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# Encapsulation of rice bran oil in tapioca starch-soya protein isolate complex using spray drying

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ABSTRACT

Microencapsulation of rice bran oil was done using spray drying technique for different oil concentrations (20, 25 and 30%), drying inlet air temperatures (140, 150 and 160 ºC) and a combination of wall material (tapioca starch and soya protein isolate) at different ratios (1:1, 3:1 and 5:1). The total solid content in the mixture used for spray drying was kept constant at 30%. A three-factor, three-level Box-Behnken design was employed for conducting the experiments in order to maximize encapsulation efficiency and gamma oryzanol content, and minimize peroxide value of the encapsulated powder. A polynomial regression model was fitted and the optimum conditions obtained were, 20% oil concentration, 2.6:1 starch-protein ratio (wall material) and 140ºC drying inlet air temperature. At optimum conditions, encapsulation efficiency was found to be 76.97%, total gamma oryzanol content was 12240 ppm and peroxide value was 1.49 meq/kg oil.

**Key words:** Encapsulation efficiency, Gamma oryzanol, Peroxide value, Response surface methodology, Spray drying

Rice is one of the most important food cereals in human nutrition, consumed by more than half of global population (Van Hoed *et al*. 2006). Rice bran oil (RBO), a byproduct obtained through milling of rice, was found to be a rich source of vitamin E complex, i.e. tocopherols, tocotrienols, polyphenols, squalene and phytosterols (Van Hoed *et al.* 2006). It also contains a functional ingredient called gamma oryzanol (GO), which is a natural antioxidant (Vieno *et al.* 2000, Juliano *et al.* 2005). RBO has been reported to possess hypocholesterolemic, neuroprotective and anticancer properties (Sen *et al.* 2007). GO from RBO reported to regulate the expression of antioxidant and stress related genes in rats (Ismail *et al.* 2010). These nutritional and functional properties of RBO evoked interest in incorporating it intowide variety of food products.

In order to obtain the health benefits associated with RBO, it should remain stable physically and chemically in the food processing operations, but GO which acts as a natural antioxidant can be lost by thermal oxidation in the process. Therefore, special care is to be taken to protect it against thermal oxidative degradation. Moreover, GO (from RBO), a water-insoluble material, characterized with poor absorption and lower bioavailability limits its application in food systems (Lee *et al.* 2009). The problem of oxidative degradation and solubility limitations could be partially overcome by entrapping RBO within a biopolymeric material through microencapsulation technique. This technique would protect RBO from unfavorable conditions andalso increase its solubility and bioavailability.

Various techniques are followed for encapsulation of food ingredients, i.e. spray drying, freeze drying, extrusion, coacervation, fluidized bed coating, liposome entrapment, inclusion complexation to name a few (Desai and Park 2005). Among them, spray drying is the most economical and flexible technique, as it uses the equipment that is readily available and also produces good quality powder. It has traditionally been used for the encapsulation of oil based vitamins and fatty acids. So far encapsulated oils produced by spray drying technique are citrus oils, essential oils, tuna oil, fish oil, soy oil and sunflower oil (Hogan *et al.* 2001, Hogan *et al.* 2003, Ascheri *et al.* 2003).

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Wall material selection is one of the important factors in encapsulation of any food material. The best wall material must have good emulsion and film forming properties; have lower viscosity even at higher solid content and should give best protection to the encapsulated oil (Desai and Park 2005). Single wall material alone cannot meet all the properties listed above; therefore, they are used in combination with each other. Protein-polysaccharide complexes have been shown to satisfy the ideal wall material requirements (Young *et al.* 1993). The combination of soya proteins with maltodextrin (Liu and Re 1995, Re and Liu 1996) and whey protein isolate or skim milk powder with maltodextrin (Bylaite *et al.* 2001) have been shown to improve the volatiles retention and encapsulation efficiency during spray drying operation.

In this present study, response surface methodology (RSM) was used to optimize the process conditions of rice bran oil encapsulation using with protein-polysaccharide complex as wall material by spray drying.

## MATERIALS AND METHODS

Physically refined rice bran oil sample was purchased from Shiv Sales Corporation (Delhi, India). Soya proteins isolate (> 95% purity) and tapioca starch was purchased from the local market. HPLC grade methanol, ethanol, acetonitrile, hexane and acetone were received from Merck, India. Carbon dioxide was purchased from Amit Lab Equipments and Services, New Delhi, India. Reference standard of gamma oryzanol (minimum assay 98%) was procured from Wako Pure Chemicals Industries, Ltd (Japan) and stored at 4°C.

According to the RSM setup (Table 1), feed emulsions were prepared. For wall material complex preparation, initially tapioca starch was added to distilled water, then soya protein isolate was added gradually to prepare protein-polysaccharide complex (at different starch-protein ratios 1:1, 3:1, 5:1) and mixture was thoroughly stirred. The total solid content of emulsion (oil and wall material) was fixed at 30%. Oil was then loaded to the wall material solution at a concentration of 20, 25 and 30% with respect to fixed total solids. Emulsions were homogenized for 3 min at 10 000 rpm using a homogenizer (IKA®T25 digital ultra-turrax, India). The temperature of the feed mixture was maintained at 30°C before drying.The feed mixtures were spray dried (Sono Dry 1000) using three inlet/outlet temperatures (140/68, 150/76 and 160/84 ± 2ºC). The following parameters were kept constant; aspirator air flow rate (50 $m^3$/hr), feed flow rate (2.5 ml/min), compressor pressure (115 psi) and atomization/injection pressure (2.5 kg/$cm^2$). The resultant microencapsulated oil powder was stored in airtight amber colour vials at –20ºC for further analysis.

Encapsulation efficiency (EE) was calculated by the following method of Bae and Lee (2008). Fifteen milliliters of hexane were added to 1.5 g of powder in a sealed glass bottle, which was shaken gently for extraction of free oil for 2 min, at room temperature. Whatman number 1 filter paper was used to filter the solvent mixture and then the powder collected on the filter paper was rinsed thrice with 20 ml of hexane. The solvent was evaporated initially at room temperature and thereafter at 60ºC until constant weight. The surface oil (non-encapsulated) was determined by mass difference between the initial empty flask and that of flask containing extracted oil residue (Jafari *et al.* 2008). Total oil content was presumed to be equal to the initial oil content; since preliminary tests have revealed that all the oil used was retained during encapsulation. Encapsulation efficiency

Table 1 Box-Behnken experimental design matrix and responses

| Runs | Oil concentration | Starch-protein | Temp (°C) (C) | Encapsulation efficiency (%) | | Peroxide value (meq/kg oil) | | Gamma oryzanol (ppm × 1000) | |
|---|---|---|---|---|---|---|---|---|---|
| | % (A) | ratio (B) | | Actual | Predicted | Actual | Predicted | Actual | Predicted |
| 1 | 20(-1) | 5(+1) | 150(0) | 76.13 | 76.83 | 1.72 | 1.71 | 12.01 | 11.98 |
| 2 | 30(+1) | 3(0) | 160(+1) | 59.99 | 61.34 | 2.58 | 2.54 | 11.32 | 11.30 |
| 3 | 25(0) | 3(0) | 150(0) | 72.47 | 70.40 | 1.75 | 1.90 | 11.93 | 11.89 |
| 4 | 30(+1) | 3(0) | 140(-1) | 55.55 | 54.38 | 2.31 | 2.31 | 11.67 | 11.65 |
| 5 | 25(0) | 1(-1) | 160(+1) | 68.56 | 67.90 | 1.87 | 1.90 | 11.78 | 11.76 |
| 6 | 25(0) | 5(+1) | 140(-1) | 64.07 | 64.73 | 1.85 | 1.82 | 11.97 | 11.99 |
| 7 | 30(+1) | 5(+1) | 150(0) | 53.81 | 54.32 | 2.38 | 2.41 | 11.36 | 11.36 |
| 8 | 20(-1) | 1(-1) | 150(0) | 73.44 | 72.93 | 1.53 | 1.50 | 12.05 | 12.05 |
| 9 | 25(0) | 3(0) | 150(0) | 71.33 | 70.40 | 1.92 | 1.90 | 11.91 | 11.89 |
| 10 | 25(0) | 5(+1) | 160(+1) | 74.12 | 72.26 | 2.05 | 2.06 | 11.61 | 11.62 |
| 11 | 20(-1) | 3(0) | 140(-1) | 78.12 | 76.77 | 1.51 | 1.55 | 12.19 | 12.21 |
| 12 | 20(-1) | 3(0) | 160(+1) | 82.10 | 83.27 | 1.84 | 1.84 | 11.83 | 11.85 |
| 13 | 25(0) | 3(0) | 150(0) | 70.40 | 70.40 | 1.93 | 1.90 | 11.85 | 11.89 |
| 14 | 25(0) | 3(0) | 150(0) | 69.38 | 70.40 | 1.99 | 1.90 | 11.91 | 11.89 |
| 15 | 25(0) | 3(0) | 150(0) | 68.41 | 70.40 | 1.92 | 1.90 | 11.84 | 11.89 |
| 16 | 30(+1) | 1(-1) | 150(0) | 51.81 | 51.11 | 2.25 | 2.26 | 11.52 | 11.55 |
| 17 | 25(0) | 1(-1) | 140(-1) | 60.11 | 61.97 | 1.63 | 1.62 | 12.12 | 12.11 |

(EE) was calculated from the following equation:

$$EE = \frac{(TO - SO)}{TO} \times 100$$

where, TO is the total oil content and SO is the surface oil (non-encapsulated oil) content

Peroxide value is a measure of the oxidative rancidity of oil and expressed as milliequivalent of peroxide oxygen combined with 1 kg of oil (meq/kg oil). To measure peroxide value, oil extraction was carried out following the method described by Partanen *et al.* (2008). The peroxide value was determined spectrophotometrically according to the IDF standard method 74A:1991 (International Dairy Federation 2005) using the V-670 UV-VIS/NIR spectrophotometer. All measurements was done in triplicate. Hydroperoxide concentrations were determined using a $Fe^{+3}$ standard curve with iron concentration varying from 1 to 24 µg, as described by Shantha and Decker (1994).

A new method was optimized for quantification of total gamma oryzanol of RBO using an integrated Acquity Ultra Performance Convergence Chromatogram ($UPC^2$) system, was integrated with a Waters photodiode array detector (PDA). Empower software 3 pro (Waters Corporation) was used for data collection and analysis. Separation of GO was carried outusing Acquity $UPC^2$ BEH 2 - Ethyl Pyridine (BEH 2 EP) column, 2.1×150 mm with particle size 5 µm. Carbon dioxide: methanol in the ratio of 80:20 (v/v) was used to carry out a run on isocratic mode. The flow rate of mobile phase was maintained at 1 ml/min through out the run and the column temperature was kept at 35°C. The sample injection volume was 2 µl at fixed wavelength of 330 nm. Active back pressure regulator (ABPR) was maintained at 2000psi and sample temperature was kept at 20°C. Samples and standards were prepared by diluting RBO sample and GO standard in acetone. Prepared samples and standard solution were passed through PVDF membrane filters 0.22µ before injection into the system.

A three-factor, three-level Box-Behnken design (BBD) was used to optimize the encapsulation of RBO for maximum encapsulation efficiency and GO content and minimum peroxide value. The design setup consisted of 17 experimental points, including 5 centre points. The three process conditions examined as independent variables include: A= Oil concentration (%), B = Wall material ratio (starch-protein) and C = Drying inlet air temperature (°C). The actual values of each process variable were coded at three levels for analysis (Table 1). The quadratic model for predicting the optimal solution was expressed using the following equation:

$$R = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC$$

where, R is the predicted response (EE or PV or GO) and $\beta_n$ are constant regression coefficients.

The response variables such as encapsulation efficiency, peroxide value and total gamma oryzanol content were analyzed for all the 17 experimental points. Analysis of variance (ANOVA) was done, to test lack of fit and the significance of the linear and interaction effects of the variables on the quality parameters. The ratio of the mean square due to regression and the mean square due to real error is called F-value. Predicted error sum of squares (PRESS) and predicted $R^2$ was considered to check model adequacy. Design Expert Statistical Software package 9.0.0 trial version was used to perform statistical analysis.

Second-order polynomial equation of RSM was used to optimize the microencapsulating conditions of RBO. A numerical optimization technique was applied to find a point which maximizes the encapsulation efficiency and total gamma oryzanol content, and minimizes peroxide value. The optimal conditions were selected on the basis of desirability at which experiments were carried out and the experimental and predicted values were compared to validate the model. To confirm the results, runs were carried out in triplicate under the optimized conditions.

## RESULTS AND DISCUSSION

RSM was employed to optimize microencapsulation of rice bran oil. Design expert software used to obtain predicted values by a model fitting technique and was found to be sufficiently correlated with the actual values. The actual and predicted values are presented in Table 1. The actual values were analyzed and fitted to various regression models (linear, interactive, quadratic and cubic). Generally, exploring with fitted response surface without checking model adequacy may give poor or unreliable results (Omwamba and Hu 2009). Hence, checking of model adequacy is necessary. The sequential model sum of squares and model summary statistics are performed to decide about the adequacy of models among different models to represent response parameters of microencapsulated oil powder (Prakash *et al.* 2013) and the results are shown in Table 2. Based on the adequacy of model summary output, the quadratic model was found to be statistically significant. The sequential model sum of square also indicates that the p-value was lower than 0.01 for quadratic model and have maximum "Predicted $R^2$" and "Adjusted $R^2$" values. Therefore, the quadratic model was selected for further analysis.

### *Model fit*

Results showed that the encapsulation efficiency varied from 51.81 to 82.1% and peroxide value varied from 1.51 to 2.58 meq/kg oil and total gamma oryzanol content value varied from 11.32 to 12.19 (ppm × 1 000). The following second-order polynomial equation in terms of coded units was generated to obtain the empirical relationship between the experimental results on the basis of Box-Behnken design.

$$EE\ (R_1) = +70.4 - 11.08A + 1.78B + 3.37C - 0.17AB + 0.12AC + 0.4BC - 2.19A^2 - 4.41B^2 + 0.73C^2$$

$$PV\ (R_2) = +1.90 + 0.37A + 0.09B + 0.13C - 0.015AB - 0.015AC - 0.01BC + 0.14A^2 - 0.071B^2 + 0.019C^2$$

Table 2 Adequacy of the model

| Source | Sum of squares | df | Mean square | F Value | P-value | Prob> F |
|---|---|---|---|---|---|---|
| | | | *Sequential model sum of squares* | | | |
| *Encapsulation efficiency* | | | | | | |
| Mean vs Total | 77767.06 | 1 | 77767.06 | | | |
| Linear vs Mean | 1097.74 | 3 | 365.91 | 35.52 | < 0.0001 | |
| 2FI vs Linear | 0.81 | 3 | 0.27 | 0.020 | 0.9958 | |
| Quadratic vs 2FI | 107.27 | 3 | 35.76 | 9.69 | 0.0069 | Suggested |
| Cubic vs Quadratic | 15.68 | 3 | 5.23 | 2.06 | 0.2482 | Aliased |
| Residual | 10.15 | 4 | 2.54 | | | |
| Total | 78998.72 | 17 | 4646.98 | | | |
| *Peroxide value* | | | | | | |
| Mean vs Total | 64.18 | 1 | 64.18 | | | |
| Linear vs Mean | 1.27 | 3 | 0.42 | 38.53 | < 0.0001 | |
| 2FI vs Linear | 2.200E-003 | 3 | 7.333E-004 | 0.052 | 0.9833 | |
| Quadratic vs 2FI | 0.10 | 3 | 0.034 | 5.95 | 0.0244 | Suggested |
| Cubic vs Quadratic | 7.200E-003 | 3 | 2.400E-003 | 0.30 | 0.8266 | Aliased |
| Residual | 0.032 | 4 | 8.070E-003 | | | |
| Total | 65.58 | 17 | 3.86 | | | |
| *Gamma oryzanol content* | | | | | | |
| Mean vs Total | 2373.46 | 1 | 2373.46 | | | |
| Linear vs Mean | 0.89 | 3 | 0.30 | 40.82 | < 0.0001 | |
| 2FI vs Linear | 3.725E-003 | 3 | 1.242E-003 | 0.14 | 0.9361 | |
| Quadratic vs 2FI | 0.080 | 3 | 0.027 | 16.80 | 0.0014 | Suggested |
| Cubic vs Quadratic | 4.625E-003 | 3 | 1.542E-003 | 0.95 | 0.4960 | Aliased |
| Residual | 6.480E-003 | 4 | 1.620E-003 | | | |
| Total | 2374.44 | 17 | 139.67 | | | |
| | *Std. Dev.* | *Actual* $R^2$ | *Adjusted* | *Predicted* | *PRESS* | |
| | | | *Model summary statistics* | | | |
| *Encapsulation efficiency* | | | | | | |
| Linear | 3.21 | 0.8913 | 0.8662 | 0.8102 | 233.74 | |
| 2FI | 3.65 | 0.8919 | 0.8271 | 0.6020 | 490.20 | |
| Quadratic | 1.92 | 0.9790 | 0.9521 | 0.7834 | 266.80 | Suggested |
| Cubic | 1.59 | 0.9918 | 0.9670 | | + | Aliased |
| *Peroxide value* | | | | | | |
| Linear | 0.10 | 0.8989 | 0.8756 | 0.8162 | 0.26 | |
| 2FI | 0.12 | 0.9005 | 0.8408 | 0.6044 | 0.56 | |
| Quadratic | 0.075 | 0.9720 | 0.9359 | 0.8824 | 0.17 | Suggested |
| Cubic | 0.090 | 0.9771 | 0.9083 | | + | Aliased |
| *Gamma oryzanol content* | | | | | | |
| Linear | 0.085 | 0.9040 | 0.8819 | 0.8308 | 0.17 | |
| 2FI | 0.095 | 0.9078 | 0.8525 | 0.6581 | 0.34 | |
| Quadratic | 0.040 | 0.9888 | 0.9743 | 0.9148 | 0.084 | Suggested |
| Cubic | 0.040 | 0.9934 | 0.9738 | | + | Aliased |

$$GO\ (R_3) = +11.89 - 0.28A - 0.065B - 0.18C - 0.03AB + 0.0025AC - 0.005BC - 0.14A^2 - 0.018B^2 - 0.0002C^2$$

The ANOVA results for the BBD are shown in Table 3. The model is highly significant as evident from F-test value being 36.30, 26.96 and 68.39 for encapsulation efficiency, peroxide value and total gamma oryzanol content, respectively with a P-value less than 0.0001. The coefficient of determination ($R^2$ value), which is a best measure of degree of fit, for encapsulation efficiency, peroxide value and total gamma oryzanol content) was 0.979, 0.972 and 0.989, respectively and the corresponding adjusted $R^2$ values were 0.952, 0.936 and 0.974, respectively. Generally, higher is the value of coefficient of variation (CV), lower is the reliability of the experiment. In present design, a low value of CV (2.87, 3.87 and 0.34) indicated a greater reliability of the experiments performed (Li *et al.* 2007). The adequacy precision values for the model were 21.823, 18.056 and 29.625 indicating a significant result. It is implied from very

Table 3 Analysis of variance and statistical parameters of the model

| Source | Sum of squares | df | Mean square | F value | P-value | Prob> F |
|---|---|---|---|---|---|---|
| *Encapsulation efficiency* | | | | | | |
| Model | 1205.82 | 9 | 133.98 | 36.30 | < 0.0001 | Significant |
| A | 981.91 | 1 | 981.91 | 266.06 | < 0.0001 | |
| B | 25.24 | 1 | 25.24 | 6.84 | 0.0347 | |
| C | 90.59 | 1 | 90.59 | 24.55 | 0.0016 | |
| AB | 0.12 | 1 | 0.12 | 0.032 | 0.8626 | |
| AC | 0.053 | 1 | 0.053 | 0.014 | 0.9081 | |
| BC | 0.64 | 1 | 0.64 | 0.17 | 0.6896 | |
| $A^2$ | 20.15 | 1 | 20.15 | 5.46 | 0.0521 | |
| $B^2$ | 81.99 | 1 | 81.99 | 22.22 | 0.0022 | |
| $C^2$ | 2.24 | 1 | 2.24 | 0.61 | 0.4612 | |
| Residual | 25.83 | 7 | 3.69 | | | |
| Lack of fit | 15.68 | 3 | 5.23 | 2.06 | 0.2482 | Not significant |
| Mean | 67.64 | | | | | |
| CV % | 2.84 | | | | | |
| PRESS | 266.80 | | | | | |
| Adeq precision | 21.823 | | | | | |
| *Peroxide value* | | | | | | |
| Model | 1.37 | 9 | 0.15 | 26.96 | 0.0001 | Significant |
| A | 1.07 | 1 | 1.07 | 188.97 | < 0.0001 | |
| B | 0.065 | 1 | 0.065 | 11.49 | 0.0116 | |
| C | 0.14 | 1 | 0.14 | 23.97 | 0.0018 | |
| AB | 9.000E-004 | 1 | 9.000E-004 | 0.16 | 0.7015 | |
| AC | 9.000E-004 | 1 | 9.000E-004 | 0.16 | 0.7015 | |
| BC | 4.000E-004 | 1 | 4.000E-004 | 0.071 | 0.7977 | |
| $A^2$ | 0.081 | 1 | 0.081 | 14.42 | 0.0067 | |
| $B^2$ | 0.021 | 1 | 0.021 | 3.76 | 0.0935 | |
| $C^2$ | 1.520E-003 | 1 | 1.520E-003 | 0.27 | 0.6197 | |
| Residual | 0.039 | 7 | 5.640E-003 | | | |
| Lack of fit | 7.200E-003 | 3 | 2.400E-003 | 0.30 | 0.8266 | Not significant |
| Mean | 1.94 | | | | | |
| CV % | 3.87 | | | | | |
| PRESS | 0.17 | | | | | |
| Adeq precision | 18.056 | | | | | |
| *Gamma oryzanol content* | | | | | | |
| Model | 0.98 | 9 | 0.11 | 68.39 | < 0.0001 | Significant |
| A | 0.61 | 1 | 0.61 | 384.83 | < 0.0001 | |
| B | 0.034 | 1 | 0.034 | 21.31 | 0.0024 | |
| C | 0.25 | 1 | 0.25 | 156.65 | < 0.0001 | |
| AB | 3.600E-003 | 1 | 3.600E-003 | 2.27 | 0.1757 | |
| AC | 2.500E-005 | 1 | 2.500E-005 | 0.016 | 0.9036 | |
| BC | 1.000E-004 | 1 | 1.000E-004 | 0.063 | 0.8090 | |
| $A^2$ | 0.077 | 1 | 0.077 | 48.55 | 0.0002 | |
| $B^2$ | 1.327E-003 | 1 | 1.327E-003 | 0.84 | 0.3909 | |
| $C^2$ | 2.632E-007 | 1 | 2.632E-007 | 1.659E-004 | 0.9901 | |
| Residual | 0.011 | 7 | 1.586E-003 | | | |
| Lack of fit | 4.625E-003 | 3 | 1.542E-003 | 0.95 | 0.4960 | Not significant |
| Mean | 11.82 | | | | | |
| CV % | 0.34 | | | | | |
| PRESS | 0.084 | | | | | |
| Adeq precision | 29.625 | | | | | |

low P-value (<0.0001) and higher $R^2$ value that the selected quadratic polynomial model is highly significant and sufficient to represent the relationship between the response and independent variables.

*Effect of process parameters on response variables*

*Effect of oil concentration*

Fig 1 shows that when the oil loading increased from 20 to 30%, encapsulation efficiency (EE) decreased at all temperatures and starch-protein ratio. The maximum EE (78.12%) was observed at 20% oil concentration, 3:1 starch-protein ratio and 140ºC inlet air temperature. At higher oil concentration, lower encapsulation efficiency was observed which might have resulted from greater amount of oil close to the drying surface; thereby, shortening the diffusion path length to the air/particle interface, leading to increase in the surface oil content. Similar behaviour was observed for microencapsulation of fish oil (Hogan *et al*. 2003, Tan *et al.* 2005), for lemon myrtle oil (Huynh *et al*. 2008) and for flax seed oil (Tonon *et al*. 2012).

In general, higher oil loads led to higher peroxide values at all temperature and starch-protein ratio studied and lowest PV (1.51 meq/kg oil) was observed at 20% oil loading, 3:1 starch protein ratio and 140ºC temperature. This can be attributed to the availability of excess oil over and above the maximum that can be adsorbed by the wall material. This non-encapsulated oil (surface oil) when comes in contact with oxygen, is easily susceptible to lipid oxidation.Tonon *et al*. (2012) had observed similar results for flax seed oil encapsulation.

Fig 2 reveals that as the oil concentration increased from 20 to 30%, total gamma oryzanol (GO) content decreased correspondingly and the maximum retention of GO (12190 ppm) was observed at 20% oil loading with 3:1 starch-protein ratio at 140ºC inlet temperature. Also it was observed that EE and total gamma oryzanol content were positively correlated. When the oil concentration is low, EE is higher which means a very small amount of oil in the surface of powder particles are exposed to heat, there by minimum degradation of total gamma oryzanol in the powder.

*Effect of starch-protein ratio (wall material)*

Increase in starch-protein ratio increased EE till 3:1 ratio however no significant difference was observed thereafter (Fig 1). At 3:1 starch-protein ratio, maximum EE observed for all the temperature and oil concentration studied. This may be attributed to the more stable emulsion formed at 3:1 starch-protein ratio, which in turn determines the effectiveness of encapsulation, and at lower ratio the amount of starch may not be sufficient to interact with all the protein molecules present in the emulsion that could provide steric stabilization at emulsion droplet interfaces. Similar results were reported by Akhtar and Dickinson (2003) for whey protein-dextran combination for emulsion stability. Benichou *et al.* (2002) reported that protein-polysaccharide complex enhance the functional properties of food formulations including emulsion stability.

Powder lipid oxidation was strongly influenced by proportion of starch and protein in wall material combination. There was an increase in the PV as the starch-protein ratio increased irrespective of oil concentration and temperatures. This could be due to decrease in amount of protein in the starch-protein complex as the ratio increased. Previous studies have reported that soya proteins and their hydrolysates have antioxidant properties (Elias *et al*. 2008, Castro *et al*. 2014). In this sense, the presence of soya protein isolate may have contributed to the protection of the oil from oxidation, as well as providing better oxidative stability. This result corroborates the findings of Rascón *et al.* (2011), who had reported that combination of maltodextrin and soya protein isolate have the ability to protect the paprika oleoresin against oxidation.

Fig 2 reveals that wall material effect on total gamma oryzanol is similar to its effect on EE.

*Effect of drying inlet air temperature*

Fig 1 shows that increase in temperature (140–160ºC), increased EE for all the oil concentration and starch-protein ratio. Higher inlet air temperature leads to rapid formation of semi-permeable membrane on the droplet surface, thereby stopping further leaching of volatiles towards the surface. This leads to maximum volatiles retention and decreased surface oil content. Similar results were reported for

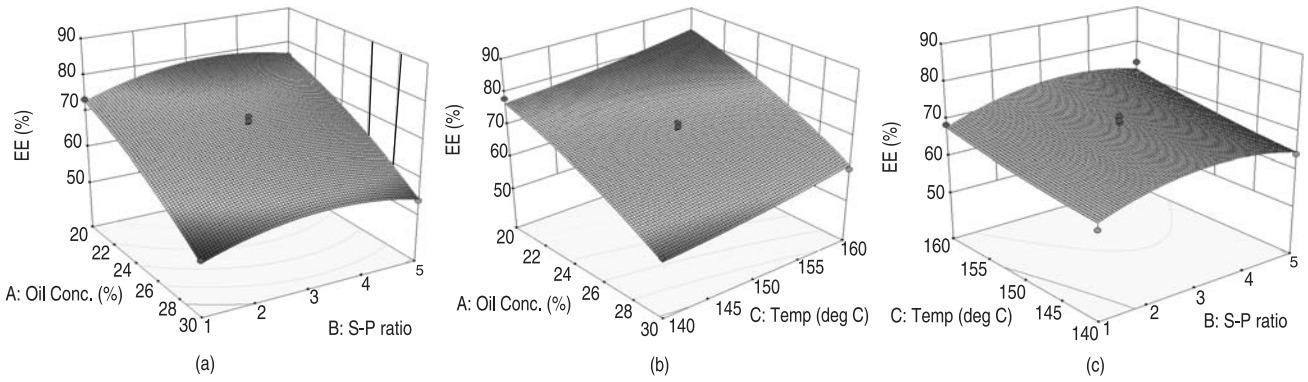


Fig 1 Response surface plots showing the effects of process parameters on encapsulation efficiency

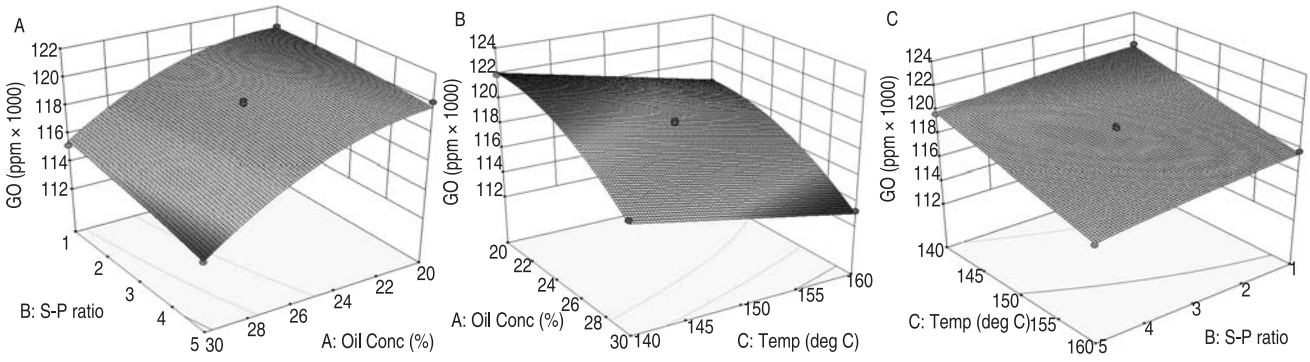


Fig 2 Response surface plots showing the effects of process parameters on total gamma oryzanol content

Table 4 Experimental runs for optimized conditions of RBO encapsulation

| Oil Conc % | starch-protein ratio | Temp (ºC) | Encapsulation efficiency (%) | | Peroxide value (meq/kg oil) | | Total gamma oryzanol content (ppm ×1 000) | | Desirability |
|---|---|---|---|---|---|---|---|---|---|
| | | | Actual | Predicted | Actual | Predicted | Actual | Predicted | |
| 20 | 2.6 : 1 | 140 | 76.97 | 76.27 | 1.49 | 1.52 | 12.24 | 12.21 | 0.927 |

encapsulation of volatiles and flavours by spray drying (Drusch *et al.* 2006, Bhandari *et al.* 1992).

The inlet air temperature was positively correlated with PV at all oil concentration and starch-protein ratio. The use of higher inlet air temperatures caused peroxide formation as more energy available for the lipid oxidation process. This result is in line with that of Serfert *et al.* (2009), who had observed that fish oil prepared at higher inlet temperature had three times higher hydroperoxide content than that produced at lower temperature. Thomsen *et al.* (2005) reported quality deterioration of milk powder due to lipid oxidation at high temperature.

The temperature effects on degradation of total gamma oryzanol content in microencapsulated RBO powder were shown in Fig 2. The gamma oryzanol content in powder decreased as the temperature increased at all oil concentration and starch-protein ratio used. This decrease in gamma oryzanol content at higher inlet air temperature may be due to oxidation of the compound by oxidation products of oil formed during the heating process. Similar results were reported for degradation of olive oil antioxidants (α-tocopherol, hydroxytyrosol derivatives and tyrosol derivatives) at 60º and 100ºC (Nissiotis and Tasioula-Margari 2002).

*Determination of optimum conditions*

The Derringer's desirability function was applied to optimize the independent variables. For optimization purpose, oil concentration (20–30%), starch-protein ratio (1:1, 3:1 and 5:1) and drying inlet air temperature (140–160ºC) were kept within range; EE and GO were set for maximum and PV for minimum. The optimum conditions selection was set for maximum desirability. The methodology of desired function was employed and the optimum level of all three variables were obtained, to indicate that 20% of oil concentration, 2.6:1 starch-protein ratio and 140ºC drying inlet air temperature provides maximum of 76.97% encapsulation efficiencyand 12240 ppm of total gamma oryzanol content and 1.49 meq/kg oil minimum peroxide value with overall desirability value of 0.927.

*Verification of the model*

Process variables of microencapsulation of RBO were optimized by BBD of RSM. In order to validate the model, actual and predicted values of the responses at optimized conditions were further processed. The results (Table 4) indicate that there is no significant difference between predicted value generated and actual value measured, confirming reliability of the model.

In this work it was possible to evaluate the performance of tapioca starch-soya protein isolate complex as wall material in rice bran oil encapsulation by spray drying.Rice bran oil-in-water emulsions prepared with 20% oil concentration and 2.6:1 starch-protein ratio, and spray dried at 140ºC inlet air temperature produced microcapsules of higher encapsulation efficiency and gamma oryzanol content with lower peroxide value.

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