

Production of Protein/Pectin Complexes Using a Microfluidic Device

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Protein/pectin (P/P) interactions can be used as an important tool to modify the microstructure of the composite systems to produce stable systems (Dickinson, 2008), because P/P complexes can show better qualities than single components (Ye, 2008). In our study, an initial P/P solution was prepared at complexation pH (pH_c) using a microfluidic device adopting the flow focusing technique (Utada, 2007); such device permits a precise flow rate control of each fluid using syringe micropumps. The complex stability was investigated measuring particle size over time and using different co-focusing fluids. While P/P complex obtained using standard production techniques (manual mixing) showed precipitation and aggregations, in the case of particles obtained using microfluidic techniques results showed a significant particle size stability after 10 h after complex production. Following these results the complex formation was investigated using a buffer acetate solution to set pH at pH_c to ensure the optimal conditions for the P/P complexation and, varying the flow rate. Best results were obtained by using a P/P ratio which is slightly different from the ratio presented in Di Pierro, et al., 2013, suggesting that mixing conditions play an important role.

Another set of experiments was also run using sucrose as focusing flow to study the influence of density and others physical parameters on the extrusion stage and stability of P/P complexes. Results demonstrated that varying the flow rate of focusing flow do not significantly affect the complex size distribution.

1. Introduction

Proteins and polysaccharides (pectin) are often used by food technologists to control structure, texture, stability, and when they are mixed together in appropriate formulations (Tolsoguzov, 1991). they are able to produce stable complexes showing better qualities than single components (Dickinson, 2008)

Proteins and polysaccharides can be associated with each other under conditions of opposite electrical charge (Jones et al., 2009). The titration of a polysaccharides protein mixture is able to charge the net charge of protein as a function of their pK_a (Hattori et al., 2001).

At specific pH value, called complexation pH (pH_c), the molecules have opposite charges leading to the formation of electrostatic interactions (Weinbreck and de Kruif, 2003).

These complexes may be further stabilized through other intermolecular forces like hydrophobic ones (Hallberg & Dubin, 1998) and/or hydrogen bonds (Girard et al., 2002). The formation of primary soluble complexes is usually a reversible process, generally occurring at low ionic strength and at a pH value corresponding to the pH_c (Weinbreck et al., 2003). It is worthy to note that the pH_c may shift to lower values with an ionic strength increase that is able to shield the Prot/Pect (P/P) attractive interactions (Di Pierro et al., 2013).

Several parameters affect the size and stability of the biopolymer particles formed by the Prot/Pec interactions: the ratio between proteins and pectins and the type of polysaccharides involved (Jones and

Clements, 2010). Traditional methods to produce complexes includes heating(protein denaturation), manual mixing and then casting, however they could not be controlled accurately.

A possible solution to this problem is the use microfluidic techniques, in which an accurate control of mixing can be obtained, for example in micromixers, and P/P complex extrusion can be obtained using focusing flow configurations. Important features that are associated with these techniques are the narrow particle size distribution, particle separation which can be obtained in one step without additional purification steps, and a mild mechanical stress (Martin-Banderas et al., 2005).

In the specific case, particle size is strictly linked to bond saturation between protein and pectin.

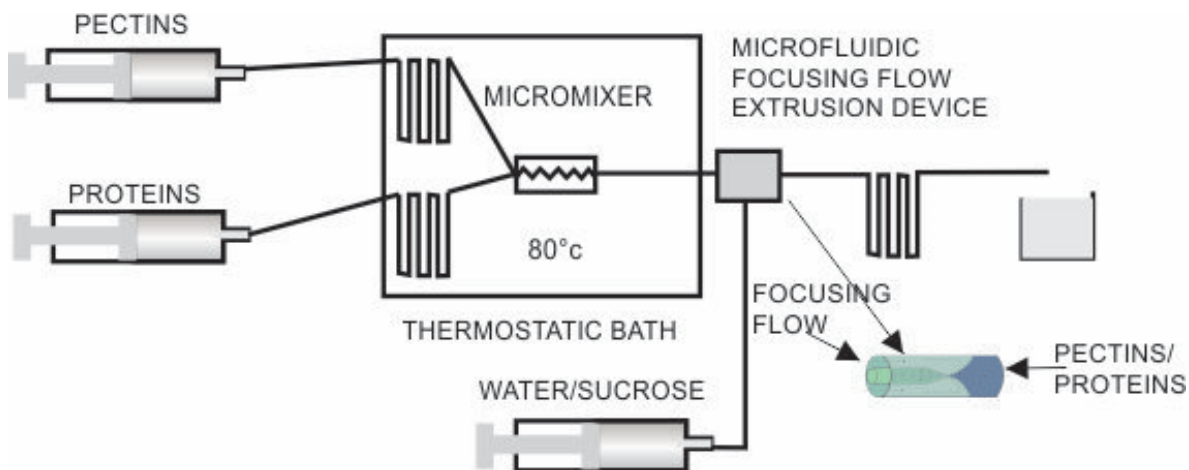


Figure 1: Microfluidic Device

Aim of the work is to test the possibility of developing a microfluidic based method; to this purpose, a device to control P/P production was assembled, providing accurate flow rates and thermal control of the all process (see Figure 1).

2. Materials and Methods.

Commercial (WP) isolate was obtained from BioLine (New Zealand), and low methoxyl pectin (LM-Pec) obtained from Citrus fruits, sucrose and all other reagents were purchased from Sigma (Steinheim, Germany). Three AI-300 infusion syringe pumps were used to control protein, pectin and focusing flow mass flow rates. Protein and pectin basic solution solutions were prepared as described by Di Pierro et al. (2013). The protein solution was prepared by dissolving 1 g of protein powder in 50 mL of distillate water, and then heated at 80 °C for 25 min. Meanwhile the pectin solution was prepared by mixing 0.4 g of pectin powder with 50 mL of distillate water, and then heated at 80 °C for 2 min.

These basic solutions were introduced into the microfluidic assembly (Figure 1) using two syringe pumps, and they were let flowing using two 30 cm length Teflon tubes (diameter 0.50 mm) for other 20 min into a thermostatic bath set to 80 °C.

This preliminary heating was necessary to induce protein's denaturation,. After this preliminary heating period, the two solutions were collected at Y junction and introduced into the micro-mixer, to start the complexation. The static micromixer was made by using a helicoidal polymeric microstructure introduced into part of the tubing behind the Y junction.

Final extrusion of the complexes was then obtained by using a focusing flow device positioned at short distance from the mixer to maintain the correct temperature, where the focusing flow consisted in a solution of sucrose 66 % w/v solution to increase the flow density and viscosity.

After the microfluidic extrusion stage, the complexes were let to flow into the sucrose solution for other 10 minutes to complete the stabilization step (saturation of sulphur bridges), and then collected.

Particle size was measured. using a light scattering system (Mastersizer 3000, Malvern, England, UK) adding 500 mL distilled water as a dispersant medium using a refraction index of 1.330, adding the required amount of sample until obtain a laser obscuration minimum of 15 %.

3. Results and Discussion

One of the advantage of microfluidic approach relies on the possibility to obtain a more detailed control of mixing rate into the micromixer, where mixing is performed at a smaller scale with respect to the manual mixing. This new technique provides an alternative to the traditional batch mixing where consistent mechanical stress can damage the particle structure, a feature that is significantly important for the possible use of complexes in controlled release systems.

In the paper of Di Pierro et al. (2013) the optimal ratio in weight protein/pectin was set to 4:1; this ratio depends on the several parameters, including mixing efficiency.

To investigate the mixing effects between pectin and protein solutions, in the microfluidic approach different flow rates of pectin solution were tested, keeping a constant protein flow rate set at 1.0 mL/min.

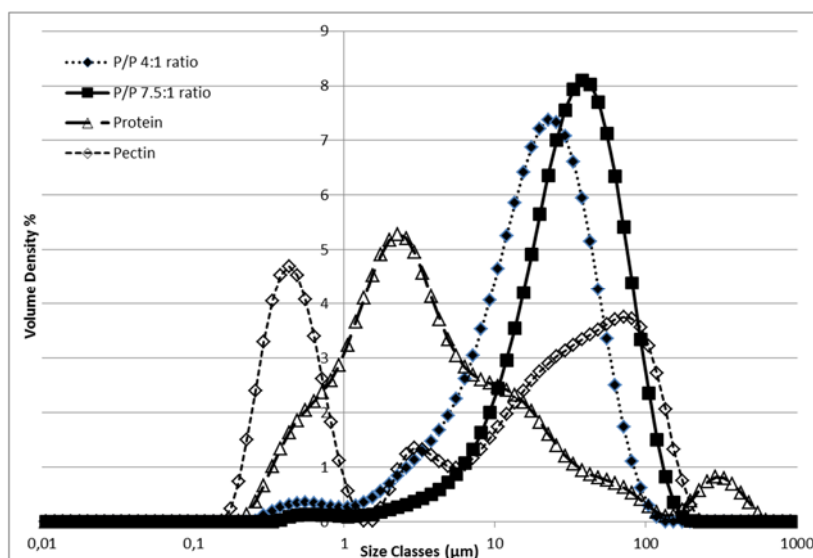


Figure 2: Particles size distribution at different P/P mixture ratio (4:1 and 7.5:1)

In Figure 2 results at different P/P ratio in weight are presented, with a focusing flow rate equal to 1 mL/min: the presence of a small peak corresponding the pectin's maximum in size distribution at approximately 0.6 μm shows that in the case of 4:1 P/P ratio some pectin do not participate in complexation process, while at 7.5:1 ratio all pectin seems to participate in the process. Although the difference is not so significant, these results suggest that the effect of mixing rate is an important parameter to be considered, requiring further investigation. The initial presence of significant percentage of pectin and protein with an average size bigger than P/P complexes size is due to the presence of pectin-pectin and proteins-proteins agglomerates generated by weak ionic interactions and hydrogen bonds, that are broken during the complex formation process. As a matter of fact the pure pectin and protein curves were obtained at room temperature, when pectin/pectin and protein/protein interactions are already present, so that the real dimension of both of them is much smaller.

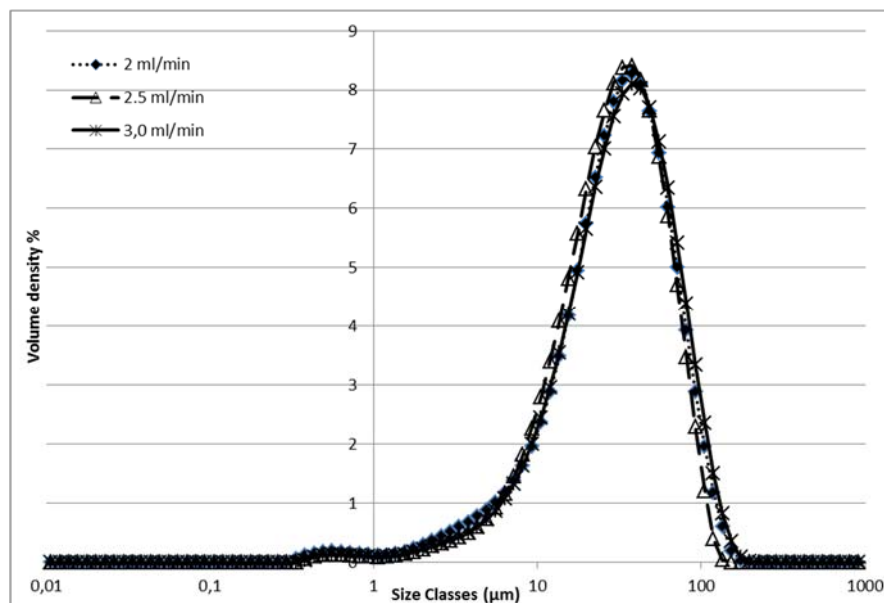


Figure 3: Effect sucrose solution (focusing flow) flow rate on complex (particle size).

In this study, varying the sucrose/water flow rate did not cause a significant change of results. Thus this inferred that the physical-chemical interactions as expected are the driving factors of complexes size distribution rather than mechanical stress occurring during the flow focusing extrusion process (Figure 3). This assumption was confirmed by changing the ratio between P/P from 2.5:1 up to 7.5, in this case with focusing flow rate equal to 2 mL/min, as shown in Figure 4: the variation of D_{50} size distribution was approximately of $\pm 5 \mu\text{m}$ from the average value of $20 \mu\text{m}$.

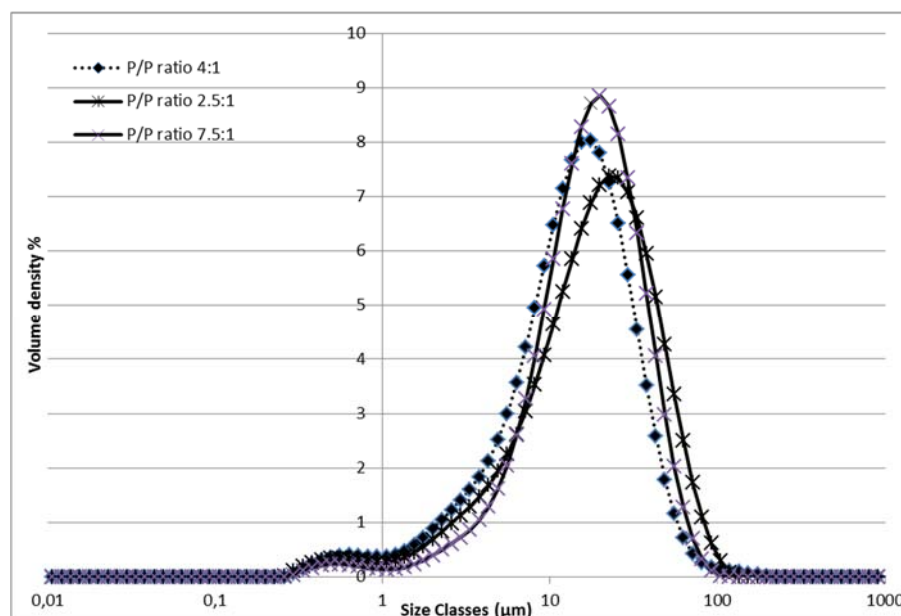


Figure 4: Complexes particle size variation related to P/P ratio (sucrose/water flow rate equal to 2 mL/min).

3.1 Complexes stability

Complex stability in time represents an important issue to be addressed in order to scale up laboratory manual mixing to industrial production.

From this study it showed that Prot//Pect complexes obtained by using manual mixing according the method illustrated in Di Pierro et al. (2013) was unstable with time, due to complexes agglomeration and interactions, as highlighted in Figure 5.

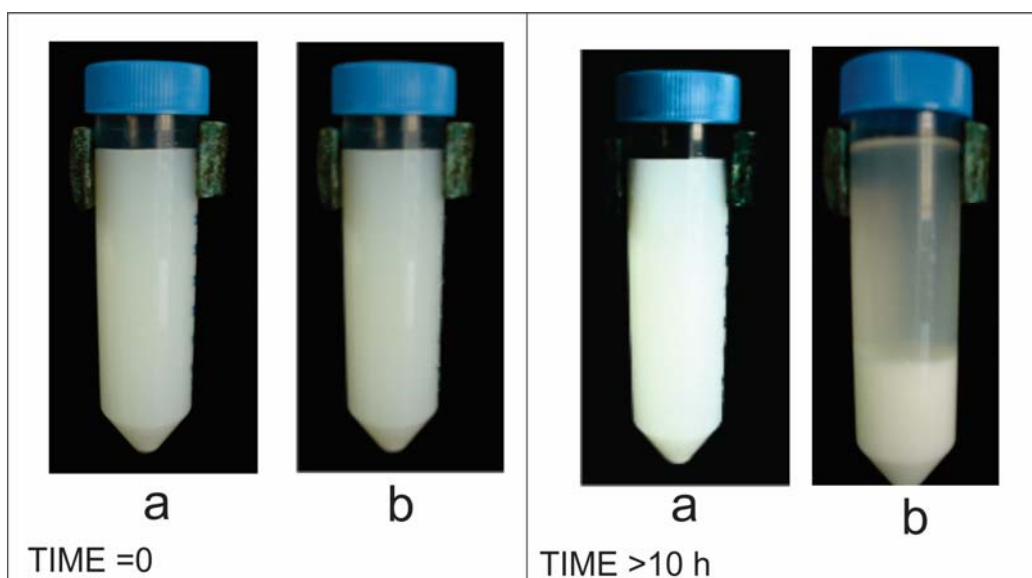


Figure 5: P/P complexes obtained using microfluidic techniques (a) and manual mixing (b) at time $t = 0$ and $t > 10$ h.

At time $t = 0$ both the solutions obtained by microfluidic technique and manual mixing appeared as a milky solution without precipitate. After 10 hours, the solution prepared manually demonstrated a separation of phases, indicating the instability of the solution. Instead the one prepared using microfluidics is constant over time.

This augmented stability is probably connected to the stabilization step obtained by flowing separately each complex after the extrusion phase in tubing for 10 minutes into the sucrose/water solution.

The size distribution of complexes obtained by using microfluidic technique remained unchanged after several days.

4. Conclusions

In this study the possibility of using microfluidic devices to improve traditional methods for mixing solutions and to generate complexes of proteins and polysaccharides was demonstrated. Several interesting features are introduced respect to traditional manual mixing method: minimal mechanical stress, reproducibility of results (an important feature in the industrial perspective), and a better control of the P/P mixing. The use of sucrose/water solution as focusing flow contribute to stabilize the complexes, providing a time stability of extruded complexes which is not present in manual mixing procedure.

The augmented stabilization is connected probably to the fact the complexes are generated (or better separated) one by one, and ionic link can be saturated before complexes start to interact each other as it happens if a stirred tank reactor, producing agglomerates and eventually generating a precipitate.

As a matter of fact, the use of microfluidic represents an important step ahead towards industrial production of such complexes, providing a relatively simple industrial scale up, allowing a precise control of the quantities involved in the process.

A further insight is required to characterize the mixing effects on complex production, as the assumption of perfect mixing and low mechanical stress cannot probably be assumed at the same time in manual mixing or in the equivalent CSTR industrial system.

A detailed investigation is also required to characterize the effect of different microfluidic device geometrical configurations of both the micromixer and the extrusion head on complex production, which is out of the purpose of the present work.

References

- Dickinson E., 2008, Stability and rheological implications of electrostatic milk-protein-polysaccharide interactions. *Trends Food Sci. Tech.* 9, 347-354.
- Di Pierro P., Rossi-Marquez G. Mariniello L., Sorrentino A., Porta R., 2013, Effect of transglutaminase on the mechanical and barrier properties of whey protein/pectin films prepared at complexation pH (pHc). *J. Agric. Food Chem.*, 61, 4593-4598.
- Girard M., Turgeon S.L., Gauthier S.F., 2002, Interbiopolymer complexing between beta-lactoglobulin and low- and high-methylated pectin measured by potentiometric titration and ultrafiltration. *Food Hydrocoll.*, 16, 585-591.
- Hattori T.H., Kimura K., Seyrek E., Dubin P.L., 2001, Binding of bovine serum albumin to heparin determined by turbidimetric titration and frontal analysis continuous capillary electrophoresis. *Anal. Biochem.*, 295, 158-167.
- Hallberg R.K., Dubin P.L., 1998, Effect of pH on the binding of beta-lactoglobulin to sodium polystyrenesulfonate. *J. Phys. Chem.*, 102, 8629-8633.
- Jones, O. G.; Decker, E. A.; McClements, D. J. 2009, Formation of biopolymer particles by thermal treatment of b-lactoglobulin-pectin complexes. *Food Hydrocolloids*, 23, 1312-1321.
- Martín-Banderas L., Flores-Mosquera M., Riesco-Chueca, P., Rodríguez-Gil A., Cebolla A., Chavez S., Gañan-Calvo A.M., 2005, Flow Focusing: A Versatile Technology to Produce Size-Controlled and Specific-Morphology Microparticle, *Small*, 1, 688-692.
- Tolsoguzov V.B., 1991, Functional Properties of food proteins and role of protein-polysaccharide interactions. *Food Hydrocolloid*, 4, 429-468
- Utada A.S., 2007, Dripping, Jetting, Drops, and Wetting: The Magic of Microfluidics. *MRS Bulletin*, 32, 702-708.
- Ye A., 2008, Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications- a review. *J. Food Sci Tech.* 406-415.
- Weinbreck F., de Vries R., Schrooyen P., de Kruif C.G., 2003, Complex coacervation of whey proteins and gum Arabic. *Biomacromolecules*, 4, 293-303.