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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(11): 207-211

www.thepharmajournal.com
Received: 14-09-2019
Accepted: 18-10-2019

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A review on extraction of glucose from rice

Anindya Dhar, Poulami Chakraborty, Shalini Chanda and Dr. Anju Paul

Abstract
Carbohydrates are one of the most essential macro-nutrient required for the maintenance of life. Glucose and Fructose act as building blocks of energy. The Human Body consumes carbohydrates in the form of glucose. Glucose is converted to glycogen, a polysaccharide and it acts as a readily available source of energy. Approximately 130 g of glucose is required to nourish the brain cells throughout the day.
Glucose is widely used in food manufacturing industries which include beverage, ice cream, alcohol, confectionary and other fermentation plants.
Studies and researches have shown that extraction of glucose from rice is a cost effective method. Rice is widely consumed and important cereal crop. The three variants of Indian rice HMT, Swarna and basmati have high starch contents. In a nutshell the paper is focused to concentrate about the different methods of glucose production from rice.
The major researches included in the paper are:

1. Glucose obtained from Rice bran by Ultrasound-Assisted enzymatic hydrolysis
2. Isolation of starch from Rice (*Orzya Sativa* L.) by alkali extraction method
3. Efficient Recovery of Glucose via enzymatic saccharification of rice straw with soft carbohydrates
4. Glucose production from rice husk by solid state fermentation method.

Keywords: Rice starch, enzymatic hydrolysis, rice amylase, Solid state fermentation, Saccharification

Introduction
Rice starch isolation is a bit different from other sources of extraction because of it's unique protein compostition. The isolation process mainly consists of the separation of other components like protein, fiber, and lipid. The key factor that one has to keep in mind is that the mechanical damage or amyoletic destruction of the starch granules must be avoided.

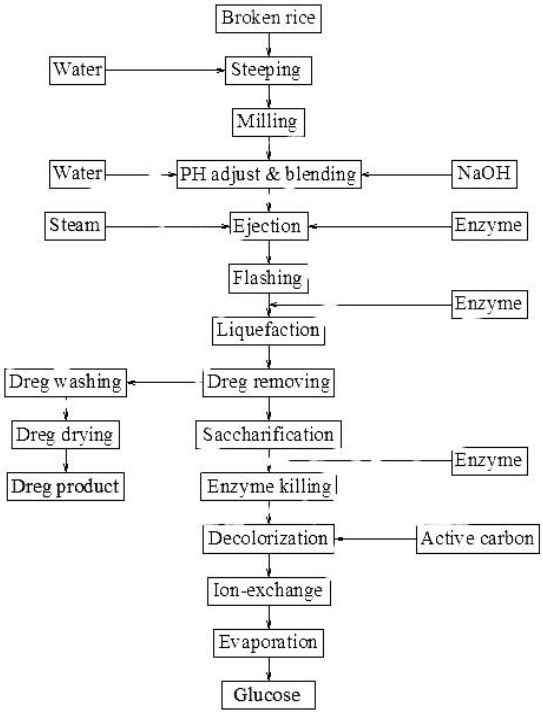

Fig: Steps of Rice Starch extraction

Rice can be used an essential raw material for producing glucose. This process of production of glucose from rice is a bio-chemical process, involving a series of chronological steps. Out of the many steps the key unit is liquefaction and saccahrifaction. These two steps hold the key of the quality of the glucose manufactured. As per as the physical characteristics are concerned, rice starch is a finely textured, gluten free powdered substance that is produced by the seed of the plant or more precisely the endosperm of rice seed. Another added advantage of rice starch over other plant sources of starch is it's easy digestibility. The different method of extraction has been proposed by many researches and they have also been implemented in some cases:

Glucose obtained from rice bran by ultrasound-assisted enzymatic hydrolysis [1]

A new concept of ultrasound technique was introduced by Ultrasound-assisted solid-state enzymatic hydrolysis of rice bran in order to obtain starch/glucose was investigated in this process. The physical variables at the optimum level such as temperature, enzyme concentration and moisture content were evaluated during the enzymatic hydrolysis with and without ultrasound irradiation. The enzyme used is a mixture of different amylases derived from genetically modified strains of the species *Trichoderma reesei*. Kinetics of the enzymatic hydrolysis of rice bran at the constant-reaction rate period (CRR) was measured.

The prime evaluation of this study is the feasibility of ultrasound-assisted enzymatic hydrolysis at solid-state of rice bran to manufacture starch/glucose. The optimum physical conditions were found out. The best results for the ultrasound-assisted enzymatic hydrolysis was obtained using 3 wt % of enzyme, 60 ◦C and moisture content of 65 wt %, yielding 0.38 g sugar/g rice starch, On the other hand, for the hydrolysis in the absence of ultrasound the highest yield was 0.20 g sugar/g rice starch using 3 wt% of enzyme, 60 ◦C and moisture content of 50 wt%. i.e. same physical conditions as before. The catalytic constant for the CRR (Constant Reaction Rate) period was always higher in the assistance of ultrasound technique, and thus indicating the fact that the ultrasound intensified the enzymatic hydrolysis in the step of constant-reaction rate period. The use of ultrasound-assisted enzymatic hydrolysis of rice bran was intensified, which obtaining about 74% more reducing sugars (Glucose/Fructose) in due presence of Ultrasound technique than in the absence of Ultrasound assisted irradiation, pointing towards the fact this process has better prospects and that the use of ultrasound is a promising technology to be used in enzymatic reaction as an alternative of process intensification.

Isolation of starch from rice (*Orzya sativa* L.) by alkali extraction method [2]

When it comes to rice-producing countries in the world, India is among the best. Rice (*Oryza Sativa* L.) is popularly consumed and important cereal crop in India. This study focuses on three Indian rice cultivars namely- HMT, Swarna and Basmati were investigated thoroughly and starch was isolated by alkali extraction method and characterized for morphological studies by Scanning Electron Microscope. Rice (*Oryza sativa* L.) viz. Basmati, HMT and Swarna were investigated.

Materials used: Three different rice cultivars viz. *HMT, Swarna and Basmati* were used. Three cultivars of Rice (*Oryza sativa* L.) viz. Basmati, HMT and Swarna were being investigated.

Procedure

The rice grains were properly cleaned, grinded in a mixture grinder and stored properly at room temperature before they are to be used in actual experiment. The next step is the isolation of Starch (Alkali Extraction Method). The residue obtained after the extraction of protein was sequentially extracted with 1 liter of each distilled water and 2% NaCl (each for 24 hrs at 4 ◦C) followed by extraction with 300 ml of 0.1 N NaOH solution twice (48 hrs at 4 ◦C). Subsequent to each of above the extraction, the slurry was being centrifuged at 10000 rpm for 30 min. at 4 ◦C and supernatant was discarded. The residue from the second NaOH solution was then extracted with 80% aqueous ethanol (100 ml) at 80 ◦C for 1hr then cooled to room temperature and allowed to settle for 4 hr at 4 ◦C. The supernatant solution was discarded, and the residue was dehydrated and powdered and as a result starch was obtained.

Starch yield was found to be in the range 81-84% on fresh weight basis method. The highest starch yield of 84% was obtained from Basmati Rice and the lowest yield was of 81% and it was obtained by HMT while that of Swarna variety was 82%.

Table: Starch contents of cultivars of rice

Sr. No.	Variety of Rice	Yield of Starch (%)
1.	Basmati	84
2.	HMT	81
3.	Swarna	82

Efficient recovery of glucose via enzymatic saccharification of rice straw with soft carbohydrates [3]

Soft carbohydrates can be defined as readily-recoverable carbohydrates via the process of extraction from the biomass or brief enzymatic saccharification, were found in significant amounts in rice straw as forms of free glucose, free fructose, sucrose, starch, and. The detailed studyon this investigated the amounts of all these components amounts in rice straw (defined as culm and leaf sheath), and this led to the develoment of a very simple method that was implemented for the extraction of glucose and fructose recovery from them with heat-pretreatment and then subsequent 4-hours enzymatic saccharification with an enzyme mixture of cellulase and amyloglucosidase.

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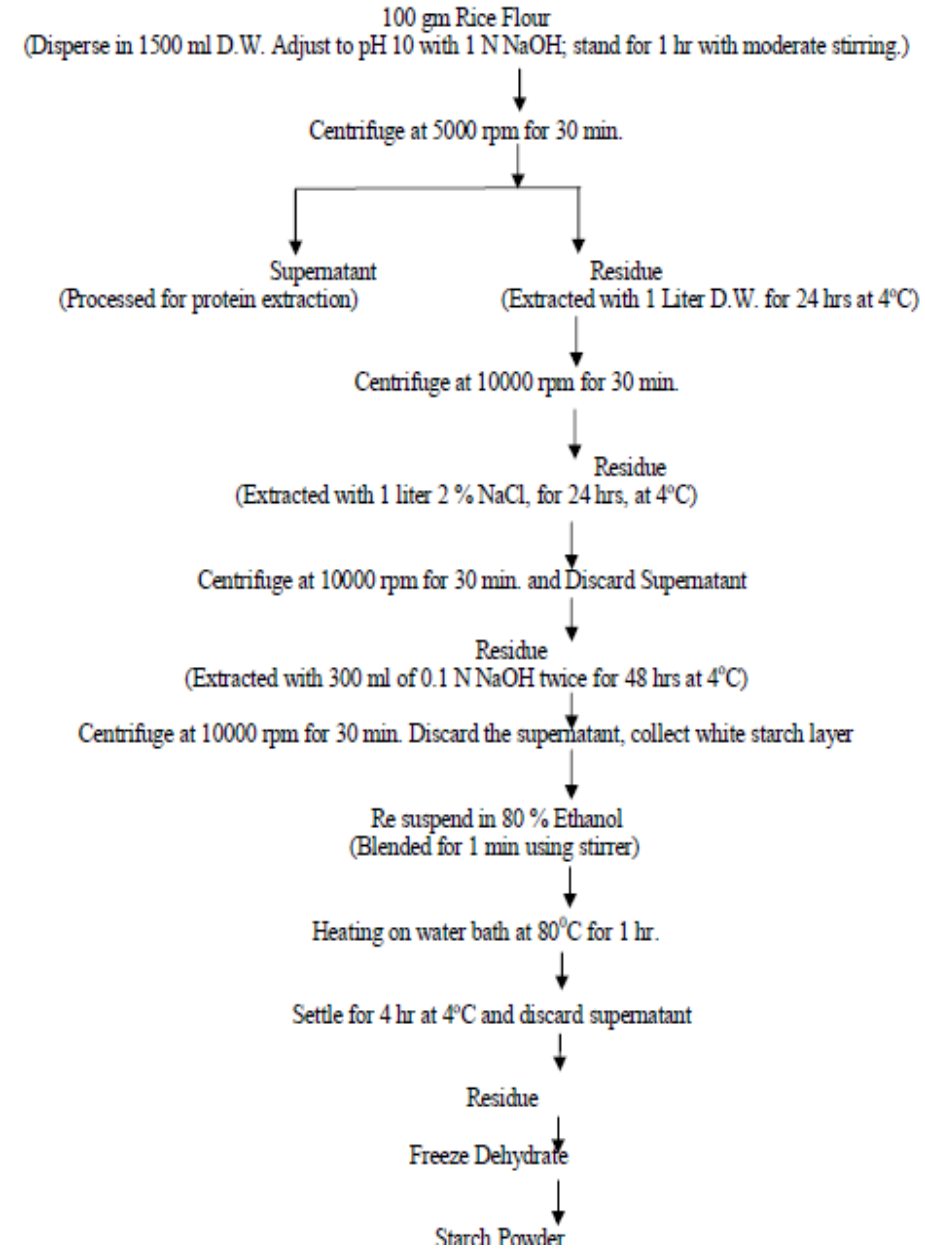

Component analysis of rice straws. The total starch content of each sample was determined by the enzymatic hydrolysis of starch into glucose, using a instrument Total Starch Kit. The amounts of glucose and sucrose were estimated with a Glucose C-II Test Wako with and without invertase treatment. The free fructose content was calculated as the same amount of free glucose contained in each sample, because it is a characteristic component of rice straw that consists of free fructose and glucose are almost of same concentration. The contents of cellulose and xylan were determined by the two-step H_2SO_4 hydrolysis method. The mixture was centrifuged at 10.00 *g* for 3 min, and the supernatant solution was neutralized with 10% (w/v) NaOH. The total glucose content in the sample was determined by Glucose C-II Test Wako, while the xylose content was determined with a D-Xylose Assay Kit.

Rice straw has been reported to contain significantly larger amounts of starch than other cereal straws, and in some cases, the amount of starch in the rice straw reaches over 20% of the dry weight. It has been re-established in terms of the increase in content of rice yield and feeding biomass. The amounts of starch varied from 9.3% to 24.0% of the dry weights at the heading stage and from 0 to 15.4% at the mature stage. The variants of rice chosen like for example- cvs. Leafstar, Habataki, and Sasanishiki accumulated relatively large amounts of starch at both stages of presence and absence of invertase enzyme, suggesting high potential as feedstocks for bioethanol production. These results could deviate depending not only on the genetic characteristics of a cultivar, but also other determinantinig factors such as cultivation conditions, date of harvest, preservation period, and drying process for the sample. Thus it can be concluded, that development of a method for stable accumulation of starch in rice straw, based on information on both endogenous and exogenous determinants, is a prerequisite for industrial utilization of rice straw with high soft carbohydrates content.

Glucose production from rice husk by solid state fermentation method [4]

Ligno-cellulosic substrates, such as rice husk are the agricultural wastes and by-products of rice producing plants. The major part of lingo-cellulosic substrates is cellulose

polymerase, hemi cellulose and lignin. Solid state fermentation means the control or inhibition of growth of microorganism on wet solid substrate in absence of free water method does not require large space, so these operations can be performed in a pan bioreactor or even in an Erlenmeyer Flask. The greatest advantage of solid state fermentation is that, this method does not to make the substrate diluted or soluble rather a little bit of moisture is enough. The desired product is concentrated so that its purification is becomes easier and also S.S.F. significantly reduces the contamination of microorganisms by eliminating them due to the low humidity of the humidity of medium. However, the SSF method is associated with some drawbacks, including limited use of microorganisms capable of growth in semi-humid environment and information about the increasing scale of production in this way is negligible. The purpose of this study is to optimize of growth medium of *Trichoderma reesei* and production of glucose via enzymatic hydrolysis of lingo-cellulosic waste by solid state fermentation

Materials and Methods

For this experimental research fungal strain *Trichoderma reesei* as lyophilized ampoule was purchased from industrial scientific research organization and transferred to physiologic serum under the sterile hood. Strains were cultured in steep medium of Malt extract Agar (MEA), and incubated at 4 °C for long-term maintenance. Rice husk was used in this study as substrate and as carbon source.

Preparation of substrate

For preparation of substrate, rice husk was being incubated at 40 ◦C and then grinded, the resulting rice husk particles size was found to be less than 2 mm.

Preparation of pre culture

After elementary operation, 12.5 g of rice husk powder was mixed with 17 c.c. of water in the Erlenmeyer Flask (500 ml). After preparation of solid bed, sterilization step 121 ◦C and 15 psi in the autoclave was achieved. Next, the temperature of the contents of the flasks was brought to room temperature.

Fermentation culture

Spore suspension prepared from slants containing 3 strains with sterile distilled water, and using sterile pipette at near the flame, impregnation operation from slant to pre culture media took place and then, pre culture flasks were incubated 30 ◦C for 5 days.

Preparation of main culture

After the flasks were placed in the autoclave and cooled at room temperature in the completely sterile conditions, impregnation from pre culture to main culture took place. With sterile inoculating loop, a part of growth fungi colony on a solid bed was transferred to flask that contained main culture and was mixed and this process was repeated 3 to 4 times till 5% growth fungi colony was transferred. Then, after preparing fungi suspension with physiologic serum, 10 micro liter of this suspension was put between lam and Lamel. Using optical microscope, the number of fungal spores in each square was detected and the dilution factor that depends on the volume of physiological serum was applied. Number of spores were calculated.

When the numbers of spores on a main culture reached to 8×105 wet weight, again cultures were incubated at 30 ◦C for 5-7 days. After the mentioned time finished, the culture was taken out of the incubator.

Separation of enzymatic solution

For the separation of raw enzymatic solution, first buffer citrate at pH= 4.8 was prepared, and then, about 5 fold of solid substrate weight, buffer citrate was added to flasks (5×12.5=125 ml). Next to that, the flasks were put in the shaker for 15 mins, until the contents found were to be equal. Then the solution was passed from Watman filter paper No.1. to assure zero. presence of fungal spores, then the passed solution from filter was centrifuged at 4 ◦C and 4000 rpm for 20 min. This prevented the turbidity in the experiment. In this way, the enzymatic solution that contained a lot of liquid sugar (glucose), can be separated. Infact, during fermentation, oozed cellulose enzyme from *Trichoderma* fungi, hydrolyzed the already existing cellulose in the rice husk and convert it to glucose (lump sugar).

Measurement of glucose and protein concentration in raw enzymatic solution

For the measurement of glucose concentration in raw enzymatic solution, kit of glucose oxidase that is an enzymatic method for lump sugar measurement was used. According to the existed direction in a kit, produced glucose concentration in crude enzymatic solution (mg/dl), was calculated. For the measurement of amount solute protein, Loury method has adopted. Bovine Serum Albumin (BSA) solution is as a standard solution and at the end the amount of samples absorption read at 578 wave length and using the help of BSA standard curve, protein concentration in the unknown sample (mg/ml) was obtained.

Measurement of enzyme activity

This research was solely based upon the calculation of filter paper activity and after that calculation of endogluconase activity. With respect to the references in this field, experiments related to activity measurement were done and at last, the amount of activity (unit/ml) was calculated.

Investigation of fungal growth in different period of time

Optimization of incubation time had been done by preparing 6 cultures. For this purpose, the different times (3-8 days) have been investigated.

Investigation of fungal growth in different incubation temperature

For the optimization of incubation temperature, 5 cultures have been studied in different temperatures (26-34 ◦C).

Table: Results of produced glucose in six medium of *Trichoderma* fungi after 3 to 8 days respectively and at 30◦C

Time (days)	3	4	5	6	7	8
Glucose concentration (mg/dl)	124.0	112.7	93.0	114.4	56.5	92.5

Table: Results of produced glucose in 5*Trichoderma reesei* medium at different temperatures after 4 days.

Temperature	26	28	30	32	34
Glucose concentration (mg/dl)	96.6	60.2	103.2	57.7	71.8

According to the previous tests, the highest degree of endogluconase enzymatic activity and filter paper activity was observed after four full days. In another phase study was done on the *Trichoderma reesei* fungi in 5 medium containing rice

husks for 4 days at different\temperatures 26 ◦C, 28 ◦C, 30 ◦C, 32 ◦C and 34 ◦C in incubator. Results have shown that in the prepared crude enzymatic solution from the cultures, the amount of glucose produced at a temperature of 30 ◦C (103 mg per dl) is the highest value, that did not show any significant 95 difference with the amount of glucose at 26 and 34 ◦C, but it was significantly more than the amount of glucose at 28 and 32 o *c* (P< 0.05). The lowest amount of produced glucose was 58 mg per dl that was obtained at 32 ◦C. According to the results of previous experiments, the optimum conditions in terms of glucose production on fourth day and at 30 ◦C were determined. In these conditions, the amount of protein concentration was found out to be 23.4 (mg/ml) and Carboxyl Methyl Cellulose activity and the paper filter activity was 680.3 and 125.7 (U/ml) respectively. It can be concluded that rice husk can be suitable substrate for *Trichoderma reesei* fungal strain in order to produce liquid glucose. The optimum conditions for liquid glucose production with used mentioned substrate and strain are through solid state fermentation (SSF) and incubation of media at 30 ◦C to 96 hours.

Conclusion

In a nutshell, it can be concluded that all the four the methods of extraction has their own benefits and disadvantages, so it's up to the manufacturer that what method is feasible depending upon the socio-economic and geo-chemical factors.

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