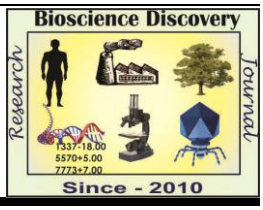
Bioscience Discovery, 9(2):227-231, April - 2018

Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Research Article



Optimization studies on the liquefaction of cassava and sweet potato starch using Liquezyme X

Regy Johnson

Central Tuber Crops Research Institute, Thiruvananthapuram
regyjohnson@gmail.com

Article Info

Received: 01-01-2018,
Revised: 06-03-2018,
Accepted: 19-03-2018

Keywords:
Starch hydrolysis, cassava starch, sweet potato starch, alpha amylase

Abstract

Liquefaction of cassava and sweet potato starch were studied using microbial alpha amylase (Liquezyme X). The optimized parameters for the liquefaction of cassava and sweet potato starch were found as 0.10% (v/v) Liquezyme X at pH 5.5, temperature 90°C and reaction time 1.0 h. Among the three starch concentrations, maximum starch conversion to reducing groups occurred in 20% (w/v) starch slurry. By increasing the concentration of enzyme from 0.10 %(v/v) to 0.40 %(v/v), only 4 -7% increase in starch hydrolysis was achieved in the three starch concentrations.

INTRODUCTION

Enzymatic degradation of starch on an industrial scale has been practiced for many years and has replaced to a considerable extent the traditional acid-catalysed processes. Starch is hydrolyzed to maltodextrins by alpha amylase and then saccharified to glucose by amyloglucosidase (Mac Allister, 1979; Graff and Shetty, 1999). Liquefaction is a process of dispersion of insoluble starch granules in aqueous solution followed by partial hydrolysis using thermostable α-amylases. The objective of the liquefaction process is to convert a concentrated suspension of starch granules into a solution of soluble dextrins of low viscosity for convenient handling and for easy conversion to glucose by glucoamylase. Although amylases can be derived from several sources such as plants, animals and microbes, the microbial amylases meet industrial demands; literature contains a large number of such amylases available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industries (Pandey et al., 2000a). Nevertheless, no optimization studies were conducted to economize the use of enzymes, substrates etc. A study was done to find out the optimum conditions for the first step in starch hydrolysis *viz.*, liquefaction of cassava and sweet potato starch using alpha amylase (Liquezyme X) and the results are discussed in this paper.

MATERIALS AND METHODS

Materials: Cassava (variety: M4) and Sweet potato (variety: Kanjangad) starch were used

Enzymes: Liquezyme X (thermostable α- amylase; EC 3.2.1.1) with an activity of 200 kilo novo units (KNU); as specified in the product catalogue, procured from M/s. Novo Industries, Denmark, was used for the liquefaction experiments.

Experimental Procedure

Optimization of parameters in the liquefaction of starch: The effect of pH, temperature, time of incubation, enzyme / substrate concentration on the liquefaction of cassava and sweet potato starch were studied. Effect of concentration of Liquezyme X at liquefaction stage on subsequent saccharification was also studied.

Effect of pH on liquefaction: A 20% (w/v) starch (cassava or sweet potato) slurry was prepared and the pH was adjusted to 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5. The other parameters like Liquezyme concentration, temperature and incubation time were kept constant for all the samples. Liquezyme X concentration of 0.10% (v/v) was added to the slurry, mixed well and incubated at 90°C for 1 h. The reducing groups formed were estimated in an aliquot by the method of Nelson and Somogyi (Nelson, 1944 and Somogyi, 1952). Effect of temperature on liquefaction: A 20% (w/v) slurry was prepared and pH was adjusted to 6.5. Liquezyme X concentration of 0.10% (v/v) was added to each slurry and incubated at different temperatures *viz*., ranging from 70°C to 110°C for 1 h. The reducing sugars formed were immediately estimated by the above method.

Effect of substrate / enzyme concentration: Three concentrations of starch slurry were prepared *viz*., 20 (%w/v), 25 (%w/v) and 30 (%w/v). Five different concentrations of Liquezyme X *viz*., 0.025% (v/v), 0.05% (v/v), 0.10% (v/v), 0.20% (v/v) and 0.40% (v/v) were added to separate flasks of each starch concentration and incubated at 90°C for 1 h. The percentage conversion to reducing groups was calculated on dry starch basis. Effect of time of incubation on liquefaction: Five different concentrations of Liquezyme X *viz*., 0.025% (v/v), 0.05% (v/v), 0.10% (v/v), 0.20% (v/v) and 0.40% (v/v) were added to separate flasks of 20% starch slurry and incubated at 90°C for 2 h. Samples were drawn at 30, 60, 90 and 120 min. and the reducing groups were estimated.

Viscosity profile analysis during liquefaction of cassava and sweet potato starch: The pattern of change in the viscosity of cassava and sweet potato starch during liquefaction by Liquezyme X was monitored in Rapid Visco Analyzer (M/s. New Port, Australia). A starch slurry was prepared in 25.0 ml distilled water (1:10 w/v) in special canisters. To this, different concentrations of Liquezyme X ranging from 1.2 mg to 60 mg were added and the viscosity changes monitored. Three replications were maintained in each case. A control set without enzyme was also run to compare with the test samples.

Statistical analysis: Four replicates were maintained for liquefaction studies and duplicate analysis was performed on each replicate. The data were analysed statistically using the software package GenStat (GenStat 7th, DE3 2007) to perform the Analysis of Variance (ANOVA). The treatments are considered significantly different at 5% level of significance ($p<0.05$). Statistical analysis was done using one way Analysis of Variance (ANOVA) for comparison of mean values among different treatments. All pair wise comparison of mean values of different treatments was analysed with Duncan's multiple range test (direction=ascending; PROB=0.05).

RESULTS AND DISCUSSION

When the liquefaction of starch was carried out at different pH (ranging from 4.5 to 7.5) pH optima for Liquezyme X was found in the range of pH 6.5-7.0 (Table 1). Highest concentration of reducing groups and maximum percentage conversion were observed at pH 6.5, with only slight reduction at pH 7.0. Antrim et al. (1991) found that saccharification of starch which had been liquefied at various pHs demonstrated that at pH 5.9 or lower no maltulose was formed. Furthermore, the high pH of liquefaction and isomerisation causes by-product formation, sugar breakdown, color formation and an overall decrease in product yield. It was observed that by increasing the pH from 5.5 to 6.5, only about 2% increase in starch hydrolysis was achieved. The effect of temperature on liquefaction by Liquezyme X has been shown in Table 2. The optimum temperature was found to be 90°C, where 22% hydrolysis occurred.

The pH and temperature optima of the alpha amylases depend on the origin of the enzyme and these properties of enzymes are of value for industrial processes. Crabb and Mitchinson (1997) found that amylase from *Bacillus licheniformis* has a pH and temperature optima of 6.0 and 95°C – 105°C, respectively. In another study, alpha amylase from *Bacillus licheniformis* has been found to have a temperature and pH optima of 95°C and 5.5-7.0, respectively. *Bacillus stearothermophilus* was found to function at a temperature (Taylor and Driber, 1994) of 105°C and pH 5.9. It was also found that the process could not be performed below 5.9 as the activity of alpha amylase decreases at lower pHs. In presence of 2% soluble starch amylase from *Bacillus subtilis* functioned best at pH 6.0 and 40˚C. However, in presence of 10% – 50% of soluble starch pH 6.5-7.5 and temperature above 70°C was found as optimal. Labout (1985) also reported that the α-amylase isolated from *Aspergillus niger* for the liquefaction of corn starch had a pH optimum of 3.5-5.0 and a temperature optimum of 65-70°C, indicating that wide differences exist among the sources from which α-

Table 1: Effect of pH on liquefaction of cassava and sweet potato starch

pH	Reducing groups formed (g/100 ml slurry)		Percent conversion to reducing groups	
	Cassava	Sweet potato	Cassava	Sweet potato
4.5	3.87±0.02[a]	3.73±0.02[a]	19.54±0.13[a]	19.45±0.09[a]
5.0	4.15±0.02[b]	3.99±0.01[b]	20.96±0.09[b]	20.84±0.08[b]
5.5	4.23±0.02[c]	4.03±0.01[c]	21.40±0.08[c]	21.03±0.03[c]
6.0	4.37±0.02[e]	4.13±0.01[d]	22.08±0.09[e]	21.57±0.08[d]
6.5	4.62±0.02[f]	4.42±0.01[g]	23.36±0.12[f]	23.08±0.06[g]
7.0	4.60±0.02[f]	4.39±0.01[f]	23.26±0.09[f]	22.91±0.07[f]
7.5	4.27±0.01[d]	4.15±0.01[e]	21.60±0.08[d]	21.67±0.08[e]
LSD(5%)	0.0280	0.0194	0.1433	0.1025

$p < 0.001$; Mean ± SD from four replicates; Means with the same superscripts in a column are not significantly different

Table 2: Effect of temperature on liquefaction of cassava and sweet potato starch

Temperature (˚C)	Reducing groups formed (g/100 ml slurry)		Percent conversion to reducing groups	
	Cassava	Sweet potato	Cassava	Sweet potato
70	1.54±0.02[a]	1.39±0.03[a]	7.78±0.09[a]	7.27±0.16[a]
80	4.15±0.02[c]	3.99±0.01[c]	20.95±0.10[c]	20.85±0.03[c]
90	4.43±0.02[e]	4.22±0.01[e]	22.41±0.11[e]	22.08±0.08[e]
100	4.25±0.02[d]	4.07±0.01[d]	21.47±0.10[d]	21.25±0.04[d]
110	4.11±0.03[b]	3.92±0.01[b]	20.77±0.14[b]	20.46±0.05[b]
LSD (5%)	0.0325	0.0236	0.1665	0.1276

$p < 0.001$; Mean ± SD from four replicates; Means with the same superscripts in a column are not significantly different

amylase is isolated. Among the three starch concentrations, maximum starch conversion to reducing groups occurred in 20% (w/v) starch slurry. By increasing the concentration of enzyme from 0.10 %(v/v) to 0.40 %(v/v), only 4 -7% increase in starch hydrolysis was achieved in the three starch concentrations (Fig. 1 & 2). Considering the cost of the enzyme, it was thus concluded that a lower enzyme concentration of 0.10 %(v/v) was sufficient to bring about appreciable liquefaction. An incubation time of 60 min. was optimum for the hydrolysis and further increase in the contact time did not significantly improve the percent conversion as depicted in Fig. 3 & 4. Ghildyal et al. (1989) reported that pulverized cassava flour without extracting starch can be used for HFS production, thus reducing the cost of production. These workers have used a starch concentration of 35% with 0.03 ml Termamyl and a liquefaction time of 2 h and compared with 30% slurry of cassava flour. When compared to acids, liquefaction of starch using α-amylase has the advantage that machinery corrosion can be avoided as also the light colour of the liquefied slurry avoids excessive use of decolorizing agents (Pancoast and Junk, 1980). The hydrolysis of starch by acid gives, besides D-glucose, other substances like oligosaccharides, furfural, hydroxymethyl-furfural, levulinic acid, formic acid and undesired color substance. Berghofer and Sarhaddar (1988) reported that cassava starch differs only insignificantly from starch of other origin like corn or potatoes.

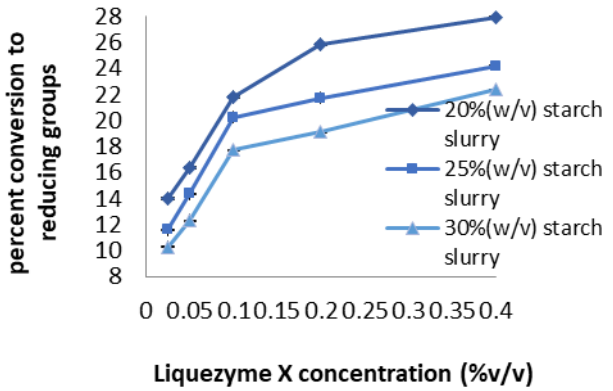


Figure 1: Percentage of hydrolysis of cassava starch as a function of Liquezyme X concentration at different substrate concentrations of cassava starch

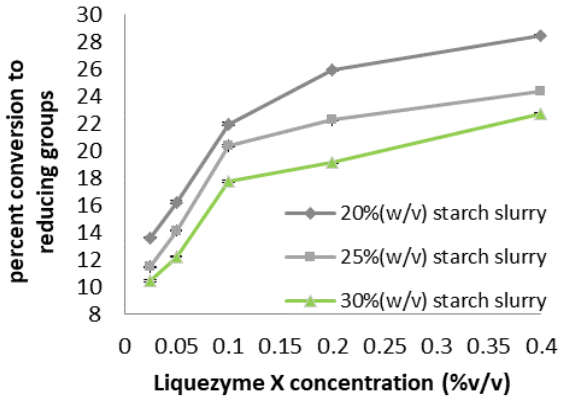


Figure 2: Percentage of hydrolysis of cassava starch as a function of Liquezyme X concentration at different substrate concentrations of sweet potato starch

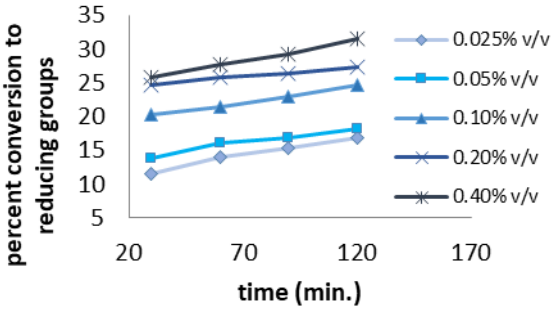


Figure 3: Percentage of hydrolysis of cassava starch as a function of the incubation time at various concentrations of Liquezyme X

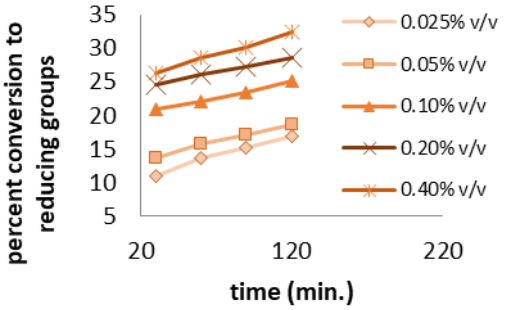


Figure 4: Percentage of hydrolysis of sweet potato starch as a function of the incubation time at various concentrations of Liquezyme X

These workers also observed that temperature of 85-95°C and average residence time of 15-30 min. was sufficient to cause complete liquefaction of cassava root slurry by α-amylase. On the contrary, Labout (1985) reported that α-amylase had a pH optimum of 3.5-5.0 and temperature optimum of 65-70°C for the hydrolysis of corn starch. Miller and Benjamin (2002) reported maximum liquefaction of sweet potato starch by α-amylase at 90°C for 5 h. The results presented in Figure 5 indicate that even with the lowest enzyme concentration of 1.2 mg (in the 25 ml assay system), there is substantial reduction in the viscosity of cassava starch. The viscosity reduction was appreciable from 7.5 mg enzyme in 25 ml assay system (equivalent to 30 mg (0.025% v/v) enzyme for the 20% slurry concentration), reaching very low levels at 60 mg enzyme in 25 ml assay system (equivalent to 240 mg (0.20% v/v) enzyme in the 20% slurry). Pasting temperature readings could be obtained in the RVA only up to an enzyme concentration of 7.5 mg for M4 starch, which indicated rapid hydrolysis of starch with the 15 mg enzyme level onwards.

Similarly the pattern of change in viscosity of sweet potato starch during liquefaction was studied by adding different concentrations of Liquezyme X ranging from 1.2 mg to 60 mg in a 25 ml starch slurry (1:10 w/v). The results (Figure 6) indicated that even with the lowest concentration of Liquezyme *viz*., 1.2 mg, there was a substantial reduction in the viscosity of starch. Viscosity was reduced to negligible levels when 30 mg – 60 mg Liquezyme X was added. Pasting temperature readings could not be recorded for higher concentrations of Liquezyme X, indicating that thinning down of the slurry was achieved before this stage itself.

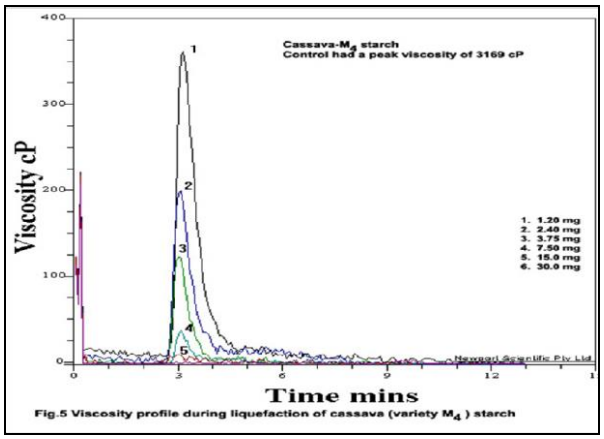


Figure 5: Viscosity reduction of cassava starch by adding α - amylase as measured using Rapid Visco Analyser

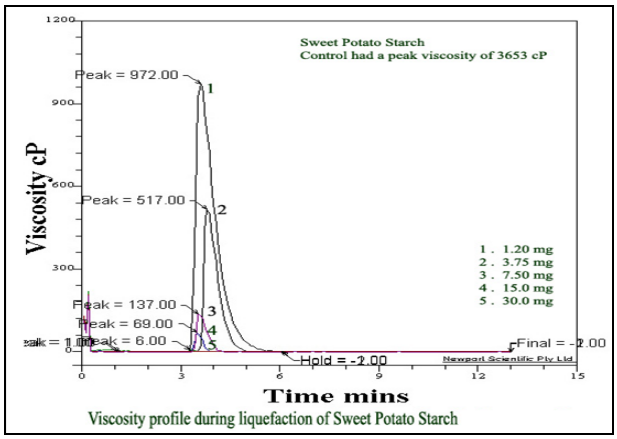


Figure 6: Viscosity reduction of sweet potato starch by adding α - amylase as measured using Rapid Visco Analyser

CONCLUSION

In the enzyme-enzyme process of starch hydrolysis, starch is liquefied and partially hydrolyzed in an aqueous suspension containing 20 to 30 percent starch and a liquefying enzyme, such as bacterial alpha-amylase enzyme at a temperature of about 90˚C to about 105˚C. The optimized parameters for the liquefaction of cassava and sweet potato starch were found as 0.10% (v/v) Liquezyme X at pH 5.5, temperature 90°C and reaction time 1.0 h.

Acknowledgement

The author is grateful to the CSIR for providing necessary financial support for this research work.

REFERENCES

Antrim RL, Solheim BA, Solheim L, Auterinen AL, Cunefare J and Karppelin S, 1991. A new *Bacillus licheniformis* alpha-amylase capable of low pH liquefaction. *Starch/ Stärke,* **43**(9): 355-360.

Berghofer E and Sarhaddar S, 1988. Production of glucose and high fructose syrup by enzymatic direct hydrolysis of cassava roots, *Process Biochem,* **23** (6): 188 – 194.

Crabb WD and Mitchinson C, 1997. Enzymes Involved in the Processing of Starch to Sugars. *Tibtech,* 15: 349. GenStat 7th, DE 3, Service Pack 1, Version 7.2.0.220, Lawes Agricultural Trust, VSN International Ltd., UK., 2007.

Ghildyal NP, Ramakrishna M and Lonsane BK, 1989. Comparative economics of the production of high fructose syrup from cassava chips and cassava starch. *Starch/ Starke*, **41** (2): 64-68.

Graff WD and Shetty SK, 1999. Commodity Scale Production of Sugars from Starches. *Current Opinion in Microbiology*, **2**: 252.

Labout JM, 1985. Conversion of liquefied starch into glucose using a novel glucoamylase system, *Starch/ Starke*, **37** (5): 157-161.

Mac Allister RV, 1979. Nutritive sweeteners made from starch. In *Advances in Carbohydrate Chemistry and Biochemistry*; Tipson, S., Horton, D., (Eds.), Academic Press: New York, 36: 15-56.

Miller SA and Benjamin ACB, 2002. Processing and evaluation of a sweet potato based sweetener / syrup, *Paper presented at the Annual Meeting and Food-Expo*, Anaheim, California,.

Nelson N, 1944. A photometric adaptation of the Somogyi method for determination of glucose, *J. Biol. Chem*: 153375-380.

Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D and Mohan R, 2000a. Advances in microbial amylases, *Biotechnol. Appl. Biochem*. **31**: 135-152.

Pancoast HM and Junk WR, 1980. *Handbook of Sugars*, AVI Publishing Company, Westport, Connecticut, **157**-169.

Somogyi M, 1952. Notes on sugar determination. *J. Biol. Chem*., **195**:19-23.

Taylor JR N and Driber KH, 1994. Malting and Brewing of Sorghum. *Journal of Institution of Brewing*, 68.

How to cite this article

Regy Johnson, 2018. Optimization studies on the liquefaction of cassava and sweet potato starch using Liquezyme X. *Bioscience Discovery*, **9**(2): 227-231.