Influence of emulsion composition and spray-drying conditions on microencapsulation of tilapia oil

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Abstract The influence of processing conditions on the microencapsulation of tilapia oil by spray drying was studied. Trehalose, gelatin, sucrose and xanthan were used as emulsion composition. The experimental parameters of spray drying such as inlet air temperature, solid content, drying air flow rate and atomizing pressure were optimized using a central composite design. Encapsulation efficiency and lipid oxidation were determined. Bulk density, powder morphology and particle size were also analyzed. Trehalose improved the glass transition temperature of wall material significantly and prevented the oxidation of the fish oil. Encapsulation efficiency reached a maximum of 90 % under optimum conditions with an inlet air temperature of 121 °C, a drying air flow rate of 0.65 m³/min and a spray pressure of 100 kPa.

Keywords Tilapia oil · Spray drying · Emulsion composition · Microencapsulation

Introduction

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 fatty acids that have been shown to be important for the maintenance of good health and prevention of a range of human diseases and disorders (Shibasaki

et al. 1999; Uauy and Valenzuela 2000; Wanasundara and Shahidi 1998). Tilapia oil, made from the tilapia fish, could be a useful dietary supplement as it contains considerable amounts of EPA and DHA. However, long-chain polyunsaturated fatty acids in tilapia oil are highly unsaturated and therefore are highly susceptible to oxidation. Oxidation leads to the formation of unpleasant tastes and odors and reduces the shelf-life of products. Oxidation promotes the generation of free radicals, which may have negative physiological effects on living organisms (Aim et al. 2008; Siriskar et al. 2011).

Lipid oxidation can be reduced by microencapsulation of the oil (Velasco et al. 2003; Heinzelmann et al. 2000; Kagami et al. 2003). Microencapsulation is a process that covers particles of sensitive materials with a crust of wall material (Dziezak 1988; Sankarikutty et al. 1988). Spray drying is a process that dehydrates and atomizes emulsions in a drying medium at high temperature, resulting in faster water evaporation and the rapid formation of a crust on the powdered core material (Gharsallaoui et al. 2007; Sagar and Kumar 2010).

Gelatin is one of the wall materials used in the microencapsulation of oils. It is a natural gum with solubility and low viscosity in aqueous solution. Xanthan, another potential material, has good emulsifying and antioxidant properties. These wall materials are considered ideal for the encapsulation of lipid droplets.

Trehalose, a natural disaccharide used in food and medicine, has a high glass transition temperature (T_g). Trehalose has been used to increase the T_g of wall materials (Truong et al. 2005). The glass transition temperature (T_g) is an index which can largely determine the processing conditions of spray drying, and the quality (such as stickiness and hygroscopicity) and stability of the final product (Bhandari and Howes 1999). Once the temperature of the particles rises above their sticky-point temperature, amorphous materials change from a glassy state to a liquid-like rubbery state and become sticky. Normally, the sticky-point temperature is 10–

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23 °C higher than the glass transition temperature (Bhandari et al. 1997; Hennings et al. 2001; Roos and Karel 1991). Once particles become sticky they clump together, adversely affecting the free-flowing conditions of spray drying. If the drying conditions are fixed to a temperature that lies between the particles' sticky-point temperature and their T_g , their stickiness will be limited.

Tilapia oil is one of the most abundant aquatic sources of polyunsaturated fatty acids. However, up to now, no studies have explored the effects of variable processing conditions and emulsion properties on the microencapsulation by spray drying of this oil.

The objective of this work was to study the influence of liquid emulsion, atomizing pressure, inlet air temperature, total solid content and drying air flow rate on the microencapsulation of tilapia oil by spray drying, using gelatin, sucrose and trehalose as wall materials. Encapsulation efficiency, lipid oxidation and bulk density were determined. The effect of inlet air temperature, solid content and drying air flow rate on powder morphology and particle size distribution was also evaluated.

Materials and methods

Materials

Tilapia oil was extracted from viscera by light alkaline hydrolysis method. The initial peroxide values and acid value of the tilapia oil are 1.46 mmol/kg and 1.25 mg/g. Analytical grade gelatin and sucrose were purchased from Guangzhou Chemical Factory (Guangdong, China). Analytical grade trehalose was purchased from Nanning Zhongnuo bioengineering Co. Ltd (Guangxi, China). Xanthan gum was obtained from Guangzhou Tianguan Food Additive Co. Ltd (Guangdong, China). Distilled and deionized water was prepared for all solutions.

Liquid emulsion preparation

There are four wall materials was used: trehalose, gelatine, xanthan and sucrose. Preliminary experiments had conducted to determine the suitable proportion of three materials, which were 4.5 wt% gelatin, 0.3 wt% xanthan, 13.2 wt% sucrose and 7.0 wt% tilapia oil. In this study trehalose was used to replace part of sucrose to increase T_g of wall materials. Tilapia oil-in-water emulsions were prepared containing 7.0 wt% tilapia oil, 4.5 wt% gelatin, 0.3 wt% xanthan and 13.2 wt% sucrose and trehalose combined. The ratio of sucrose to trehalose was changed incrementally from 10:0 to 6:4. All the materials and tilapia oil was dissolved in hot water about 60 °C. The emulsion was stirred for about 10 min to ensure complete dissolution, then

homogenized by a high-pressure valve homogenizer (IKA Ultra-Turax T25 System, IMLAB, Germany) at 11,000 rpm for 10 min at room temperature. The emulsion was spray dried immediately following homogenization.

Microencapsulation by spray drying

The spray-drying process was performed in a laboratory scale spray dryer Eyela SD-1000 (Tokyo Rikakikai Co. Ltd, Japan). The double-current nozzle was 0.4 mm in diameter. The emulsion was fed into the main chamber through a peristaltic pump, and the feed flow rate was 450 mL/h. The dryer was operated at an air inlet temperature of 110 to 120 °C, while the drying air flow rate varied from 0.70–0.80 m³/min.

Atomizing pressure

A single-factor experiment was designed to select the suitable level of atomizing pressure, using encapsulation efficiency and lipid oxidation as indexes.

Rotatable central composite design

A rotatable central composite design was used to test the microencapsulation of tilapia oil. Three factors were considered as independent variables: inlet air temperature, total solid content and drying air flow rate. Five levels of each variable were chosen for the trials, including the three central points and two axial points, giving a total of 17 combinations. Encapsulation efficiency, lipid oxidation and powder bulk density were determined. The analysis of variance, regression coefficients and three-dimensional graphs were generated with SAS 9.0 software (StatSoft, USA).

Powder analysis

Encapsulation efficiency (EE)

Surface oil (SO) was measured using the following steps. Thirty-milliliters of petroleum ether were added to 2.5 g of the oil powder and shaken with a vortex mixer for 2 min. The mixture was then filtered through a filter paper and the powder on the filter was washed twice with 20 mL of petroleum ether. The powder free of surface oil was dried at 60 °C to a constant weight. Surface oil was determined gravimetrically.

Total oil (TO) was extracted as follow. Two grams of powder were dissolved in 20 mL of hot water (at 60 °C). The solution was then extracted with 80 mL ethyl alcohol/ether/petroleum ether (2:1:1 v/v). The conical flask was shaken for 5 min. The solution was then transferred to a separating funnel, the clear organic phase was collected and

the aqueous phase was re-extracted with the solvent mixture. All of the organic phase was transferred to a clean beaker, was left to evaporate and then was dried at 60 °C to a constant weight. Total oil was calculated based on the difference between the empty beaker and that containing the extracted oil.

Encapsulation efficiency (EE) was calculated as follows:

$$EE = [\text{TO}(\text{g/g powder}) - \text{SO}(\text{g/g powder})] \times 100 / [\text{TO}(\text{g/g powder})] \quad (1)$$

Lipid oxidation

Lipid oxidation was evaluated by a modified method of Partanen et al. (2008). A visual-spectrophotometry method for hydroperoxides of oil-in-water emulsions was used. A sample of 0.5 g of powder was weighed into a test tube and suspended in 5 mL of water. The tube was shaken with a vortex mixer until the powder had completely dissolved. A 300 µL portion of the resulting solution was added to 1.5 mL of isooctane/isopropanol (2:1 v/v) and was then subjected to vortexing three times for 10 s each and centrifuging for 2 min at 3,400 g. A 600 µL of clean organic phase was added to 2.8 mL of chloroform/methanol (7:3 v/v). For color formation, 30 µL of ammonium thiocyanate/iron (II) chloride solution was added. The thiocyanate/Fe²⁺ solution was prepared immediately prior to use by combining equal volumes (1 mL each) of 3.94 mol/L thiocyanate solution and Fe²⁺ solution. Ferrous iron solution was prepared by mixing 0.132 mol/L BaCl₂ and 0.144 mol/L FeSO₄ in acidic solution. The sample was vortexed, and the absorbance at 500 nm was measured after 20 min. Hydroperoxide concentrations were determined using a Fe³⁺ standard curve with iron concentration.

Bulk density

The powder was poured into a 10 mL graduated cylinder. Bulk density was calculated from the weight of powder contained in the cylinder.

Glass transition temperature (*T_g*)

Differential scanning calorimetry, DSC (204F1, NETZSCH, Germany) was used to determine the glass transition temperature of spray-dried powders. The purge gas used was dry nitrogen (20 mL/min). The onset, mid-point and endset values for glass transition temperature of samples were calculated from the DSC thermogram.

Particle morphology

Surface morphology of the powders was evaluated by scanning electron microscopy (SEM). The images were viewed by scanning electron microscope at 10.0 kV (JSM-6330F, JEOL, Japan).

Statistical analysis

Data were analyzed by the analysis of variance (ANOVA). All statistical analyses were performed using the Statistical software, SPSS Version 12.0 for windows (SPSS Inc., Chicago, IL, USA). Value of *P*<0.05 was used to indicate significant differences. Data were expressed as mean ± SD (mean of at least three determinations for each sample).

Results and discussion

Emulsion composition

Table 1 shows the encapsulation efficiency and bulk density of the different microencapsulated fish oil products. Encapsulation efficiency of the samples containing sucrose and trehalose was higher than that of the corresponding samples containing only sucrose. However, raising the content of trehalose beyond the ratios used in this experiment would not improve the encapsulation efficiency. Encapsulation efficiency was highest when the ratio of sucrose to trehalose was 7 to 3. Bulk density showed a similar trend to encapsulation efficiency with changing particle size, probably because moisture

Table 1 Encapsulation efficiency and bulk density of microcapsulated fish oil with different ratios of sucrose to trehalose and atomizing pressures

Ratio of sucrose to trehalose	Encapsulation efficiency (%)	Bulk density (g/mL)	Atomizing pressure (kPa)	Encapsulation efficiency (%)	Bulk density (g/mL)
10:0	80.4±1.13 ^a	0.201±0.011 ^a	90	85.2±0.69 ^b	0.213±0.008 ^a
9:1	82.7±0.51 ^a	0.205±0.005 ^a	100	89.6±0.35 ^c	0.232±0.011 ^b
8:2	84.9±0.44 ^b	0.216±0.006 ^b	110	86.4±1.25 ^b	0.251±0.005 ^c
7:3	87.0±0.74 ^c	0.230±0.005 ^c	120	82.8±0.93 ^a	0.253±0.005 ^c
6:4	81.6±0.42 ^a	0.221±0.008 ^b			

Different superscripts in a column differ significantly (*p*<0.05) (*n*=3)

Fig. 1 DSC thermograms of spray-dried microcapsules with either sucrose (**a**) or the mixture of sucrose and trehalose (**b**). The ratio of sucrose to trehalose was 7 to 3

was slightly increased when trehalose was present in the wall material.

Glass transition temperature

Trehalose has a high glass transition temperature. The addition of trehalose to emulsion can improve the glass transition temperature of microencapsulation. Figure 1 shows the glass transition temperature of microencapsulated fish oil with different wall materials. The glass transition temperature of the sample containing sucrose was 83.8 °C while it was 96.0 °C in the corresponding samples containing sucrose and trehalose. Thus, the glass transition temperature of microencapsulation improved significantly with the addition of trehalose. Duddu and Dal Monte (1997) also found that

the Tg of trehalose and sucrose mixture increased and trehalose could increase the Tg.

Atomizing pressure

Table 1 shows the effects of different atomizing pressures on microcapsules. Bulk density increased with increasing atomizing pressure, presumably because the size of the emulsion droplet decreases with increasing pressure and the moisture in small droplets evaporates faster than in big ones. Al-Kahtani and Hassan (1990) also observed that higher nozzle pressures decrease droplet and therefore particle size, thus increasing bulk density in spray drying *Hibiscus sabdariffa* (Roselle) powder. Encapsulation efficiency was highest when the atomizing pressure was 100 kPa. The

Table 2 Encapsulation efficiency and lipid oxidation for the 17 trials of the experimental design

Tests	Solid content (%)	Inlet air temperature (°C)	Drying air flow rate (m ³ /min)	Encapsulation efficiency (%)	Lipid oxidation (mmol/kg)
1	20 (−1)	110 (−1)	0.70 (−1)	73.5±0.32	1.95±0.22
2	30 (1)	110 (−1)	0.70 (−1)	76.6±1.56	2.08±0.14
3	20 (−1)	120 (1)	0.70 (−1)	88.4±0.60	2.35±0.18
4	30 (1)	120 (1)	0.70 (−1)	87.0±0.54	2.13±0.12
5	20 (−1)	110 (−1)	0.80 (1)	61.4±0.72	2.73±0.09
6	30 (1)	110 (−1)	0.80 (1)	59.4±0.96	2.85±0.14
7	20 (−1)	120 (1)	0.80 (1)	70.4±0.53	2.46±0.21
8	30 (1)	120 (1)	0.80 (1)	68.2±1.12	2.65±0.15
9	16.59 (−1.682)	115 (0)	0.75 (0)	79.8±0.64	2.19±0.12
10	33.41 (1.682)	115 (0)	0.75 (0)	81.6±1.21	2.13±0.23
11	25 (0)	106.59 (−1.682)	0.75 (0)	61.3±1.18	2.42±0.16
12	25 (0)	123.41 (1.682)	0.75 (0)	88.2±0.66	1.88±0.19
13	25 (0)	115 (0)	0.6659 (−1.682)	91.4±0.42	1.73±0.15
14	25 (0)	115 (0)	0.8341 (1.682)	63.2±1.38	2.54±0.21
15	25 (0)	115 (0)	0.75 (0)	83.6±1.56	2.06±0.15
16	25 (0)	115 (0)	0.75 (0)	80.1±0.78	2.09±0.17
17	25 (0)	115 (0)	0.75 (0)	81.1±0.52	2.17±0.11

Results were given as mean ± SD, (n=3)

Table 3 Values of regression coefficients calculated for the tilapia oil microencapsulation

Independent variable	Regression coefficient	
	Encapsulation efficiency (%)	Lipid oxidation (mmol/kg)
Constant	−2753.63	52.97
X1	7.47	−0.12
X2	35.60	−0.49
X3	1821.61	−59.41
X1*X1	−0.05	0.003
X2*X1	−0.02	0.0002
X2*X2	−0.13	0.002
X3*X1	−2.99	−0.045
X3*X2	−3.77	−0.035
X3*X3	−986.50	47.11
R ²	0.95	0.90
F	14.43	6.71

moisture content was lower when the droplet size was smaller. However, low moisture can lead to cracks on the surface of particles, which then lowers the encapsulation efficiency.

Response surface analysis

The values of encapsulation efficiency and lipid oxidation are shown in Table 2. Table 3 shows the regression coefficients for the coded second-order polynomial equation, the F values and the determination coefficients (R^2). The fitted models are suitable, showing significant regression, low residual values, no lack of fit and satisfactory determination coefficients.

According to Tables 2 and 3, encapsulation efficiency varied from 59.40 to 91.38 % and was significantly influenced by inlet air temperature and drying air flow rate.

Fig. 3 Microphotograph of microencapsulated fish oil

Figure 2a shows the influence of these variables on the encapsulation efficiency.

Inlet air temperature was the factor that most affected encapsulation efficiency. It showed a positive effect, where an increase in inlet air temperature resulted in a higher encapsulation efficiency. Higher air temperature implies a shorter time to form a crust, making it difficult for the oil to diffuse into the drying particle surface.

Drying air flow rate had a negative effect on encapsulation efficiency. The higher the drying air flow rate, the lower the encapsulation efficiency.

To determine the optimal conditions for spray drying and the relationship between the efficiency (EE) and the chosen variables, the regression coefficients were calculated for EE by RSREG analysis. The optimum conditions were 26.60 % solid content, 121 °C inlet air temperature and 0.65 m³/min for the drying air flow rate.

Figure 2a shows the response surface of encapsulation efficiency. Higher inlet air temperature and lower drying air flow rate lead to high encapsulation efficiency for better drying at those conditions. Figure 2b shows the response surface of lipid oxidation. Lower solid content and higher inlet air temperature lead to higher peroxide values because of the lower encapsulation efficiency obtained in these

Fig. 2 Response surface for encapsulation efficiency with the inlet air temperature and drying air flow rate (a); response surface for lipid oxidation with solid content and inlet air temperature (b)

conditions, which leads to poorer oil protection against oxidation. More oil is present in the particle surface when the encapsulation efficiency is low. This unencapsulated oil, once exposed to air, is more easily oxidized than encapsulated oil. As shown in Table 2, the trial conditions that resulted in the highest encapsulation efficiency (91.38 %) were the same as those that generated the lowest lipid oxidation (1.73 mmol/kg).

One reason for peroxide value increased with increasing inlet air temperature is the higher inlet air temperature providing more energy for the lipid oxidation process. Thomsen et al. (2005) observed similar results. Another reason may be due to rapid particle shrinkage during the early stage of the drying process (Shu et al. 2006). High temperature at the early stage usually lead to rapid wall solidification (Rosenberg and Sheu 1996; Sheu and Rosenberg 1998). Serfert et al. (2009) observed that the peroxide value of microencapsulation was three times higher at inlet temperatures of 210 °C than that produced at 160 °C.

Particle morphology

Figure 3 shows the SEM microphotograph of the powder produced at 121 °C, with 26.6 % total solids. All the other samples had a similar morphology.

The resulting powders had particles of various sizes. Most of the particles showed a rounded external surface with a continuous wall and no apparent fissures or cracks, which is important to ensure a low permeability for oils and gases. Thus, these microcapsules offered better protection and core retention. Moreover, their surfaces were slightly concave, which is typical of microcapsules produced by spray drying. This type of morphology was also observed by Trindade and Grosso (2000).

The absorption of moisture increased in higher relative humidity conditions for the compounds containing trehalose. In these conditions, the particles could become sticky.

Conclusion

Trehalose increased the glass transition temperature of microencapsulated fish oil. Encapsulation efficiency was affected by inlet temperature and drying air flow rate. Lower solid content which was attributed to the lower encapsulation efficiency led to higher peroxide value. And peroxide value was also affected by the inlet air temperature. The particles showed a rounded external surface with a continuous wall and no apparent fissures or cracks.

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