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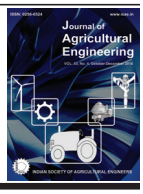

Optimization of Oil Loading and Starch-Protein Ratio for Encapsulation of Flaxseed Oil Using Response Surface Methodology

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ABSTRACT

Microencapsulation of flaxseed oil (FSO) was studied with tapioca starch (TS)-whey protein isolate (WPI) combination as a shell material using spray drying to optimize the process variables for preparation of spray dried microcapsules of flaxseed oil using TS-WPI coating material combination. Box Behnken design (3^k) was used to design the experimental runs of selected independent variables (three level), which included starch: protein ratio (1:1, 3:1 and 5:1) and oil loading (20 %, 25 %, and 30 %) of total solids in feed mixture and inlet air temperature (IAT:160 ^oC, 170 ^oC, and 180 ^oC) of spray dryer. Response surface methodology (RSM) was used for response analysis and for optimization of process variables for maximizing encapsulation efficiency and minimizing peroxide value (PV). Quadratic models were significantly fitted to the responses with high coefficient of determination (R^2). The optimum conditions predicted based on numerical optimization were 20 % oil loading, 2.40:1 TS: WPI ratio and 170 ^oC drying air temperature, respectively. The results of responses observed after confirmatory experiment were in good agreement with the predicted responses of the model.

Consumption of functional foods is popular among the consumers as they are nutritive and salubrious to health. The increasing demand for functional food encourages the food industry to put their efforts in development of foods enriched with bioactive ingredients. The essential fatty acids (EFAs), especially polyunsaturated fatty acid (PUFA), alpha linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are also termed as active ingredients due to their health promoting benefits and their role in reducing the risk of hypertension, coronary heart diseases, inflammatory bowel diseases and prevention of breast and prostate cancers (Rodriguez-Leyva *et al*., 2010; Singh *et al.,* 2011; Carraro *et al*., 2012). Although fishes are the largest source of omega-3 fatty acids, the Indian diet does not include enough fish to meet dietary recommendations of ω-3 fatty acids. This issue is even of more concern in case of vegetarians and non-fish lovers. Flaxseed oil (FSO) is rich in polyunsaturated oils extracted from the seeds of flax plant (*Linum usitatissimum L.*), which are found to be enriched with 52-57% α-linolenic acid (ALA) of its total fatty acids. Flaxseed oil might resolve the problem of ω-3 fatty acids for those who cannot consume fish due to religious belief and practices.

Direct incorporation of flaxseed oil in food items has the problem of its high and rapid susceptibility to oxidative deterioration, rancidity and consequent off-flavour development. The practice of microencapsulation using spray drying has been proposed as an alternative to avoid oxidative deterioration, off-flavour development and controlled diffusion. It has been a widely used approach by researchers for protection of oils/flavours against lipid oxidation, and is helpful in increasing shelf life of products (Drusch *et al*., 2007; Charve and Reineccius, 2009).

The effectiveness of spray drying in encapsulation is critically dependant on selection of appropriate wall

material for developing microcapsules as efficiency of core protection or controlled release largely depend on it (Young *et al*., 1993; Narsaiah *et al*., 2015, Bibwe *et al*., 2017). Incorporation of polysaccharides helps to improve the drying properties of microcapsules, while proteins help to improve the emulsifying and stabilising properties of spray feed emulsion. The use of tapioca starch in specialty applications like encapsulating agent (Kapusniak and Tomasik, 2006; Loksuwan, 2007), food additives, and thickener and has received attention as a replacement for gum Arabic. Whey protein concentrate and isolate exhibit excellent microencapsulating properties for volatile and non-volatile core materials. Whey protein isolates (WPI) matrices have been reported to provide effective protection against oxidation of encapsulated lipids in storage conditions that promote lipid oxidation (Shpigelman *et al*., 2010). Further, Monica and Herrera (2014) also reported that the continuous phase of mixed gels can be made of either using protein or starch or by interpenetrating whey protein and starch complex.

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). Work of most of researchers on microencapsulation using protein-starch complex reported that these complexes as coating material exhibit better functional properties in encapsulation than that of the polysaccharides and proteins alone (Young *et al*., 1993; Bylaite *et al.,* 2001; Carneiro *et al*., 2013). The use proteins and/or polysaccharides as coating agent allows protection of a sensitive core material from environmental factors such as temperature, relative humidity (RH), moisture, oxygen, pH; controlled release of core, uniform dispersion of the active ingredient (Desai and Park, 2005). Hari *et al*. (2012) used various concentrations of jackfruit seed starch with gelatine as coating material to prepare microspheres for use in oral drug delivery.

However, meagre information is available on concentration or proportion of starch or protein to be mixed for emulsion preparation in microencapsulation to maximize encapsulation efficiency, and to retard lipid oxidation. Hence, use of tapioca starch-WPI (starch-protein) combinations as coating material were investigated in this study with an aim to optimize the oil concentration and starch- protein ratio for preparation of spray dried microcapsules of flaxseed oil.

MATERIALS AND METHODS

Materials

Fresh, cold-pressed and physically refined flaxseed oil (*Linum usitatissimum L.*) was used as core material for microencapsulation. The oil was procured from M/S Shiv Sale Corporations, New Delhi. Tapioca starch (TS) as a source of starch, and whey protein isolate (WPI) as a source of protein were selected as encapsulating materials based on literature review mentioned earlier and preliminary experiment. The materials were procured from M/S AM labs, New Delhi. Analytical grade chemicals such as chloroform, methanol, ammonium thiocyanate, barium sulfate, hydrochloric acid, Fe (II) solutions, petroleum ether, acetone and hexane were procured from M/S Sigma-Aldrich Chemical Co., St. Louis, USA.

The present study was carried out in the Division of Agricultural Engineering, ICAR-Indian agricultural research institute, New Delhi.

Experimental Design

A three-factor Box and Behnken design (BBD), with three levels for each factor, was used to design the experimental plan for optimization of process variables with goals of maximization of encapsulation efficiency and minimization of lipid oxidation.

The design consisted of 17 experimental points with 5 centre points. Three factors (independent variables) used in this study were *X*= Oil loading (20 %, 25 % and 30%) of total solids, *Y*= Starch: protein ratio (1:1, 3:1 and 5:1), and *Z* = Inlet air temperature (160 ^{o}C, 170^{o}C and 180^{o}C). The actual and coded values (in parenthesis) of each level used for experimentation are presented in Table 1. The response variables (encapsulation efficiency and peroxide value) were analyzed in triplicate for all 17 set of experiments.

Response surface methodology was used to optimize the process variables using Design Expert software (9.0-trial version), as it emphasizes on statistical and mathematical modelling and analysis of responses of interest that are influenced by process variables.

The quadratic model for prediction of responses was expressed using second order polynomial equation as follows:

$$R_p = \beta_0+\beta_1 X+\beta_2 Y+\beta_3 Z+\beta_{11} X^2+\beta_{22} Y^2+\beta_{33} Z^2+\beta_{12} XY+\beta_{13} XZ+\beta_{23} YZ \quad \ldots(1)$$

Table 1. Experimental design and observed responses of encapsulated FSO powder with TS: WPI coating material combination

Test/ Run	Actual and coded (in parenthesis) design			Observed response	
	Oil loading, % of total solid	TS: WPI Ratio	IAT, °C	Encapsulation efficiency,	Peroxide value, meq.kg^{-1} oil
	X	*Y*	*Z*	%	
1	30 (1)	5:1 (1)	170 (0)	81.88	2.65
2	30 (1)	1:1 (-1)	170 (0)	74.77	2.24
3	20 (-1)	5:1 (1)	170 (0)	84.66	2.05
4	20 (-1)	1:1 (-1)	170 (0)	81.83	1.61
5	30 (1)	3:1 (0)	180 (1)	80.33	2.72
6	30 (1)	3:1 (0)	160 (-1)	73.67	2.37
7	20 (-1)	3:1 (0)	180 (1)	85.53	2.03
8	20 (-1)	3:1 (0)	160 (-1)	83.06	1.65
9	25 (0)	5:1 (1)	180 (1)	84.93	2.48
10	25 (0)	5:1 (1)	160 (-1)	82.53	2.12
11	25 (0)	1:1 (-1)	180 (1)	81.66	1.98
12	25 (0)	1:1 (-1)	160 (-1)	76.46	1.92
13	25 (0)	3:1 (0)	170 (0)	84.66	2.10
14	25 (0)	3:1 (0)	170 (0)	83.67	1.98
15	25 (0)	3:1 (0)	170 (0)	85.12	1.82
16	25 (0)	3:1 (0)	170 (0)	82.13	2.08
17	25 (0)	3:1 (0)	170 (0)	83.45	1.89

Note: TS: Tapioca starch, WPI: Whey protein isolate, IAT: Inlet air temperature

Where,

R_p = Predicted response,

X = Oil loading, %,

Y = Tapioca starch-whey protein isolate ratio,

Z = Inlet air temperature, °C,

β_0 = Constant,

β_1, β_2, β_3 = Linear product regression coefficients,

β_{11}, β_{22}, β_{33} = Quadratic product regression coefficients, and

β_{12}, β_{13}, β_{23} = Cross product regression coefficients.

Feed Sample Preparation

Feed sample emulsions were manually prepared in accordance to the set levels (Table 1). The total solid contents of feed emulsion preparation were fixed at 30 % based on preliminary trial experiment and literature review (Pedro *et al*., 2011) for all sets of experiment, and oil loading was varied at 20 %, 25 % and 30 % with respect to total solid content. Tapioca starch was slowly mixed in lukewarm distilled water with continuous stirring, and then WPI of said proportion was added gradually to form starch: protein complex with respect to the proportions of designed level. The proportionate oil content of a particular level (run) was gradually loaded with continuous stirring, and emulsion set for homogenization at 10,000 rpm for 3-4 min using an Ultra-Turrax homogenizer (Model: IKA ®T25, range 3000-25000 rpm, Bengaluru, India). Emulsion stability testes were carried out before each set of experimental trial to ensure no phase separation occurred before feeding emulsion to the spray dryer.

Spray Drying Process

A Sono Dry 1000 (Make: Sono-Tek Corporation, USA), a vertical co-current twin cyclone type dryer (water evaporation rate: up to 1000 ml.h^{-1}) was used for this study, Fig. 1. Feed temperature was set at 40°C

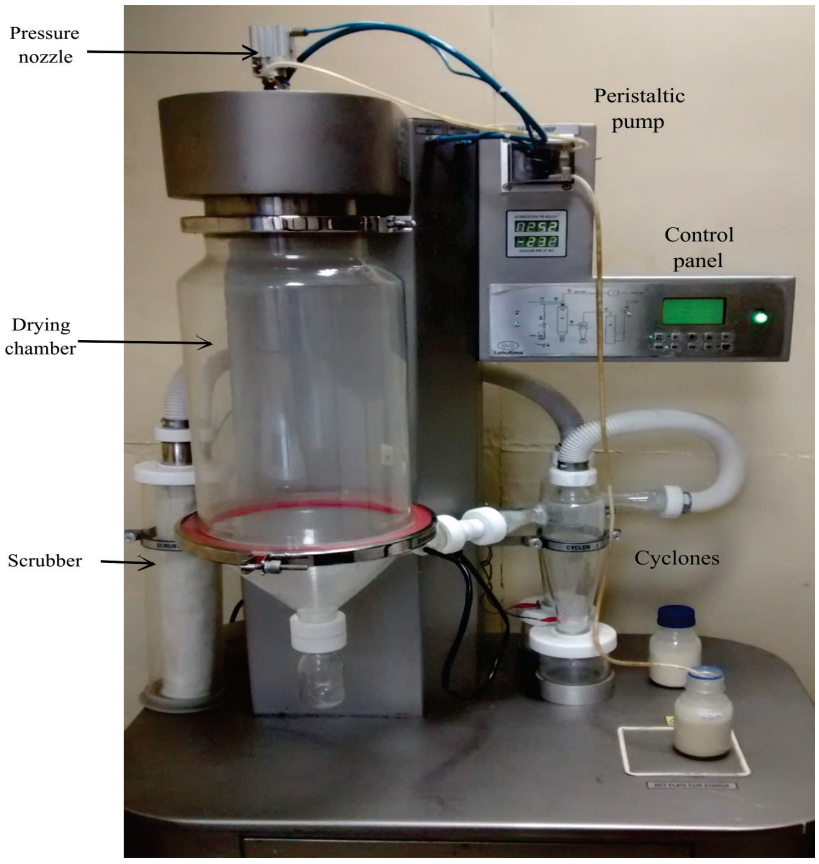


Fig. 1: Laboratory-scale spray dryer and experimental setup (Model: Sono Dry 1000, Sono-Tek Corporation, USA)

with continuous steady stirring of feed sample before feeding the emulsion to nozzle using the feed pump at a flow rate of 2ml.min^{-1}. The operating conditions of spray dryer and response variables are very important to produce highest microencapsulation efficiency and powder yield. The spray dryer operating parameters were decided after preliminary trials (Table 2) to obtain uniform, fine powder at set feed rate. The spray dried microencapsulated powder was collected immediately after each set of experiment, and stored in airtight amber colour bottles at (-) 4°C for further analysis.

Table 2. Spray dryer parameters used for encapsulation experiment

Sl. No.	Parameter	Set value
1.	Inlet temperature, °C	160, 170, 180
2.	Outlet temperature, °C	60, 70, 80
3.	Inlet high temperature, °C	200
4.	Outlet high temperature, °C	100
5.	Flow rate, ml.min^{-1}	2
6.	Atomization pressure, kg.cm^{-2}	2.51

Measurement of Response Variables

Encapsulation efficiency (EE)

Encapsulation efficiency is defined as the ratio of core material in the prepared microcapsules to core material that was originally used for preparation. It is the fraction of encapsulated oil over the total oil content used for preparation of microcapsule, and it was calculated using the following equation (Bae and Lee, 2008):

$$EE\ (\%) = \frac{(\text{Total oil} - \text{Surface oil})}{\text{Total oil}} \times 100 \quad \ldots(2)$$

Total oil

Total oil content of microencapsulated flaxseed oil powder was calculated using Soxhlet extraction method given by AOCS (2000) with hexane: petroleum ether (1:1) as solvents.

Surface oil

Surface oil content was determined by the method described by Tan *et al*. (2005). The amount of surface oil of microsphere was determined by adding 50 ml of n-hexane to 5.0 g weighed amount (ME 204 analytical

balance, Mettler-Toledo, Mumbai, 0.1 mg readability) of microencapsulated flaxseed oil powder, followed by stirring for 1 min. The suspension was then filtered, and the residue was rinsed thrice by passing 20 ml of hexane each time. The residual powder was then air dried for 30 min, and accurately weighed. The surface oil was calculated using the following equation:

$$\text{Surface oil (SO)} = W^{br} - W^{ar} \quad \ldots(3)$$

Where,

W_{br} = Weight of microspheres before rinsing, g, and
W_{ar} = Weight of microspheres after rinsing, g.

Peroxide value (PV)

Peroxide value of oil is a measure of oxidative rancidity of the oil. It is an important chemical characteristic for assessing the degree of deterioration of fats and oils. To determine peroxide value of flaxseed oil in microcapsules, the oil was extracted using Soxhlet extraction method given by AOCS (2000). The peroxides present was determined in triplicate (IDF standard method 74A:1991) using an UV-VIS/NIR spectrophotometer (JASCO, JAPAN, Model: V-670, accuracy ± 0.3 nm). Peroxide present in the extracted fats were determined using IDF, 1991 method (Shantha and Decker, 1994). The test is based on the co-oxidation of Fe (II) to Fe (III) by hydroperoxides from sample (fat) and the formation of the reddish Fe(III)-thiocyanate complex read at 500 nm using a spectrophotometer. The extracted flaxseed oil (0.3 g) was mixed with 9.8 ml of chloroform-methanol (7:3 v/v) solution ina vortex mixer (Range 500-2500 rpm, IKA, Bengaluru) for 4-5 s. Fifty µl of 3.94 M ammonium thiocyanate solution (30 g in 100 ml aqueous solution) and Fe (II) solutions (1:1) were added, and the solution was vortexed for 2-4 s. The resultant solution was incubated (Standard Scientific Instrument, Delhi incubator: 455 × 455× 605 mm) in dark for 5 min at room temperature, and the absorbance was measured in triplicate at 500 nm against blank by using the spectrophotometer. The entire procedure was conducted in subdued light, and was completed within 10 min. The Fe^{3+} standard curve with varying (1 to 25 µg) iron concentration was drawn for determination of hydroperoxide concentration as described by Shantha and Decker (1994). Peroxide value (meq.kg^{-1} oil) was calculated using the following equation:

$$\frac{dm_4}{dt} = \frac{\left[-m_1 + 6m_2 - 18m_3 + 10m_4 + 3m_5\right]}{1200\,\Delta t} \quad \ldots(4)$$

Where,

A_s = Absorbance of sample,
A_b = Absorbance of blank,
m = Slope, obtained from the calibration curve = 41.55,
m_o = Mass of oil taken, g, and
Atomic weight of iron = 55.874.

The division by factor 2 was to express the peroxide value as milliequivalents of peroxide instead of milliequivalents of oxygen.

Optimization and Model Validation

Response surface methodology was used for optimization of process conditions with the goals of maximization of encapsulation efficiency and minimization of peroxide value of spray dried encapsulated powder. Numerical optimization was done for selection of optimal conditions on the basis of desirability. Experimental runs were carried out in triplicate under the optimized conditions to validate the model by comparing the experimental and predicted values of selected responses.

Characterisation of Optimized Microcapsules

The moisture content of the microencapsulated powder produced under optimized process condition was determined by using standard hot air oven method (AOAC, 2000). The water activity of microcapsules was measured by a digital water activity analyser (Rotronics, USA, Model: Hygrolab-3, accuracy: ± 1.5% RH at 23 °C). The particle size distribution was measured using a Laser Scattering PSD Analyzer (Horiba Scientific, Japan, Model: LA-950, Range: 0.01 to 3,000 micron). The morphological characteristics of optimized microcapsules were analysed using a scanning electron microscope (Carl Zeiss, Germany, EVO/MA10 model, accelerating voltage: 0.5 kV to 30 kV) at 20 kV with 2000× magnification.

RESULTS AND DISCUSSION

The important response variables, namely, encapsulation efficiency and peroxide values were analysed for the 17 sets of experiment in triplicate. The experimental design and observed values of encapsulation efficiency and peroxide value achieved under different experimental runs are presented in Table 1.

Response surface methodology was used for analysis of observed responses. After eliminating some non-significant terms ($p \geq 0.10$), the predicted models were tested for adequacy and fitness using analysis

Table 3. Analysis of variance and coded regression coefficients of quadratic order (Second order polynomial model) for observed responses

Parameter	Encapsulation efficiency, %		Peroxide value, meq.kg^{-1} oil	
	p-value (Prob > F)	Coefficient estimate	p-value (Prob > F)	Coefficient estimate
Intercept	-	83.806	-	1.974
X:Oil loading, %	0.0001	-3.054***	<0.0001	0.314***
Y:TS-WPI ratio	0.0005	2.410***	0.0021	0.179***
Z:IAT, °C	0.0011	2.091***	0.0061	0.145***
XY	0.0943	1.070*	0.5053	-0.037
XZ	0.1001	1.048	0.8880	-0.008
YZ	0.2462	-0.700	0.1938	0.076
X^2	0.0101	-1.884**	0.0893	0.102*
Y^2	0.0730	-1.137*	0.5385	0.033
Z^2	0.0501	-1.274*	0.0574	0.117*
Lack of Fit		0.5643^{NS}		0.6833^{NS}
Model (F Value)		17.900***	13.374***	
C.V. %		1.3528	5.0701	
R^2		0.9584	0.9450	
Adjusted R^2		0.9048	0.8744	
Adequate Precision		12.8988	13.0772	

Note: X: oil loading (%), Y: Tapioca starch-whey protein isolate ratio, Z: Inlet air temperature (°C)
**Significant at .05<= p <0.10; **Significant at .01≤p <.05 and ***Significant at p <0.01, NS- non significant*

of variance (ANOVA). The fit summary statistics and sequential model sum of squares were performed to check model adequacy. It was done to avoid poor and unreliable results by the predicted model. The ANOVA and estimated regression coefficients of quadratic models (second order polynomial model) for selected responses are shown in Table 3. The calculated F values of models were higher than tabulated F values for encapsulation efficiency, peroxide value and moisture content indicating that models found highly significant ($p \leq 0.01$), with high coefficient of determination ($R^2 \geq 0.94$). Lack of fit relative to pure error was non-significant for these responses, which also indicated the suitability of these respective models. The coefficient of variation (% CV) was also found to be less than 10 %, which was 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 the model could be used to navigate the design space.

Encapsulation Efficiency (EE)

Under different experimental conditions, encapsulation efficiency using TS-WPI coating material combination varied from 73.67 % to 85.53 per cent. Different models (Linear, quadratic, cubic, 2 FI) were tested for model adequacy. The response surface quadratic model for encapsulation efficiency was significant with $R^2 = 0.96$ and lack of fit F-value of 0.78, implying non-significant lack of fit relative to the pure error. Adequate precision of 12.899 indicated that this model could be used to navigate the design space. ANOVA showed significant linear effect of oil loading, TS-WPI ratio, temperature ($p < 0.01$), interaction effect of oil loading and TS-WPI ratio ($p < 0.1$), quadratic effects of oil loading ($p < 0.05$), TS-WPI ratio, and inlet air temperature ($p < 0.1$) on encapsulation efficiency (Table 3). The results of regression analysis could be expressed in the form of a second-order polynomial equation as following:

$$EE = 83.81 - 3.05X + 2.41Y + 2.09Z + 1.07XY - 1.88X^2 - 1.14Y^2 - 1.27Z^2 \quad \ldots (5)$$

The increase in oil loading resulted in decreased encapsulation efficiency (Fig. 2a). Similar results were also reported by Tan *et al*. (2005) in microencapsulation of fish oil using modified starch suspensions, Omar *et*

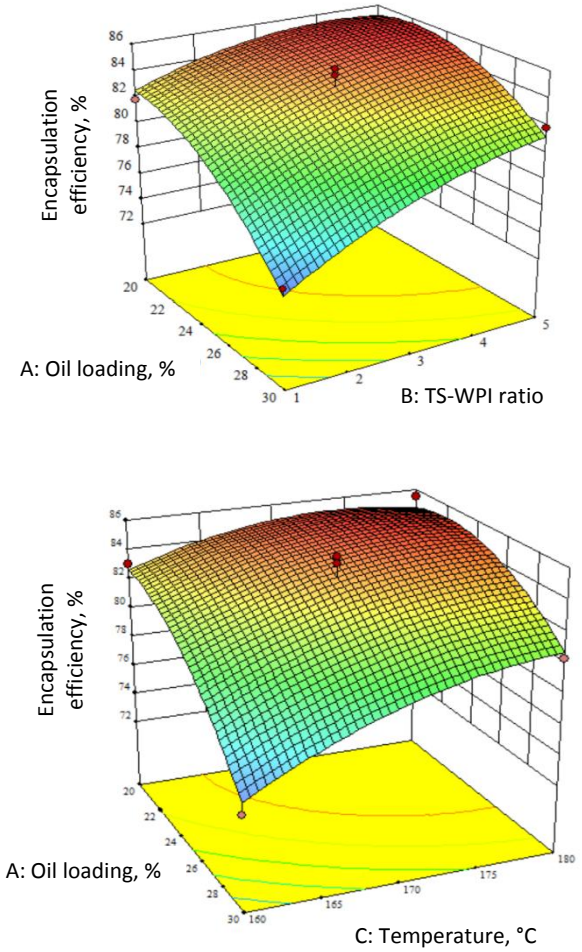


Fig. 2: Response surface 3D plots showing effects of process variables (a) Oil loading and TS-WPI ratio, and (b) Oil loading and inlet air temperature on encapsulation efficiency

al. (2009) reported work on FSO encapsulation using gum Arabic-maltodextrin materials, and by Quispe-condori *et al*. (2011) for flaxseed oil encapsulation with corn protein. This might be due to the fact that the amount of coating material was not sufficient to cover oil droplets at higher loading, and higher oil loading presumably led to more amount of core material close to drying surface resulting in increasing surface oil due to short diffusion path to air/particle interface (Jafari *et al.,* 2008).

Regression coefficients also indicated the negative linear effect of oil loading on encapsulation efficiency. In general, with increase in oil loading (%), the encapsulation efficiency decreased. It was observed that the encapsulation efficiency slightly increased with increase in oil loading and TS-WPI ratio till 24 % oil loading due to positive interaction effect of oil with TS-WPI matrix. The positive linear effects of TS-WPI ratio and inlet air temperature were also depicted on encapsulation efficiency by regression coefficients at higher oil loads (24 % to 30 %).This might be due to availability of more starch material for rapid crust formation on droplet surface, which in turn increased the encapsulation efficiency at higher oil loads. Similar results were reported by Young *et al.* (1993) and Sheu and Rosenberg, (1995) for encapsulation of volatiles.

Response surface plots (Fig. 2a, 2b) also revealed that with increase in TS-WPI ratio and inlet air temperature, encapsulation efficiency increased. Above 24 % oil loading, increase in encapsulation efficiency was also affected by negative quadratic effects of oil loading, TS-WPI ratio and inlet air temperature.

Peroxide Value (PV)

The peroxide values showed variations from 1.61 meq. kg^{-1} oil to 2.72 meq.kg^{-1} oil for different experimental runs with TS-WPI wall material combinations. Using model fitting technique, the adequacy of fitted (Linear, quadratic, cubic, 2FI)) models were tested. The quadratic model showed suitability for response prediction with R^2= 0.95, and highly non-significant lack of fit. The quadratic model for peroxide value could be used for prediction as it possessed adequate precision of 13.07, which was well above the desirable value of 4. ANOVA results exhibited significant positive linear effect of oil loading, TS-WPI ratio, inlet air temperature ($p < 0.01$) and quadratic effects of oil loading and inlet air temperature ($p < 0.1$) on peroxide value for TS-WPI wall material combinations (Table 3).

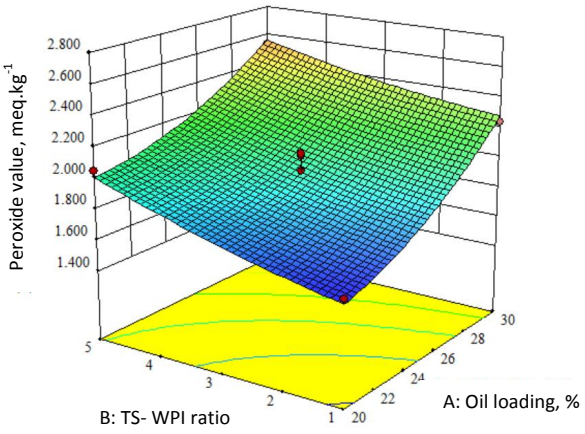
The following equation was developed based on regression analysis for prediction of peroxide value after eliminating the non-significant terms:

$$PV = 1.974 + 0.31X + 0.18Y + 0.15Z + 0.10X^2 + 0.12Z^2 \quad ...(6)$$

The increase in oil loading into the feed mixture resulted into increase in peroxide value of final products at a given ratio of TS-WPI complex. The increase in oil loading might have resulted in more oil on the surface of microcapsules, which in turn became more susceptible to oxidation. Similar results were also reported by Omar *et al.* (2009) and Tonon *et al.* (2012) in their studies on microencapsulation of flaxseed oil using gum Arabic and maltodextrin as coating materials.

Regression analysis coefficients estimates indicated

that the effect of increase in oil loading was higher as compared to the effect of increase in TS-WPI ratio (1:1, 3:1 and 5:1) on peroxide values. Response surface plots (Fig. 3a, b) also showed a positive linear effects of TS-WPI ratio and inlet air temperature on peroxide value. Hence, lower PV value was observed at high protein content in the mixture, which meant that WPI provided better emulsion stabilization and protection towards lipid oxidation. Similar results were also stated by Jimenez *et al.* (2004) in a study on microencapsulated linoleic acid with whey protein concentrate over gum Arabic, and by Tonon *et al.* (2012) in microencapsulation od flaxseed oil using gum Arabic. Interaction effect of independent variables was found to be non-significant. With increase in inlet air temperature, PV was found to be increased at given oil loading. Fig. 3(b) shows that increase in PV between 25 % and 30 % oil loading was higher compared to increase in PV between 20 % and 25 % oil loading. This effect was due to availability of more non-encapsulated oil on the surface of microcapsules, which was more susceptible to oxidation.



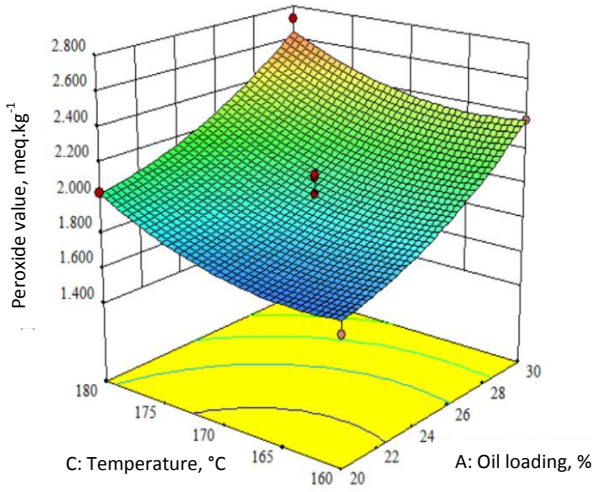


Fig. 3: Response surface 3D plots showing effect of process variables (a) Oil loading and TS-WPI ratio, and (b) Oil loading and inlet air temperature on peroxide value

Process Optimization and Model Validation

Numerical optimization of three independent variables (*viz.* oil loading, TS-WPI ratio and inlet air temperature) was carried out by setting goals on significant dependent variables. Optimization of process conditions was done with goals of maximization of encapsulation efficiency, and minimization of peroxide value. The optimal conditions were selected on the basis of desirability. To confirm the results, experimental runs were carried out in triplicate under the optimized conditions and the response variables were measured. The validation of the model was tested by comparing the experimental and predicted values of selected responses.

The predicted optimized process conditions using TS-WPI wall materials were 20 % oil loading, 2.40:1 TS-WPI ratio and 170°C drying temperature with desirability of 0.92. The spray dried FSO microcapsules produced using optimal conditions are shown in Fig. 4. The observed mean value of responses (EE = 83.2 ± 1.1 % and PV = 1.78 ± 0.13 meq.kg^{-1} oil) and relative mean error with respect to predicted response are shown in Table 4. It is evident that the model could be considered to be suitable for prediction of the response factors.

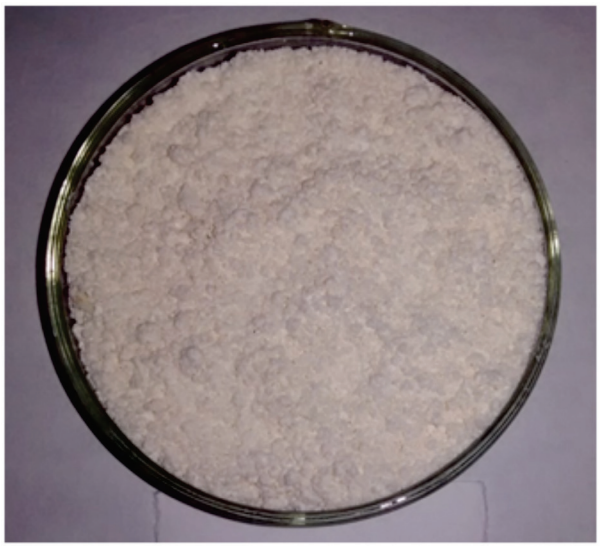


Fig. 4: FSO microcapsules prepared using TS-WPI coating combination under optimized process conditions

Table 4. Model confirmation report with predicted and experimental values of responses at optimal process condition using TS-WPI as coating material

Two-sided	Name	Confidence = 95 %			n = 3	
Factor		Optimised level	Low level	High level	Standard deviation	Coding
X	Oil loading, %	20.00	20	30	0	Actual
Y	TS-WPI ratio	2.40	1	5	0	Actual
Z	Inlet air temperature, °C	170	160	180	0	Actual
Response	**Predicted value**	**Observed value**	**Relative mean error, %**	**SE Pred. n=3**	**95% PI Low**	**95% PI High**
Encapsulation efficiency, %	84.43	83.2 ± 1.1	1.45	0.93	82.23	86.65
Peroxide value, meq.kg^{-1} oil	1.70	1.78 ± 0.13	4.71	0.090	1.49	1.91

Characteristics of Optimized Microcapsule

The moisture content and water activity values of microcapsules produced under optimized process condition were 5.18 ± 0.03 (% w.b.) and 0.11± 0.01, respectively. Lower water activity values are desirable since lipids oxidation is lowest at water activity of 0.2 - 0.3 for most of the dried foods (Polavarapu *et al.,* 2011). Particle size distribution analysis showed that the size of microcapsules ranged between 5.12 - 451.55 µm, with mean particle size of 29.48 µm and mode size of 21.207 µm. The D_{90}, D_{50} and D_{10} values of microcapsules were 43.31, 21.51 and 12.22 µm, respectively, implying that particles were more homogeneous with respect to its shape and structure. Surface morphology (Fig. 5) of encapsulated microcapsules obtained at optimized process conditions showed that most of the particles had smooth, continuous and spherical surface. However, some large-sized microcapsules had surface shrinkage and dents, which might be due to expansion or ballooning process occurred during the surface smoothing mechanism during early stages of drying (Jafari *et al*., 2008; Rodea-González *et al*., 2012).

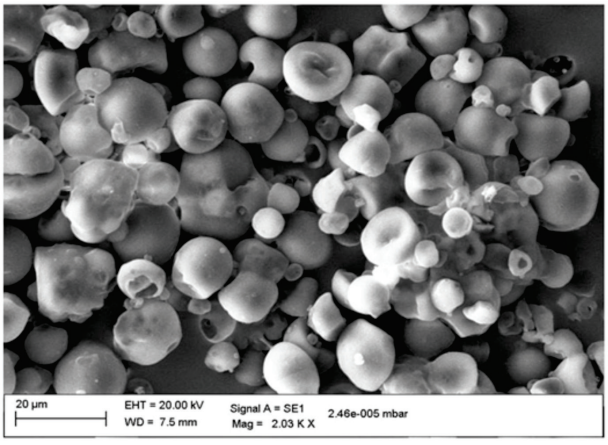


Fig. 5: SEM micrograph of optimized TS-WPI complex-based FSO microcapsules at 2000× magnification

CONCLUSIONS

Second-order polynomial equations for prediction of responses (encapsulation efficiency and peroxide value) were found to be suitable with higher desirability (0.92) and coefficient of determination ($R^2 \geq 0.94$). Encapsulation efficiency and peroxide value were significantly influenced by the changes in oil concentration. Increase in oil loading and inlet air temperature resulted in higher peroxide value of final products at given ratio of TS-WPI complex. The optimum condition for encapsulation of flaxseed oil using starch-protein combination as coating materials to achieve maximum encapsulation efficiency and minimum peroxide value at given process variables were 20% oil loading, 2.40:1 TS-WPI ratio and 170°C drying air temperature. Hence, TS-WPI in ratio of 2.40:1 could be suggested as a good coating material for encapsulation of flaxseed oil, which would provide higher encapsulation efficiency and provide stability against lipid oxidation.

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