*Int. J. Chem. Sci.* : 2 (3), 2004, 450–460

# OPTIMIZATION OF PROCESS CONDITIONS FOR PREPARATION OF GLUCOSE SYRUP FROM *SORGHUM BICOLOR L MONECH* STARCH USING IMMOBILIZED α–AMYLASE AND GLUCOAMYLASE ENZYMES

**V. V. SHINDE, A. S. JHALA, M. Y. SAYYAH[a], Y. S. JHALA[b], N. VIJAYVERGIVA[c], P. P. DIWANI and G. K. MATHUR**

Department of Dairy and Food Technology, College of Dairy and Food Science Technology, M.P. University of Agricultural and Technology, UDAIPUR–313 002 (Raj) INDIA

[a]Department of Food Biochemistry and Applied Human Nutrition, College of Food Technology, Marathwada Agricultural University, PARBHANI (M.S.) INDIA

[b]Department of Chemistry, B.N. (P.G.) College, UDAIPUR – 313 002 (Raj) INDIA.

[c]Department of Chemistry, M.L. Sukhadia University, UDAIPUR – 313 002 (Raj) INDIA

## ABSTRACT

Attempts were made to explore the production of starch from sorghum grains (Var. CSH–18) for utilization in the production of liquid glucose. Process parameters were standardized for dextrinization of starch by α–amylase. Maximum dextrinized product was formed at starch concentration of 30 per cent and enzyme concentration of 60 units at temperature of 95°C and pH 6.0 for 90 minutes reaction time. Saccharification of liquefied starch using immobilized glucoamylase resulted in maximum amount of glucose liberated at dry solid concentration of 30 per cent, enzyme concentration of 115 units, at pH 4.8 and temperature 60°C after 48 hours reaction time. Use of immobilized enzymes exhibited advantages over the conventional soluble forms such as efficient use of the enzyme, less contamination of the reaction mixture with the enzyme and impurities usually associated with the enzyme, and lower residence times in reactor and consequently, results in less by–product formation.

**Key words:** Glucose syrup, Immobilized α–amylase, Glucoamylase, Sorghum starch.

## INTRODUCTION

Sorghum (*Sorghum bicolor* L Monech) is one of the major cereal crops in India. Although low in food value, it is used as an important food grain for alternate use in poultry and cattle feed, livestock forage, production of starch, sugar, alcohol, glucose and high fructose syrups and polyols.

Hybrid sorghum can beneficially be utilized on industrial scale for production of different value added products like starch, malt, sweetners like glucose and high fructose syrups, and beverages. Processing can be economical as hybrid sorghum is available on large scale at low cost.

Studies have been carried out in the field of production of starch and starch–based sweetners like glucose, high fructose syrup using maize raw material by different workers. Maize and sorghum starch have similar characteristics and hence, sorghum starch can also be effectively converted into glucose syrup with good amount of D.E. Glucose syrup production from sorghum starch is beneficial as sugar, a major food ingredient can be replaced wholly, or in part. It is used in dietary foods, in confectionery, jams, jellies, canned foods, food drinks, and ice–creams. It is also used in bakery fermentation and infant foods, in manufacture of gelatin gum and for pharmaceutical purposes.

Starch is one of the most important naturally occurring carbohydrate compounds. Starch finds use for many purposes i.e. glucose syrup about 55.6 per cent, for food 10 per cent, in textile 8.0 per cent, paper industry 7.0 per cent and for other products 1.9 per cent.

When starch is hydrolysed by acid or enzyme, various dextrose, maltose and dextrins are formed. In acid conversion, the starch is attacked in a relatively random fashion. If the conversion is carried to completion, the bulk of the material is converted to dextrose. At the same time, an undesirable side reaction occurs. Some of the dextrose polymerizes, forming higher molecular weight material that are known as reversion products. When conversion reaches to 55–58 DE, they begin to impart a bitter taste to syrup.

Use of enzymes instead of acid hydrolysis has advantage due to the specific mode of action of the enzyme and much smaller amounts of undesirable byproduct formation. Amylolytic enzymes contain alpha and beta amylase. The enzymes are specific in their hydrolytic attack. Because of the specificity of the activity in attack, their hydrolytic product pattern is different than that of acid conversion, being much richer in maltose fraction. Glucosidases may be used to supplement acid or amylolytic conversions and give higher portions of dextrose in the product mix.

In the present investigation, an attempt has been made to optimize the conditions for production of starch and glucose syrup using the sorghum variety CSH 18.

## EXPERIMENTAL

Sorghum, (*Sorghum bicolor* L. Moench) grain of the genotype CHS–18 was procured from Sorghum Research Station, Mahathwada Agricultural University, Parbhani. The isolation and purification of starch from sorghum grains were achieved essentially by the method of Wankhede *et al*[1].

The bacterial $\alpha$–amylase, Termamyl–120 L (*Bacillus licheniformis*, E.C. 3.2.1.1, 1,4–glucan and glucanohydrolase) was obtained from Novo Nordisk Co., Denmark. The $\alpha$–amylase had an activity of 120 KNU/g. One Kilo Novo $\alpha$–amylase Unit (1KNU) is the amount of enzyme that breaks down 5.26 g starch per hour at Novo Nordisk's standard method for determination of α–amylase.

Glucoamylase, AMG E, (*Aspergillus* sp. E.C. 3.2.1.3., Exo–1, 4–α–glucosidase 1,4–α–D–glucan glucohydrolase) was procured from Novo Nordisk Co., Denmark, which had an activity of 5000 units/g solid.

## Standardization of dextrinization of Sorghum starch using α–amylase

In the present investigation, standardization of dextrinization of sorghum starch at different concentrations (i.e. 25, 30 and 35 per cent, w/v) as a function of α–amylolytic dextrinization, using various concentration of α–amylase (55, 60 and 65 units), various temperatures (i.e. 75, 85, 95, 105, and 110$^{o}$C) for different time intervals (i.e. 15, 30, 60, 90, 120 and 150 min) at various pH (i.e. 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0) was achieved.

## Standardization of saccharification of dextrinized starch using glucoamylase

The immobilization of glucoamylase on DEAE–cellulose was performed as per the procedure described[1]. The standardization of the production of glucose syrup from dextrin syrup of sorghum starch at various concentration (20, 30 and 40 per cent, w/v) as a function of gluco–amylase saccharification, using various concentrations of gluco–amylase (110, 115 and 120 units), various temperatures (i.e. 50, 60, 70, 80 and 90$^{o}$C) for different time intervals (i.e. 6, 12, 24, 36, 48 and 72 hrs) at various pH (i.e. 4.0, 4.4, 4.8, 5.0, 6.0 and 7.0) was achieved.

## Estimation of glucose and fructose

The glucose and fructose were estimated by paper chromatography, gas liquid chromatography, thin layer chromatography and carbon–celite chromatography.

For paper chromatography, butanol: acetic acid: distilled water (4: 1: 5, v/v) was used as solvent and saturated $AgNO_3$ in acetone solution and 0.1 M NaOH in methanol 3– 0.5 M sodium thiosulphate solution as spraying solution.

Gas liquid chromatography was carried out by method of Bjorndal *et al.*[2] with a Netel chromatograph equipped with a flame ionization detector, fitted with a glass column (0,32 × 150 cm) packed with 3 per cent of ECNSS–M on Gas Chrom–W (100–200 mesh), at al column temperature of 170$^{o}$ C.

Carbon–celite chromatography was performed according to the method of Whistler and BeMiller[3].

# RESULTS AND DISCUSSION

The starch content in the grain sample of CSH–18 was found to be 71.90 per cent. Starch was isolated from sorghum grains by wet–milling process using optimal conditions. The optimal conditions for obtaining maximum yield of starch from sorghum grain are: soaking time

20 hrs., pH 4.5 and soaking temperature 55°C. Unless otherwise mentioned, soaking was performed using cleaned grains and distilled water (1:2, w/v) and soaking in 750 ppm $K_2S_2O_5$ and 0.1 M $HgCl_2$.

### Standardization of dextrinization of starch by α–amylase

The α–amylase is endo–enzyme which hydrolyses α–1,4 linkages in the starch to form a less viscous solution of shorter oligosaccharides.

Dextrinization of sorghum starch was undertaken using α–amylase with addition 60 ppm $Ca^{+2}$. In the typical experiment, attempts have been made to standardize various parameters viz. starch concentration, α–amylase concentration, temperature, reaction time and pH for the dextrinization of starch by α–amylase. The hydrolysis of sorghum startch by α–amylase is done up to a DE of 15 to 25.

### Effect of starch concentration on the dextrinization of starch

The effect of starch concentration on the per cent hydrolysis and the yield of dextrinized product is presented in Table 1.

**Table 1. Effect of starch concentration on the dextrinization of starch by α–amylase**

| Starch (%) | Hydrolysis (%) | Dextrinized product (g) |
|---|---|---|
| 25 | 28.62 | 7.16 |
| **30** | **47.51** | **14.25** |
| 35 | 32.28 | 11.30 |

It is evident from results that the per cent hydrolysis and yield of the dextrinized product were optimum (47.51 per cent, 14.25 g) at 30 percent starch concentration. It is also clear from the results that, when the starch concentration is increased from 25 to 30 per cent, the per cent hydrolysis of starch was also increased. Further increase in the starch concentration is found to decrease the per cent hydrolysis and the concentration of dextrinized product. Hence, 30 per cent starch concentration was selected as optimum. These results are in close agreement with the results reported by Lindroos *et al.*[4], Novo[5], MacAllister[6] and Marc *et al.*[7].

### Effect of α–amylase concentration on the dextrinization of starch

The effect of various concentrations of α–amylase on the percent hydrolysis and the yield of dextrinized product is presented in Table 2. It is evident from the result that the per cent hydrolysis and yield of the dextrinized product was optimum (45.78 per cent, 13.73 g) at 60 units of enzyme. It is also clear from the results that when the enzyme concentration is increased from 55 to 60 units, the per cent hydrolysis and the concentration of dextrinized product was also increased. Further increase in the enzyme concentration is found to decrease the per cent hydrolysis and the concentration of dextrinized product. Hence, 60–unit enzyme concentration was selected as an optimum. These results are in close agreement with the results reported earlier[4-8].

**Table 2. Effect of α–amylase concentration on the dextrinization of starch**

| Enzyme concentration (Units) | Hydrolysis (%) | Dextrinized product (g) |
|---|---|---|
| 55 | 37.16 | 11.15 |
| 60 | 45.78 | 13.73 |
| 65 | 41.20 | 12.36 |

## Effect of temperature on the dextrinization of the starch

The results of effect of temperature on the hydrolysis of starch and the yield of dextrinized product indicate that the per cent hydrolysis was optimum (45.70 per cent) at 95° C (Fig.1). It can also be seen that as the temperature increases from 75 to 95° C, the per cent hydrolysis also increased. Further decrease in the percentage of hydrolysis was noted above 95° C. Hence, the temperature 95° C was selected as optimum. This result is in close proximity with the results reported by Lindroos *et al.*[4], Novo[5], Marc *et al.*[6] and Kulkarni *et al.*[9].

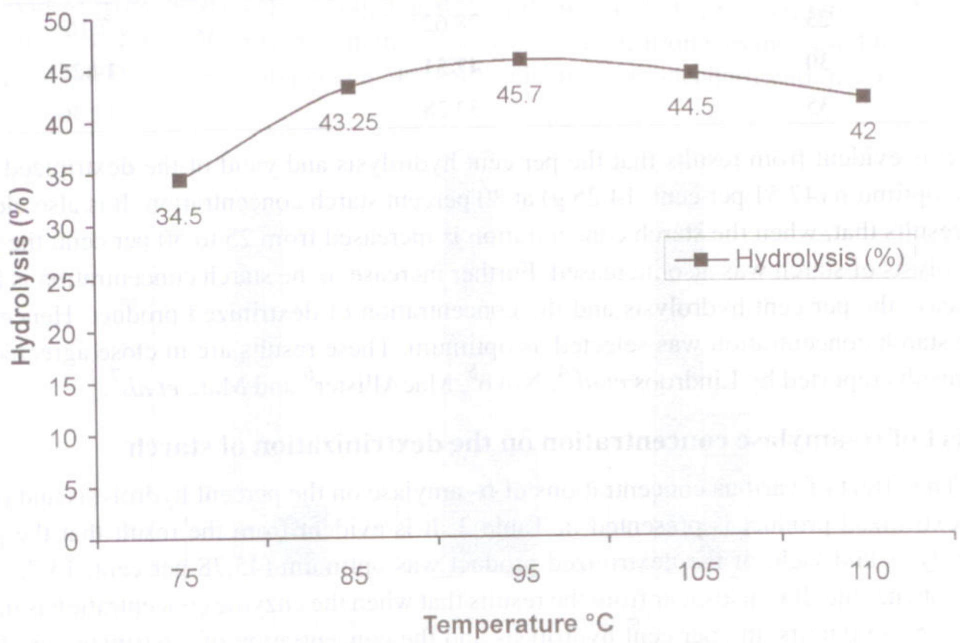


Fig. 1. Effect of temperature on dextrinization of starch by α–amylase.

## Effect of pH on the dextrinization of starch

The data pertaining to effect of pH on dextrinization of starch by α–amylase are given in Table 3. As the pH was increased from 4.5 to 6.0, the per cent hydrolysis was also found to increase (34.6 to 45.8). However, when the pH exceeds 6.5 per cent, then the hydrolysis was found to decrease. The maximum dextrinization was obtained at pH 6.0. These results are in close agreement with the results reported by MacAllister[6], Marc et al.[7] and Kshirsagar[8]; however, the result (pH 6.0) was comparatively lower as reported by Lindroos et al.[4] and Novo[5], i.e. pH (6.5–7).

**Table 3. Effect of pH on dextrinization of starch by α–amylase**

| pH | Hydrolysis (%) | Dextrinized product (g) |
|---|---|---|
| 4.5 | 34.60 | 10.38 |
| 5.0 | 37.47 | 11.24 |
| 5.5 | 43.53 | 13.06 |
| 6.0 | 45.80 | 13.74 |
| 6.5 | 44.29 | 13.29 |
| 7.0 | 44.08 | 13.22 |

## Effect of reaction time on the dextrinization of starch

The data on effect of reaction time on the yield of dextrinized product are presented in Fig. 2. It can be seen from the results that the maximum yield of dextrinized product (46.30 per cent)

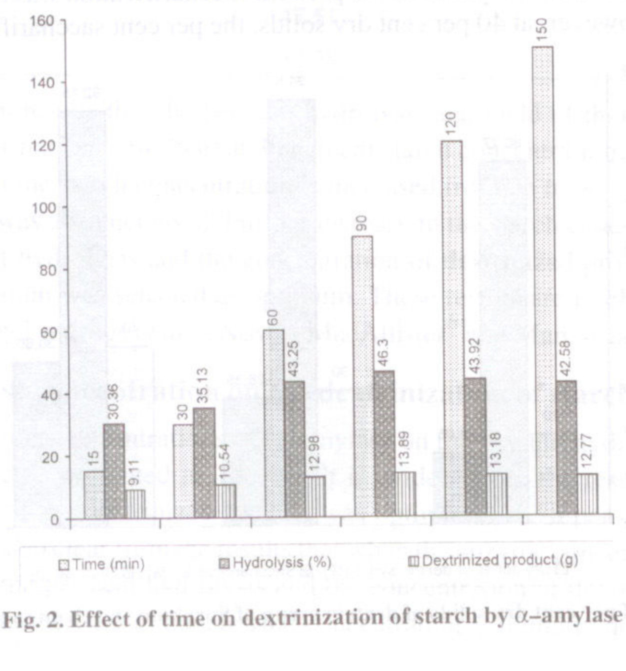


**Fig. 2. Effect of time on dextrinization of starch by α–amylase**

was obtained at 90 min reaction time. Beyond that, there was a decrease in the yield of dextrinized product. Hence, reaction time of 90 min was considered optimum, and kept constant throughout the study. These results are in close agreement with the reported by Novo[5], Marc et al.[7] and Kshirsagar[8]; however, these results are comparatively lower as reported by Kulkarni et al.[9], i.e. 2 hrs, but it is higher as observed by MacAllister[6] i.e. 10–20 min.

## Standardization of saccharification of the starch by immobilized glucoamylase

The sorghum starch treated with α–amylase may optionally be autoclaved for a short period of time to improve filtration after the subsequent treatment with glucoamylase.

The saccharification of above obtained dextrinized sorghum starch was undertaken using immobilized glucoamylase, an exo–enzyme which sequentially removes glucose units from the non–reducing ends of the maltodextrins. Glucoamylase is produced extracellularly by *Aspergillus niger*. It has high temperature optima. $Ca^{2+}$ was added (60 ppm) because of the $Ca^{2+}$ dependency of the enzyme. The final product of glucoamylase action is glucose syrup with DE of 97–98 percent. This consists of 95–97 percent glucose with 3–5 percent higher oligosaccharides. In the typical experiment, attempts have been made to standardize various parameters viz. per cent dry solids of dextrin syrup, immobilized glucoamylase concentration, temperature and reaction time.

## Effect of percent dry solids of dextrin syrup of Sorghum starch on saccharification

It is evident from the data (Fig. 3) that as the sorghum starch dextrin syrup (dry solids per cent) was increased from 20–30 per cent, the per cent saccharification was increased from 80.27 to 94.50 per cent. However, at 40 per cent dry solids, the per cent saccharification was found to

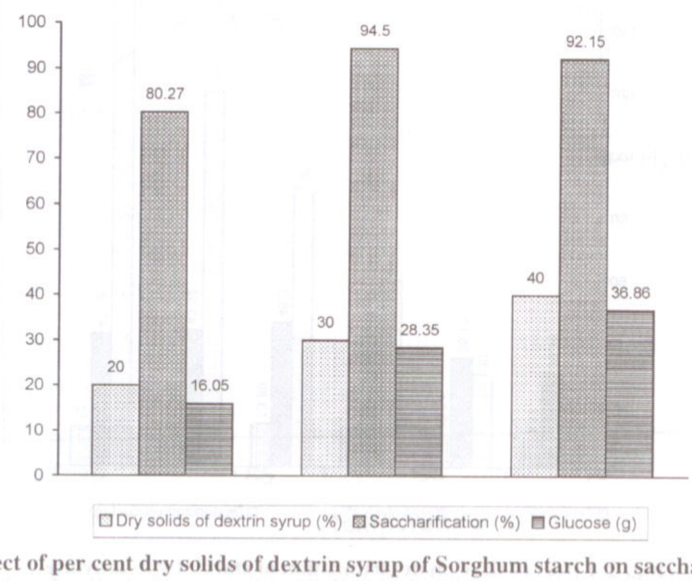


**Fig. 3. Effect of per cent dry solids of dextrin syrup of Sorghum starch on saccharification.**

decrease. Therefore, 30 per cent dry solids was found to be optimum. These results substantiate the data reported by Kshirsagar[8].

## Effect of glucoamylase concentration on the saccharification of starch

The effect of glucoamylase concentration on the per cent saccharification are presented in Table 4. It can be seen from the results that as the concentration of enzyme was increased from 110 units to 115 units, the per cent saccharification of dextinized starch was also increased (90.58 to 93.40). It is also observed that further increase in concentration showed an adverse effect on hydrolysis. Therefore, 115 units of enzyme were found to be optimum for saccharification starch.

**Table 4. Effect of glucoamylase concentration on the saccharification of the starch**

| Enzyme concentration (Units) | Saccharification (%)[a] | Glucose (g) |
|---|---|---|
| 110 | 90.58 | 27.17 |
| 115 | 93.40 | 28.02 |
| 120 | 92.45 | 27.74 |

[a] Unless otherwise stated, the presence of the only sugar detected was D–glucose in the hydrolysate.

## Effect of pH on the saccharification of starch

Effect of pH ranging from 4.0 to 7.0 on saccharification of dextinized starch by gluco–amylase was also investigated (Fig 4).

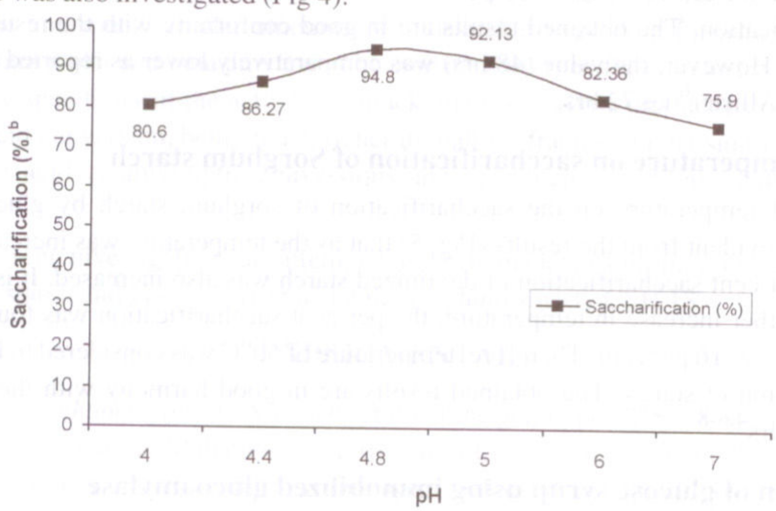


**Fig. 4. Effect of pH on saccharification of Sorghum starch**

It is evident from the data that as pH was increased from 4 to 4.8, there was increase in percentage of saccharification of dextinized starch, but on subsequent increase in pH, it adversely affects the per cent saccharification. Therefore, pH 4.8 was found to be optimum for saccharification of dextinized starch (94.80 percent). The obtained results are in good harmony with those reported by Lindroos et al.[4], but it was greater than the results given by others[5-7].

## Effect of reaction time on saccharification of Sorghum starch

**Table 5. Effect of time on saccharification of Sorghum starch**

| Time (hrs) | Saccharification (%)[b] | Glucose (g) |
|---|---|---|
| 6.0 | 52.12 | 15.64 |
| 12.0 | 65.61 | 19.68 |
| 24.0 | 80.25 | 24.07 |
| 36.0 | 89.68 | 26.90 |
| 48.0 | 93.60 | 28.08 |
| 72.0 | 93.05 | 28.09 |

[b] The dextrose equivalent (DE) for final product 97 per cent.

It is evident from the data (Table 5) that as the time was increased from 6 to 48 hrs., the per cent saccharification of dextrinized starch was also increased from 52.12 to 93.60 per cent. It is also observed that with further increase in time, there is a decrease in the per cent saccharification from 93.60 to 93.05 per cent. Therefore, 48 hrs time was considered optimum for saccharification. The obtained results are in good conformity with the results reported by Marc *et al.*[7]. However, the value (48 hrs) was comparatively lower as reported by Lindroos *et al.*[4] and MacAllister[6] i.e 72 hrs.

## Effect of temperature on saccharification of Sorghum starch

Effect of temperature on the saccharification of sorghum starch by glucoamylase was studied. It is evident from the results (Fig. 5) that as the temperature was increased from 40 to 60°C; the per cent saccharification of dextinized starch was also increased. It is also observed that with further increase in temperature, the per cent saccharification was found to decrease from 93.30 to 88.16 per cent. Therefore, temperature of 60°C was considered to be optimum for saccharification ot starch. The obtained results are in good harmony with those reported by earlier workers[4,6-8].

## Preparation of glucose syrup using immobilized glucoamylase

The results on the per cent saccharification using immobilized glucoamylase (*Aspergillus niger*) at different time intervals (up to 6 Days) are presented in Table 6. The data indicated that the per cent of saccharification was higher to the extent of 94.5 per cent. However, it has been observed that as time of saccharification increased from 2 to 6 days, there was a declining trend

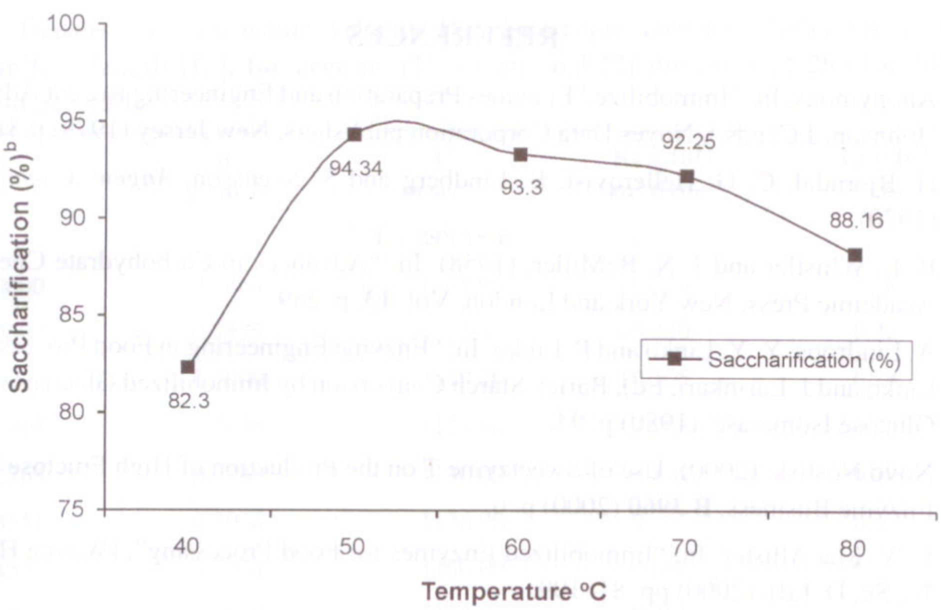


**Fig. 5. Effect of temperature on saccharification of sorghum starch**
**[b] The dextrose equivalent (DE) for final product 97 percent.**

in the saccharification (per cent) of dextrinized sorghum starch syrup. It may be attributed to the fact that this decline may be due to the lowering effect of the activity of glucoamylase. The declining trend in the saccharifying activity, at least to some extent, can be correlated to the formation of transglycosidation product especially isomaltose.

**Table 6. Effect of time interval on percent saccharification using immobilized glucoamylase at optimum experimental condition**

| S.No. | Period (Day) | Saccharification (%)[b] | Glucose (g) |
|---|---|---|---|
| 1. | 2 | 94.52 | 28.36 |
| 2. | 3 | 93.08 | 27.92 |
| 3. | 4 | 92.80 | 27.84 |
| 4. | 5 | 92.83 | 27.85 |
| 5. | 6 | 85.08 | 25.51 |

[b] Unless otherwise mentioned, the production of glucose syrup was undertaken with batch type laboratory scale using pharmacia double jacketed glass (quartz) column without peristelic pump equipped with other accessories.

## REFERENCES


1. Anonymous. In: "Immobilized Enzymes Preparation and Engineering Recent Advances", (Johnson, J.C. eds.), Noyes Data Corporation publishers, New Jersey (1979) p.310.
2. H. Bjorndal, C. G. Hellerqvist, B. Lindberg and S. Svensson, *Angew. Chem.*, **9**, 610 (1970).
3. R. L. Whistler and J. N. BeMiller, (1958). In: "Advances in Carbohydrate Chemistry", Academic Press, New York and London. Vol. **13**, p. 289.
4. A. Lindroos, Y. Y. Linko and P. Linko. In: "Enzyme Engineering in Food Processing", (P. Linko and J. Larinkari, Ed), Barley Starch Conversion by Immobilized Glucoamylase and Glucose Isomerase. (1980) p. 93.
5. Novo Nostisk. (2000). Use of Sweetzyme T on the Production of High Fructose Syrup in Enzyme Business. **B 3960** (2000) p. 6.
6. R. V. MacAllister. In: "Immobilized Enzymes for Food Processing", (Wayne H. Pitcher Jr., Sc. D. Ed). (2000) pp. 81–109.
7. J. E. C. Marc, van der Maarel, Bart van der Veen and C. M. Joost Uitdehaag. Properties and Applications of Starch Converting Enzymes of the Alpha amylase Family. *J. Biotechnol.*, **94**, 137 (2002).
8. S. S. Kshirsagar, Studies on Suitability of CSH 5 Sorghum Starch for Enzymatic Production of Glucose M. Tech. (Food Science) Thesis submitted to Marathwada Agricultural University, Parbhani (M.S.) India. (1986)
9. D. N. Kulkarni, U. M. Ingl and S. S. Thorat, Technology and Applications for Alternative Uses of Sorghum, Proceedings of the National Seminar, held at Marathwada Agricultural University, Parbhani (1987).
10. D. B. Wankhede, S. S. Rathi, B. B. Gunjal, H. B. Patil, S. G. Walade, A. B. Rodge and A. R. Sawte. Studies on Isolation and Characterization of Starch from Pearl Millet (*Pennisetum americanum* L. Leeke) grains *Carbohydrate polymers*, **13** : 17 – Anonymous (1990). Glucose Syrup Inhancers, *Food Industries*, **47 (3)**, 16 (1994).


*Accepted : 25.6.04*