Using plant macromolecules to produce adjustable microcapsules by spray-drying

A. Gharsallaoui, O. Chambin and R. Saurel
ENSBANA-Dijon, France (remi.saurel@u-bourgogne.fr)

Introduction

The production of foods often requires the addition of functional ingredients that control flavor, color or texture properties. Microencapsulation is a useful tool to improve the delivery of sensory and bioactive compounds into foods, particularly flavors, minerals, fatty acids, vitamins and antioxidants. So, the main challenge of microencapsulation is to preserve stability of the encapsulated ingredients during processing and storage, and to release these ingredients at given physicochemical conditions. Conventional oil-in-water emulsions should be considered as the more important delivery system of lipophilic molecules because of their relative ease of preparation and low cost. However, these conventional emulsions generally suffer from physical instability when exposed to environmental stresses such as heating, chilling, freezing, drying, pH or ionic strength variation. At present there is a lack of natural emulsifiers that can be used to stabilize emulsified food products against environmental stresses occuring during manufacture, storage, transport and utilization. One strategy that has been proved particularly effective is to create a coating around oil droplets that consists of multiple layers of emulsifiers and/or polyelectrolytes using a layer-by-layer (LBL) electrostatic deposition technique (*Guzey, 2006*). The polyelectrolytes used in food products may be either proteins and/or ionic polysaccharides. The LBL technology can be used to create emulsion-based products that have better resistance to the environmental stresses. The following step in the microencapsulation process is to transform multilayered emulsion into dry powder, which can be done by spray-drying. Spray-drying is the most common technique applied in the food industry for the microencapsulation of functional ingredients because of its good productivity and low cost. Dry emulsions are powders from which an oil-in-water emulsion can easily be reconstituted when exposed to an aqueous medium. Dry emulsions are prepared by drying liquid oil-in-water emulsions containing a soluble solid carrier in the aqueous phase. Carbohydrates such as maltodextrins, starches, corn syrup solids, and acacia gums have been widely used as encapsulating agents. By drying, the aqueous phase is removed causing the solid carrier to encapsulate the dispersed lipid phase where a bioactive component could be added (*Gharsallaoui, 2007*). Such encapsulation provides several advantages in further handling of the dry material such as the minimization of the lipophilic compound oxidation and the packaging and storage requirements. There are a lot of attempts to identify novel emulsifiers and encapsulating agents or to improve effectiveness of the existing ones in order to improve their emulsification and microencapsulation properties.

In order to produce improved dry emulsion form, the work investigate the effect of multilayered matrix of pea (*Pisum sativum L.*) protein, pectin and maltodextrins on the pre-drying emulsion properties and on the ability of the spray-dried microcapsules to be reconstituted into stable oil-in-water emulsion. The plant macromolecules used provide alternative encapsulating agents to animal proteins such as gelatin and milk proteins or to gum Arabic for microencapsulation of food ingredients and drugs.

Material and methods

Materials. Powdered pea protein isolate and maltodextrin (DE 28) were obtained from Roquette-frères SA, (Lestrem, France). As measured by Kjeldahl method, the total protein content of the pea protein powder was 84.4% (with globulin fraction up to 95% as stated by the manufacturer) and the moisture content was below 8%. Miglyol 812 Neutraloel, a triglyceride containing 50-65% of caprylic acid ($C_{8:0}$) and 30-45% capric acid ($C_{10:0}$), was obtained from Sasol (Germany GmbH). Analytical grade imidazole ($C_3H_4N_2$), acetic acid, sodium azide (NaN_3), sodium hydroxide (NaOH), hydrochloric acid (HCl), and pectin (DE 60) were purchased from Sigma Chemical Co (Germany). Distilled and deionized water was used for the preparation of all solutions and emulsions.

Emulsion preparation. An aqueous emulsifier solution containing 0.558 wt% pea protein isolate was prepared by dispersing powdered pea protein isolate into imidazole/acetate buffer (5mM, pH 2.4) containing 0.044 wt% NaN_3 (as an antimicrobial agent). This protein solution was then stirred for at least 6 h to ensure complete hydration of the protein, and the pH was adjusted if necessary. 10 wt% Miglyol 812N was then blended with 90 wt% aqueous emulsifier solution using an Ultra-Turrax T25 high-speed blender (IKA, Staufen, Germany) operated at 17500 rpm for 90 s. The resultant pre-emulsion (10 wt% oil, 0.5 wt% protein isolate, and 0.04 wt% NaN_3) was further homogenized at 500 bar with three recirculations using a high pressure homogenizer (Niro Soavi NS 1001 L, Parma Italy). Pectin solution (0.5 wt%, imidazole/acetate buffer (5mM, pH 2.4)) was slowly added to the primary emulsion to give secondary emulsion (5 wt% oil, 0.25 wt protein, 0.02 wt% NaN_3) with different pectin concentrations (0-0.25 wt%).

Particle size measurement. Emulsion particle size distributions were measured by a laser diffraction instrument (Malvern Mastersizer S, Malvern Instruments, Worcs., UK). To avoid multiple scattering effects, the emulsions were diluted with imidazole/acetate buffer (pH 2.4 and pH 7) prior to making the measurements. The emulsions were stirred continuously throughout the measurement to ensure the samples were homogeneous. The particle diameter was calculated from three injections of three separate samples with two reading per sample.

Zeta potential measurement. The electrical charge (ξ-potential) on the oil droplets was measured using a particle electrophoresis instrument (Zetacompact, CAD Instruments, France). The ξ-potential is determined by measuring the direction and velocity of droplet movement in the applied electric field. The oil-in-water emulsions were diluted to a droplet concentration of approximately 0.015 wt% oil with imidazole/acetate buffer 5 mM adjusted to the suitable pH prior to measurements. The diluted emulsions were mixed thoroughly and then injected into the measurement chamber of a particle electrophoresis instrument. The ξ-potential measurements are reported as the average and standard deviation of measurements made on three freshly prepared samples, with three readings made per sample.

Spray-drying. The emulsions (5 %wt oil, 0.25 %wt protein, 0.02 %wt NaN3, with or without 0.2 %wt pectin, 11 %wt maltodextrin DE 28) were dried in a laboratory scale spray-drier equipped with a 0.5 mm nozzle atomizer (Mini spray-dryer B-290, BUCHI, Switzerland). Emulsions were pumped to the spray-drier at room temperature and dried at an inlet temperature of 180 °C and an outlet temperature of 90 °C. The dried powder was collected and stored in airtight containers at 4 °C.

Scanning electron microscopy. Scanning electron microscopy was used to study the surface and internal structures of the spray-dried powders. Powder particles were attached to a sample stub with double-sided sticky tape and fractured with a scalpel (Swann Morton, England B. S). The specimens were sputter coated with gold using a ION sputter coater JFC-1100 (JEOL FINE COAT) and examined using a Philips scanning electron microscope (FW 6800/70) at an accelerating voltage of 10 or 15 kV.

Emulsion reconstitution and stability measurement. Dry emulsion powder was weighted and mixed with imidazole/acetate buffer (5 mM, pH 2.4) to obtain reconstituted emulsion with the same dry matter as before drying. After 1 h of rotation at approximately 20 rpm, samples were withdrawn for stability measurement. Reconstituted emulsions were transferred into a cylindrical glass test tube (internal diameter 15 mm, height 180 mm) until an emulsion height of 135 mm, tightly sealed

with a plastic cap, and then stored at room temperature. The extent of creaming was characterized by a creaming index (CI) that represents the serum layer formed at the bottom of the tubes expressed as a percentage of the total volume of emulsion in the tube. The creaming index provided indirect information about the extent of droplet aggregation in an emulsion: the higher the creaming index, the greater the aggregation. Measurements were carried out on three separate samples (replicates) and reported as the mean and standard deviation.

Statistical analysis. All experiments were performed using at least three freshly prepared samples (replicates). The results presented are the averages and standard deviations that were calculated from these replicate measurements. Statistical differences between samples were calculated using Student's *t* test for independent samples (Microsoft Excel, Microsoft Corporation, Redmond, WA).

Results and discussion

Effect of pectin concentration on oil droplet properties. The addition of pectin to the homogeneized primary emulsion (mean particle size ~ 1.7 µm and positively charged droplets) at pH 2.4 gives a negatively charged oil droplets having a size of about 2.08 µm at highest pectin concentrations (**Figure 1**) and a stable secondary emulsion from 0.2 wt% pectin (data not shown). At low pectin concentration, the particles should be susceptible to bridging flocculation because there is insufficient polyelectrolyte present to completely saturate the surfaces of all the particles. Bridging flocculation takes place when a pectin molecule adsorbs to the surface of several droplets and links them together. Above 0.25 wt% pectin, the free pectin concentration in the continuous phase increases and attractive osmotic force becomes strong enough to overcome the various repulsive forces and consequently, depletion flocculation occurs (data not shown). This osmotic force is caused by the exclusion of pectin molecules from a narrow region surrounding the droplet surfaces (*Guzey, 2006*).

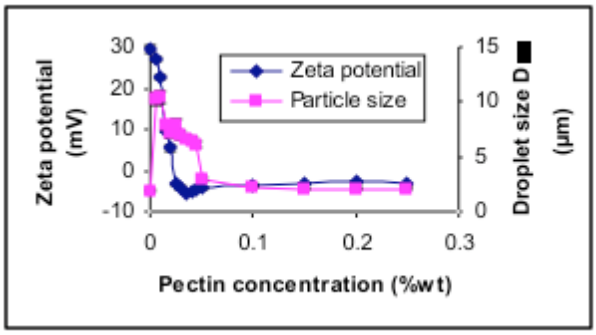

Figure 1 : Effect of pectin concentration on oil droplet zeta potential and particle size

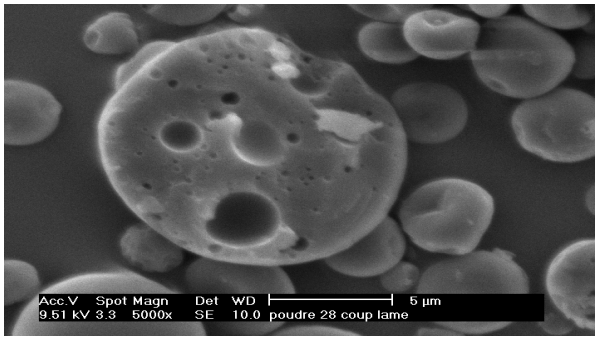

Figure 2 : Surface and inner structure of spray-dried microcapsules

Structure of spray-dried powder. The freshly prepared spray-dried microcapsules consisted of well separated spherical particles, rounded shape, smooth surface with no obvious dents. The internal structure of obtained microcapsules showed that oil was located in the form of small droplets embedded in the shell of wall matrix as shown in **Figure 2**. A thicker shell matrix was observed and microcapsule showed an air bubble (so called void) at the centre of the microcapsule. It has been reported that void formation is caused by a shrinking process that occurred after hardening of the outer surface followed by expansion of air bubbles trapped inside the droplet. The mechanisms associated with the formation of these central voids are related to the expansion of the particles during the latter stages of the drying process (*Teixeira, 2004*).

Stability of reconstituted emulsions (influence of pectin). The success of the microencapsulation process is measured by the degree of preservation of the original emulsion structure (droplet size distribution and creaming stability). The LBL technology used in the present work was used to create emulsion-based microcapsules that have better stability to aging (**Figure 3**) and to pH changes (**Figure 4**).

Conclusions

A newly application of the LBL technique was used to produce spray-dried microencapsulated oil droplets. 0.2 wt% pectin gave a stable secondary emulsion with a particle size slightly higher than that of the primary emulsion and reconstituted secondary emulsion was more stable than reconstituted primary emulsion to aging and pH changes. To interpret these results, we propose that pectin, an anionic polysaccharide, formed a protective layer around the protein interfacial film surrounding the oil droplets that could improve their stability to spray-drying. In fact, the emulsions formed consist of oil droplets surrounded by multilayer interfacial coatings, which are comprised of an inner protein layer and an outer pectin layer. This second pectin layer could be effective to

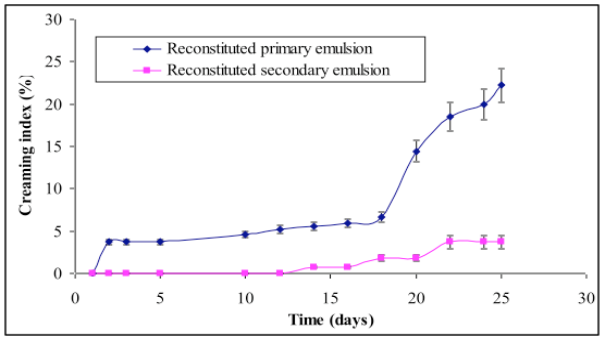

Figure 3 : Stability of reconstituted primary and secondary emulsions to aging

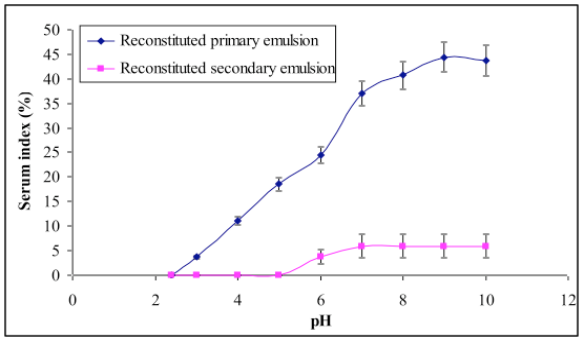

Figure 4 : Stability of reconstituted primary and secondary emulsions to pH changes

improve the protective efficiency or the release control of encapsulated ingredients.

References

A. Gharsallaoui et al. (2007) *Applications of spray-drying in microencapsulation of food ingredients: An overview*. Food Research International 40 (9), 1107-1121.

D. Guzey et al. (2006) *Formation, stability and properties of multilayer emulsions for application in the food industry.* Advances in Colloid and Interface Science. 128-130, 227-248.

M. I. Teixeira et al. (2004) *Characterization of short chain fatty acid microcapsules produced by spray drying.* Materials Science and Engineering: C 24 (5), 653-658.