IOP Conf. Series: Materials Science and Engineering **87** (2015) 012034 doi:10.1088/1757-899X/87/1/012034

Whey protein isolate modified by transglutaminase aggregation and emulsion gel properties

Weiwei Qi, Chong Chen, Mujun Liu, Guoping Yu[1], Xinghang Cai, Peipei Guo, Yuxiu Yao and Sijie Mei

College of Food Science, Northeast Agricultural University, No. 59 Mucai Street, Xiang Fang District, Harbin 150030, China

E-mail:neauqiww@163.com

Abstract. Whey protein isolate and commercial soybean salad oil were used to produce the WPI emulsion dispersions. The properties of TG-catalyzed emulsion gelation produced from WPI emulsion dispersions were investigated by the amount of TG, temperature, pH and reaction time. Specifically, the texture properties (hardness and springiness), water-holding capacity and rheological properties (G 'and G ") were assessed. The result of Orthogonal tests showed WPI emulsion can form better hardness and springiness gel when the ratio of TG and WPI was 20U/g, pH 7.5, treatment temperature and time were 50℃ and 3 h, respectively. The microstructure of TG emulsion gels was more compact, gel pore is smaller, distribution more uniform, the oil droplets size smaller compared with untreated emulsion gels. Compared to the control of rheological properties, G ' and G " were significantly increased and G ' > G ", results showed that the gel was solid state, and TG speeded up the process of gelation.

1. Introduction

Whey protein isolate (WPI) is an economically important by-product of the cheese manufacturing process, used as an ingredient by the food industry because of its high nutritional value and functional attributes. Whey proteins comprise of a mixture of proteins, dominated by β-lactoglobulin (β-Lg) and α-lactalbumin with minor amounts of proteose-peptones, serum albumin and immu-noglobulins [1]. β-Lg is the major protein present in bovine milk whey. It exists, as a dimer with a molecular weight of 36,400 Da. Two disulfide bonds and a free thiol group exist within β-lactoglobulin [2]. Due to whey protein small molecular weight and more spherical shapes, it has the ability to form a stable functional properties and good emulsion gelation, therefore can prepare emulsified gel [3], and the gel matrix can retain a lot of water and other non-protein components, and can effect on texture and sensory properties of different food [4].

As the active factors, droplets can improve the emulsion gel strength and water-holding capacity. Yogurt, fresh cheese, gelatin, pudding, milk, snacks, hot dogs and other food products belong to these emulsion gel. Fat plays an important role to improve the texture characteristics of emulsion gel food. Rheological properties of such composite gel are strongly dependent on the volume fraction of filler

[1] Address for correspondence: Guoping Yu, College of Food Science, Northeast Agricultural University, No. 59 Mucai Street, Xiang Fang District, Harbin 150030, China. Tel.: 0451-55190637; E-mail: yuguopingneau@hotmail.com.

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

particles and the interaction with the gel matrix and oil droplets. Therefore, the research about emulsion gel to improve the textural properties of the food is necessary. In the present work, there are few papers published concerning with whey protein emulsion gel.

To be competitive in food ingredient markets, the functionality of whey proteins must be continuously improved and designed for specific end uses. Chemical and physical methods are commonly used. Food proteins can have their functionality altered by temperature and other chemical means. Specific functional attributes could be obtained by enzymatic polymerization of proteins. Here the enzymatic reaction could be controlled by time to enhance the functionality to the desired level [5]. The enzyme transglutaminase (EC 2.3.2.13) catalyzes an acyl transfer reaction between the γ-carboxyamide group of peptide-bound, glutamine residues as acyl donors and primary amines as receptors [6]; when transglutaminase acts on protein molecules, intra- and inter-molecular ε-(γ-glutamyl) lysine crosslinks are formed by the enzyme reaction [7]. The inter-molecular cross-linking, which increases the weight average molecular weight of the protein, may improve the gelation and emulsifying properties [8]. Many studies have been carried out the TG-induced polymerization of modified whey protein to obtained different functional properties of gels, such as high hardness [9], the changes temperature of the gel point [10], the reduction of syneresis cracking of gel catalyzed by TG [11], but the properties of whey protein emulsified gel catalyzed by transglutaminase rarely reported. The effect of the amount of TG, pH, temperature, heating time on emulsion gel were observed by texture analysis, water-holding capacity and rheology, and provide the reference about theoretical studies to improve whey protein gel emulsion properties.

2. Materials and methods

2.1. Materials

The whey protein isolate powder used in this study was a commercial product provided by Columbia company (USA), with an approximate composition of >90% protein, <5.1% moisture, <1.5% minerals as per experiment's data. Soybean salad oil (JIUSAN Oil Industry Group Co. Ltd. Harbin, China). Ca^{2+}-independent microbial transglutaminase (TG-B) was provided by Yiming Biological Products Co. Ltd. (Jiangsu, China). The enzyme (composed of lactose, maltodextrin and transglutaminase) presented mean enzymatic activity of 100 U/g, as per manufacturer's data. The enzyme was used in the original form without further purification. All other chemicals were of analytical grade.

2.2. WPI emulsion dispersions preparation

Preparation of 8% (w/v) whey protein isolate emulsion dispersions. WPI solutions were prepared by dispersing the WPI powder in distilled water adjusted to 7.0 pH using either 1.0 M HCl or 1.0 M NaOH, followed by mechanically stirring at room temperature for 120 min, then left overnight at 4℃ to equilibrate and ensure completely hydration. Commercial soybean salad oil was added to whey protein isolate solution, and the ratio of oil is 15% (v/v). This system was stirred and premixed for 15 min at 60℃. Emulsions were obtained using an YQ-3 high speed homogenizer (Scientific research machinery work, Jiangsu, China) at 10000 rpm for 120 s. Emulsions were cooled and stored at 4℃ until further analyses. All samples were prepared in triplicate.

2.3. The effects of TG on the characteristics of emulsion gel

The basic parameters were setting for the temperature of 45℃, cross-linking of time 3 h, pH of 7.5, TG-B for 30 U/g. The sample was heated at 90℃ for 30min, and then gel was rapidly cool to room temperature in ice-water bath. Finally, left overnight at 4℃ before the analysis.

2.3.1. Single factor experimental design. In order to investigate the effects of TG on emulsion gel characteristics, texture properties and water-holding capacity were determined. The amount of TG (0,

5, 10, 15, 20, 25, 30 U/g protein), pH of the reaction (5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5), the temperature (35, 45, 55, 65, 75℃), reaction time (1, 2, 3, 4, 5, 6, 7 h) were presented.

2.3.2. The orthogonal design. According to Univariate tests, four variables the amount of TG, pH, temperature, reaction time were selected to carry out L_9 (3^4) orthogonal test in table 1. Gel hardness, gel elasticity, water-holding capacity as index. Assistant Ⅱ by orthogonal design software for analysis to determine the transglutaminase catalyzed gel emulsion optimal conditions. The form of factors and levels are shown as followed:

Table 1. The factors and levels of orthogonal test.

Number	Amount of TG A(U/g)	pH B	T C(℃)	Time D(h)
1	15	7.0	40	2
2	20	7.5	45	3
3	25	8.0	50	4

2.4. Texture analysis of emulsion gel

The texture of emulsion gel was determined according to the method of Xin Gu [12] with minor modification by TA-XT plus Texture Analyzer (Stable Micro System, England). The gel (25 mm in height and 50 mm in diameter) was equilibrated at room temperature for 30min before measured. Using P/0.5 cylindrical probe, which moves downward twice during measuring. setting forward speed: 10 mm/s, measuring speed: 10 mm/s; retreating speed: 10 mm/s, compression depth: 10 mm. Three measurements were conducted for each replication, and there were three replications in all treatments. Means and standard deviations were calculated from nine data.

2.5. Water-holding capacity

The water-holding capacity (WHC) of emulsion gel was determined according to the method of Vale'rie Leung Sok Line [13] with minor modification (centrifugal machine, Medicine centrifuge works, Beijing). The WPI emulsion gel was weighed and placed into 50 mL centrifugal tubes, and centrifuged at 9000 rpm for 20 min, then measured the weight of overflow water. Values are means of three replications with duplicate measurements. Water-holding Capacity equation is as follows:

$$\text{WHC } \% = (W_t - W_r)/W_t \times 100$$

Where W_t (g) is the weight of total moisture of gel and W_r (g) is the weight of overflow water after centrifuging.

2.6. Scanning electron microscopy

The gel was spiced into squares of 3×3×3 mm^3, and were determined according to the method of Chin K B [14]. Accelerating voltage 5 KV, observing and photographing the sample particle morphology with Japan JME-100CXII TEM.

2.7. Rheology

The Rheology of emulsion gel was determined according to the method of Li [15] with slight changes by MALI038384 Rotational rheometer (Malvern Instruments Ltd. England). Rheological measurements carried out using parallel-plate with 20 mm diameter and 0.3 mm gap. In order to prevent evaporation of moisture during the test, the sample was sealed around paraffin oil. The gelation conditions were the same as those previously described in Section 2.3. Heating temperature from 30℃ to 85℃ by 3℃/min constant heating rate and maintain 10 min. Oscillation frequency of 1

Hz, the stress amplitude of 0.122, which is the linear viscoelastic region, as measured by the initial trial. The parameter G' (storage modulus) and G" (loss modulus) were measured.

2.8. Statistical analysis

Each experiment was repeated three times. The data are the average of three times, standard deviation of the error term; Analysis of variance was used ($p<0.05$) and when the effect of the factors was significant. Data were statistically analyzed using SPSS13.0 software.

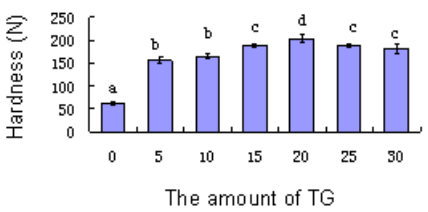


Figure 1. Effect of enzyme dosage on hardness of TG-WPI emulsion gels.

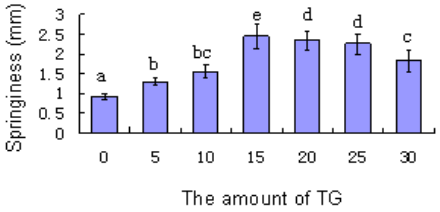


Figure 2. Effect of enzyme dosage on springiness of TG-WPI emulsion gels.

3. Results and discussion

3.1. The effects of the amount of TG on texture and water-holding capacity

The gel strength increased significantly at TG dosage (0-20 U/g).The emulsion gel strength reached maximum with the amount of enzyme 20 U/g; over 20 U/g, the gel strength decreased with the amount of TG increasing (figure 1). Figure 2 showed that amount of TG was 0-15 U/g, the gel springiness significantly enhanced; and then decreased when the dosage was over 15 U/g.

The α-lactalbumin structure has 8 glutamine residues and 12 lysine residues; while β-lactoglobulin has 16 glutamine residues and 15 lysine residues in its protein chain [16]. However, not all of these residues are available for enzymatic reaction with transglutaminase, due to the globular structure of whey proteins. The rate of crosslinking by transglutaminase is dependent on the macromolecular structure of each protein substrate; reactive glutamine residues, i.e., reside in flexible regions of the polypeptide chain or in regions with reverse turns [17]. Nieuwenhuizen et al. [18] investigated the accessibility of the lysine and glutamine residues of a-lactalbumin to the microbial transglutaminase reaction and showed that a maximum of five lysines and five glutamines can be modified by transglutaminase depending on the temperature, pH, and the presence or absence of calcium (Ca^{2+}).

Transglutaminase concentrations above 20 U/g of protein may be higher than the quantities required for the reaction with the available residues of α-lactalbumin and β-lactoglobulin, saturation of the protein substrate may occur, without altering, even decreasing the hardness and springiness of the gel at transglutaminase concentrations higher than 20 U/g of protein, this probable due to excessive crosslinks. Similar results were observed by Han Cui-ping et al. [19]. The polymerization of whey proteins, the gel strength reached maximum at the amount of 10 U/g of TG, over 10 U/g the strength decreased.

The water-holding rate was high at 94-98% at the of 5-30 U/g, Figure 3 showed when TG dosage was 0-5 U/g, the water-holding capacity of gel was significantly increased; subsequently slowly improved with 5-15U /g; enzyme dosage is 15 U/g, the WHC was maximum.As a consequence, when TG dosage was a range of 5-20 U/g, emulsion gel strength, springiness and WHC were at optimum values.

3.2. The effects of pH on texture and WHC of emulsion gel

Emulsion gel strength increased with increasing pH.The gel strength significantly increased at pH 5.0-7.5, and reached maximum when the pH is 7.5, whereas when the pH continues increasing, gel hardness declined (figure 4).Tang Chuan-he [20] also confirmed the optimum pH of TG catalyzed β-

lactoglobulin was 7.5, however the pH over 7.5, the catalysis of TG was weakened, TG was almost inactivation, when pH was over 8.5. Figure 5 described the effect of pH on gel springiness of TG-WPI emulsion gels. Like hardness, gel elasticity increases with increasing pH. Gel elasticity increases slowly at pH values 5.0-6.5, but at pH 6.5-7.5, the elasticity increases significantly and reached maximum at pH 7.5, the treatment of pH exceeded 7.5 was not found to significantly increase.

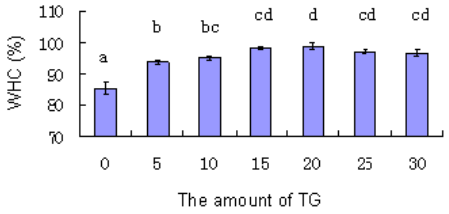


Figure 3. Effect of enzyme dosage on water-holding capacity of TG-WPI emulsion gels.

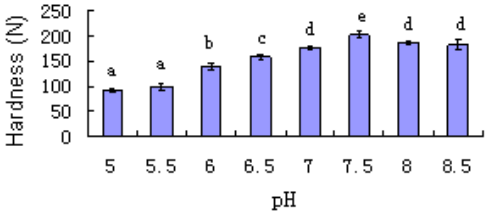


Figure 4. Effect of pH on gel hardness of TG-WPI emulsion gels.

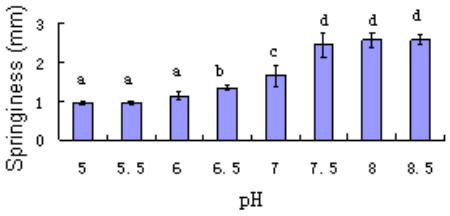


Figure 5. Effect of pH on gel springiness of TG-WPI emulsion gels.

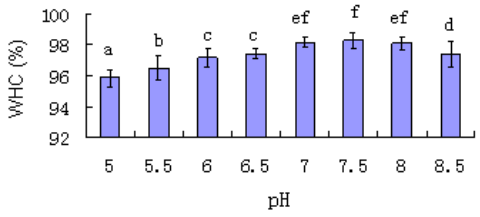


Figure 6. Effect of pH on water-holding capacity of TG-WPI emulsion gels.

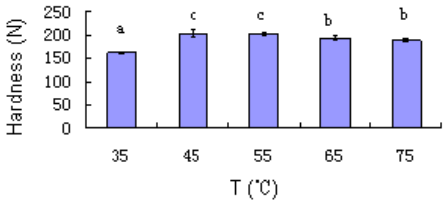


Figure 7. Effect of temperature on hardness of TG-WPI emulsion gels.

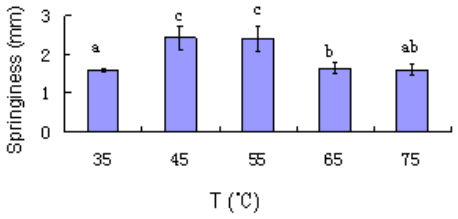


Figure 8. Effect of temperature on springiness of TG-WPI emulsion gels.

As shown in figure 6, WHC of emulsion gel significantly increased at pH 5.0-7.0. When the pH was 7.0-8.0, the WHC was not significant increased. Overall, the emulsion gel strength, elasticity and water-holding capacity are at optimum values at pH 7.0-7.5.

3.3. The effects of temperature on texture and WHC of emulsion gel

Emulsion gel strength increased with increasing temperature. Specially at 35-45℃, the strength is significantly increased. Each of enzymes itself has optimal temperature and pH. The optimal temperature of TG is at the range of 45-55℃, when the temperature is higher 55℃, the TG is passivated even inactivated. Therefore, as the temperature continues to rise, the emulsion gel strength is slightly decreased (figure 7).

As shown in figure 8, Emulsion gel elasticity increases with increasing temperature, the gel elasticity is significantly improved at 35-45℃; however, the elasticity has no significant change at 45-55℃; as the temperature continues to rise, the elastic gel is significantly reduced. The effect of temperature on WHC is the same like to hardness. When the temperature is 45℃, WHC of emulsion gel is maximum (figure 9). According to above analysis, the optimum values of gel strength, elasticity, WHC are at the range of 45-55℃.

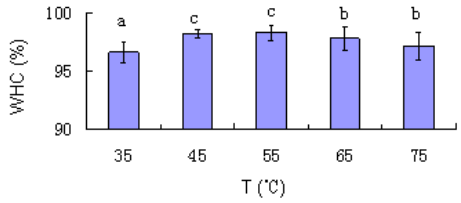


Figure 9. Effect of temperature on water-holding capacity of TG-WPI emulsion gels.

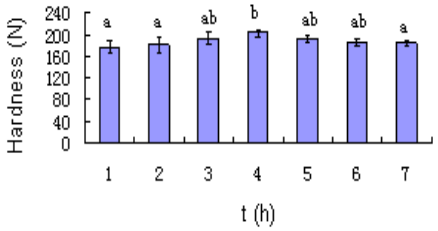


Figure 10. Effect of time on hardness of TG-WPI emulsion gels.

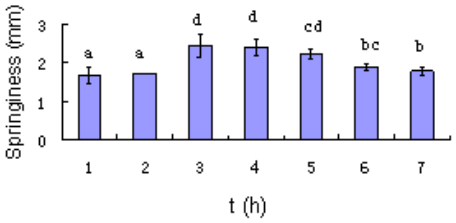


Figure 11. Effect of time on springiness of TG-WPI emulsion gels.

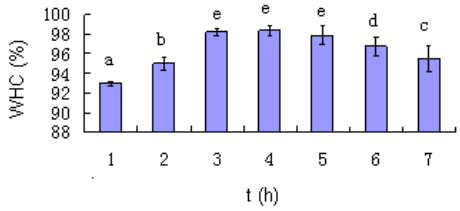


Figure 12. Effect of time on water-holding capacity of TG-WPI emulsion gels.

3.4. The effects of heating-time on texture and WHC of emulsion gel

The emulsion gel strength showed no significant change as heating-time extended, gel strength was maximized when heated 3-4 h (figure 10). As shown in figure 11, gel elasticity significantly increased when the reaction 2-3 h; at the time of 5-7 h, the emulsified gel elasticity decreased. As reaction time extended, the WHC increased firstly, then decreased, when the reaction was at 3-4 h, WHC reached the maximum (figure 12).This could be related that with heating-time extending, whey protein occured so excessive crosslinks that it is not prone to aggregate and form gel. Another reason maybe the mechanism of TG catalysis, the TG enzyme can catalyze and decomposite glutamine at the present of H_2O, thereby as the reaction extended, TG probably promotes reverse reaction and parts of aggregation are resolved.

3.5. Result of orthogonal design

According to single factor experimental results, L_9 (3^4) orthogonal test was carried out by optimum levels of the amount of enzyme added (A), pH (B), temperature (C), reaction time (h) in table 2 and table 3. The characteristics of gel were evaluated by lining-up score method to determine the optimum process conditions of the emulsion gel of whey protein isolate.

Table 2. L_9 (3^4) Orthogonal experiment design.

Test number	TG Amount	pH	T	Time
	A(U/g)	B	C(℃)	D(h)
1	1(15)	1(7.0)	1(40)	1(2)
2	1(15)	2(7.5)	2(45)	2(3)
3	1(15)	3(8.0)	3(50)	3(4)
4	2(20)	1(7.0)	2(45)	3(4)
5	2(20)	2(7.5)	3(50)	1(2)
6	2(20)	3(8.0)	1(40)	2(3)
7	3(25)	1(7.0)	3(50)	2(3)
8	3(25)	2(7.5)	1(40)	3(4)
9	3(25)	3(8.0)	2(45)	1(2)

Table 3. Data of $L_9(3^4)$ orthogonal experiment.

Number	Hardness (g)	springiness	WHC (%)
1	180.891±8.091	1.921±0.291	96.321±1.029
2	190.221±5.093	1.322±0.383	94.928±4.039
3	179.391±3.023	2.019±0.198	94.338±8.043
4	193.981±9.004	1.823±0.284	95.271±5.093
5	211.169±4.907	2.113±0.377	98.029±4.972
6	201.135±5.095	2.531±0.163	97.832±3.672
7	198.327±6.784	2.939±0.185	95.021±1.091
8	200.223±2.907	2.312±0.330	96.406±3.903
9	195.147±4.096	2.011±0.023	95.987±5.001

Table 4. Result data of lining-up score.

Number	Hardness scores	Springiness scores	WHC scores	comprehensive scores
1	1.441	1.000	5.875	8.316
2	4.080	4.889	5.054	14.023
3	1.000	3.856	1.720	6.576
4	5.055	5.411	3.578	14.044
5	10.000	7.733	10.00	27.733
6	7.012	10.000	5.898	22.910
7	6.371	6.517	3.161	16.049
8	6.907	4.844	5.875	17.626
9	5.566	7.900	1.000	14.466

Table 5. The range value analysis of $L_9(3^4)$ Orthogonal experiment.

	Factors			
	TG Amount A(U/g)	pH B	T C(℃)	Time D(h)
K1	28.915	38.409	48.852	50.515
K2	64.687	59.382	42.533	52.982
K3	48.141	43.952	50.358	38.246
k1	9.638	12.803	16.284	16.838
k2	21.562	19.794	14.178	17.661
k3	16.047	14.651	16.786	12.749
Range	11.924	6.991	2.608	4.912

Table 6. The variance value analysis of $L_9(3^4)$ Orthogonal Experiment.

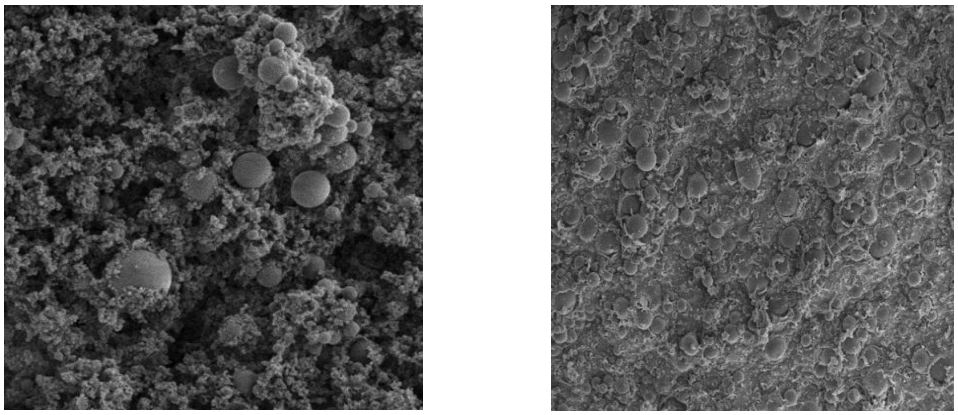
Factors	DEVSQ(sum of square of deviations)	DF(degree of freedom)	FR(F ratio)	FCV(F critical value)
TG Amount	213.672	2	2.474	4.46
pH	78.742	2	0.912	
T	11.492	2	0.133	
Time	44.529	2	0.481	
Range	345.440	8		

Lining-up score method [21]: For the No. i (i = 1,2, ..., 9) test, Xmax represents the best, in the first place, marked 10 scores; Xmin means the worst, in the last, marked 1 score; for the remaining number of other indicators points, according to proportion of their degree of difference between the outstanding value of the index, namely:

$$\Delta X = (X_{max} - X_{min}) / 9;\ A\ (score) = (X_{max} - X_i);\ final\ score = 10\text{-}A;$$

Then the sum of each number test score for all indicators was comprehensive scores in table 4.

TG has the potential to improve gelation through the formation of covalent intra-and intermolecular bonds. Covalent bonds, such as disulfide and ε-(γ-Glu)-Lys bonds, can restrict the flow of protein chains, thereby enhancing the elasticity and hardness of the network [22]. Orthogonal test results in table 5 showed primary and secondary relationship of the four factors on the impact of the emulsion were A> B> D> C, that is the amount of TG impacts the most, and temperature the least. Optimal conditions established by the test results for the main parameters $A_2B_2C_3D_2$, namely transglutaminase catalyzed emulsion gel processing as follows: enzyme dosage 20 U/g, pH 7.5, temperature 50℃, reaction time 3 h. By variance analysis in table 6, the main factor was enzyme dosage, consistent with the range analysis in table 5. Be verified experimentally, the measured value of the gel strength of 211.927±2.433 g; gel elasticity was 2.018± 0.234, WHC was 98.357± 5.903%.



(a) the control (b) TG

Figure 13. Microstructure of TG-WPI emulsion gel observed by scanning electron microscopy. a: 8% (w/v) whey protein isolate emulsions, pH 7.5, heated at 90℃ for 30 min b:enzyme additives 20 U/g, pH 7.5, temperature 45℃ and heating-time 3 h, then heated at 90℃ for 30 min.

[a]Both the micrographs was 4000×magnified.

3.6. The microstructure of TG emulsion gels

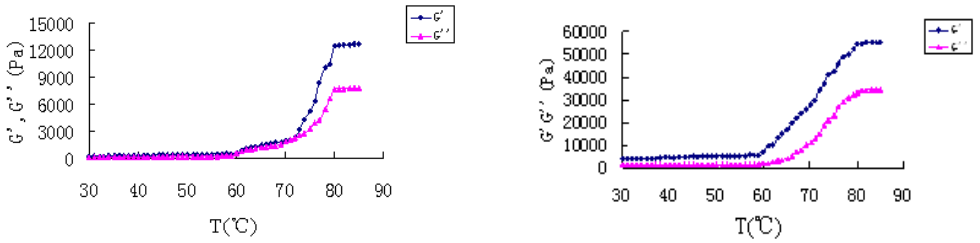
Electron micrographs were taken using SEM in order to get insight into the structure of emulsion gel. The optimum conditions were tested rather than the full experimental design. Scanning electron microscopy (SEM) showed that there were differences in the microstructure of the control and experimental gel (figures 13(a) and 13(b)). The differences were mainly associated with the compactness of the protein matrix and the size of the void spaces containing the aqueous portion of the gel. Compared to the control (figure 13(a)), the network structure is more compact, led to emulsion gels with higher density and less porosity, more uniform and smoothly distribution, smaller particle size of droplets.

3.7. Rheology

Rheological properties of gels can be described by the storage modulus (G ') and loss modulus (G' '). When the G ' value is much greater than G' ' values, indicating the status of the sample exhibits solid-like behaviour with high elasticity; the performance characteristics similar to liquid (G' '>G '), on the contrary, has a high viscosity [23].

The control WPI emulsion gel (figure 14(a)) the maximum G 'values and G "values were 12670.6 Pa and 7840.8 Pa. TG catalyzed WPI forming emulsion gel (figure 14(b)), G ' value and G " values were increased by 334.85% and 338.81%, which implied that TG-WPI emulsion gels were more compact than WPI emulsion gels, this agreed with the micrographs (figure 13).

The gel point was about at 70℃ (figure 14a), probable due to the denatured temperature of whey protein was over 65℃. Although not the true definition, the gel point represented quite accurately the transition point from what was perceived to be a liquid to a more solid-like material. Gel point temperatures were lower for samples that had more extensive cross-linking [24]. The control of G ' increased rapidly at 60℃(figure 14(b)), the gel point decreased by 10℃ compared to figure 14(a), which showed the gel point temperature was decreased, indicating that TG enhanced the ability of the emulsion solutions forming gel.



(a) control (b) TG

Figure 14. The temperature ramps of WPI emulsion gel. a: 8% (w/v) whey protein emulsions, pH 7.5; b:enzyme additives 20U/g, pH 7.5, temperature 50℃ and heating-time 3 h.

4. Conclusion

This study demonstrates that the optimum condition of forming emulsion gels catalyzed TG, enzyme dosage 20 U/g, pH 7.5, temperature 50℃, reaction time 3 h. Emulsion gels with high G ' and G " and good water-holding capacity were obtained. SEM show TG leads to changes in the structure of emulsion gels from a particulate to a mixed-type gel, where both fine-stranded and random aggregates are found. TG-WPI emulsion results in emulsion gels of unique properties that can be tailored to specific food applications.

Acknowledgment

The authors gratefully acknowledge the Ministry of Science and Technology of China (Project #2013BAD18B07-03) for funding this work. Thanks are also due to Yiming Biological Products Co. Ltd for providing enzyme samples.

References

[1] Schokker E P, Singh H and Creamer L K 2000 Heat-induced aggregation of β-lactoglobulin A and B with α-lactalbumin *International Dairy Journal* **10** 843-53
[2] Sawyer L and Kontopidis G 1999 β-lactoglobulin–A three-dimensional perspective. *International Journal of Food Science and Technology* **34** 409-18
[3] Han Xue and Sun Bing 2003 The functions and main applications of whey protein *China Dairy Industry* **3** 28-30
[4] Singh H 1991 Modification of food proteins by covalent cross linking *Trends in Food Science and Technology* **2** 196-200
[5] Mangino M E 1992 Gelation of whey protein concentrates *Food Technology* **46** 114-8
[6] Motoki M and Seguro K 1998 Transglutaminase and its use for food processing *Trends Food Sci Technol* **9** 204-10
[7] Kuraishi C, Yamazaki K and Susa Y 2001 Transglutaminase: Its utilization in the food industry *Food Rev Int* **17** 221-46
[8] Liu, M X and Damodaran S 1999 Effect of transglutaminase-catalyzed polymerization of β casein on its emulsifying properties *Journal of Agricultural and Food Chemistry* **47** 1514-9

[9] Eissa A S，Bisram S，Khan S A 2004 Polymerization and gelation of whey protein isolates at low pH using transglutaminase enzyme *J Agric Food Chem.* **52** 4456-64
[10] Truong V D, Clare D A, Catignani G L and Swaisgood H E 2004 Cross-linking and rheological changes of whey proteins treated with microbial transglutaminase *J Agric Food Chem.* **52** 1170-6
[11] Gauche C, Tomazi T, Barreto P L M and Bordignon-Luiz M T 2009 Physical properties of yoghurt manufactured with milk whey and transglutaminase *LWT–Food Science and Technology* **42** 239-43
[12] Xin G U, Lydia J. Campbell and Stephen R Euston 2009 Influence of sugars on the characteristics of glucono-δ-lactone-induced soy protein isolate gels *Food Hydrocolloids* **23** 314-26
[13] Vale´rie Leung Sok Line, Gabriel E. Remondetto and Muriel Subirade 2005 Cold gelation of β-lactoglobulin oil-in-water emulsions *Food Hydrocolloids* **19** 269-78
[14] Chin K B, Goa M Y and Xiong Y L 2009 Effect of soy protein substitution for sodium caseinate on the transglutaminate-induced cold and thermal gelation of myofibrillar protein *Food Research International* **4** 1-8
[15] Li J, Ould Eleya M M and Gunasekaran S 2006 Gelation of whey protein and xanthan mixture: Effect of heating rate on rheological properties *Food Hydrocolloids* **20** 678-86
[16] Morr C and Ha E W 1993 Whey protein concentrates and isolates processing and functional: Properties critical reviews *Food Sci Nutr.* **33** 431-76
[17] Dickinson E 1997 Enzymatic crosslinking as a tool for food colloid rheology control and Interfacial stabilization *Trends Food Sci Technol.* **8** 334-49
[18] Nieuwenhuizen W F, Dekker H L, Koning L J, Gro neveld T, Koster C G and Jong G A H 2003 Modification of glutamine and lysine residues in holo and apo-a-lactalbumin with microbial transglutaminase *J Agric Food Chem.* **51** 7132-9
[19] Han Cui-ping, Huo Gui-cheng, Duan Cui-cui 2009 Study on texture characteristic of whey protein polymerized by MTGase with different temperature and amount of MTGase *China Dairy Industry* **33** 28-30
[20] Tang Chuan-he,Yang Xiao-quan, Peng Zhi-ying 2002 Polymerization of whey protein catalyzed by microbial transglutaminase *China Dairy Industry* **30** 11-4
[21] Yuan Yufeng 2005 Analysis of multi-target orthogonal experiment *Journal of Hubei Automotive Industries Institute* **19** 53-6
[22] Chanyongvorakul Y, Matsumura Y, Nonaka M, Motoki M and Mori T 1995 Physical properties of soy bean and broad bean 11S globulin gels formed by transglutaminase reaction *Journal of Food Science* **60** 483-8
[23] Vale´rie Leung Sok Line, Gabriel E. Remondetto and Muriel Subirade 2005 Cold gelation of β-lactoglobulin oil-in-water emulsions *Food Hydrocolloids* **19** 269-78
[24] Wilcox C P and Swaisgood H E 2002 Modification of the rheological properties of whey protein isolate through the use of an immobilized microbial transglutaminase *J. Agric. Food Chem.* **50** 5546−51