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Research Article

Effect of drying methods on the physicochemical characteristics of dried mucilage from *Dictyophora indusiata*

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ABSTRACT

Dictyophora indusiata known as bamboo mushroom is an edible mushroom in Genus *Dictyophora*, Family *Phallaceae* that could produce highly viscous mucilage (DIM) encased in a peridium of egg-shaped immature fruiting body. In this study, DIM were dried by three different techniques; lyophilization, spray-drying and heat-drying. The physicochemical properties of dried DIMs including morphology, crystallinity, viscosity after reconstituted and swelling index were evaluated. The effect of mannitol as an additive in each process was also investigated. The dried DIMs showed amorphous nature with different morphology depended on the drying processes. The viscosity of 50%w/w fresh DIM solution was 10890±135.07 cps at 25 ºC. After reconstitution, the viscosity decreased. Lyophilization with mannitol was found to be the most suitable technique to maintain DIMs viscosity. The swelling index values of the dried DIMs from lyophilization and spray-drying techniques were clearly higher than other DIMs from heat-drying. Therefore, heat-drying may affect the structure conformation of substances in DIMs. The lyophilization and spray-drying were seem to be the promising methods. According to the physicochemical properties, especially high swelling ability and high viscosity nature, dried DIMs showed the possibility to be a novel pharmaceutical excipient.

1. INTRODUCTION

Dictyophora indusiata is a kind of edible mushroom in family *Phallacae*. It is well known in traditional Chinese food and medicine as veiled lady mushroom or bamboo sprouts. It has a conical to bell-shape cap where its mucilage and spores are located. The fruiting body of the mushroom is egg-shape before the phallic-like basidioma emerges over the course of a few hours. Its fruiting bodies provide high nutrients including, amino acids, metallic ions, vitamins and polysaccharides[1]. There were several studies reporting about bioactivities of its fruiting body such as, antioxidants[1], antimicrobial[2] and antitumor[3]. However, there was no report about the use of *Dictyophora indusiata* as a pharmaceutical excipient.

Besides the fruiting body, *Dictyophora indusiata* has a thick layer of mucilage under its peridium. The *Dictyophora indusiata* mucilage, so called DIM, shows high swelling ability in water, high viscosity and adhesive nature which are crucial properties in many pharmaceutical excipients. However, there are some limitations to use the DIM as freshly prepared mucilage due to its stability, purity and biological contamination problems. Therefore the effective drying process should be developed to preserve and maintain DIM properties.

The drying processes were proven to be one of the most significant parts influenced the physicochemical properties of natural product[4,5]. Among several techniques have been used, heat-drying or oven-drying was the most common one. However, significant quality changes of dried products often occur. Lyophilization and spray-drying are also the general procedures for drying natural products. Lyophilization is based on the dehydration by sublimation of a frozen product. The lyophilized products showed the less changes of physical properties compared to the heat-dried one[6]. Lyophilization was also an effective process for drying of some natural products to maintain their chemical properties[4,7]. However, lyophilization requires a long drying time, which leads to high energy consumption and high production cost. On the contrary, the spray-drying technique is a cost effective drying method. It has the characteristics of high temperature and short contact time hence, the products are not associated with prolonged heat. However, the major problems of spray-drying are stickiness and hygroscopicity of the dried products. The spray-drying was also successfully used for drying of natural products[8,9].

In this study, the DIM was dried by three different methods and characterized in order to get the appropriate drying method. Moreover, the primary properties of DIM were also investigated for the purpose of further development as a novel pharmaceutical excipient.

2. MATERIALS AND METHODS

2.1. Materials

Dictyophora indusiata was purchased from a local farm in Bangkok, Thailand. Mannitol was purchased from Merck (Darmstadt, Germany). All chemicals used in this study were analytical grade.

2.2. Preparation of DIM solution

The mucilages (DIMs) were isolated from the mucilage layer under the peridium of two weeks-old fruiting bodies. The DIMs were homogenized and diluted with deionized water to obtain 50% w/w solutions then vacuumed to remove bubbles.

2.3. Drying of DIMs

2.3.1. Lyophilization

Various concentration mannitol (0.5, 1, 2, 3, and 5% w/w) was directly added to 50% w/w DIM solutions as a cryoprotectant in the lyophilization process. The mixtures were frozen in a freezer (Scancool Snowbird Ultra-Freezer, Labogene, Denmark) at -80 ºC for 6 hours before lyophilization (Alpha 2-4 LCSplus, Christ, Germany) for at least 24 hours. The dried products were pulverized by a hand blender (MQ100, Braun, Germany). The dried powder, so call lyophilized DIMs, were then kept in a desiccator. The selected ratio of dried DIM/mannitol (1:1) by dried weight was used in the further physicochemical studies.

2.3.2. Spray-drying

Aqueous solutions containing 10% w/w DIM were spray-dried using a Büchi Labortechnik AG, Postfash, Switzerland) with a 0.7-mm nozzle orifice and sprayed condition as following: inlet temperature 150℃, nozzle air flow 40 mm, feeding speed 10 and aspirator 100%. The effects of mannitol on dried DIMs prepared by spray-drying process were also investigated. Mannitol was added into DIM solutions in dried DIM/mannitol, weight ratio of 1:1 as freeze-dried DIM/mannitol, before spray-dried. Spray-dried DIMs powders were separated with high performance cyclone then kept in a polypropylene tube under a desiccator.

2.3.3. Heat-drying

An appropriate amount of 50% w/w DIM solutions were poured into a glass petri dish before dried in a hot-air oven (FD 115, Binder, Germany) at 60 °C for 12 hours. The effects of mannitol on dried DIMs prepared by heat-drying process were also investigated in the same dried DIM/mannitol ratio as two previous methods. The dried products were carefully removed from the petri dish then kept in a desiccator.

2.4. Morphology

The morphology of dried DIMs was observed by scanning electron microscope (Mira3, Tescan, Czech Republic). The dried DIMs were fixed on SEM stubs before coated with a thin gold layer before investigation. The SEM micrographs were taken at a magnification of ×5000.

2.5. Fourier Transform Infra-Red (FTIR) spectroscopy

The FT-IR spectra of all samples were detected by Attenuated Total Reflection-FTIR (ATR-FTIR) (Nicolet 6700, Thermo Scientific, USA) in the range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. FTIR spectral parameters of the samples were obtained using a software package (OMNIC FT-IR Software, version 7.2a, Thermo Electron Corporation, USA).

2.6. NMR analysis

The ^{1}H NMR spectroscopic studies were carried out using a Bruker NEO™500 MHz NMR instrument at 500 MHz. All four samples as pure mannitol, DIM, spray dried DIM/mannitol (1:1) and freeze dried DIM/mannitol (1:1) were dissolved in deuterated water (D2O) which showed resonance at 4.70 ppm. Chemical shifts (Δδ) for the protons of mannitol were determined according to Eq. (1):

$$\Delta\delta = \text{DIM:mannitol} - \text{Mannitol} \quad (1)$$

2.7. PXRD analysis

PXRD analysis of all dried DIMs was done using the powder X-ray diffractometer (Model Miniflex II, Rikagu, Japan) at 40 kV, 40 mA over the range of 5–45° 2θ using Cu Ka radiation wavelength of 1.5406 Å.

2.8. Rheology and viscosity

To obtain the rheogram, 50% w/w DIM solution was sheared continuously at 25 °C with a maximum shear rate of 1.74 s^{-1} using a SC4-16 spindle (Model DV-II+, Brookfield, USA), and the corresponding forward and backward rheograms were recorded.

The viscosity of freshly prepared DIM solutions contained various amounts of mannitol and the reconstituted solutions of lyophilized DIM with mannitol were determined at 25 °C by a viscometer (Model DV-II+, Brookfield, USA) using a SC4-16 at the rotational speed of 6 rpm. Those lyophilized DIMs were reconstituted to their prior concentration before the measurement. Suitable amount of mannitol was selected for further study. The reconstituted viscosity of 0.5% w/w to 2% w/w dried DIMs (with or without mannitol) obtained by different methods in deionized water were also investigated.

2.9. Swelling properties

The swelling studies of mucilage were modified from the study of Muñoz[10]. Briefly, an accurate amount of dried DIMs were individually placed in a pre-soaked dialysis bags before weighing (W_0). The dialysis bags were immersed in 300 mL deionized water for 10 minutes. Then, the excess water was wiped out with cellulose paper and the dialysis bags were weighed (W_t). The swelling index was calculated by equation (2).

$$Swelling\ index = \frac{(W_t - W_0)}{Dried\ powder\ weight\ (mg)} \quad (2)$$

3. RESULTS AND DISCUSSION

3.1. Morphology

The DIMs isolated from the fresh mushrooms were clear-to-slightly yellowish viscous liquid. Drying processes extremely influenced on the characteristic of dried DIMs. Lyophilized DIMs were found to be a highly porous cake-like product, while the spray-dried DIMs were spherical particle and heat-drying process provided thin-film. Addition of mannitol remarkably affected morphology of dried DIMs from those three processes. Figure 1 showed the morphology under SEM of lyophilized DIMs with various ratios of mannitol. Lyophilized DIMs (Figure 1a) showed smooth-surface film. Addition of mannitol tremendously affected the lyophilized DIMs morphology. The needle-like crystal of lyophilized mannitol (Figure 1 g) were observed in all lyophilized DIMs. At low concentrations, the mannitol crystals embedded in the DIM film homogeneously. Increasing of mannitol concentration to an excess amount (i.e. higher than 3% w/w) could destroy DIM structure as shown in Figure 1e and 1f.

Figure 2 showed the morphology of dried DIM prepared by spray-drying (Figure 2a and 2b) and heat-drying (Figure 2c and 2d). Spray-dried DIMs were smooth-surface spherical particles with high size distribution ranged from nano- to micro size. Needle-shape crystals appeared on the particles surface when 1% w/w mannitol was added (Figure 2b), related to the crystal found on the spray-dried mannitol particles. The thin-films obtained by heat-drying showed scatter undissolved particles in their structure. These particles might be due to the

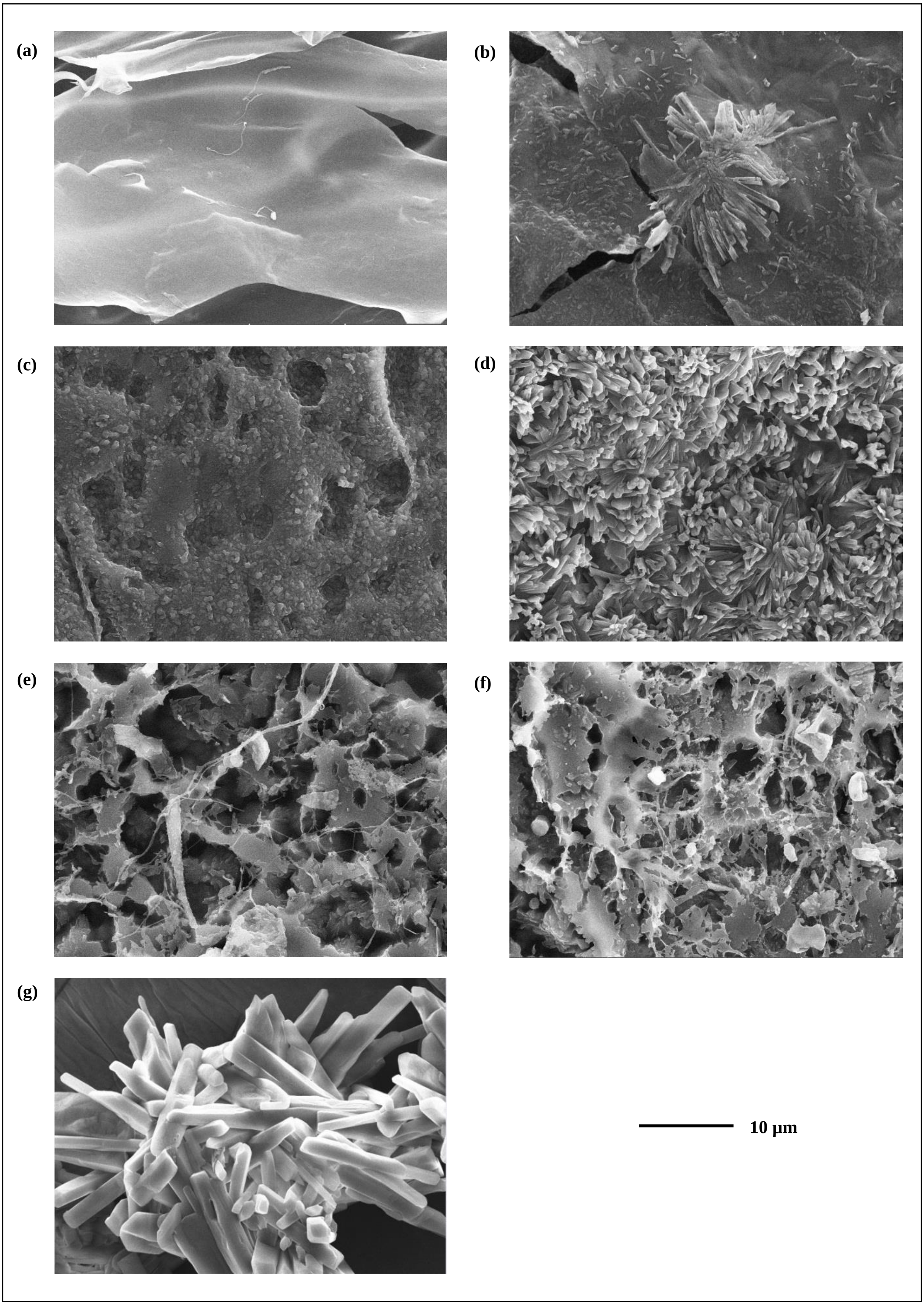

Figure 1. SEM images of lyophilized DIMs without mannitol (a), lyophilized DIMs with (b) 0.5% w/w, (c) 1.0% w/w, (d) 2.0% w/w, (e) 3.0% w/w, (f) 5.0% w/w mannitol, and lyophilized mannitol (g). The magnifications are 5000×.

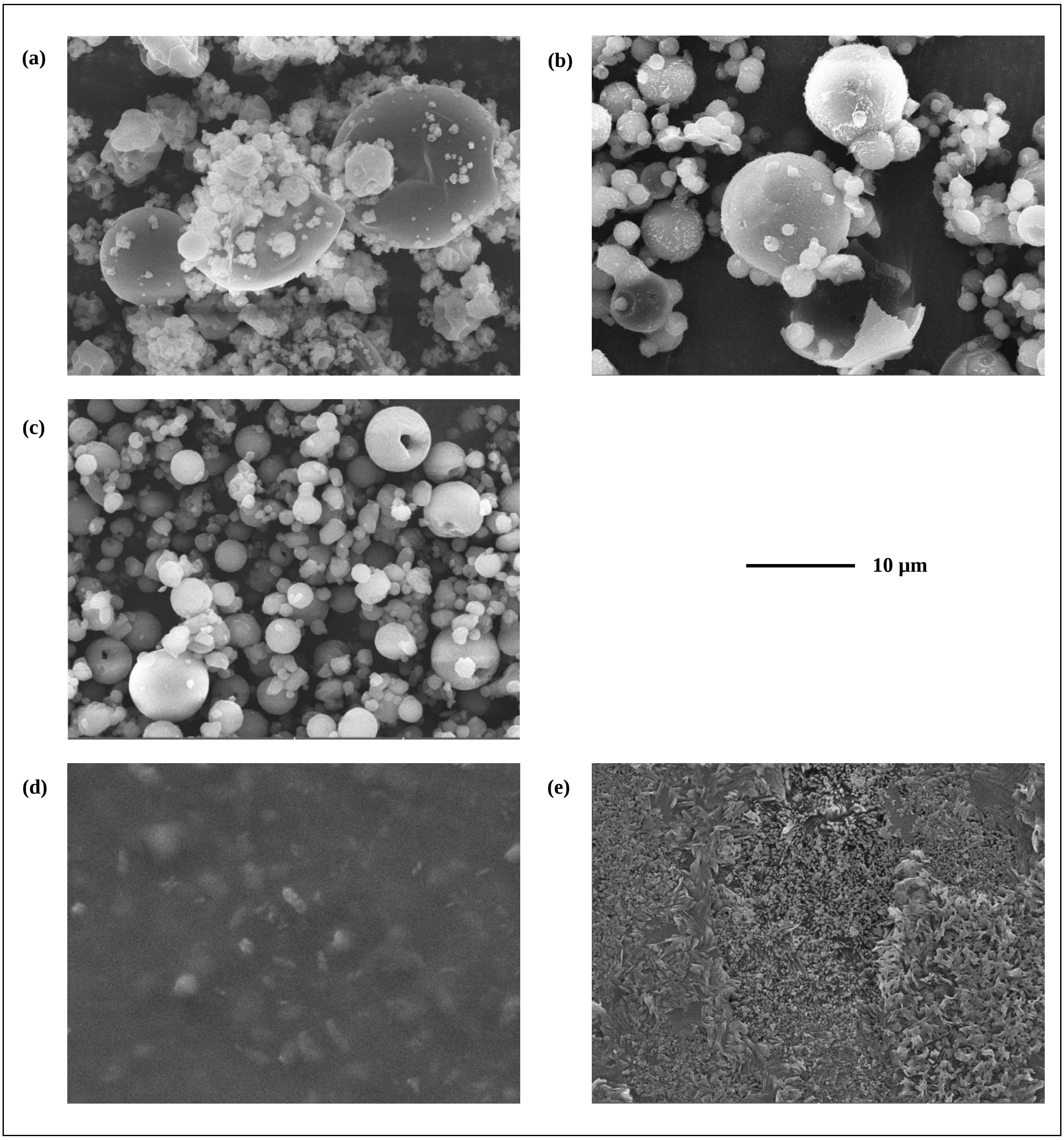

Figure 2. SEM images of dried DIMs obtained by spray-drying method; without mannitol (a), and with 1.0% w/w mannitol (b), spray-dried mannitol (c), dried DIMs obtained by heat-drying method; without mannitol (d), and with 1.0% w/w mannitol (e). The magnifications are 5000×.

hydrophobic part associated in the DIMs during segregation that mucilage from peridium. Similar to the previous two processes, the crystals of mannitol were also found in the DIM film consisted of mannitol.

3.2. Fourier Transform Infra-Red (FTIR) spectroscopy

The FT-IR spectra of lyophilized DIMs and spray-dried DIMs were shown in Figure 3a and 3b, respectively. There was no difference between the FT-IR spectra of the three samples by different drying methods. The spectrum of dried DIMs showed the characteristic peaks corresponding to cellulose. The broad peak at 3296 cm^{-1} is characteristic for stretching vibration of O-H and C-H bonds in polysaccharides. This peak includes also inter- and intra-molecular hydrogen bond vibrations in cellulose. The band at 2906 cm^{-1} is assigned to be C-H stretching vibration of hydrocarbon in polysaccharides. The absorption bands at 1597 was designated as C=O stretching cm^{-1}. The bands at 1409, and 1022 cm^{-1} belong to stretching and bending vibrations of C-O bonds in polysaccharides[11]. The weak bands at 1083.0 cm^{-1} and more intense 1022 cm^{-1} may also be assigned to C-O and C-C stretching vibrations of a pyranose ring.

With the presence of mannitol, there was no new absorption peak or shifting found from the FT-IR analysis, indicating no or minor interaction between DIMs and mannitol molecules. However, there were some differences in the FT-IR spectra between physical mixture and the dried DIM products. There was peak shifting at 1416 cm^{-1}, referred to O-H bending of an alcohol group. These observations suggested the possibility of weak physical interactions between DIMs and mannitol[12] or molecular level dispersion of mannitol in DIMs[13].

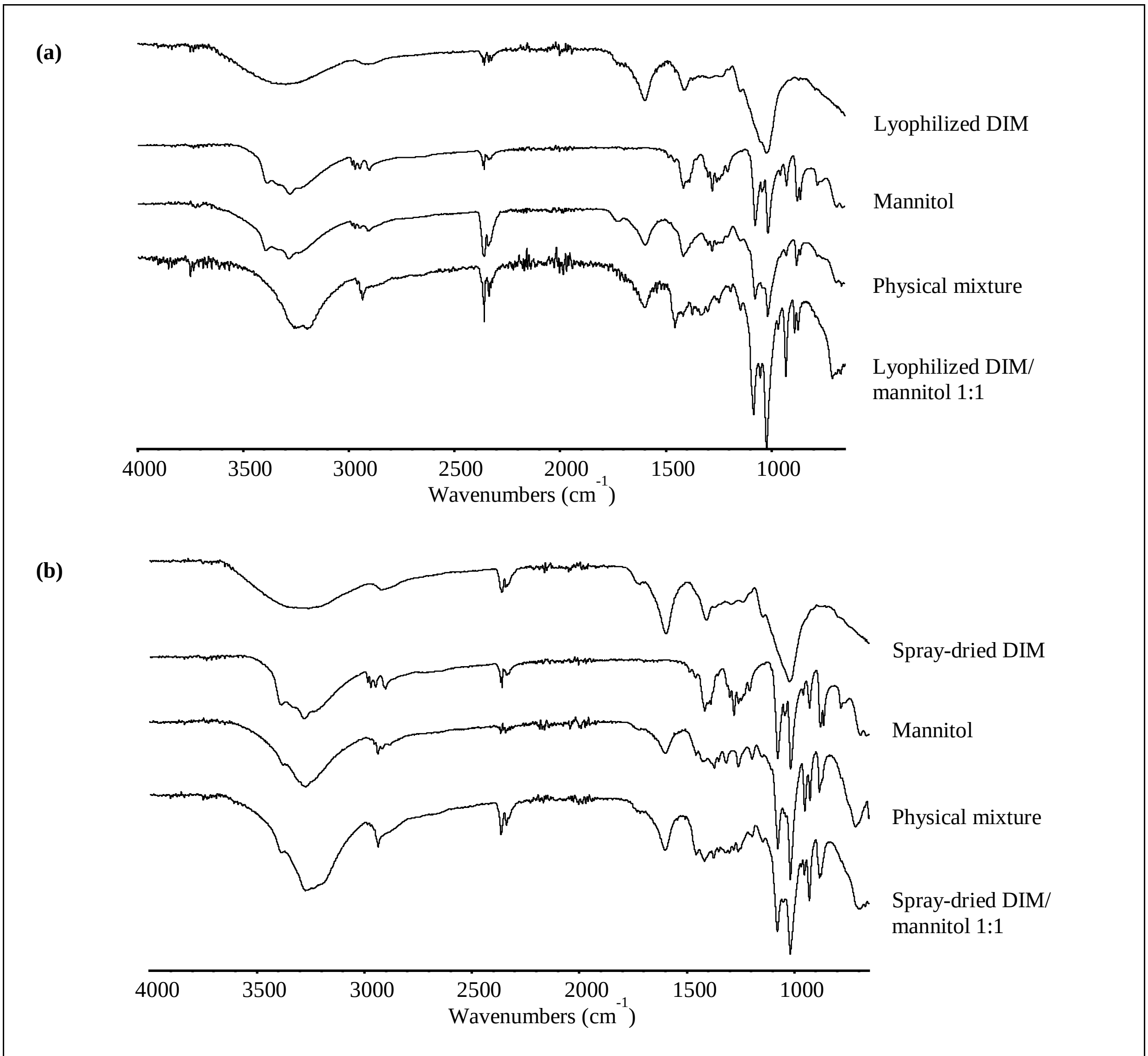

Figure 3. FT-IR spectra of redispersed DIMs prepared by lyophilization (a), and spray-drying (b).

3.3. ^{1}H NMR spectroscopy

Chemical shifts were determined from protons signals of mannitol because of low-intensity signals from pure DIM (data not shown). The arrangement of hydrogen atoms in mannitol was shown in Figure 4. The resonance signals of pure mannitol in ^{1}H NMR spectrum show doublet of doublets (dd) at 3.56-3.60 ppm, multiplet (m) at 3.65-3.68 ppm, doublet (d) at 3.70-3.72 ppm and doublet of doublets at 3.76-3.79 ppm assigned as M04, M01, M02 and M03, respectively. Chemical shifts of each peak (Table 1) indicated conformation change of mannitol during both drying processes. Since mannitol play as cryoprotectant agent, the higher chemical shifts from lyophilization, compared to spray drying, were observed.

Table 1. ^{1}H-chemical shifts (δ) corresponding to mannitol in spray-dried and lyophilized DIM/mannitol (1:1).

Resonance peaks	$\delta_{Mannitol}$ (ppm)	$\Delta\delta_{Lyophilized\ DIM\ :\ mannitol\ (1:1)}$ (ppm)	$\Delta\delta_{Spray\text{-}dried\ DIM\ :\ mannitol\ (1:1)}$ (ppm)
M04a	3.7891	0.0035	0.0022
M04b	3.7836	0.0038	0.0027
M04c	3.7655	0.0035	0.0022
M04d	3.7602	0.0038	0.0027
M01a	3.7154	0.0040	0.0028
M01b	3.6984	0.0036	0.0024
M02a	3.6807	0.0038	0.0026
M02b	3.6754	0.0042	0.0031
M02c	3.6686	0.0039	0.0027
M02d	3.6632	0.0042	0.0030
M02e	3.6515	0.0036	0.0024
M02f	3.6462	0.0040	0.0029
M03a	3.5993	0.0036	0.0024
M03b	3.5870	0.0037	0.0025
M03c	3.5757	0.0036	0.0025
M03d	3.5636	0.0037	0.0025

3.4. PXRD analysis

The dried DIM crystallinity was investigated by powder x-ray diffraction method, as shown in Figure 5. All dried DIMs exhibited halo-pattern indicating their amorphous nature, while mannitol showed very high crystallinity. PXRD pattern of mannitol revealed the characteristics peak at 2θ values of 10.5, 14.6, 18.8 and 23.4°[14]. The PXRD of dried DIMs containing mannitol still showed the sharp peaks with straight base lines referred to crystallinity, however, the extremely decrease of intensity was observed. The reduction of crystallinity was more clearly observed in case of lyophilized DIMs. These might be due to the more decreasing of mannitol crystallinity after lyophilized.

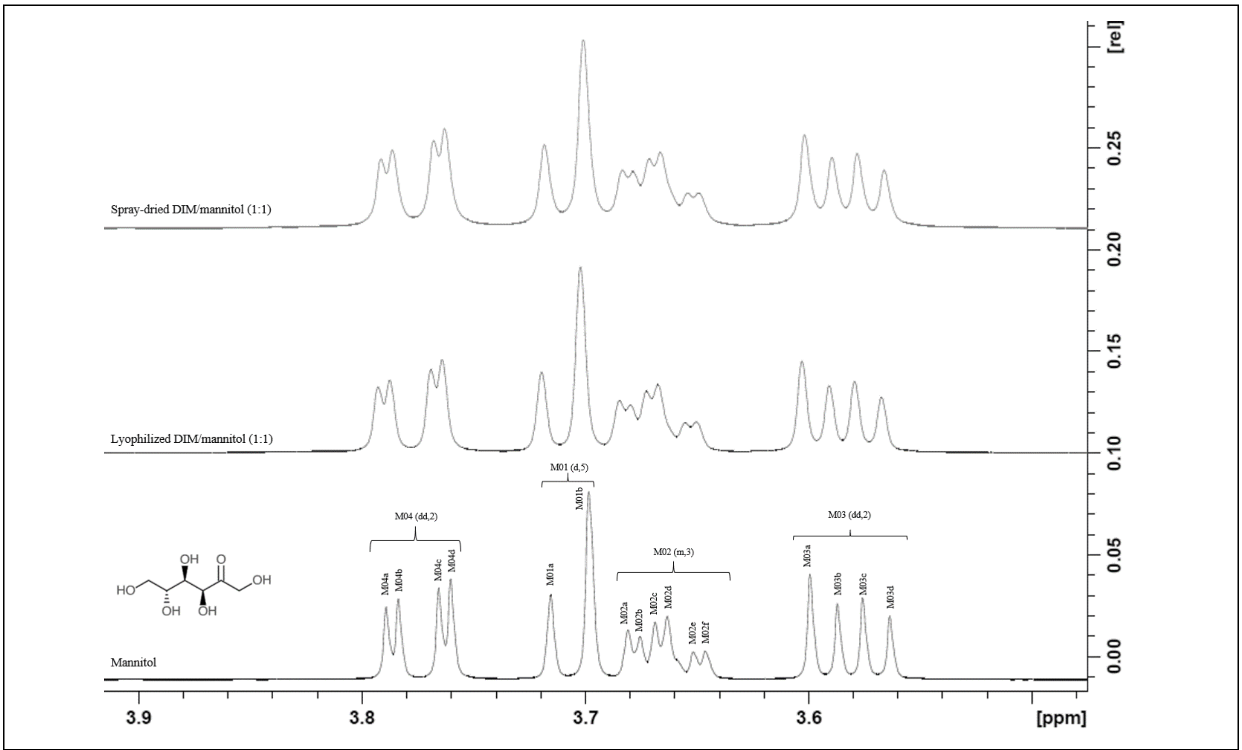

Figure 4. NMR spectra of lyophilized DIM: mannitol (1:1), spray-dried DIM: mannitol (1:1), and mannitol.

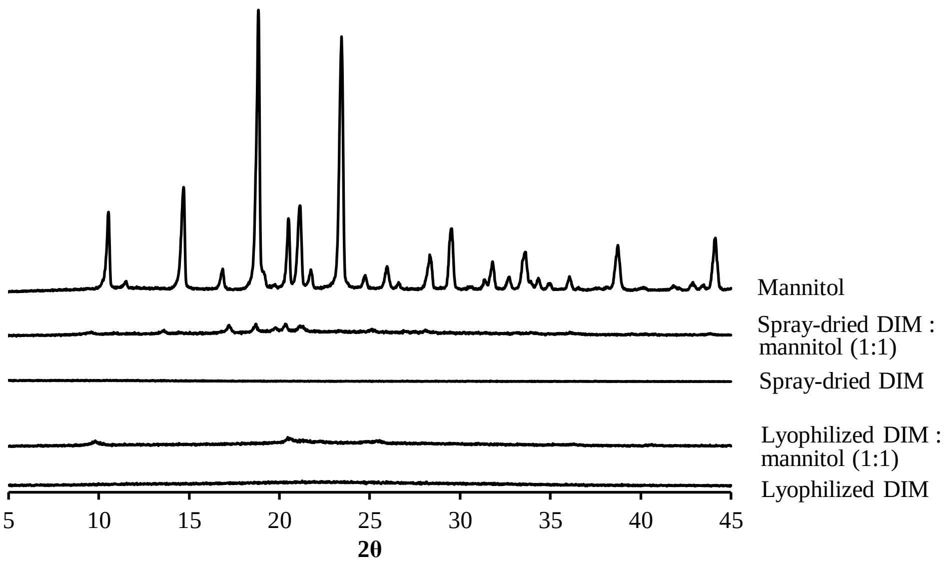

Figure 5. PXRD patterns of lyophilized DIM, lyophilized DIM: mannitol (1:1), spray-dried DIM: mannitol (1:1), and mannitol.

3.5. Rheology and viscosity

The thixotropic characteristic of DIM solution was revealed (Figure 6). It was found that viscosity of 50% w/w DIM solution decreased when shear rate was increased. Addition of mannitol and the drying process did not significantly affect the rheology of both freshly and redispersed DIMs (data not shown). However, the drying process and presence of mannitol tremendously affected viscosity of the redispersed dried DIMs.

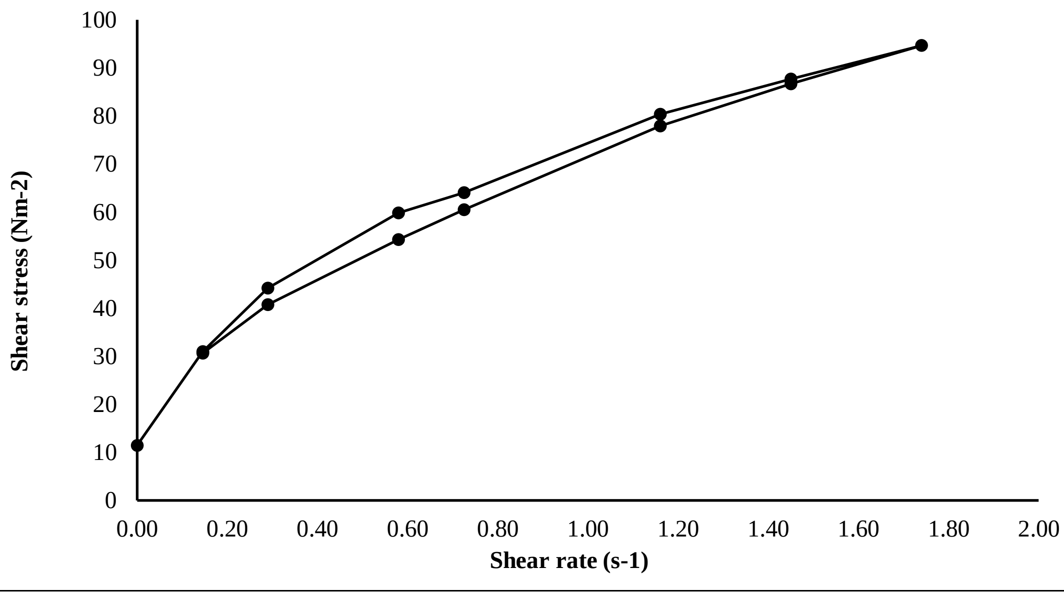

Figure 6. Rheogram of 50% w/w DIM solution.

Although lyophilization was a typical procedure used in mucilage drying, the formation of ice crystals during freezing can destroy the polysaccharides structure of mucilages[15]. Even polysaccharides from plants had been reported to potentially be a cryoprotectant[16] by themselves, lyophilized DIM showed cake shrinkage during the drying process. Also these dried DIM provided the poor ability to maintain its viscosity since the reconstituted viscosity extremely decreased. The addition of a suitable amount of mannitol as a cryoprotectant clearly improved the reconstituted solution viscosity. The usage of 0.5% - 2.0% w/w mannitol could better maintain reconstituted viscosity of DIMs than that with no mannitol, while the viscosity of reconstituted DIMs with 3.0% - 5.0% w/w mannitol decreased (Figure 7). These were in agreement with the morphology from SEM. Excess amount of mannitol could disrupt the fibrous structure or water solubility of dried DIMs hence, the viscosity decreased[17].

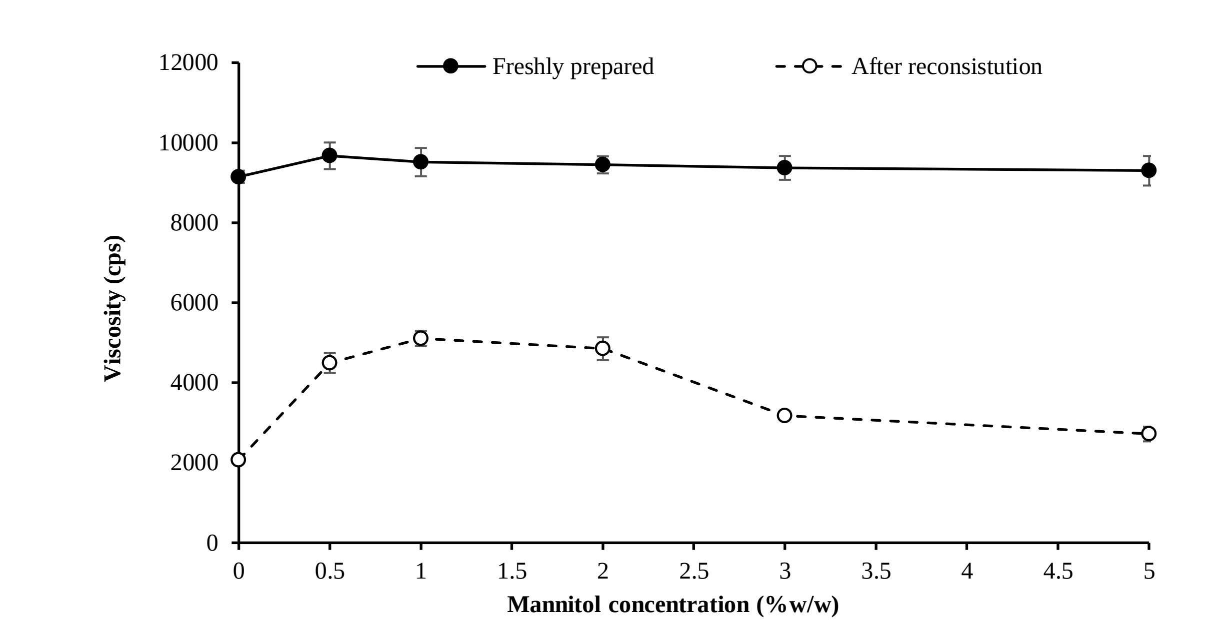

Figure 7. Viscosity of freshly prepared 50% w/w DIMs solutions with various concentration mannitol compared to the reconstituted solutions.

Aqueous DIM solution with 1% w/w mannitol, according to dried DIM/mannitol ratio of 1:1, was selected for further studies due to its performance in maintaining DIMs viscosity. The viscosity of redispersed DIM solutions (with or without mannitol) increased regarding to concentration in a nonlinear manner (Figure 8). Without mannitol, spray-dried DIM provided a higher reconstituted viscosity compared to lyophilized and heat-dried DIMs especially when concentration of redispersion DIM was higher than 1% w/w. The presence of mannitol caused extremely decreasing of redispersed viscosity from spray-dried and heat-dried DIMs meanwhile increased of viscosity was observed in lyophilized DIM with mannitol. Therefore, the addition of mannitol might not be suitable for drying processes, either flash-heat or intense-heat.

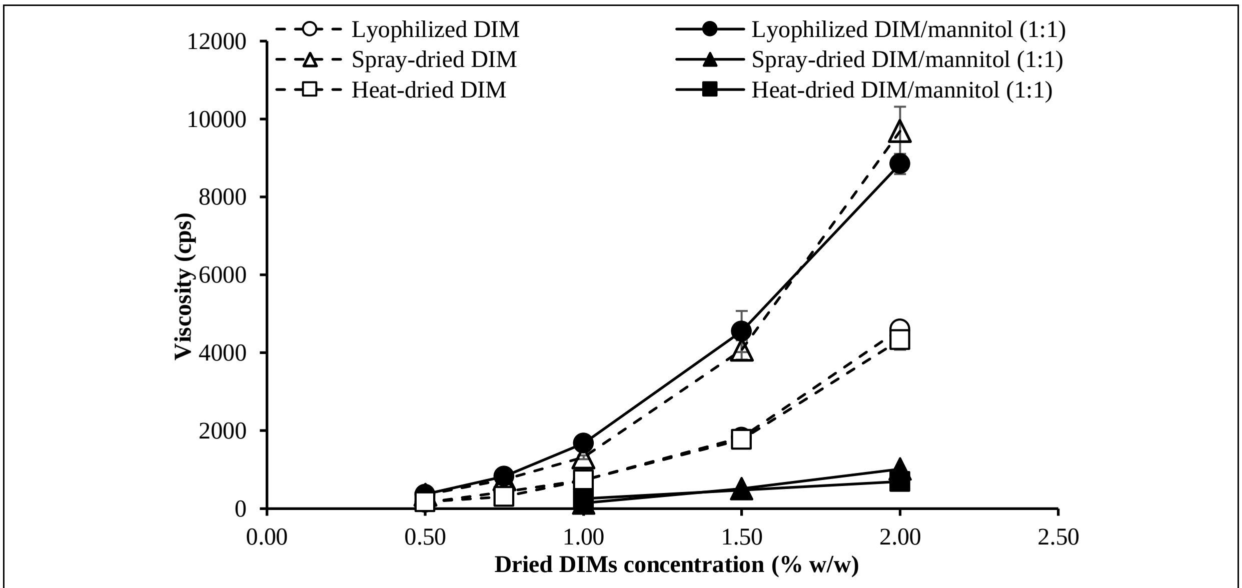

Figure 8. Viscosity of redispersed DIMs prepared by heat-drying, lyophilization and spray-drying**.**

3.6. Swelling index

Swelling index of dried DIM was found to depend on the drying technique and the addition of mannitol (Table 2). Because weight gain corresponding to the rate of hydration[18], the lowest swelling value from heat-dried DIM indicated the slowest hydration rate. According to SEM photogram, heat-dried DIM was solidified to be dense film which interrupted water uptake from the test procedure. While spherical particles and fibrous structure of spray-dried and lyophilized DIMs, respectively, showed high surface area which promoted water uptake resulting in high values of swelling index. Moreover the presence of mannitol in lyophilized and spray-dried DIMs delayed penetration of water resulting in the decreasing of swelling index values suggested the chemical reaction between mannitol and DIMs corresponded to the result from conformation analysis (section 3.3). Water absorption of heat-dried DIM/mannitol (1:1) increased dramatically (i.e. 1.5 times compared to heat-dried DIM). It was possible that instantly dissolved mannitol provided spaces which facilitate water penetration into dried DIMs then hydration rate was induced. These swelling properties of dried DIM/mannitol emphasized the role of mannitol and drying techniques on dried DIMs.

Table 2. Swelling index values of dried DIMs prepared by different drying techniques.

Drying techniques	Swelling index	
	Without mannitol	With mannitol
Lyophilization	23.25	20.46
Spray-drying	37.86	22.61
Heat-drying	16.85	26.84

4. CONCLUSIONS

DIM is a novel mucilage with little published information on its characterization. In this study, different drying techniques (lyophilization, spray-drying and heat-drying) for mucilage extracted from peridium of immature *Dictyophora indusiata* (DIM) were studied. Lyophilization and heat-drying showed a benefit over spray-drying in terms of yield but lyophilized DIMs required mannitol as a cryoprotective agent while brittle dried films were obtained from heat-drying which were not impractical for further studies. FT-IR and PXRD analysis showed that dried DIMs from spray-drying and heat-drying was insignificantly difference. Chemical reaction between DIM and mannitol was confirmed by NMR. Furthermore the effect of mannitol on physical properties of dried DIMs was studies. It was found that a suitable amount of mannitol (as 1% w/w) influenced on fibrous structure of lyophilized DIM. Crystals of mannitol were also observed on the particle surface of spray-drying DIM. Presence of mannitol decreased redispersed viscosity of spray-dried DIM but inversely affected on viscosity from lyophilized DIMs. Also mannitol interfered water absorption of lyophilized and spray-dried DIMs resulting in the decreasing of swelling index values. Finally, the promising drying technique for DIM was lyophilization and a suitable amount of cryoprotectant required to maintain redispersed viscosity and swelling property. Thus, the lyophilized DIMs showed an ability to be developed as a novel pharmaceutical excipient.

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Conflicting Interests
The authors declare that they have no conflict of interests.

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Ethical approval
None to declare.

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