2446 J. Agric. Food Chem. 2010, 58, 2446–2454
DOI:10.1021/jf903464s

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Chitosan Coating Improves Retention and Redispersibility of Freeze-Dried Flavor Oil Emulsions

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Flavor oils are often encapsulated as emulsions by drying processes such as freeze-drying or spray-drying, using mainly macromolecular emulsifiers such as gums and proteins to stabilize the emulsions during drying. The objective of the present study was to examine whether a combination of a charged small-molecule emulsifier and an oppositely charged polysaccharide adsorbed to the emulsion droplet surface can substitute commonly used encapsulation materials for the drying of flavor oil emulsions. To this end, polysaccharide-coated flavor oil emulsions were prepared by high-pressure homogenization of mixtures consisting of a flavor oil (*R*-carvone), a negatively charged citric acid ester small-molecule emulsifier (citrem), and various concentrations of a positively charged polysaccharide (chitosan) in acetate buffer at pH 4.0. Nanoemulsions with average particle diameters of ≈100 nm in the absence and ≈230−250 nm in the presence of chitosan coating were obtained. These emulsions were subsequently freeze-dried with different concentrations of maltodextrin, which served as the main encapsulation material. It was demonstrated that coating the oil droplet surface with a small amount of chitosan resulted in remarkably improved retention levels and redispersibility properties of the freeze-dried carvone emulsions. Maltodextrin content also affected both retention and redispersibility. At optimal chitosan and maltodextrin concentrations ≈95% retention levels were obtained, and the average particle sizes of freeze-dried and redispersed emulsions were ≈270−300 nm, as compared to ≈230−250 nm before freeze-drying. The results demonstrate that charged small-molecule emulsifiers used in combination with oppositely charged polymers are viable alternatives to macromolecular emulsifiers for freeze-drying of flavor oil emulsions.

KEYWORDS: Freeze-drying; spray-drying; carvone; chitosan; maltodextrin; emulsion; nanoemulsion; encapsulation; electron microscopy; retention; dry emulsion

INTRODUCTION

Carvone is a volatile flavor oil that is present in many different essential oils. It exists in both *S*- and *R*-enantiomeric forms, which smell of caraway and spearmint, respectively, and both forms are used as flavoring agents in the food industry. The spearmint variety, for instance, is often used as a flavor in chewing gum. Rather than using the liquid flavor oils, encapsulation techniques are often employed to convert the flavor oils into dry powders that may have controlled release properties or improved storage stability and are easier to handle and incorporate into food products (*1*).

Common techniques for encapsulating flavor oils involve the preparation of an emulsion of the flavor oil in an aqueous phase containing water-soluble encapsulation materials, followed by a drying process such as spray-drying or freeze-drying. In both techniques, the flavor oil becomes trapped as small droplets within a glassy matrix of encapsulation materials, which forms as the water evaporates during drying (*2*, *3*). The majority of published literature on encapsulation of flavor oils concerns spray-drying, as this technique is much more commonly employed than freeze-drying. On the basis of spray-drying studies using different emulsion preparation techniques and encapsulation materials, the successful preparation of dried flavor oil emulsions has been linked to a number of key properties of the initial emulsion as well as the encapsulation materials. With respect to emulsion properties, it has been found that stable emulsions (*4*) and emulsions with small particle sizes (*5*−*8*) generally result in improved retention levels. For this reason, good emulsifying properties are a key requirement that encapsulation materials must satisfy, whereas the formation of amorphous glassy states during dehydration is another desirable property (*2*, *9*). Among encapsulation materials, gum arabic has been most commonly used, as it possesses both emulsifying properties and good retention of volatile flavors during drying (*10*−*13*). Gum arabic is a complex mixture of polysaccharides, proteins, and glycoproteins. It consists predominantly of polysaccharide, but also contains a glycoprotein fraction believed to be responsible for its emulsifying properties (*14*). The prevailing view of the emulsifying mechanism of gum arabic is that hydrophobic protein fragments of the glycoproteins are embedded in the oil phase, whereas the hydrophilic carbohydrates extend into the

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 Published on Web 01/25/2010

water phase (*15*, *16*). However, as gum arabic is both expensive and subject to fluctuations in supply, efforts toward substituting gum arabic with alternative encapsulation materials have received considerable attention in recent years (*17*, *18*). Other materials used as flavor oil encapsulation materials include food proteins such as whey protein isolates, soy protein isolates, and caseinates (*19*−*22*) and carbohydrates such as hydrolyzed starches (e.g., maltodextrin) (*23*). Carbohydrates have poor emulsifying properties, however, and are mainly used in combination with emulsifiers or as modified starches that have been given emulsifying properties by covalent attachment of hydrophobic groups to the carbohydrate polymer backbone (*5*, *24*−*26*).

Fewer studies on freeze-dried flavor oil emulsions have been published, and the current understanding of encapsulation of flavor oil emulsions by freeze-drying consequently rests on less solid ground than is the case for spray-drying. A recent study by Kaushik and Roos (*27*) investigated the effect of wall material composition and high-pressure homogenization pressure on retention levels of freeze-dried limonene emulsions containing different blends of gelatin, sucrose, and gum arabic as encapsulation materials. Gum arabic was found to be the best of the tested encapsulation materials, as these emulsions resulted in high retention levels (up to 75%) as well as good drying properties, whereas emulsions with high gelatin contents retained high levels of limonene but collapsed during drying and were difficult to convert into a powder. Sucrose alone resulted in very low retention levels. Tobitsuka et al. (*28*) have also reported good retention levels in freeze-dried flavor oil emulsions when gum arabic was used as encapsulation material, whereas hydrophobically modified starch resulted in good retention of limonene in another recent study by Lee et al. (*29*). Furthermore, results with freeze-dried nonvolatile oil emulsions have shown improved encapsulation efficiency when using stable emulsions with smaller particle sizes (*3*). Hence, it appears that the encapsulation materials and emulsion characteristics used in spray-drying generally work well for freeze-drying, too. It must be emphasized, however, that despite the similarities with respect to dehydration, there are also important differences between freeze-drying and spray-drying, and emulsions are exposed to different stresses in the two techniques. Most importantly, spray-drying involves atomization and heating, whereas freeze-drying, on the other hand, involves freezing.

In the present study, we have investigated the potential of using a small amount of a charged polysaccharide deposited onto flavor oil droplets by electrostatic attraction to promote retention of volatile flavor oils during freeze-drying. Electrostatic deposition of polysaccharides and proteins onto the surface of nonvolatile triglyceride-based oils has previously been employed as a method for stabilizing emulsions in general and, in particular, to improve the stability toward environmental stresses such as high ionic strengths, freezing, and freeze-drying (*30*−*35*). The creation of an interfacial layer of polysaccharides electrostatically bound to small-molecule emulsifiers at the emulsion droplet surface results in a molecular arrangement that resembles that of both hydrophobically modified starches and gum arabic, which are known to be two of the best encapsulation materials for volatile flavor oils. We therefore envisioned that electrostatic deposition of polysaccharides to flavor oil emulsion droplets would result in favorable drying properties. To this end, carvone flavor oil emulsions were prepared by high-pressure homogenization of mixtures consisting of carvone, a negatively charged food grade citric acid ester small-molecule emulsifier, citrem, and the positively charged polysaccharide chitosan. Before freeze-drying, the emulsions were mixed with maltodextrin, which served as a glass-forming encapsulant and constituted the vast majority of the solids content (*36*). Retention levels and redispersibility were measured for freeze-dried emulsions containing chitosan adsorbed to the emulsion droplet interface as well as emulsions that were stabilized by the citrem emulsifier alone and did not contain a chitosan surface layer.

The aim of the present study is two-fold. At the practical level the study examines whether a combination of a small-molecule emulsifier and a polysaccharide adsorbed to the emulsion droplet surface is a viable alternative to gum arabic and other commonly used encapsulation materials for the drying of flavor oil emulsions. At the conceptual level the study examines the importance of a steric stabilization mechanism of emulsifiers for obtaining good retention and redispersion properties of dried flavor oil emulsions.

MATERIALS AND METHODS

Materials. *R*-Carvone (99.4% pure by GC) and maltodextrin with a dextrose equivalent of 20 (dextrin from maize starch, BioChemika, 20) were obtained from Sigma-Aldrich, Brøndby, Denmark. Chitosan (viscosity = 16 cP as a 1% solution in 1% acetic acid, deacetylation degree = 95%; product name Chitoclear fg95LV) was obtained from Primex ehf, Siglufjordur, Iceland. Citrem LR10 was kindly provided by Danisco A/S, Brabrand, Denmark. Citrem LR10 is produced by reacting citric acid with mono- and diglycerides made from refined sunflower oil, which results in emulsifiers that contain a citric acid headgroup linked to one or two hydrophobic fatty acid chains by ester bonds.

Preparation of Emulsions. A stock solution consisting of 16.7 wt % citrem in carvone was prepared by dissolving 1.6 g of citrem LR10 in 8 g of carvone. Another stock solution of 2 wt % chitosan in acetate buffer (100 mM, pH 4.0) was prepared by dissolving 2 g of chitosan in 98 g of acetate buffer at room temperature under magnetic stirring overnight and subsequently readjusting the pH to 4.0 with 1 M HCl. By mixing appropriate amounts of these two stock solutions with appropriate amounts of pure acetate buffer, mixtures containing carvone (5 wt %), citrem LR10 (1 wt %), and various concentrations of chitosan (0−1 wt %) were prepared in acetate buffer (100 mM, pH 4.0). The total weights of the mixtures were 40 g. Emulsions were prepared by first homogenizing the mixtures using an Ultra-Turrax T25 (IKA, Staufen, Germany) at 20000 rpm for 2 min, followed by five passes through a high-pressure homogenizer at 10000 psi (69 MPa) (Emulsiflex-C5, Avestin Inc., Ottawa, Canada).

Particle Size and Zeta Potential Measurements. Emulsion particle size and zeta potential were measured by dynamic light scattering and phase analysis light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, U.K.), respectively, using 1 mL emulsion samples diluted 100× with acetate buffer (100 mM, pH 4.0). Measurements were performed after 1, 8, and 15 days of storage. Redispersed emulsions were measured on the day of redispersion. Average particle sizes are reported as intensity-weighted mean hydrodynamic sizes (*z*-average), and particle size distributions are shown as intensity-based distributions.

Freeze-Drying and Redispersion. Three emulsion compositions were selected for freeze-drying experiments. All three compositions contained 5 wt % carvone and 1 wt % citrem, whereas the chitosan contents were 0, 0.5, and 1 wt %, respectively. Before freeze-drying, 8 g of each of these three emulsions was mixed with appropriate amounts of a 40 wt % maltodextrin stock solution and pure acetate buffer. The total amount of maltodextrin solution and acetate buffer was kept constant at 12 g, whereas the ratio of maltodextrin stock solution and acetate buffer was varied to obtain emulsions with various maltodextrin contents. In this way, a series of emulsions containing 2 wt % carvone, 0.4 wt % citrem, and various concentrations of chitosan (0, 0.2, and 0.4 wt %) and maltodextrin (0, 2, 5, 8, 10, and 12 wt %) in acetate buffer (100 mM, pH 4.0) were prepared. Samples of these emulsions (5 mL) were transferred to 30 mL glass bottles and frozen at −80 °C, followed by drying for 24 h in a laboratory-scale freeze-dryer (Benchtop 4K ZL, Virtis, Gardiner, NY), with a condenser temperature of −100 °C and a chamber pressure of 10 mTorr (1.33 Pa). The freeze-dried emulsions were ground to coarse powders with a spatula, and these powders were used for retention,

redispersion, and electron microscopy studies. The freeze-dried emulsion powders were redispersed by addition of acetate buffer (100 mM, pH 4.0) to a weighed amount of powder (≈30−70 mg). The amount of buffer added was proportional to the amount necessary to achieve the same volume as before freeze-drying if all of the powder had been used for redispersion. For example, if half the freeze-dried powder obtained from 5 mL of emulsion was used for redispersion, then 2.5 mL of buffer would be added.

Retention Measurements. Retention of carvone was determined by dispersing approximately 30 mg of freeze-dried powder in 5 mL of Milli-Q H_2O in capped glass vials. Ten milliliters of heptane was added and the mixture heated at 70 °C for 10 min under vigorous stirring to extract carvone into the heptane phase. The UV absorbance of carvone in the heptane phase was measured at 236 nm (after appropriate dilution to achieve absorbance values lower than 1.0), and the amount of extracted carvone was calculated from a standard curve. Carvone was also extracted from samples of the wet emulsions before freeze-drying by subjecting 0.5 g of these emulsions to the same extraction procedure. The carvone contents before freeze-drying were between 86 and 95% of the amount of added carvone, with the lowest values obtained from emulsions that did not contain the chitosan coating. This indicates that some evaporation occurs during the emulsion preparation process, especially for the uncoated emulsions. To correct for evaporation during emulsion preparation, the retention was calculated as the amount of carvone in the freeze-dried powder relative to the amount of carvone in the emulsion before freeze-drying:

$$\text{retention (\%)} = 100\% \times \frac{\text{carvone in powder}}{\text{carvone in emulsion before freeze-drying}}$$

Electron Microscopy. A small amount of freeze-dried powder was spread onto the surface of an aluminum stub and excess material removed using dry compressed air. The sample was then sputter-coated with a thin gold layer in a Polaron SC7640 sputtercoater. The aluminum stub containing the gold-coated sample was then placed in a Zeiss FEG-SEM Ultra 55 scanning electron microscope and imaged using 5 kV accelerating voltage.

RESULTS AND DISCUSSION

Preparation of Chitosan-Coated Emulsions. The objective of the present study was to examine the potential of using a small amount of charged polymer deposited onto flavor oil droplets by electrostatic attraction to improve retention of volatile flavor oils during freeze-drying. *R*-Carvone was selected as the volatile flavor oil, citrem as the small-molecule emulsifier, and chitosan as the polymer. The molecular structures of *R*-carvone, chitosan, and citrem are depicted in panels **a**, **b**, and **c**, respectively, of **Figure 1**. Citrem emulsifiers are produced by reacting mono- and diglycerides with citric acid, which results in emulsifiers that contain a citric acid headgroup linked to one or two hydrophobic fatty acid chains. The first and second pK_a values of citric acid are 3.1 and 4.8, respectively, and citrem is therefore negatively charged at basic and moderately acidic pH values. Chitosan, on the other hand, contains amino groups with pK_a values in the 6.3−7.0 range and is therefore positively charged at acidic pH values. **Figure 1d** shows the zeta potential for chitosan solutions and citrem-stabilized carvone emulsions as a function of pH. As expected, the zeta potential graph illustrates that chitosan and citrem-stabilized emulsions carry opposite charges at moderately acidic pH values. In this pH range, it should therefore be possible to prepare chitosan-coated carvone emulsions by electrostatic deposition of positively charged chitosan onto the droplet surface of negatively charged citrem-stabilized carvone emulsions.

Chitosan-coated carvone emulsions were prepared by high-pressure homogenization of carvone, citrem, and chitosan mixtures. The emulsions were characterized by measuring the zeta potential and particle size and by evaluating the visual appearance after storage, as shown in **Figure 2**. The zeta potential

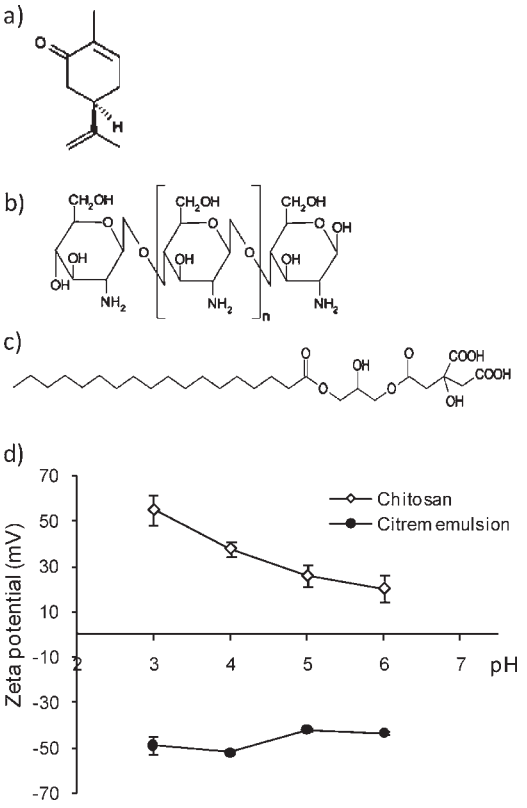

Figure 1. (**a**) Molecular structure of *R*-carvone. (**b**) Molecular structure of chitosan (100% deacetylated). At acidic pH values the amino groups are positively charged. (**c**) Representative molecular structure of citrem LR10, shown here with a single saturated C18 fatty acid chain. However, citrem LR10 consists of a mixture of citric acid esters containing both one and two fatty acid chains of different lengths and degrees of unsaturation. (**d**) Zeta potential as a function of pH for 0.02 wt % chitosan solutions and 5 wt % carvone emulsions containing 1 wt % citrem LR10, both in 100 mM acetate buffer (mean ± SD, $n = 2$). The carvone emulsions were diluted 100× in acetate buffer before the measurements.

changed from a negative value of ≈−52 mV for carvone emulsions containing citrem alone to a positive value of ≈46 mV when the chitosan concentration was increased from 0 to 1 wt % (**Figure 2a**). At approximately 0.25−0.50 wt % chitosan, the zeta potential reaches a plateau and only a marginal increase in zeta potential occurs when the chitosan concentration is increased further. The increase in zeta potential with chitosan concentration indicates that the carvone emulsion droplets become progressively more coated with chitosan until reaching saturation around 0.25−0.50 wt % chitosan. Similar findings have been reported in other studies on chitosan-coated emulsions based on nonvolatile oils and different emulsifiers (*35*, *37*). The zeta potential did not depend significantly on storage time.

The emulsion stability was evaluated by particle size measurements and by visual inspection of the emulsions after different storage times at room temperature. The particle size measurements (**Figure 2b**) showed that emulsions containing citrem but no chitosan had an average particle diameter of ∼100 nm, which apparently did not change significantly over the 15 day storage period. However, visual inspection revealed a small degree of oiling off after 8 and 15 days of storage for the emulsion that did not contain chitosan, indicating that this emulsion was not completely stable toward droplet coalescence, although undetected by the particle size measurements. At low chitosan concentrations (0.02−0.04 wt %) the emulsions were highly unstable to the extent that it was not possible to obtain reliable particle size

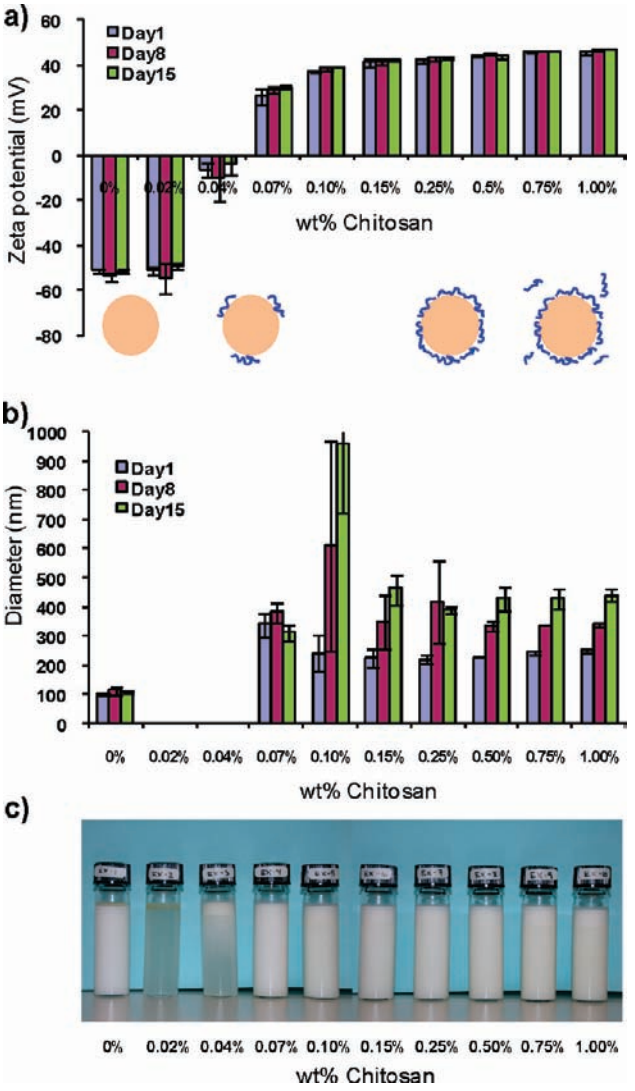

Figure 2. (**a**) Zeta potential of 5 wt % carvone emulsions containing 1 wt % citrem and increasing concentrations of chitosan (mean ± SD, $n = 2$). The emulsions were measured at 1, 8, and 15 days after preparation and diluted 100× before measurements. The zeta potential changes from negative to positive values as the chitosan concentration increases. This indicates that the positively charged chitosan adsorbs to the chitosan surface, as shown schematically in the drawing below the zeta potential graph. (**b**) Average particle size of the same emulsions (mean ± SD, $n = 2$). At 0.02 and 0.04 wt % chitosan, the emulsions were too unstable to obtain reliable particle size measurements and are therefore omitted. At higher chitosan concentrations, the particle size measurements indicate an increase in emulsion stability until reaching 0.50 wt % chitosan, whereupon the emulsion stability is similar for 0.75 and 1.0 wt % chitosan. (**c**) Photograph of the emulsions after 15 days of storage at room temperature. The instability of the emulsions containing 0.02 and 0.04% chitosan is clearly visible.

measurements by dynamic light scattering. The instability of these emulsions is also clearly visible in the photograph shown in **Figure 2c** and is most likely related to the fact that the net charge at these chitosan concentrations is close to neutral because the oil droplets are only partially covered by chitosan. The electrostatic repulsion between oil droplets is hereby lost and the emulsions become unstable. Bridging flocculation caused by individual chitosan molecules binding to more than one oil droplet may also play a role in destabilizing these emulsions (*35*, *38*). Higher chitosan concentrations increased the emulsion stability. At 0.5 wt % chitosan no additional improvement in emulsion stability was achieved when the concentration was increased to 0.75 and 1.0 wt % chitosan, as indicated by similar visual appearances and similar changes in particle size during storage.

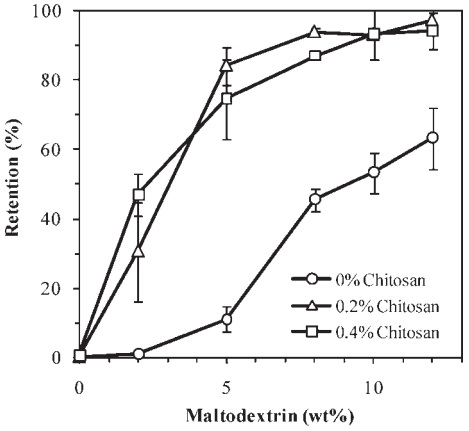

Figure 3. Retention of carvone in emulsions consisting of 2 wt % carvone, 0.4 wt % citrem, and different chitosan and maltodextrin concentrations (mean ± SD, $n = 3$). It is evident that coating the emulsions droplet surface with chitosan improves retention significantly. Chitosan in excess of the amount needed to coat the droplet surface, as is the case for the 0.4 wt % emulsions, did not result in notable further improvements of retention.

Retention of Carvone during Freeze-Drying. On the basis of the zeta potential and emulsion stability data in **Figure 2**, three different emulsions were selected to test the hypothesis that coating the surface of volatile oil droplets with a polysaccharide layer increases retention upon freeze-drying, namely, the emulsions containing 0, 0.5, and 1.0 wt % chitosan. The 0 wt % chitosan emulsion represents an emulsion that is fairly stable but does not contain a polysaccharide layer, whereas the 0.5 wt % emulsion represents an emulsion with a polysaccharide surface coating. To examine whether unadsorbed chitosan in the water phase affected retention of carvone during freeze-drying, the emulsion containing 1.0 wt % chitosan was included as a surface-coated emulsion that was equally stable as the 0.5 wt % chitosan emulsion, but also contained a larger excess of unadsorbed chitosan. In the case that surface coating of the oil droplets was the important parameter affecting carvone retention during freeze-drying, one would expect the 0.5 and 1.0 wt % emulsions to give similar retention results, whereas retention would be different if factors relating to chitosan present in the water phase were important.

Before freeze-drying, the three emulsions were mixed with a maltodextrin stock solution to obtain emulsions containing various concentrations of chitosan (0−0.4 wt %) and maltodextrin (0−12 wt %). The dilution resulting from mixing in maltodextrin means that the emulsions containing 0, 0.5, and 1.0 wt % chitosan before mixing correspond to 0, 0.2, and 0.4 wt % chitosan after mixing. The dilution does not change the amount of chitosan per emulsion droplet, however, as the carvone content is diluted by the same factor. The emulsions were freeze-dried, and the retention of carvone in the freeze-dried powder was analyzed as shown in **Figure 3**.

The results show that 0.2 wt % chitosan significantly improved retention as compared to emulsions not containing chitosan. However, doubling the chitosan concentration to 0.4 wt % did not result in significant additional increases in retention, and the improved retention can therefore be ascribed to the creation of an interfacial layer of chitosan around the oil droplets. The increased retention for chitosan-coated emulsions as compared to uncoated

emulsions was largest at low maltodextrin concentrations and became less pronounced as the maltodextrin concentration was increased. At 5 wt % maltodextrin, for example, high retention values of 84.1 ± 5.4 and 74.5 ± 11.6% were obtained for emulsions containing 0.2 and 0.4 wt % chitosan, respectively, whereas it was only 11.2 ± 3.9% for emulsions not containing chitosan. At 12 wt % maltodextrin, on the other hand, the retention of uncoated emulsions was considerably higher at 63.3 ± 8.9%, whereas the retentions of 0.2 and 0.4 wt % chitosan containing emulsions were 97.2 ± 4.1 and 94.1 ± 5.4%, respectively. This shows that it is possible to retain appreciable amounts of carvone emulsions stabilized by citrem alone, provided that the maltodextrin content is high, but a small amount of chitosan deposited onto the droplet surface significantly lowers the amount of maltodextrin needed to achieve acceptable retention levels.

A recent spray-drying study by Jafari et al. (*39*) using maltodextrin as wall material compared the retention of limonene emulsions stabilized by the small-molecule emulsifier, Tween 20, to the retention obtained when the macromolecular emulsifier whey protein concentrate or modified starch was used to stabilize the emulsions. These researchers found that Tween 20 resulted in lower encapsulation efficiencies than both whey protein concentrate and modified starch despite the fact that Tween 20 produced emulsions with smaller particle sizes and suggested that the poor encapsulation properties were related to the lack of film-forming properties of Tween 20, resulting in a lower stability against changes occurring to the emulsions during atomization and drying. The lower retention levels for the emulsions not containing a chitosan coating that we observe in the present freeze-drying study are in agreement with the spray-drying study by Jafari et al. (*39*) and demonstrate that the formation of an interfacial coating around the emulsion droplets improves the retention significantly. Exact retention levels are shown in Table S1 of the Supporting Information.

Structure of the Freeze-Dried Powder. To evaluate the microstructure of the freeze-dried emulsions, they were ground into powders and analyzed by scanning electron microscopy. No powder was obtained for the sample containing only carvone and citrem, whereas sheets containing microscopic holes were obtained for the chitosan-coated emulsions that did not contain any maltodextrin (Figure S1 of the Supporting Information). As none of these preparations retained any carvone, they will not be considered any further.

In the case of emulsions containing maltodextrin, white and brittle powders were obtained, with macroscopic appearances as shown in the photograph in **Figure 4a**, and microscopic appearances as shown in the representative electron microscopy images in panels **b** and **c** of **Figure 4** for a freeze-dried emulsion containing 0.2 wt % chitosan and 5 wt % maltodextrin. **Figure 4b** is a low-magnification electron microscopy image showing a flaky powder, and is similar to other reported images of freeze-dried flavor oils (*27*). **Figure 4c** is a higher magnification image focusing on edges where the flakes were broken when the sample was ground to a powder and gives insight into the interior microstructure of the powder flakes. A large number of dark round features are visible within the powder flakes, most likely reflecting encapsulated carvone oil droplets in a maltodextrin matrix. To be precise, the round features correspond to holes where carvone droplets used to be, as the SEM images are obtained under vacuum, which causes evaporation of the exposed oil droplets at the interface.

Whereas the overall powder appearance was similar for all powders containing maltodextrin, there were distinct differences with respect to the size and distribution of the encapsulated oil

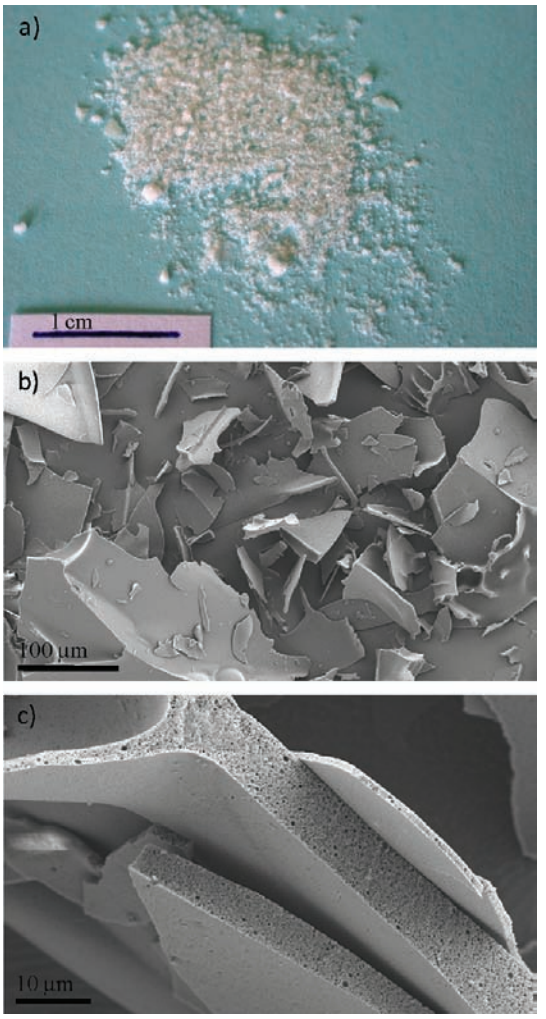

Figure 4. (**a**) Photograph of ground freeze-dried chitosan-coated carvone emulsion consisting of 2 wt % carvone, 1 wt % citrem, 0.2 wt % chitosan, and 5 wt % maltodextrin. (**b**) SEM micrograph of the same powder sample showing a flaky appearance, which is characteristic of freeze-dried emulsions. (**c**) Higher magnification micrograph focusing on the edge of the powder flakes, where the interior structure is visible. Several dark round features corresponding to encapsulated carvone oil droplets are visible in the powder interior.

droplets within the powders. **Figure 5** shows high-magnification electron microscopy images of the interior structure of freeze-dried carvone emulsions containing different amounts of chitosan and maltodextrin. A number of conclusions can be drawn from these images. With respect to chitosan concentration, the most striking observation is that the interior droplet sizes are markedly larger than the sizes measured before drying (≈100 nm; **Figure 2b**) for the emulsions not containing chitosan (top row). In the presence of chitosan (middle and bottom rows), in contrast, the droplet sizes are quite close to the sizes measured before drying (≈240 nm; **Figure 2b**), especially for the emulsions containing 10 wt % maltodextrin. This suggests that substantial droplet coalescence occurs during the freeze-drying process in the absence of chitosan coating. Both the uncoated and the chitosan-coated emulsions carry surface charges (see **Figure 2a**) and are thereby stabilized by electrostatic repulsion. However, the chitosan coating confers additional steric stabilization to the oil droplets, and it seems likely that this steric stabilization is essential for protection against coalescence when the oil droplets are brought into close contact during freeze-drying, whereas steric stabilization is less

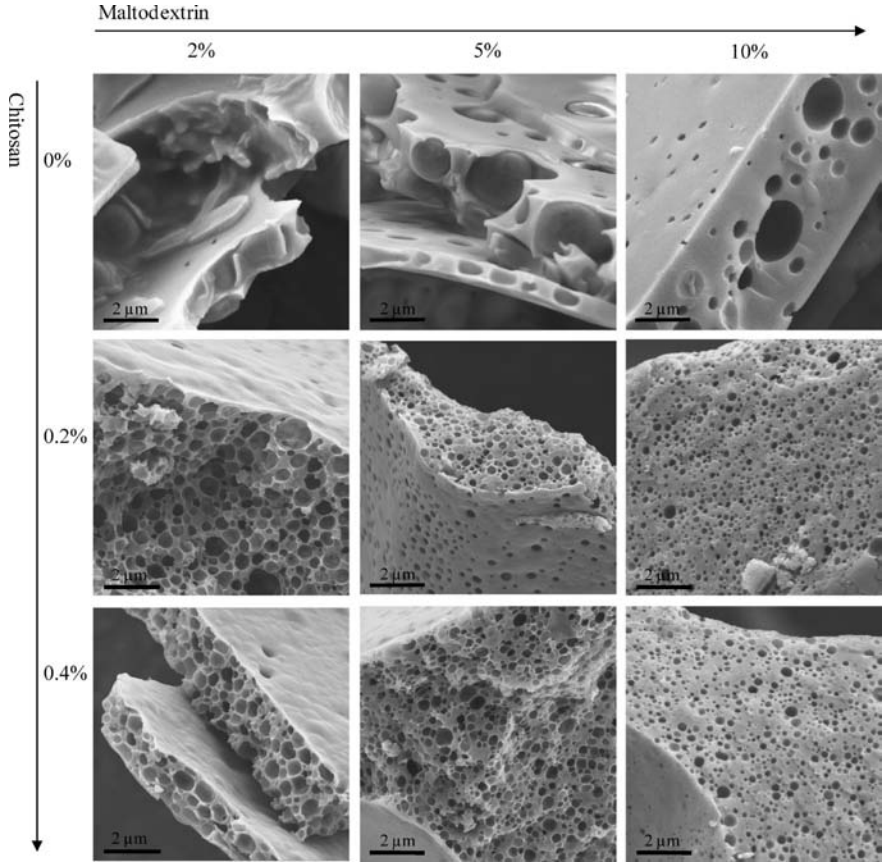

Figure 5. SEM micrographs showing the interior structure of freeze-dried chitosan-coated carvone emulsions containing different amounts of chitosan and maltodextrin. In the absence of chitosan (top row), the interior droplet sizes are much larger than for the chitosan-coated emulsions (middle and bottom rows), indicating that substantial droplet coalescence occurs during the freeze-drying process in the absence of chitosan coating. Increasing the maltodextrin concentration results in a lower density of carvone droplets within the maltodextrin matrix.

important in the wet state. It is also interesting to compare the interior structures of the freeze-dried emulsions containing 0.2 wt % chitosan (middle row) to the emulsions containing 0.4 wt % (bottom row). No clear differences between the two levels of chitosan are seen, which indicates that the stabilizing effect is related to the chitosan coating at the droplet surface, whereas excess chitosan in the water phase does not provide further improvement of the freeze-drying stability.

With respect to maltodextrin content, it is evident that the density of droplets within the maltodextrin matrix is lower at high maltodextrin contents than at lower contents. At 2 wt % maltodextrin (left column), for example, the droplets are located very close to one another with only a small amount of maltodextrin between droplets, whereas the spacing between encapsulated droplets is much higher at 10 wt % maltodextrin (right column). Furthermore, the droplet sizes within the powder are noticeably larger at low maltodextrin concentrations than at higher concentrations for both chitosan-coated and uncoated emulsions. In the absence of a chitosan coating (top row) several submicrometer-sized droplets are visible in the sample containing 10 wt % maltodextrin, whereas the droplet size is generally larger at 5 wt % maltodextrin. In fact, at 5 wt % maltodextrin the droplet size is in many cases larger than the thickness of the freeze-dried powder flakes, which may explain the low retention levels measured for this sample (see **Figure 3**), as these droplets evaporate during drying. At 2 wt % maltodextrin no encapsulated droplets are seen in the absence of chitosan coating, indicating that all of the oil has evaporated, in accordance with the close to 0% retention level measured for this sample. As far as the chitosan-coated emulsions are concerned (middle and bottom rows), the droplet sizes are also larger at lower maltodextrin contents, although the difference is less pronounced than for the uncoated emulsions.

Overall, the SEM images suggest that the steric stabilization effect provided by chitosan coating is essential for avoiding droplet coalescence during the freeze-drying process, which, apart from better preserving the droplet size of the original emulsion, results in significantly improved retention levels. Moreover, the images show that maltodextrin also plays a role in preventing droplet coalescence during freeze-drying.

Redispersion of Emulsions. It is often desirable to obtain dry emulsions that are redispersible so that the original emulsion is restored upon the addition of water or buffer. To examine redispersibility, the freeze-dried emulsion powders were redispersed in acetate buffer to the same volumes as before drying, and the particle sizes and zeta potentials of the redispersed emulsions were measured. In parallel, emulsion samples were frozen and rethawed without being subjected to the drying stage. This experiment was done to discriminate between instability arising from the freezing stage or the drying stage of the freeze-drying process.

Figure 6 shows particle size distributions of the original emulsions, the frozen and rethawed emulsions, and the freeze-dried and redispersed emulsions for different chitosan and maltodextrin concentrations (average particle sizes are shown in Table S2 of the Supporting Information). The zeta potentials after freeze-thawing and freeze-drying were also measured and found to be practically identical to the values obtained for the original emulsions, showing that the chitosan coating is retained during freeze-drying and redispersion (Figure S2 and Table S2 of the Supporting Information). In some cases,

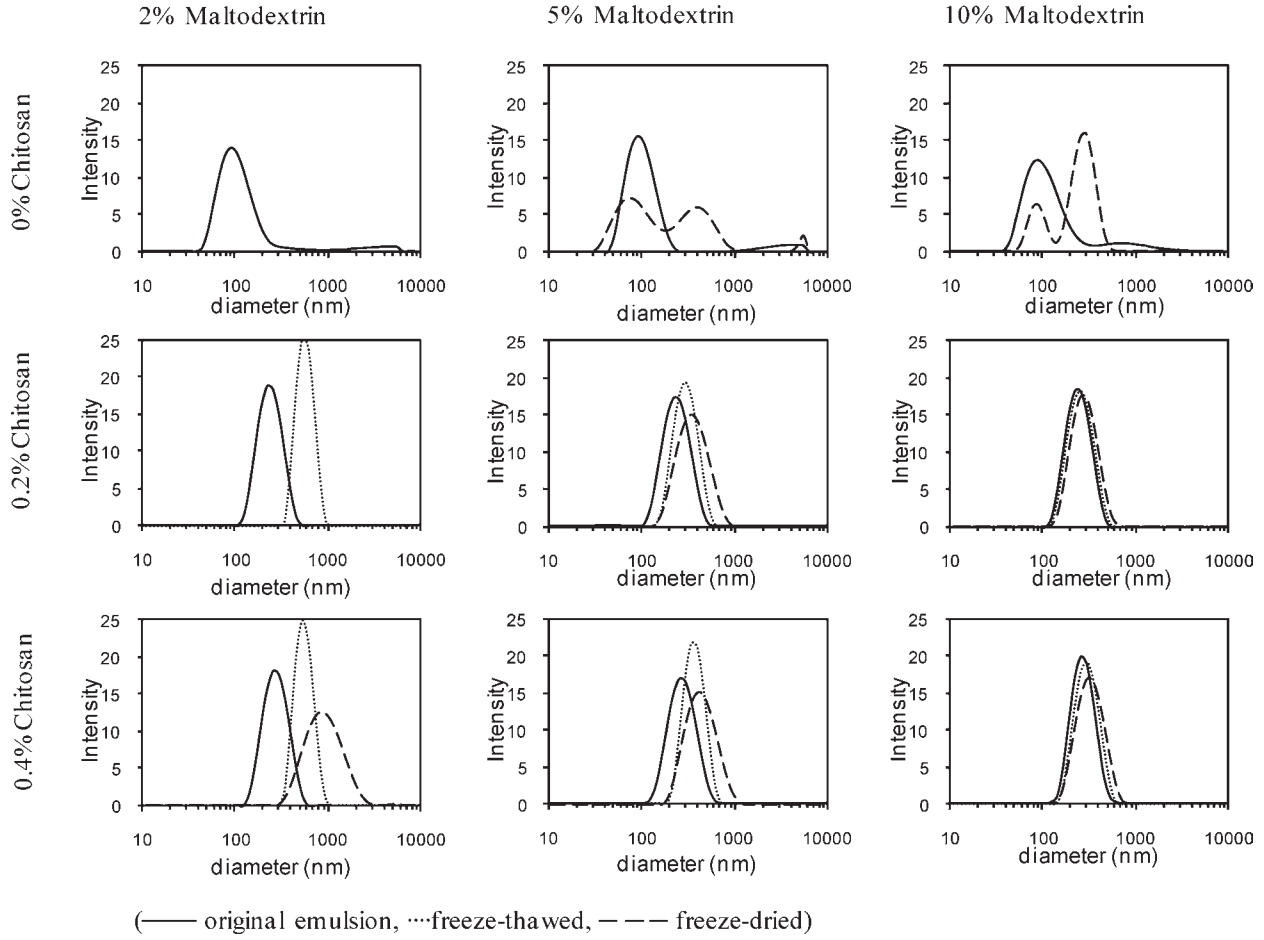

Figure 6. Particle size distributions of original emulsions (—), freeze-thawed emulsions (· · ·), and redispersed freeze-dried emulsions (— — —), containing different amounts of chitosan and maltodextrin. In cases where reliable size measurements could not be obtained, the size distributions are omitted.

particle sizes of the emulsions obtained after freeze-thawing or freeze-drying could not be measured reliably by dynamic light scattering, and these size distributions are therefore not shown in **Figure 6**. This typically indicates that the samples contain emulsion droplets larger than 4−6 μm, which is the approximate upper cutoff for measuring emulsion particle sizes by dynamic light scattering. In general, the size distributions obtained for the freeze-dried emulsions, as well as the cases when sizes were most likely too large to be measured, appear to be in good agreement with the electron microscopy images in **Figure 5**.

In the absence of a chitosan coating (top row) the emulsions were highly unstable to freeze-thawing and it was not possible to obtain reliable dynamic light scattering measurements for any of these emulsions, which was also the case for the freeze-dried sample that contained only 2 wt % maltodextrin. At 5 and 10 wt %, the emulsions could be measured after freeze-drying, but the size distributions changed significantly toward larger particle sizes than before freeze-drying. This shows that droplet coalescence has occurred during freeze-drying and agrees with the electron microscopy observations in **Figure 5**. It may seem surprising that the freeze-dried emulsions could be measured although the freeze-thawed emulsions could not, because freeze-drying can be considered to be a harsher treatment than freeze-thawing. However, as the retention measurements showed (**Figure 3**), a large percentage of carvone evaporates during freeze-drying, which means that only a fraction of the original emulsion is left for measurement in the freeze-dried and redispersed sample. This is not the case for the freeze-thawed sample in which the entire carvone content remains in the sample that is measured after thawing. We therefore speculate that large carvone droplets are formed by droplet coalescence during freezing in the emulsions not containing chitosan but that it is predominantly these large droplets that evaporate during the drying step, whereas smaller droplets are retained.

For the chitosan-coated emulsions (middle and bottom rows) all emulsions were sufficiently stable for dynamic light scattering measurement except for the emulsion containing 0.2% chitosan and 2% maltodextrin, which was not measurable after freeze-drying and redispersion. Apart from that difference, the results obtained with 0.2 and 0.4 wt % chitosan were almost identical, and chitosan-coated emulsions are clearly much more stable toward freeze-thawing and freeze-drying than the emulsions not containing chitosan. In terms of maltodextrin content, it is evident that maltodextrin also has a stabilizing effect toward both freeze-thawing and freeze-drying, as the size distributions of both the freeze-thawed and freeze-dried emulsions become increasingly more similar to that of the original emulsion as the maltodextrin concentration is increased. At 10 wt % maltodextrin, the size distributions of the freeze-dried and redispersed emulsions are highly similar to those of the original emulsions, showing that very good redispersibility can be achieved by selecting appropriate amounts of chitosan and maltodextrin.

It is interesting to note that for chitosan-coated emulsions the size distributions of the freeze-thawed emulsions are consistently

located between those of the original emulsions and the freeze-dried and redispersed emulsions. This demonstrates that both the freezing step and the drying step contribute to changes in emulsion droplet sizes of freeze-dried flavor oil emulsions. The fact that the chitosan coating confers stability toward the drying step also suggests that electrostatic deposition of charged polymers onto emulsion oil droplets may be a promising method for obtaining good retention and redispersibility properties of spray-dried emulsions, too.

Probable Mechanisms for Improved Retention and Redispersibility of Chitosan-Coated Emulsions. Several mechanisms may be involved in the increased stability and retention of freeze-dried chitosan-coated emulsions. During the freezing step, the formation of ice crystals divides the emulsion into two phases consisting of ice crystals and an unfrozen matrix phase of dispersed emulsion droplets and dissolved maltodextrin (*40*,*41*). As the ice crystals grow by recruiting water molecules from the unfrozen phase, the volume of the unfrozen matrix phase decreases. This forces the emulsion droplets into close proximity and increases the rate of droplet coalescence. Likewise, the buffer ions are also excluded from the ice crystals and concentrated in the unfrozen phase, causing the ionic strength of the matrix phase to increase. At high ionic strength the electrostatic repulsion between the charged emulsion droplets is reduced because the charges are shielded by the ions, and this further increases droplet coalescence. It has also been suggested that crystallization of either the water phase or the oil droplets generates crystals that disrupt the emulsifier structure on the droplet surface whereby droplet coalescence increases (*30*, *42*). Furthermore, the freezing-induced decrease in water content of the matrix phase dehydrates the droplet surfaces, which may be an additional factor that destabilizes emulsions during the freezing step. Together, all of these stresses promote droplet coalescence during the freezing step. The superior preservation of emulsion droplet size observed for the chitosan-coated emulsions suggests that the steric stabilization mechanism that the chitosan polymer coating confers to the emulsion in addition to electrostatic stabilization is essential for avoiding substantial droplet coalescence during freezing.

As outlined above, ice crystal formation and growth during freezing result in a progressive increase in the concentration of solutes in the unfrozen matrix phase. Depending on the freezing temperature and the nature and concentration of solutes, the matrix phase may either remain as an unfrozen phase in coexistence with the ice phase or vitrification of the matrix phase may occur, whereby the emulsion droplets become trapped in the vitrified glassy matrix (*40*, *43*). In either case, higher solute concentrations will result in larger volumes of the matrix phase compared to the volume of the ice phase. In the present study, this means that the volume of the matrix phase coexisting with the ice phase increases as the maltodextrin concentration is increased. At high maltodextrin concentrations the droplets are consequently less densely packed in the matrix phase, which is a likely reason why maltodextrin protects against droplet coalescence during the freezing step.

The fact that chitosan coating and addition of maltodextrin protect against droplet coalescence during freeze-drying does not necessarily explain why this also results in improved retention. However, a link between reduced droplet coalescence and improved retention can be rationalized by considering the events that occur during drying (*40*). During the drying step, most of the water of the frozen emulsions disappears by sublimation of the ice crystals. This leaves open pores where the ice crystals used to be, and subsequent removal of the water in the matrix phase takes place by evaporation from the pore walls. Emulsion droplets that are located adjacent to the ice crystals will be exposed to the pores and evaporate after the ice crystals disappear. Larger sized emulsion droplets have a higher probability of being located next to ice crystals, and as droplet coalescence increases the droplet size, it is therefore not surprising that the protection from droplet coalescence provided by chitosan coating and maltodextrin addition results in improved retention levels. Furthermore, as the water in the matrix phase evaporates from the pore walls, the emulsion droplets trapped within the matrix are forced into closer proximity, which may explain why droplet coalescence is observed for the drying step as well.

In conclusion, this study has demonstrated that coating the surface of carvone flavor oil droplets with a small amount of chitosan protects the emulsions against droplet coalescence during freeze-drying and results in significantly improved retention levels when freeze-dried with maltodextrin as a glass-forming encapsulation material. In addition, it was shown that increasing the maltodextrin content also improved both retention and redispersibility and that the protective effects of chitosan and maltodextrin were related to both the freezing step and the drying step of the freeze-drying process. Thus, the principle of stabilizing emulsions with a charged small-molecule emulsifier in combination with an oppositely charged polymer has been proven to be a viable alternative to macromolecular emulsifiers for freeze-drying of flavor oil emulsions, and the importance of using emulsifiers with a steric stabilization mechanism that protects the oil droplets against coalescence during drying has been demonstrated.

ACKNOWLEDGMENT

We thank laboratory technicians Pia Friis Jensen and Kathrine Bjørneboe for assistance with preparation of emulsions and acquisition of electron microscopy images, respectively.

Supporting Information Available: Tables with exact retention values corresponding to **Figure 3** and average particle sizes corresponding to **Figure 6**. Figure with zeta potential distributions of original, freeze-thawed, and freeze-dried and redispersed emulsions. Additional electron microscopy images of freeze-dried chitosan coated emulsions without maltodextrin. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review October 2, 2009. Revised manuscript received December 16, 2009. Accepted December 31, 2009. This work was supported by a grant to the Innovation Consortium 'BioEnCap' from the Danish Ministry of Science Technology and Innovation. We thank Tina Ahmt and Anne Maria Hansen for obtaining funding for the work.