Gluconic Acid Production by *Aspergillus Niger* from Banana Must.

Anjali Anil Sharma, Dr. Ashok Gomashe, Harsha Bawane.
Shri Shivaji Science College Department of Microbiology Nagpur, MH, India.

Abstact:

Gluconic acid is an important organic acid resulting from the oxidation of glucose. Banana must were evaluated as the cheaper carbohydrate source for gluconic acid production from Aspergillus niger in surface culture fermentation process. The banana must was found to be a better source with significant gluconic acid production. Gluconic acid and its derivatives, the principal being sodium gluconate, have wide applications in food and pharmaceutical industry. The present work made attempt to increase gluconic acid production using banana must as sole carbon source by using A.niger.

Keywords: *Gluconic acid, Banana Must, Aspergillus niger.*

Introduction:

Gluconic acid is an oxidative product of D-gluconic acid.[1] Gluconic acid is abundantly available in plants, fruits and other foodstuffs such as rice, meat, dairy products, wine (up to 0.25 %), honey (up to 1 %), and vinegar. Gluconic acid is a noncorrosive, nonvolatile, nontoxic, mild organic acid. It imparts a refreshing sour taste in many food items such as wine, fruit juices, *etc*. Gluconic acid has found its extensive use in pharmaceutical and food industries. As stated above, it is a natural constituent in fruit juices and honey and is used in the pickling of foods. It is used in meat and dairy products, particularly in baked goods as a component of leavening agent for preleavened products. It is used as a flavoring agent and it also finds application in reducing fat absorption in doughnuts and cones. Foodstuffs containing D-glucono-d-lactone include bean curd, yoghurt, cottage cheese, bread, confectionery and meat.

Generally speaking, gluconic acid and its salts are used in the formulation of food, pharmaceutical and hygienic products. They are also used as mineral supplements to prevent the deficiency of calcium, iron, *etc*. and as buffer salts. Different salts of gluconic acid find various applications based on their properties. Sodium salt of gluconic acid has the outstanding property to chelate calcium and other di- and trivalent metal ions. It is used in the bottle washing preparations, where it helps in the prevention of scale formation and its removal from glass. It is well suited for removing calcareous deposits from metals and other surfaces, including milk or beer scale on galvanised iron or stainless steel. Its property of sequestering iron over a wide range of pH is exploited in the textile industry, where it prevents the deposition of iron and for desizing polyester and polyamide fabrics. It is also used in metallurgy for alkaline derusting, as well as in the washing of painted walls and removal of metal carbonate precipitates without causing corrosion. It also finds application as an additive to cement, controlling the setting time and increasing the strength and water resistance of the cement. It helps in the manufacture of frost and crack resistant concretes. It is also used in the household cleaning compounds such as mouthwashes.

Calcium gluconate is used in pharmaceutical industry as a source of calcium for treating calcium deficiency by oral or intravenous administration. It also finds a place in animal nutrition. Iron gluconate and iron phosphogluconate are used in iron therapy. Zinc gluconate is used as an ingredient for treating common cold, wound healing and various diseases caused by zinc deficiencies such as delayed sexual maturation, mental lethargy, skin changes, and susceptibility to infections.[2]

Thus, the demand for gluconic acid ia about 50,000-60,000 t/y and still growing year after year. The microbial fermentation process is exclusively used for commercial gluconic acid production. It leads to select an effective system for increased and economical production of gluconic acid. [3]

Material and Method:

Microorganism:

The microbial strain used was *Aspergillus niger*.* the strain was maintained on potato dextrose agar (PDA) slant.

Preparation of banana must:

Market refuses ripened bananas that did not meet the quality norms were utilized as a cheap carbohydrate source for gluconic acid fermentation. The fully ripened, peeled ground and blanched banana juice was clarified by heating and after that by filtration and centrifugation. [4]

Fermentation media:

Fermentation media was prepared by adding banana must in concentration equivalent to 12 percent reducing sugar(glucose). The salt mixture containing $(HN_4)_2HPO_4$ 0.1 %, KH_2PO_4 0.05%, $MgSO_4.7H_2O$ 0.015% and $CaCO_3$ 4.0% was added in media and was sterilized.

Fermentation:

Fermentation was carried out I Erlenmeyer flask of 500ml containing 100ml media. The inoculums was prepared from aseptically harvested spores of the subcultured *Aspergillus niger* at 30 ^{0}C for 5 days and suspended in 5ml sterile 0.05M phosphate buffer (pH 6.8) containing 0.1 percent Tween 80.

Recovery of gluconic acid:

After 5 days of incubation the fermentation media was diluted with distilled water to dissolve gluconic acid. From the fermentation media mycelia was removed by filtration through Watmann No. 1 filter paper. The filtrate was concentrated to the half of its volume. Then about one quarter of its volume of ethanol was added and the precipitate was discarded. The supernatant was then concentrated and about 4 times of its volume of ethanol was added whereby precipitate of gluconic acid was obtained. This precipitate was further purified to get purified gluconic acid.

Identification of gluconic acid by thin layer chromatography:

Slurry of silica gel was prepared by dissolving 30-35grms of silica powder in 65ml distilled water. Spread the slurry over the glass slide to get thin layer of gel. Allow the gel to dry at 80^{o}C for 30mins in hot air oven. After the plate is dried mark a line 1cm above at the bottom of the plate and spot the gluconic acid solution on the line three times. Allow the spot to dry. Keep the plate in chromatographic chamber which contain solvent for separation. The solvent is allowed to completely evaporate off. Glass slide was deepen into the chamber containing solvent, to a depth of less than 1 centimeter, and the lid is closed. Solvent was allowed to travel before it reaches the top of the plate, plate was taken out of chamber and dried. Analysis of spot was done by keeping chromatogram into a chamber containing iodine vapors or spray with a 50 g/l solution of potassium dichromate in a 40 per cent m/m solution of sulphuric acid. After 5 min, the spot in the chromatogram obtained. .[5]

Result :

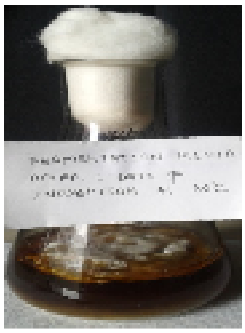


Figure1:- fermentation media after 5 days of incubation at 30^{o}C.

Figure 2:-Precipitate of gluconic acid in the form of its calcium salt.

In the present study, gluconic acid production was observed by *A .niger* from banana must as a cheaper carbohydrate source. The study was carried out at optimum conditions. The fermentation media was incubated for 5 days at 30°c.

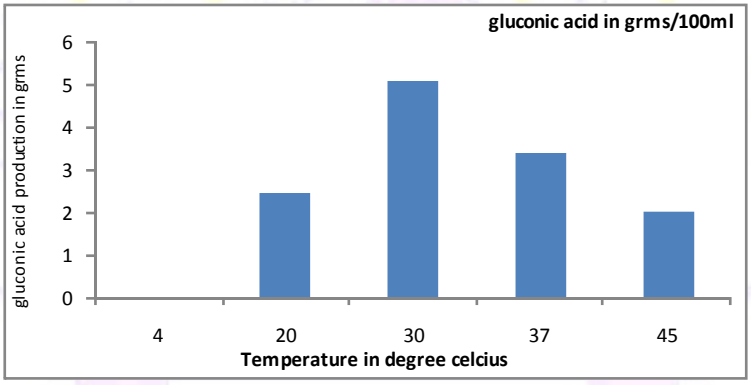
Effect of different parameter on gluconic acid production.

Effect of temperature:

To achieve the significant level of gluconic acid production by employing cheap carbohydrate source influence of different temperature range was evaluated.(table no. 1). Fermentation was carried out for 5 days at 4°C to 45°C using *A.niger* in surface culture. High gluconic acid production was observed at optimum temperature of 30°C and it was found to be 5.14 grms/100ml. Gluconic acid production beyond 30°C was found to be low.[6][7]

Table no 1:- Table showing effect of different temperature on the gluconic acid production at range of 4°C, 20°C, 30°C, 37°C and 45°C for 5days.

Sr.no	Temperature	Gluconic acid in grms/100ml
1	4°C	-
2	20°C	2.94
3	30°C	5.14
4	37°C	3.43
5	45°C	2.04



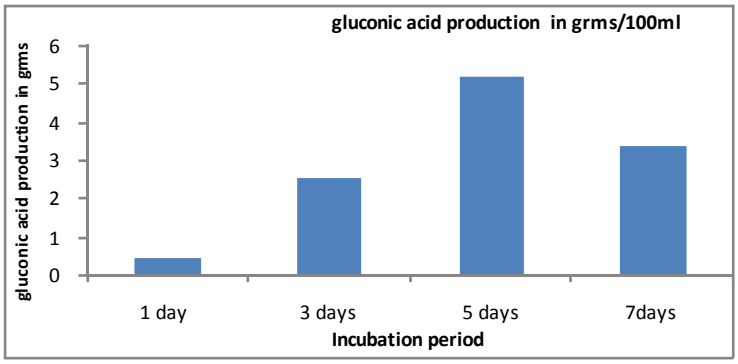
Graph no1:- Gluconic acid production in grms/100ml at different temperatures range 4, 20, 30, 37and 45 in °C.

Effect of incubation period:

Production of gluconic acid were determined during the incubation period of 7 days. The result shown in table no. 2 it was found that after 5 days of incubation there is maximum yield of gluconic acid i.e. 5.23 grms/100ml. Before optimum incubation period the yield was found to be low i.e. after 1 day it was 0.5 grms/100ml and after 3 days it was 2.60grm/100ml. After optimum incubation period i.e. after 7 days it was 3.3 grms/100ml. [8][9]

Table no:-2. Table showing effect of incubation period on gluconic acid production at range of (1,3,5,7) days of incubation at 30° C.

Sr. no	Incubation period (in days)	Gluconic acid in grms/100ml
1	1	0.5
2	3	2.60
3	5	5.23
4	7	3.39



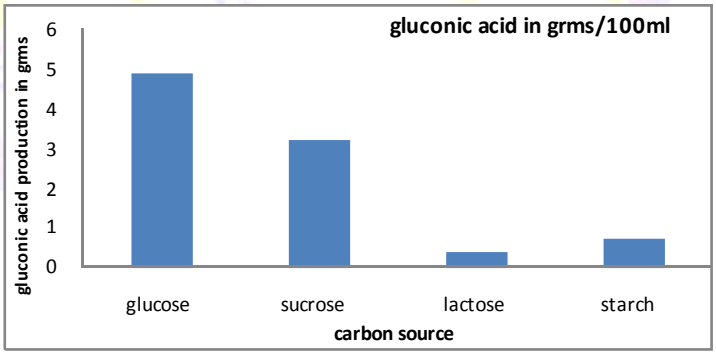
Graph no:-2 Gluconic acid production in grms/100ml at different incubation period 1, 3 , 5and 7 days.

Effect of carbon source:

The results of fermentation using other carbon source are shown in table no. 3 from the result we get to know that glucose and sucrose were found to be the best carbon source for gluconic acid production whereas, lactose and starch was unsuitable for gluconic acid production. In presence of glucose as a carbon source 4.9 grms/100ml of gluconic acid was produced and in presence of sucrose 3.21 grms/100ml.[10][11]

Table No:-3 Table showing effect of carbon source on gluconic acid production.

Sr. no	Carbon source	Gluconic acid in Grms/100ml
1	Glucose	4.9
2	Sucrose	3.21
3	Lactose	0.41
4	Starch	0.74



Graph no:-3 -Gluconic acid production in grms/100ml at different carbon source glucose , sucrose, lactose and starch.

Calculation of Rf Value

Actual value of gluconic acid is 0.18.

Formula for Rf value:-

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$$= \frac{1.1}{6.3}$$

$$= 0.174$$

Discussion

The production of gluconic acid is a simple oxidation process carried out by electrochemical, biochemical or bioelectrochemical method production by fermentation process by different fungi is well established commercially. In the present study attempt were made to increase gluconic acid production using banana must as a sole source of carbon from *A.niger*. Surface culture process can be used for gluconic acid production by *A.niger* from banana must. To get significance level of gluconic acid different parameter evaluated.

From the above study by evaluating the different parameter that is temperature, incubation period and carbon source we get to know that from out of different temperature ranges from 4^0c to 45^0c. 30^0c was found to be optimum for high gluconic acid production and it was 5.14grms/100ml but at 37^0c it was 3.43 grms/100ml. Our this result coincide with O.V. Singh and R.P. Singh (2002) in which 39.60g/L of gluconic acid was obtained.

At the different incubation period from 1-7 days of incubation and out of that 5 days of incubation period was found to be optimum. The production was found to be 5.23grms/100ml. our results coincide with A.A. Shindia, et.al.(2006) in which they found 57.30g/L 0f gluconic acid after 5 days of incubation.

Out of different carbon source used that is glucose , sucrose, lactose and starch, glucose and sucrose was found to be more efficient for high gluconic acid production which was 4.9 grms/100ml and 3.21 grmS/100ml resp. our results are similar with A.A. Shindia, et.al.(2006) in which glucose and fructose was found to be the best carbon source followed by sucrose for gluconic acid production and lactose and starch are unsuitable for gluconic acid production.

References:

1] **Om Vir Singh and R P Singh, (2002)** utilization of Agro-food by-products for gluconic acid production by aspergillus niger ORS-4 under surface culture cultivation. Journal of Scientific and Industrial Research. Vol-61 356-360

2]**Sumitra Ramachandran, Pierre Fontanille, Ashok Pandey and Christian Larroche; (2006)** Gluconic Acid: Properties, Applications and Microbial Production, Food Technol. Biotechnol. 44 (2) 185–195.

3] **Grasssin and Fouquenbergue, 1997** Fruit juices, *In Industrial Enzymology*,. Pp 227-260.

4] **A. A. Shindia, G. A. El-Sherbeny, A. E. El-Esawy and Y. M. M. M. Sheriff, (2006)** Production of Gluconic Acid by Some Local Fungi, Mycobiology 34(1): 22-29 .

6] **Moresi, M., Parente, E. and Mazzatura, A. (1991).** Effect of dissolved oxygen concentration on repeated production of gluconic acid by immobilized mycelia of *Aspergillus niger*. Appl. Microbiol. Biotechnol. 36: 320-323.

7] **Subba-Rao, D., Panda, T. and Roa, D. S. (1994).** Comparative analysisof different whole cell immobilized *Aspergillus niger* catalystsfor gluconic acid fermentation using pretreated can molasses. Bioproc. Eng. 11: 209-212.

8] **El-Naghy, M. A. and Megalla, S. E. (1975).** Gluconic acid production by *Penicillium pubenulum*. Folia Microbiol. 20: 504-508.

9] **Madhavi, C. H., Subramani, S. and Datta, M. D. (1999)** Fermentative production of gluconic acid using cheese whey. J. Food sci. Technol. 36: 361-364.

10] **Rosenberg, M., Svitel, J., Rosenbergova, I. and Sturdik, E. (1992).**Gluconic acid production by *Aspergillus niger* with oxygen supply by hydrogen peroxide. Bioprecess. Eng. 7: 309-301.

11] **Singh, O. V., Pereira, B. M. J. and Singh, R. P. (1999).** Isolation and characterization of a potent fungal strain *Aspergillus niger* ORS-4 for gluconic acid production. J. Sci. Industrial Res. 58:594-600.