# Investigation of Phenyl Glucosazone Compound Using Jaggery by Enzyme

**Pa Pa San***

## Abstract

In the present study, preparation and spectroscopic identification of phenyl glucosazone compound obtained from the hydrolyses of jaggery by invertase extracted from yeast was investigated. When the selected reaction mixture was boiled with Benedict's solution, a marked reduction took place due to glucose (reducing sugar) formation. The presence of glucose was confirmed by the reaction of reaction product with phenyl hydrazine forming bright yellow crystals of phenyl glucosazone, $C_6H_{10}O_4(N–NHC_6H_5)_2$. In FT–IR spectrum of phenyl glucosazone compound, it contains alcohol group, NH group, $sp^3$ hydrocarbon, $sp^2$ hydrocarbon, C = C aromatic benzene ring, C = N imine group, allylic hydrocarbon, C – C – O alcohol group, C – N amine group, trans or E alkene and cis or z alkene functional groups respectively.

**Keywords** : jaggery, phenyl glucosazone, invertase enzyme, FT–IR

## Introduction

Enzymes are protein in all living things and act as biological catalysts that increase the rate of specific chemical reactions. Each enzyme catalyzes chemical reaction between substrate and the enzyme. The substrates are converted into different molecules, called products. Enzymes are given names ending in–ase. Enzymes called amylases break down starches into sugar molecules, proteases break down proteins into amino acids and lipase break down fat into its component parts (Clark, D., Sokoloff, L., 1999). They are manufactured by all plant and animal cells. All cells require enzymes to survive and function. Enzymes are an absolute necessity to live. They form solutions in water and in dilute salt solutions, and are precipitated when such solutions are saturated with ammonium sulphate. They are highly specific in their reactions, e.g, maltose attacks the β–glucosidic link (which occurs in maltose), but without action on the β–glucosidic links (Clark, D., Sokoloff, L., 1999).

The effect of temperature is complex, but the majority of enzymes are most active at about 45°C and all are completely destroyed at 100°C. At 0°C, the activity is reduced considerately but the enzyme is not destroyed. Inhibitors are molecules that decrease enzyme activity; activators are molecules that increase activity. The enzyme used in this study was invertase extracted from yeast (Mnn, F, G. Saunders, B.C., 1975).

* Lecturer, Dr, Department of Chemistry, Yadanabon University

Invertase is a yeast derived enzyme. Invertase is a carbohydrate digesting enzyme that splits sucrose (common table sugar) into its components parts, glucose and fructose. It is one of the most widely used enzymes in food industry where fructose is preferred especially in the preparation of jams and candies because it is sweeter and does not crystallize easily. Sucrose is a non–reducing disaccharide produced by crystallization from syrups derived from processing sugar cane and sugar beets. It is found naturally in many food plants along with the monosaccharide fructose. In many fruits, such as pineapple and apricot, sucrose is the main sugar. In others, such as grapes and pears, fructose is the main sugar. Sucrose is very rich in cane sugar. Cane sugar looks rather like a bamboo cane. Jaggery, containing sucrose was hydrolysed by the enzyme invertase to give glucose and fructose. In this study, hydrolysis action of jaggery containing sucrose by invertase extract from yeast was investigated (Website 1).

## Aim and Objectives

### Aim

The aim of this research work is to study the reaction of hydrolysis of jaggery (sucrose) with invertase from yeast.

### Objectives

- To carry out the prepared solution of invertase from yeast
- To perform the reaction product by hydrolysis of jaggery (sucrose) with invertase from yeast
- To carry out phenyl glucosazone by the action of reaction product with phenyl hydrazine
- To confirm the prepared phenyl glucosazone by FT–IR

### Invertase

Yeast contains a number of enzymes more particularly invertase and zymase. Invertase, also known as beta–fructofuranosidase, is an enzyme that helps break down sucrose. It is essential to the body's ability to digest sugar. Invertase is usually derived from yeast, but it is also contained in honey and produced in a number of other microorganisms. Invertase has a protective function against a number of metals poisonous to the body, such as lead and mercury. It has antioxidant, antibacterial, and antiseptic properties and is a natural immune booster (Website 2).

### Sucrose

Sucrose, also known as table sugar, is a common disaccharide. It is composed of two monosaccharides: D–glucose (left) and D–fructose (right). Disaccharides are produced

by the condensation of either two identical molecules of the same monosaccharide or of two different monosaccharides by glycosidic bonds with the elimation of one molecule of water. Sucrose is the most abundant disaccharide, and the main form in which carbohydrates are transported in plants (Mnn, F, G. Saunders, B.C., 1975).

**Glucose**

Glucose, also known as $C_6H_{12}O_6$ D–glucose, dextrose, or grape sugar, is a simple form of natural sugar and a carbohydrate. Glucose is easily regulated by our body, if we eat it regularly. It is found in fruit and honey and is the major free sugar circulating in the blood of higher animals. It is the source of energy in cell function, and the regulation of its metabolism is great importance. Molecules of starch, the major energy–reserve carbohydrate of plants consist of thousands of glucose units, as do those of cellulose. Glucose is stored in mainly the liver and muscles as glycogen. It is a common medical analyte measured in blood sample. Eating or fasting prior to taking a blood sample has an effect on the result. A high fasting glucose blood sugar level may be a sign of prediabetes or diabetes mellitus. Glucose exists in several different molecular structures, but all of these structure can be divided into two families of mirror imaged (stereoisomers) (Website 3).

**Fructose**

Fructose is also a natural sugar. It is found throughout nature as a components of many of the foods we eat. Fructose is a monosaccharide, a simple natural sweetener. It is the sweetest of the naturally occurring nutritive sweeteners and has many unique functional and nutritional properties that make it a valuable food ingredient. Fructose is a high quality ingredient with many marvellous physical and functional properties. Fructose is derived from sugar cane, sugar beets, corn, there are three commercially important forms. High–fructose corn syrup is a mixture of glucose and fructose as monosaccharides (Website 3).

## Material and Methods

**Sample Collection**

Jaggery were obtained from local market. Yeast and Benedict's solution were purchased from Able Chemical Shop, Mandalay, Myanmar.

**Preparation of an Invertase Solution**

Commercial yeast (25 g) and an equal weight of sand were ground together in a mortor and 10 mL of water was gradually added into mixture. After well ground up and the yeast cells effectively ruptured, about 80 mL of water was slowly added with thorough

mixing. The mixture was allowed to stand for about 20 minutes and was stirred from time to time. The mixture solution was filtered through a funnel until a reasonably clear filtrate was obtained. This solution did not contain any zymase.

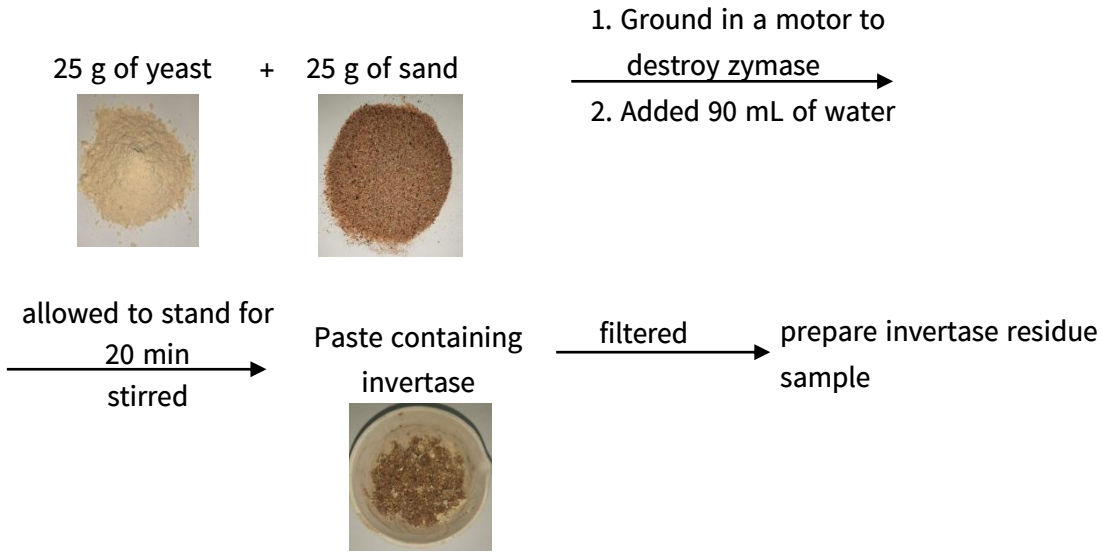



**Figure (1) Flow chart of prepare invertase residue sample**

**Hydrolysis of Jaggery by Invertase Extracted from Yeast**

Jaggery (10 g) was dissolved in water and made up to approximately 100 mL. Three conical flaks A, B and C were labelled. 20 mL of sugar solution and 2 mL of the invertase solution were added in conical flask A. 10 mL of sugar solution and 1 mL of the invertase solution which has previously been boiled thoroughly for 2–3 minutes. The mixture was added in conical flask B. 10 mL of sugar solution, 1 mL of the invertase solution and 1 mL of 10 % NaOH solution were added in conical flask C.

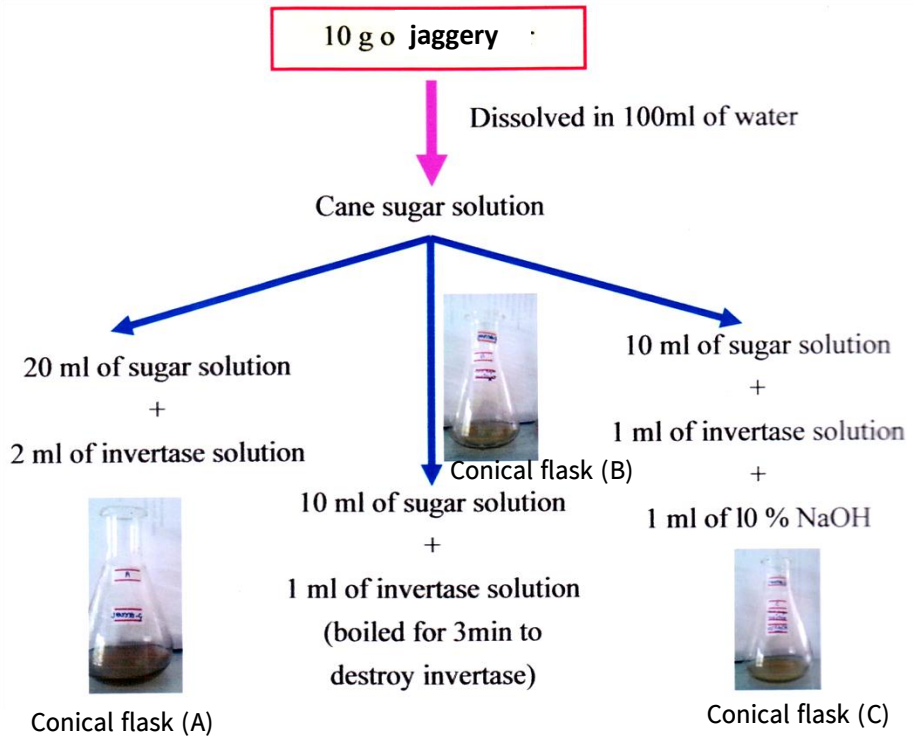



**Figure (2) Flow chart of hydrolysis of jaggery by invertase extracted from yeast**

**Testing of Conical Flask A, B and C with Benedict's Solution**

Each of above conical flask was placed in water bath and temperature was maintained at 50°C. After 10 minutes, 1 mL of each of the reaction mixture was transferred to separate test–tubes. 2 mL of Benedict's solution was added to each and boiled.

Conical flask A (20 mL of sugar solution + 2 mL of invertase solution) → maintained at 50°C in water bath for 10 min → take 1 mL of solution → added 2 mL of Benedict's solution, boiled → brick–red colored with precipitate

Conical flask B (10 mL of sugar solution + boiled for 3 min to destroy investase) → maintained at 50°C in water bath for 10 min → take 1 mL of solution → added 2 mL of Benedict's solution, boiled → No reduction

Conical flask C (10 mL of sugar solution + 1 mL of invertase solution + 1 mL of 10 % NaOH) → maintained at 50°C in water bath for → take 1 mL of solution → added 2 mL of Benedict's solution, boiled → very slightly reduction

**Figure (3) Flow chart of testing of conical flask A, B and C with Benedict's solution**

**Confirmation of the Presence of Glucose in Reaction Product**

Phenyl hydrazine (4 mL) was added into 8 mL of glacial acetic acid in 10 mL of water and they were mixed well in order that a clear solution is obtained. The resultant mixture solution was added to test tube A and mixed thoroughly. A slightly cloudy solution was obtained. The mixture solution was placed in boiling water–bath. After one hour, the mixture solution was cooled, and the precipitate was filtered off. And then it was dried and weighed.

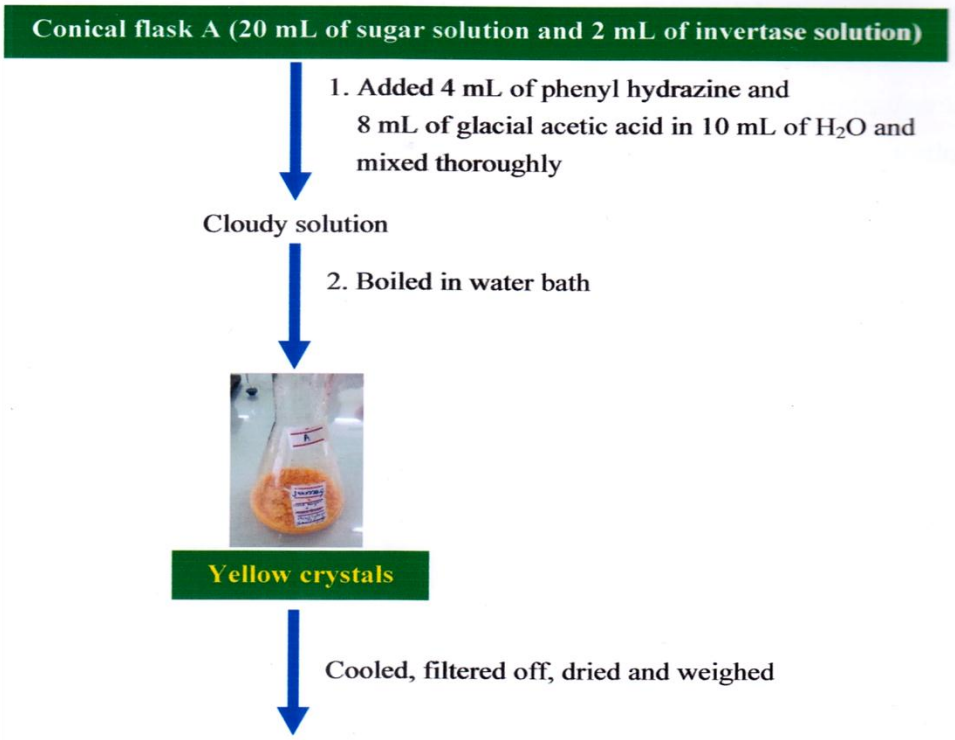


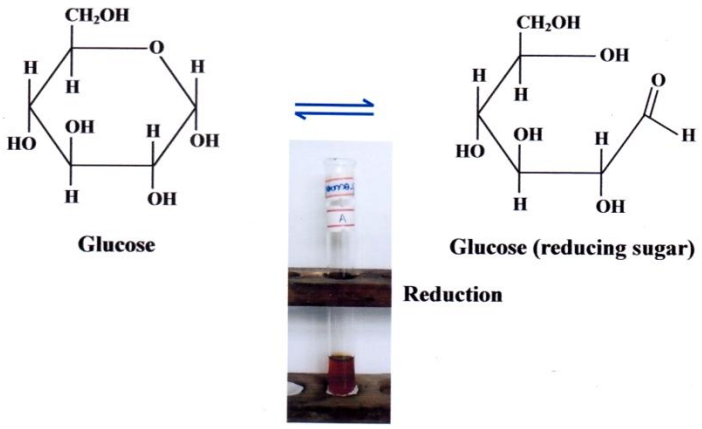

**Figure (4) Fl** **:t**

## Results and Discussion

**Testing of Reaction Products (Test Tubes A, B and C) with Benedict's Solution**

The presence of glucose (reducing sugar) was confirmed by the reaction of test tubes A, B and C with Benedict's solution.

Benedict's solution is a mixture of copper (II) sulphate and filtered mixtured of hydrated sodium citrate and hydrated sodium carbonate. Benedict's solution is added to the test solution and boiled. A high concentration of reducing sugar includes the formation of red precipitate, a lower concentration produces a yellow precipitate.




A marked reduction (brick–red colored with precipitate) took place in test tube A. This coloration is due to the presence of glucose and no change in color was observed in slightly changes in colour. No reduction is obtained in test tube B. Very slightly reduction is obtained in test tube C because the activity of invertase in test tube B and C was destroyed by heat and alkali. The presence of glucose can be further demonstrated by the reaction of glucose (reducing sugar) with phenyl hydrazine.

**Confirmation of the Presence of Glucose (Reducing sugar)**

4 mL of phenyl hydrazine was added in 8 mL of glacial acetic acid in 10 mL of water and they were mixed well in order obtain a clear solution. The resultant mixture solution was added to the solution in A and they were mixed thoroughly. A slightly cloudy solution was obtained. The mixture solution was placed in a water–bath. After placing one hours, the mixture solution was cooled, and the precipitate was filtered off. And then it was dried and weighed. Yellow crystal of phenyl glucosazone $C_6H_{10}O_4$ $(N–NHC_6H_5)_2$ was obtained. Melting point of it was found to be 204°C.

$C_6H_{12}O_6$ + 2 $C_6H_5NH–NH_2$ $\xrightarrow[10\text{ mL distilled water}]{8\text{ mL }CH_3COOH}$ Cloudy solution

glucose phenyl hydrazine (4 mL)

$\xrightarrow[\text{water bath}]{\Delta,\ 1hr}$ $\xrightarrow[\text{filtered, dry}]{\text{cooled}}$ $C_6H_{10}O_4(N–NHC_6H_5)_2$ + $3H_2$

phenyl glucosazone (yellow crystal)

**Confirmation of Phenyl Glucosazone by FT–IR Assignments**

The yellow crystal of the reaction product, phenyl glucosazone could be confirmed by FT–IR spectrum at Mandalay University. In this spectrum, the bands at 3296.46 $cm^{-1}$,

3246.31 $cm^{-1}$, indicated the O–H stretching vibration of alcohol group and N–H stretching vibration band. The peak at 3063.06 $cm^{-1}$ showed the C–H stretching vibration of $sp^2$ hydrocarbon. The peak at 2912.61 $cm^{-1}$ and 2870.17 $cm^{-1}$ were due to asymmetrical and symmetrical C–H stretching vibration of $sp^3$ hydrocarbon. Moreover, the peak at 1547.11 $cm^{-1}$ indicated the C = C ring skeletal stretching vibration of aromatic benzene ring. The band which showed at 1570.11 $cm^{-1}$ implied the C = N stretching vibration band. The peaks at 1498.74 $cm^{-1}$ are due to C–H in plane bending vibration of allylic hydrocarbon. The observation of O–H stretching could be confirmed by C–C–O stretching vibration at 1259.56 $cm^{-1}$. The C–N stretching vibration bands of amine group could be observed at 1165.04 $cm^{-1}$, 1080.17 $cm^{-1}$, 1041.60 $cm^{-1}$. The C–H out of plane bending vibration of trans or E and cis or z alkenic group could be observed at 950.94 $cm^{-1}$ and 877.64 $cm^{-1}$.

**Table (1) FT–IR assignment of phenyl glucosazone**

| Absorption band ($cm^{-1}$) | Assignment |
|---|---|
| 3296.46 $cm^{-1}$, 3246.31 $cm^{-1}$ | O–H stretching vibration band of alcohol group and N–H stretching vibration band of NH group |
| 3063.06 $cm^{-1}$ | C–H stretching vibration of $sp^2$ hydrocarbon |
| 2912.61 $cm^{-1}$, 2870.17 $cm^{-1}$ | Asymmetric and symmetric C–H stretching vibration of $sp^3$ hydrocarbon |
| 1597.11 $cm^{-1}$ | C=C ring skeletal stretching vibration of aromatic benzene ring |
| 1570.11 $cm^{-1}$ | C=N stretching vibration of imine group |
| 1498.74 $cm^{-1}$ | C–H in plane bending vibration of allylic hydrocarbon |
| 1259.56 $cm^{-1}$ | C–C–O stretching vibration of alcohol group |
| 1165.04 $cm^{-1}$, 1080.17 $cm^{-1}$, 1041.60 $cm^{-1}$ | C–N stretching vibration of amine group |
| 950.94 $cm^{-1}$, 877.64 $cm^{-1}$ | C–H in plane bending vibration of trans or E and cis or Z alkene group |

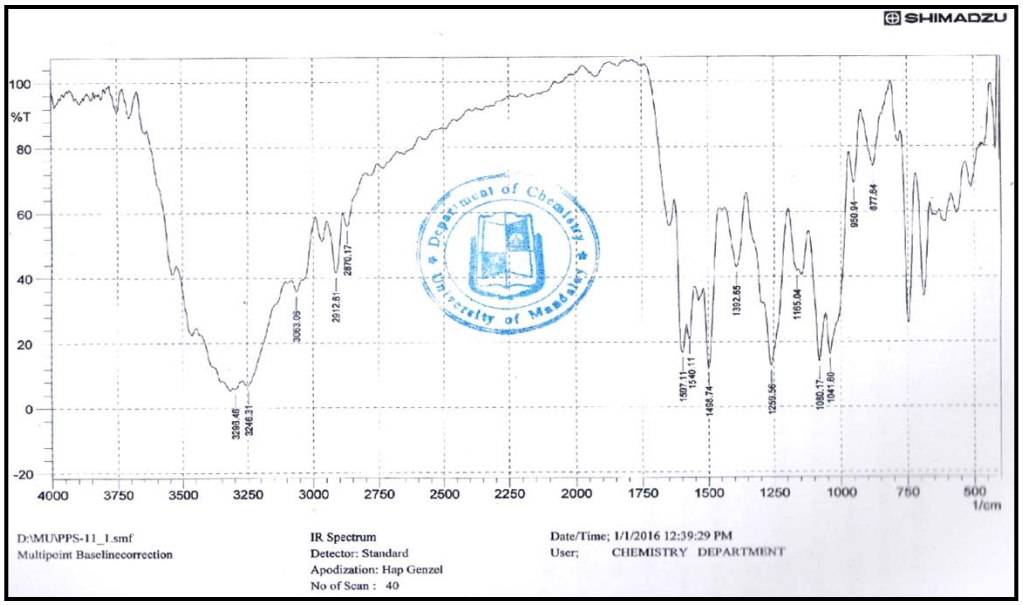



**Figure (5) FT–IR assignments of phenyl glucosazone**

According to FT–IR spectrum, this compound consisted of alcohol group, NH group, $sp^3$ hydrocarbon, $sp^2$ hydrocarbon, trans or E alkene and cis or Z alkene functional groups respectively. FT–IR assignments showed that the compound obtained was phenyl glucosazone.

## Conclusion

In this study, the used enzyme was invertase extracted from yeast. Hydrolysis action of jaggery containing sucrose by extracted invertase was investigated. The selected solution was tested with Benedict's solution, a marked reduction took place due to glucose formation. The presence of glucose was further confirmed by the reaction with phenyl hydrazine. Yellow crystals of phenyl glucosazone (353 mg), $C_6H_{10}O_4(N–NHC_6H_5)_2$ was obtained. The melting point was 204°C. The formation of phenyl glucosazone was confirmed by FT–IR spectrum. According to FT–IR spectrum, this compound contained alcohol group, NH group, $sp^3$ hydrocarbon, $sp^2$ hydrocarbon, C = C aromatic benzene ring, C=N imine group, allylic hydrocarbon, C–C–O alcohol group, C–N amine group, trans or E and cis or z alkene functional groups. The FT–IR results were consistent with phenyl glucosazone.

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http:www.global healing center.com/natural health/invertasel (Website 2)

http://www.kew.org/plant…/plants sugar cane profile htm (Website 3)