

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 radiata*), kidney bean (*Phaseolus vulgaris*), black-eyed bean (*Vigna unguiculata unguiculata*), and rice bean (*Vigna umbellata*). The assays were also performed against an increasing starch concentration in order to determine whether the inhibitor found in the bean extract is competitive in nature. Finally, different pre-treatment tests were also conducted to characterize the properties of the alpha-amylase inhibitors. Of the 4 beans tested, only kidney bean showed significant inhibitory activity against alpha-amylase, and the inhibitor was determined to be non-competitive in nature. In addition, both soaking and boiling reduced 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

Beans are known to be a rich source of defensins, which are small cysteine rich proteins known to have digestive enzyme inhibitory properties. There is a need to characterise these digestive enzyme inhibitors as they have the potential to be utilized as a form of pesticide and as a medicine to control diabetes and weight gain. In addition, greater understanding of these inhibitors will enable us to reduce flatulence that may be caused by their consumption.

Objectives

- To determine the level of alpha-amylase inhibition by the bean extracts
- To determine the nature of enzyme inhibition caused by the bean extracts
- To find out the effect of different treatments on the inhibitory activity of the bean extracts.

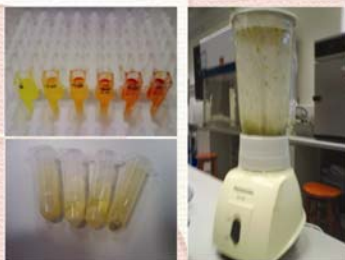
Hypothesis

- Different bean extracts will show varying levels of amylase inhibitory activity.
- The inhibitors found in the bean extracts are non-competitive.
- Certain pre-treatments before consumption would decrease the amylase inhibitory activity of the bean extract

Methods

Maltose Standard Curve

1. Pipette 1ml, 0.8ml, 0.6ml, 0.4ml and 0.2ml of 2mg/ml maltose standard solution into respective containers.
2. Make up the solution to 2ml by adding deionized water.
3. Add 1ml of 1% 3,5-Dinitrosalicylic acid (DNS) solution to each container.
4. Place the containers in a boiling water bath for 10 minutes.
5. Add 8ml of deionized water to each container.
6. Record their absorbance value at 540nm.



Extract Preparation

1. Homogenize 25g of beans in 50ml of buffer.
2. Centrifuge at 8000rpm for 10 minutes.
3. Collect the supernatant in Eppendorf tubes and centrifuge again at 12,000 rpm for 20 minutes.

Starch Concentration Test

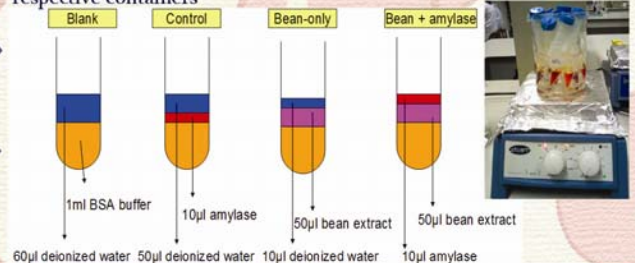
1. Repeat the amylase inhibition assay, but with varying concentrations of starch: 1%, 2%, 3%, 4%, 5% and 6%

Treatment Test

1. Pre-soaking (Soak the beans in water overnight)
2. Boiling (Heat the beans in boiling water for 30 minutes)
3. Dehulling

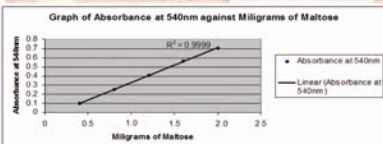
Amylase Inhibition Assay

1. Add the following reagents to the respective containers



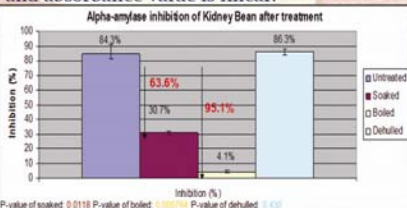
2. Incubate the reaction mixture for 15 minutes at 25°C.
3. Add 250µl of reaction mixture to 500µl of starch solution and 250 µl of de-ionized water before incubating at 37°C for 10 minutes.
4. Add 500µl DNS solution and heat in a boiling water bath for 15 minutes.
5. Add 3.5ml of de-ionized water to the reaction mixture.
6. Record the absorbance values at 540nm.

Results



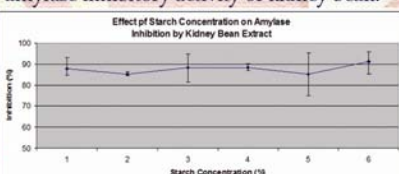
Maltose Standard Curve

Relationship between maltose concentration and absorbance value is linear.



Treatment Test

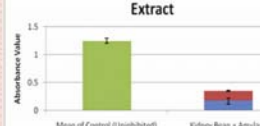
Both presoaking and boiling reduced the alpha-amylase inhibitory activity of kidney bean.



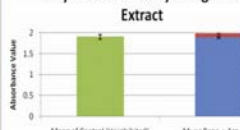
Starch Concentration Test

An increase in starch concentration had negligible effect on the alpha-amylase inhibition by the kidney bean extract.

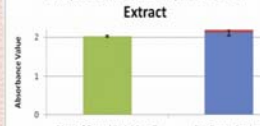
Amylase Inhibition by Kidney Bean Extract



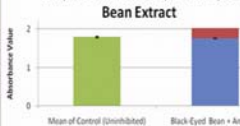
Amylase Inhibition by Mung Bean Extract



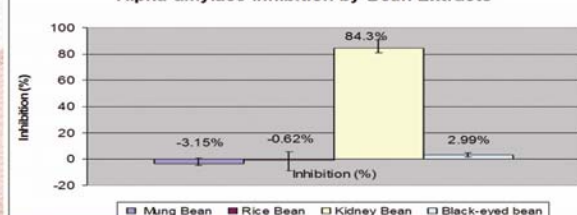
Amylase Inhibition by Rice Bean Extract



Amylase Inhibition by Black-Eyed Bean Extract



Alpha Amylase Inhibition by Bean Extracts



Alpha Amylase Inhibition Assay

Of the 4 beans, Mung, Rice, Kidney and Black-eyed bean, only the kidney bean extract displayed significant and consistent inhibition against alpha-amylase.

Conclusion

-The kidney bean extract exhibited the highest amylase inhibitory activity. Thus, it may be used as a pesticide against starch digesting pests.

-Unlike previous findings (Liu, 2006), the mung bean extract did not show any significant alpha-amylase inhibition. This may be due to different varieties of the same species of bean exhibiting varying alpha-amylase inhibitory activity.

-The inhibitors found in the kidney bean extract is non-competitive in nature, providing a possibility for it to be used as a novel medicine to combat diabetes and weight gain.

-The inhibitor is most likely a heat-labile protein.

Future Research

-Isolation and identification the fragments responsible for the alpha-amylase inhibition by the kidney bean extract via HPLC

-*In vivo* tests to assess the potential of kidney bean extract in inhibiting growth of storage pests

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