Microencapsulation Properties of Gum Arabic and Several Food Proteins: Liquid Orange Oil Emulsion Particles

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The comparative suitability of gum arabic (GA), soy protein isolate (SPI), whey protein isolate (WPI), and sodium caseinate (SC) for use as food flavorant encapsulants was investigated in this study by determining their ability to form small-sized, physically stable orange oil emulsion particles by high-pressure homogenization. The resulting emulsion particles were evaluated for their microstructural properties, physical stability, and droplet size distribution as a function of oil content and homogenization pressure. SPI-emulsified orange oil droplets were most stable and GA-emulsified orange oil droplets were least stable against creaming during 10 days of storage at room temperature. Light scattering results revealed that SC was most effective and SPI was least effective for producing orange oil emulsion droplets of ≤ 4 μm diameter by high-pressure homogenization. Transmission electron microscopy images revealed that SPI-emulsified orange oil droplets were surrounded by the thickest membrane structures but that GA-stabilized emulsion particle membranes did not fully surround the orange oil droplets. Statistical analysis revealed a significant interaction between several independent variables, i.e., encapsulant type and percent oil load, and two of the dependent variables, i.e., droplet size and depth of cream layer. No interaction was observed between emulsifier/encapsulant type and homogenization pressure at $\alpha = 0.05$.

Keywords: *Emulsification; encapsulation; proteins; gum arabic; microstructure*

INTRODUCTION

Microencapsulation is an industrially important process for physically coating liquids, solids, or gases with a thin, protective layer or wall of material to inhibit their loss by volatilization and to protect them against chemical deterioration (Balassa and Fanger, 1971; Dziezak, 1988; Rosenberg and Young, 1993). This process is widely used to retain and protect chemically reactive and volatile oils and flavor compounds in commercial food flavorants. The two major processing steps in microencapsulation of liquid flavorants are (1) emulsification of the flavoring materials into an aqueous dispersion of the microencapsulant that functions as an emulsifier and (2) drying the microencapsulated emulsion under conditions that minimize loss of the encapsulated material by volatilization and that enhance the chemical stability of the encapsulated materials.

The selection of the microencapsulant for each application is important; i.e., each encapsulant possesses unique emulsifying and film-forming properties that affect its ability to function as an encapsulant. Although gum arabic (GA) and other food hydrocolloids are widely used as flavor microencapsulants, food proteins, i.e., sodium caseinate (SC), whey protein isolates (WPI), and soy protein isolates (SPI), have apparently not been used extensively for this purpose. On the basis of the chemical and physicochemical properties of these proteins, i.e., array of different chemical groups, amphiphilic properties, ability to self-associate and interact with a variety of different types of substances, large molecular weight, and molecular chain flexibility, as well as their excellent functional properties, i.e., solubility, viscosity, emulsification, and film-forming properties (Kinsella, 1990; Morr and Ha, 1993), one would expect that they would be highly capable of being used as microencapsulants.

The ability of proteins to interact with water, small ions, and other polymers and groups at the oil/water interface allows them to stabilize emulsion droplets that are formed during homogenization (Walstra, 1988; Graham and Phillips, 1976). Most food hydrocolloids are high molecular weight polysaccharides that can also stabilize emulsion droplets against creaming by interacting with water, small ions, and other polymers and groups residing at the interfaces (Dickinson, 1988).

A number of factors would likely affect the ability of the protein to function as microencapsulant, i.e., protein concentration, proportion of dispersed and dispersion phases, processing conditions with respect to homogenizer type and pressure, and properties of the material to be encapsulated. Moreover, certain intrinsic properties of the proteins and hydrocolloids affect their ability to interact water, small ions, other macromolecules, or with the dispersed phase. These factors that control their ability to interact via hydrogen bonding, van der Waals forces, dipole and electrostatic interactions, hydrophobic association, and formation of covalent disulfide bonds (Kinsella, 1990) are believed to affect their emulsification properties. Several environmental factors, i.e., temperature, pH, ionic composition, ionic strength, and proportions of dispersed and dispersion phases, also affect the ability of the protein and hydrocolloid to function as emulsifiers.

This study was conducted to investigate the ability of GA and the three food protein isolates, i.e., SC, SPI, and WPI, to function as orange oil microencapsulants on the basis of their ability to form small-sized, physically stable orange oil emulsion droplets with good microstructural properties. The relative abilities of these microencapsulants to retain volatile flavor com-

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S0021-8561(95)00391-8 CCC: $12.00

pounds and inhibit their chemical deterioration in spray-dried microencapsulated orange oil particles will be considered in the accompanying paper (Kim and Morr, 1996).

EXPERIMENTAL PROCEDURES

Materials. Commercial WPI, BiPRO, from Davisco International Inc. (Le Sueur, MN); SPI, FXP 950, from Protein Technology International (St. Louis, MO); SC, Alanate 110, from New Zealand Milk Products Inc. (Santa Rosa, CA); and GA from Sigma Chemical Co. (St. Louis, MO) were used as enacapsulants. Redd natural orange cold pressed oil pera, 95012, was obtained from Tastemaker (Cincinnati, OH).

Emulsion Preparation. The microencapsulants were reconstituted in 50−55 °C deionized water at a 10% w/v concentration and mixed overnight at 20−25 °C with a magnetic stirrer to enhance their hydration. Orange oil was warmed to 50−55 °C and emulsified into each protein solution, which had also been warmed to 50−55 °C. Orange oil was used at concentrations of 10, 20, and 30% on a solids basis.

Homogenization. Coarse emulsions were prepared by blending each of the oil/encapsulant mixtures for 1 min at 12 000 rpm with a polytron PT 3000 homogenizer (Brinkmann, Westbury, NJ). These emulsions were subjected to a partial vacuum to deareate them and homogenized at 70, 140, or 210 kg cm^{-2} (first stage) and 35 kg cm^{-2} (second stage) with a Niro-Soavi NS 1001-L, two-stage piston homogenizer (Niro Hudson, Hudson, WI).

pH and Viscosity. The pH of microencapsulant solutions was determined with an Accumet 10 pH meter (Fisher Scientific, Cincinnati, OH). Viscosity was determined at 50 °C with a Brookfield viscometer, Model DV-II (Brookfield Engineering Laboratories, Inc., Stoughton, MA) equipped with a No. 1 spindle before and after the solutions were homogenized.

Emulsion Stability and Light Scattering Spectrophotometry (LSS). The size distribution of emulsion droplets was determined by LSS using a Coulter, LS 130, laser light scattering spectrophotometer (Hialeah, FL) equipped with several diffraction sizing components in the optical module including laser light source, spatial filter and projection lenses, diffraction sample cell, 126 photodiode detectors, and Fourier lens. Laser light at 750 nm was used. The physical stability of the emulsions was determined by storing 8 mL samples for 10 days at 20−25 °C in replicate, round-bottom borosilicate culture tubes (15 × 85 mm) (Fisher Scientific, Pittsburgh, PA). The depths of free oil and cream layers that formed were measured with calipers. Sodium azide (Fisher Scientific, Fair Lawn, NJ) was added to the emulsions at a concentration of 0.01% as a preservative.

Transmission Electron Microscopy (TEM). About 0.5−0.7 mL samples of each of the 10% w/v orange oil emulsions were placed in 4 mm flat-width, Spectra/Por, molecular porous dialysis membrane bags (Spectrum Medical Industries, Inc., Houston, TX) and suspended in 10 mL of 2.5% w/v glutaraldehyde (Sigma) in distilled water solution (Sigma) for 6 h at 0−5 °C. The dialysis bags were sequentially suspended in five 10 mL aliquots of 0−5 °C distilled water for 30 min each. Dialysis bags were then suspended in 10 mL of 1% w/v, 0−5 °C osmium tetraoxide (OsO_4) (Jenneile Chemical Co., Cincinnati, OH) in water solution overnight at 0−5 °C and sequentially in five 10 mL aliquots of 0−5 °C distilled water as before. Specimens were dehydrated by suspending the dialysis bags in a series of ethanol in distilled water solutions for 30 min each to progressively increase their ethanol concentrations from 30 to 70% v/v. Specimens were removed from the dialysis bags and further dehydrated by placing them in 80, 95, and 100% (two times) ethanol solution (Midwest Grain Product Co., Pekin, IL) for 30 min each. Specimens were treated twice for 15 min each in propylene oxide (Sigma) and then in 25, 50, and 75% SPURR solutions made in propylene oxide for 30 min each. Specimens were suspended twice in 10 mL of SPURR for 3 h each. Specimens were placed in separate Beem Capsules (Polyscience Inc., Warrington, PA), covered with 2 mL of freshly prepared SPURR and held in a vacuum oven (25 °C) for 4 h and at 60−70 °C overnight in a vacuum oven.

Table 1. pH and Viscosity of Microencapsulant Solutions and Orange Oil Emulsions[a]

	WPI	SPI	SC	GA
pH of 5% encapsulant solutions at 20 °C[b]	7.34	7.70	6.60	4.50
pH of 10% encapsulant solutions at 50 °C	6.83	7.25	6.30	4.35
viscosity of 10% encapsulant solutions (cP)	2.00	5.76	15.50	6.01
viscosity of emulsions[c] (cP)				
70 kg cm^{-2}	2.25	5.51	12.50	6.76
210 kg cm^{-2}	2.67	5.76	13.00	7.76

[a] Means of duplicate determinations at 50 °C. [b] Provided by the supplier. [c] First-stage homogenization pressures.

Table 2. Physical Stability of Microencapsulated Orange Oil Emulsions[a]

	cream layer depth[b] (cm)			
sample	WPI	SPI	SC	GA
70 kg cm^{-2} [c]				
10% of total solids	ND[d]	ND	ND	0.25
20% of total solids	ND	ND	0.10	0.30
30% of total solids	0.25	ND	0.25	0.40
140 kg cm^{-2}				
10% of total solids	ND	ND	ND	0.20
20% of total solids	ND	ND	ND	0.30
30% of total solids	0.25	ND	0.15	0.45
210 kg cm^{-2}				
10% of total solids	ND	ND	ND	0.20
20% of total solids	0.20	ND	ND	0.30
30% of total solids	0.30	ND	0.10	0.50

[a] Results are means of duplicate determinations. [b] Ten days of storage at 20−25 °C. [c] First-stage homogenization pressures only. [d] No cream layer was detected.

Prepared specimens were cut on Reichert Ultra Cut E (Reichert Co., Knosxville, TN) and stained with uranyl acetate (2%) and lead citrate. A Phillips CM-12 STEM microscope (Eindhoven, The Netherlands) was operated at 60 kV.

Statistical Analysis. Physical stability and emulsion droplet size results were analyzed by analysis of variance (ANOVA) of SAS. The tests of the hypotheses on the three independent variables (encapsulant, oil load, and homogenization pressure) were tested against two dependent variables (droplet size and depth of the cream layer) for variable interactions.

RESULTS AND DISCUSSION

pH and Viscosity of Microencapsulated Orange Oil Emulsions. The pH of the three protein microencapsulant solutions ranged from 6.30 for SC to 7.25 for SPI, whereas the pH of GA solutions was 4.35 (Table 1), these values are all several tenths of a pH unit lower than reported by the respective manufacturers. The low pH of GA was not unexpected since it is a complex mixture of the calcium, magnesium, and potassium salts of arabic acid (Sharma, 1981). This low pH value of GA solutions, i.e., pH 4.35, that would be expected to lower the zeta potential of the orange oil emulsion droplets was probably responsible for their enhanced tendency to coalesce and cream during the physical stability test (Table 2).

Viscosity of microencapsulant solutions and emulsions is important, since this parameter affects the size of microencapsulated particles and the thickness of their walls (Risch and Reineccius, 1988; Rosenberg et al., 1990). In the present study, SC microencapsulated emulsions exhibited the highest viscosity value of 15.5 cP, followed in decreasing order by GA > SPI > WPI = 2.0 cP. Surprisingly, high pressure homogenization resulted in only minor changes in the viscosity of microencapsulated orange oil emulsions.

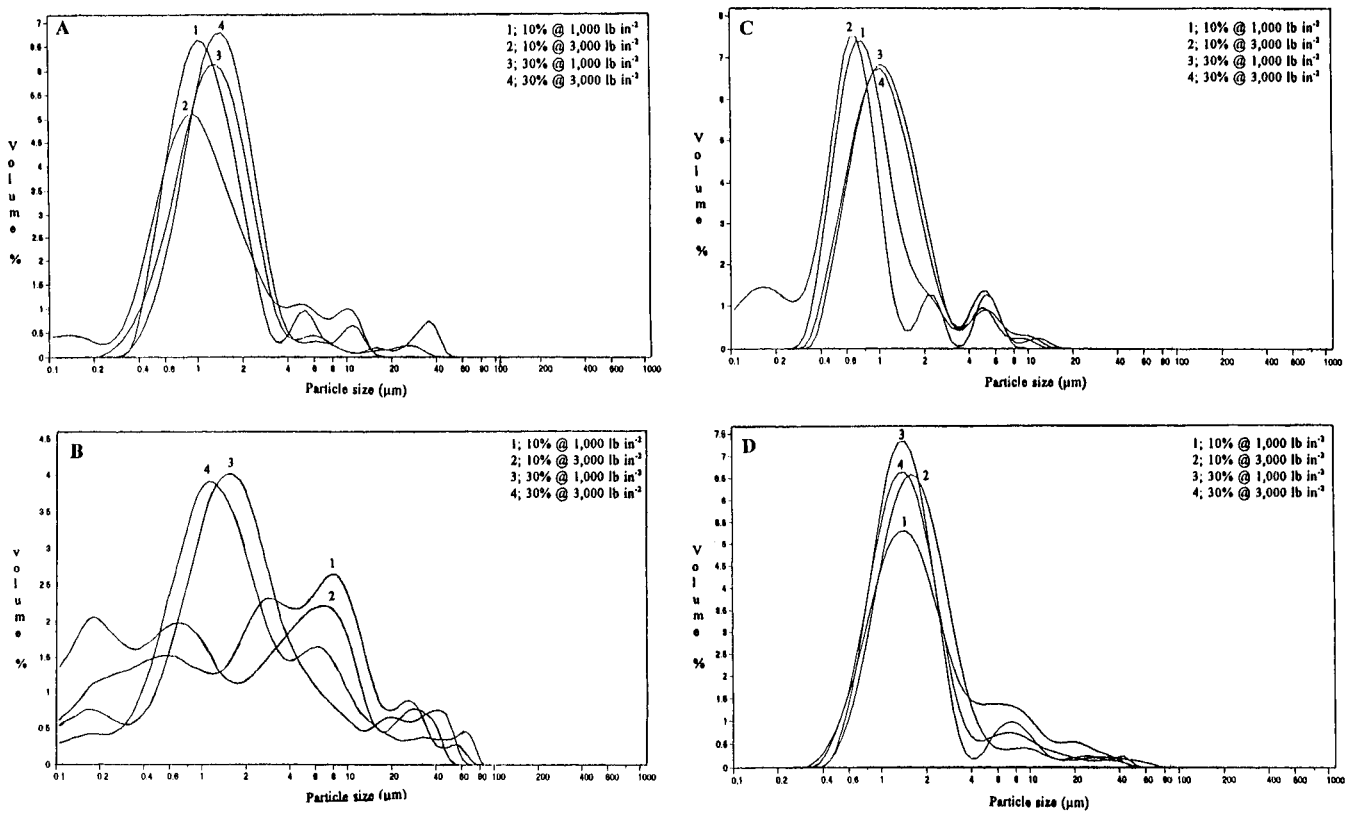

Figure 1. LSS size distribution spectra of orange oil emulsion droplets stabilized by microencapsulant: (A) WPI; (B) SPI; (C) SC; (D) GA.

High-pressure homogenization resulted in only minor changes in the viscosity of coarse microencapsulant orange oil emulsions. The viscosities of WPI and GA were increased slightly, whereas SPI and SC solutions exhibited no change or slight reductions in their viscosity values due to homogenization. The viscosities of emulsions subjected to 210 kg cm^{-2} pressure homogeniztion were slightly higher than those processed at 70 kg cm^{-2}.

Physical Stability of Microencapsulated Orange Oil Emulsion Particles. A preliminary study was made to determine the physical stability of emulsions prepared with the four microencapsulants. Orange oil was emulsified into aliquots of each 10% encapsulant solution to provide concentrations of 10, 20, and 30% w/w on a total solids basis. The emulsions were formulated, blended, homogenized, and evaluated for physical stability by measuring the depth of the cream layer that formed during storage for 10 days at 20−25 °C as described under Experimental Procedures.

Results in Table 2 indicate that SPI-stabilized emulsions were most stable, SC, and WPI-stabilized emulsions had intermediate stabilities, and GA-stabilized emulsions were least stable to creaming. WPI functioned well for emulsions that contained ≤20% orange oil on a solids basis. As expected, physical stability results indicated that SC and GA did not encapsulate orange oil as effectively at the higher oil concentrations as at lower oil concentrations. Higher homogenization pressures also resulted in emulsions with slightly improved physical stability properties. GA, however, did not provide acceptable physical stability to 10% oil emulsions even at the highest homogenization pressures of 210 kg cm^{-2}.

Size Distribution of Orange Oil Emulsion Particles. Emulsion globule size is an important parameter that affects its physical stability, i.e., creaming rate and flocculation. The ability of each of these microencapsulants to provide small and uniformly sized globules is related to their ability to completely coat the oil droplets during homogenization and to prevent their coalescence after homogenization (Walstra, 1988).

Table 3. Droplet Sizes of Microencapsulated Orange Oil Emulsions Determined by LSS[a]

% oil[b]	homogenization pressure[c] (kg cm^{-2})	median particle diameter (μm)			
		WPI	SPI	SC	GA
10	70	1.130	2.804	0.846	1.700
	210	1.074	1.305	0.649	1.684
30	70	1.310	1.687	1.115	1.484
	210	1.409	1.517	1.072	1.473

[a] Means of duplicate experiments. [b] Percent of total solids. [c] First-stage homogenization pressure.

Oil globule sizes of the orange oil emulsions produced with two homogenization pressures and two oil concentrations were determined by light scattering spectrophotometry. Size distribution spectra are displayed in Figure 1 for each of the four microencapsulants as a function of oil concentration and homogenization pressure. Although emulsion droplet sizes ranged from 0.1 to 80 μm for emulsions stabilized with WPI, SPI, and GA and from 0.1 to 10−20 μm for SC-stabilized emulsions, most of the droplets were in size ranges of 0.4−4 μm. Variations in homogenization pressure and oil concentration resulted in relatively minor affects on the globule size distribution spectra of all but the SPI-stabilized emulsions, where it was observed that only the 30% oil SPI-stabilized emulsions exhibited size distribution spectra that were somewhat more typical of the emulsions that were stabilized by the other microencapsulants.

Median particle size results for microencapsulated orange oil particles in Table 3 revealed that SPI-stabilized emulsions contained 1.3−2.8 μm globules, whereas GA-stabilized emulsions contained 1.5−1.7 μm globules. WPI- and SC-stabilized emulsions exhibited

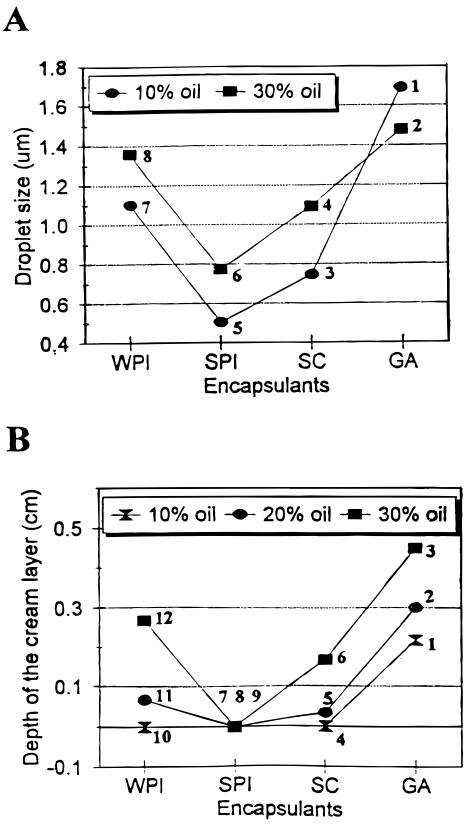

Figure 2. Plots of droplet size (A) and depth of cream layer (B) for orange oil emulsions as a function of oil content and encapsulant.

generally smaller emulsion particles in the range of about 0.6−1.4 μm.

Size distribution spectra of homogenized 10% SPI solutions without oil were determined by LSS before and after homogenization at 210 cm kg^{-2}. Fully hydrated SPI solutions before and after homogenization provided median particle sizes of 40 and 2 μm, respectively. These results indicated that high-pressure homogenization completely dispersed the fully hydrated SPI particles. However, SPI-stabilized emulsions that contained 10% oil on a solids basis exhibited a much broader size distribution spectrum than the emulsions produced by the other three microencapsulants (Figure 1). The 10% oil concentration SPI-stabilized emulsions in Figure 1B revealed six poorly resolved peaks ranging in size from approximately 0.2 to 60 μm, whereas the 30% oil concentration emulsions exhibited a single major peak centered between 1 and 1.6 μm. The ≥2 μm peaks in Figure 1B were determined to be residual SPI protein aggregate peaks. Results in Table 3 and Figure 1B also indicate that oil concentration as well as homogenization pressure affects median particle size and particle size distribution spectra of SPI-stabilized oil emulsion droplets. It was evident that 10% oil emulsions homogenized at 70 kg cm^{-2} resulted in a larger median particle size than the 30% oil emulsions homogenized at 210 kg cm $^{-2}$ (Table 3). Results in Figure 1B confirmed that emulsions containing 30% oil exhibited smaller concentrations of the larger, 3−10 μm, particles than in the 10% oil emulsions. It is believed that the more complete interaction of SPI proteins with the emulsified oil droplets during homogenization at higher oil concentrations and homogenization pressures aids in dispersing their larger sized aggregates into smaller sized particles.

The generally accepted principle that smaller emulsion droplets (Table 3) are more physically stable than

Table 4. Statistical Analysis of Variance of Independent Variables on Orange Oil Emulsion Droplet Size

source	DF	sum of squares	mean squares	*F* value	Pr > *F*[a]
I. Dependent Variable: Droplet Size (Model with Interaction)					
model	12	2.315	0.193	117.02	0.0011*
error	3	0.005	0.002		
corrected total	15	2.320			
R-square	***0.998***				
encapsulants	3	1.987	0.662	401.76	0.0002**
oil load	1	0.109	0.109	66.06	0.0039*
pressure	1	0.005	0.005	3.28	0.1679
encap × oil	3	0.196	0.065	39.55	0.0065*
encap × pressure	3	0.011	0.004	2.20	0.2673
oil × pressure	1	0.007	0.007	4.33	0.1288
II. Dependent Variable: Droplet Size (Model with Main Effect Only)					
model	5	2.101	0.420	19.23	0.0001**
error	10	0.219	0.022		
corrected total	15	2.320			
R-square	***0.906***				
encapsulants	3	1.987	0.662	30.30	0.0001**
oil load	1	0.109	0.109	4.98	0.0497*
pressure	1	0.005	0.005	0.25	0.6298

[a] * $P < 0.05$; ** $P < 0.001$.

Table 5. Statistical Analysis of Variance of Independent Variables on the Physical Stability of Microencapsulated Orange Oil Emulsions

source	DF	sum of squares	mean squares	*F* value	Pr > *F*[a]
I. Dependent Variable: Depth of Cream Layer (Model with Interaction)					
model	23	0.798	0.035	17.13	0.0001**
error	12	0.024	0.002		
corrected total	35	0.823			
R-square	***0.970***				
encapsulants	3	0.523	0.174	86.08	0.0001**
oil load	2	0.178	0.089	43.92	0.0001**
pressure	2	0.003	0.001	0.72	0.5066
encap × oil	6	0.068	0.011	5.61	0.0055*
encap × pressure	6	0.023	0.004	1.91	0.1604
oil × pressure	4	0.003	0.001	0.36	0.8326
II. Dependent Variable: Depth of Cream Layer (Model with Main Effect Only)					
model	5	0.360	0.072	11.68	0.0006**
error	10	0.062	0.006		
corrected total	15	0.421			
R-square	***0.854***				
encapsulants	3	0.245	0.082	13.29	0.0008**
oil load	1	0.114	0.114	18.50	0.0016*
pressure	1	0.000	0.000	0.03	0.8766

[a] * $P < 0.05$; ** $P < 0.001$.

large emulsion droplets was evidenced in this study for all by SPI-stabilized emulsion droplets. For example, SC-stabilized emulsion droplets, which were smallest in size, exhibited the highest physical stability among these three microencapsulants. However, SPI-stabilized emulsion droplets, which exhibited the largest particle sizes by light scattering measurements (Table 3), exhibited the highest physical stability of all of the microencapsulants (Table 2). This apparent inconsistency indicates the possibility that light scattering data for SPI-emulsified oil droplets may be unreliable or that factors other than particle size may be responsible for these unexpected results. Light scattering particle size results in Table 3 indicate that homogenization pressures of ≥70 kg cm^{-2} were not required to obtain complete emulsification of the orange oil droplets for all microencapsulants other than SPI.

Statistical analysis of these data was performed to determine which, if any, of the independent variables, i.e., encapsulants, oil concentration or load, and homog-

Table 6. Table of Probabilities for Comparison of Means[a]

Droplet Size (Least-Squares Means for Effect Encapsulants × Oil Load)

i/*j*	1	2	3	4	5	6	7	8
1		0.0134	0.0002	0.0007	0.0001	0.0002	0.0007	0.0038
2	0.0134		0.0004	0.0025	0.0002	0.0004	0.0027	0.0610
3	0.0002	0.0004		0.0034	0.0093	0.5536	0.0032	0.0006
4	0.0007	0.0025	0.0034		0.0007	0.0043	0.8476	0.0072
5	0.0001	0.0002	0.0093	0.0007		0.0069	0.0007	0.0002
6	0.0002	0.0004	0.5536	0.0043	0.0069		0.0040	0.0007
7	0.0007	0.0027	0.0032	0.8476	0.0007	0.0040		0.0079
8	0.0038	0.0610	0.0006	0.0072	0.0002	0.0007	0.0079	

Depth of Cream Layer (Least-Squares Means for Effect Encapsulants × Oil Load)

i/*j*	1	2	3	4	5	6	7	8	9	10	11	12
1		0.0426	0.0001	0.0001	0.0003	0.1986	0.0001	0.0001	0.0001	0.0001	0.0015	0.1986
2	0.0426		0.0015	0.0001	0.0001	0.0035	0.0001	0.0001	0.0001	0.0001	0.0001	0.3822
3	0.0001	0.0015		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0003
4	0.0001	0.0001	0.0001		0.3822	0.0007	1.0000	1.0000	1.0000	1.0000	0.0947	0.0001
5	0.0003	0.0001	0.0001	0.3822		0.0035	0.3822	0.3822	0.3822	0.3822	0.3822	0.0001
6	0.1986	0.0035	0.0001	0.0007	0.0035		0.0007	0.0007	0.0007	0.0007	0.0186	0.0186
7	0.0001	0.0001	0.0001	1.0000	0.3822	0.0007		1.0000	0.0000	1.0000	0.094	0.0001
8	0.0001	0.0001	0.0001	1.0000	0.3822	0.0007	1.0000		1.0000	1.0000	0.0947	0.0001
9	0.0001	0.0001	0.0001	1.0000	0.3822	0.0007	1.0000	1.0000		1.0000	0.0947	0.0001
10	0.0001	0.0001	0.0001	1.0000	0.3822	0.0007	1.0000	1.0000	1.0000		1.0947	0.0001
11	0.0015	0.0001	0.0001	0.0947	0.3822	0.0186	0.0947	0.0947	0.0947	0.0947		0.0001
12	0.1986	0.3822	0.0003	0.0001	0.0001	0.0186	0.0001	0.0001	0.0001	0.0001	0.0001	

[a] (Pr > |*T*|, where Ho:LSMEAN (*i*) = LSMEAN (*j*).

enization pressure, were related to the dependent variables, i.e., median droplet size or physical stability, as determined by the depth of the cream layer during storage.

If there was no interaction between two independent variables, the lines connecting the means of the dependent variables should be parallel or nearly parallel (Stevens, 1992). Figure 2 shows the interaction between encapsulants and oil load. In Figure 2A, WPI-, SPI-, and SC-stabilized emulsions containing 30% oil showed significantly larger droplet sizes than those that contained 10% oil, whereas GA-stabilized emulsions that contained 10% oil resulted in larger droplet sizes than those that contained 30% oil (Table 6). Consideration of Figure 2B and Table 6 reveals that for SPI-stabilized emulsions, variations of oil concentrations of 10, 20, and 30% did not significantly affect particle sizes. For both WPI- and SC-stabilized emulsions, the particle size means for 10 and 20% oil emulsions were not significantly different; however, particle size means for 30% oil emulsions were significantly higher than for the 10 and 20% oil emulsions. For GA-stabilized emulsions, all particle size mean values were significantly different as a function of oil concentration or load (Table 6). Results in Table 4 revealed that there is significant interaction (0.0065) between microencapsulants and percent oil concentration or load at $\alpha = 0.05$ which affects emulsion droplet size. However, there was no interaction between encapsulants and homogenization pressure and no significant affect of percent oil load and homogenization pressure on emulsion droplet size. Results in Table 5 reveal that microencapsulants and percent oil concentration or load significantly affect the physical stability of the emulsions. These results confirmed that physical stability and droplet size of the emulsions depend significantly on the microencapsulant and percent oil load, but not on homogenization pressure.

The Pearson product moment correlation coefficient of the droplet size and the depth of cream layer was determined to be 0.776. This finding further confirms the previous observation that the depth of the cream layer and the emulsion droplet size are directly related.

Table 7. Comparison of Emulsion Droplet Sizes Determined by TEM and LSS Methods

	mean diameter (μm)			
method	WPI	SPI	SC	GA
TEM				
8000×	0.62	0.31	0.54	1.39
22000×	0.56	0.28	0.53	1.09
35000×	0.77	0.22	0.35	ND[b]
mean[a]	**0.65**	**0.28**	**0.48**	**1.24**
LSS	1.07	0.48	0.65	1.69
% TEM/LSS	**60.6%**	**58.3%**	**73.3%**	**73.8%**

[a] Mean values for all three TEM magnifications. [b] Not determined.

TEM Examination. TEM images at three different magnifications of the four orange oil emulsions prepared with the three proteins and GA encapsulants are studied (Table 7) and micrographs at magnification 22000× are presented in Figure 3. Results indicate that all microencapsulants result in formation of spherically shaped, smooth surfaced, 0.2−2.0 μm emulsion particles.

Mean emulsion droplet sizes were determined by averaging the sizes of three to six droplets in each of the 22000× magnification micrographs. These means were then compared with data obtained from the light scattering spectrophotometer (Table 7).

Mean TEM droplet size values were determined to be 0.65, 0.24, 0.48, and 1.24 μm for WPI-, SPI-, SC-, and GA-stabilized emulsions, respectively. These particle size values were 60.6, 58.3, 73.3, and 73.8% of the particle sizes that had been determined by LSS for WPI-, SPI-, SC-, and GA-stabilized emulsion particles, respectively. Differences in the data from the two methods may be due to the fact that the 50−90 nm thick TEM sections were not always made through the center of the particles.

The thickness of the membranes surrounding the particles varied among the different microencapsulants (Figure 3). Walstra (1988) reported that the amount of protein adsorbed onto emulsion droplets, i.e., surface load, was affected by (1) the ability of the protein to unfold and cover the interface, (2) the size of the adsorbed protein particles being adsorbed onto the

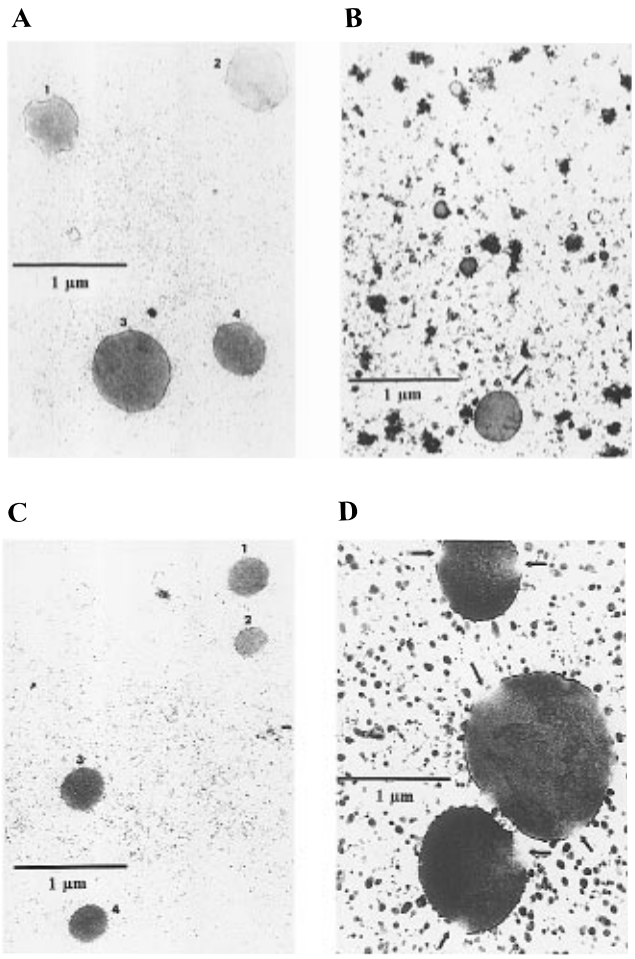

Figure 3. TEM micrographs (22000×) of orange oil emulsions as a function of microencapsulant: (A) WPI; (B) SPI; (C) SC; or (D) GA.

interface, and (3) the concentration of the protein that functions as the surfactant. It appeared that SPI-stabilized emulsion droplets had the strongest membrane, which was presumably due to the larger size of the SPI molecular complexes that were adsorbed onto the emulsion particles during homogenization. Unlike the continuous membranes that surrounded the three protein-stabilized emulsions, GA-stabilized emulsion particle membranes appeared to be incomplete. Regions on the membrane surrounding GA-stabilized oil droplets were observed (see arrows in Figure 3D) which would likely not be able to prevent aggregation or coalescence of individual oil droplets. This factor is believed to be responsible in part for the relatively poor physical stability of GA-stabilized emulsion particles and would likely also render them susceptible to chemical degradation during subsequent drying and storage.

Conclusion. LSS results revealed that SC was most effective and GA least effective for functioning in the first phase of orange oil microencapsulation, i.e., producing smallest sized, emulsified orange oil droplets by high-pressure homogenization. WPI and SC solutions exhibited the lowest and highest viscosity values of 2 and 15.5 cP, respectively.

The microstructure, i.e., size, shape, and membrane structure, of orange oil emulsion droplets was examined with TEM. Although the sizes of orange oil emulsion droplets determined by TEM were smaller than those determined by light scattering spectrophotometry, their size orders were the same, i.e., GA- > WPI- > SC- > SPI-stabilized emulsions. SPI-stabilized emulsions exhibited the most pronounced membranes, whereas GA-stabilized emulsion droplet membranes were less distinct and exhibited a discontinuous structure. TEM micrographs did not indicate evidence of oil droplet coalescence or aggregation in any of the emulsions. SPI-stabilized emulsion droplets were most stable and GA-encapsulated emulsions least stable against creaming during 10 days of storage at room temperature. WPI solutions provided stable orange oil emulsions at oil concentrations of 20% of the solids.

The relationships between two major dependent variables, i.e., emulsion droplet size and physical stability, and three independent variables, i.e., encapsulant, percent oil concentration or load, and homogenization pressure, were statistically analyzed by analysis of variance. It was concluded that the droplet size and the depth of the cream layer were significantly affected by encapsulant and percent oil load but not by homogenization pressures of 70 and 210 kg cm^{-2}.

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Received for review June 27, 1995. Revised manuscript received February 28, 1996. Accepted March 18, 1996.[⊗] This research was supported by Haas Chair Endowment funds of The Ohio State University.

JF950391E

[⊗] Abstract published in *Advance ACS Abstracts*, May 1, 1996.