Optimization of Microencapsulation of Human Milk Fat Substitute by Response Surface Methodology

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Abstract: Human milk fat substitutes (HMFS) are rich in polyunsaturated fatty acids which upon microencapsulation, can be used as a source of high quality lipids in infant formula. The response surface methodology (RSM) was employed to optimize the microencapsulation condition of HMFS as a functional product. The microencapsulation efficiency (MEE) of microencapsulated HMFS was investigated with respect to four variables including concentration of soya lecithin (A), ratio of demineralized whey powder to malt dextrin (B), HFMS concentration (C), and homogenizing pressure (D). The optimum conditions for efficient microencapsulation of HMFS by the spray drying technique were determined as follows: the amount of soybean lecithin-0.96%, ratio of desalted whey powder to malt dextrin-2.04:1, oil content-17.37% and homogeneous pressure-0.46MPa. Under these conditions, the MEE was 84.72%, and the basic indices of the microcapsules were good. The structure of the microcapsules, as observed by scanning electron microscopy (SEM), revealed spherical, smooth-surfaced capsules with diameters ranging between 10-50 μm. Compared with HFMS, the peroxide value (POV) and acid value (AV) of the microcapsule were significantly lower during storage indicating that the microencapsulation process increases stability and shelf life. Infrared spectroscopic analyses indicated that HFMS had the same characteristic functional groups as the oil extracted from microcapsules. Simulated in vitro digestion revealed that the microcapsules were digested completely within 2h with maximum lipid absorption rate of 64%. Furthermore, these results advocate the embedding process of HFMS by RSM due to its efficacy.

Key words: human milk fat substitute, microcapsule, response surface experiment, scanning electron microscopy, infrared spectrum, vitro digestion

1 INTRODUCTION

Human milk, with a composition of approximately 9 to 12 g/L for protein, 32 to 36 g/L for fat, 67 to 78 g/L for lactose and energy content 650 to 700 kcal/L, is considered to be the most ideal food for newborns. Long chain polyunsaturated fatty acids (PUFAs) found in human milk, mainly linoleic acid, α-linolenic acid and arachidonic acid (AA), are essential for the physical and cognitive development of infants and play an important role in promoting healthy eyesight. The n-3 PUFAs include α-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which help in the development of the central nervous system and also protect against cardiovascular and cerebrovascular diseases. Some studies have found fat to be the most variable nutrient of human milk and the fatty acid (FA) profile of human milk particularly that of the long chain polyunsaturated fatty acids, varies with respect to maternal diet. The fat content in human milk is influenced by many factors such as genetics, diet, gestational age, lactation and any underlying disease. Since an increasing number of infants are unable to receive breast milk due to various factors, much attention recently has been focused on the development of lipid substitutes for breast milk to ensure and enhance infant health. These human milk fat substitute (HFMS) can be made from various sources. Most experiments on HFMS have focused on enhancing the percentage of n-3PUFA by making use of soybean oil, palm oil, rape-seed oil and other basic oils. Numerous studies have reported the use of different raw materials and procedures for the synthesis of various HMFS that differ in chemical composition. However, the HFMS is actually an oil derivative and is therefore prone to oxidation and difficult to eat, store and transported. Therefore HMFS preparation.
should ideally be delivered in microencapsulated form.

Microcapsule technology is a process wherein tiny spheres of bio active materials such as lipids, enzymes and even whole cells are surrounded by a coating or wall of soluble film to form microcapsules. Spray drying, extensively used in the food industry due to the ease of production and consumption, involves the suspension or dissolution of the bio active substance (the core material) in a polymer solution (the wall material) which is then subjected to high temperature gas atomization, thus trapping the substance in a microcapsule. Since the physical and chemical properties of the microencapsulated products are influenced by the wall material to a great extent, the selection of a suitable polymer is vital to optimum microcapsule. The basic requirement of spray drying is the lack of interaction between the core and wall material due to differences in their solubility, flow and permeability. Malt dextrin is a hydrolyzed starch commonly used as wall material in microcapsule of food ingredients. It offers advantages such as relatively low cost, neutral aroma and taste, low viscosity at high solids concentrations and good protection against oxidation. However, the biggest problem of malt dextrin is its low emulsifying capacity. Therefore, it is desirable to use malt dextrin in combination with other surface active biopolymers, such as desalted whey powder which is one of the most common wall materials. The selection of wall material combinations affects both the emulsion properties and the particles’ characteristics after drying and during storage. The different ratio between them may lead to the deterioration of emulsion stability and low surface strength, thus reducing the efficiency of microcapsules. The microcapsule of lipids therefore requires an aqueous base as the wall material. Since lipids and water are immiscible, an appropriate emulsifier is added so that the lipid phase can be stably and evenly distributed in the aqueous film. And soybean lecithin is a widely used emulsifier with remarkable effect. But too much emulsifier will result in excessive viscosity of emulsion and even flocculation; the lower emulsifier content will lead to the oil and water phase can not be stably dissolved. The microencapsulated lipids are then processed into a powder form so that they can later be dissolved in water or other aqueous solution. Sub-optimal physical-chemical conditions employed during spray drying milk fats, e.g. involving homogenization pressure and lipid content, can lead to the loss of functional PUFAs. High oil content may lead to the wall sticking phenomenon, while the microcapsule oil content is too low, which makes the microcapsule meaningless. On the other hand, the homogeneous pressure is too low to be able to obtain stable emulsion; the high homogenization pressure may produce a large amount of heat energy, leading to the rise of temperature, which may affect the unsaturated fatty acids (UFAs). It is important therefore to optimize the process to maximize the yield and quality of PUFAs in the microencapsulated HMFS. In this regard, microcapsule efficiency (MEE) has been used as an important parameter to assess the quality of microencapsulated samples.

The aim of this study was to produce HMFS from raw materials like coconut oil, soybean oil, corn oil, sunflower oil and fish oil, with FA composition similar to human milk as per the acceptable ratios in China, and an enhanced percentage of PUFAs (especially DHA, EPA and AA). The microcapsules were prepared by the spray drying method. The MEE of microencapsulated HMFS was investigated with respect to four variables including concentration of soy lecithin (A), ratio of demineralized whey powder to malt dextrin (B), HFMS concentration (C), and homogenizing pressure (D) and the optimum conditions of the procedure were determined. The quality of the microcapsules was assessed on the basis of their basic properties (moisture, ash content, solubility, microcapsule efficiency and yield) and structural characteristics were observed through scanning electron microscopy (SEM) and infrared spectroscopy. The stability and in vitro digestion of the microcapsules were also determined for their intended use in infant formula.

2 MATERIALS AND METHODS

2.1 Materials

Soybean oil, coconut oil, corn oil, sunflower oil and fish oil were purchased from a supermarket in Haikou, China. Desalted whey powder, maltodextrin (DE = 15 ~ 20) and food grade soybean lecithin were purchased from Shandong Xiwang Food Co. Ltd. Potassium hydroxide, calcium chloride, phosphate buffer, hydrochloric acid, sodium thiocyanate, phenolphthalein, ethanol, potassium iodide, ammonia water and petroleum ether (boiling range is 30-60°C) were purchased from Sinopharm Chemical Reagent Co., Ltd. China. Pepsin was derived from pig stomach at ≥ 2500 units/mg dry weight, pig bile salts were purchased from China Yuanye Biotechnology Company and pancreatic lipase was derived from bovine pancreas (15-35 units/mg in powdered form).

2.2 Preparation of HMFS

The starting HMFS sample was prepared by mixing 10.01 g soybean oil, 16.59 g coconut oil, 31.04 g corn oil, 4.81 g sunflower seed oil and 37.55 g fish oil to a total of 100 g. Then HMFS were stirred at 4000 rpm/min at high speed for 3 min to obtain a stable mixture. The samples were homogenized by a disintegrator and stored at −4°C until used.

2.3 Microencapsulation process

The microcapsules were prepared as previously described and the process is outlined in Fig. 1.
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(1) Oil phase: the HMFS was emulsified with soy lecithin at room temperature (25°C).
(2) Aqueous phase: the wall material which includes the desalination of whey powder and maltodextrin was dissolved in the distilled water (65°C).
(3) Oil and water phases were emulsified at 1500 rpm/min for 5 min.
(4) After a stable emulsion was obtained by homogenization, the microcapsule was prepared by spraying and drying with inlet air temperature of 180°C and outlet temperature of 90°C. The microcapsules were then sealed with nitrogen and stored at −20°C.

2.4 Optimization of microencapsulation process
Response surface methodology (RSM) was used to study the variations in MEE with respect to operating parameters including soy lecithin concentration (A), ratio of demineralized whey powder: malt dextrin (B), oil concentration (C) and homogenizing pressure (D). A Box–Behnken design (BBD) was used to establish the experimental conditions and the effects were evaluated on the basis of the factors and their interactions. The factors and their concentration ranges were: A from 0.75 to 1.25 (% w/w), B from 3:1 to 1:1, C from 10 to 20 (% w/w) and D from 20 to 40 MPa. The actual factor was coded to facilitate multiple regression analysis (Table 1). The RSM of the microcapsule samples are shown in Table 2.

### Table 1
| Factor                      | level |
|-----------------------------|-------|
|                             | −1    | 0    | 1    |
| A. Concentration of soy lecithin (% w/w) | 0.75  | 1    | 1.25 |
| B. Ratio of demineralised whey powder: malt dextrin (w/w) | 3:1   | 2:1  | 1:1  |
| C. Concentration of HFMS (% w/w) | 10    | 15   | 20   |
| D. Homogeneous pressure (MPa) | 20    | 30   | 40   |

2.5 Moisture and ash content
Moisture and ash content were determined according to protocol recommended by the Association of Official Analytical Chemists (29, 30).

2.6 Solubility
To calculate solubility, 5 g of the microcapsules (accurate to 0.01 g) was mixed with 40 mL water till the milk (whey) powder dissolved completely. The solution was then incubated at 30°C in a water bath for 5 min, shaken for 3 min, and then centrifuged (3000 rpm/hr, 10 min) to precipitate the insoluble matter. The supernatant was drained, re-suspended in 38 mL water warmed between 25 to 30°C, mixed thoroughly and centrifuged again. After draining the supernatant, the precipitate was rinsed with a small amount of water and transferred into a weighing dish of known weight. The weighing dish was then placed on a boiling water bath to evaporate any remaining water in the dish and then dried at 100°C to a constant weight (the difference between the last two measurements should not exceed 2 mg). The solubility was calculated as follows:

\[
\text{Solubility} (g/100 \text{ g}) = \frac{\text{undissolved substance (g)}}{1 - \frac{\text{moisture (g/100 g)}}{\text{microcapsule sample (g)}}} \times \frac{100}{100}
\]
2.7 Microencapsulation efficiency (MEE) and yield (MEY)

The MEE and MEY were determined as previously described\(^{23,24}\).

The surface oil content in the microcapsules were first measured using the following method: 5 g of the microcapsules was weighed in a triangular bottle of known weight and the oils were extracted with 50 mL petroleum ether upon shaking for 10 min. The extracted mixture was separated through filter papers and the extraction and filtration steps were repeated for 3 times. The filtrates were combined into the flask of known weight, evaporated by rotation, and then vacuum dried to a constant weight, following which the surface oil content was calculated.

To determine the total oil content of the microcapsules, a series of extractions were performed as described below: 5 g of the microcapsule powder was first dissolved in 25 mL water and then added 5 mL ammonia (25\%, \(v/v\)). The mixture was incubated in a water bath at 65\(^\circ\)C for 20 min, followed by organic phase extraction. After cooling down the mix to room temperature, the organic phase was sequentially extracted with 25 mL ethyl alcohol absolute, 62.5 mL ether, 62.5 mL petroleum ether and shaken for 1 min. The mixture was kept static and layered, and then the organic layer was transferred to another flask. To the remaining aqueous phase, 7.5 mL ethanol, 37.5 mL ether and 37.5 mL petroleum ether were added and the above steps

| Std | Run | A  | B  | C  | D  | MEE(%) |
|-----|-----|----|----|----|----|--------|
| 1   | 15  | -1 | -1 | 0  | 0  | 68.66  |
| 2   | 1   | 1  | -1 | 0  | 0  | 64.13  |
| 3   | 8   | -1 | 1  | 0  | 0  | 68.38  |
| 4   | 11  | 1  | 1  | 0  | 0  | 63.1   |
| 5   | 7   | 0  | 0  | -1 | -1 | 66.27  |
| 6   | 29  | 0  | 0  | 1  | -1 | 77.24  |
| 7   | 5   | 0  | 0  | -1 | 1  | 69.22  |
| 8   | 9   | 0  | 0  | 1  | 1  | 75.87  |
| 9   | 25  | -1 | 0  | 0  | -1 | 71.56  |
| 10  | 13  | 1  | 0  | 0  | -1 | 66.48  |
| 11  | 12  | -1 | 0  | 0  | 1  | 75.43  |
| 12  | 16  | 1  | 0  | 0  | 1  | 70.64  |
| 13  | 22  | 0  | -1 | -1 | 0  | 62.78  |
| 14  | 4   | 0  | 1  | -1 | 0  | 61.66  |
| 15  | 18  | 0  | -1 | 1  | 0  | 74.83  |
| 16  | 21  | 0  | 1  | 1  | 0  | 75.16  |
| 17  | 20  | -1 | 0  | -1 | 0  | 68.17  |
| 18  | 2   | 1  | 0  | -1 | 0  | 63.28  |
| 19  | 6   | -1 | 0  | 1  | 0  | 77.72  |
| 20  | 19  | 1  | 0  | 1  | 0  | 74.25  |
| 21  | 23  | 0  | -1 | 0  | -1 | 64.31  |
| 22  | 26  | 0  | 1  | 0  | -1 | 72.56  |
| 23  | 24  | 0  | -1 | 0  | 1  | 69.64  |
| 24  | 17  | 0  | 1  | 0  | 1  | 70.44  |
| 25  | 14  | 0  | 0  | 0  | 0  | 84.55  |
| 26  | 27  | 0  | 0  | 0  | 0  | 81.63  |
| 27  | 10  | 0  | 0  | 0  | 0  | 84.76  |
| 28  | 28  | 0  | 0  | 0  | 0  | 82.67  |
| 29  | 3   | 0  | 0  | 0  | 0  | 82.85  |
were repeated. For the second remaining phase, only 20 mL petroleum ether was added. The filtrates were combined into the flask of known weight, evaporated by rotation, and then vacuum dried to a constant weight, following which the total oil content was calculated.

The MEE and MEY values were then calculated as follows:

\[
\text{MEE} (\%) = \left[ 1 - \frac{\text{microcapsule sample surface oil (g)}}{\text{total microcapsule sample oil (g)}} \right] \times 100
\]

\[
\text{MEY} (\%) = \frac{\text{total microcapsule sample oil (g)}}{\text{total HMFS (g)}} \times 100
\]

2.8 Scanning electron microscopy (SEM)

The surface structure of the microcapsules was observed using SEM (JEM 2100, Japan optical electron (JOEL)). A small amount of the microcapsule sample was fixed on the sample table with double adhesive and any excess powder was gently swiped off. The sample was then gold plated for 15-20s treatment of gold spraying and observed under the SEM at an acceleration voltage of 20 KV.

2.9 Infrared Spectroscopy

Spectroscopic absorption spectra of both the microcapsule oil and the HMFS in the infrared region were obtained with a resolution of 2 cm\(^{-1}\), 18 scans and within the frequency range of 4000 to 400 cm\(^{-1}\). The infrared spectra were pretreated by point smoothing, normalization and baseline correction as previously described\(^{26}\).

2.10 Storage stability of microcapsules

A Schaal oven test was used to accelerate the oxidation process in oils\(^{26, 27}\). In total, 500 g of oils was weighed into 500 mL glass bottles that were loosely capped. Bottles were kept randomly inside an oven maintained at (62 ± 1)\(^{\circ}\)C without light to accelerate the oxidation (WGS201, Hongzhan Products Inc., Shanghai, China). After 6 day intervals of heating, the samples were removed from the bottles and were detected. The method used for extracting microcapsule oil was the same as that used for total oil (method 2.7). Sample oils were topped immediately with nitrogen, capped tightly, and kept frozen (−20\(^{\circ}\)) until analysis. The oxidized oils was removed to determine the acid value (AV) and the peroxide value (POV). The values for HMFS were determined in the same way as the microcapsules.

The AV and POV were calculated according to the American Oil Chemists Society (AOCS) official methods\(^{28, 29}\).

2.11 Determination of in vitro digestion of the microencapsulated samples

The digestion rate of the microencapsulated samples was determined by the pH-stat method which measured the release rate of free fatty acids (FFAs)\(^{30-32}\).

1) Preparation of simulated gastric juice: 10 g pepsin (accurate to 0.01 g) was dissolved in 50 mL water and the pH of the solution was adjusted to 2 with 0.1 mol/L hydrochloric acid and 2 g NaCl with constant stirring. The solution was diluted in a 1000 mL volumetric flask with deionized water.

2) Digestive efficacy of simulated gastric juice: to digest the microcapsules, 1.1 g of the microcapsule sample was first incubated in 100 mL of the simulated gastric juice at 37\(^{\circ}\)C for 2 h with constant stirring at 100 rpm/min.

3) Simulated intestinal fat digestion in vitro: to simulate the conditions of the small intestine, pH of above digestive juice was adjusted to 7 with phosphate buffer and then 1 mL CaCl\(_2\) solution (1.7 mg/mL), 5 mL pig bile salt (5 mg/mL) and 1.5 mL pancreatic lipase steapsin (1.6 mg/mL) were added to 30 mL of the microcapsule solution and the pH was always kept at 7.0. The reaction mix was incubated at 37\(^{\circ}\)C in a water bath for 2 hours with constant stirring at 100 rpm/min.

4) The concentration of released fatty acids was measured indirectly by neutralizing the FFAs with 0.0113 mol/L NaOH solution. The volume of NaOH (\(V_{NaOH}\)) was recorded during titration and the percentage of FFAs was calculated as follows.

\[
\text{FFA} (\%) = \frac{0.0113 \times \text{V}_{\text{NaOH}} (\text{mol}) \times M_{\text{mol}} (g/mol)}{m_{\text{oil}} (mg) \times 3} \times 100
\]

2.12 Statistical Analysis

All assays were conducted in three parallel experiments and analyzed using Origin9.0 software. Data are expressed as the mean value ± standard deviation.

3 RESULTS AND DISCUSSION

3.1 Optimization of microcapsule sample by RSM

RSM was used to fit the experimental data into two multinomial regression equations of microcapsule efficiency and 4 factors (A, B, C and D).

\[
y = 83.29 - 2.344 + 0.58B + 5.31C + 1.07D - 0.19AB + 0.36AC + 0.072AD + 0.36BC - 1.86BD - 1.08CD - 7.33A^2 - 9.35B^2 - 5.50C^2 - 5.10D^2
\]

In variance analysis, the p value of model (<0.0001) means high significance while for lack of fit of the model, a p value of 0.2681 is not significant. Similarly, the \(R^2\) value of 0.9687 indicates a good fit and a linear relation between actual and predicted values. In our study, the \(R^2_{\text{Adj}}\) was 0.9375 meaning that the model could match the experimental data at 93.75% accuracy. As can be seen in Fig. 2, the measured values and the predicted values are evenly distributed on both sides of the line indicating that the
The predicted values of the model are near real values. The high degree of fit seen in the regression equation means that this model can be used for analyzing and predicting the MEE of HMFS by spray drying.

As shown in Fig. 3, the surface maps of each interacting factor were made and the effect of each factor on the microencapsulation efficiency was analyzed using a variance table. Table 3 and Fig. 3 show that the A and C factors of the model are very significant, B and D are not significant, the BD interaction is significant, the other is not significant, and the two items are very significant. The Design-Expert software was used to analyze the regression equation and the optimum conditions for HMFS microencapsulation, and then the optimum technological conditions were determined to be: 0.96% of soybean lecithin, ratio of desalted whey powder to malt dextrin 2.04:1, oil content of 17.37% and homogeneous pressure of 30.46 MPa. The MEE under these conditions was predicted to be 84.76%. Using these optimum conditions, three groups of tests were carried out and the average MEE was $(84.72 \pm 0.69)$ %, which was very
close to the predicted value. These results further prove that this model could be used to predict and analyze the MEE of HMFS by spray drying.

### 3.2 Analysis of basic indices of microcapsule samples

Microcapsule samples prepared according to the optimum conditions showed no agglomeration, impurity or peculiar smell, and had milky white color and a slight milky flavor. The basic index included that water content \( \mu = 0.87 \pm 0.25 \) %, ash content \( (2.41 \pm 0.33) % \) and solubility \( (87.38 \pm 0.62) \) (g/100 g). No bleaching oil was observed after storage and neither was any obvious delamination seen. The above basic indices meet the requirements of microencapsulated products (Table 4).

### 3.3 SEM analysis

The structure of a microcapsule is an important index of its quality as it influences several properties of the microcapsules. For example, the integrity of the microcapsule wall material will affect the encapsulation and protection of the core material, whereas the overall morphology of the microcapsule particles will affect its fluidity and solubility\(^2\). As shown in Fig. 4, the shape of the microcapsule is spherical with partial depression and shrinkage and the particle size is relatively uniform with diameter ranging between 10-50 \( \mu \)m. A small number of the particles also displayed adhesion towards each other. On the basis of these structural features, the microcapsule was deemed to be relatively good.

### 3.4 Infrared Spectroscopy analysis

The spectroscopic behavior of the individual oils and the microcapsule oil in the infrared region is shown in Fig. 5. The spectroscopic profiles show that the microencapsulation process does not interfere with IR absorbance of the oils. Twelve characteristic peaks were displayed in the spectrum including sharp-pointed and high peaks around the band range of 2950 cm\(^{-1}\) and 1745 cm\(^{-1}\), three medium intensity peaks with band ranges of about 3450 cm\(^{-1}\),1461 cm\(^{-1}\) and 722 cm\(^{-1}\), and other peaks with low signal.

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**Table 3** Values of regression coefficients calculated for the HMFS microencapsulation.

| Source | R\(^2\) | df | Mean square | F value | P value | Significance |
|--------|--------|----|-------------|---------|---------|--------------|
| Model  | 1301.19| 14 | 92.94       | 31.00   | < 0.0001| **          |
| A      | 65.52  | 1  | 65.52       | 21.85   | 0.0004  | **          |
| B      | 4.03   | 1  | 4.03        | 1.34    | 0.2660  |             |
| C      | 338.03 | 1  | 338.03      | 112.73  | < 0.0001| **          |
| D      | 13.70  | 1  | 13.70       | 4.57    | 0.0507  |             |
| AB     | 0.14   | 1  | 0.14        | 0.047   | 0.8317  |             |
| AC     | 0.50   | 1  | 0.50        | 0.17    | 0.6880  |             |
| AD     | 0.021  | 1  | 0.021       | 0       | 0.9345  |             |
| BC     | 0.53   | 1  | 0.53        | 0.18    | 0.6818  |             |
| BD     | 13.88  | 1  | 13.88       | 5.63    | 0.0494  | *           |
| CD     | 4.67   | 1  | 4.67        | 1.56    | 0.2327  |             |
| A\(^2\) | 348.37 | 1  | 348.37      | 116.18  | < 0.0001| **          |
| B\(^2\) | 566.73 | 1  | 566.73      | 189.00  | < 0.0001| **          |
| C\(^2\) | 196.02 | 1  | 196.02      | 65.37   | < 0.0001| **          |
| D\(^2\) | 168.45 | 1  | 168.45      | 56.18   | < 0.0001| **          |
| Residual | 41.98 | 14 | 3.00        |         |         |             |
| Lack of Fit | 34.90 | 10 | 3.49        |         | 0.2681  |             |
| Pure Error | 7.08 | 4  | 1.77        |         |         |             |
| Cor Total | 1343.17 | 28 |             |         |         |             |
| R\(^2\) = 0.9687 | R\(^2\)Adj = 0.9375 | | |

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**Table 4** Basic indexes of microcapsule samples.

| Sample                             |       |
|------------------------------------|-------|
| Moisture (%)                       | 0.87 ± 0.25 |
| Ash (%)                            | 2.41 ± 0.33 |
| Solubility (g/100g)                | 87.38 ± 0.62 |
| Microencapsulation yield (%)       | 82.33 ± 1.05 |
| Microencapsulation efficiency (%)  | 84.72 ± 0.69 |
wide bands at 2926 cm\(^{-1}\), 2855 cm\(^{-1}\), 1462 cm\(^{-1}\) and 722
\[\text{cm}^{-1}\] are characteristic of the methylene\((\text{-CH}_2)\) group and
have symmetric, antisymmetric, shear, bending and rocking
vibration, respectively\(^{34}\). For compounds having the
\((\text{-CH}_2)\)_n– group, a signal at 722 cm\(^{-1}\) indicates a chain of n
> 4\(^{35}\). Furthermore, the band range of about 1746 cm\(^{-1}\) to
1162 cm\(^{-1}\) is characteristic of functional esters\((\text{-C-O})\).

A comparison of the IR spectral profile of the oils with
the microcapsule oil showed no changes in the position of
the infrared bands and the signal strengths, indicating that
microencapsulation does not affect the behavior of the
band characteristic of long chain FAs and functional esters
in HMFS\(^{36}\).

3.5 Storage stability analysis

Lipid oxidation, one of the main reasons for their
decrease in quality over time, can occur due to high tempera-
ture, light exposure, contact with air or oxidizing enzymes.
The initial 2-step oxidation of lipids produces hydrogen
peroxide which further oxidizes the FAs into aldehydes,
ketones, aromatic compounds etc., all of which make oils
and fats rancid and reduce their quality\(^{30}\). The POV there-
fore is widely used to evaluate the degree of lipid oxidation
when the production rate, oxygen concentration and tem-
perature are known\(^{37}\). AV is a measure of the FFA content
of oils; FFAs are produced upon the slow hydrolysis of oils
due to heat and/or microbial and enzymatic activity and
serve as the main substrates of lipid oxidation. A low value
of POV and AV indicates better oil quality. As shown in Fig.
6, in the initial stage of oxidation, the AV and POV of the
microcapsule lipids and the HMFS are relatively close.
However, over a period of 18 days, the AV of the microcap-
sules increased gradually and after 18 days, the increase in
AV was steep. The AVs of the HMFS increased steadily,
reaching the peak on the 30th day\((35.32 \pm 1.22\) (mg KOH/
kg). The POV increased slowly in the initial stage of oxida-
tion\((0-12\) days\), then accelerated as the oxidation rate
sharply increased and finally plateaued as the reaction also
reached moderate levels. The POV was highest on the 30th
day of oxidation\((54.63 \pm 0.88\) mmol/kg for the original
HMFS sample and\((13.03 \pm 0.25)\) mmol/kg for the microen-
capsulated sample. Therefore, in the absence of microcap-
sules, the unshielded lipids underwent rapid oxidation as
their AV and POV increased by 28.5 (mg KOH/kg) and 48.2
mmol/kg respectively. In contrast, the AV and POV of the
microencapsulated sample increased only by 10.5 (mg
KOH/kg) and 8.56 mmol/kg respectively. Possible reasons
for the higher stability of microencapsulated lipids are even
dispersion of the HMFS in the wall material through the
high speed shear emulsification, and the rapid atomization
of the core material solution by spray drying at high tem-
perature air flow, and the solidified wall material that pro-
tects the core material. According to the Arrhenius empiri-
cal formula, the shelf life of microcapsules is at least 24
months, which is significantly longer than that of un-cap-
sulated lipids\(^{38}\). To conclude, microcapsule of HMFS protects
it against spontaneous oxidation and increases its shelf life.
3.6 Experimental analysis of in vitro digestion of the microencapsulated samples

The digestion of fat takes place in the stomach and small intestine, with steapsin acting as the main hydrolytic pancreatic lipase. For infants, 5% to 30% of ingested milk is digested in the stomach by gastric lipases and the remaining in the small intestine by the coordinated actions of gall-bladder and pancreatic secretions (bile and lipases). The FA released by the lipase action are then absorbed or hydrolyzed further\(^{39-42}\). As shown in Fig. 7, the rate of FA release was slow in the initial stage of simulated digestion (0-10 min), then increased steeply during the rapid digestion phase and finally reached a plateau between 90-110 min. Studies have shown that short, medium and long chain FAs hydrolyze at different rates, with the short chain FAs showing the fastest rates. Amongst the short chain FAs, the C8:0 have the highest rate of FA release and digestion; lower the carbon number, the lower is the rate of digestion\(^{43}\). The UFAs in the HMFS start hydrolyzing only after 10 min, corresponding with the surge in FA release seen after that time point. Furthermore, the microcapsule samples were digested within 2 hours and up to 64% of the HMFS were absorbed (Fig. 7), indicating that the wall material does not prevent the intestinal breakdown and absorption of lipids and that HMFS microcapsules are suitable for human consumption.

4 Conclusions

In this study, the optimum conditions for efficient microencapsulation of HMFS were analyzed and the influence of microencapsulation on the quality of HMFS was determined. According to the results obtained, the amount of emulsifier (soybean lecithin), the ratio of individual wall materials (desalting whey powder: malt dextrin), oil content and homogenization pressure had significant influence on MEE. As per the surface response experiments, the optimal conditions for microencapsulation were as follows: amount of soybean lecithin - 0.96%, ratio of desalted whey powder to malt dextrin - 2.04:1, oil content - 17.37% and homogeneous pressure - 30.46 MPa. Under these conditions, the MEY was (82.33 ± 1.05)% and the MEE was (84.72 ± 0.69 )%. Infrared spectrum analysis showed that the process of microencapsulation did not destroy the characteristics of raw materials. In addition, the storage stability of the microencapsulated lipids was better than that of the unprocessed oils since microencapsulation protected the lipids from oxidation. The microcapsules could be digested completely in 2 hours and the maximum absorption rate of the lipids reached 63% proving that microencapsulated lipids are suitable for consumption.

Fig. 6 Storage stability of microencapsulated samples.

Fig. 7 In vitro simulated digestion of microencapsulated samples.
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