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Application of Oven-Dried Durian Seed Powder and Freeze-Dried Durian Seed Powder on Extending Probiotics Longevity

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Abstract*— Durian seed is often wasted but it can be utilized as a biopolymer and encapsulating agent for probiotics. This is good since it is a natural and sustainable biopolymer source. However, durian seed contains a lot of impurities that may hinder the biopolymer's functional properties. This study aims to extract gum from durian seeds, subject them to oven-drying and freeze drying to obtain durian seed powder, study both oven-dried and freeze-dried durian seed's characteristics and the potential to use them to enhance the growth of probiotics. Freeze-dried durian seed powder has higher yield percentage, higher water holding capacity, and higher oil holding capacity than oven-dried durian seed powder. Freeze-dried durian seed powder also has more pores and more uniform structure compared to oven-dried durian seed powder, which can be seen through scanning electron microscope. Both oven-dried durian seed powder and freeze-dried durian seed powder manage to support probiotics' growth. The probiotics' concentration can be seen to increase after 3 days of incubation upon encapsulation via dried durian seed flour for both encapsulated probiotics with oven-dried durian seed powder and encapsulated probiotics with freeze-dried durian seed powder. Freeze-dried durian seed powder support probiotics' growth better than oven-dried durian seed powder since the concentration of probiotics after being encapsulated with freeze-dried durian seed powder is higher than the concentration of probiotics after being encapsulated with oven-dried durian seed powder. Based on observation via scanning electron microscope, encapsulated probiotics with freeze-dried durian seed powder also has better appearance, less wrinkles and less dents compared to encapsulated probiotics with oven-dried durian seed powder.

***Keywords*— durian seed; biopolymer, drying techniques, sustainable**

I. INTRODUCTION

Hydrocolloid is from combination of Greek terminology 'hydro' and 'kolla' which mean water and glue respectively [1]. Scientists describe hydrocolloid as a compound that is rich with hydroxyl groups (-OH) and multiple functional groups such as amino groups and carbonyl groups that may display a certain amount of water affinity, leading to its unique colloidal behaviours [2]. The hydrocolloid description is supported by other research papers which state that hydrocolloids are a complex polysaccharides chain that are easily spread in water, water soluble, and susceptible to swelling reaction with water [3]. Their hydrophilic nature and behaviour allow it to be further utilized as thickening agent, emulsifier, coating agent and stabilizer, gelling agent, texture improver and many more [4]. Hydrocolloids can be obtained from multiple sources be it from plants, animals and microbial origins. For example, gum Arabic, pectin, starch, cellulose, gum karaya, gum tracanth, pectin, guar gum, locust bean gum, gelatine, chitosan, caseinate, whey protein, soy protein, egg white protein, xanthan, agar-agar, carrageenan, alginate, and gellan gums [4], [5]. The multiple sources and uses of hydrocolloids make it easily available to be utilized in food industry [4]. Most fruit seed can serve as food hydrocolloid but there are not many studies yet covers the potential of durian seed as a hydrocolloid.

Food preservation technique is a method or application used to extend the food product's shelf-life and retain its quality to certain degree [6]. Food preservation technique is important in modern society to help solving logistics issue and develop trade across the globe [7]. In addition to, it also enhances food security and food availability to the mass [8], [9]. Food waste can be reduced and minimized through food preservation technique since it slows down food spoilage. A study has shown that food waste from fresh food products is 6 times the amount of frozen food products among Austrian households [10]. There are a lot of food preservation techniques such as drying, pasteurization, fermentation, vacuum packaging, pickling, refrigeration, and etc [11], [12]. Drying technique removes water content from food product making less water available for microbial growth. Dried food product is less susceptible to microbial spoilage, making it last longer [13], [14]. There are a lot of drying techniques available as of current, but hydrocolloid is sensitive to heat treatment, hence there is necessity to study the suitable drying technique for durian seed.

Hydrocolloid is made up of complex sugar polymers and contain certain amount of fibre [3]. Therefore, hydrocolloid can help probiotics growth since it serves as prebiotics and can prevent probiotics loss due to heat treatment such as drying to certain degree [15]. Probiotics are sensitive to heat since it is mostly composed of protein. Hence, high exposure to heat may result in denaturation of probiotics [1], [16]. High content of fibre and complex sugar in durian seed may indicates its potential as a hydrocolloid source [17], [18].

Durian is a native fruit to Southeast Asia region and a popular fruit especially, in Malaysia, Indonesia, Thailand and Philippines [19]. It is also well received and high in demand for global export especially to China [20]. It is the top 3 fruits with highest revenue and commercial value, amounting to RM9.29 billion according to data from Department of Agriculture Malaysia [21]. The edible part of the durian is its flesh while the durian seed and husk, which composed 2/3 of the whole durian is often discarded [22]. The extracted durian seed gum however does have sugar group like rhamnose, galactose, and glucose. In addition to, it has a rich amount of carbohydrate, fiber, moisture and protein, as shown in Table 1 [22]. Durian seed powder also shows a certain degree of viscosity upon interaction with water molecules [23], [22].

Table 1. Nutritional Content Of Durian Seed Powder [22].

Parameter	Content (%)
Protein	6.0 ± 0.13
Fat	0.4 ± 0.03
Ash	3.1 ± 0.03
Moisture	6.5 ± 0.05
Carbohydrate	73.9 ± 0.02
Fiber	10.1 ± 0.10

A study has found that durian seed powder contains oligosaccharide and shows better prebiotic potential compared to commercial inulin [24]. Moreover, durian is shown to have polyphenol content and antioxidant [25]. Another research records that durian seed gum can encourage the growth of lactic acid bacteria and safekeep stable shelf-life of lactic acid bacteria [26].

Application of dried durian seed to provide optimum surrounding for growth of probiotics is possible via encapsulation method. This is made possible since the durian seed itself holds prebiotics potential, has high affinity towards water and has a rich amount of nutrient content itself. This paper aims to discuss further on application of both oven-dried durian seed powder and freeze-dried durian seed powder on extending longevity of probiotics. There has been a lot of studies made on hydrocolloid as prebiotic source but there are not many studies yet made on durian seed potential. This study aims to extract gum from durian seeds, process them via oven-drying and freeze drying to obtain durian seed powder, study both oven-dried and freeze-dried durian seed's characteristics and the potential to use them to enhance the growth of probiotics.

II. THE MATERIAL AND METHOD

A. *Extraction of Durian Seed Powder*

Figure 1 shows summary of methods used in this experiment. Firstly, Durian Kampung (*Durio zibethinus*) samples were used in this study. Durian seeds were removed and cleaned properly with distilled water. Then, they were partially air dried before further process. Durian seeds were then washed again, cut into tiny pieces, air-dried again, and finally milled [27]. Durian seed powder is the purified to remove its oil content via treatment with a mixture of hexane and isopropanol with ratio of 60:40 respectively at room temperature (25 ± 1°C). The obtained durian seed powder is then centrifuged at 1400 g for 15 min and soaked in ethanol solution for 2 hours. Next, it is vacuum filtered and then soaked in 1% aqueous acetic acid for 1.5 hour at room temperature. The mixture is then filtered with Nylon cloth filter and precipitated with 95% ethanol. The precipitated durian seed powder is then washed thrice with absolute ethanol (99%). Finally, it is oven-dried at temperature of 40°C, overnight [23]. This extraction was carried out in triplicate (n = 3).

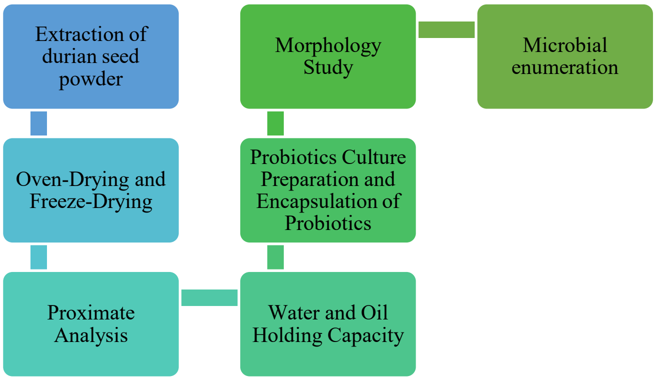

Figure 1. Flow diagram of method used in this experiment

B. Oven-Drying and Freeze-Drying

Durian seed powder solution (10% w/v) is first homogenized before subjected to oven-drying process at 105°C for 3 hours. It is then milled and sieved with a 1.0 mm sieve, before storage in chiller for further steps [28].

Another durian seed powder solution (10% w/v) is first homogenized before subjected to freeze-drying process. It was pre-frozen at -20°C for 24 hours followed by freeze-drying at -50°C for 48 hours. It is then milled and sieved with a 1.0 mm sieve, before storage in chiller for further steps [28].

C. Proximate Analysis of Oven-Dried Durian Seed Powder and Freeze-Dried Durian Seed Powder

Proximate analysis of both oven-dried durian seed powder and freeze-dried durian seed powder is carried out via methods based on AOAC to find out the nutritional value of the dried durian seed powder from moisture, ash, crude protein, crude fat and crude fiber contents [29]. Paired t-test is carried out to evaluate the different significance between proximate composition of oven-dried durian seed powder and freeze-dried durian seed powder.

D. Water Holding Capacity and Oil Holding Capacity of Oven-Dried Durian Seed Powder and Freeze-Dried Durian Seed Powder

Water holding capacity and oil holding capacity (WHC and OHC) for both oven-dried durian seed powder and freeze-dried durian seed powder were evaluated based on procedures by researcher with modifications [30]. The samples (10% w/v) were carefully weighed into a clean conical flask and were mixed rigorously with distilled water/oil using a warring mixer for 1 minute. The samples were let to stand for 30 minutes at ambient temperature, which were then centrifuged at speed of 5000 rpm for 30 minutes. The free water or oil (supernatant) was recorded directly from the graduated centrifuge cuvette. The absorbed water/oil was converted to weight (in grams) by multiplying with the respective density (water, 1g/ml and soybean oil, 0.924g/ml). The water and oil absorption capacities will be recorded in grams of water/oil absorbed per gram of dried durian seed powder.

Water holding capacity = (Swollen sample weight (g)-Sample weight (g))/(Sample weight (g))

Oil holding capacity = (Oil adsorbed sample weight (g)-Sample weight (g))/(Sample weight (g))

E. Probiotics Culture Preparation and Encapsulation of Probiotics

A colony of *S. thermophilus* and *L. d. bulgaricus* were prepared and cultured overnight in MRS media. They served as the inoculum during fermentation. After fermentation, the entire culture medium was taken for freeze-drying to encapsulate the probiotics culture with oven-dried durian seed powder and freeze-dried durian seed powder as encapsulating agent [31]. 5ml of sunflower oil is added to each sample before freeze-drying to facilitate encapsulation process. The encapsulated probiotics, in powdered form, were collected and stored in a polyethene bag and kept at 4°C before further analysis [26], [32].

F. Morphology Study of Encapsulated Probiotics

Encapsulated probiotics with oven-dried durian seed powder and freeze-dried durian seed powder were evaluated and observed using Scanning Electron Microscope (SEM). The powder was spread thinly on a carbon tape and was coated with gold. Images were taken at a voltage of 5kV and magnification of x500 [26].

G. Microbial Growth of Encapsulated Probiotics

This is the final step as shown in Figure 1. 10 mg of encapsulated probiotics powder with oven-dried durian seed powder and encapsulated probiotics powder with freeze-dried durian seed powder were administered in 1 ml of 0.5% sterile saline water. Additional probiotics strains were also added together in the sterile saline water. The proper serial dilutions were performed, and the colonies were enumerated on MRS agar. A blank was prepared by enumerating the probiotics strains on MRS agar without ongoing any encapsulation process. The plates were incubated at 30°C overnight. The number of probiotics colonies formed on MRS agar with encapsulated probiotics powder with oven-dried durian seed powder and encapsulated probiotics powder with freeze-dried durian seed powder were counted every 24 hours and recorded [26].

III. RESULTS AND DISCUSSION

A. Extraction of Durian Seed Powder,Oven-Drying and Freeze-Drying

The oven-dried and freeze-dried durian seed powder produced upon extraction, purification and drying processes are as shown in Figure 2 and Figure 3. Dried durian seed powder yield obtained for oven-dried durian seed powder is 49.83% ± 1.14% while for freeze-dried durian seed powder is 53.24% ± 0.35%.

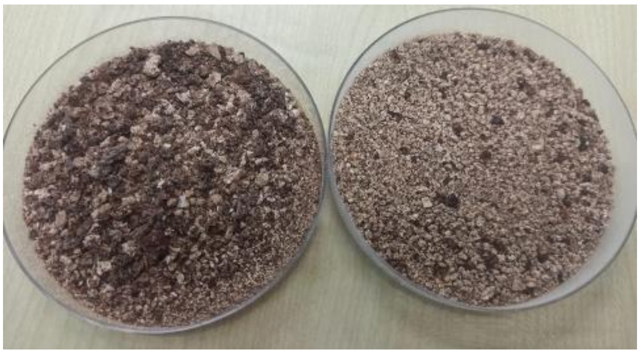

Figure 2. Oven-dried durian seed powder

Figure 3. Freeze-dried durian seed powder

The mass yield of freeze-dried durian seed powder (53.24% ± 0.35%) is higher than oven-dried durian seed powder (49.83% ± 1.14%). Freeze-drying process eliminates moisture via sublimination compared to oven-drying process which depends on thermal convection via hot air. Freeze-drying process can maintain volatile and heat sensitive compounds in durian seed powder while oven-drying process cannot maintain those substances as much as freeze-drying process can [33]. Hence, the mass yield of freeze-dried durian seed powder obtained is higher than oven-dried durian seed powder.

B. Proximate Analysis of Oven-Dried Durian Seed Powder and Freeze-Dried Durian Seed Powder

Based on data shown in Figure 4 and Table 2, freeze-durian seed powder has smaller moisture content percentage, 13.00% compared to the moisture content percentage of oven-dried durian seed powder, 32.75%. Freeze-durian seed powder has bigger mineral content percentage, 3.77% compared to the mineral content percentage of oven-dried durian seed powder, 2.59%. Freeze-durian seed powder has bigger fiber content percentage, 7.47% compared to the fiber content percentage of oven-dried durian seed powder, 2.35%. Freeze-durian seed powder has bigger fat content percentage, 8.42% compared to the fat content percentage of oven-dried durian seed powder, 5.82%. Freeze-durian seed powder has bigger carbohydrate content percentage, 67.00% compared to the carbohydrate content percentage of oven-dried durian seed powder, 56.30%.

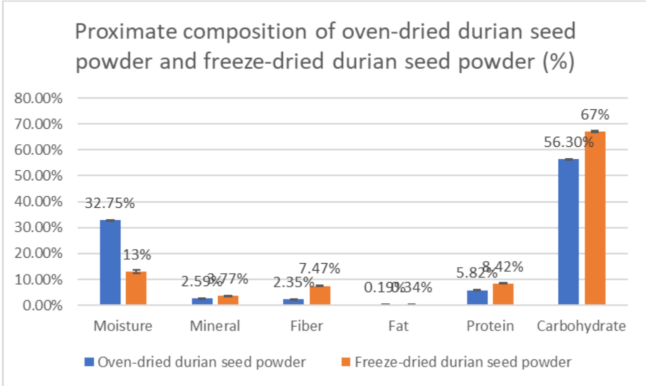

Figure 4. Proximate composition of oven-dried seed powder and freeze-dried durian seed powder (%)

Table 2. Proximate Composition Of Oven-Dried Seed Powder And Freeze-Dried Durian Seed Powder (%)

	Oven-dried durian seed powder	Freeze-dried durian seed powder
Moisture	32.75% ± 0.2%	13.00% ± 0.7%
Mineral	2.59% ± 0.1%	3.77% ± 0.1%
Fiber	2.35% ± 0.1%	7.47% ± 0.1%
Fat	0.19% ± 0.1%	0.34% ± 0.1%
Protein	5.82% ± 0.2%	8.42% ± 0.2%
Carbohydrate	56.30%± 0.2%	67.00%± 0.3%

Paired t-test is carried out, and it is found that the p-value between oven-dried durian seed powder and freeze-dried durian seed powder is lower than α value (0.05) for moisture, mineral, fat and protein content percentage. This shows significant difference between the mentioned composition of oven-dried durian seed powder and freeze-dried durian seed powder. However, p-value between oven-dried durian seed powder and freeze-dried durian seed powder is higher than α value (0.05) for fibre content percentage. This indicates no significant difference between the fibre of oven-dried durian seed powder and freeze-dried durian seed powder.

Freeze-dried durian seed powder has lower moisture content percentage than oven-dried durian seed powder. However, freeze-dried durian seed powder has higher mineral, fiber, fat, protein, and carbohydrate content percentage than oven-dried durian seed powder.

Freeze-dried food product retains as much nutritional content possible while getting its moisture content removed in a much larger percentage relatively to oven-dried food [33]. The high loss of nutritional content of oven-dried durian seed powder is highly due to the high temperature involved during oven-drying process, which caused degradation [34], [35]. Moreover, high temperature during oven-drying can also expose protein content in the durian seed powder to denaturation, which leads to large decrease in protein content percentage of oven-dried durian seed powder. In addition to, presence of moisture, amine groups of amino acid in the protein composition and reducing sugar in the durian seed powder, with addition of heat during oven-drying process, eventually contributes to Maillard reaction which further lower down protein content percentage of oven-dried durian seed powder [35], [36]. Maillard reactions are induced when amino groups available on protein, peptides, and amino acids, under presence of moist heat, react with carbonyl from reducing sugar [37], [38]. It is important to note, Maillard reactions can cause significant changes regarding food quality parameters such as organoleptic properties, colour and functionality [37].

C. *Water Holding Capacity and Oil Holding Capacity of Oven-Dried Durian Seed Powder and Freeze-Dried Durian Seed Powder*

Oven-dried durian seed powder has lower water and oil holding capacity (155.5 g/100g and 198.7 g/100g) than freeze-dried durian seed powder (352.2 g/100g and 392.2 g/100g).

Paired t-test is carried out, and it is discovered that the p-value between oven-dried durian seed powder and freeze-dried durian seed powder is lower than α value (0.05) for water and oil holding capacity. This proves significant difference between the water and oil holding capacity of oven-dried durian seed powder and freeze-dried durian seed powder.

Oven-dried durian seed powder has lower water and oil holding capacity (155.5 g/100g and 198.7 g/100g) than freeze-dried durian seed powder (352.2 g/100g and 392.2 g/100g). The data from this study differs with another study performed by another group of researchers [23]. He extracted gum from durian seed and found out that freeze-dried durian seed gum has lower water holding capacity than oven-dried durian seed gum, but higher oil holding capacity. This phenomenon occurs due to different setting of extraction method and experimental conditions of carbohydrate study [23].

Some researchers studied about water holding capacity of dried fruit peels and point out that freeze-dried orange peels, mango peels and prickly pear peels have better water holding capacity compared to other dried fruit peels with different high heat drying processes [39]. Water holding capacity of a food product can be affected by change in its microstructure after drying process. The changes in dried food's microstructure occur due to thermal damage and contact hours with heat during drying process [33], [40]. Freeze-dried food products have uniform and tiny pores which enable efficient water and oil holding whereas oven-dried and hot air-dried food products have less uniform and bigger pores [33]. Therefore, water and oil holding capacity of freeze-dried durian seed powder are higher and better than water and oil holding of oven-dried durian seed powder.

Table 3. Water Holding Capacity of Oven-Dried Durian Seed Powder and Freeze-Dried Durian Seed Powder

Oven-dried durian seed powder	Freeze-dried durian seed powder
(155.5g/100g) ±3.61g	(352.2g/100g) ±2.19g

Table 4. Oil Holding Capacity of Oven-Dried Durian Seed Powder and Freeze-Dried Durian Seed Powder

Oven-dried durian seed powder	Freeze-dried durian seed powder
(198.7g/100g) ±4.45g	(392.2g/100g) ±11.97g

D. *Probiotics Culture Preparation and Encapsulation of Probiotics*

The encapsulated probiotics using oven-dried durian seed powder and freeze-dried durian seed powder obtained are as seen in Figure 5. The colour of encapsulated probiotics with freeze-dried durian seed powder appears to be a bit bright while the colour of encapsulated probiotics with oven-dried durian seed powder appears to be a bit dark.

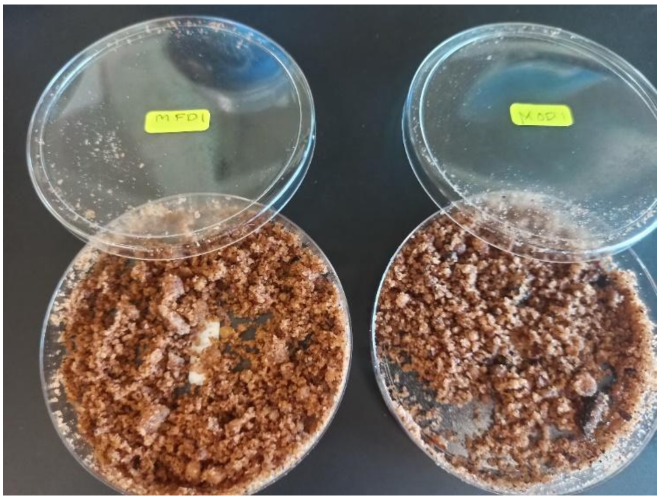

Figure 5. Probiotics encapsulated with freeze-dried durian seed powder (left) and oven-dried durian seed powder (right)

E. *Morphology Study of Encapsulated Probiotics*

Figure 6 and Figure 7 show the scanning electron microscopic (SEM) images of encapsulated probiotics with oven-dried durian seed powder and encapsulated probiotics with freeze-dried durian seed powder. Encapsulated probiotics with oven-dried durian seed powder appears to have numerous irregular pores, wrinkles and dents compared to encapsulated probiotics with freeze-dried durian seed powder.

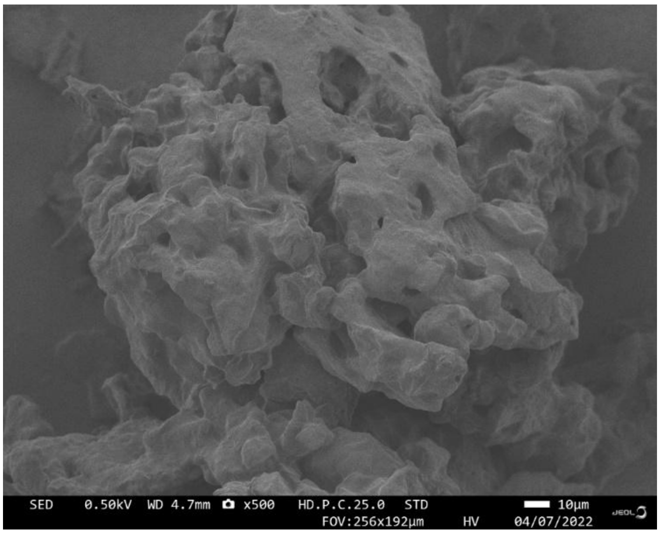

Figure 6. Scanning electron microscopic image of encapsulated probiotics with oven-dried durian seed powder (magnification x500)

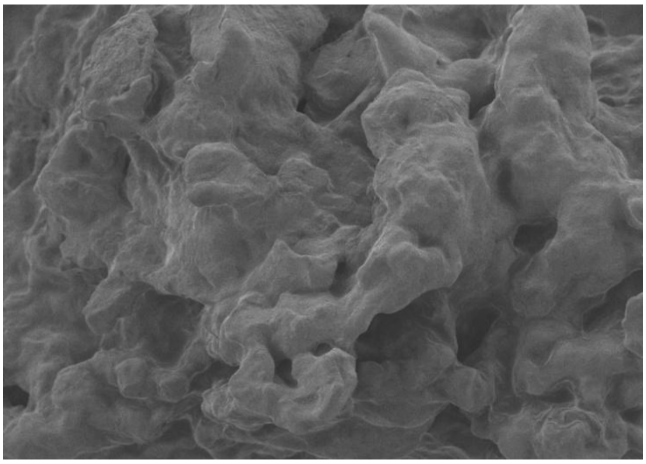

Figure 7. Scanning electron microscopic image of encapsulated probiotics with freeze-dried durian seed powder (magnification x500)

F. *Microbial Growth of Encapsulated Probiotics*

The probiotics used are *Streptococcus thermophilus (S. thermophilus)* and *Lactobacillus delbrueckii subsp. bulgaricus (L. bulgraricus)*. The data of the microbial growth of encapsulated probiotics from this experiment is as shown in Figure 8. The initial concentration of probiotics before encapsulation process is 7.55 log CFU/mL. The growth of probiotics which are encapsulated with oven-dried durian seed powder is 7.73 log CFU/mL after 24 hours, which then increased to 8.47 log CFU/mL after 48 hours, and 9.58 log CFU/mL after 72 hours. The growth of probiotics which are encapsulated with freeze-dried durian seed powder is 7.91 log CFU/mL after 24 hours, which then rose up to 8.65 log CFU/mL after 48 hours, and 10.69 log CFU/mL after 72 hours. The concentration of probiotics encapsulated with oven-dried durian seed powder after 72 hours, 9.58 log CFU/mL is lower than the concentration of probiotics encapsulated with freeze-dried durian seed powder after 72 hours, 10.69 log CFU/mL.

Previous studies have shown that probiotics can survive for up to 24 months in vacuum conditions at temperatures as low as 4°C. However, at 30°C, probiotics typically only last for 3 months [41]. This finding show that dried durian seed powder can be utilized as a good encapsulating material for probiotics as well as their nutrient source to support their growth. This aligns with the findings of a group of scientists [26] who also discovers the potential of durian seed as nutrient source for *L. plantarum*, which is also a probiotic which proves beneficial for gastrointestinal tract. The study used samples from durian seed and extracted it as durian seed gum. The durian seed gum is used to encapsulate *L. plantarum* and incubated for a few days. The findings from the study showed that *L. plantarum* concentration increase after incubation upon being encapsulated with durian seed gum [26].

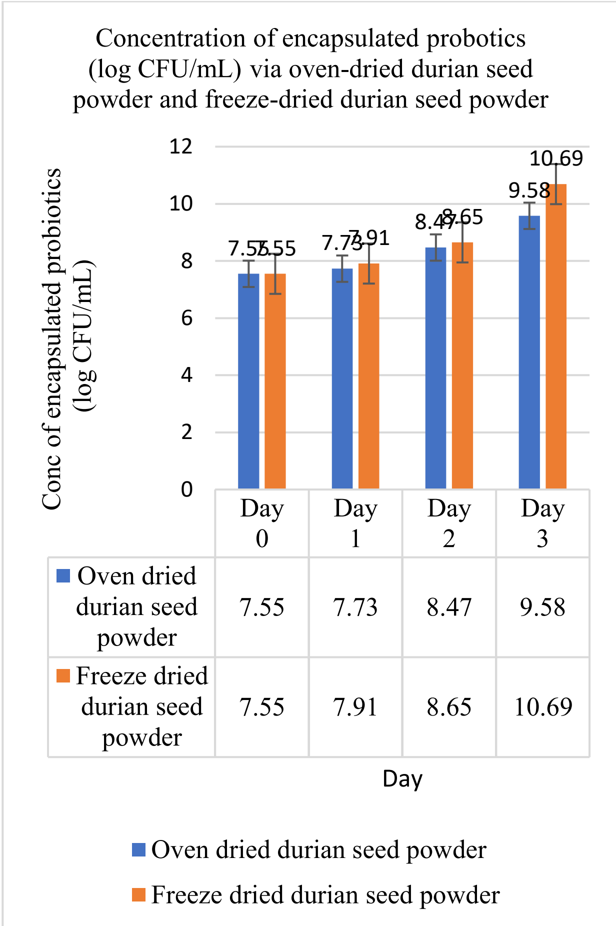

Figure 8. Concentration of encapsulated probotics (log CFU/mL) via oven-dried durian seed powder and freeze-dried durian seed powder

IV. CONCLUSION

Durian seed powder can extend probiotics longevity to a certain degree.

Freeze-drying process is better than oven-drying process in preparing dried durian seed powder since freeze-drying process retains the durian seed's functional properties better than oven-drying process does. Freeze-dried durian seed powder has higher yield percentage, higher water holding capacity, and higher oil holding capacity than oven-dried durian seed powder.

Freeze-dried durian seed powder also has more pores and more uniform structure compared to oven-dried durian seed flour, which can be seen through scanning electron microscope.

Durian seed powder can be used as encapsulating material for probiotics to support probiotics' growth. Both oven-dried durian seed powder and freeze-dried durian seed powder manage to support probiotics' growth. The probiotics' concentration can be seen to increase after 3 days of incubation upon encapsulation via dried durian seed flour for both encapsulated probiotics with oven-dried durian seed powder and encapsulated probiotics with freeze-dried durian seed powder.

Freeze-dried durian seed powder support probiotics' growth better than oven-dried durian seed powder since the concentration of probiotics after being encapsulated with freeze-dried durian seed powder is higher than the concentration of probiotics after being encapsulated with oven-dried durian seed powder. Encapsulated probiotics with freeze-dried durian seed powder also has better appearance, less wrinkles and less dents compared to encapsulated probiotics with oven-dried durian seed powder.

Future research could explore the application of findings available here to be used as reference to study characteristics and potential of other sources of hydrocolloid available since there are a lot of fruits and plant-based hydrocolloids in Malaysia, such as, banana, papaya, pineapple, jackfruit and many more.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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