

## RESEARCH ARTICLE

# Standardization of protocol for development of synbiotic milk pudding using co-encapsulation of probiotic culture and ascorbic acid

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**Abstract:** Interest in probiotics and its products has grown recently among consumers and researchers alike. The current concept aims to create a non-fermented milk product preparation that can be consumed at every meal. It can be taken after lunch or dinner as a dessert or savoury supplement. Such product will fully satisfy the body's minimal needs by supplying ascorbic acid (AA) and a daily dose of probiotic bacteria in encapsulated form. The concept of such product development was standardized and optimized to have sufficient isabgol, a prebiotic component to provide the host with more dietary content. The present investigation a recipe for preparation of synbiotic milk pudding. The current study focuses on optimizing the technique for making synbiotic milk pudding. In this study, addition rate of sugar, agar-agar, isabgol, co-encapsulated beads were optimized and rates of addition of sugar, agar-agar, isabgol @ 16%, 1.5%, 0.5% respectively gained better sensory scores. For microcapsules, it was @1% that showed optimum cell viability and ascorbic acid concentration along with higher sensory scores. The finalized product had a probiotic viability of  $9.457 \pm 0.08 \log$  cfu/g that is within the range of effective probiotic dosage. The product also has  $38.21 \pm 0.80$  mg/100 g of ascorbic acid concentration that means as per the serving size of 25 g it confers 9.55 mg of ascorbic acid that can fulfil about 11.6 % of RDA (for an average adult human being) of vitamin C. The developed synbiotic non-fermented milk pudding could be better option to have three-in-one effect of a probiotic culture, ascorbic acid and benefits of isabgol for better health.

**Keywords:** Probiotics, ascorbic acid, co-encapsulation, pudding, synbiotic

## Introduction

Vitamin C, a water-soluble vitamin commonly recognised as L-ascorbic acid, is added to many foods and presented as a dietary supplement. Very similar to the most animals, humans are unable to produce vitamin C, making it a crucial part of the human diet. In addition to being required for the production of collagen, L-carnitine, and certain neurotransmitters, vitamin C plays a function in protein metabolism. In a system, one particular dose-dependent, active transporter controls the intestinal assimilation of vitamin C. Vitamin C is up taken by cells *via* a second unique transport protein (Na<sup>+</sup>-dependent vitamin C transporters -SVCTs) (Doseděl et al. 2021; Wang et al. 2023). According to *in vitro* research, oxidized vitamin C (dehydroascorbic acid), enters inside of cell by facilitated glucose transporters before it is being internally reduced to ascorbic acid. Uncertainty exists regarding the physiological function of dehydroascorbic acid absorption and its contribution to the overall vitamin C economy (Wang et al. 2023).

According to Xu et al. (2024), 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. The microencapsulation technology is used to help ascorbic acid overcome some of these drawbacks and increased stability.

*Plantagoovata*, the scientific name for isabgol (Psylliums), is derived from the Persian terms "isap" and "ghol," which mean "horse ear" and "seed," respectively. The husk of psyllium seeds is made up of mucilaginous polysaccharides with a xylan backbone embedded in a highly branched acidic arabinoxylan. It is well known as a dietary fiber that aids in promoting laxation and increasing stool volume. It is among the most popular natural treatments for constipation. Isabgol promotes weight loss by making you feel full and preventing overeating.

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Synbiotics were developed to help probiotics dealing with any potential problems associated with host. The justification for using synbiotics appears to be supported by observations demonstrating an enhancement in the probiotic bacteria's survival during transit through the part of upper digestive tract *i.e.* esophagus, stomach and duodenum (Govaert et al. 2024). Maintaining intestinal homeostasis and a healthy body requires a more effective colon implantation as well as a rousing influence on the viability of probiotics and common bacteria.

Pudding is a type of food that can be either a dessert or a savoury (salty or spicy) dish that is part of the main meal (Singh et al. 2022). In North America, pudding characteristically denotes a sweet, milk-based dessert similar in consistency to egg-based custards, instant custards or a mousse, often commercially set using corn starch, gelatin or similar coagulating agent such as Jell-O (Ysewijn, 2016).

In the United Kingdom and some of the Commonwealth countries, the word pudding is still used to describe both sweet and savory dishes. Unless qualified, however, the term in everyday usage typically denotes a dessert; in the United Kingdom, pudding is used as a synonym for a dessert course. Dessert puddings are rich, fairly homogeneous starch- or dairy-based desserts such as rice pudding, steamed cake mixtures such as treacle sponge pudding with or without the addition of ingredients such as dried fruits as in a Christmas pudding (Anon, 2022).

In the preparation of many functional probiotic dairy products, including yoghurt, ice cream, goat cheese, kulfi and many others, many research works reported using encapsulated probiotics and prebiotics (Khosravi Zanjani et al. 2017; Kataria et al. 2018; Kavas et al. 2021; Kaur et al. 2021). Their findings were noteworthy and revealed that well-defined product improvements with excellent sensory, physico-chemical, and microbiological qualities were possible when incorporated encapsulated probiotics and prebiotics. The present work is for the development of non-fermented milk product preparation which can be consumed after each meal. It will have daily dose of encapsulated probiotic bacteria and ascorbic acid content to full fill minimum basic requirement of the body. Based on scientific findings, this study is a development of routine milk-based pudding type non-fermented product that contains probiotics and ascorbic acid in encapsulated form, with prebiotic in the form of psyllium husk, was prepared.

## Materials and Methods

### Materials

Pasteurized full cream milk was used for pudding preparation, agar powder (By nature-vegetarian gelatin alternative) was purchased from Amazon India and Isabgol was purchased from

Apolo Pharmacy, Amul Dairy Road, Anand. Co-encapsulated beads were added in product preparation to fulfil minimal recommended dietary allowance (RDA) requirements. For preparation of co-encapsulated beads *Lactobacillus helveticus* MTCC 5463 (V3) cultures was obtained from the culture collection of Dairy Microbiology Department, SMC College of Dairy Science, Kamdhenu University, Anand and L-ascorbic acid food grade was purchased from Hi-media, India. Details protocol for preparation of micro-encapsulated beads is cited and taken from Das and Gawai (2024).

### Optimization of ingredients in milk pudding preparation (based on sensory parameters)

Following mentioned ingredients were used in the range of addition provided to choose best combination for sensorily good quality of milk pudding. Details of these variable are as follows: Sugar addition - 12, 14, 16 and 18%, Agar-agar addition - 1, 1.25, 1.5 and 1.75%, Isabgol addition - 0.25, 0.5, 0.75 and 1%, Microcapsule addition - 0.5, 1, 1.5 and 2%. After addition of micro capsules to product, each sample was analyzed for probiotic viability and ascorbic acid concentration.

### Preparation of milk pudding

Pasteurized full cream milk (6 % fat and 9 % SNF) was taken and caramelized sugar was added in to milk. Then milk was kept at 75 °C/10 min with intermittent stirring. After heat treatment it was cooled to 60 °C and optimized level of agar-agar, isabgol and microcapsules were added. Then the product was heated on low flame till the body turned viscous. Further product was poured in PET cups and allowed to cool. Settled product was kept in refrigerated temperature ( $7 \pm 2$  °C). The details flow chart for preparation of milk pudding is given in Figure 1.

### Cell viability

Lactobacilli count of *Lactobacillus helveticus* MTCC 5463 (V3) was enumerated by MRS Agar plating method (37 °C for 24-48 h). The encapsulated cells of lactobacilli were released from the beads according to the procedure of Hossain et al. (2021) by mixing milk pudding sample with phosphate buffer (pH  $7.2 \pm 0.1$ ) containing 0.1 % peptone and incubated at 37 °C for 1 hour, and depolymerised by vortexing until a uniform cloudy solution is obtained followed by preparation of suitable dilutions using 9 ml sterile phosphate buffer tubes. Phosphate ions in the solution react with sodium in sodium-alginate and cause de-polymerisation of sodium-alginate, which leads in to the release of encapsulated lactobacilli cells from the alginate-maize capsules. MRS agar was used for enumeration by pour plating and colonies developed were counted after 48 h at 37 °C (Das et al. 2025).

**Ascorbic acid concentration**

The ascorbic acid content was determined by the AOAC (967.21), 2006 method. This method is 2,6-dichlorophenolindophenol titration method. It depends upon the stoichiometric reduction of the dye 2, 6-dichlorophenol indophenol to a colorless compound by ascorbic acid (Ismillayli et al. 2024).

**Sample preparation**

To determine ascorbic acid concentration, grind an accurately weighed sample (about 5 g) in a mortar with acid washed sand using TCA reagent or metaphosphoric acid and transfer into 100-ml graduated flask. Shake the mixture thoroughly and make up the volume to 100 ml with TCA reagent or metaphosphoric acid. Filter immediately through a fluted filter paper. The final concentration of ascorbic acid in the extract should be 10 to 15 µg per ml.

**Determination of vitamin C (ascorbic acid)**

Take 10 ml of the filtrate, and titrate rapidly with the indophenol solution.

Carry out a blank determination with 11 ml of the reagent along with water sufficient to make the volume of the mixture equivalent to 15 ml plus the volume of the indophenol solution required in the direct titration. Calculate the vitamin C content in the sample

as follows:

$$\text{Ascorbic acid (mg/100g of sample)} = \frac{(A \times B \times 1000)}{W}$$

A = volume in ml of the indophenol solution used for titration,

B = weight in mg of the ascorbic acid equivalent to one millilitre

of the indophenol solution

W = weight in g of the sample taken for the test

**Sensory analysis**

Sensory evaluation of the milk pudding was conducted throughout the standardization procedure of respective ingredients. Evaluation was carried out by 10 trained panelist judges in the morning and afternoon hour of the day. Evaluation was carried out for flavour, body and texture, color and appearance and overall acceptability score. 9-point Hedonic scale was provided to judges for evaluation purpose (Addo-Preko et al. 2023). Evaluation was carried out till product was fully standardized.

**Statistical analysis**

Statistical analysis was carried out using statistical design FCRD. All the experiments were conducted in required numbers of

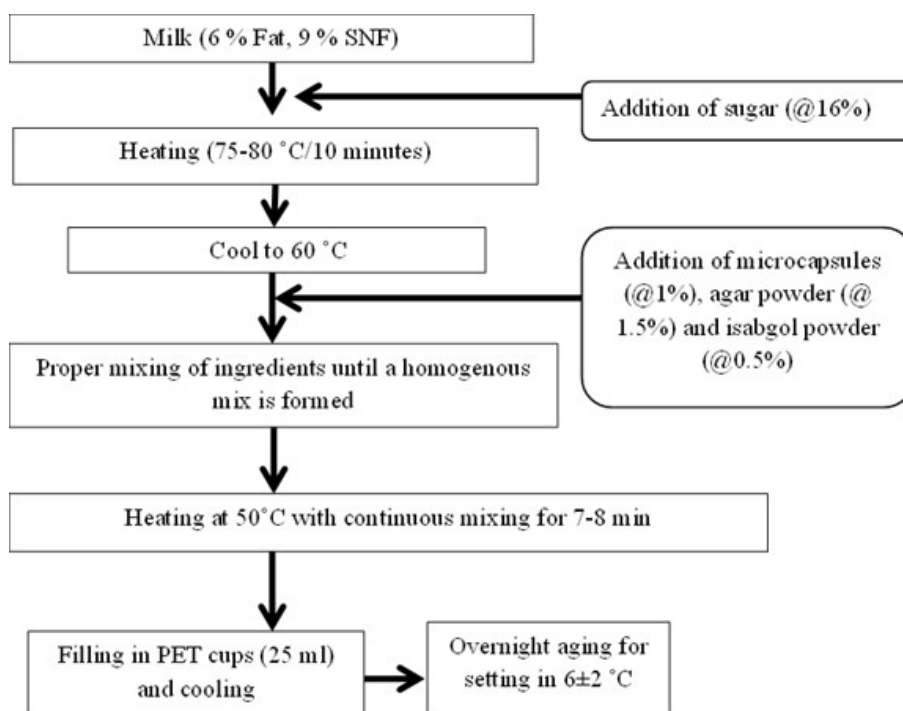


Figure 1: Method for preparation of synbiotic milk pudding

replications and the results are expressed as mean  $\pm$  standard deviation (SD).

## Results and Discussion

### Development of synbiotic milk pudding using standardized co-encapsulated beads

#### Optimization of rate of addition of Sugar

For sugar optimization four different rates of addition of sugar were taken *i.e.* 12 % (A), 14 % (B), 16 % (C) and 18 % (D). Milk pudding had been prepared by keeping addition rate of agar at 1.2% and isabgol at 0.5% constant. In Table 1 responses from different panelists for flavour, body & texture, sweetness, color & appearance and overall acceptability has been shown.

Comparing the flavour scores, sample C and D showed higher scores that were non-significant. Sample A & B showed lower scores that were significantly different from C and D sample. In terms of body and texture, C sample showed highest scores followed by D, B and A samples. All showed significantly different values. In terms of sweetness, similar trend was observed - C sample showed highest scores followed by D, B and A samples. All showed significant different values. For color and appearance, C and D sample showed higher values but were non-significant. Significantly lower values were observed for sample B and A respectively. For overall acceptability scores, C sample showed highest scores followed by D, B and A samples. All showed significantly different values.

Chandan and Kilara (2015) stated about consumption pattern of milk-based puddings and custards. Authors have also depicted their market value and different ingredients used in preparation of milk-based puddings. According to them sugar should be used within the range of 13-16% for getting proper body and texture and optimum sweetness. Singh et al. (2015) prepared misti dahi by using soy milk and milk combinations and evaluated its efficacy. Authors used different sugar concentrations (10, 12, 14 and 16%) and evaluated these samples for sensory attributes. Sample with 12 % sugar showed maximum values for sweetness, color and appearance and body and texture. Saikia and Mishra (2017) prepared misti dahi using three different sugar concentrations- 10, 15, 18%. While products were subjected to sensory evaluation, product with 18 % sugar got highest scores in terms of sweetness, flavour and color and appearance.

Comparing to experimental product sample C showed significantly higher values for body & texture, sweetness and over all acceptability score. Hence, sample C (@16%) was considered as optimum concentration for sugar.

#### Optimization of rate of addition of agar-agar

For agar-agar optimization four different rates of addition of agar-agar were taken *i.e.* 1% (A), 1.25 % (B), 1.5 % (C) and 1.75 % (D). Milk pudding has been prepared by keeping addition rate of sugar at 16 % (previously optimized) and isabgol at 0.5% constant. In Table 2 responses from different panellists for flavour, body & texture, sweetness, color & appearance and overall acceptability has been shown.

Comparing the flavour scores, sample A and B displayed statistically similar scores with sample C as well as D. But mean flavour score for sample C was significantly higher than other samples. In terms of body and texture, C sample showed significantly higher scores than other samples. Sample A, B and D were statistically non-significant. Sample A had a weak body, B had a semi soft body and D had a hard or rubbery body. In terms of sweetness, color and appearance all of the samples showed non-significant difference. For overall acceptability scores, C sample showed significantly higher scores than other samples. Sample A, B and D were statistically non-significant.

Kour (2022) described about mostly used gelling concentrations of some hydrocolloids that are being used in food preparation. According to the data, agar shows good gelling properties within 1-2 % concentration and doesn't impart any negative impact on the mouth feel of the product prepared. Riedel et al. (2015) developed some formulations for preparing agar-based fruit jellies. While comparing preference responses, products prepared with sugar and without sugar (having various agar concentrations), were having non-significant responses. This indicates that optimum level of agar-agar addition did not affect the mouth feel and sweetness of the product. Menaka and Wijesekara (2025) used hot water extraction to extract agar from *Gracilariopsis longissima* (formerly *G. verrucosa*) and the highest yield was displayed at 90 °C. Moreover, 1.5% agar gel prepared from native agar powder extracted at 90 °C acquired higher gelling temperature, melting temperature, viscosity, and pH value and lower syneresis than the other two gels prepared from agar extracted at 70 °C and 50 °C and recommended 12 g of agar powder added plant-based food jellies were accepted as the same as gelatine-added food jellies after conducting the sensory evaluation. Suebsaen et al. (2019) demonstrated effect of different hydrocolloids on rheological, textural and sensory properties a of banana dessert. Dessert made from agar showed lowest elastic behaviour. Through linear regression analysis, a significant linear model was found for sensory and mastication properties. Agar with different hardness scored more than carrageenan and gelatin.

In the experimental product similar observations were noticed. Sample C (@ 1.5%) showed significantly higher values for flavour, body & texture and overall acceptability score. Sample C was considered as optimum concentration for agar-agar. Different agar concentration did not affect the sweetness of the product.

**Optimization of rate of addition of Isabgol**

For isabgol optimization four different rates of addition of isabgol were taken *i.e.* 0.25 % (A), 0.5 % (B), 0.75 % (C) and 1% (D). Milk pudding has been prepared by keeping addition rate of sugar at 16 % and agar-agar at 1.5 % (previously optimized) constant. In

Table 3 responses from different panelists for flavour, body & texture, sweetness, color & appearance and overall acceptability has been shown.

Flavour score of all the four samples were non-significant and as per the comments by the panelists no difference was perceived.

**Table 1:** Comparison of sensory scores for sugar optimization

Sample	Sensory score (9 point hedonic scale)				
	Flavour	Body and texture	Sweetness	Color and appearance	Overall acceptability
A	7.12 <sup>c</sup> ±0.09	7.12 <sup>d</sup> ±0.10	6.66 <sup>d</sup> ±0.42	7.05 <sup>c</sup> ±0.05	7.03 <sup>d</sup> ±0.03
B	7.50 <sup>b</sup> ±0.31	7.64 <sup>c</sup> ±0.18	7.46 <sup>c</sup> ±0.09	7.56 <sup>b</sup> ±0.13	7.65 <sup>c</sup> ±0.15
C	8.07 <sup>a</sup> ±0.15	8.34 <sup>a</sup> ±0.17	8.26 <sup>a</sup> ±0.04	8.10 <sup>a</sup> ±0.07	8.37 <sup>a</sup> ±0.15
D	8.06 <sup>a</sup> ±0.04	8.01 <sup>b</sup> ±0.01	7.90 <sup>b</sup> ±0.15	8.08 <sup>a</sup> ±0.02	8.07 <sup>b</sup> ±0.14
SEm±	0.088	0.067	0.114	0.038	0.065
CD (0.05)	0.27	0.209	0.351	0.121	0.199
CV (%)	2.276	1.74	3.012	1.018	1.662

A= 12% Sugar, B= 14% Sugar, C= 16% Sugar, D= 18% Sugar

Each observation is mean ± standard deviation of four replicates

Values with different superscripts within a column differ significantly (p<0.05)

**Table 2:** Comparison of sensory scores for usage level of agar-agar

Sample	Sensory score (9 point hedonic scale)				
	Flavour	Body and texture	Sweetness	Color and appearance	Overall acceptability
A	7.5 <sup>ab</sup> ±0.57	6.86 <sup>b</sup> ±0.16	7.75±0.50	7.50±0.41	7.00 <sup>b</sup> ±0.82
B	7.38 <sup>ab</sup> ±0.48	7.25 <sup>b</sup> ±0.20	7.75±0.50	7.25±0.29	7.25 <sup>b</sup> ±0.28
C	8.37 <sup>a</sup> ±0.47	8.56 <sup>a</sup> ±0.43	8.00±0.82	7.50±0.41	8.25 <sup>a</sup> ±0.50
D	6.75 <sup>b</sup> ±0.96	7.13 <sup>b</sup> ±0.85	7.75±0.58	7.13±0.25	6.87 <sup>b</sup> ±0.63
SEm±	0.326	0.247	0.306	0.173	0.295
CD (0.05)	1.007	0.763	NS	NS	0.91
CV (%)	8.714	6.644	7.902	4.713	8.044

A= 1%, Agar-agar, B= 1.25%, Agar-agar, C= 1.5%, Agar-agar, D= 1.75% Agar-agar

Each observation is mean ± standard deviation of four replicates

Values with different superscripts within a column differ significantly (p<0.05)

**Table 3:** Comparison of sensory scores for level of Isabgol

Sample	Sensory score (9 point hedonic scale)				
	Flavour	Body and texture	Sweetness	Color and appearance	Overall acceptability
A	7.625±1.10	7.438 <sup>b</sup> ±0.31	7.75±0.5	7.675 <sup>b</sup> ±0.24	7.625 <sup>ab</sup> ±0.47
B	8.125±0.25	8.375 <sup>a</sup> ±0.48	8±0	8.875 <sup>a</sup> ±0.25	8.375 <sup>a</sup> ±0.48
C	7.25±0.5	6.750 <sup>bc</sup> ±0.64	7.5±0.57	7.75 <sup>b</sup> ±0.38	6.875 <sup>bc</sup> ±0.63
D	7.375±0.37	6.250 <sup>c</sup> ±0.5	7.5±0.57	6.875 <sup>c</sup> ±0.63	6.25 <sup>c</sup> ±0.5
SEm±	0.332	0.249	0.239	0.193	0.262
CD (0.05)	NS	0.768	NS	0.595	0.81
CV (%)	8.762	6.923	6.227	4.959	7.216

A= 0.25%, Isabgol, B= 0.5%, Isabgol, C= 0.75%, Isabgol, D= 1% Isabgol

Each observation is mean ± standard deviation of four replicates

Values with different superscripts within a column differ significantly (p<0.05)

In terms of body and texture, B sample showed significantly higher scores than other samples. Sample A and D were statistically non-significant. Sample C was statistically significant to both of A and D. As isabgol contributes to viscosity of the product, difference in isabgol concentration can show variation in products body and texture (Arora et al. 2016). In terms of sweetness all of the samples showed non-significant difference.

For color and appearance, sample B showed significantly higher scores whereas sample A and C were non-significant and sample D showed significantly lowest scores. As per comments received from the panelist, as increase in the quantity of isabgol in product preparation, increases the visible particles on the products and upper layers.

For overall acceptability scores, B sample showed significantly higher scores than other samples. Sample A displayed statistical similarity to sample B and C. Sample C showed statistical similarity with sample D. Sample B showed significantly higher values for body & texture, color and appearance and overall acceptability score.

Suryawanshi et al. (2020) prepared rasogolla by incorporating isabgol @ 0.5, 1 and 1.5%. Rasogolla prepared with 0.5% isabgol powder received higher sensory scores in terms of body and texture, overall acceptability, flavour, sweetness. Hemanth et al. (2021) prepared milkshake using acid modified psyllium husk @ 0.4, 0.6 and 0.8 %. When prepared products were taken for sensory evaluation, product prepared with 0.6% of psyllium, scored highest amongst the panellists.

Similarly, the experimental product (sample B) obtained better scores in terms of body and texture, color and appearance and overall acceptability scores than other formulations. Sample B (@ 0.5%) was considered as optimum concentration for isabgol.

### Optimization of rate of addition of microcapsules

As per the objectives of this study, a synbiotic milk pudding has to be developed. Optimizing addition rate of microcapsules should be such that in the final product cell count should remain  $10^8$  cfu/g and ascorbic acid concentration should fulfill minimal requirement of RDA of ascorbic acid.

For this purpose, four different addition rates of freeze-dried microcapsules have been studied for sensory scores as well as probiotic count and ascorbic acid concentration. Addition rates were 0.5 % (A), 1% (B), 1.5% (C) and 2% (D). In Table 4 responses from different panelists for flavour, body and texture, sweetness, color & appearance and overall acceptability has been shown.

Comparing flavour scores it was observed that Sample B had significantly higher values compared to other three samples. Similar pattern of score was observed for overall acceptability score, where sample B showed significantly different values than other samples. While comparing body and texture scores sample A and B showed significantly higher values than other two samples. Score for sample B was higher than sample A and D. Sample C showed statistical significance with sample A and D. As the addition rate of the microcapsules was increased an unpleasant, mouth feel was observed. For overall acceptability sample B had significantly higher scores than other samples. Non-significant difference was found for sweetness and color and appearance scores. Difference in microcapsule addition did not affect sweetness and color and appearance of the product.

Similarly, these four samples were analyzed for probiotic count and ascorbic acid content. In Table 5 data is shown for these attributes. Sample B showed a mean viability of  $9.457 \pm 0.08$  log cfu/g and a mean ascorbic acid concentration of  $38.21 \pm 0.80$  mg. For probiotic viability no significant difference was observed

**Table 4:** Comparison of sensory score for microcapsule optimization

Sample	Sensory score (9 point hedonic scale)				
	Flavour	Body and texture	Sweetness	Color and appearance	Overall acceptability
A	7.583 <sup>b</sup> ±0.31	7.583 <sup>b</sup> ±0.12	7.830±0.17	7.583±0.19	7.418 <sup>b</sup> ±0.13
B	8.583 <sup>a</sup> ±0.37	8.250 <sup>a</sup> ±0.20	7.750±0.22	7.750±0.21	8.330 <sup>a</sup> ±0.25
C	7.668 <sup>b</sup> ±0.24	7.330 <sup>bc</sup> ±0.24	7.750±0.27	7.583±0.33	7.500 <sup>b</sup> ±0.20
D	7.330 <sup>b</sup> ±0.25	7.083 <sup>c</sup> ±0.11	7.750±0.24	7.250±0.35	7.418 <sup>b</sup> ±0.31
SEm±	0.137	0.088	0.093	0.131	0.135
CD (0.05)	0.426	0.272	NS	NS	0.416
CV (%)	3.547	2.338	2.398	3.494	3.522
A= 0.5%, Microcapsule					
B= 1%, Microcapsule					
C= 1.5%, Microcapsule					
D= 2% Microcapsule					
Each observation is mean ± standard deviation of four replicates					
Values with different superscripts within a column differ significantly (p<0.05)					

**Table 5:** Effect of varying levels of microcapsules on probiotic count and ascorbic acid concentration of synbiotic milk pudding

Sample	Probiotic count (log cfu/g)	Ascorbic acid concentration (mg/100 g)
A	9.30±0.05	18.66 <sup>d</sup> ±0.51
B	9.46±0.08	38.21 <sup>c</sup> ±0.80
C	9.50±0.28	56.18 <sup>b</sup> ±2.92
D	9.65±0.17	75.79 <sup>a</sup> ±1.56
SEm±	0.086	0.863
CD (0.05)	NS	2.661
CV (%)	1.826	3.658

A= 0.5%, B= 1%, C= 1.5%, D= 2% of microcapsules

Each observation is mean ± standard deviation of four replicates

Values with different superscripts within a column differ significantly (p<0.05).

but a significant increase in ascorbic acid concentration was observed. Highest value was observed for sample D whereas lowest value was observed for sample A. We could not come across any research literature where similar results regarding different addition rates of microcapsules were reported.

Pinto et al. (2019) used spray-dried microcapsules carrying the probiotic *Bifidobacterium lactis* BB-12 to make a lactose-free Greek-style yogurt. Maltodextrin, inulin, and gum arabic were used as wall components to create three distinct microcapsule formulations. While preparation of yoghurt they stated that microcapsule added to assured that the cell concentration must be more than 10<sup>8</sup> cfu/g of final product. Researchers reported that microcapsules were added @ 2.5% in product preparation. Da Silva et al. (2022) investigated the effects of co-encapsulating guaraná seed extract (GSE) and *Lactocaseibacillus paracasei* BGP-1 on the stability of phenolic components and bacterial viability during microcapsule preservation in a yoghurt beverage. Co-encapsulation improved the survivability of *L. paracasei* BGP-1 (greater than log 7 cfu/ml at 7 °C for up to 28 days) during storage and boosted the retention of phenolic compounds by about 88%. The overall acceptability of yoghurt containing microcapsules was higher than that of yoghurt containing free GSE because microencapsulation avoided post-fermentation alterations and covered up the bitter taste of GSE.

As per these references if addition rate of microcapsules remained > 2% (w/v), those were not considered as of superior sensory quality. For this reason, capsule addition rates were selected between 0.5-2 %. Based on sensory scores, probiotic viability and ascorbic acid concentration sample B (@1%) was selected for incorporating in final product.

Flavour of goat milk limit the acceptability by the consumers. To overcome this, Bhanu and Heremath (2020) prepared vitamin-C microcapsules using 3% sodium alginate and 0.2M CaCl<sub>2</sub> through extrusion process. Encapsulated vitamin-C enriched flavoured goat milk prepared by using 8% sugar, 0.75% Pista flavour was reported the best on 9-point hedonic scale. Similarly Thangaraj and Seethalakshmi in 2015 used two percentage of alginate as wall material and 0.1m cacl2 as hardening bath were optimized for

encapsulation (bead formation) through extrusion process. Encapsulated ascorbic acid beads had significantly higher the content vitamin C with 354±0.5mg to 318±0.8mg per 100g of beads at both ambient and refrigeration stage of storage. They reported throughout the storage period unencapsulated formulated ice cream samples had a decreasing manner of vitamin c in comparison with encapsulated ice cream.

According to FSSR (2016) the viable number of added probiotic organisms in food shall be e”10<sup>8</sup> cfu in the serving size on daily basis and for AA, the recommended daily intake is 90 mg (male) and 75 mg (female) on daily basis (<https://ods.od.nih.gov/factsheets/VitaminC-HealthProfessional/>).

Based on this optimized parameters. the synbiotic milk pudding contained 16% sugar, 1.5% agar-agar, 0.5% isabgol and 1% microcapsules (w/v basis) was prepared. According to Table 5, the finalized product had a probiotic viability of 9.457±0.08 log cfu/g that is within the range of effective probiotic dosage. The product also has 38.21±0.80 mg/100 g of ascorbic acid concentration that means as per the serving size of 25 g it confers 9.55 mg of ascorbic acid that can fulfil about 11.6 % of RDA (for an average adult human being) of vitamin C.

## Conclusions

In optimization study, it was revealed that sugar, agar-agar, isabgol addition @ 16%, 1.5%, 0.5% gained better sensory scores (flavour, body and texture, sweetness, color and appearance and overall acceptability scores in 9-point hedonic scale). Addition of microcapsules @ 1% showed optimum sensory scores than other samples. But different addition rates (0.5, 1, 1.5, 2 %) of microcapsules showed non-significant difference for probiotic viability and were significantly different for AA concentration. Based on sensory scores and ascorbic acid concentration 1% rate of addition for microcapsules were selected. Together with the food and beverage industry, the demand for synbiotic products from the dietary supplement and animal feed industries is also propelling the global market’s expansion. Considering this, trend for synbiotic milk pudding prepared with a probiotic culture and ascorbic acid protected by a microencapsulated form

could be more significance considering health aspects of community.

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