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Physicochemical Properties of Xylitol Crystals from Oil Palm Empty Fruit Bunches Hydrolysate

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***Abstract*— Xylitol, a low-calorie sugar made up of five carbon atoms, had the valuable characteristics suitably applied for pharmaceutical and food industries. This sugar can be produced from oil palm empty fruit bunches (OPEFB) through hydrolysis and followed by fermentation. The xylitol in the fermentation broth requires the downstream process to obtain the final product with high purity and yield. Among a series of xylitol downstream process, crystallization becomes a critical step since this process determines the properties of final products. The objective of this study was to evaluate the effect of evaporation temperature (55°C and 70°C) and seeding addition (0%, 0.1%, 0.5%, 1 %) in the crystallization step on the physicochemical properties of xylitol crystals obtained from the OPEFB hydrolysate. The main evaluation criteria were crystal contents, purity, melting point, water content, hygroscopicity, solubility, caloric content, and crystal xylitol yield. The result showed that the crystal form obtained was relatively sticky and had lower purity than commercial ones because the concentration of xylitol solution increased after evaporation. The differences of physicochemical properties of the crystals such as the purity, porosity, yield and crystal form were influenced by evaporation temperature. The crystals formed by 70°C evaporation temperature produced the crystals with higher caloric value and purity, but it had lower hygroscopicity and moisture content than crystals formed by 55°C. However, the percentage of seeding gave an insignificant impact on xylitol crystal properties.**

***Keywords*— crystallization; oil palm empty fruit bunches; seeding; temperature; xylitol.**

I. INTRODUCTION

Xylitol, an alcohol sugar consisting of five carbon atoms and five hydroxyl groups, has the attractive and applicable characteristics for food and pharmaceutical application. These characteristics enhance the value of xylitol, so this sugar group is potentially developed in industrial scale by certainly considering aspects of economic feasibility. The most exciting attribute of xylitol as compared to the other sugar substitutes is that this sugar is unable to be metabolized by human insulin [1]. Thus, both the diabetics and the hypoglycemics are allowed to consume this sugar safely [2].

Xylitol can be produced through chemical route by hydrogenation reaction of pure D-xylose [3]. Because the initial step for D-xylose purification and high energy consumption for the reaction is imperative, the use of this method is costly, and it must be thoroughly evaluated, both in term of economic and environmental aspects [4]. One of the promising alternatives for xylitol production is the biological conversion of agricultural wastes. This process brings about several advantages; for instance, it is needless of highly pure xylose or substrate, performed at mild condition, and environmentally friendly [5].

In general, xylitol production from agricultural residues via this process route comprises pretreatment [6] followed by either enzymatic [7] or acid hydrolysis [8], and lastly hydrolysate fermentation using specific microbial strains [9]. Several studies have shown that yeasts were able to produce xylitol with a higher yield than bacteria and fungi [10]. One of that yeast species that has been previously investigated and proven to give the maximum product yield and productivity was *Debaromycess hansenii* produces [11].

Several studies have reported that xylitol fermentation by *D. hansenii* could employ oil palm empty fruit bunches (OPEFB) hydrolysate as its substrate. The OPEFB material is one of the biomass feedstocks generated from activities of the palm oil industries. The composition of this material includes cellulose (32-43%), hemicellulose (23-25%), and lignin (11-23%) and other extractives [12]. The particular monomeric sugar utilized for xylitol production is xylose obtained from hemicellulose degradation. Nevertheless, the hydrolysate from hemicellulose hydrolysis contains not only xylose, but also the other pentose (L-arabinose) and hexose

sugars (D-galactose, D-glucose, and D-mannoses) [11]. Sometimes, the xylanase used, xylan-hydrolyzing enzyme, has low selectivity because its enzyme solution still contains cellulose with high enzyme activity. The high glucose content from cellulose hydrolysis become a challenge. This is because the high presence of glucose could inhibit the fermentation [13]. Moreover, various by-products also will be detected in the fermentation broth and cause more complex downstream process.

Aqueous xylitol in fermentation broth requires a series of the downstream process for separation and purification. Xylitol with high purity and crystal form is obtained after passing through the stage of crystallization. However, before crystallization, the initial process, such as filtration and then adsorption by activated charcoal, is needed to remove all of both insoluble and soluble compounds [14]. Purification of crude xylitol from this broth also can apply chromatography [15], ion exchange resins [16], [17], or membrane filtration [18] such as the combination between ultrafiltration and electro deionization process [19] or just nanofiltration [20]. These methods, however, tend to be very expensive for scaling up to the industry. The most efficient and effective technique for xylitol purification is the activated charcoal followed by vacuum evaporation and ultimately crystallization [21]. Thus, this study implemented this process steps.

Research on the purification of xylitol by activated charcoal has been widely reported. For instance, its is pointed out that the optimum xylitol yield reached to 43.31% when the clarification process was performed at 30 °C with 15 g/L of activated charcoal concentration [22]. The lower concentration of activated charcoal caused the rapid saturation of adsorbent. However, mass transfer will be a problem when the high concentration of charcoal is employed [23]. Furthermore, before crystal formation in the following step, the aqueous xylitol also must be concentrated up to supersaturation condition achieved. The sufficient concentration of xylitol to form crystal successfully is approximately 40% using a rotary evaporator. Apart from water, the bioethanol, by-products of xylitol fermentation, also evaporate in the vapor phase. [24]. This phase is then further condensed to recover the bioethanol.

The last downstream process is crystallization to increase the xylitol purity and to alter the phase from xylitol liquid to crystal. This xylitol phase has high purity, reaching up to nearly a hundred per cent, and enables easier storage and distribution than xylitol liquid [25]. The success of crystallization is affected by several influencing factors such as evaporation temperature and the addition of crystal seeding at the concentration step [26]. The low temperature of evaporation is preferable because this condition will save more energy of the overall process and give the effective performance showed from its yield. On the contrary, the crystals purity degree will show the opposite behavior to be lower when the evaporation is conducted at a lower temperature [2]. In addition, this condition also needs a longer evaporation duration to evaporate all water and volatile compounds in broth fermentation. Thus, another factor, the addition of crystal seeds, is offered to cope with this problem by shortening the evaporation time, so the process will be more efficient and give high xylitol productivity [26]. Hence, the objective of this study was to evaluate the effect of evaporation temperature and the percentage of crystal seed at concentration step on the physicochemical properties and the final xylitol yield.

II. Materials and Method

A. Raw Material

PTPN VIII, Bogor, Indonesia, provided the OPEFB used in this research. The provided OPEFB was initially prepared by washing and followed by oven drying at 105°C for 24 h. The dried material with 4.6% moisture content was ground using a disc mill to be smaller particles. To uniform the particle size, the smaller OPEFB was sieved with the size of less than 80 mesh [27].

Before the OPEFB particles were used, its initial lignocellulose composition was analyzed following the Chesson method [28]. The result analysis showed that the OPEFB used in this study consisted of 39.47% cellulose, 17.31% hemicellulose, 23.25% lignin, 4.78% ash content, and 4.83% (dB) moisture content.

B. Acid Hydrolysis

In this study, 10 g OPEFB particles were mixed with 4% sulfuric acid solution (250 mL working volume). After all of this particle surface was thoroughly wet, the hydrolysis was conducted using autoclave at 121°C for 15 min [29]. The hot solution was then cooled down up to room temperature and separated into two fractions, residual solid and hydrolysate. The latter was adjusted until the pH value of 5.0 by adding 2 M NaOH solution. Subsequent to pH adjustment, the 15 g/L activated carbon was added to this liquor and mixed under a controlled temperature of 30°C for 60 min. The pure hydrolysate was further used for fermentation. The result of hydrolysate purified by activated carbon was compared with the control (without purification by activated charcoal). Although xylose was the primary sugar obtained from hemicellulose, other by-products such as glucose, acetic acid, and furfural were also produced in low amounts during hydrolysis [29]. The higher the concentration of sulfuric acid, the more by-products were produced.

C. Inoculum Preparation

The inoculum of *Debaryomyces hansenii* R85 obtained from the laboratory of Microbiology and Bioprocess Technology, Chemical Engineering, Institut Teknologi Bandung was prepared in the formulated medium. This medium consisted of 2 g synthetic xylose, inorganic salts (9.438 g $(NH_4)_2SO_4$, 2.5 g KH_2PO_4, 0.05 g $CaCl_2.2H_2O$, 0.5 g $MgSO_4.7H_2O$, 0.5 g citric acid, 0.035 g $FeSO_4.7H_2O$, 0.0092 g $MnSO_4.4H_2O$, 0.011 g $ZnSO_4.7H_2O$, 0.001 g $CuSO_4.7H_2O$, 0.002 g $CoCl_2.6H_2O$, 0.0013 g $Na_2MoO_4.2H_2O$, 0.002 g H_3BO_3, 0.0035 g KI, and 0.00005 $Al_2(SO_4)_3$), and vitamins (0.1 g Myo-inositol, 0.02 g calcium-pantothenate, 0.005 g thiamine hydrochloride, 0.005 g pyridoxal hydrochloride, 0.005 nicotinic acids, 0.001 g aminobenzoic acid, and 0.0001 g D-biotin) in 1 L distilled water [30]. The inoculum was then incubated at 30°C for 48 h using rotary shaker incubator until the number of *D.hansenii* cell reached around 10^8 cell/mL.

D. Fermentation

The medium used for fermentation was slightly similar to the inoculum preparation step. The difference was that the carbon source for fermentation was obtained from the hydrolysate. The volume ratio of hydrolysate to inoculum to fermentation medium was 2:2:3 [31]. Prior to fermentation, the pH value of this solution mixture needed to be adjusted to 5.0. The hydrolysate was fermented using *D. hansenii* at 30°C with 200 rpm agitation for 96 hours in shaker incubator [12].

E. Purification and Crystallization

Purification was initiated by centrifugation of fermentation broth to remove cells and insoluble solid that had high density. The supernatant resulted from centrifugation was then filtered to obtain the solution with free insoluble solid. After filtration, 15 g/L activated charcoal was added into the solution and stirred at 30°C for 60 min. The charcoal was separated by filtration, and the purified solution was subsequently crystallized.

Before crystallization, the purified solution was mixed with xylitol powder, and it was then evaporated to increase the concentration of a solution by rotary vacuum evaporator until the solution reached supersaturation point. In this research, the evaporation temperature was varied from 55°C to 70°C. The initial concentration of the solution was 346.67 g/L, and the final concentration of the solution was 1155.56 g/L indicated that the concentrate solution was in the labile zone of crystallization. After the evaporation step, commercial xylitol crystals were added into concentrate samples and then stored at 10°C for two days as a seeding step in crystallization. This step used four various variations of seeding, 0%, 0.1%, 0.5%, and 1%. Finely ground commercial xylitol (1.0 g/L) was added to induce nucleation of crystals [2]. The formed crystals were separated by filtration after two days and dried at room temperature for further analysis.

F. Analysis

1) Xylitol and by-product concentration: The xylose, glucose, acetic acid, xylitol, and bioethanol were measured by NREL method [32] using HPLC (RID Detector, Column type HPX-87H, column temperature 60 °C, detector temperature 40 °C, flow rate 0.6 mL/minutes, wavelength 560 nm, injection volume 20 µl, and using 5mM H_2SO_4 as eluent). The product yield and sugar utilization in the fermentation step was expressed by formulation as follows:

Yield or $Y_{xyt/xyl}$ (g/g) = (final xylitol concentration – initial xylitol concentration)/(initial xylose concentration – final xylose concentration) (1)

Glucose or Xylose Utilization (%) = (initial glucose or xylose concentration – final glucose or xylose concentration) × 100/(initial glucose or xylose concentration) (2)

2) The concentration and purity of crystal xylitol: Xylitol crystal was diluted in distilled water and filtered. The dilute xylitol was then analyzed its composition by NREL method [32]. Furthermore, the purity of crystals could be determined by this formula [28].

Purity (%) = (Concentration of dissolved xylitol crystals (g/L) x Volume of dissolved xylitol crystals (L))/(mass of xylitol crystals (g)) x 100% (3)

3) The moisture content of xylitol crystal: the moisture content of xylitol crystals was determined by thermogravimetry method. The crystal sample was measured approximately 2 g in the constant aluminum dish. The sample was dried at 105°C for 3 h until its mass was constant. The moisture content of the sample was calculated:

Moisture content (%) = (initial weight of sample (gram)-final weight of sample (gram))/(initial weight of sample (gram)) x 100% (4)

4) Melting point and caloric value of xylitol crystal: the melting point was determined based on [33] using the capillary method. Dried crystal samples were mashed and inputted into the capillary pipe (diameter was around 1.5 – 2 mm). The capillary pipe was tied with the thermometer. The temperature was recorded when all samples were melted. The caloric value of crystal was determined by measured 1 g of samples to automatically analyzed using a bomb calorimeter.

5) Xylitol crystal hygroscopicity: The hygroscopicity of crystal was determined by GEA Niro Method No. A14a [34]. Xylitol sample was measured 0.5 g in the aluminum dish and placed at RH 79.5%. The increase of sample mass was recorded every 10 min for 40 min, then every 20 min until it reached a constant mass (± 4 h):

Hygroscopicity (%) = ((%Wi+%FW))/(100+%Wi) x100% (5)

%Wi = (mass of absorbed water (g))/(mass of sample (g)) x 100% (6)

%FW = moisture content of the sample (%)

6) Xylitol crystal solubility: The crystal sample was measured 0.75 g and diluted in 100 mL distilled water. The sample was filtered using constant filter paper, and its residue was then dried at 105°C for 3 hours until the weight was constant.

Solubility (%) = 1-(c-b)/((100-%KA)/100-a) x 100% (7)

a = initial mass of sample (g)
b = mass of dried filter paper (g)
c = mass of dried filter paper with residue (g)
%KA = moisture content of sample (%)

7) Xylitol crystal yield: it was the ratio between the mass of crystals and volume of evaporated solution [22]:

Yield = (mass of crystals (g))/(volume of evaporated solution (mL)) (8)

III. Results and Discussion

A. Hydrolysate Composition

The presence of by-products during hydrolysis and fermentation could inhibit the crystallization process [35]. Hence, the composition of hydrolysate and fermentation broth also needed to be analyzed and evaluated. In the hydrolysate obtained from heat-assisted acid hydrolysis, apart from monomeric sugars, organic acids and furan groups such as furfural and 5-Hydroxymethyl furfural (5-HMF) also were detected [36]. Organic acids such as acetic acid was formed during hemicellulose deconstruction by acids. On the other hand, the furan formation was caused by the further decomposition of monomeric sugars due to more severe condition. For instance, the effect of more concentrated sulfuric acids, temperature increase, and the extension of hydrolysis duration led to dehydration of xylose and glucose to be furfural and 5-HMF, respectively.

In this study, acid hydrolysis at 121 °C for 15 min with 4% sulfuric acid concentration gave 2.89 g/L xylose prior to hydrolysate purification. Hydrolysis with the same condition has been applied to corncob by [37]. The result showed that this condition could give the yield (xylose/initial hemicellulose) up to 55% (9.22 g/L). This study, however, had a lower xylose yield (only 47.6%) because of high OPEFB lignin content, lower OPEFB hemicellulose amount, no pretreatment process. Lignin is composed of a compact and firm structure hindering hydrolysis access [38]. Each biomass feedstock has its appropriate pretreatment techniques to dissolute lignin due to the different lignocellulose structure and composition. The corncob used by [37] reached 41.17% hemicellulose as compared to this in OPEFB (17.31%).

On the other hand, it has been reported that reported that the increase of sulfuric acid hydrolysis from 4% to 6% could decrease the xylose yield because of the decomposition reaction of xylose [29]. The consequence of this reaction was the reduction of xylose yield. The more furfural was formed, the lower xylose was obtained. The study reported by also had proven that the increase of acid concentration, time, and temperature significantly influenced the decrease of reducing sugars, including xylose, for other acids type such as oxalic, formic, and citric acid [39]. proposed two-stage treatment to optimize the reducing sugar yield at the mild condition [40].

Acid randomly and not selectively degrades the lignocellulose [41]. Consequently, glucose was also detected in the hydrolysate despite its concentration was low, only 0.71 g/L. Besides glucose, this hydrolysate also contained 0.65 g/L acetic acid, and other compounds such as furan showed from a lot of chromatogram peaks detected. Wei [21], activated carbon could bind inhibitory compounds of fermentation such as phenolic groups, acetic acid, and furan. Thus, to minimize the concentration of such compounds, adsorption of hydrolysate by activated carbon was conducted. Adsorption by activated carbon also could accelerate the rate of xylose consumption and increase xylitol productivity for fermentation [42].

The result of this study showed that the addition of activated carbon could give a clearer solution of hydrolysate. This indicated that several substances as a cause of dark hydrolysate color such as lignin-derivative compounds had partly removed. In addition, the concentration of glucose slightly decreased by 0.03 g/L, but the moderate decline occurred in xylose, approximately 0.16 g/L. The different trend, however, occurred in acetic acid. Purification with 15 g/L activated carbon was unable to reduce the amount of acetic acid.

B. Components in Fermentation Broth

After hydrolysis, the following process was fermentation. The fermentation broth obtained in this study consisted of 0.10 g/L xylitol, 0.04 g/L xylose and 0.003 g/L glucose after 96 h fermentation. According to this result, not all xylose, however, was consumed and converted to xylitol during the fermentation. Even though the xylose utilization reached 98%, the low product yield was obtained, only 0.034 g xylitol/g xylose. This was because the remained oxygen in the flask used for fermentation could induce *D. hansenii* to transform xylose into cells and energy. No oxygen controlling system led to high oxygen availability in the flask. The oxygen was the essential factor in xylitol formation [7]. Xylitol was optimally produced under micro-aerobic condition. When there was no oxygen available (anaerobic condition), the main product produced by this yeast was bioethanol. Conversely, the substrate would be dominantly converted to cells under aerobic condition.

Nearly all glucose in hydrolysate was consumed by *D. hansenii*, going to 97%. This yeast had the ability to utilize glucose for cell growth and formation [43]. High glucose content could increase the utilization of xylose [9]. When the initial fermentation liquid contained high glucose concentration and was performed under anaerobic condition, *D. hansenii* was also able to convert this sugar to bioethanol [7]. Nevertheless, the ratio of xylose and glucose in hydrolysate further used for fermentation was 4: 1. In addition, the presence of oxygen was high enough. This caused no bioethanol detected in the broth after fermentation completed.

Besides remained sugars such as xylose and glucose, there were also several by-products most likely present in the fermentation broth. The by-products included solvents and organic acids secreted as extracellular metabolites and also the other unconsumed macro- and micro-nutrients [14]. The by-products of hydrolysis, for example, acetic acid, furan, and phenolic acids was also still available in the fermentation broth. Such compounds could affect the characteristics of crystal obtained.

C. Crystal Components

Fig. 1 showed the components in the xylitol crystal samples. Apart from xylitol, other components, for instance, glucose and xylose were still present in the samples. These components could be inhibitory substances during the crystallization process [35].

According to Fig. 1, xylitol crystals formed by 70°C of evaporation temperature without crystal seeding tended to produce xylitol crystals with higher xylitol content. However, when the crystal seeding concentration was increased to 1%, the xylitol content remained constant and then decreased.

The effect of temperature also influenced the xylose content. The increase of temperature from 50°C to 70°C

could drop the amount of xylose as the impurity, and even no xylose was detected in the sample after evaporation. Conversely, the glucose content levelled off with the increase of evaporation temperature. Crystal seeding did not have any impact on the components of the final product in this research. It just affected crystallization time [2].

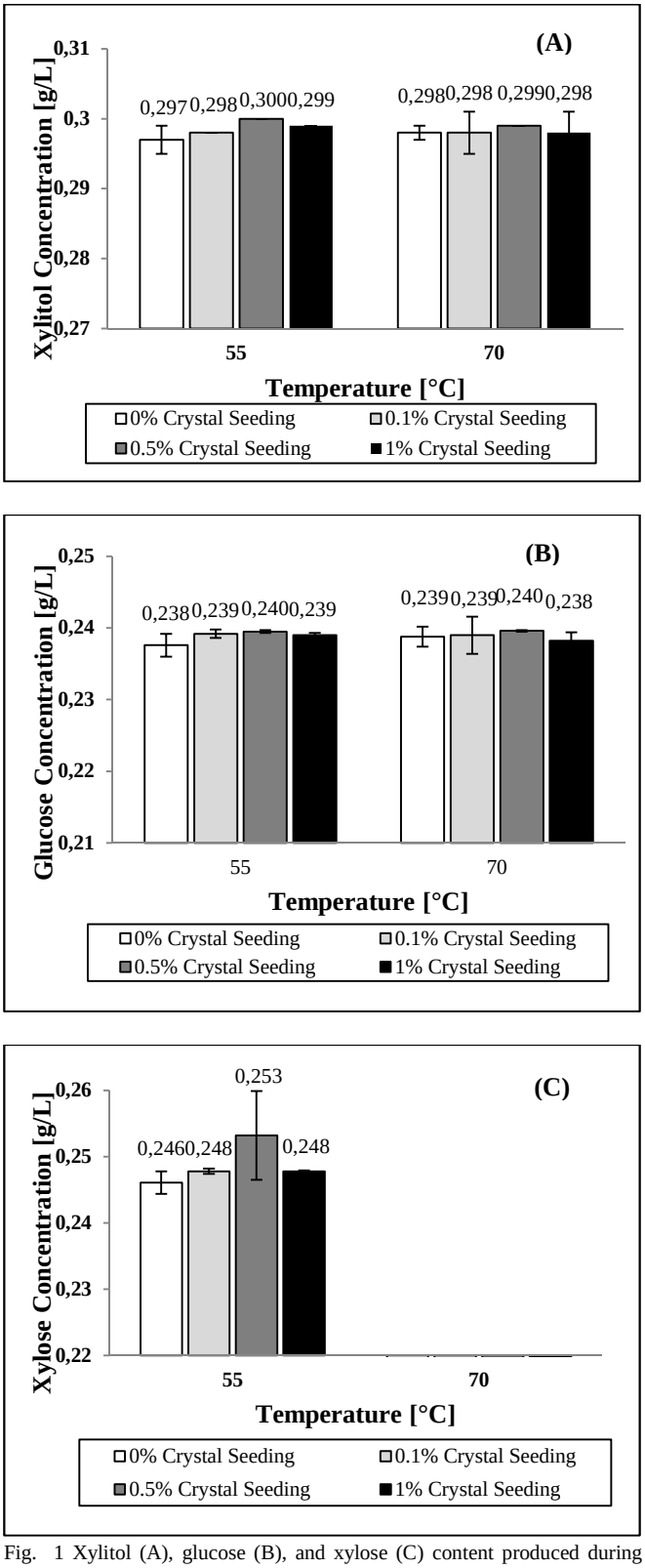

Fig. 1 Xylitol (A), glucose (B), and xylose (C) content produced during crystallization process at 55 and 70 °C using 0, 0.1, 0.5, and 1% crystal seeding

Although purification of solution using activated charcoal had been conducted, by-products still arose in the final product. Activated charcoal absorbed components such as phenolic compound, acetic acid, aromatic compounds, and colorant. The best-activated charcoal in purification was 4% activated charcoal with M1 type at 60°C [21]. This study gave the high decolorizations ratio (96%) and low xylitol loss ratio (4%). In comparison, the decolorizations and xylitol loss ratio was up to 50% and 25%, respectively, for activated charcoal with LH type [21]. It was concluded that types of activated charcoal also affected components of the final product. However, the xylitol component in crystals was still higher than residual sugars such as xylose and glucose, where the average glucose levels in crystals were 0.24 g/L and xylose 0.25 g/L.

D. Physicochemical Properties of Crystals

The factor that affected the melting point of crystal was the differences of crystals form. In previous research, it was reported that the stable crystal form melting at 61°C was found to be orthorhombic, whereas the metastable form melting at 61°C was monoclinic [33]. Furthermore, the presence of by-products, such as glucose and xylose, could increase the melting point of the product.

TABLE I
PHYSICOCHEMICAL PROPERTIES OF XYLITOL CRYSTALS

Sample		Melting Point [°C]	Caloric Value [cal/g]	Moisture Content [%]
T [°C]	Seeds [%]			
55	0	68 ± 0.00	2.74 ± 0.11	23.85 ± 2.19
55	0.1	70 ± 0.00	2.77 ± 0.03	24.54 ± 0.22
55	0.5	74 ± 0.00	2.79 ± 0.05	23.06 ± 1.51
55	1	73 ± 0.71	2.85 ± 0.02	24.43 ± 2.08
70	0	74 ± 0.71	2.87 ± 0.06	21.16 ± 0.89
70	0.1	73 ± 0.00	2.86 ± 0.04	21.97 ± 1.42
70	0.5	73 ± 0.00	2.89 ± 0.07	20.61 ± 0.44
70	1	73 ± 0.71	2.77 ± 0.09	21.93 ± 1.60
Sample		**Hygroscopicity [%]**	**Purity [%]**	**Solubility [%]**
T [°C]	**Seeds [%]**			
55	0	24.32 ± 1,59	29.67±0.25	99.33 ± 0.04
55	0.1	24.75 ± 0,49	29.81±0.07	99.35 ± 0.00
55	0.5	23.44 ± 2,04	29.94±0.06	99.38 ± 0.01
55	1	25.04 ± 1,25	29.85±0.03	99.87 ± 0.02
70	0	22.71 ± 2,67	29.77±0.12	99.45 ± 0.03
70	0.1	22.25 ± 1,06	29.81±0.31	99.05 ± 0.72
70	0.5	20.91 ± 0,07	29.86±0.03	99.53 ± 0.01
70	1	22.72 ± 0,57	29.76±0.29	99.62 ± 0.00

The caloric value of xylitol crystal commercial was 2.40 cal/g [34], which was lower than sugar (sucrose), 4.0 cal/g. All of the xylitol crystals in this study had a higher caloric value than commercial xylitol (Table I). Crystallization conducted at high evaporation temperature (70 °C) tended to produce the crystal xylitol with the higher caloric value than that of 55 °C. The purity level, the presence of inhibitory by-products, influenced the caloric value. The purer solution that would be crystallized was used; the higher caloric value of xylitol crystal was obtained. Thus, glucose and xylose present in the solution as inhibitors could decline the crystal purity of xylitol. Consequently, the caloric value of xylitol in this study was lower than that of sucrose (4 Cal/g).

The maximum moisture content for sugar crystals was 0.1 (% w.b) [34] while the moisture content of xylitol crystal samples was higher than commercial ones. The moisture content of xylitol crystal that formed by 70°C evaporation temperature, 20.61-21.97% was lower than 55°C ones, 23.06-24.54% (Table I) because crystallization in lower temperature would produce crystals with higher porosity. Moisture content was also affected by crystal form in which xylitol crystals produced in this research were sticky.

Hygroscopicity of commercial xylitol was lower than fructose, sorbitol, and corn starch in RH between 60-80% [27]. Hygroscopicity of a powder material was divided into 5 categories: non-hygroscopic (<10%), slightly hygroscopic (10.10-15%), hygroscopic (15.10-20%), very hygroscopic (20.10-25%), and extremely hygroscopic (> 25%) [44]. The hygroscopicity of xylitol crystal that was formed by 70°C evaporation temperature, 20.91-22.72%, is lower than 55°C ones, 23.44-25.04% (Table I) which was related to crystal form and porosity as reported above. The xylitol crystals of the test were sticky, owing to relatively large viscosity of the residual sugar such as glucose.

The effect of evaporation temperature and seeding on the physicochemical properties of xylitol crystals were investigated (Table I). All of xylitol crystal samples had low purity since its purity was lower than 98% [16] because the concentration of the solution was so high, 1155.56 g/L. The higher the concentration of the solution in crystallization, the crystallization yield was improved, but the purity decreased. In particular, crystallization was more quickly at 908.27 g/L xylitol than 630 g/L – 750 g/L, but the purity was the lowest [21]. Xylitol crystals that were formed by 70°C evaporation temperature tended to produce xylitol crystals with higher purity because the higher the temperature led to the simultaneous increase of purity of crystals [2].

The solubility of xylitol below 30°C was lower than that of sucrose. Above that temperature, the xylitol was more soluble than sucrose [33]. In this study, all samples gave a high solubility, above 99%, for all evaporation temperature. Xylitol was highly soluble in water and was difficult to dissolve in bioethanol [34]. Overall, the variation of crystal seeds did not impact the properties of xylitol crystals in this research. This happened because there was mixing step with commercial xylitol powder into the solution before the evaporation, so that the seeding step with xylitol crystals commercial after the evaporation did not have a significant impact on physicochemical properties of the final product. stated the phenomenon involved behind the addition of xylitol crystals in the metastable zone during the process of crystallization helped in preferential crystallization [22]. It was also reported that the inhibition to crystallize xylitol in the fermentation medium containing wheat straw hemicellulose hydrolysate was overcome by adding commercialized xylitol crystals to the medium [45].

E. The Yield of Xylitol Crystal

The theoretical yield of xylitol crystallization was 3.32 g xylitol/50 mL fermented broth or 0.07 g /mL. The yield of xylitol crystals formed by 70°C evaporation temperature tended to be higher, 0.0366-0.0380 g/L than 55°C ones, 0.0362-0.0271 g/mL (Table II). According to Table II, the xylitol crystals that formed by 55°C evaporation temperature produced more by-products such as glucose and xylose. In contrast, the xylitol crystals formed by 70°C did not contain xylose and contained more xylitol component.

The yield of xylitol crystallization was lower than the theoretical yield because the concentration of xylitol solution after the evaporation process was so high, 1155.56 g/L. [22] reported that the xylitol solution was concentrated at 55°C±5°C on xylitol crystal production until the concentration was 637.08 g/L with -20°C crystallization temperature to produce crystal with 43.37% of yield. In addition, [46] also reported that the highest yield (56%) could be obtained when the xylitol solution as fermentation result of hardwood hemicellulose hydrolysate was concentrated up to 730 g/L and crystallized at -5°C.

TABLE II
THE YIELD OF CRYSTAL SAMPLES

Sample		**Yield [g/mL]**
T [°C]	**Crystal Seeding [%]**	
55	0	0.0362 ± 0.0133
55	0.1	0.0366 ± 0.0025
55	0.5	0.0371 ± 0.0272
55	1	0.0371 ± 0.0158
70	0	0.0366 ± 0.0071
70	0.1	0.0370 ± 0.0184
70	0.5	0.0374 ± 0.0002
70	1	0.0380 ± 0.0079

The increasing xylitol concentration and the decreasing temperatures of crystallization improved xylitol recovery through crystallization [47] using synthetic solution. It was found that the optimal concentration of xylitol solution was 728 g/L with cooling temperature -6°C led to 54% of xylitol crystallization yield. It was supported by in the concentration of xylitol solution until 750 g/L in order to increase xylitol crystallization yield [21]. The yield of crystal xylitol obtained was 60% of clarified xylitol with purity up to 95%.

IV. CONCLUSIONS

The concentration of the solution after the evaporation process was so high, 1155.56 g/L that caused crystal form was sticky and affected on its moisture content and hygroscopicity. In this research, evaporation temperature and seeding percentage in the crystallization process affected the physicochemical properties of xylitol crystal OPEFB. The higher the temperature would increase porosity, purity and crystal yield. It could be seen that the evaporation temperature at 70 °C could produce crystal xylitol with higher caloric value and purity than that at 55 °C. However, this crystal xylitol gave lower hygroscopicity and moisture content. This was because the xylitol crystal using 70 °C of evaporation temperature had higher porosity. Furthermore, the seeding step did not make any crucial effect on physicochemical crystals in this research because xylitol commercials that were added in the mixing step were much higher than the seeding step. However, the optimization of xylitol purification was still needed in this research to obtain xylitol-rich crystals and higher yield.

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