



## New insight on the formation of whey protein microbeads by a microfluidic system

Robi Andoyo, Fanny Guyomarc'H, Hervé Tabuteau, Marie-Hélène Famelart

### ► To cite this version:

Robi Andoyo, Fanny Guyomarc'H, Hervé Tabuteau, Marie-Hélène Famelart. New insight on the formation of whey protein microbeads by a microfluidic system. 1. International Conference and exhibition on powder technology INDONESIA (ICePTi) 2017, Aug 2017, Bandung, Indonesia. pp.030022, 10.1063/1.5021215 . hal-01709342

**HAL Id: hal-01709342**

**<https://hal.science/hal-01709342>**

Submitted on 29 May 2018

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# New insight on the formation of whey protein microbeads By a microfluidic system

Robi ANDOYO<sup>a\*</sup>, Fanny GUYOMARC'H<sup>bc</sup>, Hervé TABUTEAU<sup>d</sup>,  
Marie-Hélène FAMELART<sup>bc</sup>

<sup>a</sup>*Faculty of Agro- Industrial Technology, Universitas Padjadjaran, Indonesia*

<sup>b</sup>*INRA, UMR1253, Science et Technologie du Lait et de l'Œuf, 65 rue de St Brieuc, F-35  
042 Rennes cedex, France*

<sup>c</sup>*AGROCAMPUS OUEST, UMR 1253, Science et Technologie du Lait et de l'Œuf, 65 rue  
de St Brieuc, F-35 042 Rennes cedex, France*

<sup>d</sup>*Institut de Physique de Rennes, Université Rennes 1, France*

\* Corresponding author; Tel: +62 85723350400; email address: [r.andoyo@unpad.ac.id](mailto:r.andoyo@unpad.ac.id)

**Abstract.** The current paper describes the formation of whey protein microbeads (WPM) having a spherical shape and a monodisperse size distribution. A microfluidic flow-focusing geometry was used to control the production of whey protein microdroplets in a hydrophobic phase. The microfluidic system consisted of two inlet channels where the WPI solution and the lipophilic phase were separately injected towards the flow-focusing (FF) junction where they eventually meet, then co-flow. A whey protein isolate (WPI) solution at 150 g kg<sup>-1</sup> protein and two types of hydrophobic phases, i.e. sunflower oil or n-dodecane, were tested as the continuous phase. The formation of WPM was observed microscopically. The aim of the present study was to describe the production of stable monodisperse WPM in suspension in milk ultrafiltrate using a microfluidic system. Hints to perform the control of the running parameters, i.e. choice of the hydrophobic phase or fluids flowrates, are provided. The results showed that in the sunflower oil, microdroplets had a large polydisperse size distribution, while in n-dodecane, microdroplets with narrow size distribution were obtained. Stabilization of the whey protein microdroplets through heat-gelation at 75 °C for 20 min in n-dodecane produced WPM and did change neither their shape nor size. Meanwhile replacing the n-dodecane by MUF using centrifugation and washing caused the swelling of the WPM, but dispersity remained low. From this study, microfluidic system seemed to be a suitable method to be used for producing small quantities of monodisperse WPM.

## 1. INTRODUCTION

Whey proteins are used in many applications in food as texturing agents, fat replacers or amino-acid supply in infant formula or other specific diets (de la Fuente et al., 2002; Foegeding et al., 2002; Dawson et al 2008; Madureira et al., 2010; Nicolai et al., 2011), due to their functional properties in gelation or emulsification. Heating milk at temperatures above ~60°C for ~5-10 min induces the unfolding and assembling of the whey proteins into aggregates (WPA) of ~0.1 µm size through thiol-disulfide and hydrophobic interactions (Donato and Guyomarc'h, 2009). Meanwhile, heating pure whey protein systems or whey products produces aggregates of size diameter >1µm (Patocka et al., 1993; Beaulieu et al 1999; Marangoni et al., 2000). The size of the WPA is thought to have an impact on the way the proteins are occupying space upon gelation, thus affecting the final gel properties. In a recent paper, we studied the effect of whey protein particle size on acid gelation using emulsification as a means to produce WPM of heat-gelled droplets of whey proteins. The results showed that the smaller the size of WPM, the more elastic the acid gel and the smaller the pore size of the gel. A more significant effect was evidenced at small size microbeads (diameter ~2.5 µm) as the number of particles in this system was higher than in the other systems (diameter >4.2 µm). It was hence suggested that more junctions and bonds were formed between particles upon gelation, yielding stronger gels. Other works have also investigated this field using the shear force (Saglam et al., 2011; van Riemsdijk et al., 2011) or a microparticulation approach (Janhoj and Ipsen 2006; Torres et al., 2011) to produce heat-denatured whey protein particles. Other works have modulated the size of whey protein aggregate particles using changes in pH, ionic or protein compositions and protein concentration. But all these factors would also affect other physico-chemical properties of the particles, such as the composition, isoelectric pH or the free thiol content, thus introducing a bias, as already explained in the previous study. In those studies, the particles produced were highly polydisperse. In model systems, polydispersity has been evidenced to play a role in the phase transition and structural packing of particles (Santiso and Mueller 2002; Nogawa et al., 2010; Sollich and Wilding 2011). Hence, when using whey protein aggregate particles, it may be difficult to distinguish the respective effects of size and polydispersity of microbeads on the building of the acid gel. Furthermore, only few studies deal with the particle size distribution as a way to control the microstructure of dairy products. For example, Doherty et al. (2011) have studied the production of narrow size distribution of whey protein microbeads as a mean to control their mechanical properties, in the context of the encapsulation of probiotic bacteria and not acid gelation.

An alternative way to reduce the polydispersity of the particles is to use a microfluidic device to generate monodisperse WPM. In the present study, a flow-focusing microfluidic system was used to modulate the size of whey protein particles without changing their other properties. Microfluidic systems are widely used in non-food contexts, especially when the size of particles can be prescribed in micro scale and described as a monodisperse distribution. The aim of the present paper was therefore to describe the production of stable monodisperse WPM in suspension in milk ultrafiltrate using a microfluidic system. Hints to perform the control of the running parameters, i.e. choice of the hydrophobic phase or fluids flow rates, are provided.

## 2. MATERIALS AND METHODS

### 2.1. Materials

A commercial whey protein isolate powder (WPI) (Prolacta 95, Lactalis Ingredients, Bourgbarré, France) was used as the source of protein. The protein content of the powder was determined using the Kjeldahl method and a conversion factor 6.38 and was  $\sim 939 \text{ g kg}^{-1}$  dry matter. Milk ultrafiltration permeate (MUF) was produced from micro then ultrafiltration of raw skim milk at pH 6.7 and was stored at  $5^\circ\text{C}$  after addition of  $0.2 \text{ g L}^{-1}$  sodium azide ( $\text{NaN}_3$ ). Polyglycerolpolyricinoleate (Grindsted PGPR 90, Denmark) was purchased from Danisco and contained apolyglycerol ester of polycondensedricinoleic acid with added antioxidants  $\alpha$ -tocopherol (E 307). N-Dodecane was purchased from Merck. Its density is  $0.748 \text{ kg L}^{-1}$  and its viscosity is  $1.72 \text{ mPa s}$  at  $20^\circ\text{C}$ . All other chemicals were of analytical grade. The sunflower oil (viscosity  $64 \text{ mPa s}$  at  $20^\circ\text{C}$ , density at  $25^\circ\text{C}$  is  $918.8 \text{ kg m}^{-3}$ ) was a commercial product purchased from a local supermarket. Polymer polydimethylsiloxane (PDMS) (SU8 SA2002) and its curing agent were purchased from Dow Corning (Midland-Michigan USA).

### 2.2. Solutions

A  $150 \text{ g kg}^{-1}$  protein WPI solution was produced by diluting the WPI powder in deionized water with  $0.2 \text{ g kg}^{-1}$   $\text{NaN}_3$ , stirring for 2 h, filtering on  $0.45 \mu\text{m}$  filter syringe. The solution was left at  $4^\circ\text{C}$  overnight for complete hydration. PGPR was dissolved in n-dodecane at  $25 \text{ g kg}^{-1}$  by stirring at  $40^\circ\text{C}$  for 1 h.

### 2.3. Formation of whey protein microbeads by microfluidic system

The microfluidic system consisted of two inlet channels where the WPI solution and the lipophilic phase were separately injected towards the flow-focusing (FF) junction where they eventually meet, then co-flow. A scheme of the FF channel is shown in Fig. 1.

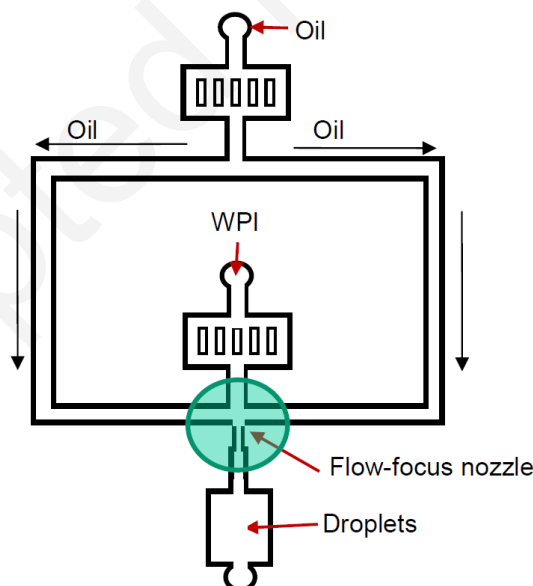


Figure 1. Scheme of the flow-focusing microfluidic channel with nozzle dimension: width  $15 \mu\text{m}$ , depth  $15 \mu\text{m}$  and length  $300 \mu\text{m}$ .

The microfluidic channels were made in resin using the standard techniques of lithography which allows replication of the microchannel design (Xia and Whitesides 1998). The manufacture of the microfluidic device started with the design of the channels on a computer-aided design software (CleWin, WieWeb Software, the Netherlands). The resin was spread on a flat surface of silicon wafer, with the desired thickness determining the height of the channel, and hence the diameter of the microbeads. A UV light was shined across a chrome mask that had been placed on top and in contact with the resin. The 2D design of the channel corresponded to the transparent part of the mask. In this way, the resin which was insulated was also cured. This part corresponded to the desired design. In a next step, the wafer and the resin were placed in a bath of developer in order to remove

the uncured resin. The PDMS was poured on the resin and is cured by the reafter heating. The microchannels molded in PDMS were finally bonded to a glass slide flat surface. In the current study, the dimensions of the flow focus junction were 15  $\mu\text{m}$  wide, 300  $\mu\text{m}$  long and 15  $\mu\text{m}$  height.

The used fluids consisted of the WPI solution as the dispersed phase and n-dodecane or sunflower oil both containing 25 g  $\text{kg}^{-1}$  PGPR as the continuous phase. The fluids were injected into the microfluidic devices at various controlled volumetric flow rates, as discussed in the result section, using syringe pumps. The dispersed phase met the carrier phase in the flow-focus junction, then the two immiscible liquids co-flow in the nozzle, where the microdroplets were formed and flowed into a reservoir. Directly after the microdroplets were produced, the w/o emulsion was dispensed in sealed 5-g glass tubes and heated at 75  $^{\circ}\text{C}$  for 20 min in a temperature-controlled waterbath, then rapidly cooled in ice water to room temperature, in order to stabilize the WPM through heat-gelation of the microdroplets. It was checked that the heat-load applied was sufficient to gel a 150 g  $\text{kg}^{-1}$  WPI solution as showed by a maximal increase of firmness as compared to the gels produced with the other temperature and time combinations (results not shown). Centrifugation at 5000g for 10 min (Heraeus, Biofuge Primo R 7500 5440) through an oil/water interface was applied to the heat-treated emulsion to remove the continuous phase and pellet the beads into water. The centrifugation step was repeated twice, by using distilled water to rinse and re-dispersed the beads. The final dispersing step was performed using MUF.

## 2.4. Optical microscopy

Samples were taken from three different steps during the formation of WPM, namely: before heat treatment, after heat treatment in n-dodecane and after re-dispersion in MUF. The WPM was analyzed using an optical microscope (Olympus, BX51). Samples were diluted 5x by using the appropriate solvent phase, i.e. n-dodecane or MUF, depending on which step the measurements were performed. A drop of diluted sample was transferred onto the microscope slide and covered with a cover slip prior to observation.

## 2.5. Confocal laser scanning microscopy (CLSM)

WPM dispersed in MUF was observed by CLSM. Samples were labelled using 0.06  $\mu\text{L}$  of rhodamine B isothiocyanate (RITC) per gram protein (from a 85 g  $\text{L}^{-1}$  RITC solution prepared in dimethylsulfoxide, Sigma-Aldrich, St Quentin Fallavier, France) and stirred for 15 min prior to observation. Samples were then imaged at 543 nm using a TE2000-E Nikon C1i inverted confocal laser scanning microscope (CLSM, Nikon, Champigny-sur-Marne, France), objective 60x. Each image was digitized in grey levels as a 512 x 512 pixel matrix (127.3 x 127.3  $\mu\text{m}^2$ ).

# 3. RESULTS AND DISCUSSION

During microdroplet production, the respective flow rates of the two immiscible liquids are the main factors that determine the stability of the droplets production (Engl et al 2008). Here, the stability is defined as the particles' ability to keep their size during a week of storage. The sunflower oil was chosen for its food grade properties and because it is widely used in emulsification studies (Saglam et al 2011). The n-dodecane was chosen because of its lower density (0.748  $\text{kg L}^{-1}$  at 20  $^{\circ}\text{C}$ ) and low viscosity value (1.72  $\text{mPa s}$  at 20  $^{\circ}\text{C}$ ).

With the two different continuous phases and flow rates, we observed a wide range of microdroplets patterns. Using sunflower oil and a flow rate ratio of sunflower/WPI of 2 / 0.8  $\text{mL h}^{-1}$ , microdroplets with a very large size polydispersity were produced (Fig. 2).

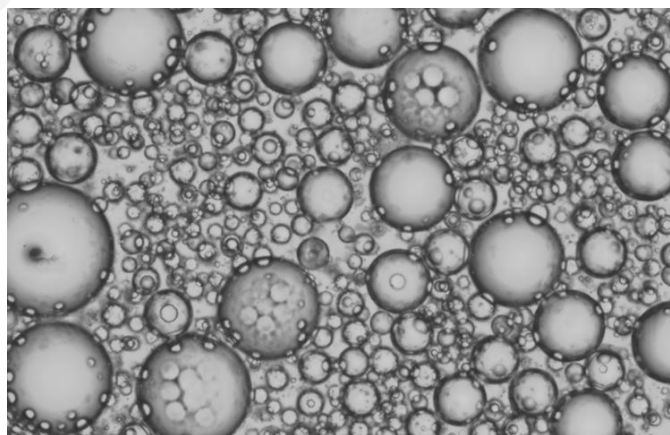


Figure 2. Whey protein microdroplets suspended in sunflower oil, produced by microfluidic system at the flow rate of 2 and 0.8  $\text{mL h}^{-1}$  for sunflower oil and whey protein isolate solution, respectively.

A lower flow rate ratio ( $R$ ), i.e. a higher flow rate of the WPI or a lower flow rate of oil led to microdroplets that could not be produced as the oil phase failed to stretch the flow of the WPI solution. We suspected that the polydispersity of the microdroplets produced at  $R = 2/0.8 \text{ mL h}^{-1}$  was due to the high dynamic viscosity difference between the sunflower oil and the WPI solution, i.e.  $64 \text{ mPa s}$  and  $5.2 \text{ mPa s}$ , respectively. The high viscosity and flow rate of the sunflower oil, as compared to WPI solution, led to an excessive pressure increase inside the flow-focusing nozzle. As the pressure increased, the tip of the WPI stream in the nozzle gradually retracted thus producing polydisperse microdroplets. Furthermore, for the same reason, the production of microdroplets could not last for more than 1 h with this setting, as the micro channel was damaged due to excessive pressure. To circumvent this issue, a continuous oil phase of lower density was tested. Microdroplets of relatively monodisperse distribution could be produced in *n*-dodecane and a  $R$  value of  $1/0.15 \text{ mL h}^{-1}$ . The microdroplets produced with this configuration had a size which was comparable with the width of the flow-focusing nozzle ( $15 \text{ }\mu\text{m}$ ) which indicates that break-up, i.e. microdroplet formation, did occur inside the flow-focus nozzle, as expected, and in a regular and reproducible manner (Fig. 3).

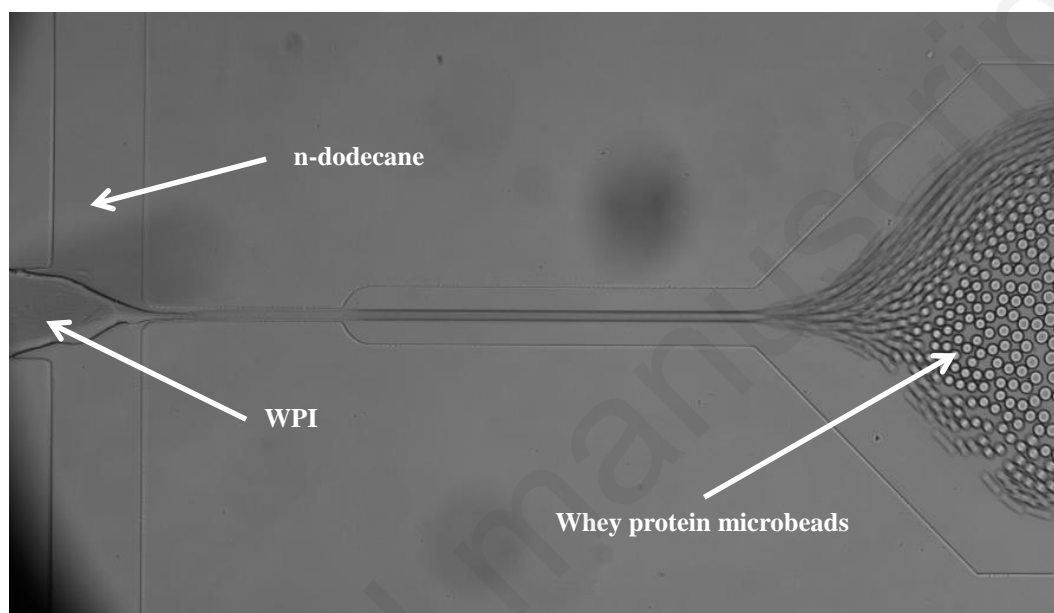


Figure 3. Whey protein microdroplets formation in the microfluidic channel. The flow rate of the two liquids were  $1$  and  $0.15 \text{ mL h}^{-1}$  for *n*-dodecane and whey protein isolate (WPI) solution, respectively

Microdroplets were spherical with a diameter of  $\sim 15 \text{ }\mu\text{m}$  as measured optically (Fig. 4A) and did not coalesce after 7 days of storage at  $20^\circ\text{C}$  without stirring. Therefore, the low viscosity of the continuous phase and the high flow rate ratio between the hydrophobic phase and the WPI solution seemed favourable for the formation of monodisperse microdroplets. When setting the flow rates at  $1$  and  $0.2 \text{ mL per h}$  for *n*-dodecane and WPI solution respectively, it was found that microdroplets of higher size could be produced ( $\sim 25 \text{ }\mu\text{m}$ ) but that they showed a polydisperse size distribution (Fig. 5), probably because the increased flow rate also increased the pressure inside the channels, as discussed above. To some extent, it therefore seemed that the size of the microdroplets could be varied by changing the setting conditions of the microfluidic system, with the risk of an increasing polydispersity of the beads. Anna et al. (2003) proposed that the size of the microdroplets could be tuned depending on the ability of the continuous phase to form narrow stream of the dispersed phase, on the size of the flow-focusing nozzle and also on the flow rates of the two liquids.

After production of the microdroplets in conditions where a monodisperse size distribution was observed, a heat treatment at  $75^\circ\text{C}$  for  $20 \text{ min}$  was applied in order to stabilize them through heat-gelation. The resulting whey protein microbeads (WPA), yet dispersed in *n*-dodecane, were observed by light transmission optical microscopy (Fig 4B). It seemed that the heat treatment did affect neither the size nor the shape of the microbeads, the same as observed in the previous study using emulsification (Andoyo et al., 2015, not listed). After heating, the microbeads exhibited slightly different optical properties than before heating, as shown by more translucent microbeads after their heat treatment than before. This change in optical properties was probably due to the denaturation of the whey protein, which may slightly affect their refractive index (Mc Meekin et al 1964) or to the increased molecular mass of the aggregated whey protein inside the particles, which affects intensity of the scattered light (Le Bon et al 1999).

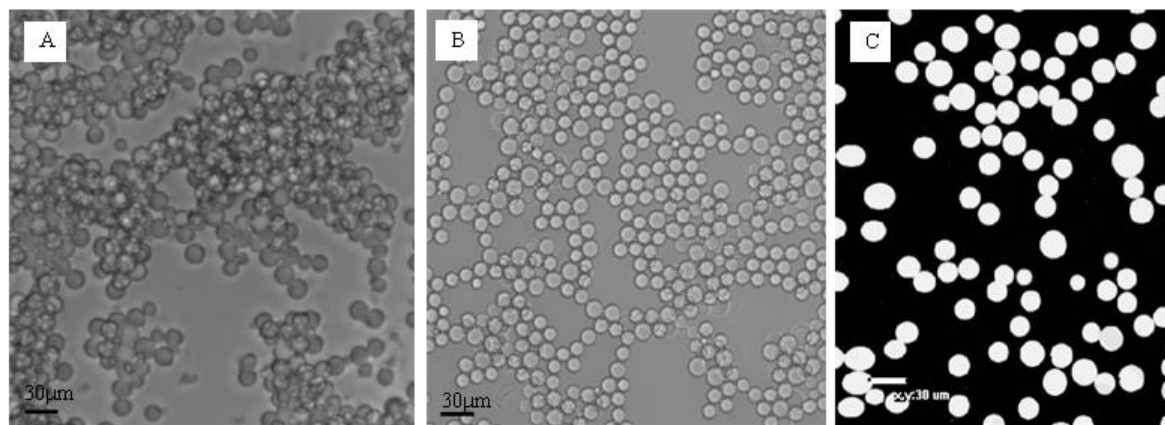


Figure 4. Whey protein microbeads in n-dodecane before heat treatment (A), after heat treatment at 75°C for 20 min suspended in n-dodecane (B) and confocal image of whey protein microbeads after heat treatment suspended in a milk ultrafiltration permeate (C).

Centrifugation followed by washing of the WPM was performed in order to replace the n-dodecane with MUF. The microbeads were then observed by confocal microscopy (Fig 4C). The size of WPM slightly increased as the solvent phase was changed from n-dodecane to MUF at pH 6.7 as shown in the confocal image in Fig. 4C. This size enlargement was probably due to the osmotic swelling of the WPM when dispersed in an aqueous environment, as reported in the previous study. When transferred into an aqueous environment, due to a higher osmotic pressure outside the beads than inside, water has a tendency to be transferred inside the beads.

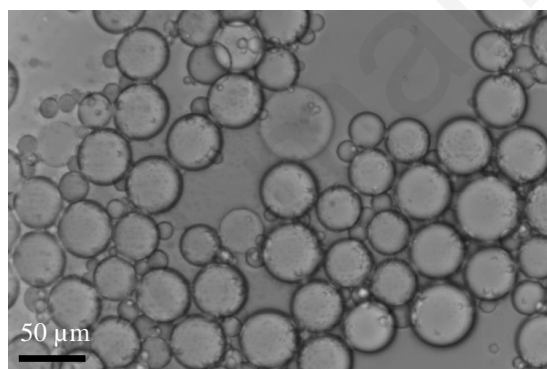


Figure 5. Whey protein microdroplets suspended in n-dodecane with 2.5 % PGPR, produced at the flow rate of n-dodecane and WPI solution and of 1 and 0.2 mL h<sup>-1</sup>, respectively.

Whey protein microbeads, produced by microfluidic system which described in previously, were adjusted to the total protein concentration of 45 g kg<sup>-1</sup> by using milk ultrafiltrate (pure WPA). Acid gelation of WPA was performed at 35 °C with glucono-δ-lactone (GDL) at the concentration of 11 g kg<sup>-1</sup>. Acid gelation was also performed in the system with the presence of casein micelles at the weight ratio of casein/whey protein of 80/20 (mixture) and pure casein system at the same total protein concentration of 45 g kg<sup>-1</sup>. The concentration of GDL used for acid gelation in these systems was proportionally adjusted with their buffering capacity to have the same kinetics, which are 19 and 21 g kg<sup>-1</sup> for mixture and pure casein system, respectively. The sample was stirred for 1 min after GDL addition and the model system was then transferred to the rheometer (AR2000 rheometer, TA Instruments, Guyancourt, France) equipped with a plane-plane geometry (diameter 2 cm) using the oscillatory mode at 35°C, with a frequency of 1 Hz and 0.1% strain. Low density paraffin n-dodecane was added around the sample to prevent evaporation. The gelation pH was defined as the moment when  $G' > 1$  Pa,

Fig. 6 shows storage modulus (Pa) as a function of time (min) recorded during acid gelation at 35°C of three model systems namely pure WPA, mixture and pure casein system, respectively. The gel formation of the three systems seemed to occur at the same time as indicated by the  $G'$  value  $> 1$  Pa occurred at the same moment. Acid gelation of pure WPA system shows a lowest final  $G'$  value among the other two systems. Furthermore, the final  $G'$  value of the mixture system was lower than the pure casein system, this indicate that the presence of WPA in the system reduces the gel firmness. This phenomenon is probably due to the fact that WPA has low functional properties or if the functional properties of WPA are exists, they were not optimally used due to large size of microbeads which mean low contact points between microbeads.

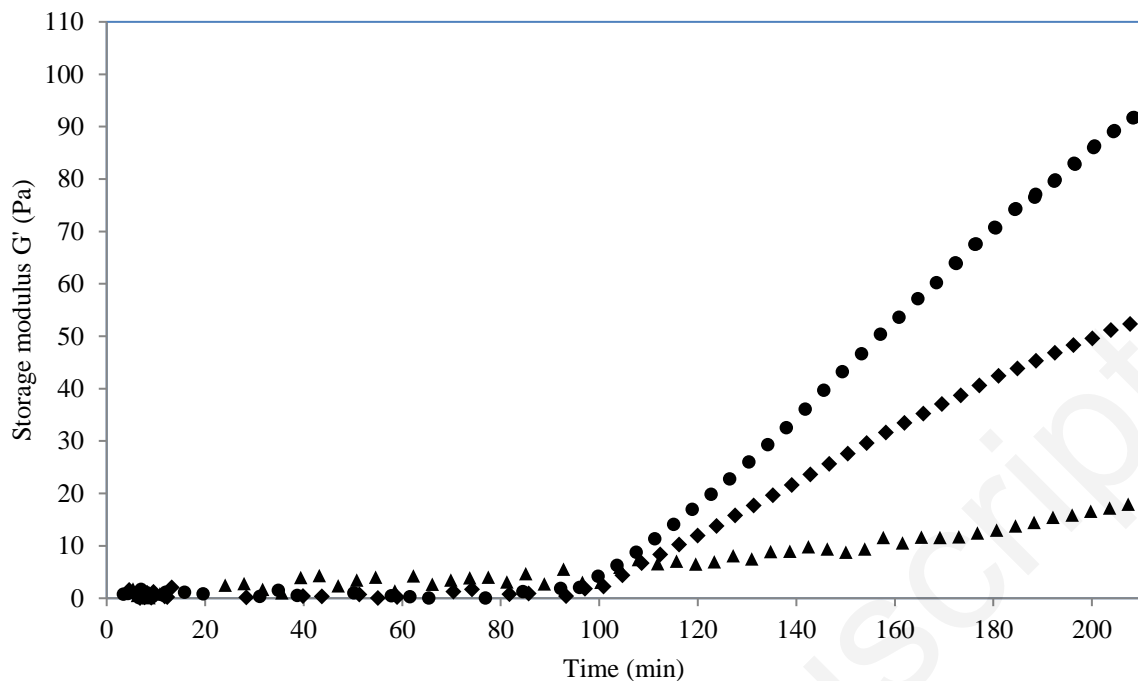


Figure 6. Storage modulus (Pa) as a function of time (min) recorded during acid gelation at 35°C of different systems of protein particles. Acid gelation of pure whey protein microbeads (▲), mixture of whey protein microbeads with casein micelles at the ratio of casein/whey protein of 80/20 (◆) and pure casein system (●) at constant total protein concentration of 45 g kg<sup>-1</sup>.

In conclusion, this findings show that it is possible to design a flow-focusing microchannel to produce monodisperse WPM that were stable in an aqueous medium, namely in the solvent phase of milk at pH 6.7. Two types of continuous phases with different viscosities were tested and the results show that the size and polydispersity of WPM depended on the viscosity and flow rate ratio of the two immiscible liquids. This proof of concept therefore opens perspectives to design model objects to investigate the physical behaviour of protein particles as they form a gel. It could also open perspectives to generate bioencapsulation products, colloidal beads for mechanical or single interaction studies by nano-indentation or atomic force spectroscopy, standard protein beads to calibrate light scattering equipment or to graft surfaces, etc. Changing the protein concentration, or reticulating the proteins inside the beads may provide a mean to prepare droplets of similar dimension but different firmness. However, producing WPM by microfluidic system is time-consuming and do not seem appropriate for applications where large amounts are needed.

## REFERENCES

- Akkermans C, van der Goot AJ, Venema P, et al (2008) Properties of protein fibrils in whey protein isolate solutions: microstructure, flow behaviour and gelation. *Int Dairy J* 18:1034–1042.
- Alting AC, Hamer RJ, de Kruif CG, Visschers RW (2000) Formation of disulfide bonds in acid-induced gels of pre-heated whey proteins isolate. *J Agric Food Chem* 48:5001–5007.
- Anna SL, Bontoux N, Stone HA (2003) Formation of dispersions using “flow focusing” in microchannels. *Appl Phys Lett* 82:364–366. doi: 10.1063/1.1537519
- Beaulieu M, Pouliot Y, Pouliot M (1999) Thermal aggregation of whey proteins in models solutions as affected by casein/whey protein ratios. *J Food Sci* 64:776–780.
- Dawson B, Taylor J, Favalaro EJ (2008) Potential benefits of improved protein intake in older people. *Nutr Diet* 65:151–156. doi: 10.1111/j.1747-0080.2008.00250.x
- De la Fuente MA, Singh H, Hemar Y (2002) Recent advances in the characterisation of heat-induced aggregates and intermediates of whey proteins. *Trends Food Sci Technol* 13:II.
- Doherty SB, Gee VL, Ross RP, et al (2011) Development and characterisation of whey protein micro-beads as potential matrices for probiotic protection. *Food Hydrocoll* 25:1604–1617.
- Donato L, Guyomarc’h F (2009) Formation and properties of the whey protein/casein complexes in heated skim milk - a review.
- Engl W, Backov R, Panizza P (2008) Controlled production of emulsions and particles by milli- and microfluidic



- techniques. *Curr Opin Colloid Interface Sci* 13:206–216.
- Foegeding EA, Davis JP, Doucet D, Mc Guffey MK (2002) Advances in modifying and understanding whey protein functionality. *Trends Food Sci Technol* 13:151–159.
- Janhoj T, Ipsen R (2006) Effect of pre-heat treatment on the functionality of microparticulated whey protein in acid milk gels. *Milchwissenschaft* 61:131–134.
- Le Bon C, Nicolai T, Durand D (1999) Growth and structure of aggregates of heat-denatured  $\beta$ -Lactoglobulin. *Int J Food Sci Technol* 34:451–465. doi: 10.1046/j.1365-2621.1999.00310.x
- Lucey JA, Tamehana M, Singh H, Munro PA (1998) Effect of interactions between denatured whey proteins and casein micelles on the formation and rheological properties of acid skim milk gels. *J Dairy Res* 65:555–567.
- Madureira AR, Tavares T, Gomes AMP, et al (2010) Invited review: Physiological properties of bioactive peptides obtained from whey proteins. *J Dairy Sci* 93:437–455. doi: 10.3168/jds.2009-2566
- Marangoni AG, Barbut S, McGauley SE, et al (2000) On the structure of particulate gels - the case of salt-induced cold gelation of heat-denatured whey protein isolate. *Food Hydrocoll* 14:61–74.
- Mc Meekin TL, Groves ML, Hipp NJ (1964) Refractive Indices of Amino Acids, Proteins, and Related Substances. In: Stekol JA (ed) *Amino Acids Serum Proteins*. AMERICAN CHEMICAL SOCIETY, WASHINGTON, D.C., pp 54–66
- Morand M, Dekkari A, Guyomarc'h F, Famelart MH (2012) Increasing the hydrophobicity of the heat-induced whey protein complexes improves the acid gelation of skim milk. *Int Dairy J* 25:103–111.
- Morand M, Guyomarc'h F, Famelart MH (2010) Changing the isoelectric point of the heat-induced whey protein complexes affect the acid gelation of skim milk.
- Morand M, Guyomarc'h F, Famelart MH (2011) How to tailor heat-induced whey protein/-casein complexes, as a means to investigate the acid gelation of milk - a review. *Dairy Sci Technol* 91:97–126.
- Nicolai T, Britten M, Schmitt C (2011)  $\alpha$ -Lactoglobulin and WPI aggregates: Formation, structure and applications. *Food Hydrocoll* 25:1945–1962.
- Nogawa T, Ito N, Watanabe H (2010) Dynamical study of a polydisperse hard-sphere system. *Phys Rev E* 82:021201. doi: 10.1103/PhysRevE.82.021201
- Patocka G, Jelen P, Kalab M (1993) Thermostability of skimmilk with modified casein/whey protein content. *Int Dairy J* 3:35–48.
- Saglam D, Venema P, de Vries R, et al (2011) Preparation of high protein micro-particles using two-step emulsification. *Food Hydrocoll* 25:1139–1148.
- Santiso E, Mueller EA (2002) Dense packing of binary and polydisperse hard spheres. *Mol Phys* 100:2461–2469. doi: 10.1080/00268970210125313
- Sollich P, Wilding NB (2011) Polydispersity induced solid-solid transitions in model colloids. *ArXiv11034734 Cond-Mat*
- Torres IC, Janhoj T, Mikkelsen BO, Ipsen R (2011) Effect of microparticulated whey protein with varying content of denatured protein on the rheological and sensory characteristics of low-fat yoghurt. *Int Dairy J* 21:645–655.
- Van Riemsdijk LE, Snoeren JPM, van der Goot AJ, et al (2011) New insights on the formation of colloidal whey protein particles. *Food Hydrocoll* 25:333–339.
- Xia YN, Whitesides GM (1998) Soft lithography. *Angew Chem-Int Ed* 37:551–575.