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Determination of Optimum Parameters Ofβ-AmylaseObtained From Red Dragonfruit and to Test Inhibitory Activity of *C. Igneus* on the Amylase Obtained.

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Abstract : *Enzymology studies are essential mediators to comprehend the complex reactions that occur in the domain of biology. B-amylase EC (3.2.1.2) is employed popularly in the brewing industry and its new applications are ever so growing. β-amylase (an exoamylase) can employ starch, glycogen and oligosaccharides as substrate and hydrolyze their alpha-D-glucosidic linkages yielding maltose units as reaction output. Sources of. B-amylase range from simple microorganisms to higher plants: Dragon fruit was screened for presence of B-amylase and gave positive results for the same. Furthermore, optimum pH and optimum temperature were determined as 4.8 pH units and 37degree Celsius respectively through experimentation. Various literature site instances defining the role of costus extract as an inhibitor of amylases and these claims were put to test.*

Keywords *–Red Dragonfruit, β-amylase, Costus igneus, Inhibitor*

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I. Introduction

1.1 .Dragon Fruit [5]

Dragon fruit refers to fruit of the genus Hylocereus, in the family Cactaceae. The dragon fruit is cultivated in Southeast Asia, Florida, the Caribbean, Australia, and throughout tropical and subtropical world regions. These fruits are commonly known in English as "dragon fruit", a name used since around 1993, apparently resulting from the leather-like skin and prominent scaly spikes on the fruit exterior. The names pitahaya and pitaya derive from Mexico, and pitaya roja in Central America and northern South America, possibly relating to pitahaya for names of tall cacti species with flowering fruit. The fruit may also be known as a strawberry pear or thang.

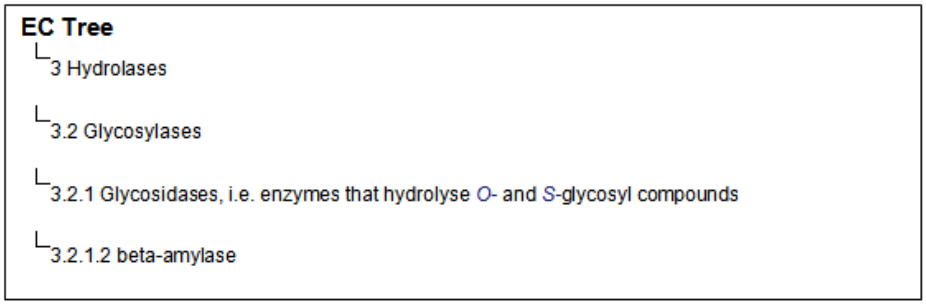
Taxonomic classification[10][11]
Botanical name- Hylocereus costaricensis
Kingdom- Plantae
Phylum- Tracheophyta

Class- Angiosperm
Order- Caryophyllales
Family- Cactaceae
Subfamily- Cactoideae
Genus- Hylocereus
Species- H. costaricensis

1.2 Beta Amylase and its optmimum parameters:

βamylase claims its existence in a majority of the biodiversity. The number of amino acids in the protein range from as low as 80s to as high as 1100s depending upon the organism containing it. The isoelectric point of the protein has range of pH 4- pH 6. The sources include stem, pericarp, petals, pulp, rhizome, pericarp etc. For an enzyme to function with its full capacity, it must be provided with optimum conditions of pH and temperature. This article explores the mentioned domains of enzyme's function. βamylase is important since it is involved in the sweet taste of the mature fruit.

EC Classification of β amylase[3]



1.3. Inhibitors

Two types of molecules exist in nature that affect the way an enzyme functions-an activator and an inhibitor. Both these molecules serve exactly opposite functions. They can be either inorganic or organic in terms of their chemical nature. An inhibitor as a justification to its name renders the enzyme less function in some or the other way. $HgCl_2$, urea ,potassium ferricyanide are known inhibitors of beta-amylase. An enzyme will exhibit a particular value of Michaleis constant(k_m) (substrate concentration at half V_{max}) which is indirectly proportion to the affinity of the enzyme to bind substrates. Inhibitors will tend to increase this value thus lowering affinity.

1.3.1.*Costus igneus* [1] [2] [7][9]

Costus igneus belongs to the family Costaceae, commonly known as insulin plant in India because its leaves help to build up insulin in the human body. Since oral hypoglycemic agents possess various side effects, there is a growing demand for herbal remedies for the treatment of diabetes mellitus. Many plant preparations are used in folklore and traditional system of medicine to manage diabetes mellitus. Investigation on new oral hypoglycemic compounds from medicinal plants will set a milestone for the development of pharmaceutical entities or as a dietary adjunct to existing therapies in the future. Insulin plant is one such traditional plant which is getting global acceptance nowadays and is now widely used as an ayurvedic medicinal herb. Consumption of the leaves are believed to lower blood glucose levels, and diabetics who consumed the leaves of this plant said to have a fall in their blood glucose levels. Insulin plant is native to Southeast Asia, especially on the Greater Sunda Islands in Indonesia. It is relatively a new entrant to India and is being grown as an ornamental plant in Kerala. In the Ayurvedic system of medicine, diabetes is traditionally treated by chewing the plant leaves for a period of one month to get a controlled blood glucose level.

Costus igneus has shown inhibition to alpha amylases and pancreatic amylase, but no studies have been done to test its role on β-amylases.[8]

Taxonomic classification[9]
Botanical name- *Costus igneus*
Domain- Eukaryota
Kingdom- Plantae
Phylum- Tracheophyta
Subphylum- Euphylophitina
Infraphylum- Radiotopses
Class- Liliopsida
Subclass- Commelinidae
Superorder- Zingiberane
Order- Zingiberales
Family- Costaceae
Subfamily- Asteroideae
Genus Costus
Specific epithet igneus

1.3.2 Practical Application

The aim of this study is to determine optimum parameter of functioning for β amylase extracted from dragon fruit and assessment of *C.igneus* inhibitory role on the same. The data summarized can have potential use in nutrition, food industry.

II. Material And Methods

2.1 Extraction of crude β amylase enzyme from pulp of dragon fruit

1) Collect the inner portion of the material without the outer skin.
2) Cut it into small pieces.
3) Weigh 1.0 gm of the material, add little chilled water (to prevent the inactivation of enzyme) in the mortar and pestle and grind thoroughly.
4) Filter the extract through cotton using funnel and collect the filtrate in a 100 ml standard flask.
5) Make up the volume to 100ml by adding chilled distilled water over the cotton, taking care that the fibers do not pass through the cotton.
6) This filtrate is 1gm% β amylase extract from a suitable source.
7) Perform confirmatory tests given below. Store the enzyme extract in the refrigerator.

CONFIRMATORY TEST:-

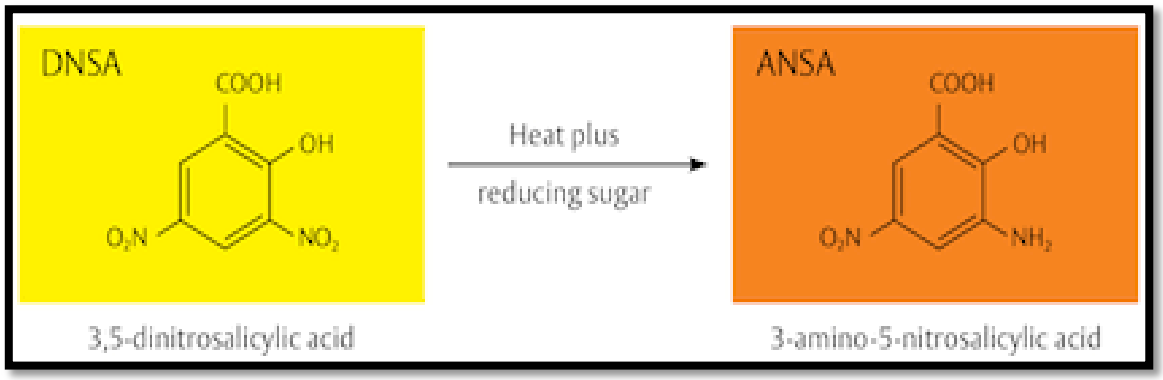
TEST	OBSERVATION	INFERENCE
2ml starch solution + 1ml enzyme extract (Incubate at 37ºC for 10mins) Add 5ml benedict's reagent. Heat.	Colour of Benedict's reagent changes to yellow/green	Reducing sugars present
2ml starch solution + 1ml enzyme extract (Incubate at 37ºC for 1min) + add 2 drops dilute Iodine solution	Violet colour is obtained	Amylase converts starch to dextrin

2.2.Determination of optimum pH and optimum temperature of β amylase

2.2.1) PRINCIPLE: Substrate concentration is one of the factors influencing enzyme action. Above a certain substrate concentration, the rate of enzyme action ceases to increase. Michaelis' Constant (Km) is characteristic of each enzyme. It is independent of both enzyme or substrate concentration and can be obtained as the substrate concentration at half-maximum velocity of the reaction. The activity of β-amylase is determined in terms of the amount of reducing sugars produced at the end of the hydrolysis of the substrate (starch). These sugars reduce three, 5-dinitrosalicylic acid to 3-amino, 5-nitrosalicylic acid. The colored complex can be measured colorimetrically at 530-540 nm. **Highest absorbance will be obtained in the tube where maximum reaction takes place thereby implying the optimum pH and optimum temperature**.

2.2.2)REQUIREMENTS: 1gm% enzyme extract, 2% starch, DNSA reagent, 0.5% NaCl, 0.2 M Acetate buffer (OF RANGE 4-5).

2.2.3)REACTION:



2.2.4) PROCEDURE(FOR OPTIMUM pH DETERMINATION):

1) Prepare the following set of tubes:

Tube No.With pH	2% Starch (ml)	Acetate Buffer (ml)	0.5%NaCl (ml)	1% Amylase	Distilled water
1 (4.0)	2.0	1.0	1.0	1.0	2.0
2 (4.2)	2.0	1.0	1.0	1.0	2.0
3 (4.4)	2.0	1.0	1.0	1.0	2.0
4 (4.6)	2.0	1.0	1.0	1.0	2.0
5 (4.8)	2.0	1.0	1.0	1.0	2.0
6 (5.0)	2.0	1.0	1.0	1.0	2.0
7 (5.2)	2.0	1.0	1.0	1.0	2.0
8 (5.4)	2.0	1.0	1.0	1.0	2.0
Blank(4.6)	---	1.0	1.0	1.0	4.0

2) Mix the contents of the tube and incubate at 370 C for 20 minutes.

3) After incubation, remove the beads if added and add 1ml of 2N NaOH in all the tubes to stop the enzyme reaction. Mix well.

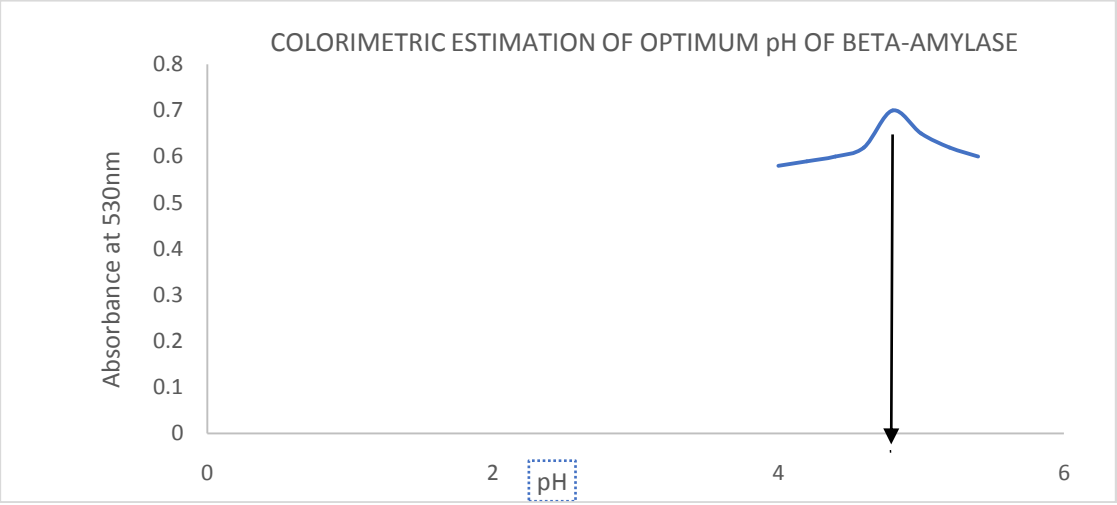
4) In another set of the tubes, keep 0.5 ml DNSA ready.

5) Transfer 0.5 ml of the incubated enzyme-substrate reaction mixture into the DNSA tubes numbered respectively. Mix and keep in boiling water bath for 5 minutes. Cool and add 9.0 ml distilled water to all tubes.

6) Read absorbance at 525 nm against blank.

Observation Table

Tube No.	pH	ABSORBANCE AT 530 nm
1	4.0	0.58
2	4.2	0.59
3	4.4	0.60
4	4.6	0.62
5	4.8	0.70
6	5.0	0.65
7	5.2	0.62
8	5.4	0.60
Blank	4.6	0.00



Interpretation:
From the graph it can be intrepreated that the optimum pH of β amylase is **4.8** .
2.2.5)REQUIREMENTS: 1gm% enzyme extract, 2% starch, DNSA reagent, 0.5% NaCl, 0.2 M Acetate buffer (pH 4.8).

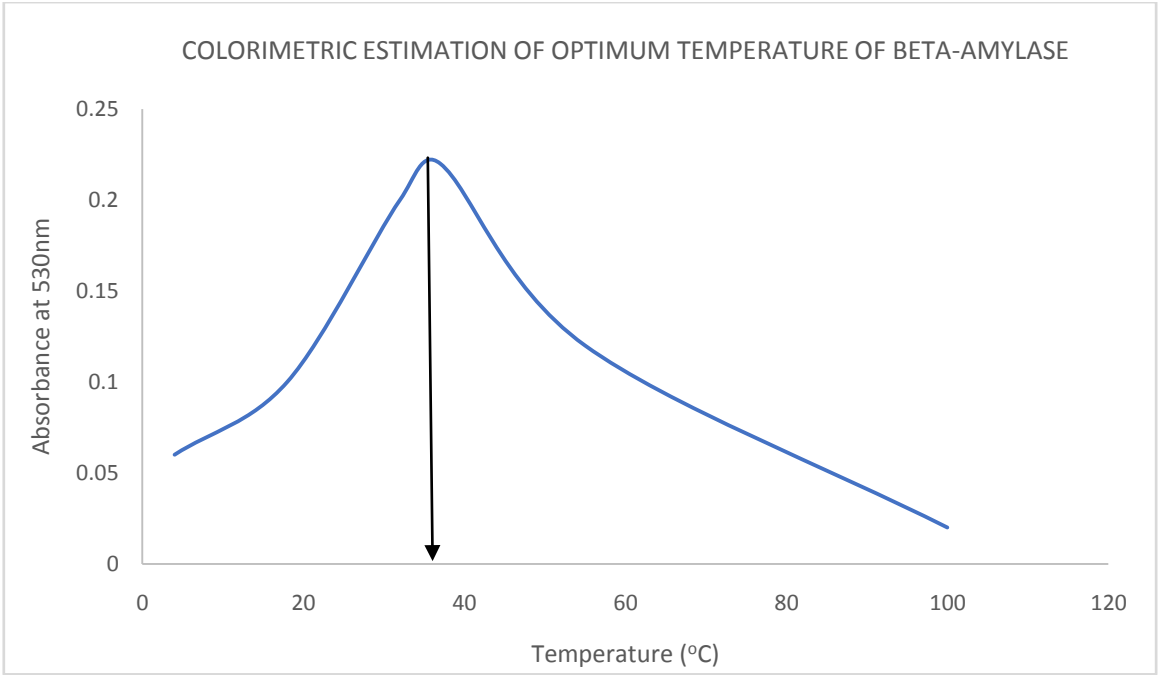
2.2.6) PROCEDURE (OPTIMUM TEMPERATURE DETERMINATION):
1) Prepare the following set of tubes:

Tube No.	Temperature	2% Starch (ml)	Acetate Buffer (ml)	0.5%NaCl (ml)	1% Amylase	Distilled water
1	4	2.0	1.0	1.0	1.0	2.0
2	18	2.0	1.0	1.0	1.0	2.0
3	RT	2.0	1.0	1.0	1.0	2.0
4	37	2.0	1.0	1.0	1.0	2.0
5	55	2.0	1.0	1.0	1.0	2.0
6	100	2.0	1.0	1.0	1.0	2.0
Blank	RT	2.0	1.0	1.0	1.0	2.0

2) Mix the contents of the tube and incubate at 37^0 C for 20 minutes.
3) After incubation, remove the beads if added and add 1ml of 2N NaOH in all the tubes to stop the enzyme reaction. Mix well.
4) In another set of the tubes, keep 0.5 ml DNSA ready.
5) Transfer 0.5 ml of the incubated enzyme-substrate reaction mixture into the DNSA tubes numbered respectively. Mix and keep in boiling water bath for 5 minutes. Cool and add 9.0 ml distilled water to all tubes.
6) Read absorbance at 525 nm against blank.

OBSERVATION TABLE:

Tube No.	TEMPERATURE	ABSORBANCE AT 530 nm
1	4	0.06
2	18	0.10
3	RT (32)	0.20
4	37	0.22
5	55	0.12
6	100	0.02
Blank	RT	0.00



Interpretation:
From the graph it can be intrepreated that the optimum temperature of β amylase is **37°C**

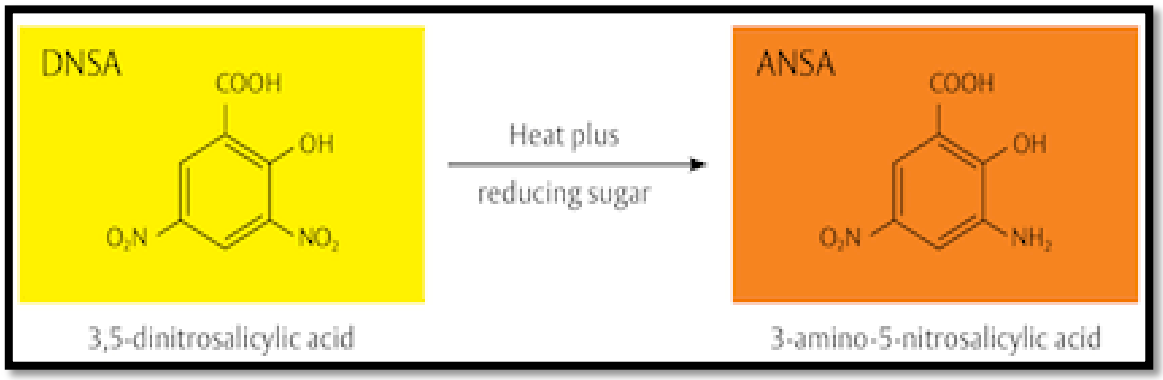
2.3. Assesment of inhibitory activity of costus igneus on β amylase:-
2.3.1) PRINCIPLE: (Same as mentioned above)

2.3.2)REQUIREMENTS: 1gm% enzyme extract, 2% starch, DNSA reagent, 0.5% NaCl, 0.2 M Acetate buffer (pH 4.8).

2.3.3)PREPARATION OF PLANT EXTRACT:

- Oven dry the leaves of costus igneus at 50 degree Celsius
- Weigh one gram of dried leaves
- With the help pf mortar and pestle,homogenise the leaves into a powder.
- To the powder add 50 ml of distilled water and mix it properly and filter using a water soaked cotton and funnel into a 100 ml volumetric funnel.
- Give aliquots of washings and make up the volume to 100ml with distilled water.
- The solution obtained is 1% plant extract.This extract is used as the inhibitor.

2.3.4) REACTION:



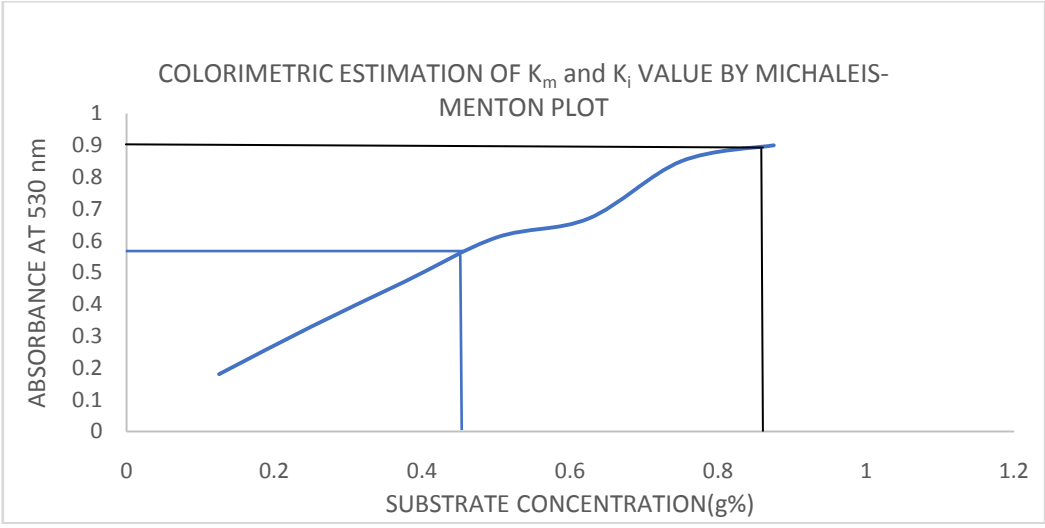
PROCEDURE: 1) Prepare the following set of tubes:
Prepare the following set of tubes:

Tube No.	2% Starch (ml)	Acetate Buffer (ml)	0.5% NaCl (ml)	Distilled Water (ml)	1% Amylase (ml)
1	0.5	1.0	1.0	4.5	1.0
2	1.0	1.0	1.0	4.0	1.0
3	1.5	1.0	1.0	3.5	1.0
4	2.0	1.0	1.0	3.0	1.0
5	2.5	1.0	1.0	2.5	1.0
6	3.0	1.0	1.0	2.0	1.0
7	3.5	1.0	1.0	1.5	1.0
8	4.0	1.0	1.0	1.0	1.0
Blank	---	1.0	1.0	5.0	1.0
Inhibitor	2.0	1.0	1.0	3.0(C. igneus extract)	1.0

2) Mix the contents of the tube and incubate at 370 C for 20 minutes.
3) After incubation, remove the beads if added and add 1ml of 2N NaOH in all the tubes to stop the enzyme reaction. Mix well.
4) In another set of the tubes, keep 0.5 ml DNSA ready.
5) Transfer 0.5 ml of the incubated enzyme-substrate reaction mixture into the DNSA tubes numbered respectively.
6) Mix and keep in boiling water bath for 5 minutes. Cool and add 9.0 ml distilled water to all tubes.
7) Read absorbance at 525 nm against blank.

OBSERVATION:-
Source – Red Dragon Fruit

Tube	Substrate Concentration (g%)	Absorbance at 525nm
Blank	---	0.04
1	0.125	0.22
2	0.250	0.37
3	0.375	0.51
4	0.500	0.65
5	0.625	0.71
6	0.750	0.89
7	0.875	0.94
8	1.000	0.96
Inhibitor	0.500	0.57



Interpretation:-
1.K_m= 0.28125 g% (from Michaelis-Menton plot)
2. K_i= 0.4375g% (from Michaelis-Menton plot)

III. Discussion

Dragon fruit refers to fruit of the genus *Hylocereus*, in the family Cactaceae.β-amylase was extracted from red dragon fruit and was investigated for its kinetic profile. To provide insight, DNSA method was used. Experimental observations support that optimum pH and optimum temperature as 4.8 pH units and 37^{o}C respectively. *C.igneus* is truly a nature's gift to mankind covering a spectrum of rectifying properties. *C.igneus* inhibitory property was confirmed by Michaleis-Menton plot.

IV. Conclusion

Our findings strive to establish experimental claims of optimum parameters for β-amylase. The chromogenic DNSA was used as colorimetric assay to determine all aims of this research. The optimum pH and optimum temperature were found out to be 4.8 pH units and 37^{0}C since at these respective conditions highest absorbance was obtained. Michaleis-Menton plot was employed to put into certain terms that *Costus igneus* does show inhibition properties.

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