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PRODUCTION OF HIGH FRUCTO-OLIGOSACCHARIDE SYRUP WITH TWO ENZYME SYSTEM OF FRUCTOSYLTRANSFERASE AND GLUCOSE OXIDASE

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SUMMARY

A novel two enzyme system of fructosyltransferase and glucose oxidase to enhance the content of the net fructo-oligosaccharide (FOS) fractions in the industrial production of FOS syrup from sucrose was devised. The net FOS content in the commercial FOS syrup has been limited only to 55-60 % due to the accumulation of glucose which acts as a feedback inhibitor of the fructosyltransferase. By supplementing glucose oxidase to the conventional FOS reaction system, we could convert the glucose to gluconic acid readily separable from neutral sugars by simple ion exchange operation in the next step. The simultaneous removal of glucose was proved effective in proceeding the reaction by fructosyltransferase further by relieving the product inhibition caused by glucose. By this way, we could raise the net FOS content as high as 90 %.

INTRODUCTION

The fructo-oligosaccharides (FOS), in which 1-4 fructose units are bound at the β-(2→1) position of sucrose, have recently attracted attentions of many investigators (Gupta and Bhatia, 1982; Smith *et al.*, 1982; Adachi *et al.*, 1983, Jung *et al.*, 1987) for their special properties such as non-digestibility, non-cariogenicity, and especially the intestinal flora modulating effect (Oku *et al.*, 1984; Hidaka *et al.*, 1986). However, these merits of FOS are curtailed by the presence of glucose, fructose, and sucrose which normally account for 40-45 % of total sugars in the commercial FOS syrup. Glucose, which accumulates as a major byproduct, acts as the feedback inhibitor of fructosyltransferase (FT) (Jung *et al.*, 1989), thus stopping the enzyme reaction leaving about 10 % of sucrose unconverted.

As a first attempt to increase the net FOS content, we have applied the chromatographic principle, proved effective for the separation of glucose from

fructose in the corn syrup industry (Hirota *et al.*, 1981), to isolate FOS from mono- and di-saccharides. Although this technique is another field yet to be developed, our tentative conclusion at this stage is that development of new chromatographic matrix effectively separating FOS from other sugars must be premised to make this method commercially viable.

Another promising way of producing FOS syrup with a high net FOS content is to convert mono- and di-saccharides selectively into another form which can be separated readily from FOS. This concept has frequently been applied in food industry for the removal of glucose from several foodstuffs such as egg white, low glucose corn syrup, and high fructose invert sugar using glucose oxidase (GO, EC 1.1.3.4.) (Reed, 1975). GO converts glucose to gluconic acid which can be readily separated from neutral sugars by simple ion exchange operation.

In this investigation, we have examined if this concept can be applied to the production of high FOS syrup containing a much higher ratio of net FOS compared with the conventional FOS syrup.

MATERIALS AND METHODS

Enzyme preparation

FT was prepared from *Aureobasidium pullulans* by the methods as previously described (Jung *et al.*, 1987) and GO (from *Aspergillus niger*, 25000 units/g stated activity) was purchased from Sigma Chemical Co. (USA).

Enzyme Assay

The activity of FT was determined by measuring the released glucose as previously described (Jung *et al.*, 1989). One unit is defined as the amount of enzyme activity required to produce one μmole of glucose per minute at 55 ℃ and pH 5.5. One unit of GO is defined as the amount of enzyme activity required to oxidize one μmole of glucose to gluconic acid per minute at 35 ℃ and pH 5.1.

Analytical methods

All reaction products were analyzed by HPLC as previously reported (Jung *et al.*, 1989).

Reactor operation

A 2 L fermentor (Biostat M, B.Braun, Germany) was used for the enzyme reactions. Unless otherwise stated, the reactions were carried out using 1 L of 40 %(w/v) sucrose containing 10 units of FT and 5 units of GO per gram sucrose at 40 ℃ and pH 6.0 for 24 hours. Aeration at the rate of 1 vvm was applied to supply oxygen required for the reaction by GO.

RESULTS AND DISCUSSION

As a preliminary experiment, we have carried out two stage reaction: the usual fructose transferring reaction by FT in the first stage followed by the glucose oxidation by GO. This scheme has advantage in that each reaction can be carried out at its individual optimum condition. However, this method has an important setback in that the maximum FOS content is limited by the unconverted sucrose, which usually accounts for 10–15% of total sugars in the product solution of the first reaction, due to the feedback inhibition caused by glucose. An effective way of overcoming this limitation is immediate removal of glucose as it is formed to allow the fructose transferring reaction to proceed further to completion. This can be fulfilled by conducting the reaction by FT and the reaction by GO simultaneously in one reactor.

The two enzyme reaction was partly optimized in terms of GO dosage, temperature, sucrose concentration, and agitation speed.

Effect of GO dosage

GO concentration was varied from 5 to 15 units per gram sucrose and sugar concentrations in the final solution were analyzed. As shown in Fig. 1, the FOS content increased from 55 % to as high as 89 % as the dosage of GO increased. The result proved that the two enzyme system is an effective way of producing high FOS syrup. In the industrial operation, the dosage of GO should be optimized considering both the reaction rate and GO cost.

Effect of temperature

The optimum temperature of FT and GO are 55 ℃ (Jung *et al.*, 1989) and 40 ℃ (Kobayashi *et al.*, 1978), respectively. The optimum reaction temperature of the two enzyme system should lie somewhere between these two temperatures. In our experiment, the best result was attained at 40 ℃, as shown in Fig. 2. At this temperature, FT exhibits only 60 % of its full activity. Above 40 ℃, however, GO lost its activity very rapidly. Another important factor influenced by the reaction temperature is the solubility of oxygen, frequently acting as the limiting substrate of air oxidation reaction. The solubility of oxygen in water decreases about 10 % as temperature rises 10 ℃. In general, the optimum temperature must be determined taking into account many factors such as the ratio of FT and GO activities, substrate concentration, and the oxygen supply rate.

Effect of substrate concentration

FT exhibits its full activity even for a highly concentrated sucrose solution of 86 %(w/v) (Yun *et al.*, 1992). On the other hand, the upper range of sucrose concentration for the two enzyme reaction was not so high: the FOS content decreased gradually at sucrose concentrations above 40 %(w/v) as can be seen in Fig. 3. This phenomenon is attributable most probably to the reduced oxygen transfer rate at high sucrose concentrations.

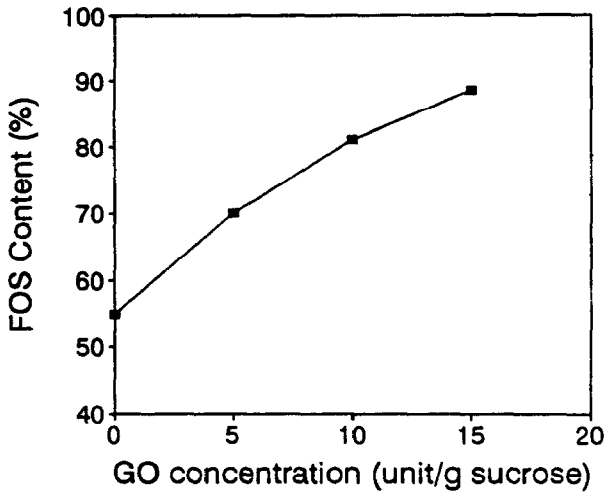

Fig. 1 Effect of GO dosage on the FOS content

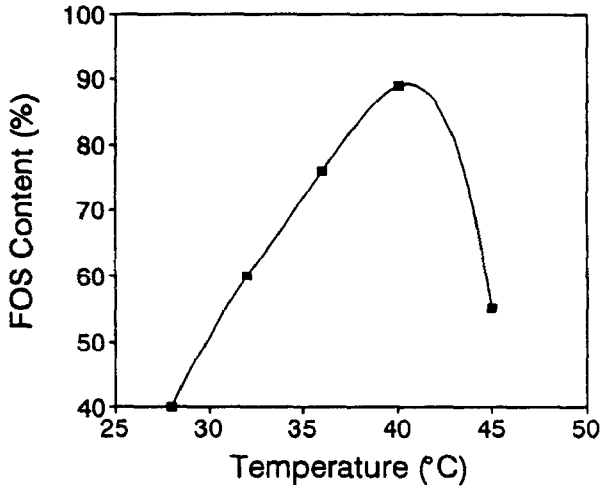

Fig. 2 Effect of temperature on the FOS content

Effect of Agitation speed

Agitation and aeration plays very important role in the glucose oxidation by determining the supply rate of oxygen. In Fig. 4 are plotted the FOS content and oxygen transfer coefficient, k_La, against the agitation speed. It is interesting to see that the FOS content changes at the almost the same pattern as k_La. This fact strongly suggests that under the experimental conditions we carried out, the two enzyme reaction is controlled by the oxygen transfer rate. More detailed study on this topic is necessary.

Still another interesting area of further study is the addition of a third enzyme, catalase, known to reduce the oxygen demand of the system by half by generating oxygen from the hydrogen peroxide produced by GO.

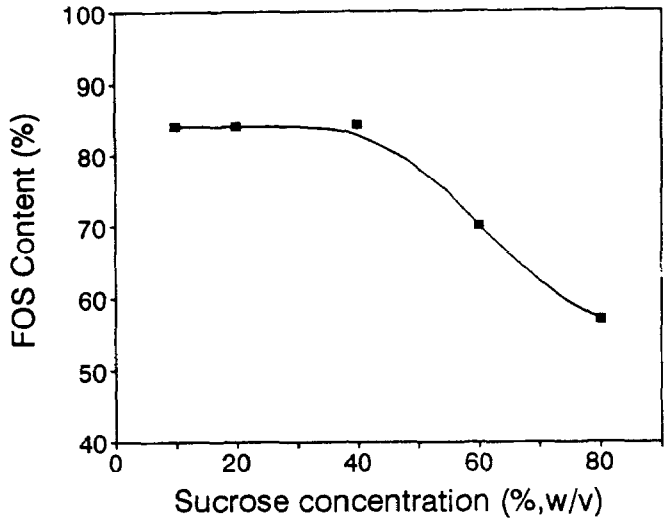

Fig. 3 Effect of sucrose concentration on the FOS content

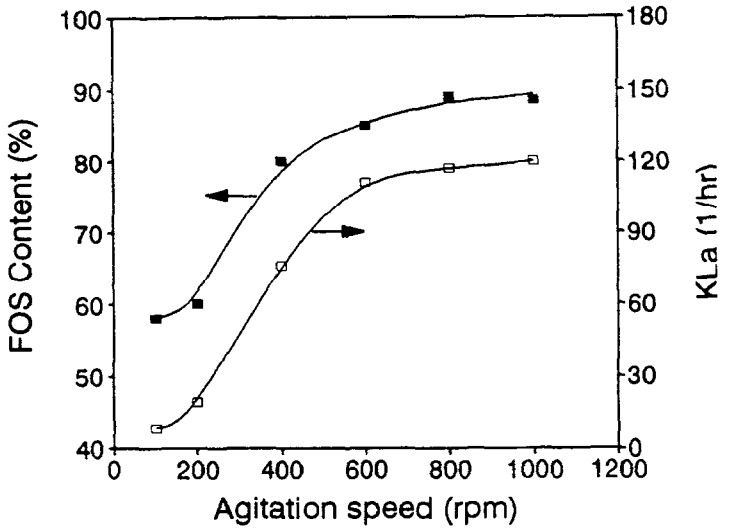

Fig. 4 Effect of agitation speed on the FOS content

Product composition

In Table 1 are presented the composition of high FOS syrup produced under the optimum condition described so far as well as that of conventional FOS syrup. The FOS content of 88.8 % was attained by the novel two enzyme system compared with 57.4 % of conventional method. The increase of FOS content is due mainly to the removal of glucose and partly to the decrease of unconverted sucrose.

Table 1. Sugar compositions of conventional FOS syrup and high FOS syrup

unit: %(w/w)

Sugar	Conventional FOS	High FOS
Fructose	1.0	3.7
Glucose	26.6	3.0
Sucrose	15.0	4.5
GF_2	35.0	44.0
GF_3	19.0	40.0
GF_4	3.4	4.8
$GF_2+GF_3+GF_4$	57.4	88.8
Total	100.0	100.0

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