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Development of Flaxseed Fortified Synbiotic Flavoured Dahi (Yoghurt) Using Response Surface Methodology

Manju Tiwari[1], Dinesh Chandra Rai[1], Dev Bukhsh Singh[2], Dipti Rai[3, *]

[1]Department of Dairy Science and Food Technology, Banaras Hindu University, Varanasi, India

[2]Department of Biotechnology, Siddharth University, Siddharth Nagar, India

[3]Department of Food Technology, Chhatrapati Shahu Ji Maharaj University, Kanpur, India

Email address:

drmanju.bhu@gmail.com (M. Tiwari), dcrai.bhu@gmail.com (D. C. Rai), answer.dev@gmail.com (D. B. Singh), deeptirai10@gmail.com (D. Rai)

[*]Corresponding author

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Abstract: Response surface methodology was employed to find out optimum proportions of flaxseed powder, mango pulp, synbiotic microcapsules to develop flaxseed fortified synbiotic mango *Dahi*. The quadratic model was fitted to antioxidant activity, firmness (g), cohesiveness (gs), whey separation (%), and probiotic viable count (CFU/g) of runs as the responses. Analysis of variance revealed that the models were well adjusted to predict the experimental data. Determination coefficients (R^2) were higher than 90% which showed that the developed models were well fitted to the experimental data. The optimized product constitutes flaxseed powder (2.65%), mango pulp (5.28%), and synbiotic microcapsules (4.16%). The desirability of the model was found to be 0.80. The current study would be helpful to food industries for the development of disease-specific health-beneficial dairy foods by incorporating flaxseed and synbiotic capsules at an optimized level. Future research may stress on limitations of research for scaling-up processes at the industry level. The incorporation of flaxseed, mango pulp, and microencapsulated probiotic bacteria has not been incorporated together previously to develop a health-beneficial functional dairy product. The developed product may be a good option as a refreshing dairy product with enhanced health-promoting functional properties as well as improved product characteristics.

Keywords: *Dahi* (Yoghurt), Flaxseed, Synbiotic, Antioxidant Activity, Optimization, Mango Pulp, Response Surface Methodology

1. Introduction

Scientific knowledge of the beneficial role of various food ingredients for the prevention and treatment of specific diseases is rapidly accumulating resulting in the development of disease-specific foods like diabetic foods. Dietary modification contributes significantly to reducing cardiovascular disease (CVD) risk factors including lowering cholesterol and atherosclerosis. In many recent studies, dairy fermented foods have emerged as a possible vehicle for developing health foods with therapeutic effects for combating CVD diseases.

Probiotics have been shown to have beneficial health effects as anti-diabetic, anti-cholesterol, anti-pathogenic, and anticarcinogenic properties. However, studies indicate that a food product must contain a minimum of 10^7 colony-forming units per gram of food (CFU/g) to assure sufficient bioavailable bacteria to exert a functional effect within the body. Dairy products like yoghurt and similar other products with low pH, pose reduced survivability of probiotic bacteria [23]. To overcome this problem many microencapsulation techniques have been developed in past few years for substantial protection for probiotics under low pH and aerobic conditions and could therefore be responsible for higher survival rates of encapsulated cells during storage in yogurt.

Dahi also known as curd is a widely consumed dairy

product in India prepared by fermentation of milk by lactic acid bacteria (LAB). Dahi can be used as a potential vehicle for the delivery of nutraceuticals by fortifying it with many probiotic organisms [27] cereals, fruits, and honey [7] to combat chronic and non-communicable diseases like hypertension.

Flaxseed, the seed from the plant (*Linumusitatissimum* variety Linott) is widely accepted as a nutraceutical due to its health-beneficial components such as lignans, α-linolenic acid, and soluble dietary fibre or mucilage/gum [10]. The flaxseed contains both soluble and insoluble fibers. About one-third of the fiber in flaxseed is soluble and it may help to lower cholesterol and to regulate levels of blood sugar. The remaining two-thirds of the fiber in the flaxseed is insoluble which aids digestion by increasing bulk and preventing constipation [12].

Secoisolariciresinol diglycoside (SDG) being the major lignan of flaxseed. In the human body, the (SDG) lignans are acted upon by the gastrointestinal microflora to release secoisolariciresinol (SECO), a non-sugar moiety of SDG. SECO plays an important role in the reduction of hypercholesterolemia, atherosclerosis, hypertension, and diabetes [21]. Velez-Ruiz *et al*. [26] analyzed the influence of added calcium and flaxseed fiber on the physicochemical and flow properties of low-fat yogurt. Unfortunately, the use of flaxseed as a nutraceutical in dairy products is limited due to its bland flavor and grainy texture which has been overcome in the present study by the incorporation of the strong sweet flavor of mango pulp with high antioxidant properties.

In past researches, flaxseed, mango pulp, and synbiotic microcapsules were previously added to dairy products but singularly and not in combination to produce a disease-specific health effect. *Dahi*, an indigenous dairy product of India with inherent anti-hypertensive bioactive peptides has not been much studied to exploit its health beneficial properties as carrier dairy food for nutraceuticals. Synbiotic capsules and flaxseed have not been optimized previously in *Dahi* to develop disease-specific therapeutic food. Therefore, the purpose of this work was to prepare *Dahi* fortified with optimum proportions of flaxseed powder, mango pulp, and synbiotic microencapsulated bacteria and to analyze the influence of the added ingredients on the textural profile, whey separation, antioxidant properties, and total probiotic viable count in the developed product.

2. Materials and Methods

2.1. Preparation of Mango Pulp and Flaxseed Powder

Fresh mango fruits of *the Totapuri* variety were obtained from the local market of Varanasi, India. The mango pulp was prepared as per the method given by Ghosh *et al.* [8]. Fruits were cleaned washed in running water, peeled, cut into small pieces, and deseeded. These cut pieces were pulped in a pulper (HL/1631/00, Philips, New Delhi, India). Pulp with total soluble solids of 17° Brix, acidity 0.5%, and pH 4.3 was obtained. The pulp was then pasteurized at 80-83°C for 30 s, rapidly cooled to 35°C, and stored in glass bottles with a stopper at -23°C until use.

Flaxseeds were purchased from the local market of Varanasi, India. After cleaning of flaxseed manually, it was roasted at 65°C and then milled using a grinder (HL 1632, Philips, New Delhi, India) sieved through 0.25 mm mesh, and stored in air-tight plastic containers at 4°C until used [18].

2.2. Preparation and Maintenance of Starter Culture for Dahi

The mixed Dahi starter culture NCDC-167 and *L. plantarum* NCDC 221 were procured from the National Collection of Dairy Cultures, NDRI, Karnal, Haryana. The culture was mixed thoroughly with reconstituted skim milk (13g of skim milk powder added to 100g of distilled water) and incubated at 37°C for 12–14 h thereafter stored in the refrigerator at 4°C for further use and treated as the seed culture. Mother culture (50mL) was prepared using sterilized reconstituted skim milk inoculated with 1ml of seed culture in the same way and the procedure was repeated four times. Further, bulk culture (200 mL) was prepared using 2ml of mother culture as inoculums following the same method and stored (4°C) for further use.

2.3. Preparation of Probiotic Bacteria for Microencapsulation

Cultures of *Lactobacillus acidophilus* NCDC 195 and *Bifidobacterium bifidum* NCDC 235 were purchased in lyophilized form from the National Collection of Dairy Cultures, NDRI, Karnal, Haryana. *L. acidophilus* 195 and *B. bifidum* 235 strains were reconstituted in 10 mL MRS broth and incubated for 24 h at 37°C. Cells were then cultured in the same conditions for three successive transfers in MRS broth at 37°C for 20-24 h to get pure colonies. Then, they were grown in a fermenter (BioFlo® /CelliGen® 115, New Brunswick, Germany) using MRS broth for 48 h. The cells were harvested by centrifugation (3-30 k, Sigma laborzentrifugen, Osterode, Germany) at 3000 x g for 10 min at 4°C and then washed twice with sterile 0.1% peptone solution and re-suspended with peptone water to obtain the final concentration of inoculums as 9.25 to 9.98 log cfu/ml to be used further in microencapsulation process.

2.4. Preparation of Synbiotic Microcapsule

Alginate beads or microcapsules were produced using a modified extrusion technique reported by Liserre *et al*. [15]. Solutions of sodium alginate (1.8% w/v) containing approximately 10^9cfu/mL of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, with 1% of prebiotic (FOS), were atomized in 0.1 M calcium chloride. The atomization was achieved by forcing the sodium alginate solution to the stainless steel tube with the aid of a peristaltic pump at a flow rate of 2.5 mL/min and compressed air at a flow rate of 2.5 m^3/min. The solution of calcium chloride

remained under constant magnetic stirring until the end of the encapsulation. Alginate beads remained at rest for 30 minutes. The beads were then separated from the calcium chloride solution with stainless steel sieves (mesh of 250, 355, and 500 mm), washed with distilled water, and were coated with chitosan by the method reported by Krasaekoopt *et al.* [13].

2.5. Preparation of Flaxseed Fortified Synbiotic Mango Dahi (FFSMD)

Fresh, milk was procured from the market of Varanasi, India, and was standardized to contain 1.5% fat and 9% SNF. Different levels of flaxseed powder (1-3%), mango pulp (3-7%), and synbiotic microcapsules (2-6%) were the variables used for the preparation of FFSMD, according to the experimental design outlined in Table 1.

Table 1. *Experimental runs (Central Composite Design) for the optimization of FFSMD.*

Run	Flaxseed Powder (g/100g)	Mango Pulp (%)	Synbiotic Micro-Capsules (%)	Antioxidant activity (%)
1	0.318	5	4	78.26
2	1	7	2	80.25
3	1	3	6	74.49
4	1	3	2	74.02
5	1	7	6	82.51
6	2	5	4	83.59
7	2	1.63	4	73.23
8	2	5	4	84.85
9	2	5	4	83.29
10	2	5	4	83.36
11	2	5	4	83.68
12	2	5	0.63	81.25
13	2	5	4	84.12
14	2	8.36	4	84.94
15	2	5	7.36	84.54
16	3	3	2	76.25
17	3	3	6	78.21
18	3	7	2	82.32
19	3	7	6	85.75
20	3.68	5	4	84.26

Table 1. *Continued.*

Run	Firmness (g)	Cohesiveness (gs)	Probiotic viable count (cfu/g)	Whey Separation (%)
1	238.61	29.56	7.46	6.45
2	234.38	25.59	7.15	8.09
3	235.19	30.45	9.25	6.29
4	230.87	26.74	7.23	4.02
5	237.56	33.29	7.98	7.30
6	240.26	34.56	9.45	5.15
7	235.20	30.09	9.25	4.25
8	243.45	34.32	8.51	5.36
9	243.60	35.60	9.02	5.25
10	243.64	34.98	8.49	5.54
11	242.92	34.57	9.12	5.47
12	238.29	28.59	5.06	6.02
13	245.78	35.71	8.76	5.36
14	241.35	32.35	6.92	7.46
15	246.89	38.36	9.85	5.21
16	232.36	30.87	7.05	4.06
17	240.53	35.25	9.64	5.03
18	240.45	30.12	7.32	7.42
19	250.34	38.24	9.46	5.45
20	243.57	36.59	9.35	4.45

A central composite rotatable design (CCD) was used to design the experiments comprising of three independent variables. Twenty experiments were performed taking into account three factors viz., flaxseed powder, mango pulp, and synbiotic microcapsules to study their effects at different levels on the following responses viz. antioxidant activity, firmness, cohesiveness, whey separation, and probiotic viable count.

Flaxseed fortified synbiotic mango *Dahi* (FFSMD) was prepared using standardized milk, which was heat-treated at 95°C for 5 min, at this point 3% sugar was added, mixed well, and then placed into clean, autoclaved-stoppered wide-mouth glass jars (100 g) and cooled to 37°C without exposing it to the atmosphere. In the above mixture mango pulp, synbiotic microcapsules, and flaxseed powder were then added and with the high-speed stirrer mixed thoroughly. In the above

blend 2% (v/v, Dairy Starter (*Dahi*) and *L. plantarum* 1:1 ratio) culture was inoculated. After inoculation, glass jar samples were transferred to a water bath; pH was continuously measured and recorded by a pH meter for evaluating the acidification rate along each run. Batch fermentations were carried out in replicates of six and stopped when the pH reached 4.6 obtained (pH of fresh Dahi: 4.6). Process flowchart for the preparation of FFSMD is presented in Figure 1. After incubation, *Dahi* samples were stored at 4°C for 1 h and then used for texture profile analysis, whey separation, antioxidant activity, and probiotic viable count.

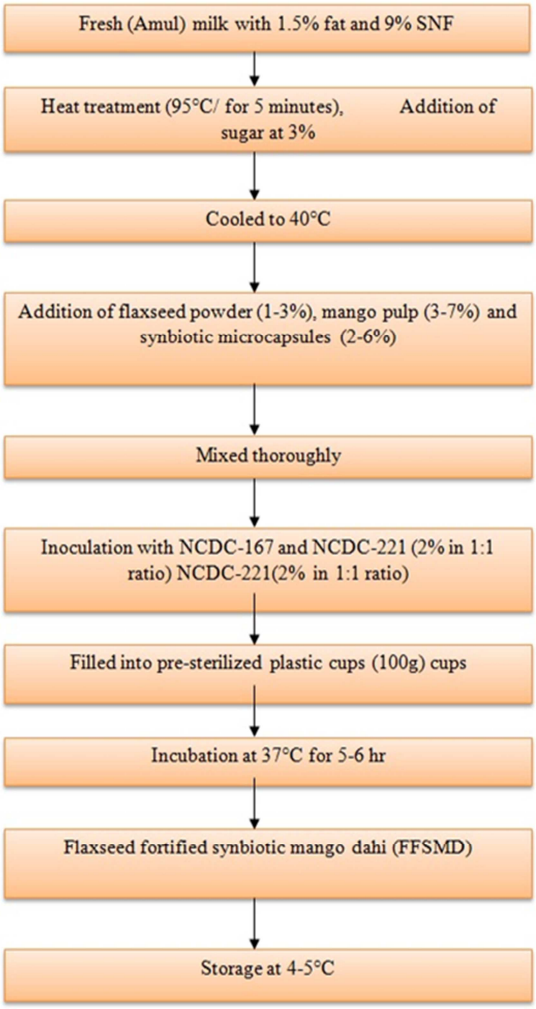

Figure 1. *Flow diagram for the manufacture of FFSMD.*

2.6. Antioxidant Activity (DPPH Inhibition)

The antioxidant activity in *Dahi* was estimated as radical scavenging activity by using a stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) which shows a strong absorption at 517 nm (deep violet colour) because of its odd electron. When the DPPH radical accepts an electron or H radical, the absorption vanishes. The decolorization is stoichiometric for the no. of electron accepted. The DPPH inhibition activity was measured by following the protocol given by Nishino *et al.* [19]. The antioxidant activity was expressed as:

$$\text{Percentage (\%) DPPH scavenging} = [(\text{absorbance of blank} - \text{absorbance of sample}) / (\text{absorbance of blank}) \times 100]$$

2.7. Texture Profile Analysis (TPA), Whey Separation and Total Solids

Textural attribute such as firmness and cohesiveness was determined by back extrusion using a texture analyzer, (TA-XT plus, M/s Stable Micro Systems Ltd, Godalming, United Kingdom) fitted with a 25 kg load cell and was calibrated with 5 kg standard deadweight before use. The firmness and cohesiveness have been determined using the probe (A/BE 35) that penetrated up to 10 mm (20% compression) into the set FFSMD at a cross head speed of 1.0 mm/s. The *Dahi* was set in a pre-sterilized container, before analysis, the beaker was tempered at 25°C for 2 h. The probe displaced the material by compression followed by back-extrusion so that the fluid flowed upwards through the concentric annular space (Exponent Lite XT PLUS, Ver. 4.0.13.0 lite). All measurements were done in replicates of eight per sample.

Syneresis was determined following the drainage method as described by Chawla *et al.* [3]. The syneresis was expressed as the percent weight of the whey over the initial weight of the yoghurt sample.

2.8. Enumeration of Probiotic Viable Bacteria

The samples were suspended in phosphate buffer solution and mixed by mechanical shaking for 10 min at room temperature to ensure complete dissolution of the *Dahi*. Serial dilutions were prepared from the initial suspension and pour plated on MRS agar (MRS Agar, Himedia, Mumbai, India) in replicates of eight. *L. acidophilus* was enumerated on MRS agar at 37°C under aerobic conditions for 72 h, while *B. bifidum* was cultured on reinforced clostridia medium agar (RCM) (Oxoid, Australia) at 37°C under anaerobic conditions for 72 h. The numbers of colonies were counted and results were expressed as log colony forming units per g (log cfu/g).

2.9. Experimental Design and Statistical Analysis

Response surface methodology (RSM), Minitab 17 was used to optimize the various parameters for the products in the present study. A five-level three factor Central Composite Design (CCD) with 6 central points and 6 axial points was obtained to find out the interactive effect of three variables viz; flaxseed powder, mango pulp, and synbiotic microcapsules on antioxidant activity, firmness, cohesiveness, whey separation, and probiotic viable count (Table 1). The three factors, flaxseed powder (A), mango pulp (B), and synbiotic microcapsules (C) were coded into five levels (−1.68, −1, 0, 1, 1.68). The coded and uncoded independent variables used in the RSM design are shown in Table 2. Ranges of flaxseed powder, mango pulp, and synbiotic microcapsules were selected based on preliminary experimental results.

The response function (Y) was partitioned into linear, quadratic, and interactive components, and the experimental data were fitted to the second-order polynomial equation as shown in Eq. 1:

$$Y=\beta_0 + \beta_1A_1 + \beta_2B_2 + \beta_3C_3 + \beta11A_1^2 + \beta22B_2^2 + \beta33C_3^2 + \beta12A_1B_2 + \beta13A_1C_3 + \beta23B_2C_3 \quad (1)$$

Where, Y=responses; β_0=constant; β_1, β_2, β_3=linear regression;

β_{11}, β_{22}, β_{33}=interaction regression; A_1, B_2, C_3=variables.

The three-dimensional surface response plots were generated showing the relationship between the response and independent variables [2]. The significance level was based on a confidence level of 95%.

Table 2. *Coded and actual levels of factors used.*

Variables	Actual factor level at the coded factor level					
	Symbol	-1.68	-1	0	1	1.68
Defatted flaxseed powder (g)	A	0.318	1	2	3	3.68
Mango juice (%)	B	1.63	3	5	7	8.36
Synbiotic microcapsules (%)	C	0.63	2	4	6	7.36

2.10. Fitting the Models

For each model main, linear, quadratic, and interactive effects were calculated. The adequacy of the model was tested using p value, coefficient of correlation (R^2), and lack of fit test (LoF). The R^2 value was more than 80%, and LoF was non-significant for each response [9]. The ANOVA, correlation coefficient (R^2), LoF, and coefficient estimate are given in Table 3 and 4. The adequacy of the model to fit the experimental data was verified by the lack of fit testing; ANOVA for the lack of a fit test for all the responses was non-significant ($p>0.05$) indicating that the model was adequately fitted the experimental data. The larger the regression coefficient in a model with a significant p-value indicates a more significant effect on the respective response variables [28]. The correlation coefficients for the responses, i.e. antioxidant activity, firmness, cohesiveness, whey separation, probiotic viable count were 96.64%, 97.72%, 99.12%, 98.43%, and 96.08% respectively, indicating that all the values were more than 90%.

3. Result and Discussion

3.1. Effect of Flaxseed Powder, Mango Pulp, and Synbiotic Microcapsules on Antioxidant Activity

The relationship between the antioxidant activity and flaxseed powder (A), mango pulp (B), and synbiotic microcapsules of bacteria (C) are shown in Eq. 2:

$$\text{Antioxidant activity}=52.56 + 6.14\,A + 6.485\,B + 1.021\,C - 1.260\,A^2 - 0.5072\,B^2 - 0.1704\,C^2 - 0.040\,AB + 0.166\,AC + 0.1019\,BC \quad (2)$$

The antioxidant activity range varied from 74.02 to 85.75%. The coefficient estimate for the variables has been presented in Table 4. All the three variables i.e. flaxseed powder, mango pulp, and synbiotic microcapsules of bacteria had significant ($P<0.01$) positive effect on antioxidant activity of Dahi sample at the linear level, and all the variables had significant ($P<0.01$) negative effect at the quadratic level. Interactive effect ($P<0.05$) of flaxseed powder with mango pulp and mango pulp with synbiotic microcapsules was positive, while between flaxseed powder and synbiotic microcapsules, it was observed negative. This data analysis was carried out for the rest of the measured responses as shown in Table 3.

Figure 2 shows the response surface plot of flaxseed and synbiotic capsules with mango pulp at a hold value of 5% on antioxidant activity. The antioxidant activity increased highly when the level of mango pulp increased in *Dahi*, which may be due to the high quantity of antioxidant activity present in mango pulp. As the level of flaxseed powder increased from 1% to about 3%, higher antioxidant activity was detected, as flaxseed powder is also rich in antioxidant activity. Antioxidant activity ranged from 0.56 to 0.86 mmol TE g^{-1} of flaxseed flour [22]. There was a very slight increase in antioxidant activity when the level of synbiotic microcapsules increased. The ANOVA data showed that the model is significant and lack of fit is non significant (Table 3). The value of R^2=96.28% indicates that 3.72% of the total variation was not explained by the model. A lack of fit value of 0.425 is found to be non significant relative to the pure error.

Table 3. *Analysis of variance (ANOVA).*

Responses	Source	F- value	P value	R^2 (%)
Antioxidant activity (%)	Model	28.74	0.000	96.28
	Linear	62.64	0.000	
	Square	22.92	0.000	
	Interaction	0.67	0.591	
	Lack of fit	5.59	0.425	
Firmness (g)	Model	5.15	0.004	97.72
	Linear	8.68	0.003	
	Square	6.29	0.006	

Responses	Source	F- value	P value	R^2 (%)
	Interaction	0.48	0.701	
	Lack of fit	3.25	0.111	
	Model	44.30	0.000	97.55
	Linear	103.01	0.000	
Cohesiveness (gs)	Square	25.65	0.000	
	Interaction	4.23	0.036	
	Lack of fit	2.66	0.154	
	Model	18.83	0.000	94.43
	Linear	44.97	0.000	
Probiotic Viable Count (cfu/g)	Square	10.06	0.002	
	Interaction	1.45	0.287	
	Lack of fit	1.60	0.310	
	Model	34.17	0.000	96.85
	Linear	75.65	0.000	
Whey Separation (%)	Square	4.62	0.028	
	Interaction	22.24	0.004	
	Lack of fit	7.17	0.025	

All main, linear, quadratic, and interactive effects were calculated for each model. The adequacy of the model was tested using p value, coefficient of correlation (R^2), and lack of fit test (LoF). The R^2 value was more than 80%, and LoF was non significant ($p>0.05$) for each response implying that the models were accurate enough to predict the responses. The larger the regression coefficient in a model with a significant p-value ($p<0.05$) indicates a more significant effect on the respective response variables.

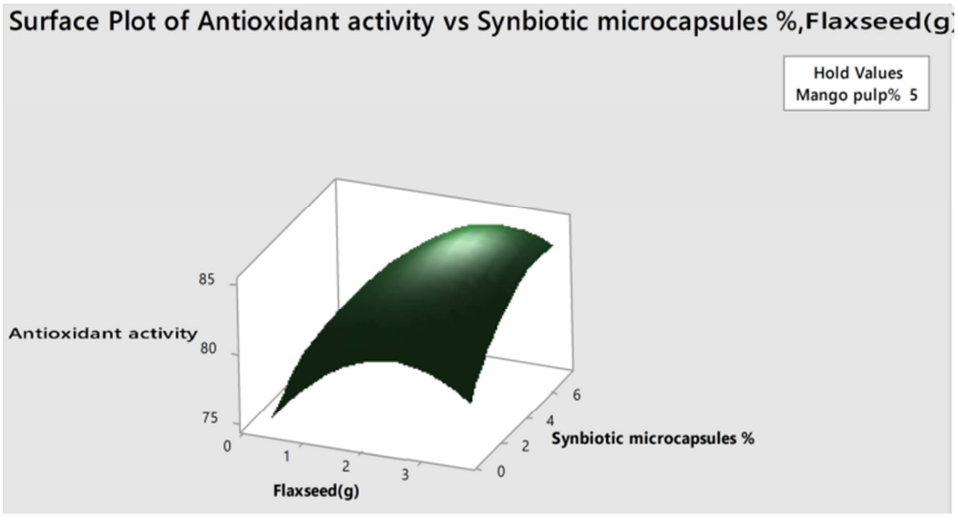

Figure 2. *Response surface plot showing the effect of flaxseed powder and synbiotic microcapsules on antioxidant activity of flaxseed fortified synbiotic mango Dahi.*

The maximal antioxidant activity predicted by response surface analysis was 85.75% with flaxseed powder of 3%, mango pulp of 7%, and synbiotic microcapsules of 6%. The data obtained show that the three components (viz. flaxseed, mango, and synbiotic microcapsules) of FFSMD contribute to the higher antioxidant activity of FFSMD. Eliana Pereira *et al.* [5] reported that yoghurt fortified with mango pulp had high antioxidant activity (42.47%). Hu *et al*. [11] assessed 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities of flaxseed lignans and found that the flaxseed lignans SDG and SECO exhibited strong antioxidant and protective effects in quenching the DPPH. Similar findings are reported by Madhu *et al.* [17] who showed that the antioxidant activity (85%) in synbiotic yoghurt containing *L. plantarum* and fructooligosaccharide was significantly higher in comparison with that of control yoghurt (72%).

3.2. Effect of Flaxseed Powder, Mango Pulp, and Synbiotic Microcapsules on the Firmness

The model that explained the relationship between the firmness of the Dahi and the variables i.e. flaxseed powder (A), mango pulp (B), and synbiotic microcapsules (C) is shown in Eq. 3.

$$\text{Firmness (g)} = 202.79 + 6.29\,A + 7.80\,B + 4.09\,C - 1.571\,A^2 - 0.641\,B^2 - 0.260\,C^2 + 0.251\,AB + 0.160\,AC - 0.232\,BC \quad (3)$$

The firmness of the samples ranged from 238.61 to 246.89 g (Table 1). The magnitude of the p value (Table 3) indicates that all the variables of linear terms have a significant effect at a 1% level of significance ($P<0.01$) on the firmness of *Dahi*. The data analysis of the measured response was carried out similarly as given for equation (1). The optimal

conditions were predicted to be the flaxseed powder of 3% mango pulp of 7% and synbiotic microcapsules of 6% for the maximal firmness of 250.34g.

The firmness of *Dahi* significantly ($P<0.01$) increased when the levels of three components increased in FFSMD. This indicates that with the increase in total solid content of yoghurt like products there is a significant increase in firmness value. Yoghurt with firmspoonable texture has high consumer acceptance. As shown in Table 3, firmness increased when mango pulp increased up to 5% (Figure 3) thereafter decreased drastically with increasing mango pulp level (shown in run no. 2, 5, 16, and 17). This could be possibly due to increased acidity attributed by mango pulp which results in weakening of the gel network leading to decreased water holding capacity.

Total solids, protein content, and the type of protein determine the firmness of *Dahi* and yoghurt. The denser and rigid gel structure is formed when the protein content is higher due to a greater degree of cross-linkage. [24]. The fibre content and high total solids content improved the firmness of FFSMD, this result is in agreement with the results of Aportela-Palacios *et al.* [1], as stated the particular structure of the fiber may be involved in the entrapment of water molecules as part of the three dimensional network thereby increasing the firmness of the yogurt gel [20].

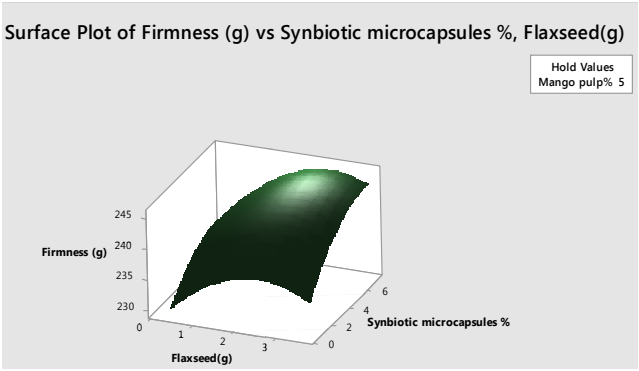

Figure 3. *Response surface plot showing the effect of flaxseed powder and synbiotic microcapsules on the firmness of flaxseed fortified synbiotic mango Dahi.*

3.3. Effect of Flaxseed Powder, Mango Pulp, and Synbiotic Microcapsules on Cohesiveness

The equation of the model fitted for the cohesiveness of *Dahi* in the actual form of process variables i.e. flaxseed powder (A), mango pulp (B), and synbiotic microcapsules (C) is shown in Eq. 4.

$$\text{Cohesiveness (gs)}=12.59 + 5.40\,A + 3.121\,B + 1.663\,C - 0.906\,A^2 - 0.3905\,B^2 - 0.1912\,C^2 + 0.041\,AB + 0.061\,AC + 0.2450\,BC \quad (4)$$

The cohesiveness range of *Dahi* samples varied from 25.59 to 38.36g. The coefficient estimate for the variables has been presented in Table 4. The data analyses of the measured response were carried out similarly as given for equation (1).

As the level of mango pulp increased from 1% to about5% (Figure 4), cohesiveness was increased thereafter decreased significantly. There was a very slight increase in cohesiveness when the level of synbiotic microcapsules increased compared to flaxseed powder and mango pulp. The maximal cohesiveness predicted by response surface analysis was 38.36g with flaxseed powder of 2g, mango pulp of 5%, and synbiotic microcapsules of 7.36%.

Cohesiveness increased with the increase in flaxseed powder and synbiotic microcapsules. The high protein content of the flaxseed and polysaccharides of microcapsules might have contributed to the increased cohesiveness or tendency to adhere to itself [14]. Cohesiveness decreased with an increase in mango pulp level beyond 5%, this could be due to the high acidity attributed to mango pulp which results in a disturbance of the gel network of *Dahi*.

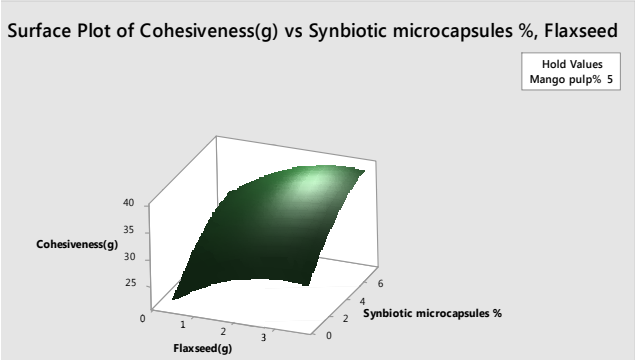

Figure 4. *Response surface plot showing the effect of flaxseed powder and synbiotic microcapsules on the cohesiveness of flaxseed fortified synbiotic mango Dahi.*

3.4. Effect of Flaxseed Powder, Mango Pulp, and Synbiotic Microcapsules on Probiotic Viable Count

The quadratic equation obtained by the response surface analysis of the data showing the effect of flaxseed powder (A), mango pulp (B), and synbiotic microcapsules (C) resulted in the following equation:

$$\text{Probiotic viable count (cfu/g)}=3.71 - 0.075\,A + 0.257\,B + 1.778\,C - 0.076\,A^2 - 0.0694\,B^2 - 0.1446\,C^2 + 0.1462\,AB + 0.0362\,AC - 0.0231\,BC \quad (6)$$

The probiotic viable count range of *the Dahi* sample varied from 5.06to 9.85 cfu/g. The coefficient estimate for the variables has been presented in Table 4. All the three variables i.e. flaxseed powder, mango pulp, and synbiotic microcapsule had significant ($P<0.01$) positive effect on the probiotic viable count of *Dahi* sample at the linear level. At the quadratic level, mango pulp shows a significant ($P<0.01$) negative impact on the probiotic viable count. Figure 5 shows the response surface plot of flaxseed powder and synbiotic capsules with mango pulp at a hold value of 5% on the probiotic viable count. The probiotic viable count increased highly when the level of synbiotic microcapsules increased in *the Dahi* sample. As the level of flaxseed powder increased from 1 to about 3%, the probiotic viable count also increased.

This indicates that flaxseed powder might have the prebiotic potential for the probiotic bacteria.

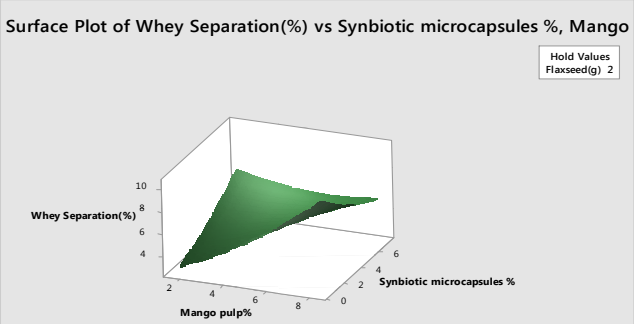

Figure 5. *Response surface plot showing the effect of flaxseed powder and synbiotic microcapsules on the probiotic viable count of flaxseed fortified synbiotic mango Dahi.*

The maximal probiotic viable count predicted by response surface analysis was 9.85cfu/g with flaxseed powder of 2%, mango pulp of 5%, and synbiotic microcapsules of 7.36%. The increase in probiotic viable count in FFSMD is in accordance with the findings of Madhu *et al.* [17] who indicated that microcapsules of bacteria supplemented with FOS resulted in a significant increase in the total count of *L. plantarum* and *L. fermentum* from 9.16 and 9.17 log $cfug^{-1}$ to 9.52 and 9.45 log $cfug^{-1}$, respectively. Chen *et al.* [4] observed that blending of prebiotics FOS and peptides in the coating materials can provide the carbon and nitrogen source for microencapsulated probiotics, modify the unfavorable environmental influences, resulting in improved probiotic viability. Prebiotics, stimulate the metabolism of probiotics, by the release of an increased level of fructose as a result of its partial hydrolysis, which gets metabolized as an additional carbon and energy source [25].

3.5. Effect of Flaxseed Powder, Mango Pulp, and Synbiotic Microcapsules on Whey Separation

The model that explained the relationship between the whey separation of the Dahi and the variables i.e. flaxseed powder (A), mango pulp (B), and synbiotic microcapsules (C) is shown in Eq. 5.

$$\text{Whey Separation (\%)} = 0.61 + 0.118\,A + 0.834\,B + 0.905\,C + 0.0969\,A^2 + 0.0600\,B^2 + 0.0388\,C^2 - 0.0812\,AB - 0.1550\,AC - 0.1875\,BC \quad (5)$$

The whey separation of Dahi samples ranged from 2.01 to 7.56% (Table 1). The magnitude of the p value (Table 3) indicates that all the variables of linear and quadratic terms have a significant effect at a 1% level of significance ($P<0.01$) on the whey separation of Dahi. The coefficient estimate for the variables has been presented in Table 4. The data analyses of the measured response were carried out similarly as given for equation (1). Figure 6 shows the effect of the surface plot of flaxseed powder and synbiotic capsules with mango pulp at a hold value of 5% on whey separation. The whey separation of Dahi decreased highly when the level of flaxseed powder increased which may be due to the high quantity of protein and dietary fibre present in flaxseed. As the level of mango pulp increased beyond 5% whey separation was also increased. There was a slight decrease in whey separation when the level of synbiotic microcapsules increased.

Table 4. *Coefficient of estimates for different factors.*

Factor	Coefficient estimate				
	Antioxidant activity (%)	**Firmness (g)**	**Cohesiveness (gs)**	**Whey separation (%)**	**Probiotic viable count (cfu/g)**
Intercept	83.870	243.40	34.994	5.345	9.331
Linear					
A	1.563	1.905	2.222	0.520	0.496
B	3.482	1.913	0.558	-1.044	0.474
C	1.000	2.345	2.962	0.064	1.157
Quadratic					
A^2	1.260	1.571	0.906	0.096	0.076
B^2	2.029	-2.566	-1.562	-0.240	-0.278
C^2	0.682	1.040	-0.765	0.155	0.578
Interaction					
AB	0.080	0.502	0.083	-0.163	0.292
AC	0.332	0.320	0.123	0.310	0.072
BC	0.408	-0.927	0.980	-0.750	-0.093

The linear, quadratic, and interaction effects of each factor on each response model were calculated. The larger value of the coefficient of estimate indicates a larger impact of a particular factor on each response at linear, quadratic, and interaction levels. The negative values indicate a negative impact on the particular response while positive values are indicative of the positive impact of the factor. Flaxseed mango pulp and synbiotic microcapsules had a positive impact on the antioxidant activity at linear, quadratic, and interaction levels. However, mango pulp had a negative impact on whey separation at a linear, quadratic and interactive level.

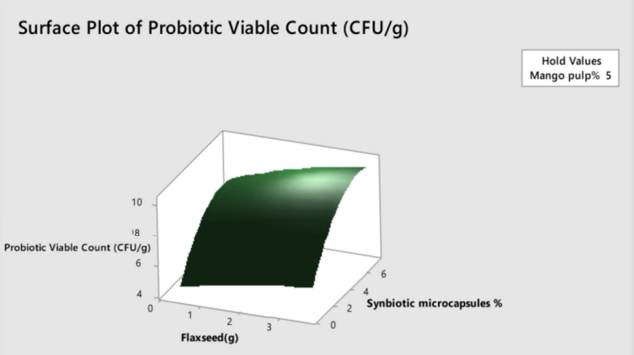

Figure 6. *Response surface plot showing the effect of flaxseed powder and synbiotic microcapsules on the probiotic viable count of flaxseed fortified synbiotic mango Dahi.*

The increase in total solids content along with protein content contributed by flaxseed results in reduced wheying-off. As the protein and fibre content increases in the gel network system, there is the formation of additional bonds between protein particles, rearrangements in the protein network, and possible attachment of dangling gel strands to the network [16]. Garcia-Perez *et al.* [6] reported that the addition of orange fiber at 0.6% and 0.8% had a loosening effect on the gel network which disturbs the gel structure of yoghurt leading to an increase in syneresis. Further, when added at a 1% level, the syneresis decreased due to increased water holding of fiber that absorbed the whey released by the gel structure. As the mango pulp concentration increases beyond 5% there is a significant increase in whey expulsion. This could be explained by the fact that increased acidity in the gel network leads to the weakening of the protein protein bonds and disturbs the orientation of the strands in the matrix. This results in the creation of large pores in a gel which facilitates a more rapid flow of serum through the matrix. The optimal conditions were predicted to be the flaxseed powder of 1%, mango pulp of 3%, and synbiotic microcapsules of 2% for the minimum whey separation of 4.02%.

3.6. Verification of Predictive Model

It can be concluded that the flaxseed fortified synbiotic mango Dahi dependent on the interaction of the different variables. The fitness of the model equations for predicting optimum response values were tested using flaxseed powder 2.65%, mango pulp 5.28%, and synbiotic microcapsules 4.16% (Table 5). The predicted values under the optimum condition of process parameters are antioxidant activity 85.12%, firmness 243.40gs, cohesiveness 34.36g, whey separation 5.34%, and probiotic viable count score 9.33cfu/g, respectively. The desirability for this formulation was 0.80. This set of conditions was also used to verify experimentally. The experimental values (Table 5) are antioxidant activity 83.67%, firmness 245.54g, cohesiveness 35.13gs, whey separation 5.14%, and probiotic viable count score 9.20cfu/g, respectively were found to be in close agreement with the predicted values and were within the acceptable limits showed the adequacy of selected models.

Table 5. *Experimental data for the verification of predicted values.*

Responses	Predicted value	Experimented value
Antioxidant activity (%)	83.12	83.67±0.22
Firmness (g)	243.40	245.54±0.20
Cohesiveness (gs)	34.36	35.13±0.13
Whey separation (%)	5.34	5.14±0.04
Probiotic viable count (cfu/g)	9.33	9.20±0.10

Values are Mean ± Standard deviation (SD) of eight replicates. The table shows that there was a satisfactory agreement between experimented and predicted values of responses for any combination of independent variables of experimental design.

4. Conclusion

The response surface methodology was successfully employed to optimize the flaxseed fortified synbiotic mango Dahi. There was a satisfactory agreement between experimented and predicted values of responses for any combination of independent variables of experimental design. An acceptable quality of flaxseed fortified synbiotic mango *Dahi* can be prepared by using the optimized level of flaxseed powder 2.65%, mango pulp 5.28%, and synbiotic microcapsules 4.16%. The developed product could be used for the development of commercial flaxseed fortified synbiotic Dahi with improved health benefits besides an option as a flavourful product of *Dahi*. *Dahi* is known in India for its health benefits and providing flavored *Dahi* along with advanced therapeutic health benefits, can attract many health-conscious consumers which would ultimately profit Dahi manufacturers in India as well as in the global market.

Conflict of Interest

The authors declare that they have no competing interests.

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