Camel milk-derived probiotic strains encapsulated in camel casein and gelatin complex microcapsules: Stability against thermal challenge and simulated gastrointestinal digestion conditions

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ABSTRACT

Probiotics have received increased attention due to their nutritional and health-promoting benefits. However, their viability is often impeded during food processing as well as during their gastrointestinal transit before reaching the colon. In this study, probiotic strains *Lactobacillus rhamnosus* MF00960, *Pediococcus pentosaceus* MF000967, and *Lactobacillus paracasei* DSM20258 were encapsulated within sodium alginate, camel casein (CC), camel skin gelatin (CSG) and CC:CSG (1:1 wt/wt) wall materials. All 3 strains in encapsulated form showed an enhanced survival rate upon simulated gastrointestinal digestion compared with free cells. Among the encapsulating matrices, probiotics embedded in CC showed higher viability and is attributed to less porous structure of CC that provided more protection to entrapped probiotics cells. Similarly, thermal tolerance at 50°C and 70°C of all 3 probiotic strains were significantly higher upon encapsulation in CC and CC:CSG. Scanning electron microscope micrographs showed probiotic strains embedded in the dense protein matrix of CC and CSG. Fourier-transform infrared spectroscopy showed that CC- and CSG-encapsulated probiotic strains exhibited the amide bands with varying intensity with no significant change in the structural conformation. Probiotic strains encapsulated in CC and CC:CSG showed higher retention of inhibitory properties against α -glucosidase, α -amylase, dipeptidyl peptidase-IV, pancreatic lipase, and cholesteryl esterase compared with free cells upon

exposure to simulated gastrointestinal digestion conditions. Therefore, CC alone or in combination with CSG as wall materials provided effective protection to cells, retained their bioactive properties, which was comparable to sodium alginate as wall materials. Thus, CC and CC:CSG can be an efficient wall material for encapsulation of probiotics for food applications.

Key words: camel milk, *Lactobacillus*, microencapsulation, in vitro digestion, bioactive properties

INTRODUCTION

Probiotic lactic acid bacteria have been employed in the design of various functional foods. To retain the viability of probiotic microorganism during food processing and storage, as well as in the gastrointestinal transit, probiotics are encapsulated in various matrices (Fernanda et al., 2016). The encapsulation methods as well as nature, type, or characteristic of encapsulating matrices are critical in maintaining the viability of probiotic bacteria and their subsequent health effects (Ahmad et al., 2019b).

Commonly used encapsulation matrices such as carbohydrates, proteins, and their combinations have been examined to maintain probiotic viability and stability. Polysaccharides, such as sodium alginate, chitosan, starch, methylcellulose, and sodium carboxymethylcellulose, exhibit several advantages, including better chemical stability, film-forming ability, stabilization, and better protection to the encapsulated probiotics in the adverse environmental conditions, especially under the gastrointestinal condition (Kanmani et al., 2011). In this regard, some studies have indicated the efficacy of gelatin as a potential encapsulating matrix because of its properties such as thermally reversible gelling behavior, membrane-forming ability, biocompatibility, and nontoxicity (Petraitytė and Šipailienė, 2019). Simi-

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larly, milk proteins such as caseins and whey proteins are also recognized as valuable biological macromolecules with high nutritional and excellent functional properties. These milk proteins have properties such as binding small molecules, self-assembly, excellent gelation, pH-responsive gel swelling behavior, and ability to interact with other polymers for the formation of complexes (Singh et al., 2019). In addition, probiotics proliferate well in dairy-based media because of the lactose-hydrolyzing enzyme and proteolytic system involved in casein utilization, which nourishes the probiotic cells with essential source of carbon and amino acids for their development (Yeo et al., 2011). Among different sources of proteins, bovine milk proteins and bovine or porcine gelatin are widely used as an encapsulating material. However, studies pertaining to camel milk proteins and camel skin gelatin (CSG) are limited (Ahmad et al., 2019b). Until today, predominant number of studies related to gelatin and its utilization for encapsulation are concentrated upon porcine, bovine and fish gelatin. The present study aims to use camel casein (CC) proteins and camel gelatin as potential alternative wall material for encapsulating novel camel milk-derived probiotic strains and compared the results with widely used encapsulant sodium alginate (SA). The probiotic cell viability during encapsulation upon their transit through simulated digestion and their thermal tolerance are thoroughly investigated. Subsequently, the effect of the probiotic strains encapsulated in casein and gelatin matrices on in vitro inhibition of enzymatic biomarkers related to diabetes [α -glucosidase (AG), α -amylase, dipeptidyl peptidase (DPP-IV)] and with obesity [pancreatic lipase (PL) and cholesteryl esterase (CE)] after simulated gastrointestinal digestion were also investigated. This study will facilitate future applications of encapsulated probiotics using novel source of protein (CC and gelatin) as wall material and may find wide scope in the functional food industry.

MATERIALS AND METHODS

Materials

The probiotic strains *Lactobacillus rhamnosus* MF00960 and *Pediococcus pentosaceus* MF000967 isolated from raw camel milk obtained from local farms of Al Ain, Abu Dhabi, United Arab Emirates. A standard probiotic strain of *Lactobacillus paracasei* DSM20258 was procured from the Leibniz Institute DSMZ, German Collection of Microorganisms and Cell Cultures GmbH. The medium de Man, Rogosa, and Sharpe (MRS) was purchased from HiMedia. All other reagents and chemicals used in this study were of analytical grade and procured from Sigma-Aldrich.

Isolation and Identification of Probiotic Strains

Isolation of probiotic strains *Lactobacillus rhamnosus* MF00960 and *Pediococcus pentosaceus* MF000967 was carried out from raw camel milk obtained from healthy female camels (mid-lactation stage, 4–6 mo), from local farms located in eastern region of Abu Dhabi, United Arab Emirates. All samples were collected in sterile glass bottles containing MRS broth, samples were brought immediately to laboratory under refrigeration conditions and incubated at 37°C, overnight that allowed the enrichment of lactic acid bacteria. Thereafter, serial dilutions were prepared and pour plated with MRS agar supplemented with (0.05 µg/mL) of bromocresol purple and L-cysteine hydrochloride as described by Ahmad et al. (2019b) and incubated at 37°C for 48 h under anaerobic conditions. Well-isolated colonies with different colony morphologies were selected, whereas gram-positive and catalase negative isolates were further purified and identified using 16S rRNA gene amplification using universal eubacteria primers: 27 F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). Upon PCR amplification, the PCR product was sequenced through an external DNA sequencing service (1st Base). Identification of isolated strains was carried out through sequence analysis by performing Basic Local Alignment Search Tool for Nucleotides (BLAST-N) against existing sequences in the National Center for Biotechnology Information (NCBI) database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and sequences were submitted to NCBI database.

Extraction of Casein and Gelatin

For the extraction of CSG, a composite sample of skins from healthy camels (*Camelius dromedaries*) was procured from a local slaughterhouse located in Al Ain, Abu Dhabi, United Arab Emirates. Camel skin gelatin was extracted using an alkali-based pretreatment and extraction method that has been previously optimized by our group (Abuibaid et al., 2020; Fawale et al., 2021). Extracted CSG samples were lyophilized using freeze dryer (Lypho-II, Telstar) and stored at -20°C until further analysis.

Camel milk caseins were separated from skimmed camel milk sample using an isoelectric acid precipitation of caseins with adjustment of pH to 4.2 as previously described by Kamal et al. (2018) with slight modifications. Briefly, a composite sample of raw milk obtained from young healthy female camels was skimmed twice by centrifugation at $4,255 \times g$ at 15°C for 15 min. Thereafter, the pH was adjusted to 4.0 using 6 N HCl and the mixture was allowed to rest at 4°C for 2 h

to ensure better precipitation of CC. The casein precipitates were separated using centrifugation at $4,255 \times g$ at 4°C for 15 min. The pellet obtained was washed thrice with deionized water and pH was readjusted to 7.0 using 1 *N* NaOH. The neutralized casein samples were then freeze-dried and stored at -20°C until their further use in encapsulation experiments.

Preparation of Probiotic Cell Suspension for Encapsulation

For encapsulation, 24-h-old cultures of probiotic strains which were freshly activated after propagation by 3 successive transfers in MRS broth were used. All the probiotic strains used in the study were grown at 37°C and cells were harvested at their late log phase through centrifugation at $5,000 \times g$ at 10°C for 5 min and cell pellets were washed thrice with sterile distilled water and recentrifuged to ensure the complete removal of spent MRS broth. The cells obtained were resuspended in sterile distilled water. The cell suspensions thus obtained were immediately used for microencapsulation and one portion from each strain was kept as free cells control.

Encapsulation of Probiotic Cells

Encapsulation of probiotic strains within different wall materials was carried out as described by Ahmad et al. (2019b) with slight modifications. Briefly, SA was used as a base material in all encapsulation procedure. Sodium alginate (2%) was used as a control, whereas other wall materials such as CC, CSG, and CC:CSG gelatin (1:1 wt/wt) at 2% were prepared by mixing them with 0.5% SA as base solutions. Upon complete dissolution of wall materials in sterile deionized water at 50°C under constant stirring using magnetic stirrer, the mixtures were homogenized at 15,000 rpm for 2 min to obtain a homogeneous solution. The solutions were then filtered using $0.2\text{-}\mu\text{m}$ filters and stored at 4°C until further use. The mixtures obtained above were then mixed with probiotic cell suspensions of *L. rhamnosus*, *P. pentosaceus*, and *L. paracasei*. Cell counts in the final mixture were enumerated using pour plate technique on MRS agar after incubation at 37°C for 48 h under aerobic conditions. The mixtures were then poured as minute droplets into calcium lactate (4%) solution to obtain hardened cell suspensions in the form of microcapsules. The microcapsules were freeze-dried using Telstar Freeze dryer (Telstar) at temperature -80°C , 0.1 bar pressure. The resulting freeze-dried powder were subsequently stored at -20°C in airtight sterile containers for further studies.

Bacterial Death Cycle and Encapsulation Efficiency

Viability of probiotic cells upon encapsulation was determined following the protocol described by Vaziri et al. (2018). Briefly, the powdered samples were suspended in sterile peptone water at a concentration of 2% and cells were allowed to release through homogenization at 15,000 rpm for 1 min. Thereafter, viable cells were enumerated on MRS agar using pour plating technique and colony forming units were determined following an incubation at 37°C for 48 h under aerobic conditions. Bacterial death cycles and encapsulation efficiency (EE) were determined using the following equations (Vaziri et al., 2018):

$$\begin{aligned} \text{Bacterial death cycles} = \\ \text{Log cfu/g (before)} - \text{Log cfu/g (after)}, \end{aligned} \quad [1]$$

$$\text{EE (\%)} = \frac{\text{Log cfu/g (after)}}{\text{Log cfu/g (before)}} \times 100, \quad [2]$$

where log cfu/g (before) and log cfu/g (after) refer to the number of viable cells before and after encapsulation.

Cell Viability upon Simulated Gastrointestinal Digestion Conditions

A 2-step static model of simulated gastrointestinal digestion (SGID) was adapted from Ahmad et al. (2019a,b). Simulated gastric juice (SGJ) was prepared by dissolving 3 g/L pepsin (cat. no. P7012, $\geq 2,500$ units/mg protein, Sigma) in sterile NaCl solution (9 g/L) and the pH was adjusted to 2.0 with 1.0 *M* HCl. Simulated intestinal juice (SIJ) was prepared by dissolving 3 g/L bile salts (cat. no. B3883, Bile bovine, dried, unfractionated, Sigma) and 10 g/L of pancreatin (cat. no. P7545, ≥ 3 USP specification, Sigma) in phosphate-buffered saline, pH was maintained at 8.0 with NaOH solution (0.1 *M*). Further, encapsulated and free cells corresponding to an approximate 10 log cfu/g were placed in sterile glass test tubes ($n = 3$) to which 10 mL of SGJ was added and incubation was carried out at 37°C for 2 h under constant stirring conditions at 100 rpm. Following this, the tubes were centrifuged at $5,000 \times g$ for 10 min at room temperature to remove spent SGJ and to recover the sediments with released cells. The pellets were replenished with 10 mL of SIJ and tubes were then incubated again at 37°C under constant agitation for 4 h. The viable count (log cfu/g) was determined by plating onto MRS agar after homogenization and after serial dilutions in sterile peptone

water with incubation at 37°C for 48 h under aerobic conditions.

Thermal Resistance

The thermal resistance of encapsulated probiotics was determined according to protocol previously described by Praepanitchai et al. (2019). Briefly, encapsulated and free cells corresponding to an approximate 8 log cfu were transferred into sterile glass tubes containing 10 mL of sterile peptone water and subjected to heat treatments at 50 and 70°C for 15 min. Viable cell counts were determined following homogenization for 2 min followed by plating with MRS agar and plates were then incubated at 37°C for 48 h under aerobic conditions.

Fourier-Transform Infrared Spectroscopy

Fourier-transform infrared spectroscopy (FTIR) spectra of encapsulated freeze-dried samples were recorded with Spectrum2 FTIR spectrophotometer (Perkin Elmer) with mid-IR range with triglycine sulphate (MIR-TGS) detector for improved signal-to-noise ratio and increased interferometer scanning speed. All the spectra were collected with Perkin Elmer Spectrum software at a scanning range of 500 to 4,000 cm^{-1} at a resolution of 1 cm^{-1} and the spectra were baseline corrected before analysis (Ahmad et al., 2019a). The peaks belonging to different functional groups were identified using tool IR Wizard 2019³ available at <http://www.science-and-fun.de/tools/>.

Scanning Electron Microscopy

The microstructure of the freeze-dried encapsulated probiotic strains was evaluated using a scanning electron microscope (JEOL Neo Scope JCM-SEM, model MP-19020NCTR). Encapsulated bacterial strains were fixed on aluminum stubs through the carbon tape and DC sputtered with the gold of 8 nm under the atmosphere of argon. The microscopic observations were performed at 15 kV and 1,000 $\times$ magnification.

Effect of SGID on Bioactivities of Free and Encapsulated Cells

Preparation of Cell Extracts. In vitro health-related bioactive properties of probiotic strains (*L. rhamnosus*, *P. pentosaceus*, and *L. paracasei*) were studied by subjecting free and encapsulated cell mass to SGID conditions. Untreated free cells were kept as control.

The cell mass obtained upon complete SGID treatment were suspended in 5% sterile peptone water followed by vortexing the cell suspensions, which were further incubated at 37°C for 6 h to produce the metabolites and disrupted ultrasonically using 3- to 5-s pulses for 5 min in an ice bath using Double-Step 1/8" Microtip Probe-based Ultra-sonifier (Branson SFX550 Sonifier). Cell debris was removed by centrifugation at 10,000 $\times g$ for 15 min at room temperature, and the supernatant obtained was stored at -20°C for performing bioactivity assays.

Bioactive Properties of Bacterial Cell Extract as Affected by SGID and Encapsulation. The bioactive properties of cellular extracts from probiotic strains were determined for in vitro antidiabetic activities via inhibition of enzymatic markers such as AG (0.2 U/mL), pancreatic α -amylase (PAA; 1 mg/mL), and DPP-IV (0.01 U/mL) inhibition activity using protocols from Zhang et al. (2015), Mudgil et al. (2019), and Nongonierma et al. (2018), respectively, with p-nitrophenyl α -D-glucopyranoside (10 mM), p-nitrophenyl α -D-maltohexaoside (5 mM), and Gly-pro-p-nitroanilide (5 mM) as AG, PAA, and DPP-IV substrates, respectively. In vitro anti-obesity activities were determined via inhibition of PL (1 mg/mL) and CE (10 $\mu\text{g/mL}$) following the protocol of Ajayi et al. (2021), with p-nitrophenyl butyrate (5 mM) as substrate. Briefly, 50 μL of sample was first mixed with 50 μL of substrate and reaction was initiated by addition of 50 μL of enzyme. The microplates were incubated at 37°C for 30 min and absorbance was measured at 405 nm using microplate reader (Multiskan Sky, Thermo-Fisher Scientific).

Individual sample blanks were also run by replacing enzyme volume with same amount of the respective buffer to eliminate the background absorbance produced from samples. Enzyme inhibition assays were performed, and percentage of enzyme inhibition was calculated using Equation 3.

$$\text{Enzyme Inhibition (\%)} = \left[1 - \left(\frac{C - D}{A - B} \right) \right] \times 100, \quad [3]$$

where *A* is control, *B* is control blank, *C* is sample, and *D* is sample blank, referring to the absorbance values of reaction vials containing enzyme, without enzyme, enzyme with sample, and without enzyme but with sample, respectively. Substrate was present in all reactions. When enzyme and samples were not present in a reaction the same amount was replaced with respective buffer for each assay.

Statistical Analysis

All experiments and analysis were run in triplicate. Three different batches of microcapsules for each probiotic strain using each wall material were produced. Data were recorded as mean $\pm$ standard deviation and were subjected to one-way ANOVA. Multiple comparisons were performed by Tukey's multiple comparison test with statistical significance set at $P < 0.05$. All analyses were performed using SPSS 26.0 (IBM Corp.).

RESULTS AND DISCUSSION

Effect of Different Wall Materials on Survival Percentage, Bacterial Cell Death Cycle, and EE

Effect of different wall materials such as SA, CC, CSG, and CC:CSG on the cell viability and bacterial death cycle of free and encapsulated probiotic strains *L. rhamnosus*, *P. pentosaceus*, and *L. paracasei* after the encapsulation process was investigated and results obtained are presented in Table 1. Encapsulation efficiency for 3 probiotic strains upon encapsulation were found in range of 91.3 to 97.4% for *L. rhamnosus*, 85.5 to 91.6% for *P. pentosaceus*, and 90.3 to 99.2% for *L. paracasei*. Although the cell death cycle remained in the range of 0.08 to 1.7 log cfu/g. Maximum reduction of cell log count was recorded for *P. pentosaceus* encapsulated in SA (1.70 ± 0.05), whereas least was seen for *L. paracasei* encapsulated in CC (0.08 ± 0.001 ; $P < 0.05$). Overall, it was observed that maximum protection (lower cell death cycle) to all 3 probiotics was provided upon probiotic encapsulation within CC followed closely by encapsulation in the CC:CSG. Cell death cycle counts demonstrated by probiotic cells encapsulated in CC and CC:CSG were significantly lower than those encapsulated in SA and CSG ($P < 0.05$). Using CSG alone for encapsulation results in cell death cycle which was very close to SA encapsulated cells ($P < 0.05$).

Milk proteins are known to be a good choice for microencapsulation of bioactive compounds and probiotics (Abd El-Salam and El-Shibiny, 2015), as they are flexible to entrap different types of probiotic cells, thus enhancing their viability. In addition, intrinsic properties of milk proteins such as better gelation and high viscosity provides better dispersion and higher protection for the entrapped probiotics. Milk proteins also possess better buffering capacity to protect cells from harsh environments. Moreover, milk proteins are known to have wide range of functional and bioactive properties with synergistic benefits for cell viability. In a study, Burgain et al. (2013) reported 99.0% of bacterial survival with an EE of 97.0% for *L. rhamnosus*

encapsulated with micellar casein and denatured whey protein, which corroborates with the results of present study. Moreover, Prasanna and Charalampopoulos (2018) also reported that encapsulation of *Bifidobacterium longum* in SA-bovine casein and SA-goat casein resulted in entrapment and survival of approximately 94.9 and 95.3% cells, respectively.

Gelatin is also considered to be an excellent macromolecule for encapsulation of bioactive compounds and probiotic cells due to their amphoteric nature (Li et al., 2009). The results obtained from the present study indicated that CSG was able to protect probiotic cells (*P. pentosaceus* and *L. paracasei*) more effectively with lower cell death cycle and higher EE, compared with that of cells encapsulated in SA ($P < 0.05$). With CSG encapsulation, the survivability of *L. rhamnosus*, *L. pentosaceus*, and *L. paracasei* upon freeze-drying was 91.3, 87.0 and 97.9%, respectively. The results are comparable to survival of *L. rhamnosus* GG ($81.02 \pm 0.19\%$) upon encapsulation using fish gelatin (Jiang et al., 2020). Furthermore, microencapsulation of *Bifidobacterium longum* in gelatin conferred better survivability than a combination of gum arabic and starch, as wall material (Lian et al., 2003). Survivability percent of *Saccharomyces cerevisiae* var. *boulardii* encapsulated in gelatin was reported to be 91.55% upon encapsulation (Arslan et al., 2015).

Moreover, synergistic application of CC to CSG for encapsulation proved to further improve the EE in *L. rhamnosus*, *L. pentosaceus*, and *L. paracasei* to the level of 96.0, 91.0, and 98.2%, respectively. These EE values for all 3 strains of probiotics encapsulated in CC:CSG were higher compared with those encapsulated in CSG alone and SA ($P < 0.05$).

The EE of CSG reported in the current study (87–97%) is higher than the values reported when *Bifidobacterium pseudocatenulatum* was encapsulated in porcine gelatin and bovine gelatin conjugated with SA and genipin which resulted in EE of 45.94 and 57.66%, respectively (Khalil et al., 2019). Zhao et al. (2018) reported that whey protein isolate/gum arabic at pH 4.5 resulted in substantial reduction in viability during spray drying and concluded that gelatin, an alkaline protein as an ideal protein matrix for encapsulation by coacervation with optimum pH (8.0). Sodium caseinate when used in encapsulation of probiotic bacteria might be able to decrease heat stress of spray drying and render a hydrophobic barrier during storage and digestion of probiotics (Zhao et al., 2020). It has been reported that gelatin in conjugation with casein as wall material for probiotic bacteria (*Lactobacillus reuteri*) demonstrated the higher protection compared with casein alone, gelatin/gum arabic conjugate and gelatin alone when probiotic was subject to gastrointestinal diges-

Table 1. Effect of different wall materials on cell death cycle, encapsulation efficiency, total survivability upon simulated gastrointestinal digestion (SGID) and their thermal tolerance at 50 and 70°C¹

Probiotic strain	Wall material ²	Cell death cycle (log cfu/g)	Encapsulation efficiency (%)	Survivability upon SGID (%)	Thermal tolerance (log cfu/g)	
					At 50°C	At 70°C
<i>Lactobacillus rhamnosus</i> MF00960	FC	—	—	43.91 ± 0.09 ^a	ND	ND
	SA	0.74 ± 0.02 ^c	93.3 ± 0.21 ^b	71.39 ± 0.21 ^c	7.57 ± 0.05 ^b	5.90 ± 0.62 ^a
	CC	0.27 ± 0.02 ^a	97.4 ± 0.19 ^d	99.01 ± 1.25 ^e	7.72 ± 0.02 ^b	7.09 ± 0.04 ^b
	CSG	0.96 ± 0.01 ^d	91.3 ± 0.11 ^a	69.32 ± 0.12 ^b	6.22 ± 0.4 ^a	6.11 ± 0.42 ^a
	CC:CSG	0.44 ± 0.03 ^b	96.0 ± 0.28 ^c	87.54 ± 0.76 ^d	7.67 ± 0.29 ^b	6.71 ± 0.06 ^{ab}
<i>Pediococcus pentosaceus</i> MF00096	FC	—	—	54.26 ± 0.16 ^a	5.47 ± 0.26 ^a	4.51 ± 0.11 ^a
	SA	1.70 ± 0.05 ^c	85.5 ± 0.44 ^a	89.34 ± 0.65 ^b	6.87 ± 0.11 ^b	5.28 ± 0.11 ^{ab}
	CC	0.98 ± 0.01 ^a	91.6 ± 0.08 ^c	97.93 ± 0.36 ^c	7.65 ± 0.03 ^c	6.14 ± 0.39 ^c
	CSG	1.52 ± 0.01 ^b	87.0 ± 0.09 ^b	99.66 ± 0.16 ^d	6.98 ± 0.01 ^b	5.64 ± 0.46 ^{bc}
	CC:CSG	1.05 ± 0.01 ^a	91.0 ± 0.06 ^c	98.55 ± 0.31 ^c	7.62 ± 0.17 ^c	6.01 ± 0.19 ^{bc}
<i>Lactobacillus paracasei</i> DSM 20258	FC	—	—	72.44 ± 0.12 ^a	5.66 ± 0.19 ^a	5.68 ± 0.13 ^a
	SA	1.09 ± 0.01 ^d	90.3 ± 0.10 ^a	87.39 ± 0.11 ^b	6.67 ± 0.04 ^b	6.89 ± 0.02 ^b
	CC	0.08 ± 0.001 ^a	99.2 ± 0.17 ^d	92.62 ± 0.25 ^e	7.92 ± 0.02 ^d	8.35 ± 0.32 ^c
	CSG	0.25 ± 0.01 ^c	97.7 ± 0.14 ^b	89.88 ± 0.11 ^c	7.51 ± 0.22 ^c	5.86 ± 0.25 ^a
	CC:CSG	0.19 ± 0.01 ^b	98.2 ± 0.09 ^c	90.79 ± 0.22 ^d	7.39 ± 0.13 ^c	7.48 ± 0.47 ^b

^{a-e}Means with different superscript letters in a column represent significant difference ($P < 0.05$) between different samples within each probiotic strain.

¹Values represent mean ± SD (n = 3). ND = not detected (no inhibitory activity detected).

²FC = free cells; SA = sodium alginate; CC = camel casein; CSG = camel skin gelatin; CC:CSG = 1:1 (wt/wt) encapsulated probiotic strains.

tion, and heat stress (Zhao et al., 2020). These results strongly indicate the advantage of using hetero-protein conjugations and effective role of caseins in enhancing probiotic resistance against external factors such as drying, heating, and digestion conditions.

Viability of Encapsulated Probiotics Under Simulated Digestion

Probiotics are often challenged by their inactivation during their passage through the gastrointestinal tract and pH fluctuations and hence do not reach the absorption site, the colon in an intact form and enough numbers (Gu et al., 2019). For any encapsulating wall material, it is essential that they keep probiotic cells protected under gastric conditions while releasing the probiotic cells under intestinal conditions, thus enabling probiotics to reach the colon intact to exhibit their bioactive properties.

Upon SGID transit, the viable cells percentage was higher in the encapsulated probiotics than the free cell suspension of *L. rhamnosus*, *P. pentosaceus*, and *L. paracasei* ($P < 0.05$; Table 1). The present study shows that the viability of free probiotic cells greatly reduced when compared with the encapsulated probiotics which exhibited greater resistance under simulated gastrointestinal conditions. The present findings revealed that CC is an efficient encapsulant for *L. rhamnosus* with the viable cells percentage of 99.01% followed by CC:CSG mixture with 87.54% cell viability. On the other hand, maximum cell viability of 99.66 and 98.55% was achieved for *P. pentosaceus* when encapsulated with

CSG and CC:CSG as encapsulating matrix. Similarly, CC-based encapsulation of *L. paracasei* produced the microcapsules with highest viability of 92.62%. The findings suggest that the probiotic cells inside the matrices of CC and CC:CSG remained more resistant to the detrimental effects of hydrogen ions and pepsin from the gastric fluids. Similarly, bile salts present in the SIJ affect the living cells by disrupting the structure and integrity of the cell membrane, thereby triggering the DNA damage and decreasing survival rate of free cells (Sannasiddappa et al., 2017). Very similar findings were recently reported for *Lactobacillus reuteri* encapsulated in gelatin/casein mixture which displayed significantly higher resistance against the bactericidal effect of SGJ and intestinal juice compared with cell encapsulated in casein or gelatin alone (Zhao et al., 2020). It was concluded that the protection efficiency of the microcapsules of *Lactobacillus reuteri* subjected to SGID, thermal treatment, and ambient storage always followed the following order: gelatin-casein ≥ casein > gelatin-gum arabic > gelatin (Zhao et al., 2020). Guimarães et al. (2013) also reported that free cells of *L. rhamnosus* and *Bifidobacterium animalis* failed to survive at low pH 2.0 and 2.5 after 180 min in SGF, whereas encapsulated cells in Ca-alginate beads showed the viability of 3.4 and 5.7 log cfu/g, respectively, at pH 2.0. Moreover, recent report on *Lactobacillus acidophilus* La-14 encapsulation in SA and bovine whey proteins revealed that only 33.41% of free cells were able to survive, whereas encapsulation in multiple layer alginate-whey matrix resulted in 77.45% viability of encapsulated cells upon their transit through SGID

(de Araújo Etchepare et al., 2020). Alginate, pectin and gelatin-based microencapsulation of *L. plantarum* strain also reported to increase the cell viability by 90.02% (Vaziri et al., 2018). Furthermore, SA as wall material for probiotics, has certain limitations such as encapsulant leaching and burst release or rapid dissolution in the intestinal pH and in the presence of sodium ions (Fathi et al., 2014). Various studies reported that casein proteins have sponge-like structure with more internal cavities connected to each other, which offer better EE for probiotic cells (Tavares et al., 2014). Gelatin is amphoteric in nature with better crosslinking activity and hence efficient encapsulation of probiotic strains and protect them from the harsh environment in gastrointestinal conditions (Paula et al., 2019). These results suggest that CC and CC:CSG had a better protective effect than SA alone on cell viability when exposed to the harsh conditions of SGID.

Thermal Tolerance of the Encapsulated Probiotic Strains

Studies suggest that extreme temperature beyond 65°C are highly damaging to probiotic cells due to the thermal stress. Microencapsulation is recommended to shield the probiotics as it can reduce the heat transfer from the surrounding medium to the cell interiors (Ahmad et al., 2019b).

Pasteurization is a common thermal process applied to many types of food and beverages. The viability of free and encapsulated probiotics before and after heating at 50°C and 70°C for 15 min was therefore monitored. Encapsulation of *L. rhamnosus*, *P. pentosaceus*, and *L. paracasei* within SA, CC, CSG, and CC:CSG exhibited significant differences in the cell viability when exposed to different levels of thermal treatment (Table 1). Free cells of *L. rhamnosus* were not able to survive the high temperature treatments at all, thus showing zero log cfu/mL. However, upon encapsulation in different wall materials survival of probiotic cells displayed a significant increase. Overall, CC and CC:CSG encapsulation of *L. rhamnosus* were found to be more protective than SA and CSG alone. Free cells of *P. pentosaceus* showed survival of 5.47 and 4.51 log cfu/g at 50 and 70°C, respectively, whereas cells encapsulated in CC showed the highest viability (7.65 and 6.14 log cfu/g) after being exposed to both temperatures. Similar results were obtained for encapsulated cells of *P. pentosaceus*, where higher tolerance was exhibited in the presence of CC with 7.65 and 6.14 log cfu/g at 50°C and 70°C respectively. Lowest survival was noticed for SA encapsulated cells with 6.87 and 5.28 log cfu/g at 50°C and 70°C. Furthermore,

L. paracasei encapsulated with CC was found to be highly tolerant toward heating even at 70°C for 15 min with 8.35 log cfu/g of viable cells. On the contrary, SA encapsulated probiotics displayed lower survivability when subjected to gastrointestinal conditions compared with CC- and CSG-encapsulated probiotics, but at the same time it enhances the viability of probiotic cells at 50 and 70°C. Zhao et al. (2020) have recently demonstrated that gelatin: casein complex was effective in providing higher thermal protection (80°C for 1 h) to *Lactobacillus reuteri* cells when compared with gelatin and casein alone. The findings are consistent with several studies where encapsulated bacterial cells are more stable to heat treatment than the free cells (Ahmad et al., 2019b). Moreover, previous studies have shown that the alginate-gelatin microgels significantly enhanced the heat resistance of *Lactobacillus salivarius* (Li01) when compared with the alginate alone (Yao et al., 2017). Similarly, Yao et al. (2018) concluded that *P. pentosaceus* encapsulated in alginate-gelatin microgels showed the best heat tolerance at 63°C for 5 min with 7 to 8 log cfu/mL. Therefore, encapsulating probiotic cells in microcapsules prepared from CC and combination of CC and gelatin can be a more efficient strategy to enhance the thermal tolerance of the probiotic cells.

Structural Characterization with FTIR Spectroscopy

The FTIR spectrum were employed to determine the effect of encapsulating matrices on the molecular structure of the microcapsules encapsulating probiotic strains. The FTIR spectra for free cells of *L. rhamnosus*, *P. pentosaceus*, and *L. paracasei* and cell encapsulated in microcapsule prepared from SA-, CC-, CSG-, and CC:CSG-based wall materials are presented in Figure 1a–c. Careful observation of the FTIR spectra of free probiotic cells shows the distinct bands between 530 and 550 cm^{-1} corresponding to the characteristics of stretching and bending of the glycosidic bond COC (the bond that connects the 2 groups of sugars). *L. paracasei* and *L. rhamnosus* both showed a clear band at 857 and 870 cm^{-1} corresponding to P–O bond from phosphorus present in the cell wall of bacteria (Figure 1a, c). Interestingly this band was not very prominent in *P. pentosaceus* strain possibly owing to differences in their cell wall composition (Figure 1b). Specific peaks at 1,236 cm^{-1} and 1,052 cm^{-1} relates to the phosphate stretch of the phosphodiester backbone of the nucleic acids (Lopes et al., 2017). In addition, all vibrations and stretching related to amide II and amide I were observed for all 3 probiotic strains in the range of 1,540 and 1,635 cm^{-1} . The peak related to C–O of carbonyl group in the ester linkages of fat molecules

Figure 1. Fourier-transform infrared spectroscopy spectra of free cells (FC), and probiotic cells encapsulated in sodium alginate (SA), camel casein (C), and camel skin gelatin (G) in their corresponding wall matrix. (A) LR = *Lactobacillus rhamnosus* MF00960, (B) PP = *Pediococcus pentosaceus* MF00096, and (C) LP = *Lactobacillus paracasei* DSM 20258.

and CH₂ symmetric stretch were observed only in *L. rhamnosus* at 1,748 cm⁻¹ and 2,852 cm⁻¹, respectively.

The FTIR spectra of pure polysaccharide SA showed the peaks that correspond to major functional groups such as OH, CH, and monosaccharides. The wide absorption band obtained between 3,000 and 3,600 cm⁻¹ is attributed to hydrogen bonds related stretching vibrations of hydroxyl groups. The sharp peaks around 1,400 cm⁻¹ and 1,600 cm⁻¹ corresponded to symmetric and asymmetric stretching of carboxyl group as also explained by Vaziri et al. (2018). These bands are used as common distinguisher between alginate and its conjunction products (Daemi and Barikani, 2012). The peak at 1,026 cm⁻¹ is related to C–O–C stretching from guluronic acid and the peak at 813 cm⁻¹ is attributed to the C–O stretching vibration of pyranosyl ring, with contributions from the deformation modes of C–C–H and C–O–H or α -configuration of the guluronic units.

The FTIR spectra gelatin protein displayed prominent amide I, II, and III as well as amide A and B peaks in the wave number ranged from 1,635 to 1,641 cm⁻¹, 1,520.05 to 1,560.0 cm⁻¹, and 1,240 to 1,247 cm⁻¹, respectively. Amide I vibration mode was primarily a

C=O stretching coupled to contributions from the C–N stretch, CCN deformation, and in-plane N–H bending modes (Kittiphattanabawon et al., 2010). Amide II bands is usually caused by an out-of-phase combination of the C–N group stretch vibration and N–H group distortion modes of the peptide group (Nagarajan et al., 2012). Amide A peak detected at wavelengths ranging from 3,250 to 3,352 cm⁻¹ corresponds to stretching vibration of the N–H group linked with hydrogen bonding (Abuibaid et al., 2020). Similar, FTIR spectra for camel gelatin have been reported by Abuibaid et al. (2020). Observation of CCs through FTIR spectra revealed the presence of a characteristic band at 721 cm⁻¹ which is attributed to C–C or N–N skeleton vibrations and served as the fingerprint band for casein protein (Socrates, 2004). Camel casein protein displayed a band around 1,098 cm⁻¹ representing complex C–O–C ether stretching and symmetric C–H deformation vibration, 1,170 cm⁻¹ (C–H deformation vibration), 1,240 cm⁻¹ (amide III group), 1,380 cm⁻¹ (amide III bands), 1,465 cm⁻¹ (CH₂ bending), 1,525 cm⁻¹ (amide II group), 1,628 cm⁻¹ (amide I group), 1,739 cm⁻¹ (C=O stretching vibrations), 2,850 cm⁻¹ (symmetric C–H stretching

vibration), $2,914\text{ cm}^{-1}$ (asymmetric C–H stretching vibration) and $3,284\text{ cm}^{-1}$ (O–H stretching and N–H stretching vibrations) were observed (Sarabandi et al., 2018).

Crosslinking of SA by calcium ion caused a shift in band at $1,301\text{ cm}^{-1}$ to $1,298$, $1,285$, and $1,238\text{ cm}^{-1}$ for *L. rhamnosus*, *P. pentosaceus*, and *L. paracasei*, respectively. The band at $1,407\text{ cm}^{-1}$ present in SA showed a shift in wavenumber to $1,420$, $1,476$ and $1,465\text{ cm}^{-1}$, respectively, for *L. rhamnosus*, *P. pentosaceus*, and *L. paracasei*, respectively. It is reported that shifting of peaks toward lower or higher wave numbers usually happen through intermolecular hydrogen bonding and electrostatic force interaction among the participating groups such as O–H, N–H, and CO. These interactions could form and shorten the bond length of the –NH functional groups, thus increasing the wavenumbers. This indicates that alginate and probiotic cells formed intermolecular hydrogen bonding and electrostatic force interactions. Moreover, some studies related these shifts to the uniform placement of core structures (probiotics or bioactive) within the coating material such as caseins (Sarabandi et al., 2018). Meanwhile, a new narrower peak at $2,873\text{ cm}^{-1}$ in *P. pentosaceus* and 2 sharp peaks at $2,853$ and $2,900\text{ cm}^{-1}$ for *L. paracasei* were observed. These new peaks in FTIR spectra of probiotics encapsulated in SA are attributed to the addition of C=O stretching vibrations and symmetric C–H stretching vibration generated from probiotic.

Moreover, the peaks for probiotics encapsulated in CC also showed the similar changes as were observed for probiotics encapsulated in SA. The presence of peaks in the region close to 880 cm^{-1} , which is a fingerprint region for probiotics attributed to the P–O bond from phosphorus confirmed the embedding of probiotics within casein matrix. In addition, the sole presence of peaks in region closer to $1,025\text{ cm}^{-1}$ indicated the presence of C–O stretching vibration arising due to saturated aliphatic esters present in the cell membrane of microorganism. Similar observations were also noticed for cells encapsulated within CSG and CC:CSG matrix. Additionally, the encapsulation of probiotic cells in CSG alone led to the development of small new peaks in the region of $620\text{--}630\text{ cm}^{-1}$ and for both CSG- and CC:CSG-based encapsulation new peaks were observed in the region spanning 815 to 820 cm^{-1} . These changes can be because of the effect of placement and distribution of probiotics and probably some of the hydrogen bonds between them. Overall, CC- and CSG-encapsulated probiotic strains exhibited the amide bands with varying intensity, which could be attributed to the hydrogen bonding with the dehydrated protein owing to freeze-drying with no significant change in the skeletal

conformation. These findings provide evidence that SA, CC, CSG, and CC:CSG were proficient in encapsulating the probiotics bacteria with no significant change in their structural conformation.

Microstructural Features

Scanning electron microscopic observations of the surface characteristics of different microparticles encapsulating probiotic strains are presented in Figures 2, 3, and 4. Micrographs of the free cells displayed the characteristic shape of the bacterial cells (rod-shaped and cocci-shaped or spherical) for all 3 probiotics used (Huang et al., 2018; Martin et al., 2019; Jiang et al., 2021). *Pediococcus pentosaceus* appeared to be in cluster of tetrads attach to each other with smooth surface morphologies, whereas *L. rhamnosus* and *L. paracasei* appeared as rod-shaped cells clustered together in chains (Figures 2 and 4, respectively). Entrapment of probiotic cells in different wall material led to change in their microstructural characteristics. The encapsulated *P. pentosaceus* cells were seen embedded inside the wall materials and were visible as small round particles in the different coating material (Figure 3). Furthermore, the surface of particle prepared with SA and CSG appeared to be more porous than the ones prepared with CC and combination of CC:CSG. Similar results were also obtained by Prasanna and Charalampopoulos (2018) where entrapment of *Bifidobacterium longum* inside SA microcapsules revealed presence of more porous structure than those obtained by mixture of SA and bovine and goat milk proteins. The less porous structure obtained with CC and CC:CSG might explain higher viability of entrapped bacteria in these capsules as microcapsules with cracks or more voids on their surface do not provide much protection to entrapped probiotics cells from adverse environmental conditions of freeze-drying and SGID. The microstructural images obtained for the CC and CGS matrices were similar to the previous reports that represented uniformly distributed probiotic strains within the interconnected protein matrices (Heidebach et al., 2010). Several studies also concluded that freeze-drying resulted in shrunken and wrinkled matrices with rough surfaces and low porosity, which is in line with our current findings (Ptiček Siročić et al., 2016). Camel skin gelatin alone or in combination with CC displayed a microstructure consisting of crosslinked protein network somehow close to the microstructure of gelatin gels. To sum up, CC and CC:CSG offered structural integrity and rigidity as encapsulating matrices for probiotics which correlate with viability and bacterial cell death data.

Figure 2. Scanning electron micrographs of *Lactobacillus rhamnosus* MF00960 as free cells (FC) or encapsulated in camel casein (CC), camel skin gelatin (CSG), CC:CSG (1:1 wt/wt), and sodium alginate (SA).

Figure 3. Scanning electron micrographs of *Pediococcus pentosaceus* MF00096 as free cells (FC) or encapsulated in camel casein (CC), camel skin gelatin (CSG), CC:CSG (1:1 wt/wt), and sodium alginate (SA).

Figure 4. Scanning electron micrographs of *Lactobacillus paracasei* DSM 20258 as free cells (FC) or encapsulated in camel casein (CC), camel skin gelatin (CSG), CC:CSG (1:1 wt/wt), and sodium alginate (SA).

Effect of SGID on the Bioactive Properties of Free Cells and Encapsulated Probiotic Cells

Inhibition of carbohydrate-hydrolyzing enzymes such as AG and PAA aids in managing type II diabetes by decreasing postprandial hyperglycemia. Similarly, inhibition of enzyme DPP-IV involved in degradation of incretin hormones is also advocated (Ashraf et al., 2021; Mudgil et al., 2021). Previous work also suggested that inhibitors of obesity-related enzymatic markers such as PL and CE are among the important therapeutic measure to manage complications associated with obesity (Mudgil et al., 2018; Ahmad et al., 2019a,b). Some previous reports suggested that cell-free extracts obtained from probiotic organisms possess inhibitory activity against AG, PAA, and DPP-IV, predicting this as one of the key mechanisms of action for their antidiabetic properties. The study conducted by Zeng et al. (2016a) showed that exopolysaccharides in the cell-free excretory supernatant of *L. rhamnosus* and *L. paracasei* showed 27.6 and 28.3% of inhibitory potential against AG, respectively. Similarly, Panwar et al. (2016) also reported that Lactobacilli showed an inhibition of DPP-IV in the range of 20 to 80%. Overall, to the best of our knowledge limited studies are available in this direction and warrants further exploration.

The inhibitory activities against enzyme related to complication of diabetes and obesity for all 3 probiotics,

before and after SGID under free cells and encapsulated conditions are presented in Table 2. The results showed that inhibition for AG displayed by the free probiotic cells before SGID transit were 85.43, 67.15, and 78.03% for *L. rhamnosus*, *P. pentosaceus*, and *L. paracasei*, respectively. When free probiotics cells were subjected to the gastrointestinal conditions, AG inhibitory activity declined for all the probiotic strains with levels reaching up to 32.53, 30.21, and 56.01% for *L. rhamnosus*, *P. pentosaceus*, and *L. paracasei*, respectively. However, probiotic strains within the encapsulating matrices CC and CC:CSG contributed positively toward retention of the inhibitory activity of AG upon their transit through the gastrointestinal tract, and displayed higher AG inhibitory activities compared with free probiotic cells subjected to SGID ($P < 0.05$) (Table 2). Similar results were obtained for PAA inhibitory activities of all 3 strains of probiotics. Before the gastrointestinal transit AG inhibition, varied between 58.86 and 73.03% with *L. paracasei* (73.03%) being the most proactive probiotic followed by *L. rhamnosus* (63.91%) and *P. pentosaceus* (58.86%; Table 2). Upon SGID transit, the PAA inhibitory properties of all the probiotic strains declined with maximum reduction observed for *L. rhamnosus* where it nearly disappeared. However, encapsulation of probiotic strains within CC enhanced the PAA inhibitory potential of probiotics upon gastrointestinal transit, when compared with the free cells

Table 2. Effect of encapsulation on the retention of bioactivity of 3 probiotic strains upon in vitro simulated gastrointestinal digestion (SGID)¹

Parameter ²	AG	PAA	DPP-4	PL	CE
<i>Lactobacillus rhamnosus</i> MF00960					
FC_NoSGID	85.43 ± 0.76 ^d	63.91 ± 3.01 ^d	70.1 ± 3.72 ^c	64.49 ± 3.09 ^e	48.00 ± 2.84 ^{bc}
FC_SGID	32.53 ± 1.36 ^a	1.00 ± 0.32 ^a	ND	15.00 ± 2.89 ^a	27.39 ± 1.92 ^a
EC_SA	61.13 ± 0.68 ^b	46.95 ± 2.16 ^c	30.58 ± 2.15 ^a	46.52 ± 1.00 ^d	46.35 ± 2.42 ^b
EC_CC	69.27 ± 0.24 ^c	52.25 ± 1.97 ^c	53.37 ± 0.15 ^b	44.05 ± 3.84 ^{cd}	53.10 ± 0.68 ^c
EC_CSG	62.16 ± 2.31 ^b	36.55 ± 2.79 ^b	30.58 ± 2.15 ^a	25.70 ± 2.32 ^b	47.90 ± 3.03 ^{bc}
EC_CC:CSG (1:1 wt/wt)	85.43 ± 0.76 ^d	52.11 ± 2.59 ^c	48.69 ± 0.55 ^b	37.63 ± 1.21 ^c	54.13 ± 2.31 ^c
<i>Pediococcus pentosaceus</i> MF000967					
FC_NoSGID	67.15 ± 0.93 ^e	58.86 ± 0.78 ^b	43.74 ± 0.6 ^c	53.24 ± 0.99 ^c	64.03 ± 0.72 ^e
FC_SGID	30.21 ± 1.19 ^a	44.66 ± 0.59 ^a	27.66 ± 0.3 ^a	12.9 ± 0.84 ^a	32.24 ± 0.36 ^a
EC_SA	43.39 ± 0.21 ^c	69.96 ± 0.18 ^d	34.62 ± 0.46 ^b	41.64 ± 0.5 ^b	49.47 ± 0.4 ^d
EC_CC	49.1 ± 0.12 ^d	79.34 ± 0.1 ^f	65.94 ± 0.9 ^e	71.52 ± 0.18 ^d	49.56 ± 1.03 ^d
EC_CSG	35.89 ± 0.15 ^b	63.06 ± 0.16 ^c	29.21 ± 0.61 ^a	40.83 ± 0.88 ^b	40.85 ± 0.12 ^b
EC_CC:CSG (1:1 wt/wt)	41.67 ± 0.96 ^c	71.14 ± 0.03 ^e	47.03 ± 0.99 ^d	71.4 ± 0.12 ^d	45.81 ± 1.32 ^c
<i>Lactobacillus paracasei</i> DSM 20258					
FC_NoSGID	78.03 ± 1.03 ^c	73.03 ± 0.79 ^b	48.69 ± 0.55 ^d	67.91 ± 0.35 ^d	56.88 ± 1.45 ^d
FC_SGID	56.01 ± 0.94 ^a	52.3 ± 0.41 ^a	7.692 ± 0.31 ^a	42.28 ± 1.58 ^a	18.13 ± 0.42 ^a
EC_SA	71.76 ± 0.54 ^b	52.19 ± 4.76 ^a	45.96 ± 0.78 ^c	65.17 ± 0.67 ^c	38.41 ± 0.24 ^b
EC_CC	81.99 ± 0.43 ^d	91.33 ± 2.98 ^d	50.59 ± 0.07 ^e	55.01 ± 0.79 ^b	50.83 ± 0.37 ^c
EC_CSG	73.59 ± 0.21 ^b	81.51 ± 3.62 ^c	25.24 ± 0.81 ^b	54.27 ± 0.29 ^b	57.88 ± 2.55 ^d
EC_CC:CSG (1:1 wt/wt)	81.61 ± 1.03 ^d	77.04 ± 1.02 ^{bc}	66.13 ± 0.71 ^f	76.07 ± 0.58 ^e	64.66 ± 0.06 ^e

^{a-f}Means with different letters in a column represent significant difference ($P < 0.05$) between different samples within each probiotic strain.

¹Results are represented as percent inhibition of α -Glucosidase (AG), pancreatic α -amylase (PAA), Dipeptidyl peptidase (DPP-IV), Porcine pancreatic lipase (PPL), and cholesteryl esterase (CE). Data expressed as mean $\pm$ SD ($n = 3$). ND = not detected (no inhibitory activity detected).

²FC = free cells; EC = encapsulated cells, SA = sodium alginate; CC = camel casein; CSG = camel skin gelatin; CC:CSG = 1:1 wt/wt encapsulated probiotic strains.

subjected to SGID ($P < 0.05$). Among 3 strains, higher retention of the PAA inhibitory activity were observed for encapsulated *P. pentosaceus* in all wall matrices, whereas for *L. paracasei* significant increase in PAA inhibitory activity was obtained for probiotics cells encapsulated in CC, CSG, and CC:CSG. In summary, encapsulated probiotic strains displayed better inhibitory activity toward AG and PAA and could serve as a dietary intervention in managing type II diabetes. The results obtained in this study corroborate well with previous reports of Ahmad et al. (2019b) where encapsulation of probiotic strain of *P. acidilactici* in bovine and camel whey proteins resulted in significant retentment of AG and PPA inhibitory properties.

With regards to DPP-IV inhibitory activity of the *L. rhamnosus*, *P. pentosaceus*, and *L. paracasei*, all the free probiotics cell showed varying levels of DPP-IV inhibitory activity, with *L. rhamnosus* (70.1%) showing the highest activity, followed by *L. paracasei* (48.69%) and *P. pentosaceus* (43.74%). The results obtained corroborates those obtained from previous studies by Zeng et al. (2016a,b), who reported that *Lactobacillus* (7.2–33.3%) and *Bifidobacterium* strains (7–27%) possessed inhibitory activity against DPP-IV. Similarly, Panwar et al. (2016) also reported that different *Lactobacillus* isolates displayed different levels of inhibition against DPP-IV (10–32%). In the present study, when different probiotic strains were exposed to the gastrointestinal conditions, DPP-IV inhibitory activity was not detected for *L. rhamnosus*, whereas 27.66 and 7.692% of inhibitory potential were observed with *P. pentosaceus* and *L. paracasei*, respectively. However, CC:CSG-encapsulated *L. paracasei* showed the greatest inhibitory potential (66.13%) against DPP-IV, followed by CC-encapsulated *P. pentosaceus* (65.94%) and *L. rhamnosus* (53.37%) upon their transition through SGID (Table 2). These findings confirm the importance of probiotic encapsulation that could protect the beneficial bioactive properties.

Inhibition of PL reduces the efficiency of fat absorption in the small intestine resulting in the long-term reduction of BW. Similarly, CE plays a key role in regulating the plasma cholesterol concentration and inhibition of CE results in decreasing the levels of cholesterol (Jafar et al., 2018). Inhibition percentage displayed by the free probiotic strains (*L. rhamnosus*, *P. pentosaceus*, and *L. paracasei*) were 64.49, 53.24, and 67.91% for PPL and 48.00, 64.03, and 56.88% for CE, respectively. The trend suggests a decrease in PL and CE inhibitory activities when the free cells were subjected to the simulated digestion whereas encapsulation with SA, CC, CSG, and CC:CSG markedly retained the activities within varying levels. Overall, *L. rhamnosus* encapsulated in SA displayed higher PL inhibitory

activity (46.52%) followed by cells encapsulated in CC (44.05%; $P > 0.05$), and for *P. pentosaceus* and *L. paracasei* highest PL inhibitory activity was demonstrated by cells encapsulated in CC:CSG with an inhibitory percentage of 71.4 and 76.07%, respectively.

Thus, *L. paracasei* strain encapsulated with CC:CSG exhibited the highest inhibiting potential against PL and CE, whereas CC- and CC:CSG-encapsulated *P. pentosaceus* displayed higher levels of inhibitory activity against PL and CE (Table 2). This could be due to the encapsulating matrices protecting the probiotic strains from harsh conditions of SGID thereby retaining their inhibitory potential against PL and CE. Recently, Ahmad et al. (2019b) reported increased PL and CE inhibitory activity of *P. acidilactici* S30–4C when encapsulated with bovine and camel whey protein.

The loss of enzyme inhibitory property by the probiotic strains upon SGID could be possibly attributed to the loss in viability and induced stress among cells that limited the production of inhibitory components by probiotic cells. Thus, it is suggested that encapsulation of cells within different wall matrices could maintain the high viability of the probiotic cells in addition to keeping their cell membrane intact and also protect the cells from external stress. These protections imparted by wall material matrix help probiotic cells in effectively producing inhibitors at a faster and higher level. It is also possible that the probiotic cells use different wall materials such as CC and CSG as nutrient substrate and degrade them, resulting in production of potent bioactive molecules which might also contribute to the enzyme inhibitory activities. More studies at molecular level are needed to gain deeper insight into the reasons behind these variation among probiotic cells before and after encapsulation.

CONCLUSIONS

This study demonstrated the survival and functionality of probiotic strains (*L. rhamnosus*, *P. pentosaceus*, and *L. paracasei*) encapsulated in SA and CC and CSG. The CC-based matrices were efficient in protecting the probiotic strains during freeze-drying and thermal treatment showing higher cell viability and survival compared with probiotics encapsulated in CSG and CC+CSG. Furthermore, probiotic strains encapsulated with CC and CC:CSG also displayed enhanced cell viability under simulated gastrointestinal conditions. Probiotic strains in the encapsulated form were also found to maintain or enhance the inhibitory properties against α -glucosidase, α -amylase, DPP-IV activity, pancreatic lipase, and cholesterol esterase, which supports the future investigation on probiotics as a functionally bioactive ingredients with specific health-

promoting properties. However, it is still unclear and further work is necessary to understand the key mechanisms involved in maintaining these bioactivities by probiotics strains.

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