Journal of
Food Process Engineering

Journal of Food Process Engineering ISSN 1745–4530

EFFECT OF ULTRASOUND ON THE STABILITY OF TURMERIC OLEORESIN MICROENCAPSULATED IN GELATIN-COLLAGEN MATRICES

LARISSA A. C. ZUANON[1,3], CASSIA R. MALACRIDA[2] and VÂNIA R. N. TELIS[1]

[1]Department of Engineering and Food Technology, São Paulo State University, 15054000, São José do Rio Preto, São Paulo, Brazil
[2]Department of Biology Science, São Paulo State University, 19806900, Assis, São Paulo, Brazil

[3]Corresponding author.
TEL: + 55 17 32212255;
FAX: + 55 17 32212299;
EMAIL: larizuanon@gmail.com

Received for Publication August 19, 2015
Accepted for Publication January 25, 2016

doi:10.1111/jfpe.12360

ABSTRACT

This study evaluated the effect of sonication on the stability of turmeric oleoresin microcapsules produced by spray and freeze-drying using blends of gelatin-collagen as encapsulating matrices. Samples prepared with different wall matrix formulations presented solubility between 82.7 and 86.9%, yield above 88% and encapsulation efficiency between 17.41 and 56.21%. The wall matrix composed of 2% gelatin and 28% collagen (C2G28), which presented the highest yield (94.7%) and good encapsulation efficiency (42.83%), was adopted to investigate the effects of ultrasound in the emulsification step (C2G28US), to compare the performance of spray and freeze-drying, as well as to study stability of the microcapsules during storage under incident light. Ultrasound combined with spray drying resulted in the smallest particle size, according to SEM analyses and the highest curcumin retention (342.84 mg/g). Sonication also showed positive effects on product stability, with better curcumin color retention observed for spray-dried microcapsules.

PRACTICAL APPLICATIONS

Encapsulation of turmeric oleoresin by spray drying in protein matrices composed of gelatin and collagen hydrolysate was enhanced by using ultrasound in the emulsification step, which resulted in the smallest particle size, higher curcumin retention and greater color stability under light incidence. The produced curcumin capsules may be used as a food supplement or as a natural dye.

INTRODUCTION

Food colorants have always been subject to criticism, whereas the replacement of artificial food dyes with naturally derived colorants may fit the current market requirements of functional foods and healthy products, using natural additives that add nutritional value to the final product (Mattea *et al.* 2009; Wrolstad and Culver 2012). Turmeric (*Curcuma longa* L.) is a tropical plant native to southern Asia that belongs to the *Zingiberaceae* family. Turmeric rhizomes are the raw material used to extract the phenolic pigment called curcumin, a natural yellow dye considered as a potent antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic agent. Despite being widely used as a natural lipophilic colorant and providing a spicy aroma to certain foods, curcumin is very susceptible to light and heat, has a short shelf life if not properly stored, is unstable at high pH and is sensitive to oxidative degradation. In addition it is insoluble in aqueous media, which inhibits its use in water-based food systems (Yanishlieva *et al.* 2006; Martins *et al.* 2013; Malacrida *et al.* 2014; Sari *et al.* 2015).

Microencapsulation of the turmeric oleoresin is a possible alternative to enhance the stability of curcumin and facilitate its use in hydrophilic systems. Microencapsulation involves the coating or entrapment of small particles of solid, liquid or gas inside biopolymer capsules or matrices, in order to protect the inner material from adverse environmental conditions such as light, moisture and oxygen and also to prevent interactions with other compounds. The encapsulating agent is basically a material capable of forming films and its composition is the main determinant of the functional

properties of the microcapsule (Jafari *et al.* 2007; Charve and Reineccius 2009; Malacrida *et al.* 2014). Therefore, microencapsulation helps stabilize the product, increasing its shelf life and in some cases, promoting the controlled release of the active material (Madene *et al.* 2006; Goula and Adamopoulos 2012).

Several techniques can be used to microencapsulate hydrophobic compounds such as oleoresins, including spray drying and freeze-drying. The spray drying technique has been widely used to dry heat-sensitive foods, pharmaceuticals and other substances, because of the rapid solvent evaporation from the droplets. Although mostly considered as a dehydration process, spray drying is also used as an encapsulation method, because it promotes entrapment of the active material by the encapsulating matrix. Freeze-drying, although involving long dehydration periods has been used as a simple technique to encapsulate natural aromas, colorants and drugs. During this process, the active compound and a biopolymer solution are homogenized and then freeze-dried to produce a dry matrix capable of protecting the core material (Khazaei *et al.* 2014; Mahdavi *et al.* 2014). The choice of encapsulation method depends on the core ingredient, the wall material and the desired characteristics of the microcapsules.

Efficient encapsulation is especially dependent on the performance of the encapsulating agent, which should present emulsifying properties, high water solubility, film-forming ability, low viscosity at high solid concentrations and low hygroscopicity, besides being economic, palatable and easily available. A single encapsulating material does not cover all these properties, and hence, in practice, many wall matrices are actually blends of more than one encapsulating compounds (Jafari *et al.* 2008). Gelatin is widely used in the pharmaceutical industry for the encapsulation of drugs due to its excellent film-forming properties, good water solubility and biodegradability, forming a thin and dense network after drying (Wang *et al.* 2009). Hydrolyzed collagen is a protein compound that undergoes a degree of hydrolysis different from that used in the production of gelatin. Unlike gelatin, hydrolyzed collagen does not form a gel, but it has good emulsifying properties, is water soluble and has low viscosity, even at high concentrations (Williams and Phillips 2009).

Most microencapsulation techniques rely on the initial emulsification of the core material in the solution containing the wall biopolymers, and this step has a direct influence on the encapsulation efficiency and properties of the resulting capsules. Generally, emulsification is carried out using high-energy input techniques, which are based on mechanical devices for generating the intense forces needed for the breakdown of the macroscopic phases. These emulsification methods include high shear mixing, high-pressure homogenization, microfluidization and ultrasonication (Abbas *et al.* 2013). Ultrasonic emulsification results from cavitation, where bubbles collapse at or near the oil–water interface causing disruption and mixing, and the ability of ultrasound to promote emulsification depends on the wave frequency used. The shear forces generated are very strong at low frequencies (16–100 kHz) due to the violent nature of the bubble collapse. Conversely, the shear forces generated at high frequencies are relatively weaker and are not useful for emulsification. The main advantages of using ultrasound for emulsion preparation are good emulsion stability, achieved with or without the addition of small amounts of surfactants, the production of small droplets with a narrow size distribution, and lower energy requirements than those needed in conventional emulsification methods (Chandrapala *et al.* 2012). Some studies have shown that the use of ultrasound as the emulsifying technique is able to produce emulsions with improved textural attributes and decreased oxidative deterioration (Shanmugam and Ashokkumar 2014), as well as increased stability of the encapsulated material and reduced microcapsule size (Mongenot *et al.* 2000; Lertsutthiwong *et al.* 2008). Jafari *et al.* (2007) studied the encapsulation of d-Limonene by spray drying comparing three emulsification techniques, and reported that ultrasound was able to produce submicron emulsions in spite of resulting in encapsulated powders with higher surface oil contents than those of microfluidized samples.

The present study aimed to evaluate the effect of ultrasound emulsification on the stability and morphological properties of turmeric oleoresin microcapsules produced by spray and freeze-drying using blends of gelatin-collagen as the encapsulant matrix.

MATERIAL AND METHODS

Material

Turmeric oleoresin OC-50 (Baculerê Corantes Naturais, Olímpia, Brazil) was used as the core material. Food-grade, type B, bloom number 240 gelatin, extracted from bovine hides by alkaline treatment, and collagen hydrolysate Hidrogel supplied by Gelita do Brasil S.A. (Mococa, Brazil), were used as the wall materials for the microcapsules.

Preparation of Emulsions

Six systems were prepared, varying the total concentration of wall polymers in the solutions and the respective proportions of gelatin (G) and collagen (C). Systems containing 30 g of total wall polymers/100 g of solution were formulated with 0, 2 or 4 g of gelatin/100 g of solution, and 30, 28 or 26 g of collagen/100 g of solution, being identified as G0C30, G2C28 and G4C26, respectively. In systems containing 20 g of total wall polymers/100 g of solution, the formulations were 0, 2 or 4 g of gelatin/100 g of solution and 20, 18 or

16 g of collagen/100 g of solution, and these were identified as G0C20, G2C18 and G4C16, respectively. The proportion of core material to the total mass of wall polymers was held constant at 15 g of oleoresin/100 g of total wall material (Cano-Higuita *et al.* 2015).

The required amount of gelatin was dissolved in deionized water at 60C, whereas the collagen was dissolved in deionized water at room temperature. The hydrated biopolymers were then mixed and deionized water added to complete to 100 g, which caused the temperature of the solution to fall to around 40C. Finally the turmeric oleoresin was emulsified in the biopolymer solution using a rotor-stator homogenizer Ultra-Turrax T25 Basic (IKA, Wilmington) operating at 15,000 rpm for 5 min (Malacrida *et al.* 2015).

Rheological Behavior of the Encapsulating Solutions

Rheological characterization of the biopolymer solutions used as encapsulants, without the addition of the turmeric oleoresin, was carried out using an AR2000EX controlled stress rheometer (TA Instruments, Delaware) with cone and plate geometry (60 mm, gap 52 μm). Mechanical spectra were determined along frequency sweeps between 0.1 and 10 rad/s with constant strain of 1% at 20C. Measurements were carried out in duplicate.

Freeze-Drying

For freeze-drying, the emulsions were placed in stainless steel trays previously lined with aluminum foil, covered with aluminum lids and frozen at -40C for 24 h in an upright freezer (model VF-500, Liotop, São Carlos, Brazil). The trays were then opened and placed in a bench top freeze dryer (model L101, Liobras, Sao Carlos, Brazil) for 72 h at a temperature below -40C (temperature of the compressor) and pressure of around 7×10^{-3} kPa. The dried samples, converted into powder using a pestle and mortar, were packaged in polyethylene bags covered by aluminum foil to prevent the incidence of light and stored over silica gel in a desiccator at room temperature ($\cong$ 25C) for subsequent analyses.

Powder Characterization

Water Content. The water content of the powder was determined in triplicate by a gravimetric method, drying in an oven at 105C for 6 h according to the AOAC method 31.1.02 (AOAC 1997).

Water Solubility. Water solubility of the powder was determined according to Cano-Chauca *et al.* (2005). Aliquots of 1 g of powder, in triplicate, were added to 100 mL of distilled water at 40C and stirred for 5 min on a magnetic stirrer. The solutions were then filtered and 20 mL aliquots of the filtrate were transferred to previously weighed Petri dishes, which were dried in an oven at 105C for 5 h. The percentage solubilized was calculated by mass difference.

Yield (%Y). The yield in powder was calculated as the ratio between the final amount of solids contained in the powder produced (FS) and the amount of solids present in the initially prepared emulsion before drying (IS), according to Eq. (1):

$$\%Y = 100(\mathrm{FS}/\mathrm{IS}) \quad (1)$$

Encapsulation Efficiency (EE%). Encapsulation efficiency was determined according to Kshirsagar et al. (2009), being calculated as the amount of curcumin present in the powder (FC) as compared to the total amount of curcumin present in the mass of oleoresin initially used in to produce the powder [IC; Eq. (2)]. To quantify the curcumin, aliquots of 7 mg of powder, in triplicate, were placed in 25 mL volumetric flasks and the volume completed with methanol. The contents were stirred for 5 min to extract the core material from the powder and then allowed to stand for 2 h in the dark, for precipitation of the wall material. The absorbance of the supernatant at 425 nm was determined in a spectrophotometer (model SP-22, Biospectro, São Paulo, Brazil), and the curcumin content determined using a previously prepared standard curve.

$$\%\mathrm{EE} = 100(\mathrm{FC}/\mathrm{IC}) \quad (2)$$

Optical Microscopy. The morphology of the rehydrated powder was observed under an optical microscope (model L – 2000 A, Bioval, São Paulo, Brazil), coupled to a video camera, and using the Image Pro Plus 6.0 software (Media Cybernetics, Inc., Bethesda, MD). Small samples of dried powder were spread over glass slides and few drops of water were added for rehydration. The rehydrated samples were covered with a coverslip and subjected to image analysis under magnification of 640×.

Application of Ultrasound

As an alternative to using high shear mixing in the Ultra Turrax device, samples containing 2 g of gelatin and 28 g of collagen/100 g of solution were subjected to ultrasound emulsification. The sonicated samples were coded as G2C28US. Ultrasound was applied for 3 min using the equipment Omni Ruptor 4000 (Omni International, GA) fitted with a 9.5 mm-diameter probe, with a power input of 180 W, frequency of 20 kHz and temperature controlled around 40C by water circulation in a double-jacketed cell. These parameters were set after preliminary tests.

Spray Drying

Spray drying was carried out in a pilot scale apparatus (B-290, Büchi, Flawil, Switzerland) equipped with a drying chamber with dimensions of 61 cm in height and 20 cm in diameter and a 0.7 mm two-fluid spray nozzle. The air compressor pressure was set at 588.4 kPa and the emulsion fed to the nozzle using a peristaltic pump. The spray dryer operated with an airflow rate of 420 L/h, feed flow rate of 5 mL/min, inlet air temperature of 170C and aspiration of 90%. The powder samples collected from the base of the cyclone were packaged in airtight low-density polyethylene bags, covered with aluminum foil and stored in a desiccator containing silica gel for further characterization according to item 2.5.

Scanning Electron Microscopy

Particle morphology was also analyzed by scanning electron microscopy (SEM) (Digital Scanning Microscope 960, Zeiss, Munich, Germany) carried out at 20 kV with work distance of 12 mm. Images were obtained digitally using the software Digital Image Transfer 1.0 (PUC, Rio de Janeiro, Brazil). Samples of dried capsules were immobilized in an appropriate support with the aid of conductive adhesive tape, and coated with gold in a sputter coater (SCD 050, Bal-Tec, Balzers, Liechtenstein).

Color Measurement

Color attributes (Hunter *L, a* and *b* values) were measured, in triplicate, in a colorimeter (ColorFlex EZ, HunterLab, Reston) with a D65 illuminant and 10° observation angle. Samples were placed in the glass sample cup so as to completely cover the bottom, which was placed at the measurement port. The sample cup was then covered with an opaque lid to exclude the interference of external light, with color being measured through the glass bottom. Three measurements were made in each sample, with rotation of the sample between readings. Total color difference (ΔE) was calculated [Eq. (3)] with reference to the powder before storage under light, and the hue angle (h) and chroma (C) were determined according to Eqs. (4) and (5), respectively.

$$\Delta E=[(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{1/2} \quad (3)$$

$$h=\text{arctg}(b/a) \quad (4)$$

$$C=[(a)^2+(b)^2]^{1/2} \quad (5)$$

Light Stability

Samples of the powder were packaged in low-density polyethylene bags, sealed and stored in a controlled temperature chamber at 25C under incident light of 3,500 lux using fluorescent 15 W lamps. The lamps were placed at a distance of 16 cm from the samples and divided uniformly across the chamber, ensuring that illumination reached all the samples for a period of 35 days. Aliquots were removed at 7-day intervals and analyzed in triplicate for their curcumin content as described in item 2.5.4, and color according to item 2.9.

Statistical Analysis

The results of the analytical determinations were subjected to an analysis of variance, and a comparison of the replicate means using Tukey's test at 5% probability using the Minitab 15 Statistical Software (MINITAB, State College, PA).

RESULTS AND DISCUSSION

Rheological Behavior of the Encapsulating Solutions

The rheological behavior of the encapsulating solutions is a relevant factor that can affect the stability of the emulsions and their drying performance, especially when using spray drying. It can also influence the resulting microcapsule morphology. The results obtained for the storage modulus (G′) and loss modulus (G″) of the encapsulating solutions showed that the total biopolymer content, as well as the gelatin content influenced the rheological properties (Fig. 1). Although all the samples analyzed presented gel behavior, with $G' > G''$ and a constant value for G′ with variation in the frequency (Chamberlain and Rao 2000), systems containing 20% total solids (Fig. 1a) showed lower values for both the storage and loss moduli when compared with the samples prepared with 30% total solids (Fig. 1b). Regarding the gelatin content, the samples with higher proportions of gelatin/collagen, that is, samples G4C16 and G4C26, showed higher values for G′ and G″ than samples G2C18 and G2C28, respectively. The important effect of gelatin is also evidenced by the values of G′ and G″ for sample G4C16, which were higher than for sample G2C28, although the latter presented a higher total solid content. The plateau observed for G′ reflected the structure of the network formed by the noncovalent intermolecular interactions in the gelatin solutions (Bohidar *et al.* 2003). This network structure can contribute to emulsion stabilization, in addition to better entrapment of the core material in the encapsulating matrix. Conversely, solutions containing only the collagen hydrolysate showed no viscoelastic behavior (data not shown), demonstrating the gel-forming ability of gelatin. Nevertheless, although not being able to form gels, the presence of collagen contributed for increasing the elastic modulus of the system when combined with gelatin.

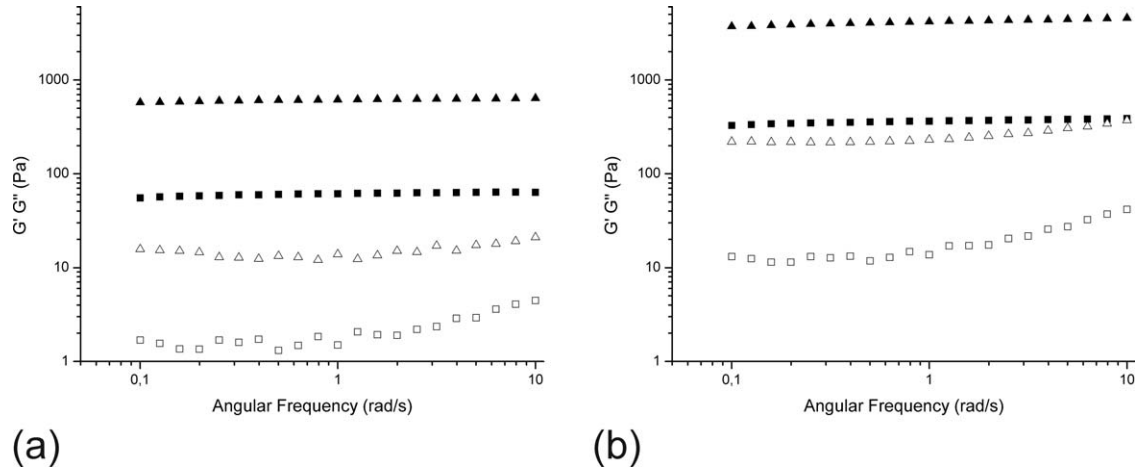

FIG. 1. MECHANICAL SPECTRA OF DIFFERENT ENCAPSULANT SOLUTIONS
(a) Systems with 20 g of total wall polymers/100 g of solution: (■, □) (2 g of gelatin + 18 g of collagen)/100 g of solution (G2C18); (▲, Δ) (4 g of gelatin + 16 g of collagen)/100 g of solution (G4C16). (b) Systems with 30 g of total wall polymers/100 g of solution: (■, □) (2 g of gelatin + 28 g of collagen)/100 g of solution (G2C28); (▲, Δ) (4 g of gelatin + 26 g of collagen)/100 g of solution (G4C26). G′ (closed symbols); G″ (open symbols).

Freeze-Drying

An initial set of the systems G0C30, G2C28, G4C26, G0C20, G2C18 and G4C16 (formulations described in item 2.2) were prepared using high shear mixing emulsification and freeze-drying. The resulting turmeric oleoresin particles were characterized for their water content, yield, water solubility and encapsulation efficiency (Table 1).

Water Content. The water contents observed ranged from 1.12 to 2.02% (dry basis) with significant higher values corresponding to samples prepared from encapsulating solutions containing 30% total wall polymers. The higher solids concentration could have hindered water vapor diffusion through the matrix during freeze-drying, although, the water contents observed were of the same magnitude usually found in freeze-dried powders (Telis and Martínez-Navarrete 2009). The observed water contents were similar to curcumin encapsulated by freeze-drying in modified starch/gelatin (Malacrida *et al.* 2015) and carbohydrate blends (Cano-Higuita *et al.* 2015).

TABLE 1. MOISTURE, YIELD AND SOLUBILITY OF FREEZE-DRIED POWDERS

System	Water content* (% dry basis)	Yield (*Y*%)	Solubility* (%)	Encapsulation efficiency* (EE%)
G0C30	2.02 ± 0.02^{a}	92.30	83.9 ± 0.50^{a}	25.07 ± 1.13^{d}
G2G28	1.99 ± 0.04^{a}	94.70	86.7 ± 1.69^{a}	42.83 ± 1.10^{b}
G4C26	1.70 ± 0.16^{a}	89.80	82.7 ± 1.95^{a}	56.21 ± 1.12^{a}
G0C20	1.12 ± 0.03^{c}	92.90	84.7 ± 0.95^{a}	17.41 ± 1.08^{e}
G2C18	1.47 ± 0.04^{b}	94.50	86.6 ± 1.95^{a}	34.20 ± 1.10^{c}
G4C16	1.47 ± 0.06^{b}	88.90	86.9 ± 1.15^{a}	37.97 ± 1.41^{c}

*Mean values ± standard error (n = 3).

Means followed by different letters in the same column differ statistically (Tukey *T* test, $P \leq 0.05$).

Solubility. The solubility results indicated that the microencapsulation of turmeric oleoresin using gelatin and collagen hydrolysate as the wall matrices was able to confer high water solubility on this active agent. Independent of the matrix formulation, the solubility of the powder was above 82% and no significant differences were found between samples. The values obtained were similar to those reported for materials encapsulated with maltodextrin (Cano-Chauca *et al.* 2005). After solubilization solid particles were not observed in the solution and the resulting color was bright yellow. The solubility results were also in agreement with those of Santos *et al.* (2005), who observed the dissolution of paprika oleoresin microencapsulated in gum Arabic and starch/gelatin mixtures. Zuanon *et al.* (2013) obtained values higher than 86% for water solubility at 40C of curcumin capsules coacervated with gelatin and Arabic gum. That encapsulating matrix as well as the used in the present work have emulsifying properties, low viscosity and ability to form films that increase particle solubility (Sarkar *et al.* 2013).

Yield. Yield values ranged from 88.9 to 94.7%. Higher gelatin contents in the wall material formulation led to lower yields (%*Y*), independent of the total polymer concentration in the encapsulating solution. The trend for lower yields in samples containing higher proportions of gelatin may be related to the ability of gelatin to form a sponge-like structure when submitted to mechanical agitation and subsequent freeze-drying (Shyamkuwar *et al.* 2010), which caused

adherence of the material to the aluminum foil used to line the drying tray. Yield values above 80% were reported by Zuanon *et al.* (2013) for turmeric oleoresin encapsulated by a complex coacervation method using gelatin and Arabic gum as the encapsulants, consistent with the results obtained in the present study. Malacrida *et al.* (2015) also reported yields ranging from 88 to 93% for curcumin encapsulated by freeze-drying in modified starch/gelatin matrices, although they have observed that increasing that in samples containing the same amount of modified starch, the increase in the concentration of gelatin provided higher yield. Nevertheless, the maximum gelatin content tested by those authors was lower than in the present work. This fact may indicate that there is a limiting content, above which gelatin causes yield reduction.

Encapsulation Efficiency. The encapsulation efficiency varied between 17.41 and 56.21%. The higher the solids concentration used, the higher the encapsulation efficiency. In addition, systems G0C30 G2C28 and G4C26 differed significantly ($P < 0.05$) from each other. It should be noted that the increase in the proportion of gelatin contributed to a significant increase in encapsulation efficiency, probably due to the structure of the network formed by noncovalent intermolecular interactions in the gelatin solutions. Nevertheless, although system G4C26 showed higher encapsulation efficiency, it formed a very rigid material which was difficult to handle after freeze-drying, the same problem being identified in system G4C16, which presented the highest efficiency amongst the systems with 20% total solids. These results could be correlated with the rheological characteristics observed for these samples (Fig. 1), since the corresponding mechanical spectra revealed the important contribution of gelatin to the gel-forming ability of the encapsulating solutions, which, in turn, is a result of the structure of the network formed due to intermolecular interactions in gelatin solutions. Kaushik and Roos (Kaushik and Roos 2008) studied the encapsulation of limonene using different proportions of gum acacia/sucrose/gelatin, and found that the greater the amount of gelatin in the encapsulating matrix, the greater the limonene retention after the freeze drying process. However, the use of pure gelatin as the encapsulating material was not feasible, since although pure gelatin retained large amounts of limonene, the sponge-like structure was not completely dehydrated and it was practically impossible to produce a powder. According to these authors, this behavior is caused by the formation of a gelatin film around the ice crystals, preventing the complete sublimation of water during freeze-drying. These authors observed limonene retention varying from 4.2 to 75.3% and the best values were obtained for the higher gelatin concentrations, as also observed in the present study.

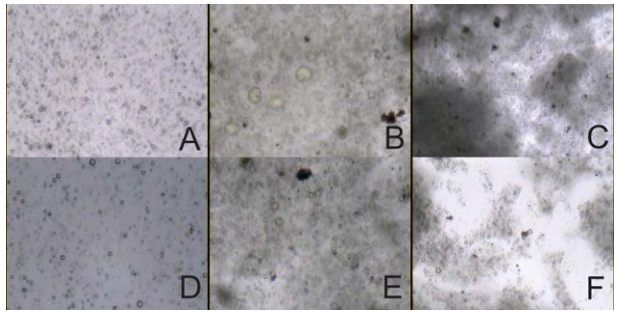

FIG. 2. OPTICAL MICROGRAPHS (MAGNIFICATION OF 640×) OF REHYDRATED FREEZE-DRIED PARTICLES
A: G0C30; B: G2C28; C: G4C26; D: G0C20; E: G2C18; F: G4C16.

Optical Microscopy. The morphological analysis carried out by optical microscopy of the rehydrated freeze-dried powder (Fig. 2) showed the presence of spheres in some systems, particularly those containing 2% gelatin (Fig. 2B,E), although it was also possible to observe droplets of free, non-encapsulated oleoresin. Conversely, the increase in gelatin concentration resulted in highly heterogeneous systems (Fig. 2C,F). The powder produced by freeze-drying is constituted of broken flakes (Fig. 3a,b), instead of individual spheres as in the case of spray-dried particles (Fig. 3c,d). Thus, when this powder is rehydrated it is only possible to observe a shapeless mass of biopolymers, in which the most noticeable characteristic is the absence of a continuous structure when the higher gelatin content was used (Fig. 2C,F).

Selection of the Systems

The results obtained for the encapsulation efficiency of turmeric oleoresin and the yield in powder were taken as the basis to select the most appropriate encapsulating formulation for use in the subsequent assays, for which the objectives were to evaluate the effect of using ultrasound emulsification and spray drying as compared to high shear mixing and freeze-drying. Despite the fact that the highest value for %EE was presented by the sample G4C26, the formulation G2C28 was selected, since this system was easier to handle and also showed relatively high encapsulation efficiency.

Microencapsulation using Ultrasound Emulsification and Spray Drying

The properties of the turmeric oleoresin powders formulated with 2 g of gelatin and 28 g of collagen/100 g of solution produced by spray and freeze-drying, with high shear mixing or ultrasound homogenization, before being subjected to light stability tests (Table 2), indicated that both the emulsification and drying methods affected the product characteristics. Independent of the drying technique, the particles produced using ultrasound (G2C28US) showed significantly lower

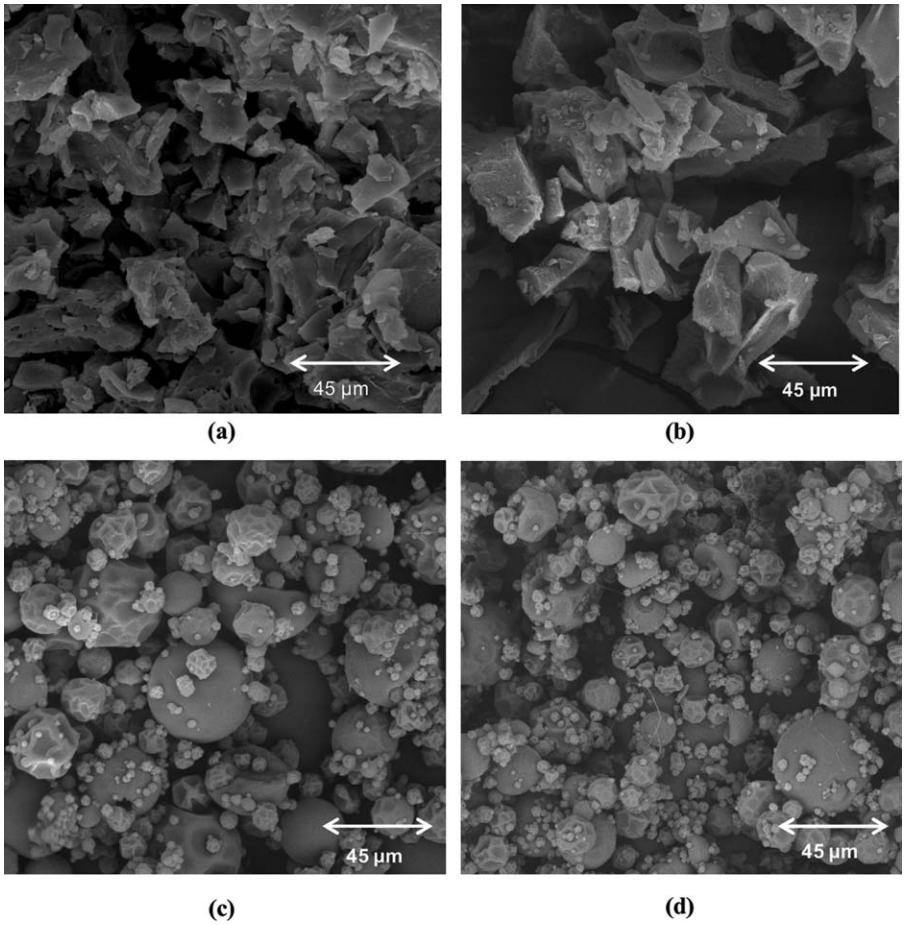

FIG. 3. SCANNING ELECTRON MICROGRAPHS (MAGNIFICATION OF 500×) OF POWDERS
(a): freeze-dried G2C28; (b): freezedried G2C28US; (c): spray dried G2C28; (d): spray dried G2C28US.

water content, higher solubility and higher curcumin content than those prepared with high shear mixing (G2C28), whereas the yields were of the same order of magnitude. The great increase in curcumin retention when using ultrasound emulsification instead of high sheer mixing permitted the inference that the degree of emulsification of turmeric

TABLE 2. PROPERTIES OF POWDERS PRODUCED BY SPRAY AND FREEZE-DRYING WITH ULTRASOUND HOMOGENIZATION (G2C28US) AND HIGH SHEAR MIXING (G2C28)

	Freeze-dried		Spray dried	
System	G2C28	G2C28US	G2C28	G2C28US
Water content (% dry basis)[a]	1.99 ± 0.04^{c}	1.34 ± 0.04^{b}	6.22 ± 0.08^{a}	3.49 ± 0.04^{b}
Curcumin content (mg/g)[a]	119.10 ± 2.85^{d}	177.02 ± 4.53^{c}	266.42 ± 2.43^{b}	342.84 ± 13.58^{a}
Color attributes[a]				
L	81.52 ± 0.00^{c}	81.16 ± 0.00^{d}	91.58 ± 0.00^{a}	91.23 ± 0.03^{b}
a	11.92 ± 0.01^{a}	11.08 ± 0.00^{b}	2.42 ± 0.01^{d}	4.54 ± 0.01^{c}
b	70.08 ± 0.02^{a}	66.23 ± 0.03^{b}	43.13 ± 0.05^{d}	44.10 ± 0.09^{c}
h	80.34 ± 0.01^{c}	80.51 ± 0.00^{c}	86.79 ± 0.02^{a}	84.11 ± 0.03^{b}
C	71.09 ± 0.02^{a}	67.15 ± 0.03^{b}	43.19 ± 0.05^{d}	44.34 ± 0.09^{c}
Solubility (%)*	86.70 ± 1.69^{d}	88.30 ± 0.38^{c}	96.8 ± 1.10^{b}	99.0 ± 0.50^{a}
Yield (%)	94.70	93.30	61.75	65.07

*Mean values ± standard error (n = 3).
Means followed by different letters in the same row differ statistically (Tukey *T* test, $P \leq 0.05$).

oleoresin in the protein matrix was positively affected by sonication, which improved the protection of the core material during drying. Telis and Malacrida (Telis and Malacrida 2013) evaluated the influence of ultrasound homogenization on the encapsulation of turmeric oleoresin using different combinations of modified starch, maltodextrin and gelatin as encapsulating matrices and different drying processes: freeze and spray drying. Their results showed that the use of ultrasound homogenization only gave positive results for spray drying, that is, the application of ultrasound homogenization resulted in significant improvements in the retention of curcumin during spray drying when comparing with mechanical agitation. Conversely, in the case of freeze-drying, the retention of curcumin decreased when ultrasound was used as the homogenization method. The different trend observed in the present work concerning the freeze-dried particles may be attributed to differences in the composition of the encapsulating matrix used, since the application of ultrasound may cause depolymerization in polysaccharides, as well as altering the functional properties of proteins (Arzeni *et al.* 2012), such that these results deserve further investigation.

Scanning Electron Microscopy

As expected, the drying methods resulted in very different types of particle morphology due to the inherent differences between freeze and spray drying (Fig. 3). The spray dried particles were mostly spherical and regular in shape, with smooth surfaces and the absence of fractures. Conversely, the freeze-dried powder consisted of irregular particles resembling flakes with sharp and broken glass-like surfaces, which are associated with the grinding procedure after drying. Che Man *et al.* (1999) and Chen *et al.* (2013) observed similar structures for spray and freeze-dried microcapsules.

Some of the spray dried particles (Fig. 3C,D) presented a dent-like formation, indicating collapse or shrinkage suffered by the liquid droplets during the initial stages of spray drying (Ré 1998). Similar morphologies for spray dried particles prepared with different encapsulation matrices were found by other authors (Krishnan *et al.* 2005a,b; Kanakdande *et al.* 2007; Rocha *et al.* 2012). Based on the SEM images, it is possible to infer that the powder produced by ultrasound homogenization and spray drying (Fig. 3D) presented the lowest average particle diameter when compared to the other treatments. Abismail *et al.* (1999) found that the use of ultrasound improved emulsion stability, producing smaller droplets and less size dispersion, thus providing smaller particle sizes after drying.

Color Measurement

Regarding the color attributes, although there were significant differences between samples obtained with and without sonication, the greatest influence was due to the drying method employed. Spray dried particles were more yellow than freeze-dried ones, which was evidenced by the higher values obtained for the hue angle (h), although the corresponding yellow was less vivid (lower value for chroma, C) and lighter (higher value for L). The color attributes of the spray-dried powder may be related to the higher curcumin content observed when comparing with that obtained by freeze-drying. In addition, spray drying resulted in greater solubility, although the water content of the product was higher and the yield was lower. Cano-Higuita *et al.* (2015) have shown that great color differences in curcumin microencapsulated in carbohydrate blends resulted because of the drying method, as well as the encapsulating matrix formulation. These authors also observed that samples produced by spray drying were significantly brighter and showed lower values of chroma than freeze-dried particles, indicating that freeze-dried capsules had a more vivid yellow color, whereas the spray dried powder had a duller or less saturated color, which was attributed to the high temperatures used during spray drying.

Stability of Turmeric Microcapsules Under Light Exposure

The stability study carried out during storage of the powder for 35 days at 25C under incident light showed that both the drying process and the emulsification method affected curcumin retention (Fig. 4). Independent of the drying process, the particles produced with ultrasound-assisted emulsification presented higher curcumin retention during storage under light. Considering the freeze-dried powder, after 35 days the G2C28US samples lost 28% of the initial curcumin content, whereas the G2C28 samples lost 61% of the initial pigment content (Fig. 4a), and the same trend was observed for the spray-dried microcapsules (Fig. 4b). These results highlight the advantage of using ultrasound in the emulsification step as an alternative to high shear mixing. The effects of sonication on the powder properties were able to provide greater curcumin retention immediately after drying as also after several weeks of storage under intensive light incidence.

Regarding the drying method, two different profiles of curcumin retention can be seen during the storage time. The samples produced by spray drying, independent of the emulsification method, showed considerable degradation of the curcumin during the first week of storage, followed by a slower decrease over the next 4 weeks. Conversely, for particles obtained by freeze-drying, curcumin retention decreased continuously during the 5-week storage period. These different profiles may be related to differences in the microstructure of the particles obtained by each drying method. The high rate of degradation observed in the first week for the spray-dried powder is probably due to the

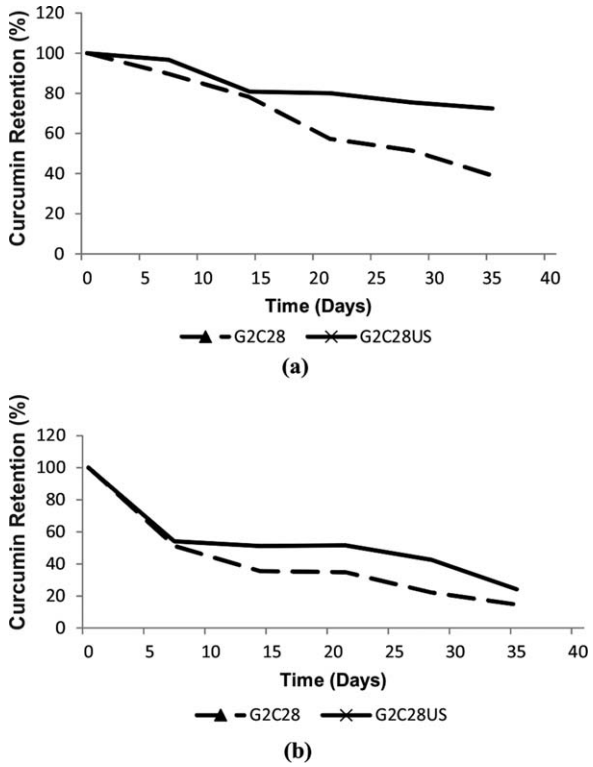

FIG. 4. CHANGES IN TOTAL CURCUMIN RETENTION AFTER 35 DAYS OF STORAGE AT 25C UNDER LIGHT INCIDENCE IN POWDERS PRODUCED WITH ULTRASOUND HOMOGENIZATION (G2C28US), AND WITH HIGH SHEAR MIXING (G2C28) DRIED BY (A) FREEZE-DRYING AND (B) SPRAY DRYING

higher degradation rates of the curcumin that remained on the particle surface, whereas the subsequent lower rates of degradation reflected the good protection offered by the encapsulation matrix, which retarded the degradation reactions. Saénz *et al.* (2009) and Pitalua *et al.* (2010) also observed two distinct slopes in the degradation curves of betalains microencapsulated by spray drying, and attributed this behavior to different degradation rates of superficial and internal betalains. The observation of only one slope throughout the complete storage time observed in the case of freeze-drying, could be associated with a more even distribution of curcumin through the powdered material.

The pigment degradation observed during storage can also be detected by changes in the color attributes of the powders. Table 3 presents the initial (after drying and before storage) and final (after 35 days storage) values for the color parameters of the samples G2C28 and G2C28US produced by spray and freeze-drying, whereas Fig. 5 shows the overall color changes (ΔE) observed during storage under light incidence. Except for the spray dried particles in the last week of storage (Fig. 5b), the samples produced with the application of ultrasound showed smaller values for ΔE. In addition, it is important to note that the order of magnitude for the ΔE values was lower for the spray dried samples than for the freeze dried G2C28 samples, and similar to those corresponding to the freeze dried G2C28US systems. This indicates that the use of both ultrasound and spray drying are both beneficial for color stability of the turmeric microcapsules using the protein matrix studied.

In order to investigate the color changes in a more detailed way, it is useful to observe the evolution of lightness, L, hue angle, h and chroma (C) with time. After 35 days of storage, the freeze-dried samples showed a significant increase ($P \leq 0.05$) in the parameter L, indicative of sample discoloration, independent of the use of ultrasound, whereas for spray-dried capsules the lightness decreased slightly.

The values for a and b – red and yellow, respectively – decreased significantly. The combined changes in these parameters resulted in decreased color saturation (C), which suffered significant losses in all samples, although the most intense decrease in C was observed for the freeze-dried samples (G2C28). The hue angle showed the opposite behavior according to the drying method: for freeze-dried particles h decreased after storage, whereas for spray dried powders the value for h increased. Hue

TABLE 3. COLOR ATTRIBUTES* OF FREEZE-DRIED AND SPRAY DRIED POWDERS BEFORE (0 DAY) AND AFTER (35 DAYS) STORAGE AT 25C UNDER LIGHT INCIDENCE

	L		*a*		*b*		*h*		*C*	
System	0 day	35 days	0 day	35 days	0 day	35 days	0 day	35 days	0 day	35 days
Freeze-dried										
G2C28	81.52^{Ab}	84.07^{Ba}	11.92^{Aa}	8.88^{Bb}	70.08^{Aa}	47.75^{Bb}	80.34^{Ba}	79.46^{Ab}	71.09^{Aa}	48.57^{Bb}
C2G28US	81.16^{Bb}	84.50^{Aa}	11.08^{Ba}	9.92^{Ab}	66.23^{Ba}	54.60^{Ab}	80.51^{Aa}	79.69^{Ab}	67.15^{Ba}	55.49^{Ab}
Spray dried										
C2G28	91.58^{Aa}	90.72^{Ab}	2.42^{Ba}	0.69^{Bb}	43.13^{Ba}	37.06^{Ab}	86.79^{Ab}	88.93^{Aa}	43.19^{Ba}	37.07^{Ab}
C2G28US	91.23^{Ba}	89.88^{Bb}	4.54^{Aa}	1.61^{Ab}	44.10^{Aa}	36.28^{Bb}	84.11^{Bb}	87.45^{Ba}	44.34^{Aa}	36.32^{Bb}

*Mean values.

Means followed by different lowercase letters in the same row differ significantly (Tukey test, $P < 0.05$).

Means followed by different capital letters in the same column differ statistically (Tukey test, $P < 0.05$).

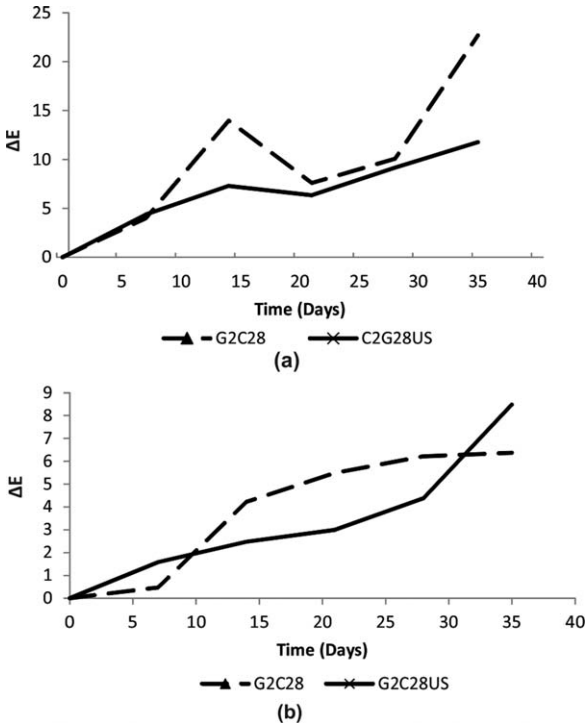

FIG. 5. OVERALL COLOR DIFFERENCE AFTER 35 DAYS OF STORAGE AT 25C UNDER LIGHT INCIDENCE IN POWDERS PRODUCED WITH ULTRASOUND HOMOGENIZATION (G2C28US), AND WITH HIGH SHEAR MIXING (G2C28) DRIED BY (A) FREEZE-DRYING AND (B) SPRAY DRYING

angles of 90 ° correspond to yellow, whereas 0 ° represents the red color. In this way, it is possible to say that, in general, turmeric microcapsules prepared by spray drying were yellower, although the yellow color was less saturated than the freeze-dried powder, which tended to present a slightly orangey color.

Gandía-Herrero *et al.* (2010) detected a degradation of more than 80% of encapsulated indicaxanthin after 6 months of light incidence at 20C. These authors observed that encapsulation greatly increased the stability of the pigment in the absence of light at different temperatures (-20.4 and 20C) but, conversely, degradation of the pigment during exposure to light was very intense. To the contrary, the present results showed that the color of the turmeric microcapsules was relatively stable to light incidence. Zuanon *et al.* (2013) studied curcumin microencapsulation by complex coacervation in gelatin-gum Arabic followed by freeze-drying, and observed that the chroma decreased from 59.34 just after production, to 39.91 after 35 days of storage under light incidence, showing more color degradation than the freeze-dried microcapsules produced in the present study. At the same time, the hue angle decreased from 79.09 to 75.05, a greater decrease than that observed in the present work for the freeze-dried product.

CONCLUSION

This work showed that blends of gelatin-collagen hydrolysate were suitable for turmeric oleoresin encapsulation. Following an initial screening between different concentrations of the proteins, the characterization of the powders produced with high shear emulsification and freeze-drying showed that the encapsulating blend consisting of 2% gelatin and 28% collagen hydrolysate resulted in the best combination for water solubility, curcumin encapsulation efficiency and product yield. In a subsequent step, in which ultrasound emulsification and spray drying were evaluated as alternatives to high shear mixing and freeze-drying, the results indicated that the use of ultrasound was efficient for both drying methods, improving curcumin retention and color stability. Nevertheless, in a more specific way, it was possible to observe that sonication combined with spray drying provided the best results, with the smallest particle size, higher curcumin retention and greater color stability under light incidence. This study suggests that the use of ultrasound positively enhance the desired characteristics of curcumin capsules that might be used as a food supplement or as a natural dye.

ACKNOWLEDGMENT

The authors acknowledge the Sao Paulo Research Foundation, FAPESP for its financial support (Grants 2010/04269-6, 2010/09614-3 and 2009/16847-7).

REFERENCES

ABBAS, S., HAYAT, K., KARANGWA, E., BASHARI, M. and ZHANG, X. 2013. An overview of ultrasound-assisted food-grade nanoemulsions. Food Eng. Rev. *5*, 139–157.

ABISMAÏL, B., CANSELIER, J.P., WILHELM, A.M., DELMAS, H. and GOURDON, C. 1999. Emulsification by ultrasound: Drop size distribution and stability. Ultrason. Sonochem. *6*, 75–83.

AOAC. 1997. *Offical Methods of Analysis International*, 16th Ed., Assoc. of Offical Analytical Chemists International, Gaithersburg.

ARZENI, C., MARTÍNEZ, K., ZEMA, P., ARIAS, A., PÉREZ, O.E. and PILOSOF, A.M.R. 2012. Comparative study of high intensity ultrasound effects on food proteins functionality. J. Food Eng. *108*, 463–472.

BOHIDAR, H., DUBIN, P. and OSADA, Y. 2003. *Polymer Gels: Fundamentals and Applications*, American Chemical Society, Washington, DC.

CANO-CHAUCA, M., STRINGHETA, P.C., RAMOS, A.M. and CAL-VIDAL, J. 2005. Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. Innovative Food Sci. Emerging Technol. *6*, 420–428.

CANO-HIGUITA, D.M., MALACRIDA, C.R. and TELIS, V.R.N. 2015. Stability of curcumin microencapsulated by spray and freeze drying in binary and ternary matrices of maltodextrin, gum arabic and modified starch. J. Food Process. Preserv. *39*, 2049–2060.

CHAMBERLAIN, E.K. and RAO, M.A. 2000. Effect of concentration on rheological properties of acid-hydrolyzed amylopectin solutions. Food Hydrocoll. *14*, 163–171.

CHANDRAPALA, J., OLIVER, C., KENTISH, S. and ASHOKKUMAR, M. 2012. Ultrasonics in food processing – food quality assurance and food safety. Trends Food Sci. Technol. *26*, 88–98.

CHARVE, J. and REINECCIUS, G.A. 2009. Encapsulation performance of proteins and traditional materials for spray dried flavors. J. Agric. Food Chem. *57*, 2486–2492.

CHE MAN, Y.B., IRWANDI, J. and ABDULLAH, W.J.W. 1999. Effect of different types of maltodextrin and drying methods on physico-chemical and sensory properties of encapsulated durian flavour. J. Sci. Food Agric. *79*, 1075–1080.

CHEN, Q., ZHONG, F., WEN, J., MCGILLIVRAY, D. and QUEK, S.Y. 2013. Properties and stability of spray-dried and freeze-dried microcapsules co-encapsulated with fish oil, phytosterol esters, and limonene. Drying Technol. *31*, 707–716.

GANDÍA-HERRERO, F., JIMÉNEZ-ATIÉNZAR, M., CABANES, J., GARCÍA-CARMONA, F. and ESCRIBANO, J. 2010. Stabilization of the bioactive pigment of opuntia fruits through maltodextrin encapsulation. J. Agric. Food Chem. *58*, 10646–10652.

GOULA, A.M. and ADAMOPOULOS, K.G. 2012. A new technique for spray-dried encapsulation of lycopene. Drying Technol. *30*, 641–652.

JAFARI, S.M., HE, Y. and BHANDARI, B. 2007. Encapsulation of nanoparticles of d-limonene by spray drying: Role of emulsifiers and emulsifying techniques. Drying Technol. *25*, 1069–1079.

JAFARI, S.M., ASSADPOOR, E., HE, Y. and BHANDARI, B. 2008. Encapsulation efficiency of food flavours and oils during spray drying. Drying Technol. *26*, 816–835.

KANAKDANDE, D., BHOSALE, R. and SINGHAL, R.S. 2007. Stability of cumin oleoresin microencapsulated in different combination of gum arabic, maltodextrin and modified starch. Carbohydr. Polym. *67*, 536–541.

KAUSHIK, V. and ROOS, Y.H. 2008. Lipid encapsulation in glassy matrices of sugar-gelatin systems in freeze-drying. Int. J. Food Prop. *11*, 363–378.

KHAZAEI, K.M., JAFARI, S.M., GHORBANI, M. and HEMMATI KAKHKI, A. 2014. Application of maltodextrin and gum Arabic in microencapsulation of saffron petal's anthocyanins and evaluating their storage stability and color. Carbohydr. Polym. *105*, 57–62.

KRISHNAN, S., BHOSALE, R. and SINGHAL, R.S. 2005a. Microencapsulation of cardamom oleoresin: Evaluation of blends of gum arabic, maltodextrin and a modified starch as wall materials. Carbohydr. Polym. *61*, 95–102.

KRISHNAN, S., KSHIRSAGAR, A.C. and SINGHAL, R.S. 2005b. The use of gum arabic and modified starch in the microencapsulation of a food flavoring agent. Carbohydr. Polym. *62*, 309–315.

LERTSUTTHIWONG, P., NOOMUN, K., JONGAROONNGAMSANG, N., ROJSITTHISAK, P. and NIMMANNIT, U. 2008. Preparation of alginate nanocapsules containing turmeric oil. Carbohydr. Polym. *74*, 209–214.

MADENE, A., JACQUOT, M., SCHER, J. and DESOBRY, S. 2006. Flavour encapsulation and controlled release – a review. Int. J. Food Sci. Technol. *41*, 1–21.

MAHDAVI, S.A., JAFARI, S.M., GHORBANI, M. and ASSADPOOR, E. 2014. Spray-drying microencapsulation of anthocyanins by natural biopolymers: A review. Drying Technol. *32*, 509–518.

MALACRIDA, C.R., FERREIRA, S., ZUANON, L.A.C. and TELIS, V.R.N. 2015. Freeze-drying for microencapsulation of turmeric oleoresin using modified starch and gelatin. J. Food Process. Preserv. *39*, 1710–1719.

MARTINS, R.M., PEREIRA, S.V., SIQUEIRA, S., SALOMÃO, W.F. and FREITAS, L.A.P. 2013. Curcuminoid content and antioxidant activity in spray dried microparticles containing turmeric extract. Food Res. Int. *50*, 657–663.

MATTEA, F., MARTÍN, Á. and COCERO, M.J. 2009. Carotenoid processing with supercritical fluids. J. Food Eng. *93*, 255–265.

MONGENOT, N., CHARRIER, S. and CHALIER, P. 2000. Effect of ultrasound emulsification on cheese aroma encapsulation by carbohydrates. J. Agric. Food Chem. *48*, 861–867.

PITALUA, E., JIMENEZ, M., VERNON-CARTER, E.J. and BERISTAIN, C.I. 2010. Antioxidative activity of microcapsules with beetroot juice using gum Arabic as wall material. Food Bioprod. Process. *88*, 253–258.

RÉ, M.I. 1998. Microencapsulation by spray drying. Drying Technol. *16*, 1195–1236.

ROCHA, G.A., FÁVARO-TRINDADE, C.S. and GROSSO, C.R.F. 2012. Microencapsulation of lycopene by spray drying: Characterization, stability and application of microcapsules. Food Bioprod. Process. *90*, 37–42.

SAÉNZ, C., TAPIA, S., CHÁVEZ, J. and ROBERT, P. 2009. Microencapsulation by spray drying of bioactive compounds from cactus pear (Opuntia ficus-indica). Food Chem. *114*, 616–622.

SANTOS, A.B., FÁVARO-TRINDADE, C.S. and GROSSO, C.R.F. 2005. Preparo e caracterização de microcápsulas de oleoresina de páprica obtidas por atomização. Cienc. Tecnol. Aliment. *25*, 322–326.

SARI, T.P., MANN, B., KUMAR, R., SINGH, R.R.B., SHARMA, R., BHARDWAJ, M. and ATHIRA, S. 2015. Preparation and characterization of nanoemulsion encapsulating curcumin. Food Hydrocoll. *43*, 540–546.

SARKAR, S., GUPTA, S., VARIYAR, P.S., SHARMA, A. and SINGHAL, R.S. 2013. Hydrophobic derivatives of guar gum hydrolyzate and gum Arabic as matrices for microencapsulation of mint oil. Carbohydr. Polym. *95*, 177–182.

SHANMUGAM, A. and ASHOKKUMAR, M. 2014. Functional properties of ultrasonically generated flaxseed oil-dairy emulsions. Ultrason. Sonochem. *21*, 1649–1657.

SHYAMKUWAR, A.L., CHOKASHI, K.P., WAJE, S.S. and THORAT, B.N. 2010. Synthesis, characterization, and drying of absorbable gelatin foam. Drying Technol. *28*, 659–668.

TELIS, V.R.N. and MALACRIDA, C.R. 2013. Influence of homogenization processes on curcumin encapsulation by freeze and spray drying. Proceedings of the XXI International Conference on Bioencapsulation, pp. 104–105, Berlin, Germany.

TELIS, V.R.N. and MARTÍNEZ-NAVARRETE, N. 2009. Collapse and color changes in grapefruit juice powder as affected by water activity, glass transition, and addition of carbohydrate polymers. Food Biophys. *4*, 83–93.

WANG, Y., LU, Z., LV, F. and BIE, X. 2009. Study on microencapsulation of curcumin pigments by spray drying. Eur. Food Res. Technol. *229*, 391–396.

WILLIAMS, P.A. and PHILLIPS, G.O. 2009. Gum arabic. In *Handbook of hydrocolloids*, (P.A. Williams and G.O. Phillips, eds.) pp. 252–273, CRC Press, Boca Raton.

WROLSTAD, R.E. and CULVER, C.A. 2012. Alternatives to those artificial FD&C food colorants. Annu. Rev. Food Sci. Technol. *3*, 59–77.

YANISHLIEVA, N.V., MARINOVA, E. and POKORNÝ, J. 2006. Natural antioxidants from herbs and spices. Eur. J. Lipid Sci. Technol. *108*, 776–793.

ZUANON, L.A.C., MALACRIDA, C.R. and TELIS, V.R.N. 2013. Production of turmeric oleoresin microcapsules by complex coacervation with gelatin–gum Arabic. J. Food Process Eng. *36*, 364–373.