Encapsulation of Nanoparticles of d-Limonene by Spray Drying: Role of Emulsifiers and Emulsifying Techniques

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Submicron emulsion particles of d-limonene prepared by a microfluidizer and ultrasound were spray dried to produce nanoparticle encapsulated powders. Maltodextrin combined with a surface-active biopolymer (modified starch or whey protein concentrate) or a small molecule surfactant (Tween 20) was used as the wall material. Results showed that microfluidization was an efficient emulsification technique resulting in a powder with the highest retention (86.2%) of d-limonene, mainly due to its capability to produce emulsions with fairly small droplets ($d_{43}$ of 700–800 nm) and narrow distributions, which had a good stability during the process. Among different emulsifiers used, although Tween 20 significantly reduced the emulsion size ($d_{43} < 200$ nm), the resulted powder had the poorest encapsulation efficiency.

Keywords Emulsification; Encapsulation efficiency; Microfluidization; Nano-emulsion; Retention

INTRODUCTION

Encapsulation of food ingredients with spray drying is a popular and common technique nowadays and it has been applied for many different food materials such as flavors, edible oils, essential oils, and spice resins. During spray-drying encapsulation, it is vital to prevent volatile losses and extend the shelf life of the products by minimizing the amount of unencapsulated oil at the surface of powder particles.1–4 For volatiles in particular, improving the retention of core material in final encapsulated powder is very important, as much of these ingredients can be lost during the process. Factors that can affect the encapsulation efficiency of food flavors and oils are the properties of wall and core materials as well as the emulsion characteristics and drying parameters.5–8 Emulsification and the emulsifying ingredients play a key role in this regard.9,10 There are some reports confirming that smaller oil droplets will be retained more efficiently within the encapsulated product and the resulted microcapsules will have lower unencapsulated oil at their surface.11–13 Much of the work in this area has been done by emulsions having a droplet size of more than one micron and the application of submicron (nano) emulsions in encapsulation of oils and flavors is scant in the literature.

Recently, nano-emulsions have attracted considerable attention in various industrial fields including cosmetics, pharmaceuticals, and agrochemicals.14–17 These emulsions are mainly produced by modern emulsification systems such as microfluidization and the resulting submicron emulsions can have a high potential application in encapsulation of food ingredients, since the stability and other features of the infeed emulsion such as droplet size and distribution play a critical role on the retention and surface oil content of the product. However, there is no clear-cut evidence on how submicron emulsion droplets can improve the encapsulation efficiency of food flavors and oils. Emulsions prepared for encapsulation purposes are a unique class of food emulsions due to existence of a high concentration of biopolymers (wall materials), which are essential for encapsulation. At the same time, because of slow adsorption kinetics of surface-active biopolymers and extreme conditions of high-energy emulsification techniques, it is not possible to reduce the droplet size of these emulsions to very small sizes (<500 nm).17–19 Application of small molecule surfactants in the encapsulation field is another issue that has not been studied extensively, since these ingredients do not have desirable encapsulation properties such as film-forming abilities. Therefore, the objectives of this work are to determine the influence of different emulsification methods and various surface-active ingredients including small surfactants on encapsulation efficiency and some other powder properties during spray drying.

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MATERIALS AND METHODS

Materials
The wall material was an aqueous solution of a modified starch (Hi-Cap 100, National Starch and Chemical, NSW, Australia) and/or whey protein concentrate (WPC; ALACEN, New Zealand Milk Products, Auckland, New Zealand) in a combination with maltodextrin (DE 16–20, Fieldlose 17-C AP, Penford Limited, NSW, Australia). d-Limonene (Orange Terpen 07231 TT, Quest International, NSW, Australia) was used as the core material (ρ = 840 kg/m^3, η = 8.8 mPa.s [25°C], RI = 1.487). A non-ionic surfactant, i.e., Tween 20 (LabChem, NSW, Australia) was used as a single emulsifying agent in some stages of this work. Analytical-grade hexane and petroleum ether (BP 40–60°C) were purchased from Sigma Chemicals Company (Sydney, Australia). Distilled water was used for the preparation of all solutions. All general chemicals used in this study were of analytical grade.

Preparation of Emulsions
One day before emulsification, wall material powders were dissolved in distilled water and kept overnight in a shaking water bath (Ratek Instruments, Melbourne, VIC, Australia) to warrant a full saturation of the polymer molecules. Total concentration of dissolved solid was 40% (w/w) composed of 30 wt% maltodextrin and 10 wt% emulsifying ingredients (Hi-Cap or WPC). Pre-emulsions (oil-in-water) were produced by a rotor-stator system (Model L2R, Silverson Machines Ltd., London, UK), and the core material (d-limonene) in the ratio of 1:4 (core:wall) was progressively added to the continuous phase during pre-emulsion preparation and stirred for 10 min at the highest speed. These coarse emulsions were then further emulsified using a microfluidizer (Model M-110 L, Microfluidics, Newton, MA) at 60 MPa for one cycle or a 24-KHz ultrasound probe (Dr Hielscher series, Model UP 400S) with 22 mm diameter at the highest power for 100 s. More details about emulsification conditions were presented in our previous works.[17,20]

Spray Drying
A pilot-plant spray dryer (Model SL 20, Saurin Group of Companies, Victoria, Australia) was used to convert infeed emulsions into encapsulated powders, as described in our earlier studies.[20] The operational conditions of the spray drying were air inlet temperature of 180°C, air outlet temperature of 65°C, and air pressure at the nozzle of 310 kPa. The dried powder was collected and stored in opaque, airtight containers at 4°C awaiting further analysis.

Emulsion Droplet Size and Powder Particle Size Analysis
Laser light scattering method via Mastersizer 2000 (Malvern Instruments, Worcestershire, UK) was used to determine the size distribution of oil droplets in infeed emulsions, as described in our previous study.[17] In order to investigate the change in emulsion size during the encapsulation process, droplet size of the reconstituted emulsion prepared from the encapsulated powders was also determined by Mastersizer 2000. Redispersion properties were studied by producing 10% (w/w) aqueous solutions of the powders by mixing the reconstituted emulsions at room temperature with a magnetic stirrer for 30 min. The analysis of powder particle size and specific surface area of the powder particles was performed using the laser light scattering method by an analyzer with a batch cell unit (Mastersizer E, Malvern Instruments, Worcestershire, UK). Encapsulated powders were dispersed in propan-2-ol for the particle size analysis. In all cases, volume mean diameter or d3,4 was reported as the emulsion size or powder particle size.

Encapsulation Efficiency Analysis
One of the important parameters of the encapsulated powders is the encapsulation efficiency (EE) during the process. By definition, it is the amount of core material encapsulated inside the powder particles and can be obtained from the following formula:

$$EE = \left[\frac{(\text{Total oil} - \text{Surface oil})}{\text{Total oil}}\right] \times 100$$

Total oil includes the internal and surface oil content of the powders, while surface oil is related to the unencapsulated oil at the surface of the particles. Accordingly, surface oil has been named free or extractable oil, as it is measured mostly through solvent extraction. In the case of volatiles, because there are some loss during the process, the term “retention” has been proposed, which is the proportion (expressed as %) of the core material remained in the powder. It is calculated as:

$$\text{Retention} = \left[\frac{\text{Actual oil content (Total oil)}}{\text{Theoretical oil content (Initial oil)}}\right] \times 100$$

We used surface oil content and retention data as an indication of the encapsulation efficiency.

Total Oil Measurement
Total oil was determined by Clevenger distillation method. A 500-mL round-bottomed flask was used to dissolve 10 g (±0.02 g) of the spray-dried powder in 250 mL of deionized water. Each flask was stoppered and manually shaken for approximately 1 min in order to break down the clumps and facilitate dissolution. Then, about 0.5 mL of antifoam solution (silicon oil) was added and the Clevenger apparatus was fitted to the top of flask equipped with a water-cooled condenser at the top of apparatus. The solution inside the flask came to boiling through underneath
heater and distillation was continued for 3 h. The volume of the oil (d-limonene), read directly from the graduated oil collection arm was converted to the oil mass via multiplying by the oil density (0.856 g/mL at 20°C), which was measured with a hydrometer.

**Surface Oil Content Measurements**

To determine the amount of extractable oil on the surface of d-limonene–encapsulated powders, 1.0 g of sample, 9 mL of hexane, and 1 mL of a stock solution of cyclohexane in hexane (about 10 μg/mL) as an internal standard were added into a glass bottle. The mixtures were slowly mixed on a rotary shaker for 30 s at ambient temperature. The extracted mixtures were centrifuged at 3000 rpm for 10 min to separate the powder from extracted solution. The oil content in the organic phase was then measured by gas chromatography (GCMS model GC-17A V3, Shimadzu Corp., Kyoto, Japan). Approximately 2 μL of the organic phase was injected into the GC equipped with an EC-5 packed column (30 m × 0.53 mm; 1.5-μm film coating). The chromatographic conditions were flame ionization detector (FID) at 275°C with helium as the carrier gas; column head pressure 45 kPa; split injection; initial temperature 60°C with initial time of 2 min; program rate 8°C/min to 100°C, and 10°C/min to 200°C was controlled at constant value of 120°C. The external standard method was used to calculate the quantity of d-limonene. Three bottles of the standard d-limonene in hexane (25, 50, and 100 μg/mL) were prepared, and 2 μL of these standards were injected into GC. The average peak area was used to calculate the amount of d-limonene.

**Analysis of Moisture Content and Water Activity of Powders**

Moisture content of the encapsulated powders was determined gravimetrically by oven drying (Vord-460-D, Thermoline Scientific Equipment Pty Ltd, NSW, Australia) at 70°C to constant weight. The sample size was 5.0 g powder widened inside glass plates and the vacuum drying time was 72 h. The results were reported on the wet basis as (weight loss/sample weight) × 100. The water activity of spray dried powders was measured by an Aquafab system (Model 3 TE, Decagon Devices Inc., Washington).

**Scanning Electron Microscopy of Encapsulated Powders**

A JSM 6400F model scanning electron microscope (JEOL Co. Ltd., Tokyo, Japan) was used to investigate the microstructural properties of the spray-dried encapsulated powders. The samples were placed on the SEM stubs using a two-sided adhesive tape (Nisshin EM Co. Ltd., Tokyo, Japan). The specimens were subsequently coated with Pt using a magnetron sputter coater (Model EIKO IB-5, Eiko Inc., Tokyo, Japan). The coated samples were then analyzed using the SEM operating at an accelerating voltage of 15 kV. The micrographs representing the microstructure of the encapsulated powders were taken by the instrument’s software installed on a PC connected to the system.

**Experimental Design and Statistical Analysis**

The parameters considered affecting the encapsulation efficiency were the emulsification method, the type of surface-active ingredient, and emulsion droplet size. The effect of each operational and compositional parameter on the encapsulation efficiency (surface oil content and retention) was studied using only one system (emulsifying method or wall material). All the experiments were performed based on a fully factorial design and the results represent the means of two replicates. A general linear model of MINITAB (Version 14, 2004) was used to conduct an analysis of variance (ANOVA) to determine differences between treatments means. Treatments means were considered significantly different at P ≤ 0.05 and the difference was considered very significant at P < 0.01. Some of the graphs were drawn by Excel (Microsoft Office 2003) and some by MINITAB 14.

**RESULTS AND DISCUSSION**

**Encapsulation Efficiency for Different Emulsification Systems**

Microfluidized and sonicated emulsions were submicron emulsions with a d₄₃ of 740 nm and 750 nm, respectively (Table 1). On the other hand, emulsions made with rotor-stator system (Silverson) had a d₄₃ of about 1.2 μm. It was found that total retention of d-limonene in spray-dried powders increased slightly from 82.7 to 86.2% when the emulsification method changed from Silverson to microfluidization, which was directly correspondent to a reduction in emulsion size.

In general, reducing emulsion size below one micron through ultrasonication and microfluidization did not improve the encapsulation efficiency significantly (P > 0.05), as similar results were obtained for all the samples (Table 1). Sonicated emulsions, however, resulted in encapsulated powders with significantly (P < 0.05) higher surface oil contents (103.1 mg/100 g powder), almost two times higher than that of microfluidized samples, in spite of having the same emulsion size and using identical spray-drying conditions. Two emulsification systems can produce two different encapsulated powders, even if they produce emulsions with the same size and if exactly the same emulsion composition is used. This can be explained by other parameters such as size distribution of the emulsion, stability of emulsions during spray drying, powder particle size, and influence of the emulsification itself on emulsion properties. Considering particle size, powders from ultrasound emulsions had a d₄₃ of about 30.9 μm, significantly (P < 0.05) smaller than their microfluidized...
counterparts (37.3 μm). Although there is controversy on the role of particle size in spray-drying encapsulation, some workers, such as Fang et al.,[21] have reported that larger particles result in improved flavor retention and lower surface oil contents due to more efficient encapsulation of oil droplets. Recently, Soottitantawat et al.[13] showed that face oil contents due to more efficient encapsulation of oil particles result in improved flavor retention and lower release of encapsulated flavor if the initial emulsion has a small size.

Another reason can be directly related to the difference between emulsion size and particle size of the spray-dried microcapsules. Re and Liu[22] defined a parameter in this regard as [X = (powder size – emulsion size)/powder size]. They showed that by increasing X from 0.2 to 0.8, retention of volatiles (Eugenol) increased significantly, so it could be possible to improve the encapsulation efficiency by increasing the difference between emulsion and powder particle size. In our case, since there is a smaller difference between emulsion size and powder size for ultrasound samples compared with microfluidized ones, final powders will have higher surface oil contents. This might be explained by the fact that there is a more chance for some emulsion droplets (particularly the bigger ones) to come onto the surface of particles due to smaller diameter of particles. In other words, fine oil droplets will be covered inside wall material more efficiently in bigger particles than smaller ones, and the spray-drying process conditions including atomization will have a minimal effect on emulsion droplets if the emulsion has a small size and narrow distribution, such as microfluidized emulsions.

Stability of emulsion droplets during encapsulation process could be another important factor. In fact, it is crucial that emulsion droplets not only have the minimum size but also be stable enough without any coalescence or flocculation so that they can be embedded in the shell of powder particles inside the capsules with maximum protection. By reconstituting (redispersing) the encapsulated powders in distilled water, we investigated the stability of emulsion droplets during the spray-drying process through comparing the reconstituted emulsion size distribution with the original emulsions. Our results (Fig. 1) showed that redispersion behavior of encapsulated powders from ultrasound samples and microfluidized emulsions are not similar. There is a small change in size distribution of microfluidized emulsions (Fig. 1b), while with ultrasound samples, it is shifting toward the larger area more obviously (Figure 1c). For example, d_{43} of reconstituted emulsions for ultrasound and microfluidized samples was equal to 1.3 and 1.0 μm, respectively (d_{43} for initial emulsions before spray drying were 0.75 and 0.74 μm, respectively). This confirms that microfluidized emulsions have been more stable during atomization and spray drying. This could be related to the influence of emulsification method on the emulsifying capabilities of the biopolymers. Emulsions with weaker stability result in encapsulated powders with higher surface oil contents and lower retention of volatiles[23–26] since encapsulation efficiency is negatively correlated with reconstituted emulsion size, in agreement with the results of Hogan et al.[27–29] Mongenot et al.[30] claimed that ultrasound improves the emulsifying properties of weak emulsifiers such as maltodextrin or modified starch and results in encapsulated powders with higher encapsulation efficiencies than rotor-stator emulsification system, although there was not a significant difference between emulsion size of both systems. They did not provide any data on emulsion size distributions and any other supporting reasons.

Since emulsification by ultrasound is not uniform across the emulsion volume and there is more energy in the vicinity of the probe, some fractions of the surface-active biopolymer (Hi-Cap) could lose their emulsifying capabilities or become weakened due to extreme emulsification conditions and thereby cannot protect oil droplets efficiently during spray drying. In microfluidization, on the other hand, emulsification is very quick inside the interaction chamber and significantly uniform as only a small volume of emulsion passes through the emulsification zone. The other reason could be influence of microfluidization on improving the emulsifying abilities of Hi-Cap by exposing more hydrophilic sited of its polymer toward oil droplets via shear and inertial stress along with cavitation inside interaction

<table>
<thead>
<tr>
<th>Emulsifying device</th>
<th>Emulsification conditions</th>
<th>Emulsion size (d_{43}, μm)</th>
<th>Powder size (d_{43}, μm)</th>
<th>Powder moisture (wt %)</th>
<th>Surface oil (mg/100 g powder)</th>
<th>Retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silverson</td>
<td>Highest speed, 10 min</td>
<td>1.2\textsuperscript{a}</td>
<td>27.8\textsuperscript{a}</td>
<td>2.6\textsuperscript{a}</td>
<td>55.6\textsuperscript{a}</td>
<td>82.7\textsuperscript{a}</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Highest power, 100 s</td>
<td>0.75\textsuperscript{b}</td>
<td>30.9\textsuperscript{b}</td>
<td>1.8\textsuperscript{b}</td>
<td>103.1\textsuperscript{b}</td>
<td>85.8\textsuperscript{b}</td>
</tr>
<tr>
<td>Microfluidizer</td>
<td>60 MPa, one cycle</td>
<td>0.74\textsuperscript{b}</td>
<td>37.3\textsuperscript{b}</td>
<td>1.5\textsuperscript{b}</td>
<td>55.4\textsuperscript{a}</td>
<td>86.2\textsuperscript{a}</td>
</tr>
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</table>

Means within the same column followed by different letters are significantly (P < 0.05) different.
chamber, making more stable emulsions. While in sonication, only cavitation in microturbulent flows is happening.

Our results revealed that there was a shift in size distribution of redispersed emulsions from Silverson samples toward the left, i.e., smaller size (Fig. 1a), which was opposite to the normal trend obtained for microfluidizer and ultrasound samples (shift to the right). This could be related to the larger size of Silverson-prepared emulsions, which makes them unstable during the spray-drying process. Soottitantawat et al.\[12\] showed that atomization of emulsions during spray drying leads to a decrease in droplet size of coarser emulsions but has no effect on the finer emulsions. This phenomenon may be explained by the shearing effect during atomization that can break down the larger emulsion droplets and ultimately result in powders with less encapsulated core material and more loss of volatiles. Our results (Table 1), however, showed no significance difference ($P > 0.05$) between Silverson and microfluidized samples in terms of total retention of d-limonene and surface oil content of encapsulated powders, possibly because of much finer emulsion droplet sizes compared to the work of Soottitantawat et al.\[12\]

Another important reason could be the occurrence of coalescence and Ostwald ripening within the encapsulated matrix, i.e., an increase in the number of small droplets due to growth of big droplets, as they are close to each other and merge together. In fact, mobility of core droplets within the encapsulated particles in a dried matrix is an unclear issue that needs more work to be understood. In microfluidized samples, and particularly ultrasound ones, coalescence is more prevalent since the volume (number) of big droplets is increasing due to merging of smaller droplets into together, and so the whole emulsion size distribution is shifting to the right (bigger size). It is interesting that Silverson emulsions were also more stable than sonicated ones as their size did not increase and some of the droplets even become smaller as a result of Ostwald ripening and/or atomization (Fig. 1a). This could be due to smaller volume of the emulsion in the emulsification zone (rotor-stator gap) of the system and more uniform emulsification with more frequent forces such as shear and inertial stress.

It is traditional that surface oil data are given based on weight; e.g., mg/100 g powder. The more reasonable way can be to represent the surface oil data in terms of specific surface area of the powder particles, since there are various sizes of particles present and the results can be different depending on the extractable surface of particles. For instance, the surface oil content of ultrasound samples was 103.1 mg/100 g powder and this powder had a specific surface area of 0.124 m$^2$/g. Therefore, it can be calculated that
Surface oil content is equal to 8.5 mg/m² of powder particles:

\[
\text{Surface oil content} = 1.03 \text{ (mg/g powder)} \times 1/0.124 \\
\times \text{(m}^2/\text{g powder)} = 8.5 \text{mg/m}^2 \text{ of powder}
\]

Surprisingly, this is comparable with 9.0 mg/m² for microfluidized emulsions, so there is no significant difference \((P>0.05)\) between these two emulsifying methods if surface oil content is given based on specific surface area of powder particles (Fig. 2). It can be justified by having the same emulsion size and similar spray-drying conditions and emulsion composition, which should result in powders with relatively similar encapsulation efficiencies.

Observations of powders via SEM (Fig. 3) showed that there were individual spherical particles in Silverson and microfluidizer samples with some dents and shrinkage that probably has happened during early stages of drying due to high drying rates. On the other hand, there were aggregated particles in ultrasound samples, possibly because of higher surface oil on these particles. Occasionally, some cracks were observed in all samples (Fig. 3d) that could be due to weak viscoelastic properties of the wall material during expansion at the final stages of spray drying. In almost all of these cases, powder particles showed a mixture of smooth and indented surface morphology, which could be attributed to the exposure of individual particles to different drying conditions, similar to the results of Hogan et al.\cite{29} In fact, surface morphology is the function of wall material properties and drying conditions and could be independent from the emulsification method used to prepare the infeed emulsions.

**FIG. 2.** Surface oil content of encapsulated powders produced from emulsions by different emulsifying devices. Emulsion preparation and composition details are given in Table 1.

**FIG. 3.** SEM of encapsulated powders produced from emulsions of different emulsifying techniques: (A) Silverson, (B) microfluidizer, (C) and (D) ultrasound. Core material was d-limonene (20 wt%) and wall materials were maltodextrin and Hi-Cap in the ratio of 3:1.
Surface-Active Biopolymers and Encapsulation Efficiency

We investigated the role of different surface-active biopolymers (Hi-Cap vs. WPC) on encapsulation efficiency during spray drying and compared them with a small molecule surfactant (Tween 20). Emulsions were produced by microfluidization and were spray-dried in the same conditions. Results (Table 2) showed that powders consisted of Hi-Cap had significantly ($P < 0.05$) higher encapsulation efficiency than their WPC counterparts. For example, total retention of d-limonene for Hi-Cap emulsions was about 86.2%, significantly higher than that of WPC emulsions (76.3%). Also, surface oil of Hi-Cap samples was 55.4 mg/100 g powder (9.0 mg/m² powder), which was significantly lower than that of WPC samples (170.1 mg/100 g = 18.5 mg/m² powder).

Hi-Cap emulsions had a smaller size and narrower dispersion as a single-peak lognormal distribution, while emulsions containing WPC were bimodal with significantly bigger $d_{43}$. So it is not surprising that encapsulated powders made with WPC will have higher unencapsulated oil at the surface of their particles and lower retention of the volatiles (d-limonene) during spray-drying encapsulation. This is in agreement with the earlier results reported by Risch and Reineccius,[11] Liu et al.,[9,10] and Soottitantawat et al.[12] A smaller emulsion droplet not only will be encapsulated more efficiently into the powder particles but the resulting emulsion will also be more stable during the process.

Another possible reason could be the larger powder size of Hi-Cap samples (37.3 µm) compared with WPC samples (21.3 µm). In fact, since the difference between emulsion and powder size is bigger in Hi-Cap samples, the resulting powder could retain more oil droplets inside the microcapsules and as a result there would be less unencapsulated oil (surface oil) on the powder particles.

Emulsion stability during encapsulation process is also important, as mentioned in the previous section. Original Hi-Cap emulsions had a single-peak lognormal distribution with narrow dispersion ($\text{span} = 1.8$), and after spray drying there was a little change in their droplet size (Fig. 4). For instance, emulsion size of reconstituted powders containing Hi-Cap was about 986 nm ($d_{43}$) compared with 739 nm before spray drying. On the other hand, emulsions made with WPC had a bimodal distribution with a wide range of droplet sizes ($\text{span} = 6.5$), and after spray drying there was a significant increase in their size. This shows that WPC emulsions were not as stable as Hi-Cap emulsions during atomization and spray drying and as a result the final encapsulated powders had a lower retention of d-limonene and a higher content of surface oil. It has been well documented that unstable emulsions will lead to lower encapsulation efficiencies.[9,10,31]

The possible explanation could be denaturation and structural change of WPC polymers during emulsification and spray-drying processes and, therefore, less capability of these biopolymers to retain and encapsulate core materials, whereas Hi-Cap molecules are more resistant to changes in temperature, pH, and other environmental conditions such as ionic strength of the original emulsion. Additionally, poorer volatile retention and higher surface oil content observed for WPC particles could also be related to the surface morphology and microstructure of powders particles. The SEM results shown in Fig. 5 revealed that indentation, shrinkage, invaginations, and particles with rough surfaces were more prevalent in WPC samples than those with HI-Cap, which had relatively spherical shape with smoother surfaces. This could be attributed to the effects of the drying rate and viscoelastic properties of the wall material on the structure of particles. In fact, the damage of surface integrity (fissures, shrinkage) and surface imperfections observed mainly for WPC particles, which result in an increase of their surface area, may contribute to the increase of the unencapsulated or surface oil.

The presence of dents is probably related to shrinkage of atomized droplets during early stages of the spray-drying process[32,33] Thermal expansion of air or vapor inside the drying particles (ballooning, associated with high drying rates) may smooth out dents, which can occur only prior to solidification of the wall material, when it is elastic enough to enable such structural changes. There were some spherical particles in WPC powders with smooth surfaces that exhibited some holes (Fig. 5b) and as a result they cannot help to prevent losses of encapsulated volatiles (d-limonene).

### TABLE 2

<table>
<thead>
<tr>
<th>Emulsifying agent</th>
<th>Emulsion size ($d_{43}$, µm)</th>
<th>Powder size ($d_{43}$, µm)</th>
<th>Powder moisture (wt. %)</th>
<th>Surface oil (mg/100 g powder)</th>
<th>Surface oil (mg/m² powder)</th>
<th>Retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hi-Cap</td>
<td>0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WPC</td>
<td>0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>53.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>270.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.9&lt;sup&gt;b&lt;/sup&gt;</td>
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Means within the same column followed by different letters are significantly ($P < 0.05$) different.
Another important result was very poor encapsulation efficiency of powders consisting of Tween 20 as the emulsifier, with the highest surface oil content (270.2 mg/100 g powder = 43.6 mg/m² powder), almost 2 and 5 times higher than WPC and Hi-Cap samples, respectively (Table 2). Surface-active biopolymers have a slow adsorption rate and cannot stabilize the newly formed droplets as fast as they are disrupted during extreme conditions of high-energy emulsification, whereas with small surfactants such as Tween 20 we could reduce the emulsion size very significantly to the range of real nano-emulsions (about 200 nm). Many workers have shown that smaller emulsion size will lead to better encapsulation efficiency, but the resulting powders from Tween 20 samples had the highest surface oil and a low retention.

These unexpected results could be explained by the poor stability of Tween emulsions and the lack of film-forming properties of this surfactant, which is vital for encapsulation. As can be seen in Fig. 6, there was a major shift in size distribution of Tween samples to bigger droplets after spray drying. For instance, $d_{43}$ of emulsions before spray drying was about 215 nm, which was increased very significantly to 4.5 μm within the encapsulated powder. It is shown that free biopolymers (maltodextrin in our case) and surfactant micelles could induce some “depletion flocculation” and “bridging flocculation,” which may cause instability in the emulsion via flocculation, coalescence, or even phase separation. As the amount of maltodextrin used in these emulsions is relatively high (40 wt%), it leads to an increase in the viscosity of the emulsion continuous phase and thereby, reduces the chance of coalescence. Also, because we spray-dried these emulsions within one hour after their production, this instability could not be justified. Furthermore, an emulsion size analysis immediately before spray drying confirmed that emulsion size did not change significantly. If emulsion size is not changing in the solid state of the encapsulated particles, then possibly the reconstituting process itself can influence emulsion size, as functionality of surface-active agents can be totally different after spray drying compared with initial emulsions.

The more reasonable explanation can be the poor stability of surfactant-made emulsions compared with biopolymer ones. Although small surfactants adsorb at the oil–water interface faster than surface-active biopolymers, the protective membrane formed by biopolymers around oil droplets is much stronger than surfactant layer and prevents droplets from coming close together by some repulsive forces such as electrical charges more efficiently. So oil droplets covered by biopolymers will undergo fewer changes during atomization and spray drying than with surfactants (Figs. 4 and 6). The combination of both surfactants and surface-active biopolymers can be a possible solution to have the minimum emulsion size and maximum

**FIG. 4.** Emulsion size distribution before spray drying and after reconstituting the encapsulated powder for two different surface-active biopolymers: Hi-Cap and WPC. Emulsification was done by microfluidizer (60 MPa, one cycle).

**FIG. 5.** SEM of encapsulated powders containing (A) Hi-Cap and (B) WPC. Core material was d-limonene (20 wt%) and wall material consisted of maltodextrin and one of the emulsifying biopolymers in the ratio of 3:1.
stability during the process, but the problem is the competition between these emulsifiers and displacement of large molecule biopolymers from the interface by surfactant molecules.

SEM examination showed that powders containing maltodextrin and Tween 20 mainly resemble the structure of encapsulated powders made from maltodextrin as the wall material and Tween molecules had no influence on their structure (Fig. 7). It can be seen that particularly the small particles had a smooth surface with spherical shape. The big particles, however, had large dents or concavities mostly presented with cracks, and sometimes with “caps within dents,” suggesting that solidification of the wall material happened prior to expansion of the microcapsules. Sheu and Rosenberg[38] reported similar results for microcapsules containing a high amount of maltodextrin. This could be related to the high drying rates, associated with large particles, that usually lead to rapid wall solidification and dent smoothing cannot occur. At the same time, the wall material (maltodextrin) has not been elastic enough, so structural changes such as cracking will happen, especially if there is extensive ballooning (Fig. 7). The core material will be lost in these situations, confirming the low retention of d-limonene (76.9%). The presence of a high amount of surface oil in these powders could be misleading. Since d-limonene is a volatile compound, it should be lost during spray drying, even if it is on the surface of particles. In other words, much of the surface oil content could have come from inside the powder particles through cracks, as we used a solvent extraction method. Therefore, reliability of surface oil data is still questionable. In order to have a better understanding of surface oil content, nondestructive analytical methods with no solvent extraction such as X-ray photoelectron spectroscopy (XPS) can be applied.

CONCLUSION

Emulsification method can influence and determine the final properties of the encapsulated powder in a number of different ways, including emulsion size, emulsion stability, powder particle size, size distributions, and other parameters, such as water activity of the spray-dried powders. Our results revealed that microfluidization was the best emulsification method to achieve minimum amounts of unencapsulated oil at the surface of particles and maximum retention of volatiles (d-limonene). The variation of results for different emulsifying devices could be well explained by the droplet size of produced emulsions. The smaller the emulsion size, the better the encapsulation efficiency. Since emulsion droplets were retained and encapsulated more efficiently in larger particles, the higher difference between emulsion size and powder size was preferred to have higher retention and lower surface oil content. Although the emulsification system can also affect
other parameters such as powder particle size, more works need to be done on the influence of each emulsification technique on the molecular structure of surface-active biopolymers and their emulsifying capabilities, because it was found that even two emulsions with the same size from two different emulsifying devices will result in different encapsulation efficiencies.

Our results showed that, in general, biopolymers such as Hi-Cap and WPC are more efficient than small molecule surfactants, as they produce more stable emulsions, and the process conditions including atomization and reconstitution have a smaller effect on their emulsion size distributions. Considering two different surface-active biopolymers, we found that Hi-Cap was better than WPC since it led to encapsulated powders with less surface oil and more retention of d-limonene. Interaction of wall materials with encapsulated cores materials is another factor that needs to be investigated.

REFERENCES


32. Teixeira, M.I.; Andrade, L.R.; Farina, M.; Rocha-Leao, M.H.M. Characterization of short chain fatty acid microcapsules produced


