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RESEARCH ARTICLE

Evaluation of performance of co-encapsulated beads formed with a probiotic culture and L-ascorbic acid against pathogens and simulated gastric conditions

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Abstract: The present investigation was conducted on encapsulated beads formed from extrusion and emulsion methods having a co-encapsulated probiotic culture and L-ascorbic acid (AA). These were tested against pathogens and its responses in simulated gastric conditions. The results of study for the survival of probiotic viability as well as changes in ascorbic acid concentration in simulated gastro-intestinal conditions showed that treatment wise there was a significant difference ($p<0.05$) and mean concentration was significantly higher for emulsion method compared to extrusion method. For antimicrobial activity, four samples were prepared - control sample (C), encapsulated culture by emulsion method (Em), extrusion method (Ex), heat treated free cells (H). Em sample showed highest inhibition zone (16.33±2.08 mm) against *Enterococcus faecalis* ATCC 29212 and lowest inhibition zone (12.7± 0.58 mm) was observed against *E. coli* MTCC 1687 and *Staphylococcus aureus* MTCC 7373. For Ex sample, highest inhibition zone (14.00±1.0 mm) was seen against *Enterococcus faecalis* ATCC 29212 and lowest inhibition zone (9.07± 1.15 mm) was observed against *Staphylococcus aureus* MTCC 7373. The beads prepared by emulsion method survived better ($p<0.05$) in simulated gastro intestinal conditions than beads formed by extrusion method in terms of cell viability, AA concentration and in acidic solution. Hence, emulsion method could be the better methods for protecting sensitive ingredients and such prepared co-encapsulated beads offer better option in delivering functional items to individuals *via* foods.

Keywords: Probiotics, Microencapsulation, Co-encapsulation, Ascorbic acid, Gastric acid, Antimicrobial activity

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Introduction

Milk is a common and healthy food, but it is almost entirely devoid of vitamin C and has very little iron (Anon, 2019, Afeiche et al. 2024). Probiotic, prebiotics and their preparation as synbiotics confers many health benefits to human and extensive research work have already proven this in the past few decades. Functional foods are ultimately a food supplements that promise to transform, modify and restore the body's natural intestinal flora. The robustness and survival of the probiotics during manufacturing, storage and their passage through the gastrointestinal tract have to bear significant obstacles. These bacteria need to survive and establish in specific numbers in the gut environment to have a good impact on health (Kailasapathy and Chin, 2000). Probiotics have been studied for enhancing their vitality and viability in food products along with their transit through GI tract by micro-encapsulating in hydrocolloid beads (Krasaekoopt et al. 2003; Annan et al. 2008; Safi et al. 2021). Microencapsulation guard cells from harmful environments and low-intensity heat exposure *via* potentially reducing cell damage and ultimately death (Ding, and Shah, 2009; Das and Gawai, 2024).

In addition of inherent robustness of probiotics, it can be further effectively protected from deterioration using microencapsulation (Cook et al. 2012). Probiotics should be stable throughout storage, secured from severe circumstances in the upper GIT, released into the colon and then encouraged to colonize the mucosal surfaces using an efficient microencapsulation mechanism (Tripathi et al. 2013; Anselmo et al. 2016). Ascorbic acid (AA) is unstable in air and solution due to interactions with copper, iron, tin, heat, light, and oxygen. It is destroyed during cooking and can be improved by low pH values (Teucher et al. 2004). Hence, a dairy product with low to medium acidity can be better carrier medium for ascorbic acid.

According to Mehta et al. (2022), ascorbic acid microencapsulation could delay its core release rate, prevent ascorbic acid from changing color, and generally cover up its acidic taste. Ascorbic acid's stability is influenced by environmental conditions like temperature, pH, oxygen concentration, presence of metal ions, UV and X-rays (Yin et al. 2022; Das and Gawai, 2024). The microencapsulation technology is helpful to overcome some of these drawbacks associated with ascorbic acid.

In the preparation of many functional probiotic dairy products, including yoghurt, ice cream, goat cheese, kulfi and many others, many research works reported the use encapsulated probiotics and prebiotics (Ahmadi et al. 2014; Kavas et al. 2021; Kaur et al. 2021). LAB are used in a variety of fermented food products preparations, mostly in yogurt or dairy based beverages. It can be available in various forms like capsules, tablets, packets, or powders for ready to use (Matera, 2024). For the purpose of incorporating vitamins in dairy products, literature reveals that among the several heat-sensitive vitamins, ascorbic acid is more stable during frying in comparison to pressure cooking and boiling (Das, et al. 2025). Encapsulation of probiotics and ascorbic acid hides the acid taste, stops the product from changing colour and keeps it stable. As a result, microencapsulation lessens the chance of any interaction between ascorbic acid and other food product constituents (Anselmo et al. 2016).

Material and methods

Encapsulation of lactic acid bacterial cells and ascorbic acid using suitable medium

This work was focused on encapsulation of lactic acid bacteria and ascorbic acid. Two methods of encapsulation (Emulsion and Extrusion) wherein coating material Sodium alginate (4 %) and Maize starch (2 %) (Hi-media) were taken and autoclaved. For core material, 12-hour old lactic culture and L-ascorbic acid (Hi-media) were taken. Probiotic cell culture was activated to keep minimum cell concentration of more than 10^8 log cfu/g.

The ratio of coating to core material was kept at 5:1 ratio (20 ml). L- ascorbic acid was added in different quantity - 60, 80, 100, 120 mg/ 100 ml of coating material. Both coating and core material were mixed vigorously to get a uniform mixture. Among these rates of addition 100 mg /100 ml addition by finalized for preparation of encapsulated beads (Das and Gawai, 2024).

Encapsulation by extrusion method

For this technique, method described by Krasaekoopt et al. (2003) was followed, where insulin syringe having needle thickness of 28G and having internal diameter of 0.18 mm was purchased from local medical store. Mixture of the materials (20 ml) were extruded through the needle drop by drop to a 100 ml hardening solution of 0.1 M $CaCl_2$ and kept undisturbed for 1 hour. Afterwards the beads were collected and stored in refrigerator.

Encapsulation by emulsion method

This method was carried out as described by Mandal et al. (2014), where 100 ml of pre-autoclaved soyabean oil containing 0.5 % Tween 80 as emulsifier was taken (acts as continuous phase). The coating-core material mixture (20 ml) was then added drop wise to the continuous phase that was magnetically stirred. Within 5 minutes a uniformly turbid emulsion was obtained into which, 0.1 M calcium chloride (100 ml) that was previously cooled was added quickly to break the emulsion and for hardening of alginate-starch microcapsules. Then it was kept undisturbed for 20 minutes. The capsules were harvested by gentle centrifugation at 350 g for 10 minutes and washed thrice with chilled $CaCl_2$ solution for proper hardening and removal of odour from oil. The beads were separated by filtration and stored in refrigerator.

Microcapsules formed by these two methods were freeze dried in a bench top freeze drier at -72ÚC under vacuum and analysed for particle size. Microcapsules made with extrusion method particle diameter ranged from 91.42±1.27 to 138.27±1.30 µm and for emulsion method it ranged from 15.88±1.99 to 54.8±2.55 µm.

Antimicrobial activity

Evaluation of the antimicrobial activity was performed using the agar well diffusion assay as described by Pupa et al. (2021). 1g of sample of freeze-dried microcapsules was taken to depolymerize in 9 ml phosphate buffer. Then, 10 µL of the liquefied suspension was cultivated on MRS broth at 37 °C for 18-24 h. The overnight culture of microencapsulated product was centrifuged at 7000 g for 5 min at 4 °C, and the supernatant was harvested and filtered through a sterile filter (0.22 µm pore-size, Millex®-HV, MERK, Ireland) to achieve a cell free supernatant (CFS).

Supernatant (100 µl) of decapsulated probiotic culture and free cells (used as control) were placed in wells 15 mm in diameters punched in cooled Nutrient agar plates with sterile Aluminium borer (Hi-media) and were tested against *Staphylococcus aureus* MTCC 7373, *Escherichia coli* MTCC 1687, *Salmonella typhimurium* ATCC 14028, *Bacillus cereus MTCC 1272*, *Enterococcus faecalis* ATCC 29212. To check activity, 100 µL of each indicator strains were poured into petri dishes followed by adding 15 to 20 ml of nutrient agar. Upon solidification, wells were punched out and aliquots were placed. Then the plates were placed at 5 ÏC for 1-2 h to facilitate diffusion of supernatant. The plates were incubated at 37 °C for 24 h and 48 h, respectively. The sizes of the inhibition zones were measured by Hi-media antibiotic zone scale in mm.

Survival in simulated gastrointestinal conditions

This analysis was performed according to the method described by Liserre et al. (2007) with modifications. For that, 0.1 g of freeze-dried microparticles and 100 ml 1M HCl (pH 1.8) were mixed. Then, 3 g l^{-1} pepsin (pepsin from porcine gastric mucosa P7000, Sigma-Aldrich) and 0.9 mg l^{-1} lipase (lipase from porcine pancreas L3126, Sigma-Aldrich) were added, and the mixture was incubated at 37 °C/20 rpm/min for 2 h under continuous stirring in a shaker water bath.

Subsequently, the pH of the samples was adjusted to 5.0, and 1 g l^{-1} bile (Hi-media) and 0.1 g l^{-1} pancreatin (pancreatin from porcine pancreas P3292, Sigma-Aldrich) were added and incubated in same conditions as described earlier. Finally, pH was adjusted to 7.5, and the bile and pancreatin concentrations were kept constant. Incubation was done as described earlier to a total of 6 hours of analysis. Counts were performed after 5, 30, 120, 125, 150, 240, 245, 270, and 360 min of incubation.

Survival in acidic condition

The survival of the encapsulated probiotic culture in the hydrogel beads under acidic conditions was performed by protocol described by Praepanitchai et al. (2019) with slight modification. The viability of co-encapsulated probiotic and ascorbic acid under acidic environment was evaluated at pH 6.5, 3.0, and 2.0. Free cells were used as control. Microcapsules (1 g) were added to 9 ml of MRS broth that was adjusted to the desired pH with 5 M HCl or 1 N NaOH. Then the samples were kept in incubation at 37 ÚC for 3 h. After that the samples were centrifuged (5000 rpm for 10 min at 4 ÚC) and the hydrogel beads were further allowed to disintegrate in 9 ml phosphate buffer solution (pH 7.0) to release the cells from the beads. The survival of the co-encapsulated probiotic and ascorbic acid was evaluated.

Statistical analysis

Statistical analysis was carried out using statistical design FCRD. All the experiments were conducted in required numbers of replications and the results are expressed as mean± standard deviation (SD).

Results and Discussion

Results of analyzed microcapsules made from both of the methods are presented as follows.

Antimicrobial activity

Antimicrobial activity was performed against five pathogens (*Staphylococcus aureus* MTCC 7373, *Escherichia coli* MTCC 1687, *Salmonella typhimurium* ATCC 14028, *Bacillus cereus* MTCC 1272, *Enterococcus faecalis* ATCC 29212), which were made available from the Culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Kamdhenu University, Anand.

In Table 1, antimicrobial activity of co-encapsulated probiotic culture with ascorbic acid is shown. Antimicrobial activity was

Table 1: Antimicrobial activity of co-encapsulated beads

	Zone of inhibition (mm) against pathogens (P)					
Treatment(T)	*Bacillus cereus* MTCC 1272	*E. coli* MTCC 1687	*Staphylococcus aureus* MTCC 7373	*Salmonella typhimurium* ATCC 14028	*Enterococcus faecalis* ATCC 29212	Treatment mean (T)
C	21.70± 0.58	17.33±0.58	15.00±1.00	16.33±0.58	19.70±2.31	18.00^{d}
Em	19.33± 0.58	12.70± 0.58	12.70±0.58	14.33±0.58	16.33±2.08	15.07^{c}
Ex	15.33± 0.58	11.00±1.00	9.07±1.15	13.00±2.65	14.00±1.00	12.6^{b}
H	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0^{a}
Pathogen mean(P)	14.08^{D}	10.25^{B}	9.33^{A}	10.91^{B}	12.5^{C}	
Source		SEm±			CD (0.05)	
T		0.397			0.732	
P		0.444			0.819	
T x P		0.888			1.637	
CV (%)				8.678		

C : 3% citric acid
Em : Microcapsules by emulsion method
Ex : Microcapsules by extrusion method
H : Heat treated free cells
Each observation is mean ± standard deviation of three replicates
Mean with different superscripts (a,b,c,d) within column and superscripts (A,B,C,D) within row differ significantly ($p<0.05$)

performed on four samples which includes two microcapsules, 3 % citric acid (control) and heat-treated free cells.

Treatment wise, there was a significant decrease in zone of inhibition from Control sample (C) followed by encapsulated culture by emulsion method (Em), extrusion method (Ex), heat treated free cells (H). Comparing the pathogen means, values for *E. coli* MTCC 1687 and *Salmonella typhimurium* ATCC 14028 were non-significant. Rest values showed significant difference. For Em sample highest inhibition zone (16.33±2.08 mm) was seen against *Enterococcus faecalis* ATCC 29212 and lowest inhibition zone (12.7± 0.58 mm) was observed against *E. coli* MTCC 1687 and *Staphylococcus aureus* MTCC 7373. For Ex sample highest inhibition zone (14.00±1.0 mm) was seen against *Enterococcus faecalis* ATCC 29212 and lowest inhibition zone (9.07± 1.15 mm) was observed against *Staphylococcus aureus* MTCC 7373. For H sample there was no inhibition zone and for C sample inhibition zones were higher than other samples.

Immobilization of *Lactobacillus plantarum* in alginate covered with chitosan and gelatin was examined by Trabelsi et al. (2014) for its implications on antibacterial activity. According to their findings, immobilizing the chitosan-coated alginate enhanced its antibacterial efficacy against particular microorganisms. *Salmonella enterica* ATCC 43972, *Listeria ivanovii* BUG496 and *E. coli* ATCC 8739, respectively, had inhibition zones of 13, 13 and 11 mm.

The antibacterial efficacy of *Lactobacillus plantarum* TISTR1465 capsuled with *Eleutherine americana* oligosaccharide extract were studied by Phoem et al. (2019). Aliquots from the co-encapsulated beads revealed inhibitory zones for *S. aureus* ATCC25923, *C. perfringens* ATCC 13124, and *E. coli* ATCC25922 were of 13.0 mm, 12.8 mm, and 9.8 mm, respectively.

Kostelac *et al* (2021) developed a lyophilized probiotic co-encapsulate with the addition of L-ascorbic acid. L-ascorbic acid was tested against *Salmonella enterica* serovar *typhimurium* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 2356 in order to determine whether it has potential antimicrobial activity or not. It was reported that addition of L-ascorbic acid concentration @50 mg/ml significantly inhibited the growth of these pathogens.

Survival in simulated gastrointestinal conditions

Gastric and intestinal juice tolerances are one of the major characters of a probiotics. To facilitate probiotics survival through gastrointestinal conditions microencapsulation can be an important tool. Its barrier properties can help in reducing the loss of probiotic cells and maintaining a balanced micro flora. In Table 2 and 3, Ex and Em represents extrusion and emulsion respectively.

In Table 2, gastric juice tolerance of encapsulated probiotic culture has been shown. Comparing treatment means, there was a significant difference ($p<0.05$) and mean cell viability was significantly higher for emulsion method compared to extrusion method.

At zero hour, periodically mean cell viability was 10.15 log cfu/g. As pH of the solution was decreased to 1.8 after 30 minutes viability was reduced to 7.24, followed by 6.96 after 120 minutes. Then pH was increased to 5 and after 150 minutes viability slightly decreased to 6.69 log cfu/ml followed by an increase to 6.97 at 240 minutes. Then pH was further increased to 7.5 and at 270 minutes viability increased to 7.72 log cfu/ml, obtaining final viability of 8.05 log cfu/ml at 360 minutes of observation. Within a total 360 minutes of observation period as pH was reduced a significant reduction was observed to 150 minutes at pH 5. Afterwards viability significantly increased in pH 7.5.

Initial probiotic viability for extrusion method was 10.15 ±0.09 log cfu/g and after surviving in gastric and intestinal juice it was

Table 2: Comparative cell viability of encapsulated probiotic culture under simulated gastrointestinal conditions

Treatment(T)	Cell viability (log cfu/g) at varying time intervals (P, min)							Treatment mean(T)
	0	30	120	150	240	270	360	
	pH							
	6.5	1.8		5.0		7.5		
Ex	10.15 ±0.09	7.19 ±0.03	6.87 ± 0.07	6.59 ±0.07	6.92 ±0.05	7.63 ±0.05	7.91 ±0.06	7.61^{a}
Em	10.15 ±0.03	7.29 ±0.01	7.05 ±0.04	6.79 ±0.05	7.02 ±0.08	7.81 ± 0.09	8.19 ±0.09	7.76^{b}
Period mean(P)	10.15^{F}	7.24^{C}	6.96^{B}	6.69^{A}	6.97^{B}	7.72^{D}	8.05^{E}	
Source		SEm±			CD (0.05)			CV (%)
T		0.014			0.040			
P		0.026			0.075			0.765
T x P		0.036			0.106			

Each observation is mean ± standard deviation of three replicates

Mean with different superscripts (a,b) within column and superscripts (A,B,C,D,E,F) within row differ significantly ($p<0.05$)

reduced to 7.91 ±0.06 log cfu/g (2.24 log reduction). For emulsion method, initial viability was 10.15 ±0.03 log cfu/g that was reduced to 8.19 ±0.09 log cfu/g (1.96 log reduction).

Lactic acid bacteria (LAB) isolated from the digestive tract of broilers and native Thai chickens were examined for probiotic properties by Musikasang et al. (2009). They measured viability of encapsulated as well as free cells and reported that the cell viability was decreased by 6.83 log cfu/ml in the extrusion approach at pH 2.5 from an initial viability of 8.22 log cfu/ml while reduced by 6.91 log cfu/ml in the emulsion method from an initial viability of 8.35 log cfu/ml.

The impact of resistant starch (Hi-maize) on the survival of microencapsulated *Lactobacillus acidophilus* with sodium alginate was examined by de Araújo et al. (2016). Microcapsules were produced by extrusion and freeze-drying method and survivability was measured in stimulated gastrointestinal conditions. After 360 mins, the freeze-dried sodium alginate microparticles (ALGL) and the freeze-dried sodium alginate microparticles plus hi-maize (AHML) microparticles, showed significant 3.67 and 3.01 log reduction respectively. In the imitated conditions of the gastrointestinal tract, the AHML therapy showed a slight reduction of viability. Conclusively, prebiotics added boosted the bacteria's resilience towards low pH and bile salts concentrations in the simulated digestive tract which led to more viable cells in the prebiotic-containing microparticles.

Similarly, in Table 3, gastric juice tolerance of encapsulated (by two methods) ascorbic acid (mg /100 mg) has been provided. Comparing treatment means there was a significant difference ($p<0.05$) and mean concentration was significantly higher for emulsion method compared to extrusion method.

Table 3: Comparative concentration of encapsulated ascorbic acid under simulated gastrointestinal conditions

Treatment(T)	Ascorbic acid concentration (mg/ 100 mg) at varying time intervals (P, min) 0	30	120	150	240	270	360	Treatment mean (T)	Reduction (%)
				pH					
	6.5	1.8	1.8	5	5	7.5	7.5		
Ex	14.27 ±0.11	9.74 ±0.10	9.45 ±0.14	8.26 ±0.13	7.96 ±0.08	4.87 ±0.53	1.12 ±0.15	7.95[a]	92.15
Em	15.75 ±0.12	10.63 ±0.06	10.5 ±0.08	9.58 ±0.07	9.34 ±0.08	6.09 ±0.08	1.94 ±0.06	9.12[b]	87.68
Period mean(P)	15.01[G]	10.19[F]	9.98[E]	8.92[D]	8.65[C]	5.48[B]	1.53[A]		
Source		SEm±			CD (0.05)			CV (%)	
T		0.038			0.109				
P		0.07			0.205			1.798	
T x P		0.099			0.29				

Each observation is mean ± standard deviation of three replicates
Mean with different superscripts (a,b) within column and superscripts (A,B,C,D,E,F,G) within row differ significantly ($p<0.05$)

Table 4: Survivability of probiotics encapsulated by extrusion method in acidic condition

Treatment (pH, T)	Probiotic count (log cfu/g) at varying incubation periods (P, min) 0	3	Treatment mean (T)
2.0	10.09±0.08	5.86±0.23	7.97[a]
3.0	10.03±0.09	7.27±0.09	8.65[b]
6.5	10.07±0.03	8.19±0.03	9.13[c]
Period Mean (P)	10.06[B]	7.12[A]	
Source	SEm±	CD (0.05)	CV (%)
T	0.046	0.144	
P	0.038	0.117	1.334
T x P	0.065	0.203	

Each observation is mean ± standard deviation of three replicates
Mean with different superscripts (a,b,c) within column and superscripts (A,B) within row differ significantly ($p<0.05$)

At zero hour, periodically mean concentration was 15.01 mg. As pH of the solution was decreased to 1.8 after 30 minutes, concentration was reduced to 10.19 mg, followed by 9.98 mg after 120 minutes. Then pH was increased to 5 and after 150 minutes concentration decreased to 8.92 mg followed by a decrease to 8.65 mg at 240 minutes. Then pH was further increased to 7.5 and at 270 minutes viability decreased to 5.48 mg, obtaining final concentration of 1.53 mg at 360 minutes of observation. Within a total 360 minutes of observation period as pH was reduced significantly.

Initial ascorbic acid concentration for extrusion method was 14.27 ±0.11mg that was reduced to 9.74 ±0.10 mg (31.75% reduction) after 30 minutes in pH 1.8, which further reduced to 9.45 ±0.14 mg after 120 minutes in pH 1.8. At pH 5 after 150 minutes concentration was 8.26 ±0.13 mg (12.6 % reduction), followed by 7.96 ±0.08 mg after 240 minutes. The pH was increased to 7.5 and at 270 minutes concentration decreased to 4.87 ±0.53 (39 % reduction), followed by 1.12 ±0.15 mg at 360 minutes.

Similarly, initial ascorbic acid concentration for emulsion method was 15.75 ±0.12 mg that was reduced to 10.63 ±0.06 mg (32.6 % reduction) after 30 minutes in pH 1.8, which further reduced to 10.5 ±0.08 mg after 120 minutes in pH 1.8. At pH 5 after 150 minutes, concentration was 9.58 ±0.07 mg (8.77 % reduction), followed by 9.34 ±0.08 mg after 240 minutes. The pH was increased to 7.5 and at 270 minutes, concentration decreased to 6.09 ±0.08 (34.8 % reduction), followed by 1.94 ±0.06 mg at 360 minutes.

In vitro release of ascorbic acid and its effect on iron bioavailability was investigated by Lee et al. (2003). The release of ascorbic acid was observed lowering down at each incubation time point in stimulated gastric fluid with a pH change from 2 to 5, showing that microcapsules were more stable at higher pH levels. At pH 2, ascorbic acid that had been microencapsulated by MCT (medium-chain triacylglycerol) was released from the microcapsules at a rate of 12.5% after 20 minutes and significantly increased to 13.2% after 60. The ascorbic acid release at pH 5 was the least of all the treatments at 4.7% at 20 minutes and 6.3% at 60 minutes. At pH 2, ascorbic acid was microencapsulated by PGMS (polyglycerol monostearate), and after 20 minutes, 13.5% of the ascorbic acid was liberated from the microcapsules, and at 60 minutes, 16.0%. The release of ascorbic acid at pH 5 was similar to other treatments, 8.5% after 20 minutes and 10.0% after 60 minutes. 28.9 and 46.2% of the product was released after 20 minutes of incubation at pH 7 and 8, and 77.8 and 83.8% after 60 minutes, respectively.

Through the do-it-yourself type of prototype of salecan and chitosan (coating material), Hu et al. (2020) created a novel polyelectrolyte complex (PEC) hydrogel that functioned as a safe carrier that could shield vitamin C from the gastric fluid and maintainable release it in the gut. They observed that release of the vitamin C was Less than 10% from various salecan/chitosan ratios of the PEC hydrogels in SGF (stimulated gastric fluid) within 2 hours. The maximum quantity of vitamin C that could be released after 10 hours, which was higher than that of other vitamin C carriers, was 92.3% when the release environment was altered to SIF (simulated intestinal fluid). The release amount increased when the salecan/chitosan ratio increased from 4/6 to 7/3, from 4.1% to 10% in SGF after 2 hours and from 74.9% to 92.3% in SIF in the next 8 hours.

Survival in acidic condition

The survival of the probiotic beads encapsulated by extrusion and emulsion methods in three different pH 2.0, 3.0, 6.5 by adjusting pH of MRS broth were evaluated. Microcapsules (1 g) were added to 9 ml of MRS broth that was adjusted to the desired pH with 5 M HCl or 1 N NaOH. Then the samples were kept in incubation at 37 ÚC for 3 h and viability was calculated.

In Table 4 and 5 survivability of encapsulated probiotics are shown in terms of log cfu/ml. For extrusion method while comparing treatment means there was a significant increase in mean values as pH was increased. Periodically mean values decreased significantly. In case of extrusion method of encapsulation at pH 2, a reduction of 4.23 log was observed,

Table 5: Survivability of probiotics encapsulated by emulsion method in acidic condition

Treatment (pH, T)	Probiotic count (log cfu/g) at varying incubation periods (P, min)		Treatment mean (T)
	0	3	
2.0	10.23±0.09	6.17±0.06	8.2^{a}
3.0	10.36±0.12	7.56±0.18	8.96^{b}
6.5	10.32±0.20	8.74±0.06	9.53^{c}
Period Mean (P)	10.30^{B}	7.49^{A}	
Source	SEm±	CD(0.05)	CV (%)
T	0.053	0.134	
P	0.043	0.109	1.168
T x P	0.075	0.189	

Each observation is mean ± standard deviation of three replicates
Mean with different superscripts (a,b,c) within column and superscripts (A,B) within row differ significantly ($p<0.05$)

whereas at pH 3 and pH 6.5, a log reduction of 2.76 and 1.88 was observed respectively. Highest viability was found at pH 6.5 (8.19±0.033 log cfu/g). Similar patterns of changes were observed for emulsion method. A significant increase in treatment mean values was observed as pH was increased and periodically mean values decreased significantly.

For emulsion method of encapsulation at pH 2, a reduction of 4.06 log was observed, whereas at pH 3 and pH 6.5, a log reduction of 2.8 and 1.58 was observed respectively. Highest viability was found at pH 6.5 (8.74±0.06 log cfu/g). Higher viability was found in case of emulsion method.

In their study, Ding and Shah (2009) enclosed several microorganisms in coatings made of alginate, guar gum, xanthan gum, locust bean gum, and carrageenan gum. The acid tolerance of the organisms in capsules was investigated. Probiotic organisms' ability to withstand acid was examined at pH 2 throughout the course of a 2-hour incubation period. Results of this study revealed that probiotic bacteria in alginate, xanthan gum, and carrageenan gum survived in better way in acidic environment ($p < 0.05$) than free probiotic bacteria.

Hybrid alginate-soy protein isolate-based hydrogel beads were prepared by Praepanitchai et al. (2019) using an extrusion method and the level of probiotic survival in the encapsulated beads was assessed under acidic circumstances (pH 2, 3, and 6.5). It was observed that with a decrease in acidity from pH 2 to 6.5, the survivability of the free and the encapsulated probiotics (in SA and SA/SPI beads) increased. After 3 hours of incubation at pH 6.5, the majority of the free and encapsulated probiotics were discovered to be live and active. The viability of the encapsulated probiotics was considerably ($p > 0.05$) higher at pH 2 and 3 than that of the free probiotics. This is due to its protection from direct interaction with the acidic medium. The SA/SPI beads (bead A-E formulations) had viable cell counts of 5.04 0.05, 5.88 0.06, 5.97 0.06, 6.23 0.04, and 6.15 0.05 log CFU ml-1, respectively, at pH 2. When immersed in NGYC medium pH 2 and 3 for 3 hours, respectively, the probiotic in bead D formulation had the best survival rate ($p< 0.05$) with 6.23 0.04 and 7.62 0.10 log CFU ml-1.

Table 6: Stability of ascorbic acid encapsulated by extrusion method in acidic condition

Treatment (pH, T)	Ascorbic acid concentration (mg/ 100 mg) at varying time intervals (P, h)		Treatment mean (T)
	0	3	
2.0	14.13±0.04	3.04±0.08	8.58^{a}
3.0	14.24±0.16	3.62±0.11	8.93^{b}
6.5	14.23±0.07	9.88±0.08	12.06^{c}
Period Mean (P)	14.20^{B}	5.51^{A}	
Source	SEm±	CD(0.05)	CV (%)
T	0.04	0.125	
P	0.033	0.102	0.94
T x P	0.057	0.176	

Each observation is mean ± standard deviation of three replicates
Mean with different superscripts (a,b,c) within column and superscripts (A,B) within row differ significantly ($p<0.05$)

Table 7: Stability of ascorbic acid encapsulated by emulsion method in acidic condition

Treatment (pH, T)	Ascorbic acid concentration (mg/ 100 mg) at varying time intervals (P, h)		Treatment mean (T)
	0	3	
2.0	15.20±0.18	3.37±0.16	9.29^{a}
3.0	15.27±0.20	3.79±0.13	9.53^{b}
6.5	15.30±0.03	10.96±0.08	13.13^{c}
Period Mean (P)	15.26^{B}	6.04^{A}	
Source	SEm±	CD(0.05)	CV (%)
T	0.057	0.179	
P	0.047	0.146	1.405
T x P	0.081	0.253	

Each observation is mean ± standard deviation of three replicates
Mean with different superscripts (a,b,c) within column and superscripts (A,B) within row differ significantly ($p<0.05$)

Lb. acidophilus was extruded into capsules by Zeashan et al. (2020) utilizing two wall materials and its combination *i.e*. sodium alginate, soy protein isolate, and SA-SPI. Probiotics were evaluated for survivability under various simulated technical and gastrointestinal situations using free and capsuled probiotics. Free cells displayed the lowest viability at pH 2, while sodium alginate, soy protein isolate, and sodium alginate-soy protein isolate all demonstrated viability of 6.10-7.75 log cfu/ml. However, sodium alginate–soy protein isolate demonstrated the highest viability of 7.75 ±0.39 log cfu/ml. Both pH 3 and pH 6.5 showed the same trend. The maximum trend of sodium alginate-soy protein isolate encapsulated was 8.6 ±0.43 log cfu/ ml at pH 6.5. Combining sodium alginate and soy protein isolate appears to have a synergistic effect on the existence of probiotics in microcapsules.

Similarly, stability of ascorbic acid in acidic solutions for the same pH was carried out. Results are shown in Table 6 and 7. For extrusion method while comparing treatment means there was a significant increase in mean values as pH increased. Periodically mean values decreased significantly. For this method of encapsulation at pH 2, about 78.48 % (3.04/14.13) ascorbic acid was released, whereas at pH 3 and pH 6.5, a release of 74.58% (3.62/14.24) and 30.57% (9.88/14.23) was observed respectively. Lowest release occurred at pH 6.5 of 30.57%.

Similar patterns of changes were observed for emulsion method. A significant increase in treatment mean values was observed as pH increased and periodically mean values decreased significantly. For this method of encapsulation at pH 2, about 77.83 % (3.37/15.2) ascorbic acid was released, whereas at pH 3 and pH 6.5, a release of 75.19% (3.79/15.27) and 28.37% (10.96/ 15.3) was observed respectively. Lowest release occurred at pH 6.5 of 28.37%. In acidic conditions, comparatively more release occurred in case of emulsion method, but near neutral pH lesser release of ascorbic acid was found.

Pea protein concentrate (PPC) was used by Pierucci et al. (2006) to create a new microencapsulation method for ascorbic acid. They investigated the retention of ascorbic acid inside the core by HPLC, overall morphology by SEM, size distribution by light scattering, and release kinetics. For comparative analyses, carboxymethylcellulose (CMC) and blends with maltodextrin (M) were created. Free AA was released practically linearly, plateauing at 100% dissolution in low pH and at 80% in high pH, however release of AA from micro particles was slow, and a plateau was not seen within 120 minutes. Low dissolving rates, whether or not they were combined with M, were seen in CMC microparticles, which released 40% of AA at low pH. PPC microparticles released 55% more ascorbic acid than before. 92 % of ascorbic acid was liberated into the medium when PPC and M were combined.

The physicochemical properties, thermal stability, and release profile of ascorbic acid (AA) in microcapsules formed via complex coacervation was studied Cruz et al. (2019). For using as a wall material, gelatin and gum arabic in concentrations of 2.5, 5.0, and 7.5% (w/v) were utilized. Following freeze-drying, the coacervate microcapsules were evaluated for physicochemical properties, thermal behavior, and stability over the course of 60 days of storage. At various pH levels (1.1, 2.2, 5.4, 7.4, 9.6, and 12), the release profile was assessed. After 90 minutes of examination, 94.69% of the AA content was released at pH 1.1, followed by 82% between pH 2.2 and 12.0, and 38% at a pH near neutral. Throughout the 120 min of the analyses, there was no apparent difference in the release profiles of the microcapsules that were in contact with solutions of pH 5.4, 7.4, and 9.6. It was also observed that at pH 2.2 and 12.0, almost 98% of the microencapsulated AA was released. The release of the microcapsules was the slowest when it kept in a solution of pH 7.4, out of all the pH values examined in the study.

Emulsion method in the presented research work showed comparatively better results in the above parameters in terms of better probiotic viability and ascorbic acid retention. Similarly, microcapsules made by this method have better effective carrier of ascorbic acid concentration up to 100mg/100ml.

Conclusions

The study examined beads created using emulsion and extrusion techniques that contained L-ascorbic acid and a co-encapsulated probiotic culture. In simulated gastrointestinal circumstances, the emulsion technique demonstrated noticeably improved probiotic cell viability and survival. Additionally, the study discovered that the emulsion approach had superior antibacterial effectiveness against *Staphylococcus aureus* and *Enterococcus faecalis*. The emulsion method may be a superior way to preserve sensitive substances and provide people functional ingredients through meals because the beads from this method managed better in acidic solutions and stimulated gastrointestinal environment.

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References

Afeiche MC, Zimmermann D, Donato-Capel L, Achakzai BK, Mak TN (2024) Consumption of milk beverages reduces iron, vitamin A, vitamin C, calcium, and B vitamins inadequacies in Pakistani school-aged children from Sindh and Punjab: a diet modeling study. Curr Dev Nutr 8(9): 104435

Ahmadi A, Milani E, Madadlou A, Mortazavi SA, Mokarram RR, Salarbashi D (2014) Synbiotic yogurt-ice cream produced *via* incorporation of microencapsulated lactobacillus acidophilus (la-5) and fructooligosaccharide. J Food Sci Tech 51: 1568-1574.

Annan NT, Borza AD, Hansen LT (2008) Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. Food Res Int 41(2): 184-193

Anon (2019) U.S. Department of Agriculture, Agricultural Research Service. Food Data Central, 2019

Anselmo AC, McHugh KJ, Webster J, Langer R, Jaklenec A (2016) Layer by layer encapsulation of probiotics for delivery to the microbiome. Adv Mater 28(43): 9486-9490

Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV (2012) Microencapsulation of probiotics for gastrointestinal delivery. J Controlled Release 162(1): 56-67

da Cruz MCR, Dagostin JLA, Perussello CA, Masson ML (2019) Assessment of physicochemical characteristics, thermal stability and release profile of ascorbic acid microcapsules obtained by complex coacervation. Food Hydrocoll 87: 71-82

Das K, Gawai KM (2024) Comparison study of co-encapsulation beads formed by extrusion and emulsion method using a probiotic culture with L-ascorbic acid. Int J Vet Sci Anim Husb 9(3): 409-414

Das K, Gawai KM, Hati S, Shendurse AM (2025) Evaluation of thermal tolerance of co-encapsulated beads incorporated with probiotic culture and L-ascorbic acid. Int J Agric Food Sci 7(4): 85-89

de Araújo Etchepare M, Raddatz GC, Cichoski AJ, Flores ÉMM, Barin JS, Zepka LQ, de Menezes CR (2016) Effect of resistant starch (Hi-maize) on the survival of *Lactobacillus acidophilus* microencapsulated with sodium alginate. J Funct Foods 21, 321-329

Ding WK, Shah NP (2009) Effect of various encapsulating materials on the stability of probiotic bacteria. J Food Sci 74(2): M100-M107

Hu X, Wang Y, Zhang L, Xu M (2020) Formation of self-assembled polyelectrolyte complex hydrogel derived from salecan and chitosan for sustained release of vitamin C. Carbohydr Polym 234: 115920

Kailasapathy K, Chin J (2000) Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium spp*. Immunol Cell Biol 78(1): 80-88.

Kaur N, Kaur A, Sridhar K, Sharma M, Singh TP, Kumar S (2021) Development and quality characteristics of functional Kulfi fortified with microencapsulated betalains. Int J Food Sci Technol 56(10): 5362-5370

Kavas N, Kavas G, Kýnýk Ö, Ateþ M, Þatýr G, Kaplan M (2021) The effect of using microencapsulated pro and prebiotics on the aromatic compounds and sensorial properties of synbiotic goat cheese. Food Biosci 43: 101233

Kostelac D, Jurić V, Frece J (2021) Microencapsulation of probiotic bacteria with ascorbic acid for increased antioxidant activity. Curr Top Biotechnol 12: 75-81

Krasaekoopt W, Bhandari B, Deeth H (2003) Evaluation of encapsulation techniques of probiotics for yoghurt. Int Dairy J 13(1): 3-13

Lee JB, Ahn J, Lee J, Kwak HS (2003) The microencapsulated ascorbic acid release *in vitro* and its effect on iron bioavailability. Arch Pharm Res 26:874-879

Liserre AM, Ré MI, Franco BDGM (2007) Microencapsulation of *Bifidobacterium animalis subsp. lactis* in modified alginate-chitosan beads and evaluation of survival in simulated gastrointestinal conditions. Food Biotechnol 21(1): 1-16

Mandal S, Hati S, Puniya AK, Khamrui K, Singh K (2014) Enhancement of survival of alginate encapsulated *Lactobacillus casei* NCDC 298. J Sci Food Agri 94(10): 1994-2001

Matera M (2024) Bifidobacteria, Lactobacilli... when, how and why to use them. Global Pediatrics 8: 100139

Mehta N, Kumar P, Verma AK, Umaraw P, Kumar Y, Malav OP, Sazili AQ, Domínguez R, Lorenzo JM. (2022) Microencapsulation as a noble technique for the application of bioactive compounds in the food industry: a comprehensive review. *Applied Sci 12*(3): 1424

Musikasang H, Tani A, H-kittikun A, Maneerat S (2009) Probiotic potential of lactic acid bacteria isolated from chicken gastrointestinal digestive tract. World J Microbiol Biotechnol 25: 1337-1345

Phoem AN, Mayiding A, Saedeh F, Permpoonpattana P (2019) Evaluation of *Lactobacillus plantarum* encapsulated with *Eleutherine americana* oligosaccharide extract as food additive in yoghurt. Brazilian J Microbiol 50(1): 237-246

Pierucci APT, Andrade LR, Baptista EB, Volpato NM, Rocha-Leão MHM (2006) New microencapsulation system for ascorbic acid using pea protein concentrate as coat protector. J Microencapsul 23(6): 654-662

Praepanitchai O, Noomhorm A, Anal AK (2019) Survival and behavior of encapsulated probiotics (*Lactobacillus plantarum*) in calcium-alginate-Soy protein isolate-based Hydrogel beads in different processing conditions (pH and temperature) and in pasteurized mango juice. BioMed Res Int 1-8

Pupa P, Apiwatsiri P, Sirichokchatchawan W, Pirarat N, Muangsin N, Shah AA, Prapasarakul N (2021). The efficacy of three double-microencapsulation methods for preservation of probiotic bacteria. Sci Rep 11(1): 1-9

Safi ME, Memlawal R, Banuree SAH (2021) Materials and techniques for microencapsulation of probiotics. Biosc Biotech Res Comm 14(3):

Teucher B, Olivares M, Cori H (2004) Enhancers of iron absorption: ascorbic acid and other organic acids. Int J Vitam Nutr Res 74(6): 403-419

Trabelsi I, Ayadi D, Bejar W, Bejar S, Chouayekh H, Ben SR (2014) Effects of *lactobacillus plantarum* immobilization in alginate coated with chitosan and gelatin on antibacterial activity. Int J Biol Macromol 64: 84-89

Tripathi P, Beaussart A, Alsteens D, Dupres V, Claes I, Von Ossowski I, Dufrene YF (2013) Adhesion and nanomechanics of pili from the probiotic *Lactobacillus rhamnosus* GG. ACS Nano 7(4): 3685-3697

Wilson N, Shah NP (2007). Microencapsulation of vitamins. ASEAN Food J 14(1): 1

Yin X, Chen K, Cheng H, Chen X, Feng S, Song Y, Liang L (2022) Chemical stability of ascorbic acid integrated into commercial products: A review on bioactivity and delivery technology. Antioxidants (Basel) 11(1): 153

Zeashan M, Afzaal M, Saeed F, Ahmed A, Tufail T, Ahmed A, Anjum FM (2020) Survival and behavior of free and encapsulated probiotic bacteria under simulated human gastrointestinal and technological conditions. Food Sci Nutr 8(5): 2419-2426