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Comparative Analysis of Microencapsulation Efficiency for Viability of *Lactobacillus sp.* and *Enterococcus sp.* Using Extrusion and Emulsion Technique

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A B S T R A C T

Milk and milk products are the main sources of beneficial probiotic strains. Probiotics are enriched with good microorganisms for better activity of gastrointestinal tract. *Yogurt, dahi*, kefir, lassi, pickles, sourdough bread and some cheeses are example of some popular probiotic products obtained after fermentation. Not all the species have probiotic properties, certain species of, *Bifidobacterium Lactobacilli, Enterobacter* and *Bacilli* are known to have potentiality to be used as probiotics. Among the major challenges in preparation of probiotics, survival rate in the gastrointestinal tract during its consumption is one of the major concerns. Since the pH of gastric fluid is too acidic, many microorganisms cannot remain viable. There are many encapsulation methods, which are useful to maintain viability and characteristics of probiotic microorganisms when used with correct coating materials. Alginate, silica, starch, chitosan, and vegetable oil are good matrices for encapsulation. In this study, viability of five encapsulated microorganisms namely to encapsulate three *Lactobacilli* and two *Enterobacter* species isolated from varieties of sources. These species are *Enterobacter faecium* MW561229, *Enterococcus faecium* MW561231, *Lactobacilli plantarum* MW561232, *Lactobacilli fermentum MW561233 and Lactobacilli plantarum MW561234.* Alginate, alginate with starch, alginate with chitosan and alginate with vegetable oil and tween 80 were used as encapsulation matrices. Microorganisms with 1.0%, 2.0% and 3.0% density were subjected to simulated gastric and intestinal conditions up to 3 hours to determine the viability. Viability of cells was counted as CFU/ml. Based on the obtained results, it was found that after exposure of 3 hours, low concentrations of alginate/starch, alginate/chitosan and alginate/vegetable oil/tween 80 were having good viability.

***Key words*:** Probiotics, *Lactobacilli species, Enterobacter species*, Encapsulation, Simulated gastro, Intestinal conditions

Many species of *Lactobacilli* and *Bifidobacterium* are known to have potential properties to be used as probiotics. They can be obtained from varieties of sources including milk and milk-based products [1-5]. A potential strain should have antibacterial-antifungal activities, bile salt hydrolase activity, ability to survive at low pH and high salt concentration, adhesion ability, gamma amino butyric acid (GABA) production efficiency, β-galactosidase assay, mucin digestion ability and hemolytic activity [6-10]. These characters are more commonly known as probiotic properties. These various metabolic activities of microorganisms are responsible for better enhancement health benefits [11-13]. The term probiotic is derived from the Greek word "probios" which means "life". Many other researchers have given various definitions from time to time for probiotics but the current definition was given by World health organization (WHO) in 2001 mentioning that them as live microorganisms which when administered in adequate amounts confer a health benefit on the host [14]. Yogurt, curd, buttermilk and other fermented food are good source for isolation of probiotics [15]. Selective media like MRS (deMan, Rogosa and Sharpe) and M17 enables easy and fast screening from multiple sources [16-17].

Before the evolution of the term probiotic, people are already using curd, yogurt, buttermilk, kefir, lassi, boruga as part of food. Studies have shown that probiotics enables better gastrointestinal track (GIT) activity [18-19]. The major challenge for development of any probiotic is to maintain their viability under extreme gastrointestinal conditions. It was seen that majority of microorganisms cannot survive in gastric juice at pH near 2.0. Effect of probiotic can only be seen if they reached to intestine and reproduce [20-22]. To protect the microorganisms from such an extreme conditions multiple methods have tried and it was found that encapsulation with suitable matrix is easy and very effective approach [23-26]. Encapsulation can be done by extrusion, emulsion, spray-drying and hydrogels [27-28]. Selection of method for

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encapsulation is depends on the type of matrix to be used for encapsulation. Numbers of matrices like alginate, gelatin chitosan, agar, starch are available for encapsulation. Selection of matrix is depends on the type of microorganisms and its application [29-30]. Many a time, combination of more than one matrix was also carried out for better results [31-32]. Encapsulated microorganisms are known to maintain the viability above the minimum requirement after exposure to harsh condition of stomach. Previous studies have proved that starch and chitosan are the most stable compounds among most of the tested matrices [33-34]. In the present study determination of viability of encapsulation of five isolates obtained from milk samples were carried out using multiple matrices. Total five different encapsulation matrices were used and microorganisms were exposed to simulated gastric and intestinal juice for different time duration. After suitable interaction time viability of probiotics were determined. Results of the study have enabled to screening the most efficient matrix and method for encapsulation for the selected microorganisms.

MATERIALS AND METHODS

Encapsulation with alginate

1.0%, 2.0% and 3.0% alginate solution were prepared in distilled water and sterilized by autoclaving at 110°C for 10 minutes. Active bacterial culture (0.1%) was added to alginate solutions. Beads were prepared by using sterile syringe. From the syringe mixture was dropped from the height of around 15 cms into cold calcium chloride (0.5%). Prepared beads were further hardened for 30 minutes in the same solution and finally washed with 0.85% NaCl solution to remove unbounded microorganisms. Beads were stored at 4°C until further use.

Encapsulation using alginate and starch

1.0%, 2.0% and 3.0% solution of alginate and 2.0% solution of starch were prepared and sterilized by autoclaving at 110°C for 10 minutes. Active bacterial culture (0.1%) was added to this sterile mixture. The mixture was dispensed drop wise in oil having tween 80 (0.2%). Solution was stirred well for 20 minutes to allow droplet formation. After 20 minutes, 0.1 M calcium chloride solution was added from side of wall of glassware and allowed to rest for 30 minutes. After 30 minutes, beads settled at bottom were collected and excess oil was drained. Beads were further washed with 0.85% saline and stored at 4°C until further use.

Encapsulation using chitosan

Low molecular weight chitosan is preferred matrix to be used for encapsulation. Here 0.4 gm of chitosan was suspended in 90mL of distilled water. The mixture was activated by adding 0.4 mL of glacial acetic acid. After activation, with the help of 0.1N NaOH pH was adjusted to 5.6 and make up to 100ml. The solution was filtered and sterilized by autoclaving. In this sterile solution, 10gm of 1.0%, 2.0% and 3.0% alginate pre-prepared beads with bacteria were mixed and incubated for 50 minutes with low rotation. The resulted alginate-chitosan coated beads were washed with 1.0% peptone water and stored are 4°C until further use.

Encapsulation with vegetable oil and tween 80 using emulsion technique

Alginate, vegetable oil and tween 80 were mixed in the proportion of 3:3:0.5 with 1.0%, 2.0% and 3.0% microorganism. Beads were prepared by filling the solution in a sterile syringe with narrow opening and dropping from the height of 15 cms into freeze cold solution of sodium chloride (0.5%) and calcium chloride (0.05%). Beads were further hardened by incubating for 30 minutes in the same mixture. After 30 minutes, it was washed with 0.85% NaCl solution to remove unbounded microorganisms. Beads were stored at 4°C until further use.

Determination of viability under simulated gastric condition

Simulated gastric juice was prepared with NaCl 25 mM, KCl_7 mM, $NaHCO_3$ 45 mM, and pepsin 3 g/L with pH 2.5. 1.0% encapsulated (all types) and free active culture of probiotic microorganisms were inoculated and incubated for 3 hours in this simulated gastric juice. For determination of viability, aliquots were taken at regular interval of 0 hour, 1 hour and 3 hours. From that 50µL of sample were spreaded on MRS agar and incubated for 24 hours at 37°C. Based on the obtained colonies, total microbial count was calculated in form of log CFU/gm.

Determination of viability under simulated intestinal condition

Simulated intestinal juice was prepared with NaCl 0.5% w/v, bile salt 0.5% w/v, and pancreatin 1.0 g/L with pH 8.0. 1.0% encapsulated (all types) and free active culture of probiotic microorganisms were inoculated and incubated for 3 hours in this simulated intestinal juice. For determination of viability, aliquots were taken at regular interval of 0 hour, 0.5 hour, 1 hour, 1.5 hours and 2.0 hours. From that 50µL of sample were spreaded on MRS agar and incubated for 24 hours at 37°C. Based on the obtained colonies, total microbial count was calculated in form of log CFU/gm.

RESULTS AND DISCUSSION

Study results of simulated gastric juice have shown that all the selected species follow almost similar pattern of growth. Detail observations were mentioned in the (Table 1-3) and expressed as graphs (Fig 1-3). It was seen that *Lactobacilli* species have comparatively better viability after three hours of incubation in simulated gastric juice. Among all tested encapsulation matrices, combination of alginate/chitosan (A/C) and alginate/vegetable/tween 80 (A/V/T) have better efficiency to protect microorganisms. *L. plantarum* MW561234 encapsulated with 3.0% concentration of A/C have given highest 5.64±0.53 CFU/gm followed by 5.48±0.41 with 2.0% concentration. Species of Enterobacter have shown comparatively less viability. It was also seen the species encapsulated with 1.0% concentration have least viability.

Results of simulated intestinal condition have shown highest viability with 2.0% concentration with 7.28±0.24 CFU/gm with (A/V/T) rather than 3.0% concentration which is only 5.21±0.32 CFU/gm with *L. fermentum* MW561233. Detail observations were mentioned in the (Table 4-6) and expressed as graphs (Fig 4-6). Similar to simulated gastric viability results, specie of *Enterobacter* has also shown lower viability as compare to all the three *Lactobacilli*. Overall results have proved that microorganisms encapsulated with 2.0% and 3.0% alginate have better survival rate. Since the difference of viability between 2.0% and 3.0% alginate is not significant, it is advisable to use 2.0% alginate instead of 3.0% alginate for better release the microorganisms.

It was seen that in the initial phase of encapsulation, alginate and gelatin were most common matrix. These matrices are weak and not suitable for long term storage. Previous studies have given emphasized on addition of other compounds for enhancement of binding efficiency and for better viability with longer duration. Chitosan is one of such compounds which

can provide maximum stability to encapsulated microorganisms. Microencapsulated *Lactobacilli casei* with calcium alginate, fructooligosaccharide and chitosan have shown very high resistance under adverse condition of GI tract. These particles have released higher number of cells than the therapeutic value [35]. Not limited to the microorganisms, combination of chitosan-alginate is also very useful in controlled released for vitamins and drugs like [36]. Enzymes like cellulase can also be immobilized and characterized using magnetic chitosan microspheres [37].

Table 1 Viability of 1.0% alginate encapsulated microorganisms under stimulated gastric juice condition

S. No.	Microorganisms	Time (Hour)	Free	Alginate	Alginate + Starch	Alginate + Chitosan	Alginate + Vegetable oil + Tween 80
1	*Enterococcus faecium*	0	6.23±0.2	6.08±0.28	6.07±0.18	6.11±0.29	6.09±0.24
	MW561229	1	5.22±0.18	5.63±0.26	5.36±0.14	5.76±0.17	5.75±0.13
		3	3.43±0.14	4.45±0.14	4.61±0.2	4.83±0.26	4.75±0.16
2	*Lactobacillus fermentum*	0	6.24±0.27	6.11±0.19	6.2±0.15	6.15±0.25	6.15±0.27
	MW561233	1	5.12±0.2	5.28±0.37	5.3±0.18	5.28±0.2	5.3±0.2
		3	3.51±0.18	4.43±0.19	4.52±0.23	4.53±0.35	4.5±0.28
3	*Enterococcus faecium*	0	6.34±0.4	6.3±0.29	6.15±0.22	6.11±0.6	6.15±0.19
	MW561231	1	5.13±0.29	5.33±0.22	5.3±0.14	5.42±0.55	5.58±0.22
		3	3.51±0.2	4.54±0.28	4.69±0.24	4.68±0.63	4.75±0.53
4	*Lactobacillus plantarum*	0	6.24±0.3	6.18±0.37	6.15±0.25	6.14±0.15	6.13±0.18
	MW561232	1	5.24±0.19	5.47±0.48	5.3±0.35	5.39±0.24	5.33±0.28
		3	3.65±0.36	4.45±0.18	4.53±0.18	4.45±0.2	4.67±0.1
5	*Lactobacillus plantarum*	0	6.35±0.18	6.2±0.26	6.14±0.23	6.28±0.18	6.3±0.25
	MW561234	1	5.3±0.19	5.54±0.4	5.51±0.23	5.59±0.33	5.46±0.3
		3	3.56±0.27	4.45±0.27	4.44±0.28	4.66±0.43	4.65±0.28

Table 2 Viability of 2.0% alginate encapsulated microorganisms under stimulated gastric juice condition

S. No.	Microorganisms	Time (Hour)	Free	Alginate	Alginate + Starch	Alginate + Chitosan	Alginate + Vegetable oil + Tween 80
1	*Enterococcus faecium*	0	6.36±0.28	4.81±0.22	4.86±0.22	5.17±0.14	5.18±0.16
	MW561229	1	5.56±0.28	4.96±0.22	5.48±0.22	5.69±0.14	5.78±0.16
		3	4.96±0.27	5.03±0.31	5.57±0.25	5.93±0.21	6.05±0.19
2	*Lactobacillus fermentum*	0	7.66±0.24	5.8±0.22	5.85±0.26	6.22±0.13	6.24±0.27
	MW561233	1	6.7±0.18	5.98±0.22	6.6±0.19	6.86±0.23	6.96±0.21
		3	5.98±0.19	6.06±0.22	6.71±0.32	7.14±0.29	7.28±0.24
3	*Enterococcus faecium*	0	6.07±0.24	4.59±0.33	4.64±0.21	4.93±0.34	4.95±0.28
	MW561231	1	5.31±0.21	4.74±0.3	5.23±0.16	5.43±0.2	5.52±0.15
		3	4.74±0.17	4.8±0.16	5.32±0.23	5.66±0.3	5.77±0.19
4	*Lactobacillus plantarum*	0	7.32±0.22	5.54±0.24	5.59±0.12	5.94±0.24	5.96±0.33
	MW561232	1	6.4±0.32	5.71±0.32	6.3±0.54	6.55±0.31	6.65±0.18
		3	5.71±0.22	5.79±0.48	6.41±0.14	6.82±0.35	6.95±0.27
5	*Lactobacillus plantarum*	0	7.41±0.32	5.61±0.22	5.66±0.18	6.02±0.29	6.04±0.32
	MW561234	1	6.48±0.24	5.78±0.44	6.39±0.21	6.63±0.24	6.73±0.23
		3	5.78±0.21	5.86±0.22	6.49±0.27	6.91±0.41	7.04±0.33

Table 3 Viability of 3.0% alginate encapsulated microorganisms under stimulated gastric juice condition

S. No.	Microorganisms	Time (Hour)	Free	Alginate	Alginate + Starch	Alginate + Chitosan	Alginate + Vegetable oil + Tween 80
1	*Enterococcus faecium*	0	7.21±0.23	7.04±0.32	7.03±0.21	7.07±0.33	7.06±0.27
	MW561229	1	6.04±0.21	6.51±0.29	6.21±0.16	6.67±0.2	6.66±0.15
		3	3.97±0.17	5.16±0.16	5.33±0.22	5.59±0.29	5.5±0.19
2	*Lactobacillus fermentum*	0	7.22±0.31	7.07±0.21	7.17±0.18	7.12±0.28	7.11±0.31
	MW561233	1	5.92±0.23	6.11±0.43	6.14±0.21	6.11±0.23	6.14±0.22
		3	4.06±0.21	5.13±0.21	5.23±0.26	5.24±0.4	5.21±0.32
3	*Enterococcus faecium*	0	7.34±0.46	7.29±0.33	7.12±0.25	7.07±0.69	7.11±0.21
	MW561231	1	5.94±0.33	6.17±0.25	6.13±0.17	6.28±0.64	6.46±0.25
		3	4.06±0.22	5.25±0.32	5.43±0.27	5.42±0.72	5.5±0.61
4	*Lactobacillus plantarum*	0	7.22±0.34	7.15±0.43	7.12±0.28	7.1±0.18	7.09±0.21
	MW561232	1	6.07±0.21	6.33±0.55	6.13±0.4	6.24±0.27	6.17±0.32
		3	4.22±0.41	5.16±0.21	5.24±0.21	5.15±0.22	5.4±0.12
5	*Lactobacillus plantarum*	0	7.35±0.21	7.17±0.3	7.1±0.26	7.27±0.21	7.29±0.28
	MW561234	1	6.14±0.21	6.42±0.46	6.38±0.26	6.47±0.38	6.32±0.34
		3	4.12±0.31	5.16±0.31	5.14±0.32	5.39±0.5	5.38±0.32

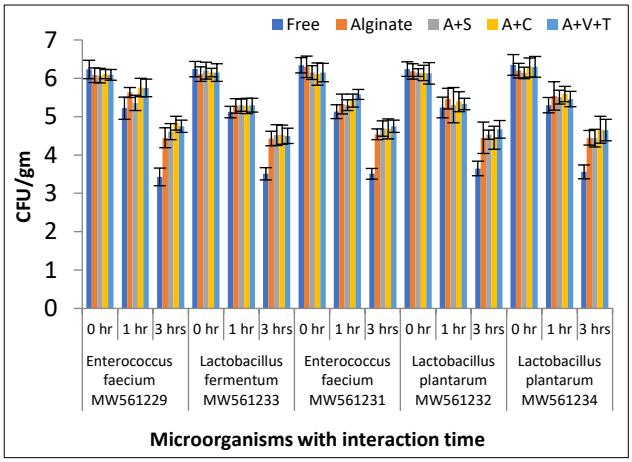

Fig 1 Viability of 1.0% alginate encapsulated microorganisms under stimulated gastric juice condition

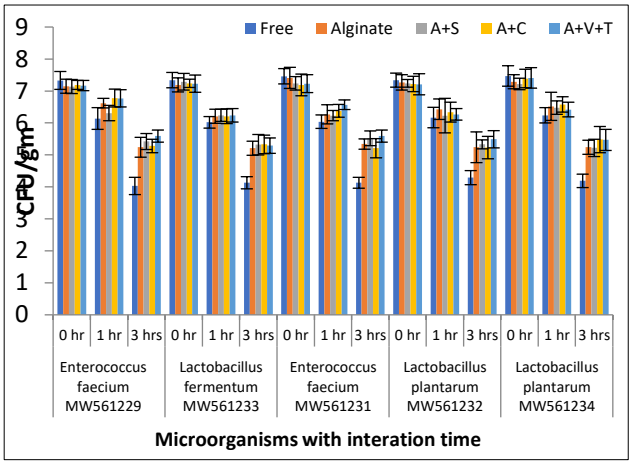

Fig 2 Viability of 2.0% alginate encapsulated microorganisms under stimulated gastric juice condition

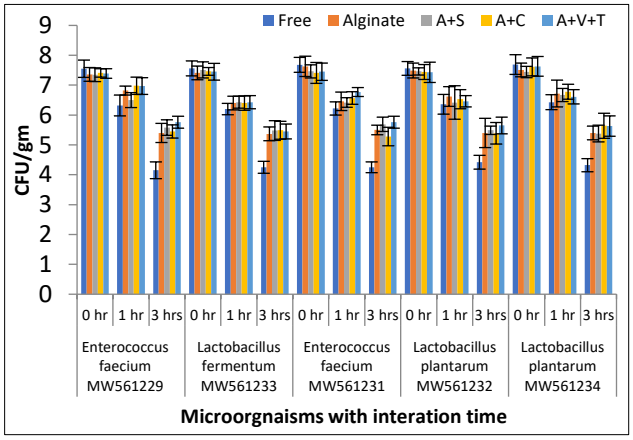

Fig 3 Viability of 3.0% alginate encapsulated microorganisms under stimulated gastric juice condition

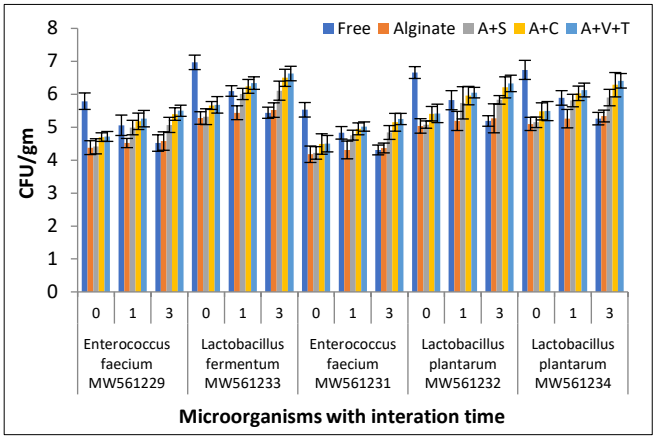

Fig 4 Viability of 1.0% alginate encapsulated microorganisms under various conditions of simulated intestinal juice

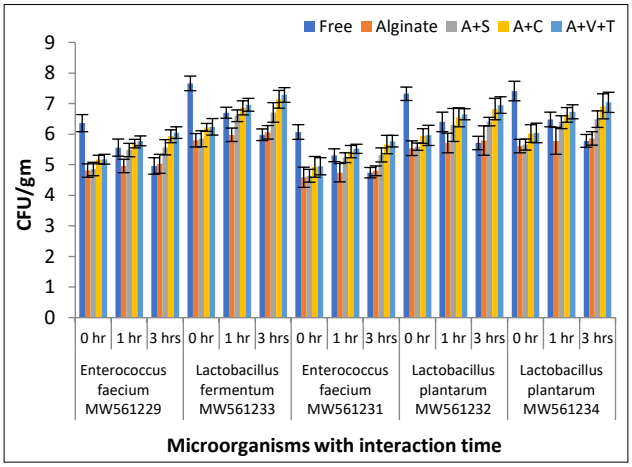

Fig 5 Viability of 2.0% alginate encapsulated microorganisms under various conditions of simulated intestinal juice

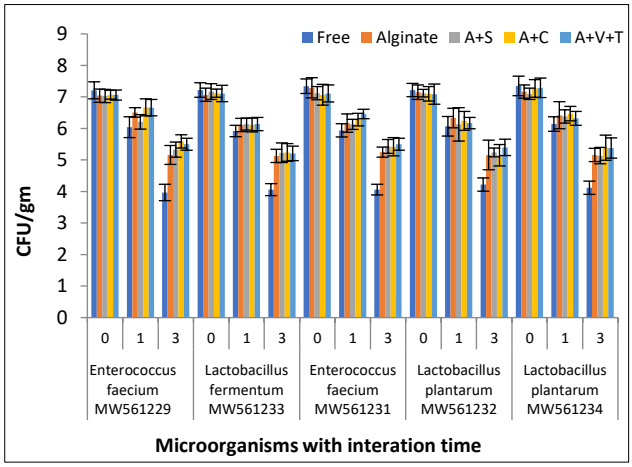

Fig 6 Viability of 3.0% alginate encapsulated microorganisms under various conditions of simulated intestinal juice

Based on the overall results of simulated intestinal conditions, it was found that the initial period of up to 1 hour is good growth of free microorganisms. But as the time passes and reached to 3 hours, the viability of encapsulated microorganisms was decreased as compare to all encapsulated microorganisms. Good growth of microorganisms was seen with 1.0% and 2.0% as compared to 3.0%. The low rate of release of microorganisms is considered as principal reason behind this [38]. Simulated intestinal fluid has pH near to 8.0, which generally doesn't inhibit the growth of microorganisms significantly. Encapsulation with starch and alginate enhances the viability of cells under simulate intestinal conditions [38]. Encapsulated microorganisms have retained their viability in simulated gastric juice (pH 2.0) and intestinal juice (pH 7.4) [39], modified amaranth starch provided better stability and allow the microorganisms to reach large intestine [40]. In a study carried out on *L. lactis* by Yeung *et al.* [41], they have found that hydro gel beads prepared using calcium, alginate and soy protein are stable at temperature upto 72°C with under extreme acidic condition of pH 2. They have also used these

hydro gel beads for mango juice fortification. Upon pasteurization, viability of *L. plantarum* was retained significantly. Al-Furain *et al.* [42] have determined effect of various encapsulation materials on *L. plantarum* DSM 20174. Encapsulation material involved combination of alginate with sodium chloride, canola oil, olive oil and chitosan. Results of their study have shown that, olive oil capsules have provided maximum stability at pH 2 ever after incubating for 24 hrs. Addition of bile salt up to concentration of 0.5% has enhanced the stability of capsules prepared using chitosan and olive oil. Combination of sodium chloride and chitosan had given highest stability at higher temperature as compared to other combinations. Based on the overall study, they have recommended using chitosan with NaCl for long term stability probiotic strains. Jimenez-Fernandez *et al*. [43] have tried gum Arabic and pectin mixture for encapsulation of *L. paracasei* and studied various physicochemical parameters. In the study, they have found that size of microcapsule can greatly influence the texture, quality and sensory properties of the product. No doubt encapsulation with gum arabic and pectin has protected *L. paracasei* against harsh condition of simulated gastrointestinal conditions.

Table 4 Viability of 1.0% alginate encapsulated microorganisms under various conditions of simulated intestinal juice

S. No.	Microorganisms	Time (Hour)	Free	Alginate	Alginate + Starch	Alginate + Chitosan	Alginate + Vegetable oil + Tween 80
1	*Enterococcus faecium*	0	5.79±0.22	4.38±0.25	4.42±0.19	4.7±0.31	4.72±0.25
	MW561229	1	5.06±0.19	4.52±0.27	4.99±0.15	5.18±0.18	5.26±0.14
		3	4.52±0.15	4.58±0.15	5.07±0.21	5.4±0.27	5.5±0.17
2	*Lactobacillus fermentum*	0	6.97±0.29	5.28±0.2	5.32±0.16	5.66±0.26	5.68±0.29
	MW561233	1	6.1±0.22	5.44±0.28	6.01±0.19	6.24±0.22	6.34±0.21
		3	5.44±0.19	5.52±0.18	6.11±0.25	6.5±0.37	6.63±0.22
3	*Enterococcus faecium*	0	5.53±0.43	4.18±0.31	4.22±0.24	4.49±0.65	4.5±0.17
	MW561231	1	4.83±0.31	4.31±0.24	4.76±0.15	4.95±0.59	5.02±0.24
		3	4.31±0.21	4.37±0.21	4.84±0.25	5.15±0.67	5.25±0.56
4	*Lactobacillus plantarum*	0	6.66±0.32	5.04±0.28	5.08±0.26	5.41±0.16	5.42±0.19
	MW561232	1	5.82±0.14	5.19±0.51	5.74±0.37	5.96±0.25	6.05±0.3
		3	5.19±0.38	5.27±0.19	5.83±0.19	6.21±0.21	6.33±0.11
5	*Lactobacillus plantarum*	0	6.74±0.19	5.1±0.28	5.15±0.25	5.48±0.19	5.49±0.26
	MW561234	1	5.89±0.17	5.26±0.43	5.81±0.25	6.03±0.35	6.13±0.32
		3	5.26±0.29	5.34±0.29	5.9±0.22	6.29±0.46	6.41±0.21

Table 5 Viability of 2.0% alginate encapsulated microorganisms under various conditions of simulated intestinal juice

S. No.	Microorganisms	Time (Hour)	Free	Alginate	Alginate + Starch	Alginate + Chitosan	Alginate + Vegetable oil + Tween 80
1	*Enterococcus faecium*	0	6.36±0.28	4.81±0.22	4.86±0.22	5.17±0.14	5.18±0.16
	MW561229	1	5.56±0.28	4.96±0.22	5.48±0.22	5.69±0.14	5.78±0.16
		3	4.96±0.27	5.03±0.31	5.57±0.25	5.93±0.21	6.05±0.19
2	*Lactobacillus fermentum*	0	7.66±0.24	5.8±0.22	5.85±0.26	6.22±0.13	6.24±0.27
	MW561233	1	6.7±0.18	5.98±0.22	6.6±0.19	6.86±0.23	6.96±0.21
		3	5.98±0.19	6.06±0.22	6.71±0.32	7.14±0.29	7.28±0.24
3	*Enterococcus faecium*	0	6.07±0.24	4.59±0.33	4.64±0.21	4.93±0.34	4.95±0.28
	MW561231	1	5.31±0.21	4.74±0.3	5.23±0.16	5.43±0.2	5.52±0.15
		3	4.74±0.17	4.8±0.16	5.32±0.23	5.66±0.3	5.77±0.19
4	*Lactobacillus plantarum*	0	7.32±0.22	5.54±0.24	5.59±0.12	5.94±0.24	5.96±0.33
	MW561232	1	6.4±0.32	5.71±0.32	6.3±0.54	6.55±0.31	6.65±0.18
		3	5.71±0.22	5.79±0.48	6.41±0.14	6.82±0.35	6.95±0.27
5	*Lactobacillus plantarum*	0	7.41±0.32	5.61±0.22	5.66±0.18	6.02±0.29	6.04±0.32
	MW561234	1	6.48±0.24	5.78±0.44	6.39±0.21	6.63±0.24	6.73±0.23
		3	5.78±0.21	5.86±0.22	6.49±0.27	6.91±0.41	7.04±0.33

Based on the previously carried out studies, it was found that encapsulation is definitely a good method for protection of important probiotic microorganisms against harsh condition of digestive tract. Wide range of encapsulation matrices are now a day available for encapsulation. Selection of suitable matrix is highly depending on the type of probiotic microorganisms and its applications. Single matrix or combinations of matrices can be used at a time. For each study, it was recommended to optimize all the relevant parameters for better outcome of the study [44].

CONCLUSION

Now days, the food sector has been developing a growing variety of probiotic containing products. This is due to a growing consumer awareness of the need of eating well and the benefits of probiotic bacteria consumption. Major difficulties facing the food industries are ensuring that products contain an acceptable level of live bacterial cells on the shelf and maintained during the storage. Microencapsulation is one of the most effective ways to preserve live probiotic strains from industrial processing, storage, and the gastrointestinal environment, hence boosting their stability and life. To achieve the best possible protection of microorganisms without affecting the end product's features, materials and microencapsulation procedures should be carefully chosen. In this study, *Enterococcus faecium* MW561229, *Lactobacillus fermentum* MW561233, *Enterococcus faecium* MW561231, *Lactobacillus plantarum* MW561232, *Lactobacillus plantarum* MW561234 were successfully encapsulated in alginate/starch

and alginate/chitosan microspheres prepared by extrusion method. *Lactobacillus sp.* showed all over good viability than *Enterococcus sp.* In a simulated gastrointestinal tract, *Lactobacillus sp.* Cells encapsulated in microspheres with 2% alginate/chitosan and 2% alginate/starch survived better than free cells. Encapsulation has once again proven to be a successful method of maintaining probiotics in the gastrointestinal environment. Microspheres made of alginate/chitosan and alginate/starch potential application as a new encapsulating material for sustaining probiotic viability during oral administration.

Table 5 Viability of 3.0% alginate encapsulated microorganisms under various conditions of simulated intestinal juice

S. No.	Microorganisms	Time (Hour)	Free	Alginate	Alginate + Starch	Alginate + Chitosan	Alginate + Vegetable oil + Tween 80
1	*Enterococcus faecium*	0	7.21±0.23	7.04±0.32	7.03±0.21	7.07±0.33	7.06±0.27
	MW561229	1	6.04±0.21	6.51±0.29	6.21±0.16	6.67±0.2	6.66±0.15
		3	3.97±0.17	5.16±0.16	5.33±0.22	5.59±0.29	5.5±0.19
2	*Lactobacillus fermentum*	0	7.22±0.31	7.07±0.21	7.17±0.18	7.12±0.28	7.11±0.31
	MW561233	1	5.92±0.23	6.11±0.43	6.14±0.21	6.11±0.23	6.14±0.22
		3	4.06±0.21	5.13±0.21	5.23±0.26	5.24±0.4	5.21±0.32
3	*Enterococcus faecium*	0	7.34±0.46	7.29±0.33	7.12±0.25	7.07±0.69	7.11±0.21
	MW561231	1	5.94±0.33	6.17±0.25	6.13±0.17	6.28±0.64	6.46±0.25
		3	4.06±0.22	5.25±0.32	5.43±0.27	5.42±0.72	5.5±0.61
4	*Lactobacillus plantarum*	0	7.22±0.34	7.15±0.43	7.12±0.28	7.1±0.18	7.09±0.21
	MW561232	1	6.07±0.21	6.33±0.55	6.13±0.4	6.24±0.27	6.17±0.32
		3	4.22±0.41	5.16±0.21	5.24±0.21	5.15±0.22	5.4±0.12
5	*Lactobacillus plantarum*	0	7.35±0.21	7.17±0.3	7.1±0.26	7.27±0.21	7.29±0.28
	MW561234	1	6.14±0.21	6.42±0.46	6.38±0.26	6.47±0.38	6.32±0.34
		3	4.12±0.31	5.16±0.31	5.14±0.32	5.39±0.5	5.38±0.32

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