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Optimization of jackfruit seed starch-soya protein isolate ratio and process variables for flaxseed oil encapsulation

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ABSTRACT

The attribution as functional food ingredient of omega-3 fatty acid encouraged food researchers and industry for development of foods enriched with omega-3 fatty acids. Flaxseed oil (FSO) is the prolific vegetarian source of alpha linolenic acid (ω-3) for food enrichment, but its direct incorporation in food is problematic due to its high oxidation susceptibility. Microencapsulation of FSO using spray drying with jack fruit seed starch (JSS)-soya protein isolate (SPI) coating combination was studied as it is widely used approach to address the issue. The study was aimed at optimization of JSS-SPI ratio and process variables for microencapsulation of FSO with goals of maximization of encapsulation efficiency (EE), minimization of peroxide value (PV) and desired range of moisture content (MC:3-5, % wb) using Response surface methodology (RSM). The Box Behnken design (3^k) was used to plan the experiments with three independent variables, viz. JSS-SPI ratio (1:1, 3:1 and 5:1), oil loading (20, 25 and 30%) of total solids in emulsion and inlet air temperature (160, 170 and 180°C) of spray dryer. Response surface methodology was used for analysis of responses (EE, PV and MC) and second order polynomial models were found significantly fitted to the responses with high coefficient of determination. The JSS:SPI ratio of 3.24:1, 23.8% oil loading and 175°C drying air temperature were selected as the optimum conditions after numerical optimization and model validation.

Key words: Encapsulation, Flaxseed oil, Omega-3, Response surface methodology, Starch-protein complex

Essential fatty acids (EFAs) specially omega-3 polyunsaturated fatty acids (PUFA) like alpha linolenic acid, EPA, DHA are termed as essential as body requires them for several biological processes but cannot synthesis them so these must be ingested through diet. The nutritional and health benefits of omega- 3 in reducing the risk of hypertension, coronary heart diseases, depression, inflammatory bowel diseases and prevention of prostate and breast and cancers (Rodriguez-Leyva *et al.* 2010, Carraro *et al.* 2012) attributes it as active ingredients of functional foods.Fishes are rich sources of omega-3 fatty acids, however, Indian diet does not include enough fishes particularly vegetarians and non-fish eaters.

Similarly, flaxseed oil is a rich vegetarian source of EFAs as it contains high PUFA especially enriched with 52-57% alpha linolenic acid of its total fatty acids. Its consumption could help to improve intake of ω-3 fatty acids for those, who do not consume fish due to prevailing belief and traditions. However, its direct incorporation in food products has issues of its high oxidation susceptibility and consequent off flavour development. The microencapsulation using spray drying has been widely used approach adopted by many researchers for protection of oils/flavours against lipid oxidation and for controlled release of bioactive compounds (Hogan *et al.* 2003, Charve and Reineccius 2009, Tonon *et al.* 2012). However, the selection of encapsulating material is very important as it affects the effectiveness of spray drying encapsulation.

Some of the commonly used coating materials for the encapsulation of ω-3 fatty acids are proteins, polysaccharides, gums and cellulose (Sanguansri and Augustin 2007).The use of polysaccharides in encapsulation helps to improve film forming properties of microcapsules and proteins improves emulsifying and stabilising properties of spray feed emulsion. Jackfruit seed starch (JSS) is a natural starch with potential applications in pharmaceutical and food industry (Narkhede *et al.* 2011, Rengsutthi and Charoenrein 2011). JSS also showed greater stability to thermal and mechanical shear and could be used as alternative to modified starches in a system that requires high stability to severe heat or mechanical shear, can be utilized for controlled drug delivery system (Mukprasirt and Sajjaanantakul 2004, Nayak and Pal 2013, Kittipongpatana and Kittipongpatana 2015). Soy

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protein isolate (SPI) has a good emulsifying, gelling, water binding and fat absorbing properties and has potential role as substrate for the development of delivery systems (Caillard *et al.* 2009, Pereira *et al.* 2009). Protein and polysaccharide molecules can link together by a covalent bond giving a specific, strong and essentially permanent conjugate (Benichou *et al.* 2002, Chobert *et al.* 2006).

Microencapsulation using protein-polysaccharide (starch) complex as coating material exhibits better functional properties in encapsulation than that of the polysaccharides and proteins alone (Young *et al.* 1993, Carneiro *et al.* 2013). However, very less information is available on starch or protein proportions to be used for efficient encapsulation process. Hence, use of JSS-SPI combinations for flaxseed oil encapsulation was planned to be studied in the present investigation to optimize JSS-SPI ratio and process conditions (oil loading and inlet air temperature) of microencapsulation. Response surface methodology was used for optimization of process variables as it emphasizes on statistical and mathematical modeling and determines relationships between various independent variables to optimize process parameters.

MATERIALS AND METHODS

Cold pressed, physically refined flaxseed oil was used as core material. Jackfruit seed starch and soya protein isolate were selected as encapsulating materials based on thorough literature review and preliminary experiments. The experimental design for flaxseed oil microencapsulation with JSS-SPI wall material combinations using spray drying method was planned with Box Behnken design (3^k) using Design expert software (9.0, Trail version). The independent variables selected for the design were oil loading, JSS: SPI ratio, and inlet air temperature with three levels of each factor (Table 1).

The proportionate of wall material and oil loadings were taken as per the designed levels (Table 2) after keeping total solid content of emulsion fixed to 30% based on preliminary experiments and thorough literature review for all sets of experiments. The jackfruit seed starch was slowly mixed in lukewarm distilled water with continuous stirring and then SPI of said proportion was added gradually to form polysaccharide: protein complex. The proportionate oil content of particular level was loaded gradually with continuous stirring and coarse emulsion was set for homogenization at 10000 rpm for 5-6 minutes using ultra-Turrax homogenizer. Spray drying process was carried out using lab-scale spray dryer**.** Feed temperature was set to 40°C with gradual stirring before feeding the emulsion to pressure nozzle using peristaltic pump at 2 ml/min flow rate.The spray dryer operating conditions (atomizer pressure: 2.5 kg/cm^2, aspirator air pressure: 0.80 MPa, inlet and outlet high temperature: 200 and 100°C, respectively) for encapsulation were finalized after preliminary experiments. The spray dried microencapsulated FSO powder was collected immediately after each set of experiment and stored in airtight amber colour bottles at -4°C for further analysis. The response variables (encapsulation efficiency and peroxide value and moisture content) were analyzed in triplicate for all 17 set of experiments.

Table 1 Levels of independent variables used in experimental design

Independent variables	Independent variable codes	Coded and actual levels		
		-1	0	+1
Oil loading (%)	X_1	20	25	30
JSS: SPI ratio	X_2	1:1	3:1	5:1
Inlet air temperature (°C)	X_3	160	170	180

Encapsulation efficiency is the fraction of encapsulated oil over the total oil content used for preparation of microcapsule. It can also be defined as ratio of core material in the prepared microcapsules to the initial core material used for preparation and calculated by method as described by Bae and Lee (2008).

$$\text{EE (\%)} = \frac{\text{(Total oil - Surface oil)}}{\text{Total oil}} \times 100$$

Total oil content of microcapsules was calculated using Soxhlet extraction method given by AOCS (2000). The surface oil was determined by the method as described by Bae and Lee (2008). It was estimated by adding 15 ml of hexane to an accurately weighed sample (2 g) of microencapsulated FSO powder in sealed glass tube followed by stirring for 1-2 minutes. The suspension was filtered through Whatman filter paper (No. 1) and residues rinsed thrice by passing 20 ml of hexane through each. The resultant solvent was evaporated at 60ºC until constant weight. The surface oil was calculated by weighing empty flask weight and weight of flask containing extracted oil residues.

Peroxide value is a measure of degree of deterioration of fats and oils. It is an indicator for assessing the oxidative rancidity of the oil. To determine peroxide values, oil was extracted using Soxhlet extraction method given by AOCS (2000). The peroxides present were determined in triplicate by spectrophotometrically by IDF standard method (74A:1991) using UV-VIS/NIR spectrophotometer as described by Shantha and Decker (1994). The moisture content of FSO microcapsules was determined in triplicate using standard procedure of hot air oven method (AOAC 1997).

Response surface methodology was used for optimization of process conditions with goals of maximization of encapsulation efficiency, minimization of peroxide value and desired (3-5% wb) moisture content (Gallardo *et al.* 2013, Tuyen *et al.* 2014) using design expert 9.0 (trail version). The quadratic model for prediction of responses was expressed using coded second order polynomial equation as follows.

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} X_i X_j + \sum_{i=1}^{3}\beta_{ii} X_i^2$$

where, Y: Predicted response, X_i and X_j are coded independent variables, β_0, β_{ij}, β_{ij}, are regression coefficient

The coding of process variables brings simplification in computational procedures. It helps in bringing the same range of independent variables irrespective of their actual magnitudes. The non-significant terms were excluded from coded second order polynomial equation to get the simple and best-fitted predictive equation (Mahawar *et al.* 2016). Numerical optimization was done for selection of optimal conditions on the basis of Derringer's desirability function. To confirm the results, experimental runs were carried out in triplicate under the optimized conditions and prediction models were validated by comparing the experimental and predicted values of selected responses.

RESULTS AND DISCUSSION

The important response variable, viz. encapsulation efficiency, peroxide value and moisture content were analyzed for all 17 set of experiments in triplicate. The experimental design and observed values of encapsulation efficiency, peroxide value and moisture content determined under different experimental runs are depicted in Table 2.

Response surface methodology was used for analysis of observed responses after eliminating some non-significant terms ($P \geq 0.10$) and predicted models were tested for adequacy and fitness using analysis of variance. The fit summary statistics and sequential model sum of squares were analyzed to check model adequacy. The ANOVA and estimated regression coefficients of quadratic models for selected responses are depicted in Table 3. The calculated F values of models were higher than tabulated F values for encapsulation efficiency, peroxide value and moisture content indicated that selected models found highly significant ($P \leq 0.01$), with high coefficient of determination ($R^2 \geq 0.93$). Lack of fit relative to pure error was observed non-significant for these responses, also indicated suitability of the models. The coefficient of variation (%CV) was less than 10%, which is a good indicator of reasonable accuracy and reproducibility of models. Adequate precision which is a measure of signal to noise ratio, greater than 4 was desirable so that model can be used to navigate the design space.

Effect of process variables on encapsulation efficiency

Encapsulation efficiency of microcapsules using JSS-SPI wall material complex varied from 73.10 to 86.71%, under different experimental runs. Model adequacy was tested for different models like linear, quadratic, cubic, quartic etc. ANOVA of quadratic model for EE showed significant model with high $R^2 = 0.97$. The results of regression analysis can be expressed in the form of second order polynomial equation as follows.

$$EE = 85.25 - 3.67X_1 + 2.99X_2 + 1.90X_3 - 3.12X_1^2 - 2.52X_2^2 - 1.82X_3^2$$

ANOVA (Table 3) revealed significant negative linear and quadratic effect of oil loading on EE ($P < 0.01$). With increase in oil loading, the EE was decreased, regression coefficients also indicated that, oil loading showed negative linear effect on EE (Fig 1a).Similar results were reported by Hogan *et al.*(2001), Tan *et al.* (2005), Quispe-condori *et al.* (2011). This may be due to amount of shell material might not sufficient to cover oil droplets at higher loading and which might resulted in more surface oil and decreased

Table 2 Experimental design and observed responses of encapsulated FSO powder with JSS:SPI coating combinations.

Tests/ Run	Actual and coded (in parenthesis) design			Observed responses		
	Oil loading (% of total solid) (X_1)	JSS:SPI ratio (X_2)	Inlet air temperature (°C), (X_3)	Encapsulation efficiency (%)	Peroxide value (meq/kg oil)	Moisture content (%wb)
1	30 (1)	5:1 (1)	170 (0)	79.07	2.45	3.65
2	30 (1)	1:1 (-1)	170 (0)	73.65	2.05	4.02
3	20 (-1)	5:1 (1)	170 (0)	85.80	1.48	4.92
4	20 (-1)	1:1 (-1)	170 (0)	79.91	1.42	5.45
5	30 (1)	3:1 (0)	180 (1)	79.32	2.51	3.15
6	30 (1)	3:1 (0)	160 (-1)	73.10	2.42	3.81
7	20 (-1)	3:1 (0)	180 (1)	86.71	1.55	5.24
8	20 (-1)	3:1 (0)	160 (-1)	82.11	1.35	6.34
9	25 (0)	5:1 (1)	180 (1)	84.82	1.84	4.26
10	25 (0)	5:1 (1)	160 (-1)	83.31	1.62	5.58
11	25 (0)	1:1 (-1)	180 (1)	79.20	1.49	4.85
12	25 (0)	1:1 (-1)	160 (-1)	76.32	1.46	5.28
13	25 (0)	3:1 (0)	170 (0)	84.44	1.48	4.84
14	25 (0)	3:1 (0)	170 (0)	86.45	1.49	5.26
15	25 (0)	3:1 (0)	170 (0)	85.22	1.42	4.98
16	25 (0)	3:1 (0)	170 (0)	84.22	1.47	4.89
17	25 (0)	3:1 (0)	170 (0)	85.94	1.41	5.28

Table 3 ANOVA and coded regression coefficients of quadratic models for various responses

Parameter	Encapsulation efficiency (%)		Peroxide value (meq/kg oil)		Moisture content (%)	
	p-value (Prob> F)	Coefficient estimate	p-value (Prob> F)	Coefficient estimate	p-value (Prob> F)	Coefficient estimate
Intercept		85.25		1.45		5.05
X_1	0.0001	-3.67***	<0.0001	0.45***	0.0001	-0.92***
X_2	0.0002	2.99***	0.0011	0.12***	0.2187	-0.15
X_3	0.0032	1.90***	0.0218	0.07**	0.0053	-0.44***
$X_1 \cdot X_2$	0.8548	-0.12	0.0356	0.08**	0.8047	0.04
$X_1 \cdot X_3$	0.5289	0.41	0.4498	-0.03	0.5028	0.11
$X_2 \cdot X_3$	0.5950	-0.34	0.1970	0.05	0.1961	-0.22
X_1^2	0.0012	-3.12***	<0.0001	0.38***	0.0212	-0.45**
X_2^2	0.0039	-2.52***	0.5379	0.02	0.5666	-0.09
X_3^2	0.0185	-1.82**	0.0051	0.13***	0.8304	0.03
Lack of Fit	*0.20*^{NS}		*0.06*^{NS}		*0.11*^{NS}	
Model (F Value)	22.26***		66.72***		10.96***	
CV (%)	1.49		3.82		6.47	
R^2	0.97		0.98		0.93	
Adjusted R^2	0.92		0.97		0.84	
Adeq. Precision	14.19		23.07		11.34	

X_1: Oil loading, X_2: JSS: SPI ratio, and X_3: inlet air temperature, *Significant at .05≤ P <0.10; ** Significant at .01≤ P <.05, ***Significant at P <0.01.

efficiency. With increase in JSS-SPI ratio, EE was observed to be was increased initially nowever, but above 3:1 ratio, it decreased. This might be due to positive linear and negative quadratic effect of JSS-SPI ratio. Infact, This may also be attributed to stabilised emulsion formatoin at 3:1 ratio which in turn improved encapsulation efficiency. Similar observations were reported by Akhtar and Dickinson (2003) for Dextran-protien conjugates and by Sheu and rosenberg (1995) for WPI-maltodextrin (MD10 and MD 15), WPI-corn syrup combinations. The inlet air temperature and JSS-SPI ratios showed positive linear effect and negative quadratic effect on encapsulation efficiency. The positive linear effects of JSS-SPI ratio and drying air temperature on EE are also depicted in response surface plot (Fig 1b).

Effect of process variables on peroxide value

Under different experimental conditions, peroxide value using JSS-SPI wall material combination varied from 1.35 to

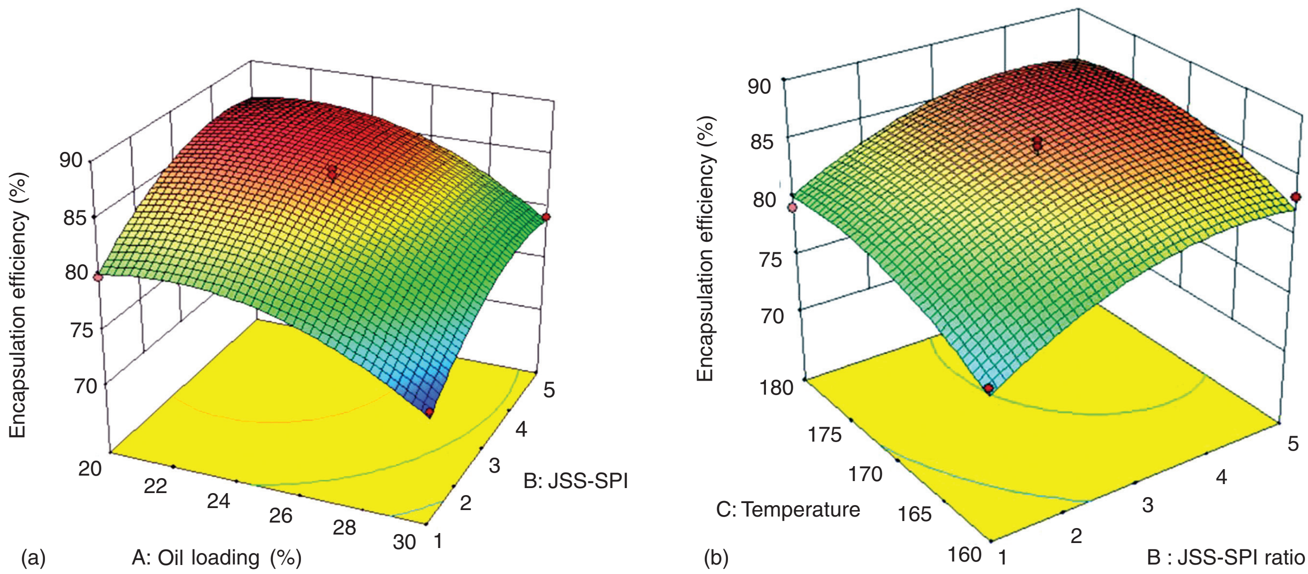

Fig 1 Response surface 3D plots showing effect of (a) oil loading and JSS-SPI ratio, and (b) JSS-SPI ratio and inlet air temperature on encapsulation efficiency.

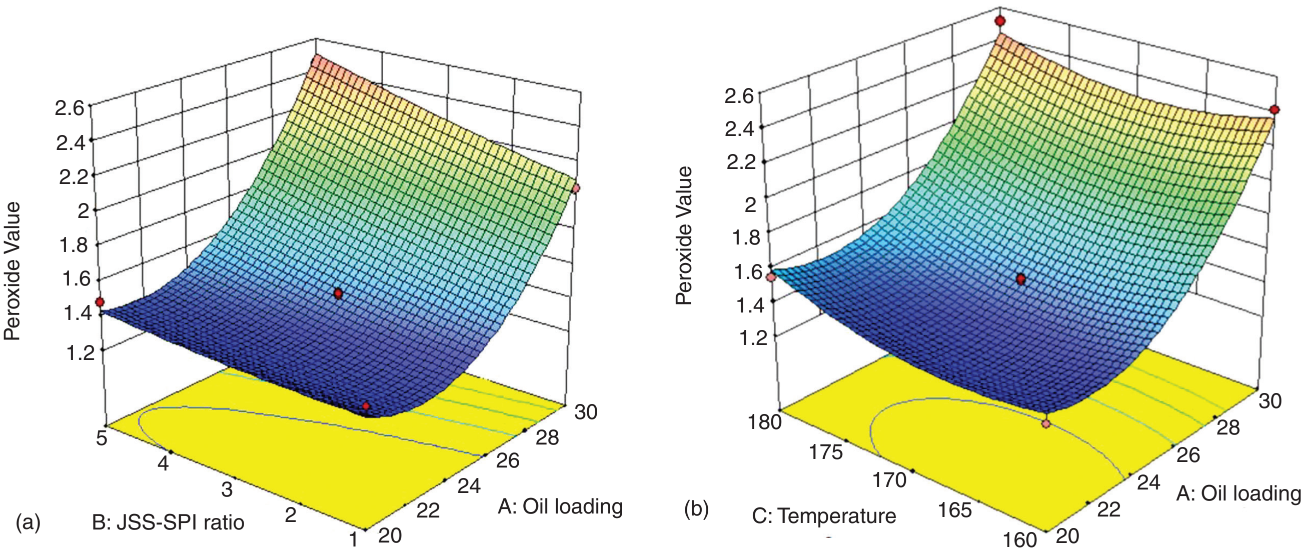

Fig 2 Response surface 3D plots showing effect of (a) oil loading and JSS-SPI ratio, and (b) oil loading and inlet air temperature on peroxide value

2.51 meq/kg oil. Different models (Linear, quadratic, cubic, etc.) were tested for model adequacy. ANOVA of quadratic model showed model F-value of 66.72, which implies the model is significant with high R^2= 0.98. Following equation was developed based on regression analysis for prediction of peroxide value after eliminating some non-significant terms.

$$PV = 1.45 + 0.45X_1 + 0.12X_2 + 0.07X_3 + 0.08X_1X_2 + 0.38X_1^2 + 0.13X_3^2$$

Peroxide value of FSO microcapsules showed significant positive linear effect of oil loading, JSS-SPI ratio ($P < 0.01$) and inlet air temperature ($P < 0.05$). Effect of increase in oil loading in feed mixture was resulted higher PV of microcapsules at given JSS-SPI complex ratio. This might be the result of higher surface oil (lower EE) due increased oil load on microcapsules which could cause oxidation (Tonon *et al.*2012). It is also evident from Fig 2a that, increase in PV above 26% oil loading was higher as compared to its increase between 20% and 26% oil loading.The increase in JSS content and drying air temperature was resulted higher peroxide value. Increasing air inlet temperature showed positive linear and quadratic effect on Peroxide value which resulted in high PV values at high drying temperature (Fig 2b). It might be due the high drying inlet air temperature that have imbalanced the rate of water evaporation and film formation and resulted in rapid oxidation of surface oil before the formation of crust at higher temperature. (Shu *et al.* 2006).Oil loading of 26% and up to 4:1 JSS-SPI ratio, better protection against lipid oxidation was observed, however lower PV value was observed at high protein content (1:1 ratio) in mixture signifies role of SPI in lipid protection. The results agree with findings of Rascon *et al.* (2011) and Elias *et al.* (2008) on role of SPI in protection against oxidation.

Effect of process variables on moisture content (MC)

The moisture content is an important parameter for oil powder because oil oxidation can quickly take place at high MC. Moisture content of most dried powders used in the food industry is less than 5% (Tuyen *et al.* 2014). The moisture content of FSO microcapsules showed variation from 3.15 to 5.45% under different experimental runs with JSS-SPI coating combination. Using model fitting technique, significant quadratic model was fitted (R^2= 0.94). The coded equation to identify the relative impact of process factors can be given as follows after eliminating some non-significant terms.

$$MC = 5.05 - 0.92X_1 - 0.44X_3 - 0.45X_1^2$$

With increase in oil loading and drying air temperature, MC of resultant microcapsules decreased. Coefficient estimates also confirmed the negative linear effect of oil loading and drying temperature ($P < 0.01$) on MC of microcapsules. It is evident from Fig 3 that, above 172ºC inlet air temperature, the decrease in moisture content was

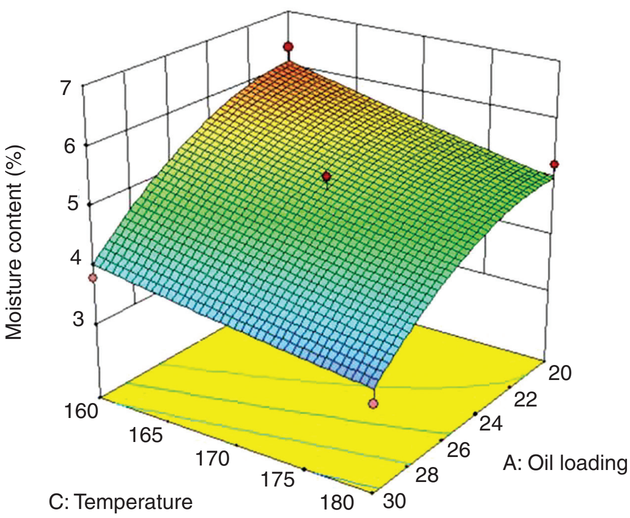

Fig 3 Response surface 3D plots showing effect of oil loading and inlet air temperature on moisture content.

Table 4 Confirmation report with predicted and observed values of responses at the optimal conditions

Factor	Name	Optimized level	Low Level	High evel	Coding	
X_1	Oil loading	23.80	20.00	30.00	Actual	
X_2	JSS-SPI ratio	3.24	1.00	5.00	Actual	
X_3	Temperature	174.68	160.00	180.00	Actual	
Response	*Predicted values*	*Observed values*	*Relative mean error (%)*	*SE Pred n=3*	*95% PI Low*	*95% PI high*
Encapsulation efficiency	86.71	82.56	4.77	0.000	*86.71*	*86.71*
Peroxide value	1.45	1.51	4.82	0.047	1.33	1.56
Moisture content	5.00	4.79	4.2	0.000	*5.00*	*5.00*

more at given oil loading. This might be due to decrease in relative humidity air with increased inlet air temperature which could have driven up more moisture from the microcapsules (Hogan *et al.* 2001). Oil loading also showed negative quadratic effect on MC of microcapsules

Optimization of process variables and model validation

Numerical optimization of three independent variables was carried out by setting goals of maximization of encapsulation efficiency, minimization of peroxide value and desired moisture content (3-5%, wb) of spray dried encapsulated powder using RSM. Selection of optimum process conditions were based on model desirability. The predicted optimized process conditions using JSS-SPI wall materials were 23.80% oil loading, 3.24:1 JSS-SPI ratio and 175^{o}C drying temperature with desirability of 0.96. The predicted values of EE, PV and MC after numerical optimization were 86.71%, 1.45 meq/kg oil and 5.00%, respectively.

Models were validated by comparing the predicted and observed responses of confirmation experiment.The confirmation report with predicted and observed values of responses at the optimal process conditions and relative mean error represents suitability of model for prediction of responses (Table 4).

The optimum conditions for flaxseed oil encapsulation using Jackfruit seed starch-soya protein isolate coating combination to achieve desired responses at given process variables were 23.80% oil loading, 3.24:1 JSS-SPI ratio and 175^{o}C drying air temperature. The quadratic models for prediction of responses (encapsulation efficiency, peroxide value and moisture content) were found suitable with higher desirability (0.96) function. Encapsulation efficiency and peroxide value, both responses were significantly influenced by the change in oil loading. The Increase in oil loading and inlet air temperature resulted in higher peroxide value and lower moisture content of final products at given ratio of JSS-SPI complex. The results also provided insights on importance of proportionate use of starch-protein ratio for efficient encapsulation process.

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