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Production, optimization and characterization of glucoamylase from agricultural residues using *Aspergillus niger*

Rana Kumbha Singh, Ajay Kumar Singh, Yashab Kumar, Harison Masih and Kunal Singh

Abstract
Filamentous fungi have been widely used to produce hydrolytic enzymes for industrial applications. Fungal enzyme glucoamylase was produced using a specific culture *Aspergillus niger* under solid state fermentation and the enzyme obtained was partially purified. The use of agro by-products present a great potential as substrate and support the low production costs for glucoamylase production. Screening of five agricultural residues viz. tea waste, mango peel, jack fruits, groundnut peel, pulse bran was done to produce glucoamylase. From these, tea was selected as substrate for further study as it yielded maximum glucoamylase (33.84 IU/ml) activity. Various cultural conditions such as temperature, pH, spore suspension and incubation period, carbon and nitrogen sources were optimized. Temperature of 30 °C, pH 6.0, spore suspension (4 ml) and incubation period of 10 days were found optimum for maximum production of glucoamylase enzyme. Glucoamylase from a tea waste culture of *Aspergillus niger* was partially purified by Ammonium Sulphate precipitation, dialysis and ion exchange chromatography. The partially purified enzyme had an activity of 64.44 IU/ml which was 2.05 folds of the activity of the crude culture filtrate. Hydrogen ion concentration as well as temperature had profound influence on enzyme activity of the partially purified enzyme while glucoamylase activity increased progressively as pH was increased from 5 to 6 reaching a maximum of 68.40 IU/ml at pH 6.0. A rapid decrease in glucoamylase activity was observed as pH was increased from 7 to 9. The glucoamylase activity increased with increase in temperature from 20 °C to 30 °C and reached a maximum of 50.04 IU/ml at 30 °C. Subsequent increase in temperature resulted into decrease in activity of the glucoamylase enzyme. The enzyme activity of glucoamylase was enhanced by Ca^{2+}, Mn^{2+}.

Keywords: *Aspergillus niger*, agro byproducts, glucoamylase, solid state fermentation

1. Introduction
Amylases are among the most important enzymes and are of great significance in present day biotechnology, having approximately 25% of the enzyme market. The amylase family has two major classes, namely amylase (EC 3.2.1.1) and glucoamylase (EC 3.2.1.3). Alpha amylase can hydrolyze starch into maltose, glucose and malto triose by cleaving the 1, 4-D-glucosidic linkages between adjacent glucose units in the linear amylose chain. Glucoamylase (GA) hydrolyzes single glucose units from the non-reducing ends of amylose and amylopectin in a stepwise manner and produce glucose as the sole end-product from starch and related polymers. Glucoamylases had manifolded the applications in industry. This enzyme is used in dextrose production, in the baking industry, in the brewing of low-calorie beer and in whole grain hydrolysis for the alcohol industry. The glucoamylase is added to the starch paste at the saccharifying step when it is around 60 ºC, thus the enzyme must be stable at this temperature for a long time. The principal industrial use of glucoamylase is therefore, the production of glucose, which in turn serves as a feed stock for biological fermentations in the production of ethanol or high fructose syrups (Zambare, 2010) [15]. Glucoamylase is also used to improve barleymash for beer production. It is a key enzyme in the production of sake and soy sauce (Zambare, 2010) [15]. Glucoamylase also has applications in confectionery, baking and pharmaceutical industries (Pandey *et al*., 2000) [12].
Traditionally, glucoamylases have been produced by submerged fermentation. A recent focus of the research and development effort is the application of glucoamylases in the enzymatic degradation of carbohydrate rich polysaccharides for production of energy syrup. The development of microbial strains, media composition and process control all have contributed to the achievement of high levels of extracellular glucoamylases. However, the glucoamylase

costs are still too high for the establishment of a cost effective production of energy syrup. One approach to overcome this obstacle is to employ solid state fermentation. The SSF process has the potential to significantly reduce the enzyme production costs because of lower energy requirements, increased productivity, smaller effluent volumes and simpler fermentation equipment (Ellaiah *et al.* 2002) [3]. In the SSF process, the solid substrate not only supplies the nutrients to the culture, but also serves as an anchorage for the microbial cells. Agro-industrial residues are generally considered best substrates for the SSF processes and enzyme production by SSF is not an exception to that (Ellaiah *et al.,* 2002) [3]. The production of amylolytic enzymes, particularly glucoamylase on [solid] substrate is more advantageous for the fermentation industry.

2. Material and Methods

2.1 Place of Work

The present study entitled "Production optimization and characterization of Glucoamylase from Agricultural residues using *Aspergillus niger*" was carried out in the Department of Microbiology and Fermentation, Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Deemed- to-be-university), Allahabad.

2.2 Procurement of Microorganism and its maintenance

The fungal species *Aspergillus niger* was isolated from the bread sample and maintained as pure on potato dextrose agar (PDA). The fungal isolates were stored at 4 °C in a refrigerator and were regenerated on freshly prepared PDA medium.

2.3 Isolation of microorganism

The fungal culture *Aspergillus niger* was isolated from the bread sample. The infected bread sample was taken from the Food shops and crushed in powder form and the serial dilution was performed. An Aliquot (1ml) of 10^{-3} and 10^{-4} diluted sample was taken and transferred onto the starch agar plates and incubated it at 25 °C for 4 to 5 days. Then after proper growth iodine was flooded on the starch agar plate and zone of starch hydrolysis was observed. A clear zone was obtained around growth of the fungal colony.

2.4 Preparation of substrate or Processing of substrate

The Mango peel (MP), Pulse bran (PB), Jack fruits (JF), Tea waste (TW), Groundnut peel (GP) were collected from local market and houses. The five substrates were separately dried and milled to 0.5 mm particle sizes**.**10 grams of each substrate was mixed with 20 ml of Aspergillus Complete Medium (ACM) (Appendix-1.4) in a 250mL Erlenmeyer flask. The ACM was comprised of: $(NH_4)_2SO_4$5.0; Na_2HPO_4.12 H_2O3.8; KH_2PO_4 3.5; $MgSO_4$0.5and yeast extract 0.1 at pH 5**.** The ACM was provided with the needed mineral solutions and was autoclaved at a temperature of 121 °C for 25 minutes before it was inoculated with the appropriate organism.

2.5 Solid state Fermentation

Agro residues (10g) were kept separately in a 250mL Erlenmeyer flask and then it was moistened with 20mL of water and sterilized at 121ºC for 30 min. The fermentation process was started by adding 1mL of spore suspension. The whole content was mixed thoroughly and then incubated at 25 ºC for 10 days under stationary condition.

2.6 Enzyme extraction

The fermented dough was added with 50mM citrate buffer (pH 5) (1:10) and homogenized for 2 hrs with a constant stirring at room temperature. This suspension was filtered through Whatman filter paper No.1 and the filtrate was again centrifuged at 6000 rpm for 15min.The solid-free supernatant was used as enzyme source for assaying glucoamylase activity.

2.7 Production optimization

Glucoamylase production was optimized with respect to various nutritional and environmental parameters such as 10g of different agro-residues (mango peel, pulse brain, ground nut, jack fruits, tea waste); initial moisture content (50-100%, v/w); inoculums size (1-10%, v/w); nitrogen sources [inorganic (0.25% w/w)-ammonium sulphate, ammonium phosphate, ammonium nitrate, sodium nitrate, urea]; carbon inducers (1% w/w) such as glucose, fructose, lactose, pH (4-9) and temperature (20-40 ºC). All the experiments was conducted independently in duplicate.

2.8 Assay of Glucoamylase

The enzyme activity was determined when the reaction mixture containing 0.9 mL of 50 mM citrate buffer (pH 5), 1.0mL starch solution (1%, w/v) and 0.1mL of crude enzyme was incubated at 50 ℃ for 20 min and then release of reducing sugars is measured with 3, 5-dinitrosalicyclic acid (DNSA) reagent using glucose as a standard. Glucoamylase activity unit (U) was expressed as the amount of enzyme releasing one µmole of glucose equivalent per minute under assay condition and enzyme activity was expressed in terms of micromole per mililitre per minute.

2.9 Time course of glucoamylase production

Glucoamylase was produced at optimum conditions of nutritional as well as environmental parameter. Samples were withdrawn aseptically after every 24 hours and up to 10daysand are analyzed for glucoamylase activity and biomass.

2.10 Partial purification of Glucoamylase

The crude extract of glucoamylase was saturated with 60 and 80% ammonium sulphate and then centrifuged at 6000rpm for 15 min at 4 °C is, re-dissolved in acetate buffer (0.1 mol L^{-1}, pH 4.6) and dialyzed against phosphate buffer (5 mmol L-1, pH 6.9). The sample was loaded into a column with anionic exchange resin (DEAE cellulose); 4.0 mL fractions was collected at a 60 mL h^{-1} flow, using as eluents, in the sequence, phosphate buffer 5, 20 and 200 mmol L-1 (pH 6.9), then a 0.0-1.0 mol/ L^{-1}NaCl gradient followed by elutions with constant NaCl concentrations at 1, 2 and 3 mol L^{-1}, all NaCl solutions in phosphate buffer (200 mmol L^{-1}, pH 6.9). These samples were read at 550 nm for each fraction.

2.10 Characterizations of Glucoamylase

2.10.1Effect of temperature on glucoamylase enzyme activity

The temperature effect on enzyme was determined by adding 1ml of enzyme + 1ml phosphate buffer (pH 5) and then incubated it at different temperature range (20-50) °C for 24hrs. The assay for glucoamylase activity of each tube was performed and measured absorbance at 550nm.

2.10.2 Effect of pH on glucoamylase enzyme activity
he pH effect on the glucoamylase enzyme was determined by adding 1ml of glucoamylase enzyme + 1ml of phosphate buffer of different pH (5-9) and incubated it at 35 °C for 24 hrs. The assay for glucoamylase activity of each tube was performed and measured absorbance at 550nm.

2.10.3 Effect of metal salts on glucoamylase enzyme activity
The effect of metal salts in the enzyme was determined by adding 1ml of enzyme +1ml of metal salt concentration ($MgSO_4$, $MnSO_4$, $FeSO_4$, $CaCl_2$)+ 1ml of phosphate buffer (pH 5) incubated it at 35 °C. The assay for glucoamylase activity of each tube was performed and measured absorbance at 550nm.

2.11 Statistical analysis
The data obtained was subjected to statistical analysis such as single factor ANOVA and the data were interpreted and analyzed to calculate significant differences in the same by using one way classification and conclusion was drawn on the basis of analysis of variance technique (ANOVA) (Fischer, 1968) [4] at 5% level of significance.

3. Results and Discussions
The aim of present research was to produce glucoamylase enzyme by solid state fermentation of agricultural residues using *Aspergillus niger*.

3.1 Screening of Glucoamylase producing fungal strain from
Fungal species *Aspegillus niger* was isolated from waste bread sample.

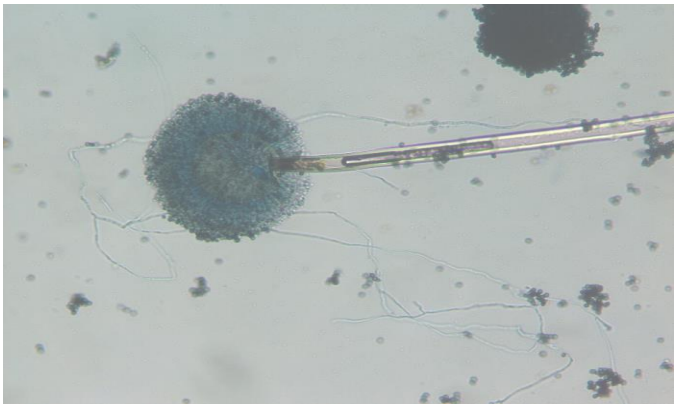

Plate 1: Growth of *Aspergillus niger sp.* on Potato dextrose agar media

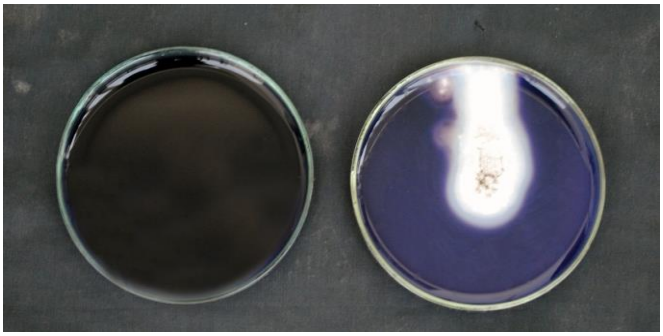

Plate 2: Glucoamylase production by a strains of *Aspergillus niger* (S1)

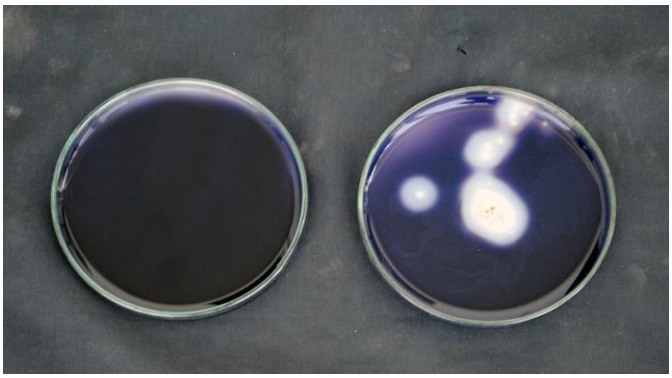

Plate 3: Glucoamylase production by a strains of *Aspergillus niger* (S3)

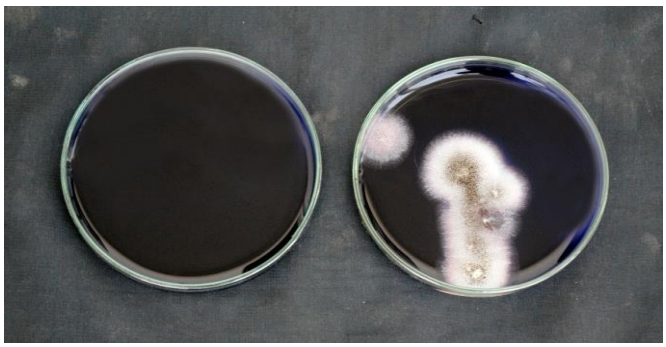

Plate 4: Glucoamylase production by a strains of *Aspergillus niger* (S2)

3.1 Optimization of processing and nutritional parameters for glucoamylase production The glucoamylase productions with different substrates were conducted and maximum glucoamylase production was observed in the fermentation medium having tea waste as carbon source.

3.1.1 Effect of fermentation periods on glucoamylase production using *Apergillus niger*
Incubation period plays an important role in substrate utilization and its protein enrichment for enzyme production. The effect of incubation period was evaluated by checking enzyme activity after 2, 4, 6, 8, 10 and 12 days of incubation at 30 °C. The maximum yield of glucoamylase (34.56 IU/ml) was observed on the 10th day of incubation. Further increase in incubation period decreased the production of glucoamylase sharply. On analysis of the data obtained in the present experiment by using ANOVA one way and found significant at 5% level.

Table 1: Effect of fermentation periods on Glucoamylase production

S. No	Fermentation periods (Days)	Glucoamylase production (IU/ml)
1	4	17.10
2	6	24.48
3	8	25.74
4	10	34.56
5	12	9.00

Kaur *et al* (2015) [7] reported maximum glucoamylase production by *Aspergillus niger* after an incubation period of 5 days. Similarly Nyamful *et al*. (2014) [11] reported that glucoamylase production was highest on 5th day of incubation period.

3.1.2 Effect of Different pH on production of glucoamylase from tea waste by using *Aspergillus niger*

The effect of pH on glucoamylase activity of *Aspegillus niger* was studied by varying the pH from 4 to 9. The results indicated that the increase in the pH value from 4 to 6, the activities of glucoamylase enzyme reached to the Maximum (22.86 IU/ml) followed by a sharped decline thereafter. The optimum pH for glucoamylase production was found at 6.0.

Table 2: Effect of different pH on glucoamylase production by using *Aspergillus niger*

S. No	Different pH	Glucoamylase production (IU/ml)
1	4	11.88
2	6	22.86
3	7	7.74
4	8	6.84
5	9	4.86

Similarly fermented deoiled rice bran for the production of glucoamylase enzyme by *Rhizopus oryzae* yielded maximum activity as reported by Kaur *et al*. (2015) [7]. The finding are comparable to the reported of Keera *et al.* (2014) [8] who obtained maximum glucoamylase activity at pH 5.5 In line with the present work the culture condition for glucoamylase production at pH 5 as reported by

3.1.3 Effect of different temperature on production of glucoamylase in Tea waste medium using *Aspergillu niger*

The maximum glucoamylase production was observed at 30 °C. A decrease in enzyme production was observed from 35 °C to 40 °C. It clear from the results that a temperature of 30 °C was found to be suitable for glucoamylase activity and maximum activity was 33.84 IU/ml. It was observed that at 20 °C enzyme activity was low and showed a gradual increase with the increase in temperature up to 30 °C. Further increase in temperature resulted in decrease in production of enzyme.

Table 3: Variation in glucoamylase production with the different temperature

S. No	Different Temperature (°C)	Glucoamylase production (IU/ml)
1	20	7.92
2	25	30.06
3	30	33.84
4	35	12.42
5	40	3.60

It is widely known that at high temperature enzyme activity can be destroyed because enzyme are protein aceous molecules. Puri *et al*. (2013) [13] also observed that at 20 °C enzyme activity were low and showed a gradual increase with the increase in temperature to 30 °C. The maximum glucoamylase synthesis was observed in the medium incubated between 28-31 °C as reported by Keera *et al*. (2014) [8]. The increasing incubation temperature resulted in decreasing enzyme synthesis by the fungus that may due to the production of large amount of metabolic heat and the fermenting substrate temperature shoots up therefore by inhibiting microbial growth and enzyme formation. *Aspergillus sp*. produce glucoamylase titres at 30 °C under optimum SSF process condition as reported by Ellaiah *et al.* (2012) [3].

3.1.4 Effect of inoculum concentration on the glucoamylase production

The present experiment was conducted to evaluate the production level of glucomaylase at various inoculums concentrations from 1ml to 5ml of *Aspergillus niger*. The maximum glucoamylase production was observed at 4ml inoculums (Table 4; Fig 1). The production of glaucoamylase was found increasing with initial increasing of inoculum concentration i.e from 1ml to 4ml and reached maximum at 4ml of inoculums concentration. Afterwards the production decreased. In a similar research conducted by Imran *et al*. (2012) [5] the maximum production was observed at 5ml of inoculum concentration.

Table 4: Effect of inoculums of *Aspergillus niger* on the glucoamylase production in tea waste medium

S. No	Inoculum concentration (ml)	Glucoamylase production (IU/ml)
1	2	39.96
2	3	41.76
3	4	43.56
4	5	28.44

Similar result were reported by Puri *et al*. (2013) [13] who used agricultural byproducts like wheat bran and rice bran substrate for the synthesis of glucomaylase by solid state fermentation using *Aspergillus niger*. A higher inoculum size may increase moisture content and lead to a decrease in growth and enzyme production. Kaur *et al*. (2015) [7] also reported increase of glucoamylase production by increasing inoculum concentration of *Rhizopus oryzae* there by nhibiting microbial growth and enzyme formation.

3.1.5 Effect of inorganic nitrogen sources on glucoamylase production

The present research was conducted for the production of glucoamylase with the supplementation of various inorganic nitrogen sources. The inorganic nitrogen sources such as Ammonium sulphate, Ammonium phosphate, Ammonium nitrate, Sodium nitrate and Urea were used. The maximum glucoamylase production was observed in urea supplemented medium. The data showed in Table 5 that Urea, Ammonium sulfate, Ammonium nitrate, Sodium nitrate, shown varying positive effects on production of glucoamylase and the highest activity was recorded by supplementation of Urea followed by ammonium nitrate and sodium nitrate respectively. Low negative effect on the production of glucoamylase was achieved by ammonium phosphate.

Table 5: Effect of inorganic nitrogen sources on glucoamylase production

S. No	Different Inorganic nitrogen source	Glucoamylase production (IU/ml)
1	Ammonium sulphate	42.66
2	Ammonium phosphate	9.54
3	Ammonium nitrate	61.20
4	Sodium nitrate	57.60
5	Urea	64.98

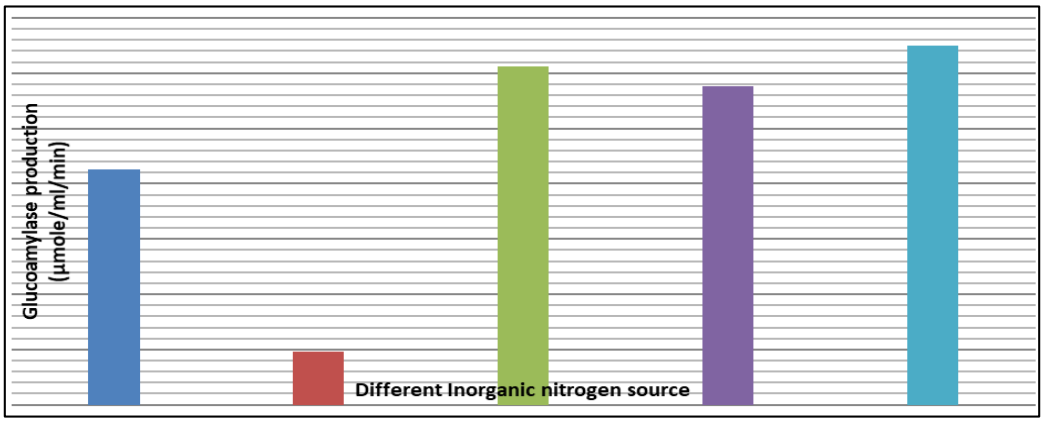

Fig 1: Variation in Glucoamylase production by *Aspergillus niger* due to supplementation of inorganic nitrogen sources

The obtained result confirmed that the effect of nitrogen source positively or negatively may be depended on two factors firstly organisms and secondly the substrate. Keera *et al.* (2014) [8] reported that the urea stimulated the production of glucoamylase using *A. niger*. Among the nitrogen sources used to inverse the production of glucoamylase enzyme by *Candida famata*, urea was the best nitrogen source as reported by Nahar *et al.* (2008) [8].

3.1.5 Effect of different carbon source supplementation on production of glucoamylase

The present investigation was conducted to evaluate the supplementation of carbon sources such as glucose, fructose and lactose on the glucoamylase production. The maximum enzyme production was observed when glucose was supplemented in the fermentation medium followed by fructose and the least production was found in lactose supplemented medium.

Table 6: Variation in glucoamylase production with the supplementation of different carbon sources

S. No.	Different Carbon Source	Glucoamylase production (IU/ml)
1	Glucose	36.18
2	Fructose	15.30
3	Lactose	7.74

Anto *et al.* (2006) [1] also reported enhanced enzyme production with glucose supplementation. Keera *et al.* (2014) [8] also reported that highest production was observed with glucose when *Aspergillus oryzae* FK 923 was used.

3.1.6 Production of glucoamylase enzyme under optimized condition of processing and Nutritional parameters

Summary of the various optimized fermentation conditions and glucoamylase production is given in table.

Table 7: Optimum glucoamylase production obtained under different fermentation conditions

S. No.	Fermentation conditions	Optimum glucoamylase production (IU/ml)
1	Substrate (Tea Waste)	33.84
2	Fermentation period (10 days)	34.56
3	pH (6)	22.86
4	Temperature (30 °C)	33.84
5	Inoculum Conc. (4ml)	43.74
6	Nitrogen sources (Urea) (0.25%)	64.98
7	Glucose (1%)	36.18

For the optimization of production of the glucoamylase, different parameters of optimization gave maximum enzyme activity such as substrate (Tea waste); fermented period (10 days); pH (6); temperature (30 °C); inoculum conc. (4ml); nitrogen sources (urease); carbon sources (glucose).

3.2 Partial purification of glucoamylase enzyme

The glucoamylase enzyme was partially purified by ammonium sulphate precipitation (80%), dialysis (10kd) and ion exchange chromatography (DEAE cellulose)

In the partial purification, the activity of crude enzyme from selected substrate (tea waste) was 31.32IU/ml. Further ammonium sulphate precipitation (80%) and dialysis (10kd) of crude enzyme gave enzymatic activity 40.68 IU/ml. Ion exchange chromatography of the dialyzed enzyme showed enzymatic activity of 64.44IU/ml. The glucoamylase yield was decreased to 51.43%.

3.6 Characterization on glucoamylase enzyme

3.6.1 Activity of partially purified glucoamylase enzyme at different pH.

The activity of partially purified glucoamylase enzyme at different pH was tested. Extremely high or low pH values generally resulted in complete loss in activity for most enzymes. The pH is also factor in the stability of enzyme. As with activity for each enzyme there is also a region of pH optimal stability. The enzyme exhibited maximum activity in a pH range of pH 6 to 7 and optimum was recorded at pH 6. The stability was declined at extreme acidic or alkaline condition.

The results are in agreement with the report of Keera *et al.* (2014) [8]. The effect of pH in enzyme structure lead to denaturation of enzyme molecule or change the ionic state of active site also its effect on the secondary and tertiary structure of enzyme lead to losing the activity in buffer that far away from optimum pH (Segel, 1976) [14].

3.6.2 Activity of partially purified glucoamylase enzyme at different temperature

The partially purified glucoamylase was found highly stable at 30 °C further other temperatures showed the decreasing activity. The partially purified enzyme was incubated in tubes in different temperature (20, 30, 40, 50) °C residential enzyme activity was determined. The maximum activity of glucoamylase was found at 30 °C. The residential activity was decreased at further increase of temperature. The data obtained were analyzed using correlation and found non significance.

The enzyme was maintained the activity when it was incubated at 20-30 °C. The study was agreed with Nguyen *et al.* (2002) [10] when they found the optimum temperature of glucoamylase stability purified from thermophillic fungus ATCC34626 ranging between 20-30 °C for 30 minutes.

3.6.3 Effect of metal ions on the activity of glucoamylase enzyme

The partially purified glucoamylase was tested for its activity with different metal ions namely $MgSO_4$, $MnSO_4$, $FeSO_4$ and $CaCl_2$. The activity of enzyme was determined after 24 hrs. Maximum enzyme activity was found with Mn^{2+} ions followed by Ca^{2+} and then Mg^{2+}. Therefore glucoamylase was found highly stable with $MnSO_4$. Further other ions showed the decreasing activity

Table 8: Effect of different metals ion on the activity of glucoamylase enzyme

S. No	Different metals ions	Glucoamylase activity (IU/ml)
1	$MgSO_4$	44.46
2	$MnSO_4$	80.46
3	$FeSO_4$	41.76
4	$CaCl_2$	67.14

Due to metal ions: $F_{(cal)} = 39.63987 > F_{(tab)} = 5.987377584$ (S) at 5%

Mean value due to different metal ion;
$MnSO_4$ $CaCl_2$ $MgSO_4$ $FeSO_4$
80.46 > 67.14 > 44.46 > 41.76
After comparing the mean of two enzymatic activity together significant difference was observed between ($MnSO_4$, $CaCl_2$); ($MnSO_4$, $MgSO_4$); ($MnSO_4$, $FeSO_4$). The maximum enzymatic activity was found in $MnSo_4$ which is observed above.

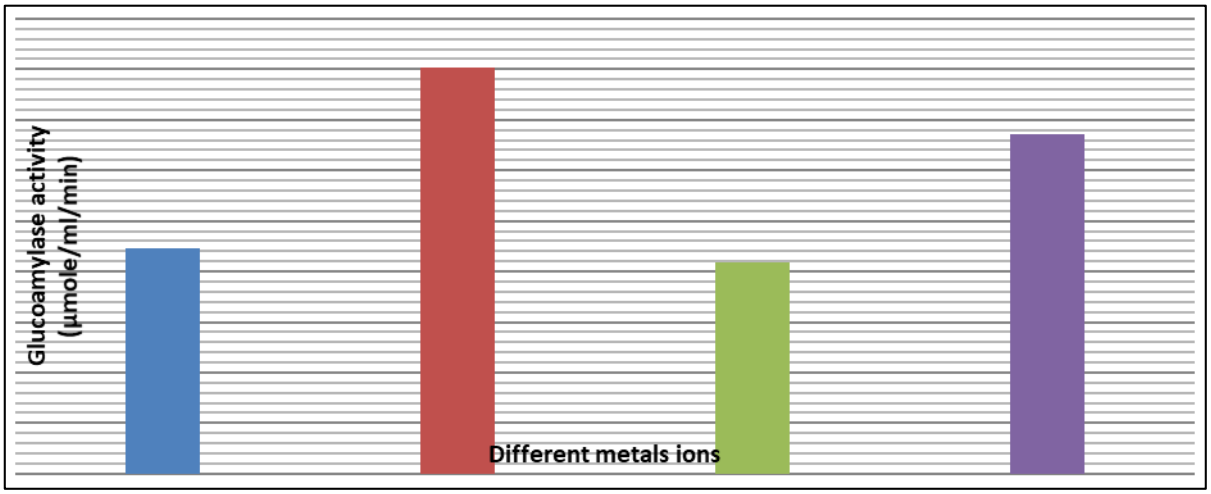

Fig 2: Activity of glucoamylase enzyme in different metal ions

Most of glucoamylase are known to be metal ion dependent enzyme (Deb *et al.* 2013) [2]. The effect of metal ion on glucoamylase activity showed that glucomaylase was enhanced by Mn^{2+}, Ca^{2+}, Fe^{2+}, Mg^{2+} since glucoamylases are used together with α-amylase which require Ca^{2+}ions in the liquefaction process, stimulation of glucoamylase activity by Ca^{2+}, Fe^{2+}, $Mn^{2/+}$ ions can make it more suitable for use in industrial starch bioconversions process. Kareem *et al*. (2014) [6] also reported that Mn^{2+}, Ca^{2+} and Fe^{2+} increase activity for glucoamylase obtained from *Rhizopus oligossporus* SKS mutant.

4. Summary and Conclusion

The present work entitled "Production, optimization and characterization of glucoamylase from agricultural residues using *Aspergillus niger"* was carried out in the Department of Microbiology and Fermentation Technology, Jacob School of Biotechnology and Bioengineering, SHUATS, Allahabad U.P. The fungal cultures were isolated from waste bread samples collected from local shops. The substrates such as tea waste, ground nut peel, jack fruits, pulse bran, mango peel was used for glucoamylase production. Among all the agricultural residues tea waste was found as highest glucoamylase producing medium. Further optimization of glucoamylase production with different parameters like incubation period, temperature, pH, inorganic sources, carbon sources, inoculums concentration was conducted. Partial purification of glucoamylase enzyme was conducted by ammonium sulphate precipitation (80%), dialysis and ion exchange chromatography and characterized under different pH, temperature and metal ions.

From the study, among all the substrate Tea waste was found as supportive glucoamylase production 33.84 IU/ml. Further optimization of glucoamylase was found at fermented period i.e. 10 days (34.56 IU/ml), pH 6 (22.86 IU/ml), 30 ˚C (33.84 IU/ml), 4ml (43.56 IU/ml), urease (64.98 IU/ml), glucose (36.18 IU/ml). After partial purification the glucoamylase was characterized at pH 6 (68.40 IU/ml), 30 ˚C (50.04 IU/ml), metal ions $MnSO_4$ (80.46).

From the above study it was concluded that tea waste was the best for glucoamylase producing substrate among different agro residues under solid state fermentation using *Aspergillus niger.* Overall *Aspergillus niger* was found to be compatible for glucoamylase production with cheaper nutritional media.

Therefore the present study can further be optimized at fermenter level so that the processing technology could be tested at pilot scale.

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