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Physicochemical property of oil palm leaves and utilization of cellulose microfiber as probiotic encapsulant

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Abstract. *Pato U, Ayu DF, Riftyan E, Restuhadi F, Pawenang WT, Firdaus R, Rahma A, Surono IS, Jaswir I. 2021. Physicochemical property of oil palm leaves and utilization of cellulose microfiber as probiotic encapsulant. Biodiversitas 22: 2937-2944.* The vast land of oil palm (*Elaeis guineensis*) in Indonesia has a huge potential for oil palm solid waste, which can be used for various human needs. The physiochemical analysis performed was proximate analysis and fiber content, FTIR and X-ray diffraction analysis, an in vitro test on viability and resistance to acid and bile during storage at room and refrigerated temperatures. The main content of oil palm leaves was carbohydrates, especially fiber and followed by ash, protein, and fat. Fiber from oil palm leaves is mainly composed of lignin followed by cellulose and hemicellulose. X-ray diffraction analysis showed that the crystal index of cellulose from oil palm leaves was 10.1%. FTIR analysis showed that the enormous absorption value, which was the stretching vibrations of the -OH group ranging from 2919.17 to 2914.82 cm^{-1}. Cellulose microfiber from oil palm leaves maintained the viability of *L. fermentum* InaCC B 1295 for up to 28 d of storage at room and refrigerated temperatures. The survival of strain B1295 at low pH and presence of bile was very high, characterized by a decrease in the number of cells by less than 0.5 log CFU/mL during storage of 35 d at room and refrigerated temperatures. Therefore, Cellulose microfiber from oil palm leaves has the potential to be used as an encapsulant for probiotics.

Keywords: Cellulose, CMF, oil palm leaves, physicochemical properties, probiotic

INTRODUCTION

Oil palm (*Elaeis guineensis*) is among the best known and most extensively cultivated plant families, especially in Indonesia and Malaysia. Many standard products and foods are derived from oil palm, making them one of the most economically important plants. Indonesia is the largest palm oil-producing country globally, with an area of 14,724,600 ha and a CPO production of 45.8 million tonnes in 2019. With a vast oil palm plantation, the potential for palm oil waste is also very abundant, namely empty fruit bunch, oil palm trunk, oil palm frond, and oil palm leavess. The report shows that for one oil palm tree that is already producing, only 10% of oil will be produced, and the remaining 90% is biomass in the form of trunk, frond, leaves, empty fruit bunches, and palm oil shells (Abdullah and Sulaim 2013). Oil palm biomass waste has shown the potentiality of producing various types of value-added products such as medium density panels, blockboard, laminated veneer lumber, mineral-bonded particleboard, plywood, chipboard, and chipboard thermoset and thermoplastic composites, nanocomposites, pulp, and paper manufacturing. Conversion oil palm trunk can be converted into plywood, particleboard, and rubberwood (Khalil et al. 2010; Dungani et al. 2013).

In Indonesia, oil palm leaves (OPL) has been used as raw material for making animal feed and compost (Rizali et al. 2018; Daryono and Alkas 2017). However, OPL also can be used as raw material for making cellulose microfiber (CMF) because they contain a lot of cellulose. Cellulose microfiber (CMF), also called microfibrillated cellulose, is cellulose that undergoes fiber separation treatment into microfibrils with a diameter range of 10-100 nm and a length of several micrometers. In addition, CMF has characteristics, including specific surface area, high strength, and stiffness, low weight, biodegradable and renewable properties (Risnasari et al. 2012). These characteristics make cellulose microfibrils have good mechanical properties, so they have the potential to be used in the composite, automotive, pulp and paper, electronics, paint and coatings industries, films, papermaking additives, thickening agents for food and cosmetic products, and various medical applications (Fahma et al. 2010; Chinga-Carrasco 2011; Lavoine et al. 2012).

In the food industry, CMF can be used as an encapsulation material for probiotic and bioactive compounds in food. Encapsulation is aimed at maintaining probiotic viability with a minimum amount of 10^7 log CFU/mL when it passes through the digestive tract to perform its therapeutic function (Sarvari et al. 2014; Abbasiliasi et al. 2017). The encapsulant materials can be

selected from a variety of natural and synthetic polymers, such as carbohydrates in the form of starch, dextrins, pectins, sucrose, cellulose, chitosan, alginates, and carrageenan, while lipids are in the form of wax, paraffin, monoglycerides, and diglycerides, as well as proteins in the form of milk, gluten, casein. , gelatin, and albumin (Ayoub et al. 2019; de la Cruz Pech-Canul et al. 2020). Encapsulation with different encapsulating materials has successfully been shown to protect probiotic bacteria in many fermented dairy products (Abghari et al. 2011). Probiotics' viability in all food products is affected by many intrinsic and extrinsic aspects such as oxygen, post acidification in fermented products, pH, storage temperature, production of hydrogen peroxide, and processing conditions (Tripathi and Giri 2014). The purposes of the present study were to characterize the physic-chemical properties of cellulose from OPL as raw material for cellulose microfiber and to evaluate the hydrogel CMF as an encapsulant on the viability and probiotic properties of *Lactobacillus fermentum* InaCC B1295 during storage at room and refrigerated temperatures.

MATERIALS AND METHODS

Study area

The study was conducted in the Laboratory of Agricultural Product Analysis, Faculty of Agriculture, Universitas Riau, Pekanbaru City, Riau Province, Indonesia (0°25'-0°45' N and 101°14'-101°34' E).

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Raw materials

The materials used in this research were solid waste such as OPL and OPF, from palm oil variety Tenera were obtained by PT. Multi Plasma Sejahtera, Bandar Sikijang District, Pelalawan Regency, Riau Province.

Characterization of oil palm solid waste

Characterization of OPL and OPF included analysis of moisture, ash, and fiber content (Gravimetric method), protein content (Kjeldahl method), fat content (Soxhlet method), and carbohydrates are calculated by difference (AOAC, 2012). In addition, hemicellulose, lignin, and cellulose contents were analyzed according to Jung et al. (2015).

Isolation of cellulose

Cellulose was isolated using the same method as Fahma et al. (Fahma et al. 2010). The OPL and OPF were cut to 0.5-1 cm length before they were exposed to any treatment. Hemicellulose was extracted by bleaching the fibers with 6 wt% KOH solution for 12 h and rinsing with deionized water until pH 7. Next, the extracted fibers were soaked $NaClO_2$ solution under acidic conditions (pH 4-5) for 5 h, then washed with deionized water to remove lignin. The resulting cellulose fibers were stored in a refrigerator before further analysis.

FTIR analysis

Cellulose was characterized using an FTIR spectrometer to study the chemical composition and bonding present. The FTIR spectra of cellulose samples were recorded using the Thermo Nicolet Nexus FTIR spectrometer coupled with an ATR detector (Thermo Scientific Nicolet iS10). All spectra were recorded in the spectral range 7800-350 cm^{-1} at 4 cm^{-1} with 32 scans. Analysis of the spectral data was performed by using OMNIC version 6.0 (Thermo Scientific Nicolet) software.

X-ray diffraction analysis

XRD analyzes were carried out to determine the molecular groups and degree of crystallinity in cellulose. XRD testing used the X'Pert PRO PANalytical instrument machine. The XRD test conditions used CuK α radiation with a wavelength (λ = 0.154). The power and current used in the XRD test are 40 kV and 30 mA. The diffraction rate ranges from 5º to 50º, with an increment rate of 2θ-0.02º. The crystallinity index (Ic) of the biocomposite film was calculated according to the following formula:

$$Ic = [(I002-I\ am) / I002] \times 100$$

Where: I002 is a diffraction intensity of 2θ-22.6º which indicates the crystalline region of the material. Iam is the diffraction intensity 2θ-18º which indicates the amorphous region of the material (Yasim-Anuar et al. 2017).

Preparation of cellulose microfiber

The preparation of CMF was carried out using the Lestari method (2016). First, OPL was cut into small pieces with a length of 0.5-1 cm, then washed with water, then boiled in boiling water (100ºC) for 1 h which they were filtered. The OPL that has been boiled was then washed with water until clean, then dried in an oven at 60ºC for 4 h. Next, the dry fiber was put into a beaker, 1000 mL of 6% KOH was added then soaked at room temperature for 12 h. After that, the fibers are washed with water for three rinses. Furthermore, the washed fibers are soaked using a hypochlorite solution for 5 h, and then the empty bunches are filtered and washed with water to a neutral pH (pH 7). Furthermore, OPL was dried and mashed in a blender until smooth, then filtered using an 80 mesh sieve. Finally, the cellulose flour of OPL was sent to Nano Center Indonesia, Tangerang Banten, Indonesia, to be processed into cellulose microfiber (CMF). CMF was processed by milling the cellulose flour using a Planetary Ball Mill machine at a speed of 8,000 rpm for 60 min with a run time of 15 sec and a rest time of 2 min to avoid sample damage caused by heat during milling. The results of the milling were then sieved to obtain CMF.

Viability, acid and bile resistance of *Lactobacillus fermentum* InaCC B1295

Preparation of MRS Broth medium

Preparation of MRS broth (MRB) medium was carried out by weighing 13.78 g of MRSB put in a beaker glass and dissolved with distilled water to a volume of 250 mL. The solution was distributed into test tubes, with each test tube containing 5 mL and closed using a setup. Then put in

an autoclave to be sterilized at a temperature of 121°C, at 1 atm for 15 min. Finally, the MRSB medium is ready to be used.

Preparation of MRS Agar medium

Preparation of MRS agar (MRSA) medium was carried out by weighing 68.2 g of MRSA, and then it was put into Erlenmeyer and dissolved with distilled water to a volume of 1000 mL and stirred. Next, the media was heated on a hot plate and stirred using a magnetic stirrer until homogeneous. Furthermore, sterilization was carried out by autoclave at a temperature of 121°C, 1 atm for 15 min. Finally, the homogeneous MRS agar solution was poured into ± 15 mL sterilized Petri dishes. The pouring of the media is carried out in laminar airflow. Then the petri dish containing the media is closed and allowed to solidify.

Activation of Lactobacillus fermentum *InaCC B1295 culture*

The *Lactobacillus fermentum* InaCC B1295 culture was activated by inoculating one loop needle into a test tube containing 5 mL MRSB medium and then stirring with vortex. Media was then incubated at 37°C for 24 h in an incubator so that the active culture stock was obtained, marked by a change in the color of media to become cloudy. The active cultures were stored in the refrigerator and ready to use.

Separation of cells and supernatant

The active culture of *L. fermentum* InaCC B1295 was separated between cells and supernatant by centrifuge at 4500 rpm for 15 min. First, the supernatant is discarded, then the cells are washed 2 times using sterile distilled water until clean cells are obtained. Then the cells are removed plus a phosphate buffer with a ratio of 1: 1 (w/v), then put in a clean container and stored at 4°C refrigerators.

Preparation of sterile microfiber cellulose hydrogel

A sterile CMF hydrogel was prepared by first making 8% polyvinyl alcohol (PVA). Weighed 96 g of PVA, then added 1104 mL of distilled water and then heated until dissolved at 100 ° C using a magnetic stirrer hot stirrer. The PVA solution was allowed to cool at room temperature. After that, 8% PVA was mixed with and CMF by inserting 250 g of CMF in 250 mL of PVA, then heating it with a hot plate magnetic stirrer assisted until the CMF dissolved utterly to form a CMF hydrogel. Then the CMF hydrogel was measured for pH and viscosity with a pH meter and viscometer, then sterilized by autoclave at 121°C with 1 atm pressure for 15 min. The sterile CMF hydrogel is cooled and ready for use as a lactic acid bacteria (LAB) encapsulant (Fung et al. 2011).

Preparation of encapsulated lactic acid bacteria

The preparation of encapsulated LAB was carried out according to Yasim-Anuar et al. (2017) with minor modifications. First, the encapsulation process is carried out by adding 40 mL of cell biomass to 40 mL of sterile CMF hydrogel, then stirring using a stirring rod until well blended. The encapsulated LAB was then tested for its probiotic properties.

Treatment at various temperatures and storage time

Storage was carried out by inserting 2 mL of each of the encapsulated LAB into 5 mL cryovial, then stored at room temperature and refrigerated temperature (4°C) for 0, 7, 14, 21, 28 and 35 d. Then the encapsulated LAB was tested for its viability according to Pato et al. (2020) and resistance to acid and bile, according to Pato (2003) and Nuraida et al. (2012).

RESULTS AND DISCUSSION

Physicochemical property of oil palm leaves, cellulose and cellulose microfiber

Flours derived from whole dried OPL and dried cellulose of OPL were shown in Figure 1. Flour derived from cellulose of OPL was generally light brown, and flour from the whole OPL was dark green because it still contained chlorophyll which was not fully damaged during leaf drying. However, after being processed into cellulose flour, the color changed to light brown. This change was because OPL was immersed in a 6% KOH solution then soaked again in hypochlorite solution to obtain cellulose. Sodium hypochlorite solution is a compound used for the bleaching process that removes chlorophyll, the green pigment in OPL (Muneer et al. 2014).

OPL is mainly composed of carbohydrates, especially crude fiber, followed by protein, water, fat, and ash (Table 1). Nurhaita and Ruswendi (2007) reported several components in OPL, such as 8.51% protein and 28.48% crude fiber. The crude fiber content in our study was much higher than that reported by Hurhaita, possibly because, in our study, we did not separate the leaf sticks containing high crude fiber and analyzed with the leaves. OPL is also rich in minerals, especially Ca and Mg, which cause the ash content to be higher than OPL. In order to make CMF, it is necessary to know the fiber content in OPL, and the result was shown in Table 2.

Table 1. Chemical composition of OPL

Chemical compounds	Amount (%)
Water	7.75
Ash	4.41
Fat	5.36
Protein	9.93
Crude fiber	43.01
Carbohydrate	29.54

Table 2. Cellulose, hemicellulose, and lignin content from OPL

Components	Amount (%)
Cellulose	43.2
Hemicellulose	5.2
Lignin	51.6

A **B**

Figure 1. Flours derived from whole dried OPL (A) and flours from the dried fiber from OPL (B)

The main content in OPL fiber is lignin, followed by cellulose and hemicellulose. Lignin is a substance found in vascular plants, usually within the cell walls and between cells. Rosli et al. (2017) have reported moisture content of 9.51% and lignin 27.7%, whereas, in this study, it was found that moisture content was 8.24% and lignin 23.2% in the OPEFB. Lignin is primarily a supportive structure and is part of the secondary thickening of tall plants. This fact is why the amount of lignin in the stem is more than other parts of a plant to support it so that it stands firm and strong and does not collapse easily (Shadle et al. 2002).

The cellulose isolated from OPL was then analyzed for crystallinity using X-Ray Diffraction (XRD), and the results can be seen in Figure 2. XRD pattern of OPL celluloses has seven diffraction peaks at 2θ = 15°, 17°, 22°, 34°, 42°,43° and 72°. The XRD diffractogram of cellulose nanofiber from oil palm mesocarp fiber showed intense reflection at 2θ = 21 to 22°, and other small peaks at 2θ = 18 to 19° and 35 to 42° (Yasim-Anuar et al. 2018). Crystallinity analysis using X-Ray Diffraction (XRD) was also performed to determine the degree of crystallinity in the cellulose sample (Table 3).

According to Gümüskaya et al. (2003), the degree of crystallinity is the essential parameter in a crystalline structure. The higher the value of the degree of crystallinity, the stronger the rigidity of fiber. The degree of crystallinity of OPL of 10,1% was lower than that of oil palm empty fruit bunch at 58.1% (Lestari 2016).

FTIR spectroscopy is an analytical method used for polymer material characterization and functional group analysis. By determining and recording the residual spectra results with energy absorption by organic molecules in infrared light. Infrared is defined as an area having a wavelength of 1-500 cm^{-1}. The results of FTIR analysis of cellulose from OPL are presented in Figure 3.

It can be seen that the dominant absorption peak which was the stretching vibrations of the -OH group on the cellulose in OPL at 3419.71 cm^{-1}. The same finding was found in the cellulose of oil palm empty fruit bunch (Fahma et al. 2010). The next absorption peak was CH stretching in OPL at 2902.28 cm^{-1}. The small peaks with sizes between 1637.01-1637.49 cm^{-1} in OPL-cellulose were characteristic of the carbonyl groups in hemicellulose or lignin. The peak with a size of 1427.43-1055.32 cm^{-1} is thought to be a C-C aromatic loop of bound lignin or C-H and C-O ester bonds stretching vibration from the partial acetylation process of the hydroxyl group of carbohydrates and lignin residues (Sun et al. 2000).

Table 3 Crystal index of cellulose from OPL

	Value
I_{002}	88.41
I_{AM}	79.45
Crystal index (%)	10.1

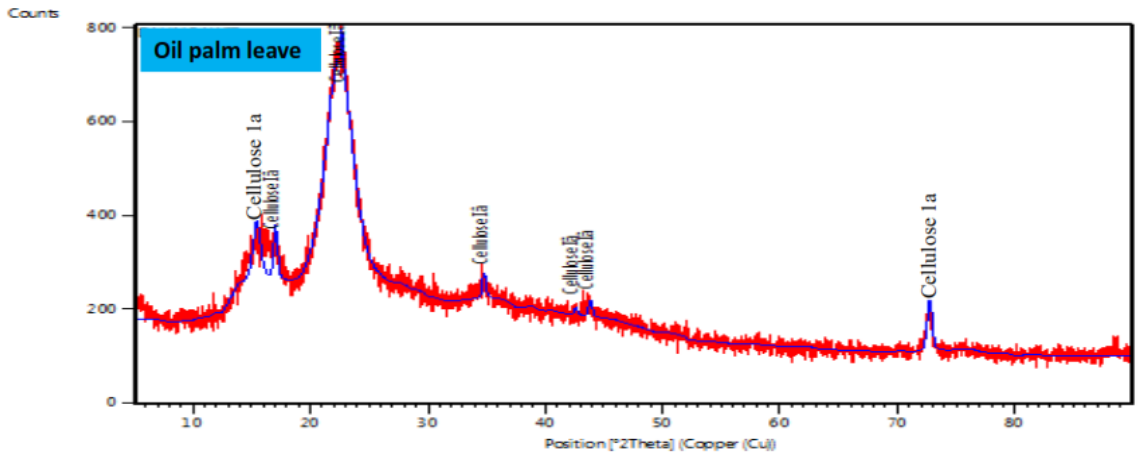

Figure 2. XRD diffractogram of cellulose of OPL

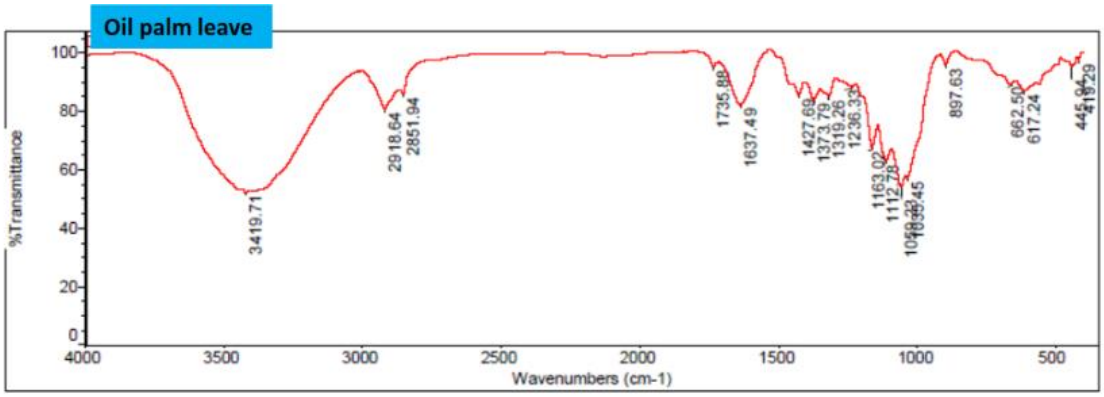

Figure 3. FTIR spectra of cellulose from OPL

Viability, acid and bile resistance of *Lactobacillus fermentum* InaCC B1295

Viability is one of the requirements for probiotics to be able to perform their therapeutic function when consumed. Analysis of variance showed that storage time significantly affected (P> 0.05) LAB viability, but storage temperature did not significantly influence (P <0.05) LAB viability (Table 4).

The decrease in the amount of LAB occurred at both storage temperatures from 7 to 35 d encapsulated with CMF hydrogel from OPL. However, the most significant decrease occurred at room temperature of 7.37 log CFU/mL, while at temperature, the decrease was only 4.72 log CFU/mL. This fact is because the longer the storage time, the CMF hydrogel layer covering the bacterial cells has decreased its stability due to the increased humidity in the CMF hydrogel as the length of storage increases. CMF hydrogel, which has increased humidity, will reduce its tensile strength and flexibility, causing the gel bond to stretch, and bacteria quickly get out of the coating so that the bacteria come into direct contact with temperature, which causes cell damage and affects bacterial growth. This statement follows Sianturi (2018), which stated that the robustness of hydrogels containing PVA depends on humidity. The higher the humidity, the more water is absorbed, reducing tensile strength and flexibility.

The results of this study are in line with Bilang et al. (2018), which states that the robustness of the encapsulant matrix formed will decrease with increasing storage time. The CMF hydrogel from OPL maintained LAB viability at both storage temperature conditions for 28 d. The viability of LAB using CMF hydrogels in the study was much longer than the finding reported by Fung et al. (2011) that the viability of *L. acidophilus* encapsulated with a hydrogel of cellulose nanofiber (CNF) from okra lasted for only 5 d, CNF oil palm trunk for 5 d and CNF oil palm frond for 21 d. Different results were reported by Hossain et al. (2021) that probiotics encapsulated using a mixture of cocoa powder with Na-alginate or FOS retained viability of more than 10^7 CFU/g for up to 180 and 120 d of storage at 4 and 25°C, respectively. The cell count of probiotics encapsulated with sodium alginate and carrageenan was 9.91 log CFU/mL and 9.89 log CFU/mL respectively at 0 day that decreased to 8.74 log CFU/mL and 8.39 log CFU/mL respectively after 120 d at frozen storage (Afzaal et al. 2019). Based on this, it is necessary to carry out further research on CMF of OPL added with Na-alginate or other encapsulants to increase the storage time of probiotics that have viability above 10^7 CFU/g.

Probiotics consumed will pass through the stomach, which has a very acidic pH, i.e., about 2. Therefore, the probiotic must be resistant to acid to reach the small intestine and colon to perform its therapeutic function. The resistance of *L. fermentum* InaCC B1295 encapsulated using CMF hydrogel stored at various temperatures and storage time and treated at pH 2 for 5 h is presented in Table 5.

Table 4. Viability of *Lactobacillus fermentum* InaCC B1295 encapsulated using CMF hydrogel stored at various temperatures and times

Storage time (d)	Number of LAB (log CFU/mL)	
	Room temperature	Refrigerated temperature (4°C)
0	$^{x}10.09^{d}$	$^{y}10.08^{e}$
7	$^{x}7.78^{c}$	$^{y}8.27^{d}$
14	$^{x}7.37^{b}$	$^{y}7.59^{b}$
21	$^{x}7.66^{c}$	$^{y}7.98^{c}$
28	$^{x}7.49^{b}$	$^{y}7.75^{c}$
35	$^{x}2.72^{a}$	$^{y}5.36^{a}$

Note: ^{a,b,c,d,e}Means in the same column with different superscript letters differ (P < 0.05). ^{x,y}Means in the same row with different superscript letters differ (P < 0.05)

Table 5. Number of *Lactobacillus fermentum* InaCC B1295 encapsulated using CMF hydrogel stored at various temperatures and times then treated at pH 2

Storage time (d)	Number of LAB (log CFU/mL)	
	Room temperature	Refrigerated temperature (4°C)
0	$^{x}9.86^{c}$	$^{x}9.87^{d}$
7	$^{x}9.39^{b}$	$^{x}9.44^{c}$
14	$^{x}9.16^{ab}$	$^{x}9.27^{bc}$
21	$^{x}9.12^{a}$	$^{x}9.18^{ab}$
28	$^{x}9.11^{a}$	$^{x}9.07^{ab}$
35	$^{x}8.94^{a}$	$^{x}9.06^{a}$

Note: ^{a,b,c,d}Means in the same column with different superscript letters differ (P < 0.05). ^{x,y}Means in the same row with different superscript letters differ (P < 0.05)

In this study, *L. fermentum* InaCC B1295 was treated at pH 2 for 5 h to mimic gastric pH and the length of time food was in the stomach (Hill et al. 2014). The results showed that although this LAB has been stored at room and refrigerated temperatures for 35 d, it could still be alive in acidic conditions with high numbers ranging from 8.94-9.87 log CFU/mL. This finding means that if this LAB is consumed, it will still have more than 7.0 log CFU/mL as a condition for performing its therapeutic function properly.

The high number of LAB treated with low pH was due to the CMF hydrogel from OPL still covering the LAB cells in acidic conditions. In addition, the reduction in cell number was minimal, with a range of 0.02 to 0.47 log CFU/mL stored at room and refrigerated temperatures (Table 6). The decrease in the number of LAB is minimal, indicating that the CMF hydrogel from OPL can maintain the survival of this LAB in very acidic conditions. This fact was probably due to the CMF hydrogel from OPL enveloping the LAB cells while they were treated at pH 2 for 5 h at 37°C. Thus, the cells of *L. fermentum* InaCC B1295 might not come in direct contact with HCl, so that the decrease in the number of cells was less than 0.50 log CFU/mL at various storage times and storage temperatures. It is also the use of sodium alginate, which is widely used as an encapsulant because of its ability to form hydrogels with divalent cations in moderate conditions. The hydrogel that is formed maintains cells from various environmental conditions that are less favorable for LAB cells, for example, at very low pH (Simpson et al., 2004). Our results showed that *L. fermentum* InaCC B1295 showed a slight reduction in cell numbers than the findings of some researchers.

The encapsulated *L. acidophilus* and Bifidobacterium spp showed decreased cell count of about 0.5 log CFU/g for 8 weeks, while free cells decreased by about 1 log CFU/g (Rizqiati et al. 2009). The results of other studies showed that the viability of co-encapsulated bacteria decreased by 1.62 log CFU/g, encapsulated cells decreased by 3.9 log CFU/g and free cells decreased by 4.5 log CFU/g after 3 h of incubation at pH 2 (Ngov et al. 2014). During the simulated gastrointestinal test, the encapsulated bacteria decreased only 3 log CFU/g, while for free cells decreased 7 log CFU/g (Afzaal et al. 2019). *L. rhamnosus* encapsulated with chitosan coating of microbeads and treated under acidic conditions for 40 to 120 min could maintain a decrease in cell numbers of only 0.94 log CFU/g (Sohail et al. 2011).

Resistance to bile is one of the requirements for probiotics because probiotics must pass through the upper small intestine before performing their therapeutic function in the digestive tract (De Smet et al. 1995). Bile resistance of *L. plantarum* InaCC B1295 stored at various storage times and temperatures is presented in Table 7. The results showed that even though this LAB was stored in a refrigerated room and temperature for 35 d, it was still resistant to bile with a high number of din above 9.0 log CFU/mL. Figures that have met the requirement of at least 7.0 log CFU/mL as a requirement to carry out its therapeutic functions in the digestive tract. The high amount of LAB treated with low pH was due to the CMF hydrogel from OPL covering the LAB cells when treated with bile for 5 h. Bifidobacterium spp and *L. acidophilus* encapsulated with sodium caseinate, fructooligosaccharide, D-glucose, and mannitol have high bile resistance characterized by the amount of LAB between 8.3 and 9.2 log CFU/g at room temperature (Dianawati et al. 2016). Dikit et al. (2015) reported that the encapsulated *L. plantarum* D6SM3cells treated in simulated gastric juice was higher than free cells. The viability of the free cells and the encapsulated cell showed a gradual decrease during the storage period at 4°C. However, their viability drastically decreased at room temperature.

The high resistance to bile was indicated by reducing the small number of cells in the range of 0.10-0.75 log CFU/g at various duration and storage temperatures (Table 8).

Table 6. The amount of reduction in *Lactobacillus fermentum* InaCC B1295 encapsulated with CMF hydrogel stored at various temperatures and time and treated with pH 2.

Storage time (d)	Number of LAB (log CFU/mL)	
	Room temperature	Refrigerated temperature (4°C)
0	$^{x}0.21^{ab}$	$^{x}0.02^{ab}$
7	$^{x}0.18^{ab}$	$^{x}0.17^{ab}$
14	$^{x}0.41^{b}$	$^{x}0.38^{b}$
21	$^{x}0.47^{b}$	$^{x}0.24^{ab}$
28	$^{x}0.20^{ab}$	$^{x}0.12^{ab}$
35	$^{x}0.03^{a}$	$^{x}0.04^{a}$

Note: ^{a,b}Means in the same column with different superscript letters differ ($P < 0.05$). ^{x,y}Means in the same row with different superscript letters differ ($P < 0.05$)

Table 7. Number of *Lactobacillus fermentum* InaCC B1295 encapsulated using CMF hydrogel stored at various temperatures and times then treated with bile

Storage time (d)	Number of LAB (log CFU/mL)	
	Room temperature	Refrigerated temperature (4°C)
0	$^{x}9.84^{b}$	$^{x}9.93^{c}$
7	$^{x}9.69^{b}$	$^{x}9.75^{ab}$
14	$^{x}9.65^{b}$	$^{x}9.60^{b}$
21	$^{x}9.75^{b}$	$^{x}9.81^{bc}$
28	$^{x}9.24^{a}$	$^{x}9.55^{b}$
35	$^{x}9.24^{a}$	$^{x}9.08^{a}$

Note: ^{a,b,c}Means in the same column with different superscript letters differ ($P < 0.05$). ^{x,y}Means in the same row with different superscript letters differ ($P < 0.05$)

Table 8. The amount of reduction in *Lactobacillus fermentum* InaCC B1295 encapsulated with CMF hydrogel stored at various temperatures and time and then treated with bile

Storage time (d)	Number of LAB (log CFU/mL)	
	Room temperature	Refrigerated temperature (4°C)
0	$^{x}0.32^{a}$	$^{x}0.75^{a}$
7	$^{x}0.28^{a}$	$^{x}0.33^{a}$
14	$^{x}0.19^{a}$	$^{x}0.18^{a}$
21	$^{x}0.16^{a}$	$^{x}0.22^{a}$
28	$^{x}0.16^{a}$	$^{x}0.17^{b}$
35	$^{x}0.10^{a}$	$^{x}0.12^{a}$

Note: ^{a,b}Means in the same column with different superscript letters differ ($P < 0.05$). ^{x,y}Means in the same row with different superscript letters differ ($P < 0.05$)

Survival of *L. fermentum* InaCC B1295 against bile was very high, indicated by a minimal decrease in the amount of LAB, which was less than 1 log CFU/mL. This fact shows that the CMF hydrogel from OPL was able to protect this LAB in bile. The protective mechanism may be due to the CMF hydrogel from OPL, which envelops the LAB cells to not come into direct contact with the bile. A similar mechanism also occurs in sodium alginate, which also forms hydrogels with divalent cations in moderate conditions. The hydrogel that is formed protects the cells from various environmental conditions that are less favorable for LAB cells, for example, in the bile, which is secreted into the upper part of the small intestine to emulsify fat (Tripathi and Giri 2014).

Our findings indicate that *L. fermentum* InaCC B1295 showed a slight reduction in cell numbers than the findings of other researchers. Eight species of *Lactobacillus* were exposed to ox gall, the viability of free cells was reduced by 6.51 log CFU/mL, while the encapsulated LAB only decreased by 3.36 log CFU/mL (Ding and Shah, 2007). The encapsulated LC 1463 strain showed only a 1.4 log CFU/mL reduction after exposure to 1% bile salts for 3 h. Under the same conditions, free cells decreased by 3.7-4.7 log CFU/mL. The encapsulated LA 1338 strain only decreased by 2.2 log CFU/mL, while free cells decreased by around 4.75-4.84 log CFU/mL (Ngov et al. 2014). Survival of free cell of *L. gasseri* SBT0274 was stable for 7 d of storage at 4°C and decreased significantly after this period. On the other hand, the amount of free cell of *L. gasseri* SBT0270 decreased continuously through storage for 28 d at 4°C after exposure to bile salt (Usman and Hosono 1999). The survival rate of probiotics encapsulated in SA/SPI beads did not decrease after 6 h of incubation in bile salts 0.5 and 1.0% (Praepanitchai et al. 2019).

In conclusions, the main content of OPL was carbohydrates, especially fiber, and it also contained ash, protein, and fat in small amounts. Fiber from OPL is mainly composed of lignin followed by cellulose and hemicellulose. XRD analysis showed that the crystal index of cellulose from OPLwas 10.1%. FTIR analysis showed that the most considerable absorption value which was the stretching vibrations of the -OH group ranging from 2919.17 to 2914.82 cm^{-1}. CMF from OPL maintained the viability of *L. fermentum* InaCC B 1295 for up to 28 d of storage at room and refrigerated temperature. The survival of *L. fermentum* InaCC B1295 at low pH and presence of bile was very high, characterized by a decrease in the number of cells by less than 0.5 log CFU/mL during storage of 35 d at room and refrigerated temperatures.

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