Microencapsulation Properties of Gum Arabic and Several Food Proteins: Spray-Dried Orange Oil Emulsion Particles

Young D. Kim† and Charles V. Morr*

Department of Food Science and Technology, The Ohio State University, Columbus, Ohio 43210-1097

The encapsulation properties of several commercial food proteins and gum arabic (GA) were evaluated by conventional analytical procedures and dynamic headspace analysis (DHA). The microstructural properties of spray-dried microencapsulated orange oil emulsion particles were investigated by scanning electron microscopy (SEM) and confocal scanning laser microscopy (CSLM). Soy protein isolate (SPI) was most effective and whey protein isolate (WPI) least effective for retaining orange oil during spray-drying of the liquid orange oil emulsions. Spray-dried SC-microencapsulated particles exhibited the largest sizes, and the sizes of the other microencapsulated orange oil particles were in decreasing order of WPI > SPI > GA. SEM and CSLM results revealed that spray-dried GA-microencapsulated orange oil particles had undergone more shrinkage during drying than the protein-microencapsulated products. A modified DHA technique was developed to determine the rate of release of volatiles from the spray-dried, microencapsulated orange oil emulsion particles. DHA results revealed that GA-microencapsulated particles had the highest volatile release rate and SPI-microencapsulated particles the lowest release rate as determined by DHA. WPI- and SPI-microencapsulated orange oil products were more stable against oxidation than SC- and GA-microencapsulated orange oil products. It was concluded that GA and SC were least effective as orange oil microencapsulants on the basis of DHA results and WPI and GA were least effective as orange oil microencapsulants on the basis of total oil retention and surface oil results.

Keywords: *Encapsulation; flavor release; protein; microstructure; dynamic headspace analysis*

INTRODUCTION

Microencapsulation is an important process in the food industry for (1) improving the chemical stability of volatile flavor compounds, (2) providing controlled release of volatile flavor compounds from microencapsulated flavorant products and (3) providing a free-flowing powder with improved handling properties (Anandaraman and Reineccius, 1986; Shahidi and Han, 1993; Sheu and Rosenberg, 1993). Encapsulant materials must retain and protect the encapsulated volatiles from loss and chemical damage during manufacture, storage, and handling and must subsequently release them into the final food product during its manufacture or consumption.

Although carbohydrates, i.e., starch, gum arabic, and other gums, are commonly used as food flavor encapsulants, protein ingredients, i.e., sodium caseinate (SC), soy protein isolate (SPI), and whey protein concentrates (WPC) and isolates (WPI), would also be expected to function well as flavor microencapsulants (Morr, 1979, 1985; Morr and Ha, 1993). WPC (Sheu and Rosenberg, 1993; Rosenberg and Young, 1993) and skim milk concentrate (Onwulata et al., 1994) have been reported to function well for encapsulating anhydrous milkfat, and WPC has been shown to effectively encapsulate volatile esters (Sheu and Rosenberg, 1993; Rosenberg and Young, 1993). Gum arabic (GA) is a branched galactose, rhamnose, arabinose, and glucuronic acid with a molecular mass of 250 kDa (Whistler and Daniel, 1985).

Traditional methods for evaluating the ability of encapsulants to retain volatiles include extraction and distillation of oils and gas chromatographic analysis of solvent extracts of the volatile compounds (Anandaraman and Reineccius, 1986; Chang et al., 1988; Anker and Reineccius, 1988). Dynamic headspace analysis (DHA) is an optional method that may be more suitable than the conventional analytical methods for evaluating the microencapsulation properties of proteins and gums. This method involves purging the volatiles from the samples with an inert carrier gas (Horwood, 1989); adsorption of the purged volatiles onto a chemical trap; and desorption, cryofocusing, and analysis of the volatiles by a gas chromatograph equipped with a mass selective detector. Although DHA is rapid, reproducible, and straightforward, it is most useful for analyzing those volatile compounds that are most effectively recovered by the purge and trap concentrator.

We previously reported on the size, microstructure and physical stability properties of liquid orange oil emulsion particles stabilized by gum arabic (GA) or one of three food proteins, i.e., WPI, SC, and SPI (Kim et al., 1996). The objectives of this study were to (1) investigate the physical and chemical properties of spray-dried orange oil microencapsulated particles that were stabilized by the same four microencapsulants, i.e., GA, WPI, SPI, and SC; (2) compare DHA and conventional analytical procedures for determining the release rate of volatile compounds from spray-dried, microencapsulated orange oil products; and (3) examine the microstructure of spray-dried, microencapsulated orange oil particles by scanning electron microscopy (SEM) and confocal scanning laser microscopy (CSLM).

EXPERIMENTAL PROCEDURES

Emulsion Preparation and Homogenization. Emulsions were prepared according to the procedures described in our previous paper (Kim et al., 1996), except that the orange

* Address correspondence to this author at P.O. Box 1063, Westcliffe, CO 81252-1063.

† Permanent address: Tastemaker, Cincinnati, OH 45216.

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oil concentration in the liquid emulsions was maintained at 30% on a total solids basis.

Spray-Drying the Emulsion. The high-pressure homogenized orange oil emulsions (Kim et al., 1996) were dried in a Lab S-1 spray dryer (Anhydro Inc., Attleboro Falls, MA) equipped with a rotary atomizer nozzle with a product feed temperature of 50−55 °C; a nozzle speed of 33 000−37 000 rpm; and inlet and outlet air temperatures of 120 and 80−84 °C, respectively. The dried, microencapsulated products were removed from the dryer, dispensed into 120 mL serum bottles, sealed with teflon-coated septa and aluminum caps (Supelco Inc., Bellefonte, PA), and stored in a laboratory freezer until they were evaluated.

Total Oil Determination. Total oil contents of the liquid emulsions and spray-dried, microencapsulated products were determined in duplicate using a Clevenger trap (Kontes, Vineland, NJ) according to the method of Risch and Reineccius (1988). Twenty grams of spray-dried, microencapsulated orange oil product dispersed in 150 mL of deionized water or 150 mL of liquid orange oil emulsion was placed in a 500 mL round-bottom boiling flask. Boiling stones and three drops of Trans-30, 30% silicone food grade antifoam (Chemco Inc., Bristol, WI) were added. The Clevenger trap and a water-cooled condenser were fitted into the top of the boiling flask, and the liquid was heated to a boil with an electromantle range (Fisher Scientific, Pittsburgh, PA) and refluxed for 4 h. The volume of oil collected in the trap was multiplied by a density factor of 0.843 g/mL to calculate the weight of oil recovered from the sample.

Surface Orange Oil Determination. The surface oil contents of the spray-dried, microencapsulated products were determined according to the method of Westing et al. (1988). One milliliter of HPLC grade pentane containing 0.1 mg mL^{-1} 2-heptanone internal standard, 150 mL of HPLC grade pentane, and 7.5−12 g of microencapsulated product were refluxed for 14 h in a Soxhlet extractor and then evaporated to a volume of 2−3 mL at 30 °C under a stream of nitrogen. The concentration of oil in the pentane solution was determined by injecting duplicate 2 μL samples into a 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a 30 m × 0.25 mm DB-5 fused silica capillary column (J&W Scientific, Rancho Cordova, CA) and a flame ionization detector. The carrier gas was helium. The column temperature was programmed from 75 to 150 °C at a rate of 6 °C min^{-1} and then to 220 °C at a rate of 10 °C min^{-1}; the injector port and detector were maintained at 220 and 280 °C, respectively.

Moisture Content Determination. The moisture contents of the spray-dried, microencapsulated products were determined according to the modified toluene distillation method (AOAC, 1990). Duplicate 30 g samples of each product were refluxed for 3 h with 175 mL of toluene in a 500 mL boiling flask fitted with a Bidwell-Sterling trap (Kontes, Vineland, NJ) and a water-cooled condenser.

Bulk Density Determination. The bulk density (grams per milliliter) of each spray-dried, microencapsulated product before and after packing was determined according to the method of Hall and Hedrick (1971). These determinations were based on the weight and volume of each product in a 100 mL graduated, glass cylinder before and after tapping for 3 min at a rate of 120 taps min^{-1}.

Dynamic Headspace Analysis of Volatiles. Triplicate 30 mg samples of microencapsulated product that had been extracted with pentane as for the surface oil determination were weighed into separate 50 mL serum bottles, sealed with Teflon-coated septa and aluminum caps (Supelco), and examined by DHA according to the method of Ha et al. (1992). The serum bottles were prepurged for 10 min with helium carrier gas at a flow rate of 40 mL min^{-1} with a Model LSC 2000 purge and trap concentrator (Tekmar, Cincinnati, OH) to remove the volatiles that accumulated in the headspace. The serum bottles were immediately purged for 1−8 min with helium carrier gas at a flow rate of 40 mL min^{-1} to recover those volatiles that accumulated in the headspace during the timed interval following prepurging. The purged volatiles were adsorbed onto a Tenax TA chemical trap, desorbed by heating the trap for 4 min at 160 °C, cryogenically focused at the capillary interface, and thermally injected into a 5890 Series II gas chromatograph (Hewlett-Packard) by heating for 1 min at 180 °C. The volatiles were then fractionated on a 30 m × 0.25 mm × 0.25 μm film thickness, fused silica DB-5, capillary column (J&W Scientific) that was programmed for an 8 min initial holding period, followed by heating from 75 to 150 °C at a rate of 4 °C min^{-1} and then to 220 °C at a rate of 10 °C min^{-1}. A 5971A mass selective detector (Hewlett-Packard) was used to provide a total ion chromatograph (TIC). Compounds were identified by their retention time and retention index (Ha et al., 1992) and by computer-matching their full or partial mass spectra with mass spectrum reference NBS49K.L (Hewlett-Packard). Concentrations of recovered volatiles (nanograms per gram) were determined according to the direct injection method of Laye et al. (1995) and Kim and Morr (1996).

Encapsulated Product Size Distribution. The size distribution of spray-dried microencapsulated products was determined by dispersing them in 2-propanol and analyzing them with the laser light scattering spectrophotometer Model LS 130 (Hialeah, FL) as by Kim et al. (1996).

SEM. A JSM 820 model JEOL (Akishima, Tokyo, Japan) scanning electron microscope was used to investigate the microstructural properties of spray-dried microencapsulated products. Microencapsulated specimens were loaded onto a specimen stub with two-sided adhesive tape (Ted Pella, Redding, CA). Specimens were coated with 60% gold and 40% palladium with a sputter coater, Model Desk II (Denton Vacuum Inc., Cherry Hill, NJ). The conditions used to operate the electron microscope were as follows: objective aperture, 10 μm; sample distance, 18−23 mm; accelerating voltage, 20 kV; and tilt angle, 0°. Examinations were made at 700×, 1200×, and 2200× magnifications.

CSLM. A MRC 600 Model confocal scanning laser microscope (Bio-Rad, Cambridge, MA) connected to a Nikon Optiphot microscope (Japan) was used. The CSLM equipment was equipped with a mixed-gas, argon−krypton (Ar/Kr) laser (Bio-Rad) with a reflectance mode. Encapsulated powders were attached to a glass slide with two-sided adhesive tape (3M Inc., St. Paul, MN) and examined with a 10× objective lens and a 4.3× zoom lens.

Shelf Life. Representative 0.5 g samples of each spray-dried microencapsulated product that had been extracted with pentane to remove their surface oil were placed in separate 150 mL serum bottles, sealed with Teflon-coated septa and aluminum caps (Supelco), and stored at 50 °C in the dark. Five milliliters of HPLC grade water was added to each serum bottle, and then the bottles were resealed and shaken vigorously for 1 min with a vortex mixer (Fisher Scientific). Ten grams of HPLC grade acetone (Sigma Chemical Co., St. Louis, MO) containing 2.0 mg of 2-heptanone as internal standard was added to each bottle, and then the bottles were shaken as before and stored overnight at 0−5 °C. Five grams of anhydrous sodium sulfate (Mallinkrodt Inc., St. Louis, MO) was added to each bottle, and then the bottles were shaken for 1 min as before and centrifuged 10 min at 3000*g* with a GSA Sorvall Superspeed RC2-B centrifuge (Ivan Sorvall Inc., Norwalk, CT). Two microliters of each clear supernatant layer was analyzed by GC as for the surface oil determination.

The amounts of limonene 1,2-epoxide and L-carvone that were produced by autoxidation of D-limonene during the 50 °C storage treatment were used as an indication of the chemical stability of the orange oil in the spray-dried microencapsulated orange oil products.

Statistical Analysis. Data were analyzed by ANOVA using the general linear model (GLM) procedure of SAS Institute, Inc. (1988). Means separation was conducted according to the technique of Duncan (1955), where $\alpha = 0.05$. Pearson correlation analysis was conducted with three variables, i.e., release rate (limonene), shelf life (limonene oxide), and spray-dried particle size by ANOVA using SAS.

RESULTS AND DISCUSSION

Composition and Properties of Liquid Emulsion and Spray-Dried Microencapsulation Products.

Table 1. Composition and Properties of Liquid Emulsion and Spray-Dried Microencapsulated Orange Oil Products[a]

	WPI	SPI	SC	GA
liquid microencapsulated product				
total oil content (g 100 g^{-1})	29.8[1]	27.5[3]	28.7[2]	28.9[2]
emulsification efficiency[b] (%)	99.3[1]	91.7[3]	95.7[2]	96.3[2]
spray-dried, microencapsulated product				
moisture (mL 100 g^{-1})	4.9[2]	4.0[3]	5.1[2]	5.7[1]
total oil content (g 100 g^{-1})	21.8[2]	25.7[1]	24.5[1,2]	22.8[1,2]
surface oil content (mg 100 g^{-1})	2.76[1]	1.79[3]	2.16[2]	2.50[1]
particle size (μm)	40.04[1]	36.11[2]	40.49[1]	33.26[3]
bulk density, unpacked (g mL^{-1})	0.13[3]	0.22[1,2]	0.19[2,3]	0.27[1]
bulk density, packed (g mL^{-1})	0.21[3]	0.34[2]	0.31[2]	0.46[1]
encapsulation efficiency[b] (%)	72.7[2]	85.7[1]	81.5[1,2]	75.9[1,2]

[a] Mean of duplicate values. [b] Computed on the basis of a theoretical oil content of 30% of the solids.[1–3] Means with the same number are not significantly different at $\alpha < 0.05$.

Total oil content of liquid, homogenized orange oil emulsions ranged from 27.5 g 100 g^{-1} for SPI-emulsified orange oil to 29.8 g 100g^{-1} for WPI-emulsified orange oil (Table 1). Emulsification efficiency of orange oil in the liquid, homogenized emulsions ranged from 91.7% for SPI-emulsified orange oil to 99.3% for WPI-emulsified orange oil.

The moisture contents of the four spray-dried, microencapsulated products ranged from 4.0 mL 100 g^{-1} for SPI-microencapsulated orange product to 5.7 mL 100 g^{-1} for GA-microencapsulated orange oil product (Table 1). Bhandari et al. (1992) studied the relationship between moisture content of the powder and viscosity of the liquid emulsion prior to spray-drying and reported a proportional relationship between them. However, the structure and porosity of the particles are additional parameters that determine their water-holding properties during drying (Kneifel and Seiler, 1993), as are the drying rate and drier inlet and exit air temperature differentials (Anker and Reineccius, 1988).

The total oil contents of the spray-dried, microencapsulated products ranged from 21.8 g 100 g^{-1} for WPI-microencapsulated product to 25.7 g 100 g^{-1} for SPI-microencapsulated product (Table 1). Theoretical recovery of orange oil in the spray-dried products ranged from 72.7% for WPI-microencapsulated product to 85.7% for SPI-microencapsulated product (Table 1).

Surface oil contents of the spray-dried, microencapsulated products ranged from 1.79 mg 100 g^{-1} for SPI-microencapsulated products to 2.76 mg 100 g^{-1} for WPI-microencapsulated products (Table 1). Surface oil content is strongly related to the emulsion droplet size (Risch and Reineccius, 1988), and a low surface oil content is important for providing storage stability to the encapsulated materials (Anandaraman and Reineccius, 1987). Although the larger spray-dried, microencapsulated particles would be expected to contain less surface oil due to their smaller surface area, results in this study for the four different microencapsulants did not follow this relationship. Spray-dried SPI-microencapsulated product, which had the second smallest mean particle size (Table 1), exhibited the lowest surface oil content of 1.79 mg 100 g^{-1}, whereas GA-microencapsulated product, which that had the smallest mean particle size of 33.26 μm, contained 2.50 mg 100 g^{-1} surface oil. Similarly, WPI-microencapsulated product with a high mean particle size of 40.04 μm contained the highest surface oil content of 2.76 mg 100 g^{-1}. These results indicate that factors other than particle size are controlling the surface oil content of spray-dried microencapsulated orange oil particles.

Unpacked bulk density of the spray-dried microencapsulated products (Table 1) ranged from 0.13 g mL^{-1} for WPI-microencapsulated product to 0.27 g mL^{-1} for GA-microencapsulated product, and packed bulk density values ranged from 0.21 g mL^{-1} for WPI-microencapsulated product to 0.46 g mL^{-1} for GA-microencapsulated product. Although particle size distribution of spray-dried products would be expected to be a function of the total solids content of the liquid orange oil emulsion prior to drying this parameter was not a factor in the present study as all four emulsions contained 30% orange oil and 10% microencapsulant. Although liquid WPI-stabilized orange oil emulsions exhibited the lowest viscosity values of 2.67 kg cm^{-2} and SC-stabilized orange oil emulsions exhibited the highest viscosity values of 13.00 kg cm^{-2} (Kim et al., 1996), they both provided the largest mean spray-dried particle sizes of about 40 μm.

The orange oil encapsulation efficiency for the emulsification and spray drying steps of the microencapsulation processs ranged from 72.7% for WPI-microencapsulated product to 85.7% for SPI-microencapsulated product (Table 1).

Table 2. Volaltile Compound Release Rate of Microencapsulated Orange Oil Determined by DHA[a]

compound	purge time (min)	concn (ng g^{-1}) WPI	SPI	SC	GA
limonene[b,c]	1	6.08	9.45	8.02	7.76
	2	17.26	16.16	28.63	30.04
	4	49.60	27.42	56.03	66.40
	6	66.53	36.34	86.01	97.35
	8	90.81	55.8	118.42	137.47
	slope[d]	12.11[3]	6.29[4]	15.40[2]	18.09[1]
	R^2	0.99	0.98	0.99	0.99

[a] Means of triplicate determinations from a single trial with 30 mg of encapsulated product. [b] Retention index: limonene 1192. [c] Response factor: limonene 3.00×10^{-7}. [d] Linear graph of concentration vs purge time (ng g^{-1} min^{-1}) from Figure 1.[1–3] Means within the same row with the same superscripts are not significantly different at $\alpha < 0.05$.

Release of Orange Oil Volatiles by Microencapsulated Product. The recovery of limonene compounds that were released into the headspace of each of the spray-dried microencapsulated products was determined as a function of purge times for up to 8 min. Results in Table 2 and Figure 1 reveal that limonene was released at different rates by each of the microencapsulated products. For example, limonene, which accounts for ≥90% of the total orange oil volatiles, was released during the 1 min of purge treatment at concentrations ranging from about 6 ng g^{-1} by WPI-microencapsulated product to about 9.5 ng g^{-1} for SPI-microencapsulated product. The range of concentrations of limonene released during the 8 min purge time period was from about 55.8 ng g^{-1} for SPI-microencapsulated product to about 137.5 ng g^{-1} for GA-micro-

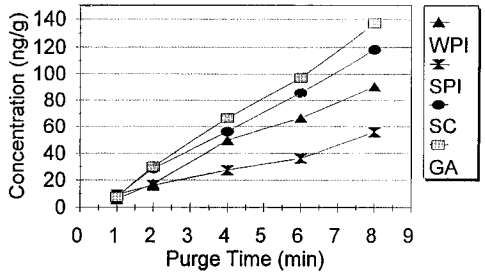

Figure 1. Concentrations of limonene released from pentane-extracted microencapsulated orange oil products as a function of time and determined by the DHA method.

encapsulated product. Values for the slope of limonene release vs purge time ranged from about 6.3 for SPI-microencapsulated product to about 18.1 for GA-microencapsulated product. R^2 values for the graphs in Figure 1 reveal that limonene release was proportional to purge time throughout the entire 8 min purge treatment for all four microencapsulated products.

Microstructural Properties of Spray-Dried, Microencapsulated Particles by SEM. SEM results in Figure 2 depict quite dramatically the microstructural differences between spray-dried SPI-microencapsulated and GA-microencapsulated orange oil particles. SEM results for SC- and WPI-microencapsulated orange oil particles resemble those for SPI-microencapsulated orange oil particles in size, shape, and surface features. Protein-encapsulated particles (Figure 2A) exhibited spherically shaped, smoothed surfaced particles that varied greatly in size and were free of visible cracks and pores. On the other hand, Figure 2B revealed that GA-microencapsulated particles had highly dented surfaces that were likely due to rapid shrinkage of the liquid droplets during the early stages of the drying process (Rosenberg et al., 1985). Microstructural details (Figure 3) reveal that the larger sized SPI- and GA-microencapsulated orange oil particles consist of large, hollow vacuoles that are surrounded by porous walls of ≤ 10 μm in thickness. The interior regions of the spray-dried particle walls exhibited a porous structure that contained numerous ≤ 1 μm diameter air cells. The GA-microencapsulated particle in Figure 3B appeared to have a slightly thicker and more highly dented particle wall than the SPI-microencapsulated particle in Figure 3A. These results revealed only a slight apparent degree of agglomeration among the individual spray-dried particles.

Microstructural Properties of Spray-Dried, Microencapsulated Particles by CSLM. CSLM has several advantages over conventional light microscopy and electron microscopy. For example, CSLM allows one to do a disturbance-free observation of the three-dimensional internal structure of the sample (Heertje, 1987). This technique allows one to examine a series of optical sections taken at selected distances from the top to the bottom of the specimen. A computer selects a section thickness of 1−5 μm that will provide 10−20 optical images of each specimen.

Figure 4 shows the microstructural properties of four sequential optical sections made from the top to the bottom of several spray-dried SPI-microencapsulated orange oil particles. The different sizes of white spots that were evident within the center optical sections of the particles (Figure 4B,C), but absent from top and bottom optical sections (Figure 4A,D), were assumed to indicate the locations and sizes of orange oil droplets.

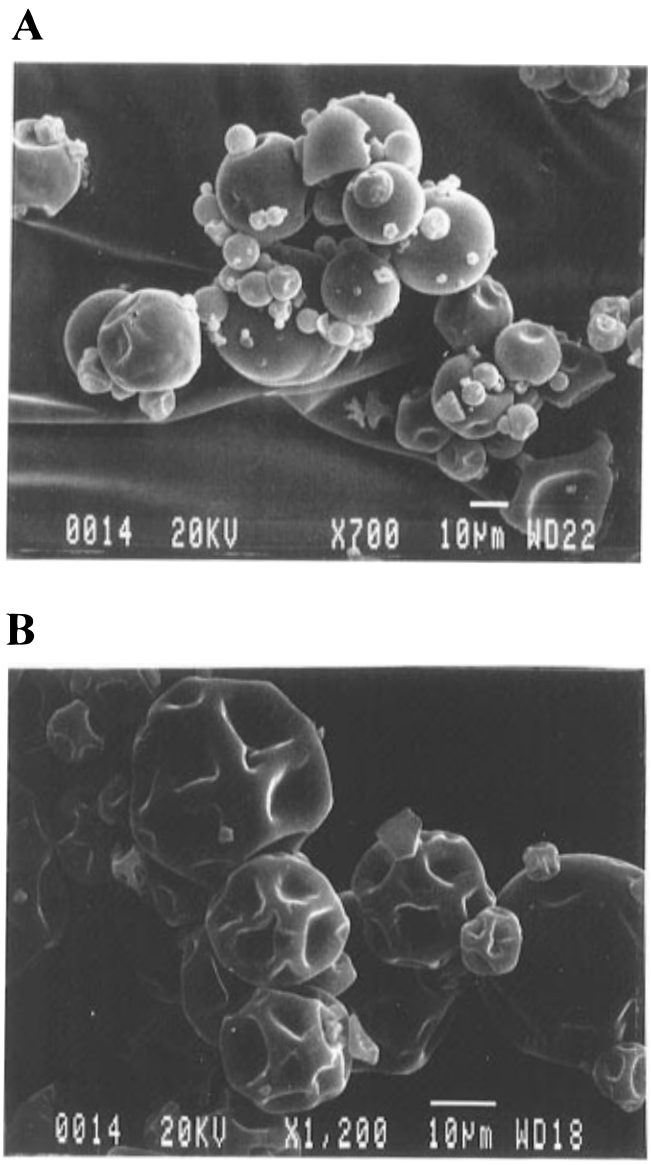

Figure 2. SEM micrographs of spray-dried microencapsulated orange oil particles: (A) SPI-microencapsulated particles at 700×; (B) GA-microencapsulated particles at 1200×.

Shelf Life. Citrus essential oils consist largely of monounsaturated and sesquiterpene hydrocarbon compounds that degrade rapidly to form oxidation products consisting mainly of limonine oxide and carvone (Westing et al., 1988).

Results for limonene oxide formation during storage of the four microencapsulated orange oil products for up to 5 weeks in the dark at 50 °C are in Table 3 and Figure 5. WPI- and SPI-microencapsulated orange oil products exhibited a 2 week induction period during which no limonene oxide was produced. In contrast, SC- and GA-microencapsulated orange oil products exhibited rapid limonene oxide formation throughout the entire storage period. Similar results were exhibited for the formation of carvone and carveol during storage of the microencapsulated products at 50 °C in the dark (Figures 5−7); i.e., WPI- and SPI-microencapsulated orange oil products were consistently more stable against oxidation than SC- and GA-microencapsulated products. Although the rate of limonene oxidation is a function of several factors other than encapsulant porosity, i.e., water activity, availability of oxygen, trace mineral composition, and the presence of antioxidants

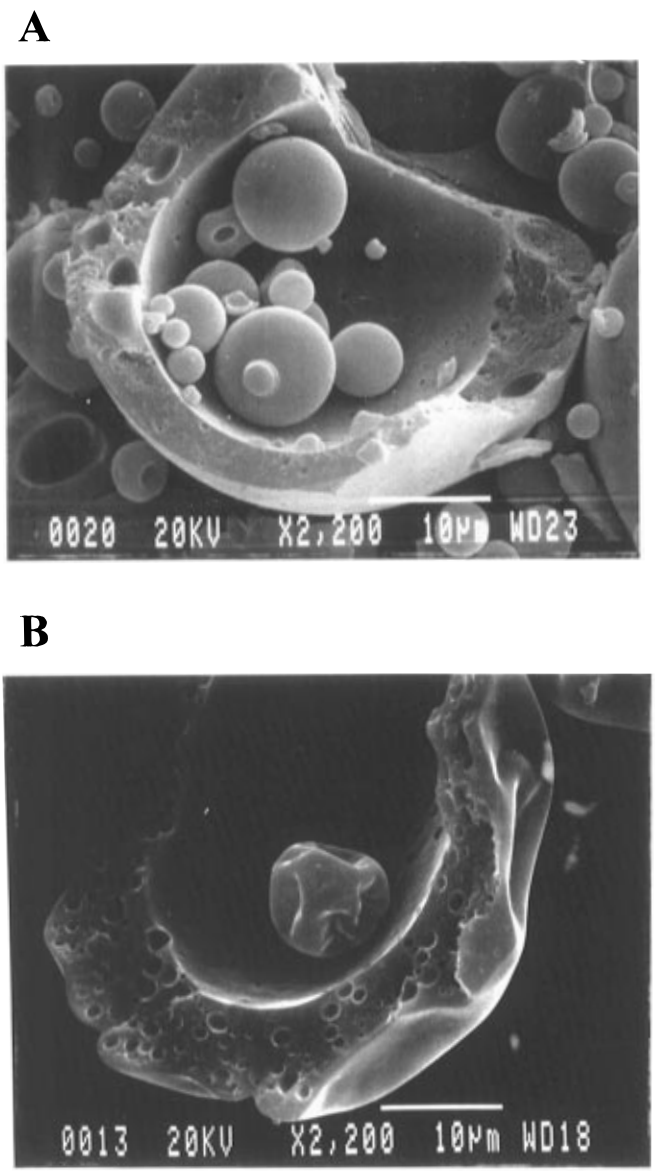

Figure 3. SEM micrographs of shattered microencapsulated orange oil particles at 2200×: (A) SPI-microencapsulated particles; (B) GA-microencapsulated particles.

(Anker and Reineccius, 1988), we interpreted our results as indicating that WPI and SPI function better as orange oil microencapsulants than SC and GA. Our results agreed with those of Anandaraman and Reineccius (1986), who concluded that limonene oxide is produced at a higher rate than carvone in encapsulated orange oil products.

In addition, Pearson correlation analysis was conducted with three variables: (1) the release rate of limonene by the microencapsulated products determined by DHA; (2) production of limonene oxide by the microencapsulated products during the shelf life studies; and three mean particle size of spray-dried microencapsulated orange oil products (Table 4). This analysis indicated a strong correlation (0.92592) between limonene release rate and limonene oxide production during storage at 50 °C in the dark. However, no linear correlation was observed between limonene release rate or limonene oxide production and the size of microencapsulated orange oil particles.

Conclusions. Total oil content data revealed that SPI was most effective and WPI least effective for retaining the volatiles during spray-drying of micro-

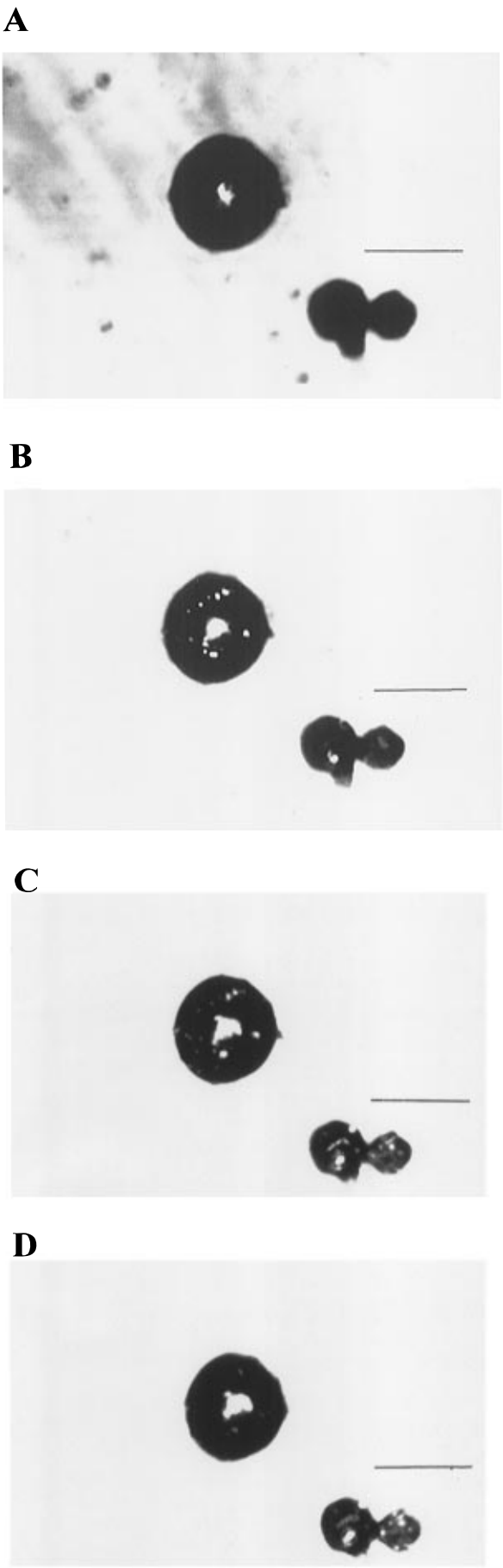

Figure 4. CSLM micrographs of SPI-microencapsulated particles. (A → D) Top to bottom optical sections of the powder particles (bar = 50 μm).

Table 3. Formation of Limonene Oxide, Carvone, and Carveol during the Storage of Encapsulated Orange Oil at 50 °C[a]

compound	storage time (weeks)	concn (mg g^{-1}) WPI	SPI	SC	GA
limonene oxide	1	ND[b]	ND	0.20	0.43
	2	ND	ND	0.58	0.93
	3	0.20	0.09	0.88	1.34
	4	0.29	0.15	1.02	1.78
	5	0.42	0.21	1.36	2.21
	slope[c]	0.11^{3}	0.06^{4}	0.28^{2}	0.44^{1}
	R^2	0.95	0.95	0.98	0.99
carvone	1	ND	ND	0.17	0.36
	2	ND	0.07	0.34	0.50
	3	0.14	0.08	0.52	0.74
	4	0.21	0.11	0.79	0.93
	5	0.28	0.14	0.99	1.16
	slope	0.08^{2}	0.03^{2}	0.21^{1}	0.20^{1}
	R^2	0.95	0.93	0.99	0.99
carveol	1	ND	ND	ND	0.12
	2	ND	ND	0.08	0.19
	3	0.05	ND	0.13	0.29
	4	0.07	ND	0.16	0.41
	5	0.10	0.02	0.20	0.56
	slope	0.03^{2}		0.05^{2}	0.11^{1}
	R^2	0.94		0.96	0.98

[a] Mean of duplicate determinations [b] Not detected [c] Linear graph of concentrarion vs storage time (mg g^{-1} week^{-1}).$^{1-4}$ Means within the same row with the same superscripts are not significantly different at $\alpha < 0.05$.

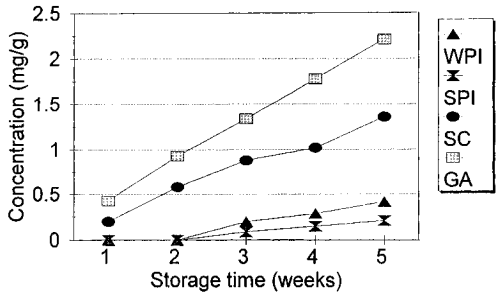

Figure 5. Concentrations of limonene oxide formed in microencapsulated orange oil products as a function of time of storage in the dark at 50 °C.

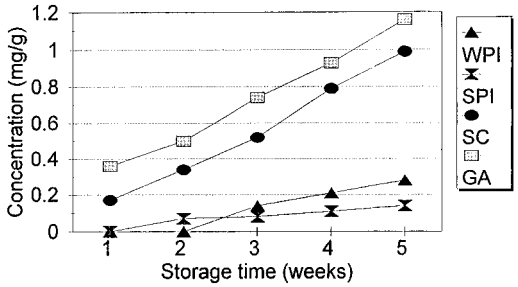

Figure 6. Concentrations of carvone formed in microencapsulated orange oil products as a function of time of storage in the dark at 50 °C.

encapsulated orange oil. Viscosity of the liquid emulsion was not an important factor for determining the mean particle size of the spray-dried microencapsulated products. SEM results revealed that the spray-dried microencapsulated particles were hollow spheres with porous walls that were ≤10 μm in thickness. Spray-dried GA-microencapsulated particles appeared more shrunken than the protein-microencapsulated products by SEM, which presumably accounted for their higher bulk density compared to that for the protein-microencapsulated products. The higher surface oil contents

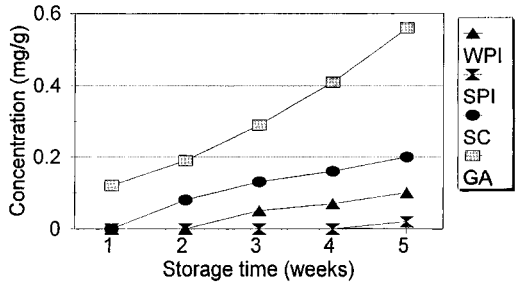

Figure 7. Concentrations of carveol formed in microencapsulated orange oil products as a function of time of storage in the dark at 50 °C.

Table 4. Correlation Analysis: Pearson Correlation Coefficients[a]

	release rate[b]	shelf life[c]	particle size[d]
release rate	1.00000	0.92592	−0.13934
	0.0	0.0001	0.6658
shelf life	0.92592	1.00000	−0.42700
	0.0001	0.0	0.1662
particle size	−0.13934	−0.42700	1.00000
	0.6658	0.1662	0

[a] Prob > |R| under Ho:Rho = 0. [b] ng released limonene g^{-1} min^{-1} determined by DHA. [c] mg limonine oxide produced g^{-1} week^{-1} determined by DHA. [d] Size of spray-dried particles, μm.

of WPI- and GA-microencapsulated products indicated that they were least able to encapsulate the orange oil during spray-drying. Higher encapsulation efficiency results further confirmed that SPI and SC were better able to retain the orange oil volatiles during spray-drying than GA and WPI.

A newly devised application of the DHA technique was used to determine the rate of release of volatiles from spray-dried microencapsulated orange oil products. DHA results demonstrated that GA had the highest volatiles release rate and SPI the lowest. Shelf life studies based on the rates of production of limonene oxide, carvone, and carveol by oxidation of limonene in the microencapsulated orange oil revealed that SPI-microencapsulated orange oil was most stable against oxidation and GA-encapsulated orange oil least stable. The general conclusion, based on DHA results, was that GA and SC are least effective and WPI and SPI are most effective as orange oil microencapsulants. Results based on total oil retention and surface oil content indicated that SPI and SC were most effective and WPI and GA were least effective as orange oil microencapsulants.

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