WFL Publisher
Science and Technology

Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.11 (1): 172-177. 2013 *www.world-food.net*

Kinetics of heat-induced microgels of whey proteins and casein micelles

Zhen-Yan Liu [1], Hong-Hua Xu [1]*, Guo-Ping Yu [1], Bao-Hua Kong [1] and Yan-Jie Zhang [2]

*[1] Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, 150030 Harbin, People's Republic of China. [2] Chenguan Dairy Co., Ltd., 201401, Shanghai, People's Republic of China. *e-mail: xhh3161@126.com*

Received 19 October 2012, accepted 28 January 2013.

Abstract

In this paper we studied the microgels of whey protein aggregates and whey protein coated casein micelles upon heating 1.0 wt% (protein basis) of skim milk at 90°C under mild acidic pH condition (pH 6.2) for 25 min. The results from kinetic and thermodynamic parameters (order of reaction *n* and rate constant *k*) of microgels were different from the routine of casein micelles and whey protein aggregates at pH 6.7. Different orders of reaction were determined as the function of heat treatment and pH for systems of skim milk samples heated at 90°C for 25 min. The reaction order of microgels was higher at pH 6.2 ($n = 1.64$) than that of the protein aggregation at pH 6.7 ($n = 1.36$). While, the free SH and surface hydrophobicity contents of microgels were increased between soluble and insoluble proteins aggregates which were prepared from whey protein and casein micelles, and the microgels were a good alternative to improve emulsion stability and foaming stability of milk protein.

Key words: Microgel, microstructure, kinetics, whey protein, casein micelle, cross-linking.

Introduction

Nanogel (1–1000 nm) and microgel (1–1000 μm) particles are gel particles prepared from synthetic or natural polymers [1-4] and exhibited the feature of being 'intramolecularly cross-linked macromolecules which constitute a new form of polymer molecule' [5]. So far, nanogel and microgel have been successfully prepared with whey proteins or caseins, for example, in slightly acidic pH conditions (pH 5.7-5.9) and very low ionic strength, β-lactoglobulin was shown to form spherical and monodispersed individualized microgels [6-8]. Schmitt *et al.* [9] studied the formation of whey protein microgels (at a constant protein concentration of 4 wt%) upon heat treatment for 15 min at 85°C in the pH range of 5.7-6.2. Huppertz *et al.* [10] showed that cross-linking of casein micelles with transglutaminase resulted in forming microgels. We are interested in the heat-induced aggregation of whey protein and casein micelles. In normal, heat-treatment of milk causes denaturation of whey protein, leading to a complex mixture of whey protein aggregates and whey protein coated casein micelles. It is established that when the whey protein is denatured, there is a reaction between them and the κ-casein originally presents on the surfaces of the casein micelles in the milk [11]. Upon heat treatment of milk above 60°C several processes take place, the denaturation of whey proteins is the most obvious [12]. During heat-treatment, the denatured whey proteins interact with themselves and with κ-casein to yield whey protein/κ-casein complexes through hydrophobic interactions and thiol/disulphide exchanges [13-18]. These aggregates are located both on the surface of the casein micelles, where κ-casein is usually found. This has been attributed to the formation of cross-linked between the denatured whey proteins and casein micelles.

In this paper, the stable microgel suspensions of whey proteins and casein micelles were generated in aqueous solvent with controlling the aggregation conditions, and the behaviors of microgels in the emulsification and foaming properties was investigated. The heat-induced reaction kinetics of microgels in skim milk samples was described by measuring the levels of native whey protein as a function of heating time. Our main aim was to understand the microgels aggregation pathway and kinetics of aggregation during heat treatment leading to formation microgels.

Materials and Methods

Skim milk powder (SMP) was purchased from Fonterra Ltd (9 Princes Street, Auckland, New Zealand) and without further purification. The composition of the SMP was 34.67 wt% protein (measured from Kjeldahl analysis: N×6.38) with the major proteins; 27.92 wt% casein protein, 6.75 wt% whey protein determined by AOAC Method 998.05 [19]. All other reagents and chemicals were of analytical grade except the soybean oil, which was purchased from the local market.

Heat treatment: The protein dispersion was prepared by dropping 1 wt% (protein basis) with the skim milk powder in Millipore water, and stirring at room temperature for 2 h. The samples were adjusted to 6.2 and 6.7 by stepwise addition of 0.1 M HCl, then transferred to glass tubes and heated for 0-30 min at 90°C in a water bath. After the heat-treatment, the milk samples were immediately cooled to room temperature by immersion in an ice bath.

Microscopic observations: The microstructure of heated protein

dispersions was investigated by transmission electron microscopy (H-7650, Hitachi High-Technologies Corporation) according to the procedure of Akkermans *et al.* [20] with some modifications. A drop of the protein dispersion diluted to 0.02 wt% in Millipore water was deposited onto an amorphous carbon films supported by Cu grid. The excess product was removed after 15 min using a filter paper. After drying the grid at room temperature for 10 min, the samples were studied in TEM, operating at 80 kV.

Analysis of the amount of nature whey protein: The protein dispersions (1 wt%, protein basis) with pH 6.2 and 6.7 were heated at 90°C for 0, 2, 4, 5, 10, 15, 20, 25 and 30 min. To induce precipitation of denatured/aggregated whey protein in certain samples according to a modification of the method of Croguennec *et al.* [21]. 0.5 ml of acetic acid/sodium acetate buffer (0.5 M, pH 4.7) was added to 0.5 ml of sample. Samples were transferred into 1.5 ml plastic Eppendorf containers (Sigma, Dublin, Ireland) and then centrifuged at 14,000 g for 30 min in an Eppendorf centrifuge 5417C (Unitech, Dublin, Ireland). The absorbance of the supernatant (appropriately diluted) was determined at 280 nm in an UV spectrophotometer (UV-2401 PC, Shimadzu Corporation, Japan) as a measurement of protein concentration. All measurements were performed in triplicate.

The fractionation of protein aggregations: Milk samples with two pH values were centrifuged to obtain different levels of protein aggregations. After heating, the dispersions were centrifuged by 3000 g for 30 min at room temperature and in order to remove the precipitation which was the concentration of all the insoluble protein aggregates (Isp). The supernatant was again centrifuged by 5000 g for 30 min, the precipitation was the microgels (Mg) and the supernatant was the soluble protein (Sp).

The concentration of remaining soluble protein including native and soluble aggregates was determined by UV-visible spectrophotometer at 280 nm using the same apparatus described above. After centrifuging, all the precipitation was collected into 25 ml volumetric flask. Then the concentration of microgels and insoluble protein were determined by Kjeldahl analysis: N×6.38. All measurements were performed in triplicate.

Surface hydrophobicity measurements: The surface hydrophobicity of the protein samples was determined by the method of Hayakawa and Nakai [22]. Protein dispersions were diluted to 0.05-0.4% (w/v, protein basis) with 0.01 M phosphate buffer (pH 6.7), then, aliquots (20 µl) of ANS (8.0 mM in the same buffer) were added to 4 ml of the sample solutions, vortexed and kept in the dark for 15 min. The fluorescence intensities of the sample solutions were measured with a Hitachi F4500 fluorescence spectrometer (Tokyo, Japan) at wavelengths of 390 nm (excitation) and 470 nm (emission). The initial slope of the plot of fluorescence intensity vs. protein concentration, calculated by linear regression (in all cases, R^2 values of > 0.95), was used as an index of the surface hydrophobicity of the protein sample evaluated. All measurements were performed in triplicate.

Determination of -SH: Free sulfydryl group (-SH) contents of protein samples were determined by the method of Beveridge *et al.* [23]. For SH contents determination, 5 ml of the Tris-Gly buffer (0.086 M Tris, 0.09 M glycine, 0.004 M EDTA, pH 8.0) containing 8 M urea was added to 1.2 ml of protein samples (1 g/l). Then 30 µl of 2,2'-dinitro-5,5'-dithiodibenzoate (DTNB, Merck, Darmstadt, Germany) was added, and absorbance was measured at 412 nm with UV spectrophotometer (UV-2401 PC, Shimadzu Corporation, Japan) after 15 min. The supernatants in buffer without DTNB were used as blanks. The calculation was as follows:

$$\mu\text{mol SH}/g = (73.53 \times A_{412} \times D) / C \quad (1)$$

where A_{412} is the absorbance at 412 nm, C is sample concentration (mg/ml), D is the dilution factor, 73.53 is derived from $10^6/(1.36\times10^4)$; 1.36×10^4 is the molar absorptivity [24] and 10^6 is for the conversion from molar basis to mM/ml basis and from mg solids to g solids. All measurements were performed in triplicate.

Determination of emulsifying properties: The emulsifying activity index (EAI) and emulsion stability index (ESI) were determined by the turbidimetric technique described by Pearce and Kinsella [25] with some modifications. To prepare the emulsions, 1.0 ml of refined soybean oil and 3.0 ml of protein solution (1 mg/ml), which diluted by 0.1 M sodium phosphate buffer (pH 6.7), were mixed at room temperature using a high-speed dispersing and emulsifying unit (Fs-2, Jiantan City Union Instrument Research Institute, Jiantan, China) at 20,000 rpm for 2 min. After the homogenisation, the emulsions were kept undisturbed for 0 and 2, 4, 6, 8, 10, 20, 30, 60 and 120 min at room temperature and 100 µl aliquots were taken from the bottom of the beaker and dispersed into 5 ml of 0.1% (w/v) SDS solution. The absorbance was measured at 500 nm against 0.1% (w/v) SDS solution as blank in an UV spectrophotometer (UV-2401 PC, Shimadzu Corporation, Japan). Emulsifying activity and emulsion stability expressed as indexes, EAI (m^2/g) and ESI (%), respectively, of the homogenized samples were defined as:

$$\text{EAI } (m^2/g) = \{(2\times2.303) / [C\times(1-\varphi)\times10^4]\}\times A_{500}\times\text{dilution} \quad (2)$$

where A_{500} represents the absorbance at 500 nm, C the protein concentration (g/ml) before emulsification, and φ the oil volume fraction (v/v) of the emulsion (φ = 0.25);

$$\text{ESI } (\%) = 100\times A_t/A_0 \quad (3)$$

where A_t and A_0 represent the absorbance after t min and time zero, respectively, at 500 nm. All measurements were performed in triplicate.

Determination of foaming properties: The foaming capacity (FC) and foam stability (FS) of the protein samples were evaluated by the method of Motoi *et al.* [26] with some modifications. The evaluated protein sample solution (0.1%, w/v) was prepared in 0.1 M sodium phosphate buffer (pH 6.7). Then, 200 ml sample solution was placed in a cylindrical glass cup with a volume scale and agitated at 10,000 rpm for 1 min with a blade-type mixer (DS-1 Waring Blender, Shanghai Jingke Industrial Co. Ltd., Shanghai, China). The foam volume was measured immediately after agitation and again after different times of setting.

Foaming capacity was expressed in terms of the relative overrun, and the foam stability was expressed as the ratio of the foam volume after 0, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h to the initial foam

volume (0 min) [27]. They were calculated with Eqs. (4) and (5), respectively.

$$\text{Relative overrun} = V_0 / V_i \quad (4)$$

$$\text{Foam stability} = V_t / V_0 \quad (5)$$

where V_0 and V_t are the foam volumes at 0 and t h, respectively; V_i is the initial liquid volume before foaming. All measurements were performed in triplicate.

SDS-PAGE analysis: The fractionation of protein aggregations were confirmed by SDS-PAGE analysis of the modified products with 12% (w/v) separating gels and 5% (w/v) stacking gels with β-mercaptoethanol (β-ME) to determine their composition, as described by Laemmli [28]. The heat-treatment samples (1 wt%, protein basis) were diluted 6-fold in the buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0), a portion (30 µl) of this sample was mixed with 50 µl 20% (w/v) SDS solution, 20 µl β-ME, and 20 µl 0.1% (w/v) bromophenol blue, then loaded at a level of 5 µl. The stacking gels were run at 60 V, while the separating gels were run at 90 V. SDS–PAGE was performed using a Bio-Rad mini-gel slab electrophoresis unit (Bio-Rad Laboratories, Richmond, CA). The gels were stained with a 0.1% (w/v) Coomassie blue R-350 solution (PhastGel Blue R, Pharmacia).

Statistical Analysis: Analysis of variance (ANOVA) was conducted using Microsoft Excel (version 2003 software, USA). Replicate means were considered significantly different at $P<0.05$ unless stated differently. When significant differences were indicated by ANOVA, Tukey pair-wise comparisons were performed to indicate where the differences between properties existed.

Results and Discussion

The microgels of whey proteins and casein micelles: The amount of whey protein associated with the casein micelles at pH 6.2 and pH 6.7 along heating process is presented in Fig. 1. The results described a very pH dependent behavior which higher levels of association at pH 6.2 were identified especially as prolonging the heating time. The degree of denaturation of whey proteins was observed at pH 6.2 and 6.7, i.e. 30.14% and 25.11% of the total whey protein (2.15 g/l), after heat treatment of 25 min at 90°C. The aggregates in denatured whey proteins and coating of casein micelles at pH 6.2 were analyzed by TEM. The morphology of the small fine stranded microgels with an average diameter lower than 5 µm in width and 10 µm in length were observed (Fig. 2). It was shown that controlled aggregation of whey proteins and casein micelles could be induced leading to the formation an interesting aggregation pattern for microgels under specific conditions of pH 6.2 and temperature 90°C for 25 min.

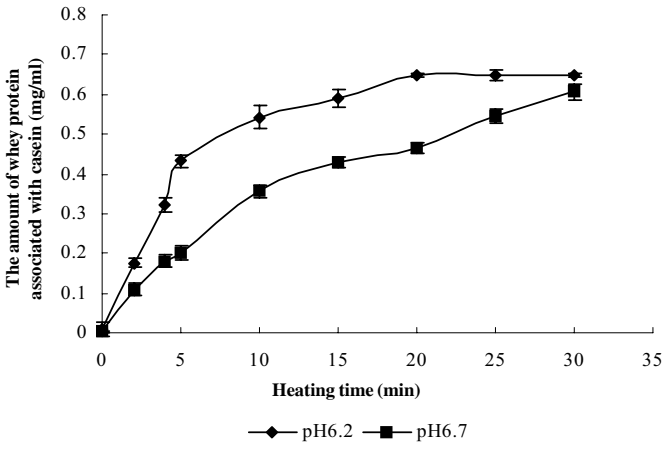

Figure 1. The amount of whey protein associated with the casein micelles by heating skim milk samples (1 wt% protein basis) with two pH values at different heating times. Reaction conditions: The skim milk samples (1 wt%, protein basis) were adjusted to 6.2, 6.7 and heated for 0, 2, 4, 5, 10, 15, 20, 25 and 30 min at 90°C in a water bath. Data are expressed as the mean±SD of three replicates.

Kinetics of the protein aggregates after heat treatment: The kinetic parameters (order of reaction and rate constant) of disappearance of native whey protein were determined using non-linear regression after integration of the general rate equation [29].

$$-d_c/d_t = k_n \times C^n \quad (6)$$

After a heating time t (s), the initial concentration C_0 falls to C_t (g/l) and the ratio of C_t/C_0 can be obtained by integration:

$$n \neq 1 \quad (C_t/C_0)^{1-n} = 1+(n-1)\times kt \quad (7)$$

$$n = 1 \quad \ln(C_t/C_0) = - kt \quad (8)$$

where C_t is the native protein concentration at the time t, $k = k_n C_0^{n-1}$ is reaction rate constant (s^{-1}), and n is the reaction order. The reaction order n should be adjusted to a value that makes it possible to plot Eq. (7) or (8) as a straight line. The rate constant k is then calculated from the slopes of straight lines [6]. The apparent reaction rate constant k_n $[(gl^{-1})^{1-n}s^{-1}]$ represents the kinetic of disappearance of native whey protein.

The degree of denatured whey protein as a function of heating time is shown in Fig. 3, which is given the degree of residual native whey protein (C_t/C_0) in %. The order of reaction (n) and the apparent reaction rate constant k_n $[(gl^{-1})^{1-n}s^{-1}]$ of whey protein were obtained from non-linear regression after integration of the general rate equation as described previously [29]. As shown in the Table 1, the rate of disappearance of native whey proteins at pH 6.2 in the time range studied was approximately 4 times as high as that at pH 6.7. While, the reaction order of whey protein was increased from 1.36 to 1.64 as the pH was decreased from 6.7 to 6.2. High orders of reaction indicated high complexity of protein denaturation and aggregation reactions that could be linked to parallel or serial reactions or formation of intermediates [30]. The kinetic parameter variations provided information on the

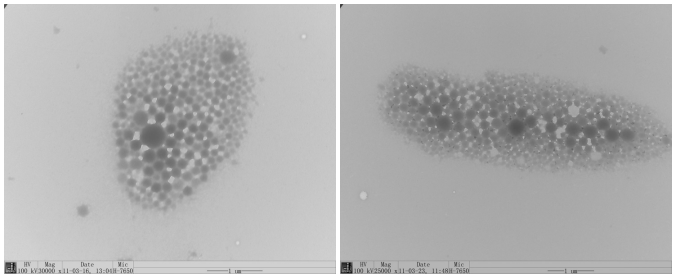

Figure 2. TEM micrographs of microgels with 1 wt% protein basis of skim milk (pH 6.2) heated for 25 min at 90°C.

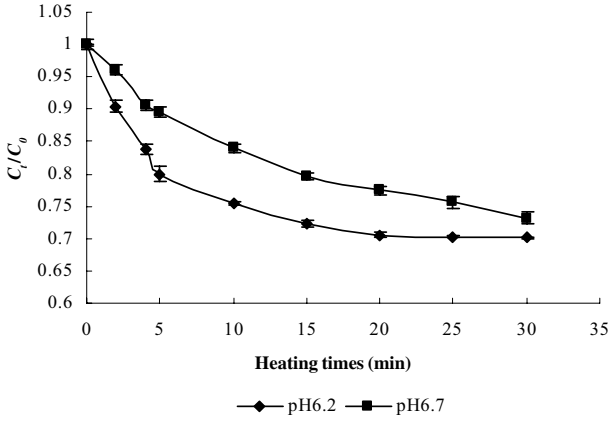

Figure 3. Fractional concentration of non-aggregated whey protein by heating skim milk samples (1 wt% protein basis) versus heating time at 90ºC with two pH values; ■, pH 6.7; •, pH6.2. C_t/C_0 is the ratio of whey protein concentration at time t and time zero. Reaction conditions: The skim milk samples (1 wt%, protein basis) were adjusted to 6.2, 6.7 and heated for 0, 2, 4, 5, 10, 15, 20, 25 and 30 min at 90°C in a water bath. Data are expressed as the mean±SD of three replicates.

denaturation and aggregation rate suggested that the microgels probably was formation of intermediate aggregates of whey proteins and casein micelles by limiting effect upon pH and heat treatment.

Heat-induced changes in surface hydrophobicity and S-S of protein aggregates: The surface hydrophobicity values indicate the number of hydrophobic groups on the surface of a protein in contact with the polar aqueous environment [31]. The surface hydrophobicities of milk protein at pH 6.2 and pH 6.7 measured by ANS fluorescence probe were significantly different from each other. A marked increase in surface hydrophobicities upon heating of milk protein could be observed in Table 2, and the values were correlated well with the pH of skim milk samples at the same heat treatment, which higher surface hydrophobicities were observed as the skim milk samples at pH 6.2. It was also found that the variation of the free SH had similar trend. More disulfide bonds were produced when the milk solution at pH 6.2. In addition, pH and heat load (temperature/time) strongly affected the aggregation pattern of milk proteins, and the protein aggregates from different aggregation pattern owned different values of surface hydrophobicity and the free SH, especially, the values of microgels were between soluble and insoluble proteins aggregates at the same heat-induced conditions (Table 2).

These results had also relationships with their protein composition which was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. It was different levels of whey proteins among in the microgels, soluble protein and insoluble proteins aggregates, which the quantity of whey protein of microgels was in the middle of soluble protein and insoluble proteins aggregates (Fig. 4). Whey protein contains free sulfhydryl, the reactivity of the free thiol group can be markedly increased by protein unfolding induced by heat treatment [32]. Therefore, these experimental studies demonstrated that microgels from heat-induced treatment with whey protein and casein micelles probably were intermediate aggregates in the protein aggregation pathway.

Table 1. Kinetic parameters of native whey proteins disappearance by heating the skim milk samples.

pH-adjusted	Reaction order n	Rate constant k_n ($g^{1-n}Ln^{-1}.s^{-1}$)
pH 6.7	1.29±0.05	(1.252±0.030)×10^{-3}
pH 6.2	1.65±0.03	(4.235±0.121)×10^{-3}

Reaction conditions: The skim milk samples (1 wt%, protein basis) were adjusted to 6.2, 6.7 and heated for 0, 2, 4, 5, 10, 15, 20, 25 and 30 min at 90°C in a water bath. Data are expressed as the mean±SD of three replicates.

Table 2. The properties of the different milk protein aggregates by heating skim milk samples (1 wt% protein basis) with two pH values at 90°C for 25 min.

Samples	s-s	H_0	FC (%)	FS (%)					EAI (m^2/g)	ESI (%)				
				0	0.5	3	6	8 h		0	10	30	60 min	
Sp (6.2)	6.24±0.08^{e}	2203.80±16.93^{e}	73.17±1.25^{c}	100±1.72^{a}	66.06±1.97^{b}	39.86±1.97^{b}	15.72±1.18^{a}	-	17.12±0.37^{b}	100±2.17^{a}	43.11±1.37^{b}	34.50±1.66^{b}	32.72±2.01^{ab}	
Mg(6.2)	3.11±0.02^{c}	1416.67±13.22^{b}	66.17±2.47^{b}	100±3.73^{a}	93.20±2.18^{d}	81.36±1.90^{d}	64.23±3.78^{d}	44.08±5.77^{a}	15.27±0.09^{a}	100±0.61^{a}	55.68±1.22^{d}	46.06±2.57^{d}	37.85±4.29^{b}	
Isp (6.2)	2.43±0.03^{b}	1229.40±14.82^{c}	58.33±2.89^{a}	100±4.95^{a}	94.00±0.49^{d}	79.99±4.95^{d}	57.14±4.95^{c}	43.71±5.94^{a}	14.77±0.27^{a}	100±1.86^{a}	53.06±0.51^{d}	44.58±0.44^{d}	37.53±0.43^{b}	
Sp (6.7)	5.72±0.08^{d}	1806.40±7.59^{d}	79.83±2.02^{d}	100±2.53^{a}	60.54±1.81^{a}	26.29±3.76^{a}	12.52±1.08^{a}	-	17.96±0.87^{c}	100±4.82^{a}	37.21±2.79^{a}	30.24±1.79^{a}	27.54±0.72^{a}	
Isp (6.7)	2.31±0.01^{a}	1018.33±17.16^{a}	70.00±5.00^{bc}	100±7.14^{a}	79.52±2.30^{c}	66.67±4.12^{c}	47.62±4.12^{b}	39.28±3.57^{a}	15.41±0.07^{a}	100±4.88^{a}	49.09±1.39^{c}	38.63±2.09^{c}	33.96±4.36^{b}	

Sp (6.2), the solution protein with pH 6.2; Mg (6.2), the microgels with pH 6.2; Isp (6.2), the insolution protein with pH 6.2; Sp (6.7), the solution protein with pH 6.7; Isp (6.7), the insolution protein with pH 6.7. Different superscript characters (a–e) indicate the significant difference at $P < 0.05$.

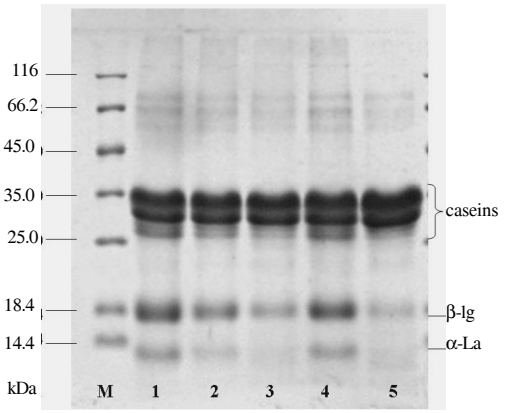

Figure 4. The SDS-PAGE electrophoretograms of protein aggregations by heating skim milk samples (1 wt% protein basis) with two pH values at 90°C for 25 min as a function of different centrifuges. Lane M, standard protein markers; Lane 1, Soluble protein with pH 6.2 ; Lane 2, Micregels with pH 6.2; Lane 3, Insoluble protein with pH 6.2; Lane 4, Soluble protein with pH 6.7; Lane 5, Insoluble protein with pH 6.7.

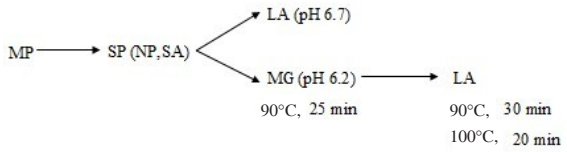

Figure 5. General proposed model of microgels aggregation pathway. LA, insoluble aggregates; MG, microgels aggregates; NP, native protein; SA, soluble aggregates; MP, milk protein.

Proposed model: We proposed a model of microgels aggregation pathway as described in Fig. 5, which was similar as β-lactoglobulin microgels aggregation pathway in the literature [6]. The two kind of heat-induced microgels all were formation of intermediate aggregates. Upon heating, whey protein lost its native state, and aggregated with casein micelles into intermediate oligomers that ultimately led to larger soluble aggregates (SA). As controlling heating conditions and pH, SA interacted further to form MG. With prolonged heating time or altering pH, the formation of MG was promoted a change in the aggregation to form LA.

It has been shown in our results that covalent (disulphide bonds) and non-covalent (hydrophobic) interactions play a major role in the stability of these assemblies [33]. The TEM images shown in Fig. 6 after the heat-treatment for 5 to 30 min at 90°C could confirmed the proposed model was correct that the microgels could be prepared by self-limitation formation of intermediate aggregates of whey proteins and casein micelles. Controlling the pattern of aggregation could be attributed to control of physical and chemical factors including heat load (time/temperature) and pH variations.

Stabilization of foams and emulsions: The improvement of the emulsifying and foaming properties of milk protein after heat treatment was already reported in several studies [34-36]. However, the use of specific protein aggregates with well characterized structures and physicochemical properties for foam and emulsion stabilization seems to have been considered only very recently for food applications [37]. The best foam and emulsions stability were obtained for the microgels in the different milk protein aggregates with whey protein and casein micelles at pH 6.2 or 6.7, 90°C for 25 min (Table 2). Compared with the solution protein, the foam stability of microgels was higher by approximately 4 times when the foam films silent 6 h. Foaming properties were found to be significantly improved after formation microgels and the emulsifying stability were also increased slightly. The same behaviors of microgels in the emulsification and foaming properties, it showed that molecular structure characteristics were probably benefit to the formation of a gel at the interface.

Conclusions

The results presented here had shown that, controlling the aggregation of whey proteins and casein micelles could be induced an interesting aggregation pattern for microgels under specific conditions of pH 6.2 and temperature 90°C for 25min. Compared with the routine of casein micelles and whey protein aggregates at pH 6.7, kinetics of reaction order of microgels was higher than one of the normal milk protein aggregation, and the free SH and surface hydrophobicity contents of microgels were in the middle

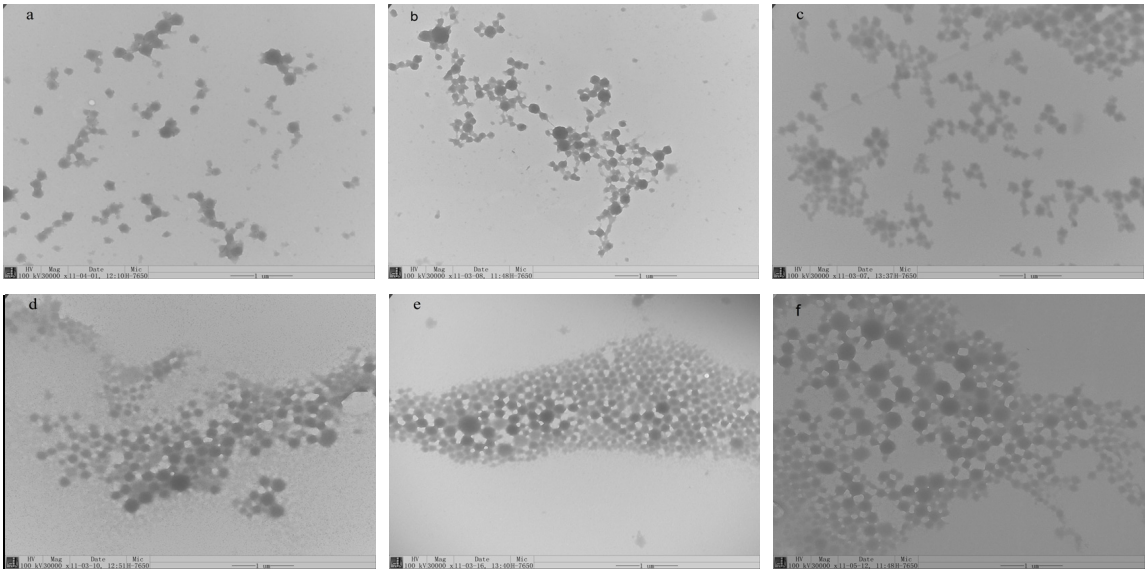

Figure 6. Transmission electron micrographs of the microgels by heating 1 wt% (protein basis) of skim milk samples with pH 6.2 at 90°C for different heating times (min): (a) 5, (b) 10, (c) 15, (d) 20, (e) 25 and (f) 30.

of soluble and insoluble proteins aggregates from whey protein and casein micelles. Microgels had been found to be useful for food applications, the characteristics of the molecular structure are beneficial to improve emulsion stability and foaming stability of milk protein.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 31071572) and the Specialized Research Fund of New Teachers for the Doctoral Program of Ministry Education of China (No. 20092325120018) and Innovative Research Team of Higher Education of Heilongjiang Province (No. 2010td11).

References

[1]Hans, M. and Lowman, A. 2002. Biodegradable nanoparticles for drug delivery and targeting. Curr. Opin. Solid State Mater. Sci. **6**:319-327.

[2]Stieger, M., Pedersen, J. S., Lindner, P. and Richtering, W. 2004. Are thermoresponsive microgels model systems for concentrated colloidal suspensions? A rheology and small-angle neutron scattering study. Langmuir **20**:7283-7292.

[3]Stieger, M. and Richtering, W. 2003. Shear-induced phase separation in aqueous polymer solutions: Temperature-sensitive microgels and linear polymer chains. Macromolecules **36**:8811-8818.

[4]Stieger, M., Richtering, W., Pedersen, J. S. and Lindner, P. 2004. Small-angle neutron scattering study of structural changes in temperature sensitive microgel colloids. J. Chem. Phys. **120**:6197-6206.

[5]Graham, N. B. and Cameron, A. 1998. Nanogels and microgels: The new polymeric materials playground. Pure Appl. Chem. **70**:1271-1276.

[6]Donato, L., Schmitt, C., Bovetto, L. and Rouvet, M. 2009. Mechanism of formation of stable heat-induced β-lactoglobulin microgels. Int. Dairy J. **19**:295-306.

[7]Jung, J. M., Savin, G., Pouzot, M., Schmitt, C. and Mezzenga, R. 2008. Structure of heat-induced β-lactoglobulin aggregates and their complexes with sodium-dodecyl sulfate. Biomacromolecules **9**:2477-2486.

[8]Santipanichwong, R., Suphantharika, M., Weiss, J. and McClements, D. 2008. Core-shell biopolymer nanoparticles produced by electrostatic deposition of beet pectin onto heat-denatured β-lactoglobulin aggregates. J. Food Sci. **73**:23-30.

[9]Schmitt, C., Bovay, C., Vuilliomenet, A. M., Rouvet, M. and Bovetto, L. 2011. Influence of protein and mineral composition on the formation of whey protein heat-induced microgels. Food Hydrocolloids **25**:558-567.

[10]Huppertz, T., Smiddy, M. A. and De Kruif, C. G. 2007. Biocompatible micro-gel particles from cross-linked casein micelles. Biomacromolecules **8**:1300-1305.

[11]Jang, H. D. and Swaisgood, H. E. 1990. Disulfide bond formation between thermally denatured β-Lactoglobulin and κ-Casein in casein micelles. J. Dairy Sci. **73**:900-904.

[12]Dannenberg, F. and Kessler, H.-G. 1988. Reaction kinetics of the denaturation of whey proteins in milk. J. Food Sci. **53**:258-263.

[13]Donato, L., Guyomarćh, F., Amiot, S. and Dalgleish, D. G. 2007. Formation of whey protein/κ-casein complexes in heated milk: Preferential reaction of whey protein with κ-casein in the casein micelles. Int. Dairy J. **17**: 1161-1167.

[14]Guyomarćh, F., Andrew, J. and Dalgleish, D. G. 2003. Formation of soluble and micelle-bound protein aggregates in heated milk. J. Agric. Food. Chem. **51**:4652-4660.

[15]Jang, H. D. and Swaisgood, H. E. 1990. Disulfide bond formation between thermally denatured β-lactoglobulin and κ-casein in casein micelles. J. Dairy Sci. **73**:900-904.

[16]Noh, B., Richardson, T. and Creamer, L. K. 1989. Radiolabelling study of the heat-induced interactions between α-lactalbumin, β-lactoglubulin and κ-casein in milk and in buffer solutions. J. Food Sci. **54**:889-893.

[17]Singh, H. and Creamer, L. K. 1991. Aggregation and dissociation of milk protein complexes in heated reconstituted concentrated skim milks. J. Food Sci. **56**:238-246.

[18]Smits, P. and van Brouwershaven, J. H. 1980. Heat-induced association of β-lactoglobulin and casein micelles. J. Dairy Res. **47**:313-325.

[19]AOAC 2000. Official Methods of Analysis. 17th edn. Association of Official Analytical Chemists International, Arlington, Gaithersburg, MD.

[20]Akkermans, C., Van der Goot, A., Venema, P., Van der Linden, E. and Boom, R. 2008. Properties of protein fibrils in whey protein isolate solutions: Microstructure, flow behaviour and gelation. Int. Dairy J. **18**:1034-1042.

[21]Croguennec, T., O'Kennedy, B. T. and Mehra, R. 2004. Heat-induced denaturation/aggregation of β-lactoglobulin A and B: Kinetics of the first intermediates formed. Int. Dairy J. **14**:399-409.

[22]Hayakawa, S. and Nakai, S. 1985. Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. J. Food Sci. **50**:486-491.

[23]Beveridge, T., Toma, S. and Nakai, S. 1974. Determination of SH-and SS-groups in some food proteins using Ellman's reagent. J. Food Sci. **39**:49-51.

[24]Ellman, G. L. 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. **82**:70-77.

[25]Pearce, K. N. and Kinsella, J. E. 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. J. Agric. Food. Chem. **26**:716-723.

[26]Motoi, H., Fukudome, S. and Urabe, I. 2004. Continuous production of wheat gluten peptide with foaming properties using immobilized enzymes. Eur. Food Res. Technol. **219**:522-528.

[27]Hammershøj, M. and Qvist, K. B. 2001. Research Note: Importance of hen age and egg storage time for egg albumen foaming. Lebensm. -Wiss. -Technol. **34**:118-120.

[28]Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**:680-685.

[29]Dannenberg, F. and Kessler, H. G. 1988. Reaction kinetics of the denaturation of whey proteins in milk. J. Food Sci. **53**:258-263.

[30]Roefs, S. P. F. M. and Kruif, K. G. 1994. A model for the denaturation and aggregation of β-lactoglobulin. Eur. J. Biochem. **226**:883-889.

[31]Sava, N., Van Der Plancken, I., Claevs, W. and Hendrickx, M. 2005. The kinetics of heat-induced structural changes of β-lactoglobulin. J. Dairy Sci. **88**:1646-1653.

[32]Anema, S. G. and McKenna, A. B. 1996. Reaction kinetics of thermal denaturation of whey proteins in heated reconstituted whole milk. J. Agric. Food. Chem. **44**:422-428.

[33]Schmitt, C., Bovay, C., Vuilliomenet, A.M., Rouvet, M., Bovetto, L., Barbar, R. and Sanchez, C. 2009. Multiscale characterization of individualized β-lactoglobulin microgels formed upon heat treatment under narrow pH range conditions. Langmuir **25**:7899-7909.

[34]Dissanayake, M. and Vasiljevic, T. 2009. Functional properties of whey proteins affected by heat treatment and hydrodynamic high-pressure shearing. J. Dairy Sci. **92**:1387-1397.

[35]Kim, D. A., Cornec, M. and Narsimhan, G. 2005. Effect of thermal treatment on interfacial properties of β-lactoglobulin. J. Colloid Interface Sci. **285**:100-109.

[36]Moro, A., Gatti, C. and Delorenzi, N. 2001. Hydrophobicity of whey protein concentrates measured by fluorescence quenching and its relation with surface functional properties. J. Agric. Food. Chem. **49**:4784-4789.

[37]Wierenga, P. and Gruppen, H. 2010. New views on foams from protein solutions. Curr. Opin. Colloid Interface Sci. **15**:365-373.