Paper Code: sp004

Separation of Prebiotics Compounds From Extract of Jackfruit

Thitipong Rugwong[1*], Pakamas Chetpattananondh[1], Kulchanat Prasertsit[1]

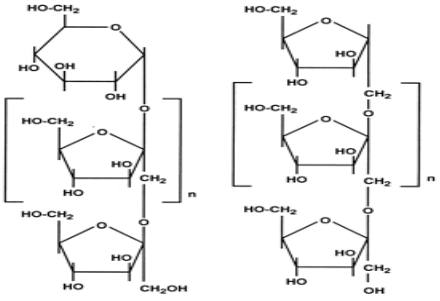
[1] Department of Chemical Engineering, Faculty of Engineering,
Prince of Songkhla University, Songkhla, 90112, Thailand
*e-mail: 5210120070@email.psu.ac.th

Abstract – Purification of prebiotics by crystallization is an accessible and low cost. That is able to extend to the higher production level. Therefore, should be the aim of this research is to separate prebiotics from extract of jackfruit seed using crystallization method. Evaporated extract of jackfruit seed was crystallized in laboratory scale in order to study the effects of crystallizing temperatures and mixing speed on crystallizing yield. The work found that range of crystallizing temperature of prebiotics is 55-64 °C (by using Differential Scanning Calorimeter) and the best temperature to obtain the highest percent non-reducing sugar is 58 °C. Moreover, percentage of non-reducing sugar increases with increasing mixing speed and the best of mixing speed is 100 rpm. For higher than 150 mixing speed show lower amount of non-reducing sugar.

***Keyword**: Prebiotics, Crystallization, Jackfruit seed, Differential Scanning Calorimeter (DSC), Gel Permeation Chromatography (GPC)*

1. Introduction

The jackfruit *(Artocarpus heterophyllus* Lam.) is a species of tree in family *Moraceae*. It is native to India and grown wildly many parts of Southern and Southeast Asia, such as Bangladesh, Burma, Sri Lanka, Malasia, Indonesia, Phillippines and Thailand [1] including Brazil and other countries that there are humid tropical and near-tropical climates. In Thailand, we can find jackfruit all year round and the best time to find it is around the end of rainy season in October or November. The green fruit is cooked as a vegetable. Additionally, the ripe fruits are normally eaten fresh or used in ice cream and also processing into canned and snacks products [2]. The residual seeds are mostly discarded. Previous research found that the jackfruit seeds contained phenolic compounds [3] and about 6.03 mg/g extracted non-reducing sugar [4] that is Prebiotics. Prebiotics are non-digestible food ingredients. It is a part of oligosaccharide and non reducing sugar that stimulate the growth and activity of bacteria in the digestive system that beneficially affect the host by improving its intestinal microbial balance. Prebiotics are carbohydrate. The composition of food classified as prebiotics include oligosaccharides and polysaccharides, such as fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), inulin and xylo-oligosaccharide, which are non-reducing sugar. The structure of inulin and FOS are shown in Fig. 1.



(a) (b)

Fig. 1. *Structure of inulin (a) and FOS (b)*

Prebiotics can be obtained by extraction from plant or synthesis. However, verifying prebiotics is more complicated, non-reducing sugar and molecular weight of oligosaccharide have been considered instead in this work.

Should be considering only non-reducing sugar, previous work [5] found that the optimum condition for maximizing the crystal of prebiotics from an extract jackfruit seed was at crystallizing temperature of 77 ˚C for 60 minutes without mixing process.

In our work, the investigation of effect of crystallizing temperature, mixing speed and rate of cooling on the crystallization by consider amount of non reducing sugar and molecular weight have been studied.

2. Materials and methods

2.1 Materials and chemicals

Tongprasert-jackfruit seeds were used in the experiment. Fresh seeds were cleaned with water and grinding before sizing by sieve shaker. 95% Ethanol, sodium hydroxide and concentrate sulfuric acid were purchased from lab-scan analytical science (laboratory grade, Thailand). Sodium potassium tatrate and sodium carbonate were purchased from Ajax Finechem Pty Ltd. (NSW, Australia). D-glucose anhydrous and gallic acid were from Sigma-Aldrich (Steinheim, Germany). Sodium sulfite and Folin-Ciocateu reagent were purchased from Merck (Darmstadt, Germany). 3,5-dinitrosalicylic acid was from Fluka Chemie (Buchs, Switzerland). Phenol was from Fisher Sciencetific (Loughborough, UK).

2.2 Method extraction of prebiotics

Fresh seeds were cleaned with water and grinding by blender to size of 1-2 mm. The seeds were extracted with 50% ethanol using batch extractor. To concentrate the extract solution was filtered by vacuum filter (SIBATA: Circulating Aspirator WJ-20) and then was evaporated by rotary vacuum evaporator (Buchi: Vacuum pump V-700).

2.3 Crystallizing procedure and conditions

The concentrated solution was heated to 80˚C for 10 minutes and then decreased this temperature to the desired temperatures with cooling rate 1 ˚C/min at mixing speeds (0, 50, 100 and 150 rpm). The concentrated solution was crystallized for analyzing prebiotics.

2.4 Analytical techniques of prebiotics

The previous related research about analysis melting point and crystallizing point by DSC has an indistinct result. Therefore, this research study is performed the laboratory in new temperature range. Sample of crystal is prepared by crystallizing the concentrated extract solution at 61˚C, next the sampled was analyzed by DSC to verify the melting point and crystallizing temperature. The result showed the obvious range of crystallizing temperature graph. Consequently, this research has extended for new range of crystallizing temperature. GPC method has been test to verify the molecular weight. And non reducing sugar (NRS) have been tested for total sugar (TS) and reducing sugar (RS) by Modified phenol sulfuric method [6] and Modified dinitrosalicylic acid (DNS) method [7], respectively.

2.4.1 Determination of total sugar

The total sugar concentration was determined by using Modified phenol sulfuric method [6]. The assay was calibrated with D-glucose anhydrous standards from 0 ppm to 600 ppm. Sample or glucose standard solution (25 μL) was added to 5 %w/v phenol solution (25μL) and concentrate sulfuric acid (125 μL). Then solution was shaken for 30 second at 500 rpm. The samples and standard were heated to 80°C for 30 minutes and then were cooled by ice for 10 minutes before reading the absorbance at 492 nm by micro plate reader (Biotek: Power Wave XS). The concentrations of sugar in the samples were calculated by comparison with the standard curve of glucose at the same condition.

2.4.2 Determination of reducing sugar

Reducing sugar concentration was determined by using the Modified dinitrosalicylic acid (DNS) method [7]. Analyzed method was calibrated with D-glucose anhydrous standards from 0 ppm to 600 ppm. Sample or standard glucose solution (100 μL) was added to DNS solution (100 μL). Then the solution was shaken for 30 second at 500 rpm. The samples and standard were heated to 80°C for 30 minutes and then were cooled by ice for 10 min before reading the absorbance at 575 nm by micro plate reader. Also the concentrations of reducing sugar in the samples were calculated by comparison with the standard curve of glucose.

2.4.3 Determination of non-reducing sugar

Non-reducing sugar contents was determined using this equation

$$NRS = TS - RS \quad (7)$$

Where *NRS* is non-reducing sugar, *TS* is total sugar and *RS* is reducing sugar.

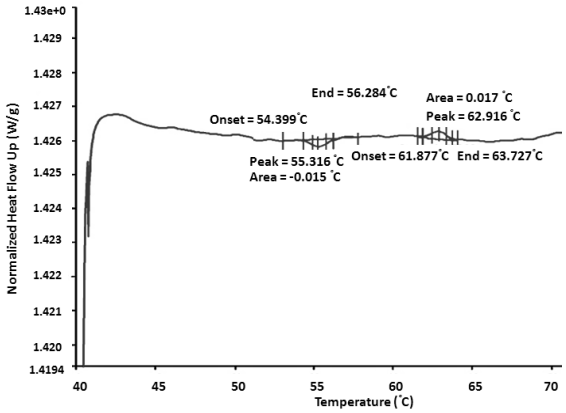


Fig. 2. Analysis of crystallization temperature by DSC at 61 °C

3. Results and Discussion

3.1 Crystallizing temperature of prebiotics

By DSC, Fig 2 shows that the melting point and crystallizing point of the sample are 64 and 55 °C, respectively. Therefore, this work has been studied the crystallizing temperature in the range of 55 to 64 °C.

3.2 The effect of mixing speed on crystallization

Fig. 3 shows percent of non-reducing sugar with mixing speed. The result shows that non-reducing sugar increase with increasing mixing speed and the maximum non-reducing sugar is 83.5% at 100 rpm, the reason of lower percent non reducing sugar at the low mixing speed is a few chance of crystal to collide with the others and the reactor wall. However, at the 150 mixing speed shows lower amount of non-reducing sugar. Hypothesis of this phenomenon is that high mixing speed will damage crystal formation. To study the effect of temperature in ranges 55°C to 64°C. The result shows highest percent non-reducing sugar at 58 °C.

3.3 The effect of temperature on crystallization

The present result shown in Fig. 3 the various speeds give high peak of % non-reducing sugar at 58°C should be performing the crystallization in the high temperature causes the % non-reducing sugar decreased owing to heat. While the temperature at 55 °C and various speeds the % non-reducing sugar are low, it may be that this temperature does not make the solution saturate.

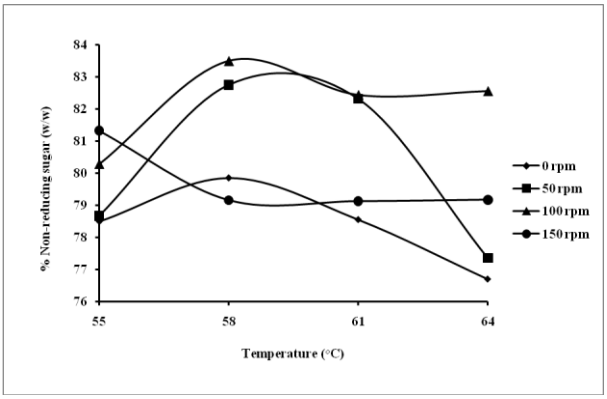


Fig. 3. Analysis of non-reducing sugar contents with respect to mixing speed and temperature.

Table. 1. Show the weight of crystal with mixing speed And temperature

Temperature (°C)	Mixing speed (rpm)			
	0	50	100	150
55	0.140	0.104	0.194	0.144
58	0.220	0.118	0.264	0.153
61	0.125	0.152	0.121	0.182
63	0.197	0.090	0.117	0.235
Crystal weight (g)	0.682	0.464	0.696	0.714
Total solid (g)	30	25.6	28.4	31.2
Crystal weight/ Total solid	0.022	0.018	0.024	0.022

3.4 Effect of operating parameter to weight of crystal

Studying about operating parameters including mixing speed and temperature was shown in Table. 1. It express the ratio of crystal weight to total solid which explain how much crystal generate per total solid. The result shows that crystal weight/total solid the maximum is 0.024 at 100 rpm then we can conclude that this mixing speed can create the most amount of crystal.

3.5 Molecular weight of prebiotics

From GPC, Fig.4 the shows that the molecular weight of crystal sample is about 1367 which is in the same range of oligosaccharide [8].

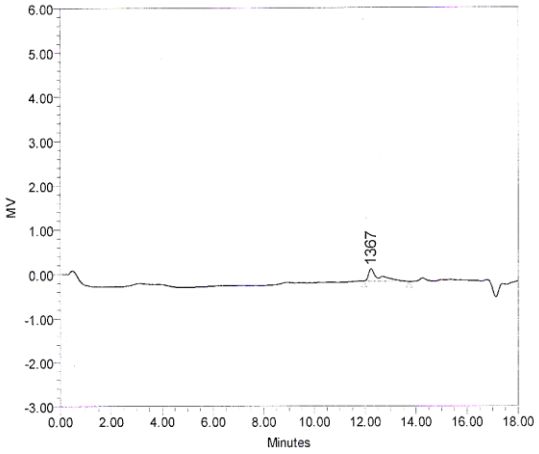


Fig. 4. Analysis of molecular weight by GPC.

4. Conclusion

The purification of prebiotics by crystallization process was investigated. The two factors affected crystallization were temperature and mixing speed. According to this study, the highest percent non-reducing sugar was obtained when using the mixing speed of 100 rpm at 58°C.

5. Acknowledgment

This research has been financially supported by Prince of Songkla University and the National Research Council of Thailand (NRCT). The Graduate School at Prince of Songkla University has provided partial funding. The Department of Chemical Engineering and the Faculty of Engineering, Prince of Songkla University are gratefully acknowledged for other supports.

Reference

[1] B. T. Ong, S.A. H Nazimah, A. Osman, S. Y. Quek, Y. Y. Voon, D. Mat Hashim, P. M. Chew, and Y. W. Kong, Chemical and Flavour Changes in Jackfruit *(Artocarpus heterophyllus* Lam.) Cultivar J3 during Ripening, *J. of Postharvest. Bioacoustics-the international journal of animal sound and its recording,* 40 (2006) 279-286.

[2] C. K. Pua, N. S. A. Hamid, C. P. Tan, H. Mirhosseini, R. B. A. Rahman, and G. Rusul, Optimization of Drum Drying Processing Parameters for Production of Jackfruit (*Artocarpus heterophyllus*) Powder using Response Surface Methodology, *J. of food science and technology series.,* 43 (2010) 343-349.

[3] Y. Y. Soong, and J. Philip Barlow, Antioxidant Activity and Phenolic Content of Selected Fruit Seeds, *J. of agricultural and food science. Chem., 88 (2004) 411–417.*

[4] S. Nualla-ong, P. Chetpattananondh, and R. Yamsaengsung, Extraction of Prebiotics from Jackfruit Seeds, *J. of Eng., 36 (2009) 213-220.*

[5] K. Prasertsit, S. Ineak, and A. Limjareonwong, Purification of Prebiotics by Crystallization, *The 8 th PSU-Engineering Conference. Prince of Songkla University, Thailand, April 22-23 (2010).*

[6] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, Colorimetric Method for Determination of Sugars and Related Substances, *J. of analytical chemistry associated with the destruction of chemical weapons., 28 (1956) 350-356.*

[7] G. L. Miller, Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar, *J. of analytical chemistry associated with the destruction of chemical weapons., 31 (1959) 426-428.*

[8] S. Wichienchot, M. Jatupornpipat, Extracttion and purification of prebiotics oligosaccharides, *35th Congress on Science and Technology of Thailand. Burapha University, Thailand, October 15-17 (2009).*