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To Utilize Fungal Beta-Glucan As An Encapsulating Agent For Delivery Of *Probiotics*

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Abstract

Background: Maintaining *probiotic* viability throughout food processing, storage, and gastrointestinal transit remains a critical limitation in the development of functional foods. Although microencapsulation is widely applied to enhance *probiotic* stability, the exploration of multifunctional wall materials that combine protective performance with added health benefits is still limited. Fungal β-glucans represent a promising alternative due to their structural robustness and inherent *prebiotic* functionality.

Objective: This study aimed to systematically evaluate fungal β-glucans derived from Agaricus, Pleurotus, Coprinus, and yeast as encapsulating agents for *probiotic* delivery, with a focus on their ability to enhance *probiotic* stability under storage, gastrointestinal, and thermal stress conditions.

Methods: Lactobacillus plantarum, Lactobacillus brevis, and Lactobacillus casei were microencapsulated using fungal β-glucans via freeze-drying. Encapsulation efficiency, refrigerated storage stability (4 °C, 45 days), survival under simulated gastric (pH 2.0) and intestinal conditions, and thermal resistance (55–75 °C) were evaluated. Microcapsule morphology and structural interactions were analyzed using SEM, ATR-FTIR spectroscopy, and particle size analysis.

Results: Encapsulation yields ranged from 63.83% to 76.63%, with Coprinus β-glucan consistently providing the highest efficiency and *probiotic* retention. Encapsulated *probiotics* exhibited significantly improved viability during storage and showed enhanced resistance to acidic, bile, and thermal stresses compared to free cells. SEM revealed porous microstructures conducive to effective cell entrapment, while ATR-FTIR confirmed molecular interactions between *probiotics* and β-glucan matrices.

Conclusion: Fungal β-glucans, particularly those derived from Coprinus and Pleurotus, function as effective and multifunctional encapsulating materials, offering both physical protection and *probiotic* potential. These findings highlight fungal β-glucan-based microcapsules as a robust delivery system for probiotics, with strong prospects for application in next-generation functional foods and nutraceutical formulations.

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INTRODUCTION

Probiotics are live microbial supplements known to play an important role in controlling gastrointestinal infections, enhancing immune function, alleviating lactose intolerance, and reducing serum cholesterol levels (Maleki, Homayouni, Khalili, & Golkhalkhali, 2016). To deliver these health benefits, probiotic-containing products must retain a minimum viable count of approximately 10^7 CFU/g at the point of consumption. Nevertheless, preserving probiotic viability remains a significant challenge due to stress conditions encountered during food processing, storage, and transit through the gastrointestinal tract (Neish, 2009). According to global market analyses, *Probiotics* accounted for nearly 30% of the functional food market, representing an annual value of about US$50 billion, with projections indicating growth from US$62.6 billion in 2014 to US$96.0 billion by 2020 at a

compound annual growth rate of 7.40% (Corcoran et all., 2008). Consequently, numerous strategies have been explored to enhance probiotic survival within intestinal microflora, including protection against gastric acidity, enzymatic degradation, and bile salt exposure in the small intestine, as probiotic viability during food processing remains a major limitation in functional food applications (Pillai et all., 2012). *Probiotics* also contribute to intestinal barrier function by stimulating mucin production, increasing the expression of tight junction proteins, and preventing pathogen-induced disruption of epithelial integrity (Johnson et all., 2008). In this regard, microencapsulation using suitable wall materials offers an effective physical barrier that enhances probiotic resistance to unfavorable environmental conditions encountered in both commercial food systems and the gastrointestinal tract (Anal & Singh, 2007). In microencapsulation systems, the probiotic cells constitute the core material, while the surrounding protective layer is referred to as the wall material; commonly used food-grade encapsulants include chitosan, alginate, xanthan gum, gellan, gelatin, and whey proteins. Recently, fungal β-glucans have attracted attention due to their prebiotic properties, and encapsulating *probiotics* within β-glucan-based matrices may provide additional benefits by promoting the growth and activity of beneficial intestinal microflora (Mitsou et al., 2010).

METHOD

Encapsulation of *probiotics* using fungal β-glucan

Microencapsulation of *probiotics* was carried out using a pilot-scale freeze dryer (Operon, IPS-55) operated at temperatures ranging from −40 to −50 °C, with the freeze-drying process completed within 20 h. Prior to freezing, skim milk powder was incorporated into the drying medium as a cryoprotectant. The freeze-dried products were packed in polyethylene bags, sealed with aluminum foil, and stored at 4 °C until further analysis. Mushroom-derived β-glucans were extracted from Agaricus bisporus, Pleurotus ostreatus, and Coprinus atramentarious following the procedure described by Smiderle et al. (2013). β-Glucan from yeast was obtained from baker's yeast (Saccharomyces cerevisiae) using the method reported by Lee et al. (2001).

Encapsulation yield of *probiotics*

To determine encapsulation efficiency, one gram of microcapsules was resuspended in 9 mL of phosphate buffer (0.1 mol/L, pH 7.0) and homogenized for 15 min. Viable cell counts (CFU/g) were estimated by plating appropriate dilutions on selective agar media followed by incubation at 37 °C for 48 h (Mandal et al., 2006). Encapsulation yield (EY), representing the survival of probiotic cells during microencapsulation, was calculated using the following equation:

$$EY(\%) = \frac{N}{No} \times 100$$

where N denotes the viable cell count (log CFU/g) recovered from the microcapsules and N_0 represents the initial viable cell count (log CFU/g) added during preparation.

Storage stability of encapsulated *probiotics*

Storage stability of encapsulated *probiotics* was evaluated by determining viable cell counts at 15-day intervals over a period of 1.5 months during storage at 4 °C.

Survival in simulated gastric juice (SGJ)

Simulated gastric juice (SGJ) was prepared using sodium chloride (9 g/L) containing pepsin (3.0 g/L), and the pH was adjusted to 2.0 with hydrochloric acid (Altman, 1961). Microcapsules (0.2 g) containing entrapped Lactobacillus cells were suspended in 10 mL of SGJ and incubated at 37 °C under constant shaking at 50 rpm for 5, 30, 60, and 120 min. Probiotic survival in SGJ was expressed as log CFU/g.

Survival in simulated intestinal juice (SIJ)

For thermal tolerance analysis, one gram of microcapsules and 1 mL of free cell suspension

were transferred into test tubes containing 10 mL of sterile distilled water. The samples were subjected to heat treatments at 55, 65, and 75 °C for 10 min, as described by Sabikhi et al. (2010).

Survival of free and microencapsulated cells under heat treatments

For thermal tolerance analysis, one gram of microcapsules and 1 mL of free cell suspension were transferred into test tubes containing 10 mL of sterile distilled water. The samples were subjected to heat treatments at 55, 65, and 75 °C for 10 min, as described by Sabikhi et al. (2010).

Morphological characterization of the microcapsules

The surface morphology of probiotic-loaded fungal β-glucan microcapsules was examined using scanning electron microscopy (SEM) (Hitachi S-300H, Tokyo, Japan). Samples were mounted on aluminum stubs with double-sided adhesive tape and coated with a thin gold layer using a sputter coater. After gold–palladium coating, micrographs were obtained at an accelerating voltage of 5 kV.

Conformational study by using ATR-FTIR

ATR-FTIR spectra of β-glucan-encapsulated *probiotics* were recorded using an ATR–FTIR spectrophotometer (CARY 630, Agilent Technologies, USA) at room temperature over a spectral range of 400–4000 cm^{-1}.

Statistical analysis

All data were expressed as mean values with corresponding standard deviations. Statistical analysis was performed using SPSS software version 10.1 (USA). Analysis of variance (ANOVA) was applied, and significant differences among means were determined using Duncan's multiple range test at a 5% significance level.

RESULTS AND DISCUSSION

Encapsulation yield of *probiotics*

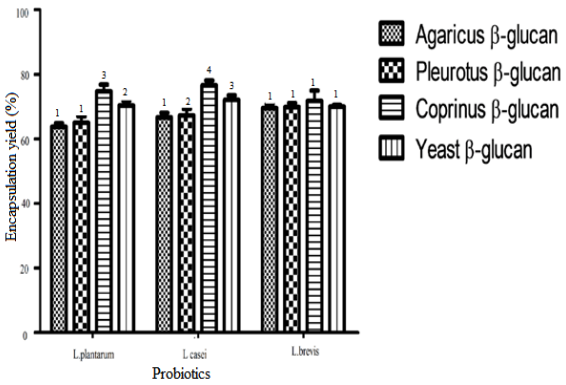


Fig. 1: Encapsulation yield of *probiotics*

The encapsulation yield (%) of L. plantarum, L. brevis, and L. casei entrapped within β-glucans derived from Agaricus, Pleurotus, Coprinus, and yeast is illustrated in Fig. 1. The encapsulation efficiency ranged from 63.83–74.83% for L. plantarum, 66.63–76.63% for L. brevis, and 69.50–71.83% for L. casei. A statistically significant difference ($p \leq 0.05$) was detected among the four β-glucan sources when used as encapsulating agents for L. casei. In contrast, no significant difference ($p > 0.05$) was observed among the fungal β-glucans for L. brevis. For L. plantarum, Agaricus and Pleurotus β-glucans showed no significant difference ($p > 0.05$), whereas Coprinus and yeast β-glucans differed significantly ($p \leq 0.05$) from each other. Encapsulation within a protective matrix is known to safeguard probiotic cells against adverse environmental conditions encountered during processing, storage, and gastrointestinal transit (Desai & Park, 2005; Heidebach et al., 2012). Fungal β-glucans have previously been recognized for their prebiotic potential and suitability as encapsulating materials (Shi et al., 2013). The present findings are consistent with Shah et al. (2016),

who reported encapsulation efficiencies of 78.95%, 71.08%, and 72.02% for L. brevis, L. plantarum, and L. casei, respectively, using barley β-glucan microspheres prepared by the emulsion technique.

Storage stability of encapsulated Probiotics

Table 1: Storage stability of free and encapsulated *probiotics* using fungal β-glucans expressed as log cfu g^{-1}.

Probiotics	**β-glucan (Wall material)**	**Storage time (Days) 0**	**1 5**	**3 0**	**4 5**
L. plantarum	*Free*	3.76 ± 0.42^{1a}	3.72 ± 0.25^{1a}	0.76 ± 0.25^{1b}	0.60 ± 0.30^{1b}
	Agaricus	5.72 ± 0.42^{2a}	4.45 ± 0.25^{2b}	1.65 ± 0.21^{2c}	1.16 ± 0.25^{3d}
	Pleurotus	6.25 ± 0.28^{3a}	4.44 ± 0.51^{23b}	2.13 ± 0.32^{4c}	1.23 ± 0.20^{3d}
	Coprinus	7.38 ± 0.18^{4a}	4.76 ± 0.25^{3b}	1.76 ± 0.25^{23c}	1.26 ± 0.35^{3d}
	Yeast	7.20 ± 0.20^{4a}	4.50 ± 0.20^{3b}	1.81 ± 0.07^{23c}	0.81 ± 0.07^{12d}
L. brevis	*Free*	7.16 ± 0.40^{1a}	3.65 ± 0.47^{1b}	2.70 ± 0.50^{1c}	1.53 ± 0.30^{1d}
	Agaricus	7.93 ± 0.50^{3a}	6.76 ± 0.32^{2b}	4.0 ± 0.50^{2c}	2.63 ± 0.15^{2d}
	Pleurotus	7.66 ± 0.15^{2a}	6.80 ± 0.26^{2b}	4.80 ± 0.26^{3c}	3.35 ± 0.39^{3d}
	Coprinus	8.66 ± 0.30^{4a}	7.03 ± 0.47^{2b}	4.70 ± 0.50^{3c}	3.46 ± 0.41^{3d}
	Yeast	8.36 ± 0.41^{34a}	6.90 ± 0.15^{2b}	4.93 ± 0.45^{3c}	3.30 ± 0.45^{3d}
L. casei	*Free*	5.83 ± 0.51^{1a}	4.13 ± 0.50^{1b}	2.56 ± 0.20^{1c}	1.63 ± 0.25^{1d}
	Agaricus	8.73 ± 0.41^{3a}	5.90 ± 0.70^{2b}	4.16 ± 0.35^{2c}	2.50 ± 0.10^{2d}
	Pleurotus	7.83 ± 0.51^{23a}	6.70 ± 0.26^{3b}	4.36 ± 0.51^{2c}	2.63 ± 0.25^{3d}
	Coprinus	12.16 ± 0.40^{4a}	8.80 ± 0.72^{4b}	5.23 ± 0.66^{3c}	3.30 ± 0.36^{4d}
	Yeast	7.81 ± 0.48^{23a}	6.76 ± 0.15^{3b}	4.26 ± 0.45^{2c}	2.66 ± 0.25^{3d}

Different alphabetical letters and the numeric indicate significant difference ($p\leq 0.05$) among samples in the same row and column respectively.

The storage stability of encapsulated *probiotics* maintained at 4 °C was evaluated at 15-day intervals over a 45-day period (Table 1). At day 0, free L. plantarum exhibited a viable count of 3.76 log cfu/g, whereas microspheres formulated with Agaricus, Pleurotus, Coprinus, and yeast β-glucans showed values of 5.72, 6.25, 7.38, and 7.20 log cfu/g, respectively. Except for Coprinus- and yeast-based β-glucans, a significant difference ($p\leq0.05$) was observed between free and encapsulated L. plantarum at the initial storage period. With prolonged storage (15–45 days), the viability of free L. plantarum declined markedly from 3.72 to 0.60 log cfu/g. A significant reduction ($p\leq0.05$) was also recorded for encapsulated L. plantarum in Agaricus (4.45–1.16), Pleurotus (4.44–1.23), Coprinus

(4.76–1.26), and yeast β-glucans (4.50–0.81 log cfu/g). Similar decreasing trends were noted for encapsulated L. brevis and L. casei as storage duration increased. Overall, encapsulation in fungal β-glucans substantially reduced the loss of cell viability during refrigerated storage by acting as a physical barrier against environmental stressors (Mitsou et al., 2010). These observations corroborate earlier reports demonstrating enhanced storage stability of encapsulated *probiotics* under refrigerated conditions (Heidebach et al., 2010; Shah et al., 2016).

Survival in simulated gastric juice (SGJ) conditions

Table 2: Viability of *probiotics* subjected to simulated gastric juices at different times (log cfu g^{-1})

Probiotics	**β-glucan (wall material)**	**Time (min.)**			
		5	**30**	**60**	**120**
L. plantarum	*Free*	5.26±0.64^{1a}	4.31±0.35^{1b}	3.90±0.36^{12c}	1.46±0.15^{1d}
	Agaricus	6.80±0.26^{23a}	5.13±0.25^{2b}	4.36±0.35^{23c}	1.90±0.40^{2d}
	Pleurotus	6.46±0.49^{2a}	5.23±0.50^{23b}	4.22±0.33^{23c}	1.50±0.24^{1d}
	Coprinus	7.60±0.20^{4a}	5.43±0.45^{3b}	4.23±0.23^{23c}	2.13±0.56^{23d}
	Yeast	7.06±0.26^{3a}	6.49±0.29^{4b}	4.31±0.45^{23c}	2.31±0.21^{23d}
L. brevis	*Free*	6.73±1.25^{1a}	6.13±0.30^{1a}	4.5±1.15^{1b}	1.73±0.30^{1c}
	Agaricus	7.76±0.32^{12a}	7.03±0.15^{3b}	6.3±0.36^{3c}	3.83±0.41^{4d}
	Pleurotus	6.96±0.34^{1a}	5.78±0.50^{2b}	3.69±0.44^{1c}	2.47±0.30^{3d}
	Coprinus	8.40±0.36^{3a}	7.36±0.45^{4b}	5.53±0.41^{2c}	2.06±0.30^{2d}
	Yeast	7.73±0.24^{12a}	6.20±0.19^{2b}	3.51±0.43^{1c}	2.07±0.25^{2d}
L. casei	*Free*	5.36±0.89^{1a}	4.56±0.06^{1ab}	3.43±1.25^{1b}	2.03±0.75^{12c}
	Agaricus	6.91±0.20^{23a}	4.95±0.17^{2b}	3.59±0.15^{1c}	2.31±0.29^{123d}
	Pleurotus	6.86±0.28^{2a}	5.38±0.35^{3b}	3.75±0.56^{12c}	2.70±0.42^{13d}

	Coprinus	6.70±0.26^{2a}	5.90±0.10^{4b}	5.30±0.36^{3c}	3.53±0.47^{4d}
	Yeast	7.00±0.41^{3a}	5.68±0.39^{34b}	3.76±0.14^{12c}	3.02±0.28^{3d}

Different alphabetical letters and the numeric indicate significant difference (p≤ 0.05) among samples in the same row and column respectively.

An in vitro gastric model was employed to assess the tolerance of encapsulated *probiotics* to acidic gastric conditions. The survival of L. plantarum, L. brevis, and L. casei encapsulated in different fungal β-glucans following exposure to SGJ is summarized in Table 2. After 5 min of incubation, free L. plantarum showed a viability of 5.26 log cfu/g, while encapsulated cells in Agaricus, Pleurotus, Coprinus, and yeast β-glucans retained higher counts (6.80–7.60 log cfu/g). Increasing incubation time up to 120 min led to a significant decline (p≤0.05) in viability for both free and encapsulated cells. However, encapsulated *probiotics* consistently exhibited improved survival compared to free cells. Comparable protective effects were observed for L. brevis and L. casei, indicating that fungal β-glucans effectively enhanced resistance to acidic stress. This protection is attributed to the acid-resistant nature of β-glucans and their resistance to gastric enzymes (Iyer et al., 2005; Shah et al., 2016). The present findings align with previous studies reporting improved probiotic survival in simulated gastric conditions following microencapsulation (Chavarri et al., 2010; Mokhtari et al., 2017).

Survival in simulated intestinal juice (SIJ) conditions

Table 3: Viability of encapsulated *probiotics* subjected to simulated intestinal juice at different times (log cfu g^{-1})

Probiotics	**β-glucan (wall material)**	**Time (min.)**		
		60	**90**	**120**
L. plantarum	*Free*	1.96±0.56^{1a}	0.99±0.08^{1b}	0.59±0.06^{1c}
	Agaricus	2.09±0.17^{1a}	1.71±0.13^{3b}	0.84±0.11^{2c}
	Pleurotus	3.16±0.15^{3a}	1.99±0.22^{4b}	1.19±0.17^{3c}
	Coprinus	2.80±0.20^{2a}	1.63±0.10^{3b}	0.79±0.06^{2c}
	Yeast	2.84±0.11^{2a}	1.50±0.07^{2b}	0.80±0.10^{2c}
L. brevis	*Free*	1.50±0.30^{1a}	1.37±0.12^{1a}	0.80±0.27^{1b}
	Agaricus	3.54±0.23^{2a}	2.03±0.06^{2b}	1.09±0.14^{2c}
	Pleurotus	3.78±0.15^{23a}	2.41±0.29^{23b}	1.18±0.18^{2c}
	Coprinus	3.48±1.19^{2a}	1.99±0.51^{2b}	1.10±0.20^{2b}
	Yeast	3.48±1.19^{2a}	2.42±0.10^{23a}	1.06±0.20^{12b}
L. casei	*Free*	1.53±0.11^{1a}	0.85±0.05^{1b}	0.44±0.02^{1c}
	Agaricus	3.20±0.20^{3a}	2.30±0.28^{3b}	1.17±0.36^{4c}
	Pleurotus	3.85±0.10^{4a}	2.47±0.25^{3b}	1.46±0.11^{4c}

	Coprinus	2.53 ± 0.11^{2a}	1.90 ± 0.10^{2b}	0.64 ± 0.02^{2c}
	Yeast	2.51 ± 0.17^{2a}	1.90 ± 0.11^{2b}	0.72 ± 0.07^{3c}

Different alphabetical letters and the numeric indicate significant difference (p≤ 0.05) among samples in the same row and column respectively.

The impact of simulated intestinal conditions on probiotic viability is presented in Table 3. Encapsulated probiotics demonstrated significantly higher survival rates than free cells, although viability declined (p≤0.05) with increasing incubation time from 60 to 120 min. Encapsulation using fungal β-glucans effectively reduced bile-induced damage, thereby preserving cell integrity. Similar observations have been reported by Rajam et al. (2015) and Mokhtari et al. (2017), who highlighted the suitability of polysaccharide-based matrices for improving probiotic survival in intestinal environments.

Survival of encapsulated Probiotics under heat treatments

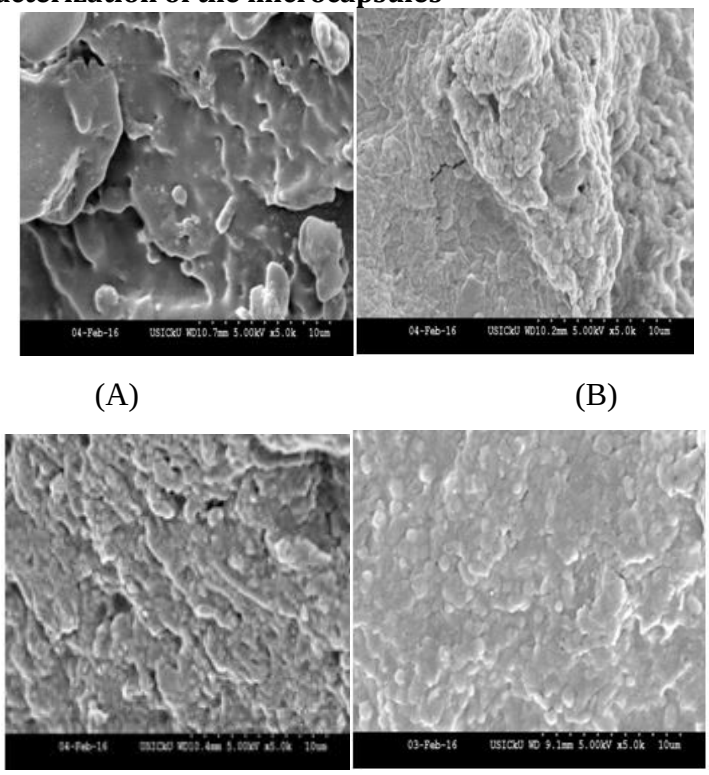
Table 4: Thermal resistance of encapsulated probiotics in fungal β-glucans (log cfu g^{-1})

Probiotics	**β-glucan (wall material)**	**Temperature (^{0}C)**		
		55	**65**	**75**
L. plantarum	*Free*	2.26 ± 0.15^{12a}	0.81 ± 0.28^{1b}	0.64 ± 0.12^{1b}
	Agaricus	2.88 ± 0.68^{2a}	2.20 ± 0.10^{3a}	0.74 ± 0.2^{1b}
	Pleurotus	3.42 ± 0.30^{23a}	2.71 ± 0.14^{4b}	1.72 ± 0.14^{3c}
	Coprinus	3.56 ± 0.15^{23a}	1.18 ± 0.30^{2b}	0.74 ± 0.4^{1b}
	Yeast	3.48 ± 0.23^{23a}	2.90 ± 0.68^{5b}	1.46 ± 0.1^{2b}
L. brevis	*Free*	0.69 ± 0.05^{1a}	0.43 ± 0.07^{1b}	0.26 ± 0.05^{1c}
	Agaricus	3.09 ± 0.07^{3a}	2.73 ± 0.05^{23b}	1.54 ± 0.20^{3c}
	Pleurotus	3.17 ± 0.25^{34a}	2.78 ± 0.09^{23b}	1.72 ± 0.13^{3c}
	Coprinus	2.96 ± 0.55^{3a}	1.81 ± 1.02^{2b}	0.27 ± 0.30^{1c}
	Yeast	2.34 ± 0.22^{2a}	2.01 ± 0.06^{2a}	0.67 ± 0.10^{2b}
L. casei	*Free*	1.28 ± 0.69^{1a}	1.14 ± 0.33^{1a}	0.97 ± 0.06^{13a}
	Agaricus	3.15 ± 0.07^{3a}	2.26 ± 0.17^{4b}	1.42 ± 0.50^{23c}
	Pleurotus	2.95 ± 0.36^{2a}	1.91 ± 0.14^{3b}	1.20 ± 0.56^{23c}
	Coprinus	3.71 ± 0.18^{4a}	1.70 ± 0.20^{2b}	1.24 ± 0.32^{23c}
	Yeast	2.72 ± 0.24^{2a}	2.19 ± 0.13^{4b}	1.04 ± 0.54^{23c}

Different alphabetical letters and the numeric indicate significant difference (p≤ 0.05) among samples in the same row and column respectively.

The thermal stability of free and encapsulated probiotics subjected to temperatures ranging from 55 to 75 °C is shown in Table 4. Encapsulated cells exhibited markedly higher thermal tolerance compared to free cells. Although viability decreased with increasing temperature, β-glucan encapsulation significantly improved heat resistance across all probiotic strains. These results support earlier findings that microencapsulation reduces heat transfer to bacterial cells and mitigates thermal damage (Corcoran et al., 2008; Shah et al., 2016).

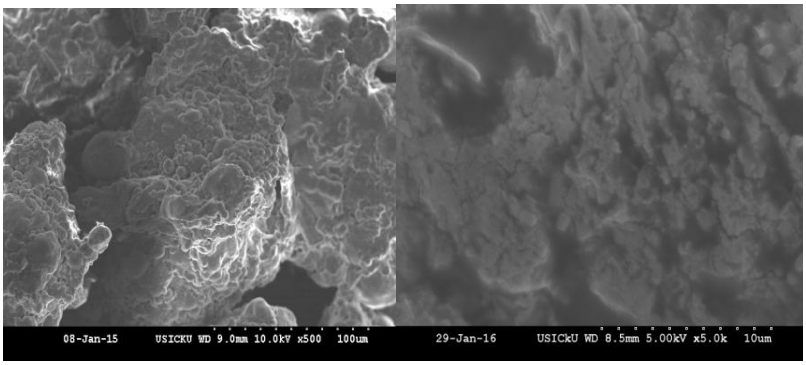
Morphological characterization of the microcapsules



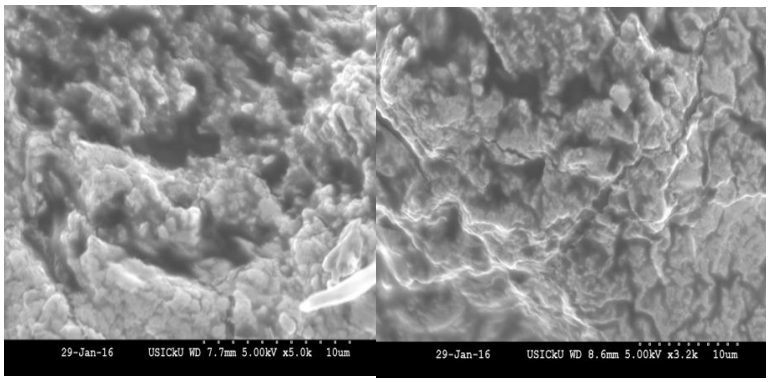
(A) (B)

(C) (D)

Fig. 2: Scanning Electron Microscopy of A) native *Agaricus* β-glucan B) Encapsulated *L. plantarum* in *Agaricus* β-glucan C) Encapsulated *L. brevis in Agaricus* β-glucan, D) Encapsulated *L. casei* in *Agaricus* β-glucan

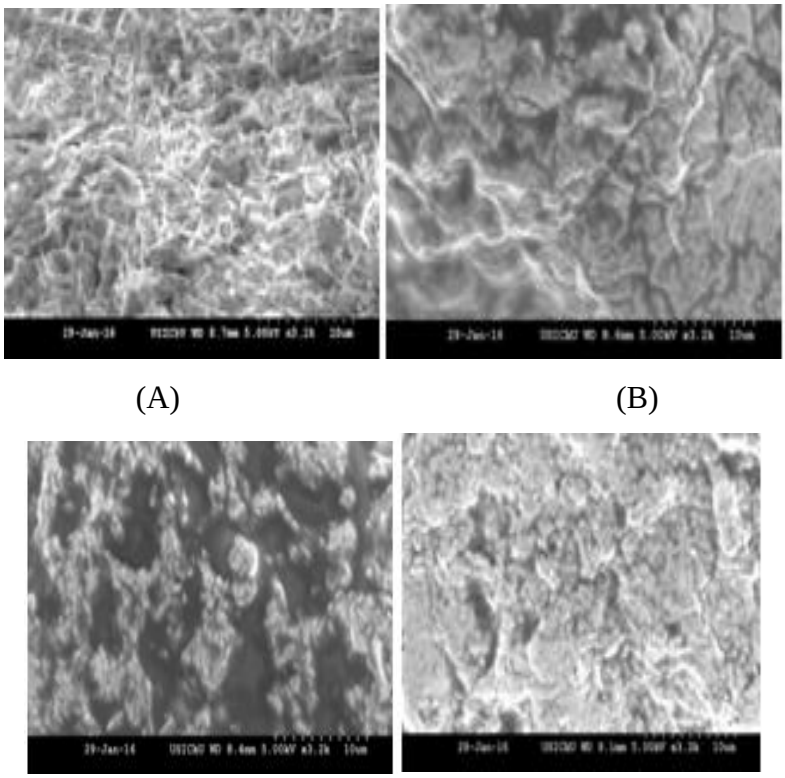


(A) (B)



(C) (D)

Fig. 3: Scanning Electron Microscopy of A) native *Pleurotus* β-glucan B) Encapsulated *L. plantarum* in *Pleurotus* β-glucan C) Encapsulated *L. brevis in Pleurotus* β-glucan, D) Encapsulated *L. casei* in *Pleurotus* β-glucan.



(A) (B)

(C) (D)

Fig.4: Scanning Electron Microscopy of A) native *Coprinus* β-glucan B) Encapsulated *L. plantarum* in *Coprinus* β-glucan C) Encapsulated *L. brevis in Coprinus* β-glucan, D) Encapsulated *L. casei in Coprinus* β-glucan.

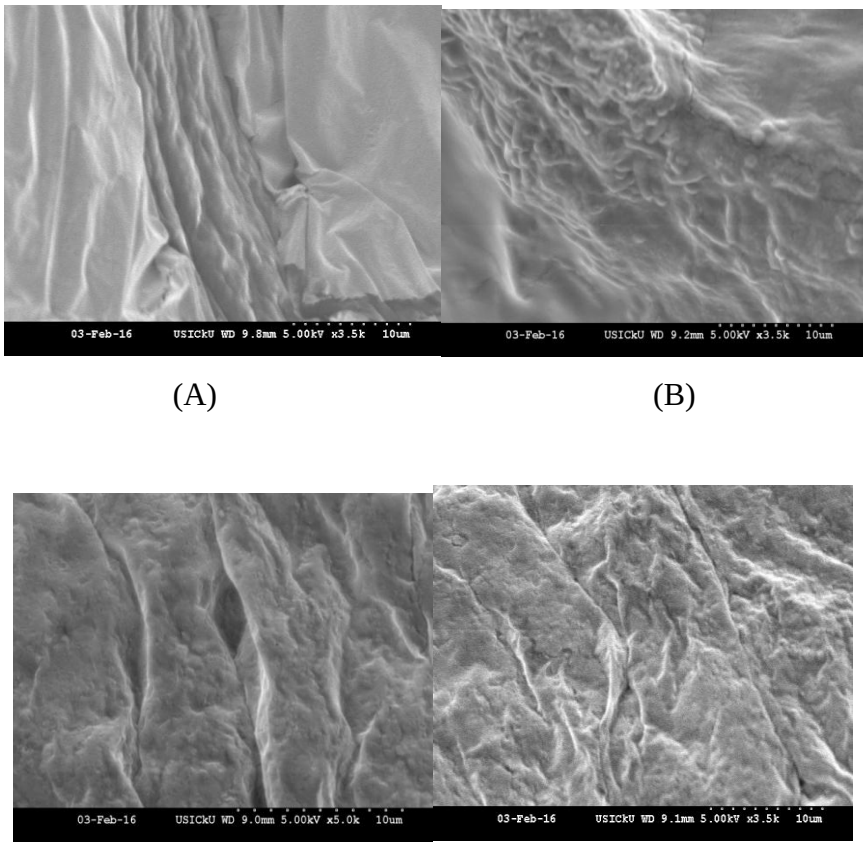


Fig. 5: Scanning Electron Microscopy of A) native yeast β-glucan B) Encapsulated *L. plantarum* in yeast β-glucan with C) Encapsulated *L. brevis* in yeast β-glucan & D) Encapsulated *L. casei* inyeast β-glucan.

SEM analysis of freeze-dried microcapsules (Fig. 2–5) revealed irregularly shaped particles with porous surface structures. The observed porosity is likely a consequence of sublimation under vacuum during freeze-drying, leading to partial structural collapse (Rathore et al., 2013). Variations in surface morphology among microcapsules may be attributed to differences in the film-forming properties of the fungal β-glucans employed. Comparable microstructural features have been reported for β-glucan-based probiotic microcapsules in previous studies (Shah et al., 2016; Rajam & Anandharamakrishnan, 2015).

Conformational study of microcapsules using ATR-FTIR

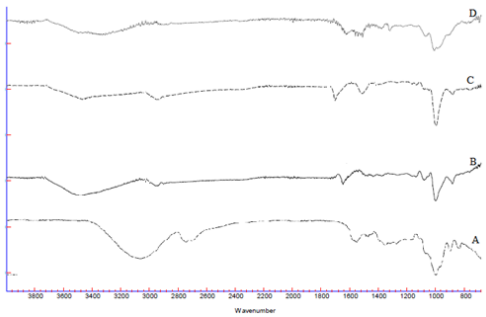


Fig. 6: ATR-FTIR spectra of (A) *Agaricus* β-glucan, (B) *Agaricus* β-glucan encapsulating *L. plantarum,* C) *Agaricus* β-glucan encapsulating *L. brevis* & (D) *Agaricus* β-glucan encapsulating *L. casei*

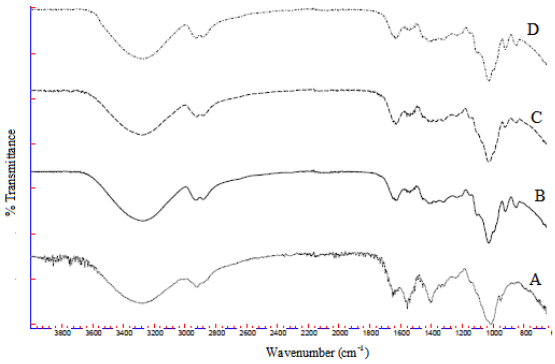


Fig. 7: ATR-FTIR spectra of (A) *Pleurotus* β-glucan, (B) *Pleurotus* β-glucan encapsulating *L. plantarum,* C) *Pleurotus* β-glucan encapsulating *L. brevis* & (D) *Pleurotus* β-glucan encapsulating *L. casei.*

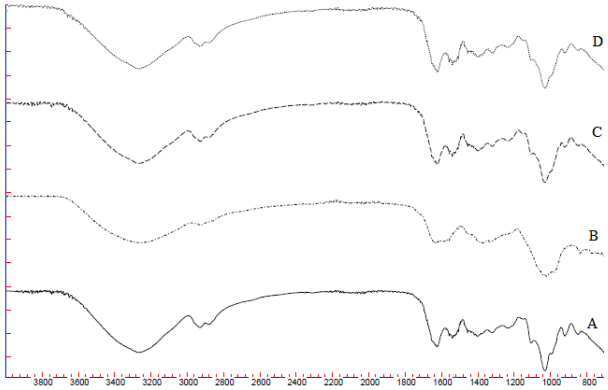


Fig. 8: ATR-FTIR spectra of (A) *Coprinus* β-glucan, (B) *Coprinus* β-glucan encapsulating *L. plantarum,* C) *Coprinus* β-glucan encapsulating *L. brevis* & (D) *Coprinus* β-glucan encapsulating *L. casei*

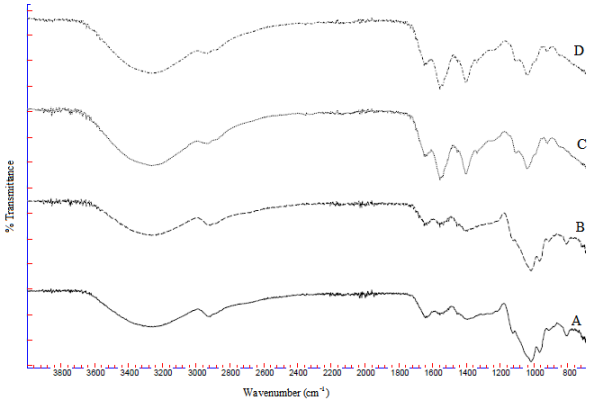


Fig. 9: ATR-FTIR spectra of (A) Yeast β-glucan, (B) Yeast β-glucan encapsulating *L. plantarum,* C) Yeast β-glucan encapsulating *L. brevis & (D)*Yeast β-glucan encapsulating *L. casei*

ATR-FTIR spectroscopy was used to examine molecular interactions between *probiotics* and fungal β-glucans (Fig. 6–9). Native β-glucans displayed characteristic absorption bands corresponding to –OH, C–H, C=O stretching, and β-glycosidic linkages. Encapsulated samples exhibited additional peaks associated with bacterial cell components, confirming successful incorporation of *probiotics* into the β-glucan matrix. The enhanced peak intensity observed in microcapsules suggests interactions between glucans and bacterial cell wall constituents, in agreement with earlier FTIR studies on probiotic encapsulation systems (Kansiz et al., 1999; Shah et al., 2016).

Particle size analysis

Particle size distribution plays a crucial role in determining the functional and sensory properties of microcapsules. The volume mean diameter of probiotic microcapsules prepared with different fungal β-glucans is summarized in Table 5. Agaricus β-glucan microcapsules exhibited the largest mean diameters (59.13–63.35 µm), while yeast β-glucan microcapsules showed the smallest sizes (43.20–47.57 µm). No significant difference ($p>0.05$) was observed between Coprinus and Pleurotus β-glucan microcapsules. The observed size variations are likely related to differences in solution viscosity during atomization, as higher viscosity tends to generate larger droplets. These findings are consistent with previous reports on spray- and freeze-dried probiotic microcapsules (Rajam et all., 2015)n

CONCLUSION

The highest encapsulation yield (%) of probiotic-loaded microspheres was obtained when Coprinus β-glucan was used as the wall material for L. plantarum, L. brevis, and L. casei, with values of 74.83±2.02%, 76.63±1.51%, and 71.83±3.25%, respectively, showing no significant difference ($p\leq0.05$). Coprinus β-glucan microspheres also exhibited the greatest probiotic viability compared to the other three fungal β-glucans, with initial counts of 7.38±0.18, 8.66±0.30, and 12.16±0.40 log cfu/g for L. plantarum, L. brevis, and L. casei, respectively, which decreased to 1.26±0.35, 3.46±0.41, and 3.30±0.36 log cfu/g after 45 days of storage. In contrast, the lowest viability was observed in *probiotics* encapsulated with Agaricus β-glucan, where counts declined from 5.72±0.42, 7.93±0.50, and 8.73±0.41 log cfu/g to 1.16±0.25, 2.63±0.15, and 2.50±0.10 log cfu/g for L. plantarum, L. brevis, and L. casei, respectively.

Free probiotic cells showed a pronounced reduction in viability during storage, with L. plantarum, L. brevis, and L. casei decreasing from 3.72±0.42, 5.00±0.40, and 5.83±0.51 log cfu/g to 0.60±0.30, 1.53±0.30, and 1.63±0.25 log cfu/g, respectively, after 1.5 months. These findings clearly demonstrate that microencapsulation significantly minimized viability losses compared to free cells. The incorporation of fungal β-glucans during microencapsulation enhanced probiotic resistance to acidic pH and bile salts in simulated gastrointestinal conditions, resulting in higher viable counts than the control (without prebiotics) across all treatments.

Following 5 min exposure to simulated gastric juice, the highest survival was recorded for Coprinus β-glucan microspheres containing L. plantarum, L. brevis, and L. casei, with viable counts of 7.60±0.20, 8.40±0.36, and 6.70±0.26 log cfu/g, respectively, which declined to 2.13±0.56, 2.06±0.30, and 3.53±0.47 log cfu/g after 120 min of incubation. Under simulated intestinal juice conditions, encapsulated *probiotics* consistently showed significantly higher viability ($p\leq0.05$) than free cells. The viable counts of free L. plantarum, L. brevis, and L. casei were 1.96±0.56, 1.50±0.30, and 1.53±0.11 log cfu/g after 60 min, further decreasing to 0.59±0.06, 0.80±0.27, and 0.44±0.02 log cfu/g after 120 min.

Among the four fungal β-glucan matrices, Pleurotus β-glucan microspheres exhibited the highest probiotic survival during intestinal simulation. The viabilities of L. plantarum, L. brevis, and L. casei were 3.16±0.15, 3.78±0.15, and 3.85±0.10 log cfu/g after 60 min, decreasing to 1.99±0.22, 2.41±0.29, and 2.47±0.25 log cfu/g after 90 min, and finally to 1.19±0.17, 1.18±0.18, and 1.46±0.11 log cfu/g after 120 min of incubation.

Encapsulation using fungal β-glucans as wall materials also improved the thermal tolerance of *probiotics* when exposed to temperatures of 55, 65, and 75 °C for 10 min. SEM analysis revealed porous surface morphology of the microspheres produced with different wall materials, resulting from moisture removal during drying. The presence of pores facilitated probiotic entrapment, leading to densely loaded microcapsules, while structural interactions between *probiotics* and β-glucans were further confirmed by ATR-FTIR analysis.

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AUTHOR CONTRIBUTION STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary material. Raw data that support the findings of this study are available from the corresponding author, upon reasonable request

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