Carbohydrate Polymers 86 (2011) 433–440

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

The influence of functional properties of different whey protein concentrates on the rheological and emulsification capacity of blends with xanthan gum

G. Panaras, G. Moatsou, S. Yanniotis, I. Mandala*

Agricultural University of Athens, Department of Food Science and Technology, 75 Iera Odos, 11855, Votanikos, Athens, Greece

ARTICLE INFO

Article history:
Received 13 March 2010
Received in revised form 23 February 2011
Accepted 28 March 2011
Available online 5 April 2011

Keywords:
Whey proteins
Xanthan
Gelation
Rheology
Emulsification capacity

ABSTRACT

The object of the study was the investigation of commercial WPCs with different protein contents (65 or 80%) and origins. The properties investigated were their solubility, oil-holding capacity, protein profiles, rheological properties and emulsification capacity in the presence of xanthan. The native protein profile of WPCs varied according to the source of the whey. Xanthan or lactose addition improved the stability of the pseudo-emulsions prepared. Adding lactose resulted in a greater amount of absorbed protein on the fat globule surface. Stabilization capacity also depended on the protein source; less pronounced effects were noticed by using proteins of different initial concentrations. The initial protein content influenced mainly the gelation process. A 15% increase in the protein amount (65 or 80%) resulted in threefold greater G' values at 85 °C. Composition and, more specifically, lactose or calcium divalent ions in the original protein samples influenced gelation temperature and further aggregation formation.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Whey proteins are composed of β-lactoglobulin, α-lactalbumin bovine serum albumin, immunoglobulins and several minor proteins and enzymes (Prasad, Butkowski, Hamilton, & Ebner, 1982). β-Lactoglobulin (β-lg) and α-lactalbumin (α-la) account for 70% of the total protein content and are responsible for hydration, gelation, emulsification and foaming properties of whey protein isolates (Tosi, Canna, Lucero, & Re, 2007). Whey proteins are globular in shape and when present in a solution in their native state they exhibit a low viscosity. Above a critical temperature (usually above 60 °C) aggregates are formed, while a gel can be formed at higher concentrations. Initially, unfolding of the protein (particularly β-lactoglobulin) takes place. Thereafter, "soluble" aggregates form a network that is stabilized by hydrophobic interactions and disulfide bonding (Aguilera & Rojas, 1997; McSwiney, Singh, & Campanella, 1994).

Whey protein concentrates (WPCs) are widely used in the food industry, because they are considered highly functional and nutritional ingredients. Their functionality is related to their protein content and composition. In recent years, whey protein ingredients isolated from bovine milk are extensively used as emulsifiers in a wide variety of emulsion-based food products, including beverages, frozen desserts, ice creams, sport supplements, infant formulae and salad dressings (McClements, 2004). However, whey proteins' sensitivity to environmental conditions, such as pH, ionic strength, and/or temperature can limit their applications (Perez, Carrara, Sánchez, Santiago, & Patino, 2009). In many foods, proteins and polysaccharides (PS) coexist and their interactions influence product quality. The parallel use of various PS could enhance protein attributes through different protein–polysaccharide interactions and problems resulting from the use of proteins alone can be overcome (Dickinson, 2003; Syrbe, Bauer, & Klostermeyer, 1998). Thus, the investigation of WPCs–PS mixtures is of high interest especially regarding the stability and texture modification that they may provide in the final products.

Among different polysaccharides, xanthan gum (XG) is widely used in many products. XG is an anionic polyelectrolyte mainly considered to be non-gelling, which is used for the control of viscosity due to the tenuous associations endowing it with weak-gel shear-thinning properties. It hydrates rapidly in cold water without lumping and gives a relatively high viscosity, which makes it useful as thickener, stabilizer, emulsifier and foaming agent. Furthermore, the relatively low viscosity at high shear rate makes it easy to mix, pour, and swallow (Kang & Petit, 1993; Nussinovitch, 1997).

XG is a non-adsorbing polysaccharide, and thus in an emulsion containing both whey proteins and xanthan gum, it does not adhere to whey protein-stabilized droplet surfaces. One of the destabilization phenomena that can occur during storage is depletion flocculation. When oil droplets come closer due to Brownian motion, the region between them is depleted of XG leaving only the solvent. The solvent between the droplets tends to dif-

* Corresponding author at: Laboratory of Engineering, Processing and Preservation of Foods, 75 Iera Odos, 11855 Votanikos, Athens, Greece. Tel.: +30 210 529 4692; fax: +30 210 529 4692.
E-mail address: imandala@aua.gr (I. Mandala).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.03.044

Table 1
Chemical composition of the WPCs used in the study, according to the manufacturers and calculated values for ash content.

Sample	Protein (%)	Fat (%)	Ash (%)/given by manufacturer	Lactose (%)
WP1	78 ± 2	Max. 8	2.74/max. 3.5	7 ± 2
WP2	Min. 65	11–17	4.01/max. 5	Max. 10
WP3	65 on d.m.	3.8	3.4/3.3	23
WP4	65 ± 1	Max. 4	3.29/max. 3.5	Min. 23
WP5	80 ± 1	Max. 8	3.4/max. 3.5	Max. 4

fuse in order to reduce the XG concentration gradient, causing aggregation of droplets by weak reversible and flexible bonds (Radford & Dickinson, 2004; Sun, Gunasekaran, & Richards, 2007). This phenomenon often occurs due to the coexistence of whey protein and XG and depends on the iso-electric point (Ip) of whey proteins. Above Ip (pH >6.0) both protein and XG are negatively charged causing an electrostatic protein–polysaccharide repulsion, while below Ip oppositely charged polymers cause an electrostatic protein–polysaccharide attraction that leads to bridging-flocculation (Blijdenstein, van Winden, van Vliet, van der Linden, & van Aken, 2004). Concerning the viscoelastic properties of an emulsion containing both whey protein isolate (WPI) and XG, shear-thinning behaviour was noticed, while emulsion viscosity was unaffected by the amount of unabsorbed WPI in the aqueous phase (Sun et al., 2007).

Concerning WPCs alone, both unheated and heat-treated WPC suspensions presented mechanical spectra similar to those of viscoelastic fluids. However, after the created aggregates are heated viscosity increases. Also, polymers with large size and effective volume fraction have higher viscosity (Bryant & McClements, 1998; Meza, Verdini, & Rubiolo, 2009). The initial mineral composition of WPs also contributes considerably to aggregates formation and to the final gel structure under heating (Schmitt, Bovay, Vuilliomenet, Rouvet, & Bovetto, 2011). Several commercial WPCs have been analysed for functionality, protein aggregation and physico-chemical properties (e.g. Morr & Foegeding, 1990). However, in order to understand the factors responsible for the variability in WPC products, it would be desirable to obtain more detailed information on their behaviour. The objectives of this study were to investigate: (a) the functional properties of WPCs having different protein contents and origins regarding milk kind and (b) their interactions with xanthan, specifically their emulsification capacity and their viscosity at different shear rates. WPCs seem to have a different behaviour than WPIs. This research is focused on WPCs and could be further used in order to understand some differences between the two WP forms.

2. Materials and methods

2.1. Whey protein concentrates (WPCs)

Commercial WPCs produced by ultrafiltration and spay drying were used. Chemical composition and coding of the samples are presented in Table 1.

- WP1, i.e. Lacprodan®-80 with 78 ± 2% protein content (Arla Foods Ingredients Amba-Denmark)
- WP2, i.e. Nutrilac®DR-7015 with 65% minimum protein content (Arla Foods Ingredients Amba-Denmark)
- WP3, i.e. WHEYPRO65 produced from sheep and goat's whey with 65% protein content on dry matter (Hellenic Protein S.A., Greece)
- WP4, i.e. PROMIL WPC 65 produced from sheep and goat's whey with 65 ± 1% protein content (Velco-Industrial Ingredients, Greece)
- WP5, i.e. PROMIL WPC 80LF with 80 ± 1% protein content (Velco-Industrial Ingredients, Greece)

2.2. Evaluation of the protein profiles of WPCs and calcium content determination

Aqueous solutions of WPCs 0.4% (WP1 and WP5) and 0.6% (WP2, WP3, and WP4) were analysed in duplicate by RP-HPLC on a Vydac C4 214 TP 5415 column (Separation Group, Hesperia, CA 92345, USA) following the methodology described by Moatsou, Hatzinaki, Kandarakis, and Anifantakis (2003). The elution times of native α-lactalbumin (α-la), β-lactoglobulin (β-lg) and caseinomacropeptide (CMP) were verified by analysis of purified standard proteins; their quantification was based on the area of the relevant peaks. The international standard method (ISO/IDF, 2007) was used for the determination of calcium content. Initially, a decomposition of organic matter including pre-ashing and ashing took place. Thereafter, a wet digestion was carried out using nitric acid and treatment with $LaCl_3$ solution to suppress phosphate interference and the ionization of elements in the flame atomic absorption spectrometric measurement (FAAS) (Atomic Absorption Spectrometer, AAnalyst 200, PerkinElmer, Waltham, MA, USA), before the final test procedure in FAAS. A blank test using the same procedure was also carried out. The wavelength of the spectrometer was set as 422.7 mm for Ca. The mean of three absorbance values was calculated and the mean absorbance value of the zero solution subtracted. For each test solution, the measurements were repeated three times and the average of the absorbance values was calculated. Calcium content was calculated as the most important macromineral influencing WP properties. To create the reference curves, analytical grade reagents were used and standard working solutions of calcium were prepared from the respective chlorides. The ash content of different WPCs was also calculated as shown in Table 1.

2.3. Oil-holding capacity and solubility

Five milliliter of olive oil was mixed with 0.25 g WPCs. The mixture was centrifuged at 4000 rpm for 5 min after vortexing for 30 s and left undisturbed for 5 min. The oil layer was carefully separated from the top of the tube using a syringe. The difference between the weight of oil added and the weight of oil separated at the top of the tube was calculated as a percentage to give oil-holding capacity.

For the determination of WPC solubility, about 0.5 g of WPC was added in a tube containing 5 ml of phosphate buffer pH 7.5. After vortexing for 10 s, the tube containing the sample solution was left undisturbed for 5 min. Then, the tube was centrifuged at 4000 rpm for 10 min and the liquid phase with the components solubilized in it was removed with a syringe (Ji & Haque, 2003). The sample tube was dried in an air drying oven for 2 h at 105 °C and weighed. Four replicas were used for each measurement. Solubility was determined by Eqs. (1) and (2).

$$\text{Insolubility}\ (\%) = \frac{\text{weight of dried insoluble sample}}{\text{sample weight}} \times 100 \quad (1)$$

$$\text{Solubility}\ (\%) = 100 - \text{Insolubility}\ (\%) \quad (2)$$

2.4. Emulsification capacity in the presence of xanthan

Stock solutions of WPC were prepared in order to determine emulsification capacity. WPC powder was dispersed into a phosphate buffer solution pH 7.5. The prepared samples (2%, w/w) were stirred for 2 h, centrifuged at 1000 rpm for 30 min to eliminate air bubbles and insoluble substances. Supernatants were collected and stored at 4 °C overnight to ensure full hydration of the biopolymers (Li, Ould Eleya, & Gunasekaran, 2006). Xanthan stock solutions (0.1%, w/w) (G1253-100g, Sigma–Aldrich ChemieGmbH, Germany)

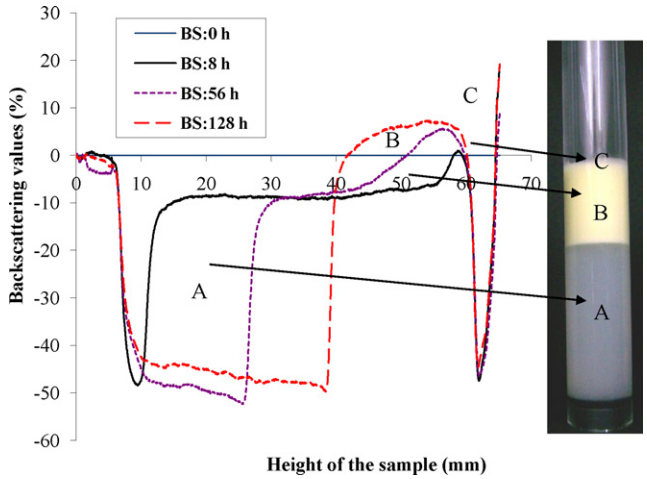


Fig. 1. Phase separation of pseudoemulsion containing WP2 at different time intervals from 0 h to 128 h. Three phases can be seen. (A) Bottom phase (serum), (B) cream separation, and (C) oiling off.

were also prepared following the same procedure as above. The pH of the final solutions was 7.5.

Blends containing WPC (2%, w/w), xanthan (0–0.1%, w/w), and olive oil (20%, v/v) were prepared by mixing all ingredients in a homogenizer (Ultra Turrax T25 Basic, IKA-WERKE, Germany) at 11,000/min for 2 min. Sodium azide (0.02%, w/v) was added as an antimicrobial agent into the final samples. The whole procedure was a modification of another procedure, in which fish oil deriving from menhaden instead of olive oil was used (Sun et al., 2007). As the prepared blends were not real emulsions, they can be characterized as pseudoemulsions and were coded as EWP*i*, where WP*i* represented the type of whey protein used. In order to investigate the effect of lactose on the emulsification capacity of the samples containing whey proteins, 1% or 2% (w/w) lactose (Hydrous lactose, Mallinckrodt Chemical Works, USA) was used. Lactose was added in pseudoemulsions containing WP1, for whose preparation the same procedure as described above was used.

Emulsion stability was measured by a technique based on multiple light scattering, using a near-infrared light source, λ_{air} = 850 nm, over a week cold storage period. The optical characterization of the samples was evaluated using a Turbiscan MA 2000 (Formulation, Toulouse, France). The samples of 6 ml each were placed in tubes. Two detector devices were used in order to monitor light transmitted through the sample (180° from the incident light, transmission sensor), and backscattered by the sample (45° from the incident radiation, backscattering detector) along the height of the tube. The transmitted and backscattered light was presented as a function of the sample height. A reference mode function was used, which subtracts the first curve from the subsequent ones. The change of backscattering profile in the bottom of the samples (serum separation) (Fig. 1) and the change in the thickness of the bottom phase (e.g. evolution change of the separated bottom phase) were observed. A non-linear regression model was fitted to the experimental results of the bottom phase volume (evolution change of lower serum phase thickness) and backscattering change (y values respectively). The models used were of first order (Eqs. (3) and (4)).

$$y = A_1 + y_1 \cdot (1 - e^{-k_1 t}) \quad (3)$$

$$y = A_0 + y_0 \cdot e^{-kt} \quad (4)$$

k_1 is a constant related to volume change rate (h^{-1}) of serum phase (Eq. (3)) and k is a constant related to backscattering change rate (h^{-1}) (Eq. (4)). A_0, A_1, y_0, and y_1 are constants. Three different replicas were used for each composition and the average value was calculated for each formula.

2.5. Rheological properties in the presence of xanthan

The rheological measurements performed were: (a) temperature sweep tests (from 25 to 85 °C) of WPC solutions (20%, w/w) at small oscillatory shear tests and (b) viscosity measurements of WPC (20%, w/w) and xanthan (0.1%, w/w) mixtures at 25 °C. Stock solutions were prepared as above. For viscosity measurements, xanthan solutions (0.1%, w/w) were mixed with WPC stock solutions (20%, w/w) at 1:1 (w/w) ratio for 30 min at 25 °C. A plate-plate rheometer was used. The samples were placed between the plates and allowed to rest for equilibration for 5 min before beginning experiments. A water trap was used in order to avoid water evaporation. Furthermore, paraffin oil was put around the sample. Temperature sweep tests were performed from 25 to 85 °C at a heating rate of 5 °C/min up to 60 °C and 1 °C/min from 60 °C to 85 °C to simulate the effect of heating causing the denaturation of proteins. A strain in the range of linear viscoelastic region was selected that changed in relation to the values found at different, preselected temperatures (25, 60, and 85 °C). Values of storage modulus (G') were recorded. All the tests were replicated three times. In order to determine the gel point, extrapolation method based on a curve fitting was used.

In viscosity measurements experimental values were fitted by the Ostwald de Waele model (Eq. (5)).

$$\sigma = K \cdot (\dot{\gamma})^n \quad (5)$$

K is a consistency index (Pa s^n) and n a flow behaviour index.

2.6. Microscopic observations

For microscopic observations a light microscope was used (Olympus BX40, Japan) to depict interactions among different ingredients. Photos were taken using a video camera (SONY, Hyper HAD, CCD-Iris). To prepare samples, a very thin layer of the diluted sample (1:30) was spread on a microscope slide and 1–2 drops of the colouring substance were added. After 5 min, before starting observations, a coverglass was placed on the stained sample.

The colouring substances used were:

Oil red O from Sigma (St. Louis, Mo, USA): It colours the oil droplets of the samples with a yellow-orange colour.
Light green SF yellowish from Sigma (St. Louis, Mo, USA): It colours the proteins of the samples.
Image-Pro Plus (Image-pro 7.0, MediaCybernetics, MD, USA) was used for the measurement of droplet diameter in random photos converting pixels to μm. Qualitative data were obtained and the mean arithmetic droplet diameter was calculated (D_a), as well as the mean volume-surface diameter defined as: $D_{vs} = \sum N_i D_i^3 / \sum N_i D_i^2$.
D_i is the diameter equal to the midpoint of the size range and N_i the number of droplets in that range (Schott & Royce, 1983).

3. Results and discussion

3.1. Protein profile and ionic calcium content

The quantification results of RP-HPLC analysis based on the chromatographic area of the relevant peaks are shown in Table 2. The calcium concentration as calculated by FAAS is shown in the same table. Greater values were noticed for WP2 samples. It is evident that the protein composition of WPCs from sheep and goat whey (WP3 and WP4) differs with regard to high CMP and low α-la contents. On the other hand, the quantification results regarding the relative protein content of WPCs from ewe's milk were similar regardless of their total protein content, i.e. 65 or 80%. Thus, the native protein profile of WPCs varied according to the source

Table 2
Quantification of native whey proteins of WPC samples by means of RP-HPLC and calcium composition. Results are expressed as percentages of the total chromatographic area of the native protein peaks of the RP-HPLC profiles.

Sample	Protein (%)	CMP[a] (%)	α-la[b] (%)	β-lg[c] (%)	β-lg/α-la	β-lg/CMP	α-la/CMP	Ca (mg/g) in wet powder
WP1	80	8.5	26.2	65.3	2.5	7.7	3.1	3.48
WP2	65	8.5	27.1	64.4	2.4	7.6	3.2	4.65
WP3	65	14.2	19.7	66.1	3.4	4.7	1.4	3.25
WP4	65	12.7	21.4	66.0	3.1	5.2	1.7	3.60
WP5	80	8.6	27.2	64.2	2.4	7.5	3.2	3.10

[a] Caseinomacropeptide.
[b] α-Lactalbumin.
[c] β-Lactoglobulin.

of whey and not according to the total protein content of the final product.

3.2. Oil-holding capacity and solubility

According to the data presented in Table 3, the greatest oil-holding capacity was observed in WP2 solution, which also had the lowest solubility value. Solubility depends on the level of protein denaturation and aggregation of the samples and less heat denaturated samples have also a high solubility (Patel & Kilara, 1990). Thus, solubility is strongly influenced by both the source and treatment of the whey used for their preparation, i.e. cheese making conditions and thermal treatment of the whey (De la Fuente, Hemar, Tamehana, Munro, & Singh, 2002).

3.3. Emulsification capacity in the presence of xanthan

Whey proteins in O/W emulsions form an interfacial protein film around oil droplets that influences the steric and electrostatic properties of the repulsive forces between lipid droplets, thus preventing flocculation and coalescence mechanisms that lead to creaming and syneresis (also known as "wheying off") (Leman & Kinsella, 1989; McClements, 2004; Prajapati, Gupta, Patel, & Patil, 1990). The phase separation kinetics and specifically the volume of the down phase (or thickness change) (Seber & Wild, 1989) presented in Fig. 2a differed in relation to: (a) the total protein concentration of the initial commercial samples and (b) the source of WPC used for the same initial concentration.

WPCs from the same producer but of different initial protein contents (i.e. 65 or 80%), exhibited differences probably due to the fact that the fraction and quantity of proteins they contain influenced their emulsification capacity in a different way. Thus, the higher the protein content of WPC (i.e. 65 or 80%), the slower the separation of the down phase (e.g. comparisons between WP1–WP2 or WP4–WP5) (Fig. 2a). However, differences were slight.

Furthermore, proteins from different production companies but of similar protein content presented a different emulsification ability in the final emulsion. EWP2, EWP3 and EWP4 presented a different stability, i.e. EWP2 presented the greatest stability and the EWP4 the lowest. In addition, the equilibrium volume of the control sample (EWP1a) prepared without xanthan was reached in different time intervals (from about 2 to 5 days) for all samples, depending on the WPC sample used. The fastest phase separation was observed in EWP4, the slowest in EWP1. Equilibrium in the presence of xanthan was not reached in any of the investigated samples for the storage period of 6 days. Furthermore, the volume of the separated phase was 65.4% for EWP1a, while it was 61% for the respective sample with xanthan (EWP1) (Fig. 2a).

According to the separation kinetics as expressed by turbidity change (backscattering values) (Fig. 2b), similarities were observed in respect to the values obtained for the volume of the down phase. EWP2 was the most stable sample and the less stable was that containing WP4 or WP5 (Fig. 2b). Destabilization kinetic constants during storage are presented in Table 4. Mean values are presented. Their standard deviations values rose from 2 to 12%.

Table 3
Oil-holding capacity and solubility of whey protein concentrates. Standard deviation values are in parentheses.

Samples	Oil-holding capacity (%)	Solubility (%)
WP1	14.5 (0.10)	97.8 (0.16)
WP2	18.3 (0.20)	82.8 (0.15)
WP3	14.0 (0.11)	96.8 (0.14)
WP4	14.3 (0.12)	97.7 (0.13)
WP5	14.9 (0.13)	99.2 (0.15)

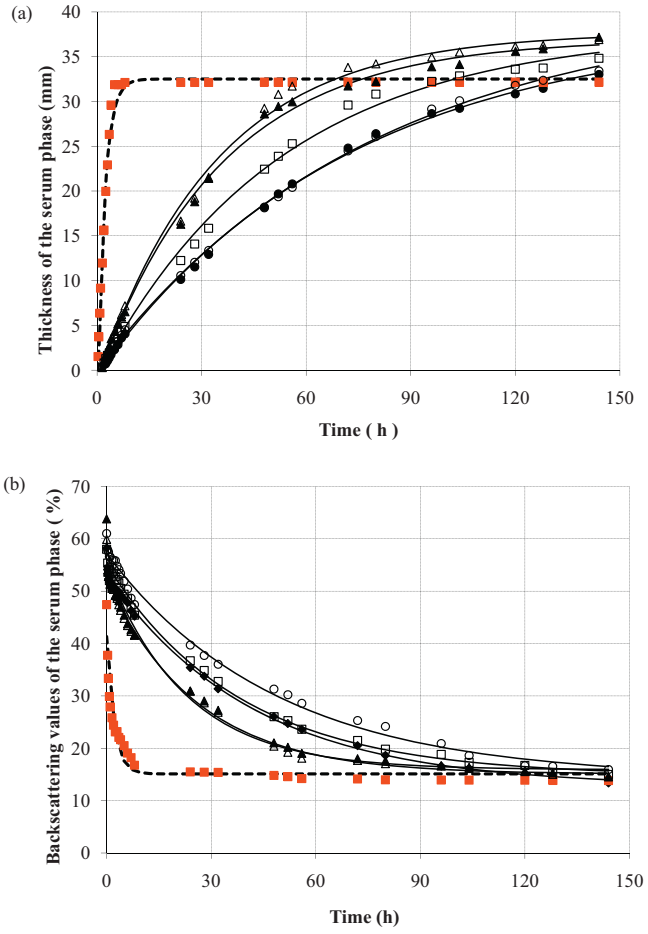


Fig. 2. (a) Volume change kinetics of pseudoemulsions (EWP*i*) containing olive oil, xanthan gum (0.1%, w/w) and various WPCs and (b) backscattering change kinetics of the same pseudoemulsions (●, WP1; ○, WP2; □, WP3; △, WP4; ▲, WP5; ■, WP1a).

Table 4
Kinetics' constants of destabilization process of pseudoemulsions containing different types of WPCs, olive oil and xanthan gum (0 or 0.1%, w/w) and lactose at two different concentrations.

Samples	Thickness				Backscattering			
	A_1	Y_1	k_1 (h^{-1})	R^2	A_0	Y_0	k (h^{-1})	R^2
EWP1a	−4.80	37.32	0.474	0.986	15.11	26.21	0.480	0.938
EWP1	−0.45	38.78	0.014	0.999	12.69	40.56	0.024	0.995
EWP1La1	0.68	35.61	0.005	0.985	27.35	24.65	0.025	0.918
EWP1La2	0.11	36.61	0.005	0.983	30.38	21.29	0.035	0.910
EWP2	0.005	40.32	0.013	0.999	14.29	42.85	0.021	0.992
EWP3	−0.76	38.70	0.019	0.997	14.72	40.33	0.026	0.996
EWP4	−1.31	39.00	0.029	0.997	15.53	38.03	0.043	0.990
EWP5	−0.96	37.94	0.028	0.998	15.90	38.46	0.044	0.982

k_1 values indicate that bottom phase thickness increased at a lower rate in samples EWP2 and EWP1, whereas approximately a double thickness increase rate was noticed in EWP4 and EWP5 samples. The presence of a stabilizer seems to be quite important, since k_1 value is only 3% of that without xanthan (comparison between EWP1 and EWP1a). Backscattering kinetics were similar to those of thickness change during storage. However, k value of EWP1 was 5% of that of control samples without xanthan, e.g. a slightly greater value was observed than that mentioned above for thickness change. Furthermore, differences of k values among samples investigated were more limited than those of k_1 values among the respective samples. This could mean that backscattering values changed a little slower than thickness ones did. Thickness change is ascribed to fast motion of oil droplets and creaming phenomenon. On the other hand, the slower change of backscattering could be linked to the remaining macromolecules in the continuous phase, e.g. xanthan or unabsorbed whey proteins may move slower than oil droplets.

Regarding samples WP1, WP2 and WP3, that had the same protein and ash content, and considering that fat content did not influence the emulsification capacity of the samples investigated, it could be hypothesized that the high lactose content of some samples (WP3 and WP4 – 23%) compared to the low lactose content of some others (WP1 and WP2) could influence the emulsification capacity of the samples investigated. Thus, the emulsification capacity of samples containing 1% or 2% lactose and WP1 was compared to that of the sample containing only WP1. Thickness and backscattering change during storage time can be seen in Fig. 3a and b. Thickness change significantly decreased by adding lactose (lower k_1 values also – Table 4). It can also be seen that the addition of lactose resulted in samples that had greater backscattering values at equilibrium (more turbid that those without lactose). However, according to k values presented in Table 4, slight differences between the sample with added lactose and that without (EWP1) can be noticed. Thus, although the backscattering values of samples with lactose were much greater than those of the sample without lactose (the constant values of the model were also greater), the rate of backscattering change was similar in all samples. This reinforces the above suggestion that protein can remain on the serum phase during the destabilization process.

A possible explanation of emulsification capacity improvement by adding lactose could be (a) a possible increase in viscosity of the continuous phase when increased protein concentration is present and (b) a greater amount of absorbed protein on the globule surface of fat (Patel & Kilara, 1990). The second assumption seems more probable, as by increasing lactose concentration, no further effect on emulsification capacity is observed. This means that after a critical concentration of protein no further amount can be absorbed by the fat surface and that a further viscosity increase by adding more protein is not observed.

Other factors that affect emulsification properties of WPCs are protein solubility, β-lg concentration, salt content (ionic strength), free sulfydryl content, fat content, pH and presence of other solutes (De Wit, Klarenbeek, & Adamse, 1986; Liao & Mangino, 1987; Patel & Kilara, 1990). Taking into account that the composition of the samples varied considerably (Table 1) their emulsifying behaviour can be attributed to several factors. The α-la, which shows better emulsifying properties than β-lg (De Wit et al., 1986), is present in higher quantity in WP2. Neither the solubility nor the oil holding capacity was related to emulsion stability; WP2 had a high oil-holding capacity and seems to be more effective in emulsion stabilization. Hydrophobicity of these samples may be related to emulsion stability. All other samples had similar oil-holding capacity values and thus differences in their emulsification ability were not evident.

Furthermore, pH values can be especially important for the stability of an emulsion. As the pH of the prepared samples was above Ip, protein–solvent interactions increase. A synergistic effect in WPI–xanthan gel at pH ≥7 for a xanthan concentration 0.1% (w/w) was found (Sanchez, Schmitt, Babak, & Hardy, 1997). However, at pH 7.0, a phase separation phenomenon is more probable due to

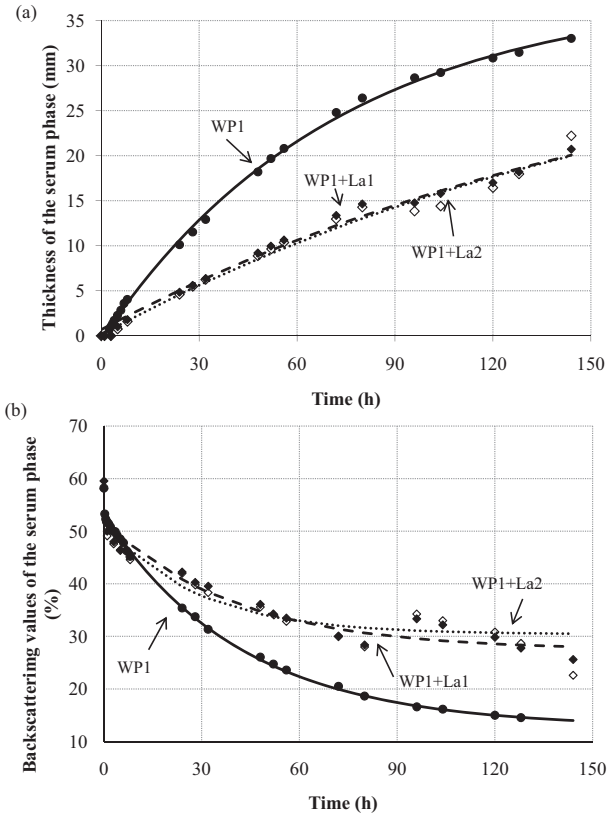


Fig. 3. (a) Volume change kinetics and (b) backscattering change kinetics of pseudoemulsions (EWP1) containing additionally 1% or 2% (w/w) lactose.

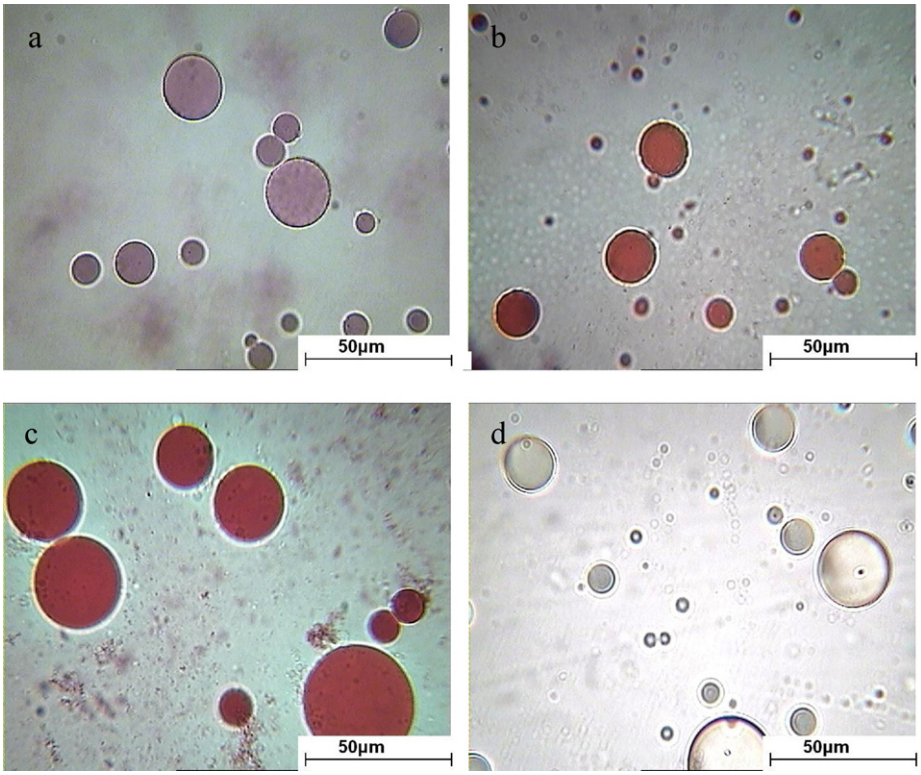


Fig. 4. LMs of pseudo-emulsions containing (a) WP1, (b) WP2, (c) WP4 and (d) WP5 stained with oil red (○) for staining fat globules (a–c) and with light green for staining the protein (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the high negative charge carried by the protein. Furthermore, when the boundary between the serum and the cream layer is very sharp, the separation could be a consequence of flocculation. A more gradual transition between serum and cream phase would be observed due to creaming of the largest droplets in the case of a broad particle size distribution (Neirynck, van Lent, Dewettinck, & van der Meeren, 2007). In our case, it is believed that flocculation was evident, thus the phase separation was very fast. Xanthan gum can cause depletion flocculation (a volume restriction effect) and result in phase separation. At higher added hydrocolloid concentrations, the depletion interactions can be stronger, but creaming is inhibited due to the viscoelastic character of the interconnected regions of emulsion droplets that can become flocculated into a gel-like network (Dickinson, 2009).

Finally, ionic strength can be altered by minerals' presence in the proteins. The presence of mineral ions can alter the net charge on the protein molecules and hence the type of interactions among different macromolecules. Thus, calcium cations at a pH of 6.5 increase the ionic strength and promote the interactions between negatively charge residues through ionic bonding (Britten & Giroux, 2001; Havea, Singh, & Creamer, 2002; Li, Hardin, & Foegeding, 1994). Increasing the ionic strength can result in a more stable emulsion. This can be the case of WP2, which had the greatest calcium concentration (Table 2) and the best emulsification capacity. Any other correlation to calcium concentration cannot be made, because values are quite similar and the previous factors affecting emulsification can dominate.

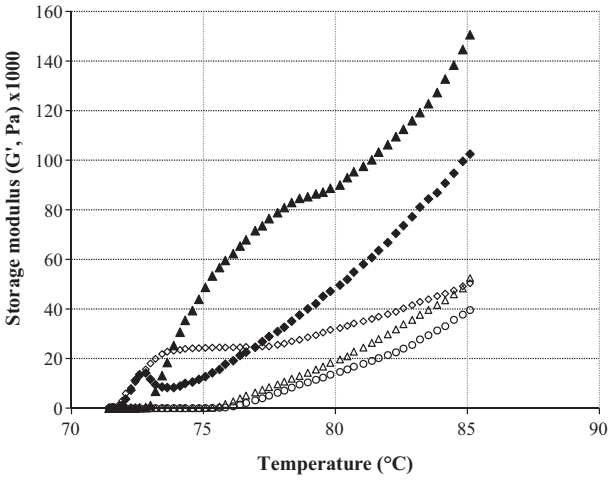
3.4. Microscopic observations

Fig. 4a–d shows the micrographs from light microscope of pseudo-emulsions prepared with different proteins. Fat globules are stained red (Fig. 4a–c) and protein around the droplets can be seen, stained light green (Fig. 4d). The droplet size differs with respect to the WPC used. According to qualitative data, the lowest droplet diameter (arithmetic mean value, $D_a = 8.87 \pm 4.85$ μm, and mean volume-surface diameter, $D_{vs} = 13.79$ μm) was noticed in samples containing WP2 and the highest in samples containing WP4 (arithmetic mean value, $D_a = 17.07 \pm 10.07$ μm, and mean volume-surface diameter, $D_{vs} = 32.38$ μm). The data found were in agreement with those of destabilization kinetics. All other samples had droplet size between the above values with D_a around 11 μm and differences were small. The destabilization is fast and the occurring droplet coalescence influences interpretations. Coalescence is clear in all samples (Fig. 4a–d). The standard deviation values, which are indicative of the distribution and the differences in size population, are greater in WP5 and WP4 and lower in WP2 or WP1 (data not shown).

3.5. Rheological properties

3.5.1. Gelation kinetics

Above a critical temperature (usually above 60 °C) gelation of whey proteins takes place (Aguilera, 1995). Gelation is a result of protein denaturation and aggregation, and its creation rate determines the rate of gelation. The rapidly rising values of storage modulus (G') of Fig. 5 indicate a structural modification of WP-solutions, e.g. a transition from a sol to a gel structure. In this research, gelation occurs at a temperature range from 71 to 76 °C, depending on the type of WPC used. The gelation temperature found by extrapolating the G' values to zero and is presented in Fig. 5. It did not depend on WPC commercial source or initial protein content (i.e. 65 or 80%), but seems to depend on the qualitative composition of samples and specifically on (a) the β-lactoglobulin ratio and (b) the CMP %. Therefore, with regard to WPC with the same protein content (i.e. WP2, WP3 and WP4), the greater the CMP % values the higher the gel point. The same was also true for the β-lg/α-la ratio.



Samples	T_{gel} (°C)	G'_{max} × 1000 at 85°C (Pa)
WP1	72.53[ab] (0.78) / 73.96 (0.70)	100.72 (1.75)
WP2	72.08[a] (0.59)	48.58 (1.77)
WP3	75.20[b] (0.88)	43.42 (3.84)
WP4	75.06[b] (1.06)	54.94 (2.48)
WP5	73.24[ab] (0.23)	152.26 (1.69)

Samples in the same column with different letters differ significantly at $p<0.05$

Fig. 5. Influence of temperature on storage modulus (G') of various WPC solutions (20%, w/w). (●, WP1; ○, WP2; □, WP3; △, WP4; ▲, WP5).

The greatest increase rate of G' was observed in WP5 samples and the lowest in WP3. WP2 presented a great increase rate of G' initially and reached plateau values thereafter. On the other hand, WP1 presented a relatively fast G' increase (at 72.5 °C). In this sample, a first peak value was observed, a drop thereafter and a second G' increase at 74 °C (Fig. 5). The greatest final G' values at 85 °C were noticed in WP5 and the lowest in WP3. Similar final G' values to WP3 were observed in WP4 and WP2. The final G' values indicate the final elastic character of the samples, which was clearly related to the protein concentration of the products. For a 15% increase in the total protein amount in the commercial sample, approximately threefold greater G' values were observed (e.g. WP4 and WP5 values). Therefore, the composition of the protein fraction of WPCs did not affect the increase rate of G', unlike what was observed with regard to gelation temperature.

The β-lg content of WPCs is linked to their gelling ability (De Wit et al., 1986), since it is the most important protein for gelation (Mulvihill & Kinsella, 1987), whereas CMP is detrimental to gel strength and water holding properties (Velth & Reynolds, 2004). Although the β-lg content of samples WP3 and WP4 was higher than that of WP2, their gelation temperature was higher. The explanation for this behaviour could be the origin of WP3 and WP4, which were prepared from sheep and goat whey. Furthermore, a possible explanation could be the process followed to isolate WPCs, which could have resulted in proteins' denaturation in the case of WP3 or WP4. Additionally, the lactose content, which protects β-lg from denaturation (De Wit, 1989), is much higher in WP3 and WP4 compared to WP2.

The role of lactose is also evident by comparing the behaviour during gelation of samples WP1 and WP5, which had the same origin and protein content. WP5, that had the lowest lactose content, had the highest G' values during heating. Solubility could be also linked to the gel strength of samples investigated (Jost, 1993). WP5 had the greatest solubility and the greatest gel strength values at heating.

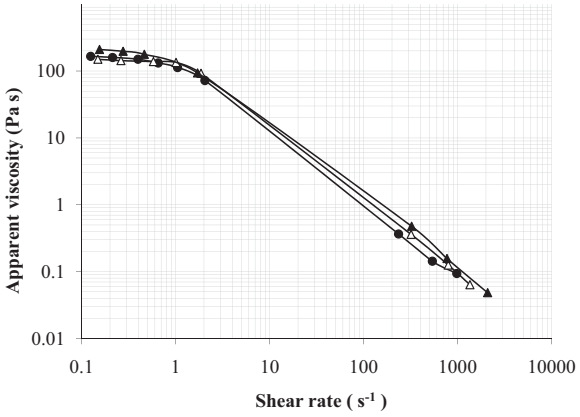


Fig. 6. Viscosity values of various WPCs (20%, w/w)–xanthan (0.1%, w/w) blends (●, WP1; △, WP4; ▲, WP5).

Concerning the role of Ca^{2+} ions, microgel formation can be attributed to the mineral composition. Furthermore, minerals contribute to the formation of homogeneous whey protein microgels (Schmitt et al., 2011).

Calcium also induces protein aggregation. This, apart from the assumptions above, can account for the low T_{gel} found in WP2 sample, which had the greatest Ca^{2+} concentration. Specifically, calcium divalent ions influence both the initial gelation step, when polypeptide chain unfolding takes place, because they stabilize them, and the gelation thereafter, when inter- and/or intramolecular cross-links between ionic carboxyl groups are bridged by divalent ions (Britten & Giroux, 2001; Havea et al., 2002; Li et al., 1994). This can justify the more pronounced elastic character of WP2 across the whole temperature range compared to that of samples (WP4 and WP3) of lower Ca^{2+} concentration.

Furthermore, when the calcium content increases, protein aggregates become more denatured. More "insoluble" aggregates are formed, whose structure is changed from fine to thicker protein strands (Barbut, 1995; Hollar, Parris, Hsieh, & Cockley, 1995), but this cannot be confirmed by the present study. The Ca^{2+} concentration in the rest of the samples is quite similar and cannot be correlated to the gelation process.

Finally, WP2 of low solubility index had a different behaviour compared to the rest of the samples as it was also observed in the case of emulsion stability. However, differences among solubility values of all other samples were limited and general conclusions should be avoided.

3.5.2. *Viscosity measurements in the presence of xanthan*

The mean viscosity values of blends containing xanthan–whey proteins are presented in Fig. 6. The mean standard deviation of the values obtained was about 8%. All samples presented a shear-thinning behaviour, but differences of viscosity evolution among samples are slight. According to Mleko (2004) protein suspensions can show shear-thinning or shear-thickening behaviour depending on the sample. The presence of xanthan is significant, since like most high-molecular-weight polymers, xanthan shows a pseudoplastic behaviour due to its semi-rigid conformation and induces pseudoplasticity in a mixture with proteins (Kang & Petit, 1993; Mandala, Savvas, & Kostaropoulos, 2004; Nussinovitch, 1997). However, at low shear rate, whey proteins–xanthan mixtures presented a Newtonian-like behaviour. Emulsions prepared containing WPC can present a Newtonian behaviour at a relatively high oil mass fraction ($\varphi = 0.20–0.60$), ascribed to non-flocculated molecules according to Manoi and Rizvi (2009).

The n values found by applying the Ostwald de Waele model confirmed that the samples investigated were pseudoplastic. Some

differences in their consistency and flow index values were found. The order of pseudoplasticity was WP1 > WP5 > WP4 and the respective n values were: 0.57 ± 0.10, 0.71 ± 0.03, and 0.84 ± 0.10. On the other hand, consistency values for the samples investigated followed the order WP5 > WP4 > WP1. The respective values were 112.24 ± 4.8, 102.9 ± 1.12 and 76.45 ± 4.93 (Pa s^n). For all samples, R^2 values of the model applied were about 0.96.

It seems that a thickening effect due to higher viscosity values did not influence the emulsification capacity of the samples investigated. WP1 had a lower consistency but still a greater emulsification capacity than WP5 or WP4.

4. Conclusions

From a technological perspective, the investigation of whey proteins of different protein profiles contributes to understanding their functional properties, i.e. differences in gelation kinetics or in their interactions with the gums. Whey protein concentrates that had similar total protein content had different emulsifying and rheological properties. These properties depended strongly on their protein profile (e.g. protein caseinomacropeptide (CMP) content, α-la/β-lg ratio). Minerals concentration and specifically Ca concentration can also be critical for both emulsification and gelation capacity. WPCs are more complicated blends than WPIs due to the presence of different components and further investigation is needed with respect to the isolation process and the origin of these proteins. The isolation process can strongly influence their denaturation properties and thus their functional properties.

Acknowledgements

The authors would like to thank the producers of WPCs mentioned in Section 2 for donating samples.

References

Aguilera, J. M. (1995). Gelation of whey protein. *Food Technology*, *10*, 83–89.
Aguilera, J. M., & Rojas, G. V. (1997). Determination of kinetics of gelation of whey protein and cassava starch by oscillatory rheometry. *Food Research International*, *30*(5), 349–357.
Barbut, S. (1995). Effect of calcium level on the structure of preheated whey protein isolate gels. *Lebensmittel-Wissenshaft and Technology*, *28*, 598–603.
Blijdenstein, T. B. J., van Winden, A. J. M., van Vliet, T., van der Linden, E., & van Aken, G. A. (2004). Serum separation and structure of depletion and bridging flocculated emulsions: A comparison. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *245*(1–3), 41–48.
Britten, M., & Giroux, H. J. (2001). Acid-induced gelation of whey protein polymers: Effects of pH and calcium concentration during polymerization. *Food Hydrocolloids*, *5*(4–6), 609–617.
Bryant, C. M., & McClements, D. J. (1998). Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. *Trends in Food Science and Technology*, *9*, 143–151.
De la Fuente, M. A., Hemar, Y., Tamehana, M., Munro, P. A., & Singh, H. (2002). Process-induced changes in whey proteins during the manufacture of whey protein concentrates. *International Dairy Journal*, *12*(4), 361–369.
De Wit, J. E. (1989). Functional properties of whey proteins in developments. In P. F. Fox (Ed.), *Dairy chemistry. 4. Functional milk proteins* (pp. 285–321). London/New York: Elsevier Applied Science.
De Wit, J. N., Klarenbeek, G., & Adamse, M. (1986). Evaluation of functional properties of whey protein concentrates and whey isolates. 2. Effects of processing history and composition. *Netherlands Milk and Dairy Journal*, *40*, 41–56.
Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, *17*, 25–39.
Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids*, *23*, 1473–1482.
Havea, P., Singh, H., & Creamer, L. K. (2002). Heat-induced aggregation of whey proteins: Comparison of cheese WPC and acid WPC and relevance of mineral composition. *Journal of Agricultural and Food Chemistry*, *50*, 4674–4681.
Hollar, C. M., Parris, N., Hsieh, A., & Cockley, K. D. (1995). Factors affecting the denaturation and aggregation of whey proteins in heated whey protein concentrate mixtures. *Journal of Dairy Science*, *78*, 260–267.
Ji, T., & Haque, Z. U. (2003). Cheddar whey processing and source. I. Effect on composition and functional properties of whey protein concentrates. *International Journal of Food Science and Technology*, *38*, 453–461.
Jost, R. (1993). Functional characteristics of dairy proteins. *Trends in Food Science and Technology*, *4*, 283–288.
ISO/IDF. (2007). Milk and milk products—Determination of sodium, potassium, calcium and magnesium contents—Method by Flame AAS. In *Joint Standard 119/ISO 8070*. Brussels, Belgium/Geneva, Switzerland: International Dairy Federation/International Standards Organisation.
Kang, K. S., & Petit, D. J. (1993). *Industrial gums. Polysaccharides and their derivatives*. New York/London: Academic Press., pp. 343–397.
Leman, J., & Kinsella, J. E. (1989). Surface activity, film formation, and emulsifying properties of milk proteins. *Critical Review in Food Science and Nutrition*, *28*, 115–138.
Li, H., Hardin, C. C., & Foegeding, E. A. (1994). Thermal denaturation and cation-mediated aggregation of β-lactoglobulin. *Journal of Agricultural and Food Chemistry*, *42*, 2411–2420.
Li, J., Ould Eleya, M. M., & Gunasekaran, S. (2006). Gelation of whey protein and xanthan mixture: Effect of heating rate on rheological properties. *Food Hydrocolloids*, *20*, 678–686.
Liao, S. Y., & Mangino, M. E. (1987). Characterization of the composition, physicochemical and functional properties of acid whey protein concentrates. *Journal of Food Science*, *52*, 1033–1037.
Mandala, I. G., Savvas, T. P., & Kostaropoulos, A. E. (2004). Xanthan and locust bean gum influence on the rheology and structure of a white model-sauce. *Journal of Food Engineering*, *64*(3), 335–342.
Manoi, K., & Rizvi, S. H. (2009). Emulsification mechanisms and characterizations of cold, gel-like emulsions produced from texturized whey protein concentrate. *Food Hydrocolloids*, *23*, 1837–1847.
McClements, D. J. (2004). *Food emulsions: Principles, practice, and techniques*. Florida: CRC Press., pp. 39–82.
McSwiney, M., Singh, H., & Campanella, O. H. (1994). Thermal aggregation and gelation of bovine β-lactoglobulin. *Food Hydrocolloids*, *8*, 441–453.
Meza, B. E., Verdini, R. A., & Rubiolo, A. C. (2009). Viscoelastic behaviour of heat-treated whey protein concentrate suspensions. *Food Hydrocolloids*, *23*, 661–666.
Mleko, S. (2004). Rheological properties of whey products dispersions. *Milchwissenschaft*, *59*, 287–290.
Moatsou, G., Hatzinaki, A., Kandarakis, I., & Anifantakis, E. (2003). Nitrogenous fractions during the manufacture of whey protein concentrates from Feta cheese whey. *Food Chemistry*, *81*, 209–217.
Morr, C. V., & Foegeding, E. A. (1990). Composition and functionality of commercial whey and milk protein concentrates and isolates: A status report. *Food Technology*, *4*, 100–112.
Mulvihill, D. M., & Kinsella, J. E. (1987). Gelation characteristics of whey protein and β-lactoglobulin. *Food Technology*, *41*, 102–111.
Neirynck, N., van Lent, K., Dewettinck, K., & van der Meeren, P. (2007). Influence of pH and biopolymer ratio on sodium caseinate–guar gum interactions in aqueous solutions and in O/W emulsions. *Food Hydrocolloids*, *21*, 862–869.
Nussinovitch, A. (1997). *Hydrocolloid applications. Gum technology in the food and other industries*. UK: Blackie., pp. 144–153.
Patel, M. T., & Kilara, A. (1990). Studies on whey protein concentrates. Foaming and emulsifying properties and their relationships with physicochemical properties. *Journal of Dairy Research*, *73*, 2731–2740.
Perez, A. A., Carrara, C. R., Sánchez, C. C., Santiago, L. G., & Patino, J. M. R. (2009). Interfacial dynamic properties of whey protein concentrate/polysaccharide mixtures at neutral pH. *Food Hydrocolloids*, *23*(5), 1253–1262.
Prajapati, P. S., Gupta, S. K., Patel, A. A., & Patil, G. R. (1990). *Brief communications of the XXIII international dairy congress, Vol. II* Montreal, October 8–12, (p. 531). Brussels, Belgium: International Dairy Federation.
Prasad, R. V., Butkowski, R. J., Hamilton, J. W., & Ebner, K. E. (1982). Amino acid sequence of rate α-lactalbumin. A unique α-lactalbumin. *Biochemistry*, *21*, 1479–1482.
Radford, S. J., & Dickinson, E. (2004). Depletion flocculation of caseinate-stabilised emulsions: What is the optimum size of the non-adsorbed protein nanoparticles. *Colloids and Surfaces A—Physicochemical and Engineering Aspects*, *238*, 71–81.
Sanchez, C., Schmitt, C., Babak, V. B., & Hardy, J. (1997). Rheology of whey protein isolate–xanthan mixed solutions and gels. Effect of pH and xanthan concentration. *Nahrung*, *41*, 336–343.
Seber, G., & Wild, C. (1989). *Methods in experimental physics*. CA: Academic Press., pp. 245–281.
Schmitt, C., Bovay, C., Vuilliomenet, A.-M., Rouvet, M., & Bovetto, L. (2011). Influence of protein and mineral composition on the formation of whey protein heat-induced microgels. *Food Hydrocolloids*, *25*(4), 558–567.
Schott, H., & Royce, A. E. (1983). Improved microscopic techniques for droplet size determination of emulsions. *Journal of Pharmaceutical Sciences*, *72*(3), 313–315.
Sun, C., Gunasekaran, S., & Richards, M. P. (2007). Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. *Food Hydrocolloids*, *21*, 555–564.
Syrbe, A., Bauer, W. J., & Klostermeyer, H. (1998). Polymer science concepts in dairy systems. An overview of milk protein and food hydrocolloid interaction. *International Dairy Journal*, *8*(3), 179–193.
Tosi, E., Canna, L., Lucero, H., & Re, E. (2007). Foaming properties of sweet whey solutions as modified by thermal treatment. *Journal of Food Chemistry*, *100*, 794–799.
Velth, P. D., & Reynolds, E. C. (2004). Production of a high strength whey protein concentrate from cheese whey. *Journal of Dairy Science*, *87*, 831–840.