Microencapsulating Properties of Whey Protein Concentrate 75

S.A. HOGAN, B.F. MCNAMEE, E.D. O’RIORDAN, AND M. O’SULLIVAN

ABSTRACT: Emulsions containing various levels of soya oil dispersed in solutions of whey protein concentrate (WPC) 75 (5% w/v) were spray-dried to yield powders with oil contents ranging from 20% to 75% (w/w). The effect of homogenizing pressure and oil/protein ratio on oil globule size distributions and protein load of the emulsions and the microencapsulation efficiency (ME) and redispersion behavior of the powders were examined. Emulsion oil droplet size decreased with increasing homogenization pressure but was not affected by oil/protein ratio. Emulsion protein load and ME of the powders were negatively correlated with increasing oil/protein ratio. Powders with an oil/protein ratio < 0.75 were least susceptible to destabilization during spray-drying.

Key Words: emulsions, whey protein concentrate, spray-drying, microencapsulation, redispersion

Introduction

MICROENCAPSULATION IS A PROCESS WHERE INGREDIENTS IN liquid or solid form may be coated or packaged in a protective wall material. Spray-drying is the most commonly used technique for the production of encapsulated materials in the food industry (Reineccius 1988), yielding powders containing the encapsulated material as tiny droplets enveloped in a polymer wall matrix. Production of microencapsulated powders by spray-drying involves the formation of a stable emulsion in which the wall material acts as a stabilizer for the core material. The emulsion is then spray-dried to yield the encapsulated powder product. Encapsulating agents used in the food industry include carbohydrates, gums, lipids, and proteins (Jackson and Lee 1991; Shahidi and Han 1993; Gibbs and others 1999). Carbohydrates lack surface active properties and so must be chemically modified or used in conjunction with emulsifying agents in order to encapsulate hydrophobic core materials. Functional characteristics of an effective wall material include good emulsion-stabilization properties, low viscosity at high concentrations, and effective redispersion behavior in order to release the core material on rehydration (Trubiano and Lacourse 1988). The physicochemical and functional properties of whey proteins have been extensively reviewed (Kinsella 1984; Leman and Kinsella 1989; Walkenström 1993) and would appear to satisfy the requirements of an encapsulating agent. Whey protein has been reported to act as an effective encapsulating agent for anhydrous milk fat (Young and others 1993; Moreau and Rosenberg 1996) and volatile esters (Sheu and Rosenberg 1993). Sodium caseinate (Kim and others 1996) and protein load and ME of the powders were negatively correlated with increasing oil/protein ratio. Powders with an oil/protein ratio < 0.75 were least susceptible to destabilization during spray-drying.

Materials and Methods

Materials

WPC 75 (protein content: 72%) was obtained from Avonmore plc. (Kilkenny, Ireland). Soya oil was obtained from Anglo Oils Ltd. (Kingston-Upon-Hull, North Humberside, England). Sodium azide, petroleum ether (bp 40 to 60 °C), propan-2-ol, Tween 20, and 2-Mercaptoethanol were of GPR grade and purchased from BDH Laboratories Ltd. (Poole, Dorset, England).

Emulsion Preparation

Soya oil was blended with aqueous solutions of WPC (5% w/v; preheated to 50 °C) using an Ultra-Turrax T25 blender (Janke & Kunkel GmbH, Staufen, Germany) operated at 13,500 rpm for 30 s to give pre-emulsions with oil/WPC 75 ratios ranging from 0.25 to 3.0. In the remainder of the text, this will be referred to as the oil/protein ratio. The resultant dispersions or pre-emulsions were further homogenized at 10 to 50 MPa with 4 recirculations using a high-pressure laboratory valve homogenizer (Niro Soavi NS 1001 L, Parma, Italy) and subsequently spray-dried to yield powders with oil contents ranging from 20% to 75% (dry wt), respectively. Sodium azide (0.01%) was added to the emulsions as a preservative (Kim and others 1996).

Emulsion Particle Size Measurements

Emulsion particle size distributions were determined by a laser diffraction technique (Malvern Mastersizer S; Malvern Instruments Ltd., Worcestershire, England). Calculation of the particle size distribution was based on a relative refractive index of 1.11 and absorption of 0.0001. Particle size was expressed as the volume average dia (D4,3):

\[ D_{4,3} = \frac{\sum n_i d_i^3}{\sum n_i d_i} \]

where \( n_i \) is the number of droplets with diameter \( d_i \). Distilled water was used as the dispersing medium. Particle size measurements were made in triplicate for each sample. This instrument also calculated the fat surface area (m²/g fat).
Microencapsulating Properties of Whey Protein

Viscosity
Apparent viscosities of emulsions at varying oil/protein ratios were measured at 25 °C using a stress-controlled rheometer (Physica Rheolab MC 100; PaarScientific Ltd., Raynes Park, London, England) fitted with concentric cylinder geometry (MS-Z1 DIN) at a constant shear rate of 150 s⁻¹.

Protein Load Measurements
This was defined as the amount of protein adsorbed on to the fat surfaces of the emulsions. It was determined by measuring the protein content of the aqueous subnatant phase following centrifugation at 17600 g × 30 min at 4 °C (Beckman J2-HS centrifuge; Beckman-RIIC Ltd., Buckinghamshire, England). An aliquot of the subnatant phase was removed by syringe, filtered through a 0.22 μm Millipore filter, and the protein content was determined by the Lowry method (1951). The surface protein concentration (Γ) (mg protein/m²) of the fat droplets was calculated from the specific fat surface area of the emulsion, and the difference between the protein concentration of the original emulsion aqueous phase and that of the subnatant following centrifugation and was expressed as protein load as follows:

\[ Γ = \frac{\text{Total protein adsorbed (mg)}}{\text{Total fat surface area (m}^2)} \]

Spray-Drying
The emulsions were dried in a laboratory scale spray-drier equipped with a 0.5-mm, 2-fluid, nozzle atomizer (LabPlant SD-04, Huddersfield, West Yorkshire, England). Emulsions, maintained at a constant temperature of 50 °C, were pumped to the spray-drier at a flow rate of about 20 mL/min and dried at an inlet temperature of 180 °C and an outlet temperature of about 95 °C. The dried powder was collected and stored in opaque, airtight containers at 4 °C.

Microencapsulation Efficiency
Total oil content of the powder was determined by a modification of the Röse-Gottlieb method (Richardson 1985). Extractable oil was determined by gently shaking 2.5 g of powder with 100 mL petroleum ether in a sealed 250-mL glass bottle at 25 °C for 15 min. The solvent was filtered (Whatman 41), and extractable fat in the filtrate was determined gravimetrically on a 50-mL aliquot following removal of the solvent by rotary evaporator (Resona Labo Rota 300) and subsequent oven drying at 103 °C for 1 h. Microencapsulation efficiency (ME) was calculated as follows:

\[ \text{ME} = \frac{[\text{Total oil} - \text{Extractable oil}]}{\text{Total oil}} \times 100 \]

Powder Particle Size
Powder particle size was determined by laser diffraction (Malvern Mastersizer) following dispersion of dried emulsion powder sample in propan-2-ol (Kim and Morr 1996).

Redispersion Behavior
Redispersion properties of the powders were determined by mixing 0.5 g powder in 150 mL water or 150 mL water containing 1% (w/v) Tween 20 (polyoxyethylene (20) sorbitan monolaurate) or 150 mL 10 mM 2-mercaptoethanol containing 1% (w/v) Tween 20. The mixtures were stirred for 30 min at room temperature, and particle size distributions of the resulting dispersions were determined by Malvern Mastersizer.

Table 1—Effect of oil/protein ratio on characteristics of WPC 75 stabilized soya oil emulsions

<table>
<thead>
<tr>
<th>Oil/protein ratio</th>
<th>Oil droplet size (D₄,₃) (m)</th>
<th>Apparent viscosity (mPa s⁻¹)</th>
<th>Protein load (mg m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.38ᵃ</td>
<td>0.83ᵃ</td>
<td>10.3ᵃ</td>
</tr>
<tr>
<td>0.38</td>
<td>0.40ᵃ</td>
<td>0.86ᵃ</td>
<td>7.1ᵇ</td>
</tr>
<tr>
<td>0.50</td>
<td>0.37ᵃ</td>
<td>0.89ᵃ</td>
<td>6.3ᵇ</td>
</tr>
<tr>
<td>0.75</td>
<td>0.36ᵃ</td>
<td>0.84ᵃ</td>
<td>5.9ᶜ</td>
</tr>
<tr>
<td>1.0</td>
<td>0.39ᵃ</td>
<td>1.14ᵇ</td>
<td>5.5ᶜ</td>
</tr>
<tr>
<td>2.0</td>
<td>0.37ᵃ</td>
<td>1.33ᶜ</td>
<td>2.1ᵈ</td>
</tr>
<tr>
<td>3.0</td>
<td>0.38ᵃ</td>
<td>1.75ᵈ</td>
<td>1.9ᵈ</td>
</tr>
</tbody>
</table>

1 Emulsions prepared at a homogenization pressure of 50 MPa, a-d Means within the same column with different superscript numbers differ significantly (P < 0.05).

Moisture Content
Moisture content of the powders was determined gravimetrically by oven drying at 103 °C to constant weight.

Scanning Electron Microscopy
Powder particles were attached to a sample stub with double-sided sticky tape and fractured with a razor blade (Young and others 1993). The specimens were sputter-coated with gold using a Polaron sputter coater E 500 (VG Microtech, East Sussex, England) and examined using a JEOL JSM-5410 LV scanning electron microscope (JEOL UK Ltd., Welwyn Garden City, Hertfordshire, England) at an accelerating voltage of 10 or 15 kV.

Statistical Analysis
All results represent the means of 4 replicates. Treatment means were analyzed using the PROC GLM of SAS (SAS Institute Inc., Cary, N.C., U.S.A.). Treatment means were considered significantly different at P ≤ 0.05.

Results and Discussion

Emulsion Particle Size
The effects of homogenization pressure on the volume average diameter (D₄,₃) of the emulsion droplets (oil/protein ratio = 1.0) are shown in Figure 1. The D₄,₃ of the emulsions decreased significantly (P < 0.05) from 0.93 to 0.38 μm on increasing the homogenizing pressure from 10 to 50 MPa. As the amount of WPC available to stabilize newly exposed droplet interfaces was not limiting at this oil/protein ratio, emulsion droplet size was determined primarily by the homogenizing pressure. The size of emulsion oil droplets homogenized at 50 MPa did not change significantly on increasing the oil/protein ratio from 0.25 to 3.0 (Table 1), and specific surface areas of these emulsions ranged from 17 to 18 m² g⁻¹. Unimodal particle size distributions showing normal distributions were observed for all emulsions. Fine oil-in-water emulsions, of sufficiently small size for microencapsulation by spray-drying, were obtained in all cases. It has been reported that smaller droplets not only confer increased emulsion stability but are also advantageous when preparing emulsions for spray-drying (Risch and Reineccius 1988).

Viscosity
Apparent viscosity of the emulsions was largely unaffected by increasing oil/protein ratio from 0.25 to 0.75 but increased significantly at ratios ≥ 1.0 (Table 1). The increase in...
apparent viscosity was most probably associated with increases in the dispersed phase volume.

Protein Load

The influence of oil/protein ratio on protein load is shown in Table 1. As the oil/protein ratio increased from 0.25 to 0.38, protein load values ($\Gamma$) decreased significantly from 10.6 to 7.1 mg m$^{-2}$. Further increases in oil/protein ratios from 0.38 to 1.0 showed a gradual but nonsignificant decrease in $\Gamma$ values, but a significant decrease was observed on increasing the oil/protein ratio from 1.0 to 2.0. $\Gamma$ values did not change significantly on further increasing the oil/protein ratio to 3.0. Previous work on the adsorption behavior of whey protein has examined the nature of the interfacial film in systems combining dilute protein solutions with high oil phase volume (Tornberg 1978; Tornberg and Ediriweera 1988; Britten and Giroux 1993). Protein load values for most studies report upper values of about ~0.3 mg m$^{-2}$ with minimum limiting surface concentrations in the region of 1.5 mg m$^{-2}$ (Hunt and Dalgleish 1994). The present study used protein concentrations and oil/protein ratios previously unreported and provides possible evidence of multilayer adsorption of WPC in 3 layers. It is possible that the lowest $\Gamma$ values correspond to saturated monolayer coverage as reported by Shimizu and others (1981). Values for $\Gamma$ at oil/protein ratios form 0.38 to 1.0 may be indicative of multilayer adsorption as such thick interfacial layers cannot be formed by whey proteins adsorbing on a monolayer basis (Tornberg 1978). A further layer of adsorbed protein at an oil/protein ratio of 0.25 may have been due to high levels of protein relative to oil. It has been shown for whey proteins that an increase in bulk protein concentration facilitates higher surface protein concentration (Yamauchi and others 1980). The trend observed is also consistent with the findings of Hunt and Dalgleish (1994) where evidence of multilayer adsorption, at higher protein concentrations, was reported, although the $\Gamma$ values observed by those researchers were considerably lower than those found in this study. The ability of whey protein layers to resist removal by centrifugation was also noted by these authors. Emulsions prepared with high protein concentrations and low oil phase volume possibly reflect the presence of thick, viscoelastic layers due to increased probability of protein-protein interactions, a result, supported by the assumption of Das and Kinsella (1990), that the nature of the interfacial film may be different at protein concentrations and oil/protein ratios higher than those previously examined.

Moisture Content

Moisture contents of spray-dried emulsions varied from 1% to 3% and were not affected by homogenization pressure or oil/protein ratio.

Powder Particle Size

$D_{4,3}$ values for powders did not differ significantly ($P > 0.05$) on increasing the oil/protein ratio from 0.25 to 0.75 ($D_{4,3} = \text{about} \sim 15 \mu m$) but increased ($P < 0.05$) at higher oil/protein ratios up to 22.5 $\mu m$ at a ratio of 3.0 (Figure 2).

Figure 1—Effect of homogenization pressure on volume average diameter of WPC 75 (5% w/v) stabilized soya oil emulsions (oil/protein ratio = 1.0). Bars represent the standard errors of the means.

Figure 2—Effect of oil/protein ratio on volume average diameter of powders spray-dried from WPC 75 (5% w/v) stabilized soya oil emulsions prepared at a homogenization pressure of 50 MPa. Bars represent the standard errors of the means.

Figure 3—Effect of homogenization pressure on ME of powders spray-dried from WPC 75 (5% w/v) stabilized soya oil emulsions (oil/protein ratio = 1.0). Bars represent the standard errors of the means.
Increasing powder particle size was associated with increasing total solids content of the emulsions and showed a high correlation \((R^2 = 0.94)\) with the apparent viscosity of the emulsions resulting from increased oil phase volumes. It is also possible that extensive agglomeration or clumping of powder particles, due to the presence of surface fat, contributed to the larger powder particle sizes observed with increasing oil/protein ratios (see Figure 6b).

**Microencapsulation Efficiency**

The ME of spray-dried powders (oil/protein ratio of 1.0) increased from 3.8% to 22.0% as emulsion homogenization pressure was increased from 10 to 40 MPa (Figure 3). Increasing pressure from 40 to 50 MPa had no significant effect \((P > 0.05)\) on the ME of powders, indicating that a homogenization pressure of 40 MPa (emulsion particle size = 0.41 \(\mu m\)) was sufficient to maximize ME values at this oil/protein ratio. Given that \(D_{4,3}\) values for emulsion particle size were in the submicron region for all homogenization pressures and that pressure-dependent changes in \(D_{4,3}\) for homogenization pressures > 30 MPa were small, the effect on ME appears dramatic. It may be that at an oil/protein ratio of 1.0 the ability of WPC 75 to stabilize emulsion droplets during the spray-drying process is approaching an upper limit and that small emulsion particle size differences have a pronounced effect at this oil load. Increasing oil/protein ratio from 0.25 to 2.0 resulted in a significant decrease in ME from 59.0% to 38.0% (Figure 4). No significant differences \((P > 0.05)\) were found on further increasing oil/protein ratios to 3.0. ME values reflect the amount of fat recovered from powder particles during the solvent extraction process, which is composed of both surface and internal fat entrapped within the wall matrix (Buma 1971). The low ME values observed, particularly at oil/protein ratios > 1.0, may be related to the high oil phase volume in the emulsion being atomized coupled with higher apparent viscosities. These factors probably resulted in a decrease in atomization efficiency during spray-drying. At high oil/protein ratios, relatively less WPC was available to form the structural matrix of the wall, and additionally such ratios may have resulted in an increase in time required for powder crust formation during the drying process. Greater disruption of emulsion droplets prior to complete drying of powder particles could have led to a decrease in ME values (Rosenberg and others 1990). A high correlation between emulsion protein load and powder ME was observed \((R^2 = 0.88)\), suggesting that emulsion stability during atomization and drying was enhanced by increased protein load. This effect is supported by the findings of Graham (1976) who showed that emulsion coalescence stability is enhanced when interfacial protein layers are thickest. The ability of whey proteins to encapsulate soya oil has been found to be low in comparison to sodium caseinate (Fält and Bergström 1996; Hogan and others 1999). Young and others (1993) also showed that WPC 75 was a poorer encapsulating agent for anhydrous milk fat than both WPI and WPC 50.

**Redispersion Behavior**

The ability to reconstitute in water is an important functional property of dried encapsulates. The redispersion behavior of spray-dried emulsions may also provide information on the stability of emulsion droplets during the drying process. Addition of Tween 20 displaces whey protein from the oil droplet surface (Dickinson 1992; Demetriades and others 1997), resulting in the dispersion of flocculated droplets with coalesced droplets remaining unaffected. The formation of disulfide bonds with whey protein stabilized emulsions or powders can prevent the displacement of surface protein using Tween 20 alone. The use of 10 mM 2-mercaptoethanol to break disulfide bonds has been shown to aid in the dissociation of such floccules (Monahan and others 1996). The \(D_{4,3}\) of spray-dried emulsions redispersed in water as a function of oil/protein ratio is shown in Figure 5. Emulsion droplet sizes following redispersion in water were greater in all cases than those of the original emulsions (about 0.38 \(\mu m\)) and increased with increasing oil/protein ratio, with values ranging from 0.5 to 16 \(\mu m\) for oil/protein ratios from 0.25 to 3.0, respectively. Significant increases \((P < 0.05)\) in droplet sizes were evident at oil/protein ratios > 0.5, suggesting that large-scale emulsion destabilization may have occurred during the drying process resulting in powders.

![Figure 4 - Effect of oil/protein ratio on ME of powders prepared by spray-drying WPC (5% w/v) stabilized soya oil emulsions prepared at a homogenization pressure of 50 MPa. Bars represent the standard errors of the means.](image)

![Figure 5 - Effect of oil/protein ratio (0.25, 0.38, 0.50, 0.75, 1.0, 2.0, 3.0) on the volume average diameter of powders prepared by spray-drying WPC 75 (5% w/v) stabilized soya oil emulsions and redispersing in water or 1% (w/v) Tween 20 or 0 mM 2-mercaptoethanol + 1% (w/v) Tween 20. Homogenization pressure = 50 MPa. Bars represent the standard errors of the means.](image)
with poor redispersion properties, possibly due to the presence of surface fat. Redispersion of powder particles in solutions of Tween 20 (1% w/v) resulted in significantly lower \( P < 0.05 \) D_{4,3} values than for powders redispersed in water at oil/protein ratios > 0.75, indicating that increases in D_{4,3} values resulting from destabilization of emulsion droplets were most pronounced at high oil/protein ratios. Particle sizes following redispersion in 10 mM 2-mercaptoethanol + Tween 20 (1% w/v) were not significantly different \( P > 0.05 \) from those of Tween 20 for oil/protein ratios < 2.0 but showed a significant decrease \( P < 0.05 \) in size for ratios of 2.0 and 3.0, indicating possible interdroplet disulfide bond formation. Intradroplet disulfide bonds may also have been cleaved by 2-mercaptoethanol, contributing further to decreases in particle size. Analysis of redispersion behavior by light microscopy confirmed that changes in particle size, for oil/protein ratios from 0.25 to 0.75, resulted solely from emulsion destabilization as no evidence of undissolved wall fragments was observed. Such fragments, however, were evident at ratios > 0.75 and would have contributed to the larger particle sizes found at high oil/protein ratios. At higher oil/protein ratios, the greater number of emulsion droplets and lower levels of bulk protein may have exposed emulsion droplets to greater stresses during the atomization stage of drying. Partial desorption of less concentrated adsorbed protein layers may have resulted in droplet disruption and release of oil on to powder surfaces. A high correlation \( R^2 = 0.72 \) was found between protein load and D_{4,3} values of redispersed dried emulsions.

**Scanning Electron Microscopy**

Representative SEM micrographs of the spray-dried powders at oil/protein ratios of 0.25 and 3.0 are shown in Figure 6a and 6b, respectively. Powder particles ranged in size from 2 to 25 \( \mu \)m for all samples, and this broad size distribution is consistent with the findings of Rosenberg and Young (1993). The surfaces of powder particles at all oil/protein ratios were free of visible pores or cracks. At an oil/protein ratio of 0.25 (Figure 6a), powder particles showed a mixture of smooth and indented surface morphology. The presence of both these morphological features is attributed to the exposure of individual particles to different drying conditions (Buma and Henstra 1971). The presence of surface dents may be due to uneven shrinkage during drying and cooling (Buma and Henstra 1971) or high surface protein content (Fäldt and

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![Figure 6](image1.png)

**Figure 6**—Scanning electron micrograph of spray-dried WPC 75 (5% w/v) stabilized soya oil emulsion at an oil/protein ratio of (a) 0.25 (\( \times 2000 \)) and (b) 3.0 (\( \times 1500 \)). Homogenization pressure = 50 MPa.

![Figure 7](image2.png)

**Figure 7**—Scanning electron micrograph showing internal structure of spray-dried WPC 75 (5% w/w) stabilized soya oil emulsion at an oil/protein ratio (a) 3.0 (\( \times 3500 \)) and (b) 0.25 (\( \times 7500 \)). Homogenization pressure = 50 MPa.
Bergenståhl 1994). Surface indentations, apparent in Figure 6a but absent in powders at an oil/protein ratio of 3.0 (Figure 6b), indicate the presence of protein at the powder surfaces. Extensive agglomeration of powder particles at a ratio of 3.0 may be attributed to high levels of surface fat. Internal structure of dried powder particles reveal a thicker capsule wall and smaller central vacuole size at an oil/protein ratio of 3.0 (Figure 7a) compared to powders at an oil/protein ratio of 0.25 (Figure 7b). Poor core retention resulted, despite the increased wall thickness observed at high oil/protein ratios, due to high surface fat levels.

Conclusions

WPC 75 offers the surface active properties required to stabilize emulsions. However, its ability to maintain oil droplet stability during spray-drying and form a protective wall matrix, with good redispersion properties, is poor at the oil/protein ratios examined. The capacity of WPC 75 to fulfill the role of a sole wall material, combining satisfactory emulsifying and structural roles, for the encapsulation of soya oil would appear to be limited.

References


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