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Evaluation of Fig Powder as Prebiotic and its Utilization for Development of Synbiotic Microcapsules

Urvi Thakkar and R. Preetha*

Department of Food Process Engineering, SRM University,
Kattankulathur Campus, Tamilnadu - 603203, India.

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Lactobacillus spp. **is known to be tolerant to bile and low pH and has probiotic effects due to multiple mechanisms. Prebiotics act as a substrate for the growth and/or activation of beneficial colonic bacteria. *Ficus carica*, commonly known as "Fig" belongs to Moraceae family. The aim of this study was to evaluate the use of fig powder as a potential prebiotic for *Lactobacillus spp.* and to develop synbiotic microcapsules. Fig powder was evaluated for its resistant starch content which was quantified to 18.04% with 2.06% of soluble fibers. Haurrsen's ratio for the prepared fig powder was found to be 1.1763 while Carr's Index was 14.99, indicating 'good' flow properties. Solubility of 60% and swelling capacity of 400-500% was observed. Also, the viability of probiotic culture in MRS medium and MRS medium incorporated of fig powder was found to be 3.3 ± 0.05 x 10^9 cfu/ml and 5.8 ± 0.05 x 10^9 cfu/ml respectively. Increased viability is attributed to presence of resistant starch. Probiotics were then encapsulated with and without prebiotic. Survivability of probiotics entrapped in alginate and synbiotic microcapsules was determined at room temperature. Probiotics entrapped in synbiotic microcapsules showed better viability, thus having better storage period.**

Keywords: Prebiotic, probiotic, *lactobacillus spp.*, resistant starch.

Prebiotics are known as "Non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health"[1]. A particular food product can be claimed as a prebiotic if; its hydrolyses or absorption does not take place in the upper part of the digestive system; it acts as a substrate for the growth and/or activation of beneficial colonic bacteria; it improves the composition of colon microflora; and it induces beneficial luminal effects to the health of the host[2]. Various categories of prebiotics include some non-digestible carbohydrates or dietary fibers (oligosaccharides and polysaccharides), some peptides and proteins, and some lipids[3]. According to the European Food Safety Authority dietary fibers are classified into four groups: Non-starch polysaccharides (cellulose, hemicelluloses, pectin and hydrocolloids), resistant oligosaccharides like galacto-oligosaccharides (GOSs) and fructo-oligosaccharides (FOSs), lignin which usually associates with dietary fibers and resistant starch such as retrograded amylose, raw starch granules, and chemically modified starches[4]. Resistant starch may not be digested because of its dense molecular configuration protected by botanical cell wall inhibits the accessibility and action of digestive enzymes. Gelatinization is a process in which starch granules are disrupted when heated under high humidity. These gels after cooling form retrograded starch crystals which are resistant to enzymatic digestion and thus are categorised under resistant starches[5].

The most significant species belonging to Ficus genera found in India are *F. bengalensis*, *F. carica*, *F. racemosa* and *F. elastica*. *Ficus carica*,

* To whom all correspondence should be addressed.
Tel.: 9176610351;
E-mail: preetha.r@ktr.srmuniv.ac.in

commonly known as "Fig" belongs to Moraceae family. The fruits and leaves parts have significant level of antioxidant and antimicrobial activity[6,7]. The antioxidant properties are attributed to the presence of total phenolics, flavonoids, alkaloids and saponins and other secondary metabolites. Consuming dried fig daily enhanced the antioxidant capacity of plasma[8]. Fig also has laxative properties. Dried fig fruit is a good source of carbohydrates and minerals while it has average protein and dietary fiber content with very low amount of fat and is hence considered as the richest nutritional source.

Encapsulation techniques can be applied to introduce viable probiotic bacteria as the wall material and can provide a physical barrier to the encapsulated material against adverse environmental conditions[9,10]. Commonly used encapsulation methods include emulsion, extrusion, spray drying, freeze drying, spray-freeze drying[11].

The natural resistant starch from green banana flour and retrograded rice flour could protect the cell and promote survival of *L. bulgaricus* (TISTR 895)[12]. In this study *Lactobacillus* strain ordered from NCIM, Pune is used as probiotic strain while the potential use of *Ficus carica* powder as a source of prebiotic, due to its resistant starch content and other therapeutic benefits, is determined. Synbiotic microcapsules were developed using fig powder as prebiotic and storage studies were conducted.

MATERIALS AND METHODS

Fresh ripened fig fruits were purchased from the local market, of Chennai, India. These were used in preparation of custard. DPPH was used for antioxidant assay. Freeze dried *Lactobacillus* strain was procured from NCIM-CSIR; National Chemical Laboratory, Pune. MRS broth and MRS agar were used for growing the probiotic. Bile salt, NaCl and pepsin were used for conducting tolerance tests on probiotics.

Preparation of Prebiotic Powder

Pulp from fresh figs was prepared (without peeling) using grinder. The pulp was lyophilised (Temperature: -40°C, Pressure: -3mbar) for 30 hr[13]. The freeze dried pulp was then ground and the powder so obtained was stored at room temperature (30°C) in sealed aluminium pouches.

Physical Parameters

Moisture Content and Water Activity

The moisture content was determined using the air oven method (AOAC 2000). The samples were weighed initially dried in the oven at 105°C. Readings were taken every half hour to determine loss in weight till constant weight is obtained. Final weight is recorded. The moisture content was calculated from the weight difference between the original and dried sample and expressed in percentage. Water activity of the prebiotic powder was calculated using Novasina Lab Swift Water Activity Meter

Moisture Content (wet basis) % = [(Initial Weight-Final Weight)/Initial Weight] x 100

Color Measurement

Colour value of the prebiotic powder was measured using Colour Quest XE Hunter Colour Meter. The L, a, b chroma system uses the corresponding value of total colour difference (DE) as dynamic parameters, was used to analyze the dynamic change in the indicator's colour[14]. The total colour difference (TCD) is expressed as follows:

$$DE = [(DL)^2 + (Da)^2 + (Db)^2]^{1/2}$$

Where,

DL = brightness difference between initiation and each time interval (value should be 0-100)

Da = redness-greenness difference between initiation and each time interval

Db = yellowness–blueness difference between initiation and each time interval

Bulk Density

The bulk density was measured[15]. 30g weight of powder was poured in to a 100ml measuring cylinder and the volume was recorded. The following formula was used to calculate bulk density:

Bulk Density (LBD) = Mass (g)/Volume (ml)

Tapped Density

The tapped bulk density was measured[15]. 30g weight of powder was poured in to a 100ml measuring cylinder and tapped on a hard surface 30 times from about 2cm height and the volume was recorded. Following formula was used to calculate tapped bulk density:

Tapped Density (TBD) = Mass (g)/Volume (ml)

Carr's Index:

Carr's Index (%) and Hausner's ratio were

calculated[15]. The values were determined using the following relationship:

C.I. = (TBD – LBD/TBD) x 100

H.R = TBD/LBD

Solubility and Swelling capacity

Solubility and swelling capacity was measured[16]. 1 g of the powder was transferred into a clean dried test tube and weighed (W_1). The powder was then dispersed in 50 ml of distilled water using stirrer. The slurry so obtained was heated for 30 min at various temperatures from 60°C. The mixture after cooling to room temperature was centrifuged for 15 min at 3000 rpm. 5 ml of the supernatant were dried to a constant weight at 110°C.

Solubility was calculated as g per 100 g of powder on dry weight basis. The residue obtained from the above experiment after centrifugation, with the water it retained was quantitatively transferred to the clean dried test tube used earlier and weighed (W_2). The Swelling capacity was calculated by the following formula:

Percentage swelling of starch= $[(W_2 - W_1)/ W_1] \times 100$

Antioxidant Activity

The free radical scavenging activity of the samples was measured in vitro by DPPH assay[17]. 1g of sample is transferred to a clean test tube and 10ml is ethanol is poured into it. The solution is then kept in water bath at 40°C for 3h to get the ethanolic extract. Same procedure is repeated to prepare methanolic extract using methanol as solvent. Methanolic and ethanolic DPPH solutions (0.04g/lit) were prepared. Working solution of different concentrations was prepared and the solution was left for 30mins at room temperature. Absorbance was measured at 517nm. Methanol or ethanol without DPPH was used as blank. Results were expressed as percentage of inhibition of the DPPH radical which was calculated according to the following equation:

% Inhibition= [(Absorbance Control-Absorbance Sample)/Absorbance Control] x 100

Resistant Starch Estimation

Resistant starch analysis was done at Chennai Testing Laboratories Pvt. Ltd. Method used was AOAC 19th edition, 2012, 993.19 for soluble fibers and AOAC 19th edition, 2012, 991.42

Microbial Analysis

Revival of Probiotics

Freeze-dried *Lactobacillus* strains were procured from NCIM-CSIR-National Chemical Laboratory, Pune. The cultures were stored at 4°C until required. DeMan-Rogosa- Sharpe broth (MRS broth) of pH- 4-5.2 was used to prepare the cell suspensions for probiotic strains[18]. The MRS agar was inoculated with active strains and incubated at 37 ºC for 24 hr under anaerobic condition[19].

Effect of Prebiotic on the Viability of Probiotic Strain

The effect of different prebiotics on viability of different probiotic strains was tested[19]. The sterilized prebiotic was added to MRS broth medium by 2% and inoculated by active probiotic strains and incubated at 37 °C for 24h under anaerobic condition. Also MRS broth inoculated with culture was used as control. The viable count was determined using spread plate method after serial dilutions of the respective broths in physiological solutions and the plates were incubated at 37°C for 48h under anaerobic condition.

Encapsulation

Extrusion method was adopted and slightly modified[20]. 2.5% sodium alginate solution and 4% calcium chloride solution was prepared. Also sodium alginate solution was mixed with prebiotic powder in the ratio 1:1. All the above solutions were sterilsed and allowed to cool. Probiotic culture was mixed and homogenized in sodium alginate solution and sodium alginate-prebiotic mixture separately. The solution was loaded in syringe without air bubble and dropped in calcium chloride solution drop wise to form beads. Beads so formed were stored at room temperature.

Encapsulation Efficiency

0.5gm of the beads was disintegrated in 4.5 ml 0.1M sodium citrate solution homogenized for 5 minutes and was used to make serial dilutions in physiological solution[19]. The viable count was determined using spread plate method the plates were incubated at 37°C for 48h under anaerobic condition. All plating was done on triplicates and encapsulation efficiency (EE), which is a combined measurement of efficacy of entrapment and survival of viable cells during microencapsulation procedure, was calculated as follows:

$EE = N/N_o \times 100$

Where, N= viable number of entrapped

cells released from microcapsules and N_o = free cells added to the biopolymer mix during production of microspheres.

Survivability Tests

The samples were diluted in solutions simulating gastric/gut and homogenized for 5 min to test tolerance of probiotic strains[21]. To obtain the viable count, 0.1 ml of the dilution of microbes were plated in MRS agar[19]. Plates were incubated at 37°C for 24 hr under anaerobic conditions. Samples were checked at at intervals of every two days of storage at room temperature for 1month. Experiments were performed in triplicates.

Statistical Analysis

Standard deviation was applied for determination of significant difference between means of moisture content, colour readings, cell counts and antioxidant activities[22].

RESULTS AND DISCUSSION

Physical Parameters

Moisture Content and Water Activity

Water content or moisture content is the quantity of water contained in a material fruit. During the drying process, moisture loss occurs due to the difference in water vapour pressure between the product and the air surrounding it. The moisture content (wet basis) of the prebiotic powder was found to be 2.05 ± 0.57 % while the water activity for the prebiotic powder was found in the range of 0.14 to 0.15 (Table 1).

Flow Properties (Density Analysis)

Bulk density is a measure of heaviness of powder and an important parameter that determines the suitability of powder for the ease of packaging and transportation of particulate foods as well as for infant formulations. Flow properties are decided based on Carr's Index (CI) and Haurrsen's ratio (HR), which is calculated based on Loose Bulk Density (LBD) as well as Tapped Bulk Density (TBD).

A Carr's CI of <10 or HR of <1.11 is considered 'excellent' flow whereas CI > 38 or HR > 1.60 is considered 'very very poor' flow[23]. There are intermediate scales for CI between 11–15 or HR between 1.12–1.18 is considered 'good' flow, CI between 16–20 or HR between 1.19–1.25 is considered 'fair' flow, CI between 21–25 or HR between 1.26–1.34 is considered passable flow, CI between 26–31 or HR between 1.35–1.45 is considered 'poor' flow, and CI between 32–37 or HR between 1.46–1.59 is considered 'very poor' flow. Based on this discussion and results as shown in Table 1; prebiotic powder had 'good' flow properties. Table 1 show that the solubility of prebiotic powder is significantly high while swelling capacity is average in the range of 400-500%.

Antioxidant Activity

Scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. The measured DPPH radical scavenging activity and the quality of the antioxidants in the extracts was determined by the percent inhibition values shown is shown in Figure 2.

The *F. carica* extract scavenging antioxidant activity was significantly increased with increasing concentration of the extract (both ethanolic and methanolic). The percent inhibition values of methanolic extract of *F. carica* were greater than the ethanolic extract of the same. Lack of hydrogen donor bioactive constituents in the extract, slow rate of the reaction between DPPH and the substrate molecules resulting in low readings for antioxidant activity probably might explain the low DPPH antioxidant activity of the *F. carica* extract

Resistant Starch Estimation

Testing was done at Chennai Testing Laboratories Pvt. Ltd. *F. carica* can be considered as a good prebiotic source since it has high

Table 1. Physico-chemical parameters for prebiotic powder

No.	Parameters	Fig Powder
1	Weight of fruit (g)	454
2	Weight of pulp (g)	329
3	Weight of powder (g)	110
4	Yield (%)	24.22
5	LBD (g/cc)	0.2535
6	TBD (g/cc)	0.2982
7	HR	1.1763
8	CI	14.99
9	Moisture Content (wb %)	2.05 ± 0.57
10	aW	0.1430 at 27.4°C
11	Solubility	60%
12	WHC/SC	446%
13	dE	41.12 ± 0.005

Photo 1. Prebiotic Powder

Photo 2. Streak plating for

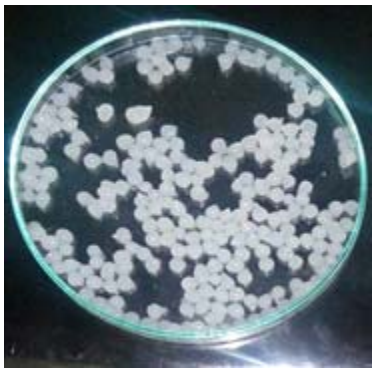

Photo 3. Encapsulated probiotics

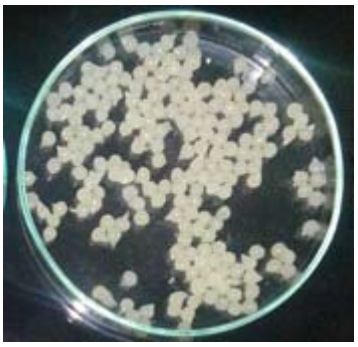

Photo 4. Synbiotically encapsulated probiotics

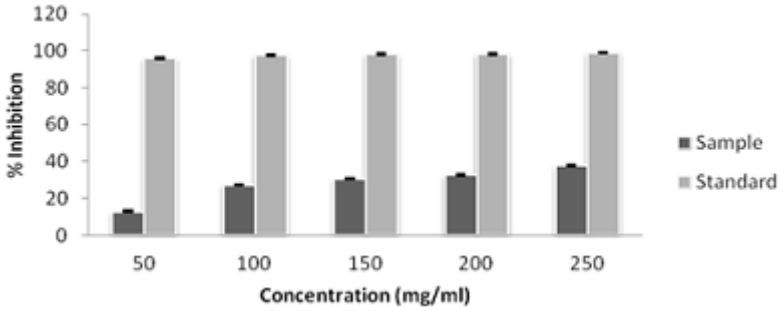

Fig. 1. Percent inhibition of DPPH activity by methanolic extract of prebiotic powder

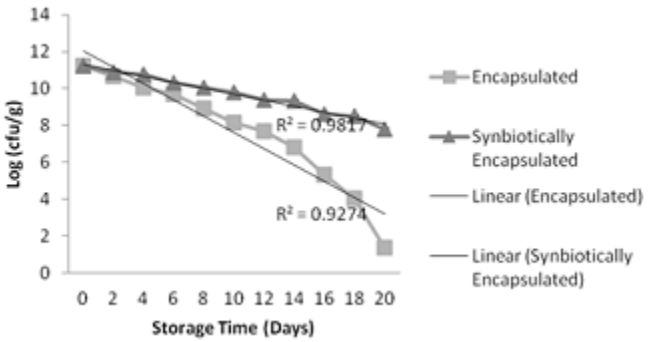

Fig. 2. Survival of probiotics in alginate microcapsules and symbiotic microcapsules in gastric environment at different time intervals

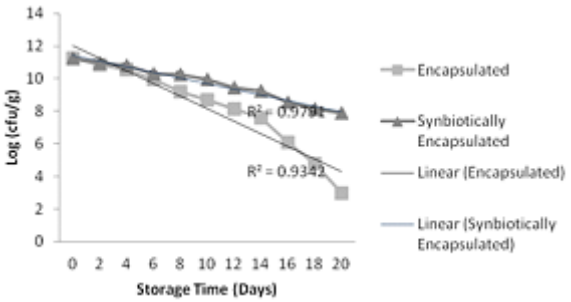

Fig. 3. Survival of probiotics in alginate microcapsules and symbiotic microcapsules in gastric environment at different time intervals

resistant starch content (18.04%) and less of soluble fibers (2.06%).

Microbial Analysis

Revival and Survivability Tests for free cells

Figure 3 shows successful revival of probiotic strains. These strains were tested for bile and NaCl tolerance. Probiotics should show good tolerance to low pH in gastric environment and should exhibit tolerance to bile released in intestinal environment. The lowest pH recorded has been pH 1.5[24,25]. A good probiotic should withstand at least pH 3.0[26].

Microbial counts in the range of 30-300 cfu/ml are considered to be viable in a given specific environment. Free probiotic strains showed a count of 11 x 10^7 cfu/ml in the NaCl environment whereas a count of as 20 x 10^7 in the bile environment. As their colony count is not in the required range, it suggests most probiotics were killed by this harsh pH. Also, upon exposure to bile acids, cellular homeostasis disruptions causes the dissociation of lipid bilayer and integral protein of their cell membranes, resulting in leakage of bacterial content and ultimately cell death; thus necessitating encapsulation.

Effect of Prebiotic on Probiotic

Prebiotic was found to be more effective for viability of the probiotic strains since the viable counts increased from 3.3 ± 0.05 x 10^9 cfu/ml to5.8 ± 0.05 x 10^9 cfu/ml. This may be attributed to resistant starch content in the sample.

Encapsulation of Probiotics and Encapsulation Efficiency

Encapsulated probiotic beads and synbiotically encapsulated beads were formed as shown in figure 4 and 5 respectively. Encapsulation efficiency for them was found to be 99.5 ± 0.05 %.

Survivability of Microencapsulated Probiotics

Survivability for probiotics was assessed in gastric as well as intestinal environment. Survival of probiotics entrapped in synbiotic microcapsules was significantly improved over those entrapped in alginate microcapsules as shown in Table 2 and 3. Microencapsulation with alginate is able to protect probiotics in food products[28]. Results of the viable counts also showed that viability decreased with increasing storage period for both alginate microcapsules and synbiotic microcapsules (Figure 6, 7).

CONCLUSION

F. carica (fig) powder was found to have around 18.04% of resistant starch and 2.06% of soluble fibers. On the basis of presence of resistant starch in higher amount in *F. carica*, it was selected as the prebiotic source for the formulation of synbiotic microcapsule. Using the prebiotic source (fig) and *Lactobacillus* strain as the probiotic, synbiotic formulation was prepared. Sodium alginate was used as carrier material for encapsulation.

The Haurrsen's ratio of 1.1763 and Carr's Index of 14.99 indicated 'good' flow properties. An increase in viability of probiotic strain was observed in the presence of fig powder in the growth medium. Thus based on the results it can be concluded that *F. carica* can be successfully used as prebiotic for *Lactobacillus* strain. Also, blending of prebiotics in the coating materials resulted in better protection for the encapsulated organisms and increased cell viability during storage, relative to the prebiotic free variants and microcapsules without prebiotic.

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