

OPTIMIZATION OF FRUCTOOLIGOSACCHARIDES PRODUCTION FROM PLANTATION WHITE SUGAR

Aris Toharisman, Hendro Santoso and Triantarti

Indonesia Sugar Research Institute
atoharis@yahoo.com

ABSTRACT

Fructooligosaccharides (FOS) produced from sucrose having a number of desirable characteristics such as low calories, non cariogenic effect, safety for diabetics, and bifidus-stimulating functionality. The aim of our research was to produce FOS from plantation white sugar (PWS) - table sugar with ICUMSA 200-300 - by using fructosyltransferase (FT-ase). The enzyme was produced by *Aspergillus* sp isolated from Wonolongan Sugar Mill area, Indonesia. The enzyme was isolated from medium using ethanol 60%(v/v). Enzyme solution was mixed with PWS and incubated at various times, pHs, and temperatures. Results showed that FOS concentration reached a maximum value of 26% after 6 h incubation when 40 g/100 ml of PWS was used as a substrate. The optimum pH and temperature of FOS production were 6.5 – 7.0 and 50°C, respectively.

Key words: fructooligosaccharides, plantation white sugar, *Aspergillus* sp

Introduction

Fructooligosaccharides (FOS) are considered as a healthy food because of their special properties such as lower serum lipid, increase intestinal calcium absorption, non cariogenic effect, indigestible ingredients (calorie-free and safe for diabetics) and having bifidus-stimulating functionality. FOS is a prospective product to be developed in Indonesia, because so far Indonesia was still importing FOS from other countries.

FOS are composed mainly of 1-kestose (GF₂), nystose (GF₃) and fructofuranosyl nystose (GF₄). Industrial scale for FOS production is done mainly using fungal enzymes from either *Aureobasidium* sp. (Yun *et al.*, 1992; 1990), *Aspergillus niger* (Hidaka *et al.*, 1988) or other fungus (Huang *et al.*, 2001).

The objectives of this research project was to produce FOS from sucrose using fructosyltransferase (FT-ase) which produced by *Aspergillus* sp isolated from Wonolongan Sugar Mill area in Indonesia.

Materials and Methods

Cultivation conditions

A fungus (*Aspergillus* sp.) in our microorganism collections was isolated from Wonolongan Sugar Mill Area, Indonesia. This fungus has been identified for its ability to produce FOS. It was inoculated into 100 ml preculture medium in 200 ml flask. Composition of preculture medium was sucrose (200 g/l), yeast extract (12 g/l), CMC (2 g/l), Magnesium sulphate (2 g/l) and pH 6.5. The flask was shaken at 200 rpm and grown for 18 h at 30°C. The preculture was subsequently inoculated into 900 ml fermentation medium in 2 l flask. Composition of fermentation medium was sucrose (200 g/L), yeast extract (12 g/l), dipotassium hydrogen phosphate (4g/l), potassium

dihydrogen phosphate (9 g/l). The flask was shaken at 200 rpm and grown for 24 h at 30⁰C. At the end of fermentation, the broth was separated from the fungal cells by filtration. Fermentation broth was used as a source of crude FT-ase enzyme.

Enzyme assay

To determine the enzyme activity, broth fermentation was added to sucrose solution to final substrate concentration 40% (w/v) and incubated at pH 5,5 and temperature 50⁰C. At the end of the reaction, the enzyme was inactivated in boiled water for 10 min. One unit of FT-ase was defined as the amount of enzyme responsible for producing 1 μ m FOS per min under above reaction conditions.

Method for FOS analysis

FOS concentration was determined using HPLC. HPLC analysis was performed with Waters carbohydrate column at room temperature. Injected samples were eluted using acetonitril 65% at 1 ml/min and detected with refractive index detector.

Effect of pH and temperature on the conversion of sucrose to FOS

The effect of temperature on the FOS production was studied by incubating the mixture of 100 ml crude enzyme (having FT-ase activity 750 U/ml) and 200 ml sucrose solution 60% (w/v) at various pH between 3.5 – 7.5 and temperature 50⁰C. PH was maintained using phosphate-citrate buffer 0.1M. The mixture was incubated for 2 hours. The final concentration of sucrose in the enzymatic reaction was 40% (w/v). Then pH 6.5 was chosen for further study to determine the effect of temperature on the FOS production which various from 40 to 70⁰C. FOS production was calculated as the amount of FOS produced per total carbohydrate in the enzymatic reaction.

Isolation of FT-ase and conversion of sucrose to FOS

Simple technique was used to separate FT-ase from broth medium. FT-ase was isolated from broth using various concentration of ethanol from 25 to 70% (v/v). The mixture of broth and ethanol during isolation was stored at 4⁰C for 5 min and enzyme was separated from supernatant using centrifuge. The yield of FT-ase activity was determined for each ethanol concentration. The optimum condition from FT-ase isolation was used to produce semi pure FT-ase. The same unit of crude FT-ase and semi pure FT-ase (230 U/ml of reaction mixture) were used for converting plantation white sugar (PWS) to FOS. The reaction was conducted at pH 6.5 and temperature 50⁰C for various incubation times (2, 4 and 6 hours). The FOS production was calculated using the same method as mentioned in the previous experiment.

Results and Discussion

This research project was aimed to determine the optimum reaction conditions for producing FOS using FT-ase produced by *Aspergillus sp.* isolated from Wonolangan SF area. Extracellular FT-ase activity was mainly produced by this isolate (Santoso *et al.*, 2007). Effect of pH and temperature on the relative FOS production was shown in Figure 1 and Figure 2, respectively. As shown in Figure 1, maximum FOS production was found at pH 7.0 but the FOS production was rapidly dropped at pH 7.4. Hence, the optimum FOS production could be considered on the range of 6.5 - 7.0. The similar result was reported by Ghazi *et al.* (2006) and Huang *et al.* (2001). Figure 2 showed that the maximum FOS production at pH 6.5 was found at temperature 50⁰C. FOS production was very similar after 2 h incubation at temperature 50 and 60⁰C. FOS

production at 40⁰C was considered 81.4% of FOS production at 50⁰C and FOS production at 60⁰C was 99.6 % of FOS production at 50⁰C. The FOS production rapidly dropped at 70⁰C. This result was supported by previous researchers, temperature around 50 - 55⁰C was used for conversion of sucrose to FOS using FT-ase (Hocine *et al.* (2000); Huang *et al.* (2001); Kim *et al.* (2000) ; Madlova *et al.* (1999) and Yun *et al.* (1997).

FT-ase in the broth medium was isolated using ethanol to separate the enzyme from other compounds in the fermentation medium. Results on Figure 3 showed that the optimum condition for FT-ase isolation was conducted using 60% (v/v) ethanol and the activity recovered by this treatment was 92%. Recovery of FT-ase was starting to increase very high when using 50% (v/v) ethanol and decreased significantly when 70% (v/v) ethanol was used.

The optimum pH and temperature were used for further conversion with longer incubation time. Figure 4 showed the FOS production using crude FT-ase compared to semi pure FT-ase (FT-ase resulted from isolation using ethanol) after 2, 4 and 6 h incubation. The results showed that FOS production continuously increased from 2 to 6 h incubation time both for crude FT-ase and semi pure FT-ase. However, FOS production of semi pure FT-ase was higher compared to crude enzyme after 2, 4 and 6 h incubation. These results indicated that the FOS production activity of semi pure FT-ase was higher compared to crude FT-ase in the reaction mixture. The crude FT-ase still contained residual compounds from broth after fermentation which might have inhibitors effect to the enzyme. Further research is needed to determine FOS production resulted from longer incubation time (> 6 h). This research project is still continued to get a higher FT-ase production. Fermentor will be needed to control fermentation conditions.

Conclusions

The optimum condition for conversion from sucrose (table sugar) to FOS using FT-ase produced by *Aspergillus sp.* (isolated from Wonolangan Sugar Mill area in Indonesia) was pH 6.5 – 7.0, temperature 50⁰C and incubated for 6 h in the reaction containing sucrose 40% (w/w). FOS production was better when using semi pure FT-ase (FT-ase resulted from isolation using ethanol 60% v/v) compared to crude FT-ase. In this experiment, the maximum FOS production was 26% (w/w) calculated as FOS / total carbohydrate.

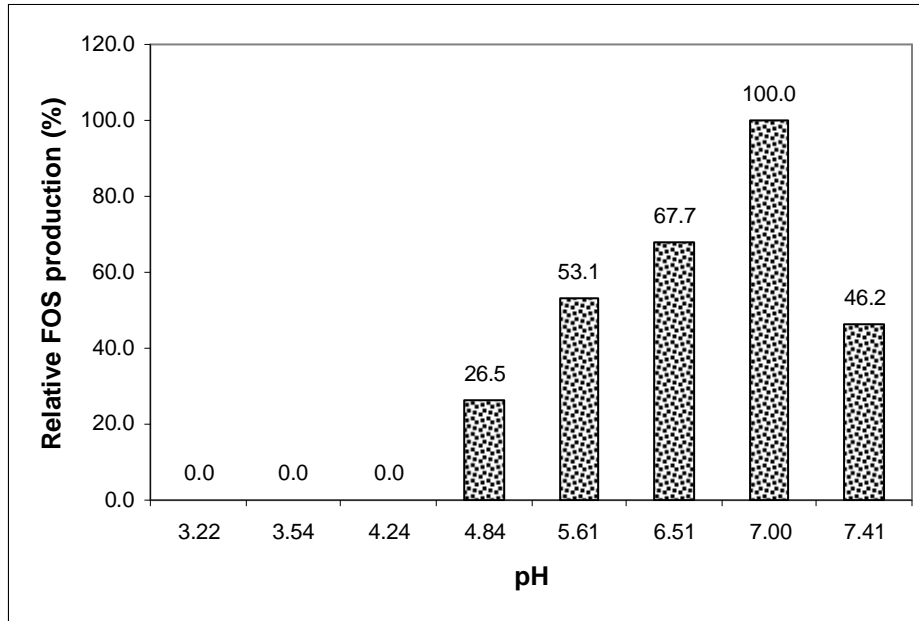


Figure 1. Relative FOS production (%) in the enzymatic reaction containing 250 U FT-ase/ml and sucrose 40% (w/v) after incubation at 50⁰C for 2 hours in various pH

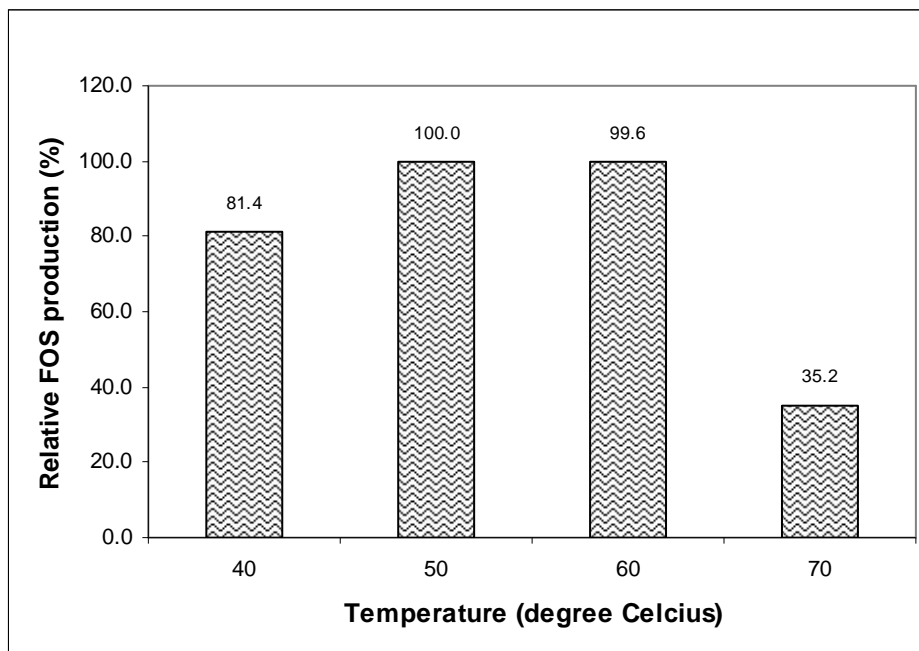


Figure 2. Relative FOS production (%) in the enzymatic reaction containing 250 U FT-ase/ml, sucrose 40% (w/v) and pH adjusted to 6.5 after incubation for 2 hours at various temperature

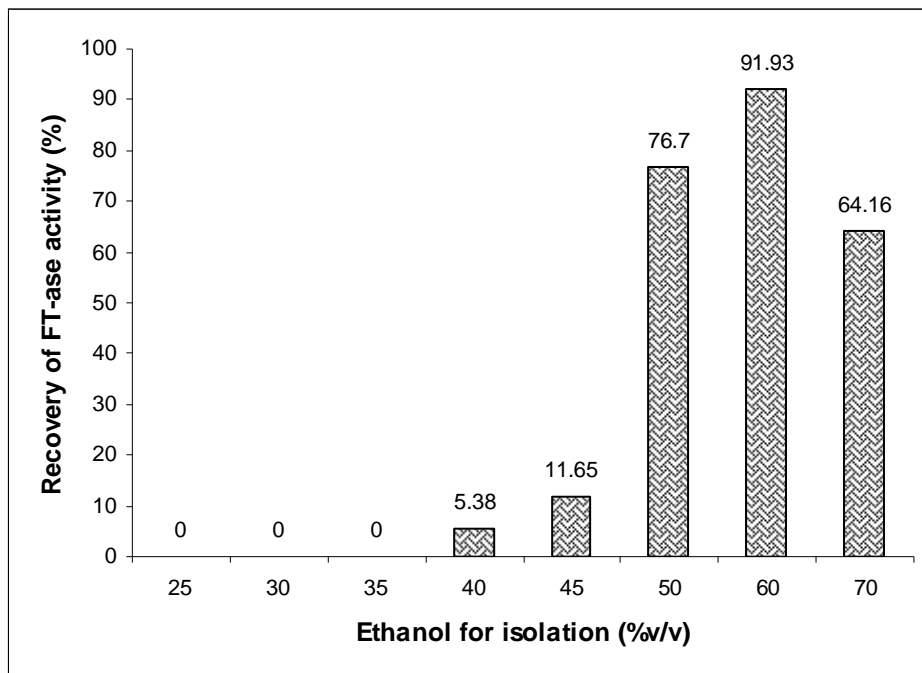


Figure 3. Recovery of FT-ase activity (%) after isolation using various concentration of ethanol

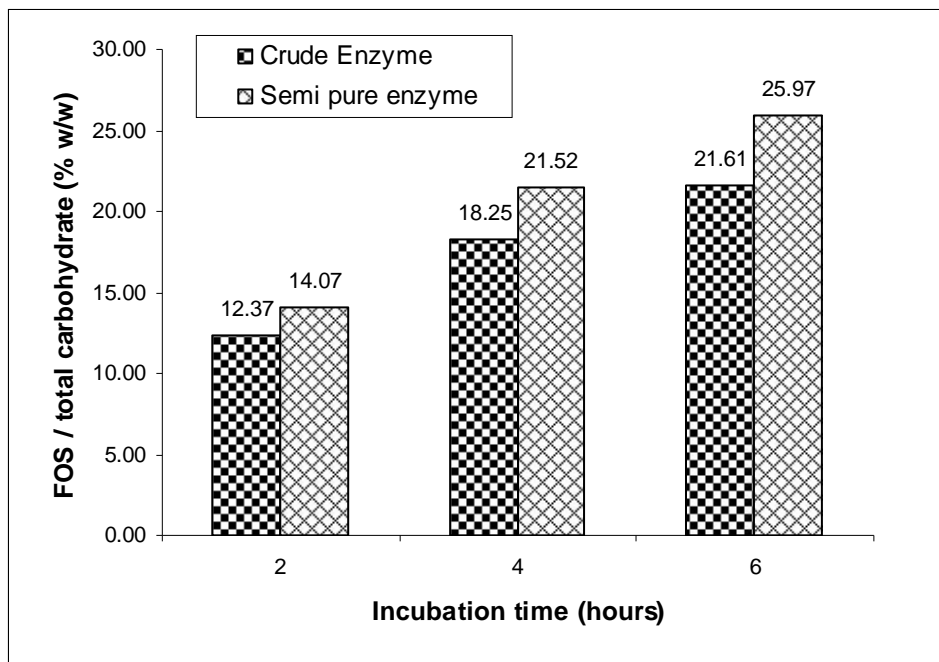


Figure 4. FOS production calculated as FOS / total carbohydrate (% w/w) using crude FT-ase and semi pure FT-ase (FT-ase resulted from isolation using ethanol 60% v/v) after incubation at 50⁰C in various time. The mixture of enzymatic reaction containing FT-ase 233 U/ml, sucrose 40% (w/v) and pH was adjusted to 6.5

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