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Short communication

Dry powder preparation of inulin fructotransferase from *Arthrobacter aurescens* SK 8.001 fermented liquor

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ABSTRACT

Difructosan anhydrides III (DFA III) are usually obtained by inulin conversion with inulin fructotransferase (IFTase). IFTase liquor is difficult to store for a long time, which could greatly restrict its application and DFA III production. To meet DFA III scale-up preparation, this work was explored to research dry powder preparation of IFTase from *Arthrobacter aurescens* SK 8.001 fermented liquor by ultrafiltration concentration, ammonium sulfate precipitation and freeze drying. IFTase powder (10.2 g) was obtained from IFTase precipitation (126.4 g) and its specific activity determined was 16.4 U/mg. Dry powder of IFTase could maintain over 120 days at different temperatures. These results showed that it is easy to scale up DFA III preparation for industrial capacity.

1. Introduction

Difructosan anhydrides III (DFA III) are a non-reducing disaccharide, which have 1/15 calories and half the sweetness of sucrose (Kikuchi et al., 2004). Low doses of DFA III could increase the absorption of calcium in small and large intestines of rats (Suzuki, Hara, Kasai, & Tomita, 1998), as the same in humans (Shigematsu, Okuhara, Shiomi, Tomita, & Hara, 2004). DFA III is prepared from inulin conversion by inulin fructotransferase (DFA III-producing) (IFTase) catalysis. IFTase is obtained from *Arthrobacter* species and other bacteria (Cho, Lee, Hwang, Jang, & Lee, 1997; Haraguchi, Yamanaka, & Ohtsubo, 2002; Haraguchi, Yoshida, & Ohtsubo, 2005; Haraguchi, Yoshida, & Ohtsubo, 2006). DFA III have only been industrialized and launched in the health food "Twintose" in Japan, which is expected to create a new market of sweetener (Hang et al., 2011). DFA III scale-up production demands a large amount of IFTase. However, the fermented IFTase liquor is easy to grow microorganisms and difficult to store for a long time, which could greatly restrict its application and DFA III production. The enzyme powder could greatly improve its stability and storage time, which would be beneficial for the DFA III scale-up production. In order to meet the scale-up preparation of DFA III, the method of making IFTase dry powder from the fermented enzyme liquor is the key.

This work was explored dry powder preparation of IFTase from *Arthrobacter aurescens* SK 8.001 fermented liquor by ultrafiltration concentration, ammonium sulfate precipitation and freeze drying. Dry powder of IFTase could greatly reduce enzyme volume, increase enzyme stability and be appropriate for conservation and transport. However, there have only been few reports for IFTase powder preparation.

2. Experimental

2.1. Materials and reagents

DFA III, 1-kestose (GF_2), nystose (GF_3) and fructofuranosylnystose (GF_4) were purchased from Wako Pure Chemical Industries (Osaka, Japan). IFTase was obtained from the extract of *A. aurescens* SK 8.001, the bacterium was maintained in this laboratory (Zhao et al., 2010). *A. aurescens* SK 8.001 was fermented and centrifugated, IFTase concentrated liquid (1:l) was obtained from crude enzyme solution (10:l) by ultrafiltration (Hang et al., 2012). All other chemicals were of analytical grade and used as received.

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2.2. Precipitation of IFTase

According to the previous research (Hang et al., 2012), IFTase was concentrated from *A. aurescens* SK 8.001 fermented liquor by ultrafiltration. The concentrated solutions (50 ml) of IFTase were taken and added ammonium sulfate by the concentrations of 20%, 25%, 30%, 40%, 45%, 50% and 60% (m/V), respectively. Ammonium sulfate was mixed sufficiently and dissolved completely. The mixture was stood (4 °C, 4 h) and centrifuged (8000 rpm, 4 °C, 20 min). The precipitation was retentated and a little precipitation was dissolved by citric acid buffer solution (pH 5.5). HPLC was used to detect IFTase activities in the supernatant and precipitation. The optimal concentration of ammonium sulfate was selected to carry out the following experiments. IFTase precipitation obtained was weighed and preserved at the temperature of 4 °C.

2.3. Freeze drying of IFTase

IFTase precipitation was put into the material tray of freeze drier at low temperature. IFTase thickness was set to 0.8 cm and carried out to freeze drying. The procedures were as follows: hot conduction oil was heated for the board; sample temperature, −35 °C; cold trap temperature, −60 °C; vacuum degree, 110 KPa. HPLC was used to detect specific activity of IFTase powder.

2.4. Enzyme assay

For the measurement of IFTase activity, IFTase solution (0.2 ml), distilled water (0.3 ml), 0.1 M citrate buffer (pH 5.5, 0.5 ml), and 20 g/l inulin solution (1.0 ml) were mixed. The mixture was reacted at 60 °C for 15 min, and the reaction was stopped by boiling water for 10 min. The DFA III produced was determined by HPLC. One unit of IFTase was defined as the amount of enzyme which could produce 1 μmol DFA III per min at pH 5.5 and 60 °C.

2.5. HPLC conditions

A Waters Sugur-PakTM 1 (6.5 mm × 300 mm, USA) column and refractive index detector (Shodex RI101) were used. Other analysis conditions were as follows: column temperature, 85 °C; mobile phase, water; flow rate, 0.4 ml/min.

3. Results and discussion

3.1. IFTase precipitation

Ammonium sulfate precipitation showed a good ability to separate proteins from phenols in cereal leaf extract (Brovko & Zagranichnaya, 1998). Enzyme proteins were precipitated by ammonium sulfate and lyophilized, which could lost little enzyme activities and be capable of conservation for a long time. Various concentrations of ammonium sulfate were used for IFTase precipitation, as shown in Table 1. When ammonium sulfate concentrations were from 25% to 60%, enzyme yields in the supernatant and precipitation were from 97.86% to 0% and from 1.08% to 84.82%, respectively. As the ammonium sulfate concentration was attained 60%, enzyme proteins were precipitated completely. Enzyme yields decreased as increasing the concentrations of ammonium sulfate. The ammonium sulfate concentrations were 25% and 60%, enzyme activity yields were degraded for 1.06% and 16.76%, respectively. The reasons might be some enzyme proteins inactivation, which were caused by decreasing acid acidity with increased concentrations of ammonium sulfate. Hence, ammonium sulfate concentration (60%) was selected to precipitate IFTase. The supernatant was removed and IFTase precipitation (126.4 g) was obtained.

Table 1
The precipitation of enzyme with different ammonium sulfate concentrations.

Ammonium sulfate concentrations (%, m/V)	Enzyme yields (%)	
	Supernatant	Precipitation
20	No precipitation	
25	97.86	1.08
30	88.74	4.25
40	47.22	42.34
45	25.68	63.54
50	11.26	77.13
55	5.12	86.11
60	0	84.24

3.2. Freeze drying of IFTase

IFTase was precipitated by ammonium sulfate, which still contained amounts of free water which could not store for a long time. Freeze drying could be a conducive method to the preservation, transportation and application of enzyme.

3.2.1. Freeze drying process

Firstly, the protein precipitation was frozen at low temperature. Pre-frozen enzyme protein aimed to solidify the free water and form to sublimation of ice crystals, which ensured that enzyme protein was kept in the same microstructure and a consistent shape. At the same time, it also could prevent foaming and solute movement from vacuum. The pre-frozen temperature of IFTase was set to −50 °C, enzyme protein was completely frozen for 8 h. Freeze drying operation was divided into two stages of sublimation and desorption. The stage of sublimation was 8–42 h, which was the key step for freeze drying. About water (90%) in the enzyme protein was removed by sublimation and the surplus moisture was further to be dried in the desorption stage for 8 h. During the freeze drying process, the temperature was not too high and should be raised slowly. A higher temperature could cause collapse, melting and other issues of enzyme protein, that to degrade IFTase activities. Dry powder (10.2 g) was obtained from IFTase precipitation (126.4 g) and its specific activity determined was 16.4 U/mg.

3.2.2. Effects of IFTase residual activity on freeze drying

Fig. 1 shows the relationship of IFTase residual activity and time. The initial IFTase residual activity was set as 100%. During the process of freeze drying, the IFTase residual activities were 95.2%, 87.6%, 83.4%, 81.2%, 80.1% and 79.6% for 8 h, 16 h, 24 h, 32 h,

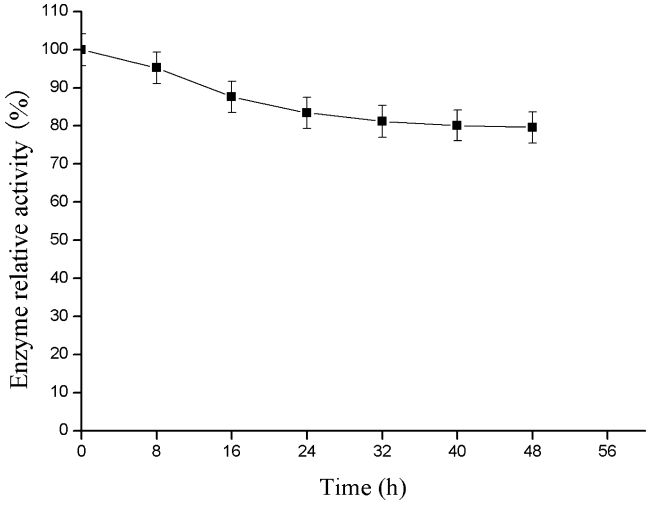

Fig. 1. The relations of IFTase activity and frozen time. Data points are means of three replicates with error bars of standard deviation.

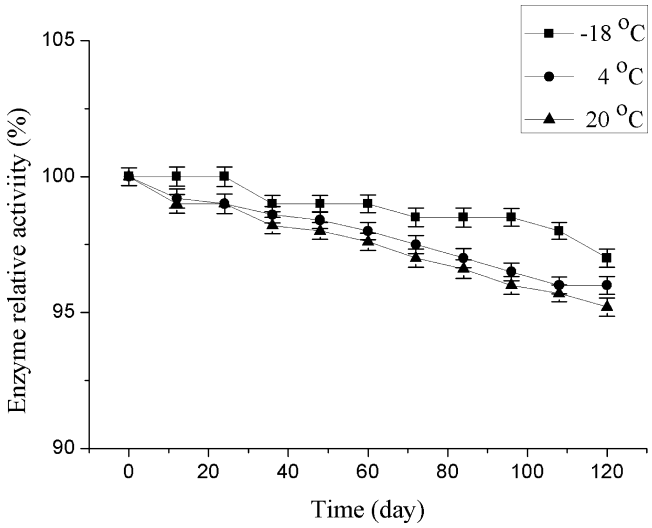

Fig. 2. The storage stability of lyophilized IFTase. Data points are means of three replicates with error bars of standard deviation.

40 h and 48 h, respectively. IFTase was significantly affected by ice crystal mechanical action for the first 8 h. As the free water existed in the surface of enzyme protein, IFTase residual activity was decreased about 5%. IFTase residual activity was degraded about 15%, which was caused by the stage of ice crystal sublimation for 8–40 h. About water (90%) was evaporated during the sublimation stage. Less water was consumed in the stage of desorption for 40–48 h, so IFTase residual activity changed little. The results were similar to the study of preparation of ferulic acid esterase (Xue, Qu, Wang, Huang, & Liu, 2006).

3.3. Stability of lyophilized IFTase

IFTase powder was separately stored at the temperatures of −18 °C, 4 °C and 20 °C for 120 days, as shown in Fig. 2. IFTase residual activities were remained over 97%, 96% and 95% at the temperatures of −18 °C, 4 °C and 20 °C, respectively. Dry powder of IFTase had better storage stability, which could maintain more than original enzyme residual activity (95%) under the different temperatures for 120 days.

4. Conclusion

In summary, the results of ultrafiltration, ammonium sulfate precipitation and freeze drying to produce dry powder of inulin fructotransferase from *A. aurescens* SK 8.001 fermented liquor had been presented in this work. IFTase concentrated liquid (1:l) was firstly obtained from IFTase solution (10:l) by ultrafiltration. Secondly, IFTase precipitation (126.4 g) was collected by ammonium sulfate. Thirdly, IFTase powder (10.2 g) was obtained and its specific activity determined was 16.4 U/mg, which had better storage stability. So it is easy to scale up DFA III preparation for industrial capacity. Future work of scale-up dry powder of IFTase production will be used in the DFA III industrial production.

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