UNIT RATIO PROFILE AND ENZYME KINETICS STUDY FOR MAXIMUM YIELD OF MALTODEXTRIN FROM BROKEN PARBOILED RICE

Saikat Mazumder[1,4*], Animesh Naskar[1], Shailesh Rai[2], Debkumar Paul[3], Sudipta Das[4]
[1]Department of Food Technology, Hemnalini Memorial College of Engineering, West Bengal, India
[2]Senior Manager, Operations, Bacardi India Private Limited, India
[3]Senior Executive, Research and Development (R&D), Diageo India, India
[4]Department of Food Technology, Techno India, Salt Lake, Sector-V, West Bengal, India
[*]E-mail: Saikat.23.1989@gail.com

Abstract
This study is based on two aims firstly, the project is to develop a relation bridge between academic and industry personnel and secondly, in this study, we have evaluated the amount of α-amylase and amyloglucosidase required for effective saccharification of parboiled rice of 18 mesh size. The reason behind the selection of broken rice was its cost-effectiveness as well as waste utilization. Another reason behind the selection of broken rice as raw material was its utilization in the industry is on a large scale. The maltodextrin production rate by α-amylase and characterization of α-amylase was also a part of the study. Three unit ratios of α-amylase and amyloglucosidase were chosen i.e. 2:2, 4:4, 6:6. The maximum yield of 85.13 % maltodextrin was obtained by saccharification with a ratio of 6:6 units of α-amylase and amyloglucosidase after 30 minutes of saccharification. The 53.52 % yield of maltodextrin was obtained with 4ml of α-amylase (concentration 1mg/ml) at 120 minutes when α-amylase act alone on rice gelatinized starch. The determined kinetic parameters for α-amylase were V_{max}- 33,333 s^{-1}, K_m- 446.66 M. The activity of α-amylase was found as 358.15 IU/mg. The kinetic model developed to predict the time perspective maltodextrin production was fitted well. The coefficient of determination (R^2) found as0.996 for experimental and predicted values of maltodextrin.

Keywords: Parboiled rice, 18 mesh, α-amylase, amyloglucosidase, Unit ratio profile, kinetics, maltodextrin

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1. INTRODUCTION

In the 21st century, it has become very important for research that it should be industry feasible and useful for both academics and industry. The era also demands that the persons belong from different industries engage on a large scale in a research and development project. This is not due to just build a relationship between academia and industry but also the involvement of different personnel from different industries will develop a focus on industry needs for day to day operations. The involvement of more persons both from academic and industry enhance the chance of brainstorming. The academic and industry personnel under "one roof" will also focus on the industry's feasible outcome-based research project. A collaborative work between academic and industry personnel will also help to diversify the academic area. Hence, different persons from different industries have involved in this research project.

The reason behind the selection of broken rice as raw material was because of its cost-effectiveness and less demand for household purposes. Hence, lots of broken rice gone to wastage in-country likes India. So, if we use broken rice, on one hand, it was waste management and on the other hand it cost-effectiveness will be feasible for industry purposes.

Maltodextrin $(C_6H_{10}O_5)_n.H_2O$ is a mixture of saccharides with a molecular weight between polysaccharides and oligosaccharides with DE lower than 20 (not sweet), which is available as white powders mostly or concentrated solution (Alexander, 1992). Its physical and functional properties such as sweetness, compressibility, and viscosity vary depending upon the extent of starch hydrolysis, which is characterized by DE determination (Storz and Steffens, 2004). It is used in the food industry, as a bulking agent, fat replacer, medical/nutritional purpose, etc.

Rice starch granules (with diameters of ca. 5 μm) consist of two α-D glucose polymers: nearly linear amylose and the highly branched amylopectin. Within starch granules, three different regions are distinguished, i.e. alternating amorphous (low electron density) and semi-crystalline growth rings (thickness 120-400 nm). The latter consist of crystalline (high electron density) and amorphous (low electron density) lamellae with a repeat distance of 9-11 nm (Cameron and Donald, 1993). Crystalline lamellae result from double helix formation of amylopectin side-chain chains which aggregate to form crystals, whereas amorphous lamellae mainly contain amorphous amylopectin branch points (Donald et. al., 1999).

Enzymatic hydrolysis of starch has distinct advantages compared to the acid process. There is no need to remove salts formed during acid neutralization and due to wider pH range and lower temperatures this process is more economic and control of the process is easier too (Haki and Rakshit, 2003). The major steps in the enzymatic conversion of starch are liquefaction and saccharification. In liquefaction, the enzyme hydrolyzes the α-1, 4-glycosidic bonds in starch, whereby the viscosity of the gel rapidly decreases and maltodextrins are produced. When maltodextrins are saccharified by further hydrolysis using glucoamylase or fungal α-amylase, a variety of sweeteners can be produced (www.novozymes.com, Placido the , 2005).

Starch can be saccharified by two enzymes such as α-amylase and amyloglucosidase. Alpha-amylase (EC 3.2.1.1), hydrolyzes the internal α-1, 4 linkages in starch in a random fashion leading to the formation of soluble maltodextrins, maltose, and glucose. This enzyme is extensively used in starch liquefaction, brewing, food, paper, textile and pharmaceutical industries (Rasiah and Rehm. 2009; Rajagopalan and Krishnan, 2008; Gangadharan et. al., 2008; Thippeswamy et. al., 2006, Akpan et. al., 2004). Amyloglucosidase (EC 3.2.1.3), (AMG; also called glucoamylase, sometimes referred to as γ-amylase in the past) is a natural side activity of many amylase preparations, but it can also be obtained in a purer form from specialized Aspergillus strains. AMG breaks down starch into its smallest subunits, namely glucose, and in contrast to α-amylase it does not stop at the branching points of amylopectin. Thus, it is important to have a thorough knowledge as is possible of the performance characteristics of enzymes if they are to be used most efficiently. The kinetic parameters V_{max} and K_m should, therefore, be determined.

The objective of the study was the optimization of saccharification time and unit profile for the production of maltodextrin by α-amylase and amyloglucosidase as well as characterization of the enzyme (α-amylase) was also considered.

2. MATERIALS AND METHODS

2.1 Collection and preparation of raw material

Broken parboiled rice was collected from the local market of Kolkata. The rice samples were ground in a ball mill and ground samples were separated by a sieve shaker based on their particle mesh size. Rice flour with particle size of18 meshes was used for further experiments as it was found earlier, best maltodextrin producing particle size (Mazumder and Bera. 2013). In this study, gelatinization of rice was done at 103421 Pa for 30 minutes because earlier it was found as the best condition for gelatinization (Mazumder and Bera. 2013).

2.2 Characterization of α-Amylase

2.2.1 Determination of α-amylase activity

α- amylase was determined by dinitrosalicylic acid method. Freeze-dried α- amylase, Siga Aldrich, US was used for this purpose. It was dissolved in Tris buffer (pH 7.0). α-amylase activity was determined by the procedure described by Sadasivam, and Manickam, (2005).

Methodology:

The principle of α-amylase activity was determined on the basis of the absorption of

iodine within the helical structure to produce a blue colored complex colorimetrically.

Preparation of iodine reagent:

- 1g iodine and 10g potassium iodide (KI) dissolved in water and volume make up done upto 500ml.

Preparation of standard:

- 100 mg pure amylase was dissolved in 10ml 1N NaOH; volume make done upto 500ml.

Preparation of control:

- 1ml iodine reagent to 50ml volume make up with distilled water was used as a blank.

Procedure:

- 100 mg of the powdered sample with 1ml of ethanol and 10ml 1N NaOH in a beaker and left for overnight soaking.
- Volume made up to 100 ml with distilled water.
- 2.5 ml sample was discarded out from beaker and 20 ml distilled water with 3 drops 0.1% phenolphthalein was added separately in a conical flask.
- 0.1N HCl was added dropwise until the pink colour just disappears.
- Then, 1ml iodine reagent was added and volume make done upto 50 ml and colour was measured in terms of absorbance read at 590 nm using the UV-Vis spectrophotometer (Perkin-Elmer, Singapore).
- The reference standard curve of different concentrations (20, 40, 60, 80 and 100 mg/ml) of the mixture.

2.2.2 Determination of kinetic parameters of α- amylase

There are two approaches to this problem using either the reaction progress curve (integral method) or the initial rates of reaction (differential method). The use of either method depends on prior knowledge of the mechanism for the reaction and, at least approximately, the optimum conditions for the reaction. If the mechanism is known and complex then the data must be reconciled to the appropriate model (hypothesis), usually by use of a computer-aided analysis involving a weighted least-square fit.

Many such computer programs are currently available and, if not, the programming skill involved is usually fairly low. If the mechanism is not known, initial attempts are usually made to fit the data to the Michaelis-Menten kinetic model.

Three ways in which the hyperbolic relationship between the initial rate of reaction and the initial substrate concentration.

$$V = \frac{V_{max}[S]_0}{K_m + [S]_0}$$

The above equation can be rearranged to give linear plots. Lineweaver-Burk (double-reciprocal) plot of 1/v against $1/[S]_0$ giving intercepts at $1/V_{max}$ and $-1/K_m$.

$$\frac{1}{V} = \frac{K_m}{V_{max}} X \frac{1}{[S]_0} + \frac{1}{V_{max}}$$

Where,

K_m-Kinetic activator constant (M)

V_{max}-Maximal velocity (S^{-1})

[S]-Substrate concentration (mg/ml)

2.3 Optimization of by α-Amylase concentration for saccharification

2 g rice flour of 18 mesh size parboiled rice was taken in different four conical flasks and 200 ml distilled water was added in each of the flasks.

After autoclaving the flasks (103421 Pa for 30 minutes), the mass was neutralized using NaOH. Afterward, 1, 2, 3, and 4 ml of α-amylase solution (1mg/ml) prepared in Tris buffer was added in four conical flasks severally and placed in a water bath at 37°C for 15 minutes and samples withdrawn after 2, 5, 10, and 15 minutes interval and placed in boiling water bath for enzyme inactivation. Maltodextrin was determined by dinitrosalicylic acid method.

2.4 Determination of maltodextrin production rate by α-Amylase

10 g, 18 mesh parboiled rice, and 100ml distilled water was taken in a conical flask and gelatinized at 103421 Pa for 30 minutes followed by cool down to 30^{o} C and followed by neutralization of pH. After that, 4ml of alpha-amylase solution (concentration 1mg/ml) prepared in Tris buffer was added to the conical flask and placed in a water bath at 37^{o} C for 2hrs, saccharified sample was picked up at 2, 5, 10, 15, 20, 25, 30, 40, 60, 75, 90, 105, 120 minutes in different test tube and placed in hot water for 2-3 minutes for inactivation of enzyme activity. Maltodextrin was determined using dextrose equivalent Dinitrosalicylic acid method as reported by Sadasivam, and Manickam, (2005).

2.5 Optimization of saccharification time and unit profile for alpha-amylase and amyloglucosidase

Optimization of saccharification time and enzyme unit profile was done based on variation of time (0 to 3h) and 2, 4, 6 enzyme unit (both for α-amylase and amyloglucosidase) basis. Amyloglucosidase extracted from *Aspergillus niger* was procured from Siga Aldrich, U.S.

2g, 18 mesh parboiled rice, and 100ml distilled water was taken thrice in three different conical flasks and gelatinized at 103421 Pa for 30 minutes followed by cool down to 30^{o} C and neutralization of pH. After that, 2:2, 4:4, 6:6 unit of α-amylase: amyloglucosidase solution prepared in Tris buffer was added to different conical flasks and placed in a water bath at 37^{o}C for 3hrs, saccharified sample was picked up at 2, 5, 10, 15, 20, 30 minutes in different test tube and after 3 hrs sample from each of the conical flask was picked up and placed in hot water for 2-3 minutes for inactivation of enzyme.

3. RESULTS AND DISCUSSION

3.1 Determination of α-amylase activity

Theα-amylase activity is defined as the amount or unit of enzyme that has to convert a certain amount of substrate (i.e. maltose) to product in a specified time frame (i.e. 5 minutes) under conditions of constant temperature (37^{0}C) and pH (7.0).

From the above standard maltose curve (Fig. 1) equation the calculated value of the activity is 358.15 IU/mg**.**

3.2 Determination of kinetic parameters of enzyme

From the above Lineweaver-Burk plot (Fig. 2) and using the Michaelis-Menten equation the calculated value of V_{max} and K_m is 33,333 s^{-1}, 446.66 M, respectively. The regression coefficient was determined by following the straight-line equation using MS-Excel. The value of K_m indicates that the enzyme has a good affinity to the substrate i.e. maltose.

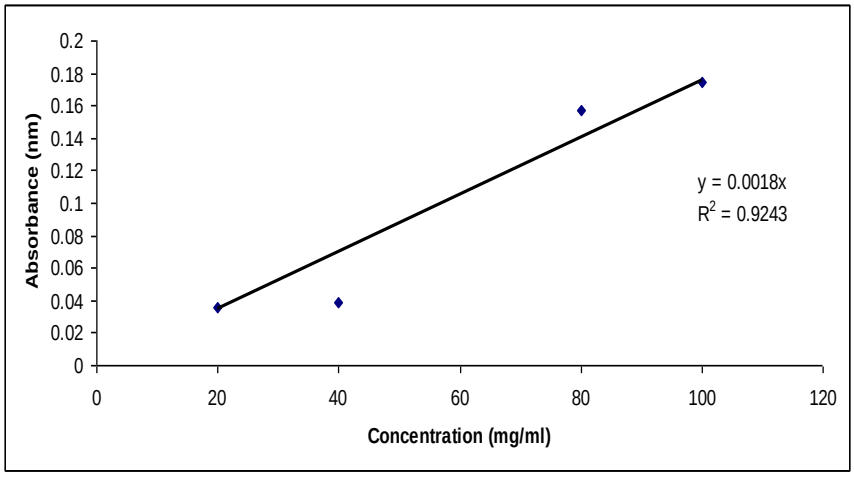

Fig 1:Standard maltose curve

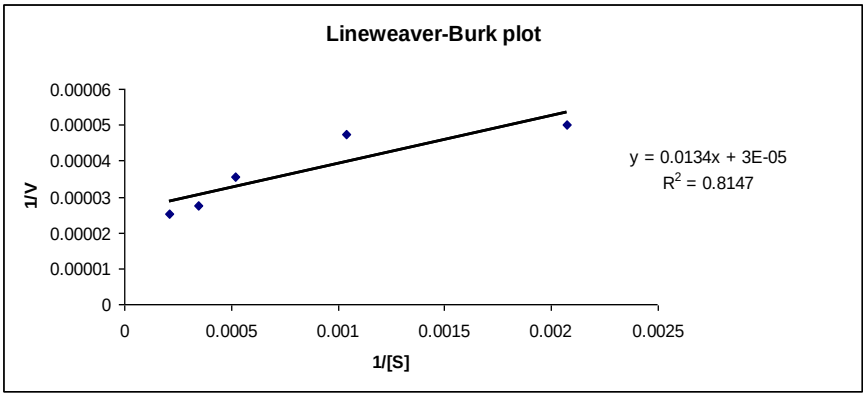

Fig 2:Graphical presentation of the Michaelis-Menten equation

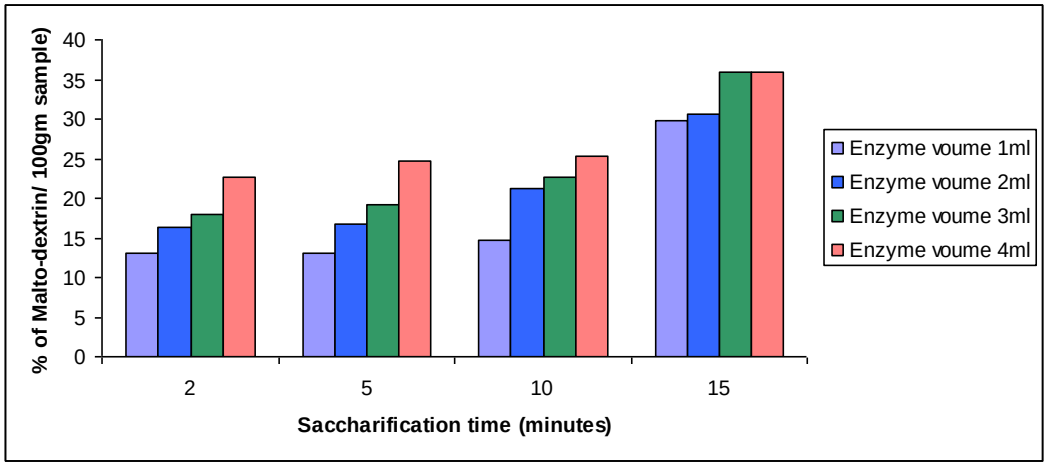

Fig 3: Percentage of maltodextrin with respect to time from different volume of α-amylase

3.3 Optimization of by α-Amylase concentration for saccharification

The α-amylase is used to break the glycoside bond of rice particles. It has been found that initially, the alpha-amylase volume makes a significant difference in maltodextrin production, but as the reaction process carried the difference in maltodextrin production by different added volume of alpha-amylase reduced, at the time 15 minutes the amount maltodextrin produced by 3ml and 4ml of alpha-amylase is same, the same result observed for 1ml and 2ml of alpha-amylase at the 15 minutes (Fig 3). So, it can be concluded that 4ml of alpha-amylase breaks down the linear linkages very quickly, whereas 3ml required some more time to that condition of linear bond breakage. Alpha-amylase addition volume has a significant effect on maltodextrin production concentration.

3.4 Determination of maltodextrin production rate by α-amylase

It is very important to predict the production of maltodextrin from optimized particle size i.e. 18 mesh parboiled rice. By the application of straight-line equation (Y=mX+C), the percentage of the production rate of maltodextrin can be evaluated which is very helpful in the case of industrial application.

From the standard curve of dextrose (Fig. 4) the values of produced maltodextrin at different time interval has been calculated (Fig. 5).

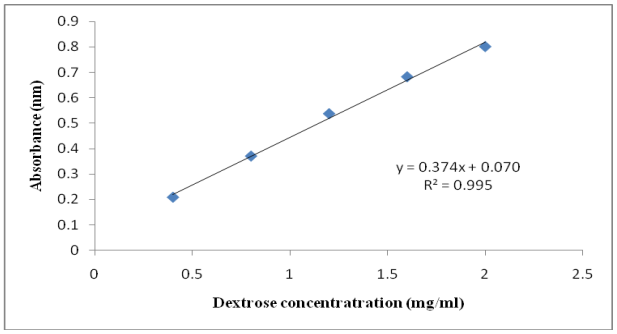

Fig 4: Standard curve of dextrose

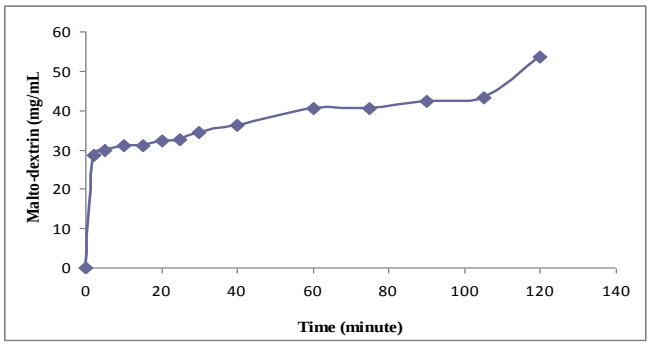

Fig 5: Produced maltodextrin with respect to time

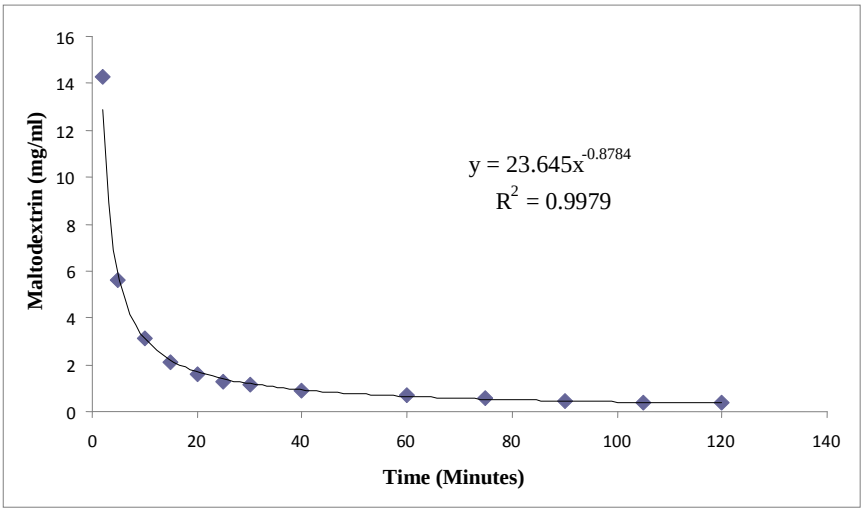

Fig 6:Production of maltodextrin per ml

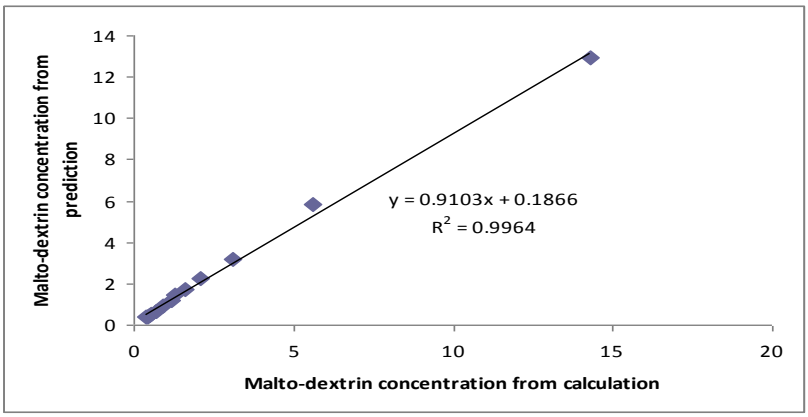

Fig 7: Correlation of experimental and predicted value

For the prediction of maltodextrin production at different time intervals an exponential model of experimental data has been generated using MS Excel. Using the equation from Fig. 6 ($y=23.645x^{-0.8784}$) the value of maltodextrin can be predicted.

It is very important to justify the model which has been developed as Fig. 6. To justify the model the predicted and experimental values were correlated which is depicted as Fig. 7. This is very clear from Fig. 7, both values effectively correlate to each other where the correlation coefficient is very closer to 1(R^2=0.996) Hence, It can be easily concluded that the equation ($y=23.645x^{-0.8784}$) is very useful for the prediction of maltodextrin production at different times.

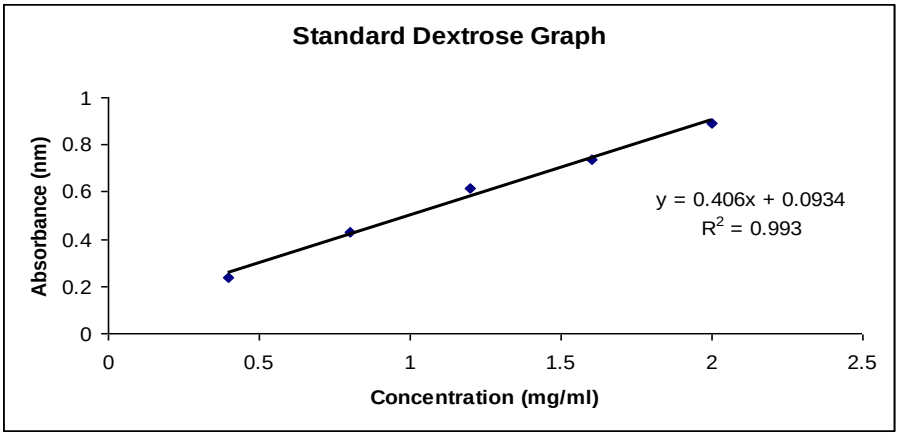

Fig 8: Standard dextrose curve

Table 1:Percentage of maltodextrin production by different ratio of α-amylase and amyloglucosidase

Unit of α-amylase: Unit of amyloglucosidase	Time (Minute)	% Of maltodextrin/100g sample
2:2	2	6.67
2:2	5	7.90
2:2	10	31.67
2:2	15	41.77
2:2	20	56.18
2:2	30	58.15
2:2	180	61.67
4:4	2	21.69
4:4	5	24.16
4:4	10	49.4
4:4	15	63.81
4:4	20	67.26
4:4	30	80.19
4:4	180	81.42
6:6	2	21.69
6:6	5	32.16
6:6	10	61.10
6:6	15	68.26
6:6	20	79.82
6:6	30	85.13
6:6	180	86.35

3.5 Optimization of saccharification time and unit profile for α-amylase and amyloglucosidase

Production of maltodextrin was done based on the variation of enzyme unit profile. Using the equation of standard dextrose curve (Fig. 8) the value of maltodextrin production by different units of alpha-amylase and amyloglucosidase was calculated. The results (Table 1) indicate that with the increment in a ratio of enzymes the rate of production of maltodextrin increases and the production of the process almost comes to end at 30 minutes as after 30 minutes there is a negligible increment maltodextrin production. It is also noticeable that when the enzyme ratio was increased from 2:2 to 4:4 then the difference between maltodextrin production rate is higher than the maltodextrin production rate when the enzyme ratio was increased from 4:4 to 6:6. So, it can be concluded that a 6:6 ratio can be used for the production of maltodextrin. This is pertinent to mention that the optimum ratio of two enzymes used in this process is indeed a novel approach. Most of the

saccharification occurs by using either a single enzyme or microbial strain (Liu et. al., 2016). Hence, a thorough study of using the cocktail enzyme system for saccharification is found to be significant for practical application.

4. CONCULSIONS

Rice starch can be saccharified by both of the enzymes α-amylase and amyloglucosidase. The α-amylase requires more time for saccharification when acts alone on rice starch and also the percentage of maltodextrin yield was low (53.52%), whereas, α-amylase and amyloglucosidase require much less time for saccharification when acts together and the percentage of maltodextrin yield was also much higher (83.13%). One probable cause may be that α-amylase can not break down the branch points (α 1→6 linkages) of starch. But more research requires for better optimization of α-amylase and amyloglucosidase unit profile for saccharification of rice starch. The future scope of this research project is the alteration of enzymes ratio.

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