dehulled using a sharp knife.

Results

Maltose Standard Curve

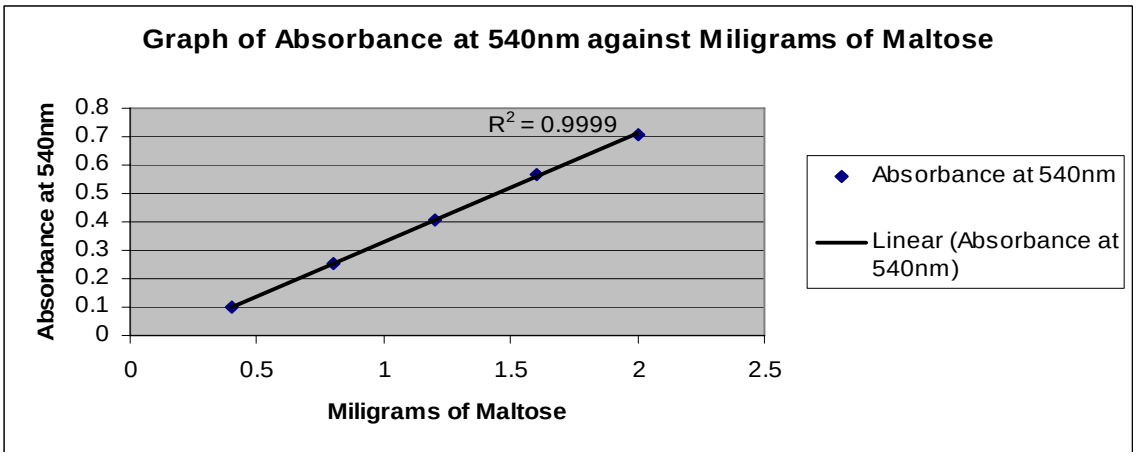

Figure 6: Maltose standard curve showing the relationship between maltose concentration and its corresponding absorbance value

From the maltose standard curve (Figure 6), it can be concluded that the relationship between the amount of maltose and its corresponding absorbance value is linear. Hence using this maltose standard curve, the amount of amylase inhibition can be quantified by the decrease in the amount of maltose liberated during the amylase inhibition assay between the control and the test solutions expressed as a percentage.

Amylase Inhibition Assay

To minimise the influence of the endogenous amylase found in the bean extract itself, the absorbance value given by the bean extract itself has to be excluded in order to calculate the actual absorbance value given by the test solution due to the inhibited amylolytic activity. The mean absorbance value of the control solution was first obtained. Next, the mean absorbance value of the bean-only solution was subtracted from the absorbance value of the test solutions in order to calculate the actual absorbance value given by the test solution after treating the amylase with the bean extract. The percentage decrease in the amount of maltose liberated was then recorded and the average of each bean extract was taken and plotted into a graph. (Figure 7, 8, 9, 10)

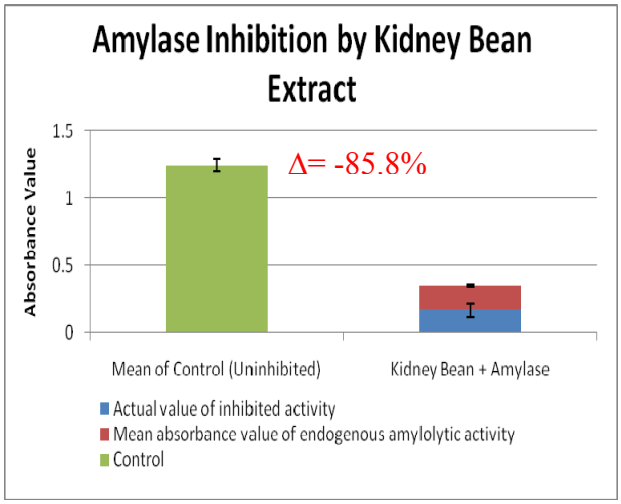

Figure 7: Amylase Inhibition by Kidney Bean

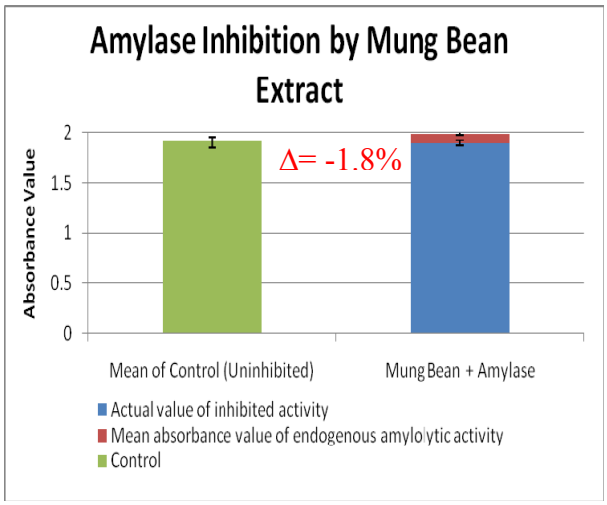

Figure 8: Amylase Inhibition by Mung Bean

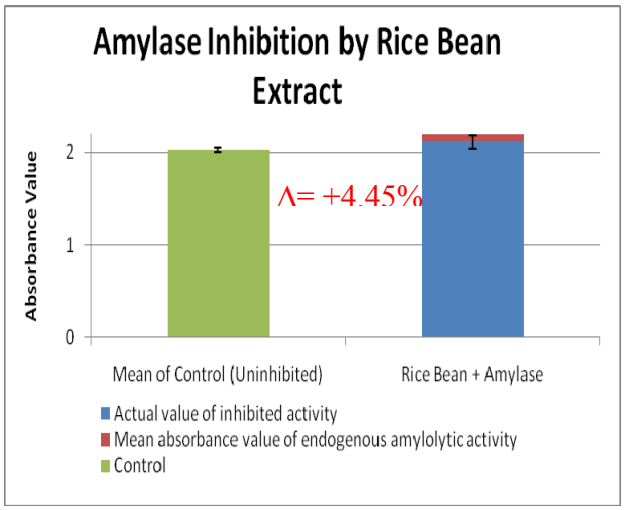

Figure 9: Amylase Inhibition by Rice Bean

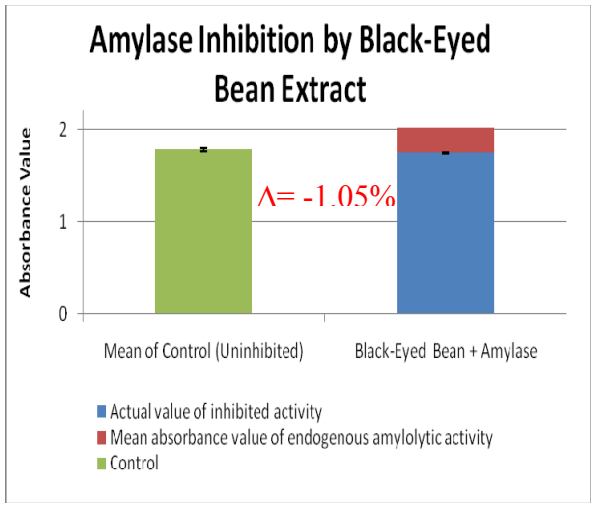

Figure 10: Amylase Inhibition by Black-Eyed Bean

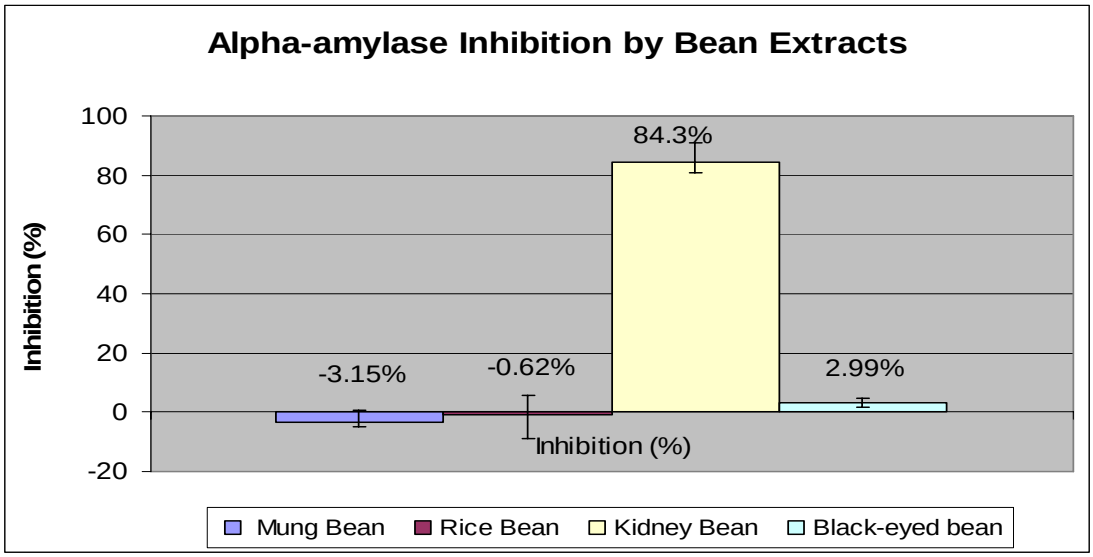

Figure 2: Graph of alpha-amylase inhibition by different bean extracts

The kidney bean was the only bean extract that consistently and significantly inhibited alpha-amylase, which was calculated to be an average of 84.3%. However, the other bean extracts showed negligible inhibition against alpha-amylase. (Figure 8)

Starch concentration Test

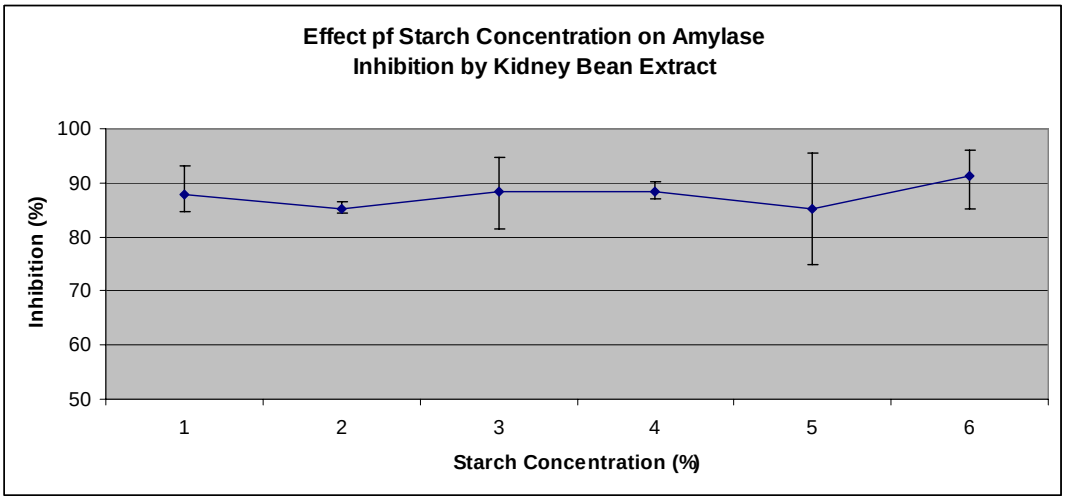

Figure 9: Graph of alpha-amylase inhibition by kidney bean extract against starch concentration

For starch concentration test, only the kidney bean extract was used as it was able to display significant alpha-amylase inhibition in the amylase inhibition assay. From the graph, an increase in starch concentration had negligible effect on the alpha-amylase inhibition by the kidney bean extract. (Figure 9)

Treatment Test

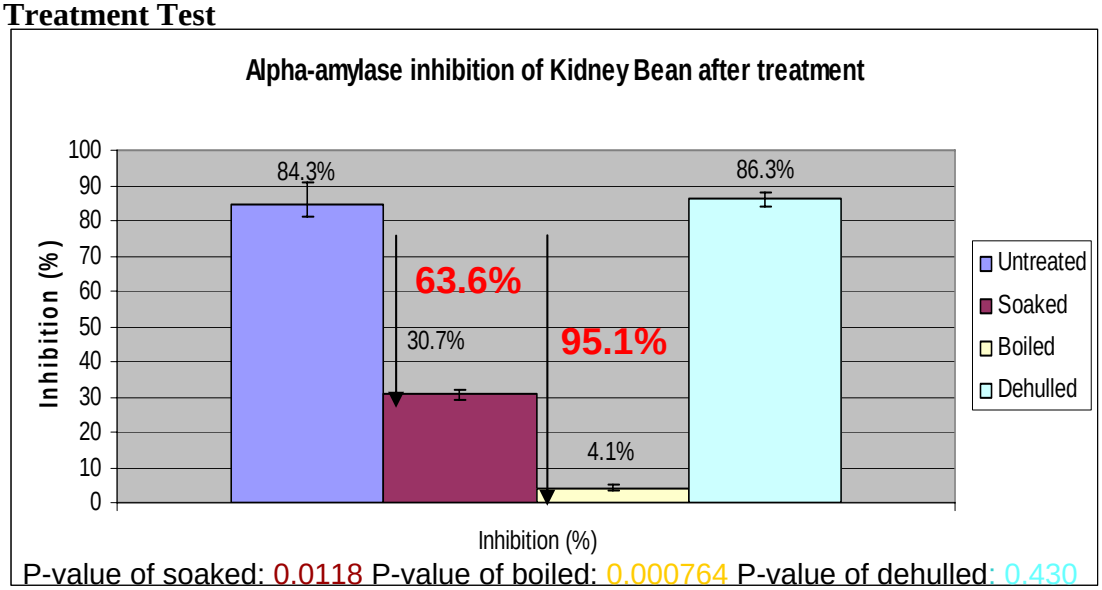

Figure 10: Graph of alpha-amylase inhibition after performing different pre-treatments on kidney bean extract

Pre-soaking and boiling the kidney bean extract prior to preparing the crude bean extract was able to significantly reduce its alpha-amylase inhibition by 63.6% and 95.1% respectively. However, dehulling the kidney bean has negligible effect in reducing the alpha-amylase inhibition by the kidney bean extract. (Figure 10)

Discussion

From the results obtained from the amylase-inhibition assay, the kidney bean extract was the only bean extract that was able to significantly inhibit alpha-amylase while the other bean extracts did not show any significant inhibition of alpha-amylase.

This does not imply that the other bean extracts do not contain any alpha-amylase inhibitors, but rather that they do not contain a high concentration of inhibitor enough to inhibit alpha-amylase, taking into account the fact that the concentration of alpha-amylase used was not adjusted. However, it can be concluded that the kidney bean extract contains the highest amount of alpha-amylase inhibitors as it displayed the highest inhibition of alpha-amylase among the four bean extracts.

Furthermore, our findings showed that the mung bean extract did not show any significant inhibition of alpha-amylase unlike previous findings that showed that the mung bean extract was able to inhibit alpha-amylase from insects. Hence it can also be concluded that different varieties of the same species of bean may contain varying contents of enzymes inhibitors.

From the results obtained from the starch concentration test, the inhibitor found in the kidney bean extract was determined to be non-competitive of nature since an increase in starch particles was unable to increase the enzyme activity of amylase, thus indicating that the inhibitor binds to a site other than the enzyme-substrate active site.

From the results obtained from the treatment test, boiling the beans was able to significantly reduce the alpha-amylase inhibition by the kidney bean extract, hence it can be concluded that the amylase inhibitor found in the kidney bean is most likely a heat-liable protein. The decrease in the alpha-amylase inhibition by the kidney bean extract can be attributed to leaching out of the inhibitor into soaking water along a concentration gradient. Thus, pre-soaking and boiling prior to consuming the bean extract would have already removed or denatured most of the alpha-amylase inhibitors found in the kidney bean extract.

However, dehulling the kidney bean had no significant effect on its alpha-amylase inhibition, suggesting that most of is alpha-amylase inhibitors are found in the cotyledon, and not the seed coat.

Considering the high levels of alpha-amylase inhibitory activity by the kidney bean extract, the kidney bean extract may have the potential to manufacture natural insecticides that do not harm the environment or human health by inhibiting major insect amylase. However, further experiments would have to be carried out to isolate and identify the fragments responsible for the alpha-amylase inhibitory activity via HPLC. *In vivo* tests would also have to be carried out to investigate whether the kidney bean extract has the potential to inhibit the alpha-amylase of storage pests.

Furthermore, the non-competitive nature of the inhibitor highlights a possibility of using the bean extracts to as a novel medicine to combat diabetes as well as weight gain by functioning as "starch blockers". The characteristics of the inhibitor found in the kidney bean extract that has been identified by the various pre-treatment tests is also key to understanding how to improve the digestibility of beans.

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