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ORIGINAL PAPER

Development of clove extract-fortified functional yoghurt powder using spray: drying

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Abstract

Spray-drying transforms functional yogurt into yogurt powder quickly and efficiently, maintaining product quality and enhancing storage stability. This process also adds value to the product. The study aimed to develop spray dried functional yoghurt powder encapsulating probiotics (*Streptococcus thermophilus* (*S. thermophilus*), *Lactobacillus bulgaricus* (*L. bulgaricus*), and *Lactococcus lactis* (*L. lactis*)) with longer shelf life. The production of functional yoghurt powder was optimized with Box–Behnken design using independent variables inlet air temperature (110–170 °C), feed temperature (10–20 °C), and pump speed (10–20%). The optimum functional yoghurt powder was obtained at 164.04 (164) °C inlet air temperature, 20 °C feed temperature, and 10.72 (11) % pump speed, respectively. The functional yoghurt powder with amorphous nature had storage viability (log CFU g^{-1}) of 8.88 ± 0.03, 6.71 ± 0.12 and 8.16 ± 0.10 for *S. thermophilus*, *L. bulgaricus*, and *L. lactis* correspondingly on 49th day at 4 °C. The functional yoghurt powder demonstrated antibacterial activity, resulting in inhibition zones measuring approximately 18.33 ± 1.15 mm and 11.00 ± 0.00 mm against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively. Functional yoghurt powder had water activity 0.35–0.52, moisture content 7.27–16.22 (%), process yield 2.29–5.33 (%), hygroscopicity 10.09–16.87 (g/100 g) and degree of caking as 37.46–59.02 (%). The bulk densities, tapped densities, particle density and porosity of yoghurt powder ranged from 242 to 425 (kg m^{-3}), 301.9–485.7 (kg m^{-3}), 1050–2500 (kg m^{-3}), and 58.67–87.85 (%) respectively. Furthermore, the solubility, dispersibility, and wettability of the powder ranged from 33 to 60%, 46.86–86.37%, and 2.13–4.28 min, respectively. The current study successfully achieved optimization at an inlet temperature of 164.04 °C (164 °C), a feed temperature of 20 °C, and a pump speed of 10.72% (11%), with desirability of 72.6%. This study makes a substantial advancement in the production of probiotic yoghurt powder infused with clove, achieving the recommended levels of viability, shelf life, powder characteristics, reconstitution capability, and antibacterial effects. Moreover, the spherical morphology of the functional yoghurt powder was revealed through scanning electron microscopy.

Keywords Functional yoghurt powder · Spray—Drying · Response surface methodology · Storage viability · SEM

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Introduction

Spray-drying is widely embraced in the functional dairy powder industry due to its cost-effectiveness. Its rapid drying capabilities and superior moisture removal rate further contribute to its widespread adoption [1]. It entails the atomization of the feed material with hot air, leading to the efficient elimination of water through effective heat and mass transfer [2]. The characteristics of the powder produced through spray-drying were primarily impacted by the inlet temperature, feed temperature, and pump speed [1]. To minimize transportation costs and streamline handling while ensuring enhanced viability, fermented foods containing lactic acid bacteria (LAB) underwent a drying process [3]. Among various drying methods, spray drying has emerged as a promising technique, primarily attributed to its ability to maintain the viability of lactic acid bacteria (LAB) during storage [4].

An investigation was conducted on the potential health benefits of probiotic herbal functional yogurt, particularly emphasizing the clove-infused variant, which exhibited superior sensory scores and functional properties [5, 6]. Thaochalee et al. [7] also studied about the feasibility of functional dairy product, incorporating herbal prebiotic and probiotics. The functional yogurt powder, produced through spray-drying, found applications in various food products such as confectioneries, instant drink mixes, soups, dips, bakery goods, sauces, and more [8]. Curcumin milk encapsulation by spray-drying were reported by Huggett et al. [9]. Ali et al. [10] also studied the development of spray dried functional yoghurt powder with higher survivability of yoghurt cultures, antioxidant and enhanced sensory properties.

Therefore, spray-drying can be effectively employed to dry functional yogurt, ensuring the increased survivability of probiotic strains [10]. The viability of lactic acid bacteria (LAB) in the food during consumption is crucial for its effectiveness, as it must withstand processing, storage, and transit through challenging intestinal conditions [11]. *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) are typically used to make yoghurt [12]. The combination of *Lactococcus lactis* (*L. lactis*) and yoghurt cultures generates lactic acid, enhancing the antimicrobial functional qualities of the fermented product [13, 14]. The recommended level of viability for its beneficial functional property is ~10^6 –10^8 CFU g^{-1} in the food at consumption [15]. Therefore, the current strategy of microencapsulation through spray-drying is of significant interest in enhancing the viability of probiotics [16].

The objective of preparing functional probiotic clove yogurt powder is the central aim or purpose behind engaging in the process. It could involve developing a convenient and innovative form of probiotic yogurt with added benefits from clove, catering to consumer preferences or addressing specific health concerns. The necessity of this preparation refers to fill the gap in the market for convenient probiotic products, and to explore the potential health benefits associated with combining probiotics and clove. In this study, we investigated the ability of functional yoghurt powder supplemented with clove (*Syzygium aromaticum*) and certain probiotics to combat the bacteria associated with antibacterial effect against *Klebsiella pneumoniae* (*K. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). The study aimed to develop functional yoghurt powder with *L. lactis, S. thermophilus,* and *L. bulgaricus* with significant improvement in consumer choices, enhanced nutritional value, or the exploration of novel health-promoting properties associated with the combination of probiotics and clove. Independent variables, such as inlet air temperature (110–170 °C), feed temperature (10–20 °C), and pump speed (10–20%) were optimized. The responses water activity, moisture content, process yield, antibacterial effect, encapsulation efficiency (EE) of probiotics, colour difference, drying rate, hygroscopicity, degree of caking, bulk densities, tapped densities, particle density, porosity, flowability, cohesiveness, solubility, dispersibility and wettability were recorded.

Material and methods

Chemicals and reagents

The chemical sodium chloride (NaCl), D-fructose, calcium chloride dihydrate, agar–agar, Tween 80 (Chemiz, Malaysia), petroleum ether, lactose, sodium acetate, Di-pottassium hydrogen phosphate (R&M chemicals, United Kingdom), D-glucose (Merck, Germany), *Streptococcus thermophilus* agar (STA), Peptone, Yeast extract, L-cysteine, uracil, GM17 agar, Muller Hinton Agar (MHA) (Himedia, India), Beef extract (Oxoid, USA), glycerol phosphate disodium salt hydrate (Sigma), chloramphenicol (Calbiochem, Germany) respectively were used in the study.

Raw materials

Low-fat UHT milk from Nestle products Sdn. Bhd. (Malaysia), *Streptococcus thermophilus* ATCC 19258 and *Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC 11842 = JCM 1002 isolated from yoghurt by Malaysia Milk Sdn. Bhd. (MariGold, Malaysia) were used in the study. *Lactococcus lactis* subsp. *cremoris* MG1363 was obtained from the culture collection at Universiti Putra Malaysia, Malaysia. All lactic acid bacteria were confirmed by 16S rRNA sequencing and data analysis by BLAST. Clove buds were purchased from the local market (Cheras, Malaysia) to prepare sterile clove aqueous extract (10% w v^{-1}) (16). Pathogenic strains *K. pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853, the widespread ecological niche plays a significant role in the prevalence of healthcare-associated infections, with a notable focus on pneumonia was obtained from culture collection at UCSI University.

Sample preparation and spray—drying

Yoghurt was prepared according to García-Gómez et al. [17] with slight modifications. For functional yoghurt production, clove aqueous extract, 7.5% (v v^{-1}) was added to low-fat UHT milk and incubated at temperature of 36.6 °C (37 °C), for 24 h with total culture concentration of 4.5% (v v^{-1}) according to the previous optimization study. Then functional yoghurt prepared with separate probiotic viability at 10 log CFU mL^{-1} was stored at low temperature (4 °C) before spray—drying. The production process of functional yoghurt through spray drying was intricately influenced by three crucial parameters: the inlet temperature, feed temperature, and pump speed. These variables hold the key to achieving optimal results in the transformation of yoghurt into a powdered form, a technique employed using the Lab Plant SD-06 Spray Dryer (Universiti Putra Malaysia). To produce functional yoghurt powder samples, the process involved the spray drying of optimized functional yoghurt using specific conditions.

Experimental design

Using Box-Behnken design in response surface methodology (RSM), the study investigated the optimization of various independent variables and their interactions to develop an optimized probiotic functional yoghurt powder.

Table 1 Independent variables and their corresponding levels for yoghurt powder optimization

Independent variables	symbols	Coded levels		
		− 1	0	+ 1
Inlet temperature (°C)	X_1	110	140	170
Feed temperature (°C)	X_2	10	15	20
Pump speed (%)	X_3	10	15	20

To fit the model, multiple regression analysis was used. Version 13.0.5.0 of the Design-Expert® programme was used to optimise the fitted polynomials. Independent variables and their corresponding levels for the responses were presented in Table 1. The limits were configured to get the variables' coded values between the lower and higher bounds. Each objective was given a specific weight in order to modify the desirability function. While retaining the third variable at its optimal level, the response surfaces plots were plotted as a function of two variables X_1X_2 and X_1X_3 [19]. To prevent errors and lessen the impact of potential external influences, 15 runs were carried out in a randomised order with three central points.

Physicochemical analysis of functional yoghurt powder

Water activity

Functional yoghurt sample (2 g) was placed in Pre AQUA LAB water activity analyser (METER, USA) at 25 °C and readings were recorded [15].

Moisture content

Functional yoghurt powder (2 g) was placed in preheated porcelain cups and were dried at 105 °C for 24 h [15]. The moisture content was calculated using the given equation:

$$\text{Percentage of moisture (\%)} = (\text{Weight after drying (g)} - \text{Weight before drying(g)}) / \text{Weight before drying (g)} \quad (1)$$

Functional yoghurt powder was optimized in terms of inlet air temperature (110–170 °C), feed temperature (10–20 °C), and pump speed (10–20%), using a three-factor BBD (Box-Behnken design) [18]. The responses recorded were water activity, moisture content, process yield, antibacterial effect, encapsulation efficiency (EE) of probiotics, colour difference, drying rate, hygroscopicity, degree of caking, bulk densities, tapped densities, particle density, porosity, flowability, cohesiveness, solubility, dispersibility and wettability of functional yoghurt powder developed.

Process yield

Spray—drying of functional yoghurt was conducted using laboratory spray drier (Lab Plant SD-06 Spray Dryer, United Kingdom). The functional yoghurt samples of 450 mL were spray dried for each run and the product was kept in sealed polyethylene bags at 4 °C. The process yield was recorded as per the equation mentioned [20].

$$\text{Process yield (\%)} = (\text{Weight of yoghurt powder (g)}/\text{Weight of yoghurt (g)}) \times 100 \tag{2}$$

Colour difference (ΔE)

Colour of functional yoghurt powder and fresh yoghurt was measured with a colorimeter (ColorFlez EZ, Hunter Associates Laboratory Inc, USA). According to the Hunter Lab format, L* (brightness), a* (+red to -green component), and b* (+yellow to—blue component), were assessed. Colour difference from normal commercial yoghurt was then computed as ΔE using the following Eq. [21].

$$\text{Colour difference } (\triangle E) = \sqrt{(\triangle L*^2) + (\triangle a*^2) + (\triangle b*^2)} \tag{3}$$

Antibacterial effect of functional yoghurt powder

The antibacterial effect of functional yoghurt powder was studied against *K. pneumoniae*, and *P. aeruginosa* was performed with slight modifications [22, 23]. The spray—dried functional yoghurt powder, were mixed in sterile UHT milk at a concentration of 0.5 g mL^{-1}. The pathogenic strains ($OD_{600\ nm}=0.1$) were prepared after 24 h of incubation at nutrient broth, was then spread over sterile MHA plates for disc diffusion assay. After being soaked in a yoghurt solution for 10 min, sterile discs were placed onto MHA plates to measure the zone of inhibition (mm). The sterile disc was impregnated with chloramphenicol (10%) and milk acting as the positive and negative controls, respectively. The plates were then incubated at 37 °C for 24 h and antibacterial activities were determining by measuring the zone of inhibition.

Encapsulation efficiency (EE) of probiotics in functional yoghurt powder

The conventional pour plate count technique was used to determine the viable counts of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. lactis* in functional yoghurt powder at a concentration of 1 g at 9 mL normal saline [15, 24]. For the enumeration of *S. thermophilus* (37 °C for 48 h), *L. bulgaricus* (37 °C for 48 h), and *L. lactis* (30 °C for 48 h), STA (HIMEDIA, India) [25], modified reinforced clostridial medium (mRCM) [26] and GM17 agar [27] were used respectively. EE (%) of each lactic acid bacteria was determined using given equation.

$$\text{Encapsulation efficiency (EE\%)} = (\text{Viability after spray} - \text{drying} / \text{viability prior to spray} - \text{drying}) \times 100 \tag{4}$$

Powder properties of functional yoghurt powder

Drying rate (DR_{ave})

The average drying rate ($kg_{water}\ h^{-1}$) was measured using the following Eq. [28].

$$DR_{ave} = (\text{amount of water in the feed (kg)} - \text{amount of water in the powder (kg)})/\text{drying time (h)} \tag{5}$$

Hygroscopicity

Functional yoghurt powder (2 g) was placed over saturated Sodium Chloride (NaCl) solution for seven days. The moisture gain was recorded [8, 29].

$$\text{Hygroscopicity (g/100 g)} = ((\text{Final weight (g)} - \text{Initial weight be (g)}) \times 100)/(\text{Initial weight (g)} \times (100 - \text{moisture}/100)) \tag{6}$$

Degree of caking

After calculating hygroscopicity, functional yoghurt powder was dried at 102 °C for 24 h in oven. The dried functional yoghurt powder was weighed and sieved at 500-µm size sieve for 5 min. The functional yoghurt powder continued in sieve were measured. The degree of caking was measured using the equation given below [8].

$$\text{Degree of caking (\%)} = (\text{Amount of the powder remained in sieve}/\text{Initial amount of powder}) \times 100 \tag{7}$$

Bulk, tapped and particle densities

Functional yoghurt powder (2 g) was taken in 10 mL measuring cylinder and the occupied volume was noted to record the bulk density (P_B; w v^{-1}). Then was tapped for 5 min at a rate of 32 taps min^{-1} and final volume occupied was recorded as tapped density (P_T; w v^{-1}). Particle density was calculated by taking (1 g) of functional yoghurt powder in 10 mL measuring cylinder. Petroleum ether (5 mL) was then added and mix properly. The wall of measuring cylinder was then rinsed again with 1 mL of petroleum ether [30].

$$\text{Particle density } (P_p) = \text{Weight of functional yoghurt powder (g)} / (\text{Final volume of suspension (mL)} - 6) \quad (8)$$

Porosity

The porosity (ε) of functional yoghurt powder was measured using the equation mentioned [8].

$$\text{Porosity } (\varepsilon) = \left(\left(P_p - P_T\right) / P_p\right) \times 100 \quad (9)$$

Flowability and cohesiveness

The flowability and cohesiveness of functional yoghurt powder was measured as Carr's Index and Hausner ratio, respectively. These were recorded using bulk and tapped densities [30].

$$\text{Carrs Index} = \left(\left(P_T - P_B\right) / P_T\right) \times 100 \quad (10)$$

$$\text{Hausner ratio} = P_T / P_B \quad (11)$$

Analysis of reconstitution properties of functional yoghurt powder

Solubility

Functional yoghurt powder (1 g) was diluted in 100 mL distilled water and centrifuged for 5 min at 3000 rpm (MIKRO 220R, Germany). After a 30-min standing period, a 25 mL supernatant was dried for 5 h at 105 °C, and the change in weight was measured to determine the solubility (%) of the sample [30].

Dispersibility

Functional yoghurt powder (1 g) was diluted in 10 mL distilled water and mixed vigorously for 15 s. The suspension was then sieved at 212 µm sieve and dried for 4 h at 105 °C. The dispersibility was recorded as per the given Eq. [30].

$$\begin{aligned}\text{Dispersibility (\%)} = & (10 + \text{yoghurt powder (g)}) \\ & \times (\text{\% total solids present in suspension aftersieve}) \\ & / (\text{yoghurt powder (g)} \\ & \times (100 - \text{moisture content})) \times 100\end{aligned} \quad (12)$$

Wettability

Time taken for 1 g of functional yoghurt powder to completely sink in 400 mL distilled water at room temperature [30].

Analysis of optimized functional yoghurt powder

Scanning electron microscopy (SEM)

The process of scanning electron microscopy (SEM) involves trimming the specimens in their location, fixing them, dehydrating with ethanol, undergoing critical-point drying, mounting on an SEM stub, sputter-coating with gold, and capturing images at an optimal accelerating voltage. The optimized functional yoghurt powder was sputter coated with gold. The characteristics morphologically at 10 kV accelerated voltage, and varying magnification 500×, 1000×, 2000× and 2500× functional yoghurt powder was detected using scanning electron microscope (UPM, Malaysia) [29].

Storage viability of optimized functional yoghurt powder

Viable counts of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. lactis* in optimized yoghurt powder were studied on day 1, 7, 14, 21, 28, 35, 42 and 49 of storage, using STA (HIMEDIA, India) [25], mRCM [26] and GM17 agar [27] respectively. One-gram optimized yoghurt powder was mixed thoroughly with 9 mL normal saline using a vortex mixer VTX-3000L (LMS, Japan), serially diluted and counted using the pour plate technique [31].

Statistical analysis

Response surface methodology was used to elaborate BBD data (Design Expert software, 13.0.5.0, Stat-Ease Inc., Minneapolis, MN, USA). The developed models were assessed for coefficient of determination (R^2), and lack of fit. The predicted values of responses were also evaluated with experimental values using paired t-test. To determine significant differences between the viability of functional yoghurt powder at storage ($p < 0.05$), all data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's post hoc test with SPSS statistics 20 version (IBM SPSS statistics 20 version).

Results and discussion

Response values for all 15 runs are listed in Table 2 and 3. For each model, the significant linear, quadratic, and 2FI interaction effects were estimated. The coefficient of correlation, P-value, and lack-of-fit test were used to evaluate the model's suitability (Table 4 and 5). The coefficients of determination among the responses ranged from 0.718 to 1.000. The lack-of-fit tests were non-significant, indicating that the models are sufficiently accurate to forecast any combination of independent factors' effects on physiochemical properties, antibacterial effect, EE, powder characters, and reconstitution responses were in the ranges under study. This implies that the models could be used to assess the relative impact of the variables, identify the best parameter combinations of desired responses, and forecast the outcomes under different circumstances.

Linear model for moisture content, antibacterial effect, EE of *L. bulgaricus*, colour difference, bulk densities, tapped densities, porosity, flowability, cohesiveness, and solubility were suggested. The 2 FI (interactive) model was suggested for drying rate and *S. thermophilus* EE, then quadratic models for water activity, process yield, EE of *L. lactis*, hygroscopicity, degree of caking, particle density, dispersibility and wettability. These models were expressed as equations in Table 6. X_1 was the coded independent factor inlet temperature, X_2 was the coded independent factor feed temperature and X_3 was the coded independent factor pump speed.

Physicochemical analysis of functional yoghurt powder

In general, the variation of independent variables had effects on the physicochemical attributes of functional yoghurt powder. Water activity and moisture content of functional yoghurt powder were measured at different runs (Table 2). Functional yoghurt powder had water activity between 0.35 and 0.52. Water activity is directly related to the amount of moisture content, and lower moisture content of yoghurt powder cause decrease in water activity [32]. Lower water activity value is vital for powder product to improve the self-life. The water activity in the functional yoghurt powder was below the minimum requirement of 0.6, for the least demanding xerophilic moulds to grow [33]. Hence, the functional yoghurt powder produced can prevent the microbial growth.

The moisture content here ranges between 7.27 and 16.22 (%). Freeze-dried yoghurt powder prepared from moisture—drained yoghurt was reported as 8.3% [31] and spray dried as 3.98—7.17% [8]. Freeze—dried goat milk yoghurt powder added with honey had 13.53% moisture according to Azemi et al. [34]. Hence the addition of aqueous clove extract can cause an increase in the moisture content of functional yoghurt powder. R^2 value for water activity and moisture content were 0.9527 and 0.8993, respectively, with non-significant lack of fit. F value with quadratic models on water activity was significantly decreases with increasing inlet air temperature ($P \leq 0.001$) and decreasing pump speed ($P \leq 0.001$), while moisture content decreases significantly with increasing inlet air temperature ($P \leq 0.001$) (Table 4). In summary, the observed effects can be attributed to the interplay of temperature and mass transfer during the drying process. Higher temperatures and effective air circulation (higher pump speed) facilitate faster evaporation and, consequently, lower water activity and moisture content in the material being dried. These findings align with common principles in drying processes, where elevated temperatures generally lead to improved drying efficiency.

The quadratic and linear regression equation for water activity and moisture content respectively was expressed in Table 6. The result was in agreement with Martins et al. [35] study on their development of viable spray—dried powder, where, the water activity decreases as inlet temperature goes up. Reduced water activity at higher temperature 160 °C, indicates stable quality and storage life [36]. The study by Iris et al. [37] also reported a decrease in moisture content with decreasing flow rate and increasing air inlet temperature according to the present study.

Process yield was significantly ($P \leq 0.001$) fitted by a quadratic model (Table 4). In comparison to pure error, lack of fit was non-significant. Process yield ranges from 2.29 to 5.33 (%), in comparison to inlet and feed temperature, the increasing inlet temperature significantly decreases process yield and increases process yield with increasing feed temperature. Similarly, yield significantly decreases with increasing pump speed. The observed effects on yogurt powder yield can be attributed to the complex interactions between temperature, processing conditions, and the specific requirements of the yogurt production process. According to El-Said et al. [38], the inclusion of plant extracts often decreases viscosity of the functional yoghurt, can reduce the process yield in support to the present study of using functional clove—fortified yoghurt.

Lower inlet temperature (40–80 °C), resulted in higher process yield. A decreased moisture content will reduce the deposition on the wall of the chamber, and thus lower the process loss. But, an increase in inlet temperature above 80 °C resulted in the reduction of process yield, the above result matches to the present investigation [39]. Lower feed temperature will reduce the drying temperature to a certain extent and which will reduce the process yield. A study by Chang et al. [40] also demonstrates

Table 2 Experimental design (Box behnken) for functional yoghurt powder optimization with independent variables and experimental values of physiochemical, antimicrobial and encapsulation efficiency responses

Run	Factor 1 A:Inlet temperature	Factor 2 B:Feed temperature	Factor 3 C:Pump speed	Response 1 Water activity (a_w)	Response 2 Moisture content	Response 3 Process yield	Response 4 Zone of inhibition *K. pneumoniae*	Response 5 Zone of inhibition *P. aeruginosa*	Response 6 Encapsulation efficiency of *S. thermophilus*	Response 7 Encapsulation efficiency of *L. bulgaricus*	Response 8 Encapsulation efficiency of *L. cremoris*	Response 9 Colour difference (ΔE)
	°C	°C	%	–	%	%	mm	mm	%	%	%	–
1	140	10	20	0.45	14.33	3.7	15	10	65.47	34.12	66.02	16.58
2	140	15	15	0.38	13.52	3.6	16	10	53.95	25.6	72.3	17.39
3	170	10	15	0.41	10.1	4.71	19	12	42.55	13.64	46.65	18.4
4	140	20	10	0.39	9.32	4.7	15	9	43.21	14.32	57.28	17.1
5	140	20	20	0.47	14.08	3.77	16	9	63.2	35.84	64.11	17.31
6	170	15	10	0.36	7.27	3.34	20	12	21.19	6.16	45.83	18.83
7	110	15	10	0.40	10.73	5.1	10	8	72.8	27.32	78.99	15.98
8	140	15	15	0.38	11.1	3.53	15	9	58.92	23.88	72.01	15.88
9	110	20	15	0.50	16.22	4.73	11	8	69.39	32.44	81.83	15.91
10	110	10	15	0.47	15.69	5.21	10	8	71.36	33.44	86.54	16.55
11	140	15	15	0.35	11.1	3.53	16	10	56.3	24.56	72.01	16.18
12	170	15	20	0.40	11	3.59	20	12	59.83	27.32	48.99	18.06
13	170	20	15	0.38	10.26	4.78	20	12	39.28	11.96	47.48	18.51
14	110	15	20	0.52	17.5	2.29	12	8	67.65	40.96	90.02	16.4
15	140	10	10	0.40	8.53	5.33	15	10	45.83	14.68	58.92	17.9

Table 3 Experimental design (Box behnken) for functional yoghurt powder optimization with independent variables and experimental values of powder characters and reconstitution properties

	Factor 1	Factor 2	Factor 3	Response 10	Response 11	Response 12	Response 13	Response 14	Response 15	Response 16	Response 17	Response 18	Response 19	Response 20	Response 21
Run	A:Inlet temperature	B:Feed temperature	C:Pump speed	Drying rate	Hygroscopicity	Degree of caking	Bulk densities	Tapped densities	Particle density	Porosity	Flowability	Cohesiveness	Solubility	Dispersibility	Wettability
	C	C	rpm	Kg/h	g/100 g	%	kg/m^3	kg/m^3	kg/m^3	%	%	–	%	%	min
1	140	10	20	0.38	11.67	42	425	485.7	1322	63.26	12.5	1.14	43	59.59	4.28
2	140	15	15	0.3	14.02	53.68	380	441.7	1250	64.66	13.97	1.16	40	58.08	3.83
3	170	10	15	0.31	15.27	56.39	353	416.7	1150	63.77	15.29	1.18	38	47.91	3.92
4	140	20	10	0.24	15.62	55.9	260	320.8	1310	75.51	18.95	1.23	47	60.97	2.37
5	140	20	20	0.49	11.64	45	400	460.7	1320	65.1	13.18	1.15	42	52.88	4.09
6	170	15	10	0.25	14.25	51.7	265	325	1110	70.72	18.46	1.23	35	46.86	2.51
7	110	15	10	0.23	15.44	55	242	303.8	2500	87.85	20.34	1.26	58	80	2.13
8	140	15	15	0.32	15.87	57	280	340.8	1300	73.78	17.84	1.22	40	61.42	3.98
9	110	20	15	0.29	13.77	48.26	246	301.9	2275	86.73	18.52	1.23	55	81.2	3.47
10	110	10	15	0.3	13.78	47.05	254	313.3	2250	86.08	18.93	1.23	60	86.37	3.63
11	140	15	15	0.32	16.87	59.02	326	388.5	1700	77.15	16.09	1.19	48	53.33	3.82
12	170	15	20	0.5	15.73	55.11	402	460.8	1115	58.67	12.76	1.15	33	47.5	4.15
13	170	20	15	0.46	15.92	55.71	350	410	1050	60.95	14.63	1.17	34	48.71	3.83
14	110	15	20	0.33	10.09	37.46	334	399.4	2500	84.02	16.37	1.2	56	84.13	3.76
15	140	10	10	0.23	14.02	52.47	257	311.5	1500	79.23	17.5	1.21	46	59.52	2.65

Table 4 Regression coefficient value of different responses in optimized functional yoghurt powder (physiochemical, antimicrobial and encapsulation efficiency)

Regression coefficients	Water activity (a_w)	Moisture content	Process yield	Zone of inhibition *K. pneumoniae*	Zone of inhibition *P. aeruginosa*	Encapsulation efficiency of *S. thermophilus*	Encapsulation efficiency of *L. bulgaricus*	Encapsulation efficiency of *L. cremoris*	Colour difference (ΔE)
	–	%	%	mm	mm	%	%	%	-
Intercept	0.3670	12.05	3.55	15.33	9.80	55.40	24.42	72.11	17.13
X_1-Inlet temperature	− 0.0433***	− 2.69***	− 0.1138***	4.50***	2.00***	− 14.79***	− 9.38***	− 18.55***	1.12***
X_2-feed temperature	0.0017	0.1537	− 0.1213***	0.3750	− 0.2500	− 1.27*	− 0.1650	− 0.9288***	− 0.0750
X_3-Pump speed	0.0350***	2.63	− 0.6400***	0.3750	0.0000	9.14***	9.47***	3.52***	− 0.1825
X_1 X_2			0.1375***					1.39***	
X_1 X_3	− 0.0187*		0.7650***			10.95***		− 1.97***	
X_2 X_3			0.1750***						
X_1^2	0.0304**		0.2546***					− 1.05***	
X_2^2	0.0409**		1.05***					− 5.43***	
X_3^2	0.0204*		− 0.2279***					− 5.10***	
R^2	0.9527	0.8993	0.9994	0.9816	0.9448	0.9916	0.9715	1.0000	0.7178
Lack of fit	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant
Model	quadratic	linear	Quadratic	linear	linear	2FI	Linear	Quadratic	linear

***p value ≤ 0.001, **p value ≤ 0.01, *p value ≤ 0.05

that the increase in pump speed reduces the contact time between the drying air and feed, which increases chamber wall deposition and causes a reduction in process yield.

Linear model fitted to colour difference was significantly increased (*P ≤ 0.001*) with increasing inlet air temperature. R^2 value observed was 0.7178 with a non-significant lack of fit and colour difference ranging from 16.18 to 18.83. To assess the overall colour changes in the yoghurt powder samples under various treatments with commercial yoghurt, the colour difference (ΔE) was employed [41]. Higher inlet temperature cause production of darker powder than low inlet temperature [42]. According to Choobkar et al. [43] clove powder had a linear significant effect on the brightness (L^* value) ($p < 0.05$) on functional product formulation. Our current study of increased colour difference in functional yoghurt powder produced from functional clove—fortified yoghurt was supported by these findings.

Antibacterial effect of functional yoghurt powder

Linear model significantly (*P ≤ 0.001*) fitted to antibacterial zone of inhibition against *K. pneumoniae* and *P. aeruginosa* respectively with non-significant lack of fit. A significant (*P ≤ 0.001*) increase in the antibacterial zone of inhibition against *K. pneumoniae* and *P. aeruginosa* was observed with increasing inlet temperature. The respective regression equation for the model were expressed in Table 6. Antibacterial zones of inhibition against *K. pneumoniae* and *P. aeruginosa* range from 10–20 mm to 8–12 mm respectively. The combination of the natural antimicrobial compounds in clove and the effects of higher temperatures may result in synergistic antibacterial effects. The temperature-induced changes in the bacterial membrane or metabolic processes could enhance the overall antimicrobial activity of the functional clove-fortified yogurt powder.

Lactic acid bacteria possess unique inherent biological properties that contribute to their antibacterial activity against pathogens [44]. Significant protection of important probiotic characteristics such as pH resilience, bile resistance, antimicrobial efficacy, and cholesterol assimilation behaviour was observed in *Lactobacillus fermentum* even after freeze and spray—drying [45]. According to Gardiner et al. [46] spray—drying will not affect antibacterial activity, as shown by the presence of antimicrobial activity before and after spray—drying. All of these findings offered substantial support for our current investigation.

Table 5 Regression coefficient value of different responses in optimized functional yoghurt powder characters and reconstitution properties

Regression coefficients	Drying rate	Hygroscopicity	Degree of caking	Bulk densities	Tapped densities	Particle density	Porosity	Flowability	Cohesiveness	Solubility	Dispersibility	Wettability
	Kg/h	g/100g	%	kg/m^3	kg/m^3	kg/m^3	%	%	–	%	%	min
Intercept	0.3300	15.07	53.87	318.27	378.71	1386.00	73.17	16.36	1.2	45.00	57.97	3.89
X_1-Inlet temperature	0.0462***	1.01*	3.89**	36.75**	36.76**	− 637.5***	− 11.32***	− 1.63**	− 0.0238**	− 11.12***	− 17.59***	0.1775***
X_2-feed temperature	0.0325***	0.2762	0.8700	− 4.13	− 4.23	− 33.38	− 0.5063	0.1325	0.0025	− 1.13	− 1.20	− 0.0900**
X_3-Pump speed	0.0938***	− 1.28**	− 4.44**	67.13***	68.19***	− 20.38	− 5.28**	− 2.56***	− 0.0363**	− 1.50	− 0.4063	0.8275***
X_1 X_2	0.0400***											
X_1 X_3	0.0375**	1.71**	5.24**									
X_2 X_3	0.0250*											
X_1^2						357.75***					7.36***	− 0.1888***
X_2^2												
X_3^2		− 1.51*	− 4.54**									− 0.5538***
R^2	0.9822	0.8381	0.8754	0.8096	0.8051	0.9489	0.8734	0.8208	0.7879	0.9207	0.9686	0.9958
Lack of fit	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant	Non significant
Model	2FI	Quadratic	Quadratic	linear	linear	Quadratic	Linear	Linear	linear	linear	Quadratic	Quadratic

*** p value ≤ 0.001, **p value ≤ 0.01, *p value ≤ 0.05

Table 6 Regression equation of responses

Responses	Regression equations	Model suggested
Water activity	1.41—0.01* X_1—0.049* X_2 + 5e-05* X_3—0.0001* X_1 X_3 + 3.375e-05* X_1^2 + 0.002* X_2^2 + 0.001* X_3^2	Quadratic
Moisture content (%)	16.24—0.09* X_1 + 0.03* X_2 + 0.53* X_3	Linear
Process yield (%)	33.52—0.17* X_1—1.52* X_2—0.67* X_3 + 0.0009* X_1 X_2 + 0.005* X_1 X_3 + 0.01* X_2 X_3 + 0.0003* X_1^2 + 0.042* X_2^{2-} 0.009* X_3^2	Quadratic
Zone of inhibition *K. pneumoniae* (mm)	− 7.92 + 0.15* X_1 + 0.08* X_2 + 0.08* X_3	Linear
Zone of inhibition *P. aeruginosa* (mm)	1.22 + 0.07* X_1—0.05* X_2—3.92523e-18* X_3	Linear
EE of *S. thermophilus* (%)	254.08—1.59* X_1—0.25* X_2—8.39* X_3 + 0.07* X_1 X_3	2FI
EE of *L. bulgaricus* (%)	40.30—0.31* X_1—0.03* X_2 + 1.89* X_3	Linear
EE of *L. cremoris* (%)	25.12—0.23* X_1 + 5.04* X_2 + 8.65* X_3 + 0.01* X_1 X_2—0.01* X_1 X_3—0.001* X_1^2—0.22* X_2^2—0.20* X_3^2	Quadratic
Colour difference (Δe)	12.68 + 0.04* X_1—0.02* X_2—0.04* X_3	Linear
Drying rate (kg/h)	1.05—0.006* X_1—0.05* X_2—0.03* X_3 + 0.0003* X_1 X_2 + 0.0003* X_1 X_3 + 0.001* X_2 X_3	2FI
Hygroscopicity (g/100 g)	23.63—0.14* X_1 + 0.06* X_2—0.03* X_3 + 0.01* X_1 X_3—0.06* X_3^2	Quadratic
Degree of caking (%)	78.85—0.39* X_1 + 0.17* X_2—0.32* X_3 + 0.03* X_1 X_3—0.18* X_3^2	Quadratic
Bulk densities (kg/m^3)	− 42.23 + 1.23* X_1—0.83* X_2 + 13.43* X_3	Linear
Tapped densities (kg/m^3)	15.26 + 1.23* X_1—0.85* X_2 + 13.64* X_3	Linear
Particle density (kg/m^3)	12,313.2—132.55* X_1—6.68* X_2—4.08* X_3 + 0.40* X_1^2	Quadratic
Porosity (%)	143.36—0.38* X_1—0.10* X_2—1.06* X_3	Linear
Flowability (%)	31.22—0.05* X_1 + 0.0* X_2—0.51* X_3	Linear
Cohesiveness	1.41—0.001* X_1 + 0.001* X_2—0.01* X_3	Linear
Solubility (%)	104.79—0.37* X_1—0.23* X_2—0.3* X_3	Linear
Dispersibility (%)	305.28—2.88* X_1—0.24* X_2—0.08* X_3 + 0.01* X_1^2	Quadratic
Wettability (min)	− 8.25 + 0.07* X_1—0.02* X_2 + 0.83* X_3—0.0002* X_1^2—0.02* X_1^2	Quadratic

EE of probiotics in functional yoghurt powder

Significant ($P \leq 0.01$) linear and quadratic models with high F values were used to fit the *L. bulgaricus* and *L. lactis* EE, respectively. Similarly, EE of *S. thermophilus* was fitted to 2FI model. Regression equation expressed for corresponding models were given in Table 6. EE of *S. thermophilus, L. bulgaricus* and *L. lactis* studied were significantly decreases ($P \leq 0.001$) with increasing inlet temperature and the EE of all three strains was also significantly decreases ($P \leq 0.001$) with decreasing pump speed. EE of *S. thermophilus,* and *L. lactis* were significantly decreased ($P \leq 0.01$, $P \leq 0.001$) with increasing feed temperature (Fig. 1). EE of probiotics *S. thermophilus*, *L. bulgaricus* and *L. lactis* ranged from 21.19–72.8 (%), 6.16–40.96 (%) to 45.83–90.02 (%), respectively. Adjusting parameters within a range that balances drying efficiency with the preservation of probiotic viability is crucial for achieving desirable encapsulation outcomes.

According to Ali et al., [10] the viability of *L. bulgaricus* is more significantly impacted by spray—drying compared to *S. thermophilus*. Moreover, increasing inlet temperatures notably decrease the survival rate in yoghurt powder. The viability of probiotic yoghurt was evaluated after spray—drying, using an inlet temperature of 170 °C ± 2 °C and an outlet temperature of 80 °C to 85 °C. The probiotic counts of the dried yoghurt were found to be 3.4×10^8 CFU/g for *Lactobacillus paracasei* NFBC 338, 1.2×10^8 CFU/g *for Streptococcus thermophilus*, and 4.0×10^5 CFU/g for *Lactobacillus bulgaricus* [47]. In concordance with our current findings higher EE of *L. lactis*, when comparing to yoghurt strains on spray—drying at ~ 130 °C was also reported by Martins et al. [35]. However, higher and lower survivability of LAB during spray—drying associated visibly with the collective toughness under oxidative and heat stress [48].

Powder properties of functional yoghurt powder

Different spray—drying conditions such as inlet temperature, flow rate and feed temperature will affect the powder characteristics of a functional yoghurt powder [8]. Table 5 (Fig. 2) revealed a significant ($P \leq 0.001$) increase in the drying rate as the pump speed and feed temperature were increased. Contrarywise, a decrease in the drying rate was noted with an increase in the inlet temperature. The drying rate, ranges from 0.23–0.5 (kg h^{-1}) in the present study. Table 6 represented the 2FI model and its regression equation for drying rate. Layusa et al. [28] reported the significant effect of pump speed on drying rate, but the non-significant effect of inlet temperature on the dry rate was inconsistent to our present findings. Directly proportional effect of feed temperature and pump speed to drying rate support of the current study. Increased pump speed cause faster feed flow, thereby shorter drying time [49]. Feed temperature, mainly affects its viscosity. Increasing feed temperature will reduce the viscosity of the feed, hence feed flow will be increased with less time requirement to finish the drying [28, 50].

The significant (*P* ≤ *0.01*) increase and decrease of hygroscopicity was observed with increasing pump speed (16%) and significantly decreases with increasing inlet temperature with non-significant (*P* ≤ *0.05*) lack of fit was presented in Table 5 (Fig. 2). Hygroscopicity, of functional yoghurt ranges from 10.09 to 16.87 (g/100 g). Table 6 represents the quadratic model and its regression equation for hygroscopicity. It represents the capacity of functional yoghurt powder to absorb moisture and can affect the shelf life. The hygroscopicity was 39 g/100 g on spray—drying without carrier and significantly decrease with carrier [51]. Nurhadi et al. [52] findings were in supportive to our current study that the acceptable of hygroscopicity should be < 20 g/100 g for powder. Higher inlet temperatures accelerate the drying process by promoting faster evaporation of water from the yogurt. This can lead to the formation of a powder with a more crystalline or less porous structure. A less porous structure may reduce the available surface area for water absorption, resulting in decreased hygroscopicity. In other words, the powder becomes less prone to absorbing moisture from the surrounding environment.

The significant (*P* ≤ *0.01*) decrease of degree of caking was observed with increasing inlet temperature and pump speed (Fig. 2) with non-significant lack of fit was presented in Table 5. Degree of caking of functional yoghurt ranges from 37.46 to 59.02 (%). Table 6 represents the quadratic model and its regression equation for degree of caking. Caking is a main problem reported during yoghurt powder processing and its degree was 30.32% [8]. Krasaekoopt and Bhatia [53] mentioned the application of anti-caking agent (silicon dioxide) in yoghurt powder to improve shelf-life. Yoghurt powder is less hygroscopic and slightly higher degree of caking as seen in Table 3. According to Tiago et al. [54] sudden temperature change of the feed can cause rapid percolation and caking, where caking also depends on matrix properties, and particle size which clarifies our findings. Higher inlet temperatures and increased pump speeds generally lead to more efficient drying processes. As the drying process becomes more effective, the moisture content of the yogurt powder decreases. Reduced moisture content is a key factor in preventing caking, as moisture is a major contributor to the agglomeration and sticking of powder particles.

Bulk, and tapped densities response significantly (*P* ≤ *0.01*) fitted to linear model and particle density significantly (*P* ≤ *0.001*) fitted to the quadratic model. Significant

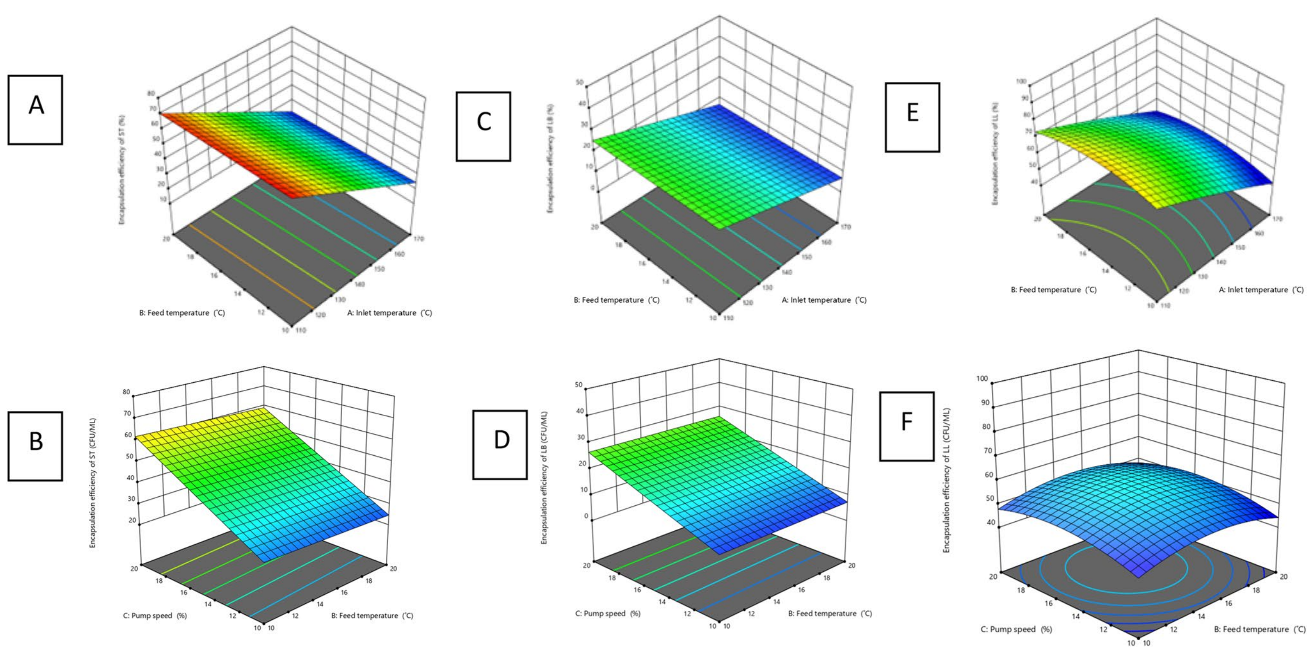

Fig. 1 Response surface plot for the effect between, (**A**) feed temperature and inlet temperature, **B** feed temperature and pump speed on encapsulation efficiency of *S. thermophilus*, **C** feed temperature and inlet temperature and **D** feed temperature and pump speed on encapsulation efficiency of *L. bulgaricus*, **E** feed temperature and inlet temperature and **F** feed temperature and pump speed on encapsulation efficiency of *L. cremoris*

change was observed in bulk and tapped densities with varying inlet temperature, and pump speed with non-significant lack of fit (Table 3). The regression equations for bulk, tapped and particle densities were expressed in Table 6. Response of bulk densities, tapped densities, particle density, and porosity, range from 242–425 (kg m^{-3}), 301.9–485.7 (kg m^{-3}), 1050–2500 (kg m^{-3}), to 58.67–87.85 (%) respectively in the 17 runs. Bulk and tapped densities significantly increase with increasing inlet temperature ($P \leq 0.01$) and pump speed ($P \leq 0.001$), however particle density significantly ($P \leq 0.001$) decreases with increasing inlet temperature (Fig. 3). Higher inlet temperatures during the drying process can lead to more effective water evaporation, resulting in a reduction in the volume of the powder particles. This reduction in volume contributes to an increase in both bulk and tapped densities. The decrease in particle density with increasing inlet temperature may be due to the reduction in the actual mass of the particles as water evaporates during drying. While bulk and tapped densities account for the occupied volume, particle density considers the actual mass of the powder particles. Higher pump speeds can result in finer and more compact powder particles. The increased energy input during drying, facilitated by higher pump speeds, may lead to the formation of particles with reduced void spaces, thereby increasing both bulk and tapped densities. According to Seth et al. [30] bulk density of yoghurt powder ranges from 344.80 to 475.41 kg m^{-3} and increases with increasing inlet temperature. Also, a similar trend was followed for tapped density (551.72–782.50 kg m^{-3}), but an increase in inlet temperature was studied with decreased particle density (1187.50–1666.67 kg m^{-3}). The size and shape of the particles in the herbal functional yoghurt powder can also contribute to its lower bulk and tap density. Herbal extracts formulation may cause finer particle sizes or irregular shapes, leading to increased air space between particles. This air space may reduce the overall density of the powder, which support our findings [55]. Tonon et al. [56] also provided similar results in juice powder drying, which is in line with our findings. However, decreasing effect of bulk density with feed rate was reported by Seth et al. [30] in their studies. Bulk density of yoghurt powder also depends on increased total solids content and decreased moisture [57].

The significant ($P \leq 0.001$) decrease of porosity was observed with increasing inlet temperature and significantly decreases with increasing pump speed ($P \leq 0.01$) (Fig. 3) with non-significant lack of fit was presented in Table 5. The reason for this may be attributed to variations in the drying process dynamics. Higher inlet temperatures could lead to more rapid evaporation and a denser material structure, while an increase in pump speed may affect the material's residence time and drying efficiency, potentially influencing porosity. Table 6 represents the linear model and its regression equation for porosity. Amorphous spray dried powder, with different particle size increase free space, which affect bulk density, tapped density, porosity and flowability [8]. According to Santhalakshmy et al. [58] porosity ranges from 90.77 to 92.60%

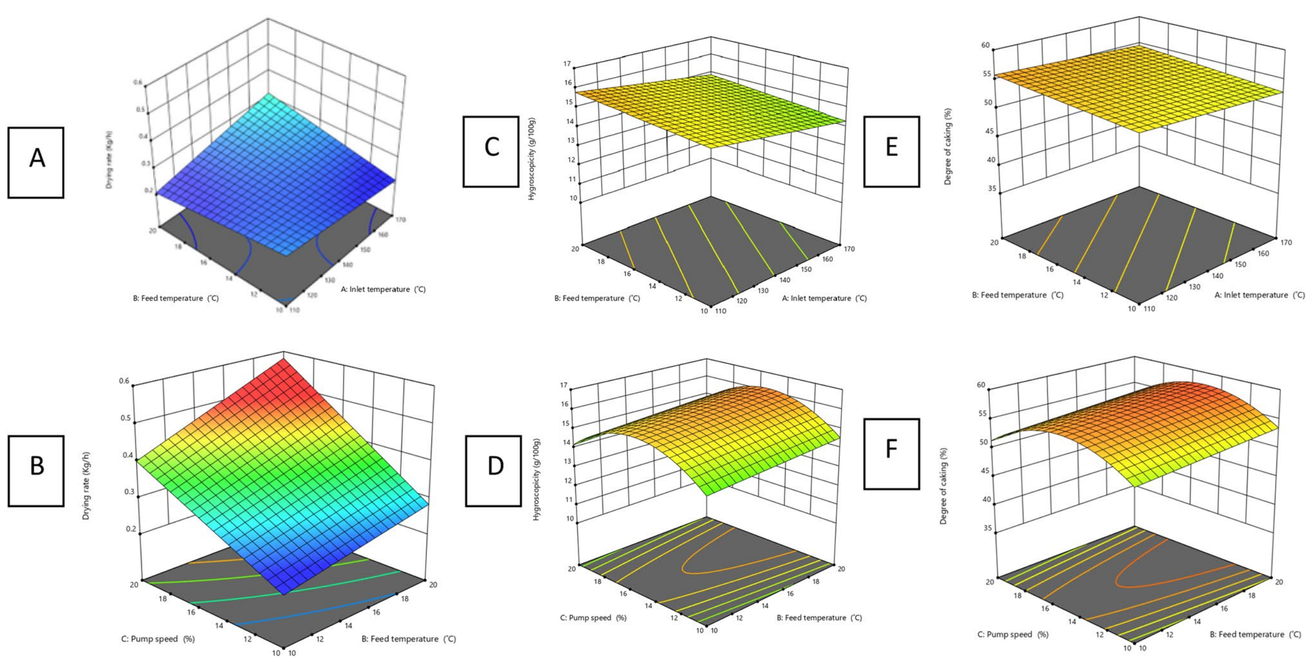

Fig. 2 Response surface plot for the effect between, **A** feed temperature and inlet temperature, **B** feed temperature and pump speed on drying rate, **C** feed temperature and inlet temperature and **D** feed temperature and pump speed on hygroscopicity, **E** feed temperature and inlet temperature and **F** inlet temperature and pump speed on degree of caking

for jamun juice this increased porosity represents more space between particles with air, which support of the present study.

The significant (*P ≤ 0.01*) decrease of flowability and cohesiveness was observed with increasing inlet temperature and increasing pump speed (Fig. 4) with non-significant lack of fit was presented in Table 5. Higher inlet temperatures and increased pump speeds generally lead to more efficient drying, resulting in lower moisture content in the yogurt powder. While low moisture content is crucial for powder stability, excessively low moisture levels can contribute to decreased flowability and cohesiveness, making the powder more prone to becoming dry, fine, and less cohesive. Higher temperatures and increased pump speeds may lead to the formation of finer powder particles. Fine particles often exhibit poorer flow properties and are more susceptible to cohesive behavior due to increased surface forces and reduced interparticle spaces. Flowability and cohesiveness, ranges from 12.5–20.34 (%) to 1.14–1.26, accordingly in functional yoghurt powder. Table 6 represented the linear model and its regression equation for flowability and cohesiveness. Factors that influence flowability were particle size, hygroscopicity, and moisture content of the powder. The flowability and cohesiveness of sweetened yoghurt powder, fell within the range of 25.74% to 47.60% and 1.39 to 1.90, respectively. A significant rise in both flowability and cohesiveness values ($p < 0.01$) was observed as the inlet air temperature increased [30]. Yoghurt powders exhibit moderate to poor flowability (27.93%) attributed to their small particle size [8]. Flowability and cohesiveness of powder with good properties should be less than 38% and 1.6 respectively [59]. Hence, our functional yoghurt powder produced was inline of better flowability and cohesiveness.

Reconstitution properties of functional yoghurt powder

The significant (*P ≤ 0.001*) decrease of solubility and dispersibility was observed with increasing inlet temperature

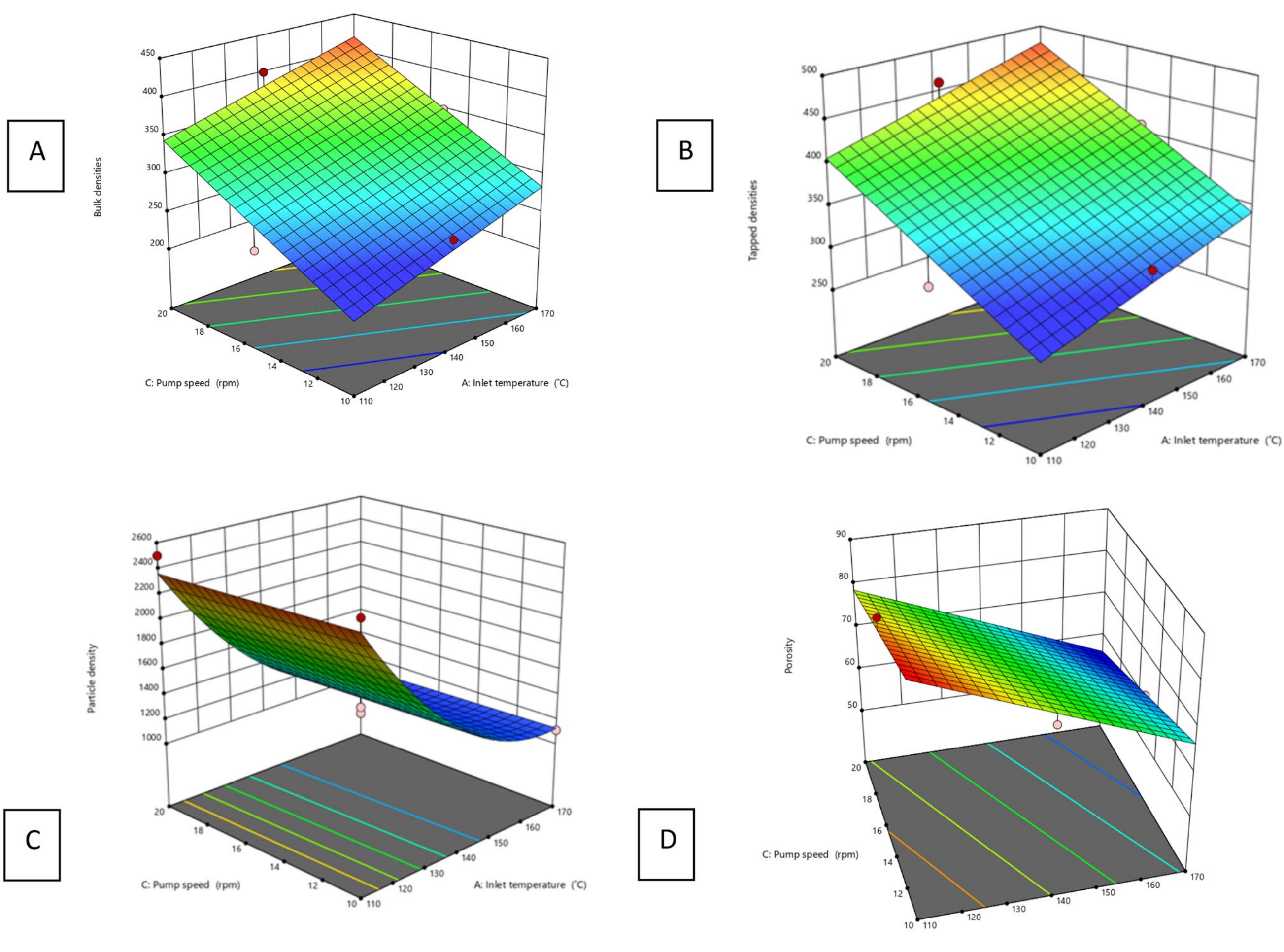

Fig. 3 Response surface plot for the effect between inlet temperature, and pump speed on **A** bulk densities, **B** tapped densities, **C** particle density, and **D** porosity

(Fig. 5) with non-significant lack of fit was presented in Table 5. Higher inlet temperatures may lead to faster drying rates, resulting in the formation of smaller and more compact particles. However, in some cases, the increased temperature might also contribute to the formation of insoluble agglomerates. These agglomerates may reduce the solubility and dispersibility of the powder in water. Table 6 represented the linear and quadratic models with corresponding regression equation for solubility and dispersibility, respectively. Solubility, dispersibility and wettability, range from 33 to 60 (%), 46.86–86.37 (%), 2.13–4.28 (min) accordingly.

Koc et al. [8] studied yoghurt powder solubility as 65.0–72.5%. The solubility of dairy powders varies according to drying and storage temperature. Seth et al. [30] reported that the solubility of yoghurt powder significantly reduced with an increase in inlet temperature due to an increased rate of protein denaturation. Similar findings were reported and the reducing solubility trend at higher inlet temperatures may be due to larger particle size on higher temperatures [8]. Also reported that the higher agglomeration of powder at low inlet temperature, can result in better solubility [60]. According to our study functional yoghurt powder dispersibility ranges between 46.86 and 86.37 (%). Denaturation of casein protein at increased inlet temperature cause reduced dispersibility. This dispersibility result of yoghurt powder was supported by the findings of Seth et al. [30] (70.62–88.74%) with quadratic model affected by inlet temperature.

The significant ($P \leq 0.001$) increase of wettability was observed with increasing inlet temperature and pump speed with a non-significant lack of fit was presented in Table 5. Wettability was significantly decreases with feed temperature (Fig. 5). The quadratic model for wettability, along with its regression equation, was presented in Table 6. Highly soluble powder required less time to sink with lower wettability. Wettability of yoghurt powder ranges from 2.2 to 6.3 min according to Seth et al. [30] and increases inlet temperature with increasing wettability ($p < 0.001$). These

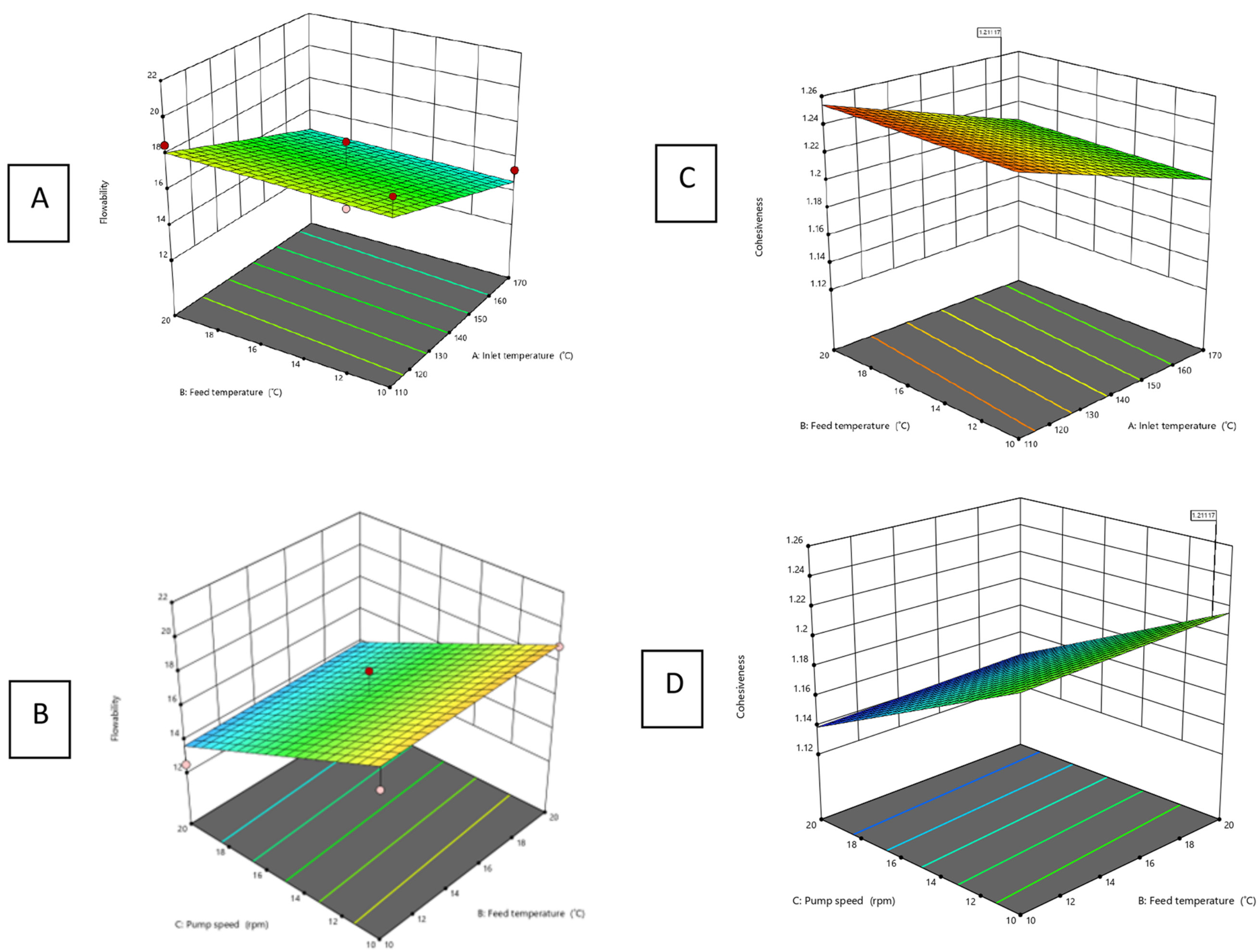

Fig. 4 Response surface plot for the effect between, **A** feed temperature and inlet temperature, **B** feed temperature and pump speed on flowability, **C** feed temperature and inlet temperature and **D** feed temperature and pump speed on cohesiveness

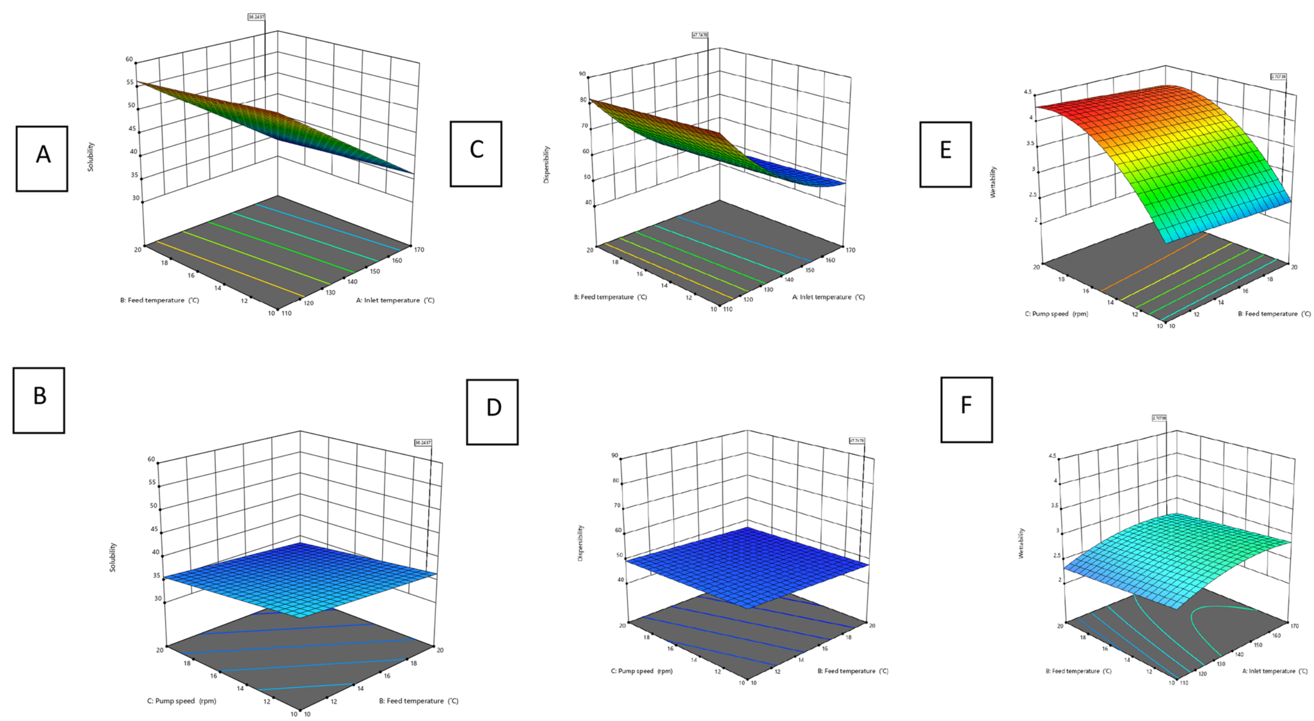

Fig. 5 Response surface plot for the effect between, **A** feed temperature and inlet temperature, **B** feed temperature and pump speed on solubility, **C** feed temperature and inlet temperature and **D** feed temperature and pump speed on dispersibility, **E** feed temperature and inlet temperature and **F** inlet temperature and pump speed on wettability

results were in line with our present study. Kim et al. [61] reported that wettability varies with particle size and porosity. Yoghurt powder wettability ranges from 2 to 4 min at different total solid content (14–25%) [57]. Increased wettability (10–5 min) with increased in inlet temperatures, and pump speed. Also reported that the lower dissolution cause poor wettability [60].

Optimization and development of functional yoghurt powder

All the responses (water activity, moisture content, process yield, colour difference, antibacterial effect, encapsulation efficiency (EE) of probiotics, drying rate, hygroscopicity, degree of caking, bulk densities, tapped densities, particle density, porosity, flowability, cohesiveness, solubility, dispersibility and wettability) were given importance and goal based on their impact on the quality and acceptability of functional yoghurt powder to optimise the level of independent variables according to Atalar and Dervisoglu [1] (Table 7). Table 8 lists the criteria employed along with expected and actual results. The model's optimal values were 164.04 °C (164 °C) for inlet temperature, 20 °C for feed temperature, and 10.72% (11%) for pump speed. The desirability of functional yoghurt powder made with probiotics and cloves aqueous extract, under ideal process conditions, was 72.6%. The measured responses were nearly matched by the levels that were predicted (Table 8).

Analysis of optimized functional yoghurt powder

Scanning electron microscopy (SEM)

The optimized functional yoghurt powder morphology at 10 kV, with magnification 1000X, and 2500X using SEM were given at Fig. 6. The functional yoghurt powder particles were observed in a spherical shape with some craters. Similar studies also reported spherical shape of yoghurt powder with a porous surface that improves wetting and solubility [8]. Irregular, crater, and wrinkled surface are due to immediate drying on spray—dryer [51].

Storage viability of optimized functional yoghurt powder

Spray-drying is used as a vital tool to preserve probiotic cultures, but its viability was affected by storage temperature. Stress due to sudden temperature change during and after drying can damage viable cells [62]. Better viability in yoghurt powder was expected at 4 °C storage [63]. Table 9 represents the viability of *S. thermophilus*, *L. bulgaricus* and *L. lactis* in functional yoghurt powder during

49 days of storage at 4 °C (> log 6 CFU g^{-1}). Significant ($p < 0.05$) reduction in viability was observed for all the three strains every week. Similarly, a significant decrease of viability was studied at 15 °C, with > log 6 CFU mL^{-1} after 42 days of storage, which meets probiotic requirement of food. *S. thermophilus* was also reported with better viability on storage than *L. bulgaricus* at 4 °C with a significant loss on storage due to membrane lipid oxidation of *L. bulgaricus* during drying and storage [47]. These findings were in accordance with the present study of functional yoghurt powder.

Conclusion

Interactive effects of inlet temperature, feed temperature, and pump speed in development of probiotic functional yoghurt powder using Box-Behnken design were studied. An optimized condition with 164.04 °C (164 °C) for inlet temperature, 20˚C for feed temperature, and 10.72% (11%) for pump speed was achieved with the desirability of 72.6%. The current work contributes to the development of probiotic clove—incorporated functional yoghurt powder with better viability, shelf life, powder properties, reconstitution ability, and broad-spectrum antibacterial activity against *K. pneumoniae* and *P. aeruginosa*. The SEM analysis indicated the clear morphology of functional yoghurt powder at different magnifications. Improved storage viability at low temperature (4 °C) along with health benefits opens the idea for further application of functional yoghurt powder in other food commodities. The spray-dried functional yoghurt powder was successfully produced under optimal conditions, resulting in improved physiochemical properties, encapsulation ability of probiotics, and powder characteristics. Positive aspects include improved shelf life, convenience in storage and transportation, enhanced flavor retention, and potential cost savings. Changes in texture or taste compared to fresh yogurt, potential nutrient losses during the drying process, or other factors that could impact overall product quality. Aspects such as sample size, experimental conditions, or constraints in resources were some of the probable limitations. Exploring different additives, or investigating novel methods for reconstituting the powder into yogurt can be further studied. However, further research is required to assess its value addition in the food industry.

Table 7 Optimization of independent variables and responses for functional yoghurt powder production

Constraints	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
X_1:Inlet temperature (°C)	Maximize	110	170	1	1	5
X_2:Feed temperature (°C)	Maximize	10	20	1	1	5
X_3:Pump speed (%)	Minimize	10	20	1	1	5
Water activity	Is in range	0.346	0.517	1	1	5
Moisture content (%)	Minimize	7.27	17.5	1	1	5
Process yield (%)	Maximize	2.29	5.33	1	1	4
Zone of inhibition *K. pneumoniae* (mm)	Maximize	10	20	1	1	5
Zone of inhibition *P. aeruginosa* (mm)	Maximize	8	13	1	1	5
EE of *S. thermophilus* (%)	Is in range	21.19	72.8	1	1	5
EE of *L. bulgaricus* (%)	Is in range	8	10	1	1	5
EE of *L. cremoris* (%)	Is in range	45.83	90.02	1	1	5
Colour difference (ΔE)	Is in range	15.88	18.83	1	1	2
Drying rate (kg/h)	Maximize	0.23	0.5	1	1	2
Hygroscopicity (g/100 g)	Minimize	10.09	16.87	1	1	1
Degree of caking (%)	Minimize	37.46	59.02	1	1	1
Bulk densities (kg/m^3)	Is in range	242	425	1	1	1
Tapped densities (kg/m^3)	Is in range	301.9	485.7	1	1	1
Particle density (kg/m^3)	Is in range	1050	2500	1	1	1
Porosity (%)	Maximize	58.67	87.85	1	1	2
Flowability (%)	Is in range	12.5	20.34	1	1	2
Cohesiveness	Is in range	1.14	1.26	1	1	2
Solubility (%)	Is in range	11.28	47.49	1	1	2
Dispersibility (%)	Is in range	46.86	86.37	1	1	2
Wettability (min)	Minimize	2.13	4.28	1	1	2

Table 8 Optimum conditions, experimental and predicted value of response at optimum conditions

Optimum conditions	Coded levels	Actual levels
Inlet temperature (˚C)	0.80	164.04 (164)
Feed temperature (˚C)	1.00	20.00
Pump speed (%)	-0.80	10.72 (11)
Responses	Predictive values (D-72.6%)	Experimental values
Water activity	0.39 ± 0.00^a	0.36 ± 0.02^a
Moisture content (%)	7.80 ± 0.00^a	7.67 ± 0.34^a
Process yield (%)	4.37 ± 0.00^a	4.32 ± 0.26^a
Zone of inhibition *K. pneumoniae* (mm)	19.00 ± 0.00^a	18.33 ± 1.15^a
Zone of inhibition *P. aeruginosa* (mm)	11.43 ± 0.00^a	11.00 ± 0.00^a
EE of *S. thermophilus* (%)	27.16 ± 0.00^a	27.21 ± 1.35^a
EE of *L. bulgaricus* (%)	8.62 ± 0.00^a	8.29 ± 0.32^a
EE of *L. cremoris* (%)	45.83 ± 0.00^a	44.61 ± 2.47^a
Colour difference (ΔE)	18.11 ± 0.00^a	18.38 ± 0.98^a
Drying rate (kg/h)	0.30 ± 0.00^a	0.32 ± 0.01^a
Hygroscopicity (g/100 g)	14.97 ± 0.00^a	14.79 ± 0.66^a
Degree of caking (%)	54.74 ± 0.00^a	53.74 ± 1.26^a
Bulk densities (kg/m^3)	286.13 ± 0.00^a	285.33 ± 5.00^a
Tapped densities (kg/m^3)	345.57 ± 0.00^a	346.34 ± 5.09^a
Particle density (kg/m^3)	1088.94 ± 0.00^a	1089.00 ± 2.22^a
Porosity (%)	68.11 ± 0.00^a	68.19 ± 0.51^a
Flowability (%)	17.37 ± 0.00^a	17.62 ± 0.38^a
Cohesiveness	1.21 ± 0.00^a	1.21 ± 0.01^a
Solubility (%)	36.24 ± 0.00^a	37.08 ± 0.82^a
Dispersibility (%)	47.75 ± 0.00^a	47.00 ± 1.06^a
Wettability (min)	2.71 ± 0.00^a	2.86 ± 0.20^a

Results are expressed as mean ± standard deviation, values are means of triplicates (n = 3)

[a]means in the same rows followed by same uppercase letters were non-significant ($p < 0.05$) via pair t-test

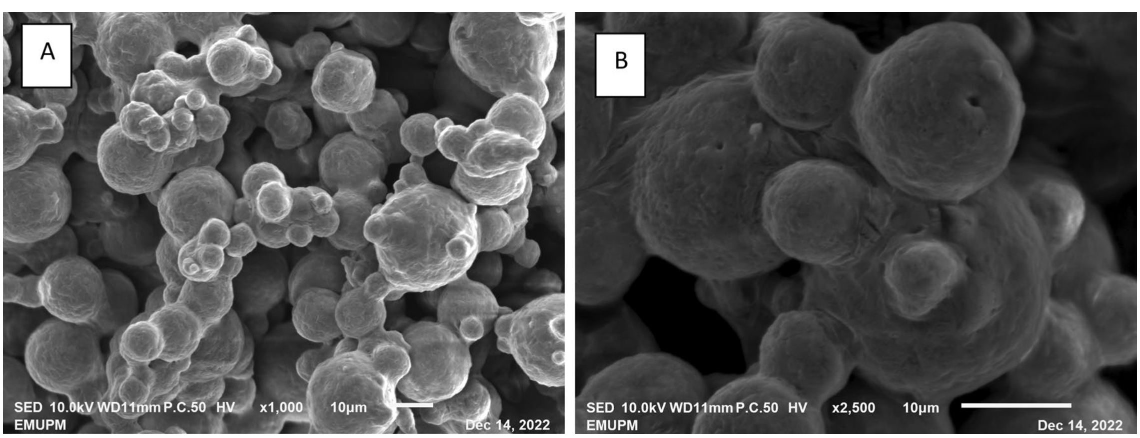

Fig. 6 Represents the Scanning electron microscopy (SEM) results on optimized functional yoghurt powder at **A** 1000× and **B** 2500× magnification

Table 9 Storage viability of optimized functional yoghurt powder

Storage duration (week)	Viability at log (CFU/g)		
	Streptococcus thermophilus	*Lactobacillus bulgaricus*	*Lactococcus cremoris*
0	10.65 ± 0.03^{A}	9.93 ± 0.01^{A}	10.08 ± 0.09^{A}
1	10.57 ± 0.01^{AB}	9.80 ± 0.02^{AB}	9.64 ± 0.16^{B}
2	10.50 ± 0.03^{B}	9.64 ± 0.03^{BC}	9.44 ± 0.03^{B}
3	10.39 ± 0.02^{C}	9.38 ± 0.04^{C}	9.14 ± 0.03^{C}
4	9.34 ± 0.04^{D}	8.10 ± 0.13^{D}	8.84 ± 0.07^{D}
5	9.30 ± 0.05^{D}	7.99 ± 0.15^{D}	8.63 ± 0.05^{D}
6	9.11 ± 0.04^{E}	7.90 ± 0.14^{D}	8.10 ± 0.17^{E}
7	8.88 ± 0.03^{F}	6.71 ± 0.12^{E}	8.16 ± 0.10^{E}

Results were expressed as mean ± standard deviation, values are means of triplicates (n = 3)

^{ABCDEF}means in the same columns followed by different uppercase letters are significantly different ($p < 0.05$) via ANOVA and Tukey's post hoc test

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Data Availability Data will be provided in accordance with genuine requests to the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest.

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