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Optimization of spray drying conditions for microencapsulation of *Lactobacillus casei* Shirota using response surface methodology

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ABSTRACT

The study aimed to determine the process conditions for microencapsulation of *Lactobacillus casei* Shirota by spray drying with reconstituted skim milk : gum arabic mix (rate constant 3:1 w/w) as encapsulating agent and to evaluate the physical properties of spray dried powder and the cell stability under gastrointestinal conditions and storage. Air inlet temperature had a major effect on the cell survival, product yield, a_w and L^* values, but the concentration of feed solution was the only significant factor on product yield, and pump rate effected a_w and L^* values ($P<0.05$). According to desirability result (0.812), the model for microencapsulation of probiotic bacteria by spray drying was obtained at following conditions: feed solution concentration of 21.16%, air inlet temperature of 119.55ºC and pump rate of 40%. Under optimized conditions, the predicted values close to the experimental values depend on deviation values except a_w. The morphological and physicochemical characteristics of the powders produced were acceptable. For the microencapsulated cells, the cell viability was detected as 7.12 log cfu/g after *in vitro* gastrointestinal treatment. The number of microencapsulated cells decreased by 1.11 and 1.77 log cycles during storage at 4 and 24 ºC, respectively.

1. Introduction

Probiotics have received increased interest by consumers due to their proven health benefits have been reported in the literature, such as improving immunity, reducing serum cholesterol, alleviating lactose intolerance, and reducing risk factors for colon cancer owing to anticarcinogenic, antibacterial and antimutagenic effects (Arslan, Erbas, Tontul, & Topuz, 2015; Reyes, Chotiko, Chouljenko, & Sathivel, 2018). To exert these beneficial efficacies on the human host, it has been recommended that the foods containing probiotics must be at least 10^6 cfu per gram or per mL at the time of ingestion (Mandal, Puniya, & Singh, 2006). Moreover, Knorr (1998) reported that a daily intake of 10^8 to 10^9 cfu of probiotics would be helpful to humans. However, many factors such as oxygen, temperature, humidity, acidity and bile affect the survival of probiotics during food processing, storage and digestion (Ainsley Reid et al., 2005; Semyonov et al., 2010). Various solutions have been developed to improve the resistance of probiotics against these adverse environmental factors. Microencapsulation is one of the most efficient methods that can be used to enhance the viability during processing and long-term storage of foods, as well as stabilizes their metabolic activity before and after consumption (Martín, Lara-Villoslada, Ruiz, & Morales, 2015; Moumita, Das, Hasan, & Jayabalan, 2018; Yao et al., 2018). Microencapsulation can be defined as the process for packaging of solids, liquids or gaseous material as called core like probiotic bacteria in an encapsulating membrane as called shell (Malmo, La Storia, & Mauriello, 2011). Spray drying is the frequently used microencapsulation method for encapsulation of probiotic bacteria due to its advantages like production of flowable powders, rapid drying, ability to control particle size, easy scale up and continuous production (Rajam & Anandharamakrishnan, 2015). However, dehydration and thermal stress during spray drying of probiotic bacteria may cause a reduction in the bacterial cell survival due to cytoplasmic membrane damage (Rajam, Karthik, Parthasarathi, Joseph, & Anandharamakrishnan, 2012; Tantratian, Wattanaprasert, & Suknaisilp, 2018). Airflow configuration, spray drying temperature conditions, concentration of feed solution, concentration of the probiotics in the feed solution, the carrier materials used in the process also known as effective factors on the cell survival (Ranadheera, Evans, Adams, & Baines, 2015). Moreover, the viability of probiotics is greatly influenced by different factors during storage period and storage conditions such as storage temperature, moisture content, etc. (Reyes, Chotiko, Chouljenko, Campbell, et al., 2018; Tripathi & Giri, 2014). Therefore, the choice of appropriate spray drying conditions such as air inlet temperature, feed flow rate, atomizer speed and encapsulating material for microencapsulation of probiotic cells are very important issue to obtain a high level of cell viability during spray drying and also long-term storage. The aim of this study were to optimize the spray drying conditions to generate the desired powder quality with maximum cell viability of *Lactobacillus casei* Shirota using a central composite design (CCD) with response surface methodology, to characterize spray dried powder under optimized conditions, to investigate the tolerance of microcapsules under simulated gastrointestinal conditions and to evaluate the viability of the spray dried probiotic cell during storage at different temperature.

2. Materials and Methods

2.1. Materials

Reconstitute skim milk (RSM) obtained from Pınar Dairy Co. (İzmir, Turkey) and gum Arabic purchased from Sigma–Aldrich (Steinheim, Germany), which were used as wall material for microencapsulation. Lyophilized *L. casei* Shirota as a probiotic strain was obtained from the Food Control Laboratory Directorate, Erciyes University (Kayseri, Turkey). The de Man, Rogosa and Sharpe (MRS) agar and broth as culture media were supplied by Merck (Darmstadt,

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Germany). All other reagents used in the study were of analytical grade and obtained from Sigma–Aldrich (Steinheim, Germany).

2.2. Preparation of probiotic culture and drying media

Lyophilized probiotic cells were activated by culturing in MRS broth supplemented with sterilized 0.5 g/l L-cysteine at 37 °C for 16 h. Afterward, an aliquot of culture was transferred and re-cultured in 50 mL MRS broth at 37 °C for 24 h. The cells were harvested by refrigerated centrifuging (Nuve–Bench Top Centrifuge, NF 1200R, Ankara, Turkey) at 2,000 x *g* for 10 min at 4 °C and washed twice in peptone solution (0.1%). Finally, the cells were suspended in peptone water to obtain a solution containing ~10 log cfu/mL.

The feed solutions were prepared following the method reported by previous study (Gul, 2017). The mixture of RSM and guar gum (3:1, w/w) was used as drying media. The media was dissolved into sterile distilled water and heat treated at 80 °C for 30 min. After the cooling to room temperature, the bacterial solution was mixed with the feed solution to obtain a desired core-to-wall ratio of 1:1.5 (v/v)

2.3. Microencapsulation by spray drying

The microencapsulation process was performed using a laboratory scale spray dryer (Buchi Mini Spray Dryer B-290, Switzerland) at operating conditions according to the experimental design. The feed solutions containing probiotic bacteria were kept under magnetic agitation at room temperature and were fed into the spray chamber through a peristaltic pump with flow rate of 6 mL/min. The drying airflow rate and aspirator rate were adjusted to 601 l/h and 35 m^3/h, respectively. The collected powders were transferred into sterile high-density polyethylene bottles and stored at 4 °C.

2.4. Determination of cell viability

The cell viability after spray drying process was determined by using the method described by Semyonov et al. (2010). The spray dried powders (0.1 g) containing cells were dispersed in 9.9 mL a sterile solution of 0.1 M phosphate buffer. The dispersed samples were subjected to serial dilutions with 9 mL of sterile peptone solution (0.1%) and plated on MRS agar. The colonies were counted after incubation at 37 °C for 48 h under anaerobic conditions. The survival of cells at each of the samples tested was calculated according to the Eq. (1).

$$Survival\ (\%) = (N/N_0) * 100 \quad (1)$$

where N_0 is the number of bacteria before drying (log cfu/g), and N is the number of bacteria after the drying process (log cfu/g).

2.5. Water activity, color properties and product yield

Water activity (a_w) of spray dried powders was measured using a water activity meter (AquaLab, Series 4TE; Washington, USA) at 25 °C.

Color properties of spray dried powders were was measured by using a Minolta Chroma Meter CR-400 (Konica Minolta, Osaka, Japan) colorimeter equipped with D65 lightning and 2° of observation angle, previously calibrated. The CIE Lab color scale was employed to measure the color L^* (lightness to darkness), a^* (greenness to redness) and b^* (yellowness to blueness).

The product yield (PY) of spray dried powder was determined according to Eq. (2).

$$PY(\%) = 100 * M/M_0 \quad (2)$$

where M is the weight of spray dried powder and M_0 is the dry weight of total solid materials.

2.6. Morphology of microcapsules

The morphology of the microcapsules obtained under optimized conditions was performed by scanning electron microscope (JSM-7001F; Jeol, Japan). The microcapsules were placed on the electron microscopy stub using double-sided adhesive disc and then gold-coated with a vacuum sputtering coater. The images were examined at an accelerating voltage of 10 and 15 kV and magnification of 5,000 times.

The particle size of spray dried powders under optimized conditions was carried out by using powder feeder in a laser diffraction particle size analyzer (Mastersizer 3000; Malvern Instruments, UK).

2.7. Simulated gastrointestinal digestion test

The gastrointestinal resistance of free and encapsulated probiotic cells was carried out according to method described by Annan, Borza, and Hansen (2008). Before each experiment, simulated gastric (SGF) and simulated intestinal (SIF) fluids were freshly prepared. SGF prepared with 2 g/L NaCl containing 3 g/L pepsin and pH adjusted to 2.0 with 1 N HCl. One mL of free cell or 1 g of microcapsules were mixed with 10 ml of SGF and incubated for 0, 30, 60, 90, and 120 min at 37 °C with agitation at 100 rpm. After SGF treatment, samples were removed by centrifugation at 6,000 x g for 15 min and resuspended with 10 mL of SIF prepared by adding 0.1% pancreatin and 0.45% oxgall (Sigma) at pH 7.4 adjusted with 0.1 M NaOH. Samples or free cells were incubated at 37 °C for 120 and 180 min. Surviving bacteria were enumerated on MRS agar after incubation at 37 °C for 48 h under anaerobic conditions.

2.8. Storage stability

Spray dried microcapsules were stored at 4 and 24 °C for 8 weeks. Viability of the probiotic cell was assessed at 1, 2, 4 and 8 weeks of storage period. The viable *L. casei* Shirota was determined by enumeration on MRS agar, as described in Section 2.4. The plot of relative viability (log N_t/N_0) versus storage time (t, day) was fitted to a first-order reaction kinetics model as described by the Eq. (3).

$$lnN_t = -kt + lnN_0 \quad (3)$$

where N_t is the total viability of bacteria at time t (day) during storage, N_0 is the viability of bacteria at time zero, and k is the cellular viability loss specific rate (day^{-1}) at the two evaluated temperature.

2.9. Experimental design and data analyses

Response surface methodology was used to process optimization for the spray drying of *L. casei* Shirota. A Central Composite Design (CCD) was applied to optimize the spray drying conditions. Air inlet temperature, pump rate and concentration of feed solution (reconstitute skim milk:gum Arabic, 3:1, w/w) were selected as independent variables depend on literature and % survival, PY, a_w and color were the dependent variables. The levels of three independent variables were shown in Table 1. Each response variable was adjusted to a second-order polynomial model shown Eq. (4):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{33}X_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (4)$$

where b_0 is the intercept; b_1, b_2, and b_3 are linear; b_{11}, b_{22}, and b_{33} are quadratic; b_{12}, b_{13}, and b_{23} are interaction terms, and X_1, X_2, and X_3 are the independent variables.

Deviation (D) from the predicted and experimental means was calculated using Eq. (5) (Keivani Nahr et al., 2015):

$$D = X_2 - X_1/X_1 \quad (5)$$

where, X_1 refers the predicted optimal value and X_2 refers the experimental value.

Design-Expert® 6.0.8 (StatEase, Inc., Minneapolis, USA) was used for regression analysis, optimization procedure and to determine the best fitting models. The significant terms in the model were determined by analysis of variance (ANOVA), and to determine the adequacy of the optimization models, lack of fit test and determination of the regression coefficients were applied.

Table 1. Level and code of variables chosen for Central Composite Rotatable Experimental Design

Variable	Code	Coded levels				
		-α	**-1**	**0**	**+1**	**+α**
Concentration of feed solution (%)	X_1	10	14	20	26	30
Air inlet temperature (ºC)	X_2	100	116	140	164	180
Pump rate (%)	X_3	20	24	30	34	40

3. Results and Discussion

3.1. Survival (%)

The influence of independent variables on the survival of probiotic cells after spray drying is illustrated in Table 2. After spray drying, the survival of probiotic cells ranged from 85.74% to 94.45%. The highest survival rate was observed at 100 ºC inlet temperature, 20% feed solution concentration and 30% pump rate. The values of the regression coefficients indicate that only air inlet temperature had significant effect on the survival of cells (P<0.01; Table 3). The cell survival significantly decreased with increase in inlet temperature whereas it was slightly increased with increase in feed solution concentration (Figure 1a). The decrease of survival depend on increase inlet temperature may be due to cellular damages such as denaturation of informational macromolecules like DNA and RNA, lipid peroxidation, dehydration of cytoplasmic membranes, rupture and collapse of cell membrane due to water removal (Behboudi-Jobbehdar, Soukoulis, Yonekura, & Fisk, 2013). This result agrees with reported by Anekella and Orsat (2013) who reported that the cell survival decrease to 55% when the inlet temperature was raised to 130 ºC. The concentration of feed solution had a positive effect on the cell survival during spray drying. This protection is due to the presence of proteins in the GA that forms a protective layer of coating on probiotic cell (Arepally & Goswami, 2019). The similar result obtained by Lira de Medeiros, Thomazini, Urbano, Pinto Correia, and Favaro-Trindade (2014) who stated that the resistance of *L. paracasei* NFBC 338 during drying increase with utilization of GA and by Arepally and Goswami (2019) who found that the cell survival increase with increase in GA concentration.

3.2. Product yield (%)

The product yield was significantly affected by air inlet temperature and feed solution concentration as displayed in Table 3 (P<0.01). The highest product yield was obtained as 63.27% at 164 ºC air inlet temperature, 26% feed solution concentration and 24% pump rate. Product yield increased with increasing air inlet temperature up to 140 ºC (Figure 1b), because the droplets were drier when they hit the cyclone wall due to the higher temperature (Goula & Adamopoulos, 2012). Similarly, Behboudi-Jobbehdar et al. (2013) found that the highest spray-drying yield is obtained at high air inlet temperatures. The product yield slightly decreased when the air inlet temperature was higher than 140 ºC. Chegini and Ghobadian (2007) reported that the air inlet temperature had significant effect on the recovery of a spray dried sugar rich product and the product yield decrease by increasing the air inlet temperature. In a study carried out by Anekella and Orsat (2013), the air inlet temperature has a minimal role in powder recovery. The product yield significantly increased with increasing feed solution concentration up to 20% and then tended to decrease (P<0.05).

Table 2. Experimental design of spray drying process according to central composite rotatable design and obtained data for each response parameters

Run	X_1	X_2	X_3	Survival (%)	Product yield (%)	a_w	*L**	*a**	*b**
1	26	164	34	89.27	51.08	0.114	93.78	-1.17	7.35
2	14	164	24	92.46	43.11	0.091	93.56	-0.6	6.42
3	14	116	34	94.37	36.71	0.432	96.21	-1.09	7.79
4	14	116	24	93.75	37.36	0.288	95.77	-1.53	7.41
5	20	140	30	91.86	49.25	0.181	94.25	-1.36	6.75
6	20	140	30	90.32	50.23	0.169	94.24	-1.25	6.18
7	20	140	30	91.28	62.86	0.159	94.16	-1.28	6.72
8	20	140	20	91.87	61.35	0.128	93.92	-1.61	9.34
9	26	164	24	88.66	63.27	0.109	93.37	-1.46	7.69
10	14	164	34	86.75	49.07	0.091	93.04	-1.5	8.64
11	26	116	34	91.51	44.06	0.370	96.10	-1.38	7.93
12	20	140	30	91.48	54.65	0.119	94.92	-1.29	7.01
13	20	180	30	85.74	57.13	0.083	91.55	-1.4	7.71
14	20	140	30	93.48	52.63	0.180	95.08	-1.19	8.8
15	10	140	30	91.56	20.3	0.101	95.20	-1.19	7.41
16	26	116	24	89.84	40.79	0.300	95.23	-1.19	7.26
17	20	140	40	90.84	46.78	0.259	96.05	-1.37	6.82
18	20	140	30	87.75	60	0.121	95.22	-1.41	7.85
19	30	140	30	90.79	49.17	0.182	95.02	-1.38	9.33
20	20	100	30	94.44	36.34	0.411	96.14	-1.25	7.85

X_1, Concentration of feed solution (%); X_2, Air inlet temperature (ºC); X_3, Pump rate (%)

Table 3. ANOVA table showing the variables as linear, quadratic and interaction terms on each response

Variance source	df	Survival (%)		Product yield (%)		a_w		*L**		*a**		*b**	
		Sum of squares	*P* value	Sum of squares	*P* value	Sum of squares	*P* value	Sum of squares	*P* value	Sum of squares	*P* value	Sum of squares	*P* value
Model	9	80.50	0.0394*	1849.75	0.0042*	0.22	< 0.0001*	26.20	0.0001*	0.27	0.8305	3.20	0.9569
X_1	1	6.41	0.1567	486.25	0.0032*	1.16E-03	0.3501	0.012	0.8152	0.048	0.3828	0.75	0.4421
X_2	1	53.25	0.0013*	499.44	0.0030*	0.17	< 0.0001*	21.91	< 0.0001*	2.67E-03	0.8339	0.021	0.8967
X_3	1	1.49	0.4774	57.87	0.2144	0.014	0.0065*	1.67	0.0174*	1.87E-04	0.9557	0.13	0.7475
X_1X_2	1	3.75	0.2690	16.22	0.4987	1.07E-03	0.3684	0.18	0.3683	0.041	0.4194	5.00E-05	0.9949
X_1X_3	1	6.82	0.1453	25.36	0.4007	5.93E-04	0.4989	0.23	0.3179	0.039	0.4273	0.66	0.4725
X_2X_3	1	6.76	0.1470	9.80	0.5973	5.49E-03	0.0587	0.25	0.2948	0.091	0.2381	0.086	0.7922
X_1^2	1	0.029	0.9203	688.32	0.0010*	2.53E-05	0.8877	0.37	0.2079	0.015	0.6255	1.18	0.3392
X_2^2	1	1.67	0.4533	102.84	0.1076	0.021	0.0018*	1.19	0.0373*	4.51E-03	0.7853	0.092	0.7855
X_3^2	1	0.17	0.8089	0.086	0.9603	5.52E-03	0.0581	0.20	0.3516	0.024	0.5346	0.49	0.5336
Residual	10	27.35		329.26		0.012		2.06		0.58		11.76	
Lack of fit	5	9.15	0.7658	180.92	0.4164	8.06E-03	0.2294	0.91	0.5980	0.54	0.0037	7.27	0.3051
Pure error	5	18.20		148.34		3.99E-03		1.15		0.032		4.49	
Model fitting													
R^2		0.984		0.9108		0.9853		0.8013		0.5478		0.6982	
R^2_{adj}		0.9695		0.8305		0.9721		0.6225		0.1408		0.4266	
Pred-R^2		0.9648		0.4478		0.9052		-0.0304		-1.8926		-0.5527	
Adeq-precision		26.582		11.715		31.573		8.167		4.109		7.581	
C.V.		1.45		6.76		7.89		0.52		75.74		10.80	
PRES		34.26		78.6		0.013		12.84		0.32		4.11	

X_1, Concentration of feed solution (%); X_2, Air inlet temperature (°C); X_3, Pump rate (%)

3.3. Water activity (a_w)

The a_w value of spray dried powder was varied from 0.083 to 0.432 at different independent variables (Table 2). The lowest a_w value of powder after spray drying was recorded at 180 °C air inlet temperature, 20% feed solution concentration and 30% pump rate. It was observed that a_w value decreased with increasing air inlet temperature ($P<0.01$) and decreasing with pump rate ($P<0.05$; Figure 1c). Similar trend was observed by Arepally and Goswami (2019) who reported that a_w value decrease with increase in air inlet temperature. In another study (Behboudi-Jobbehdar et al., 2013), it was shown that spray dried products produced with the highest air temperature and lowest feed flow rate result in the driest formulations. In general, the a_w value of powder should be lower than 0.6 considering the microbial and chemical stability (Favaro-Trindade , 2010). The water mobility reduces because of the matrix moves from the rubbery state toward the glassy state at low a_w value. Thus cell metabolic activity of microencapsulated bacteria slows down, leading to extended shelf life (Behboudi-Jobbehdar et al., 2013). According to our results, obtained spray dried powder is within the recommended limit to ensure microbiological and chemical stability. On the other hand, low a_w values are prerequisites for the commercial production of spray-dried powders with good handling characteristics, such as low tackiness and agglomeration, high fluidity, as well as for maximum probiotic viability (Hernández-López et al., 2018).

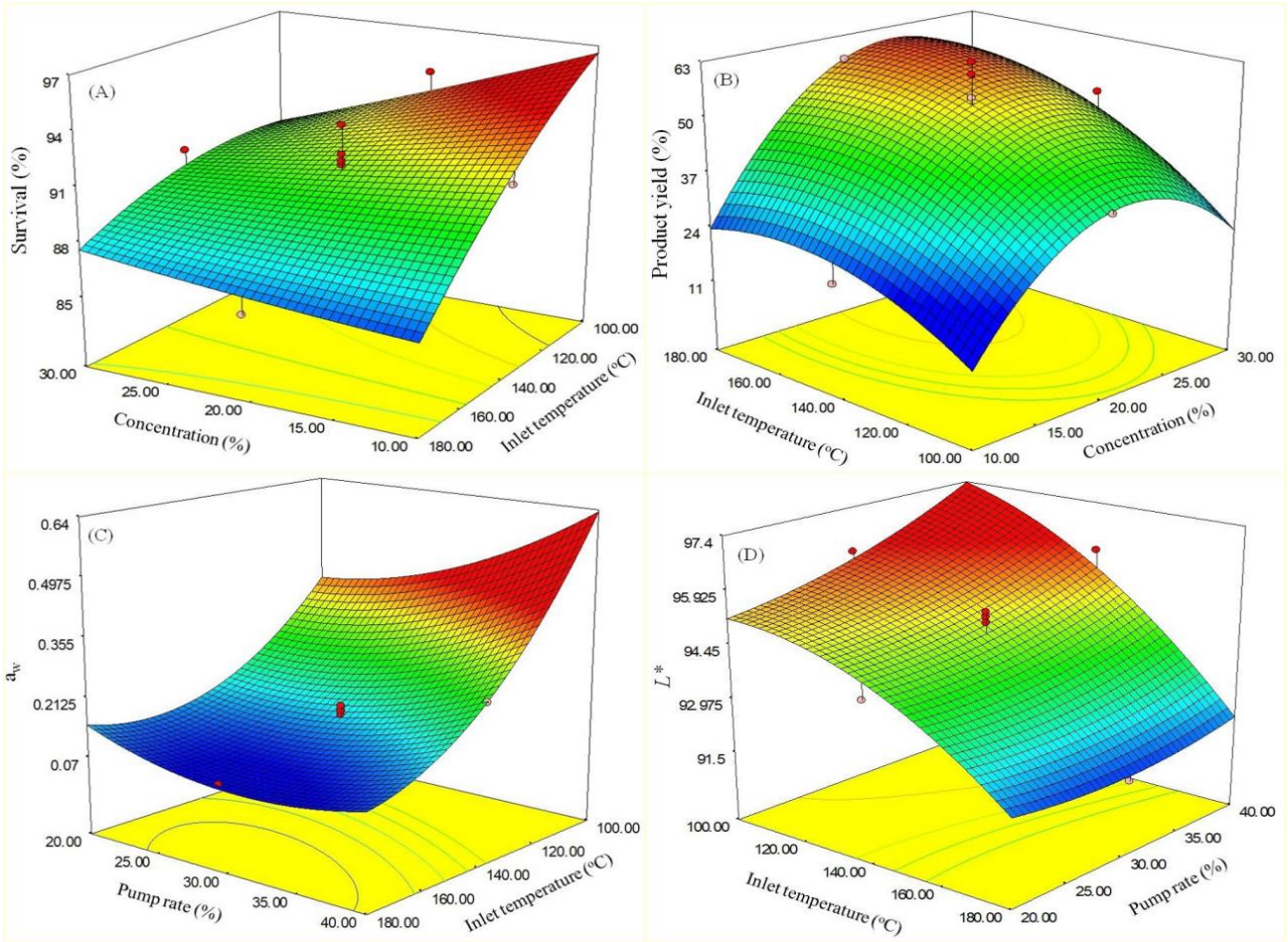

Figure 1. Response surface plots (3D). The effects of independent variables on survival (%) (a), product yield (%) (b); a_w value (c) and *L** value (d) after spray drying

3.4. Color properties

L^*, a^* and b^* values of spray dried powder determined individually were shown in Table 2. Although the color values were significantly affected by the inlet air temperature and pump rate ($P<0.05$), the a^* and b^* values were not evaluated due to model incompatibility ($P>0.05$; Table 3). As expected, L^* values of powder (ranged from 91.55 to 96.8) decreased with increase in air inlet temperature (Figure 1d) which might be due to the intensity of browning reactions. These findings agree with Atalar and Dervisoglu (2015) who determined that L^* values of kefir powders ranged from 84.04 to 90.6 and air inlet temperature is the one factor that effect negatively on the L^* value statistically important.

3.5. Optimization of the microencapsulation process

Spray dryer process conditions for microencapsulation of *L. casei* Shirota was optimized for the optimal values of variables for the production of microcapsules with a maximum survival, product yield and L^* values and acceptable a_w value. The model showed high significance ($P<0.01$) and good fit (R^2=0.8-0.985) with the experimental data of all responses (Table 3). The lack of fit for responses except a^* and b^* values were found as non-significant ($P>0.05$). The relative error (< 10%) shows that the model adequately fit the data. The responses were associated with the independent variables and the best-fit model, in terms of coded factors, is shown as follows using a polynomial equation, Eq. (4):

$$Survival\ (\%) = 91.03 - 0.69X_1 - 1.97X_2 - 0.33X_3 + 0.68X_1X_1 + 0.92X_2X_2 - 0.92X_3X_3 + 0.045X_1X_2 - 0.34X_1X_3 + 0.11X_2X_3 \quad (6)$$

$$Product\ yield\ (\%) = 54.9 + 5.97X_1 + 6.05X_2 - 2.06X_3 + 1.42X_1X_1 - 1.78X_2X_2 - 1.11X_3X_3 - 6.91X_1X_2 - 2.67X_1X_3 - 0.077X_2X_3 \quad (7)$$

$$a_w = 0.15 - 0.0092X_1 - 0.11X_2 + 0.032X_3 + 0.012X_1X_1 - 0.0086X_2X_2 - 0.026X_3X_3 + 0.0012X_1X_2 + 0.039X_1X_3 + 0.02X_2X_3 \quad (8)$$

$$L* = 94.65 - 0.029X_1 - 1.27X_2 + 0.35X_3 + 0.15X_1X_1 + 0.17X_2X_2 - 0.18X_3X_3 + 0.16X_1X_2 - 0.29X_1X_3 + 0.12X_2X_3 \quad (9)$$

Desirability function was used to determining optimum process conditions. The optimum drying conditions of the quadratic model response surface model for the selected criteria were found to be at an air inlet temperature of 119.55 °C, feed solution concentration of 21.16% and pump rate of 40%, with an overall desirability value of 0.812. The predicted values for variables at these optimum conditions were calculated and further, the probiotic bacteria was microencapsulated by spray drying under optimized conditions (Table 4).

Table 4. Predicted and experimental values of the responses at optimum condition for spray dried *L. casei* Shirota

	Survival (%)	Product yield (%)	a_w	L^*
Predicted*	93.61	45.73	0.423	96.73
Experimental**	91.31±1.9	46.74	0.168±0.01	95.55±0.2
Deviation	-0.024	0.022	0.602	0.012

* Estimated by the model at the optimum point.
** Operating conditions were set as 21.16% feed solution concentration, 119.55 °C air inlet temperature and 40% pump rate.

Obtained predicted and experimental results confirmed the validity of the model and the results showed that the experimental values were close to the predicted values with the deviation values calculated for each parameter except a_w value. The experimental a_w value of spray dried powder produced under optimized conditions was lower than predicted a_w value. This may be due to the acceptance of a_w value in the range for all trials in the optimization process and thus the predicted a_w value being close to the highest a_w value.

3.5.1. Size distribution and morphology of microcapsules

The mean particle size of microcapsules obtained under optimized condition was determined as 7.07 µm. Particle size of microcapsules was consistent with previous reports obtained by Ilha, da Silva, Lorenz, de Oliveira Rocha, and Sant'Anna (2014), De Castro-Cislaghi, Silva, Fritzen-Freire, Lorenz, and Sant'Anna (2012) and Fritzen-Freire et al. (2012) who found that the mean particle size of microcapsules obtained with spray drying using different wall material is varied up to ~20 µm which depend on the properties of feed solution (total solids and viscosity) and spray dryer parameters like nozzle type, atomization pressure and pump rate (Ying et al., 2010). It is expected that the particle size will not caused sensory problems in food samples due to small powder size. However, the capsule diameter is known to affect the encapsulated cell's resistance to environmental conditions, and it has been revealed by studies that capsules with large diameters contain more cells and protect the cell better against environmental stresses (Burgain, Gaiani, Cailliez-Grimal, Jeandel, & Scher, 2013; De Castro-Cislaghi et al., 2012).

Scanning electron microscopy images show that spray dried particles were perfectly spherical shape but their surfaces were irregular (Figure 2). Moreover, collapsed structure was visualized, which is characteristic of many spray-dried powders analyzed under vacuum, that can be described as a deflated, flat, ball-like, spherical particles (Behboudi-Jobbehdar et al., 2013). The same situation was observed by O. Gul (2017) and De Castro-Cislaghi et al. (2012) in microcapsules obtained by spray drying. Conversely, the hollow or cracks on the surface of microcapsules was not observed, which confirms good structural integrity and suggests low gas permeability, for example, oxygen and water vapor (Ilha et al., 2014).

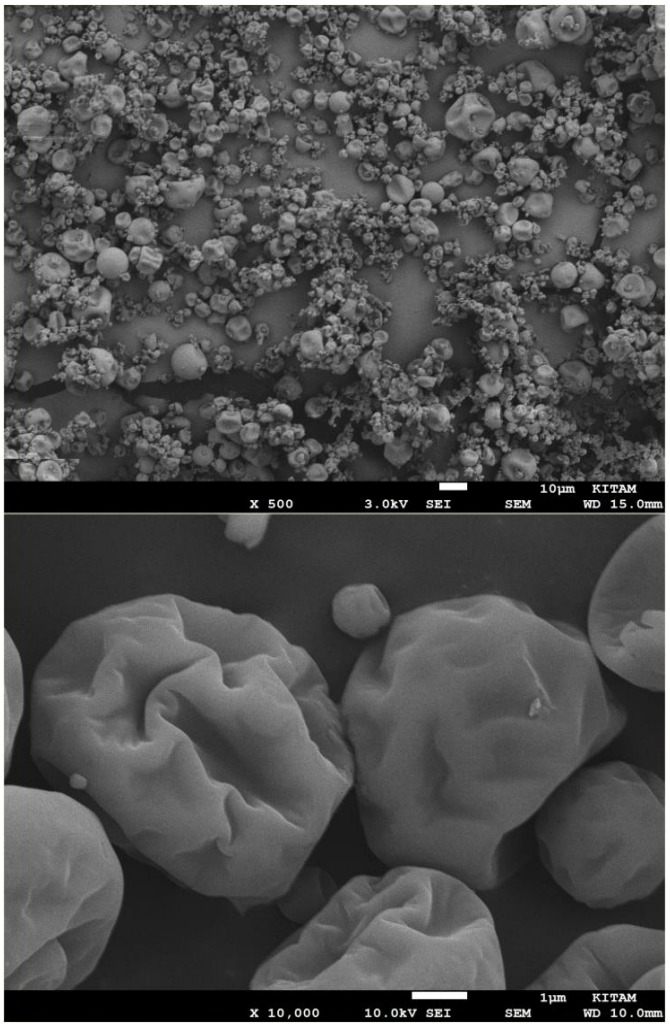

Figure 2. Micrograph of the microcapsules containing *L. casei* Shirota encapsulated under optimized condition by spray drying

3.5.2. Survival of microencapsulated *L. casei* Shirota in vitro gastrointestinal conditions

Probiotic bacteria are commonly taken with foods and thus the bacteria move from the mouth to the lower intestinal tract, and the time between entering and exiting the stomach is about 90 minutes. Almost the first cellular stress begins in the stomach with a pH of about 1.5 after being taken into the body (Khater, Ali, & Ahmed, 2010). Many studies have revealed that bacteria cannot reach the gastrointestinal tract after consumption (Dave & Shah, 1997; Hamilton-Miller, Shah, & Winkler, 1999). Therefore, it is important to protect probiotic microorganisms with a physical barrier created against environmental conditions by using microencapsulation techniques (Kailasapathy, 2002). Resistance to in vitro gastrointestinal conditions of microcapsules produced with spray drying under optimum conditions was shown in Figure 3.

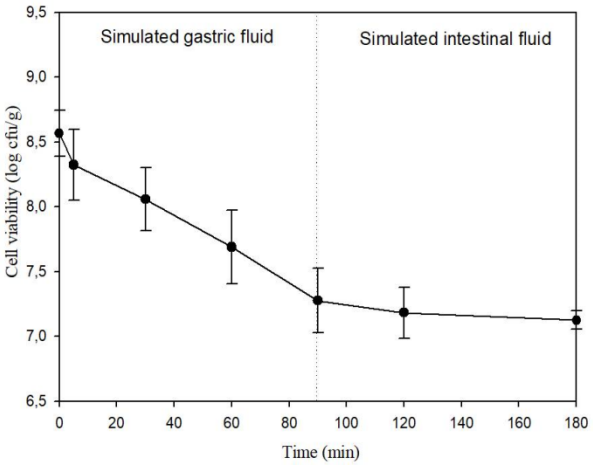

Figure 3. Survival of microencapsulated *L. casei* Shirota encapsulated under optimized condition by spray drying (SGF: simulated gastric fluid, SIJ: simulated intestinal fluid)

Free *L. casei* Shirota cell determined as ~10 log cfu/mL and a drastic decrease in the free cell was determined when cell exposed to SGF. After 60 min in gastric fluid at pH 2.0, free cell was not detected. On the other hand, the viability of cell microencapsulated with optimum condition remarkably decreased by about 1.3 log cycles at the end of treatment in SGF and then slightly decreased during exposure in the SIF at pH 7.4. The results obtained indicated that the microencapsulation process under optimum conditions protects the *L. casei* Shirota. These results are in accordance with previously published data (O. Gul, 2017). Reddy, Madhu, and Prapulla (2009) found that lactobacillus cultures encapsulated with maltodextrin exhibit good tolerance towards acid and bile. Also, Xavier dos Santos et al. (2019), Dimitrellou et al. (2016) and Fritzen-Freire, Prudêncio, Pinto, Muñoz, and Amboni (2013) who observed that the survival of microencapsulated probiotic bacteria with spray drying using encapsulating agent increase after exposure to in vitro conditions when compared with the free cells. Jantzen, Gopel, and Beermann (2013) and Arslan et al. (2015) stated that microencapsulation of probiotic bacteria by spray drying is an effective way to improve cell resistance during gastric transit. At the end of *in vitro* gastrointestinal treatment, the number of detected viable cell counts was about 7.12 log cfu/g.

3.5.3. Survival of microencapsulated *L. casei* Shirota during storage

The cell viability of *L. casei* Shirota microencapsulated under optimized conditions was observed at temperatures of 4 and 24 ºC (simulated refrigeration and room temperature) during 8 weeks of storage at, and the results were presented in Figure 4a. The cell viability of microencapsulated probiotic bacteria showed a slight decreasing trend during storage period and the viability of microencapsulated cell reduced as 1.11 and 1.77 log cycles at 4 and 24 ºC, respectively. The loss of microencapsulated cell viability during two storage condition followed first-order kinetics model with R^2 value greater than 0.994 (Figure 4b), which is in the range of those presented by other researchers in terms of dry storage of microencapsulated probiotic cells (L. B. Gul, Gul, Yilmaz, Dertli, & Con, 2020; Heidebach, Först, & Kulozik, 2010; Savedboworn et al., 2017; Tao et al., 2019). The *k* values (a specific rate of cell death, 1/d) calculated as 4.26×10^{-2} 1/d and 6.81×10^{-2} 1/d with the final cell count of 7.45 log cfu/g and 6.79 log cfu/g obtained under storage period at 4 and 25 ºC, respectively. Results showed that storing the microencapsulated cell at refrigeration temperature displayed better stability than ones stored at room temperature ($P<0.05$). Similar results have been reported by Tao et al. (2019) and Reyes, Chotiko, Chouljenko, Campbell, et al. (2018) stated the same downward trend in microencapsulated cell viability stored at higher temperature. This result may be related to the less molecular movement because of the limited kinetic energy at low temperature. Furthermore, bacterial metabolism is slowed down and consequently, bacterial cells will not only produce less waste products because of their limited metabolism, but also external detrimental reactions (such as lipid oxidation) will be limited (Broeckx et al., 2017; Heidebach et al., 2010).

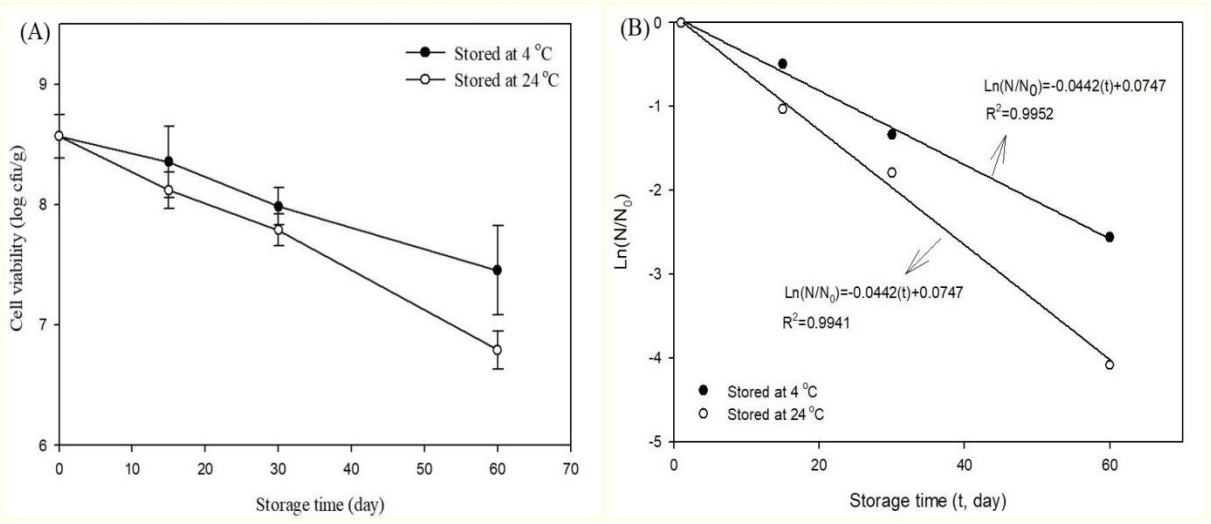

Figure 4. Survival of microencapsulated *L. casei* Shirota under optimized condition by spray drying during storage at 4 and 24 ºC (a) and rate constant of inactivation for the microencapsulated *L. casei* Shirota powders at 4 and 25°C during storage (b)

4. Conclusion

In this study, it was investigated the optimal process for the microencapsulation of probiotic *L. casei* Shirota by spray drying. Regression analysis indicated that the optimized process condition was determined as: feed solution concentration of 21.16%, air inlet temperature of 119.55 °C and pump rate of 40%, with an overall desirability value of 0.812. Under optimized condition, *L. casei* Shirota was successfully microencapsulated by spray drying with high cell viability as 8.57 log cfu/g. The powders produced at the optimized spray-dried conditions were characterized as having acceptable color, a_w values and also morphological properties. Microencapsulation of probiotic bacteria by spray drying under optimized condition offers greater protection to cell when exposed to gastrointestinal conditions and microencapsulated cells were able to maintain a satisfactory viability as 7.45 after storage at 4 °C. Overall, microencapsulated *L. casei* Shirota under optimized conditions by spray drying demonstrated promising efficiency depend on cell viability during drying, gastrointestinal treatment and refrigerated storage condition and also physicochemical and morphological properties of powders.

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