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Cellulose Microfiber Encapsulated Probiotic: Viability, Acid and Bile Tolerance during Storage at Different Temperature

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Abstract

This work aimed to analyze the physicochemical properties of cellulose from OPT used in the fabrication of CMF and evaluate the efficacy of the hydrogel CMF as an encapsulant for *L. fermentum* InaCC B1295 stored at room temperature and in the refrigerator. The Kjeldahl method was used to evaluate the protein content; the gravimetric method was used to determine OPT's ash, moisture, and fiber contents; the Soxhlet method was used to determine the fat content carbohydrates were computed using the difference method. The levels of holocellulose, lignin, and cellulose were also determined. Viability, acid and bile resistance of strain B1295 were evaluated at various temperatures for 35 days. The most abundant component of OPT fiber was cellulose, followed by hemicellulose and lignin. XRD examination revealed that OPT cellulose has a crystal index of 83.40%. FTIR analysis was used to detect the stretching vibrations of the –OH group on cellulose at 3419.03 cm^{-1}. CMF hydrogel from OPT sustained *L. fermentum* InaCC B1295 survival for up to 28 days at room and refrigerated temperatures. At acidic conditions and in the presence of bile, the viability of *L. fermentum* InaCC B1295 was excellent, with a drop in cell population of less than 0.2 log CFU/g over 35 days at room and refrigerated temperatures. CMF obtained from OPT can be used as an encapsulant to maintain viability, acid resistance and bile of probiotics. There is still a need for research into the usage of CMF from OPT in combination with other encapsulants to extend the storage life of *L. fermentum InaCC* B1295.

Keywords:

Cellulose Microfiber;
Oil Palm Trunk;
Viability;
Acid and Bile Tolerance.

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1- Introduction

Indonesia is the world's top palm oil producer, with 16.37 million hectares of land and 45.8 million tons of CPO production in 2020. There is a high likelihood of palm oil waste with such a vast oil palm plantation, including empty fruit bunches, oil palm trunks, oil palm fronds, and oil palm leaves. According to the analysis, only 10% of an oil palm tree is oil; 90% is biomass such as the trunk, frond, leaves, palm oil shells, and empty fruit bunches [1]. Oil palm trunk (OPT) waste is generally obtained during replanting when the oil palm plants have reached 25-30 years. Approximately about 2.76 million hectares of oil palm will be replanted in Indonesia [2]. Thus, OPT waste is abundant from replanting today and the next few years. OPT waste was usually burned in the past, but the new waste was difficult to burn, so it was usually left to accumulate in palm oil fields to decompose entirely within five years [3]. Today and in the future, OPT can be used to make various high-value products, including medium-density panels, laminated veneer wood, nanocomposites, pulp and paper [4-6]. OPT can also be used as a source of starch [7, 8] and feed for ruminants [9].

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Recently, OPT has been used in the production of cellulose microfibers (CMF). CMF, also known as microfibrillated cellulose, is cellulose that has been processed into microfibrils ranging in diameter from 10 to 100 µm. CMF has a high specific surface area, strength, and stiffness and is lightweight, biodegradable, and renewable [10]. CMF can be utilized in the paper, paint and coating, automotive, electronics, composites, food and cosmetics, and medical industries due to their properties [11-13]. CMF may also be used to encapsulate probiotic and bioactive substances in food. To exert therapeutic effects, a possible probiotic strain must possess specific desirable characteristics, including viability during manufacturing, storage, and marketing, as well as acid and bile tolerance [14]. Encapsulation is designed to maintain probiotic viability at a minimum of 10^7 log CFU/ml as it goes through the digestive tract to perform its therapeutic function [15, 16]. Probiotics have been shown to benefit health and wellness in various ways, including modulating the gut microbiota by inhibiting pathogenic microorganisms, generating anticarcinogenic chemicals, and modulating immunological responses [17]. The probiotic foods industry continues to grow each year, with numerous markets including functional foods and beverages, dietary supplements, and animal feed. Between 2016 and 2022, the probiotics market is expected to reach $57.4 billion, increasing at a compound annual growth rate of 7.7% [18]. Producing foods with probiotic claims, on the other hand, is challenging due to the problems inherent in the survival and retention of probiotic cells added to foods during manufacturing, storage, distribution, and consumption [19]. Simultaneously, cell encapsulation is gaining popularity to extend the life of probiotics added to a variety of food matrices [20]. Carbohydrates such as dextrins, pectins, cellulose, chitosan, carrageenan; lipids such as wax, paraffin, mono, and diglycerides; and proteins like milk gluten, casein, gelatin, and albumin are all commonly used as encapsulant materials [21, 22]. Many fermented dairy products have been effectively encapsulated with various encapsulating materials to protect probiotic microorganisms [23]. Numerous intrinsic and extrinsic parameters, including pH, storage temperature, hydrogen peroxide generation, oxygen, and fermentation conditions, affect probiotic survival in food items [24].

Cell encapsulation techniques are designed to preserve viable and functional cells within a semi-permeable matrix. The effectiveness of various encapsulating materials is also critical for the stability of the encapsulated bacterial particles, including 1) easy separation of cells from products, 2) increased productivity due to high cell concentrations achieved, 3) protection of cells from harsh environmental conditions, 4) the ability to use packed columns, and 5) reusability of the immobilized cells [25]. Additionally, these materials must be biocompatible and permeable to oxygen, nutrients, and potentially toxic metabolites to ensure cell survival [26]. As a result, they must manage the release through the human stomach and intestines [27]. A previous study found that CMF hydrogel from oil palm leave (OPL) was only able to maintain the viability of *L. fermentum* InaCC B1295 for 28 days and resistance to acid and bile after storage for 35 days at room and cold temperatures [28]. Considering the potential for OPT waste which is very abundant at the time of replanting, it is necessary to test the ability of CMF from OPT to maintain viability and resistance to acid and bile during storage at room temperature and cold temperatures.

2- Materials and Method

2-1- Materials

Palm oil solid waste used to make CMF is OPTs obtained by oil palm plantations in Riau, Indonesia. *Lactobacillus fermentum* InaCC B1295 was obtained from the Indonesian Culture Collection (InaCC), Research Center for Biology, Indonesian Institute of Sciences (LIPI), West Java, Indonesia. 89% polyvinyl alcohol was purchased from Sigma-Aldrich, Steinheim, Germany.

2-2- Characterization of OPT

The Kjeldahl method was used to evaluate the protein content; the gravimetric method was used to determine OPT's ash, moisture, and fiber contents; the Soxhlet method was used to determine the fat content carbohydrates were computed using the difference method [29]. Additionally, the levels of holocellulose, lignin, and cellulose were determined according to Jung et al. [30].

2-3- Cellulose Separation

The procedure described by Fahma et al. [11] was used to isolate cellulose. Before being exposed to any treatment, the OPT was trimmed to 0.5–1 cm in length. The holocellulose was extracted by bleaching the fibers for 12 hours with a 6% potassium hydroxide solution and then rinsing with deionized water until a pH of 7 was achieved. Following that, the extracted fibers were immersed in a $NaClO_2$ solution for 5 hours at pH 4–5. Lignin was removed by rinsing with deionized water. Before proceeding with further analysis, the cellulose fibers were refrigerated.

2-4- FTIR Analysis

The chemical composition and bonding of cellulose were determined using an FTIR spectrometer. A Thermo Nicolet Nexus spectrometer connected to an ATR detector was used to acquire the FTIR spectra of cellulose samples. All spectra were acquired at a resolution of 4 cm^{-1} using 32 scans in the spectral range 7800-350 cm^{-1}. OMNIC 6.0 (Thermo Scientific Nicolet iS10) software was used to analyze the spectrum data.

2-5- XRD Analysis

XRD analysis was used to identify the chemical groups and degree of crystallinity of cellulose. The XRD analysis was conducted on the X'Pert PRO PANalytical instrumentation. CuK radiation with a wavelength of 0.154 was employed in the XRD test settings. The XRD test is conducted at a voltage and current of 30 mA and 40 kV. The diffraction rate is between 5º to 50º per second, with an increased rate of 2θ-0.02º per second. The crystallinity index (CrI) was calculated according to the following equation:

$$\mathrm{CrI} = \frac{\mathrm{I}_{002} - \mathrm{I}_{\mathrm{am}}}{\mathrm{I}_{002}} \times 100\% \quad (1)$$

Where; I_{002} is a diffraction intensity of $2\theta = 23$, which indicates the crystalline region of the material. I_{am} refers to the peak at about $2\theta = 18$, which indicates the amorphous region of the compound [31].

2-6- Preparation of CMF

CMF was prepared according to the Lestari technique [32]. First, OPT was fragmented, ranging in length from 0.5 to 1 cm, rinsed with water, and cooked for 1 hour in boiling water (100ºC). They were then filtered. Following boiling, the OPT was thoroughly rinsed with water and dried for four h at 60ºC. The fiber was then placed in a beaker with 1000 cc of 6% (w/v) KOH and left to soak for 12 hours at room temperature. The fibers are then washed with water three times. Additionally, washed fibers are steeped for 5 hours in a hypochlorite solution before being filtered and rinsed with water pH 7. After drying and crushing OPT in a blender until smooth, it was filtered through a sieve with a screen no 80. To avoid sample damage caused by milling heat, CMF was handled by milling the cellulose flour at an 8,000 rpm speed for 60 minutes with a run time of 15 seconds and a rest period of 2 minutes. The milled product was then sieved through a sieve with a screen no 100 to get CMF. The flowchart of the research is presented in Figure 1.

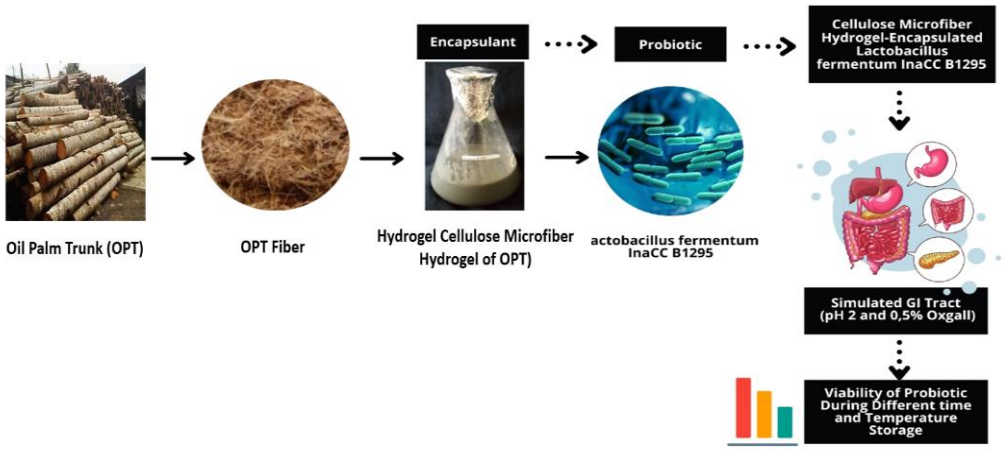

Figure 1. Flowchart of the research methodology

2-7- MRSB Medium Preparation

The MRSB medium was prepared by weighing 13.78 g of MRSB powder and diluting it with distilled water to a volume of 250 mL. The fluid was divided into 5 ml test tubes and sealed using a setup. Then place in an autoclave for 15 minutes at 121°C, 1 atm. Finally, the MRSB medium is complete and ready for use.

2-8- MRSA Medium Preparation

MRSA medium was made by weighing 68.2 g of MRSA powder and adding 1000 mL of distilled water, stirring until dissolved. The medium was then heated and swirled until homogenous. Additionally, sterilization was performed using an autoclave at 121°C, 1 atm for 15 minutes. Finally, 15 mL sterilized Petri dishes were filled with the homogenous MRS agar solution. The medium is poured in laminar airflow. The media-containing petri dish is then sealed and allowed to harden.

2-9- L. Fermentum InaCC B1295 Source and Activation

The strain B1295 was isolated from dadih, a traditional fermented from buffalo milk in West Sumatera, Indonesia. The culture was activated by inoculating 1 mL into a 5 mL MRSB medium test tube and vortex stirring. After that, the medium was incubated at 37°C for 24 hours to obtain the active culture stock. Then the active culture was prepared for research.

2-10- Preparation of CFS

Active culture of strain B1295 was inoculated into a sterile MRSB medium and incubated for 24 hours at 37°C. This mixture was centrifuged at 4500 rpm for 15 minutes following incubation to separate cells from the supernatant. After removing the supernatant, the cells were washed twice with sterile distilled water to achieve clean cells. After adding phosphate buffer at a 1:1 (w/v) ratio, the CFS was deposited in clean containers and kept at 4°C.

2-11- CMF Hydrogel Preparation

The first stage was to prepare 8% (w/v) polyvinyl alcohol. PVA was weighed at 96 g, then combined with 1104 mL distilled water and heated to 100°C until dissolved using a hot magnetic stirrer. Allow sufficient time for the PVA solution to cool to room temperature. 250 g PVA 8% was combined with 250 mL CMF and heated at 60°C until the CMF dissolved entirely, resulting in a CMF hydrogel (CMFH). The pH and viscosity of the CMFH were then determined using a pH meter and viscometer. The hydrogel was then autoclaved at 121°C for 15 minutes. Sterile CMFH was cooled to room temperature and ready to be used as a LAB encapsulant [33].

2-12- Encapsulation of L. Fermentum InaCC B1295

The process for encapsulating *L. fermentum* InaCC B1295 was somewhat modified from Yasim-Anuar et al. [34]. 40 mL of sterile CNF hydrogel was added to 40 mL of cell biomass and swirled until thoroughly mixed using a stirring rod. Following that, the probiotic features of the encapsulated LAB were evaluated.

2-13- Treatment at Various Temperatures and Storage Times

Two ml of each encapsulated probiotic was placed in a 5 mL cryovial and then kept at room and cold temperatures for 35 days. The encapsulated probiotic was then examined for viability and resistance to acid and bile at days 0, 7, 14, 21, 28, and 35 [35][36].

2-14- Data Analysis

Data on physico-chemical properties of OPT, fiber, and CMF were analyzed descriptively, while data on resistance to acid and bile and viability were analyzed statistically using ANOVA and DMRT with SPSS version 26 [37].

3- Results and Discussion

3-1- Physicochemical Characteristics of OPT and Cellulose

Whole OPT flour was analyzed to determine the moisture content and chemical constituents, and the results are presented in Table 1. The main content of OPL was crude fiber and carbohydrates, 42.55 and 37.39%, respectively. The primary carbohydrate in OPT was starch, made up of 71.24% amylopectin and 28.76% amylose. According to the amylograph investigation, the oil palm starch's final viscosity was more remarkable than commercial starch [38].

Table 1. The moisture content and chemical composition of whole OPT flour

Chemical compounds	Amount (%)
Water	10.23
Ash	6.08
Fat	0.78
Protein	2.99
Crude fiber	42.55
Carbohydrate	37.39

OPL also contained small amounts of ash, protein, fat, and water. Winarni et al. [30] similarly found that the moisture and ash content of the OPL was nearly identical to the moisture and ash content of this study, which was 10.5 and 6.73%, respectively. Compared with the composition between OPT and OPL, protein and fat levels were higher in OPL than in OPT. On the other hand, the levels of carbohydrates and ash were higher in OPT than in OPL. The crude fiber content in both samples was relatively the same in OPT and OPL [28]. It is essential to determine the fiber content of OPT to prepare CMF, which is provided in Table 2.

Table 2. Cellulose, hemicellulose, and lignin contents from OPT

Components	Amount (%)
Cellulose	52.0
Holocellulose	25.2
Lignin	22.8

OPT fiber was mainly composed of cellulose, and the rest was holocellulose and lignin in almost equal amounts. The OPT fiber content in this study was higher than the previous findings of 40.95-41.72% [40]. Because of the high cellulose content, the plant cell walls and fibers are more robust and more stable. In contrast, OPL fiber is mainly lignin, followed by cellulose and holocellulose [28]. Lignin is a substance found in the cell walls and between cells of vascular plants. Vascular and parenchymal bundles contain lignin as much as 15.7 and 20.0 of the whole chip of OPT, respectively [41]. Deli [42] similarly recorded a lignin content of 19.20% in OPT Lignin is primarily a supporting structure that contributes to tall plants' secondary thickening. This fact results in a more significant concentration of lignin content of the stem than in other regions of the plant. The presence of lignin in the stems aids the plant in remaining rigid and solid and preventing collapse [43]. The crystallinity of the cellulose extracted from OPT was then determined using X-Ray Diffraction (Figure 2).

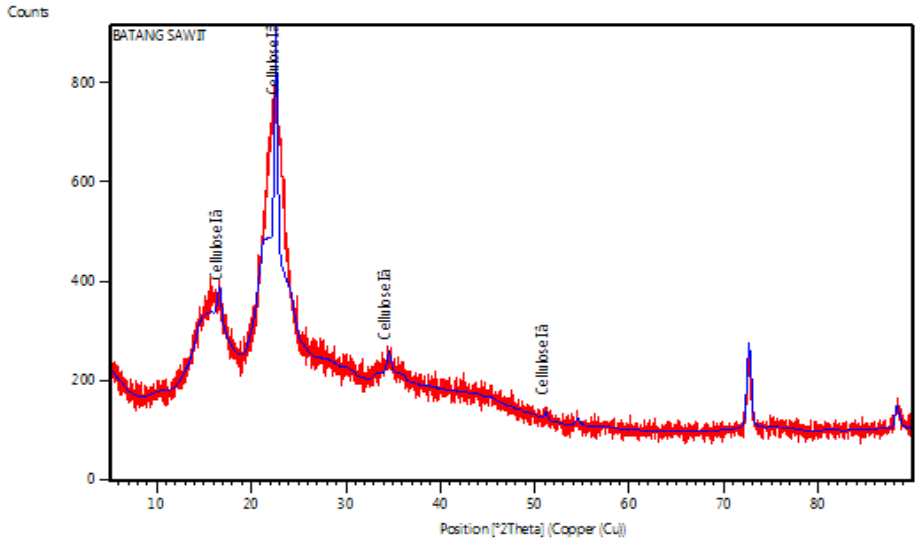

Figure 2. XRD diffractogram of cellulose of OPT

Five diffraction peaks were seen in the XRD pattern of OPT celluloses at 2θ = 16, 22, 34, 51, and 88°. This result is different from our previous finding, which found seven diffraction peaks at 2θ = 15, 17, 22, 34, 42, 43 and 72° in cellulose from oil palm leaves. The XRD diffractogram of CNF from OPM revealed a strong reflection at 2θ = 21 to 22° as well as small peaks at 2θ = 18 to 19° and 35 to 42° [34]. In this figure, potassium hydroxide was also detected at peaks at 2θ = 54° and 22°. This compound was used to separate cellulose from lignin and hemicellulose. The presence of potassium hydroxide may be due to the incomplete washing process with distilled water or because this compound bound firmly to the components in the fiber so that it was not easily separated from the cellulose during washing. Additionally, XRD analysis was used to assess the crystallinity level of the OPT cellulose (Table 3).

Table 3. Crystal index of cellulose from OPT

Parameters	Value
I_{002}	323.53
I_{AM}	53.77
Crystal index (%)	83.38

The degree of crystallinity of the OPT was relatively high in this investigation, at 83.40%. This value is significantly greater than the previously reported crystallinity of 64.66% in OPT [40]. OPT has a higher crystallinity index when compared to OPL, which is only 10.1% [28]. The stiffness of the fiber increases as the degree of crystallinity increases [44]. The crystalline structure of cellulose is crucial in determining the material's elasticity, stiffness, thermal stability, and absorption properties [45].

FTIR spectroscopy is an analytical technique used to determine the functional groups in polymer materials and characterize them. The residual spectra of energy absorption by organic molecules in infrared light can be determined and recorded. The term "infrared" refers to a wavelength range of 1-500 cm^{-1}. Figure 3 illustrates the outcome of an FTIR study of cellulose from OPT.

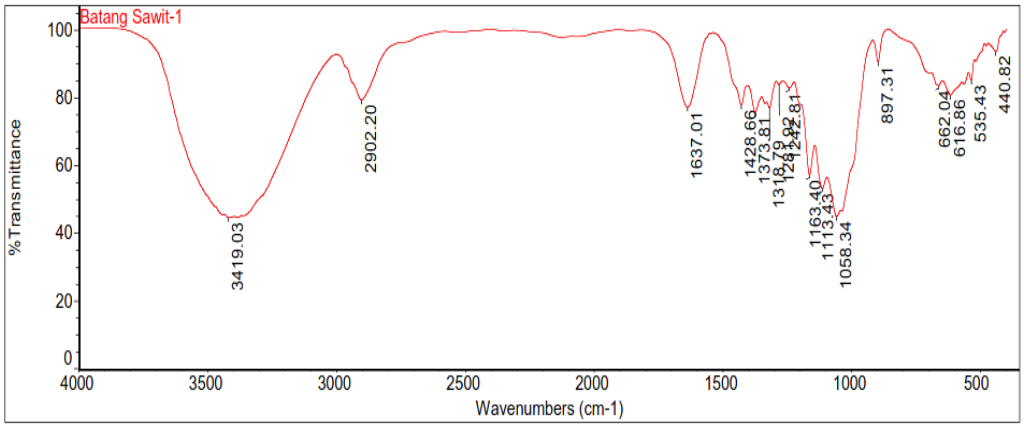

Figure 3. FTIR spectra of cellulose from OPT

The dominant absorption peak in OPT was 3419.03 cm^{-1}, which corresponded to the stretching vibrations of the –OH group on cellulose. The identical observation was made in the cellulose of an EOPFB [11]. CH stretching in OPT had the next highest absorption peak at 2902.20 cm^{-1}. The tiny peaks in OPT-cellulose with diameters ranging from 1637.01 to 1282.41 cm^{-1} were carbonyl groups in holocellulose or lignin. The peak at 1163.40-1058.34 cm^{-1} is assumed to represent a C-C aromatic loop of linked lignin or a C-H and C-O ester bond stretching vibration generated by partial acetylation of the hydroxyl group in lignin or carbohydrate residues [46]. The FTIR spectra of OPT revealed similarities to that of OPL [28].

3-2- Viability of Strain B1295

One of the conditions for probiotics to execute their therapeutic effect when taken is their viability. The Anova revealed that storage duration significantly influenced probiotic viability ($P<0.05$). However, storage temperature was not significantly affected on probiotic survival ($P<0.05$), as shown in Table 4. From days 7 to 35, LAB decreased at both storage temperatures encapsulated in OPT's CMFH. At room temperature, the probiotic concentration declined from 10.15 log CFU/g to 5.06 log CFU/g and from 10.20 log CFU/g to 5.46 log CFU/g when refrigerated. The LAB decrease was slightly more at room temperature than at refrigerated temperature, but on day 35, the number of LAB was below 10^7 CFU/g at different temperatures as a probiotic requirement. This conclusion is attributable to the fact that as storage time increases, the CMFH layer covering the probiotic cells loses its stability due to the CMFH's increased humidity. Increased humidity reduces the tensile strength and flexibility of CMFH, allowing the gel link to expand and bacteria to readily escape the coating, exposing them to direct temperature, causing cell damage, and inhibiting bacterial development. This remark aligns with Sianturi [47], who indicated that PVA-containing hydrogels' toughness depends on humidity. More water is absorbed when humidity rises, lowering tensile flexibility and strength. The current findings corroborated a prior observation that the encapsulant matrix's durability decreased as the storage period increased [48]. At both room and cold temperatures, the CMFH from OPT maintained probiotic viability for 28 d. The study discovered that probiotics encapsulated in OPT and OPF cellulose nanofiber hydrogel (CNFH) were alive for 5 and 21 days, respectively [33]. We previously discovered that strain B1295 also had a survival time of 28 days in CMFH from OPL [28]. These results demonstrate that when stored under the same conditions, different types of palm oil solid waste have no effect on the survival of LAB but are strongly reliant on the type of LAB encapsulated. LAB encapsulated in a mixture of some compounds maintained survivability of greater than 10^7 CFU/g for 180 days at low temperatures and 120 d at room temperature [49].

Table 4. Viability of strain B1295 microencapsulated with CMFH from OPT at different temperatures for 35 days storage

Storage time (d)	Number of LAB (log CFU/g)	
	Room temperature	Refrigerated temperature
0	$^{x}10.15^{d}$	$^{y}10.20^{d}$
7	$^{x}7.39^{b}$	$^{y}7.84^{bc}$
14	$^{x}7.55^{b}$	$^{y}7.67^{b}$
21	$^{x}7.82^{c}$	$^{y}7.99^{c}$
28	$^{x}7.43^{b}$	$^{y}7.93^{c}$
35	$^{x}5.06^{a}$	$^{y}5.46^{a}$

^{a,b,c,d} Different letters within a column indicate significant differences at $P < 0.05$

^{x,y} Different letters within a column indicate significant differences at $P < 0.05$

In comparison to free cells, the combination of whey protein isolate and fructooligosaccharide microcapsules considerably boosted bacteria stability in the product over 30 days at 4°C, averaging 8.57 log CFU/g for *L. acidophilus* and 7.61 log CFU/g for *L. casei* [50]. Additionally, this study discovered that strain B1295 had longer viability at cold temperatures than at room temperature. These results are consistent with those obtained previously in L. acidophilus encapsulated with maltodextrin and gum arabic [51].

3-3- Acid resistance of Strain B1295

Probiotics consumed must be acid-resistant in the stomach to reach the colon and perform their therapeutic effect [52]. Table 5 summarizes the acid resistance of strain B1295 encapsulated in CMFH and stored at different conditions before being treated for 5 h at pH 2. For 5 hours, strain B1295 was treated at pH 2 to adapt to the circumstances and food retention duration in the human stomach. *L. fermentum* InaCC B1295 was stored for 35 days in the room, and cold temperatures showed viability above 10^9 CFU/g after being treated under acidic conditions. This finding indicates that even if strain B1295 is consumed, it will still have more than the 7.0 log CFU/g required to perform its therapeutic function. Since the CMFH from OPT was still coating the probiotic cells at low pH levels, many probiotics were still alive. The viability of strain B1295 was marginally more significant when held at cold temperatures than when stored at room temperature. This finding is most likely related to the cold gel state, which directly affects the viability of the encapsulated cells. Compared to CMFH OPT and CMFH OPL, the amount of probiotic cells was comparable, ranging between 8.94 and 9.89 log CFU/g after 0–35 days of storage at room temperature and cold temperature [28].

Table 5. Number of strain B1295 microencapsulated with CMFH from OPT at different temperatures for 35 days storage and treated at pH 2

Storage time (d)	Number of LAB (log CFU/g)	
	Room temperature	Refrigerated temperature
0	$^{x}9{,}83^{d}$	$^{y}9{,}89^{d}$
7	$^{x}9{,}37^{c}$	$^{y}9{,}44^{c}$
14	$^{x}9{,}13^{b}$	$^{y}9{,}20^{b}$
21	$^{x}9{,}09^{a}$	$^{y}9{,}19^{b}$
28	$^{x}9{,}07^{a}$	$^{y}9{,}09^{ab}$
35	$^{x}9{,}05^{a}$	$^{y}9{,}03^{a}$

^{a,b,c,d} Different letters within a column indicate significant differences at $P < 0.05$

^{x,y} Different letters within a column indicate significant differences at $P < 0.05$

Additionally, the number of cells decreased insignificantly at both ambient and refrigerator temperatures (Table 6).

Table 6. The amount of reduction in strain B1295 microencapsulated with CMFH from OPT at different temperatures for 35 days storage and treated with pH 2

Storage time (d)	Number of LAB (log CFU/g)	
	Room temperature	Refrigerated temperature
0	$^{x}0{,}08^{a}$	$^{x}0{,}02^{a}$
7	$^{x}0{,}02^{a}$	$^{x}0{,}03^{a}$
14	$^{x}0{,}09^{a}$	$^{x}0{,}08^{a}$
21	$^{x}0{,}09^{a}$	$^{x}0{,}18^{a}$
28	$^{x}0{,}10^{a}$	$^{x}0{,}17^{a}$
35	$^{x}0{,}07^{a}$	$^{x}0{,}11^{a}$

^{a} Different letters within a column indicate significant differences at $P < 0.05$

^{x} Different letters within a column indicate significant differences at $P < 0.05$

The decrease in the probiotic count was slight, demonstrating that the CMFH from OPT can keep this LAB alive in extremely acidic circumstances. The reduction in Strain B1295 was also not statistically different at all temperatures and storage conditions. This finding was most likely due to the CMFH from OPT wrapping the probiotic cells for 5 h at 37°C while they were treated at pH 2. Because strain B1295 cells did not come into direct contact with **hydrogen chloride,** the amount of strain B1295 was reduced by less than 0.20 log CFU/g over a range of storage periods and temperatures. The hydrogel protects the cells from numerous environmental conditions unfavourable to LAB cells, such as extremely low pH [24]. Under the same storage conditions, the amount of strain B1295 encapsulated with CMFH from OPT decreased roughly identically to the amount of CMFH from OPL, between 0.03 and 0.47 loc cycle [28]. The present

results revealed that strain B1295 had a somewhat lower cell number than other researchers' findings. For 8 weeks, the cell count of encapsulated *L. acidophilus* and Bifidobacterium spp reduced by around 0.5 log CFU/g, while unattached cells fell by around 1 log CFU/g [53]. Other studies discovered that after 3 hours of incubation at pH 2, co-encapsulated probiotics' viability fell by 1.62 log CFU/g, encapsulated probiotics' viability declined by 3.9 log CFU/g, and free probiotics' viability decreased by 4.5 log CFU/g [54]. Encapsulated bacteria reduced 3 log CFU/g throughout the simulated gastrointestinal test, while free cells decreased 7 log CFU/g [55]. *L. rhamnosus* encapsulated in chitosan microbeads was only able to maintain a 0.94 log CFU/g drop in cell counts when exposed to acidic conditions for 40–120 minutes [56].

3-4- Bile resistance of Strain B1295

Because LAB must travel through the upper part of the small intestine to perform their therapeutic activity in the digestive system, resistance to bile is one of the probiotic selection criteria [57]. Table 7 shows the bile resistance of strain B1295 stored at various storage durations and temperatures.

Table 7. Number of strain B1295 microencapsulated with CMFH from OPT at different temperatures for 35 days storage and treated with bile

Storage time (d)	Number of LAB (log CFU/g)	
	Room temperature	Refrigerated temperature
0	$^{x}9{,}88^{b}$	$^{x}9{,}93^{c}$
7	$^{x}9{,}57^{ab}$	$^{x}9{,}71^{b}$
14	$^{x}9{,}64^{b}$	$^{x}9{,}67^{b}$
21	$^{x}9{,}70^{b}$	$^{x}9{,}68^{b}$
28	$^{x}9{,}32^{a}$	$^{x}9{,}37^{a}$
35	$^{x}9{,}32^{a}$	$^{x}9{,}40^{a}$

^{a,b,c} Different letters within a column indicate significant differences at $P < 0.05$

^{x,} Different letters within a column indicate significant differences at $P < 0.05$

The results showed that this LAB was resistant to bile during storage at room temperature and refrigerated for 35 d, with more than 9.0 log CFU/g. This amount has exceeded the minimum of 7.0 log CFU/mL required for probiotics to perform therapeutic actions in the gastrointestinal tract. After 5 h of bile treatment on probiotic cells, the CMF hydrogel from OPT covered the LAB cells, resulting in many LAB treated with low pH. *L. acidophilus* and *Bifidobacterium* encapsulated in a mixture of sodium caseinate, D-glucose, mannitol, and fructooligosaccharide displayed a high level of bile resistance, as determined by the number of cells, which, at ambient temperature, varied between 8.3 and 9.2 log CFU/g [58]. The number of encapsulated strain D6SM3 cells treated with simulated gastric HCl was significantly more significant than the number of unencapsulated cells [59]. During the storage time at 4°C, the survivability of the free and encapsulated LAB decreased gradually. Their viability, on the other hand, plummeted at ambient temperature.

The high bile tolerance was demonstrated by lowering the minimal count of Strain B1295 by 0.10-0.72 log CFU/g over a range of storage conditions (Table 8).

Table 8. The amount of reduction in strain B1295 microencapsulated with CMFH from OPT at different temperatures for 35 days storage and treated with bile

Storage time (d)	Number of LAB (log CFU/g)	
	Room temperature	Refrigerated temperature
0	$^{x}0{,}13^{a}$	$^{x}0{,}10^{a}$
7	$^{x}0{,}12^{a}$	$^{x}0{,}30^{ab}$
14	$^{x}0{,}11^{a}$	$^{x}0{,}12^{a}$
21	$^{x}0{,}10^{a}$	$^{x}0{,}14^{a}$
28	$^{x}0{,}72^{c}$	$^{x}0{,}63^{b}$
35	$^{x}0{,}43^{b}$	$^{x}0{,}49^{a}$

^{a,b,c} Different letters within a column indicate significant differences at $P < 0.05$

^{x,} Different letters within a column indicate significant differences at $P < 0.05$

The survivability of strain B1295 against bile was relatively high, evidenced by a slight drop in cell count (<1 log cycle). The number of cells encapsulated with CMFH from OPT was not significantly different between room temperature and cold temperature treatment. At the same storage circumstances, similar results were obtained using strain B1295 encapsulated in CMFH from OPL [28]. This finding demonstrates that CMFH from OPT was effective in

protecting this probiotic in bile. CMFH from OPT, which covers the probiotic cells, so they do not directly touch the bile, could be the protective mechanism. In favourable circumstances, sodium alginate creates hydrogels with divalent cations through a similar method. The hydrogel protects the cells from various less-than-ideal environmental conditions, such as those found in bile, delivered into the small intestine's upper section to emulsify fat [24]. According to our findings, strain B1295 had a modest decline in cell counts compared to other researchers' findings. When Lactobacillus were exposed to oxgall, their viability decreased by 6.51 log cycles in loose cells but only by 3.36 log cycles in encapsulated cells [60]. After 3 h exposure to 1% bile salt, the encapsulated strain 1463 decreased by just 1.4 log cycles. Free cells were decreased by 3.7–4.7 log cycles under the same circumstances. The number of free probiotic cells was reduced by 4.75 - 4.84 log cycles. However, the encapsulated LA 1338 strain was reduced by just 2.2 log cycles [54]. The survival of *L. gasseri* SBT0274 free cells was constant at 4°C for 7 days before declining dramatically after that. After exposure to bile salt, the number of free cells in *L. gasseri* SBT0270 constantly reduced throughout 28 d at 4°C [61]. The survival rate of *L. acidophilus* encapsulated in calcium-alginate-soy protein isolate-based hydrogel beads remained stable after 6 hours of incubation in bile 0.5 and 1% [62].

4- Conclusion

In this study, carbohydrates, particularly fiber, were the primary component of OPT, with ash, protein, and fat forming minor components. The most abundant component of OPT fiber was cellulose, followed by holocellulose and lignin. XRD examination revealed that OPT cellulose has a crystal index of 83.40%. The stretching vibrations of the –OH group on cellulose were detected at 3419.03 cm^{-1}, which corresponded to the significant absorption peak in OPT OPT's CMF hydrogel-protected strain B1295 life for up to 28 days at cold and room conditions. At acidic conditions and in the presence of bile, the viability of *L. fermentum* InaCC B1295 was excellent, with a drop in cell population of less than 0.2 log CFU/g over 35 days at room and cold temperatures. OPT produced during oil palm replanting can be employed as raw materials in the production of CMF, which can be used as a probiotic encapsulant. Additional research on the use of CMF from OPT combined with other encapsulants to prolong the storage life of *L. fermentum* InaCC B1295 is needed.

5- Declarations

5-1- Author Contributions

Conceptualization, U.P. and D.F.A.; methodology, U.P. and E.R.; software, F.R; validation, U.P., E.R. and I.J.; formal analysis, W.T.P. and R.F.; investigation, R.F., and A.R.; resources, U.P.; data curation, D.F.A.; writing—original draft preparation, U.P. and E.R.; writing—review and editing, I.J.; visualization, A.R.; supervision, U.P.; project administration, A.R.; funding acquisition, U.P. All authors have read and agreed to the published version of the manuscript.

5-2- Data Availability Statement

The data presented in this study are available in article.

5-3- Funding

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5-5- Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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