Properties of microcapsules based on pea protein, pectin and maltodextrins

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INTRODUCTION

Conventional oil-in-water emulsions could be considered as an important delivery system of lipophilic molecules because of their relative ease of preparation and low cost. Emulsification is one of the key steps in the preparation of microencapsulated components by spray-drying. The emulsion must be stable over a certain period of time prior to spray-drying, oil droplets should be rather small (below 2 µm) and viscosity should be low to prevent ballooning of the particles during the drying process. This ballooning leads to air inclusion in the particle, which may, for example, favor lipid oxidation. Plant proteins could be an alternative to replace animal proteins such as gelatin which is extensively used in food, pharmaceutical, and cosmetic products. Particularly, seed storage proteins could take up this function because of their emulsifying, film-forming, and gelling properties. The most extensively studied plant protein for its functional properties is soy protein. However, the use of storage proteins of pea (*Pisum sativum L.*) seeds in the food industry for the formulation of new food products is very interesting because of their highly nutritive value, non allergenic character, and good functional properties. This work aimed to develop new microcapsules of pea protein stabilized oil droplets prepared at neutral pH and entrapped into a matrix of starch hydrolysates with different DE. In order to develop emulsion-based delivery systems, the effect of the wall material properties on its microencapsulating performance was elucidated in conjunction with the effect of drying on emulsion stability. On one other hand, pea protein was used as an emulsifier at acidic conditions (pH 2.4) to form an oil-in-water emulsion containing small droplets (primary emulsions), and then the pectin was added to produce emulsions containing droplets coated with protein-polysaccharide membranes (secondary emulsions). The second purpose of this study is to present a novel system for emulsion stabilisation and to determine whether the multilayer coatings can be used to improve the stability of protein-coated lipid droplets to spray-drying. So we prepared emulsions containing lipid droplets coated with pea protein-pectin layers and determined if this system has better stability to spray-drying than oil droplets coated by pea protein alone.

MATERIAL AND METHODS

Wall and core materials. Powdered pea protein isolate (PPI), maltodextrins (DE 6, 12, 19, and 28) (referred in this work as, DE 6, DE 12, DE 19, and DE 28, respectively) were obtained from Roquette-frères SA, (Lestrem, France). As measured by Kjeldahl method, the total protein content of the PPI powder was 91.7 wt% (dry matter basis), with globulin fraction up to 95% as stated by the manufacturer. Miglyol 812 Neutraloel, a medium chain triglyceride, 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). Deionized water was used for the preparation of all solutions and emulsions.

Determination of protein content required to emulsify 10 wt% oil emulsion and emulsion preparation. An aqueous emulsifier solutions containing 0.111 – 0.888 wt% pea protein isolate was prepared by dispersing powdered PPI into imidazole/acetate buffer (5 mM) containing 0.044 wt% NaN_3 (as an antimicrobial agent). This protein solution was then stirred for at least 6 h. 10 g of Miglyol were then blended with 90 g of aqueous emulsifier solution using an Ultra-Turrax T25 high-speed blender operated at 17500 rpm for 90 s. The resultant pre-emulsion (10 wt% oil, 0.1 – 0.8 wt% PPI, 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). The mean particle size was then measured as shown in the next section. The oil droplets were washed from non-adsorbed proteins with the method described by Tsoukala, (2006) slightly modified. Thirty five milliliters of emulsion were centrifuged at 4000g for 30 minutes at 10 °C. After centrifugation, the aqueous phase was delicately collected and the cream was washed two times using imidazole/acetate buffer solution (0.005 mM). Protein concentration was assessed in the whole obtained aqueous phases containing the non-adsorbed proteins by the Kjeldahl method.

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 prior to making the measurements. The emulsions were stirred continuously throughout the measurement to ensure the samples were homogeneous.

Apparent viscosity measurements. A controlled stress (CS) rheometer (RotoVisco 1 TCL/Z, Haake GmbH, Karlsruhe, Germany) with a coaxial cylinder attachment was employed to determine the rheological behavior of emulsions at 20 ± 0.1 °C. Flow curves were measured at increasing shear rates: 0–200 s^{-1} followed by reverse flow at decreasing shear rates (200–0 s^{-1}).

Spray-drying and moisture content determination. The emulsions (5 %wt oil, 0.25 %wt protein, 0.02 %wt NaN_3, with or without 0.2 %wt pectin, 11 %wt maltodextrins) 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. The moisture contents of the spray-dried microencapsules were determined by oven-drying at 103 °C until constant weight.

Microencapsulation efficiency. The surface oil (extractable oil) was washed from 2 g of spray-dried powder in a Randall extraction apparatus (Laboratory Solvent extractor, SER148, VELP SCIENTIFICA) by using petroleum ether. To determine total oil content, 50 g of HCl (1 M) was added to 5 g of the dried powder. The mixture was then heated in a boiling water bath for 20 min. The liquid phase was filtered (Whatman 41) and extracted oil in the filtrate was determined following removal of the solvent by Randall extraction apparatus. Microencapsulation efficiency (ME) was calculated as follows (Hogan, 2001):

$$ME = \frac{Total\ oil - Extractable\ oil}{Total\ oil} \times 100$$

Emulsion reconstitution. Dry emulsion powder was weighted and mixed with imidazole/acetate buffer (0.005 mM) to obtain reconstituted emulsion with the same dry matter as before drying. After 1 h of rotation at approximately 200 rpm using a magnetic stirrer, samples were withdrawn for particle size distribution measurements.

RESULTS AND DISCUSSION

Protein concentration required to emulsify 10 wt% oil. In this work the protein isolate concentration (0.5 g/100g) allowing to stabilize emulsion containing 10g /100g oil was experimentally determined (Figure 1). This concentration, which corresponds to 0.25 g/100g after dilution with carbohydrate solutions, permits to obtain small size oil droplets and to make the oil/water interface fully covered and only a very small amount of protein remained in the bulk phase. For a fixed concentration of oil and protein, as homogenization proceeds, the size of the oil droplets decreases and the interfacial area increases. The minimum size of stable droplets (d_{43} ~ 1.7 µm) that could be produced under the used homogenization conditions is therefore governed by the protein concentration.

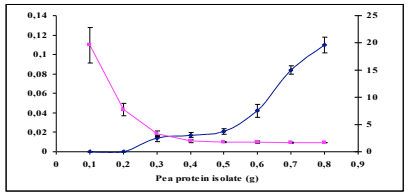

Figure 1 : Mean oil droplet size (d_{43}) (■) and non-adsorbed proteins measured in the serum fraction after emulsion centrifugation (♦)

Starch hydrolysates (11 wt%)	*d43* (µm)	SPAN	Apparent viscosity (mPa.s)
Without carbohydrates	1.69±0.11	2.11	1.67±0.15
DE 6	1.82±0.15	2.21	11.92±1.26
DE 12	1.75±0.19	2.07	9.67±1.21
DE 19	1.83±0.09	2.12	5.08±0.59
DE 28	1.71±0.12	2.07	3.69±0.66

Table 1: Influence of starch hydrolysates DE on the properties of feed emulsions (pea protein isolate: 0.25 wt%; oil: 5 wt%; starch hydrolysates: 11 wt%).

Effects of maltodextrin DE on the properties of feed emulsions. The obtained results (Table 1) indicated that homogeneous and fine oil-in-water emulsions, necessary for microencapsulation by spray-drying were obtained in most cases. Indeed, oil droplet particle size distribution affects emulsion viscosity and fat losses during atomization shearing (Gharsallaoui A., 2007). Table 1 shows also that the droplet size distribution of the liquid emulsions before spray-drying does not significantly ($P>0.05$) change after adding starch hydrolysates for all studied DE. Viscosity measurements were also used to confirm that the droplets in the feed emulsions were not flocculated. However, the apparent viscosity values of feed emulsions increased from ~3.7 to ~12 mPa.s when the DE decreased from 28 to 6 because of the increased molecular weight of carbohydrate molecules.

Effects of maltodextrin DE on the properties of dry powder and reconstituted emulsions. Table 2 listed ME data of the microcapsules obtained at different DE. These results showed that extractable fat was markedly higher in samples with low DE than in samples with high DE. Lower efficiency values were observed at lower DE (DE 6 and DE 12) and may limit the shelf-life of the microcapsules. When spray-drying process parameters are fixed, moisture content of spray-dried powder depends mainly from feed solution properties, which could influence the residual water content, and from wall material hygroscopicity. Consequently, direct fitting between the DE and moisture content has been found because lower molecular weight starch hydrolysates contained more hydrophilic groups. On one other hand, particle size distribution results showed that the d_{43} of reconstituted emulsions decreased significantly ($P<0.05$) as the DE increased (Table 2). Moreover, bidisperse systems were obtained for dry emulsions containing maltodextrins having DE 12 and 6 giving rise to larger d_{43} and SPAN-values in the reconstituted emulsions. The reason for the increase

in oil droplet diameter is possibly the facilitated mobility of the oil droplets during setting of the solid particle structure upon drying in samples with carbohydrates having lower DE.

Starch hydrolysates (11 wt%)	Microenpsulation efficiency (%)	Moisture content (%)	*d43* (µm)	SPAN
DE 6	37.54±3.89	0.915±0.13	8.09±1.84**	7.21
DE 12	52.31±2.27	2.924±0.28	6.47±0.91**	6.86
DE 19	63.48±3.22	4.006±0.29	2.61±0.29*	4.76
DE 28	84.95±2.17	5.762±0.22	2.08±0.25*	4.41

Table 2: Influence of starch hydrolysates DE on the properties of dry powder and reconstituted emulsions. *: Monodisperse distribution, **: Bidisperse distribution.

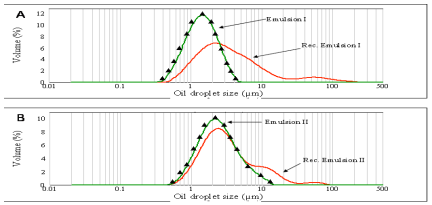

Figure 2: Droplet size distribution of primary and secondary emulsions before spray-drying and after reconstitution.

Effect of pectin addition on oil droplet size stability during drying process. Both primary and secondary emulsions containing DE 28 before drying show monodisperse droplet size distributions (Figure 2). However, the difference in oil droplet size can be attributed to pectin adsorbed onto the surfaces of protein-coated droplets at pH values where the droplets have a net positive charge (pH < pI ~ 4.3) (Guzey D, 2006). During reconstitution, the original oil-in-water emulsion was not fully reformed and a general shift in the oil droplet size of the first peak of the distribution was observed for reconstituted primary emulsion leading to an increase in the d_{43} and the SPAN. For reconstituted secondary emulsion droplet coalescence occurs also but the average droplet size of the first peak of the distribution seems to be less affected by spray-drying processing.

CONCLUSIONS

Obtained results showed that the DE of the carbohydrate wall materials had a marked influence on the microencapsulation efficiency. Therefore, glucose syrup (DE 28) could be recommended for the high oil retention during spray-drying as well as the ability to reconstitute emulsion with approximately similar droplet size. In addition, it has been shown in this study that oil droplets coated by pea protein-pectin complexes have superior stability to spray-drying than those coated by protein single-layered membrane.

REFERENCES

- Gharsallaoui A. et al. (2007) *Applications of spray-drying in microencapsulation of food ingredients: An overview*. Food Research International 40 (9), 1107-1121.
- Guzey D. 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.
- Hogan, S. A. et al. (2001) *Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends.* International Dairy Journal, 11(3), 137-144.
- Tsoukala, A. et al. (2006) *Adsorption at the air-water interface and emulsification properties of grain legume protein derivatives from pea and broad bean.* Colloids and Surfaces B: Biointerfaces, 53(2), 203-208.